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Elucidando el mecanismo de interacción entre el receptor tipo cinasa CRK10 y el transportador de poliaminas AtPUT2

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List of Abbreviations

AOs	Amine oxidases
bp	Base pair
BiFC	Bimolecular fluorescent complementation
CDS	Coding sequence
CKD	Cytoplasmic kinase domain
DAOs	Diamine oxidases
DUF26	Domain of Unknown Function 26
ECD	Extracellular domain
EPK	Eukaryotic Protein Kinases
HR	Hypersensitive response
LAT	L-type amino acid transporter
LRR	Leucine rich repeat
MAPK	Mitogen-activated protein kinase
NBD	Nucleotide-binding domain
NLR	Nucleotide-binding leucine-rich repeat receptors
ORF	Open reading frame
PAOs	Polyamine oxidases
PCD	Programmed cell death
PDLPs	PLASMODESMATA LOCALIZED PROTEINS
PRR	Pattern recognition receptor
PTI	PAMP-triggered immunity
PUT	Polyamine uptake transporter
Put	Putrescine
RBOH-D	RESPIRATORY BURST ORTHOLOG D
RLCK	Receptor-like cytoplasmic kinase
RLK	Receptor-like kinase
RLP	Receptor-like protein
ROS	Reactive oxygen species
SAR	Systemic acquired resistance
SP	Signal peptide
Spd	Spermidine
Spm	Spermine
TMD	Transmembrane domain
YFP	Yellow fluorescent protein

Summary

Plants, as sessile organisms, have evolved intricate signal transduction pathways to perceive and respond to environmental stimuli. Receptor-like kinases (RLKs) are central components of these pathways, linking external cues to intracellular signaling. Although a vast number of receptors have been identified in plants´ genomes, their recognized ligands, specific functions, and interaction partners, including membrane proteins, have not been fully described. In this study, we investigate the interaction mechanism between the cysteine-rich receptor-like kinase *At*CRK10 and the *Arabidopsis thaliana* polyamine transporter *At*PUT2, both of which are implicated in plant immune responses. Particularly, we focus on determining which of the protein domains of *At*CRK10, the extracellular, transmembrane, and intracellular kinase domains, interact with the *At*PUT2 protein through Bimolecular fluorescence complementation (BiFC) assays in tobacco leaves. BiFC assays revealed that *At*PUT2 interacts with the cytoplasmic kinase domain and the extracellular domain of *At*CRK10. Interestingly, this interaction depends on a fully functional kinase domain; a death-kinase variant, obtained by a point mutation in the conserved aspartic acid that abolishes the kinase activity of the *At*CRK10 protein, failed to produce a fluorescence signal, suggesting a loss of interaction.

Moreover, preliminary characterization of reporter lines *promCRK10*::*GFP-GUS* and *promCRK10*::*CRK0-GFP-GUS* showed that *At*CRK10 is expressed in rosette leaves, particularly in the guard cells of the stomata, while the evaluation of *promPUT2*::*GFP-GUS* and *promPUT2*::*PUT2-GFP-GUS* reporter lines showed that *At*PUT2 is also expressed in rosette leaves.

We propose a model in which activation of *At*CRK10 upon pathogen perception might induce its homodimerization and autophosphorylation, thereby facilitating its interaction with the *At*PUT2 transporter through its extracellular and kinase domains. This *At*PUT2-*At*CRK10 interaction could induce polyamine transport upon pathogen activation.

Resumen

Las plantas, como organismos sésiles, han desarrollado complejas vías de transducción de señales para percibir y responder a estímulos ambientales. Los receptores tipo quinasa (RLKs) son componentes centrales de estas vías, vinculando señales ambientales a respuestas intracelulares. Aunque se ha identificado un gran número de receptores RLK en los genomas de plantas, sus ligandos que reconocen, sus funciones particulares y las proteínas con las que interactúan, incluyendo proteínas de membrana, solo han sido descritos en pocos casos. En este estudio investigamos el mecanismo de interacción entre el receptor tipo quinasa rico en cisteína *At*CRK10 y el transportador de poliaminas de *Arabidopsis thaliana At*PUT2, dos proteínas implicadas en la respuesta de defensa de las plantas. En particular nos enfocamos en determinar cuál de los tres dominios de *At*CRK10, el extracelular, el transmembranal y el intracelular quinasa, interactúa in planta con la proteína *At*PUT2, utilizando la técnica de Complementación Bimolecular de Fluorescencia (BiFC) en células de hoja de tabaco. Los análisis de BiFC mostraron que *At*PUT2 puede interactuar con los dominios extracelulares y la quinasa de *At*CRK10. Interesantemente, esta interacción depende de que el dominio quinasa sea funcional, ya que una variante que generamos mediante una mutación puntual en el ácido aspártico conservado, diseñada para inactivar la actividad quinasa en la proteína completa de *At*CRK10, no produjo señal de fluorescencia, lo que sugiere una pérdida de la interacción.

Por otra parte, la caracterización preliminar de líneas reporteras *promCRK10*::*GFP-GUS* y *promCRK10*::*CRK0-GFP-GUS* mostró que *At*CRK10 se expresa en las hojas de la roseta, particularmente en las células guarda de los estomas, mientras que el uso de las reporteras prom*PUT2*::*GFP-GUS* y *promPUT2*::*PUT2-GFP-GUS* mostró que el gen *At*PUT2 también se expresa en hojas de la roseta.

Proponemos un modelo en el que la activación del receptor *At*CRK10 tras la detección de patógenos podría inducir su homodimerización y autofosforilación, lo que favorecería su interacción con el transportador *At*PUT2 a través de sus dominios extracelular y quinasa. Esta interacción *At*CRK10-*At*PUT2 podría inducir el transporte de poliaminas en respuesta al ataque de patógenos.

1. Introduction

Plants, unlike animals and other motile organisms, cannot move; therefore, they are constantly influenced by various environmental and physiological signals from their surroundings. To survive, plants must effectively perceive multiple simultaneous environmental cues, process the information, and activate appropriate cellular responses to adapt to new and challenging conditions.

1.1 Plant Signal Transduction in the Immune Response

Plant signal transduction refers to a series of events that enable plants to perceive intracellular and extracellular cues and integrate this information to generate appropriate cellular responses (Valls & Esposito, 2022). A generalized overview of these signal transduction processes is presented in Figure 1. The recognition and interpretation of internal and external stimuli are mediated by a complex network of signaling proteins that function at the cell membrane and in the cytoplasm (Bender & Zipfel, 2023). Specifically, at the cell surface, membrane receptors mediate the perception of exogenous molecules. Upon ligand binding to its receptor, these membrane proteins undergo conformational changes that facilitate the formation of homo- and heterodimers (Roux & Zipfel, 2011) and receptor-mediated protein modifications (Bhatla & Lal, 2018). These protein-protein interactions initiate downstream signaling processes through a series of biochemical reactions, *e.g.*, phosphorylation events (Nussinov et al., 2025), which trigger the activation of second messengers, including cytosolic calcium (Ca^{2+}), reactive oxygen species (ROS), and cyclic nucleotides (cAMP/cGMP) (Demidchik et al., 2017), and recruitment of other effector proteins important for signal amplification (Valls & Esposito, 2022). Thus, signal propagation depends on the presence of multiple molecular actors and regulatory proteins that contribute to the integration and specificity of cellular responses (Nussinov et al., 2025), which translate into a variety of physiological changes, such as altered gene expression, changes in cytoskeleton structure, and enzymatic activity (Demidchik et al., 2017). It is this principle of signal transduction that provides the fundamental basis for the plant's immune response upon pathogen perception.

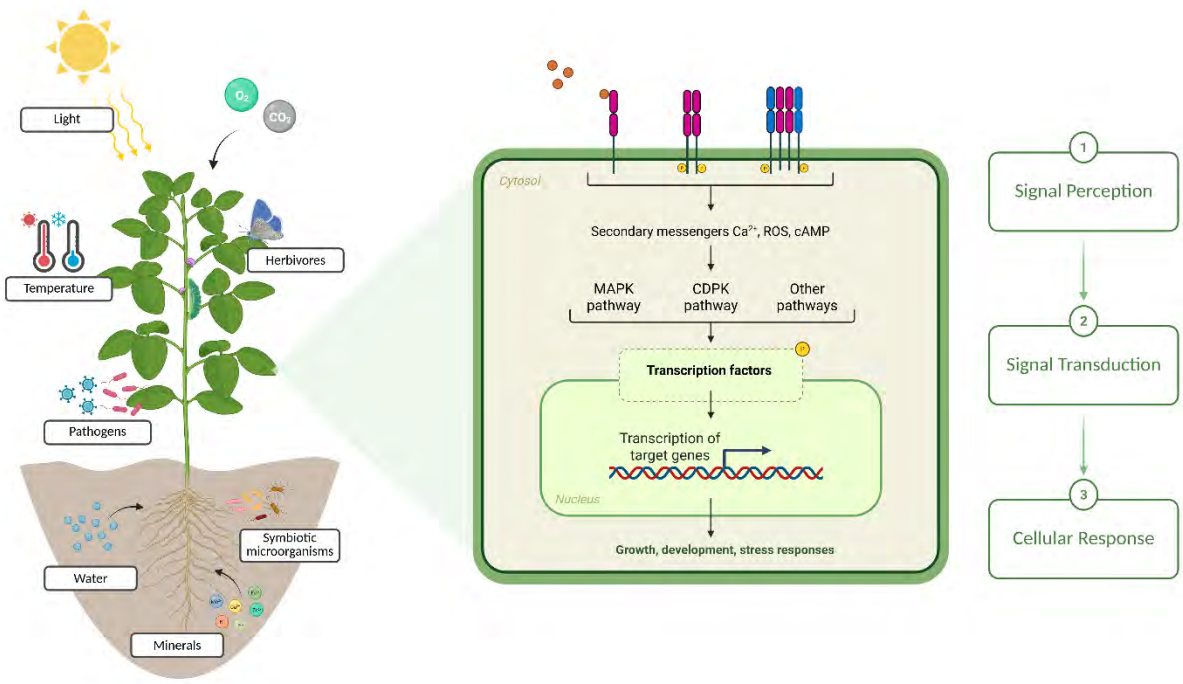

Figure 1. *General Overview of Plant Signal Transduction.* Environmental and internal stimuli are perceived by specialized receptors on the plant cell surface. This perception initiates a signal transduction cascade within the cell, involving various components like second messengers and transcription factors. These intricate pathways culminate in a specific cellular response, allowing the plant to adapt to its surroundings.

