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Decoding small peptides: Regulators of plant growth and stress resilience[oo]

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ABSTRACT

Small peptides (SPs) are pivotal signaling molecules that play essential roles in the precise regulation of plant growth, development, and stress responses. Recent advancements in sequencing technologies, bioinformatics approaches, and biochemical and molecular techniques have significantly enhanced the accuracy of SP identification, unveiling their diverse biological functions in plants. This review provides a comprehensive overview of the characteristics and methodologies for identifying SPs in plants. It highlights recent discoveries regarding the biological roles and signaling pathways of SPs in regulating plant growth, development, and plant–microbial interactions, as well as their contributions to plant resilience under various environmental stresses, including abiotic stress, nutrient deficiencies, and biotic challenges. Additionally, we discuss current insights into the potential applications of SPs and outline future research directions aimed at leveraging these molecules to enhance plant adaptation to environmental challenges. By integrating recent findings, this review lays a foundation for advancing the understanding and utilization of SPs to improve plant resilience and productivity.

Keywords: abiotic and biotic stress responses, crop improvement, plant developmental regulation, plant–microbe interactions, small peptide signaling

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INTRODUCTION

As sessile multicellular organisms, plants are continuously exposed to diverse abiotic and biotic stresses throughout their life cycle. To survive and adapt to these dynamic environments, they have evolved sophisticated signaling networks to perceive external stimuli and trigger appropriate responses. These networks include wellcharacterized components such as cell-surface receptors, mitogen-activated protein kinase (MAPK) signaling cascades, transcription factors (TFs), phytohormones, and reactive oxygen species (ROS) (Zhang et al., 2022; Zhou et al., 2022; Leisner et al., 2023). Recently, small peptides (hereafter SPs) have emerged as important modulators within these pathways, where they play roles in stress response and adaptive growth, contributing to plant resilience and reproductive success (Matsubayashi, 2014; Ge et al., 2017; Datta et al., 2024; Lalun et al., 2024; Lu and Xiao, 2024; Chang and Xiao, 2025).

Small peptides are encoded by short open reading frames (sORFs), typically fewer than 300 nucleotides in length (Hellens et al., 2016; Couso and Patraquim, 2017; Ong et al., 2022; Chen et al., 2023a). These peptides are generally

categorized into conventional peptides (CPs) and non-conventional peptides (NCPs). Conventional peptides are derived from well-characterized coding regions or conventional open reading frames (ORFs) (Tavormina et al., 2015; Olsson et al., 2019), while NCPs originate from untranslated or noncoding regions (Couso and Patraquim, 2017; Wang et al., 2020a, 2023a; Chen et al., 2023a). This distinction leads to greater structural diversity in NCPs compared to the more conserved CPs (Ong et al., 2022; Chen et al., 2023a). Biologically, SPs regulate diverse physiological processes that are essential for plant growth, development, and responses to environmental cues, as well as interactions with microbes (Takahashi et al., 2018; Laffont et al., 2020; Datta et al., 2024; Lu and Xiao, 2024; Chang and Xiao, 2025). Examples of well-known CPs within SPs include Systemin, CLAVATA3/Embryo Surrounding Region (CLE) peptides, C-terminally encoded peptides (CEP), PHYTOSULFOKINE (PSK), and RAPID ALKALINIZATION FACTOR (RALF) peptides. Acting as hormone-like signaling molecules, these peptides facilitate both intercellular communication and long-distance signaling (Tavormina et al., 2015; Takahashi et al., 2019), thereby coordinating development and enhancing resilience to stress (Tabata et al., 2014; Ohkubo et al., 2017; Takahashi et al., 2018; Yang et al., 2024; Chang and Xiao, 2025). Despite advancements in understanding CPs, the roles of NCPs, particularly those derived from untranslated regions (UTRs) or long noncoding RNAs, remain poorly understood (Andrews and Rothnagel, 2014; Yin et al., 2019; Chen et al., 2023a), especially in plant species beyond well-studied models such as *Arabidopsis thaliana* (hereafter, *Arabidopsis*) and *Oryza sativa* (rice).

Recent technological advances, particularly in omics-based sequencing techniques, mass spectrometry (MS), and gene editing technologies, have significantly enhanced our ability to identify and investigate previously unknown SPs (Wang et al., 2020a, 2020b; Chen et al., 2023a). These advancements have provided valuable insights into plant biology and present promising opportunities for improving crop resilience and productivity. Nonetheless, several practical challenges continue to hinder the full realization of bioactive peptides' potential in agriculture. These challenges include the need for efficient screening methods and rigorous functional validation, along with ensuring their stability and efficacy under field conditions.

This review explores the characteristics of SPs and recent strategies for their identification in plants. We examine their roles in regulating plant growth, development, stress responses, and the molecular mechanisms involved. Critical advances in leveraging SPs to enhance plant resilience to environmental stresses are highlighted, emphasizing their agricultural applications. We also address current challenges in SP research and propose future directions for developing innovative approaches to boost plant resilience, which is crucial for global food security in the context of climate change. This review offers a valuable resource for SP research and underscores its significance for promoting agricultural sustainability.

CHARACTERISTICS AND CLASSIFICATION OF SPs

Small peptides are short-chain biological molecules typically consisting of 2–100 amino acids linked by peptide bonds (Couso and Patraquim, 2017; Ong et al., 2022; Chen et al., 2023a; Chang and Xiao, 2025). They can be broadly classified into CPs and NCPs, also referred to as sORF-encoded peptides or micropeptides (Couso and Patraquim, 2017; Wang et al., 2020a; Chen et al., 2023a). Conventional peptides and NCPs differ not only in their origins but also in their biosynthetic pathways and their roles in various physiological processes (Chen et al., 2023a; Wang et al., 2023a; Chang and Xiao, 2025). Conventional peptides tend to be longer, typically exceeding 80 amino acids, due to their derivation from large precursor proteins that require extensive post-translational modifications (PTMs) (Aspden et al., 2014; Matsubayashi, 2014; Chen et al., 2020b). In contrast, NCPs are generally shorter, often less than 20 amino acids, as they are translated directly from sORFs without complex post-translational processing (Andrews and Rothnagel, 2014; Aspden et al., 2014; Chen et al., 2023a; Wang et al., 2023a).

Peptide size is strongly correlated with both sequence conservation and translation efficiency (Aspden et al., 2014; Matsubayashi, 2014; Tavormina et al., 2015). Longer peptides, especially CPs, exhibit greater sequence conservation and higher translation efficiency due to selective evolutionary pressure and functional specialization in plant signaling and defense (Aspden et al., 2014; Matsubayashi, 2014). In comparison, shorter NCPs are often less conserved, more diverse, and dynamically expressed, frequently lacking clear bioinformatic signatures due to their varied regulatory roles in plant growth and stress responses (Andrews and Rothnagel, 2014; Yin et al., 2019; Chen et al., 2023a). This size-related distinction may affect the stability, localization, and interaction potential of SPs, with longer peptides more likely to have specific receptor-binding capabilities, while shorter peptides are often involved in rapid, transient signaling processes (Tavormina et al., 2015; Chang and Xiao, 2025).

Conventional peptides

Conventional peptides are typically produced through the proteolytic cleavage of larger precursor proteins, a process that often involves the removal of an N-terminal signal sequence (NSS) or a pro-domain (Matsubayashi, 2014; Olsson et al., 2019; Chen et al., 2020b; Hu et al., 2021). Based on their structural characteristics, biosynthetic pathways, and biological functions, CPs can be broadly classified into three major groups: PTM peptides, cysteine-rich peptides (CRPs), and non-cysteine-rich/non-PTM peptides (Figure 1; Matsubayashi, 2014; Tavormina et al., 2015; Olsson et al., 2019; Chang and Xiao, 2025).

