Trends in Plant Science

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Feature Review

Cysteine-rich receptor-like protein kinases: emerging regulators of plant stress responses

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Cysteine-rich receptor-like kinases (CRKs) belong to a large DUF26-containing receptor-like kinase (RLK) family. They play key roles in immunity, abiotic stress response, and growth and development. How CRKs regulate diverse processes is a long-standing question. Recent studies have advanced our understanding of the molecular mechanisms underlying CRK functions in Ca^{2+} influx, reactive oxygen species (ROS) production, mitogen-activated protein kinase (MAPK) cascade activation, callose deposition, stomatal immunity, and programmed cell death (PCD). We review the CRK structure–function relationship with a focus on the roles of CRKs in immunity, the abiotic stress response, and the growth–stress tolerance tradeoff. We provide a critical analysis and synthesis of how CRKs control sophisticated regulatory networks that determine diverse plant phenotypic outputs.

Highlights

Cysteine-rich receptor-like kinases (CRKs) are evolutionarily conserved DUF26-containing receptor-like kinases (RLKs).

CRKs regulate pattern-triggered immunity and effector-triggered immunity by modulating reactive oxygen species (ROS) production, Ca^{2+} influx, mitogen-activated protein kinase (MAPK) cascade activation, phytohormone signaling, and callose deposition.

CRKs control abiotic stress response and the stress–growth balance.

Future research using CRISPR, inducible systems, single-cell omics, post-translational modification analysis, and proximity-labeling proteomics will advance our understanding of plant CRKs.

CRKs are evolutionarily conserved RLKs

CRKs belong to a large RLK family containing many evolutionarily conserved members in vascular plants but not in bryophytes and algae [1]. Forty-four CRKs in *Arabidopsis thaliana* (arabidopsis) and 1074 CRKs from 14 crops have been found through multi-omic and molecular genetic analyses, but only 63 CRKs have been shown to function in regulating plant immunity, abiotic stress (e.g., salinity, osmosis, oxidation, and heat) responses, and growth and development [2–21] (Figure 1, and Table S1 in the supplemental information online). Specifically, CRKs are involved in regulating Ca^{2+} influx, ROS homeostasis, MAPK cascade activation, and callose deposition, thereby modulating stomatal closure, pathogenesis-related (*PR*) gene expression, and PCD (Figure 1).

Most CRK genes localize in tandem arrays on chromosomes

During evolution, *CRK*s have expanded lineage specifically through relatively recent tandem duplication and preferential retention of duplicates following whole-genome duplication [1]. Some *CRK*s share a high level of sequence similarity and localize in tandem arrays on chromosome, as found in arabidopsis [22], pepper (*Capsicum annuum*) [11], cotton (*Gossypium barbadense*) [13], soybean (*Glycine max*) [15], and a halophyte pasture alkaligrass (*Puccinellia tenuiflora*) [21] (Figures 2A and S1A in the supplemental information online). The tandem duplications of *CRK* evolved mainly through unequal crossover or homologous recombination events [1]. These tandem repeats of *CRK*s have been suggested to correlate with stress responses to facilitate stress-adaptive evolution because most clustered *CRK*s function in adaptation to environmental stimuli and pathogen infection [1,11,23]. An interesting question concerns whether tandem expansion drives the evolution of *CRK* functional diversification such as subfunctionalization and neofunctionalization. Answering this question requires a large-scale functional analysis of *CRK* members. However, only 42 *CRKs* located in tandem repeats have been characterized (Table S1). How many *CRKs* are functionally redundant or unique through point mutations, chromosomal deletions, and promoter regulatory elements still needs to be established.

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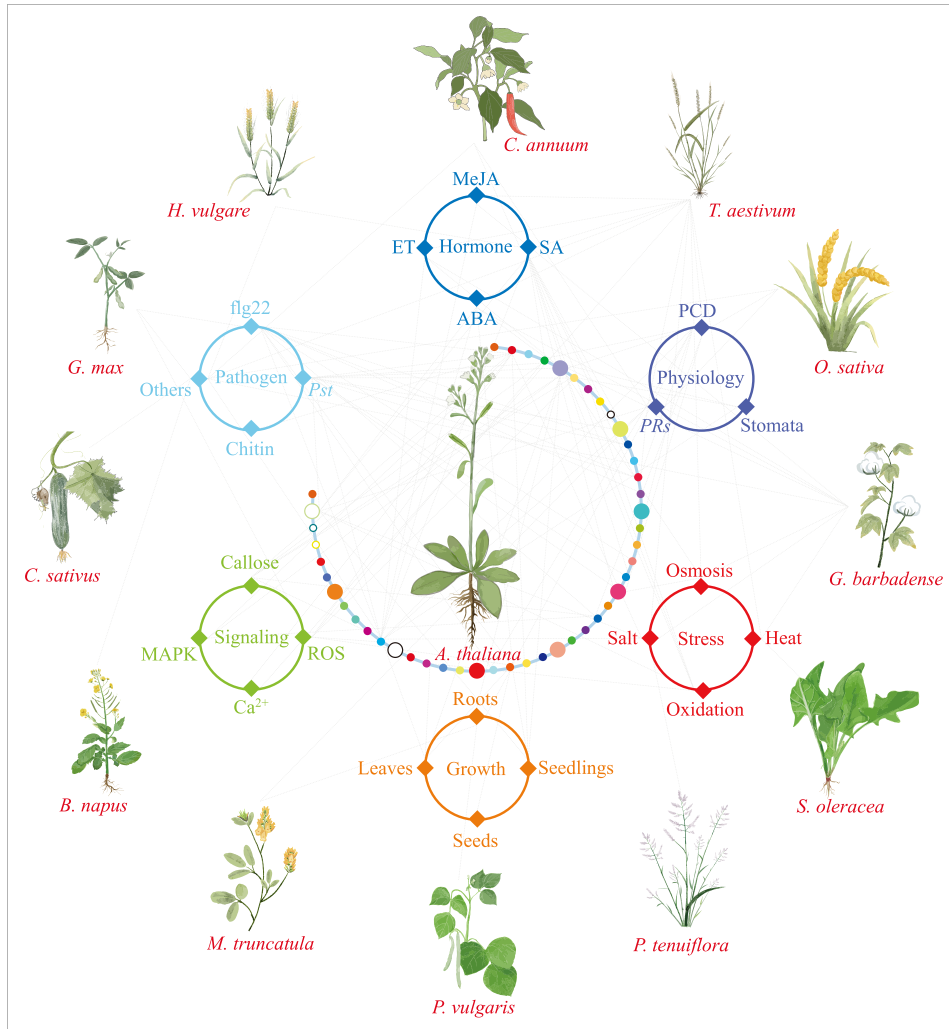

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Figure 1. Schematic diagram of the regulatory roles of cysteine-rich receptor-like kinases (CRKs) from arabidopsis and 12 other plant species. The central string of circles represents arabidopsis CRKs. Colored circles indicate the 41 typical CRKs, three colored open circles indicate the cytoplasm-localized CRKs (CRK43, CRK44, and CRK45), and two black open circles represent a truncated CRK9 and a pseudogene CRK35. The six circles with diamonds around the central circle show the reported functions of CRKs, including regulation of growth and development, modulation of signaling pathways, hormone response, pathogen defense, stress tolerance, and adjustment of various physiological and cellular processes. The gray lines linking the colored circles or plant species with the diamonds on the six circles indicate the regulatory roles of CRKs from arabidopsis and 12 other plant species. Abbreviations: ABA, abscisic acid; arabidopsis, *Arabidopsis thaliana*: *B. napus*, *Brassica napus*; *C. annuum*, *Capsicum annuum*; *C. sativus*, *Cucumis sativus*; ET, ethylene; *G. barbadense*, *Gossypium barbadense*; *G. max*, *Glycine max*; *H. vulgare*, *Hordeum vulgare*; *M. truncatula*, *Medicago truncatula*; MAPK, mitogen-activated protein kinase; MeJA, methyl jasmonate; *O. sativa*, *Oryza sativa*; PCD, programmed cell death; *PRs*, pathogen-related genes; *Pst, Pseudomonas syringae* pv. tomato; *P. tenuiflora*, *Puccinellia tenuiflora*; *P. vulgaris*, *Phaseolus vulgaris*; ROS, reactive oxygen species; SA, salicylic acid; *S. oleracea*, *Spinacia oleracea*.