Plant immunity can be broadly categorized into cell-surface and intracellular immunity. Activation of cell surface immunity depends on membrane-bound receptors, commonly referred to as Pattern Recognition Receptors (PRRs), which consist of receptor-like kinases (RLKs) and receptor-like proteins (RLPs) (Lee et al., 2021). RLPs, however, lack an intracellular kinase domain; therefore, they must associate with additional proteins to transduce signals from exogenous stimuli (Greeff et al., 2012). PRRs perceive microbe/pathogen-associated molecular patterns (M/PAMPs) through their extracellular domain, such as flagellin, peptidoglycans, and chitin, as well as damage-associated molecular patterns (DAMPs) such as pectin, cellulose, and hemicellulose from the plant cell wall, and extracellular ATP and NAD^+ (Roudaire et al., 2021). Upon ligand binding, PRRs associate with different co-receptors via homo- and heterodimerization, bringing their kinase domains into close proximity. This new configuration activates their catalytic activity, leading to *cis*- and transphosphorylation of their intracellular domains (Dodds et al., 2024). Moreover, these interactions require the subsequent recruitment of accessory proteins that serve as positive and negative regulators of complex formation and protein interactions, as well as regulators of immune outputs (Delplace et al., 2021; Zhou & Zhang, 2020). Since excessive activation can harm the host cell, the intensity and duration of the PRR immune

response must be controlled. Some regulatory mechanisms involve protein post-translational modifications, such as ubiquitination, which leads to receptor internalization via endocytosis or protein degradation (Ranf, 2017). Additionally, the presence of phosphatases and pseudokinases negatively regulates PRRs, preventing their association and autoactivation (Bentham et al., 2020; Couto & Zipfel, 2016). Another important aspect for the correct function of PRR complexes is their appropriate spatial distribution in the plasma membrane. As such, receptor protein interaction networks are localized in nanodomains, which adds another layer of specificity control (Zhou & Zhang, 2020).

The formation of PRR complexes is followed by the phosphorylation and activation of receptor-like cytoplasmic kinases (RLCKs), which act as intermediaries between extracellular perception and intracellular signal transduction (Yu et al., 2024). RLCKs, in turn, phosphorylate downstream signaling components, including the NADPH oxidase RESPIRATORY BURST ORTHOLOG D (RBOHD), cyclic nucleotide-gated calcium channels, guard cells ion channels, and mitogen-activated protein kinases, triggering a cascade of early transient responses: ROS burst, calcium influx, stomatal closure, and MAPK cascades, respectively (Dodds et al., 2024). Furthermore, activation of MAPK cascades leads to the phosphorylation of transcription factors and subsequent transcriptional reprogramming (Bernoux et al., 2022), inducing a broad-spectrum defense response that includes the production of defense hormones, antimicrobial compounds, and callose deposition (Ngou et al., 2022).

Intracellular immunity, on the other hand, is mediated by intracellular nucleotide-binding leucine-rich repeat receptors (NLRs). These proteins, encoded by resistance (*R*) genes, detect effector proteins introduced into the cell by pathogens (Bentham et al., 2020). Their basic structure consists of a central nucleotide-binding domain (NBD), a C-terminal leucine-rich repeat (LRR) domain crucial for effector recognition and mediating protein-protein interactions, and an N-terminal domain involved in signaling functions and the activation of programmed cell death (Zhou & Zhang, 2020).

Detection of effector proteins by intracellular NLRs triggers domain rearrangements, allowing the ADP-bound inactive NBD to exchange ADP for ATP and thereby transitioning the NLR receptor into its active form. This activation induces conformational changes and oligomerization of NLR proteins, resulting in the formation of a pentameric complex known as the resistosome (Dodds et al., 2024; Shi et al., 2019). The ZAR1-RKS1-PBL2 pentameric resistosome is the best-studied NRL-mediated complex to date (Wang et al., 2019). This complex is later translocated to the plasma membrane to form a Ca^{2+} permeable channel. Additional signaling events induced by NLRs activation include a more sustained calcium influx, a stronger wave of ROS production, and a longer-lasting MAPK activation. Additionally, these downstream responses lead to transcriptional reprogramming by phosphorylating

transcriptional factors (Roudaire et al., 2021). As more robust defense response, NLR-mediated immunity triggers a hypersensitive response (HR) and subsequent programmed cell death (PCD) limiting the propagation of pathogens (Maruta et al., 2022).

Although traditionally viewed as separate pathways, it is now well-established that cell-surface and intracellular immunity form an interconnected network that coordinates to perceive and respond to pathogen attack. For instance, co-activation of both PPR- and NLR-mediated immunity has been described as necessary for effective and sustained immune responses that confer complete disease resistance (Ngou et al., 2022; Ramírez-Zavaleta et al., 2022). This integration of cell-surface and intracellular signaling might be required due to shared molecular components in both responses. For example, both pathways contribute to the accumulation of key second messengers such as ROS and intracellular Ca^{2+}, which are rapidly produced upon immune activation and trigger downstream defense responses (Zhou & Zhang, 2020). This accumulation of signaling components might result from the phosphorylation of shared downstream targets, including the NADPH oxidase RBOHD and calcium channels (Kadota et al., 2018). Furthermore, it has been established that these pathways are mutually dependent. For instance, the proper functioning of PRR-mediated immunity relies on NLR signaling (Pruitt et al., 2021). Likewise, NLR-mediated immunity is impaired when PRR-signaling components are compromised (Ngou et al., 2020). Additionally, NLR-immunity complexes have been reported to induce the expression of PRR-immunity components and boost protein accumulation; conversely, PTI signaling leads to increased expression of *R* genes upon pathogen infection (Bernoux et al., 2022).

Signal transduction events of the plant immune response should be viewed not as linear, independent pathways but rather as an intricate network that integrates multiple signals into an appropriate defense response. Figure 2 illustrates the main components of cell-surface and intracellular immune signaling pathways and the convergence of their downstream signaling responses.

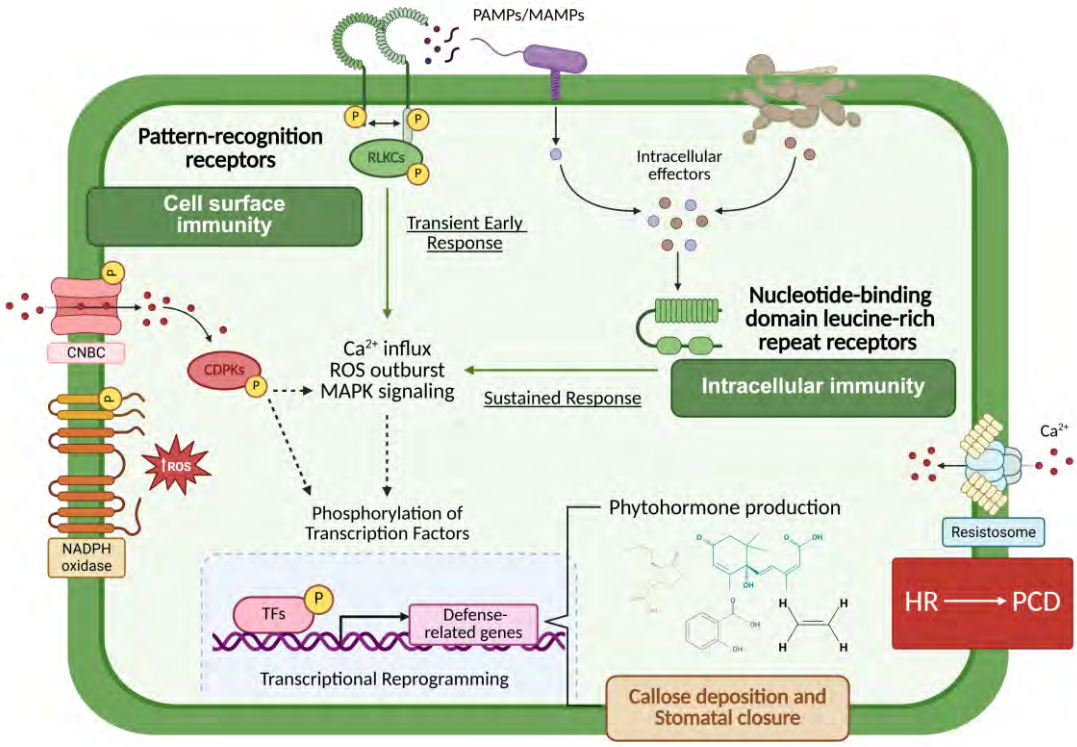

Figure 2. *Convergence of cell-surface and intracellular immunity*. Activation of cell-surface PRRs induces their association with co-receptors, leading to transphosphorylation of their kinase domains and activation of downstream molecular actors. Intracellularly, detection of pathogen effector proteins activates NLRs, allowing their oligomerization and formation of resistosomes. Both PRR and NLR signaling pathways initiate common downstream responses (ROS burst, Ca^{2+} influx, and activation of MAPK cascades), ultimately leading to transcriptional reprogramming and the induction of defense responses. Notably, NLR activation typically triggers a stronger, more robust defense, often leading to HR and PCD to limit pathogen spread. Created with https://BioRender.com.

1.2 Receptor-Like Kinases (RLKs)

Living organisms must sense their environment, interpret this information, and activate appropriate cellular responses to regulate growth, development, and responses to stress (Zhu et al., 2023). The process of perceiving and integrating internal and external cues is the basis of signal transduction, and at the top of signaling networks are receptors.

Receptor-like kinases (RLKs) comprise the detection system that senses and recognizes extracellular signals and conveys the message into the cell (Soltabayeva et al., 2022). They belong to the Eukaryotic Protein Kinases (EPKs) superfamily, a group of proteins that catalyze the transfer of the terminal phosphate group (γ-phosphate) from ATP to the free hydroxyl groups (-OH) of serine, threonine, or tyrosine residues in protein substrates (Jose et al., 2020). The typical structure of RLKs consists of a variable N-terminal extracellular domain (ECD) connected to a signal peptide, a single-pass transmembrane domain (TMD), and a conserved C-terminal serine/threonine cytoplasmic kinase

domain (CKD) (Kong & Ramonell, 2022) (Figure 3). While the ECD can have multiple architectures, the kinase domain consists of a highly conserved core that includes the activation and catalytic loops required for ATP binding and phosphate transfer (Liu et al., 2024).