Post-translationally modified peptides

Post-translationally modified peptides are small signaling molecules typically consisting of five to 20 amino acids and are distinguished by their PTMs, such as tyrosine sulfation, proline

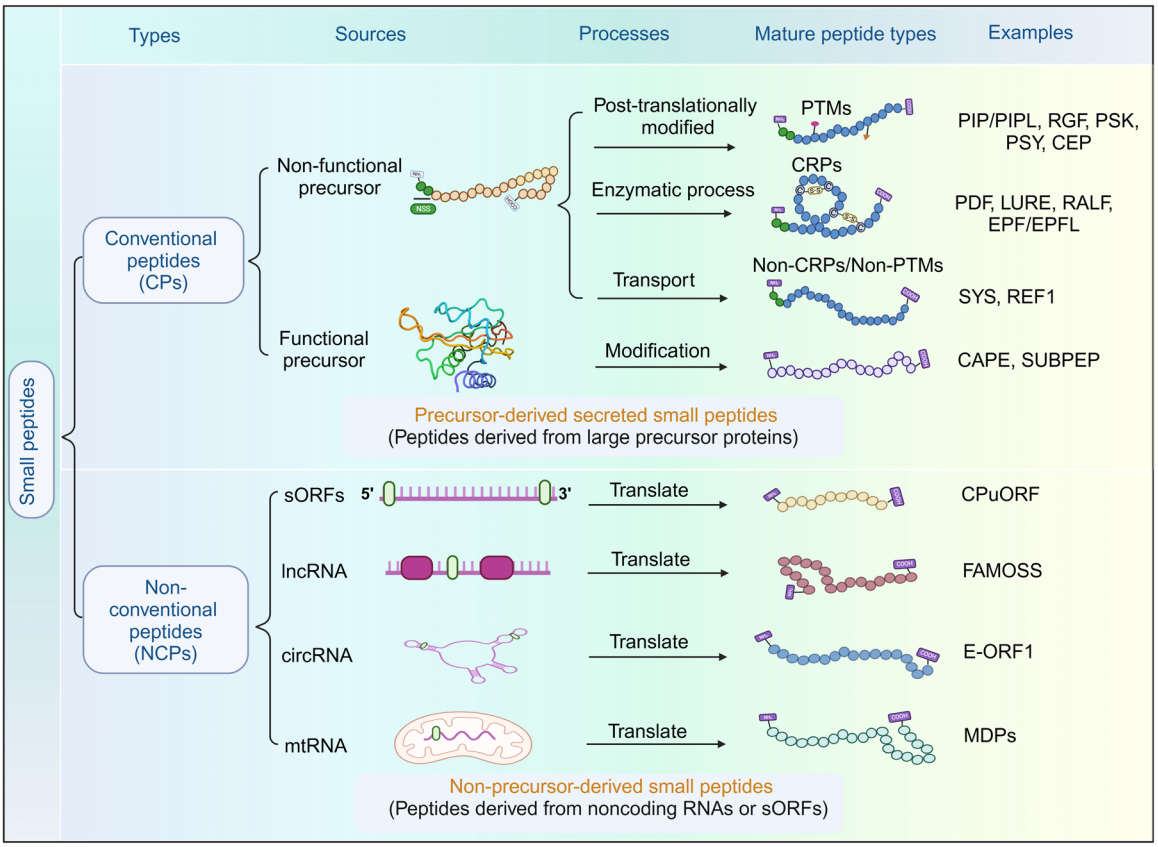

Figure 1. The diversity of small peptides (SPs) in plants

Plant SPs exhibit a wide range of origins and classifications. They are categorized into conventional peptides (CPs) and non-conventional peptides (NCPs). Conventional peptides are derived from large precursor proteins, which may be functional or non-functional. Functional precursor peptides carry out their biological roles after being transported to specific locations within the plant, whereas non-functional precursor peptides may undergo post-translational modifications and enzymatic processing. These non-functional precursor peptides are further classified into those with post-translational modifications (PTMs), cysteine-rich peptides (CRPs), and non-cysteine-rich/non-PTM peptides. In contrast, NCPs, also known as sORF-encoded or micro peptides, originate from sORFs, lncRNAs, circRNAs, and mtRNAs. Several representative SPs are listed within the different peptide categories. CEP, C-terminally encoded peptide; PDF, plant defensins; SYS, Systemin; REF1, Regeneration Factor 1; CAPE, CAP-Derived Peptide; EPF/EPFL, epidermal patterning factor/like; PIP/PIPL, PAMP-induced secreted peptide/like; PSK, Phytosulfokine; CEP, C- terminally encoded peptide; PSK, phytosulfokine; RGF, root growth factor; PSY, plant peptide containing sulfated tyrosine; SUBPEP, subtilase peptide; sORF, small open reading frame; CPuORF, conserved peptide upstream open reading frame; FAMOSS, FAst-growing MOSS; E-ORF1, ELVd-ORF1; MDPs, mitochondrial-derived peptides; lncRNAs, long noncoding RNAs; circRNAs, circular RNAs; mtRNAs, mitochondrial RNAs.

hydroxylation, hydroxyproline arabinosylation, or glycosylation (Myllyharju, 2003; Komori et al., 2009; Ogawa-Ohnishi et al., 2013; Tavormina et al., 2015). These modifications enhance the stability and specificity of peptide–receptor interactions, thereby enabling precise regulation of plant growth and defense responses (Tavormina et al., 2015; Olsson et al., 2019; Takahashi et al., 2019; Chang and Xiao, 2025). Prominent examples of PTM peptides include CLAVATA3 (CLV3), CEP1, Plant Peptide Containing Sulfated Tyrosine 1 (PSY1), and ROOT MERISTEM GROWTH FACTOR 1 (RGF1) in *Arabidopsis*. These peptides play critical roles in maintaining plant cell identity, promoting organogenesis, and enabling responses to environmental stimuli (Takahashi et al., 2018; Shao et al., 2020; Fang et al., 2024; Chang and Xiao, 2025).

Cysteine-rich peptides

Cysteine-rich peptides are a diverse class of SPs characterized by their high cysteine content, typically containing 2 to 16 cysteine residues that form disulfide bonds (Tavormina et al., 2015; Liu et al., 2017; Olsson et al., 2019). These disulfide bonds stabilize the mature, active conformations of CRPs, enabling them to maintain structural integrity under challenging extracellular conditions (Matsubayashi, 2014; Olsson et al., 2019). The formation and arrangement of disulfide bonds are key determinants of CRP structural diversity, directly influencing their functional specificity (Liu et al., 2017; Olsson et al., 2019). Prominent examples of CRPs in plants include peptides involved in defense (e.g., plant defensins, antimicrobial peptides (AMPs), and thionins), lipid transport (e.g., non-specific lipid transfer proteins, ns-LTPs), and reproduction and development (e.g., LURE peptides, RALFs, EPIDERMAL PATTERNING FACTOR (EPF), and EPF-LIKE (EPFL) peptides) (Takeuchi and Higashiyama, 2016; Ge et al., 2017; Kawamoto et al., 2020; Erdem Büyükkiraz and Kesmen, 2022; Lu and Xiao, 2024).

The structural differences in CRPs underpin their functional versatility in plant defense, reproduction, and symbiotic

interactions (Gao et al., 2000; Matsubayashi, 2014; Erdem Büyükkiraz and Kesmen, 2022). For example, the rigid structures of plant defensins, stabilized by multiple disulfide bonds, enable them to disrupt pathogenic membranes, often by forming ion-permeable pores (Broekaert et al., 1995). In contrast, CRPs like LURE peptides, with more flexible structural motifs, guide pollen tube growth by interacting with receptor kinases on the surface of pollen tubes, ensuring precise ovule targeting (Takeuchi and Higashiyama, 2016). Similarly, CRPs involved in nutrient exchange during symbiotic interactions possess finely tuned structures essential for the molecular recognition of microbial receptors and the binding specificity required for symbiosis (Sankari et al., 2022). The ability of CRPs to form and maintain disulfide bonds not only reinforces their structural stability but also allows for functional adaptability, making them essential for signaling and molecular recognition during dynamic biotic interactions.

Non-cysteine-rich/non-PTM peptides

The third category of CPs comprises non-cysteine-rich/non-PTM peptides, which lack both an NSS in their precursor proteins and extensive PTMs (Tavormina et al., 2015; Olsson et al., 2019; Chen et al., 2020b; Hu et al., 2021; Chang and Xiao, 2025). Instead, these peptides may achieve biological activity through intrinsic structural properties, compensating for the absence of external targeting signals. This category includes peptides that are either directly derived from precursor proteins through proteolytic processing or adopt bioactive conformations without requiring cysteine-rich disulfide bonds or PTM-based modifications (Olsson et al., 2019; Chen et al., 2020b; Hu et al., 2021; Chang and Xiao, 2025).

