KINGDOM OF SAUDI ARABIA
Northern Border University (NBU)
Journal of the North for Basic & Applied Sciences(JNBAS)
p - ISSN : 1658 -7022 / **e-ISSN:** 1658 - 7014
www.nbu.edu.sa
s.journal@nbu.edu.sa

Cysteine-rich receptor-like kinases in Arabidopsis thaliana: Properties and Functions in Stress Responses and Development

Fatima Omari Alzahrani
Department of Biology, Faculty of Sciences, Al-Baha University, Saudi Arabia

(**Received:** 15th July 2025; **Accepted:** 7th October 2025)

Abstract
Cysteine-rich receptor-like kinases (CRKs) constitute a significant subfamily of receptor-like kinases (RLKs) that are essential elements in numerous signal transduction pathways. This review synthesizes current knowledge on the evolutionary background, structural characteristics, and functional diversity of the CRKs in Arabidopsis thaliana. The whole genome and tandem duplications have resulted in the emergence of 44 members of the CRKs subfamily in Arabidopsis, predominantly located on chromosome 4. Recent research increasingly demonstrates that CRK has a role in various physiological processes, including activation of immunological responses to biotic stimuli, and regulation of reactive oxygen species (ROS), abiotic stress responses, plant growth and development. The concept of functional redundancy within the CRK family is explored, proposing that closely related genes may compensate for one another's responsibilities. This work develops a fundamental understanding of CRKs, offering a baseline for subsequent research on the CRK family, including other species of agricultural significance.

Keywords: Cysteine-rich receptor-like kinases, Pathogen defense, Stress responses, Reactive oxygen species (ROS), Immunological responses.

DOI: 10.12816/0062292

(*) **Corresponding Author**:
Fatima Omari Alzahrani
Department of Biology, Faculty of Sciences, Al-Baha University, Saudi Arabia; Al-Baha 65729.
Email: drfatimaomari@gmail.com

1. Introduction

Plants must navigate various challenges throughout their lives, including adapting to a dynamic environment and less-than-ideal growth conditions. Stress has a significant impact on reproduction. Typically, the onset of early flowering is commonly associated with environmental pressures, leading to accelerated seed maturation and successful reproduction [1,2]. Grain yield and biomass are key factors influenced by stress, making them the primary focus of plant research aimed at enhancing stress tolerance. Plants have developed various strategies to enhance growth in response to fluctuating environmental conditions. For instance, plants have the capacity to maintain reproductive fitness and buffer water/nutrient deficits by employing vacuolar storage and osmotic adjustment. Additionally, they are capable of adjusting to variations in light intensity and temperature. Programmed cell death (PCD) is employed as a defense mechanism against diseases, effectively inhibiting the entry and proliferation of pathogens. The process of leaf abscission is utilized as a means to remove infected leaves in order to protect the overall health of the plant [3-6].. In order to react to both biotic and abiotic challenges, the first and most crucial step is to perceive environmental signals. Various environmental factors trigger the activation or deactivation of distinct signal transduction pathways. Protein kinases play a crucial role in the perception of environmental signals and subsequent response at different stages. Plants possess a significantly higher number of kinases in comparison to other organisms. As an example, when comparing the human genome to that of Arabidopsis and rice, it is observed that the latter two have a significantly higher number of protein kinases. Arabidopsis has over 1000 protein kinases, while rice has over 1400 protein kinases, highlighting the significance of kinases in plants for sophisticated environmental sensing, stress response, and other developmental processes via distributed signaling networks essential for survival in the absence of mobility [7,8]. Plants have receptor-like protein kinases (RLKs) that are similar to the receptor protein kinases found in animal genomes, and function as part of signaling systems between cells and their surrounding microenvironment [9-11]. Studies have shown that Arabidopsis has over 600 members of RLK, while rice has approximately 1100 [12].

Recent studies have initiated the functional characterization of subclass-specific receptor-like kinases (RLKs) in other crops such as wheat and maize. For example, a comprehensive phylogenomic investigation revealed over 3,400 RLK genes in wheat. A number of genes were confirmed via qRT-PCR to be responsive to drought or Fusarium graminearum infection [13]. TaCrRLK1L16 has been demonstrated to be transcriptionally activated after Puccinia striiformis infection, and overexpression improves wheat resistance, supporting the subclass's mechanistic function in immunity [14]. Furthermore, 248 L-type lectin receptor-like kinases (LecRLKs) were identified in wheat, with subclass members such as TaL-LecRLK35-3D and TaL-LecRLK67-6B/A exhibiting stress-responsive expression patterns [15]. A comprehensive genome-wide investigation of maize found 205 LRR-RLKs, several of which were upregulated during Fusarium verticillioides infection [16]. Functional validation of the RLCK subclass member ZmBLK1 verified its role in pathogen defence by restoring immunological deficits in Arabidopsis bik1 mutants and increasing maize resistance to Clavibacter michiganensis [17]. Despite these advances, the subclass-specific signalling roles of RLKs in essential agricultural crops remain poorly understood. Numerous RLK subclasses remain poorly characterised in terms of essential mechanistic characteristics such as co-receptor interactions, ligand recognition, and downstream signalling cascades [9].

In recent years, there has been significant research dedicated to understanding their physiological and mechanistic roles in various aspects of plant biology. These include plant development, responses to abiotic stress, plant immunity, and symbiosis. Recent advances highlight the practical importance of RLK research in enhancing crop resilience and yield. The overexpression of Oryza sativa Large Spike S-domain Receptor-Like Kinase 1 (OsLSK1), enhanced rice panicle architecture and yield [18]. Similarly,the overexpression of Triticum aestivum Brassinosteroid-Insensitive 1 (TaBRI1) in wheat correlated with accelerated flowering and enhanced seed production [19]. Research has shown that genes like Oryza sativa Salt Tolerance Receptor-like Cytoplasmic Kinase 1(OsSTRK1), which regulates salt tolerance through ROS and Glycine soja Leucine-Rich Repeat Receptor-Like Protein Kinase (GsLRPK), which confers cold tolerance in soybean can be overexpressed or modified to improve resilience without significant yield losses [18,20] . Certain RLKs function as negative regulators; for instance, Oryza sativa Salt Intolerance 1 (OsSIT1) enhances salt sensitivity in rice, however, the natural haplotype Hap2 exhibits significantly reduced kinase activity compared to the standard haplotype (Hap1). Therefore, It was reported that the overexpression of Hap2-SIT1 in rice facilitated normal development and mitigated ROS accumulation under salt stress, while overexpression of Hap1-SIT1 detrimentally affected both growth and stress tolerance [21]. These findings emphasize the potential application of elite RLK alleles in marker-assisted breeding or transgenic approaches in crops improvement.

The majority of RLKs are located in the plasma membrane and act as sensors to detect environmental

signals such as hormones or Pathogen-Associated Molecular Patterns (PAMPs) [22,23]. Wall-Associated RLK (WAKs) are located in the cell wall, where their extracellular domains interact directly with pectin or oligogalacturonides. This allows them to monitor the cell wall's integrity while also controlling its growth and stress response [24,25]. A specific group comprises Receptor-Like Cytoplasmic Kinases (RLCKs), which are exclusively localised in the cytoplasm and function as signal carriers downstream of plasma membrane receptors, lacking of extracellular or transmembrane domains [26]. Furthermore, many RLKs are subjected to proteolytic cleavage, leading to the release of their intracellular kinase domains. This facilitates the translocation and regulation of alternative signalling pathways, potentially involving nuclear processes [27].

The three primary components of an RLK are a protein kinase catalytic domain (PKC), a transmembrane domain (TM), and an extracellular ligand-binding domain (ECLB) [28,29]. The PKC domain is located within the intracellular domain and plays a central role in the signaling cascade [30]. It contains the serine/threonine kinase domain that is responsible for its kinase activity. The TM domain, which usually consists of 22-28 amino acids, facilitates signal transduction between the extracellular and intracellular domains [30]. It is responsible for transferring signals to the intracellular realm. The ECLB domain of RLKs greatly contributes to their functional diversity, making them one of the most functionally versatile gene families in plants [30]. It exhibits significant variation between RLKs and commonly incorporates distinct motifs that facilitate the binding of ligands. The majority of RLKs possess the N-terminal signal peptide (SP) located in the ECLB domain, with the exception of those containing epidermal growth factor-like repeats (EGFs). SP is important for attaching the protein in the endoplasmic reticulum and detecting extracellular signals during protein synthesis [30]. SP variation categorizes RLKs into over 44 subclasses [31]. The Cysteine-Rich Receptor-Like (CRKs) are a significant subgroup of RLKs in higher plants. It is worth mentioning that the abbreviation CRK has also been used for a different group of protein kinases, namely the CALCIUM-DEPENDENT PROTEIN KINASE (CDPK/CPK)-RELATED KINASEs [32].