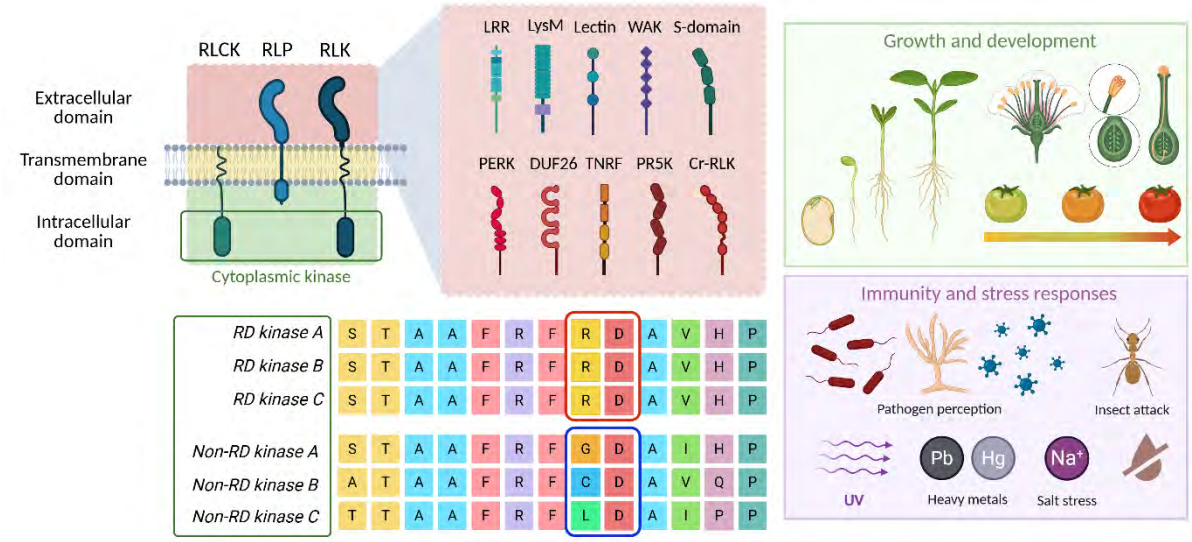

Figure 3. *Structure and classification of receptor-like kinases.* RLKs' structure consists of a variable extracellular domain, a transmembrane domain, and an intracellular kinase domain. RLPs lack a kinase domain and instead contain a short cytoplasmic tail. RLCKs, on the other hand, lack the extracellular domain and possess only a kinase domain. RLKs can be classified according to their extracellular and kinase domains and by their physiological roles. Created with https://BioRender.com

In *Arabidopsis thaliana*, RLKs comprise a multi-gene family with at least 610 members accounting for 2.5% of its genome (Jose et al., 2020). However, out of these 610 RLK-encoding genes, 170 encode receptor-like proteins (RLPs), which lack the kinase domain, and 149 encode receptor-like cytoplasmic kinases (RLCKs), which lack an extracellular domain (Liu et al., 2024). Furthermore, RLKs can also be categorized as RD kinases or non-RD kinases based on the presence or absence of an arginine residue preceding the conserved aspartate residue in the catalytic loop of the kinase domain (Greeff et al., 2012).

Plant RLKs are classified based on their extracellular domains into at least 11 subfamilies (Table 1). This structural diversity translates into the recognition of a wide array of ligands. For example, LRR-RLKs bind peptide or protein ligands, LysM-RLKs, LecRLKs, and WAKs can bind to carbohydrates, Lectin RLKs are capable of sensing fatty acids and extracellular ATP, among others (Bentham et al., 2020; Dodds et al., 2024; Jose et al., 2020; Roudaire et al., 2021).

Plant RLKs are also categorized based on their functions as those responsible for growth and development and those involved in immunity and stress responses (Shiu & Bleecker, 2001). While just a small number of RLKs have been fully characterized, many have been identified to play important

roles in developmental processes, including cell division, elongation, and differentiation, meristem development, reproductive organ development, plant growth, flowering, and fruit ripening, as well as hormone signaling (Liu et al., 2024; Manhães et al., 2020; Shumayla & Upadhyay, 2022; Zhu et al., 2023). Conversely, other RLKs have been reported in biotic stress responses, detecting bacterial, fungal, and viral infections, as well as herbivore and insect attacks, and abiotic stress responses, including drought stress, oxidative stress, and metal toxicity (Gandhi & Oelmüller, 2023; Lee et al., 2021; Saijo et al., 2017; Soltabayeva et al., 2022; Tang et al., 2017).

Table 1. *Classification of RLKs according to their cellular domain*.

Subtype of RLKs		*Extracellular Domain*
Leucine-rich repeat receptor-like kinases	LRR-RLK	Leucine-rich repeats
S-domain receptor-like kinases	S-RLK	Self-incompatibility domain
Wall-associated receptor-like kinases	WAK-RLK	Epidermal growth factor repeats
Lysin motif-type receptor-like kinases	LysM-RLK	LysM domain
Lectin receptor-like protein kinases	LecRLK	Lectin domain
Pathogenesis-related protein-5-like receptor kinases	PR5K-RLK	Thaumatin-like domain
Tumor necrosis factor receptor-like protein kinases	TNFR-RLK	Tumor necrosis factor receptor repeats
Cysteine-rich receptor-like kinase/ Domain of unknown function 26	CRK-RLK/ DUF26	Cysteine-rich domain
Proline-rich extensin-like receptor kinases	PERK-RLK	Proline-rich extensin-like domain
Catharanthus roseus receptor-like kinase 1-like	Cr-RLK	Malectin-like domain

Modified from Zhu, Q., Feng, Y., Xue, J., Chen, P., Zhang, A., & Yu, Y. (2023). Advances in Receptor-like Protein Kinases in Balancing Plant Growth and Stress Responses. *Plants*, *12*(3).

As described in Section 1.1, activation of RLKs upon ligand-binding induces a conformational change within the receptor that allows homo- and heterodimerization with co-receptors and other scaffold proteins. The formation of this protein complex allows transphosphorylation of the kinase domains, thereby initiating signal transduction via a series of phosphorylation cascades that activate downstream signaling components, such as RLCKs and G-proteins (Huang & Joosten, 2024; Smet et al., 2009).

1.3 Cysteine-rich receptor-like kinases (CRKs)

Cysteine-rich receptor-like kinases constitute the largest subfamily within the RLK family in *A. thaliana*, with 46 identified members (Liu et al., 2021). They have the typical structure of RLKs: an extracellular domain, a transmembrane domain, and an intracellular serine/threonine protein kinase domain with conserved Lys and Asp residues essential for their catalytic activity (Quezada-Rodríguez et al., 2019; Zhang et al., 2023). The distinct feature of CRKs is the presence of two copies of the Domain of Unknown Function 26 (DUF26), which contains highly conserved cysteine residues organized in a C-X8-C-X2-C configuration in their extracellular domain (Figure 4) (Shumayla & Upadhyay, 2022).

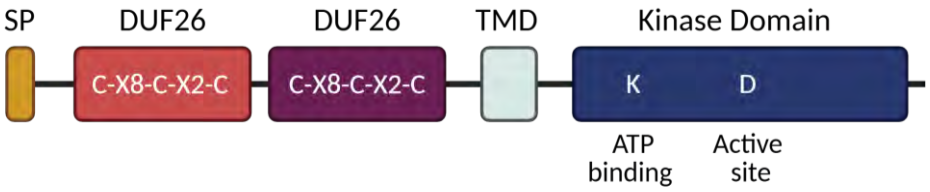

Figure 4. *Domain composition of cysteine-rich receptor-like kinases.* The basic structure of CRKs comprises a signal peptide (SP), two DUF26 domains, a transmembrane domain, and an intracellular kinase domain with conserved lysine and aspartate residues in the ATP-binding site and the active site, respectively. Created with https://BioRender.com

The specific function of the DUF26 domain remains to be elucidated, but it has been proposed that the cysteine residues contribute to the structure and ligand specificity of the extracellular domain (Czernic et al., 1999). Furthermore, the thiol group of cysteines is hypothesized to form disulfide bridges that can act as ROS sensors or serve as targets for redox modifications (Bourdais et al., 2015; Wrzaczek et al., 2010). More recently, crystallographic analysis of the structure of antifungal protein ginkbilobin-2 (Gnk2) and DUF26 domain-containing proteins PLASMODESMATA LOCALIZED PROTEINS (PDLPs) revealed the formation of three intramolecular disulfide bridges between the DUF26 cysteine residues that contribute primarily to structural stabilization of the protein rather than to redox signaling (Miyakawa et al., 2009; Vaattovaara et al., 2019). However, additional cysteine residues outside the C-X8-C-X2-C motif could serve as redox switches, and a change in the cell's redox state of could lead to changes in protein conformation and localization, affect structural stability, or impact ligand binding (Martin-Ramirez et al., 2025; Zeiner et al., 2023).

*Arabidopsis At*CRKs are located in four clusters across chromosomes I, III, IV, and V, with chromosome IV containing most of these genes arranged in tandem (Zhang et al., 2023). Since most *At*CRKs are involved in stress responses, this clustered and tandem arrangement may have arisen as a direct result of gene duplication and subsequent functional diversification, allowing these receptors

to participate in a wide array of cellular responses associated with pathogen perception (Bourdais et al., 2015; Zeiner et al., 2023; Zhang et al., 2023). For instance, the genes *AtCRK11*, *AtCRK13, AtCRK14, AtCRK18, AtCRK22, AtCRK28*, and *AtCRK29* are induced upon flg22 perception, and overexpression of *AtCRK28* confers enhanced resistance to *Pst*. Moreover, *At*CRK28 was found to associate with the FLS2/BAK1 receptor complex in a flg22-independent manner (Yadeta et al., 2016). Overexpression of *AtCRK4, AtCRK6,* and *AtCRK36* has also been shown to reduce disease symptoms and bacterial titers following *Pst* infection, and to induce a flg22-triggered oxidative burst. Furthermore, these receptors were shown to interact with FLS2 in BiFC assays and co-immunoprecipitation experiments (Yeh et al., 2015). Additionally, *At*CRK36 has been demonstrated to interact with FLS2 and the cytoplasmic kinase BIK1 and to mediate flg22-triggered BIK1 phosphorylation (Lee et al., 2017). Expression of *AtCRK4, AtCRK5, AtCRK19*, and *AtCRK20* is induced by salicylic acid (SA) treatment and pathogen perception (*Pst*), and *AtCRK5* induction leads to HR-like cell death (Chen et al., 2004). *AtCRK45* positively regulates disease resistance, with overexpression plants exhibiting increased expression of the *AtPR1, AtPR2*, and *AtAIG1* defense genes and enhanced resistance to *Pseudomonas syringae*. Conversely, *crk45* mutant plants showed increased susceptibility to the bacteria and reduced expression of defense genes (Zhang et al., 2013). *At*CRK2 exists in an inactive complex with the NADPH oxidase RBOH in the plasma membrane. Upon pathogen perception, *At*CRK2 was shown to phosphorylate the C-terminal region of the RBOH, and this phosphorylation is required for full flg22-induced ROS burst. Moreover, *crk2* mutants showed impaired defense responses, displayed reduced stomatal aperture, and enhanced MAPK activation and callose deposition (Kimura et al., 2020). Finally, some CRKs, such as *At*CRK28 and *At*CRK36, have been reported to form homodimers in response to biotic stress and can also form heterodimers with homologs such as *At*CRK28/*At*CRK29 and *At*CRK39/*At*CRK40. These interactions allow faster integration of signaling components and enhance the defense response (Zhang et al., 2023).