A notable example of this group is the REF1 peptide in tomato, which is derived from the precursor protein SlPep (Yang et al., 2024). The REF1 peptide (ATDRRGRPPSRPK VGSGPPPQNN) contains specific sequence features that are hypothesized to contribute to its biological function. Basic residues (arginine, R; lysine, K) may facilitate electrostatic interactions with the receptor PORK1, enhancing binding specificity and affinity. Additionally, proline (P) residues, particularly within the PPPQ motif, may provide localized structural rigidity, stabilizing the peptide's active conformation. In contrast, glycine (G)-rich segments (e.g., GRP, VGSG) are speculated to promote flexibility, allowing the peptide to adapt to the PORK1 binding interface. Collectively, these features are consistent with the view that REF1 may function as a local wound signal, promoting callus formation and shoot regeneration in plants (Yang et al., 2024). However, these proposed mechanisms require further experimental validation to confirm their role in REF1-mediated signaling.

Non-cysteine-rich/non-PTM peptides can also achieve biological activity through proteolytic activation of pro-peptides, a process that involves the removal of a conserved pro-domain by specific proteases. This pro-domain functions as an inhibitory region that maintains the precursor peptide in an inactive state. Proteolytic activation is catalyzed by subtilisin-like serine proteases (SBTs), metalloendopeptidases, and cysteine proteases, among others. Among these, Xylem Cysteine Peptidase 1 (XCP1) plays a pivotal role due to its strict substrate selectivity. XCP1 specifically recognizes and cleaves the conserved CNYD motif in the precursor Pathogenesis-Related Protein 1 (PR1), releasing the functional peptide CAPE9, a key regulator of plant systemic immunity (Chen et al., 2023b). This precise recognition and cleavage ensure the efficient production of active CAPE9, which triggers downstream immune responses. The activity of XCP1 is tightly controlled by environmental factors, particularly pH and calcium (Ca^{2+}) levels, which regulate its proteolytic capacity. Optimal cleavage occurs in the apoplast, where the pH is more acidic, and the Ca^{2+} concentration is higher than in the cytosol (Chen et al., 2023b). This environmental regulation ensures that XCP1-mediated cleavage occurs only under specific physiological conditions, allowing for the precise, context-dependent activation of CPs. By controlling the spatio-temporal activation of XCP1, plants can ensure a rapid and efficient response to environmental stimuli, such as pathogen attack or mechanical damage (Chen et al., 2023b).

REF1 and CAPE9 exemplify two distinct mechanisms of peptide activation. REF1 may achieve bioactivity through sequence-driven structural adaptability, while CAPE9 relies on protease-mediated pro-domain removal. These mechanisms highlight the versatility of non-cysteine-rich/non-PTM peptides in plant biology. By utilizing distinct activation pathways, these peptides facilitate crucial processes in plant development, stress response, underscoring their functional diversity and adaptive capacity (Tavormina et al., 2015; Hu et al., 2021; Chang and Xiao, 2025).

Non-conventional peptides

In contrast to CPs, NCPs are directly translated from sORFs containing fewer than 100 codons (Hu et al., 2021; Chen et al., 2023a; Chang and Xiao, 2025). These sORFs originate from previously annotated noncoding genomic regions, including intergenic regions, introns, 5' and 3' UTRs, long noncoding RNAs (lncRNAs), and circular RNAs (circRNAs) (Figure 1; Couso and Patraquim, 2017; Wang et al., 2020a; Chen et al., 2023a; Chang and Xiao, 2025). Unlike CPs, which are typically derived from larger precursor proteins (Matsubayashi, 2014; Tavormina et al., 2015; Olsson et al., 2019; Hu et al., 2021), NCPs are translated *de novo* from noncoding sequences, a feature that has garnered increasing attention due to the growing recognition that NCPs play critical regulatory roles in plant development, stress responses, and signaling pathways (Couzigou et al., 2016; Sharma et al., 2020; Chen et al., 2022, 2023a; Yu et al., 2025). Recent advances in high-throughput sequencing, ribosome profiling (Ribo-seq), and bioinformatics have facilitated the identification of numerous NCPs in plants (Aspden et al., 2014; Chen et al., 2020a, 2023a; Wang et al., 2020a; Yu et al., 2025). Despite their typically low expression levels and small molecular size, numerous NCPs have been shown to possess significant biological activity (Chen et al., 2020a, 2023a; Wang et al., 2020a; Yu et al.,

2025). Experimental evidence from MS and molecular validation supports the existence and functionality of thousands of NCPs, many of which are now cataloged in specialized databases, such as NCPbook (Sami et al., 2024). Recent studies have demonstrated that NCPs are involved in a variety of biological processes, with several well-characterized examples highlighting their regulatory potential in growth, development, and defense mechanisms (Lauressergues et al., 2022; Chen et al., 2023a; Chang and Xiao, 2025; Yu et al., 2025).

One such example is microRPG1, a 31-amino acid NCP identified in maize (*Zea mays*) using quantitative trait locus mapping (Yu et al., 2025). MicroRPG1 is unique to the Zea genus and is derived *de novo* from a noncoding sequence (Yu et al., 2025). It plays a pivotal role in regulating the kernel dehydration rate (KDR), a key trait for maize kernel maturation. Functional studies revealed that overexpression of microRPG1 slows KDR, while its knockout accelerates the process. Interestingly, the exogenous application of synthetic microRPG1 influences KDR not only in maize but also in *Arabidopsis*, suggesting a conserved regulatory mechanism for dehydration control (Yu et al., 2025). Further analysis showed that microRPG1 regulates ethylene signaling genes involved in KDR control (Yu et al., 2025), highlighting the potential of NCPs to act as key modulators of plant hormone signaling pathways.

Another prominent class of NCPs is the microRNA-encoded peptides (miPEPs). Unlike other NCPs, miPEPs are translated from sORFs embedded within the primary transcripts (pri-miRNAs) of microRNAs (Lauressergues et al., 2015, 2022; Chen et al., 2023a; Chang and Xiao, 2025). These miPEPs regulate their corresponding miRNAs through a positive feedback loop, promoting the transcription of pri-miRNAs and increasing mature miRNA levels, thereby modulating downstream gene expression (Lauressergues et al., 2015, 2022). For instance, in *Medicago truncatula* (*M. truncatula*), the miPEP miPEP171b enhances the transcription of *pri-miR171b*, leading to the accumulation of mature miR171b, which is associated with root development (Lauressergues et al., 2015). Functional studies demonstrated that overexpression of *miPEP171b* increases *pri-miR171b* transcription, while mutations in the sORF initiation codon abolish this effect. Notably, the exogenous application of synthetic miPEP171b triggers a similar response, demonstrating its role as a regulator of miRNA biosynthesis (Lauressergues et al., 2015).

Beyond miPEPs, other classes of NCPs have also been functionally characterized in plants. For example, certain NCPs, such as miPEP858a in *Arabidopsis* and miPEP172c in soybean (*Glycine max*), regulate key developmental processes, including nodulation and flavonoid biosynthesis, demonstrating their diverse regulatory roles (Couzigou et al., 2016; Sharma et al., 2020). Additionally, other NCPs derived from lncRNAs, circRNAs, and intergenic sORFs have been identified in plants, further emphasizing the structural diversity of plant NCPs (Wang et al., 2023a; Sami et al., 2024; Chang and Xiao, 2025). These NCPs exhibit a wide range of biological functions, including growth regulation, stress adaptation, and hormone signaling. Their ability to act as modulators of developmental pathways and stress responses underscores their essential role in plant biology. Collectively, these findings highlight the growing recognition of NCPs as a crucial layer of regulatory molecules in plants, offering new opportunities to explore their roles in plant growth, stress response, and environmental adaptation.

PREDICTION AND IDENTIFICATION OF SPs

Each plant genome encodes numerous peptides, yet the majority remain putative due to limited information regarding their mature structure and biological function (Andrews and Rothnagel, 2014; Yin et al., 2019; Wang et al., 2020a). Accurately predicting and identifying functional SPs is challenging for several reasons. First, current plant genome annotations frequently overlook sORFs, which are likely to encode SPs (Yin et al., 2019; Chen et al., 2023a). Second, the low conservation within peptide families reduces the effectiveness of homology-based search approaches. Third, the rules governing peptide processing, and the incorporation of PTMs are poorly defined. To address these challenges, researchers are developing systematic strategies for SP identification by combining bioinformatic prediction methods with rigorous experimental validation (Figure 2; Chen et al., 2020a, 2023a; Wang et al., 2020a). To date, several vital SPs have been identified, yet many remain to be discovered and characterized to fully understand their roles in plant biology.

