FUNCTION CONSERVATION OF PLANT CELL-SURFACE CO-REGULATORY RECEPTORS ACROSS 400 MILLION YEARS OF EVOLUTION OF THE LAND PLANTS

A Dissertation

by

ANA MARCIA ESCOCARD DE AZEVEDO MANHAES

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Chair of Committee,	Libo Shan
Co-Chair of Committee,	Ping He
Committee Members,	Michael V. Kolomiets
	Charles M. Kenerley
Head of Department,	Leland S. Pierson III

December 2020

Major Subject: Plant Pathology

ABSTRACT

Plants possess a variety of cell surface receptors that perceive endogenous and exogenous signals to regulate plant growth, development, and immunity. These receptors include receptor-like kinase (RLKs) and receptor-like protein (RLPs) that possess different extracellular domains perceiving distinct ligands and leading to the activation of downstream signaling. The signaling pathways mediated by RLKs and RLPs converge at a small group of co-receptors called somatic embryogenesis receptor kinases (SERK). These co-receptors modulate both immunity and plant development and growth. Although their homologous sequences are widespread across various plant species, it remains unknown if SERKs redundant and distinct functions are also conserved across plant kingdom. To shed light on the functional conservation of SERKs, three homologous genes were retrieved from the moss *Physcomitrella patens*, a bryophyte representative of early land plants. Their ability to complement *Arabidopsis serk* mutant deficiencies in terms of brassinosteroid responses and immunity was analyzed. Furthermore, to enlighten the physiological functions of PpSERKs in *Physcomitrella*, knockout lines of PpSERKs were generated via homologous recombination and their growth, development, and responses to *Botrytis cinerea* were assessed. In this work, we showed that SERKs homologs are present in the moss *Physcomitrella* and possess conserved function in immunity activation and brassinosteroid-mediated growth and development.

ACKNOWLEDGEMENTS

I would like to express my gratitude and appreciation for my advisors Dr. Libo Shan and Dr. Ping He for their guidance and feedback throughout this project. They continuously encouraged and challenged me to be better and do better. I also would like to give special thanks to Dr. Mike Kolomiets and Dr. Charles Kenerley, for their support throughout the course of this research.

I sincerely thank all the colleagues from Dr. Shan and Dr. He lab for all their help with this project, for keeping me company in the late nights, and for making my workplace happy and fun.

From the bottom of my heart I would like to thank my fiance Jefferson for all the love, care, patience and support through happy and difficult times. It meant a lot to me. I also would like to thank my parents, siblings and nephews for their love and excitement for my achievements.

CONTRIBUTORS AND FUNDING SOURCES

This work was supervised by a committee composed of Professors Libo Shan and Ping He from the Department of Biochemistry and Biophysics and Professors Michael Kolomiets and Charles Kenerley from the Department of Plant Pathology and Microbiology.

Dr. Shan Jiang and Dr. Jun Liu collaborated performing a few experiments to generate data on chapter VI. Dr. Fausto Andres Ortiz-Morea was involved in reviewing the chapters I, II, III, IV, and V. All other work presented in the dissertation was conducted by the student independently.

The work was supported by the Brazilian National Council for Scientific and Technological Development (CNPq) (201710/2014-5) to A.M.E.A.M; National Institutes of Health (NIH) (R01GM092893) and National Science Foundation (NSF) (MCB-1906060) to P.H.; and NIH (R01GM097247) and the Robert A. Welch Foundation (A-1795) to L.S.

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION

Plants are regularly exposed to microbial pathogen invasions and variable environmental conditions that need to be continuously monitored. Thus, plants rely on plasma membrane-localized receptors that recognize exogenous and endogenous signals and trigger proper responses to ensure a balanced modulation of growth, development, immunity, and stress adaptation (De Smet et al., 2009; Couto and Zipfel, 2016; Tang et al., 2017).

These receptors mainly consist of RECEPTOR-LIKE KINASEs (RLKs) and RECEPTOR-LIKE PROTEINs (RLPs), which have been expanded across the plant kingdom. As evidence of their biological relevance, the *Arabidopsis thaliana* genome is predicted to encode more than 600 RLKs and 57 RLPs, and the rice (*Oryza sativa*) genome encodes more than 1000 RLKs and 90 RLPs (Shiu and Bleecker, 2003; Shiu et al., 2004; Fritz-Laylin et al., 2005).

Recent studies have shown that various receptors perceive specific ligands; however, the perception could lead to converged signaling pathways that will regulate diverse physiological and biological processes (Chen et al., 2019; Zheng et al., 2019). In this review, we discuss the roles of RLKs and RLPs in regulating distinct pathways that control plant immunity, growth, and development. Moreover, we discuss the relationship between the receptors and the co-regulatory receptors that result in the activation of diverse intracellular signaling pathways.

1.1. General features of RLKs and RLPs

The plant's ability to adapt to their environment has likely resulted in the evolution of a wide variety of RLKs and RLPs that can recognize a broad range of ligands, including peptides, lipids, steroids, proteins, among others. A classical RLK consists of a unique ligand-binding extracellular domain, a single transmembrane domain, and an intracellular kinase domain, while a typical RLP shares a very similar structure with an RLK apart from the intracellular kinase domain (Figure 1) (Shiu and Bleecker, 2001; Wang et al., 2008). An RLP lacks the kinase domain. Instead, it possesses a short cytoplasmic tail (Wang et al., 2008; Jamieson et al., 2018). The perception of a ligand in the apoplast by an RLK, particularly for the leucine-rich repeat (LRR) RLKs, usually leads to its hetero-dimerization with a co-regulatory RLK that will result in phosphorylation and activation of ligand-receptor complexes (Couto and Zipfel, 2016; Hohmann et al., 2017; Tang et al., 2017). Subsequently, the kinase domains of RLKs transduce the signal through a cascade of phosphorylation events, which ultimately leads to the plant cellular response to the cognate ligands (Couto and Zipfel, 2016; Hohmann et al., 2017; Tang et al., 2017). On the other hand, a similar process occurs to RLPs, but, due to lacking the kinase domain, RLPs depend on one or several receptor kinases to activate intracellular signaling (Couto and Zipfel, 2016; Jamieson et al., 2018).

Based on the heterogeneity of their ligand-binding motifs, RLKs and RLPs are classified into 12 subgroups, including LRR, lysine motif (LysM), lectin, wall-associated kinases (WAK), malectin-like, proline-rich, cysteine-rich repeat, and self-incompatibility

locus (S-Locus) (Shiu and Bleecker, 2001; Dievart et al., 2020). LRR is the most common motif of the extracellular domain of RLKs and RLPs, comprising more than 200 and 50 members, respectively (Shiu and Bleecker, 2001; Wang et al., 2008; Lehti-Shiu et al., 2009). RLKs and RLPs can also be distinguished based on the roles they play in the plant life cycle, forming part of a remarkable surveillance system that modulates plant immune responses, growth, and development (De Smet et al., 2009; Couto and Zipfel, 2016; Tang et al., 2017).

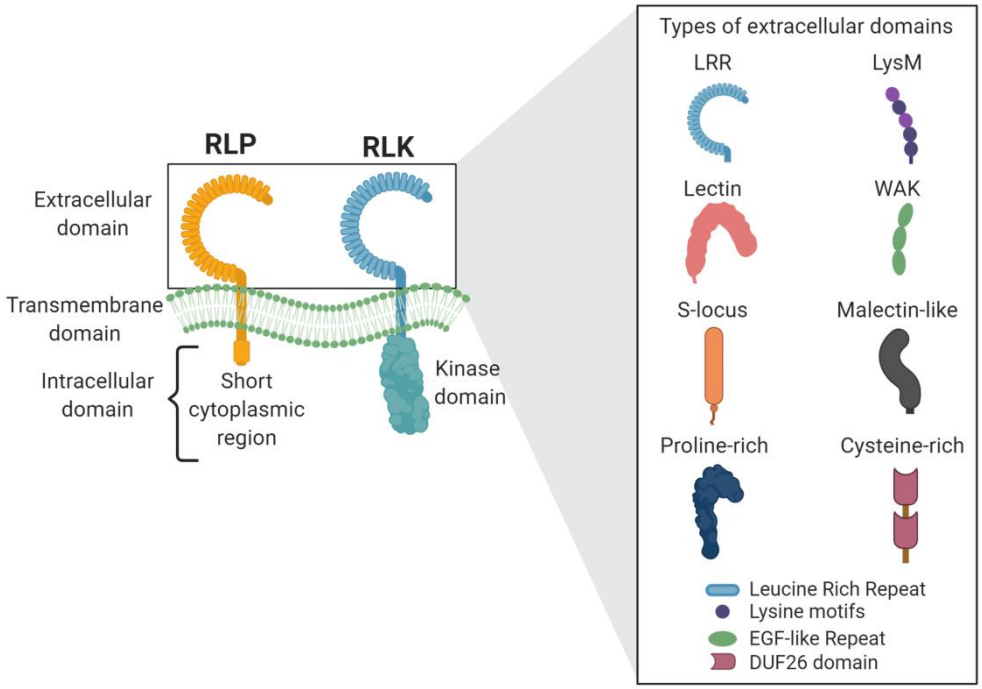


Figure 1. Schematic portrayal of plant cell-surface receptors. Plants have evolved cell surface receptors to recognize self and non-self signals to activate defense response and promote plant growth and development. A classical RECEPTOR-LIKE KINASE (RLK) contains an extracellular domain, transmembrane domain and a kinase domain. Meanwhile, the RECEPTOR-LIKE PROTEIN (PLP) possesses an extracellular domain, transmembrane domain and a short cytoplasmic region, but lacks a cytoplasmic kinase domain. RLKs and RLPs are classified in several subgroups based on the diverse composition of the extracellular domains. These include LRR domain, composed by leucine-rich repeats; LysM, constituted by lysine motifs; lectin; wall-associated kinases (WAK), comprised of EGF-like repeat; S-locus domain; malectin-like; proline-rich; and cysteine-rich repeat, consisting of DUF26 domain. Created with BioRender.com

CHAPTER II

THE ROLES OF RECEPTOR-LIKE KINASES AND RECEPTOR-LIKE PROTEINS IN PLANT IMMUNITY

2.1. Plant Immune system

Plants have a robust immune system that is modulated by a two-tiered perception system. The first layer is activated upon recognition of MICROBE/PATHOGEN-ASSOCIATED MOLECULAR PATTERNS (MAMPs/PAMPs) by PATTERN-RECOGNITION RECEPTORS (PRRs), which are plasma membrane-localized RLKs and RLPs (Figure 2a) (Jones and Dangl, 2006; Boller and Felix, 2009). Collectively, this line of immunity is known as PAMP-TRIGGERED IMMUNITY (PTI). MAMPs/PAMPs correspond to essential conserved regions of pathogen or microbial components. Likewise, PTI can also be activated upon the detection of DAMAGE-ASSOCIATED MOLECULAR PATTERNS (DAMPs), which are molecules secreted by the plants as a result of exposure to biotic and abiotic stresses (Figure 2a) (Gust et al., 2017; Tang et al., 2017; Ortiz-Morea and Reyes-Bermudez, 2019). Activation of PTI leads to an increase of intracellular calcium, activation of MAPK, a burst of reactive oxygen species (ROS), activation of CALCIUM-DEPENDENT PROTEIN KINASEs (CDPKs), massive transcriptional reprogramming, stomata closure, callose deposition, and so forth (Figure 2a) (Wu et al., 2014; Couto and Zipfel, 2016; Yu et al., 2017; Wang et al., 2020). Currently, despite the large number of genes predicted to encode RLKs and

RLPs, only a small portion have been characterized, and few ligands are identified (Figure 2b, Table 1). Most of the characterized PRRs belong to the LRR subgroup.

PTI responses contribute to plants effectively fighting off non-adapted microbes and provide restricted protection against host-adapted pathogens (Jones and Dangl, 2006; Boller and Felix, 2009). Nevertheless, most of the host-adapted microbes can overcome the first line of defense by deploying and delivering effectors into the plants that will target specific components of PTI and block its activation or interfere with host physiology and other defense barriers resulting in the EFFECTOR-TRIGGERED SUSCEPTIBILITY (ETS) (Jones and Dangl, 2006). To fend off infections, plants have evolved intracellular NUCLEOTIDE-BINDING LEUCINE-RICH REPEAT PROTEINs (NB-LRRs), also known as resistance proteins (R proteins), which can indirectly or directly recognize effectors and trigger another line of defense called EFFECTOR-TRIGGERED IMMUNITY (ETI) (Jones and Dangl, 2006; Cui et al., 2015). Activation of ETI often induces programmed cell death at the infection sites known as the hypersensitive response (HR) to reduce the pathogen growth (Jones and Dangl, 2006; Cui et al., 2015).

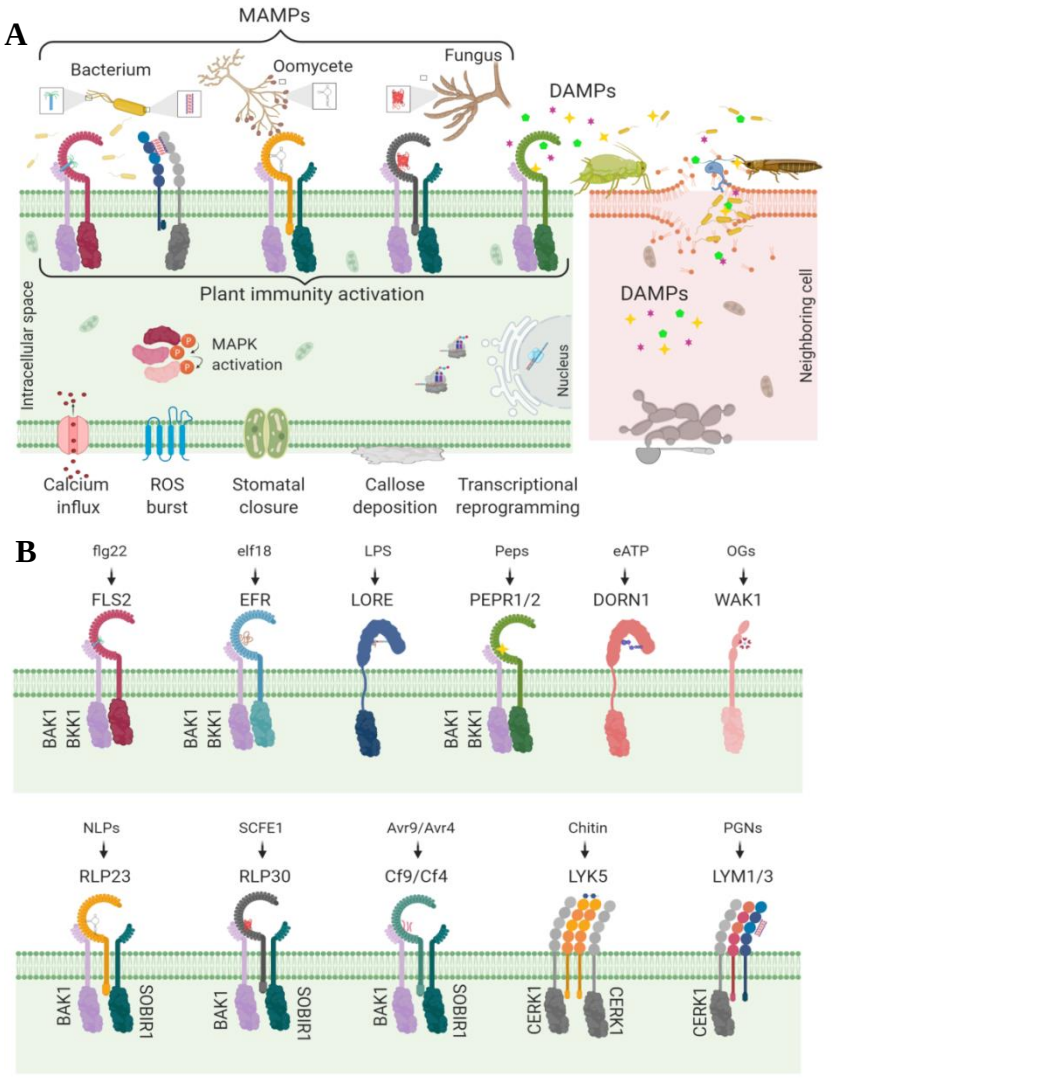


Figure 2. Receptor complexes in plant immunity. (A) Plants perceive external and internal signals to regulate diverse biological processes. Several conserved regions from microbe-associated molecular patterns (MAMPs) are recognized by RLKs and RLPs to activate plant immunity. RLKs and RLPs often recruit co-regulatory receptors to amplify and transduce the signal into downstream MAPK cascades which in turn are phosphorylated and transduce the signal into the nucleus, resulting in transcriptional reprograming. In addition, early and late immune responses are also activated upon MAMP perception, including cytosolic calcium influx, ROS burst, stomatal closure, and callose deposition.(B) Upon perception of MAMPs/ DAMPs, RLKs and RLPs associate with co-receptors such as SERKs to transduce the signals into the plant cell to activate immunity. The bacterial elicitors flg22 and elf18 are perceived by the RLKs FLS2 and EFR, respectively, which then, associate with BAK1 and BKK1 to activate PTI. Plant Lec-RLK LORE percieves bacterial LPS. Peps are perceived RLKs PEPR1 and PEPR2, which associate with BAK1 and BKK1 to amplify immune responses. Additionally, the Lec-RLK DORN1 binds to eATP in response to wounding. The WAK-RLK WAK1 recognizes oligogalacturonides (OGs) originated from fungi to activate defense. Besides, the fungal-derived MAMPs NLP, SCFE1, and Avr9/4 are regulated by LRR-RLPs RLP23, RLP30, and Cf9/4, respectively, which is then associated with LRR-RLK SOBIR and BAK1. The perception of chitin by LysM-RLP LYK5 induces the complex formation with LysM-RLK CERK1. In addition, LysM-RLK CERK1 also associates with LysM-RLPs LYM1 and LYM3 to activate immunity upon perception of bacterial PGN. Created with BioRender.com

2.2. LRR-RLKs as PRRs for the plant immune system

The first and best-characterized immune-related PRR in *Arabidopsis* is the LRR-RLK FLAGELLIN SENSING 2 (FLS2) that perceives the bacterial flagellin and the cognate peptide flg22 (Gómez-Gómez and Boller, 2000). FLS2 orthologs have been found in tomato (*Solanum lycopersicum*), a wild relative of tobacco (*Nicotiana benthamiana*), grapevine (*Vitis vinifera*), and rice (Hann and Rathjen, 2007; Robatzek et al., 2007; Takai et al., 2008; Trdá et al., 2014). The diversification of PRRs results from modifications that have occurred over time following the evolution of the pathogens (Steinbrenner, 2020). Therefore, analogous MAMPs may be recognized by distinct and/or convergent PRRs. For instance, tomato has evolved another LRR-RLK, SlFLS3 that perceives flgII-28, a variation of the flg22 epitope (Hind et al., 2016).

Another well-studied LRR-RLK PRR is ELONGATION FACTOR-TU (EF-Tu) RECEPTOR (EFR) that recognizes the bacterial EF-Tu (elf18), a very abundant and conserved protein in bacteria (Kunze et al., 2004; Zipfel et al., 2006). Similarly to what was observed for flagellin, a second EF-Tu epitope (EFa50) was found in rice, suggesting that rice may have evolved PRRs that can recognize bacterial EF-Tu. Even though elf18 is a specific MAMP for Brassicaceae species, rice plants can recognize another region of the EF-Tu epitope (Furukawa et al., 2014).

Several other LRR-RLKs have been identified as PRRs throughout the plant kingdom. For instance, csp22 is a peptide from bacterial COLD-SHOCK PROTEIN (CSP) that is perceived by LRR-RLK COLD SHOCK PROTEIN RECEPTOR (SlCORE) in tomato (Wang et al., 2016). In addition, the MAMP RaxX from

Xanthomonas oryzae pv. *oryzae* (Xoo) was reported as essential for activation of PTI responses mediated by OsXA21, an LRR-RLK from rice (Pruitt et al., 2015). Although remarkable progress has been made in identifying and characterizing new pairs of MAMPs/PRRs, there is a handful of identified PRRs, which lack recognition of their cognate ligands. For instance, NEMATODE-INDUCED LRR-RLK 1 (NLR1) is required for nematode-mediated defense response activation in *Arabidopsis;* however, its corresponding ligand is yet to be identified (Mendy et al., 2017).

Besides detecting MAMPs, LRR-RLKs can also perceive DAMPs and activate the immune response in response to physical wounding and herbivore attack. The RLK PEP1 RECEPTOR1 (PEPR1) and PEPR2 in *Arabidopsis* recognize the so-called PLANT ELICITOR PEPTIDE (PEP1 through 8) in response to wounding (Yamaguchi et al., 2006, 2010; Krol et al., 2010). Furthermore, PEP1 also acts mutually with another plant peptide family known as PAMP-INDUCED PEPTIDES (PIPs) to magnify the immune response triggered by flagellin (Hou et al., 2014). In *Arabidopsis*, PIP1 is recognized by RECEPTOR-LIKE KINASE 7 (AtRLK7) (Hou et al., 2014).

LRR-RLKs also perceive signals from parasitic plants. For instance, the obligate parasitic plant *Orobanche cumana* can infect the roots of susceptible sunflowers (*Helianthus annuus*), leading to yield losses (Duriez et al., 2019). Resistant varieties of sunflowers possess the LRR-RLK HAOR7 that prevents the parasitic plant from attaching to the roots (Duriez et al., 2019).

2.3. LRR-RLPs as PRRs for the plant immune system

In addition to LRR-RLKs, members of LRR-RLPs perceive MAMPs and regulate plant immune activation (Figure 2b, Table 1). Although RLPs lacks the kinase domain to transduce the signal from the plasma membrane, it has been suggested that LRR-RLPs constitutively associate with SUPPRESSOR OF BIR 1-1 (SOBIR1) to form a bimolecular complex with an RLK to initiate the signal transduction (Gust and Felix, 2014).

The first identified LRR-RLP belongs to tomato, SlCF-9, which confers resistance to pathogen *Cladosporium fulvum* (Cf) bearing the avirulence gene Avr9 (Jones et al., 1994). Subsequently, a few other LRR-RLPs were identified as PRRs for resistance genes, such as SlCf-2, SlCf-4, and SlCf-5 (Dixon et al., 1996, 1998; Thomas et al., 1997). Although these LRR-RLPs require binding to ligands to activate PTI, the evidence of direct binding does not occur. Instead, they perceive the inhibitory effect of the avirulence proteins on the specific plant cysteine proteases such as Rcr1, Rcr2, and Rcr3 (Hammond-Kosack et al., 1994; Rooney et al., 2005). In tomato, perception of the ETHYLENE-INDUCED XYLANASE (eix) from fungi *Trichoderma* spp. requires the LRR-RLPs LeEIX1 and LeEIX2 (Ron and Avni, 2004; Zipfel, 2008). Although LeEIX1 and LeEIX2 can bind to eix elicitor, only LeEIX2 can transduce the signal to induce plant defense, whereas LeEIX1 acts as a decoy receptor and suppresses the immune response activated by LeEIX2 (Ron and Avni, 2004; Bar et al., 2010). Unlike in tomato, the csp22 perception in tobacco is accomplished by an LRR-RLP called RECEPTOR-LIKE PROTEIN REQUIRED FOR CSP22 RESPONSIVENESS (NbCSPR) (Saur et al., 2016).

In *Arabidopsis*, LRR-RLP RLP23 confers resistance to a wide-spread microbial elicitor from bacteria, fungi, or oomycetes called NECROSIS- AND ETHYLENE-INDUCING PEPTIDE 1 (NEP1)-LIKE PROTEINs (NLPs) (Böhm et al., 2014; Albert et al., 2015). Meanwhile, LRR-RLP RLP30 triggers PTI in response to the fungal elicitor SCLEROTINIA CULTURE FILTRATE ELICITOR 1 (SCFE1) from *Sclerotinia sclerotiorum* (Zhang et al., 2013). Additionally, the fungal ENDOPOLYGALACTURONASE (PGs) was reported as MAMP perceived by the LRR-RLP RLP42, also known as RESPONSE TO BOTRYTIS POLYGALACTURONASES 1 (RBPG1) (Zhang et al., 2014). Moreover, LRR-RLPs are also able to detect MAMP elicitors from parasitic plants. For instance, the first of its kind is the tomato LRR-RLP CUSCUTA RECEPTOR 1 (SlCuRe1), which can perceive a small peptide factor from the parasitic plant, *Cuscuta reflexa* (Hegenauer et al., 2016).

Recent studies have shown that the conservation of RLKs and RLPs has taken place across land plants. Nevertheless, compared with the widespread preservation of RLKs, RLPs are less conserved but show a degree of conservation within the specific plant genus (Steinbrenner, 2020; Jamieson et al., 2018). As revealed by a recent study, most of the characterized LRR-RLPs from tomato and *Arabidopsis* are classified into different clades with no evident orthology between them (Steinbrenner, 2020). For instance, *Arabidopsis* LRR-RLPs 30, 23, and 42 are grouped within the *Arabidopsis* clade, whereas the tomato LRR-RLPs Cf-4 and Cf-5 are placed in the tomato clade. Albeit the LRR-RLPs LeEIX1, LeEIX2, and CuRe1 are not placed in a clade as specific

as the above mentioned LRR-RLPs, they are still classified within particular species groups (Steinbrenner, 2020).

2.4. RLKs and RLPs with a distinct extracellular domain as PRRs for the plant immune system

Although LRR comprises the most common extracellular domain of RLKs and RLPs, there are others with peculiar extracellular domains that also play a role in the activation of plant immune responses upon the presence of MAMPs and DAMPs (Figure 2b, Table 1). For instance, chitin, the primary component of the fungal cell wall, is perceived by a complex formed by the LysM-RLKs LYSINE MOTIF RECEPTOR KINASE 5 (LYK5), LYSINE MOTIF RECEPTOR KINASE 4 (LYK4), and CHITIN ELICITOR RECEPTOR 1 (CERK1), also known as LYK1 (Kaku et al., 2006; Miya et al., 2007; Cao et al., 2014; Xue et al., 2019). The receptors in this complex have a differential contribution to recognizing the elicitor and activation of defense response. For instance, LYK5 is the primary receptor of chitin, whereas LYK4 acts as a scaffold protein to increase the immune response (Cao et al., 2014; Xue et al., 2019). Meanwhile, CERK1 binds to LYK5 in a chitin-depend manner, and the binding is required for CERK1 phosphorylation and transduction of the signal from the membrane to the intracellular space to activate PTI (Cao et al., 2014). Additionally, a lectin S-domain RLK LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION (LORE) was reported to recognize medium-chain 3-hydroxy fatty acids (mc-3-OH-FAs) from bacterial LPS (Kutschera et al., 2019). Furthermore, a WAK domain RLK, WALL-

ASSOCIATED KINASE 1 (WAK1), can recognize the oligogalacturonides (OGs) released during fungal infection (Brutus et al., 2010). This receptor can activate MAMPs and DAMPs in response to both immunity and development. Moreover, it has been shown that the malectin-like domain RLKs ANXUR1 (ANX1) and ANX2 function as a molecular link regulating two-tiered plant immunity by association with both PRR and NB-LRR protein complexes (Mang et al., 2017); however, how ANXs differentially modulated plant PTI and ETI is not clear.

Along with the various RLKs with distinct extracellular domains, several RLPs with LysM motifs have been extensively studied. For instance, the rice receptor CHITIN ELICITOR BINDING PROTEIN (OsCEBiP) was reported to play a critical role in the perception and activation of downstream signaling in response to chitin (Shimizu et al., 2010). Later on, similarly to *Arabidopsis*, the RLK OsCERK1 was also identified as a crucial component of the chitin-mediated immune signaling in rice (Shimizu et al., 2010). Additionally to its function in chitin recognition, OsCERK1, together with LYSM DOMAIN-CONTAINING PROTEIN 4 (LYP4) and LYP6, also play a role in perceiving bacterial peptidoglycan (PGN) (Liu et al., 2012). Similarly, in *Arabidopsis*, CERK1 also associates with two LysM-RLPs called LYSIN-MOTIF 1 (LYM1) and LYM3 to activate PTI in response to bacterial PGN (Willmann et al., 2011).

In addition, RLKs with a distinctive extracellular domain plays a role in amplifying the PTI responses and activating plant defense upon DAMP perception. For instance, DOES NOT RESPOND TO NUCLEOTIDES 1 (DORN1) is an RLK with an

extracellular lectin domain, and binds to the extracellular ATP (eATP) in response to physical wounding (Choi et al., 2014).

Table 1. RLKs and RLPs in plant immunity. Receptor names are included with the extracellular domain composition, organism identified, ligand recognized, origin of the ligand, and known co-regulatory proteins.