CRKs belong to a DUF26-containing protein family with distinct domains

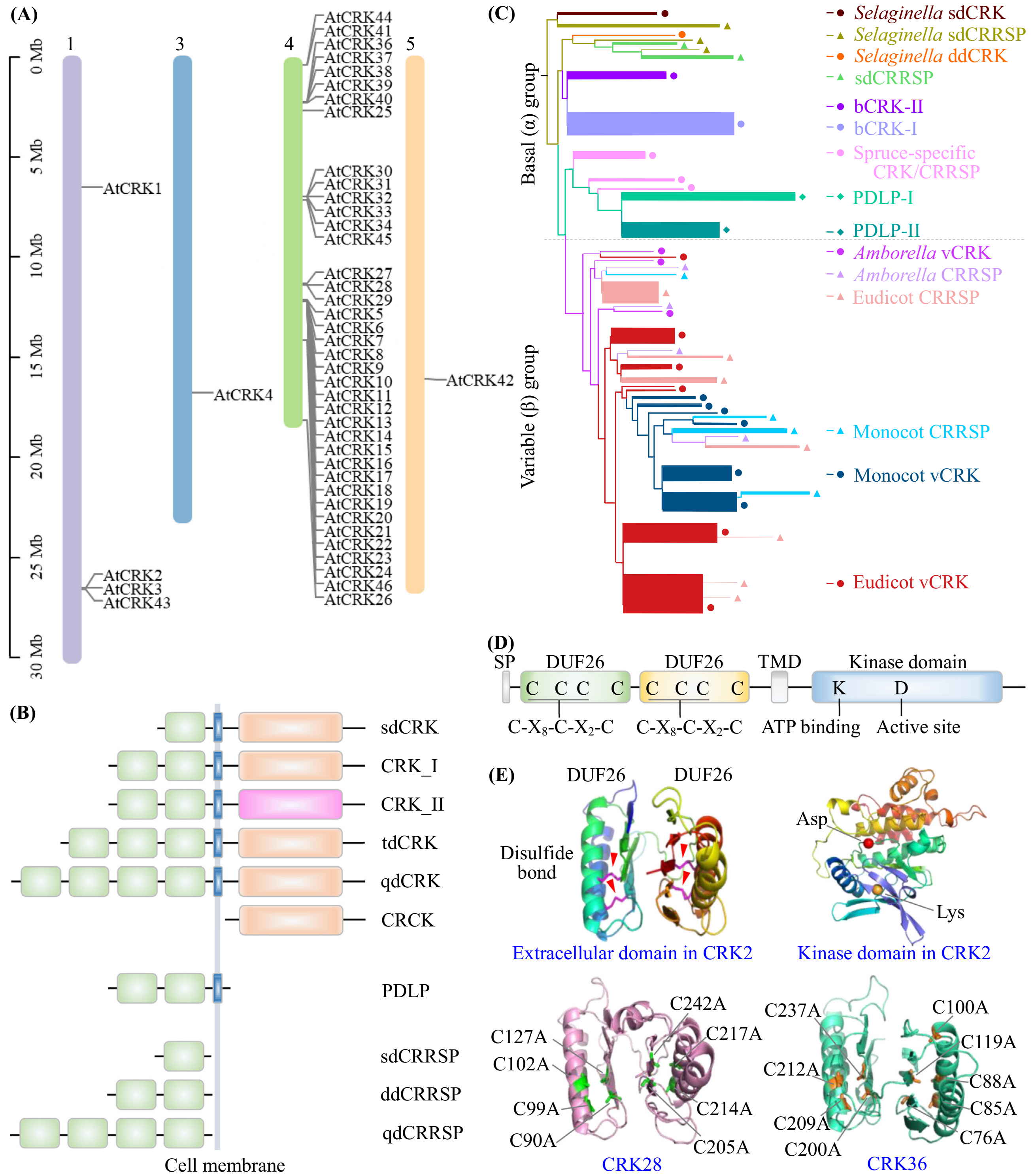
CRKs contain an extracellular domain, a transmembrane domain (TMD), and an intracellular serine/threonine kinase domain (the classic RLK structure) [24]. The extracellular domain is composed of one to four DUF26 (domain of unknown function 26) motifs [1,22]. Most CRKs contain

two DUF26s in the extracellular region, whereas CRKs from *Selaginella* uniquely have a single DUF26, and a few CRKs from eudicots contain three to four DUF26 domains (Figure 2B). In arabidopsis, most CRKs (41 out of 44) have two DUF26s [22,25]. CRK43, CRK44, and CRK45 have no DUF26 or TMD. They are localized in the cytoplasm and may physically interact with other plasma membrane (PM)-localized CRKs [22].

In addition to CRKs, DUF26 is also a crucial domain in cysteine-rich receptor-like secreted proteins (CRRSPs) and plasmodesmata-localized proteins (PDLPs) (Figure 2B). CRKs, CRRSPs, and PDLPs form a large DUF26-containing protein family, and their phylogeny is highly intermixed [1] (Figure 2C). All DUF26-containing proteins are divided into two distinct groups: a basal group and a variable group [1]. The basal group is more conserved and includes *Selaginella* CRKs, a monophyletic group of CRKs from gymnosperms and angiosperms, and spruce-specific CRKs. The variable group is less conserved and includes angiosperm variable CRKs (i.e., two eudicot-specific subgroups and one monocot-specific subgroup) (Figure 2C). The CRKs might originate from fusing a single DUF26-containing CRRSP with LRR_clade_3 RLKs containing only TMD and a kinase domain in a common ancestor of vascular plants [1,26]. A CRRSP Gnk2 from ginkgo (*Ginkgo biloba*) and two maize (*Zea mays*) proteins (AFP1 and AFP2) can bind to mannose for defense against fungal pathogens [27,28], whereas PDLPs are crucial for cell-to-cell trafficking [29,30], callose deposition [31], and pathogen response [32]. In addition, the wheat peptide harboring two DUF26s, *Ta*CRK3, has direct antifungal activity and inhibits mycelial growth of *Rhizoctonia cerealis* in the culture medium [33]. This implies that plant DUF26-containing proteins are crucial for the response to fungal pathogens, but the extracellular ligands and precise biochemical functions of DUF26 have not been elucidated [1].

The Cys residues form inter/intramolecular disulfide bonds for protein structural stability [1,34], and probably serve as switches that modulate the functions of some CRKs [35,36]. The conserved Cys residues in each DUF26 are usually located in the C-X8-C-X2-C motif [22] (Figure 2D), but their precise roles in modulating CRK structure and activity are not entirely clear. DUF26 Cys residues have long been proposed as targets for ROS sensing or redox regulation [22,37]. Single Cys-to-Ala mutations of *At*CRK28 DUF26 (i.e., C99A, C127A, C214A, or C242A) completely abolish *At*CRK28-mediated cell death in *Nicotiana benthamiana*, even though protein stability is not affected [38] (Figure 2E). In addition, hypersensitive cell death lesions and ROS production in arabidopsis are enhanced in *CRK36*-overexpressing plants but not in *CRK36*$^{C76/85/88/100/119A}$- or *CRK36*$^{C200/209/212/237A}$- overexpressing plants when exposed to either the necrotrophic pathogen *Alternaria brassicicola* or avirulent *Pseudomonas syringae* pv. tomato DC3000 (*Pst* DC3000) *AvrRpm1* [39]. However, the crystallographic structure of the extracellular regions of two proteins (PDLP5 and PDLP8) that are closely related to CRKs shows that all Cys residues for disulfide bond formation are not solvent-exposed but are buried inside the lectin fold [1]. This implies that the extracellular region in CRKs could form a lectin structure for signal perception. Therefore, studies on Cys exchanges with other amino acids, such as Ala [38,39], may not provide direct evidence for redox regulation and might instead reflect changes in protein folding and conformation [1]. Moreover, it is unclear whether Cys mutations in CRK28 and CRK36 affect protein subcellular localization [38,39]. Additional experiments to clarify the functions of the Cys residues in CRKs are needed.

The TMD affects CRK subcellular localization. Most CRKs are localized in the PM, whereas wheat *Ta*CRK2 and barley *Hv*CRK1 are localized to the endoplasmic reticulum (ER) [19,40]. It is speculated that proteins with a shorter hydrophobic TMD localize to the ER, whereas proteins with a longer TMD associate with the PM [41]. The TMDs of *Hv*CRK1 and *Ta*CRK2 consist of 17



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and 19 amino acids, respectively, which are less than the 23 amino acids in the TMD of PM-localized *At*CRK6 [19,40]. Moreover, the specific amino acid composition of TMD is also implicated in determining protein localization [42]. When the highly hydrophobic LVL motif in the TMD was replaced by the less hydrophobic AAA, the localization of PDLP1a changed from the PM to the ER [42]. *Hv*CRK1 and *Ta*CRK2 contain the less hydrophobic AAA and YLW motifs at the TMD C terminus, whereas the LVG in *At*CRK6 is more hydrophobic [19,40]. In addition, the R/K-rich motif following the TMD is an ER localization signal [43]. The RRLR motif in *Hv*CRK1 and the RKAR motif in *Ta*CRK2 adjacent to the TMDs are predicted to be a noncanonical arginine-based ER retention signal for directing protein to the ER [19,40]. It has been demonstrated that mutations in the TMD hydrophobic YLW (Y323V) and RKAR motifs (R326A/R329A and R326A/K327A/R329A) affect *Ta*CRK2 localization to the ER [40].