Identifying secreted SPs involves several key steps, as illustrated in Figure 2A. The process begins with the application of bioinformatics tools specifically designed to identify potential secreted SPs in plant genomes. These tools are crucial for screening and predicting precursor proteins or sORFs ranging from 10 to 100 amino acids, which are considered to have secretory potential (Lu and Xiao, 2024). Commonly used prediction tools include sORF Finder, Seqkit, HAltORF, and SPADA (Hanada et al., 2010; Zhou et al., 2013; Chen et al., 2023a; Wang et al., 2023a; Lu and Xiao, 2024). Among these, sORF Finder is particularly effective for detecting putative sORFs in plant genomes, particularly those ranging from 10 to 100 amino acids, which are critical for identifying SPs that may be secreted (Hanada et al., 2010). Once these proteins or sORFs are identified, further analysis is carried out using tools such as SignalP, which predicts NSS, a key feature involved in protein sorting for secretion (Petersen et al., 2011). Additional tools, such as TMMHMM, are employed to exclude transmembrane domains (Krogh et al., 2001), while analysis of ER domains helps to eliminate proteins associated with endoplasmic reticulum docking. This multi-step bioinformatics approach leads to the identification of putative secreted SPs for subsequent validation (Lu and Xiao, 2024). In addition to these computational tools, multi-omics approaches, including transcriptomics and high-resolution MS-based proteomics, are frequently utilized

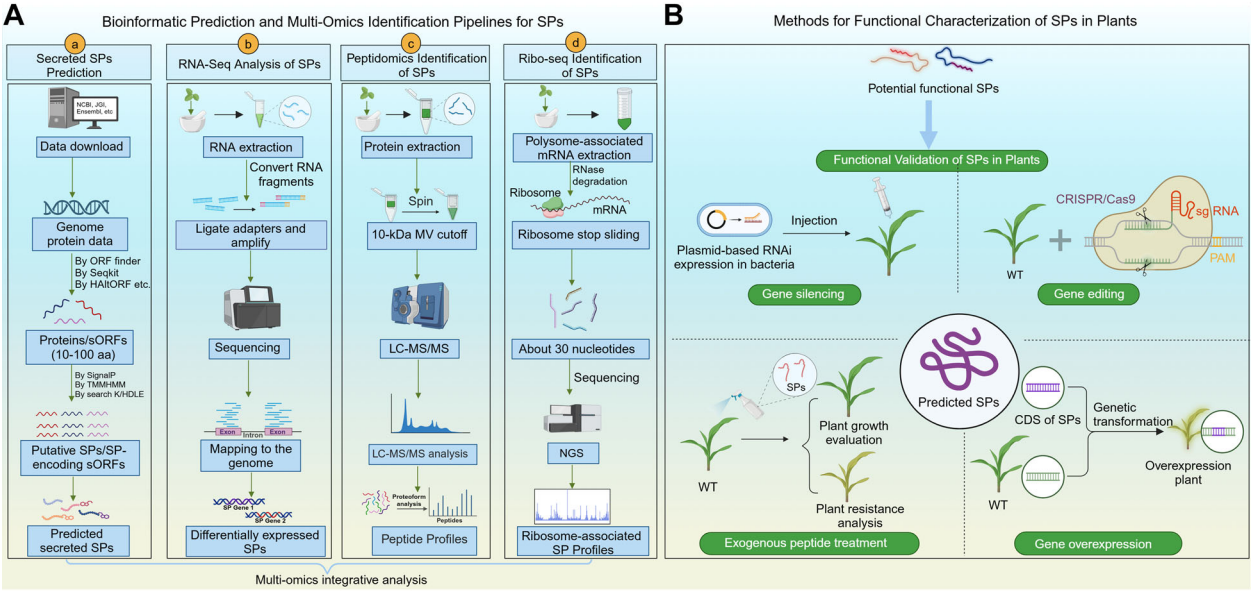

Figure 2. Strategies for prediction, identification, and functional validation of small peptides (SPs)

(A) Bioinformatic prediction and multi-omics identification pipelines for SPs. The genome of the plant species is initially sequenced. Recent bioinformatics methods for peptide identification follow a comprehensive approach. Proteins or small open reading frames (sORFs) ranging from 10 to 100 amino acids are first predicted based on size criteria using tools such as Seqkit and ORFfinder. SignalP is then used to predict N-terminal signal sequence, while TMMHMM identifies and excludes transmembrane domains. Endoplasmic reticulum (ER) domains are targeted to remove proteins associated with ER docking. This process helps identify putative secreted SPs for further validation. A similar strategy is applied to identify noncoding small secreted peptides, starting with the identification of potential SP-encoding sORFs, followed by validation using predictive tools. In the multi-omics identification pipeline, total RNA is extracted from diverse plant samples and subjected to RNA-seq to identify differentially expressed SP genes. Total proteins are extracted from diverse plant samples via centrifugation, followed by the selective isolation of SPs using a 10-kDa molecular weight cutoff. Utilizing a peptidomics approach in combination with LC-MS/MS and *de novo* search strategies, these SPs are systematically screened and characterized to determine their unique profiles and potential functional roles. For Ribo-seq, ribosome–RNA complexes are separated by centrifugation, treated with RNase I to generate ribosome-protected fragments, and subsequently analyzed for ribosome-associated SP profiles using NGS and bioinformatics tools. Finally, potential functionally active SPs are identified through the integration of multi-omics analytical strategies. **(B)** Functional validation of SPs in plants. Biochemical and molecular biology techniques, such as gene silencing, gene editing, exogenous peptide treatment, and gene overexpression, are utilized to elucidate the functions of SPs. LC-MS/MS, liquid chromatography-tandem mass spectrometry; Ribo-seq, ribosome profiling; RNA-seq, RNA sequencing; NGS, next-generation sequencing; MW cutoff, molecular weight cutoff.

to screen for functional peptides with coding potential (Wang et al., 2020a, 2020b, 2023a). For example, transcriptomics and proteomics screenings have successfully identified 236 small, secreted peptides involved in rice immunity (Wang et al., 2020b).

Ribo-seq is a cutting-edge technique for comprehensively identifying translated sORFs across the genome (Aspden et al., 2014; Wang et al., 2020a; Yu et al., 2025). This method not only measures translational regulation and efficiency but also correlates it with messenger RNA (mRNA) abundance from RNA-seq data (Aspden et al., 2014). Ribo-seq operates by isolating ribosome-protected fragments (RPFs) that are resistant to RNase degradation, which are then sequenced using next-generation sequencing (NGS). These fragments, approximately 30 nucleotides in length, are aligned with RNA sequences to precisely pinpoint ribosome positions during translation (Aspden et al., 2014). Wang et al. (2020a) integrated genomic approaches with Ribo-seq and peptidomics to identify 1,993 novel NCPs in maize, highlighting their potential roles in gene regulation and crop domestication. Their findings demonstrated that most of these NCPs were detected through ribosome profiling, underscoring the power of Ribo-seq in identifying actively translated sORFs. However, although Ribo-seq provides strong evidence of translation, it does not confirm functional protein production. Therefore, experimental validation, including MS-based identification, is essential for accurately verifying and quantifying proteins and peptides translated by sORFs (Fabre et al., 2021).

Mass spectrometry is a robust technique for the direct detection and quantification of both annotated and novel (unannotated) proteins and peptides (Fabre et al., 2021). The critical stages of MS research encompass protein extraction and enrichment, enzymatic digestion, MS identification, and the subsequent validation of SPs (Figure 2A). However, traditional MS protocols encounter challenges in detecting SPs due to their degradation susceptibility from peptidases and interference from degradation products of other proteins. To address these issues, optimized protocols, such as adjusting lysis conditions to facilitate protein precipitation and denature endogenous peptidases, have been established (Wang et al., 2020a). Additionally, selective protein fractionation techniques, such as using specific-sized filter columns or sodium dodecyl sulfate – polyacrylamide gel electrophoresis for low molecular weight fractions, are employed. For instance, Wang et al. (2020a) employed

a 10-kDa molecular weight cutoff centrifuge filter for high-resolution, accurate-mass liquid chromatography-tandem MS (LC-MS/MS) analysis, successfully identifying numerous NCPs in maize and *Arabidopsis*. Mass spectrometry is frequently coupled with RNA-seq or Ribo-seq to enhance SP identification, allowing researchers to validate SPs across various plant species using published datasets (Wang et al., 2020a, 2020b; Yu et al., 2025).