Receptor name	Extracellular domain	Plant	Ligand	Ligand origin	Co-receptors
RECEPTOR-LIKE KINASE					
FLS2	LRR	*Arabidopsis*	flg22	Bacteria	BAK1, BKK1
FLS3	LRR	Tomato	flgII-28	Bacteria	BAK1
EFR	LRR	*Arabidopsis*	elf18	Bacteria	BAK1, BKK1
CORE	LRR	Tomato	csp22	Bacteria	BAK1
Xa21	LRR	Rice	raxX	Bacteria	SERK2
NLR1	LRR	*Arabidopsis*	?	Nematode	
PEPR1/PEPR2	LRR	*Arabidopsis*	Peps	Plants	BAK1, BKK1
RLK7	LRR	*Arabidopsis*	PIP1	Plants	BAK1, BKK1
DORN1	Lectin	*Arabidopsis*	eATP	Plants	
LYK4/LYK5	LysM	*Arabidopsis*	chitin	Fungi	
CERK1	LysM	*Arabidopsis*	chitin	Fungi	
LORE	LysM	*Arabidopsis*	LPS	Bacteria	
WAK1	WAK	*Arabidopsis*	OGs	Plants	
CERK1	LysM	Rice	chitin	Fungi	
RECEPTOR-LIKE PROTEIN					
SlCf9	LRR	Tomato	Avr9	Fungus	BAK1, SOBIR1
SlCf2	LRR	Tomato	Avr2	Fungus	BAK1, SOBIR1
SlCf4	LRR	Tomato	Avr4	Fungus	BAK1, SOBIR1
SlCf5	LRR	Tomato	Avr5	Fungus	BAK1, SOBIR1
LeEIX1/LeEIX2	LRR	Tomato	EIX	Fungi	BAK1, SOBIR1
NbCSPR	LRR	Tobacco	csp22	Bacteria	BAK1
RLP23	LRR	*Arabidopsis*	NLPs	Bacteria, ungi, foomycete	BAK1, SOBIR1
RLP30	LRR	*Arabidopsis*	SCFE1	Fungi	BAK1, SOBIR1
RLP42	LRR	*Arabidopsis*	PGs	Fungi	SOBIR1
CuRe1	LRR	Tomato	Cuscuta factor	Parasitic plant	
CEBiP	LysM	Rice	chitin	Fungi	
LYP4/LYP6	LysM	Rice	PGNs/chitin	Bacteria, Fungi	
LYM1/LYM3	LysM	*Arabidopsis*	PGNs	Bacteria	

CHAPTER III

THE ROLES OF RECEPTOR-LIKE KINASES AND RECEPTOR-LIKE PROTEINS IN PLANT DEVELOPMENT

3.1. Plasticity of plant growth and development

In contrast with animals, plants have the ability to form and regenerate organs throughout their life cycle and dynamically adjust their body architecture in response to environmental conditions. Therefore, to promote their growth and development, plants rely on pluripotent stem cells that compose the meristems. These cells are not specialized, and they continuously divide to generate new cells that subsequently induce the differentiation of all cell types (Heidstra and Sabatini, 2014; Greb and Lohmann, 2016).

The embryonic phase includes the establishment of the shoot apical meristem (SAM), the root apical meristem (RAM), and the first vascular stem cells. Afterward, in the post-embryonic phase, the SAM induces the development of leaves, flower primordia, side branches, and stem tissue (Kitagawa and Jackson, 2019). Meanwhile, the RAM induces the elongation and the formation of primary and lateral roots (Kitagawa and Jackson, 2019). In addition, the vascular tissue of the primary root, hypocotyl, and cotyledons is derived from the first vascular stem cells. In contrast, the vascular tissue of newly formed organs such as leaves, lateral roots, and stem, is derived from the apical meristems (Zhu et al., 2020). The vascular tissue allows the plant to grow radially, resulting in the thickening of the stem tissue (Kitagawa and Jackson, 2019).

Moreover, plants also undergo cell elongation, where the plant grows towards a stimulus, such as light, water, physical contact, and gravity. The steady formation of new organs and tissues by meristems demands a robust control of plant growth and development processes regulated by hormones, plant growth regulators (PGR), and signals from other plant cells (Drisch and Stahl, 2015; Galli and Gallavotti, 2016). Several plant hormones function in preserving the meristem activity. For instance, auxin and cytokinin coordinate cell differentiation and division (Pierre-Jerome et al., 2018). Furthermore, ethylene and brassinosteroid (BR) are responsible for regulating senescence and the control of cell elongation by ethylene and cell division, respectively (Pierre-Jerome et al., 2018).

Interestingly, during the last decades, an increasing number of RLKs and RLPs have been found to play crucial functions in regulating different aspects of plant growth and development, with the LRR-RLKs and LRR-RLPs as the dominant group (Fig 3a, 3b, Table 2).

3.2. LRR-RLKs in plant growth and development

One of the best well-characterized LRR-RLK involved in plant growth and development is BRASSINOSTEROID INSENSITIVE 1 (BRI1) (Li and Chory, 1997). BRI1 is the receptor of brassinosteroids, and it can regulate plant response to light, cell elongation, root growth, stomata development, and stress response (Figure 3b) (Li and Chory, 1997; Gudesblat et al., 2012; Zhu et al., 2013; Nolan et al., 2020). Another key regulator of plant development is the phytohormone PHYTOSULFOKINE (PSK), which

is responsible for cell division and cell expansion and perceived by the LRR-RLK PSK RECEPTOR 1 (PSKR1) (Matsubayashi, 2002; Matsubayashi et al., 2006; Wang et al., 2015). Additionally, the peptide PLANT PEPTIDE CONTAINING SULFATED TYROSINE 1 (PSY1) also regulates the cell division and expansion in *Arabidopsis,* after being distinguished by an LRR-RLK named as PSY1 RECEPTOR (PSY1R) (Amano et al., 2007). Moreover, the LRR-RLKs ERECTA, ERECTA-LIKE 1 (ERL1), and ERL2 play a role in stomatal development, reproductive organ development, and trigger cell differentiation, upon recognition of EPIDERMAL PATTERNING FACTOR 1 (EPF1) and EPF2 (Shpak et al., 2004; Lee et al., 2012; Shpak, 2013). A recent study identified another peptide, CLAVATA3/ESR-RELATED 9/10 (CLE9/10), also plays a role in stomatal development upon being recognized by HAESA-LIKE 1 (HSL1) (Qian et al., 2018). Furthermore, a peptide called INFLORESCENCE DEFICIENT IN ABSCISSION (IDA), upon perception by HAESA (HAE) and HAESA-LIKE 2 (HSL2) receptors, triggers lateral root emergence and cell abscission of the floral organ after pollination (Figure 3a) (Cho et al., 2008; Kumpf et al., 2013).

The plant stem cells' maintenance demands a sensitive regulation of the cell division and differentiation in the meristems. Taking that into consideration, in *Arabidopsis*, a peptide named CLAVATA 3 (CLV3) is perceived by an LRR-RLK known as CLAVATA1 (CLV1), which in turn, regulates transcription factors responsible for the maintenance of SAM (Figure 3a) (Brand et al., 2000; Ogawa et al., 2008; Pierre-Jerome et al., 2018). The peptide CLE9/10, additionally to its function in stomatal patterning, is perceived by the receptors BARELY ANY MERISTEM 1

(BAM1), BAM2, and BAM3 to promote xylem development (Qian et al., 2018). Furthermore, BAM receptors are also involved in anther development and the maintenance of the meristem function (DeYoung et al., 2006; Hord et al., 2006). Another receptor, RECEPTOR-LIKE PROTEIN KINASE 2 (RPK2), was also reported to play a role in meristem maintenance and anther development (Mizuno et al., 2007; Kinoshita et al., 2010). Moreover, the CLE peptide/receptor complex also regulates the differentiation and proliferation of the vascular stem cells. Namely, the CLE peptide TRACHEARY ELEMENT DIFFERENTIATION INHIBITORY (TDIF) is recognized by the TDIF RECEPTOR (TDR), also known as PHLOEM INTERCALATED WITH XYLEM (PXY) (Fisher and Turner, 2007; Hirakawa et al., 2008). Besides, the receptors ROOT GROWTH FACTOR (RGF) RECEPTOR 1 to 5 (RGFR1 to RGFR5) recognize the peptide RGF leading to a fine-tuned control of the root meristem development (Ou et al., 2016; Shinohara et al., 2016).

Several ligand-receptor complexes have also been identified as critical components of plant reproduction. For instance, a small protein called TAPETUM DETERMINANT1 (TPD1) can induce the specialization of anther cells after being perceived by the RLK EXCESS MICROSPOROCYTES1 (EMS1) or EXTRA SPOROGENOUS CELLS (EXS) (Canales et al., 2002; Zhao et al., 2002; Yang et al., 2003; Jia et al., 2008). In tomato, the POLLEN-SPECIFIC RECEPTOR KINASES 1 (LePRK1), LePRK2, and LePRK3 family regulate pollen germination and pollen tube growth (Tang et al., 2004, 2002; Huang et al., 2014). Besides, recent studies revealed new receptors as a critical component in regulating plant growth and development

processes. For instance, the *Arabidopsis* STRUBBELIG (SUB) receptor is involved in tissue morphogenesis and controls the composition of the plant cell wall (Chaudhary et al., 2020). Meanwhile, the kinase-inactive RLKs MUSTACHES (MUS) and MUSTACHES-LIKE (MUL) can control lateral root development (Xun et al., 2020). Although MUS and MUL are in vitro kinase-inactive RLKs, they are phosphorylated by an unidentified kinase in planta (Xun et al., 2020).

3.3. LRR-RLPs in plant growth and development

Along with LRR-RLKs, several LRR-RLPs have also been reported to function as crucial components of the plant growth and development system (Figures 3a and 3b, Table 2). However, they often require another RLK to transduce the signal. For instance, the receptor TOO MANY MOUTHS (TMM) or RLP17 regulates the stomatal patterning through complex formation with ERECTA and ERL1 upon the perception of EPF1 and EPF2 in *Arabidopsis* (Lin et al., 2017). Similarly, CLAVATA 2 (CLV2) interacts with CLV1 in response to CLV3, which leads to the regulation and maintenance of SAM, RAM, and organ development (Kayes and Clark, 1998; Jeong et al., 1999). Besides this pathway, CLV2 also interacts with the RLCK CORYNE (CRN) to form a complex and independently perceive CLV3 to regulate the meristems (Figure 3a) (Müller et al., 2008; Bleckmann et al., 2010; Guo et al., 2010; Somssich et al., 2016). Meanwhile, FASCIATED EAR 2 (FEA2), a CLV2 homolog in maize, was identified to function in the shoot meristem proliferation and positively affect floral meristem (Taguchi-Shiobara et al., 2001). Furthermore, RECEPTOR-LIKE PROTEIN (RLP44) mediates the

activation of brassinosteroid to regulate plant growth and cell wall integrity upon pectin modification (Wolf et al., 2014).

3.4. RLKs and RLPs with distinct extracellular domains in plant growth and development

Apart from the LRR extracellular domain, several RLKs and RLPs with distinct extracellular domains also contribute to plant growth and development. The malectin domain RLK FERONIA (FER) belongs to the *Catharanthus roseus* RLK1-LIKE (CrRLK1L) subfamily and takes part in the hormone signaling and female fertility in *Arabidopsis* (Li et al., 2016). Besides, FER also perceives the peptide RAPID ALKALINIZATION FACTOR (RALF) in order to regulate the primary root growth and plant cell expansion (Haruta et al., 2014). Other malectin-like RLKs involved in plant reproduction are HERCULES RECEPTOR KINASE 1 (HERK1) and ANJEA (ANJ). During fertilization, HERK1 and ANJ interact with FER to regulate pollen tube reception (Galindo-Trigo et al., 2020). Additionally, the receptors ANXUR1 (ANX1) and ANX2 are the closest homolog of FER and play a role in plant reproduction by controlling the pollen tube's cell wall integrity during fertilization (Boisson-Dernier et al., 2009; Miyazaki et al., 2009).

CYSTEINE-RICH RECEPTOR-LIKE KINASE 28 (CRK28) was shown to regulates growth and development of the root and shoot system, and to fine-tune ABA signaling in germination and early root growth (Pelagio-Flores et al., 2020). In rice, several RLKs with distinct extracellular domains have been reported recently. For

instance, an S-domain receptor-like kinase OsESG1 plays a role in early crown root development through auxin regulation (Pan et al., 2020). Meanwhile, the S-RECEPTOR-LIKE KINASE 1 (OsSRK1) contributes to the salt tolerance response in rice and regulates the leaf width (Jinjun et al., 2020). Another rice RLK identified is the LECTIN RECEPTOR KINASE 5 (OsLecRK5) that controls the callose deposition, which is crucial for male gametophyte development (Wang et al., 2020).

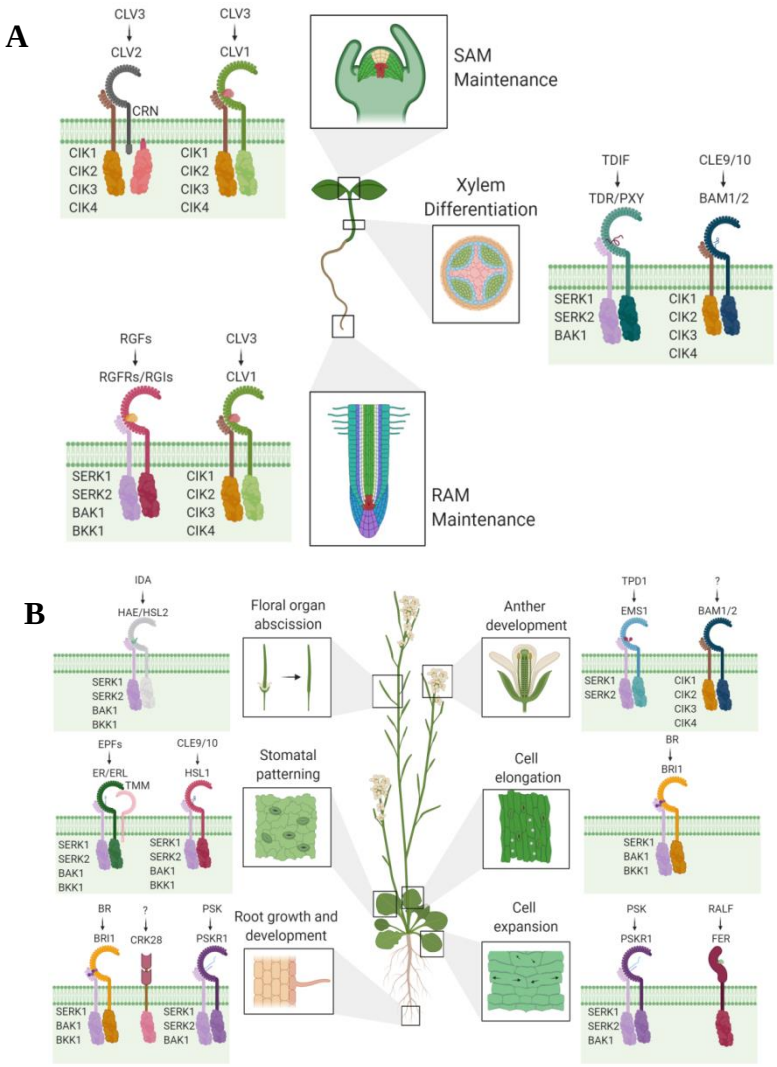


Figure 3. Receptor complexes in plant growth and development. (A) Plant stem cells are responsible for plant growth during the embryonic and post embryonic phase. To maintain SAM and RAM, LRR-RLK CLV1 perceives the peptide CLV3 and forms a complex with the co-regulatory proteins CIKs. Similarly, LRR-RLP CLV2 also associates with CIKs and a cytoplasmic RLK CORYNE (CRN) in response to CLV3 and regulate SAM and RAM. LRR-RLKs RGFRs form a complex with SERKs to control RAM upon perception of RGFs. The xylem differentiation is regulated by LRR-RLK complexes TDR/PXY-SERKs and BAM1/BAM2-CIKs after perception of TDIF and CLE9/10, respectively. (B) Hormones and peptides are also perceived by RLKs/RLPs to regulate development and growth processes. The floral organ abscission is regulated by LRR-RLK HAE/HSL2 associated with SERKs upon recognition of IDA. BR is perceived by BRI1 to regulate cell elongation and root growth and development. Root growth is regulated by cysteine-rich RLK CRK28 and LRR-RLK PSKR1-SERKs complex upon PSK perception. PSKR1-SERKs complex also control cell expansion, which is also regulated by Malectin-RLK FER in response to RALF. The stomatal patterning and development is controlled, respectively, by the complexes LRR-RLP/RLK TMM-ER/ERL-SERKs in response to EPFs and LRR-RLK HSL1-SERKs in response to CLE9/10. The peptide TPD1 is perceived by the complex LRR-RLK EMS1-SERKs. LRR-RLK BAM1/2 together with CIKs can also regulate anther development. Created with BioRender.com

Table 2. RLKs and RLPs in plant growth and development. Receptor names are included with their extracellular domain composition, cognate ligands, co-regulatory proteins and functions**.**

Receptor name	Extracellular domain	Ligand	Co-regulatory receptors	Function
RECEPTOR-LIKE KINASE				
BRI1	LRR	BR	SERK1, BAK1, BKK1	Cell elongation and plant growth
PSKR1	LRR	PSK	SERK1, SERK2, BAK1	Cell division and cell expansion
PSYR1	LRR	PSY1	SERK1, SERK2, BAK1	Cell division and cell expansion
ERECTA	LRR	EPFs	SERK1, SERK2, BAK1	Stomatal patterning
ERL1/ERL2	LRR	EPFs	SERK1, SERK2, BAK1	Stomatal patterning
HAE/HSL2	LRR	IDA	SERK1, SERK2, BAK1, BKK1	Floral organ abscision
HSL1	LRR	CLE9/10	SERK1, SERK2, BAK1	Stomatal patterning
CLV1	LRR	CLV3	CIK1, CIK2, CIK3, CIK4	SAM and RAM maintenance
RGFR1 to 5	LRR	RGFs	SERK1, SERK2, BAK1, BKK1	RAM maintenance
BAM1/2	LRR		CIK1, CIK2, CIK3, CIK4	Anther development
TDR (PXY)	LRR	TDIF	SERK1, SERK2, BAK1	Xylem differentiation
EMS1 (EXS)	LRR	TPD1	SERK1, SERK2	Male sporogenesis
LePRK1 to 3	LRR			Polen tube growth
SUB	LRR			Tissue morphogenesis
MUS/MUL	LRR			Root development
FERONIA	Malectin-like	RALF		Plant reproduction, root growth and cell expansion
CRK28	Cysteine-rich			Plant growth and root organogenesis
OsESG1	S-domain			Root development
OsSRK1	S-domain			Leaf development
OSLecRK5	Lectin			Gametophyte development
ANX1/2	Malectin-like			Plant reproduction
HERK1/ANJ	Malectin-like			Plant reproduction
RECEPTOR-LIKE PROTEIN				
TMM (RLP17)	LRR	EPFs	SERK1, SERK2, BAK1	Stomatal patterning
CLV2	LRR	CLE	CIK1, CIK2, CIK3, CIK4	SAM and RAM maintenance
FEA2	LRR			SAM maintenance
RLP44	LRR			BR regulation and plant growth

CHAPTER IV

REGULATION OF RECEPTOR-LIKE KINASES AND RECEPTOR-LIKE PROTEINS

Upon recognizing a ligand, RLKs and RLPs often rely on co-regulatory receptors to transfer the signal from the apoplast to the cell interior. The plant response to a ligand depends on the differential phosphorylation in the kinase domains of the complexes formed at the plasma membrane (Figure 4) (Perraki et al., 2018; Burgh and Joosten, 2019).

4.1. Co-regulatory RLKs

Currently, it is known that the co-regulatory RLKs can interact constitutively or in a ligand-dependent manner with a ligand-perceiving receptor (Liebrand et al., 2014). They function as co-receptors and play a role in amplifying the ligand-receptor complex signals transmitted to the downstream cascade by transphosphorylation (Liebrand et al., 2014).

4.1.1. SOMATIC EMBRYOGENESIS RECEPTOR KINASES as positive regulators in plant immunity and plant growth and development

SOMATIC EMBRYOGENESIS RECEPTOR KINASES (SERKs) are a subgroup of LRR-RLK that operate as co-receptors for several RLKs that regulate immunity, growth, and development (Figures 2b, 3a, and 3b) (Ma et al., 2016). SERK

was first identified in carrot, Daucus carota SERK (DcSERK), where it functions as a marker of embryogenesis (Schmidt et al., 1997). After that, SERK homologs were identified in other species such as *Arabidopsis thaliana*, rice, cotton, tomato, among others (Ito et al., 2005; Mantelin et al., 2011; Sakamoto et al., 2012; Gao et al., 2013; Shi et al., 2014; Aan den Toorn et al., 2015).

In *Arabidopsis*, SERK3/BRI1-ASSOCIATED KINASE 1 (BAK1) and its closest homolog SERK4/BAK1-LIKE1 (BKK1), hereafter BAK1 and BKK1 respectively, play a positive role in MAMP-triggered immunity by complexing with PRRs (Figure 2b) (Roux et al., 2011). For instance, the PRRs FLS2 and EFR, in response to bacterial MAMPs flg22 and elf18 respectively, require BAK1 and BKK1 to form a complex and subsequently activate defense response (Chinchilla et al., 2007; Roux et al., 2011). Similarly, the tomato receptors SlFLS3 and SlCORE, the tobacco NbCSPR, and the rice receptor OsXa21 also recruit SERKs to positively regulate plant response to bacterial effectors (Chen et al., 2014; Hind et al., 2016; Saur et al., 2016; Wang et al., 2016). On the other hand, ANX1 and ANX2 negatively regulate plant immune response by associating BAK1 and FLS2 and disrupting the complex formation (Mang et al., 2017). In addition to activating response to bacterial infections, SERKs are also involved in defense response to oomycetes and fungi. For instance, the activation of PTI by tomato receptors SlCf9 and SlCf4, in response to fungal elicitors, is dependent on SERKs (Liebrand et al., 2013; Postma et al., 2016). Likewise, RLP23 relies on BAK1 to activate defense response to fungi and oomycetes in *Arabidopsis* (Albert et al., 2015). The PTI activation by DAMPs also depends on SERKs. For example, PEPR1 and PEPR2 bind in

a ligand-dependent manner with BAK1 and BKK1 to form a heterodimer complex and activate intracellular signaling (Postel et al., 2010; Roux et al., 2011).

On the other hand, SERKs also play a pivotal role in plant growth and development (Figures 3a and 3b). Upon BR perception, BRI1 recruits BAK1 to form a complex and regulate plant response to light, root growth, cell elongation, and stress response (Li and Chory, 1997; Li et al., 2002; Nam and Li, 2002; Zhu et al., 2013). Later, it was reported that SERK1 and BKK1 act redundantly with BAK1 to regulate the BR-mediated signaling (Gou et al., 2012). Another example is the PSKR1 receptor that mobilizes SERK1, SERK2, and BAK1 to form a stable complex and regulate root growth and cell expansion in response to PSK (Wang et al., 2015). Furthermore, the PSY1R receptor is also able to interact with SERKs to mediate PSY1 signal transduction after phosphorylation, and subsequently regulate cell expansion and cell division (Oehlenschlæger et al., 2017). In *Arabidopsis*, SERK1 and SERK2 are also known to play a role in embryogenesis (Albrecht et al., 2005). They associate with EMS1/EXS to regulate male-gametophyte development. SERKs also carry out additional functions in growth and development. For instance, the stomatal opening and closure are vital for efficient gas and water exchange with the environment (Nadeau and Sack, 2002). The stomatal cell patterning is regulated by EPFs that are recognized by the receptors ER and ERL1. The distribution patterns in *serks* quadruple mutants are similar to er/erl1 mutants, indicating that SERKs play a redundant role in regulating stomatal patterning (Meng et al., 2015). SERKs complex with ER and ERL1 upon EPF peptide perception. Moreover, SERKs were also reported to control floral organ abscission redundantly. In

this process, the plant peptide IDA is recognized by the receptors HAE and HSL2 that recruit SERKs to activate downstream signaling and promote the detachment of floral organs (Meng et al., 2016). Besides, SERKs are also involved in the development of root meristem and xylem differentiation, acting as co-receptors of RGFR 1 through 5 and TDR, respectively (Song et al., 2016; Zhang et al., 2016)

Altogether, the co-regulatory SERKs are versatile and capable of regulating distinct signaling pathways to maintain the plant balance between immunity and growth and development.

4.1.2. Negative regulators of plant immunity and plant growth and development

Although the SERK family positively regulates plant immunity and plant growth development, negative regulators are critical for maintaining the plant homeostasis by simultaneously attenuating the responses activated by SERKs. For instance, the LRR-RLK BAK1-INTERACTING RECEPTOR-LIKE KINASE 2 (BIR2) constitutively interacts with BAK1 to avoid binding FLS2 before flg22 exposure (Halter et al., 2014; Kumar and Van Staden, 2019). Meanwhile, another RLK from the BIR family, BIR3, negatively regulates BR signaling by constitutively binding with BAK1 and BRI1, thus preventing the complex formation until the ligand perception (Imkampe et al., 2017). Furthermore, the MEMBRANE STEROID-BINDING PROTEIN 1 (MSBP1) interacts with the extracellular domain of BAK1, which rapidly induces its endocytosis, resulting in a negative impact in BR signaling (Song et al., 2009). Additionally, PROTEIN

PHOSPHATASE 2A (PP2A), a serine/threonine protein, negatively regulates plant immunity by controlling the kinase activity of BAK1 (Segonzac et al., 2014). Another example of negative regulation is the ubiquitination of FLS2 by the E3-ubiquitin ligases PLANT U-BOX 12 (PUB12) and PUB13 that culminate in the FLS2 degradation and attenuation of PTI (Lu et al., 2011).

4.1.3. The role of SUPPRESSOR OF BIR1-1 (SOBIR1) in plant immunity, growth and development

An additional co-regulatory LRR-RLK, SUPPRESSOR OF BIR1-1 (SOBIR1), was initially described as a positive regulator of the cell death pathway (Gao et al., 2009). Apart from this, SOBIR1 also constitutively interacts with other LRR-RLPs leading to the formation of functional signaling receptor complexes (Figure 2b) (Gao et al., 2009; Leslie et al., 2010; Gust and Felix, 2014; Liebrand et al., 2013, 2014). RLP23 requires SOBIR1 to form a complex together with BAK1 to activate immune responses to NLP peptides (Albert et al., 2015). Besides, tomato receptors SlCf9, SlCf4, LeEIX1, and LeEIX2 also interact with SOBIR1 homolog in tomato in response to fungus (Liebrand et al., 2013). Moreover, the tomato receptor SlCuRe constitutively associates with tomato SOBIR1 to activate defense against the parasitic plant Cuscuta reflexa. (Hegenauer et al., 2016). A recent study also described a new role of SOBIR1 in plant growth and development. Alongside with ERECTA, SOBIR1 regulates secondary xylem formation (Milhinhos et al., 2019).

4.1.4. The role of CLV3-INSENSITIVE RECEPTOR KINASEs (CIKs) in plant growth and development

Recently, a new class of co-receptors was described as regulators of stem cell homeostasis. Like the SERK family, the CLV3-INSENSITIVE RECEPTOR KINASE 1 (CIK1) to CIK4 are also members of the subclass LRR II of the RLK family. CIKs were reported to play a role as co-receptors of CLV1 and RPK2 in response to the CLV3 peptides to regulate meristem maintenance (Figure 3a) (Hu et al., 2018; Xu and Jackson, 2018). Subsequently, a new study reported that CIKs also bind to RPK2 and BAM1/2 receptors to regulate the differentiation of anther cells (Figure 3b) (Cui et al., 2018).

4.2. RECEPTOR-LIKE CYTOPLASMIC KINASEs (RLCKs) relaying the signaling

RECEPTOR-LIKE CYTOPLASMIC KINASEs (RLCKs) bear a kinase domain similar to RLKs. However, they lack the extracellular domain and the transmembrane domain (Lin et al., 2013b; Liebrand et al., 2014). RLCKs associate with the RLK complexes at the plasma membrane to transmit the intracellular signal via transphosphorylation. They play critical roles in plant immunity, growth, and development (Lin et al., 2013b).

4.2.1. The role of RLCKs in plant immunity

A well-known RCLK in plant immunity is BOTRYTIS-INDUCED KINASE 1 (BIK1) (Veronese et al., 2006; Laluk et al., 2011; Lu et al., 2010). BIK1 associates with

FLS2 and BAK1 complex. Upon flg22 perception, BIK1 is phosphorylated by FLS2 and BAK1. Subsequently, BIK1 is released from the complex to transduce the signal, leading to ROS bursts (Figure 4) (Lu et al., 2010; Zhang et al., 2010). Besides, recent studies have shown that the release of BIK1 from the PRR complex and its activation is dependent on ubiquitination by E3 ubiquitin ligases RHA3A and RHA3B (Ma et al., 2020). Also, BIK1 can also directly interact with several other PRR complexes, including EFR, PEPR1, PEPR2, and CERK1 (Zhang et al., 2010; Liu et al., 2013).