RLKs identify a variety of ligands with significant biochemical specificity; yet, their signalling outcomes may differ based on stress conditions. Examples of ligands include pathogen-associated molecular patterns (PAMPs) such as the 22–amino acid peptide epitope derived from bacterial flagellin (flg22) and the 18–amino acid peptide epitope derived from the N-terminal region of bacterial elongation factor (elf18). [25,33,34]. In Arabidopsis, under normal conditions, FLAGELLIN-SENSING 2 (FLS2) recognises the bacterial flagellin peptide flg22, while ELONGATION FACTOR Tu RECEPTOR (EFR) identifies the elongation factor elf18. In Arapidopsis, it was shown that while the specificity of ligand-receptor interactions is maintained, abiotic stresses can alter apoplastic pH, reactive oxygen species (ROS) levels, receptor concentrations, or membrane dynamics, thereby affecting the magnitude and outcomes of FLS2-flg22 and EFR-elf18 signalling without changing their specificity [33,35,36]. Further examples of ligand include peptide hormonessuch as Rapid Alkalinization Factors (RALF) peptides. FERONIA (FER) is part of the Catharanthus roseus receptor-like kinase 1-like (CrRLK1L) protein subfamily, which plays a role in plant reproduction, abiotic stress, biotic stress, and various molecular mechanisms. It mainly interacts with demethylesterified pectin. However, under stress conditions like salinity or heat, RALF peptides and pectin engage in dynamic protein interactions and phase separation. This process recruits FER along with the small co-receptor LRE-like GPI-anchored protein 1 (LLG1i) and the Leucine-Rich Repeat extensins (LRXs) [37]. This triggers various signaling pathways such as calcium influx, ROS production, and Rho-of-Plant (ROP) signaling [37].

A number of studies have addressed the roles of CRKs in various plant species; however, the majority have focused on individual members or particular stress pathways. A detailed synthesis of the evolutionary history, structural characteristics, and several functions of CRKs in Arabidopsis is currently lacking. This review will provide a detailed overview of CRKs in Arabidopsis, including their evolutionary diversification, domain organisation, and signalling roles in ROS regulation, pathogen defence, abiotic stress responses, and plant growth.

2. The evolutionary history of CRKs in Arabidopsis thaliana

CRKs are found in a wide range of vascular plants, but interestingly, they are not present in bryophytes and algae [38]. In Arabidopsis, a study revealed that a total of 38 members of the CRKs subfamily are located on chromosome 4, with 34 of them being found in tandem repeats [39]. Based on the phylogenetic relationships among CRKs genes in the tandem repeats, it was inferred that there was at least one intrachromosomal duplication event in the region containing the tandem repeats. Throughout evolution, CRKs have greatly increased their lineage, particularly through more recent tandem duplication and the selective preservation of duplicates after whole-genome duplication [38]. Several CRKs exhibit a significant degree of sequence similarity and are arranged in consecutive arrays on the chromosome, as

observed in Arabidopsis. The CRK tandem duplications primarily arose from unequal crossover or homologous recombination events. The development of CRK primarily occurred through unequal crossover or homologous recombination events [38]. These tandem repeats of CRKs have been proposed to be correlated with stress responses in order to facilitate stress-adaptive evolution. This is due to the fact that the majority of clustered CRKs are involved in the adaptation to environmental stimuli and pathogen infection.

The protein's extracellular domain consists of two copies of the DUF26 domain (the DOMAIN OF UNKNOWN FUNCTION 26) (Fig. 1). This domain contains four conserved cysteines, three of which form the C-8X-C-2X-C motif [36]. Two DUF26s are present in 41 of the 44 CRKS in Arabidopsis, while the remaining three (CRK43, CRK44, CRK45) lack either a DUF26 or a TMD and their cytoplasmic location allows them to interact with plasma membrane (PM)-localized CRKs [40]. There are two genes in the present Arabidopsis genome. The first gene, At4g11500 (CRK35), is a pseudogene. The second gene, At4g23170 (CRK9), does not have any identifiable extracellular domain, signal peptide, or full kinase domain. The DUF26 domain is present also in cysteine-rich receptor-like secreted proteins (CRRSPs) and plasmodesmata-localized proteins (PDLPs), which are grouped together with CRKs in the DUF26-containing protein family under RLKs [38]. It is highly probable that the CRKs emerged as a result of the fusion between a CRRSP containing DUF26 and LRR_clade_3 RLKs, which possess only a TM domain and a kinase domain [38]. It is widely accepted that this fusion event occurred in a shared ancestor of vascular plants [38].

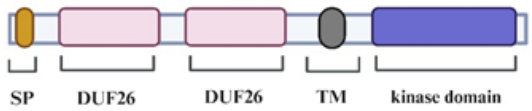

Figure 1: Typical domain structure of Cysteine-rich Receptor-like Kinases (CRKs), illustrating the extracellular DUF26 cysteine-rich motifs for redox sensing and ligand recognition, a transmembrane helix for membrane anchoring, and a serine/threonine kinase domain for signal transduction.

In various analyses, including multi-omics and molecular genetic approaches, a total of 1074 CRKs have been found in 14 different crops such as 50 CRKs have been identified in rice, while Zea mays has been found to have 30. However, a total of 63 CRKs have been identified as key players in the regulation of plant immunity, abiotic stress responses (such as salinity, osmosis, oxidation, and heat), and growth and development ([40-46].

The CRK subfamily of RLKs is often highlighted for its significant size, but this aspect has not been thoroughly explored in research. CRKs are essential for regulating a wide range of cellular processes. These include controlling the influx of Ca2+, maintaining ROS homeostasis, activating the MAPK cascade, and facilitating the deposition of callose. These processes play a crucial role in various physiological functions, including stomatal closure, expression of pathogenesis-related (PR) genes, and PCD. Based on the amino acid sequences of the coding region, the 44 Arabidopsis CRKs are categorised into five separate groups (Fig 2). Comparable groupings are recognized in evolutionary trees according to the intracellular kinase domain and the extracellular area. It is proposed that the extracellular and intracellular domains of Arabidopsis CRKs co-evolved.

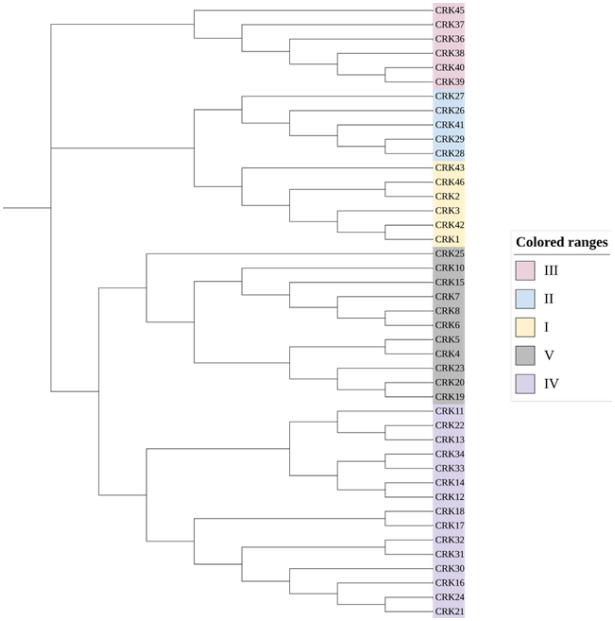

Figure. 2: Maximum-likelihood phylogenetic tree of the Arabidopsis thaliana CRK gene family. Clades are color-coded and bootstrap support values are shown at the nodes. Amino acid sequences for all A. thaliana CRKs were obtained from TAIR10 (https://www.arabidopsis.org). The tree was inferred in MEGA11 [47] using the maximum-likelihood method.

3. Structure of CRKs in Arabidopsis

3.1 Gnk2-like domain (DUF26)

In Arabidopsis, the extracellular region of CRKs is found to contain the Gnk2-like domain.

The Gnk2-like superfamily is a significant protein superfamily with a wide range of domain homology (28–31%) to the domain initially discovered in the Ginkbilobin2 (Gnk2) protein [48]. This antifungal protein is present in the endosperm of Ginkgo seeds. It specifically hinders the growth of fungus like Fusarium oxysporum [49]. Gnk2 exhibits a substantial degree of similarity, around 85%, to embryo-abundant proteins

(EAP) found in the gymnosperms Picea abies and Picea glauca [49]. Plant EAPs have a role in seed defense against stresses from the environment including drought and are expressed during the last stages of seed maturity [50]. The Gnk2-like domain is composed of two α-helices and a five-stranded β-sheet [48]. This configuration leads to a highly structured single-domain structure with an α+β-fold. The domain contains C-X (8)-C-X (2)-C motif. Cysteine residues create three intramolecular disulphide bridges: C1-C5, C2-C3, and C4-C6 [48]. DUF26 Cys residues are commonly found in the C-X8-C-X2-C motif, where they play a crucial role in the formation of inter/intramolecular disulphide bonds that contribute to the stability of protein structure [38,51]. Additionally, these residues can function as switches, effectively regulating the functions of certain CRKs [52,53]. It was suggested that Cysteine residues might have a vital function in detecting ROS and controlling redox activities [54]. Arabidopsis plants with increased CRK36 expression show enhanced hypersensitive cell death lesions and ROS production when exposed to the necrotrophic pathogen Alternaria brassicicola or the avirulent Pseudomonas syringae pv. tomato DC3000 (Pst DC3000) AvrRpm1. In a different scenario, if specific cysteine residues in CRK36 undergo mutations (CRK36C76/85/88/100/119A or CRK36C200/209/212/237A), the plants exhibit a different level of cell death or production of ROS [54].