In addition to high-throughput prediction and identification methods, validating the biological functions of SPs often necessitates detailed experiments at the individual gene level, like those used for conventional coding genes (Figure 2B). Reverse genetics is a widely utilized strategy in plants for validating predicted peptide-coding genes. This approach begins with a target gene and examines the resulting phenotypic changes following targeted gene mutations. Techniques such as gene overexpression, knockout, knockdown, and clustered regularly interspaced small palindromic repeats-mediated gene editing are commonly employed (Figure 2B). By analyzing the phenotypic alterations induced by these genetic modifications, researchers can determine the functional role of the target peptide, thereby providing critical evidence for the functionality of peptide-coding genes.

SMALL PEPTIDES REGULATE PLANT GROWTH AND DEVELOPMENT

Approximately 30 peptide families have been identified, with notable examples including RGF, PSK, and CLE peptides. These peptides are synthesized as inactive precursors and subsequently undergo PTMs, such as cleavage and sulfation, to become biologically active (Matsubayashi, 2014; Olsson et al., 2019; Chen et al., 2020b; Hu et al., 2021; Chang and Xiao, 2025). Upon binding to specific cell-surface receptors, these SPs activate signaling pathways that regulate various developmental processes, underscoring their critical roles in plant growth and adaptation (Takahashi et al., 2018, 2019; Zhang et al., 2019; Lu and Xiao, 2024). This section focuses on the roles of SPs in regulating three key aspects of plant development: root development, stomatal differentiation, and reproductive growth (Figures 3–5). It is also important to note that SPs are involved in many other developmental processes. A comprehensive overview of these additional roles is provided in Table 1.

Small peptides regulate plant root development

Small peptides are essential regulators of root development in plants, influencing critical processes such as root apical

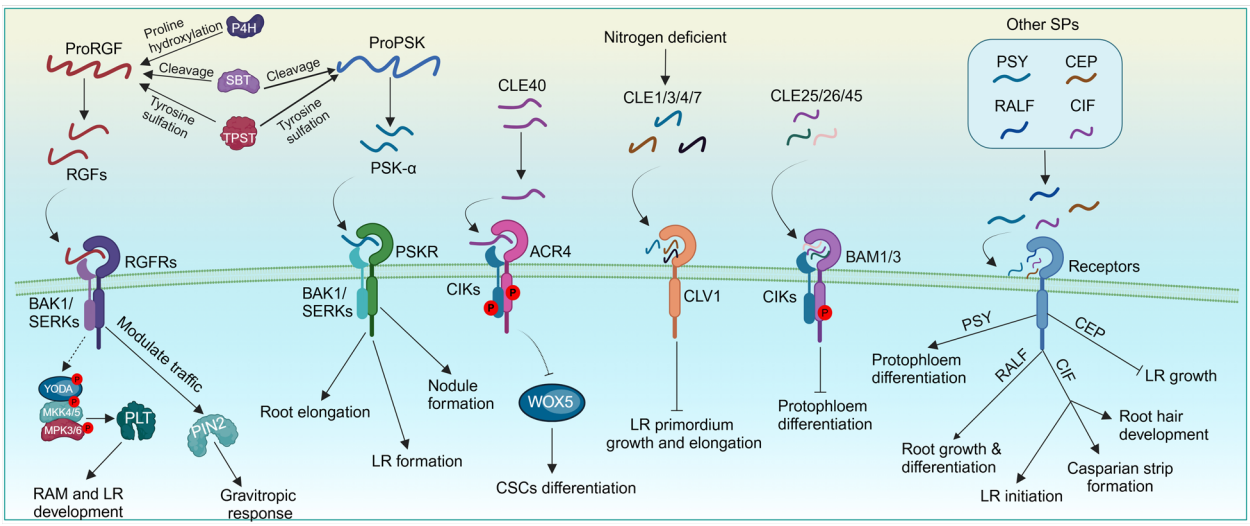

Figure 3. Signaling pathways regulating root development by small peptides (SPs)

This figure illustrates the diverse signaling pathways of SPs, including RGF/GLV, PSK, and CLE, in regulating root development. RGF peptides are synthesized as precursors that undergo key post-translational modifications (PTMs) such as cleavage by subtilases (SBTs), proline hydroxylation by P4H, and tyrosine sulfation by tyrosylprotein sulfotransferases (TPSTs) to become active. Mature RGF peptides bind to receptors (RGFR1, RGFR2, RGFR3), activating the YODA-MKK4/5-MPK3/6 cascade, which modulates PLT expression crucial for maintaining the RAM and lateral root (LR) development. Additionally, RGF/GLV signaling affects PIN2 trafficking, facilitating auxin distribution for gravitropic responses. Similarly, PSK peptides are cleaved and sulfated to become bioactive, acting through the PSKR and co-receptors to promote root elongation and nodulation, thus enhancing root system adaptability and benefiting crop growth. CLE peptides regulate stem cell maintenance and differentiation in the RAM and LR development in response to nutrient availability. Specifically, CLE25, CLE26, and CLE45 induce the heterodimerization of BAM1/3 and CIKs, activating the receptor complex through phosphorylation to suppress protophloem differentiation. Conversely, CLE40 binds to the ACR4–CIK receptor complex, leading to the phosphorylation of CIKs by ACR4, which subsequently promotes the differentiation of CSCs.These mechanisms collectively contribute to the adaptability of the root system. Additionally, peptides such as PSY, CEP, RALF and CIF regulate various aspects of root development, including elongation, LR formation, and tissue differentiation. P4H, Prolyl-4-Hydroxylase; YODA, A MAPKKK; RAM, root apical meristem; PLT, PLETHORA; PIN2, PIN-FORMED2; PSKR, PSK receptor; BAM1/3, BARELY ANY MERISTEM1/3 (BAM1/3); CIKs, CLAVATA3 INSENSITIVE KINASEs; CSCs, columella stem cells; PSY1, plant peptide containing sulfated tyrosine 1; CIF, Casparian strip integrity factor; ACR4, receptor kinase *ARABIDOPSIS* CRINKLY4.