Similarly, avrPphB SENSITIVE 1 (PBS1), PBS1-LIKE 1 (PBL1), and PBL2 belong to the same family as BIK1 and interact with the above mentioned PRRs to initiate PTI responses (Zhang et al., 2010; Laluk et al., 2011). In tomato, PTO-INTERACTIN 1 (PTI1) was reported to be a positive regulator of PTI activation in response to FLS2 and FLS3 activation upon bacterial MAMPs (Schwizer et al., 2017). Besides, CERK1 also interacts with PBS1-LIKE 27 (PBL27) to regulate PTI activation induced by chitin (Shinya et al., 2014; Yamada et al., 2016). BR-SIGNALING KINASE 1 (BSK1), a critical RLCK in the BR pathway, can positively regulate flg22-triggered immune response by associating with the FLS2 complex (Shi et al., 2013). In addition to BSK1, other members of the BSK subfamily also regulate plant immunity. For instance, BSK3 and BSK5 were reported to function together with several PRRs in response to bacterial elicitors (Xu et al., 2014; Majhi et al., 2019).

4.2.2. The role of RLCKs in plant growth and development

In addition to its role in plant immunity, BIK1 can directly associate and phosphorylate the BRI1 complex to negatively regulate the BR signaling (Lin et al., 2013a). In the presence of BR, BIK1 is released from the complex upon phosphorylation by BRI1 (Figure 4). On the other hand, in contrast with its negative role in regulating immunity, BSK1 plays a positive role in regulating the BR signaling (Tang et al., 2008). Together with RLCK CONSTITUTIVE DIFFERENTIAL GROWTH (CDG1), BSK1 positively governs plant growth and development by directly binding with BRI1 (Tang et al., 2008; Kim et al., 2011). After being phosphorylated by BRI1, BSK1 and CDG1 transduce the signal to the downstream of the BR pathway (Figure 4) (Tang et al., 2008; Kim et al., 2011). Other BSK subfamily members such as BSK3 and BSK5 are also involved in BR signaling response (Tang et al., 2008; Sreeramulu et al., 2013). Additionally, SHORT SUSPENSOR (SSP) or BSK12 was reported to be involved in embryo patterning. However, it is still unclear the receptor complex that SSP likely associates with to regulate plant growth and development (Bayer et al., 2009; Costa et al., 2014). Furthermore, another RLCK, known as CAST AWAY (CST), was reported to associate with HAE and BAK1 to inhibit floral organ abscission (Burr et al., 2011). Meanwhile, MARIS (MRI) and RPM1-INDUCED PROTEIN KINASE (RIPK) were found to regulate root hair growth and root development, respectively, in association with the FER receptor (Boisson-Dernier et al., 2015; Du et al., 2016; Liao et al., 2016).

CHAPTER V

INTRACELLULAR SIGNALING UPON ACTIVATION OF RECEPTOR-LIKE KINASE, RECEPTOR-LIKE PROTEIN, AND RECEPTOR-LIKE CYTOPLASMIC KINASE COMPLEXES

Phosphorylated RLCKs relay the signal transduction to convergent signaling hubs, including MAPK cascades, ROS production, cytosolic calcium (Ca2+) influx, and activation of CALCIUM-DEPENDENT PROTEIN KINASES (CDPKs or CPKs) (Couto and Zipfel, 2016; He et al., 2018). In particular, the MAPK cascades are critical for regulating plant immunity and development. In general, three kinases compose the MAPK module: the MAPK kinase kinase (MAPKK or MEKK), the MAPK kinase (MAPKK or MKK), and the MAPK (MPK) (Meng and Zhang, 2013). This cascade is sequentially activated through phosphorylation events, resulting in the signal transduction to the next substrate (Figure 4)(Meng and Zhang, 2013).

In plant immunity, two parallel MAPK cascades are activated upon MAMP/DAMP perception. The first one comprises MEKK3 and MEKK5 (hereafter MEKK3/MEKK5); MKK4/MKK5; and MPK3/MPK6. The second one consists of MEKK1; MKK1/MKK2; and MPK4 (Meng and Zhang, 2013). For instance, the MAPK cascade activated in response to flg22 and elf18 include MEKK3/MEKK5-MKK4/MKK5-MPK3/MPK6 and MEKK1-MKK1/MKK2-MPK4 (Asai et al., 2002; Zipfel et al., 2006; Suarez-Rodriguez et al., 2007; Meng and Zhang, 2013; Bi et al., 2018; Sun et al., 2018). Meanwhile, the MAPK cascade downstream of chitin and

CERK1 pathway consists of MEKK-MKK4/MKK5-MPK3/MPK6 (Miya et al., 2007; Meng and Zhang, 2013). Likewise, plant response to DAMPs leads to activation of WAK receptors that will transduce the signal through the MPK3/MPK6 cascade (Denoux et al., 2008; Galletti et al., 2011; Meng and Zhang, 2013). MAPK activation often leads to the expression of immune-related genes, ROS production, stomatal closure, ethylene production, and hypersensitive response (Meng and Zhang, 2013).

Besides its function in activating immunity, MAPK cascades are also crucial for signal transduction to regulate plant growth and development. For instance, the MAPK cascade comprising YDA-MKK4/MKK5-MPK3/MPK6 regulates stomatal patterning and development (Meng et al., 2015). Similarly, the above mentioned MAPK cascade was reported to play a role downstream of the RLCK SSP to control embryo patterning (Costa et al., 2014). Moreover, the MKK4/MKK5-MPK3/MPK6 cascade is phosphorylated downstream HAE and HSL2 receptors regulating floral organ abscission (Meng et al., 2016). However, the MAPKKK in this pathway is not known. Interestingly, the MAPK cascade seems not to be involved in the BR signaling; in fact, BR signaling mainly relies on RLCKs, phosphatases, and transcription factors to regulate plant development (Wang et al., 2014). The MAPK activation in plant development often leads to phosphorylation of transcription factors and enzymes that regulate the growth and development responses (He et al., 2018).

In addition to the MAPK cascade, the RESPIRATORY BURST OXIDASE HOMOLOGs (RBOHs), also known as NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE (NADPH) oxidases, are the substrate for RLCKs to

relay the signal transduction that results in the accumulation of REACTIVE OXYGEN SPECIES (ROS) (Kärkönen and Kuchitsu, 2015). As reactive molecules, ROS is able to oxidize cellular components restricting their ability to function properly (Mittler et al., 2004). Thus, ROS levels require tight regulation to prevent cell damage due to excessive ROS production (Lee et al., 2020). In *Arabidopsis*, plasma membrane-resident RBOHD is mainly responsible for ROS burst upon MAMP perception (Torres et al., 2002; Wang et al., 2020). The PRRs FLS2, EFR, PEPR1, LYK5, and CERK1, upon the perception of their cognate ligands, phosphorylate BIK1, which subsequently phosphorylates RBOHD resulting in ROS production (Figure 4) (Kimura et al., 2017). Moreover, upon the perception of eATP by the DORN1 receptor, RBOHD is also phosphorylated, leading to stomatal closure and an increase of ROS burst (Chen et al., 2017). ROS also function in controlling plant growth and development. For instance, ROS positively regulates root meristem development via BR signaling (Lv et al., 2018; Tian et al., 2018). Besides, the ROS oxidative state can also expedite the cell division of embryonic roots (de Simone et al., 2017). Further, the accumulation of ROS regulates cell expansion and elongation of roots in *Arabidopsis* (Foreman et al., 2003).

One of the plant responses to a variety of stimuli is an increase of the concentration of the cytosolic free Ca2+, which leads to two opposite reactions: calcium inflow and calcium outflow (Tuteja and Mahajan, 2007). The calcium ion influx can be perceived by Ca2+-sensors that subsequently activate CDPKs (Tuteja and Mahajan, 2007; Boudsocq and Sheen, 2013). CDPKs can decode the calcium signals into phosphorylation reactions to subsequently relay the signal to trigger plant response to a

stimulus (Boudsocq and Sheen, 2013). The flg22-FLS2 complex was reported to activate CPK4, CPK5, CPK6, and CPK11 that afterward positively regulate the expression of immune-related genes (Boudsocq et al., 2010). However, CPK28 was found to negatively regulate plant immunity by phosphorylation of BIK1, limiting the amount of BIK1 available for PTI activation (Monaghan et al., 2014). Moreover, CDPKs also regulate ROS burst through phosphorylation; namely, CPK5 was reported to positively control ROS activation after flg22 perception (Boudsocq et al., 2010; Dubiella et al., 2013). CDPKs also function in plant development. For instance, several CDPKs were reported to regulate pollen tube growth in *Arabidopsis* (Myers et al., 2009). Differing from its role in immunity, CPK28 plays a positive role in vascular development and stem elongation (Matschi et al., 2013). Nonetheless, the mechanism behind the CDPKs regulation of immunity and development is not well understood. Recent studies have provided new insights into calcium signaling. For instance, upon MAMP perception, the Ca2+ channel, OSCA1.3, regulates stomatal closure, and subsequently, it is regulated by BIK1, leading to an increase of Ca2+ influx (Thor et al., 2020). In addition, the CYCLIC NUCLEOTIDE-GATED CHANNEL 2 (CNGC2) and CNGC4 were reported to be essential in the formation of a functional calcium channel, that after MAMP perception, is phosphorylated by BIK1, resulting in an increase of calcium in the cytoplasm (Tian et al., 2019).

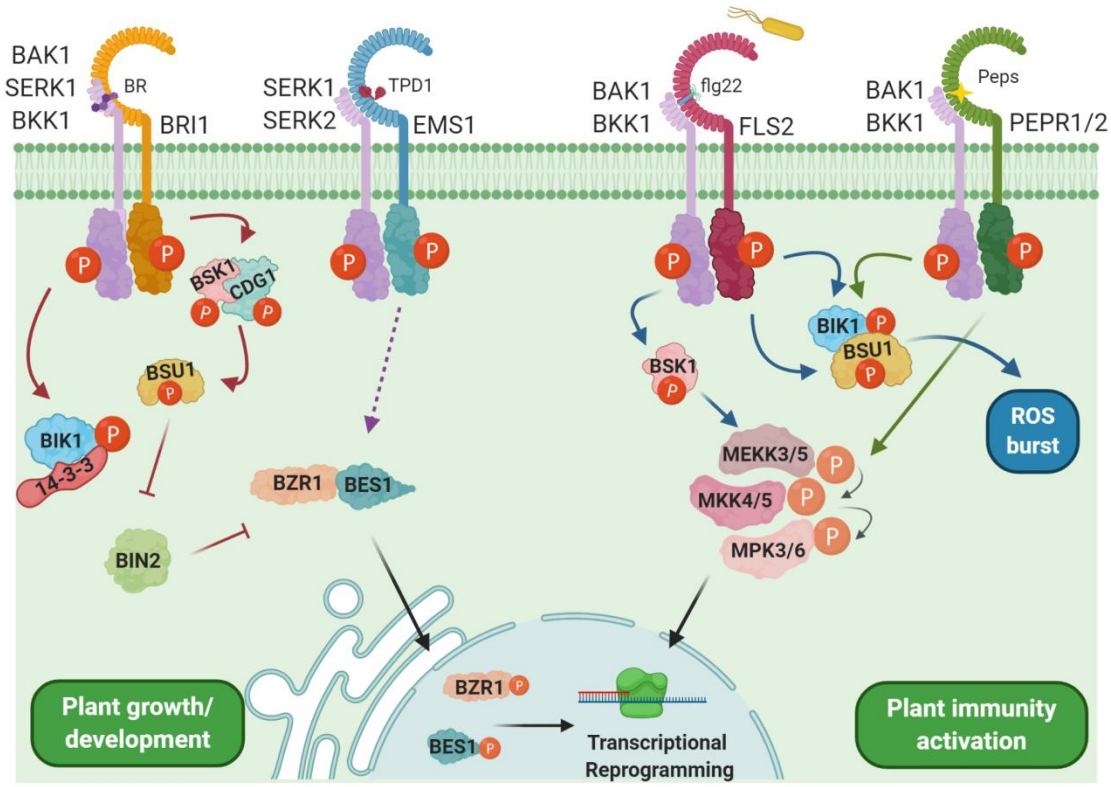


Figure 4. Shared signaling regulating plant growth, development, and immunity. Upon perception of the ligands, several RLKs and RLPs transduce the signal into the plant cell employing shared key components. For instance, upon BR perception, BRI1 associates with BAK1, which phosphorylate BSK1 and CDG1 and release BIK1 from BRI1. Subsequently, BSK1 and CDG1 phosphorylate BSU1 which in turn deactivate BIN2. Sequentially, BZR1 and BES1 induce the activation of BR-related genes. EMS1 form a complex with SERK1/2 in response to TPD1 regulating anther development. Recent studies have shown that upon EMS1-SERKs complex formation, BZR1 and BES1 activate anther development genes. In the presence of bacterial infections, FLS2 binds to BAK1/BKK1 and subsequently phosphorylate BIK1 and BSK1, which results in ROS burst and MAPK activation, respectively. Besides, PEPR1 and PEPR2 also associate with BAK1 and BKK1 in response to Peps and subsequently, phosphorylate BIK1. Later, the MAPK cascade is activated in response to both flg22 and Peps perception and results in transcriptional reprogramming to activate immunity. Maroon arrows indicate the pathway activated by BRI1; meanwhile the purple arrows represent the pathway activated by EMS1. The blue arrows indicate the pathway activated by FLS2, while the green arrows represent the pathway activated by PEPR1/2. Black arrows indicate converged pathways. Circled P represents phosphorylation events. Created with BioRender.com

CHAPTER VI

EVOLUTION AND FUNCTION CONSERVATION OF SOMATIC EMBRYOGENESIS RECEPTOR KINASE GENE FAMILY

6.1. Introduction

Layered crosstalk via convergent and shared components occurs in plant immunity, growth, and development, ensuring effective and concerted coordination between these biological processes. For instance, the co-regulatory receptors are essential for signal transduction and downstream signaling activation. The SERK family is crucial to diverse growth and development processes and immune response to different microbial elicitors. The mechanism underlying SERKs versatility to function in multiple signaling processes is yet to be understood. It also remains unknown how the SERK family has evolved in plants and whether the functional conservation of SERKs exists in early evolved plant species. Therefore, the functional characterization of SERKs in early evolved species would provide insight in the evolution of SERKs in regulating the plant growth, development and immunity.

So, due to the crucial role of SERKs in plant biological processes, we hypothesized that SERKs are conserved in early evolved species and shared similar roles as SERKs from flowering /seed plants. For this reason, this work proposed to find homolog SERKs in *Physcomitrella patens*, an evolutionary distant plant, and test their kinase functionality. In addition, we also evaluate the PpSERKs function conservation by genetic complementation of *Arabidopsis serk* mutants using PpSERKs. Subsequently,

we also test the function of SERKs in *Physcomitrella* by generating knockout lines through homologous recombination.

6.1.1. *Physcomitrella patens* as model organism for evolutionary studies

The moss *Physcomitrella patens* belongs to the group of bryophytes, alongside liverworts and hornworts. Bryophytes are non-vascular plants that adapted to the land about 400 million years ago (mya), laying the foundation for the evolution of more complex life forms (Wellman et al., 2003). Mosses diverged from unicellular aquatic algae and conquered the land, overcoming a wide range of stressors such as water availability, temperature, UV exposure, and pathogens (Rensing et al., 2008). Therefore, mosses are considered essential to study the genome evolution of plants, including the function conservation of genes involved in crucial plant biological processes (Rensing et al., 2008; Prigge and Bezanilla, 2010).

6.1.2. Genome of *Physcomitrella patens* and homologous recombination

The genome of *Physcomitrella* consists of 27 chromosomes with a total size of 480 Mb, compared with five chromosomes and 125 Mb in *Arabidopsis* (Rensing et al., 2008; "The *Arabidopsis* Genome Initiative," 2000). Evolutionary studies revealed that whole genome duplication (WGD) events occurred in both *Physcomitrella* and *Arabidopsis*. In *Physcomitrella*, two WGD events occurred 27 to 35 and 40 to 48 mya (Lang et al., 2018). Before the first WGD, *Physcomitrella* had seven chromosomes. During the first WGD, the original seven-chromosomes duplicated to yield 14 and subsequently one chromosome was lost and a chromosome fusion occurred to generate a

genome with 12 chromosomes. A second WGD event happened, followed by five chromosome breaks and two chromosome fusions, which resulted in the 27 chromosome genome of modern moss (Lang et al., 2018). In contrast, more than two WGD occurred in *Arabidopsis*, including gene losses and duplications (Ku et al., 2000; "The *Arabidopsis* Genome Initiative," 2000).

Although several genomic events modulate the evolution of *Physcomitrella*, about 30% of its non-redundant transcripts have homologs in *Arabidopsis* and rice, and only 130 transcripts do not have homologs in seed plants. In addition, studies have shown that *Physcomitrella* shares at least 66% of its genome with the model plant *Arabidopsis* (Nishiyama et al., 2003; Prigge and Bezanilla, 2010). In 1991, a protocol using gene targeting homologous recombination was described in *Physcomitrella* (Schaefer et al., 1991). In the homologous recombination process, nucleotides sequences are exchanged between homologous regions enabling the disruption or removal of specific gene targets. This approach led to the use of *Physcomitrella* as a model organism to study gene function and characterization

6.1.3. Morphology and life cycle of *Physcomitrella patens*

Similar to any Bryophytes, *Physcomitrella* displays alternation of generations, indicating they possess both diploid and haploid stages. The dominant stage of moss' life cycle is the haploid stage (gametophore), which is composed of rhizoids, leaf-like structures, and a stem-like structure (Figure 5). Upon release from the capsule, the haploid spores are dispersed and germinate into filamentous protonema with apical growth (Figure 5). Subsequently, the protonema will grow into two types of

gametophores, male and female, bearing the sexual organs antheridia and archegonia, respectively. In moist conditions, the sperm fertilizes the egg leading to the formation of diploid cells that will originate the sporophyte, which consist of seta and capsule. Inside the capsule of the sporophyte, the spores are formed through meiosis initiating the cycle again.

6.1.4. *Physcomitrella patens* response to MAMPs and pathogen

There have been a few discoveries made in regards to the *Physcomitrella* ability to activate immunity. For instance, homologs of LRR-RLK CERK1 and MAPKs were identified as components of chitin induced immunity in *Physcomitrella* (Bressendorff et al., 2016). In addition, *Physcomitrella* was also reported to induce ROS burst and programmed cell death (PCD) in response to necrotrophic fungi including *Botrytis cinerea*, *Phytium*, and bacteria *Erwinia carotovora* (Oliver et al., 2009; Ponce De León et al., 2012; Ponce de León et al., 2007).

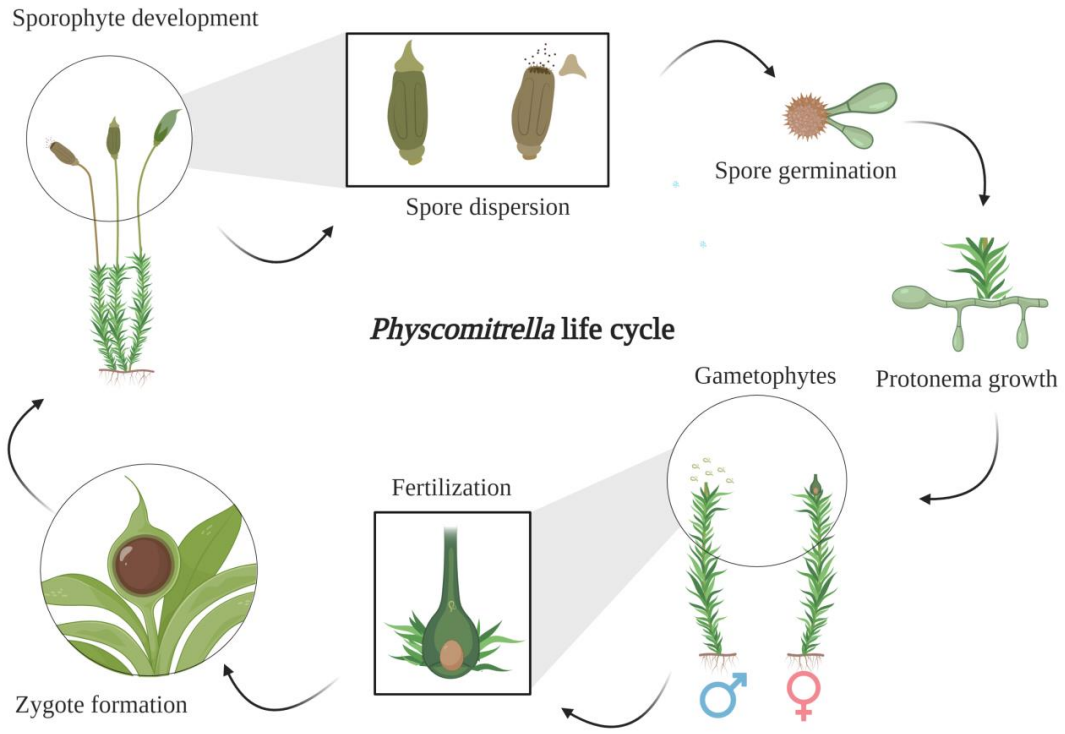


Figure 5. *Physcomitrella patens* life cycle. Created with Biorender.

6.2. Materials and methods

6.2.1. Plant material and growth conditions

Arabidopsis WT (Col-0), *bak1-4-/+serk4-/-*, *serk1-1-/-serk2-2-/+*, *bak1-4*, *serk1-1/bak1-4*, *bak1-5/serk4*, and transgenic plants were grown in pots containing a soil mix (Metro Mix 366, Sunshine LP5 or Sunshine LC1, Jolly Gardener C/20 or C/GP). The pots were placed in a growth room at 23°C, 65% relative humidity and 75 µE•m-2•s-1 light with a 12-hour photoperiod for approximately 4 weeks before protoplast isolation, cell death assay, ROS production, and *Agrobacterium* transformation. WT *P. patens* cv Gransden (Ashton and Cove, 1977) and knockout lines *ppserk1.1* and *ppserk2* were routinely grown on BCD medium plates (Roberts et al., 2011) overlaid with cellophane discs (AA Packaging). The plates were then placed in continuous lighting at 25ºC

following standard protocols (Roberts et al., 2011). After approximately 10 days, the tissue on the plates were used for elicitor treatment and re-cultivated in BCD plates containing 5 mM diammonium tartrate for transformation purposes.

6.2.2. Plasmid constructs and generation of transgenic plants

Arabidopsis 35S::BAK1, 35S::BIK1, 35S::ASR3, 35S::BRI1, and 35S::FLS2 constructs tagged with HA (hemagglutinin) tag antibodies were reported previously (Li et al., 2015; Lu et al., 2010; Shan et al., 2008). The PpSERK1.1 and PpSERK1.2 full length genes were amplified by PCR from *Physcomitrella* cDNA and introduced into a plant expression vector with or without an HA and a FLAG epitope tags at the C-terminus. Point mutation were generated by site-directed mutagenesis using Pfu DNA polymerase and 35S::PpSERK2-HA/FLAG constructs as templates. The kinase domain of BAK1 in the pMAL-c2 (New England Biolabs) and full-length BIK1 in the modified GST fusion protein expression vector pGEX4T-1 (Pharmacia) were reported previously (D. Lu et al., 2010). The kinase domain of SERK1 and SERK2 and the kinase domain of PpSERK1.1, PpSERK1.2 and PpSERK2 were amplified by PCR from *Arabidopsis* and *Physcomitrella* cDNA, respectively, and introduced into the pMAL-c2 (New England Biolabs) fusion protein expression vector. To generate *Physcomitrella* knockouts a region of approximately 1Kb upstream and downstream of each PpSERKs were amplified by PCR from *Physcomitrella* genomic DNA, and introduced into pBHRFΔLinkerG1 vector (Johansen et al., 2016), flanking the hygromycin resistance cassette. *Agrobacterium*-mediated transformation was used to introduce individual

pCB302-35S::PpSERK1.1-HA and pCB302-35S::PpSERK1.2-HA into *bak1-4-/+serk4-/-* and *serk1-1/bak1-4*. To eliminate the C-terminal tag potential effect on SERKs function, individual non-tagged pCB302-35S::PpSERK1.1 and pCB302-35S::PpSERK1.2 were introduced into *bak1-4* and *bak1-5/serk4* via *Agrobacterium* transformation. The transgenic plants were selected with BASTA and kanamycin resistance and immunoblot using α-HA (Roche) antibodies.

6.2.3. PEG-mediated protoplast transformation of *Physcomitrella*

Protoplasts isolation and PEG-mediated transformation were conducted as described by Liu and Vidalli, 2011. The transformed protoplasts were plated in PRMB medium plates covered with cellophane. After one week, the cellophane layer with the protoplasts was transferred to fresh PRMB plate containing hygromycin as the selection agent. Another week later, the cellophane with the protoplasts were transferred again to fresh PRMB plate without the selection agent. This process was repeated twice more. Subsequently the surviving plants were submitted to genotyping to confirm the transformation.

6.2.4. Protoplast transient assay

Protoplasts isolation and a transient expression assay were conducted as described by Lu et al., 2010 and Shan et al., 2008. In general, 100 µl of protoplasts at the density of 2 x 105 /ml and 20 µg DNA were used for transfection. The flagellin peptide flg22 (Felix et al., 1999) was used in a final concentration of 100 µM unless stated

otherwise. While the hormone brassinolide (BL) was used in a final concentration of 2 μM unless stated otherwise. Protoplasts transfected with empty vector were used as a control.

6.2.5. Protein Extraction, Western Blot, and Co-immunoprecipitation Assay

To detect BRASSINOSTEROID INSENSITIVE 1 (BRI1)-EMS-SUPPRESSOR 1 (BES1) proteins, 10-days-old seedlings were grown on 1/2 MS agar plate and subsequently transferred to six-well culture plates containing 1 mL of H2O per well (10 seedlings per well). The plants were left overnight on water. After incubation, the seedlings were submerged for 3h in 1 mL of 2 μM brassinolide (BL) and ground to fine powder in liquid N2. The total proteins were extracted with 2× sample buffer and a Western blot was performed using the α-bri1-Ems-Suppressor (BES1) antibody (from Y. Yin) to detect the phosphorylation of BES1. In the co-immunoprecipitation (Co-IP) assay, transfected protoplasts (2×10^5) were lysed with 0.5 mL of extraction buffer [10 mM Hepes at pH 7.5, 100 mM NaCl, 1 mM EDTA, 10% (vol/vol) glycerol, 0.5% Triton X-100, and protease inhibitor mixture from Roche]. After transfection, the samples were vortexed vigorously for about 30 s. Subsequently, the samples were centrifuged at 12,470 × g for 10 min at 4 °C. The supernatant was collected and incubated with α-HA or α-FLAG antibody for 2 h, following the incubation with protein G-agarose beads (Roche) for another 2 h at 4 °C. All the incubation periods were performed with gentle shaking. After incubation, the beads washed three times with washing buffer [10 mM

Hepes at pH 7.5, 100 mM NaCl, 1 mM EDTA, 10% glycerol, and 0.1% Triton X-100] and once with 50 mM Tris·HCl at pH 7.5. The immune-precipitated proteins were submitted to Western blot with an α-HA or α-FLAG antibody. For Co-IP using soil grown plants. approximate 15 g of leaf samples from 4-week-old *Arabidopsis* were ground in liquid N2 and subsequently ground in 10 mL of ice-cold extraction buffer 1 [20 mM Tris·HCl at pH 8.5, 150 mM NaCl, 1 mM EDTA, 20% glycerol, 1 mM PMSF, 20 mM NaF, 50 nM microcystin, and protease inhibitor mixture]. Samples were centrifuged at 7,000 × g for 15 min at 4 ℃. The supernatants were further centrifuged for 2h at 100,000 × g and 4 ℃ to precipitate the total membrane fraction. In addition, the pellet was resuspended in 1 mL buffer 2 [10 mM Tris·HCl at pH 7.5, 150 mM NaCl, 1 mM EDTA, 10% glycerol, 1% Triton X-100, 1 mM PMSF, 20 mM NaF, and protease inhibitor mixture]. The samples were used to perform Co-IP assay with the same procedures as protoplast Co-IP assay.

6.2.6. BIK1 and ASR3 phosphorylation assays

In the BIK1 phosphorylation assay, FLAG epitope-tagged BIK1 was co-transfected with individual pHBT-BAK1-HA, pHBT-PpSERK1.1-HA, pHBT-PpSERK1.2-HA or non-tagged pHBT-BAK1, pHBT-PpSERK1.1, pHBT-PpSERK1.2 into *Arabidopsis* WT and *bak1-4* protoplasts. Transfected protoplasts were incubated at room temperature overnight, and then treated with 100 nM flg22 or water for 10minutes. Protoplasts were harvested, and the total protein was separated by 10% SDS–PAGE gel followed by an α-HA immunoblot. In the ASR3 phosphorylation assay, similar

experiments were performed as in BIK1 phosphorylation assay, except that protoplasts were co-transfected, FLAG epitope-tagged ASR3.