The typical protein kinase domain has 11 conserved kinase subdomains [44]. The conserved Lys in subdomain II for ATP binding and Asp in subdomain VII for catalysis are crucial for CRK activity [45] (Figure 2D). Mutation of the conserved Lys to Glu ($CRK2^{K353E}$) or Asp to Asn ($CRK2^{D450N}$) ablates the kinase activity of CRK2 *in vitro*, but these mutations do not alter CRK2 stability and subcellular localization [6,45]. Expression of these kinase-inactive CRK2 variants in the *crk2* mutant does not restore the growth defects of the mutant [45]. Similarly, substitution of the conserved K by E in CRK36 ($CRK36^{K386E}$) or by N in CRK28 ($CRK28^{K377N}$) attenuates their functions in regulating stomatal closure, cell death, and growth and development [38,39]. How other amino acid residues in the subdomains affect CRK functions remains unclear.

In summary, although the domain structures of CRKs have been well defined, our understanding of their functional diversity is far from complete. The TMD domain and signal peptides are certainly relevant to the subcellular localization of CRKs. Based on limited information [1,28], the lectin fold may directly bind to and sense different ligands/signals, thereby playing a role in different pathways and processes. The development of AlphaFold [46] provides a good opportunity to predict the conformations of more CRKs and to better understand their functions.

CRKs function in plant immunity

The different, coordinated, and redundant functions of CRKs in plant immune responses have mainly been studied in the model plant arabidopsis using reverse genetics and phenotyping [22,38,45,47]. Most *AtCRK* genes are clustered on chromosome 4, which may facilitate the adaptive evolution of novel specificity, subfunctionalization, or coordinated gene expression [22,38]. Because loss-of-function mutants of single *CRK* genes have inconspicuous phenotypes, *CRK* higher-order mutants and *CRK*-overexpressing lines are used for functional characterization [34,38,39,47–51]. Owing to the lack of genetic resources and stable transformation systems, CRKs in several crops have been characterized using various transient expression systems. For instance, transient-induced gene silencing (TIGS) analysis indicates that barley *HvCRK1* (an

Figure 2. Chromosomal distribution, domain composition, evolution relationship, and 3D structure of cysteine-rich receptor-like kinases (CRKs). (A) Chromosomal distribution of *Arabidopsis thaliana CRK* genes. The chromosome numbers are indicated at the top of the columns. Gray lines indicate segment- and tandem-duplicated genes. (B) Domain composition of plant proteins containing DUF26. The DUF26 domain is shown in green, the transmembrane domain (TMD) in blue, and the kinase domain in orange/pink); sd (single domain), dd (double domain), td (triple domain), and qd (quadruple domain) refer to the number of DUF26 domains. (C) Sketch map of the evolutionary relationship between DUF26-containing CRRSPs, CRKs, and PDLPs. The color and thickness of the lines indicate the different families and relative protein numbers, respectively. The triangles, spheres, and diamonds represent different CRRSPs, CRKs, and PDLPs, respectively. (D) Domain composition of CRK showing cysteine sites, ATP-binding sites, and the active site. (E) General domain architecture and 3D structure of CRKs. The red arrows indicate the disulfide bonds in CRK2. The conserved ATP-binding Lys residues and Asp residues in the catalytic domain are crucial for the kinase activity of CRK2. When Cys residues are mutated to Ala, the disulfide bonds are disrupted in CRK28 and CRK36. Abbreviations: CRRSPs, cysteine-rich receptor-like secreted proteins; DUF, domain of unknown function; PDLPs, plasmodesmata-localized proteins; SP, signal peptide.

AtCRK6 homolog) is a crucial negative regulator for defense against powdery mildew pathogen *Blumeria graminis* f. sp. *hordei* (*Bgh*) [19]. In addition, virus-induced gene silencing (VIGS)-based analyses show that four wheat *CRK*s (i.e., *TaCRK2*, *TaCRK3*, *TaCRK7A*, and *TaCRK10*), pepper *CaCRK5*, and cotton *GbCRK18* are required for plant defense against diverse pathogens [8,11,13,33,40,52]. Furthermore, quantitative trait locus (QTL) mapping reveals that two cucumber *CsCRK*s (*Csa1M064780* and *Csa1M064790*) [12], wheat *TaCRK6* (*TaStb16q*) [53], and several *CRK* candidates in oilseed rape [18] may play potential roles in the defense response to powdery mildew, *Septoria tritici* blotch, and blackleg. However, the molecular mechanisms underlying CRK functions in plant immune responses remain largely unexplored.

Plants have developed pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) in their defense against microbial infections. PTI is triggered by pathogen-/microbe-associated molecular patterns (PAMPs/MAMPs) which are recognized by surface-localized pattern-recognition receptors (PRRs). ETI is initiated by plant resistance (R) proteins after recognizing pathogen effectors [54–56]. The characteristics of PTI include a series of physiological and cellular structural changes, such as rapid Ca^{2+} influx, apoplastic ROS burst, activation of Ca^{2+}-dependent protein kinase (CPK) and MAPK cascades, alteration of defense hormone networks, callose deposition, stomatal closure, and extensive transcriptional, translational, and metabolic reprogramming for defense [57–60]. By contrast, ETI usually induces a stronger and more prolonged immune response than PTI, often leading to a hypersensitive response (HR) associated with PCD at the infection site to restrict pathogen spread [60]. HR can induce a systemic acquired resistance (SAR) which exhibits plant-wide, long-lasting, and broad-spectrum resistance to subsequent pathogen infection [61]. Historically, PTI and ETI have been regarded as binary or zig-zag processes [62,63]. In reality, PTI and ETI share central hubs of signaling including ROS burst, MAPK cascades, and activation of *PR* genes [64,65]. Accumulating evidence indicates that CRKs are involved in regulating both PTI and ETI responses.

CRKs associate with PRRs in the PTI process

PRRs are pivotal cell-surface receptors that trigger PTI responses upon recognition of PAMPs [66]. The LRR-RLK FLAGELLIN-SENSITIVE 2 (FLS2) is a well-characterized PRR, and it can recognize the conserved bacterial flagellin peptide flg22 [67]. Upon flg22 perception, FLS2 recruits another LRR-RLK BRASSINOSTEROID INSENSITIVE 1 (BRI1)-associated kinase 1 (BAK1, also called SERK3), and they form a heterodimer [68–71]. Malectin-like LRR-RLK IMPAIRED OOMYCETE SUSCEPTIBILITY 1 (IOS1) can promote the FLS2–BAK1 association [72], which subsequently phosphorylates and activates their downstream targets such as receptor-like cytoplasmic kinases (RLCKs) including BOTRYTIS-INDUCED KINASE 1 (BIK1) [39,73,74] (Figure 3A). PRRs constitute a central hub of PM-localized multiprotein complexes for timely PTI responses [34,75]. A subset of CRKs interacts with the PRR FLS2. They are proposed to be potential components of the PRR complex for recognizing flg22 [34,38,39]. Specifically, PM-localized CRK4, CRK6, and CRK36 associate with the PRR FLS2 in a ligand flg22-independent manner, and overexpression of these *CRK*s enhances flg22-triggered ROS production [34] (Figure 3A). CRK36 also directly interacts with BIK1 to enhance flg22-triggered BIK1 phosphorylation [39] (Figure 3A). In addition, CRK28 is associated with BAK1 or FLS2 in a ligand-independent manner which is also independent of the kinase activity of CRK28 [38]. Importantly, the CRK28–FLS2–BAK1 module forms a PRR immune complex in a flg22-dependent manner [38] (Figure 3A). Therefore, CRKs are implicated in contributing to the structural stability and phosphorylation cascade of the PRR FLS2 complex. However, whether other CRKs form PRR complexes and the nature of the downstream signaling partners and the dynamics of the complexes remain to be investigated. Notably, CRK28 self-associates to form homodimers, or associates with its closely related CRK29 to form heterodimers [38] (Figure 3A). It will also be interesting to investigate