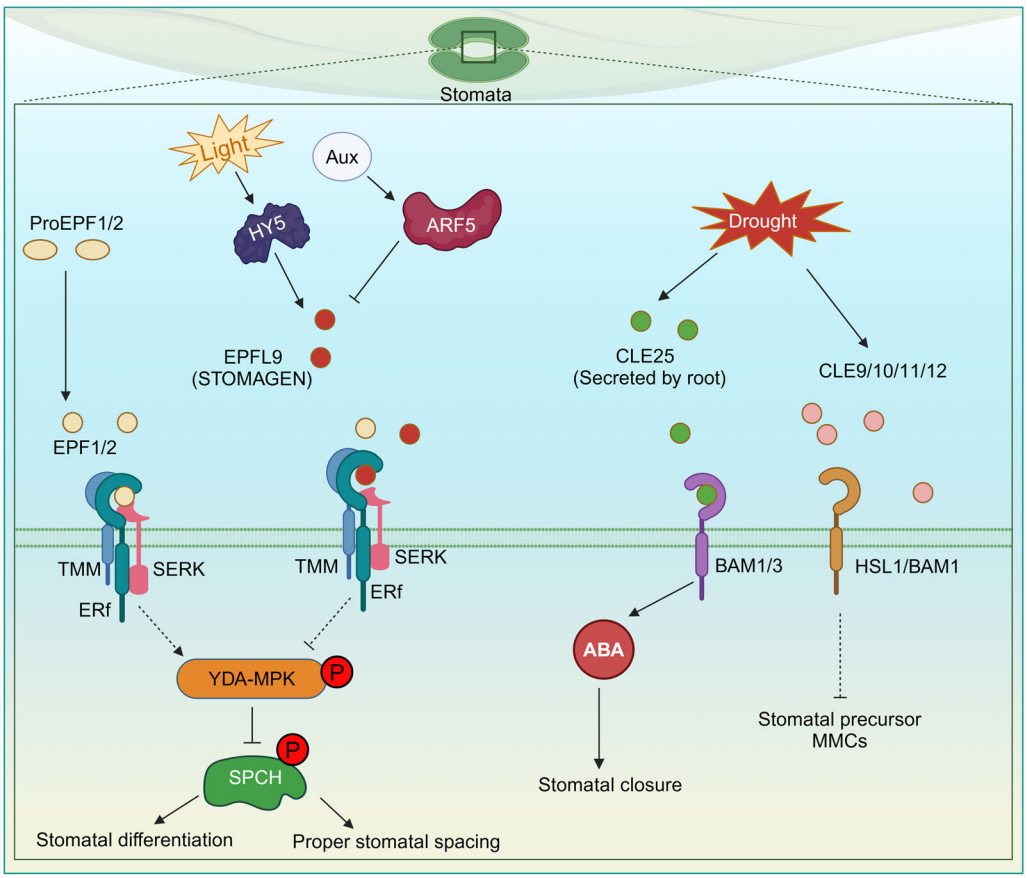

Figure 4. Signaling pathways and functions of small peptides (SPs) in plant stomatal development

This diagram illustrates the roles of epidermal patterning factor/epidermal patterning factor-like (EPF/EPFL) and CLE peptide signaling in *Arabidopsis* stomatal development. EPF1 and EPF2 act as negative regulators by binding to ERECTA family receptors (ERf) and the co-receptor, TOO MANY MOUTHS (TMM), activating the YDA-MAPK cascade, which phosphorylates and inhibits the transcription factor SPCH, thereby preventing stomatal precursor differentiation. In contrast, EPFL9 (also known as STOMAGEN) serves as a positive regulator by competing with EPF2 for ERf binding, thereby inhibiting YDA-MAPK activation and relieving repression of SPCH to promote stomatal formation. This interplay between inhibitory and stimulatory signals finely tunes stomatal density, balancing gas exchange with water conservation. Additionally, environmental factors, such as light and auxin, further modulate EPFL9 expression, integrating external cues into the regulation of stomatal development. Furthermore, CLE peptides, including CLE25, are secreted in response to drought and transported to the leaves, where they promote stomatal closure through the accumulation of ABA. CLE9/10, CLE11, and CLE12 inhibit the formation of stomatal precursor MMCs, underscoring their role in regulating stomatal density. ABA, abscisic acid; ERf, ERECTA family receptors; SPCH, SPEECHLESS; MMCs, meristemoid mother cells.

meristem (RAM) maintenance, lateral root (LR) initiation, and root elongation (Matsuzaki et al., 2010; Meng et al., 2012; Shao et al., 2020; Kaufmann et al., 2021; Lu and Xiao, 2024). Several well-characterized SP families specifically regulate each of these processes, playing distinct roles in coordinating root growth and development. Below, we discuss the functions of these SP families in modulating these critical aspects of root development.

ROOT MERISTEM GROWTH FACTOR 1/GLV/CLEL peptides

ROOT MERISTEM GROWTH FACTOR 1, also known as GOLVEN (GLV) or CLE-like (CLEL) peptides, are a family of 13-amino-acid signaling molecules critical for both RAM maintenance and LR development in *Arabidopsis* (Matsuzaki et al., 2010; Meng et al., 2012; Shao et al., 2020). These peptides undergo essential PTMs, including cleavage by signal peptidases and subtilases (SBTs), proline hydroxylation by prolyl-4-hydroxylase (P4H), and tyrosine sulfation by tyrosylprotein sulfotransferases (TPSTs) (Figure 3; Matsuzaki et al., 2010; Matsubayashi, 2014; Tavormina et al., 2015; Chang and Xiao, 2025). These modifications enable their binding to receptors such as RGFR1, RGFR2, and RGFR3, which are predominantly expressed in the RAM (Ou et al., 2016; Shinohara et al., 2016; Song et al., 2016). Upon binding, these receptors recruit co-receptors such as SERK1, SERK2, and SERK3, forming a stable receptor complex that activates downstream MAPK signaling pathways, particularly the YODA-MKK4/5-MPK3/6 cascade (Figure 3; Song et al., 2016; Lu et al., 2020; Shao et al., 2020; Ou et al., 2022). Through this pathway, RGF/GLV peptides regulate the expression of PLETHORA (PLT) TFs, which are crucial for maintaining root meristem activity and stem cell populations (Figure 3; Matsuzaki et al., 2010; Ou et al., 2016; Shinohara et al., 2016; Song et al., 2016; Lu et al., 2020; Shao et al., 2020). RGF peptides regulate both RAM

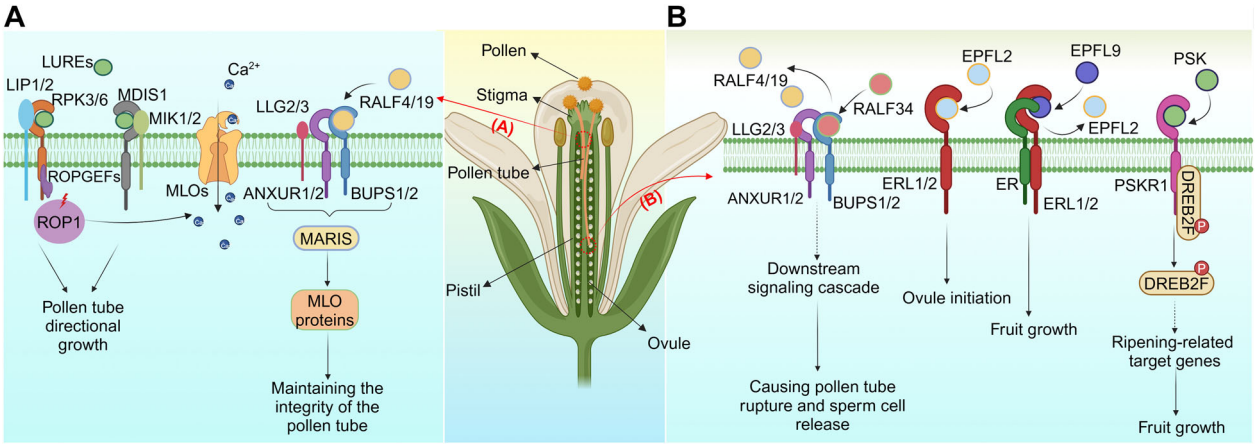

Figure 5. Signaling pathways of small peptides (SPs) in pollen tube growth, fertilization, and fruit development

(A) RALF4 and RALF19 regulate pollen tube growth by interacting with BUPS1/2 receptors, which form complexes with ANXUR1/2 (ANX1/2). LLG2 and LLG3 act as co-receptors, enhancing the receptor complex activity. This binding activates the MARIS signaling pathway and Mildew Resistance Locus O (MLO) proteins, leading to Ca^{2+} influx, which maintains pollen tube integrity and prevents premature rupture as the pollen tube elongates toward the ovule. Meanwhile, LURE peptides secreted by synergid cells guide pollen tubes to the ovule micropyle by forming a gradient sensed through the MDIS1–MIK1/MIK2 receptor complex. LURE1 binding induces receptor dimerization, activating MIK1 kinase and downstream signaling. PRK6 amplifies LURE signaling by interacting with ROPGEFs to activate ROP1, driving cytoskeletal remodeling. LIP1 and LIP2 stabilize the receptor complex for micropylar guidance and ensure efficient signal transmission to ROPGEFs and PRK6. This cascade reorganizes actin and microtubules, enabling pollen tube reorientation along the gradient. LURE signaling redistributes PRK6, MDIS1, and LIP1/2 to the pollen tube tip, enhancing sensitivity and promoting fertilization. **(B)** RALF34 competes with RALF4/19 for binding to the BUPS1/2–ANXUR1/2 complex, initiating a downstream signaling cascade that triggers pollen tube rupture and releases sperm cells for fertilization. EPFL2 and EPFL9 regulate ovule initiation and fruit growth through the ERECTA family receptors (ER, ERL1, ERL2). EPFL2 ensures proper ovule spacing, preventing twin ovule formation, while EPFL9 promotes fruit elongation. PSK signals through PSKR1 to regulate fruit development by phosphorylating DREB2F, which activates ripening-related target genes. This pathway is critical for pollen germination, tube growth, and subsequent fruit ripening. BUPS1/2, Buddha's Paper Seal 1/2; LLG2/3, LORELEI-like GPI-anchored proteins; RALF, rapid alkalinization factor; ANXUR1/2 (ANX1/2), ANXUR Receptor-like Kinase 1/2; MARIS, a receptor-like kinase critical for pollen tube integrity; EPFL2/EPFL9, EPIDERMAL PATTERNING FACTOR-LIKE 2/9; ER, ERECTA receptor kinase; ERL1/2, ERECTA-Like 1/2 receptor kinases; PSK, phytosulfokine; PSKR1/2, Phytosulfokine Receptor 1/2; DREB2F, dehydration-responsive element-binding protein 2F; MDIS1, Male Discoverer 1; MIK1/MIK2, MDIS1-Interacting Kinase 1/2; PRK6, Pollen Receptor Kinase 6; ROPGEF, Rho of plant guanine nucleotide-exchange factor; ROP1, Rho-Related GTPase from Plants 1; LIP 1/2, Lost In Pollen tube guidance 1/2.