6.2.7. In vitro kinase assay

The GST and MBP fusion proteins were purified using Pierce glutathione agarose (Thermo Scientific), and amylose resin (New England Biolabs) respectively, following the standard protocols from the company. The concentration of the proteins was measured using the BioRad Quick Start Bradford Dye Reagent and confirmed by the nanodrop ND-1000 Spectrophotometer. In the in vitro kinase assay, reactions were prepared with 30 μL kinase buffer [20 mM Tris•HCl, pH 7.5, 10 mM MgCl2, 5 mM EGTA, 100 mM NaCl, and 1 mM DTT] containing 0.1 mMcold ATP and 5 μCi [32P]-γ-ATP and 10 μg fusion proteins for 3 h and with gentle shaking. The reaction was stopped by using the 4XSDS loading buffer. The phosphorylation of fusion proteins were analyzed by autoradiography after separation with 10% SDS/PAGE.

6.2.8. MAPK assay

Arabidopsis ten-day-old seedlings (*WT, bak1-5/serk4*) were grown on ½ MS medium, and *Physcomitrella* ten-day-old protonemata tissues (WT, *ppserk1.1* and *ppserk2*) were grown on BCD medium supplemented with 5 mM diammonium tartrate. *Arabidopsis* seedlings were transferred to water and incubated overnight. Both *Arabidopsis* and *Physcomitrella* tissue were treated with 100 nM flg22 or 100nM chitin at time points of 0, 5, or 15 minutes and frozen in liquid nitrogen. The samples were

homogenized in the extraction buffer (20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1 mM EDTA, 10% glycerol, and 1% Triton X-100), and supernatant was collected after centrifugation at max speed for 10 min at 4C. Protein samples with 1x SDS buffer were denaturated at 95C for 10 min and subsequently separated in 10% SDS-PAGE gel to detect pMPK3 and pMPK6 by immunoblotting with a-pERK1/2 antibody (Cell Signaling, #9101). Protoplasts of WT and *bak1-4 Arabidopsis* were also used to detect MAPK activity. In this process the protoplasts were transfected with individual HA-tagged and non-tagged pHBT-BAK1, pHBT-PpSERK1.1, pHBT-PpSERK1.2, and pHBT-PpSERK2. Transfected protoplasts were incubated at room temperature for overnight and then treated with 100 nM flg22 or water for 10, 15 and 20 minutes. Protoplasts were harvested and suspended in lysis buffer. The subsequently steps were same for MAPK activity detection assay used for seedlings.

6.2.9. ROS Assay

Approximately 25 leaves of 5-week-old soil-grown *bak1-5/serk4* were excised into leaf discs (5-mm diameter), followed by an overnight incubation with water in 96-well plates to remove the wounding effect. ROS burst was determined by a luminol based assay. Leaf discs were soaked with solution containing 50 mM luminol and 10 mg/mL horseradish peroxidase (Sigma-Aldrich) supplemented with 100 nM flg22 or water. The measurement was performed immediately after adding the solution with a Multilabel Plate Reader (Perkin-Elmer; Victor X3) for a period of 30 min. The values for ROS production from each line were indicated as means of relative light units.

6.2.10. Genotyping of *Arabidopsis* and *Physcomitrella*

In order to determine the genotyping of *bak1-4-/+serk4-/-* and *serk1-1-/-serk2-2-/+* transgenic plants, PCR using the Left Border (LB) primer of the T-DNA and gene-specific (Left Primer) LP and (Right Primer) RP were performed. The primers were designed by T-DNA Primer Design (http://signal.salk.edu/tdnaprimers.2.html) and the PCR reactions were carried out according to its protocol. The sequence of LB is originated from the T-DNA fragment while the LP and RP are BAK1 and SERK2 gene-specific primers. The product of the amplification of LB plus RP indicates the insertion of T-DNA fragment, and subsequently if it is the only amplification this indicates the plant is homozygous for the mutation. The product of the amplification by using LP plus RP indicates the existence of BAK1 or SERK2, which means that a copy of the WT gene is still present. The presence of both PCR products indicates the phenotype is heterozygous. The genotyping to confirm PpSERKs knockouts was performed by PCR using a primer located in the C-terminal region of hygromycin (forward) and another primer (reverse) flanking the 1kb region downstream PpSERKs. The product of this amplification indicates that the PpSERKs were successfully substituted by the selection marker. Another PCR using a gene-specific primer (forward) and the same reverse primer was performed. The product of this amplification indicates that the PpSERKs are still intact.

6.2.11. Accession Numbers

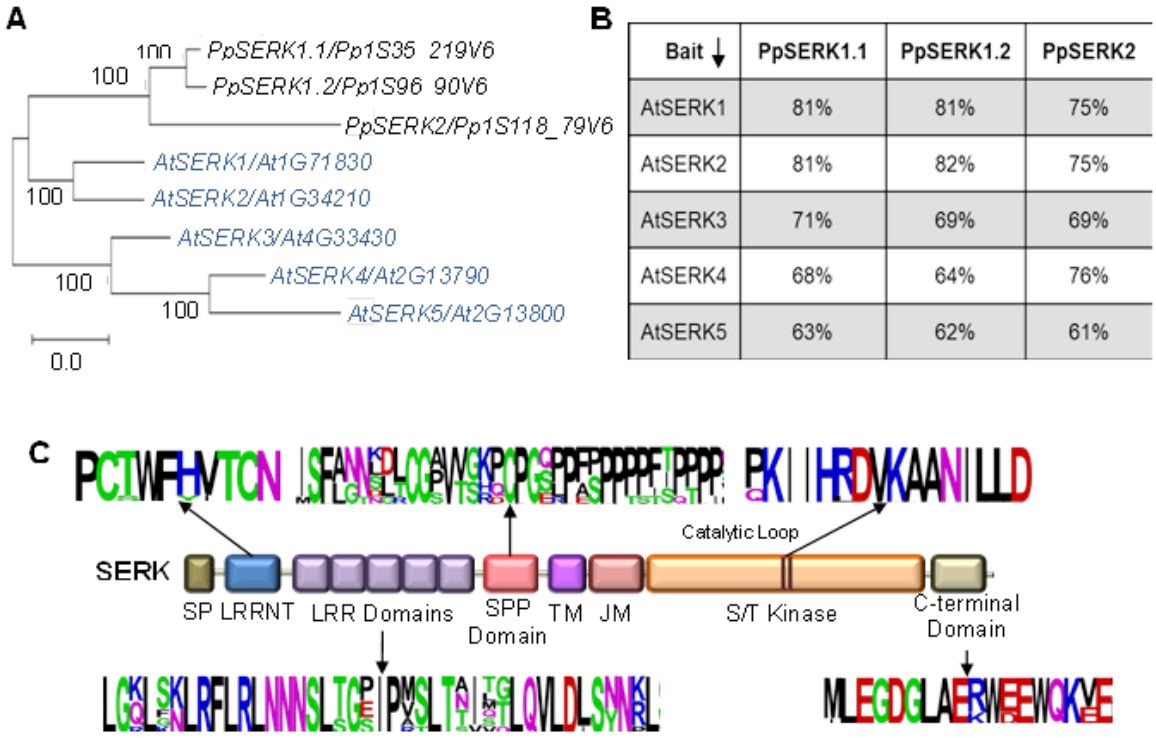
Sequence data from this article can be found in the Phytozome (https://phytozome.jgi.doe.gov/pz/portal.html) and The *Arabidopsis* Information Resource (https://www.*Arabidopsis*.org/) databases under the following accession numbers: ASR3 (AT2G33550), FLS2 (AT5G46330), BIK1 (AT2G39660), BRI1 (AT4G39400), BAK1 (AT4G33430), SERK1 (AT1G71830), SERK2 (AT1G34210), SERK4 (AT2G13790), SERK5 (AT2G13800), PpSERK1.1 (Pp1s35_219V6.1), PpSERK1.2 (Pp1s96_90V6.1), and PpSERK2 (Pp1s118_79V6.1).

6.3. Results

6.3.1. In silico identification and phylogenetic analysis of SERK family proteins in *Physcomitrella*

SOMATIC EMBRYOGENESIS RECEPTOR KINASES (SERKs) are receptor-like kinases (RLKs) that possess an amino (N)-terminal signal peptide (SP), an extracellular domain consisting of a leucine zipper domain (LZ), five leucine-rich repeats (LRRs), and a serine-proline rich (SPP) domain, a single-pass transmembrane domain (TM), and an intracellular domain consisting of juxtamembrane domain (JM), a kinase domain, and a C-terminal domain. In *Arabidopsis*, the SERK family is composed of five close homologs (SERK1 to SERK5). To identify the SERK genes in *Physcomitrella*, the full-length amino acid sequence of *Arabidopsis* SERKs were used as queries for BLASTp analysis against *Physcomitrella* in both NCBI and Phytozome databases (respectively, https://blast.ncbi.nlm.nih.gov/Blast.cgi and https://phytozome.jgi.doe.gov/pz/portal.html). Using 60% identity and 600 score points

as cutoffs, three putative SERK homologs from *Physcomitrella* (PpSERKs), including Pp1s35_219V6, Pp1s96_90V6, and Pp1s118_79V6, were retrieved. Phylogenetic tree using Muscle Alignment and Neighbor-Joining analysis revealed that PpSERKs lie in closer to the clade with SERK1 and SERK2 than the BAK1 (SERK3), BKK1 (SERK4) and SERK5 clades (Figure 6A). Pp1s35_219V6 and Pp1s96_90V6 bear considerable high homologies to each other (97% identities and 98% similarities) and carry 81% and 82% identity with SERK1 and SERK2, respectively. Meanwhile, Pp1s118_79V6 shares 75% identity with SERK1 and SERK2 (Figure 6b) and about 83% identity with Pp1s35_219V6 and Pp1s96_90V6. As a result of the similarity of PpSERKs with SERK1 and SERK2, Pp1s35_219V6 was named as PpSERK1.1, Pp1s96_90V6 as PpSERK1.2 and Pp1s118_79V6 as PpSERK2. SERKs belong to Class II family of LRR-RLKs with 14 members in *Arabidopsis*. Using these additional members of Class II LRR-RLKs as baits for BLASTp analysis, 11 genes with identities above 35% were retrieved, including the 3 putative PpSERKs. Phylogenetic tree analysis using either Neighbor-Joining (NJ) or Maximum likelihood (ML) methods including all the class II LRR-RLKs from *Arabidopsis* and *Physcomitrella* showed that PpSERK1.1, PpSERK1.2, and PpSERK2 are clustered together close to the clade of SERK1 and SERK2, further distant away from the other LRR-RLKs, suggesting that PpSERK1.1, PpSERK1.2, and PpSERK2 are *Arabidopsis* SERKs orthologs (Supplemental figure 1a and b). Moreover, PpSERKs possess all conserved domains present in *Arabidopsis* SERKs (Figure 6c).



Bait ↓	PpSERK1.1	PpSERK1.2	PpSERK2
AtSERK1	81%	81%	75%
AtSERK2	81%	82%	75%
AtSERK3	71%	69%	69%
AtSERK4	68%	64%	76%
AtSERK5	63%	62%	61%

Figure 6. Identification of SERKs in *Physcomitrella patens*. (A) Neighbor-Joining (NJ) tree based on the alignment of SERKs and PpSERKs amino acid sequence. The optimal tree with the sum of branch length = 0.85083387 is shown. Bootstrap values from 1000 replicates are shown next to the branches and indicate the percentage of replicate trees in which the associated genes clustered together. The Dayhoff matrix-based method was used to compute the evolutionary distances. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 641 positions in the final dataset. Evolutionary analyses were conducted in MEGA X. PpSERK1.1, PpSERK1.2 and PpSERK2 are grouped together due to the high similarity (around 80%) between the proteins. PpSERKs are also clustered together with SERK1 and SERK2 because they share at least 75% identity. In another way, BAK1, SERK4, and SERK5 are grouped together and share around 70% identity with PpSERKs. (B) Protein sequence comparison between PpSERKs and SERKs. The table shows the percentage of identity using SERKs as bait. (C) Domain architecture of SERK orthologs. SERK gene family possess an N-terminal signal sequence (SP), an extracellular domain that possesses a leucine zipper (LZ), five leucine-rich repeats (LRRs), and a serine-proline rich domain (SPP), a single-pass transmembrane domain (TM) and an intracellular domain that possesses a juxtamembrane domain (JM), a kinase domain, and a C-terminal domain. Those regions are highly conserved among different plant species. The sequence logo, from https://weblogo.berkeley.edu/logo.cgi, is a graphical representation of SERKs regions where the amino acids are more conserved among each other. The height of the amino acid indicates the sequence conservation of each position.

To gain insight into the evolution of three PpSERKs, neighboring genes upstream (~100 Kb) and downstream (~ 100 Kb) of PpSERKs were retrieved and compared for their similarities (Supplemental figure 2b). Interestingly, genes upstream and

downstream of PpSERK1.2 show high degree of similarities with those upstream and downstream of both PpSERK1.1 and PpSERK2 (Supplemental figure 2a). The similarities range from 60% to 95%. However, the similarities among the genes upstream and downstream of PpSERK1.1 and PpSERK2 are relatively low and not significant (Supplemental figure 2a). This indicates that PpSERK1.2 may be the ancestral gene of PpSERK1.1 and PpSERK2. This notion is further supported by the evolution of the *Physcomitrella* genome (Figure 7). In the WGD1, chromosome 3 was duplicated and originated into chromosomes 12 and 26. In the WGD2, chromosome 3 was duplicated again, one copy remained intact (chromosome 3) and the other copy was broken down into chromosomes 8, 10 and 13. Meanwhile, chromosome 12/26 was also duplicated in the WGD2, generating chromosome 4. After WGD2, the chromosome 12/26 was broken down to chromosome 12 and chromosome 26 (Figure 7). PpSERK1.2 is localized in chromosome 3, the ancestral chromosome remained after WGD1 and WGD2, supporting that PpSERK1.2 is the most ancient PpSERKs. Compared to PpSERK1.1, PpSERK2 may diverge earlier from PpSERK1.2, as it shows a lower degree of similarities with PpERK1.2 than PpSERK1.1. Taken together, it appears that PpSERK1.2 may be the ancestral PpSERK, PpSERK2 diverge out first and followed by PpSERK1.1.

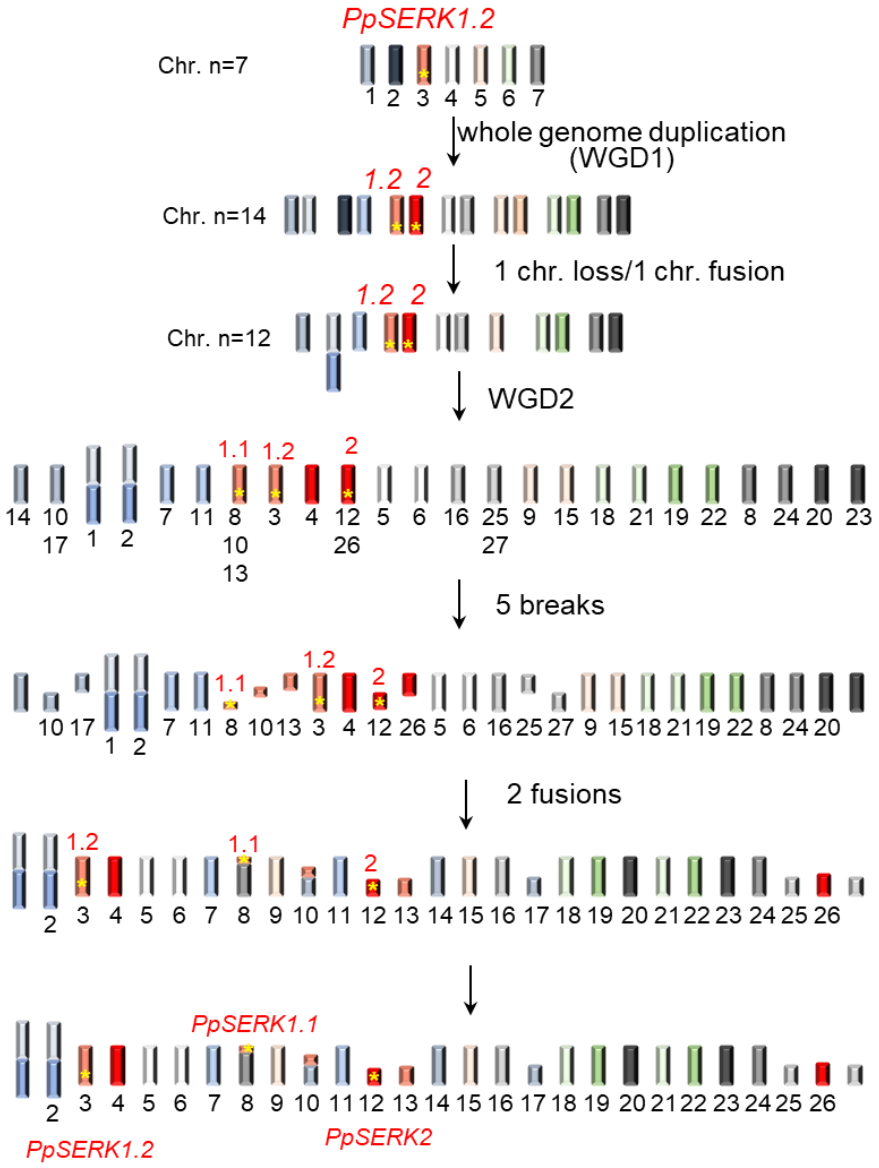


Figure 7. Evolution of SERKs in *Physcomitrella patens*. Evolution of P. *patens* genome (Adapted from Lang D et al, The P. *patens* chromosome-scale assembly reveals moss genome structure and evolution. Plant J 2017). Originally with 7 chromosomes, the P. *patens* genome has evolved to 27 chromosomes after two whole-genome duplications (WGD) over 50 million years ago (mya). In the WGD1, chromosome 3 was duplicated and originated into chromosomes 12 and 26. In the WGD2, chromosome 3 was duplicated again, one copy remained intact (chromosome 3) and the other copy was broken down into chromosomes 8, 10 and 13. PpSERK1.2 is localized in chromosome 3, the ancestral chromosome remained after WGD1 and WGD2, supporting that PpSERK1.2 is the most ancient PpSERKs. Compared to PpSERK1.1, PpSERK2, in chromosome 12, may diverge earlier from PpSERK1.2, as it shows a lower degree of similarities with PpERK1.2 than PpSERK1.1, on chromosome 8. The yellow star indicates the position of the putative copies of PpSERKs during the two WGD. The black arrows indicate the events occurred during the WGD.

6.3.2. Kinase activity of PpSERKs

SERK1, SERK2, and BAK1 bear kinase activities, which are critical for their functions (Hohmann et al., 2018). To examine whether PpSERKs also are active kinases, an in vitro kinase assay was performed using their cytosolic domains (CD) fused to maltose-binding protein (MBP) at the N-terminus. Similar to BAK1, MBP-PpSERK1.1CD and MBP-PpSERK1.2CD showed a relatively strong autophosphorylation activity, but MBP-PpSERK2CD exhibited a relatively weak autophosphorylation activity (Figure 8a). BAK1 confers serine (Ser) /threonine (Thr) /tyrosine (Tyr) kinase activities (Oh et al., 2009). Using anti-Thr antibodies which recognize phosphorylated Thr residues, we detected kinase activities in BAK1 as well as in PpSERK1.1 and PpSERK1.2 (Figure 8b). Immuno-blots using anti-Tyr antibodies revealed that BAK1 possesses strong Tyr phosphorylation activities and none of PpSERKs exhibit Tyr phosphorylation activities (Figure 8b). Consistent with P^{32}-based in vitro kinase assays (figure 8a), it appears that the kinase activities of PpSERK1.1 and PpSERK1.2 are weaker than BAK1.

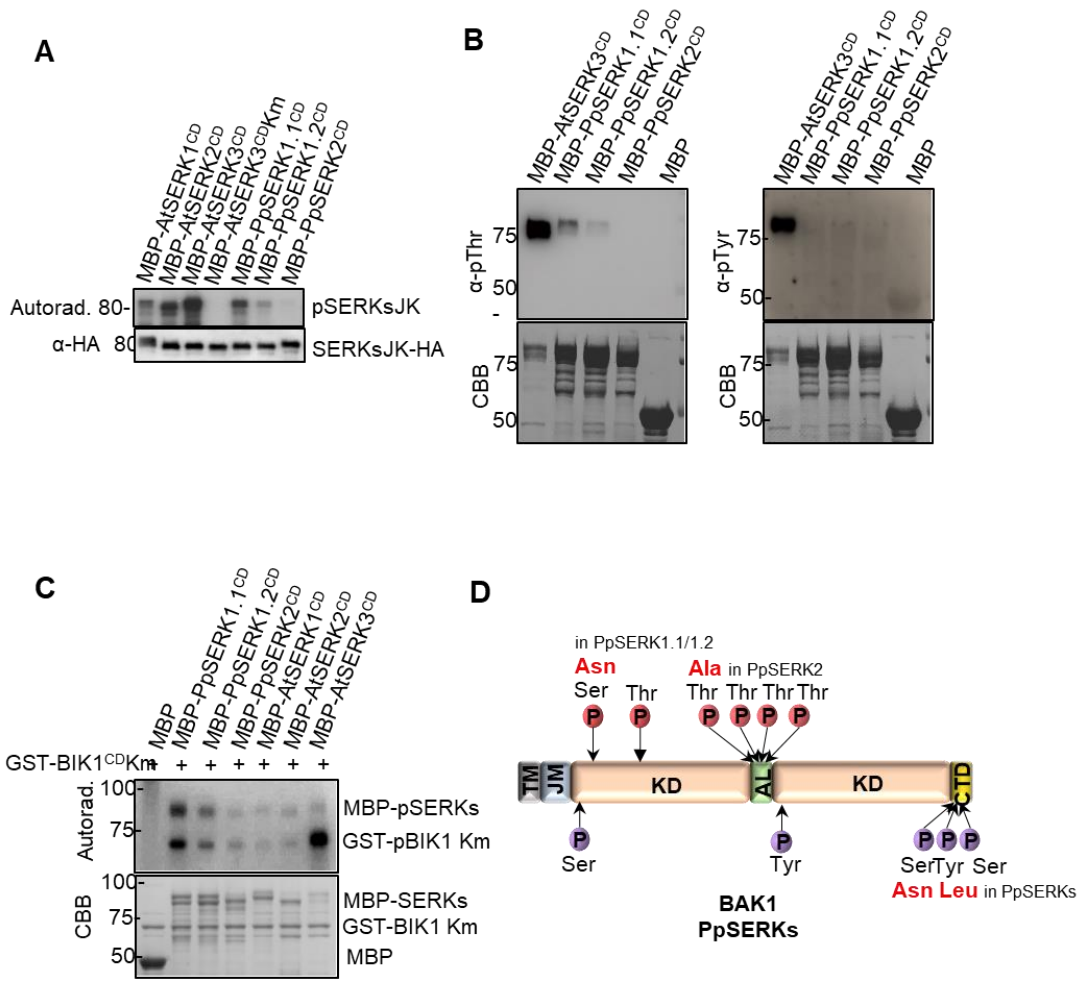


Figure 8. PpSERKs possess kinase activity. (A) The cytosolic domains (CDs) of PpSERK1.1 and PpSERK1.2, but not PpSERK2, possess autophosphorylation activities in vitro. The CD domains of SERKs, including the juxtamembrane domain and kinase domain, were fused to maltose-binding protein (MBP) and were subjected for an in vitro kinase assay. Proteins were separated by 10% SDS/PAGE and analyzed by autoradiography (upper panel). The protein loading control was shown by Coomassie Blue staining (CBS) (Lower panel). (B) Phosphorylation of PpSERKs is absent on tyrosine (Tyr) after an in vitro kinase using phosphotyrosine antibody with radiolabeled ATP. Phosphorylation of PpSERK2 is absent on threonine (Thr) after an in vitro kinase using phosphothreonine antibody with radiolabeled ATP, while PpSERK1.1 and PpSERK1.2 possess some phosphorylation on thr but not as strong as in SERK3. (C) SERK3, but not SERK1 nor SERK2, phosphorylates BIK1 and PpSERK1.1 and PpSERK1.2, but not PpSERK2, phosphorylate BIK1 in vitro. An in vitro kinase assay was performed by incubating MBP, MBP-PpSERK1.1CD, MBP-PpSERK1.2CD, MBP-PpSERK2CD, MPB-AtSERK1CD, MBP-AtSERK2CD, and MBP-AtSERK3CD with GST-BIK1CDKm. Proteins were separated with SDS/PAGE and analyzed by autoradiography (Upper). The protein loading control was shown by Coomassie blue staining (Lower). (D) PpSERK2 have polymorphisms in important phosphorylation sites in the activation loop of the kinase domain (Ala, highlighted in red). Scheme comparing the phosphorylation sites of PpSERK1.1/1.2 and SERK1/2/3 with PpSERK2. Phosphorylation sites in red indicate transphosphorylation sites in SERK3 and in purple indicate autophosphorylation sites in SERK3.

It is known that BAK1 phosphorylates BIK1, a receptor-like cytoplasmic kinase in *Arabidopsis* (Lu et al., 2010). To examine whether PpSERKs are able to phosphorylate BIK1, we performed an in vitro kinase assay using the kinase mutant variant of BIK1 (BIK1km, to avoid auto phosphorylation) fused to Glutathione S-transferase (GST-BIK1km) as substrates. MBP-BAK1CD strongly phosphorylates GST BIK1, while MBP-SERK1CD and MBP-SERK2CD display weak phosphorylation on GST-BIK1. Despite being phylogenetically closer to SERK1/2, MBP-PpSERK1.1CD and MBP-PpSERK1.2CD transphosphorylate GST-BIK1Km similar to BAK1 (Figure 8c). MBP-PpSERK2CD only showed a slight transphosphorylation of GST-BIK1Km (Figure 8c). The data indicate that PpSERK1.1 and PpSERK1.2 resembles BAK1 phosphorylating BIK1. Notably, in the activation loop of the kinase domain of PpSERK2, one of the conserved threonine residues is substituted by alanine (Ala) (Figure 3E). This may contribute to a reduced kinase activity of PpSERK2. In addition, the conserved Ser and Tyr residues in the CTD domain of SERK3 are substituted by Asn and Glu in PpSERK1.1 and PpSERK1.2, which may attribute to their relative weak kinase activities.

6.3.3. PpSERK1.1 and PpSERK1.2 play a role in MAMP-triggered immunity

In *Arabidopsis*, BAK1 together with BKK1 play a positive role in MAMP-triggered immunity via complexing with PRRs as co-receptors. For instance, BAK1 and BKK1 interact with FLS2, which recognize bacterial flagellin or flg22, a 22-amino acid

synthetic peptide corresponding to a conserved core with full elicitor activity. Perception of flg22 induces rapid phosphorylation of BIK1 by BAK1. In the *bak1-4* mutant, flg22-induced BIK1 phosphorylation, which could be readily detected as a mobility shift on the Western Blot (WB), is compromised compared to WT plants (Figure 9a) (Lu et al., 2010). To investigate if PpSERKs play a role in MAMP-triggered immunity, *PpSERK1.1* and *PpSERK1.2* genes, under the control of the CAULIFLOWER MOSAIC VIRUS (CaMV) *35S* promoter with an HA tag at their carboxyl (C)-terminus (*35S::PpSERK1.1-HA* and *35S::PpSERK1.2-HA*) were expressed in *Arabidopsis bak1-4* protoplasts. It was reported that in the presence of C-terminal tags, BAK1 is not able to fully complement the immunity-related phenotypes of the *bak1-4* mutant (Ntoukakis et al., 2011). Thus, we also generated PpSERK1.1 and PpSERK1.2 without a tag under the control of the CaMV-35S promoter (*35S::PpSERK1.1* and *35S::PpSERK1.2*). As shown in Figure 9a, BAK1 with or without the HA-tag strongly restores flg22-induced BIK1 phosphorylation of the *bak1-4* mutant to the WT level. PpSERK1.1 and PpSERK1.2 without the HA tag but not those with the HA-tag are able to restore flg22-induced BIK1 phosphorylation of the *bak1-4* mutant. The data indicate that PpSERK1.1 and PpSERK1.2 confer a similar function to BAK1 in flg22-induced BIK1 phosphorylation.

The downstream of the FLS2-BAK1-BIK receptorsome complex consists of mitogen-activated protein kinase (MAPK) cascades. Flg22 perception activates phosphorylation of MAPKs, including MPK3, MPK4, and MPK6. The conserved TXY motif of MAPKs could be detected with anti-phospho-ERK antibodies. Similar to BAK1, PpSERK1.1 and PpSERK1.2 without the HA tag, but not those with the HA tag,

restore flg22-induced MAPK activations of the *bak1-4* mutant (Figure 9c). The data suggest that PpSERK1.1 and PpSERK1.2 play a role similar to BAK1 in mediating flg22-induced MAPK activations.