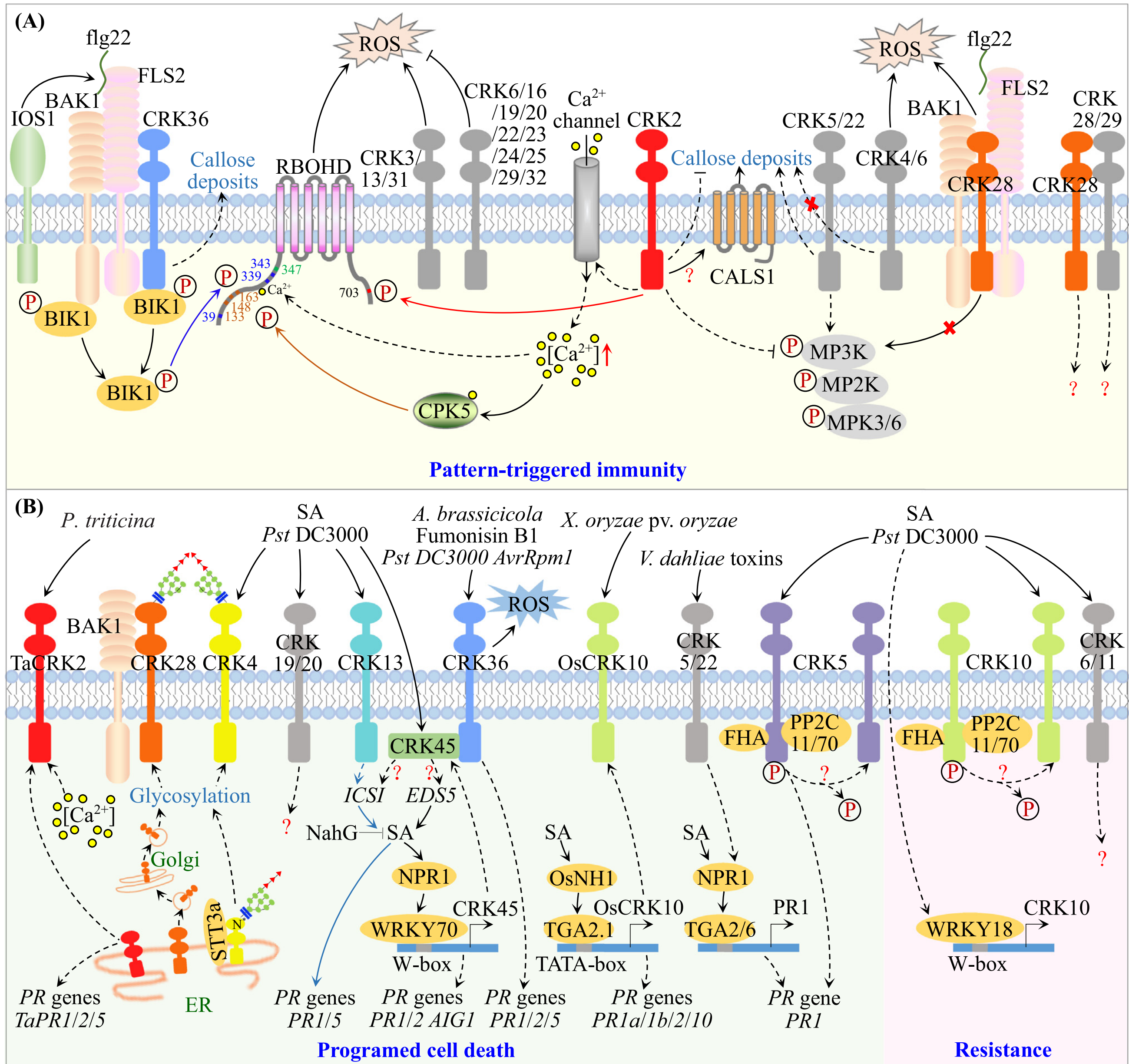

Figure 3. Schematic model for cysteine-rich receptor-like kinase (CRK) function in plant immunity. (A) CRK interacts with other RLKs to form PRR immune complex upon pattern-triggered immunity (PTI). Upon flg22 perception, CRK36 enhances the function of the FLS2–BAK1–BIK1 module to phosphorylate the N terminus of RBOHD for apoplastic ROS production. The CRK28–FLS2–BAK1 module works as a PRR immune complex in a flg22-dependent manner to trigger ROS accumulation. CRK28 can associate with another CRK28 or CRK29 to form homodimers or heterodimers, respectively. CRK2 directly phosphorylates the C terminus of RBOHD at Ser^{703} for ROS production. Ca^{2+} influx into the cytosol and the Ca^{2+}-dependent CPK activation is important for phosphorylating the N terminus of RBOHD. In addition, CRK2 mediates the inhibition of the MAPK cascade and callose deposition, possibly via CALS1. Three CRKs (CRK3, CRK13, and CRK31) induce apoplastic ROS elevation, whereas the other ten CRKs probably decrease ROS production. (B) CRK regulation of programmed cell death to defend against pathogens. CRK5, CRK6, CRK10, and CRK11 are induced by *Pst* DC3000 and SA. The W-box sequence region of CRK10 is recognized by WRKY18, and binding activity is induced by SA. CRK5 and CRK10 interact with PP2C70, PP2C11, and FHA, which probably alter CRK kinase activity by dephosphorylation. CRK19, CRK20, and *N*-glycosylated CRK4 are also induced by SA and pathogen *Pst* DC3000. *N*-glycosylated CRK28 interacts with BAK1 on PM for sensing flg22 and mediating programmed cell death. Wheat *Ta*CRK2 localized in the ER and PM positively regulates the HR cell death by modulating *PR* genes after *Puccinia triticina* infection. CRK13 promotes SA accumulation

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whether the dimerization of CRKs facilitates PRR complex formation for the rapid recruitment of downstream signaling components.

CRKs modulate the shared signaling hubs of PTI and ETI

ROS, as important signaling molecules, regulate plant development and stress responses. In plants, the PM-localized NADPH oxidase RBOH family functions in apoplastic ROS production. Arabidopsis RBOHD is a key player in the response to pathogen infection. It has been well characterized to be phosphorylated by CRK2 [45] and several other kinases such as BIK1 [73,74] and CPKs [76]. For example, Ser^{39}, Ser^{339}, Ser^{343}, and Ser^{347} of RBOHD are phosphorylated by BIK1 [73,74], whereas Ser^{133}, Ser^{148}, Ser^{163}, and Ser^{347} are phosphorylated by CPK5 [76] (Figure 3A).

CRK2 regulates RBOHD activity by interacting with the cytosolic N-terminal or C-terminal regions of RBOHD [45] (Figure 3A). CRK2 phosphorylates N-terminal Ser^{8} and Ser^{39} and C-terminal Ser^{611}, Ser^{703}, and Ser^{862} residues of RBOHD [45]. Notably, only two of the five CRK2 phosphorylation sites in RBOHD (Ser^{703} and Ser^{862}) are crucial for ROS production. Specifically, phosphorylation of Ser^{703} and Ser^{862} by CRK2 positively and negatively regulates RBOHD activity, respectively [45]. Phosphoproteomic analysis has revealed that phosphorylation of Ser^{703} and Ser^{39} in RBOHD is enhanced in arabidopsis after flg22 treatment for 5 minutes [45]. Further genetic analyses have shown that CRK2 mediates flg22-induced phosphorylation at Ser^{703} of RBOHD (Figure 3A) [6,45]. ROS production is decreased in both the *crk2* and *bik1* mutants, implying that CRK2 and BIK1 may synergistically regulate RBOHD phosphorylation for apoplastic ROS production [45] (Figure 3A). In addition to CRK2, CRK36 interacts with BIK1 and FLS2, which is also required for RBOH-mediated ROS production [34,39] (Figure 3A). RBOHD/F-mediated ROS production, flg22-induced stomatal closure, and resistance to *Pst* DC3000 are enhanced in *CRK36*-overexpressing plants but are compromised in the *crk36* mutant [39]. The CRK36–BIK1–RBOHD/F module is proposed to form a feedback activation loop that mediates rapid and transient ROS production during stomatal immunity. Similarly to *CRK36*, overexpression of *CRK4* and *CRK6* also primes ROS production upon flg22-triggered PTI, but the regulatory mechanism is unclear [34] (Figure 3A). In addition, CRK28-mediated ROS accumulation is specifically induced by treatment with flg22 but not with chitin. This suggests that CRK28 enhances the ROS burst in response to specific pathogens [38].