3.2 Serine/threonine kinase domain

The serine/threonine kinase domain is the catalytic area of enzymes that phosphorylate serine or threonine residues in proteins. The phosphorylation process is considered essential for the control of cellular, with ATP being utilized as a phosphate donor [55]. The protein kinase domain is characterized by 11 conserved subdomains, labelled I through XI, which intricately fold into distinct N-terminal and C-terminal lobes [56]. The N-terminal lobe comprises the I-IV subdomains, serving to anchor and orient ATP effectively and the linker region connecting the two lobes is represented by Subdomain V [56]. The C-terminal lobe encompasses VI-XI subdomains, facilitating substrate binding and positioning them in proximity to ATP for the phosphorylation process [56]. In subdomain II, there is a conserved Lys residue that plays a crucial role in ATP binding. On the other hand, subdomain VII contains an Asp residue that is essential for catalysis. It was revealed that mutations in specific amino acids, converting Lys to Glu and Asp to Asn, impact the kinase activity of CRK2 [45,57]. Interestingly, these mutations do not seem to have any effect on the stability and subcellular localization of CRK2. This suggests that these two subdomains play a crucial role in the kinase activities of CRKs. Similarly, when Lys is replaced with glutamic acid in CRK36 or lysine is replaced with asparagine in CRK28, it impacts their function related to stomatal closure, cell death, and development [54,58]. However, the impact of mutations in the subdomains of the kinase domain has yet to be investigated.

The transmembrane domain (TM) and the N-terminal signal peptide (SP). The localization of CRK proteins in the cell is determined by both the TM domain and the N-terminal SP. TM domain plays a crucial function in attaching CRKs to the plasma membrane. On the other hand, SP plays a crucial role in directing CRKs to a specific compartment within the cell. Prior research has demonstrated that certain CRKs are situated on the plasma membrane, whereas others are situated in the endoplasmic reticulum.

A single nucleotide mutation in the TM domain can result in the relocation of proteins from the plasma membrane to the endoplasmic reticulum [59]. There is speculation that this disparity may result in a variation in hydrophobicity, which subsequently impacts the subcellular localization of proteins. The TM domain of CRK6 in Arabidopsis is composed of 23 amino acid residues. The hydrophobicity score of these residues is 3.148, and they are located in the plasma membrane, as revealed by subcellular localization studies. The key differentiating characteristic between Arabidopsis CRK6, positioned on the plasma membrane, and barley HvCRK1, situated on the endoplasmic reticulum, was the occurrence of leucine-valine-glycine (LVG) in Arabidopsis CRK6, while barley HvCRK1 consisted of a repetition of three alanine(s) (alanine-alanine-alanine, AAA) [59]. Research has demonstrated that Arabidopsis CRK4, CRK6, and CRK36 are proteins that are specifically located in the plasma membrane [51].

4. Functionals Analysis of Arabidopsis CRKs

CRKs in Arabidopsis are essential for numerous physiological functions, especially in the plant's defense against both abiotic and biotic stress as well as in plant growth and development (Table 1).

4.1 Reactive Oxygen Species (ROS) Regulation and Pathogen Defense

Numerous CRKs have been demonstrated to participate in plant defence systems, frequently by regulating ROS generation and related immunological responses. Their preserved role in defence can be ascribed to structural characteristics, including extracellular DUF26 domains with redox-sensing C-X8-C-X2-C motifs and intracellular kinase activity, which together enhance pathogen detection and immune signalling (Vaattovaara et al., 2019; Bourdais et al., 2015). For instance, several CRKs (CRK4, CRK7, CRK11, CRK12, CRK13, CRK14, CRK18, CRK24, CRK36, CRK37, and CRK45) are transcriptionally induced in Arabidopsis following oviposition by Pieris brassicae butterflies, indicating a function in detecting egg-derived elicitors

(Little et al., 2007).

CRK2 as a central regulator: CRK2 is a crucial regulator of ROS and calcium signalling in pathogen defence among the CRKs. Phosphorylation of certain residues (Ser703 and Ser862) in CRK2 facilitates its interaction with the NADPH oxidase RBOHD, thereby enhancing ROS generation [57]. The crk2 mutant exhibits diminished cytoplasmic calcium levels during pathogen invasion and compromised stomatal closure, hence affirming its function as a positive regulator of Ca^{2+} inflow and immunological signalling. Moreover, CRK2 engages with BIK1 and additional CRKs, establishing it as a central node in PTI-related signalling. CRK2 precisely regulates defence mechanisms by altering MAPK cascades, maintaining equilibrium between immunity and growth [57].

CRKs in PTI responses: Numerous CRKs enhance pattern-triggered immunity (PTI) responses downstream of pattern recognition receptors (PRRs) like FLS2 and BAK1. Proteomic investigations demonstrated that flg22 induces the upregulation of CRK11, CRK13, CRK14, CRK18, and CRK22 [58]. The overexpression of CRK4, CRK6, and CRK36 amplifies early PTI by elevating the oxidative burst in reaction to flg22 [51].

CRKs play a role in stomatal immunity: CRK4 and CRK36 inhibit pathogen-induced stomatal reopening, but CRK6 sustains stomatal closure [51]. CRK36 significantly facilitates delayed PTI by increased callose deposition, while CRK4 and CRK6 exert a minimal influence on this process [51]. Moreover, CRK4 has been recognised as a marker gene in defence priming during encounters with Pseudomonas cannabina pv. alisalensis, with its expression in systemic leaves correlating with increased immunological preparedness [60].

CRKs in immunological receptor complexes: CRK28, CRK29, and CRK36 engage with BIK1 subsequent to flg22 detection, hence affirming their function in immune signalling complexes [54,58]. Redundancy is apparent, as the individual knockouts of CRK28 or CRK29 exhibit no significant immunological deficiencies, whereas overexpression increases resistance and may trigger cell death [58].

CRKs in pathogen resistance and hypersensitive response mechanisms: In addition to PTI, several CRKs play a role in the defence against Pseudomonas syringae pv. tomato (Pst DC3000) and fungal infections. The overexpression of CRK5 enhances resistance to Pst DC3000 by triggering (Hypersensitive Response) HR-like cell death, irrespective of EDS1, NDR1, NPR1, or salicylic acid (SA) signalling [61]. CRK13 is activated by avirulent strains of Pst DC3000, providing resistance upon overexpression, and simultaneously experiences alternative splicing in response to PAMPs, together with CRK29 [62,63]. CRK20 facilitates diminished bacterial proliferation in mutant contexts [64]. CRK45 increases resistance through the activation of WRKY transcription factors and salicylic acid-related genes [65]. In fungal immunity, CRK5 and CRK22 modulate defence against Verticillium dahliae toxins through the salicylic acid pathway, functioning upstream of MPK3 and MPK6, and engaging with WRKY70 to promote the expression of salicylic acid-responsive defence genes [44].

Although the roles of individual CRKs have been elucidated, it is increasingly evident that they operate as components of integrated networks rather than as isolated entities. Besides modulating MAPK signalling and regulating calcium influx, CRK2 serves as a a central regulator by directly phosphorylating RBOHD to begin ROS generation [57]. Additional CRKs, including as CRK4, CRK6, and CRK36, appear to function collaboratively downstream of PRRs like FLS2 and BAK1, with their overexpression enhancing ROS burst, promoting stomatal immunity, and facilitating callose deposition [51]. It was suggestd that CRK28, CRK29, and CRK36 interact with BIK1 and may contribute to the sequential amplification of signals from PRRs to downstream MAPK and ROS modules [54,58]. As has been mentioned earlier, functional redundancy have been observed, particularly between CRK28 and CRK29, where overexpression enhances PTI, although individual knockouts display only slight immunological anomalies [58]. Additional CRKs, including CRK20, CRK22, and CRK45, offer supplementary defensive mechanisms by regulating SA signalling and WRKY transcription factors through parallel pathways that intersect at common nodes [40,44,65]. These findings collectively support a model in which CRKs function at multiple PTI nodes: (i) as modulators of calcium signalling and MAPK cascades (CRK2, CRK19, CRK45); (ii) as positive regulators of ROS homeostasis (CRK2, CRK4, CRK6, CRK36); and (iii) as enhancers of HR-like responses and (Salicylic Acid) SA-mediated defence (CRK5, CRK20, CRK22, CRK45). Due to the flexible and multi-layered nature of plant immunological signalling, certain CRKs operate redundantly, others synergistically, and only a few function sequentially within receptor-co-receptor complexes. This integration of ROS, Ca^{2+}, MAPK, and hormone pathways contributes to a robust, context-dependent defense mechanism.

4.2 Abiotic Stress Responses

Recent studies demonstrate the essential functions of CRKs in Arabidopsis, especially regarding their responses to environmental stresses including ozone exposure, salinity, drought, and oxidative stress, thereby indicating their essential role in plant resilience and signaling mechanisms (Fig. 3).

Ozone and oxidative stress: The impact of ozone (O3), bright light, and pathogen/elicitor therapy on the transcriptional regulation of all Arabidopsis CRKs was demonstrated to induce the production Zhao of ROS in several cellular compartments, suggesting that CRKs play a crucial role in this process [36]. The ozone-induced response of Arabidopsis CRKs showed a similarity to the pathogen-induced response, suggesting that these CRKs play a crucial role in both biotic and abiotic responses [36]. Ozone (O3) exposure was determined to induce transcriptional regulation of many CRKs. Among the 44 CRKs analyzed, 25 exhibited mRNA abundances above twofold after one hour of O3 exposure, while a similar rise was noted in 26 CRKs after six hours of O3 exposure followed by a recovery period [36]. However, mutant plants without functional CRKs showed only minor alterations when exposed to ozone, in contrast to wild-type plants, despite the pronounced transcriptional response of CRK genes to ozone. This finding implies that the plant's response to ozone is partially determined by CRKs, even though it is influenced by them [40]. Additionally, CRK6 has been identified as a crucial component of the signaling pathways in plants responsible for ROS [42]. It aids in regulating cellular responses to extracellular ROS, particularly during oxidative stress [42]. It was demonstrated that CRK6 is a protein kinase capable of phosphorylating substrates in in vitro, showing a preference for manganese (Mn^{2}□) as a divalent cation, which plays a crucial role in its enzymatic activity [42].