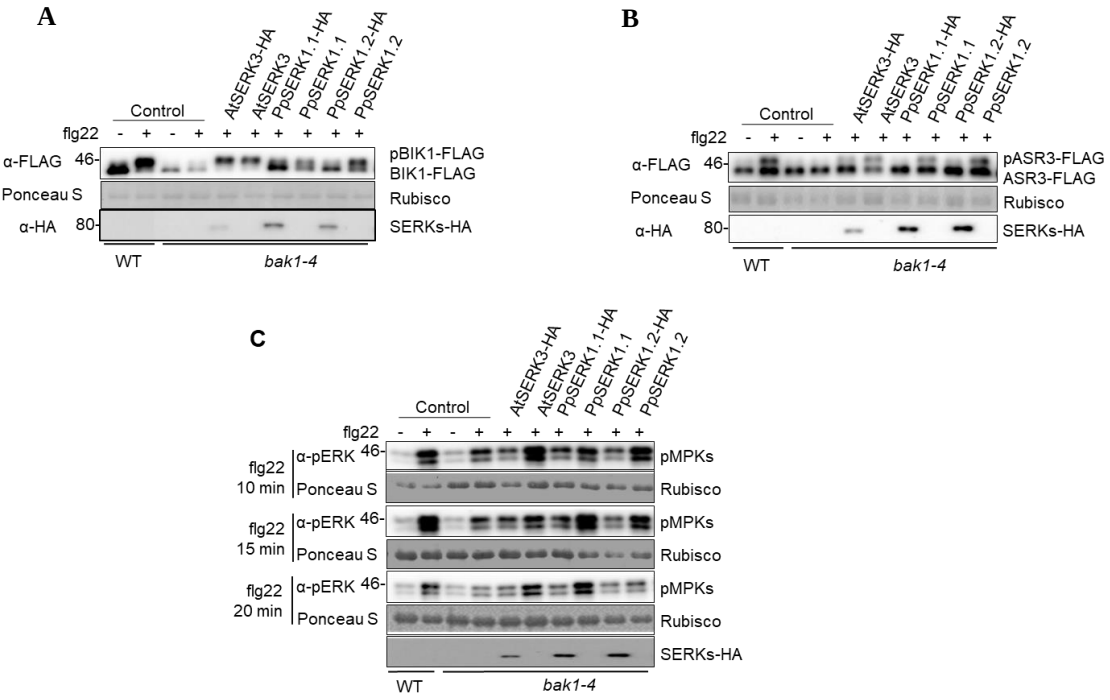


Figure 9. PpSERK1.1 and PpSERK1.2 confer functions in PAMP-triggered immunity in *Arabidopsis*. (A) Similar to SERK3, PpSERK1.1, and PpSERK1.2 were able to complement flg22-induced BIK1 phosphorylation in *Arabidopsis* bak1-4 protoplasts. Protoplasts from 4-week old plants of WT and bak1-4 were transfected with BIK1-FLAG co-expressing with the control vector, non-tagged or HA-tagged SERKs, and PpSERKs. Transfected protoplasts were treated with or without 100 nM flg22 for 15 min after six hr incubation. Flg22-induced BIK1 phosphorylation was detected as a band shift on a Western blot (WB) using an α-flag antibody (upper panel). Total protein loading was shown by Ponceau S staining for Rubisco (middle panel) and protein expressions of SERKs-HA and PpSERKs-HA were shown by WB using an anti-HA antibody (bottom panel). (B) Similar to SERK3, PpSERK1.1, and PpSERK1.2 were able to complement flg22-induced ASR3 phosphorylation in *Arabidopsis* bak1-4 protoplasts. Similar experiments were done as in (A) except that protoplasts were co-transfected with ASR3-FLAG. Flg22-induced ASR3 phosphorylation was detected as a band shift on a Western blot (WB) using an α-flag antibody (upper panel). (C) Similar to SERK3, PpSERK1.1, and PpSERK1.2 complement flg22-triggered MAPKs activation in *Arabidopsis* bak1-4 protoplasts. Protoplasts from 4-week old plants of WT and bak1-4 were transfected with the control vector, non-tagged or HA-tagged SERKs, and PpSERKs. Transfected protoplasts were treated with or without 100 nM flg22 for indicated time after six hr incubation. The proteins were analyzed through a Western blot with an α-pErk1/2 antibody. Within one set, the top panel shows flg22-triggered MAPK activation, the bottom panel shows the equal protein loading by Ponceau staining for Rubisco. The last panel shows the expression of both SERKs-HA and PpSERKs-HA.

It has been shown that upon flg22 perception, MPK4 phosphorylates *ARABIDOPSIS* SH4-RELATED 3 (ASR3), a transcriptional repressor to regulate immune gene expression (Li et al., 2015). Flg22-induced MPK4-mediated phosphorylation of ASR3 could be detected by a mobility shift by the WB (Figure 9b) (Li et al., 2015). In the *bak1-4* mutant protoplasts, flg22-induced ASR3 phosphorylation is abolished (Figure 9b). Similar to BAK1, without the HA tag, PpSERK1.1 and PpSERK1.2 restore the flg22-induced ASR3 phosphorylation of the *bak1-4* mutant (Figure 9b). The data suggest that PpSERK1.1 and PpSERK1.2 confer a function similar to BAK1 in flg22-mediated immune responses, including BIK1 phosphorylation, MAPK activations, and subsequent ASR3 phosphorylation.

In addition, a ROS burst is among the early immune response in plant, upon pathogen perception. Measurements of ROS in *bak1-5-/-serk4-/-* carrying *35S::PpSERK1.1* and *35S::PpSERK1.2*, revealed that PpSERKs were able to restore ROS bursts defect from *serk* mutants (Figure 10a). Besides MAPK restoration on *bak1-4* protoplasts, *bak1-4* and *bak1-5-/-serk4-/-* transgenic plants carrying PpSERK1.1 and PpSERK1.2 were also tested to confirm MAPK activation upon flg22 treatment. As expected, both PpSERKs without tags were able to restore MAPK activation on *serk* mutants (Figure 10 b and c).

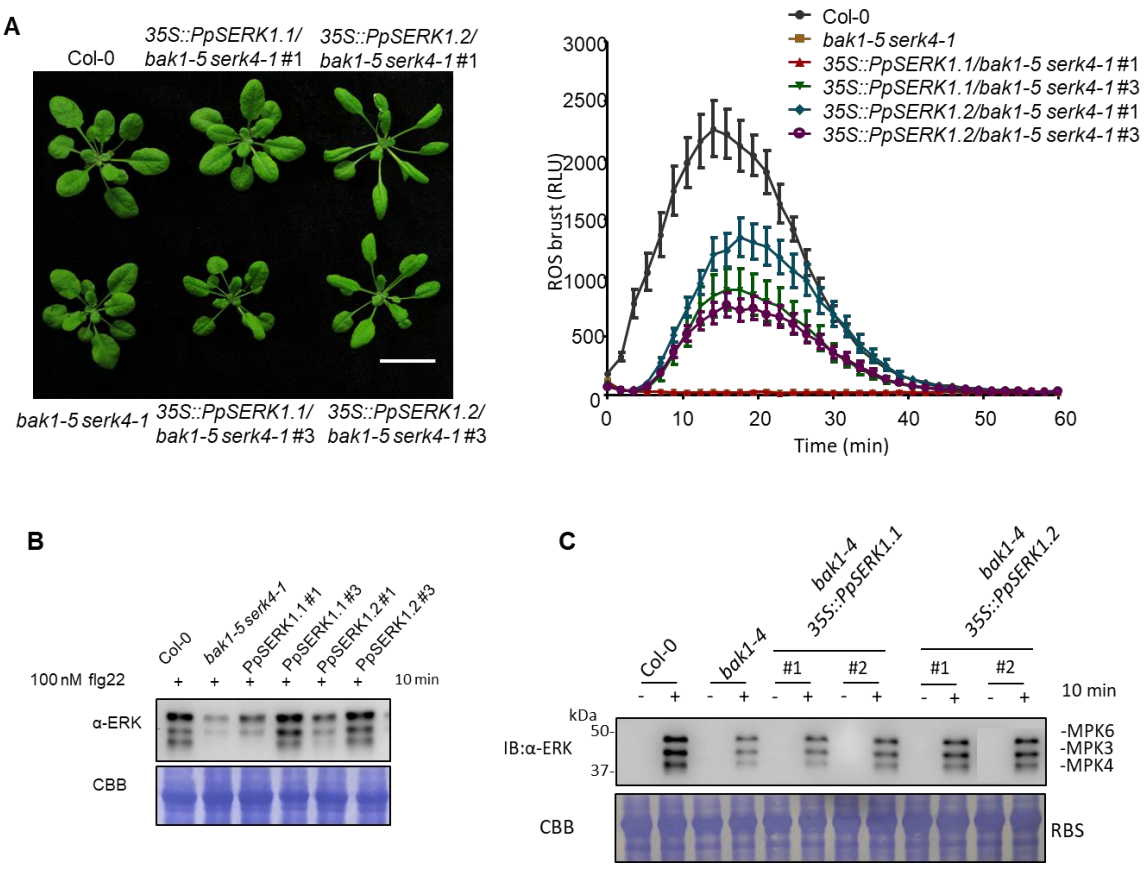


Figure 10. ROS bursts and MAPK activation in transgenic plants. (A) PpSERK1.1 and PpSERK1.2 were able to partially restore ROS production in *bak1-5-/serk4-/-* mutant platns (B) PpSERK1.1, and PpSERK1.2 complement flg22-triggered MAPKs activation in *Arabidopsis bak1-4* and *bak1-5-/serk4-/-* transgenic plants.

6.3.4. PpSERK1.1 and PpSERK1.2 play a complex role in brassinosteroid-mediated growth and development in *Arabidopsis*

In *Arabidopsis*, BAK1 together with SERK1 and BKK1 play a role in Brassinosteroid (BR)-mediated plant growth and development (Gou et al., 2012). Genetic analysis of different combinations of mutants indicates that BAK1 plays a primary role followed by SERK1 and then BKK1 in BR-mediated growth and

development. The *serk1-1bak1-4* mutant display BR-related growth defects such as shorter petioles and more compact and rounder leaves than wide-type (WT) Col-0 *Arabidopsis* plants (Figure 11a). In order to evaluate the contribution of PpSERKs for the BR-mediated growth and development, we transformed PpSERK1.1 and PpSERK1.2 genes with an HA tag at their carboxyl (C)-terminus under the control of the cauliflower mosaic virus (CaMV) 35S promoter (*35S::PpSERK1.1-HA* and *35S::PpSERK1.2-HA*) into *serk1-1bak1-4*. Multiple independent lines of transgenic plants with similar protein expression levels were obtained (Supplemental figure 3). Transgenic plants expressing PpSERK1.1 or PpSERK1.2 clearly restored the shortened petiole and compact plant architecture of *serk1-1bak1-4* mutant plants (Figure 11a). Surprisingly, transgenic plants expressing PpSERK1.1 or PpSERK1.2 displayed much longer petioles and less compact plant architecture when compared with WT plants (Figure 11a and b). These characteristics resemble those from overexpression of BRI1-GFP due to increased BR signaling (Figure 11a) (Nam and Li, 2002).

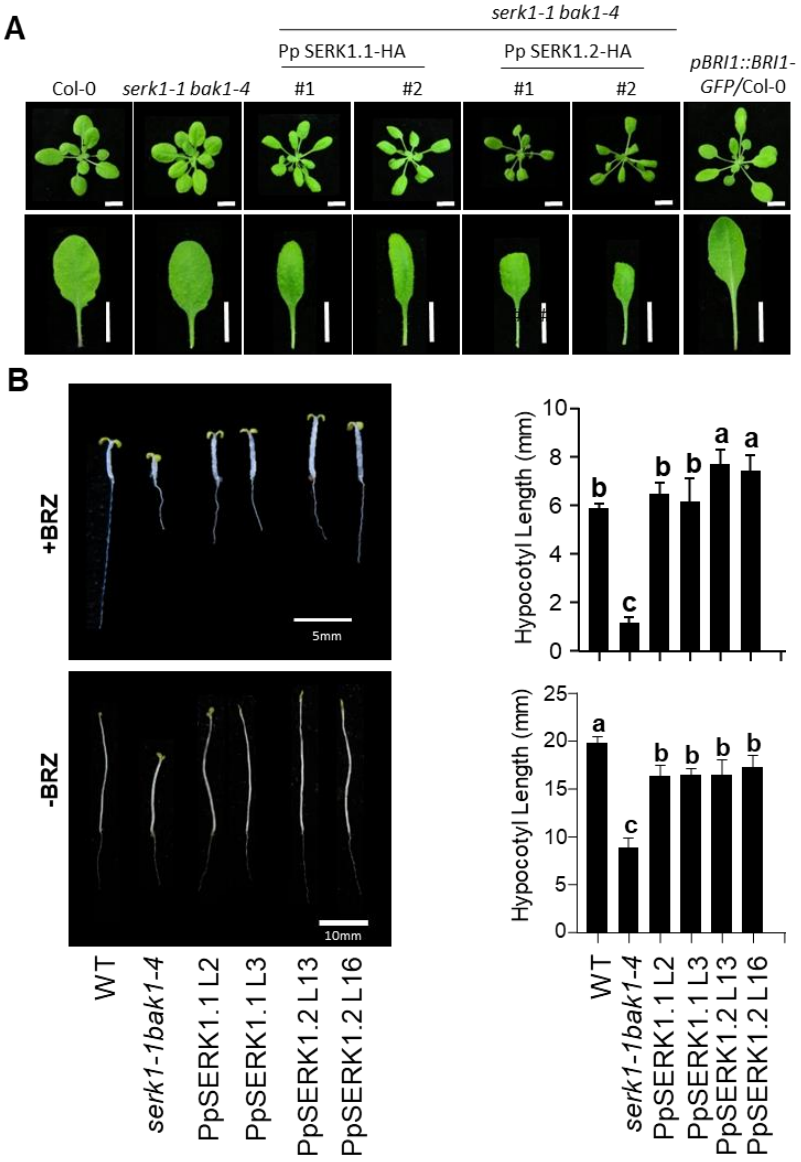


Figure 11. PpSERK1.1 and PpSERK1.2 play a role in brassinosteroid (BR)-mediated *Arabidopsis* growth and development. (A) Constitutive expression of PpSERK1.1 and PpSERK1.2 complement the growth defects of the serk1-1bak1-4 mutant and resemble the growth phenotypes of BRI1 overexpression transgenic plants. Compared to WT plants, serk1-1bak1-4 plants display shorter petioles and rounder leaves due to impaired BR signaling while BRI1-GFP transgenic plants exhibit elongated and curling petioles associated with elevated BR signaling. (B) Constitutive expression of PpSERK1.1 and PpSERK1.2 complement the hypocotyl elongation defects of the serk1-1bak1-4 mutant in the dark. The seedlings were grown on 1/2 MS plates in the dark for 8 days. Compared to WT, serk1-1bak1-4 plants display reduced hypocotyl elongation due to impaired BR signaling. Transgenic plants PpSERK1.1 or PpSERK1.2 restore the hypocotyl elongation defects of serk1-1bak1-4 with even longer hypocotyls than WT plants. In the presence of BRZ, serk1-1bak1-4 plants display reduced hypocotyl elongation under the dark due to impaired BR signaling. Transgenic plants PpSERK1.1 or PpSERK1.2 restore the hypocotyl elongation defects of serk1-1bak1-4 with even longer hypocotyls than WT plants. The data are shown as mean ± SE from at least 25 seedlings. Means with different letters are significantly different with $P < 0.05$.

BR stimulates hypocotyl elongation of *Arabidopsis* seedlings when grown in the dark (Yin et al., 2002). The *serk1-1bak1-4* mutant plants, which bear defects in the BR signaling, display shorter hypocotyls than WT plants (Figure 11b) (Gou et al., 2012). Transgenic plants expressing PpSERK1.1 or PpSERK1.2 restored the hypocotyl elongation defects of *serk1-1bak1-4* mutant plants (Figure 11b). Notably, transgenic plants expressing PpSERK1.1 or PpSERK1.2 displayed even longer hypocotyls when compared with WT plants, particularly for PpSERK1.2 plants (Figure 11b). Meanwhile, treatment of brassinazole (BRZ), an inhibitor of BR biosynthesis, inhibits hypocotyl elongation of *Arabidopsis* seedlings when grown in the dark, hence plants with a defect in the BR pathway become hypersensitive to BRZ and display further shortened hypocotyls (Figure 11b) (Yin et al., 2002,). Transgenic plants expressing PpSERK1.1 or PpSERK1.2 showed similar or even longer hypocotyls than WT plants, suggesting that PpSERK1.1 or PpSERK1.2 could restore or even reduce the hypersensitivity to BRZ of *serk1-1bak1-4* mutant plants (Figure 11b). Thus, the data reveal that PpSERK1.1 and PpSERK1.2 restore the growth defects of *serk1-1/bak1-4* and play a positive role in the BR signaling.

6.3.5. PpSERK1.2 is essential for *Physcomitrella* growth, development, and survival

To assess the role of PpSERKs in *Physcomitrella*, we generated knockout lines of PpSERK1.1, PpSERK1.2 or PpSERK2 via double homologous recombination, where two identical or similar region of nucleotide sequences are exchanged. In order to

achieve that, regions with a size of approximately 1 kilo-base pair (Kb) upstream (UP) and downstream (DS) of each PpSERKs were cloned into the pBHRF vector upstream and downstream of the hygromycin (Hyg) gene, encoding a selection marker, respectively (Figure 12a). The constructs were introduced into *Physcomitrella* by using a PEG-mediated protoplast transfection method (Liu and Vidali, 2011). The double homologous recombination in the UP and DS regions between the vector and the *Physcomitrella* endogenous genomic region will replace PpSERKs with the Hyg gene in the genome, enabling transformed *Physcomitrella* lines to gain resistance to hygromycin (Figure 12a). Such lines were further screened and confirmed by genotyping and Sanger-sequencing using multiple combinations of primers including reverse primers that anneal to a region downstream of each PpSERKs, forward primers that anneal to a region from Hyg and an internal region of each PpSERKs (Figure 12b).

Furthermore, transformation of PpSERK1.1, PpSERK1.2 or PpSERK2 KO constructs was conducted at the same time for several independent attempts. We obtained multiple lines of *ppserk1.1* and *ppserk2* bearing resistance to hygromycin. However, the transformants for *ppserk1.2* survived on plates carrying the same concentration of hygromycin for only two to three weeks and subsequently the plants displayed stunted development and became brown and yellowish, leading to death (Figure 12c). The *ppserk1.1* and *ppserk2* lines develop morphologically indistinguishable from WT untransformed *Physcomitrella* lines (Figure 12c). The data indicate that PpSERK1.2, the ancestral PpSERKs genes may play an essential role in *Physcomitrella* growth, development, and survival.

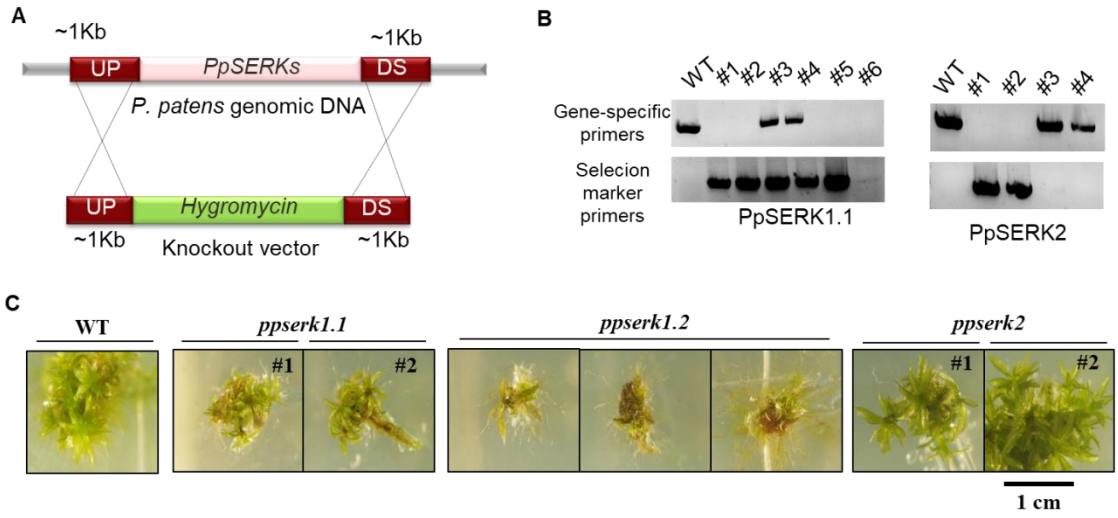


Figure 12. *Physcomitrella* transformation and putative transformants. (A) Schematic diagram of the homologous recombination-based knock-out (KO) strategy. The top scheme represents the genomic position of PpSERKs and its regions upstream (US) and downstream (DS) used in the homologous recombination (approximately 1Kb). The bottom scheme represents the vector containing hygromycin (Hyg) selection marker flanked by the corresponding US and DS of PpSERKs. The dash lines indicate potential recombination. (b) Genotyping of ppserk1.1 and ppserk2 (C) Phenotype of the knockout ppserk lines.

6.3.6. PpSERK1.1 and PpSERK2 play negative roles in chitin-mediated responses and immunity to *Botrytis cinerea*

It has been reported that *Physcomitrella* is not responsive to bacterial elicitors such as flg22, but it is responsive to fungal elicitors such as chitin in terms of MAPK activation (Bressendorff et al., 2016). Similar to this report, we observed that chitin and chitosan, but not bacterial elicitors, including flg22, lipopolysaccharide (LPS) or peptidoglycan (PGN), activate MAPKs detected by anti-phosphoErk antibodies (Figure 13a). To examine the involvement of PpSERK1.1 and PpSERK2 in immunity, we compared chitin-induced MAPK activations of *ppserk1.1* and *ppserk2* mutants with WT

Physcomitrella. The KO *ppserk1.1* and *ppserk2* lines displayed an enhanced MAPK activation compared with WT (Figure 13b). These findings suggested that PpSERK1.1 and PpSERK2 may negatively regulate chitin-triggered immunity in *Physcomitrella*. To further investigate this conclusion, *Arabidopsis serk* mutants were also treated with chitin. Surprisingly, the mutants also showed an enhanced MAPK activation, indicating that SERKs may act as a negative regulator of plant immunity mediated by chitin (Figure 13c). In addition, compared to WT plants, the KO *ppserk1.1*, and *ppserk2*, when treated with *B. cinerea*, a necrotrophic fungal pathogen, displayed delayed necrosis of the gametophyte tissues, suggesting PpSERK1.1 and PpSERK2 plays a negative role in mediating resistance to the fungal pathogen *B. cinerea* (Figure 13d and Supplemental figure 4). Furthermore, *Arabidopsis* mutants *bak1-3*, *bak1-4*, and *bak1-5* displayed an enhanced resistance to *B. cinerea* infection compared to WT plants (Figure 13e).

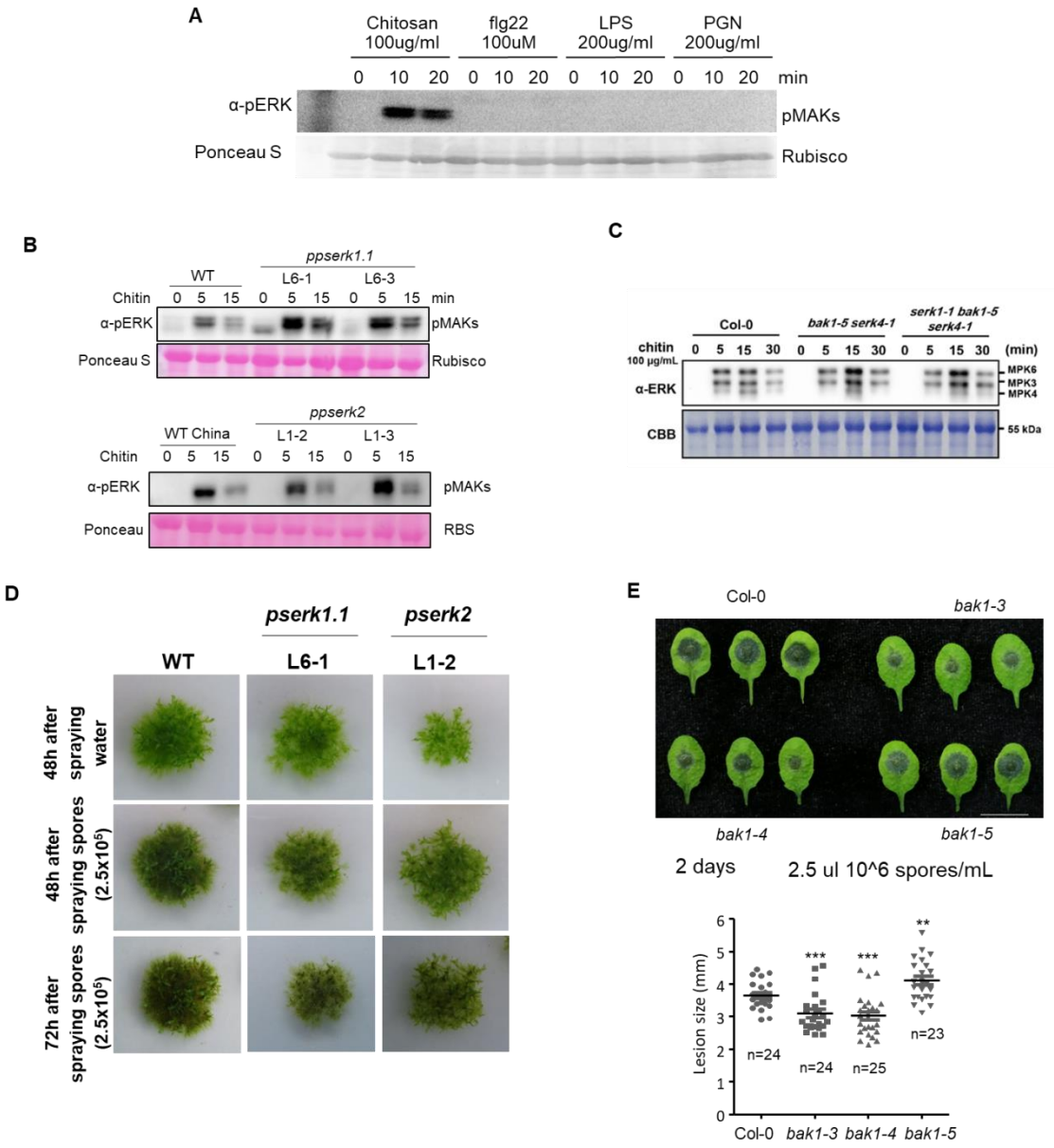


Figure 13. Characterization of PpSERKs in plant immunity. (A) MAPK activation of WT *Physcomitrella* upon different bacterial and fungi elicitors. The activation of MAPK was observed upon the treatment of chitosan, but not flg22, lipopolysaccharide (LPS) nor peptidoglycan (PGN). Protonemata tissues of 7-days old were treated with 100 µg/mL chitosan, 100 µM flg22, 200 µg/mL (LPS), and 200 µg/mL (PGN) for 0, 10 and 20 minutes. The top panel indicates the elicitor-triggered MAPK activation, the bottom panel shows the equal protein loading by Ponceau staining for Rubisco. (B) MAPK activation of knockout *ppserks* in response to chitin led to an increase of MAPK actication, indicating that *ppserks* play a negative role in chitin-mediated immunity. (C) MAPK activation of *Arabidopsis serks* mutants also displayed similar response to *ppserks*, suggesting that *Arabidopsis* SERKs may also play a negative role in chitin-induced immune response. (D) *ppserks* knockouts showed a delayed disease phenotype when sprayed with *B. cinerea* spores compared with WT. (E) *Arabidopsis bak1* mutants displayed enhanced resistance to *B. cinerea* infection

6.3.7. PpSERK1.1 and PpSERK2 function in BR-mediated growth and development in *Physcomitrella*

Although there are no reports about the existence of a brassinosteroid receptor in *Physcomitrella*, a biologically active BR named castasterone was identified in moss, but the common BR, brassinolide, was not (Yokota et al., 2017). Despite that, we tested WT *Physcomitrella* response to BRZ, the BR biosynthesis inhibitor. Interestingly, WT *Physcomitrella* displayed growth inhibition of the protonemata and a stunted gametophore growth in a dosage-dependent manner (Figure 14a). In addition, when treated with BRZ, the *ppserks* knockout displayed strong stunted gametophore growth and more severe reduction of protonemata development (Figure 14b).