maintenance and LR development, suggesting a coordinated role with PLT TFs across both processes. In the RAM, the RGF gradient aligns with the PLT gradient to stabilize PLT expression, ensuring continuous root growth. Similarly, during LR development, RGF signaling controls PLT activity to regulate root cell proliferation and differentiation (Lu et al., 2020; Shao et al., 2020).

The importance of RGF peptides in root development is further underscored by the severe defects observed in *rgi1,2,3,4,5* mutants, which exhibit impaired root meristem function and fail to respond to RGF signaling (Ou et al., 2016; Shao et al., 2020). During LR formation, eight of the 11 *GLV* genes, including *GLV6* and *GLV10*, are expressed early in LR primordium development (Meng et al., 2012; Fernandez et al., 2015). Overexpression of *GLV6* disrupts nuclear migration in LR founder cells (LRFCs), leading to abnormal cell division and reduced LR density (Fernandez et al., 2015). *GLV6*, functioning as an autocrine signal in the xylem pole pericycle (XPP), regulates cell patterning, with its overexpression in this tissue further supporting its role in LR development (Meng et al., 2012; Fernandez et al., 2015). Moreover, RGF peptides also influence root gravitropism by modulating the trafficking of PIN-FORMED2 (PIN2), a protein critical for establishing the asymmetric auxin distribution required for gravitropic responses (Figure 3; Whitford et al., 2012; Xu et al., 2023). This modulation may also contribute to the reduced LR densities observed in *GLV* overexpression lines. Collectively, RGF peptides play an integrated role in regulating both root meristem maintenance and LR development by controlling the expression and activity of PLT TFs. This dual function underscores their critical role in adapting root architecture to environmental changes, thereby optimizing nutrient acquisition through dynamic regulation of root stem cell populations and LR formation.

PHYTOSULFOKINE peptides

While RGF peptides primarily regulate root meristem maintenance and LR initiation, PSK peptides, a pentapeptide sulfated at two tyrosine residues, complement these functions by promoting root elongation and nodulation, ensuring that the root system can adapt to developmental and environmental cues (Figure 3; Matsubayashi and Sakagami, 1996; Kutschmar et al., 2009; Zhang et al., 2025). PSK peptides are also processed through tyrosine sulfation and proteolytic cleavage, catalyzed by TPSTs and SBTs (Matsubayashi and Sakagami, 1996; Kutschmar et al., 2009; Kaufmann et al., 2021). In *Arabidopsis*, PSKs enhance root elongation by binding to the PSKR receptors (Matsubayashi and Sakagami,

Table 1. Typical SPs involved in plant growth and development

Peptide	Plant	Precursor	Key receptor	Function	References
RGF	*Arabidopsis*	proRGF/ GLV/CLEL	RGFR1 RGFR2 RGFR3 RGI1-5	Regulates RAM maintenance by stabilizing PLETHORA (PLT) transcription factors via the YODA-MAPK signaling cascade; controls lateral root development by modulating PLT activity in LRFCs to coordinate cell division and differentiation; regulates root gravitropism by controlling PIN2 trafficking; regulates root elongation and cell division via a pH-dependent mechanism, enhancing these processes in acidic conditions and reducing RAM activity under alkaline conditions.	Matsuzaki et al. (2010); Shinohara et al. (2016); Song et al. (2016); Ou et al. (2016); Meng et al. (2012); Whitford et al. (2012); Fernandez et al. (2015); Lu et al. (2020); Shao et al. (2020); Liu et al. (2022); Xu et al. (2023)
PSK	*Asparagus officinalis*, *Arabidopsis*, *M. truncatula*, *S. lycopersicum*	proPSK	PSKR1 PSKR2	Induces cells proliferation; Promotes root elongation, lateral root initiation, and nodulation through enhanced cell expansion and division in the RAM; regulates pollen germination and tube growth by activating the PSKR1–BAK1 complex and synergizing with RALF signaling; facilitates fruit ripening and improves fruit quality traits via the PSKR1-DREB2F signaling pathway.	Matsubayashi and Sakagami (1996); Kutschmar et al. (2009); Stührwohldt et al. (2015); Kaufmann et al. (2021); Zhang et al. (2024, 2025); Fang et al. (2024)
CLE40	*Arabidopsis*	proCLE40	ACR4 CIKs	Regulates CSC differentiation by suppressing WOX5 expression. Binds to ACR4–CIK1-4 receptor complex to promote CSC differentiation. Loss-of-function mutants exhibit CSC overproliferation, highlighting its role in balancing stem cell maintenance and differentiation.	Stahl et al. (2009); Stahl et al. (2013); Zhu et al. (2021a);
CLE25 CLE26	*Arabidopsis*	proCLE25 proCLE26	BAM1 BAM3	Regulates protophloem development by inhibiting premature protophloem differentiation.	Ren et al. (2019); Qian et al. (2022); Hu et al. (2022); Hardtke (2023)
CLE45	*Arabidopsis*	proCLE45	BAM3	Regulates protophloem differentiation and root growth under acidic conditions; pH-dependent interaction with BAM3 promotes growth inhibition.	Diaz-Ardila et al. (2023); Hardtke (2023)
CLE1 CLE3 CLE4 CLE7	*Arabidopsis*	proCLE	CLV1	Regulates LR primordium development under nitrogen-deficient conditions. These CLEs inhibit LR growth and elongation enabling the plant to adapt its root system architecture to fluctuating nutrient availability.	Araya et al. (2014)
CLV3	*Arabidopsis*	proCLV3	CLV1 CLV2	Regulates SAM size and fruit development. Maintains stem cell homeostasis in the SAM and controls carpel and ovule development in fruits, ensuring proper fruit size and seed production.	Brand et al. (2000); Xu et al. (2015); Kwon et al. (2022)
PSY	*Arabidopsis*	proPSY	PSYR1	Promotes root elongation by binding to PSYRs, alleviating the inhibitory effects of these receptors on root growth. This signaling ensures proper cell elongation for root system development.	Amano et al. (2007); Ogawa-Ohnishi et al. (2022); Yimer et al. (2023)
CEP	*Arabidopsis*, *M. truncatula*	proCEP	CEPR1 CEPR2	Regulates LR growth under N or sucrose deficiency. By binding to CEPR1/2 receptors, CEPs inhibit LR elongation by reducing cell size and cell number,	Tabata et al. (2014); Zhu et al. (2021b)