In contrast to CRK6, CRK7 demonstrates a preference for magnesium (Mg^{2}□) as a divalent cation in its phosphorylation activities [42]. CRK7 expression was concentrated in regions commonly impacted by O□ damage, suggesting its role in the plant's reaction to oxidative stress, specifically in relation to extracellular ROS, rather than chloroplastic ROS [42]. Furthermore, CRK20 has been identified as a regulator of plant responses to ozone stress ([64]. Although CRK20 is upregulated by ozone, the loss-of-function mutants (crk20-1 and crk20-2) do not exhibit significant differences in physiological responses to ozone treatment when compared to the wild-type, suggesting that alternative mechanisms or pathways may compensate for the absence of CRK20 in mitigating ozone stress [64].

Salt and osmotic stress: Further investigations have revealed the particular contributions of several CRK mutants in improving plant resilience to salt stress. CRK2 stimulates callose deposition at plasmodesmata to enhance plant resilience to salt stress during germination. The phosphorylation of callose synthase CALS1 regulates plasmodesmata permeability in response to osmotic stress [45]. In response to several stimuli, a reduction in stomatal closure was observed in the crk2 mutant plants [40]. The crk2 mutant plants exhibit salt tolerance, evidenced by reduced root growth and germination efficiency under saline conditions [40,45]. Plants deficient in CRK5, CRK8, CRK11, CRK28, CRK29, CRK37, and CRK45 exhibit delayed germination on salt-enriched medium, suggesting additional function in salt stress responses alongside CRK2 [40]. CRK2 and CRK3 were recognized as essential in the response to salt stress. It was shown that CRK2 and CRK3 interact with the cytoplasmic kinase PBL27, which is an essential part of the signaling cascade that starts defense responses [66]. CRK2 interacts strongly with PBL27, but CRK3 has a weaker connection, suggesting that CRK2 contributes more to the signaling network than CRK3. Additionally, CRK18 and CRK36 were identified within the downstream stress-responsive networks and as ABA-responsive genes that exhibited down-regulation in the sahy9/apum23 Arabidopsis mutant under conditions of salt stress [67]. The role of the nucleolar protein SAHY9/APUM23 in ribosome production and the abscisic acid (ABA) signaling pathway is essential for Arabidopsis's response to salt stress [67].

Drought and ABA signaling: Alongside the functions of CRKs in salt stress responses, various other CRKs contribute importantly to the regulation of stomatal functions and responses to environmental stimuli such as drought, emphasizing the intricate nature of these signaling pathways. The loss of function of CRK33 has been shown to influence the stomatal density in Arabidopsis plants. This alteration subsequently impacts transpiration and water-use efficiency, thereby enhancing the potential of the plants' drought tolerance [68]. The role of CRK5 in drought tolerance was determined by the observation of ABA-hypersensitive phenotypes, such as increased stomatal closure and inhibition of stomatal opening, upon CRK5 overexpression [69]. A study demonstrated that CRK22, CRK24, CRK37, and CRK46 mutants exhibit greater ABA-induced stomatal closure than the wild type, potentially enhancing the plant's ability to respond to environmental stimuli, including drought stress and other conditions necessitating stomatal regulation [40]. The greater stomatal closure noted in these mutants is believed to be linked to the signaling pathways that regulate the effects of ABA [40]. This reaction ensures the maintenance of water balance in plants during water shortages. The exact mechanisms by which CRK22, CRK24, CRK37, and CRK46 enhance this response are not fully elucidated, yet their involvement in ABA signaling pathways is recognized as crucial for the regulation of stomatal functions.

CRK24 was recognized as a downregulated gene and a potential ethylene-response factor in transgenic Arabidopsis plants that overexpress the XERICO gene: encodes a small protein (162 amino acids) with an N-terminal trans-membrane domain and a specific

zinc-finger motif [70]. The proposed mechanism by which these plants enhance drought tolerance involves an elevation in the biosynthesis of ABA. The observed downregulation suggests that CRK24 could play a role in the regulatory network associated with ethylene signaling pathways, potentially influenced by elevated levels of ABA [70]. In related context, the expression of CRK36 is predominantly activated in Arabidopsis leaves when subjected to drought and high-salinity stress conditions [41]. The suppression of CRK36 expression via RNA interference (RNAi) leads to an enhanced response to ABA and osmotic stress, indicating that CRK36 functions as a negative regulator within these signaling pathways [41]. The reduction of CRK36 leads to elevated transcription levels of ABA-responsive genes, suggesting its involvement in the regulation of stress responses at the transcriptional level.

During the post-germinative development phase of Arabidopsis, CRK36 engages with the Receptor-Like Cytosolic Kinase (RLCK) ARCK1, leading to the creation of a complex that inhibits ABA and osmotic stress signal transduction [41].

Nutrient and ammonium stress: CRK34 has been recognized as a regulatory gene involved in phosphate homeostasis, with a potential role in sequestering the shoot-to-root mobile miRNA miR-399 through "target mimicry" [71]. Consequently, CRK34 may establish a depletion gradient that facilitates the regulation of phosphorus transport from roots to shoots and maintains phosphorus homeostasis. This suggests that plants have the ability to adjust their responses to nutrient availability and stress conditions through the utilization of mobile RNAs, which regulate the activity of miRNAs, subsequently affecting gene expression and physiological processes.

In the initial two hours of ammonium stress, CRK39 was elevated, indicating that CRKs might be involved in modulating the plant's reaction to ammonium-induced oxidative stress [72]. The expression CRK.39 exhibits sensitivity to variations in the redox state within plant cells, which are intricately linked to the redox conditions induced by ammonium treatment [72]. This shows that CRK39 could act as a sensor or mediator in signaling networks that respond to oxidative stress.

Cold and UV stress: Regarding cold stress, transgenic Arabidopsis plants that overexpress glycine-rich RNA-binding protein 2 (GRP2) and are exposed to cold stress exhibit increased expression of CRK40 [73]. This could imply that CRK40 is involved in signaling pathways that aid plants respond to environmental stress. In addition, CRK5 is involved in the response to ultraviolet (UV) radiation, the crk5 mutant exhibits considerable oxidative damage and cell death upon exposure to UV-A and UV-B radiation [40,43]. Hence, the several roles that CRKs play in Arabidopsis highlight their critical function in mediating responses to abiotic stresses, highlighting their significance in the resilience and adaptability mechanisms of plants.

4.3 Plant Growth and Development

The CRK gene family plays a crucial role in multiple developmental processes in Arabidopsis, such as flowering, root development, stress responses, and cell wall dynamics (Fig. 3). Pollen exhibits a distinct expression of CRK1, and it is identified as a member of a gene cluster that is abundant in pollen and may play a role in the signaling pathways related to pollen germination and tube elongation [74].

It is suggested that a complex regulatory mechanism involving CRK2 controls the flowering process [75]. The expression and mRNA alternative splicing of flowering-related genes, including FLOWERING LOCUS C (FLC) and its homologs (MAFs), are modulated by the FERONIA (FER) receptor-like kinase, encoded by CRK2. This kinase also regulates the transition from vegetative to reproductive growth. The timing of flowering in Arabidopsis is regulated by Rapid Alkalinisation Factor 1, a peptide that interacts with CRK2 and suppresses flowering more significantly than FER. In addition, the features of crk2 mutant phenotype involve decreased rosette size, delayed flowering, and early senescence, suggesting a role of CRK2 in growth regulation [40]. Moreover, CRK45 has been suggested to regulate bolting and early seedling development, playing a crucial role in flowering timing and overall plant growth [65].

Besides regulating seedling growth, CRK2 may also influence root formation [45]. In addition, CRK5 regulates auxin transport in plants by phosphorylating auxin efflux carriers like PIN2 and stabilizing PIN2 in the plasma membrane, which is important for appropriate gravitropic responses [76,77]. Mutant plants lacking CRK5 had abnormal PIN2 localization, which affected root gravitropic response and root development[76]. Root development and gravitropic responses depend on ROS homeostasis, which is maintained by CRK5. [78]. Mutant plants lacking CRK5 also exhibit a reduced number of stomata, decreased stomatal conductance, and accelerated senescence, increased basal ROS levels, elevated ethylene and SA levels in the leaves, along with a greater number of transcripts responsive to these compounds, are also observed [40,43]Furthermore, CRK5 influences the stability and localization of other PIN proteins, which are involved in the transport of auxin and the development of the embryo [77]. CRK5 expression has been reported to be controlled by many WRKY transcription factors, including WRKY18, WRKY40, and WRKY60, suggesting an interaction between CRK5 and ABA signaling pathways [43,69].

Additionally, CRK5 inhibits the signaling of both ethylene and salicylic acid (SA), which are crucial for growth and senescence [44]. Hence, root hair elongation, rosette leaf formation, and seedling height have been demonstrated to be influenced [44]. Previously, when AtCRK5 was overexpressed, an increase in plant biomass is observed, suggesting a potential role in growth promotion in general [61]. CRK28-deficient plants exhibit elongated, more branching roots compared to wild-type plants, while those with CRK28 overexpression display a contrasting phenotype, marked by restricted root growth and diminished lateral root development, highlighting the role of CRK28 in root organogenesis [46]. Additionally, crk28 mutants have shorter root hairs and an increased number of trichomes, whereas overexpressing lines show longer root hairs and a reduced number of trichomes. Moreover, it was found that CRK28 acts as a regulator of lateral root formation, affecting the regulation of cell division, primary root elongation, and ABA responses during seedling development and germination [46]. As a result, a positive effect on rosette diameters, inflorescence branching, and the regulation of root hair development is observed [46].