CRK-mediated ROS production via RBOHD phosphorylation is a finely tuned process. Only CRK2 has been shown to phosphorylate RBOHD [45], but whether RBOHD can be phosphorylated by other CRKs requires further study. In some *crk* mutants, ROS production is obviously perturbed under flg22 treatment, with increases in ROS production in 11 mutants (i.e., *crk6*, *crk16*, *crk19-2*, *crk20*, *crk22*, *crk23-1*, *crk23-2*, *crk24*, *crk25*, *crk29*, and *crk32*) and decreases in four mutants (i.e., *crk2*, *crk3*, *crk13*, and *crk31*) [22] (Figure 3A). The reverse genetics data from the aforementioned 11 mutants indicate that these CRKs may be negative regulators,

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by inducing *ICS1* and *PR* genes to cope with pathogens. Cytoplasm-localized CRK45 interacts with PM-localized CRK36, thus positively regulating plant resistance to pathogens and SA. Pathogen-induced SA accumulation promotes NPR1 translocation, triggering *WRKY70* expression and subsequently enhancing *CRK45* transcription. Abbreviations: *A. brassicicola*, *Alternaria brassicicola*; AIG1, AvrRpt2-induced gene 1; BAK1, BRI1-associated receptor kinase 1; BIK1, Botrytis-induced kinase 1; CALS1, callose synthase 1; CPK5, calcium-dependent protein kinase 5; EDS5, enhanced disease susceptibility 5; ER, endoplasmic reticulum; FHA, FHA domain-containing protein; flg22, bacterial flagellin peptide 22; FLS2, flagellin-sensing 2; Golgi, Golgi apparatus; HR, hypersensitive response; ICS1, isochorismate synthase 1; IOS1, impaired oomycete susceptibility 1; MPK, mitogen-activated protein kinase; MP2K, mitogen-activated protein kinase kinase; MP3K, mitogen-activated protein kinase kinase kinase; NahG, salicylate hydroxylase; NH1, NPR1 homolog 1; NPR1, nonexpressor of PR genes 1; P, phosphorylation; PM, plasma membrane; PP2C, protein phosphatase 2C; PR, pathogenesis-related protein; PRR, pattern-recognition receptor; *Pst*, *Pseudomonas syringae* pv. tomato; RBOHD, respiratory burst oxidase homolog protein D; RLK, receptor-like kinase; ROS, reactive oxygen species; SA, salicylic acid; STT3a, oligosaccharyl transferase subunit STT3A; TGA2.1, transcription factor TGA2.1; WRKY, WRKY DNA-binding protein; *X. oryzae* pv. *oryzae*, *Xanthomonas oryzae* pv. *oryzae*.

whereas the data from the other four mutants indicate that the CRKs may be positive regulators. Notably, *crk5* and *crk28* mutants exhibit normal ROS production but severe disease symptoms after *Pst* DC3000 infection, whereas *crk23* elevates flg22-induced ROS levels but does not improve defense against *Pst* DC3000. Enigmatically, *crk20* and *crk29* plants with elevated ROS levels are more susceptible to *Pst* DC3000 compared to the wild type (WT). Clearly, ROS levels in the *crk* mutants do not predict disease resistance.

Ca^{2+} is a transient cellular messenger, and an increase in cytoplasm Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) is a prominent trigger for initiating downstream immune responses [77]. $[Ca^{2+}]_{cyt}$ is rapidly induced in WT plants but is decreased in the *crk2* mutant [45]. This indicates that CRK2 is an essential component for controlling Ca^{2+} influx during the immune response (Figure 3A). In turn, CRK is probably regulated by calcium signals. The induction of *TaCRK2* transcription by *Puccinia triticina* in wheat is inhibited following treatment with the extracellular Ca^{2+} chelator EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid] [40]. This implies that *Ta*CRK2 functions in a Ca^{2+}-dependent manner in wheat defense against pathogens.

MAPK cascades are highly conserved in modulating multiple defense responses. MAPK activation is the earliest signal, even after sensing PAMPs in PTI and pathogen effectors in ETI [78]. CRK2 might be a negative regulator of the MAPK cascade because flg22-induced MAPK activation is enhanced in the *crk2* mutant [45]. Thus, CRK2 could be involved in fine-tuning the PAMP-induced MAPK signaling pathway (Figure 3A). However, flg22-induced expression of CRK28 does not significantly affect MAPK activation [38] (Figure 3A). In addition, CRK5 and CRK22 are involved in the activation of MPK3 and MPK6 to cope with *Verticillium dahliae* (Vd) toxins [79] (Figure 3A). This suggests that different CRKs differentially modulate MAPK signaling during the plant immune response.

Callose deposition between the PM and cell wall in epidermal cells, plasmodesmata, and vascular tissue can restrict pathogen ingression and spread [80]. Callose deposition is increased in *CRK36*-overexpressing plants irrespective of *Pst* DC3000 *hrcC*⁻ and flg22 treatments but is decreased in *crk36-2* mutants [34,39]. Similarly, callose deposition is increased in *CRK5*- and *CRK22*-overexpressing plants but decreased in their mutants upon Vd toxin treatment [79] (Figure 3A). By contrast, *CRK4* or *CRK6* overexpression has no effect on PTI-mediated callose deposition [34]. Notably, early callose deposition is enhanced in *crk2* mutants after 30 min of flg22 treatment, whereas callose deposits are comparable in the WT and *crk2* mutants at 12 h after flg22 treatment [45]. These results imply that CRK-mediated callose deposition is crucial for the late PTI process. Although it is reported that CRK2 interacts with callose synthase 1 (CALS1) under salt stress and phosphorylates it *in vitro* [6], the role of salt stress-triggered callose deposition and the molecular mechanism of CRK-mediated callose deposition are not completely understood.

Stomatal movement is modulated by diverse factors such as phytohormones [e.g., abscisic acid (ABA), methyl jasmonate (MeJA), brassinosteroid, salicylic acid (SA), cytokinin, and auxin], ROS, Ca^{2+} signaling, and osmotic homeostasis [22,81]. As an important part of PTI, stomatal closure prevents water loss and limits pathogen entry [82,83], but prolonged stomatal closure creates aqueous apoplasts, thus promoting colonization by pathogens [84,85]. Different members of the CRK family act as functionally diverse and/or specific regulators of stomatal immunity [22] (Figure S2 in the supplemental information online). Flg22-triggered stomatal closure is compromised in the *crk2*, *crk5*, *crk10-2*, *crk17*, *crk20*, and *crk28* mutants, resulting in increased susceptibility to *Pst* DC3000 [22,45]. Chitin-triggered stomatal closure is also retarded in the *crk2*, *crk6*, *crk10-2*, *crk10-4*, *crk12*, and *crk19-2* mutants, while only the *crk2* and *crk10-2* mutants exhibit

impaired stomatal closure in response to flg22 and chitin [22] (Figure S2). In addition, *CRK6* overexpression induces constitutive stomatal closure, whereas *CRK4* and *CRK36* overexpression counteracts *Pst* DC3000-mediated stomatal reopening in a coronatine (COR)-dependent manner [34,86] (Figure S2). *Pst*-induced stomatal reopening is completely abolished in *CRK36*-overexpressing plants but is more pronounced in the *crk36-2* mutant (Figure S2). Importantly, CRK36 phosphorylates BIK1 [39], and BIK1 subsequently phosphorylates RBOHD/F to trigger apoplastic ROS production [73,74] (Figure S3 in the supplemental information online). In the *bik1* and *rbohD/F* mutant backgrounds, CRK36-mediated stomatal closure and prevention of reopening are impaired in response to flg22 and *Pst* DC3000 treatments (Figure S2). These data suggest that CRK36 acts through the BIK1–RBOHD/F signaling module to positively regulate stomatal immunity [39].

Various phytohormones such as SA, MeJA, ethylene, and ABA play synergistic or antagonistic roles in plant pathogen immunity. Generally, SA signaling induces defense against biotrophic and hemibiotrophic pathogens, whereas MeJA and ethylene cooperatively activate resistance against necrotrophic pathogens [87]. Although ABA is known to function mainly in abiotic stress, the enhancement of ABA signaling correlates with susceptibility to disease caused by several plant pathogens [88]. The transcription levels of several *CRK*s in arabidopsis, wheat, and pepper are changed in response to exogenous SA [11,37,49–51,89], MeJA [8], ethylene [33], and ABA [90] (Figure 3B and Figure S3). In arabidopsis, nine *CRK* transcripts (i.e., *CRK 4*, *5*, *6*, *10*, *11*, *13*, *19*, *20*, and *45*) are induced upon SA treatment and pathogen infection [48–51,89] (Figure 3B). They all have a cluster of W-box elements in their promoter regions for binding WRKY transcription factors, and the binding of WRKYs to the W-box in the *CRK10* promoter is induced by SA [89] (Figure 3B). Importantly, SA-induced expression of *CRK5*, *CRK13*, and *CRK45* can promote downstream *PR1* gene expression [49–51], whereas overexpression of *CRK4*, *5*, *13*, *19*, *20*, and *22* triggers rapid PCD [48,51,79] (Figure 3B).