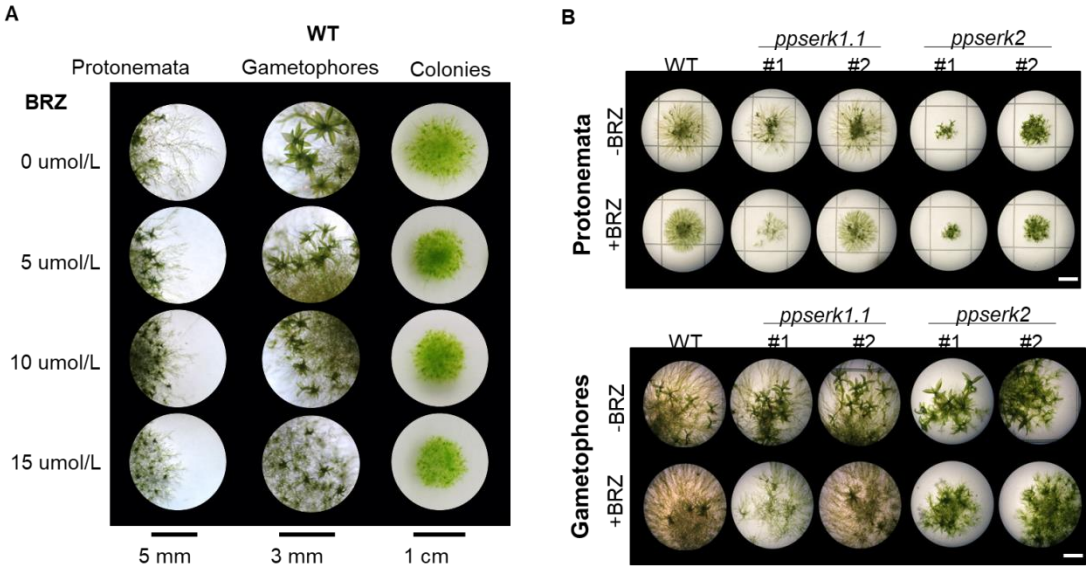


Figure 14. Characterization of PpSERKs in plant growth and development. (A) Morphology of protonemata, gametophores, and 1-month old colonies of P. *patens* WT upon the different concentrations of BRZ (0, 5, 10, 15 μM). Upon the increase of BRZ concentration, the protonemata growth was reduced and the gametophore displayed an impaired growth phenotype. (B) Both *Physcomitrella* tissues where treated with BRZ to observe the mutant phenotype. The knockout lines displayed severe stunt growth of the gametophore and reduction of development of protonemata.

6.4. Discussion

The perception of endogenous and exogenous signals by plant cell surface receptors contribute to the ability of plants to defend themselves against biotic and abiotic stress while maintaining their continuous growth and development. The tradeoff between immunity and development is tightly regulated by plants and to ensure the plant homeostasis, signaling pathways are often modulated by shared components. In *Arabidopsis*, several pathways mediated by RLKs and RLPs converge at the SERK co-regulatory protein family, which regulates a wide range of process including plant innate immunity, brassinosteroid signaling, male sporogenesis, cell death, stomata patterning, and floral organ abscission. Although SERKs are ubiquitously spread among plant species, little is known about their function conservation and evolution in early evolved species.

A phylogenetic analysis revealed the presence of three homolog SERKs in *Physcomitrella*, PpSERK1,1, PpSERK1.2, and PpSERK2. A recent study demonstrated that the current genome of *Physcomitrella* comes from two whole genome duplication (WGD) events (Lang et., al 2018). Based on chromosome localization and sequence identities, PpSERK1.2 (chromosome 3) was hypothesized to be the ancestral of the PpSERKs. During WGD1, chromosome 3 was duplicated originating chromosome 12, where PpSERK2 is localized. Meanwhile, in the WGD2, chromosome 3 was duplicated again originating chromosome 8, where PpSERK1.1 is located. The degree of similarity of PpSERKs amino acids sequences corroborated the idea that PpSERK1.2 and PpSERK2 diverged earlier, they shared 83% identity. While PpSERK1.2 and

PpSERK1.1 shared 97% identity, indicating that they diverged later on (Figure 7). Furthermore, sequence analysis of neighboring genes upstream and downstream of each PpSERKs support the hypothesis that PpSERK1.2 is the ancestral SERK (Supplemental figure 2). Homologous genes adjacent to PpSERK1.2 were found in sequence adjacent to both PpSERK1.1 and PpSERK2. Moreover, phylogenic analysis between *Physcomitrella* and *Arabidopsis* showed that PpSERKs bear a higher homology with SERK1 and SERK2. Even so, PpSERKs were clustered together with *Arabidopsis* SERK family when using either Neighbor-Joining (NJ) or Maximum Likelihood (ML) method to position all LRR-RLKs class II (Figure 6 and Supplemental figure 1). In addition, we found that all the SERK domains were conserved in PpSERKs.

The kinase activity and the conserved phosphorylation sites are crucial for SERKs to function properly. Our biochemical assays revealed that PpSERK1.1 and PpSERK1.2 displayed autophosphorylation activity similar to SERK1 and SERK2, whereas the kinase activity in PpSERK2 was almost undetectable (Figure 8a). Similar outcome was observed when testing the ability of SERKs to phosphorylate the RLCK BIK1 (Figure 8c). In addition, a differential phosphorylation pattern was observed in PpSERKs compared to BAK1 when Thr and Tyr specific antibody was used (Figure 8b). This behavior is likely due to the polymorphism present in the kinase domain of PpSERKs. For instance, PpSERK1.1 and PpSERK1.2 possess two Asn and one Glu amino acid sites instead of two Ser and one Tyr sites in BAK1 (Figure 8d). Additionally, PpSERK2 display a polymorphism in an essential phosphorylation site in the activation loop of the kinase domain, which could explain its almost undetectable kinase activity.

Taken together, we conclude that, although PpSERKs possess differential phosphorylation activity, they exhibit a functional kinase.

Subsequently, we tested the ability of PpSERKs to complement *Arabidopsis serk* mutants defective in PTI activation (*bak1-4* and *bak1-5-/-serk4-/-*) and BR-mediated growth and development (*serk1-1bak1-4*). Our findings indicate that, upon flg22 treatment, PpSERK1.1 and PpSERK2 were able to partially restore BIK and ASR3 phosphorylation in *bak1-4* protoplasts and MAPK activation in *bak1-4* protoplasts and .*bak1-4* and *bak1-5-/-serk4-/-* transgenic plants (Figures 9 and 10). Additionally, PpSERK1.1 and PpSERK1.2 were also able to partially recover ROS burst upon flg22 treatment in *bak1-5-/-serk4-/-* transgenic plants (Figure 10). Surprisingly, in BR defective mutant, PpSERK1.1 and PpSERK1.2 not only restore the short petiole mutant phenotype but also displayed a much longer petiole compared to WT plants (Figure 11). The longer petiole phenotype is typical of BRI1 overexpression, where elevated BR signaling results in elongated petioles and curling leaves. Although PpSERKs were able to restore BRI defective phenotype, it was unclear why they display a phenotype similar to BRI overexpression plants. Altogether, we were able to demonstrate that PpSERKs play a role similar to flg22-induced PTI activation and BR-mediated growth and development. Nevertheless, the role of PpSERKs in mediating male sporogenesis as well as the complementation analysis of PpSERK2 is still under investigation.

Afterwards, we demonstrated that *ppserk1.1* and *ppserk2* knockout lines in *Physcomitrella* play a negative role in chitin-based immunity activation. In addition, the knockout lines also displayed a delayed disease phenotype when treated with *B. cinerea*.

These unexpected findings prompted us to test the response of *Arabidopsis serk* mutants to chitin. Surprisingly, when treated with chitin, *bak1-5-/-serk4-/-* mutant also showed enhanced MAPK activation. Furthermore, *bak1-4* and *bak1-3* mutants exhibit enhanced resistance to the necrotrophic fungus *B. cinerea* in *Arabidopsis*. Another surprising discovery was the ability of *Physcomitrella* to perceive and respond a treatment using BR biosynthesis inhibitor, even though they do not possess a homolog of BR receptor. Our results showed that BRZ affect the protonemata growth and displayed a stunted gametophore in WT *Physcomitrella*. The knockout lines showed slightly severe phenotype when compared with WT. Further researches are necessary to address the new found function of SERK in chitin-mediated susceptibility and BR responses in *Physcomitrella*.

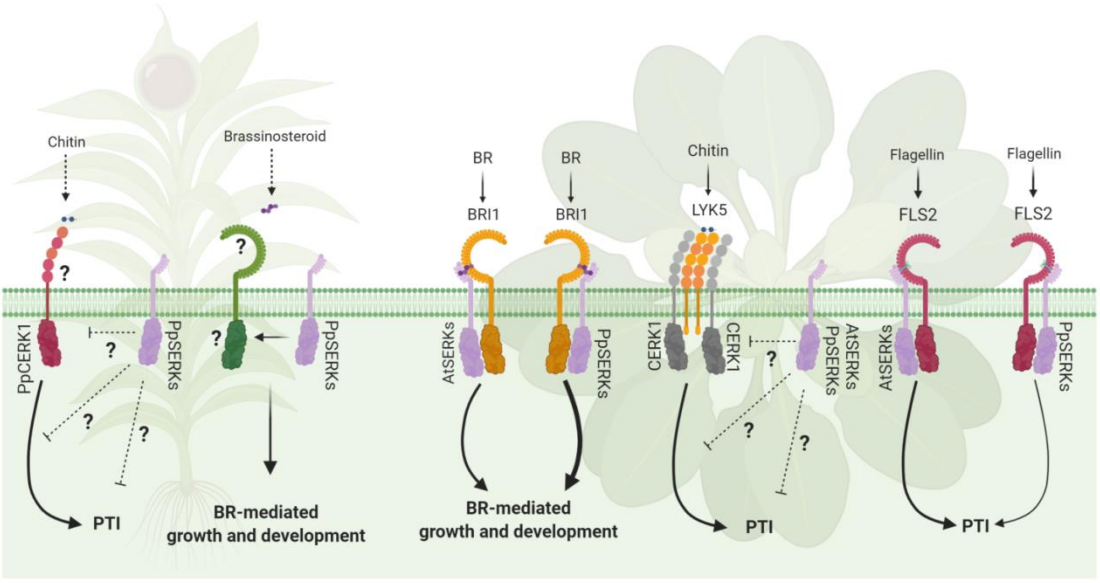


Figure 15. Model of PpSERKs function in *Physcomitrella* and *Arabidopsis*.

Our model summarizes the function conservation of PpSERKs in *Arabidopsis* and their function in *Physcomitrella* (Figure 15). In terms of brassinosteroid signaling, PpSERKs displayed stronger responses to BR indicating that PpSERKs can boost up their growth and development in response to the hormone. In fact, due to the high homology between SERKs, more research can uncover specific amino acids sites that are responsible for the magnification of BR response. This discovery can significantly impact the productivity of crop plants and could be a more practical approach to improve crop yields. Additionally, the partial recovery of flagellin-induced plant immunity activation can indicate that the ability of plants to overcome bacterial infections happened after their establishment on land. Similar to BR, the knowledge about important amino acid sites in SERK genes can fine-tune plant response to pathogen, which has a direct effect on crop growth.

Taking everything into consideration, our work suggests that the function conservation of SERKs, in response to PTI regulation and BR-mediated growth and development, dates back to more than 400 mya. This observation indicates that very likely *Arabidopsis* SERKs evolved from *Physcomitrella* SERKs. Additionally, considering how ancient the function conservation of SERKs is, we can speculate that SERK family is essential for plant survival.

CHAPTER VII

CONCLUSIONS

In the past decade, remarkable advances have been made to reveal the critical role of the plant cell surface receptors in regulating plant immunity, growth, and development. Despite the fact that the functions of a large body of RLKs and RLPs await to be ascertained, many RLKs and RLPs have been uncovered to play novel sentinel roles in perceiving diverse endogenous and exogenous signals. Subsequently, the signals perceived by the RLK and RLP complexes are relayed to the downstream signaling modules that further transduce the signaling to activate and coordinate distinct physiological responses.

The co-regulatory protein SERKs are essential for signaling perception, transduction and activation. Due to their multiple roles in plant immunity and development, our research focused on understating how SERKs evolved to modulate distinct signaling pathways. Our findings suggest that *Physcomitrella* possess three homologs SERKs sharing around 80% identity with *Arabidopsis* SERKs. In addition, we showed that PpSERKs possess kinase activity and are able to restore the function of mutants defective in flg22-induced immunity and Br-mediated growth and development. Interestingly, we also uncover a new function for SERK proteins in *Physcomitrella* and *Arabidopsis*. Our data suggests that SERKs play a negative role in chitin induced immunity activation. Besides, we showed that although *Physcomitrella* does not have a BR receptor homolog, WT and mutant display phenotypic responses to BR inhibitor.

Our work demonstrates that even though *Physcomitrella* and *Arabidopsis* are placed evolutionary distant from each other, SERKs functions are conserved between them; indicating SERKs may have evolved from a common ancestral.

Although remarkable progress has been made in understanding RLKs and RLPs together with signaling transduction, a lot more needs to be uncover downstream receptor complexes. For instance, most pathways activated by RLKs and RLPs lead to the activation of MAPK cascades, and RLCKs often act downstream of the several known PRR complexes. However, how RLCKs dir;ectly link the signaling to the MAPK cascades is not fully elucidated. Besides, the substrates downstream MAPK cascades are not fully understood. Other components that function at the different modules to regulate the activities of RLKs, RLPs, and RLCKs are emerging. Besides, recent studies have indicated that the plasma membrane is compartmentalized into microdomains that harbor PRRs and their associated co-regulatory receptors to promote rapid and optimized response to different stimuli (Ott, 2017; Bücherl et al., 2017; Jaillais 2020). It would be interesting to examine the composition and dynamics of the microdomains containing PRRs complexes with shared components in response to various stimuli in plant immunity, growth, and development. Functional characterization of plasma membrane-resident RLKs and RLPs perceiving diverse environmental and endogenous cues will be continuously a burgeoning area of investigation and offer insight into strategic development for improved crop resilience.

REFERENCES

Aan den Toorn M, Albrecht C, de Vries S (2015) On the Origin of SERKs: Bioinformatics Analysis of the Somatic Embryogenesis Receptor Kinases. **Mol. Plant** 8: 762–782.

Albert I, Böhm H, Albert M, Feiler CE, Imkampe J, Wallmeroth N, Brancato C, Raaymakers TM, Oome S, Zhang H, Krol E, Grefen C, Gust AA, Chai J, Hedrich R, Van den Ackerveken G, Nürnberger T (2015) An RLP23–SOBIR1–BAK1 complex mediates NLP-triggered immunity. **Nat. Plants** 1: 1–9.

Albrecht C, Russinova E, Hecht V, Baaijens E, De Vries S (2005) The *Arabidopsis thaliana* SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASES1 and 2 Control Male Sporogenesis. **Plant Cell** 17: 3337–3349.

Amano Y, Tsubouchi H, Shinohara H, Ogawa M, Matsubayashi Y (2007) Tyrosine-sulfated glycopeptide involved in cellular proliferation and expansion in *Arabidopsis*. **Proc. Natl. Acad. Sci.** 104: 18333–18338.

Arabidopsis Genome Initiative [WWW Document], 2000. URL https://www.nsf.gov/news/news_summ.jsp?cntn_id=103071 (accessed 10.8.20).

Asai T, Tena G, Plotnikova J, Willmann MR, Chiu WL, Gomez-Gomez L, Boller T, Ausubel FM, Sheen J (2002) MAP kinase signalling cascade in *Arabidopsis* innate immunity. **Nature** 415: 977–983.

Ashton NW, Cove DJ (1977) The isolation and preliminary characterisation of auxotrophic and analogue resistant mutants of the moss, *Physcomitrella patens*. **Mol. Gen. Genet. MGG** 154: 87–95

Bar M, Sharfman M, Ron M, Avni A (2010) BAK1 is required for the attenuation of ethylene-inducing xylanase (Eix)-induced defense responses by the decoy receptor LeEix1. **Plant J.** 63: 791–800.

Bayer M, Nawy T, Giglione C, Galli M, Meinnel T, Lukowitz W (2009) Paternal Control of Embryonic Patterning in *Arabidopsis thaliana*. **Science** 323: 1485–1488.

Bi G, Zhou Z, Wang W, Li L, Rao S, Wu Y, Zhang X, Menke FLH, Chen S, Zhou J-M (2018) Receptor-Like Cytoplasmic Kinases Directly Link Diverse Pattern Recognition Receptors to the Activation of Mitogen-Activated Protein Kinase Cascades in *Arabidopsis*. **Plant Cell** 30: 1543–1561.

Bleckmann A, Weidtkamp-Peters S, Seidel CAM, Simon, R (2010) Stem Cell Signaling in *Arabidopsis* Requires CRN to Localize CLV2 to the Plasma Membrane. **Plant Physiol.** 152: 166–176.

Böhm H, Albert I, Oome S, Raaymakers TM, Van den Ackerveken G, Nürnberger T (2014) A Conserved Peptide Pattern from a Widespread Microbial Virulence Factor Triggers Pattern-Induced Immunity in *Arabidopsis*. **PLoS Pathog.** 10.

Boisson-Dernier A, Franck CM, Lituiev DS, Grossniklaus U (2015) Receptor-like cytoplasmic kinase MARIS functions downstream of CrRLK1L-dependent signaling during tip growth. **Proc. Natl. Acad. Sci**. **U. S. A.** 112: 12211–12216.

Boisson-Dernier A, Roy S, Kritsas K, Grobei MA, Jaciubek M, Schroeder JI, Grossniklaus U (2009) Disruption of the pollen-expressed FERONIA homologs ANXUR1 and ANXUR2 triggers pollen tube discharge. **Development** 136: 3279–3288.

Boller T, Felix G (2009) A Renaissance of Elicitors: Perception of Microbe-Associated Molecular Patterns and Danger Signals by Pattern-Recognition Receptors. **Annu. Rev. Plant Biol.** 60: 379–406.

Boudsocq M, Sheen J (2013) CDPKs in immune and stress signaling. **Trends Plant Sci.** 18: 30–40.

Boudsocq M, Willmann MR, McCormack M, Lee H, Shan L, He P, Bush J, Cheng SH, Sheen, J (2010) Differential innate immune signalling via Ca 2+ sensor protein kinases. **Nature** 464: 418–422.

Brand U, Fletcher JC, Hobe M, Meyerowitz EM, Simon R (2000) Dependence of Stem Cell Fate in *Arabidopsis* on a Feedback Loop Regulated by CLV3 Activity. **Science** 289: 617–619.

Bressendorff S, Azevedo R, Kenchappa CS, León IP de, Olsen JV, Rasmussen MW, Erbs G, Newman M-A, Petersen M, Mundy J (2016) An Innate Immunity Pathway in the Moss *Physcomitrella patens*. **Plant Cell** 28: 1328–1342.

Brutus A, Sicilia F, Macone A, Cervone F, Lorenzo GD (2010) A domain swap approach reveals a role of the plant wall-associated kinase 1 (WAK1) as a receptor of oligogalacturonides. **Proc. Natl. Acad. Sci**. 107: 9452–9457.

Bücherl CA, Jarsch IK, Schudoma C, Segonzac C, Mbengue M, Robatzek S, MacLean D, Ott T, Zipfel C (2017) Plant immune and growth receptors share common signalling components but localise to distinct plasma membrane nanodomains.

eLife 6: e25114.

Burgh AM van der, Joosten MHAJ (2019) Plant Immunity: Thinking Outside and Inside the Box. **Trends Plant Sci.** 24: 587–601.

Burr CA, Leslie ME, Orlowski SK, Chen I, Wright CE, Daniels MJ, Liljegren SJ (2011) CAST AWAY, a Membrane-Associated Receptor-Like Kinase, Inhibits Organ Abscission in *Arabidopsis*. **Plant Physiol.** 156: 1837–1850.

Canales C, Bhatt AM, Scott R, Dickinson H (2002) EXS, a putative LRR receptor kinase, regulates male germline cell number and tapetal identity and promotes seed development in *Arabidopsis*. **Curr. Biol. CB** 12: 1718–1727.

Cao Y, Liang Y, Tanaka K, Nguyen CT, Jedrzejczak RP, Joachimiak A, Stacey G (2014) The kinase LYK5 is a major chitin receptor in *Arabidopsis* and forms a chitin-induced complex with related kinase CERK1. **eLife** 3: e03766.

Chaudhary A, Chen X, Gao J, Leśniewska B, Hammerl R, Dawid C, Schneitz K (2020) The *Arabidopsis* receptor kinase STRUBBELIG regulates the response to cellulose deficiency. **PLOS Genet.** 16: e1008433.

Chen D, Cao Y, Li H, Kim D, Ahsan N, Thelen J, Stacey G (2017) Extracellular ATP elicits DORN1-mediated RBOHD phosphorylation to regulate stomatal aperture. **Nat. Commun.** 8: 2265.

Chen W, Lv M, Wang Y, Wang PA, Cui Y, Li M, Wang R, Gou X, Li J (2019) BES1 is activated by EMS1-TPD1-SERK1/2-mediated signaling to control tapetum development in *Arabidopsis thaliana*. **Nat. Commun.** 10: 4164.

Chen X, Zuo S, Schwessinger B, Chern M, Canlas PE, Ruan D, Zhou X, Wang J, Daudi A, Petzold CJ, Heazlewood JL, Ronald PC (2014) An XA21-Associated Kinase (OsSERK2) Regulates Immunity Mediated by the XA21 and XA3 Immune Receptors. **Mol. Plant** 7: 874–892.

Chinchilla D, Zipfel C, Robatzek S, Kemmerling B, Nürnberger T, Jones JDG, Felix G, Boller T (2007) A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. **Nature** 448: 497–500.

Cho SK, Larue CT, Chevalier D, Wang H, Jinn TL, Zhang S, Walker JC (2008) Regulation of floral organ abscission in *Arabidopsis thaliana*. **Proc. Natl. Acad. Sci**. 105: 15629–15634.

Choi J, Tanaka K, Cao Y, Qi Y, Qiu J, Liang Y, Lee SY, Stacey G (2014) Identification

of a Plant Receptor for Extracellular ATP. **Science** 343: 290–294.

Costa LM, Marshall E, Tesfaye M, Silverstein KAT, Mori M, Umetsu Y, Otterbach SL, Papareddy R, Dickinson HG, Boutiller K, VandenBosch KA, Ohki S, Gutierrez-Marcos JF (2014) Central Cell–Derived Peptides Regulate Early Embryo Patterning in Flowering Plants. **Science** 344: 168–172.

Couto D, Zipfel C (2016) Regulation of pattern recognition receptor signalling in plants. **Nat. Rev. Immunol.** 16: 537–552.

Cui H, Tsuda K, Parker JE (2015) Effector-triggered immunity: from pathogen perception to robust defense. **Annu. Rev. Plant Biol.** 66: 487–511.

Cui Y, Hu C, Zhu Y, Cheng K, Li X, Wei Z, Xue L, Lin F, Shi H, Yi J, Hou S, He K, Li J, Gou X (2018) CIK Receptor Kinases Determine Cell Fate Specification during Early Anther Development in *Arabidopsis*. **Plant Cell** 30: 2383–2401.

de Simone A, Hubbard R, de la Torre NV, Velappan Y, Wilson M, Considine MJ, Soppe WJJ, Foyer CH (2017) Redox Changes During the Cell Cycle in the Embryonic Root Meristem of *Arabidopsis thaliana*. **Antioxid. Redox Signal.** 27: 1505–1519.

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

Denoux C, Galletti R, Mammarella N, Gopalan S, Werck D, De Lorenzo G, Ferrari S, Ausubel FM, Dewdney J (2008) Activation of Defense Response Pathways by OGs and Flg22 Elicitors in *Arabidopsis* Seedlings. **Mol. Plant** 1: 423–445.

DeYoung BJ, Bickle KL, Schrage KJ, Muskett P, Patel K, Clark SE (2006) The CLAVATA1-related BAM1, BAM2 and BAM3 receptor kinase-like proteins are required for meristem function in *Arabidopsis*. **Plant J. Cell Mol. Biol.** 45: 1–16.

Dievart A, Gottin C, Périn C, Ranwez V, Chantret N (2020) Origin and Diversity of Plant Receptor-Like Kinases. **Annu. Rev. Plant Biol.** 71: 131–156.

Dixon MS, Hatzixanthis K, Jones DA, Harrison K, Jones JDG (1998) The Tomato Cf-5 Disease Resistance Gene and Six Homologs Show Pronounced Allelic Variation in Leucine-Rich Repeat Copy Number. **Plant Cell** 10: 1915–1925.

Dixon MS, Jones DA, Keddie JS, Thomas CM, Harrison K, Jones JDG (1996) The Tomato Cf-2 Disease Resistance Locus Comprises Two Functional Genes Encoding Leucine-Rich Repeat Proteins. **Cell** 84: 451–459.

Drisch RC, Stahl Y (2015) Function and regulation of transcription factors involved in root apical meristem and stem cell maintenance. **Front. Plant Sci.** 6: 505.

Du C, Li X, Chen J, Chen W, Li B, Li C, Wang L, Li J, Zhao X, Lin J, Liu X, Luan S, Yu F (2016) Receptor kinase complex transmits RALF peptide signal to inhibit root growth in *Arabidopsis*. **Proc. Natl. Acad. Sci**. 113: E8326–E8334.

Dubiella U, Seybold H, Durian G, Komander E, Lassig R, Witte CP, Schulze WX, Romeis T (2013) Calcium-dependent protein kinase/NADPH oxidase activation circuit is required for rapid defense signal propagation. **Proc. Natl. Acad. Sci**. U. S. A. 110: 8744–8749.

Duriez P, Vautrin S, Auriac M-C, Bazerque J, Boniface M-C, Callot C, Carrère S, Cauet S, Chabaud M, Gentou F, Lopez-Sendon M, Paris C, Pegot-Espagnet P, Rousseaux J-C, Pérez-Vich B, Velasco L, Bergès H, Piquemal J, Muños S (2019) A receptor-like kinase enhances sunflower resistance to *Orobanche cumana*. **Nat. Plants** 5: 1211–1215.

Felix G, Duran JD, Volko S, Boller T (1999) Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. **Plant J.** 18: 265–276.

Fisher K, Turner S (2007) PXY, a Receptor-like Kinase Essential for Maintaining Polarity during Plant Vascular-Tissue Development. **Curr. Biol.** 17: 1061–1066.

Foreman J, Demidchik V, Bothwell JHF, Mylona P, Miedema H, Torres MA, Linstead P, Costa S, Brownlee C, Jones JDG, Davies JM, Dolan L (2003) Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. **Nature** 422: 442–446.

Fritz-Laylin LK, Krishnamurthy N, Tör M, Sjölander KV, Jones JDG (2005) Phylogenomic Analysis of the Receptor-Like Proteins of Rice and *Arabidopsis*. **Plant Physiol.** 138: 611–623.

Furukawa T, Inagaki H, Takai R, Hirai H, Che FS (2014) Two distinct EF-Tu epitopes induce immune responses in rice and *Arabidopsis*. Mol. Plant-Microbe Interact. **MPMI** 27: 113–124.

Galindo-Trigo S, Blanco-Touriñán N, DeFalco TA, Wells ES, Gray JE, Zipfel C, Smith LM (2020) CrRLK1L receptor-like kinases HERK1 and ANJEA are female determinants of pollen tube reception. **EMBO** Rep. 21.

Galletti R, Ferrari S, Lorenzo GD (2011) *Arabidopsis* MPK3 and MPK6 Play Different Roles in Basal and Oligogalacturonide- or Flagellin-Induced Resistance against

Botrytis cinerea. **Plant Physiol.** 157: 804–814.

Galli M, Gallavotti A (2016) Expanding the Regulatory Network for Meristem Size in Plants. **Trends Genet. TIG** 32: 372–383.

Gao M, Wang X, Wang D, Xu F, Ding X, Zhang Z, Bi D, Cheng YT, Chen S, Li X, Zhang Y (2009) Regulation of Cell Death and Innate Immunity by Two Receptor-like Kinases in *Arabidopsis*. **Cell Host Microbe** 6: 34–44.

Gao X, Li F, Li M, Kianinejad AS, Dever JK, Wheeler TA, Li Z, He P, Shan L (2013) Cotton GhBAK1 Mediates *Verticillium* Wilt Resistance and Cell Death. **J. Integr. Plant Biol.** 55: 586–596.

Gómez-Gómez L, Boller T (2000) FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in *Arabidopsis*. **Mol. Cell** 5: 1003–1011.

Gou X, Yin H, He K, Du J, Yi J, Xu S, Lin H, Clouse SD, Li J (2012) Genetic Evidence for an Indispensable Role of Somatic Embryogenesis Receptor Kinases in Brassinosteroid Signaling. **PLOS Genet.** 8: e1002452.

Greb T, Lohmann JU (2016) Plant Stem Cells. **Curr. Biol.** 26: R816–R821.

Gudesblat GE, Schneider-Pizoń J, Betti C, Mayerhofer J, Vanhoutte I, van Dongen W, Boeren S, Zhiponova M, de Vries S, Jonak C, Russinova E (2012) SPEECHLESS integrates brassinosteroid and stomata signalling pathways. **Nat. Cell Biol.** 14: 548–554.

Guo Y, Han L, Hymes M, Denver R, Clark SE (2010) CLAVATA2 forms a distinct CLE-binding receptor complex regulating *Arabidopsis* stem cell specification. **Plant J.** 63: 889–900.

Gust AA, Felix G (2014) Receptor like proteins associate with SOBIR1-type of adaptors to form bimolecular receptor kinases. **Curr. Opin. Plant Biol., SI: Cell signalling and gene regulation** 21: 104–111.