CRK25 was detected in proteins isolated from the cell wall of 5-day-old, not 11-day-old, etiolated hypocotyls of Arabidopsis plants, indicating that it is only involved in the early stages of growth in the elongating cells of Arabidopsis [79]. The expression of CRK25 can be influenced by plant factors such as salicylic acid (SA), jasmonic acid (JA), and ethylene (ET)[36].

CRK25was shown to have a role cell wall dynamics in Arabidopsis, specifically in the elongation of hypocotyls by rearranging and modifying components of the cell wall [79]. Global proliferative arrest (GPA) in Arabidopsis was shown to be regulated by CRK14 via a unique CRK14-dependent signaling mechanism [80]. The gain-of-function mutation in CRK10 results in a dwarf phenotype and collapsed xylem vessels in the roots and hypocotyls of Arabidopsis plants, accompanied by increased resistance to the vascular pathogen Fusarium oxysporum [81]. Transcriptomic analysis indicates that the crk10 mutant exhibits a constitutive up-regulation of genes responsive to both biotic and abiotic stress [81].

The transcriptional dynamics observed during seed germination across different seed compartments, including the testa, endosperm, and embryo, in Arabidopsis suggest that CRK11 and several other genes are involved in the germination process [82]. In CRK36 knockdown plants, cotyledon greening was reduced in response to ABA treatment, which could be associated to senescence [41]. Indeed, CRK36 overexpression causes early senescence characteristics, while a loss of function leads to delayed senescence [54].

These findings highlight the promise of CRK proteins as targets for genetic engineering and breeding strategies focused on improving plant growth, development, stress resilience, and productivity in agricultural systems.

5. Functional redundancy

The analysis of the CRKs genes reveals a complicated network of functional redundancy and regulatory relationships that are crucial for plant defense and stress responses that is most clearly documented in Arabidopsis. Research indicates that after mutating certain CRKs genes in Arabidopsis, there were no noticeable changes in the plant's defense process. This indicates the presence of afunctional redundancy, where the mutation of one gene is compensated for by closely related genes in the CRK family [83]. In Arabidopsis, CRK4 and CRK19 have the ability to compensate for the role of CRK5 in the signaling pathway of ABA [69]. The overexpression of CRK4 and CRK19 elicits identical ABA responses to those observed with CRK5, indicating a potential overlap in their functions within this signaling pathway [69]. Similar to CRK5, both CRK4 and CRK19 contribute to the enhanced drought resilience observed in plants. WRKY transcription factors, including WRKY18, WRKY40, and WRKY60, regulate the expression of CRK5, and they are also likely involved in the regulation of CRK4 and CRK19, indicating that these kinases are components of a complex regulatory system [69].

CRK6 and CRK7 were identified to have similar functions and redundancies in Arabidopsis plant defense and ROS regulation. They have significant sequence similarity, indicating that these two kinases perform similar functions [42]. This complicates the task of describing their specific roles, since the loss of one position might be seamlessly covered by the other. Indeed, there were no discernible changes in Arabidopsis plant growth or disease resistance when CRK6 was constitutively expressed [61]. It is possible that the absence of evident phenotypes in transgenic Arabidopsis plants that overexpress CRK6 is an indication that it is functionally redundant with other members of the CRK family, including CRK7. CRK31 and CRK32 collectively form a low diversified duplicate gene pair [84]. The individual suppression of each gene did not markedly affect the phenotype; nevertheless, the simultaneous suppression of both low-diversity duplicates resulted in Arabidopsis transgenic plants exhibiting abnormal phenotypes [84]. It is suggested that functional redundancy, a characteristic of low diversified duplicates, is implicated in CRK31 and its copy. Thereby, diversified duplicates, such as CRK31 and CRK23, play a significant role in preserving essential functions within the plant genome due to their conservation under purifying selection and their frequent association with critical signaling pathways.

The identification of functional redundancy within

the CRK gene family carries significant implications for genetic engineering and plant biology. Initially, due to the compensatory functions of other closely related genes, altering a single CRK gene may not yield the desired phenotypic alterations. This emphasizes the need for a more comprehensive methodology in the development of genetic modifications,

Moreover, as certain CRKs have overlapping functions, altering one gene may need concurrent modifications of others to substantially change plant responses to environmental stimuli. This interplay may complicate the engineering of plants for resilience, demanding a systems biology approach to understand the entire regulatory network involved.

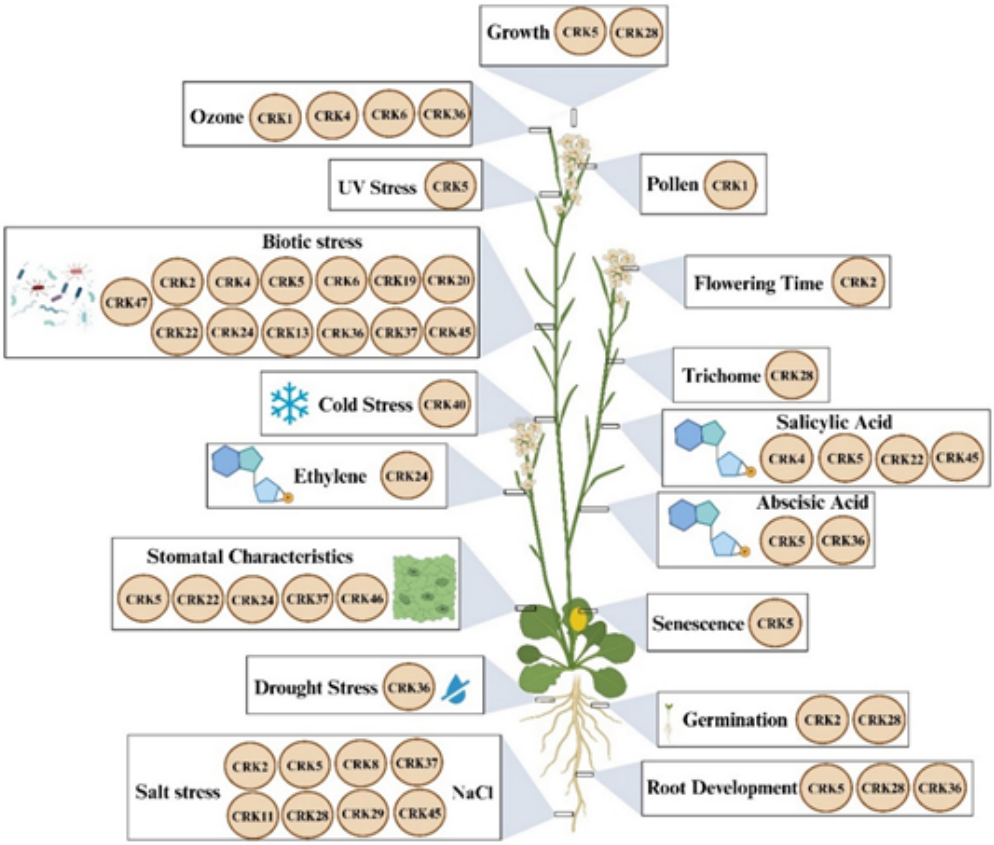

Figure 3: Functional Analysis of Cysteine-Rich Receptor-Like Kinases (CRKs) in Arabidopsis thaliana. This figure summarizes the experimentally characterized roles of CRKs in Arabidopsis, in regulating root development, seed germination, plant growth and development, and various biotic stress responses and abiotic stress.

6. Future directions

To fully understand how plants grow and develop, as well as to navigate challenges, it is essential to expand our understanding of the complete signaling pathway of CRKs in plants. Future studies should concentrate on several important areas to enhance our understanding of the significance of CRKs in plant biology. The conservation and divergence of CRKs functions in response to biotic and abiotic stresses will be determined by extending research beyond Arabidopsis to include other plant species. Investigating the complex molecular mechanisms by which CRKs affect signaling pathways, particularly regarding the interactions of each subdomain in signal transduction, can be improved through techniques such as site-directed mutagenesis, domain deletion, region exchange, and other methodologies. The manipulation of specific CRK genes, whether through overexpression or knockout, has been advanced by creating transgenic plants that exhibit modified CRK expression levels. Utilizing multi-omics approaches,

including genomics, transcriptomics, and proteomics, will enhance our understanding of CRK functions and their regulatory networks in relation to environmental stimuli, as well as evaluating their contributions to plant growth, development, and stress resilience.

7. Conclusions

The Arabidopsis CRK subfamily shows gene family evolution, expanding mainly through tandem and intrachromosomal duplications that enabled functional diversification. This evolutionary path has established a network of receptors that play both common and distinct roles in plant stress adaptation. Functional studies demonstrate that CRKs are essential regulators of ROS homeostasis, immune activation, and stress signalling, directly interacting with components like RBOHD, MAPKs, Ca^{2}□ channels, and hormone pathways. These interactions show how CRKs fit into complex signalling networks, where redundancy, synergy, and sequential activation form a strong defence against biotic and abiotic stressors. CRKs are vital targets for crop engineering, ipmroving resilience, optimising growth, and stabilising yield under environmental stress. Future research requires assessing the functional divergence of individual CRKs, their coordination within receptor complexes, and their contributions across species to facilitate targeted applications in agriculture and biotechnology.

Table 1. Functionals Analysis of Arabidopsis CRKs. The table summarizes the reported biological roles of individual CRK family members in Arabidopsis.