Some CRKs have also been found to participate in abiotic stress responses. For instance, *At*CRK5 was found to be important in ROS-related senescence and in UV susceptibility, as evidenced by increased membrane damage and cell death in *crk5* mutants (Bourdais et al., 2015). Moreover, homologs *At*CRK4 and *At*CRK5 positively regulate ABA signaling during drought conditions, leading to stomatal closure (Lü et al., 2016). *At*CRK6 and *At*CRK7 were shown to reduce the sensitivity to overaccumulation of extracellular ROS caused by O_3 in mutant plants, suggesting they play an important role in oxidative signaling (Idänheimo et al., 2014). Several other CRKs have also been shown to be transcriptionally induced in response to O_3-induced oxidative stress (Wrzaczek et al., 2010). Finally, *At*CRK2 phosphorylates CALLOSE SYNTHASE 1 (CALS1) under salt stress, promoting callose deposition and enhancing osmotic resistance (Hunter et al., 2019).

While the functions of some cysteine-rich receptor-like kinases have been elucidated, the precise roles, ligands, and protein interactors for most of them remain largely uncharacterized. Further investigation is necessary, as CRKs have been shown to play a significant role in plant immunity and responses to biotic stress.

1.4 Polyamines

Polyamines are aliphatic molecules containing two or more amine groups, which are protonated at physiological pH (Figure 5), and are ubiquitous across all three living domains: Archaea, Bacteria, and Eukarya (Michael, 2016). The most common polyamines are the diamine putrescine (Put) and the higher polyamines, the triamine spermidine (Spd), and the tetraamine spermine (Spm) (Salvi & Tavladoraki, 2020). Due to their polycationic nature, polyamines can form electrostatic interactions with negatively charged macromolecules such as DNA, RNA, proteins, and phospholipids. Furthermore, they can bind to smaller molecules, such as hydroxycinnamic acids and phenolic compounds (Igarashi & Kashiwagi, 2015; Macoy et al., 2015). This property allows them to act as regulatory molecules in various cellular processes, including replication, transcription, translation, folding and stabilization of nucleic acids and proteins, cell division and differentiation, regulation of ion channel activity, ROS signaling, and apoptosis (Kusano et al., 2008; Miller-Fleming et al., 2015; Pál et al., 2021; Despotović et al., 2020) and consequently play essential roles in the organisms´ growth, development, and stress responses (Masson et al., 2017). Given their importance in these numerous physiological processes, the intracellular levels of polyamines must be tightly regulated through their biosynthesis, catabolism, transport, and conjugation (Tiburcio et al., 2014).

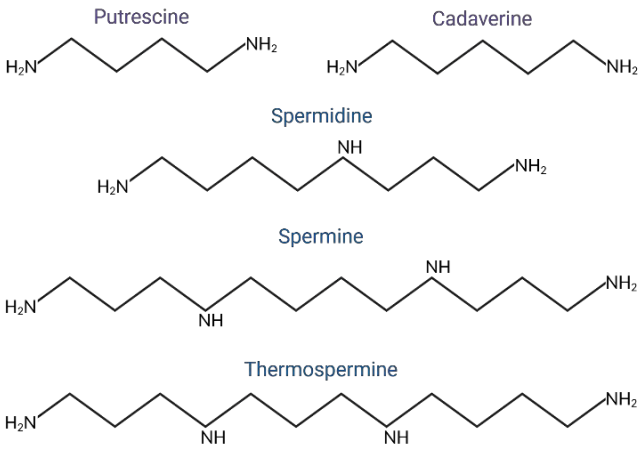

Figure 5. *Chemical structure of polyamines.* Created with https://BioRender.com

1.4.1 Polyamines in plants

Polyamines play diverse and crucial roles throughout a plant's life cycle, impacting growth, various developmental stages, and responses to environmental stresses. Growing evidence suggests that polyamines positively regulate growth and development, playing essential roles in embryogenesis and organogenesis (Jangra et al., 2022), reproductive organ development (Napieraj et al., 2023), vascular tissue development (Tiburcio et al., 2014), flowering and flower bud differentiation (Chen et al., 2019), pollen development (Masson et al., 2017), root growth (Mulangi et al., 2011), and leaf senescence (Masson et al., 2017). Beyond development, polyamines contribute to plant defense responses against pathogen attack by modulating their metabolic pathways (Gonzalez et al., 2021; Jiménez-Bremont et al., 2014). Furthermore, polyamines are equally crucial in enhancing tolerance to a wide array of abiotic stresses, including drought (Blázquez, 2024), salinity (Masson et al., 2017), extreme temperatures, and metal toxicity (Napieraj et al., 2023).

In plants, putrescine is synthesized through three distinct pathways. The first pathway involves arginine decarboxylation by arginine decarboxylase (ADC), producing agmatine and CO_2. Agmatine is subsequently converted to Put by agmatine iminohydrolase (AIH) and N-carbamoyl putrescine amidohydrolase (Joshi et al., 2024). The second pathway directly converts ornithine to Put via ornithine decarboxylase (ODC) (Liu et al., 2007). Finally, the third pathway, exclusively identified in sesame, begins with the conversion of arginine to citrulline, which is then decarboxylated by citrulline decarboxylase (CDC) to produce Put (Chen et al., 2019). Subsequently, after Put is synthesized, the enzyme Spd synthase (SPDS) adds aminopropyl groups to produce Spd. Additionally, the Spm synthase (SPMS) enzyme converts Spd into Spm. Notably, the decarboxylated *S*-adenosylmethionine molecule (SAM), synthesized by *S*-adenosyl-methionine decarboxylase (SAMDC), provides the aminopropyl groups for these reactions (Liu et al., 2007).

Catabolism of polyamines in plants is mediated by amine oxidases (AOs). Oxidation of putrescine and cadaverine is mediated by diamine oxidases (DAOs), enzymes that use copper as a prosthetic group, producing ammonia, 4-aminobutanal (4-AB), and 5-aminopentanal (5-AP) as subproducts, respectively (Gerlin et al., 2021). Spd, Spm, and thermospermine (tSpm) are oxidized by polyamine oxidases (PAOs), which utilize flavin adenine dinucleotide as a cofactor to catalyze the back-conversion of Spm and tSpm into Spd, and Spd into Put, concurrently releasing 3-aminopropanal (3AP) (Jiménez-Bremont et al., 2014). Both, DAO and PAO enzymatic reactions generate hydrogen peroxide (H_2O_2) as a byproduct. Furthermore, an alternative catabolic pathway has been identified in some plant species, in which PAOs directly convert Spm and Spd into 1,3-diaminopropane and 4-AB by terminal catabolism rather than facilitating back-conversion reactions (Gonzalez et al., 2021).

1.4.2 Polyamine transport

Given the importance of polyamines in regulating several cellular processes, precise intracellular distribution and compartmentalization are essential. However, while polyamine transport systems in bacteria, yeast, and mammals have been described in detail, the characterization of plant polyamine transporters with respect to subcellular localization, substrate specificity, and regulatory mechanisms remains incomplete (Pál et al., 2021).

The first plant polyamine transporter, *Os*PUT1 (*AK068055*), was identified in *Oryza sativa* based on its homology with polyamine transporters of *Leishmania major* and *Trypanosoma cruzi* and amino acid transporters of *Phytophthora sojae*. The coding sequence of *Os*PUT1 was cloned and evaluated in a yeast heterologous expression system, where it showed a high affinity for Spd and the herbicide paraquat. Furthermore, semi-quantitative RT-PCR revealed that this transporter is expressed in all plant tissues, except for seeds and roots (Mulangi et al., 2011).

In 2012, Fujita et al. screened for natural variations in paraquat-resistant mutants and identified an *Arabidopsis* L-type amino acid transporter (LAT) involved in the uptake of paraquat and polyamines. The gene responsible for the resistance to paraquat (*At5g05630*) was denominated *resistance to methyl viologen 1* (*RMV1*). It encodes for an amino acid permease protein with high affinity for Spm and Spd and lower affinity for Put. *At*RMV1 was found to localize to the plasma membrane and later characterized as LAT1/*At*PUT3 (Li et al., 2013). More recently, Martinis et al. (2016) characterized *At*PUT3 as a phloem thiamine transporter that mediates long-distance transport of Vitamin B1.

Following the characterization of *Os*PUT1, five additional candidate polyamine transporters from *A. thaliana* and *O. sativa* were identified based on their homology and sequence identity to *Os*PUT1, *Lm*POT1 from *L. major*, and *Tc*PAT12 from *T. cruzi*. These transporters were expressed in a yeast heterologous system for functional characterization. All the identified transporters (*Os*PUT1, *Os*PUT2, *At*PUT1, *At*PUT2, *At*PUT3) showed a high affinity to Spd in yeast expression assays, with *At*PUT2 exhibiting the highest affinity. Furthermore, *At*PUT2 was detected in leaves, stems, roots, flowers, panicles, and seeds, while *At*PUT1 was mainly expressed in leaves, and *At*PUT3 showed high expression levels in flowers. Additionally, two other transporters, *At*PUT4 and *At*PUT5, that clustered with *Os*PUT1, were also found to be involved in polyamine and paraquat transport (Mulangi et al., 2012).

Screening for paraquat-resistant mutants in *Arabidopsis* identified and characterized the *par1* mutant. Furthermore, DNA mapping and sequencing identified the *AtPAR1* gene (*At1G31830*), which encodes an amino acid permease belonging to the LAT transporter family. Moreover, sequence comparison revealed a small gene family in *A. thaliana* that shares high homology with PAR1

(LAT4/*At*PUT2), including LAT1 (RMV1/*At*PUT3) and PAR2 (LAT3/*At*PUT1), the latter being immediately adjacent to PAR1. Additionally, sub-cellular localization experiments showed that PAR1 is localized in the Golgi Apparatus, while PAR2 localizes to the endoplasmic reticulum (Li et al., 2013). The five identified *At*PUT transporters in *A. thaliana* are listed in Table 2.

Confocal analysis in *Nicotiana benthamiana* leaves revealed that the *Arabidopsis* transporter *At*PUT5 and *Oryza sativa Os*PUT1 localize to the endoplasmic reticulum, while *At*PUT2 and *At*PUT3 localize to the chloroplast (Ahmed et al., 2016). Furthermore, using GUS assays, *At*PUT5 was shown to be expressed in veins, leaves, flowers, siliques, and root shoots (Ahmed et al., 2016; Begam et al., 2020).

Table 2. *Arabidopsis thaliana Polyamine Uptake Transporters*

Locus tag	Protein	Other names
At1G31820	Polyamine Uptake Transporter 1 (PUT1)	LAT3
At1G31830	Polyamine Uptake Transporter 2 (PUT2)	PAR1/LAT4
At5G05630	Polyamine Uptake Transporter 3 (PUT3)	LAT1/RMV1/LHR1
At3G13620	Polyamine Uptake Transporter 4 (PUT4)	
At3G19553	Polyamine Uptake Transporter 5 (PUT5)	LAT5

2. Background

In 2017, Méndez-Iberri conducted a partial characterization of the *At*PUT2 transporter. A key aspect of her research was to evaluate the effects of exogenously added polyamines on the expression of the *AtPUT* genes. Her results revealed that treatment with different concentrations of polyamines transcriptionally regulated the expression of *AtPUT* genes, and this regulation was time- and concentration-dependent. Subsequent confocal analysis in *Nicotiana* leaves showed that *At*PUT2 localizes to the plasma membrane and nucleus. In contrast, *At*PUT4 showed GFP signal only in some regions of the plasma membrane, suggesting that this protein is localized in plasmodesmata. Further characterization of the *AtPUT2* gene revealed that its expression varies across plant developmental stages, with notable expression in cotyledons, young leaves, and meristems during early development, and in flower buds, petals, anthers, and siliques during the flowering stage. Additional bioinformatic analyses of the amino acid sequence predicted *At*PUT2's secondary and tridimensional structures, suggesting this transporter contains 12 transmembrane domains with cytoplasmic N- and C-terminal tails and that it could form dimers and interact with structurally similar proteins, such as *At*PUT1 (Méndez-Iberri, 2020).