Gust AA, Pruitt R, Nürnberger T (2017) Sensing Danger: Key to Activating Plant Immunity. **Trends Plant Sci.** 22: 779–791.

Halter T, Imkampe J, Mazzotta S, Wierzba M, Postel S, Bücherl C, Kiefer C, Stahl M, Chinchilla D, Wang X, Nürnberger T, Zipfel C, Clouse S, Borst JW, Boeren S, de Vries SC, Tax F, Kemmerling B (2014) The leucine-rich repeat receptor kinase BIR2 is a negative regulator of BAK1 in plant immunity. **Curr. Biol. CB** 24: 134–143.

Hammond-Kosack KE, Jones DA, Jones J (1994) Identification of Two Genes Required in Tomato for Full Cf-9-Dependent Resistance to *Cladosporium fulvum*. **Plant Cell** 6, 361–374.

Hann DR, Rathjen JP (2007) Early events in the pathogenicity of *Pseudomonas syringae* on *Nicotiana benthamiana*. **Plant J.** 49: 607–618.

Haruta M, Sabat G, Stecker K, Minkoff BB, Sussman MR (2014) A Peptide Hormone and Its Receptor Protein Kinase Regulate Plant Cell Expansion. **Science** 343: 408–411.

He Y, Zhou J, Shan L, Meng X (2018) Plant cell surface receptor-mediated signaling - a common theme amid diversity. **J. Cell Sci.** 131.

Hegenauer V, Fürst U, Kaiser B, Smoker M, Zipfel C, Felix G, Stahl M, Albert M (2016) Detection of the plant parasite *Cuscuta reflexa* by a tomato cell surface receptor. **Science** 353: 478–481.

Heidstra R, Sabatini S (2014) Plant and animal stem cells: similar yet different. **Nat. Rev. Mol. Cell Biol.** 15: 301–312.

Hind SR, Strickler SR, Boyle PC, Dunham DM, Bao Z, O'Doherty IM, Baccile JA, Hoki JS, Viox EG, Clarke CR, Vinatzer BA, Schroeder FC, Martin GB (2016) Tomato receptor FLAGELLIN-SENSING 3 binds flgII-28 and activates the plant immune system. **Nat. Plants** 2: 1–8.

Hirakawa Y, Shinohara H, Kondo Y, Inoue A, Nakanomyo I, Ogawa M, Sawa S, Ohashi-Ito K, Matsubayashi Y, Fukuda H (2008) Non-cell-autonomous control of vascular stem cell fate by a CLE peptide/receptor system. **Proc. Natl. Acad. Sci**. 105: 15208–15213.

Hohmann U, Lau K, Hothorn M (2017) The Structural Basis of Ligand Perception and Signal Activation by Receptor Kinases. **Annu. Rev. Plant Biol.** 68: 109–137.

Hohmann U, Santiago J, Nicolet J, Olsson V, Spiga FM, Hothorn LA, Butenko MA, Hothorn M 2018 Mechanistic basis for the activation of plant membrane receptor kinases by SERK-family coreceptors. **Proc. Natl. Acad. Sci. USA** 115: 3488–3493.

Hord CLH, Chen C, DeYoung BJ, Clark SE, Ma H (2006) The BAM1/BAM2 Receptor-Like Kinases Are Important Regulators of *Arabidopsis* Early Anther Development. **Plant Cell** 18: 1667–1680.

Hou S, Wang X, Chen D, Yang X, Wang M, Turrà D, Pietro AD, Zhang W (2014) The

Secreted Peptide PIP1 Amplifies Immunity through Receptor-Like Kinase 7. **PLOS Pathog.** 10: e1004331.

Hu C, Zhu Y, Cui Y, Cheng K, Liang W, Wei Z, Zhu M, Yin H, Zeng L, Xiao Y, Lv M, Yi J, Hou S, He K, Li J, Gou X (2018) A group of receptor kinases are essential for CLAVATA signalling to maintain stem cell homeostasis. **Nat. Plants** 4: 205–211.

Huang WJ, Liu HK, McCormick S, Tang WH (2014) Tomato Pistil Factor STIG1 Promotes in Vivo Pollen Tube Growth by Binding to Phosphatidylinositol 3-Phosphate and the Extracellular Domain of the Pollen Receptor Kinase LePRK2. **Plant Cell** 26: 2505–2523.

Imkampe J, Halter T, Huang S, Schulze S, Mazzotta S, Schmidt N, Manstretta R, Postel S, Wierzba M, Yang Y, van Dongen WMAM, Stahl M, Zipfel C, Goshe MB, Clouse S, de Vries SC, Tax F, Wang X, Kemmerling B (2017) The *Arabidopsis* Leucine-Rich Repeat Receptor Kinase BIR3 Negatively Regulates BAK1 Receptor Complex Formation and Stabilizes BAK1. **Plant Cell** 29: 2285–2303.

Ito Y, Takaya K, Kurata N (2005) Expression of SERK family receptor-like protein kinase genes in rice. **Biochim. Biophys. Acta BBA - Gene Struct. Expr.** 1730: 253–258.

Jaillais Y, Ott T (2020) The Nanoscale Organization of the Plasma Membrane and Its Importance in Signaling: A Proteolipid Perspective. **Plant Physiol.** 182: 1682–1696.

Jamieson PA, Shan L, He P (2018) Plant cell surface molecular cypher: Receptor-like proteins and their roles in immunity and development. **Plant Sci.** 274: 242–251.

Jeong S, Trotochaud AE, Clark SE (1999) The *Arabidopsis* CLAVATA2 gene encodes a receptor-like protein required for the stability of the CLAVATA1 receptor-like kinase. **Plant Cell** 11: 1925–1934.

Jia G, Liu X, Owen HA, Zhao D (2008) Signaling of cell fate determination by the TPD1 small protein and EMS1 receptor kinase. **Proc. Natl. Acad. Sci**. 105: 2220–2225.

Jinjun Z, Peina J, Fang Z, Chongke Z, Bo B, Yaping L, Haifeng W, Fan C, Xianzhi X (2020) OsSRK1, an Atypical S-Receptor-Like Kinase Positively Regulates Leaf Width and Salt Tolerance in Rice. **Rice Sci.** 27: 133–142.

Johansen W, Ako AE, Demko V, Perroud P-F, Rensing SA, Mekhlif AK, Olsen O-A (2016) The DEK1 Calpain Linker Functions in Three-Dimensional Body Patterning

in *Physcomitrella patens*. **Plant Physiol.** 172: 1089–1104.

Jones DA, Thomas CM, Hammond-Kosack KE, Balint-Kurti PJ, Jones JD (1994) Isolation of the tomato Cf-9 gene for resistance to *Cladosporium fulvum* by transposon tagging. **Science** 266: 789–793.

Jones JDG, Dangl JL (2006) The plant immune system. **Nature** 444: 323–329.

Kaku H, Nishizawa Y, Ishii-Minami N, Akimoto-Tomiyama C, Dohmae N, Takio K, Minami E, Shibuya N (2006) Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. **Proc. Natl. Acad. Sci**. 103: 11086–11091.

Kärkönen A, Kuchitsu K (2015) Reactive oxygen species in cell wall metabolism and development in plants. **Phytochemistry** 112: 22–32.

Kayes JM, Clark SE (1998) CLAVATA2, a regulator of meristem and organ development in *Arabidopsis*. **Development** 125: 3843-3851.

Kim TW, Guan S, Burlingame AL, Wang ZY (2011) The CDG1 Kinase Mediates Brassinosteroid Signal Transduction from BRI1 Receptor Kinase to BSU1 Phosphatase and GSK3-like Kinase BIN2. **Mol. Cell** 43: 561–571.

Kimura S, Waszczak C, Hunter K, Wrzaczek M (2017) Bound by Fate: The Role of Reactive Oxygen Species in Receptor-Like Kinase Signaling. **Plant Cell** 29: 638–654.

Kinoshita A, Betsuyaku S, Osakabe Y, Mizuno S, Nagawa S, Stahl Y, Simon R, Yamaguchi-Shinozaki K, Fukuda H, Sawa S (2010) RPK2 is an essential receptor-like kinase that transmits the CLV3 signal in *Arabidopsis*. **Dev. Camb. Engl.** 137: 3911–3920.

Kitagawa M, Jackson D (2019) Control of Meristem Size. **Annu. Rev. Plant Biol.** 70: 269–291.

Krol E, Mentzel T, Chinchilla D, Boller T, Felix G, Kemmerling B, Postel S, Arents M, Jeworutzki E, Al-Rasheid KAS, Becker D, Hedrich R (2010) Perception of the *Arabidopsis* Danger Signal Peptide 1 Involves the Pattern Recognition Receptor AtPEPR1 and Its Close Homologue AtPEPR2. **J. Biol. Chem.** 285: 13471–13479.

Ku H-M, Vision T, Liu J, Tanksley SD (2000) Comparing sequenced segments of the tomato and *Arabidopsis* genomes: Large-scale duplication followed by selective gene loss creates a network of synteny**. Proc. Natl. Acad. Sci.** 97: 9121–9126.

Kumar V, Van Staden J (2019) Multi-tasking of SERK-like kinases in plant embryogenesis, growth, and development: current advances and biotechnological applications. **Acta Physiol. Plant.** 41: 31.

Kumpf RP, Shi CL, Larrieu A, Sto IM, Butenko MA, Peret B, Riiser ES, Bennett MJ, Aalen RB (2013) Floral organ abscission peptide IDA and its HAE/HSL2 receptors control cell separation during lateral root emergence. **Proc. Natl. Acad. Sci**. 110: 5235–5240.

Kunze G, Zipfel C, Robatzek S, Niehaus K, Boller T, Felix G (2004) The N Terminus of Bacterial Elongation Factor Tu Elicits Innate Immunity in *Arabidopsis* Plants. **Plant Cell** 16: 3496–3507.

Kutschera A, Dawid C, Gisch N, Schmid C, Raasch L, Gerster T, Schäffer M, Smakowska-Luzan E, Belkhadir Y, Vlot AC, Chandler CE, Schellenberger R, Schwudke D, Ernst RK, Dorey S, Hückelhoven R, Hofmann T, Ranf S (2019) Bacterial medium-chain 3-hydroxy fatty acid metabolites trigger immunity in *Arabidopsis* plants. **Science** 364: 178–181.

Laluk K, Luo H, Chai M, Dhawan R, Lai Z, Mengiste T (2011) Biochemical and genetic requirements for function of the immune response regulator BOTRYTIS-INDUCED KINASE1 in plant growth, ethylene signaling, and PAMP-triggered immunity in *Arabidopsis*. **Plant Cell** 23: 2831–2849.

Lang D, Ullrich KK, Murat F, Fuchs J, Jenkins J, Haas FB, Piednoel M, Gundlach H, Bel MV, Meyberg R, Vives C, Morata J, Symeonidi A, Hiss M, Muchero W, Kamisugi Y, Saleh O, Blanc G, Decker EL, Gessel N van, Grimwood J, Hayes RD, Graham SW, Gunter LE, McDaniel SF, Hoernstein SNW, Larsson A, Li F-W, Perroud P-F, Phillips J, Ranjan P, Rokshar DS, Rothfels CJ, Schneider L, Shu S, Stevenson DW, Thümmler F, Tillich M, Aguilar JCV, Widiez T, Wong GK-S, Wymore A, Zhang Y, Zimmer AD, Quatrano RS, Mayer KFX, Goodstein D, Casacuberta JM, Vandepoele K, Reski R, Cuming AC, Tuskan GA, Maumus F, Salse J, Schmutz J, Rensing SA (2018) The *Physcomitrella patens* chromosome-scale assembly reveals moss genome structure and evolution. **Plant J.** 93: 515–533.

Lee D, Lal NK, Lin ZJD, Ma S, Liu J, Castro B, Toruño T, Dinesh-Kumar SP, Coaker G (2020) Regulation of reactive oxygen species during plant immunity through phosphorylation and ubiquitination of RBOHD. **Nat. Commun.** 11: 1838.

Lee JS, Kuroha T, Hnilova M, Khatayevich D, Kanaoka MM, McAbee JM, Sarikaya M, Tamerler C, Torii KU (2012) Direct interaction of ligand-receptor pairs specifying stomatal patterning. **Genes Dev.** 26: 126–136.

Lehti-Shiu MD, Zou C, Hanada K, Shiu SH (2009) Evolutionary History and Stress Regulation of Plant Receptor-Like Kinase/Pelle Genes. **Plant Physiol.** 150: 12–26.

Li B, Jiang S, Yu X, Cheng C, Chen S, Cheng Y, Yuan JS, Jiang D, He P, Shan L (2015) Phosphorylation of Trihelix Transcriptional Repressor ASR3 by MAP KINASE4 Negatively Regulates *Arabidopsis* Immunity. **Plant Cell** 27: 839–856.

Li C, Wu HM, Cheung AY (2016) FERONIA and Her Pals: Functions and Mechanisms. **Plant Physiol.** 171: 2379–2392.

Li J, Chory J (1997) A Putative Leucine-Rich Repeat Receptor Kinase Involved in Brassinosteroid Signal Transduction. **Cell** 90: 929–938.

Li J, Wen J, Lease KA, Doke JT, Tax FE, Walker JC (2002) BAK1, an *Arabidopsis* LRR Receptor-like Protein Kinase, Interacts with BRI1 and Modulates Brassinosteroid Signaling. **Cell** 110: 213–222.

Liao HZ, Zhu MM, Cui HH, Du XY, Tang Y, Chen LQ, Ye D, Zhang XQ (2016) MARIS plays important roles in *Arabidopsis* pollen tube and root hair growth. **J. Integr. Plant Biol.** 58: 927–940.

Liebrand TWH, Berg GCM van den, Zhang Z, Smit P, Cordewener JHG, America AHP, Sklenar J, Jones AME, Tameling WIL, Robatzek S, Thomma BPHJ, Joosten MHAJ (2013) Receptor-like kinase SOBIR1/EVR interacts with receptor-like proteins in plant immunity against fungal infection. **Proc. Natl. Acad. Sci**. 110: 10010–10015.

Lin G, Zhang L, Han Z, Yang X, Liu W, Li E, Chang J, Qi Y, Shpak ED, Chai J (2017) A receptor-like protein acts as a specificity switch for the regulation of stomatal development. **Genes Dev.** 31: 927–938.

Lin W, Lu D, Gao X, Jiang S, Ma X, Wang Z, Mengiste T, He P, Shan L (2013a) Inverse modulation of plant immune and brassinosteroid signaling pathways by the receptor-like cytoplasmic kinase BIK1. **Proc. Natl. Acad. Sci**. 110: 12114–12119.

Lin W, Ma X, Shan L, He P (2013b) Big Roles of Small Kinases: The Complex Functions of Receptor-Like Cytoplasmic Kinases in Plant Immunity and Development. **J. Integr. Plant Biol.** 55: 1188–1197.

Liu B, Li J-F, Ao Y, Qu J, Li Z, Su J, Zhang Y, Liu J, Feng D, Qi K, He Y, Wang J, Wang HB (2012) Lysin motif-containing proteins LYP4 and LYP6 play dual roles in peptidoglycan and chitin perception in rice innate immunity. **Plant Cell** 24: 3406–3419.

Liu Y-C, Vidali L (2011) Efficient Polyethylene Glycol (PEG) Mediated Transformation of the Moss *Physcomitrella patens.* **J. Vis. Exp. JoVE.**

Liu Z, Wu Y, Yang F, Zhang Y, Chen S, Xie Q, Tian X, Zhou JM (2013) BIK1 interacts with PEPRs to mediate ethylene-induced immunity. **Proc. Natl. Acad. Sci**. 110: 6205–6210.

Lu D, Lin W, Gao X, Wu S, Cheng C, Avila J, Heese A, Devarenne TP, He P, Shan L (2011) Direct ubiquitination of pattern recognition receptor FLS2 attenuates plant innate immunity. **Science** 332: 1439–1442.

Lu D, Wu S, Gao X, Zhang Y, Shan L, He P (2010) A receptor-like cytoplasmic kinase, BIK1, associates with a flagellin receptor complex to initiate plant innate immunity. **Proc. Natl. Acad. Sci**. 107: 496–501.

Lv B, Tian H, Zhang F, Liu J, Lu S, Bai M, Li C, Ding Z (2018) Brassinosteroids regulate root growth by controlling reactive oxygen species homeostasis and dual effect on ethylene synthesis in *Arabidopsis*. **PLOS Genet.** 14: e1007144.

Ma X, Claus LAN, Leslie ME, Tao K, Wu Z, Liu J, Yu X, Li B, Zhou J, Savatin DV, Peng J, Tyler BM, Heese A, Russinova E, He P, Shan L (2020) Ligand-induced monoubiquitination of BIK1 regulates plant immunity. **Nature** 581: 199–203.

Majhi BB, Sreeramulu S, Sessa G (2019) BRASSINOSTEROID-SIGNALING KINASE5 Associates with Immune Receptors and Is Required for Immune Responses. **Plant Physiol.** 180: 1166–1184.

Mang H, Feng B, Hu Z, Boisson-Dernier A, Franck CM, Meng X, Huang Y, Zhou J, Xu G, Wang T, Shan L, He P (2017) Differential Regulation of Two-Tiered Plant Immunity and Sexual Reproduction by ANXUR Receptor-Like Kinases. **Plant Cell** 29: 3140–3156.

Mantelin S, Peng HC, Li B, Atamian HS, Takken FLW, Kaloshian I (2011) The receptor-like kinase SlSERK1 is required for Mi-1-mediated resistance to potato aphids in tomato. **Plant J. Cell Mol. Biol.** 67: 459–471.

Matschi S, Werner S, Schulze WX, Legen J, Hilger HH, Romeis T (2013) Function of calcium-dependent protein kinase CPK28 of *Arabidopsis thaliana* in plant stem elongation and vascular development. **Plant J.** 73: 883–896.

Matsubayashi Y (2002) An LRR Receptor Kinase Involved in Perception of a Peptide Plant Hormone, Phytosulfokine. **Science** 296: 1470–1472.

Matsubayashi Y, Ogawa M, Kihara H, Niwa M, Sakagami Y (2006) Disruption and Overexpression of *Arabidopsis* Phytosulfokine Receptor Gene Affects Cellular Longevity and Potential for Growth. **Plant Physiol.** 142: 45–53.

Mendy B, Wang'ombe MW, Radakovic ZS, Holbein J, Ilyas M, Chopra D, Holton N, Zipfel C, Grundler FMW, Siddique S (2017) *Arabidopsis* leucine-rich repeat receptor–like kinase NILR1 is required for induction of innate immunity to parasitic nematodes. **PLoS Pathog**. 13.

Meng X, Chen X, Mang H, Liu C, Yu X, Gao X, Torii KU, He P, Shan L (2015) Differential function of *Arabidopsis* SERK family receptor-like kinases in stomatal patterning. **Curr. Biol.** CB 25: 2361–2372.

Meng X, Zhang S (2013) MAPK Cascades in Plant Disease Resistance Signaling. **Annu. Rev. Phytopathol.** 51: 245–266.

Meng X, Zhou J, Tang J, Li B, de Oliveira MVV, Chai J, He P, Shan L (2016) Ligand-Induced Receptor-like Kinase Complex Regulates Floral Organ Abscission in *Arabidopsis*. **Cell Rep.** 14: 1330–1338.

Milhinhos A, Vera-Sirera F, Blanco-Touriñán N, Mari-Carmona C, Carrió-Seguí À, Forment J, Champion C, Thamm A, Urbez C, Prescott H, Agustí J (2019) SOBIR1/EVR prevents precocious initiation of fiber differentiation during wood development through a mechanism involving BP and ERECTA. **Proc. Natl. Acad. Sci**. 116: 18710–18716.

Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. **Trends Plant Sci.** 9: 490–498.

Miya A, Albert P, Shinya T, Desaki Y, Ichimura K, Shirasu K, Narusaka Y, Kawakami N, Kaku H, Shibuya N (2007) CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in *Arabidopsis*. **Proc. Natl. Acad. Sci**. 104: 19613–19618.

Miyazaki S, Murata T, Sakurai-Ozato N, Kubo M, Demura T, Fukuda H, Hasebe M (2009) ANXUR1 and 2, Sister Genes to FERONIA/SIRENE, Are Male Factors for Coordinated Fertilization. **Curr. Biol.** 19: 1327–1331.

Mizuno S, Osakabe Y, Maruyama K, Ito T, Osakabe K, Sato T, Shinozaki K, Yamaguchi-Shinozaki K (2007) Receptor-like protein kinase 2 (RPK 2) is a novel factor controlling anther development in *Arabidopsis thaliana*. **Plant J.** 50: 751–766.

Monaghan J, Matschi S, Shorinola O, Rovenich H, Matei A, Segonzac C, Malinovsky

FG, Rathjen JP, MacLean D, Romeis T, Zipfel C (2014) The calcium-dependent protein kinase CPK28 buffers plant immunity and regulates BIK1 turnover. **Cell Host Microbe** 16: 605–615.

Müller R, Bleckmann A, Simon R (2008) The Receptor Kinase CORYNE of *Arabidopsis* Transmits the Stem Cell–Limiting Signal CLAVATA3 Independently of CLAVATA1. **Plant Cell** 20: 934–946.

Myers C, Romanowsky SM, Barron YD, Garg S, Azuse CL, Curran A, Davis RM, Hatton J, Harmon AC, Harper JF (2009) Calcium-dependent protein kinases regulate polarized tip growth in pollen tubes. **Plant J. Cell Mol. Biol.** 59: 528–539.

Nadeau JA, Sack FD, (2002) Stomatal Development in *Arabidopsis*. **Arab. Book Am. Soc. Plant Biol.** 1.

Nam KH, Li J, (2002) BRI1/BAK1, a Receptor Kinase Pair Mediating Brassinosteroid Signaling. Cell 110: 203–212.

Nishiyama T, Fujita T, Shin-I T, Seki M, Nishide H, Uchiyama I, Kamiya A, Carninci P, Hayashizaki Y, Shinozaki K, Kohara Y, Hasebe M (2003) Comparative genomics of *Physcomitrella patens* gametophytic transcriptome and *Arabidopsis thaliana*: Implication for land plant evolution. **Proc. Natl. Acad. Sci.** 100: 8007–8012.

Nolan TM, Vukašinović N, Liu D, Russinova E, Yin Y (2020) Brassinosteroids: Multidimensional Regulators of Plant Growth, Development, and Stress Responses. **Plant Cell** 32: 295–318.

Ntoukakis V, Schwessinger B, Segonzac C, Zipfel C (2011) Cautionary Notes on the Use of C-Terminal BAK1 Fusion Proteins for Functional Studies. **Plant Cell** 23: 3871–3878.

Oehlenschlæger CB, Gersby LBA, Ahsan N, Pedersen JT, Kristensen A, Solakova TV, Thelen JJ, Fuglsang AT (2017) Activation of the LRR Receptor-Like Kinase PSY1R Requires Transphosphorylation of Residues in the Activation Loop. **Front. Plant Sci.** 8.

Ogawa M, Shinohara H, Sakagami Y, Matsubayashi Y (2008) *Arabidopsis* CLV3 Peptide Directly Binds CLV1 Ectodomain. **Science** 319: 294–294.

Oh M-H, Wang X, Kota U, Goshe MB, Clouse SD, Huber SC (2009) Tyrosine phosphorylation of the BRI1 receptor kinase emerges as a component of brassinosteroid signaling in *Arabidopsis*. **Proc. Natl. Acad. Sci. U. S. A**. 106: 658–663.

Oliver JP, Castro A, Gaggero C, Cascón T, Schmelz EA, Castresana C, Ponce de León I (2009) Pythium infection activates conserved plant defense responses in mosses. **Planta** 230: 569–579.

Ortiz-Morea FA, Reyes-Bermudez AA (2019) Endogenous Peptides: Key Modulators of Plant Immunity, in: Jogaiah, S., Abdelrahman, M. (Eds.), **Bioactive Molecules in Plant Defense: Signaling in Growth and Stress.** Springer International Publishing, Cham, pp. 159–177.

Ott T (2017) Membrane nanodomains and microdomains in plant–microbe interactions. **Curr. Opin. Plant Biol.** 40: 82–88.

Ou Y, Lu X, Zi Q, Xun Q, Zhang J, Wu Y, Shi H, Wei Z, Zhao B, Zhang X, He K, Gou X, Li C, Li J (2016) RGF1 INSENSITIVE 1 to 5, a group of LRR receptor-like kinases, are essential for the perception of root meristem growth factor 1 in *Arabidopsis thaliana*. **Cell Res**. 26: 686–698.

Pan J, Li Z, Wang Q, Yang L, Yao F, Liu W (2020) An S-domain receptor-like kinase, OsESG1, regulates early crown root development and drought resistance in rice. **Plant Sci.** 290: 110318.

Pelagio-Flores R, Muñoz-Parra E, Barrera-Ortiz S, Ortiz-Castro R, Saenz-Mata J, Ortega-Amaro MA, Jiménez-Bremont JF, López-Bucio J (2020) The cysteine-rich receptor-like protein kinase CRK28 modulates *Arabidopsis* growth and development and influences abscisic acid responses. **Planta** 251: 2.

Perraki A, DeFalco TA, Derbyshire P, Avila J, Séré D, Sklenar J, Qi X, Stransfeld L, Schwessinger B, Kadota Y, Macho AP, Jiang S, Couto D, Torii KU, Menke FLH, Zipfel C (2018) Phosphocode-dependent functional dichotomy of a common co-receptor in plant signalling. **Nature** 561: 248–252.

Pierre-Jerome E, Drapek C, Benfey PN (2018) Regulation of Division and Differentiation of Plant Stem Cells. **Annu. Rev. Cell Dev. Biol.** 34: 289–310.

Ponce de León I, Oliver JP, Castro A, Gaggero C, Bentancor M, Vidal S (2007) *Erwinia carotovora* elicitors and *Botrytis cinerea* activate defense responses in *Physcomitrella patens***. BMC Plant Biol.** 7: 52.

Ponce De León I, Schmelz EA, Gaggero C, Castro A, Álvarez A, Montesano M (2012) *Physcomitrella patens* activates reinforcement of the cell wall, programmed cell death and accumulation of evolutionary conserved defence signals, such as salicylic acid and 12-oxo-phytodienoic acid, but not jasmonic acid, upon *Botrytis cinerea* infection. **Mol. Plant Pathol.** 13: 960–974.

Postel S, Küfner I, Beuter C, Mazzotta S, Schwedt A, Borlotti A, Halter T, Kemmerling B, Nürnberger T (2010) The multifunctional leucine-rich repeat receptor kinase BAK1 is implicated in *Arabidopsis* development and immunity. **Eur. J. Cell Biol.** 89: 169–174.

Postma J, Liebrand TWH, Bi G, Evrard A, Bye RR, Mbengue M, Kuhn H, Joosten MHAJ, Robatzek S (2016) Avr4 promotes Cf-4 receptor-like protein association with the BAK1/SERK3 receptor-like kinase to initiate receptor endocytosis and plant immunity. **New Phytol.** 210: 627–642.

Prigge MJ, Bezanilla M, (2010) Evolutionary crossroads in developmental biology: *Physcomitrella patens*. **Development** 137: 3535–3543.

Pruitt RN, Schwessinger B, Joe A, Thomas N, Liu F, Albert M, Robinson MR, Chan LJG, Luu DD, Chen H, Bahar O, Daudi A, Vleesschauwer DD, Caddell D, Zhang W, Zhao X, Li X, Heazlewood JL, Ruan D, Majumder D, Chern M, Kalbacher H, Midha S, Patil PB, Sonti RV, Petzold CJ, Liu CC, Brodbelt JS, Felix G, Ronald PC (2015) The rice immune receptor XA21 recognizes a tyrosine-sulfated protein from a Gram-negative bacterium. **Sci. Adv.** 1: e1500245.

Qian P, Song W, Yokoo T, Minobe A, Wang G, Ishida T, Sawa S, Chai J, Kakimoto T (2018) The CLE9/10 secretory peptide regulates stomatal and vascular development through distinct receptors. **Nat. Plants** 4: 1071–1081.

Robatzek S, Bittel P, Chinchilla D, Köchner P, Felix G, Shiu SH, Boller T (2007) Molecular identification and characterization of the tomato flagellin receptor LeFLS2, an orthologue of *Arabidopsis* FLS2 exhibiting characteristically different perception specificities. **Plant Mol. Biol.** 64: 539–547.

Roberts AW, Dimos CS, Budziszek MJ, Goss CA, Lai V (2011) Knocking Out the Wall: Protocols for Gene Targeting in *Physcomitrella patens*, in: Popper, Z.A. (Ed.), The Plant Cell Wall: Methods and Protocols, **Methods in Molecular Biology.** Humana Press, Totowa, NJ, pp. 273–290.

Ron M, Avni A (2004) The Receptor for the Fungal Elicitor Ethylene-Inducing Xylanase Is a Member of a Resistance-Like Gene Family in Tomato. **Plant Cell** 16: 1604–1615.