No.	TAIR	Name	Role	References
1	AT1g19090	CRK1	Pollen development role Plant's immune response	[58,74]
2	AT1g70520	CRK2	Modulation of ROS Regulation of calcium signaling Promotion of callose accumulation Immune responses Influence on flowering and growth regulation Salt tolerance	[40,45,57,75]
3	AT3g45860	CRK4	Activation in response to pathogen infection and salicylic acid Role in defense priming Enhancement of ROS generation Stomatal closure in response to pathogen invasion.	[66,85]
4	AT4g23130	CRK5	Enhancement of resistance to pseudomonas syringae. Regulation of drought tolerance. Influence on auxin transport. Impact on root growth and gravitropic responses. Response to ultraviolet radiation Regulation of stomatal conductance and senescence	[51,60,61]
5	AT4g23140	CRK6	ROS regulation Response to oxidative stress induced by ozone	[40,43,61,69,76]
6	AT4g23150	CRK7	ROS regulation Response to oxidative stress induced by ozone	[42,61]
7	AT4g23180	CRK10	Plant growth regulation Defense mechanisms against fusarium oxysporum Up-regulation of genes associated with both biotic and abiotic stress	[42]
8	AT4g23190	CRK11	Seed germination.	[82]
9	AT4g23210	CRK13	Initial defense signaling. Alternative splicing in response to PAMPs.	[62,63]
10	AT4g23220	CRK14	Regulation of global proliferative arrest (GPA)	[80]
11	AT4g23260	CRK18	ABA signaling under salt stress conditions	[67]
12	AT4g23270	CRK19	Activation in response to pathogen infection and salicylic acid Drought tolerance	[26,61,83]

No.	TAIR	Name	Role	References
13	AT4g23280	CRK20	Regulator of plant responses to pseudomonas syringae pv. Tomato dc3000 Response to ozone stress	[40,64]
14	AT4g23300	CRK22	Defense against toxins produced by the pathogen verticillium dahliae Involved in the signaling pathways that modify the salicylic acid	[44]
15	AT4g23320	CRK24	Involvement in ethylene signaling pathways Potential role in drought tolerance	[70]
16	AT4g05200	CRK25	Involvement in early growth phases Influence on cell wall dynamics	[79]
17	AT4g21400	CRK28	Germination and root development Involved in trichomes numbers. Involve in plant resistance to pseudomonas syringae Involved in salt stress.	[40,46,58]
18	AT4g21410	CRK29	Involvement in plant defense mechanism against pseudomonas syringae Alternative splicing in response to PAMPs.	[58]
19	AT4g11490	CRK33	Stomatal density regulation.	[68]
20	AT4g11530	CRK34	Phosphate homeostasis.	[71]
21	AT4g04490	CRK36	Drought and high-salinity stress response regulation. Influence on senescence	[41,54]
22	AT4g04500	CRK37	Modulation of pattern-triggered immunity (PTI)	[54,58]
23	AT4g04540	CRK39	Ammonium stress response.	[72]
24	AT4g04570	CRK40	Cold stress response	[73]
25	AT4g11890	CRK45	Resistance to pseudomonas syringae Modulation of bolting and early seedling development	[65]

Author Contributions: The author, Fatima Omari Alzahrani, participated in all stages of the research study, involving conceptualization, design, data collection, analysis, and manuscript writing.

Funding: This research received no external funding.

Data Availability Statement: All data within the review article

Acknowledgments: Not Applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

8. Abbreviations

The following abbreviations are used in this manuscript:

CRK Cysteine-rich receptor-like kinases

PCD Programmed cell death

RLKs receptor-like protein kinases

ROS reactive oxygen species

TM transmembrane domain

ECLB extracellular ligand-binding domain

SP signal peptide

EGFs epidermal growth factor-like repeats

DUF26 domain the DOMAIN OF UNKNOWN FUNCTION 26

PM plasma membrane

PDLPs plasmodesmata-localized proteins

9. References

1. *Xu, M.Y.; Zhang, L.; Li, W.W.; Hu, X.L.; Wang, M.; Fan, Y.L.; Zhang, C.Y.; Wang, L. Stress-induced early flowering is mediated by miR169 in Arabidopsis thaliana. J Exp Bot 2014, 65, 89–101, DOI https://doi.org/10.1093/jxb/ert353..*
2. *Wada, K.C.; Takeno, K. Stress-induced flowering. Plant signaling & behavior 2010, 5, 944–947, DOI https://doi.org/10.4161/psb.5.8.11826..*
3. *Williams, B.; Dickman, M. Plant programmed cell death: can't live with it; can't live without it. Molecular Plant Pathology 2008, 9, 531–544, DOI https://doi.org/10.1111/j.1364-3703.2008.00473.x..*
4. *Jones, J.D.; Dangl, J.L. The plant immune system. Nature 2006, 444, 323–329, DOI https://doi.org/10.1038/nature05286..*
5. *Coll, N.S.; Epple, P.; Dangl, J.L. Programmed cell death in the plant immune system. Cell Death & Differentiation 2011, 18, 1247–1256, DOI https://doi.org/10.1038/cdd.2011.37..*
6. *Bruggeman, Q.; Raynaud, C.; Benhamed, M.; Delarue, M. To die or not to die? Lessons from lesion mimic mutants. Frontiers in plant science 2015, 6, 24, DOI https://doi.org/10.3389/fpls.2015.00024..*
7. *The Arabidopsis Genome Initiative Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. Nature 2000, 408, 796–815, DOI https://doi.org/10.1038/35048692..*
8. *Dardick, C.; Chen, J.; Richter, T.; Ouyang, S.; Ronald, P. The rice kinase database. A phylogenomic database for the rice kinome. Plant Physiol 2007, 143, 579–586, DOI https://doi.org/10.1104/pp.106.087270..*
9. *Krasensky-Wrzaczek, J.; Wrzaczek, M. New kids on the block—cysteine-rich receptor-like kinases in pattern-triggered immunity. Curr Opin Plant Biol 2024, 81, 102619.*
10. *Zeiner, A.; Colina, F.J.; Citterico, M.; Wrzaczek, M. CYSTEINE-RICH RECEPTOR-LIKE PROTEIN KINASES: their evolution, structure, and roles in stress response and development. J Exp Bot 2023, 74, 4910–4927.*
11. *Zhang, Y.; Tian, H.; Chen, D.; Zhang, H.; Sun, M.; Chen, S.; Qin, Z.; Ding, Z.; Dai, S. Cysteine-rich receptor-like protein kinases: emerging regulators of plant stress responses. Trends Plant Sci 2023, 28, 776–794.*
12. *Shiu, S.; Karlowski, W.M.; Pan, R.; Tzeng, Y.; Mayer, K.F.; Li, W. Comparative analysis of the receptor-like kinase family in Arabidopsis and rice. Plant Cell 2004, 16, 1220–1234, DOI https://doi.org/10.1105/tpc.020834..*
13. *Yan, J.; Su, P.; Meng, X.; Liu, P. Phylogeny of the plant receptor-like kinase (RLK) gene family and expression analysis of wheat RLK genes in response to biotic and abiotic stresses. BMC Genomics 2023, 24, 224.*
14. *Zhao, G.; Qin, S.; Wei, Z.; Bai, X.; Guo, J.; Kang, Z.; Guo, J. Evolutionary characteristics, expression patterns of wheat receptor-like kinases and functional analysis of TaCrRLK1L16. Stress Biology 2025, 5, 1–16.*
15. *Zhao, W.; Zhang, F.; Wang, J.; Fang, S.; Cheng, Z.; Ma, X.; Fan, J.; Xu, Z.; Chen, X. Comprehensive Genome-Wide Characterization of L-Type Lectin Receptor-like Kinase (L-LecRLK) Genes in Wheat (Triticum aestivum L.) and Their Response to Abiotic Stress. Plants 2025, 14, 1884.*
16. *Gao, Y.; Qu, Q.; Liu, N.; Sun, M.; Liu, X.; Cao, Z.; Dong, J. Genome identification of the LRR-RLK gene family in maize (Zea mays) and expression analysis in response to Fusarium verticillioides infection. BMC Plant Biology 2025, 25, 524.*
17. *Li, W.; Liao, C.; Bluhm, B.H.; Mengiste, T.; Woloshuk, C.P. A maize (Zea mays L.) BIK1-like receptor-like cytoplasmic kinase contributes to disease resistance. Plant Mol Biol Rep 2022, 40, 28–42.*
18. *Zou, X.; Qin, Z.; Zhang, C.; Liu, B.; Liu, J.; Zhang, C.; Lin, C.; Li, H.; Zhao, T. Over-expression of an S-domain receptor-like kinase extracellular domain improves panicle architecture and grain yield in rice. J Exp Bot 2015, 66, 7197–7209.*
19. *Singh, A.; Breja, P.; Khurana, J.P.; Khurana, P. Wheat Brassinosteroid-Insensitive1 (TaBRI1) interacts with members of TaSERK gene family and cause early flowering and seed yield enhancement in Arabidopsis. PLoS One 2016, 11, e0153273.*
20. *Yang, L.; Wu, K.; Gao, P.; Liu, X.; Li, G.; Wu, Z. GsLRPK, a novel cold-activated leucine-rich repeat receptor-like protein kinase from Glycine soja, is a positive regulator to cold stress tolerance. Plant Science 2014, 215, 19–28.*
21. *Zhang, L.; Xu, D.; Zhang, N.; Gao, P.; Zhang, J.; Zhao, J.; Han, Y.; Chen, Y.; Sun, Y.; Zhao, J. Activity-directed selection of natural variants of a receptor kinase facilitates salt-tolerant rice breeding. Plant Physiol 2024, 194, 618–622.*