*At*PUT2 is important in the plant's biotic stress response. For instance, Flores-Hernandez et al. (2025) characterized the role of *At*PUT2 in systemic acquired resistance (SAR) within an *Arabidopsis-Pseudomonas* pathosystem. Following SAR induction, *put2-1* loss-of-function mutant lines exhibited compromised systemic resistance. Furthermore, these *put2-1* mutants also lost broad-spectrum SAR, evidenced by larger lesion sizes in *Botrytis cinerea*-inoculated plants compared to wild-type plants after *Pst* priming. Transcriptome analysis of WT and *put2-1* mutant plants revealed that genes involved in defense, hypersensitive response, and SAR were deregulated in the absence of *At*PUT2. Collectively, these results indicate that the *At*PUT2 transporter plays a critical role in mediating systemic acquired resistance and establishing appropriate defense responses. Concurrently, Peña-Lucio et al. (2025) investigated the role of *At*PUT2 and *At*PUT5 transporters in the defense response to *Botrytis cinerea*. This study revealed that fungal inoculation induced expression of all PUT/LAT genes at different time points during disease progression. Moreover, *put2-1* and *put5-1* single mutants, as well as the *put2-1 put5-1* double mutant, exhibited increased susceptibility to *B. cinerea* infection compared to wild-type plants, as shown by larger lesion size. At the same time, overexpression of *AtPUT2* confers enhanced resistance against *B. cinerea*. Finally, since Spd priming failed to reduce lesion size in both single mutants, it is suggested that *At*PUT2 and *At*PUT5 mediate Spd transport during pathogen attack (Peña-Lucio et al., 2025).

Recently, the *At*PUT3 transporter was found to interact with the Na^+/H^+ antiporter SOS1 and SOS2 protein kinase to modulate salt stress responses. Moreover, it was demonstrated that SOS2

phosphorylates the N-terminal region of *At*PUT3 to regulate its transport activity (Chai et al., 2020). These findings set the precedent for hypothesizing that polyamine transporters can interact with other regulatory proteins, such as kinases, thereby linking polyamine transport to specific cellular signaling networks.

Building on these findings, we evaluated the interaction between *At*PUT2 and *At*CRK10, a receptor-like kinase. Bimolecular fluorescent complementation (BiFC) assays revealed that *At*PUT2 and *At*CRK10 form both homodimers and heterodimers and that they localize to the plasma membrane of epidermal and guard cells in stomata of *Arabidopsis thaliana* and *Nicotiana benthamiana* leaves (Morquecho-Robledo, 2024). Since *At*CRK10 expression is induced by pathogen perception (Du & Chen, 2000) and has been linked to the stomatal closure of plants in response to the PAMP chitin (Bourdais et al., 2015), and also, polyamines modulate its expression in a time- and concentration-dependent manner, our findings suggest a potential link between the transport of polyamines and the perception of PAMPs in the plant's immune response. Furthermore, this interaction suggests a possible regulatory mechanism for polyamine transport.

3. Justification

Plants are susceptible to many diseases caused by microbial pathogens and pests that constantly threaten agriculture and global food security (Ahmed et al., 2023). Up to 40% of major crops, including maize, rice, wheat, potatoes, and soybeans, are lost each year due to plant diseases. This translates to a loss of approximately US$220 billion worldwide (Savary et al., 2019; Wang et al., 2024). Even more, the rapidly changing climate is driving the emergence of new pathogenic microorganisms and pests and facilitating their spread into new geographic areas, further threatening agricultural production (Rizzo et al., 2021).

While current disease control measures, such as pesticides, have proven helpful in the past, we now need to consider their negative environmental impacts in light of the climate change emergency and their adverse effects on human health (Góngora & Silva, 2024).

Consequently, to achieve efficient and ecologically sustainable agriculture, a comprehensive understanding of various factors is needed, including the effect of climate change on host susceptibility and pathogen virulence, pathogenicity mechanisms, antimicrobial resistance of microorganisms and pests, plant-microbe interactions, plant susceptibility and resistance mechanisms, and possible applications of genetic approaches to enhance plant immune responses and disease control (Wang et al., 2024).

Polyamines are crucial in plant biology and essential to growth, development, and stress responses. Their involvement in regulating several physiological processes has many potential applications, including protection against biotic and abiotic stresses. Therefore, a deeper understanding of their biological properties, mechanisms of action, and metabolic pathways is required. The *At*PUT2 polyamine transporter has been implicated in the immune response against the plant pathogens *P. syringae* (Flores-Hernandez et al. 2025) and *B. cinerea* (Peña-Lucio et al. 2025). In a previous study in our group, we also determined that this transporter physically interacts with the cysteine-rich receptor-like kinase *At*CRK10 by BiFC assays in *N. benthamiana*. This interaction was detected at the plasma membrane of epidermal cells and the guard cells of stomata on *N. benthamiana* leaves. *At*PUT2 lacks a signal peptide, but it has been reported in different cellular compartments (i.e., the Golgi apparatus (Li et al. 2013), and chloroplast (Ahmed et al. 2017); therefore, it is very likely that this multiple subcellular localization depends on protein-protein interactions and polyamine concentration. Furthermore, this interaction suggests a regulatory mechanism of polyamine transport.

Given that the *At*CRK10 receptor-like kinase has been linked to the perception of PAMPs (Du & Chen, 2000), elucidating the functional importance of its kinase domain in the interaction with *At*PUT2 would provide insights into whether this receptor is responsible for the activation or

subcellular localization of *At*PUT2, and thus mediating the direct regulation of polyamine transport upon pathogen perception.

4. Hypothesis

5. Objectives

6. Materials and Methods

7. Results

8. Discussion

9. Conclusion

10. References

Ahmed, S., Ariyaratne, M., Patel, J., Howard, A. E., Nestor-Kalinoski, A. L., Phuntumart, V., & Morris, P. F. (2016). Altered expression of polyamine transporters reveals a role for spermidine in the timing of flowering and other developmental response pathways. Plant Science, 258, 146. https://doi.org/10.1016/j.plantsci.2016.12.002

Ahmed, T., Noman, M., Shahid, M., Hameed, A., & Li, B. (2023). Pathogenesis and Disease Control in Crops: The Key to Global Food Security. Plants, 12(18), 3266. https://doi.org/10.3390/plants12183266

Allen, J. R., Wilkinson, E. G., & Strader, L. C. (2021). Creativity comes from interactions: modules of protein interactions in plants. FEBS Journal, 289(6), 1492. Wiley. https://doi.org/10.1111/febs.15847

Begam, R. A., D'Entremont, J., & Good, A. G. (2020). The *Arabidopsis* L-Type Amino Acid Transporter 5 (LAT5/PUT5) Is Expressed in the Phloem and Alters Seed Nitrogen Content When Knocked Out. Plants, 9(11), 1519. https://doi.org/10.3390/plants9111519

Bender, K. W., & Zipfel, C. (2023). Paradigms of receptor kinase signaling in plants. Biochemical Journal, 480(12), 835. Portland Press. https://doi.org/10.1042/bcj20220372

Bentham, A. R., Concepción, J. C. D. la, Mukhi, N., Zdrzałek, R., Draeger, M., Gorenkin, D., Hughes, R. K., & Banfield, M. J. (2020). A molecular roadmap to the plant immune system. Journal of Biological Chemistry, 295(44), 14916. Elsevier BV. https://doi.org/10.1074/jbc.rev120.010852

Bernoux, M., Zetzsche, H., & Stuttmann, J. (2022). Connecting the dots between cell surface- and intracellular-triggered immune pathways in plants. Current Opinion in Plant Biology, 69, 102276. Elsevier BV. https://doi.org/10.1016/j.pbi.2022.102276

Bhatla, S. C., & Lal, M. A. (2018). Plant Physiology, Development and Metabolism. In Springer eBooks. Springer Nature. https://doi.org/10.1007/978-981-13-2023-1

Birnboim, H. C., & Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Research, 7(6), 1513. https://doi.org/10.1093/nar/7.6.1513

Blázquez, M. Á. (2024). Polyamines: Their Role in Plant Development and Stress. Annual Review of Plant Biology, 75(1), 95. Annual Reviews. https://doi.org/10.1146/annurev-arplant-070623-110056

Bourdais, G., Burdiak, P., Gauthier, A., Nitsch, L., Salojärvi, J., Rayapuram, C., Idänheimo, N., Hunter, K., Kimura, S., Merilo, E., Vaattovaara, A., Oracz, K., Kaufholdt, D., Pallon, A., Anggoro, D. T.,

Głów, D., Lowe, J., Zhou, J., Mohammadi, O., … Kangasjärvi, J. (2015). Large-Scale Phenomics Identifies Primary and Fine-Tuning Roles for CRKs in Responses Related to Oxidative Stress. PLoS Genetics, 11(7). https://doi.org/10.1371/journal.pgen.1005373

Chai, H., Guo, J., Zhong, Y., Hsu, C., Zou, C., Wang, P., Zhu, J., & Shi, H. (2020). The plasma-membrane polyamine transporter PUT3 is regulated by the Na^+/H^+ antiporter SOS1 and protein kinase SOS2. New Phytologist, 226(3), 785. https://doi.org/10.1111/nph.16407

Chen, D., Shao, Q., Yin, L., Younis, A., & Zheng, B. (2019). Polyamine Function in Plants: Metabolism, Regulation on Development, and Roles in Abiotic Stress Responses. Frontiers in Plant Science, 9. Frontiers Media. https://doi.org/10.3389/fpls.2018.01945

Chen, K., Fan, B., Du, L., & Chen, Z. (2004). Activation of hypersensitive cell death by pathogen-induced receptor-like protein kinases from *Arabidopsis*. Plant Molecular Biology, 56(2), 271. https://doi.org/10.1007/s11103-004-3381-2

Chung, C. T., Niemela, S. L., & Miller, R. H. (1989). One-step preparation of competent *Escherichia coli*: transformation and storage of bacterial cells in the same solution. Proceedings of the National Academy of Sciences of the United States of America, 86(7), 2172–2175. https://doi.org/10.1073/pnas.86.7.2172