Compared to arabidopsis, wheat has a larger CRK family with 170 members. Most *TaCRK*s exhibit differential temporal expression patterns in response to different pathogens and phytohormones [8,9,33,52,90] (Figure S3). *TaCRK1* transcription is induced by ABA at 3 h and by MeJA at 12–24 h post-treatment, but is decreased by MeJA at 1–6 h post-treatment and is also downregulated after ethylene and SA treatments. This clearly shows the temporal dynamics of *TaCRK1* regulation in response to different hormones [90]. By contrast, *TaCRK3* is significantly induced by ethylene treatment, whereas *TaCRK3* silencing decreases the expression of ethylene biosynthesis and signaling genes in wheat, such as *ACO2* encoding an ethylene biosynthesis enzyme 1-aminocyclopropane-1-carboxylate (ACC) oxidase [33] (Figure S3). This suggests that *Ta*CRK3 may act as an upstream regulator of ethylene biosynthesis and signaling, and that the ethylene pathway in turn induces *TaCRK3* expression in a feedback regulation manner [33]. Furthermore, *TaCRK7A* is significantly induced after JA treatment, whereas in *TaCRK7A* knockdown wheat lines several JA-responsive genes are downregulated, such as *PR2*, *TaGluD*, and *TaChit1/3/4* [8] (Figure S3). This implies that the *TaCRK7A* regulatory loop triggered by JA is similar to that of *TaCRK3* by ethylene. In addition, *TaCRK10* is induced by SA, but is downregulated after ABA, MeJA, and ethylene treatments [52] (Figure S3). Silencing of *TaCRK10* attenuates the disease resistance of wheat cultivar 'XY6', whereas overexpression of *TaCRK10* in susceptible wheat variety 'Fielder' increases resistance by inducing the expression of *TaPR1* and *TaPR2* genes in the SA signaling pathway [52]. Upon *Puccinia striiformis* inoculation and high-temperature stress, *Ta*CRK10 physically interacts with and phosphorylates *Ta*H2A.1, and the phosphorylated *Ta*H2A.1 transfers into the nucleus for regulating immune-related gene expression (Figure S3). Histone modification has been reported to be involved in SA signal transduction in response to diverse pathogens [91], and silencing *TaH2A.1* can suppress wheat resistance [52]. Interestingly,

CaCRK5 from pepper is also induced by SA, and is directly regulated by homeodomain zipper I protein *Ca*HDZ27. Pepper *Ca*CRK5 is involved in regulating SA-mediated signaling and the expression of several downstream genes involved in defense against *Ralstonia solanacearum* [11]. In addition, *Ca*CRK5 can form a heterodimer with *Ca*CRK6 on PM, but the mechanisms of this dimerization and its biological roles are not clear [11] (Figure S3). It is also not known whether *Ta*CRK3 and *Ta*CRK7A regulate ethylene and JA signaling, respectively, through epigenetic mechanisms.

CRKs regulate ETI PCD to defend against pathogens

In plants, the PCD process is an inherent part of regular development and responses to biotic and abiotic stress [92]. Pathogen-triggered PCD (pPCD) occurs at infection sites and surrounding areas in host plants, and it is mediated by various phytohormones such as ethylene [93], JA [94], and SA [95,96]. Importantly, calcium is proposed to be both a positive regulator for the timely triggering of pPCD [97] and a negative regulator for suppressing SA-dependent defense [98]; the positive feedback loop between SA and ROS is also assumed to be a key pPCD trigger [99].

Several arabidopsis CRKs and rice *Os*CRK10 are involved in SA-mediated pPCD [10,47–49,51,79]. In *CRK13*- and *CRK45* (*ARCK1*)-overexpressing plants, the expression of multiple SA-related genes is induced, including the SA biosynthesis gene *ICS1*, the SA transporter gene *EDS5*, and several SA-responsive *PR* genes [49,51]. In addition, pathogen-induced SA accumulation promotes translocation of the transcription activator NPR1 from the cytoplasm to the nucleus where it triggers *WRKY70* gene expression and subsequently enhances *CRK45* transcription (Figure 3B) because *CRK45* expression is decreased in *npr1* and *wrky70* mutants [49] but increased in a *sni1* (NPR1 suppressor) mutant upon pathogen infection [100]. This is another example of a positive feedback loop. Similarly, Vd toxin-induced CRK5 and CRK22 positively regulate SA biosynthesis and then modulate *NPR1* expression. Subsequently, NPR1 is recruited by transcription factor TGA to induce the expression of *PR* genes [79] (Figure 3B). Consistent with this, transcription factor *Os*TGA2.1 interacts with *Os*NH1 (an NPR1 homolog) to trigger downstream *OsCRK10* expression, which induces the expression of multiple *PR* genes for the enhancement of rice resistance to infection by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) [10] (Figure 3B). Moreover, in the loss-of-function mutant of SA biosynthesis, dexamethasone (DEX)-induced expression of *DEX:CRK13* delays the phenotype of HR-like cell death [51]. Similarly, *NahG* transgenic plants (SA-deficient) exhibit obvious reductions of *CRK45* expression and disease symptoms after pathogen infection [49] (Figure 3B). This implies that pathogen-induced SA accumulation exhibits positive feedback that enhances CRK-induced PCD.

PCD is closely correlated with pathogen growth and spread [39]. *CRK36*-overexpressing plants exhibit dramatic ROS burst and cell death symptoms after infection by the necrotrophic pathogen *A. brassicicola*, avirulent *Pst* DC3000 *AvrRpm1* and *AvrRpt2*, and PCD-eliciting fungal toxin fumonisin B1, in contrast to the *crk36* mutant [39] (Figure 3B). Enhanced PCD facilitates the growth of *A. brassicicola*. Conversely, after infection by virulent *Pst* DC3000, disease symptoms and ROS accumulation are reduced in *CRK36*-overexpressing plants but become severe in *crk36-2* seedlings [39]. This indicates that CRK36-induced *PR* gene expression and PCD positively restrict virulent pathogen growth [39] (Figure 3B). Interestingly, *Ta*CRK2 also positively regulates HR by modulating several *PR* genes to cope with *P. triticina* infection, but whether this is SA-mediated is unknown [40] (Figure 3B).

Notably, some *CRK*-overexpressing plants exhibit 'dose-dependent' phenotypes of pathogen tolerance. The highly DEX-inducible and moderately constitutive overexpression of *CRK*s shows distinct functions in plant growth and stress tolerance [50,51]. For instance, 5 μM DEX-

induced high expression of *DEX:CRK13* triggers rapid cell death, but 0.05 μM DEX-induced moderate expression of *DEX:CRK13* enhances arabidopsis resistance to *Pst* DC3000 [51]. In addition, DEX-induced high expression of *DEX:CRK5* triggers HR-like cell death which is associated with H_2O_2 accumulation and nuclear DNA fragmentation, but constitutive *35S:CRK5* overexpression promotes leaf growth and resistance to *Pst* DC3000 by quickly inducing *PR1* genes [50]. This indicates that the manner and/or level of CRK induction or activation in transgenic plants can have different effects on the tradeoff between leaf growth/disease resistance and the PCD process.

The ATP-binding site (K) and the potential *N*-glycosylation site (N) in CRK4, CRK5, and CRK28 are crucial for their kinase activities or subcellular localization that are necessary for regulating PCD [38,50,101]. Kinase-inactive *DEX:CRK5^{K368E}* transgenic plants exhibit no cell death phenotype [50]. In addition, *N*-glycosylation of N^{181} in CRK4 through STT3a in the ER is crucial for its protein stability [101]. Transient expression of CRK4^{N181Q} in *N. benthamiana* leaves leads to reduced PCD intensity compared to WT CRK4 [101]. Moreover, *N*-glycosylation in the ER and Golgi apparatus and K^{377}-dependent kinase activity are required for CRK28 modulation of PCD [38]. However, the exact *N*-glycosylation sites of CRK28 and post-translational modification (PTM) regulatory mechanisms remain to be revealed. Interestingly, two rice *Os*CRK10 single amino acid substitution (V^{429} to I/L^{429}) mutants, *LIL1* and *als1*, exhibit SA accumulation, ROS burst, increased expression of *PR* genes, and HR-like cell death in leaves [61,102], while overexpression of *OsCRK10*^{V429I} induces a *LIL1* lesion phenotype in 'Nipponbare' rice [61]. However, these primary phenotype studies on lesion-mimicking mutants of rice does not explain whether a single amino acid substitution affects OsCRK10 function in regulating SA and ROS signaling to cope with rice blast [61,102].