22. *Sun, T.; Nitta, Y.; Zhang, Q.; Wu, D.; Tian, H.; Lee, J.S.; Zhang, Y. Antagonistic interactions between two MAP kinase cascades in plant development and immune signaling. EMBO Rep 2018, 19, e45324.*
23. *Chen, T. Identification and characterization of the LRR repeats in plant LRR-RLKs. BMC Mol Cell Biol 2021, 22, 9, DOI 10.1186/s12860-021-00344-y..*
24. *Kohorn, B.D.; Kohorn, S.L. The cell wall-associated kinases, WAKs, as pectin receptors. Frontiers in plant science 2012, 3, 88.*
25. *Liu, J.; Li, W.; Wu, G.; Ali, K. An update on evolutionary, structural, and functional studies of receptor-like kinases in plants. Frontiers in Plant Science 2024, 15, 1305599.*
26. *Lu, D.; Wu, S.; Gao, X.; Zhang, Y.; Shan, L.; He, P. A receptor-like cytoplasmic kinase, BIK1, associates with a flagellin receptor complex to initiate plant innate immunity. Proceedings of the National Academy of Sciences 2010, 107, 496–501.*
27. *Yu, M.; Nie, X.; Ke, S.; Li, L.; Li, B. Receptor-like kinase cleavage: molecular mechanism and regulatory functions in plants. New Phytol 2025, 246, 2478–2483.*
28. *Greeff, C.; Roux, M.; Mundy, J.; Petersen, M. Receptor-like kinase complexes in plant innate immunity. Frontiers in plant science 2012, 3, 209, DOI https://doi.org/10.3389/fpls.2012.00209..*
29. *Walker, J.C. Structure and function of the receptor-like protein kinases of higher plants. Plant Mol Biol 1994, 26, 1599–1609, DOI https://doi.org/10.1007/978-94-011-0239-1_20..*
30. *Dievart, A.; Gottin, C.; Périn, C.; Ranwez, V.; Chantret, N. Origin and diversity of plant receptor-like kinases. Annual Review of Plant Biology 2020, 71, 131–156, DOI https://doi.org/10.1146/annurev-arplant-073019-025927..*
31. *Gish, L.A.; Clark, S.E. The RLK/Pelle family of kinases. The Plant Journal 2011, 66, 117–127, DOI https://doi.org/10.1111/j.1365-313x.2011.04518.x..*
32. *Yip Delormel, T.; Boudsocq, M. Properties and functions of calcium-dependent protein kinases and their relatives in Arabidopsis thaliana. New Phytol 2019, 224, 585–604, DOI https://doi.org/10.1111/nph.16088..*
33. *Sun, Y.; Li, L.; Macho, A.P.; Han, Z.; Hu, Z.; Zipfel, C.; Zhou, J.; Chai, J. Structural basis for flg22-induced activation of the Arabidopsis FLS2-BAK1 immune complex. Science 2013, 342, 624–628.*
34. *Jose, J.; Ghantasala, S.; Roy Choudhury, S. Arabidopsis transmembrane receptor-like kinases (RLKs): a bridge between extracellular signal and intracellular regulatory machinery. International journal of molecular sciences 2020, 21, 4000.*
35. *Chinchilla, D.; Zipfel, C.; Robatzek, S.; Kemmerling, B.; Nürnberger, T.; Jones, J.D.; Felix, G.; Boller, T. A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. Nature 2007, 448, 497–500.*
36. *Wrzaczek, M.; Brosché, M.; Salojärvi, J.; Kangasjärvi, S.; Idänheimo, N.; Mersmann, S.; Robatzek, S.; Karpiński, S.; Karpińska, B.; Kangasjärvi, J. Transcriptional regulation of the CRK/DUF26 group of receptor-like protein kinases by ozone and plant hormones in Arabidopsis. BMC plant biology 2010, 10, 1–19.*
37. *Zhang, Z.; Deng, H.; Hu, S.; Han, H. Phase separation: a new window in RALF signaling. Front Plant Sci 2024, 15, 1409770, DOI 10.3389/fpls.2024.1409770..*
38. *Vaattovaara, A.; Brandt, B.; Rajaraman, S.; Safronov, O.; Veidenberg, A.; Luklová, M.; Kangasjärvi, J.; Löytynoja, A.; Hothorn, M.; Salojärvi, J. Mechanistic insights into the evolution of DUF26-containing proteins in land plants. Communications biology 2019, 2, 56.*
39. *Shiu, S.; Bleecker, A.B. Receptor-like kinases from Arabidopsis form a monophyletic gene family related to animal receptor kinases. Proceedings of the National Academy of Sciences 2001, 98, 10763–10768, DOI https://doi.org/10.1073/pnas.181141598..*
40. *Bourdais, G.; Burdiak, P.; Gauthier, A.; Nitsch, L.; Salojärvi, J.; Rayapuram, C.; Idänheimo, N.; Hunter, K.; Kimura, S.; Merilo, E. Large-scale phenomics identifies primary and fine-tuning roles for CRKs in responses related to oxidative stress. PLoS Genetics 2015, 11, e1005373.*
41. *Tanaka, H.; Osakabe, Y.; Katsura, S.; Mizuno, S.; Maruyama, K.; Kusakabe, K.; Mizoi, J.; Shinozaki, K.; Yamaguchi-Shinozaki, K. Abiotic stress-inducible receptor-like kinases negatively control ABA signaling in Arabidopsis. The Plant Journal 2012, 70, 599–613.*
42. *Idänheimo, N.; Gauthier, A.; Salojärvi, J.; Siligato, R.; Brosché, M.; Kollist, H.; Mähönen, A.P.; Kangasjärvi, J.; Wrzaczek, M. The Arabidopsis thaliana cysteine-rich receptor-like kinases CRK6 and CRK7 protect against apoplastic oxidative stress. Biochem Biophys Res Commun 2014, 445, 457–462.*

43. *Burdiak, P.; Rusaczonek, A.; Witoń, D.; Głów, D.; Karpiński, S. Cysteine-rich receptor-like kinase CRK5 as a regulator of growth, development, and ultraviolet radiation responses in Arabidopsis thaliana. J Exp Bot 2015, 66, 3325–3337.*
44. *Zhao, J.; Sun, Y.; Li, X.; Li, Y. CYSTEINE-RICH RECEPTOR-LIKE KINASE5 (CRK5) and CRK22 regulate the response to Verticillium dahliae toxins. Plant Physiol 2022, 190, 714–731.*
45. *Hunter, K.; Kimura, S.; Rokka, A.; Tran, H.C.; Toyota, M.; Kukkonen, J.P.; Wrzaczek, M. CRK2 enhances salt tolerance by regulating callose deposition in connection with PLD α 1. Plant Physiol 2019, 180, 2004–2021.*
46. *Pelagio-Flores, R.; Muñoz-Parra, E.; Barrera-Ortiz, S.; Ortiz-Castro, R.; Saenz-Mata, J.; Ortega-Amaro, M.A.; Jiménez-Bremont, J.F.; López-Bucio, J. The cysteine-rich receptor-like protein kinase CRK28 modulates Arabidopsis growth and development and influences abscisic acid responses. Planta 2020, 251, 1–12.*
47. *Tamura, K.; Stecher, G.; Kumar, S. MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol 2021, 38, 3022–3027.*
48. *Miyakawa, T.; Miyazono, K.; Sawano, Y.; Hatano, K.; Tanokura, M. Crystal structure of ginkbilobin-2 with homology to the extracellular domain of plant cysteine-rich receptor-like kinases. Proteins: Structure, Function, and Bioinformatics 2009, 77, 247–251.*
49. *Sawano, Y.; Miyakawa, T.; Yamazaki, H.; Tanokura, M.; Hatano, K. Purification, characterization, and molecular gene cloning of an antifungal protein from Ginkgo biloba seeds. 2007.*
50. *Hong-Bo, S.; Zong-Suo, L.; Ming-An, S. LEA proteins in higher plants: structure, function, gene expression and regulation. Colloids and surfaces B: Biointerfaces 2005, 45, 131–135.*
51. *Yeh, Y.; Chang, Y.; Huang, P.; Huang, J.; Zimmerli, L. Enhanced Arabidopsis pattern-triggered immunity by overexpression of cysteine-rich receptor-like kinases. Frontiers in plant science 2015, 6, 322.*
52. *Nagahara, N. Intermolecular disulfide bond to modulate protein function as a redox-sensing switch. Amino Acids 2011, 41, 59–72.*
53. *Feige, M.J.; Hendershot, L.M. Disulfide bonds in ER protein folding and homeostasis. Curr Opin Cell Biol 2011, 23, 167–175.*
54. *Lee, D.S.; Kim, Y.C.; Kwon, S.J.; Ryu, C.; Park, O.K. The Arabidopsis cysteine-rich receptor-like kinase CRK36 regulates immunity through interaction with the cytoplasmic kinase BIK1. Frontiers in plant science 2017, 8, 1856.*
55. *Pereira, S.F.; Goss, L.; Dworkin, J. Eukaryote-like serine/threonine kinases and phosphatases in bacteria. Microbiology and Molecular Biology Reviews 2011, 75, 192–212.*
56. *Taylor, S.S.; Keshwani, M.M.; Steichen, J.M.; Kornev, A.P. Evolution of the eukaryotic protein kinases as dynamic molecular switches. Philosophical Transactions of the Royal Society B: Biological Sciences 2012, 367, 2517–2528.*
57. *Kimura, S.; Hunter, K.; Vaahtera, L.; Tran, H.C.; Citterico, M.; Vaattovaara, A.; Rokka, A.; Stolze, S.C.; Harzen, A.; Meißner, L. CRK2 and C-terminal phosphorylation of NADPH oxidase RBOHD regulate reactive oxygen species production in Arabidopsis. Plant Cell 2020, 32, 1063–1080.*
58. *Yadeta, K.A.; Elmore, J.M.; Creer, A.Y.; Feng, B.; Franco, J.Y.; Rufian, J.S.; He, P.; Phinney, B.; Coaker, G. A cysteine-rich protein kinase associates with a membrane immune complex and the cysteine residues are required for cell death. Plant Physiol 2017, 173, 771–787.*
59. *Rayapuram, C.; Jensen, M.K.; Maiser, F.; Shanir, J.V.; Hornshøj, H.; Rung, J.H.; Gregersen, P.L.; Schweizer, P.; Collinge, D.B.; Lyngkjaer, M.F. Regulation of basal resistance by a powdery mildew-induced cysteine-rich receptor-like protein kinase in barley. Molecular Plant Pathology 2012, 13, 135–147.*
60. *Sistenich, A.J.; Fürtauer, L.; Scheele, F.; Conrath, U. Marker and readout genes for defense priming in Pseudomonas cannabina pv. alisalensis interaction aid understanding systemic immunity in Arabidopsis. Scientific Reports 2024, 14, 3489.*
61. *Chen, K.; Du, L.; Chen, Z. Sensitization of defense responses and activation of programmed cell death by a pathogen-induced receptor-like protein kinase in Arabidopsis. Plant Mol Biol 2003, 53, 61–74.*
62. *Acharya, B.R.; Raina, S.; Maqbool, S.B.; Jagadeeswaran, G.; Mosher, S.L.; Appel, H.M.; Schultz, J.C.; Klessig, D.F.; Raina, R. Overexpression of CRK13, an Arabidopsis cysteine-rich receptor-like kinase, results in enhanced resistance to Pseudomonas syringae. The Plant Journal 2007, 50, 488–499.*