Rooney HCE, Klooster JW van't, Hoorn RAL van der, Joosten MHAJ, Jones JDG, De Wit PJGM (2005) Cladosporium Avr2 Inhibits Tomato Rcr3 Protease Required for Cf-2-Dependent Disease Resistance. **Science** 308: 1783–1786.

Roux M, Schwessinger B, Albrecht C, Chinchilla D, Jones A, Holton N, Malinovsky

FG, Tör M, De Vries S, Zipfel C (2011) The *Arabidopsis* Leucine-Rich Repeat Receptor–Like Kinases BAK1/SERK3 and BKK1/SERK4 Are Required for Innate Immunity to Hemibiotrophic and Biotrophic Pathogens. **Plant Cell** 23: 2440–2455.

Sakamoto T, Deguchi M, Brustolini OJ, Santos AA, Silva FF, Fontes EP (2012) The tomato RLK superfamily: phylogeny and functional predictions about the role of the LRRII-RLK subfamily in antiviral defense. **BMC Plant Biol.** 12: 229.

Saur IML, Kadota Y, Sklenar J, Holton NJ, Smakowska E, Belkhadir Y, Zipfel C, Rathjen JP (2016) NbCSPR underlies age-dependent immune responses to bacterial cold shock protein in *Nicotiana benthamiana*. **Proc. Natl. Acad. Sci**. 113: 3389–3394.

Schaefer D, Zryd J-P, Knight CD, Cove DJ (1991) Stable transformation of the moss *Physcomitrella patens*. **Mol. Gen. Genet. MGG** 226: 418–424.

Schmidt ED, Guzzo F, Toonen MA, De Vries SC (1997) A leucine-rich repeat containing receptor-like kinase marks somatic plant cells competent to form embryos. **Development** 124: 2049–2062.

Schwizer S, Kraus CM, Dunham DM, Zheng Y, Fernandez-Pozo N, Pombo MA, Fei Z, Chakravarthy S, Martin GB (2017) The Tomato Kinase Pti1 Contributes to Production of Reactive Oxygen Species in Response to Two Flagellin-Derived Peptides and Promotes Resistance to *Pseudomonas syringae* Infection. **Mol. Plant-Microbe Interact. MPMI** 30: 725–738.

Segonzac C, Macho AP, Sanmartín M, Ntoukakis V, Sánchez-Serrano JJ, Zipfel C (2014) Negative control of BAK1 by protein phosphatase 2A during plant innate immunity. **EMBO J**. 33: 2069–2079.

Shan L, He P, Li J, Heese A, Peck SC, Nürnberger T, Martin GB, Sheen J (2008) Bacterial Effectors Target the Common Signaling Partner BAK1 to Disrupt Multiple MAMP Receptor-Signaling Complexes and Impede Plant Immunity. **Cell Host Microbe** 4: 17–27.

Shi H, Shen Q, Qi Y, Yan H, Nie H, Chen Y, Zhao T, Katagiri F, Tang D (2013) BR-SIGNALING KINASE1 Physically Associates with FLAGELLIN SENSING2 and Regulates Plant Innate Immunity in *Arabidopsis*. **Plant Cell** 25: 1143–1157.

Shi Y, Guo S, Zhang R, Meng Z, Ren M (2014) The role of Somatic embryogenesis receptor-like kinase 1 in controlling pollen production of the *Gossypium* anther. **Mol. Biol. Rep.** 41: 411–422.

Shimizu T, Nakano T, Takamizawa D, Desaki Y, Ishii-Minami N, Nishizawa Y, Minami E, Okada K, Yamane H, Kaku H, Shibuya N (2010) Two LysM receptor molecules, CEBiP and OsCERK1, cooperatively regulate chitin elicitor signaling in rice. **Plant J. Cell Mol. Biol.** 64: 204–214.

Shinohara H, Mori A, Yasue N, Sumida K, Matsubayashi Y (2016) Identification of three LRR-RKs involved in perception of root meristem growth factor in *Arabidopsis*. **Proc. Natl. Acad. Sci**. 113: 3897–3902.

Shinya T, Yamaguchi K, Desaki Y, Yamada K, Narisawa T, Kobayashi Y, Maeda K, Suzuki M, Tanimoto T, Takeda J, Nakashima M, Funama R, Narusaka M, Narusaka Y, Kaku H, Kawasaki T, Shibuya N (2014) Selective regulation of the chitin-induced defense response by the *Arabidopsis* receptor-like cytoplasmic kinase PBL27. **Plant J.** 79.

Shiu SH, Bleecker AB (2003) Expansion of the Receptor-Like Kinase/Pelle Gene Family and Receptor-Like Proteins in *Arabidopsis*. **Plant Physiol.** 132: 530–543.

Shiu SH, Bleecker AB (2001) Receptor-like kinases from *Arabidopsis* form a monophyletic gene family related to animal receptor kinases. **Proc. Natl. Acad. Sci**. 98: 10763–10768.

Shiu SH, Karlowski WM, Pan R, Tzeng YH, Mayer KFX, Li WH (2004) Comparative Analysis of the Receptor-Like Kinase Family in *Arabidopsis* and Rice. **Plant Cell** 16: 1220–1234.

Shpak ED (2013) Diverse roles of ERECTA family genes in plant development. **J. Integr. Plant Biol.** 55: 1238–1250.

Shpak ED, Berthiaume CT, Hill EJ, Torii KU (2004) Synergistic interaction of three ERECTA-family receptor-like kinases controls *Arabidopsis* organ growth and flower development by promoting cell proliferation. **Development** 131: 1491–1501.

Somssich M, Je BI, Simon R, Jackson D (2016) CLAVATA-WUSCHEL signaling in the shoot meristem. **Development** 143: 3238–3248.

Song L, Shi QM, Yang XH, Xu ZH, Xue HW (2009) Membrane steroid-binding protein 1 (MSBP1) negatively regulates brassinosteroid signaling by enhancing the endocytosis of BAK1. **Cell Res.** 19: 864–876.

Song W, Liu L, Wang J, Wu Z, Zhang H, Tang J, Lin G, Wang Y, Wen X, Li W, Han Z, Guo H, Chai J (2016) Signature motif-guided identification of receptors for peptide hormones essential for root meristem growth. **Cell Res**. 26: 674–685. Sreeramulu S,

Mostizky Y, Sunitha S, Shani E, Nahum H, Salomon D, Hayun LB, Gruetter C, Rauh D, Ori N, Sessa G (2013) BSKs are partially redundant positive regulators of brassinosteroid signaling in *Arabidopsis*. **Plant J.** 74: 905–919.

Steinbrenner AD (2020) The evolving landscape of cell surface pattern recognition across plant immune networks. **Curr. Opin. Plant Biol.** 56: 135–146.

Suarez-Rodriguez MC, Adams-Phillips L, Liu Y, Wang H, Su SH, Jester PJ, Zhang S, Bent AF, Krysan PJ (2007) MEKK1 Is Required for flg22-Induced MPK4 Activation in *Arabidopsis* Plants. **Plant Physiol.** 143: 661–669.

Sun T, Nitta Y, Zhang Q, Wu D, Tian H, Lee JS, Zhang Y (2018) Antagonistic interactions between two MAP kinase cascades in plant development and immune signaling. **EMBO Rep.** 19: e45324.

Taguchi-Shiobara F, Yuan Z, Hake S, Jackson D (2001) The fasciated ear2 gene encodes a leucine-rich repeat receptor-like protein that regulates shoot meristem proliferation in maize. **Genes Dev.** 15: 2755–2766.

Takai R, Isogai A, Takayama S, Che FS (2008) Analysis of Flagellin Perception Mediated by flg22 Receptor OsFLS2 in Rice. **Mol. Plant-Microbe Interactions** 21: 1635–1642.

Tang D, Wang G, Zhou JM (2017) Receptor Kinases in Plant-Pathogen Interactions: More Than Pattern Recognition. **Plant Cell** 29: 618–637.

Tang W, Ezcurra I, Muschietti J, McCormick S (2002) A Cysteine-Rich Extracellular Protein, LAT52, Interacts with the Extracellular Domain of the Pollen Receptor Kinase LePRK2. **Plant Cell** 14: 2277–2287.

Tang W, Kelley D, Ezcurra I, Cotter R, McCormick S (2004) LeSTIG1, an extracellular binding partner for the pollen receptor kinases LePRK1 and LePRK2, promotes pollen tube growth in vitro. **Plant J.** 39: 343–353.

Tang W, Kim TW, Oses-Prieto JA, Sun Y, Deng Z, Zhu S, Wang R, Burlingame AL, Wang ZY (2008) BSKs Mediate Signal Transduction from the Receptor Kinase BRI1 in *Arabidopsis*. **Science** 321: 557–560.

Thomas CM, Jones DA, Parniske M, Harrison K, Balint-Kurti PJ, Hatzixanthis K, Jones JD (1997) Characterization of the tomato Cf-4 gene for resistance to Cladosporium fulvum identifies sequences that determine recognitional specificity in Cf-4 and Cf-9. Plant Cell 9: 2209–2224.

Thor K, Jiang S, Michard E, George J, Scherzer S, Huang S, Dindas J, Derbyshire P, Leitão N, DeFalco TA, Köster P, Hunter K, Kimura S, Gronnier J, Stransfeld L, Kadota Y, Bücherl CA, Charpentier M, Wrzaczek M, MacLean D, Oldroyd GED, Menke FLH, Roelfsema, MRG, Hedrich R, Feijó J, Zipfel C (2020) The calcium-permeable channel OSCA1.3 regulates plant stomatal immunity. **Nature** 585: 569–573.

Tian Y, Fan M, Qin Z, Lv H, Wang M, Zhang Z, Zhou W, Zhao N, Li X, Han C, Ding Z, Wang W, Wang ZY, Bai MY (2018) Hydrogen peroxide positively regulates brassinosteroid signaling through oxidation of the BRASSINAZOLE-RESISTANT1 transcription factor. **Nat. Commun.** 9: 1063.

Tian W, Hou C, Ren Z, Wang C, Zhao F, Dahlbeck D, Hu S, Zhang L, Niu Q, Li L, Staskawicz BJ, Luan S (2019) A calmodulin-gated calcium channel links pathogen patterns to plant immunity. **Nature** 572: 131–135.

Torres MA, Dangl JL, Jones JDG (2002) *Arabidopsis* gp91phox homologues AtrbohD and AtrbohF are required for accumulation of reactive oxygen intermediates in the plant defense response. **Proc. Natl. Acad. Sci.** 99: 517–522**.**

Trdá L, Fernandez O, Boutrot F, Héloir MC, Kelloniemi J, Daire X, Adrian M, Clément C, Zipfel C, Dorey S, Poinssot B (2014) The grapevine flagellin receptor VvFLS2 differentially recognizes flagellin-derived epitopes from the endophytic growth-promoting bacterium *Burkholderia phytofirmans* and plant pathogenic bacteria. **New Phytol.** 201: 1371–1384.

Tuteja N, Mahajan S (2007) Calcium Signaling Network in Plants. **Plant Signal. Behav.** 2: 79–85.

Veronese P, Nakagami H, Bluhm B, AbuQamar S, Chen X, Salmeron J, Dietrich RA, Hirt H, Mengiste T (2006) The Membrane-Anchored BOTRYTIS-INDUCED KINASE1 Plays Distinct Roles in *Arabidopsis* Resistance to Necrotrophic and Biotrophic Pathogens. **Plant Cell** 18: 257–273.

Wang B, Fang R, Zhang J, Han J, Chen F, He F, Liu YG, Chen L (2020) Rice LecRK5 phosphorylates a UGPase to regulate callose biosynthesis during pollen development. **J. Exp. Bot.** 71: 4033–4041.

Wang G, Ellendorff U, Kemp B, Mansfield JW, Forsyth A, Mitchell K, Bastas K, Liu CM, Woods-Tör A, Zipfel C, de Wit PJGM, Jones JDG, Tör M, Thomma BPHJ (2008) A Genome-Wide Functional Investigation into the Roles of Receptor-Like Proteins in *Arabidopsis*. **Plant Physiol.** 147: 503–517.

Wang J, Li H, Han Z, Zhang H, Wang T, Lin G, Chang J, Yang W, Chai J (2015) Allosteric receptor activation by the plant peptide hormone phytosulfokine. **Nature** 525: 265–268.

Wang L, Albert M, Einig E, Fürst U, Krust D, Felix G (2016) The pattern-recognition receptor CORE of Solanaceae detects bacterial cold-shock protein. **Nat. Plants** 2: 16185.

Wang R, He F, Ning Y, Wang G-L (2020) Fine-Tuning of RBOH-Mediated ROS Signaling in Plant Immunity. **Trends Plant Sci.**

Wang W, Bai MY, Wang ZY (2014) The brassinosteroid signaling network—a paradigm of signal integration. **Curr. Opin. Plant Biol., SI: Cell signalling and gene regulation** 21: 147–153.

Wang W, Feng B, Zhou J, Tang D (2020) Plant immune signaling: Advancing on two frontiers. **J. Integr. Plant Biol.** 62: 2–24.

Wellman CH, Osterloff PL, Mohiuddin U (2003) Fragments of the earliest land plants. **Nature** 425: 282–285.

Willmann R, Lajunen HM, Erbs G, Newman MA, Kolb D, Tsuda K, Katagiri F, Fliegmann J, Bono JJ, Cullimore JV, Jehle AK, Götz F, Kulik A, Molinaro A, Lipka V, Gust AA, Nürnberger T (2011) *Arabidopsis* lysin-motif proteins LYM1 LYM3 CERK1 mediate bacterial peptidoglycan sensing and immunity to bacterial infection. **Proc. Natl. Acad. Sci**. 108: 19824–19829.

Wolf S, Does D van der, Ladwig F, Sticht C, Kolbeck A, Schürholz AK, Augustin S, Keinath N, Rausch T, Greiner S, Schumacher K, Harter K, Zipfel C, Höfte H (2014) A receptor-like protein mediates the response to pectin modification by activating brassinosteroid signaling. **Proc. Natl. Acad. Sci**. 111: 15261–15266.

Wu S, Shan L, He P (2014) Microbial signature-triggered plant defense responses and early signaling mechanisms. **Plant Sci., Disease resistance: molecular mechanisms and biotechnological applications** 228: 118–126.

Xu F, Jackson D (2018) Learning from CIK plants. **Nat. Plants** 4: 195–196.

Xu P, Xu SL, Li ZJ, Tang W, Burlingame AL, Wang ZY (2014) A Brassinosteroid-Signaling Kinase Interacts with Multiple Receptor-Like Kinases in *Arabidopsis*. **Mol. Plant** 7: 441–444.

Xue DX., Li CL, Xie ZP, Staehelin C (2019) LYK4 is a component of a tripartite chitin

receptor complex in *Arabidopsis thaliana*. **J. Exp. Bot.** 70: 5507–5516.

Xun Q, Wu Y, Li H, Chang J, Ou Y, He K, Gou X, Tax FE, Li J (2020) Two receptor-like protein kinases, MUSTACHES and MUSTACHES-LIKE, regulate lateral root development in *Arabidopsis thaliana*. **New Phytol.** 227: 1157–1173.

Yamada K, Yamaguchi K, Shirakawa T, Nakagami H, Mine A, Ishikawa K, Fujiwara M, Narusaka M, Narusaka Y, Ichimura K, Kobayashi Y, Matsui H, Nomura Y, Nomoto M, Tada Y, Fukao Y, Fukamizo T, Tsuda K, Shirasu K, Shibuya N, Kawasaki T (2016) The *Arabidopsis* CERK1-associated kinase PBL27 connects chitin perception to MAPK activation. **EMBO J.** 35: 2468–2483.

Yamaguchi Y, Huffaker A, Bryan AC, Tax FE, Ryan CA (2010) PEPR2 Is a Second Receptor for the Pep1 and Pep2 Peptides and Contributes to Defense Responses in *Arabidopsis*. **Plant Cell** 22: 508–522.

Yamaguchi Y, Pearce G, Ryan CA (2006) The cell surface leucine-rich repeat receptor for AtPep1, an endogenous peptide elicitor in *Arabidopsis*, is functional in transgenic tobacco cells. **Proc. Natl. Acad. Sci**. 103: 10104–10109.

Yang SL, Xie LF, Mao HZ, Puah CS, Yang WC, Jiang L, Sundaresan V, Ye D (2003) TAPETUM DETERMINANT1 Is Required for Cell Specialization in the *Arabidopsis* Anther. **Plant Cell** 15: 2792–2804.

Yin Y, Wang Z-Y, Mora-Garcia S, Li J, Yoshida S, Asami T, Chory J (2002) BES1 Accumulates in the Nucleus in Response to Brassinosteroids to Regulate Gene Expression and Promote Stem Elongation. **Cell** 109: 181–191.

Yokota T, Ohnishi T, Shibata K, Asahina M, Nomura T, Fujita T, Ishizaki K, Kohchi T (2017) Occurrence of brassinosteroids in non-flowering land plants, liverwort, moss, lycophyte and fern. **Phytochemistry** 136: 46–55.

Yu X, Feng B, He P, Shan L (2017) From Chaos to Harmony: Responses and Signaling upon Microbial Pattern Recognition. **Annu. Rev. Phytopathol.** 55: 109–137.

Zhang H, Lin X, Han Z, Wang J, Qu L-J, Chai J (2016) SERK Family Receptor-like Kinases Function as Co-receptors with PXY for Plant Vascular Development. **Mol. Plant** 9: 1406–1414.

Zhang J, Li W, Xiang T, Liu Z, Laluk K, Ding X, Zou Y, Gao M, Zhang X, Chen S, Mengiste T, Zhang Y, Zhou JM (2010) Receptor-like Cytoplasmic Kinases Integrate Signaling from Multiple Plant Immune Receptors and Are Targeted by a *Pseudomonas syringae* Effector. **Cell Host Microbe** 7: 290–301.

Zhang L, Kars I, Essenstam B, Liebrand TWH, Wagemakers L, Elberse J, Tagkalaki P, Tjoitang D, van den Ackerveken G, van Kan JAL (2014) Fungal Endopolygalacturonases Are Recognized as Microbe-Associated Molecular Patterns by the *Arabidopsis* Receptor-Like Protein RESPONSIVENESS TO BOTRYTIS POLYGALACTURONASES1. **Plant Physiol.** 164: 352–364.

Zhang W, Fraiture M, Kolb D, Löffelhardt B, Desaki Y, Boutrot FFG, Tör M, Zipfel C, Gust AA, Brunner F (2013) *Arabidopsis* RECEPTOR-LIKE PROTEIN30 and Receptor-Like Kinase SUPPRESSOR OF BIR1-1/EVERSHED Mediate Innate Immunity to Necrotrophic Fungi. **Plant Cell** 25: 4227–4241.

Zhao DZ, Wang GF, Speal B, Ma H (2002) The EXCESS MICROSPOROCYTES1 gene encodes a putative leucine-rich repeat receptor protein kinase that controls somatic and reproductive cell fates in the *Arabidopsis* anther. **Genes Dev.** 16: 2021–2031.

Zheng B, Bai Q, Wu L, Liu H, Liu Y, Xu W, Li G, Ren H, She X, Wu G (2019) EMS1 and BRI1 control separate biological processes via extracellular domain diversity and intracellular domain conservation. **Nat. Commun**. 10: 4165.

Zhu JY, Sae-Seaw J, Wang ZY (2013) Brassinosteroid signalling. **Development** 140: 1615–1620.

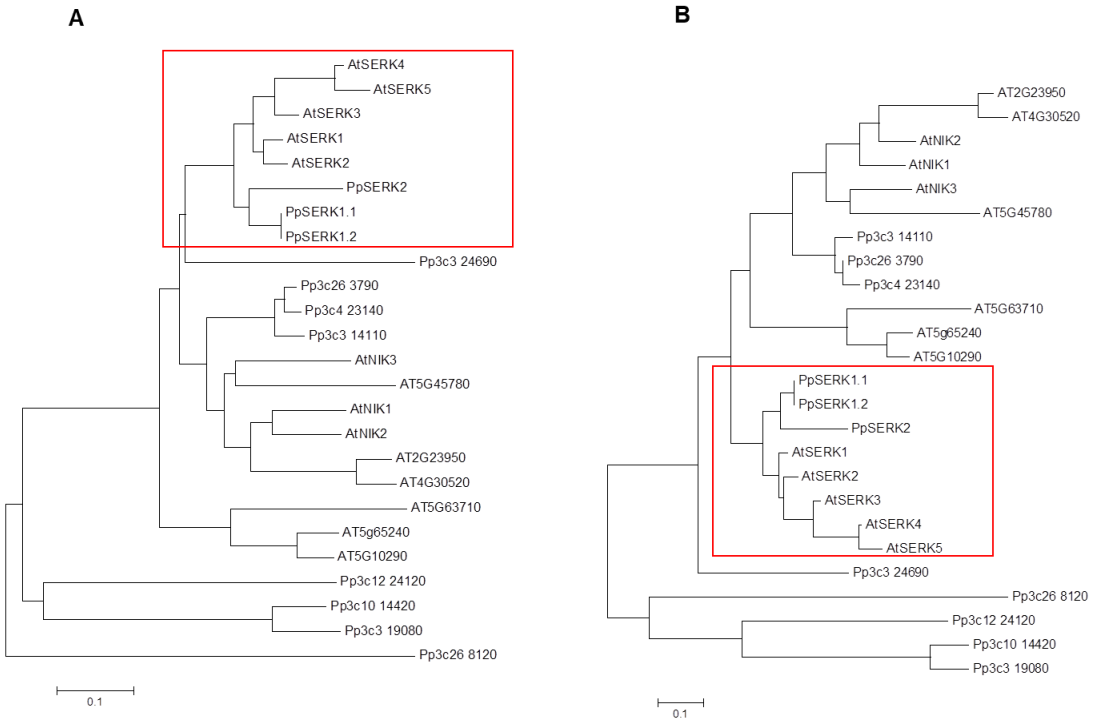
Zhu Y, Hu C, Gou X (2020) Receptor-like protein kinase-mediated signaling in controlling root meristem homeostasis. **aBIOTECH** 1: 157–168.

Zipfel C (2008) Pattern-recognition receptors in plant innate immunity. **Curr. Opin. in Immunol.** 20: 10-16.

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

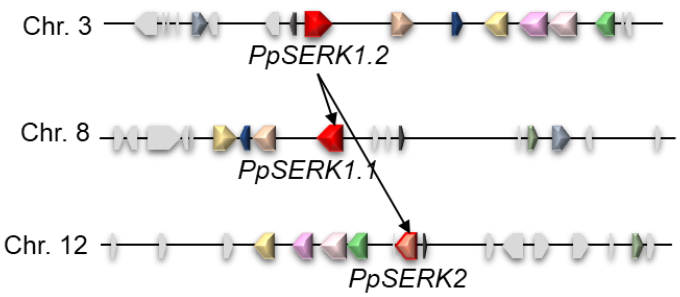
APPENDIX A

SUPPLEMENTAL FIGURES



Supplemental figure 1. Identification of SERKs in *Physcomitrella patens*. (A) Neighbor-Joining (NJ) tree based on the alignment of the LRR Class II family of *Arabidopsis thaliana* and putative LRR Class II family of *Physcomitrella patens* amino acid sequence. (B) Maximum likelihood (ML) tree based on the alignment of the LRR Class II family of *Arabidopsis thaliana* and putative LRR Class II family of *Physcomitrella patens* amino acid sequence.

A



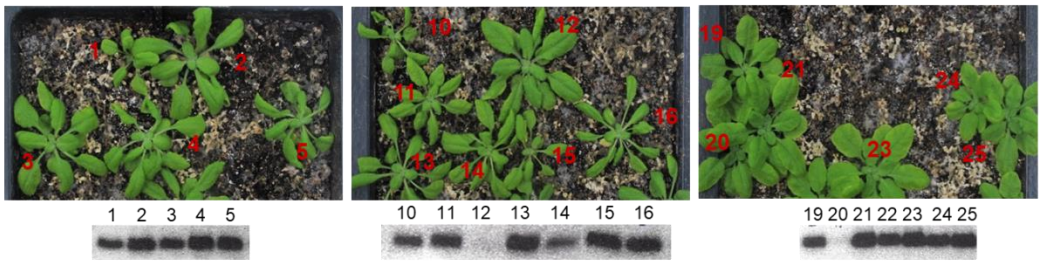
B

	PpSERK1.2	
	Gene	Homology
PpSERK1.1	1	78%
	2	93%
	3	81%
	4	95%
	5	62%
PpSERK2	4	97%
	1	74%
	7	71%
	8	75%
	9	55%

	PpSERK1.1	
	Gene	Homology
PpSERK2	6	57%
	1	71%
	4	97%

Supplemental figure 2. Evolution of SERKs in *Physcomitrella patens*. (A) Schematic diagram of PpSERKs chromosomes region. Genes upstream and downstream of PpSERKs with the same color indicate they have a high identity with each other. Genes in gray indicate that the similarity among them was not significant. (B) Summary tables indicate the percentage of identity between genes localized upstream and downstream PpSERKs. The amino acid sequence of at least 10 genes upstream and downstream of each PpSERK were retrieved and blasted against each other.

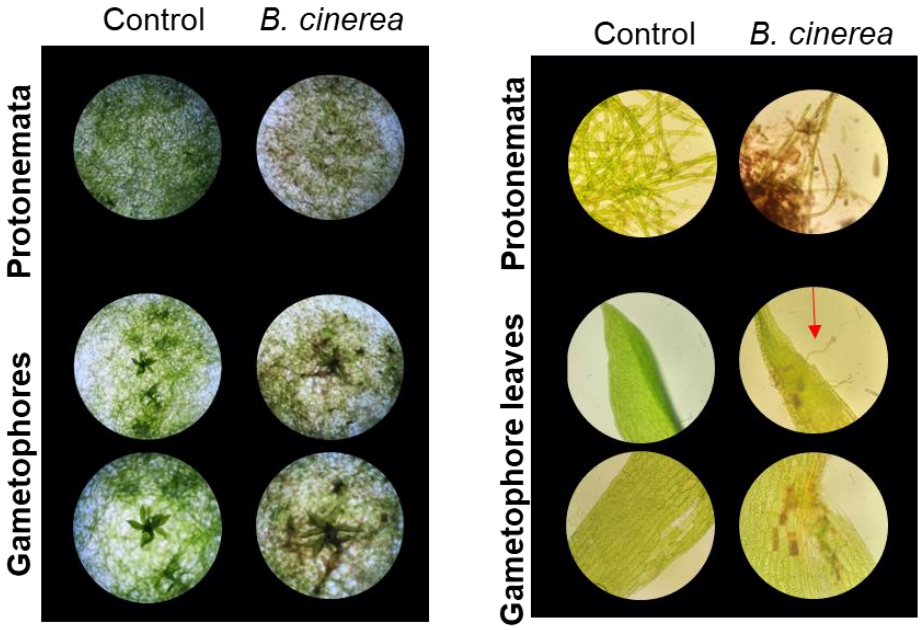
A



B

Transgenic cascades introduced into *serk1-1/bak1-4*	Numbers of T0 lines obtained after Basta spray	Numbers of lines with PpSERK protein expression positive	Numbers of lines resembling BRI1-GFP transgenic plants	% of lines resembling BRI1-GFP transgenic plants
35S::PpSERK1.1-HA	9	Yes	7	80%
35S::PpSERK1.2-HA	9	Yes	8	90%
35S::PpSERK2-HA	10	Yes	None	0%

Supplemental figure 3. PpSERK1.1 and PpSERK1.2 play a role in BR-mediated *Arabidopsis* growth and development. (A) Multiple transgenic lines carrying 35S::PpSERK1.1-HA or 35S::PpSERK1.2-HA in the serk4-1/bak1-4 background from the T0 generation grown in soil resemble BRI1-GFP overexpression phenotypes with elongated and curling petioles. Notably, 35S::PpSERK1.2-HA transgenic plants show more pronounced petiole elongation compared to 35S::PpSERK1.1-HA transgenic plants. All transgenic lines carrying 35S::PpSERK2-HA resemble the ser4-1/bak1-4 mutant with shorter petioles, rounder leaves, and dwarf architecture compared to WT plants. Protein expressions were shown below by WB using anti-HA antibodies. (B) The summary table shows percentages of T0 transgenic lines carrying 35S::PpSERKs-HA in the serk1-1bak1-4 background resembling BRI1-GFP transgenic plants. 80% to 90% of transgenic lines carrying 35S::PpSERK1.1-HA or 35S::PpSERK1.2-HA serk1-1bak1-4 with positive PpSERK protein expressions at the T0 stage show similar growth phenotypes as BRI1-GFP transgenic plants. Despite the high protein expression, all transgenic plants carrying 35S::PpSERK2-HA in the serk4-1bak1-4 background resemble the serk4-1bak1-4 mutant plants.



Supplemental figure 4. *B. cinerea* infection in *Physcomitrella* WT protonemata and gametophores. WT *Physcomitrella* tissues are susceptible to the pathogen, exhibiting a brownish tissue resembling cell death. Microscopy of the protonemata and gametophore leaves indicate that the presence of the pathogen leads to cell death.