Couto, D., & Zipfel, C. (2016). Regulation of pattern recognition receptor signalling in plants. Nature Reviews. Immunology, 16(9), 537. Nature Portfolio. https://doi.org/10.1038/nri.2016.77

Cuadrado, A. F., & Damme, D. V. (2024). Unlocking protein–protein interactions in plants: a comprehensive review of established and emerging techniques. Journal of Experimental Botany, 75(17), 5220. Oxford University Press. https://doi.org/10.1093/jxb/erae088

Czernic, P., Visser, B., Sun, W., Savouré, A., Deslandes, L., Marco, Y., Montagu, M. V., & Verbruggen, N. (1999). Characterization of an *Arabidopsis thaliana* receptor-like protein kinase gene activated by oxidative stress and pathogen attack. The Plant Journal, 18(3), 321. https://doi.org/10.1046/j.1365-313x.1999.00447.x

Das, P., & Joshi, N. (2011). Minor modifications in obtainable *Arabidopsis* floral dip method enhances transformation efficiency and production of homozygous transgenic lines harboring a single copy of transgene. Advances in Bioscience and Biotechnology, 2(2), 59. https://doi.org/10.4236/abb.2011.22010

Delplace, F., Huard-Chauveau, C., Berthomé, R., & Roby, D. (2021). Network organization of the plant immune system: from pathogen perception to robust defense. The Plant Journal, 109(2), 447. Wiley. https://doi.org/10.1111/tpj.15462

Demidchik, V., Maathuis, F. J. M., & Voitsekhovskaja, O. V. (2017). Unravelling the plant signalling machinery: an update on the cellular and genetic basis of plant signal transduction. Functional Plant Biology, 45(2), 1. https://doi.org/10.1071/fp17085

Despotović, D., Longo, L. M., Aharon, E., Kahana, A., Scherf, T., Gruic-Sovulj, I., & Tawfik, D. S. (2020). Polyamines Mediate Folding of Primordial Hyper-Acidic Helical Proteins. bioRxiv (Cold Spring Harbor Laboratory). https://doi.org/10.1101/2020.10.01.321877

Dodds, P. N., Chen, J., & Outram, M. A. (2024). Pathogen perception and signaling in plant immunity. The Plant Cell, 36(5), 1465. https://doi.org/10.1093/plcell/koae020

Du, L., & Chen, Z. (2000). Identification of genes encoding receptor-like protein kinases as possible targets of pathogen- and salicylic acid-induced WRKY DNA-binding proteins in *Arabidopsis*. The Plant Journal, 24(6), 837. https://doi.org/10.1111/j.1365-313x.2000.00923.x

Earley, K., Haag, J. R., Pontes, O., Opper, K., Juehne, T., Song, K., & Pikaard, C. S. (2006). Gateway-compatible vectors for plant functional genomics and proteomics. The Plant Journal, 45(4), 616. https://doi.org/10.1111/j.1365-313x.2005.02617.x

Flores-Hernández, E., Gonzalez, M. E., Alvarado-Guitron, P., Jasso-Robles, F. I., Ovando-Vázquez, C., Jiménez-Bremont, J. F., Ćavar Zeljković, S., Ulbrichová, M., De Diego, N., & Rodríguez-Kessler, M. (2025). Polyamine uptake transporter 2 is essential for systemic acquired resistance establishment in *Arabidopsis*. Plant Stress, 18, 101046. https://doi.org/10.1016/j.stress.2025.101046

Fujita, M., Fujita, Y., Iuchi, S., Yamada, K., Kobayashi, Y., Urano, K., Kobayashi, M., Yamaguchi-Shinozaki, K., & Shinozaki, K. (2012). Natural variation in a polyamine transporter determines paraquat tolerance in *Arabidopsis*. Proceedings of the National Academy of Sciences, 109(16), 6343. https://doi.org/10.1073/pnas.1121406109

Gandhi, A., & Oelmüller, R. (2023). Emerging Roles of Receptor-like Protein Kinases in Plant Response to Abiotic Stresses. International Journal of Molecular Sciences, 24(19), 14762. Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/ijms241914762

Gerlin, L., Baroukh, C., & Genin, S. (2021). Polyamines: double agents in disease and plant immunity. Trends in Plant Science, 26(10), 1061. Elsevier BV. https://doi.org/10.1016/j.tplants.2021.05.007

Gnanasekaran, P., & Pappu, H. R. (2023). Bimolecular Fluorescence Complementation (BiFC) Assay to Visualize Protein–Protein Interactions in Living Cells. Methods in Molecular Biology, 117. https://doi.org/10.1007/978-1-0716-3327-4_11

Góngora, C. E., & Silva, M. do C. (2024). Sustainable Strategies for the Control of Crop Diseases and Pests to Reduce Pesticides. Agronomy, 14(9), 2158. https://doi.org/10.3390/agronomy14092158

Gonzalez, M. E., Jasso-Robles, F. I., Flores-Hernández, E., Rodríguez-Kessler, M., & Pieckenstain, F. L. (2021). Current status and perspectives on the role of polyamines in plant immunity. Annals of Applied Biology, 178(2), 244. https://doi.org/10.1111/aab.12670

Greeff, C., Roux, M., Mundy, J., & Petersen, M. (2012). Receptor-like kinase complexes in plant innate immunity. Frontiers in Plant Science, 3, 209. https://doi.org/10.3389/fpls.2012.00209

Huang, W. R. H., & Joosten, M. H. A. J. (2024). Immune signaling: receptor-like proteins make the difference [Review of Immune signaling: receptor-like proteins make the difference]. Trends in Plant Science. Elsevier BV. https://doi.org/10.1016/j.tplants.2024.03.012

Hunter, K., Kimura, S., Rokka, A., Tran, H. C., Toyota, M., Kukkonen, J. P., & Wrzaczek, M. (2019). CRK2 Enhances Salt Tolerance by Regulating Callose Deposition in Connection with PLDα1. Plant physiology, 180(4), 2004–2021. https://doi.org/10.1104/pp.19.00560

Idänheimo, N., Gauthier, A., Salojärvi, J., Riccardo, S., Brosché, M., Kollist, H., Mähönen, A. P., Kangasjärvi, J., & Wrzaczek, M. (2014). The *Arabidopsis thaliana* cysteine-rich receptor-like kinases CRK6 and CRK7 protect against apoplastic oxidative stress. Biochemical and Biophysical Research Communications, 445(2), 457. https://doi.org/10.1016/j.bbrc.2014.02.013

Igarashi, K., & Kashiwagi, K. (2015). Modulation of protein synthesis by polyamines. IUBMB Life, 67(3), 160. Wiley. https://doi.org/10.1002/iub.1363

Jangra, A., Chaturvedi, S., Kumar, N., Singh, H., Sharma, V., Thakur, M., Tiwari, S., & Chhokar, V. (2022). Polyamines: The Gleam of Next-Generation Plant Growth Regulators for Growth, Development, Stress Mitigation, and Hormonal Crosstalk in Plants—A Systematic Review.

Journal of Plant Growth Regulation, 42(8), 5167. https://doi.org/10.1007/s00344-022-10846-4

Jasso-Robles, F. I., Jiménez-Bremont, J. F., Becerra-Flora, A., Juárez-Montiel, M., Gonzalez, M. E., Pieckenstain, F. L., Cruz, R. F. G. de la, & Rodríguez-Kessler, M. (2016). Inhibition of polyamine oxidase activity affects tumor development during the maize-*Ustilago maydis* interaction. Plant Physiology and Biochemistry, 102, 115. https://doi.org/10.1016/j.plaphy.2016.02.019

Jiménez-Bremont, J. F., Marina, M., Guerrero-González, M. de la L., Rossi, F. R., Sánchez-Rangel, D., Rodríguez-Kessler, M., Ruiz, O. A., & Gárriz, A. (2014). Physiological and molecular implications of plant polyamine metabolism during biotic interactions. Frontiers in Plant Science, 5. Frontiers Media. https://doi.org/10.3389/fpls.2014.00095

Jose, J., Ghantasala, S., & Choudhury, S. R. (2020). *Arabidopsis* Transmembrane Receptor-Like Kinases (RLKs): A Bridge between Extracellular Signal and Intracellular Regulatory. International Journal of Molecular Sciences, 21(11), 4000. Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/ijms21114000

Joshi, kumud, Ahmed, S., Ge, L., Avestakh, A., OLOyede, B. A., Phuntumart, V., Nestor-Kalinoski, A. L., & Morris, P. F. (2024). Spatial Organization of Putrescine Synthesis in Plants. SSRN Electronic Journal. https://doi.org/10.2139/ssrn.4856564

Kámán-Tóth, E., Pogány, M., Dankó, T., Szatmári, Á., & Bozsó, Z. (2018). A simplified and efficient *Agrobacterium tumefaciens* electroporation method. 3 Biotech, 8(3), 148. https://doi.org/10.1007/s13205-018-1171-9

Kadota, Y., Liebrand, T. W. H., Goto, Y., Sklenář, J., Derbyshire, P., Menke, F. L. H., Torres, M. Á. M., Molina, A., Zipfel, C., Coaker, G., & Shirasu, K. (2018). Quantitative phosphoproteomic analysis reveals common regulatory mechanisms between effector- and PAMP-triggered immunity in plants. New Phytologist, 221(4), 2160. https://doi.org/10.1111/nph.15523

Kerppola, T. K. (2008). Bimolecular Fluorescence Complementation (BiFC) Analysis as a Probe of Protein Interactions in Living Cells. Annual Review of Biophysics, 37(1), 465. Annual Reviews. https://doi.org/10.1146/annurev.biophys.37.032807.125842

Kimura, S., Hunter, K., Vaahtera, L., Tran, H. C., Citterico, M., Vaattovaara, A., Rokka, A., Stolze, S. C., Harzen, A., Meißner, L., Wilkens, M., Hamann, T., Toyota, M., Nakagami, H., & Wrzaczek, M. (2020). CRK2 and C-terminal Phosphorylation of NADPH Oxidase RBOHD Regulate Reactive Oxygen Species Production in *Arabidopsis*. The Plant Cell, 32(4), 1063. https://doi.org/10.1105/tpc.19.00525

Kong, F., & Ramonell, K. M. (2022). Receptor-like kinases and their role in plant innate immunity. In Elsevier eBooks (p. 39). Elsevier BV. https://doi.org/10.1016/b978-0-323-90594-7.00003-x

Kusano, T., Berberich, T., Tateda, C., & Takahashi, Y. (2008). Polyamines: essential factors for growth and survival. Planta, 228(3), 367. Springer Science+Business Media. https://doi.org/10.1007/s00425-008-0772-7

Lee, D., Lee, H., & Belkhadir, Y. (2021). Coding of plant immune signals by surface. Current Opinion in Plant Biology, 62, 102044. Elsevier BV. https://doi.org/10.1016/j.pbi.2021.102044

Lee, D. S., Kim, Y. C., Kwon, S. J., Ryu, C., & Park, O. K. (2017). The *Arabidopsis* Cysteine-Rich Receptor-Like Kinase CRK36 Regulates Immunity through Interaction with the Cytoplasmic Kinase BIK1. Frontiers in Plant Science, 8, 1856. https://doi.org/10.3389/fpls.2017.01856