63. *Bazin, J.; Mariappan, K.; Jiang, Y.; Blein, T.; Voelz, R.; Crespi, M.; Hirt, H. Role of MPK4 in pathogen-associated molecular pattern-triggered alternative splicing in Arabidopsis. PLoS pathogens 2020, 16, e1008401.*
64. *Ederli, L.; Madeo, L.; Calderini, O.; Gehring, C.; Moretti, C.; Buonaurio, R.; Paolocci, F.; Pasqualini, S. The Arabidopsis thaliana cysteine-rich receptor-like kinase CRK20 modulates host responses to Pseudomonas syringae pv. tomato DC3000 infection. J Plant Physiol 2011, 168, 1784–1794.*
65. *Zhang, X.; Han, X.; Shi, R.; Yang, G.; Qi, L.; Wang, R.; Li, G. Arabidopsis cysteine-rich receptor-like kinase 45 positively regulates disease resistance to Pseudomonas syringae. Plant Physiology and Biochemistry 2013, 73, 383–391.*
66. *Desaki, Y.; Morishima, M.; Sano, Y.; Uemura, T.; Ito, A.; Nemoto, K.; Nozawa, A.; Sawasaki, T.; Arimura, G. Cytoplasmic kinase network mediates defense response to Spodoptera litura in Arabidopsis. Plants 2023, 12, 1747.*
67. *Huang, K.; Lin, W.; Cheng, W. Salt hypersensitive mutant 9, a nucleolar APUM23 protein, is essential for salt sensitivity in association with the ABA signaling pathway in Arabidopsis. BMC plant biology 2018, 18, 1–21.*
68. *Arellano-Villagómez, F.C.; Guevara-Olvera, L.; Zuñiga-Mayo, V.M.; E. Cerbantez-Bueno, V.; Verdugo-Perales, M.; R. Medina, H.; De Folter, S.; Acosta-García, G. Arabidopsis cysteine-rich receptor-like protein kinase CRK33 affects stomatal density and drought tolerance. Plant Signaling & Behavior 2021, 16, 1905335.*
69. *Lu, K.; Liang, S.; Wu, Z.; Bi, C.; Yu, Y.; Wang, X.; Zhang, D. Overexpression of an Arabidopsis cysteine-rich receptor-like protein kinase, CRK5, enhances abscisic acid sensitivity and confers drought tolerance. J Exp Bot 2016, 67, 5009–5027.*
70. *Ko, J.; Yang, S.H.; Han, K. Upregulation of an Arabidopsis RING-H2 gene, XERICO, confers drought tolerance through increased abscisic acid biosynthesis. The Plant Journal 2006, 47, 343–355.*
71. *Thieme, C.J.; Rojas-Triana, M.; Stecyk, E.; Schudoma, C.; Zhang, W.; Yang, L.; Miñambres, M.; Walther, D.; Schulze, W.X.; Paz-Ares, J. Endogenous Arabidopsis messenger RNAs transported to distant tissues. Nature Plants 2015, 1, 1–9.*
72. *Burian, M.; Podgórska, A.; Ostaszewska-Bugajska, M.; Kryzheuskaya, K.; Dziewit, K.; Wdowiak, A.; Laszczka, M.; Szal, B. A prospective study of short-term apoplastic responses to ammonium treatment. J Plant Physiol 2023, 286, 154008.*
73. *Kim, J.Y.; Park, S.J.; Jang, B.; Jung, C.; Ahn, S.J.; Goh, C.; Cho, K.; Han, O.; Kang, H. Functional characterization of a glycine-rich RNA-binding protein 2 in Arabidopsis thaliana under abiotic stress conditions. The Plant Journal 2007, 50, 439–451.*
74. *Becker, J.D.; Boavida, L.C.; Carneiro, J.; Haury, M.; Feijó, J.A. Transcriptional profiling of Arabidopsis tissues reveals the unique characteristics of the pollen transcriptome. Plant Physiol 2003, 133, 713–725.*
75. *Wang, L.; Yang, T.; Lin, Q.; Wang, B.; Li, X.; Luan, S.; Yu, F. Receptor kinase FERONIA regulates flowering time in Arabidopsis. BMC plant biology 2020, 20, 1–16.*
76. *Rigó, G.; Ayaydin, F.; Tietz, O.; Zsigmond, L.; Kovács, H.; Páy, A.; Salchert, K.; Darula, Z.; Medzihradszky, K.F.; Szabados, L. Inactivation of plasma membrane–localized CDPK-RELATED KINASE5 decelerates PIN2 exocytosis and root gravitropic response in Arabidopsis. Plant Cell 2013, 25, 1592–1608.*
77. *Baba, A.I.; Valkai, I.; Labhane, N.M.; Koczka, L.; Andrási, N.; Klement, É; Darula, Z.; Medzihradszky, K.F.; Szabados, L.; Fehér, A. CRK5 protein kinase contributes to the progression of embryogenesis of Arabidopsis thaliana. International Journal of Molecular Sciences 2019, 20, 6120.*
78. *Cséplő, Á; Zsigmond, L.; Andrási, N.; Baba, A.I.; Labhane, N.M.; Pető, A.; Kolbert, Z.; Kovács, H.E.; Steinbach, G.; Szabados, L. The AtCRK5 protein kinase is required to maintain the ROS NO balance affecting the PIN2-mediated root gravitropic response in Arabidopsis. International journal of molecular sciences 2021, 22, 5979.*
79. *Irshad, M.; Canut, H.; Borderies, G.; Pont-Lezica, R.; Jamet, E. A new picture of cell wall protein dynamics in elongating cells of Arabidopsis thaliana: confirmed actors and newcomers. BMC plant biology 2008, 8, 1–16.*
80. *Imai, S.; Hirozawa, H.; Sugahara, S.; Ishizaki, C.; Higuchi, M.; Matsushita, Y.; Suzuki, T.; Mochizuki, N.; Nagatani, A.; Ueguchi, C. The CRK14 gene encoding a cysteine-rich receptor-like kinase is implicated in the regulation of global proliferative arrest in Arabidopsis thaliana. Genes to Cells 2024.*

81. *Piovesana, M.; Wood, A.K.; Smith, D.P.; Deery, M.J.; Bayliss, R.; Carrera, E.; Wellner, N.; Kosik, O.; Napier, J.A.; Kurup, S. A point mutation in the kinase domain of CRK10 leads to xylem vessel collapse and activation of defence responses in Arabidopsis. J Exp Bot 2023, 74, 3104–3121.*
82. *Dekkers, B.J.; Pearce, S.; van Bolderen-Veldkamp, R.P.; Marshall, A.; Widera, P.; Gilbert, J.; Drost, H.; Bassel, G.W.; Müller, K.; King, J.R. Transcriptional dynamics of two seed compartments with opposing roles in Arabidopsis seed germination. Plant Physiol 2013, 163, 205–215.*
83. *Chen, K.; Fan, B.; Du, L.; Chen, Z. Activation of hypersensitive cell death by pathogen-induced receptor-like protein kinases from Arabidopsis. Plant Mol Biol 2004, 56, 271–283.*
84. *Ezoe, A.; Shirai, K.; Hanada, K. Degree of functional divergence in duplicates is associated with distinct roles in plant evolution. Mol Biol Evol 2021, 38, 1447–1459.*
85. *Miyamoto, T.; Uemura, T.; Nemoto, K.; Daito, M.; Nozawa, A.; Sawasaki, T.; Arimura, G. Tyrosine kinase-dependent defense responses against herbivory in Arabidopsis. Frontiers in plant science 2019, 10, 776.*