A few CRK-interacting proteins have illuminated the pathogen signal sensing and transduction to regulate PCD. CRK28 interacts with the RLK coreceptor BAK1 for mediating PCD because CRK28-induced cell death is significantly decreased in *NbSerk3* (*BAK1*)-silenced tobacco leaves [38,103] (Figure 3B). In addition, although *CRK10*-overexpressing plants exhibit no inconspicuous PCD phenotypes, both CRK10 and CRK5 can physically interact with several type 2C protein phosphatases (PP2Cs) such as PP2C70, PP2C11, and an FHA domain-containing protein (*At*2g21530) [48] (Figure 3B). However, whether the activities of CRK5 and CRK10 are modulated through PP2C dephosphorylation requires further investigation. Interestingly, cytoplasm-localized CRK45 has been shown to interact with PM-localized CRK36, but how their interaction senses and transduces extracellular signals remains elusive [2].

In summary, CRKs clearly play important roles in PTI and ETI processes. Some CRKs (e.g., CRK2 and CRK36) are better studied than others, but most of the work has been descriptive and correlative, and further work will be necessary to provide a mechanistic understanding. Important questions remain unanswered, such as whether CRKs function through kinase activity, why some CRKs are positive regulators whereas others are negative regulators, and how different CRK signaling pathways crosstalk. The positive feedback loops involving *Ta*CRK3 and ethylene, *Ta*CRK7A and JA, and CRK45 and SA deserve further study. How the loops regulate is not yet known. Considering the large and diverse CRK family and the fact that many CRKs may have redundant functions, elucidating CRK molecular networks in plant immunity seems to be a daunting task. Large-scale interacting proteomics and PTM proteomics together with reverse genetics tools may shed light in the future.

CRKs are involved in abiotic stress

Drought and salinity stress lead to osmotic imbalance. Plants have evolved sophisticated mechanisms for regulating stomata movement and cell-wall dynamics to cope with osmotic stress.

Specifically, ABA is known to play an important role in modulating stomatal closure and osmotic homeostasis [104]. Some CRKs are involved in ABA signaling and biosynthesis [2,105,106]. CRK5, together with its homolog CRK4, redundantly enhances plant drought tolerance by positively regulating the ABA sensitivity of stomatal movement [106]. Interestingly, the ABA-responsive CRK5 is modulated by a feedback and feed-forward mechanism because *CRK5* expression is cooperatively inhibited by three homologous ABA-regulated transcription factors, WRKY18/40/60, and CRK5 functions upstream of ABI2 in ABA signaling [4,106,107]. In addition, cytoplasm-localized CRK45 positively regulates the expression of ABA biosynthesis genes such as 9-*cis*-epoxycarotenoid dioxygenases (*NCED3* and *NCED5*), ABA deficient (*ABA1* and *ABA2*), and abscisic aldehyde oxidase 3 (*AAO3*), and enhances tolerance to drought and salt stress upon germination and post-germination growth [105]. However, CRK45 also interacts with and is phosphorylated by CRK36 to negatively regulate ABA and osmotic stress signaling in post-germination growth [2]. In *CRK36* RNAi plants, ABA treatment induces several ABA-responsive genes such as late embryogenesis abundant (*LEA*), oleosin (*OLEO4*), *ABI4*, and *ABI5* [2]. This implies that CRK45 plays opposite roles under different experimental conditions such as stratification and ABA treatments [2,105].

Callose is a key regulator of plasmodesmata transport in response to osmotic and salinity stress [108]. CRK2 promotes callose deposition at plasmodesmata, thereby enhancing plant salt stress tolerance at the germination stage [6]. Salt stress induces an increase in cytosolic Ca^{2+} and triggers phospholipase D α1 activity, which are required for CRK2 relocalization from the PM to the nanodomain in plasmodesmata [6]. The ATP-binding K^{353} and the D^{450} in the catalytic core are essential for CRK2 activity, which phosphorylates CALS1 to promote callose deposition for the regulation of plasmodesmata permeability against osmotic stress and Na^+ toxicity during salt stress [6]. In addition, our previous transcriptomic analysis revealed that 17 alkaligrass *PutCRK*s exhibited salinity-induced expression changes in leaves and roots under NaCl, Na_2CO_3, and $NaHCO_3$ treatments [21] (Figure S1). However, their regulatory mechanisms are unknown.

CRKs may be involved in oxidative stresses caused by H_2O_2, O_3, and UV light [3,6,22,37,47]. H_2O_2-induced extracellular ROS accumulation alters the localization pattern of CRK2, possibly causing it to form microdomains [6]. The *crk2*, *crk5*, *crk40*, and *crk42* mutants exhibit a significant elevation of electrolyte leakage under oxidative stress conditions, and genetic complementation of *crk5* rescues its hypersensitivity to UV radiation [22]. In addition, more than 25 *CRK*s can redundantly function in response to O_3 [22,37]. For example, *CRK6*, *CRK7*, and *CRK20* are induced by O_3 in WT plants, but their mutants show little change in phenotype, physiology, and antioxidant gene expression [3,47]. Overall, only a small number of CRKs have been found to be involved in plant abiotic stress responses. The molecular mechanisms underlying CRK functions in plant abiotic stress have not been sufficiently studied.

CRKs balance the plant stress response and growth

In adverse environments, sessile plants have evolved sophisticated mechanisms to balance growth and stress responses [109]. Active growth inhibition is an adaptive strategy for facilitating plant survival under stress conditions [104]. Various CRK-mediated pathways such as ROS production [22,38,45], Ca^{2+} signaling [45], MAPK cascade [38,45], and ABA signaling are essential for both stress response and plant growth. Therefore, CRKs may be involved in regulating the growth–stress tolerance tradeoff.

Reverse genetics data of several CRKs suggest their potential roles in the balance of growth and stress tolerance [7,45,105,106,110]. For instance, CRK2, CRK5, and CRK36 mediate callose

deposition of immune response, whereas their mutant phenotypes imply that they might be involved in the regulation of seed germination, seedling growth, rosette leaf size, and root development [6,34,39,45,79] (Figure 4). This indicates that CRK-mediated callose deposition may also regulate cell expansion-driven growth. Similarly, CRK28, a key regulator of plant immunity, inhibits the initiation of lateral root primordia and primary root meristems, and decreases silique length and seed number [7,38] (Figure 4). CRK28 also positively controls root hair development, rosette size, and inflorescence branches [7]. In addition, CRK45 is a modulator of ABA, osmotic, and salt stresses [2,105], and may also regulate bolting and early seedling development [105] (Figure 4).

Several CRKs mediate stomatal movement in response to stress and stomata development (length and density) under normal conditions [22] (Figure S2). CRK33 is preferentially expressed in leaves and cotyledons, and regulates stomatal spacing and development by modulating the expression of several genes involved in guard cell fate such as SPEECHLESS (*SPCH*), TOO MANY MOUTHS (*TMM*), *MUTE*, and *FAMA* [110] (Figure 4). In *crk33-2* and *crk33-3* mutants, stomatal density and stomatal index are decreased in the leaves and cotyledons, leading to decreased stomatal conductance and transpiration, as well as enhanced water-use efficiency and drought tolerance [110]. In addition, ABA-induced stomatal closure is accelerated in the *crk22*, *crk24*, *crk37*, and *crk46* mutants compared to the WT [22]. In detached leaves, stomatal closure is impaired in *crk2*, *crk5*, and *crk31* mutants, leading to increased water loss and the rapid decrease of fresh weight, but water loss is less pronounced in the *crk45* mutant compared to the WT (Figure S2). These phenomena can be rescued by complementation of the *crk2*, *crk5*, and *crk45* mutations, respectively, with the corresponding WT genes [22]. CRK-mediated stomatal movement regulates the water use and the CO_2 concentration in mesophyll cells, thus contributing to photosynthetic carbon fixation and energy supply. The extent to which CRK-mediated stomatal movement contributes to the balance between the stress response and growth is not known.