Lee, S. C., Lan, W., Kim, B., Li, L., Cheong, Y. H., Pandey, G. K., Lu, G., Buchanan, B. B., & Luan, S. (2007). A protein phosphorylation/dephosphorylation network regulates a plant potassium channel. Proceedings of the National Academy of Sciences, 104(40), 15959. https://doi.org/10.1073/pnas.0707912104

Li, J., Mu, J., Bai, J., Fu, F., Zou, T., An, F., Zhang, J., Jing, H., Wang, Q., Li, Z., Yang, S., & Zuo, J. (2013). PARAQUAT RESISTANT1, a Golgi-Localized Putative Transporter Protein, Is Involved in Intracellular Transport of Paraquat. PLANT PHYSIOLOGY, 162(1), 470. https://doi.org/10.1104/pp.113.213892

Li, X., Zhang, J., Shi, H., Li, B., & Li, J. (2022). Rapid responses: Receptor-like kinases directly regulate the functions of membrane transport proteins in plants. Journal of Integrative Plant Biology, 64(7), 1303. Wiley. https://doi.org/10.1111/jipb.13274

Liu, H., & Naismith, J. H. (2008). An efficient one-step site-directed deletion, insertion, single and multiple-site plasmid mutagenesis protocol. BMC Biotechnology, 8(1), 91. https://doi.org/10.1186/1472-6750-8-91

Liu, J., Kitashiba, H., Wang, J., Ban, Y., & Moriguchi, T. (2007). Polyamines and their ability to provide environmental stress tolerance to plants. Plant Biotechnology, 24(1), 117. https://doi.org/10.5511/plantbiotechnology.24.117

Liu, J., Li, W., Wu, G., & Ali, K. (2024). An update on evolutionary, structural, and functional studies of receptor-like kinases in plants. Frontiers in Plant Science, 15. Frontiers Media. https://doi.org/10.3389/fpls.2024.1305599

Liu, Y., Feng, Z., Zhu, W., Liu, J., & Zhang, Y. (2021). Genome-Wide Identification and Characterization of Cysteine-Rich Receptor-Like Protein Kinase Genes in Tomato and Their Expression Profile in Response to Heat Stress. Diversity, 13(6), 258. https://doi.org/10.3390/d13060258

Lü, K., Liang, S., Wu, Z., Bi, C., Yu, Y., Wang, X., & Zhang, D. (2016). Overexpression of an *Arabidopsis* cysteine-rich receptor-like protein kinase, CRK5, enhances abscisic acid sensitivity and confers drought tolerance. Journal of Experimental Botany, 67(17), 5009. https://doi.org/10.1093/jxb/erw266

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. Methods, 25(4), 402. https://doi.org/10.1006/meth.2001.1262

Macoy, D. M., Kim, W., Lee, S. Y., & Kim, M. G. (2015). Biosynthesis, physiology, and functions of hydroxycinnamic acid amides in plants. Plant Biotechnology Reports, 9(5), 269. https://doi.org/10.1007/s11816-015-0368-1

Manhães, A. M. E. de A., Ortiz-Morea, F. A., He, P., & Shan, L. (2020). Plant plasma membrane-resident receptors: Surveillance for infections and coordination for growth and development. Journal of Integrative Plant Biology, 63(1), 79. Wiley. https://doi.org/10.1111/jipb.13051

Martinis, J., Gas-Pascual, E., Szydlowski, N., Crèvecœur, M., Gisler, A., Bürkle, L., & Fitzpatrick, T. B. (2016). Long-Distance Transport of Thiamine (Vitamin B1) Is Concomitant with That of Polyamines . PLANT PHYSIOLOGY, 171(1), 542. https://doi.org/10.1104/pp.16.00009

Martin-Ramirez, S., Stouthamer, J., & Smakowska-Luzan, E. (2025). More questions than answers: insights into potential cysteine-rich receptor-like kinases redox signalling in *Arabidopsis*. The Plant Journal, 122(2). Wiley. https://doi.org/10.1111/tpj.70176

Maruta, N., Burdett, H., Lim, B. Y. J., Hu, X., Desa, S., Manik, M. K., & Kobe, B. (2022). Structural basis of NLR activation and innate immune signalling in plants. Immunogenetics, 74(1), 5. Springer Science+Business Media. https://doi.org/10.1007/s00251-021-01242-5

Masson, P., Takahashi, T., & Angelini, R. (2017). Editorial: Molecular Mechanisms Underlying Polyamine Functions in Plants. Frontiers in Plant Science, 8. https://doi.org/10.3389/fpls.2017.00014

Méndez-Iberri, A. E. (2017). Caracterización parcial de los transportadores de poliaminas AtPUT2 y AtPUT4 de *Arabidopsis*: Un enfoque a su localización intracelular, regulación transcripcional y filogenia . [Bachelor's thesis, Universidad Autónoma de San Luis Potosí].

Méndez-Iberri, A. E. (2020). Estudio transcripcional y funcional del gen AtPUT2 de *Arabidopsis* en desarrollo y estrés abiótico. [Master's thesis, Universidad Autónoma de San Luis Potosí].

Michael, A. J. (2016). Polyamines in Eukaryotes, Bacteria, and Archaea. Journal of Biological Chemistry, 291(29), 14896. Elsevier BV. https://doi.org/10.1074/jbc.r116.734780

Miller-Fleming, L., Olín-Sandoval, V., Campbell, K., & Ralser, M. (2015). Remaining Mysteries of Molecular Biology: The Role of Polyamines in the Cell. Journal of Molecular Biology, 427(21), 3389. Elsevier BV. https://doi.org/10.1016/j.jmb.2015.06.020

Miyakawa, T., Miyazono, K., Sawano, Y., Hatano, K., & Tanokura, M. (2009). Crystal structure of ginkbilobin-2 with homology to the extracellular domain of plant cysteine-rich receptor-like kinases. Proteins Structure Function and Bioinformatics, 77(1), 247. https://doi.org/10.1002/prot.22494

Morquecho-Robledo, M. (2024). Bimolecular fluorescent complementation analysis of the interaction between the AtPUT2 polyamine transporter and the CRK10 receptor-like kinase in *Arabidopsis* [Bachelor's thesis]. Universidad Autónoma de San Luis Potosí.

Mulangi, V., Chibucos, M. C., Phuntumart, V., & Morris, P. F. (2012). Kinetic and phylogenetic analysis of plant polyamine uptake transporters. Planta, 236(4), 1261. https://doi.org/10.1007/s00425-012-1668-0

Mulangi, V., Phuntumart, V., Aouida, M., Ramotar, D., & Morris, P. F. (2011). Functional analysis of OsPUT1, a rice polyamine uptake transporter. Planta, 235(1), 1. https://doi.org/10.1007/s00425-011-1486-9

Murashige, T., & Skoog, F. (1962). A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiologia Plantarum*, *15*(3), 473. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Napieraj, N., Janicka, M., & Reda, M. (2023). Interactions of Polyamines and Phytohormones in Plant Response to Abiotic Stress. Plants, 12(5), 1159. Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/plants12051159

Ngou, B. P. M., Ahn, H., Ding, P., & Jones, J. D. G. (2020). Mutual Potentiation of Plant Immunity by Cell-surface and Intracellular Receptors. bioRxiv (Cold Spring Harbor Laboratory). https://doi.org/10.1101/2020.04.10.034173

Ngou, B. P. M., Ding, P., & Jones, J. D. G. (2022). Thirty years of resistance: Zig-zag through the plant immune system. The Plant Cell, 34(5), 1447. Oxford University Press. https://doi.org/10.1093/plcell/koac041

Nussinov, R., Regev, C., & Jang, H. (2025). Kinase signaling cascades: An updated mechanistic landscape. Chemical Science. Royal Society of Chemistry. https://doi.org/10.1039/d5sc04657b

Pál, M., Szalai, G., Gondor, O. K., & Janda, T. (2021). Unfinished story of polyamines: Role of conjugation, transport and light-related regulation in the polyamine metabolism in plants. Plant Science, 308, 110923. Elsevier BV. https://doi.org/10.1016/j.plantsci.2021.110923

Peña-Lucio, E. M., Pieckenstain, F. L., Gonzalez, M. E., Flores-Hernández, E., & Rodríguez-Kessler, M. (2025). The polyamine uptake transporters PUT2/LAT4 and PUT5/LAT5 contribute to *Arabidopsis* defense response against *Botrytis cinerea*. Physiology and Molecular Biology of Plants, 31(7), 1053. https://doi.org/10.1007/s12298-025-01630-1

Pruitt, R. N., Locci, F., Wanke, F., Zhang, L., Saile, S. C., Joe, A., Karelina, D., Hua, C., Fröhlich, K., Wan, W., Hu, M., Rao, S., Stolze, S. C., Harzen, A., Gust, A. A., Harter, K., Joosten, M. H. A. J., Thomma, B. P. H. J., Zhou, J., … Nürnberger, T. (2021). The EDS1–PAD4–ADR1 node mediates *Arabidopsis* pattern-triggered immunity. Nature, 598(7881), 495. https://doi.org/10.1038/s41586-021-03829-0

Quezada-Rodríguez, E. H., García-Ramírez, G. X., Arthikala, M., Govindappa, M., Lara, M., & Nanjareddy, K. (2019). Cysteine-Rich Receptor-Like Kinase Gene Family Identification in the *Phaseolus* Genome and Comparative Analysis of Their Expression Profiles Specific to Mycorrhizal and Rhizobial Symbiosis. Genes, 10(1), 59. https://doi.org/10.3390/genes10010059

Ramírez-Zavaleta, C. Y., García-Barrera, L. J., Rodríguez-Verástegui, L. L., Arrieta-Flores, D., & Gregorio-Jorge, J. (2022). An Overview of PRR- and NLR-Mediated Immunities: Conserved Signaling Components across the Plant Kingdom That Communicate Both Pathways. International Journal of Molecular Sciences, 23(21), 12974. Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/ijms232112974

Ranf, S. (2017). Sensing of molecular patterns through cell surface immune receptors. Current Opinion in Plant Biology, 38, 68. Elsevier BV. https://doi.org/10.1016/j.pbi.2017.04.011

REN, H., Ou, Q., Pu, Q., Lou, Y., Yang, X., Han, Y., & Liu, S. (2024). Comprehensive Review on Bimolecular Fluorescence Complementation and Its Application in Deciphering Protein–

Protein Interactions in Cell Signaling Pathways. Biomolecules, 14(7), 859. Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/biom14070859

Rizzo, D. M., Lichtveld, M. Y., Mazet, J. A. K., Togami, E., & Miller, S. A. (2021). Plant health and its effects on food safety and security in a One Health framework: four case studies. One Health Outlook, 3(1). BioMed Central. https://doi.org/10.1186/s42522-021-00038-7