CRK5 and CRK36 are not only necessary for the response to pathogen infection and abiotic stress (i.e., osmosis, oxidation, and salinity) but also regulate plant senescence (Figures 3 and 4). Stress-induced senescence benefits plant survival but decreases productivity [111]. CRK5 negatively regulates ethylene and H_2O_2 production through SA signaling in the senescence process [4]. The *crk5* mutant displays smaller young seedlings, rapid cell death in cotyledons, impaired stomatal conductance, and accelerated senescence, but the *CRK5* overexpressing line shows slightly larger rosettes [22] and shorter roots in young seedlings [106]. Ethylene accumulation, the expression of *ERF1* and *PDF1.2* in ethylene signaling pathways, and SA accumulation are induced in the *crk5* mutant [4]. SA accumulation correlates with the increase of the positive SA signaling regulator WRKY53 and the decrease of the negative regulator WRKY70 which can recognize the W-box elements enriched in the *CRK5* promoter region [4] (Figure 4). In addition, CRK36 is also involved in leaf senescence, which is revealed by the obvious early-senescence phenotype in *CRK36*-overexpressing plants and delayed senescence in *crk36-2* leaves [39] (Figure 4). Current data suggest that CRK5 and CRK36 may be involved in growth/senescence processes, and CRK5 seems to function by regulating stress-related hormones in a tissue-specific and temporal manner [104,112].

Clearly, the current results do not address whether the growth/senescence phenotypes represent pleiotropic effects or the direct functions of CRKs. Future studies using inducible knockdown systems will be important for determining the causal effects of CRK functions. Based on the results from a small number of CRKs, the balance between stress and growth may be modulated through CRK-mediated stomatal movement and stress hormone crosstalk.

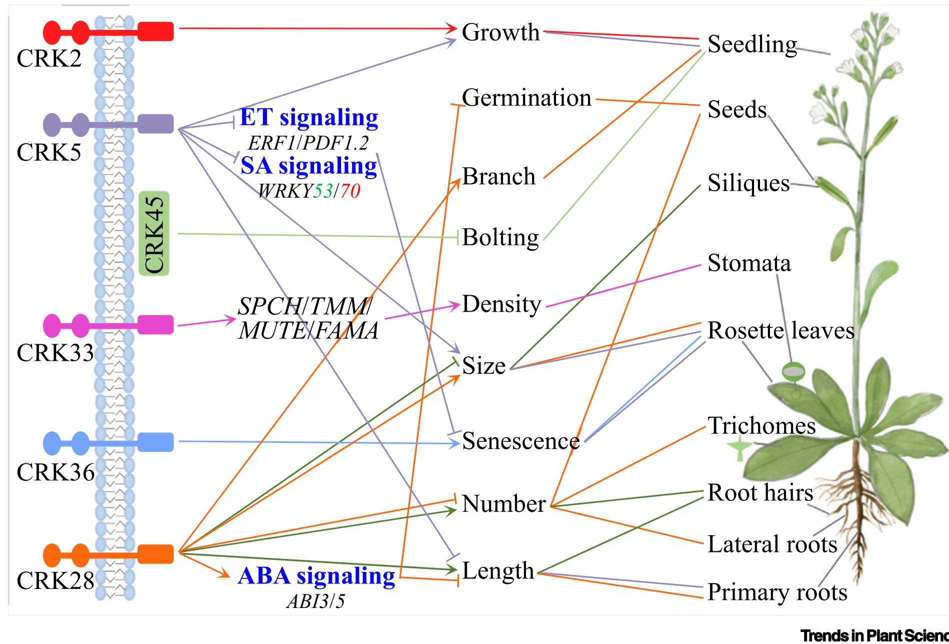

Figure 4. Participation of cysteine-rich receptor-like kinases (CRKs) in the regulation of plant growth and development. CRK2 induces seedling growth whereas CRK33 facilitates stomatal spacing and development. CRK5 negatively regulates ethylene and SA signaling, leading to increased seedling height and rosette leaf growth but decreased root hair growth. CRK36 promotes leaf senescence whereas CRK45 regulates the delay in bolting time. CRK28 is a repressor for the initiation of early lateral root primordia and primary root meristems induced by ABA signaling. CRK28 positively controls root hair development, growth delay, rosette size, and inflorescence branches, but reduces silique length and the number of seeds. Abbreviations: ABA, abscisic acid; ABI, abscisic acid-insensitive; ERF1, ethylene-responsive factor 1; ET, ethylene; FAMA, transcription factor FAMA; MUTE, transcription factor MUTE; PDF1.2, plant defensin 1.2; SA, salicylic acid; SPCH, Speechless; TMM, too many mouths; WRKY, WRKY DNA-binding protein.

Outstanding questions

How do PTMs (e.g., glycosylation and phosphorylation) regulate CRK activity and functions?

Is CRK dimerization/multimerization regulated by Cys redox in the DUF26 domain, and how does redox modification subsequently modulate CRK structure and function?

What are the extracellular ligands of CRKs during plant immune responses?

What are the signaling components downstream of CRKs in response to different environmental stresses?

What are the mechanisms and roles underlying the formation of complexes between CRKs and PRRs upon PAMP/MAMP perception?

How do CRKs regulate HR cell death, and how are SA and other hormones involved in CRK-mediated HR processes?

How do CRKs regulate cell division and differentiation in different tissues and organs during plant growth and development?

How do different CRKs modulate the sophisticated hormone signaling networks in various stress responses and growth processes?

Concluding remarks and future perspectives

Tackling the functional redundancy and diversity of CRKs

Plant CRKs can sense and transduce extracellular signals to fine-tune immune responses, abiotic stress tolerance, and growth and development. Based on the current knowledge summarized in this review, it is reasonable to conclude that CRKs are emerging regulators in many of these processes (see Outstanding questions). However, the clustered distribution on the chromosomes and the evolutionary redundancy of most CRKs make it difficult to accurately determine the unique function of each CRK through molecular genetics analyses in loss-of-function mutants [22]. Most studies on CRKs have only reported plant phenotypes related to development and/or stress responses and have not investigated their downstream target proteins and finely tuned regulatory pathways. The generation of higher-order *CRK* mutants using the CRISPR system may overcome the possible functional redundancy of closely related CRKs. In addition, an inducible expression/knockdown system together with temporal sampling and data acquisition may overcome the pleiotropic effects of *CRK*s and reveal their direct molecular functions. Moreover, different CRK members, $[Ca^{2+}]_{cyt}$ [45], apoplastic and/or cytoplasmic ROS bursts [22], and various phytohormone levels [87] form multiple complex feedback loops for positively or negatively modulating downstream gene expression and metabolism [99]. However, how CRKs respond to stress signals and regulate stress hormones to mediate energy and resource allocation during the stress growth balance requires further investigation [104].

Elucidating CRK ligands and PTMs

Some CRKs can form homodimers with themselves (e.g., CRK28 and CRK36) and/or heterodimers with closely related homologs (e.g., CRK28/CRK29, CRK39/CRK40, and *Ca*CRK5/*Ca*CRK6), and interact with PRR components on the PM (e.g., CRK4, CRK6, CRK28, and CRK36) in response to pathogens and abiotic stress [2,11,38,39]. The formation of complexes enables rapid recruitment of signaling components to facilitate a robust defense response. Whether CRKs function as scaffolds in the PRR complex and/or sensors for recognizing extracellular ligands [39], and the specific ligands of the DUF26 domain that are crucial for CRK signaling, remain largely unknown [1,33].

Specific subcellular localizations [19], protein–protein interactions [39], and PTMs [38] of CRKs result in functional alterations and diverse physiological outputs. Thus, in-depth studies on the mechanisms of kinase activation, protein interactions, the dynamics of complex formation, and PTM crosstalk can greatly enhance our understanding of the key regulatory sites, conformational changes, and biological implications of different CRKs. Furthermore, single-cell-omic analysis, cellular and subcellular imaging, and proteomics-based CRK target mining in plants with different *CRK* genetic backgrounds may provide valuable insights into CRK signaling pathways and networks at the single-cell level [113].

Enhancing crop stress resilience through CRK regulation

Given its vast genetic resources and fast life cycle, most of our knowledge about CRKs comes from studies in arabidopsis, especially regarding the roles of CRKs in immune responses. Whether this knowledge is translatable to crops is not known because the mechanisms of CRK functions might not be conserved in crops [11,33]. Therefore, testing the functions of CRKs in various crops upon disease and abiotic stress needs to be accomplished in due course. Ultimately, an improved understanding of CRK networks will facilitate synthetic biology and molecular design-based breeding toward improving crop stress resilience, yield, and quality.

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Declaration of interests

The authors declare no conflicts of interest.

Supplemental information

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