Roudaire, T., Héloir, M., Wendehenne, D., Zadoroznyj, A., Dubrez, L., & Poinssot, B. (2021). Cross Kingdom Immunity: The Role of Immune Receptors and Downstream Signaling in Animal and Plant Cell Death. Frontiers in Immunology, 11, 612452. Frontiers Media. https://doi.org/10.3389/fimmu.2020.612452

Roux, M., & Zipfel, C. (2011). Receptor Kinase Interactions: Complexity of Signalling. In Signaling and communication in plants (p. 145). Springer Nature. https://doi.org/10.1007/978-3-642-23044-8_9

Saijo, Y., Loo, E. P. I., & Yasuda, S. (2017). Pattern recognition receptors and signaling in plant–microbe interactions. The Plant Journal, 93(4), 592. Wiley. https://doi.org/10.1111/tpj.13808

Salvi, D., & Tavladoraki, P. (2020). The tree of life of polyamine oxidases. Scientific Reports, 10(1). https://doi.org/10.1038/s41598-020-74708-3

Savary, S., Willocquet, L., Pethybridge, S. J., Esker, P. D., McRoberts, N., & Nelson, A. (2019). The global burden of pathogens and pests on major food crops. Nature Ecology & Evolution, 3(3), 430. https://doi.org/10.1038/s41559-018-0793-y

Shi, X., Dong, S., & Liu, W. (2019). Structures of plant resistosome reveal how NLR immune receptors are activated. aBIOTECH, 1(2), 147. https://doi.org/10.1007/s42994-019-00012-y

Shiu, S., & Bleecker, A. B. (2001). Plant Receptor-Like Kinase Gene Family: Diversity, Function, and Signaling. Science Signaling, 2001(113). https://doi.org/10.1126/scisignal.1132001re22

Shumayla, & Upadhyay, S. K. (2022a). An overview of receptor-like kinases in plants. In Elsevier eBooks (p. 1). Elsevier BV. https://doi.org/10.1016/b978-0-323-90594-7.00005-3

Shumayla, & Upadhyay, S. K. (2022b). Cysteine-rich receptor-like kinases and stress response in plants. In Elsevier eBooks (p. 183). Elsevier BV. https://doi.org/10.1016/b978-0-323-90594-7.00016-8

Schütze, K., Harter, K., & Chaban, C. (2008). Bimolecular Fluorescence Complementation (BiFC) to Study Protein-protein Interactions in Living Plant Cells. Methods in Molecular Biology, 189. https://doi.org/10.1007/978-1-59745-289-2_12

Smakowska-Luzan, E., Mott, G. A., Parys, K., Stegmann, M., Howton, T. C., Layeghifard, M., Neuhold, J., Lehner, A., Kong, J., Grünwald, K., Weinberger, N., Satbhai, S. B., Mayer, D., Busch, W., Madalinski, M., Stolt-Bergner, P., Provart, N. J., Mukhtar, M. S., Zipfel, C., … Belkhadir, Y. (2018). An extracellular network of *Arabidopsis* leucine-rich repeat receptor kinases. Nature, 553(7688), 342. https://doi.org/10.1038/nature25184

Smet, I. D., Voß, U., Jürgens, G., & Beeckman, T. (2009). Receptor-like kinases shape the plant. Nature Cell Biology, 11(10), 1166. Nature Portfolio. https://doi.org/10.1038/ncb1009-1166

Smirnova, O. A., Bartosch, B., Zakirova, N. F., Kochetkov, S. N., & Ivanov, A. V. (2018). Polyamine Metabolism and Oxidative Protein Folding in the ER as ROS-Producing Systems Neglected in Virology. International Journal of Molecular Sciences, 19(4), 1219. Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/ijms19041219

Soltabayeva, A., Dauletova, N., Serik, S., Sandybek, M., Omondi, J. O., Kurmanbayeva, A., & Srivastava, S. (2022). Receptor-like Kinases (LRR-RLKs) in Response of Plants to Biotic and Abiotic Stresses. Plants, 11(19), 2660. Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/plants11192660

Struk, S., Jacobs, A., Martín-Fontecha, E. S., Gevaert, K., Cubas, P., & Goormachtig, S. (2018). Exploring the protein–protein interaction landscape in plants. Plant Cell & Environment, 42(2), 387. Wiley. https://doi.org/10.1111/pce.13433

Subhadarshini, S., Sahoo, S., Jolly, M. K., & Rashid, M. (2025). An integrative molecular systems approach unravels mechanisms underlying biphasic nitrate uptake by plant nitrate transporter NRT1.1. Npj Systems Biology and Applications, 11(1). https://doi.org/10.1038/s41540-025-00587-x

Tang, D., Wang, G., & Zhou, J. (2017). Receptor Kinases in Plant-Pathogen Interactions: More Than Pattern Recognition. The Plant Cell, 29(4), 618. Oxford University Press. https://doi.org/10.1105/tpc.16.00891

Tiburcio, A. F., Altabella, T., Bitrián, M., & Alcázar, R. (2014). The roles of polyamines during the lifespan of plants: from development to stress. Planta, 240(1), 1. Springer Science+Business Media. https://doi.org/10.1007/s00425-014-2055-9

Vaattovaara, A., Brandt, B., Rajaraman, S., Safronov, O., Luklová, M., Kangasjaervi, J., Loeytynoja, A., Hothorn, M., Salojaervi, J., & Wrzaczek, M. (2019). Mechanistic insights into the evolution of DUF26-containing proteins in land plants. Communications Biology, 2(1), 56. https://doi.org/10.1038/s42003-019-0306-9

Valls, P. O., & Esposito, A. (2022). Signalling dynamics, cell decisions, and homeostatic control in health and disease. Current Opinion in Cell Biology, 75, 102066. Elsevier BV. https://doi.org/10.1016/j.ceb.2022.01.011

Walter, M., Chaban, C., Schütze, K., Batistič, O., Weckermann, K., Näke, C., Blazevic, D., Grefen, C., Schumacher, K., Oecking, C., Harter, K., & Kudla, J. (2004). Visualization of protein interactions in living plant cells using bimolecular fluorescence complementation. The Plant Journal, 40(3), 428. https://doi.org/10.1111/j.1365-313x.2004.02219.x

Wang, J., Hu, M., Wang, J., Qi, J., Han, Z., Wang, G., Qi, Y., Wang, H., Zhou, J., & Chai, J. (2019). Reconstitution and structure of a plant NLR resistosome conferring immunity. Science, 364(6435). https://doi.org/10.1126/science.aav5870

Wang, N., Sundin, G. W., Fuente, L. D. L., Cubero, J., Satyanarayana, T., Brewer, M. T., Zeng, Q., Bock, C. H., Cunniffe, N. J., Wang, C., Candresse, T., Chappell, T. M., Coleman, J. J., & Munkvold, G. P. (2024). Key Challenges in Plant Pathology in the Next Decade. Phytopathology, 114(5), 837. https://doi.org/10.1094/phyto-04-24-0137-kc

Wouters, E., Vasudevan, L., Crans, R. A. J., Saini, D. K., & Stove, C. P. (2019). Luminescence- and Fluorescence-Based Complementation Assays to Screen for GPCR Oligomerization: Current State of the Art. International Journal of Molecular Sciences, 20(12), 2958. Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/ijms20122958

Wrzaczek, M., Brosché, M., Salojärvi, J., Kangasjärvi, S., Idänheimo, N., Mersmann, S., Robatzek, S., Karpiński, S., Karpińska, B., & Kangasjärvi, J. (2010). Transcriptional regulation of the CRK/DUF26 group of Receptor-like protein kinases by ozone and plant hormones in *Arabidopsis*. BMC Plant Biology, 10(1), 95. https://doi.org/10.1186/1471-2229-10-95

Yadeta, K. A., Elmore, J. R., Creer, A. Y., Feng, B., Franco, J., Rufián, J. S., He, P., Phinney, B. S., & Coaker, G. (2016). A Cysteine-Rich Protein Kinase Associates with a Membrane Immune Complex and the Cysteine Residues Are Required for Cell Death. PLANT PHYSIOLOGY, 173(1), 771. https://doi.org/10.1104/pp.16.01404

Yang, L., Liu, H., Wang, Y., Seematti, J., Grenville-Briggs, L. J., Wang, Z., & Zhan, J. (2021). Pathogen-Mediated Stomatal Opening: A Previously Overlooked Pathogenicity Strategy in the Oomycete Pathogen *Phytophthora infestans*. Frontiers in Plant Science, 12. https://doi.org/10.3389/fpls.2021.668797

Yeh, Y., Chang, Y.-H., Huang, P., Huang, J., & Zimmerli, L. (2015). Enhanced *Arabidopsis* pattern-triggered immunity by overexpression of cysteine-rich receptor-like kinases. Frontiers in Plant Science, 6. https://doi.org/10.3389/fpls.2015.00322

Yu, X., Niu, H., Liu, C., Wang, H., Yin, W., & Xia, X. (2024). PTI-ETI synergistic signal mechanisms in plant immunity [Review of PTI-ETI synergistic signal mechanisms in plant immunity]. Plant Biotechnology Journal, 22(8), 2113. Wiley. https://doi.org/10.1111/pbi.14332

Zeiner, A., Colina, F., Citterico, M., & Wrzaczek, M. (2023). CYSTEINE-RICH RECEPTOR-LIKE PROTEIN KINASES: their evolution, structure, and roles in stress response and development. Journal of Experimental Botany, 74(17), 4910. https://doi.org/10.1093/jxb/erad236

Zhang, W. J., Zhou, Y., Zhang, Y., Su, Y. H., & Xu, T. (2023). Protein phosphorylation: A molecular switch in plant signaling. Cell Reports, 42(7), 112729. Cell Press. https://doi.org/10.1016/j.celrep.2023.112729

Zhang, X., Han, X., Shi, R., Yang, G., Qi, L., Wang, R., & Li, G. (2013). *Arabidopsis* cysteine-rich receptor-like kinase 45 positively regulates disease resistance to *Pseudomonas syringae*. Plant Physiology and Biochemistry, 73, 383. https://doi.org/10.1016/j.plaphy.2013.10.024

Zhang, Y., Tian, H., Chen, D., Zhang, H., Sun, M., Chen, S., Qin, Z., Ding, Z., & Dai, S. (2023). Cysteine-rich receptor-like protein kinases: emerging regulators of plant stress responses. Trends in Plant Science, 28(7), 776. Elsevier BV. https://doi.org/10.1016/j.tplants.2023.03.028

Zhou, J., & Zhang, Y. (2020). Plant Immunity: Danger Perception and Signaling. Cell, 181(5), 978. Cell Press. https://doi.org/10.1016/j.cell.2020.04.028

Zhu, Q., Feng, Y., Xue, J., Chen, P., Zhang, A., & Yu, Y. (2023). Advances in Receptor-like Protein Kinases in Balancing Plant Growth and Stress Responses. Plants, 12(3), 427. Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/plants12030427

11. Supplementary Material