Continued

Table 1. Continued

Peptide	Plant	Precursor	Key receptor	Function	References
				thereby coordinating systemic responses to N starvation.	
RALF	*Arabidopsis*	proRALF	FER BUPS1/2 ANX1/2	Mediates calcium-dependent signaling, regulates root growth; regulates pollen tube growth, rupture, hybridization, and Ca^{2+} signaling. They also facilitate intergeneric hybridization, enabling pollen tubes from distant *Brassicaceae* species to penetrate *Arabidopsis* stigmas.	Ge et al. (2017); Gjetting et al. (2020); Gao et al. (2023); Lan et al. (2023)
EPF1 EPF2	*Arabidopsis*, *Populus tomentosa*	proEPF1 proEPF2	ER ERL1 ERL2	Inhibits stomatal development by restricting meristemoid transition and controlling asymmetric division in the stomatal lineage. Suppresses SPCH activity via the ERECTA–TMM receptor complex, ensuring optimal stomatal density and spacing.	Hara et al. (2007, 2009); Lau et al. (2014); Wang et al. (2016a)
EPFL2	*Arabidopsis*	proEPFL2	ER ERL1 ERL2	Regulates ovule spacing by controlling the pattern of ovule initiation along the carpel wall. Prevents ovule twinning by maintaining inter-ovule boundary signaling through interaction with ERL1/2 receptors. Regulates cotyledon development.	Chen and Shpak (2014); Kawamoto et al. (2020); Fujihara et al. (2021)
EPFL9	*Arabidopsis*	proEPFL9	ER ERL1 ERL2	Dual role in stomatal and fruit development. Promotes stomatal development by blocking EPF2 binding to the ER-ERL1/2–TMM complex, enabling meristemoid differentiation. In fruit development, promotes fruit elongation and cell expansion via ER-family receptors.	Lee et al. (2015)
EPFL4 EPFL5 EPFL6	*Arabidopsis*	proEPFL4 proEPFL5 proEPFL6	ER	Promotes stamen filament elongation by enhancing cell proliferation in the stamen filaments, ensuring proper anther dehiscence and pollen release for successful fertilization.	He et al. (2023a); Negoro et al. (2023)
CIF	*Arabidopsis*, *Oryza sativa*	proCIF	GSO1 GSO2	Regulates Casparian strip integrity, pollen wall formation, and embryonic cuticle formation. Promotes lignification for Casparian strip development, polarizes tapetal cells for sporopollenin deposition in pollen wall formation, and maintains embryonic cuticle integrity for seed protection.	Nakayama et al. (2017); De Giorgi et al. (2021); Truskina et al. (2022); Zhang et al. (2024, 2025)
miPEP171d1	*Vitis vinifera*	vvi-miPEP171d1	Unknown	Regulates adventitious root formation by activating its corresponding microRNA, vvi-miR171d. This promotes adventitious root formation, enhancing root plasticity and system adaptability.	Chen et al. (2020a, 2020b)
IDA	*Arabidopsis*	proIDA	HAE HSL2	Regulates floral organ abscission, lateral root emergence, and root cap sloughing. Promotes cell separation by breaking down the pectin-rich middle lamella in abscission zones, facilitating the detachment of floral organs, fruits, and leaves, as well as supporting lateral root emergence and root elongation.	Butenko et al. (2003) Stenvik et al. (2008) Kumpf et al. (2013) Meng et al. (2016) Shi et al. (2018)

Continued

Table 1. Continued

Peptide	Plant	Precursor	Key receptor	Function	References
LURE	*Arabidopsis*, *Torenia fournieri*	proLURE	MDIS1-MIK1/2; LIP1/2; PRK3/6	Guides pollen tube growth and mediates species-specific male–female communication by establishing a chemoattractive gradient secreted by synergid cells. Activates MDIS1–MIK1/2 receptor complexes, triggering kinase activity and amplifying signaling via PRK6-ROPGEF-ROP1 pathways to drive cytoskeletal reorganization at the pollen tube apex. Enhances receptor redistribution for precise navigation and successful fertilization.	Okuda et al. (2009); Takeuchi and Higashiyama (2012); Liu et al. (2013); Wang et al. (2016b); Takeuchi and Higashiyama (2016); Yang et al. (2022)

Abbreviations: ANX1/2, ANXUR1/2; BAM, barely any meristem; BUPS1/2, BUDDHA'S PAPER SEAL 1/2; CLE, CLAVATA3/embryo surrounding region; CEP, C-terminally encoded peptide; CIF, Casparian strip integrity factor; CLEL, CLE-Like; CLV1/2, CLAVATA1/2; CSC, columella stem cell; ERL1/2, ERECTA-Like Receptor Kinase 1/2; FER, FERONIA; EPF, epidermal patterning factor; EPFL, epidermal patterning factor-like; GLV, GOLVEN; HAE, HAESA; HSL2, HAESA-like 2 receptor; IDA, INFLORESCENCE DEFICIENT IN ABSCISSION; LR, lateral root; LIP 1/2, lost in pollen tube guidance 1/2; LRFCs, lateral root founder cells; MDIS1, Male Discoverer 1; MIK1/MIK2, MDIS1-Interacting Kinase 1/2; miPEP, miRNA-encoded small peptide; PSK, phytosulfokine; PSY, plant peptide containing sulfated tyrosine; PSKR, PSK receptor; ER, ERECTA; PSYR1, PSY Receptor1; PRK6, pollen receptor kinase 6; RAM, root apical meristem; RALF, rapid alkalinization factor; RGF, root meristem growth factor; RGFR, RGF receptor; ROP1, Rho-Related GTPase from plants 1; ROPGEF, Rho of plant guanine nucleotide-exchange factor; SAM, shoot apical meristem; SPCH, SPEECHLESS.

1996; Kutschmar et al., 2009; Kaufmann et al., 2021). Application of synthetic Phytosulfokine-alpha (PSK-α) promotes root growth, while mutations in *PSKR* or *TPST* result in defective root development and reduced cell expansion, highlighting the essential role of PSKs in root architecture (Kutschmar et al., 2009; Kaufmann et al., 2021). Co-receptors such as BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1) and SOMATIC EMBRYOGENESIS RECEPTOR KINASEs (SERKs) are required for receptor complex formation and effective signal transduction, although they do not directly bind PSKs (Ladwig et al., 2015; Wang et al., 2015).

Recent studies in *M. truncatula* have expanded our understanding of PSK signaling, particularly in root nodulation. Zhang et al. (2024, 2025) identified *MtTPST*, a gene highly expressed in root nodules and the RAM, as a critical regulator of root elongation and nodule formation. Mutants lacking *MtTPST* exhibited impaired root elongation and defective nodule development, with these defects partially rescued by exogenous sulfated PSK and RGF3 peptides (Zhang et al., 2025). This finding suggests that the MtTPST-PSK signaling module modulates root elongation and nodulation, likely through cytokinin and auxin pathways that are crucial for cell division and differentiation (Zhang et al., 2025). Thus, PSK peptides not only regulate root elongation but also play a critical role in nodulation, particularly in legumes where nodulation is essential for symbiotic nitrogen (N) fixation. This expanded understanding positions PSKs as key regulators of both developmental processes and environmental interactions, making them promising targets for improving crop growth and symbiotic efficiency.

CLAVATA3/Embryo Surrounding Region peptides

CLAVATA3/Embryo Surrounding Region peptides are crucial regulators of stem cell maintenance and differentiation in the RAM (Stahl et al., 2009; Stahl et al., 2013; Fletcher, 2020; Zhu et al., 2021a). For example, CLE40 plays a pivotal role in regulating columella stem cells (CSCs) at the root apex. In *Arabidopsis*, CLE40 is expressed in differentiated cells and provides negative feedback signals that modulate the expression of WOX5, a key TF involved in maintaining stem cell identity (Figure 3; Stahl et al., 2009; Stahl et al., 2013; Zhu et al., 2021a). CLE40 is recognized by the receptor kinase *ARABIDOPSIS* CRINKLY4 (ACR4), which interacts with CLAVATA-INTERACTING KINASE (CIK) proteins (Stahl et al., 2013; Zhu et al., 2021a). Upon binding to CLE40, ACR4 phosphorylates CIKs, promoting the differentiation of CSCs (Figure 3; Stahl et al., 2009; Zhu et al., 2021a). This interaction is essential for regulating both stem cell proliferation and differentiation. Loss-of-function mutants of *CLE40* exhibit overproliferation of CSC layers, underscoring its critical role in balancing stem cell maintenance and differentiation (Stahl et al., 2009; Stahl et al., 2013; Zhu et al., 2021a). In addition to CLE40, other CLE peptides such as CLE25, CLE26, and CLE45 are involved in early root development, specifically regulating protophloem differentiation and CSCs maintenance (Ren et al., 2019; Hu et al., 2022; Qian et al., 2022; Hardtke, 2023). These peptides interact with the receptor kinases BARELY ANY MERISTEM1/3 (BAM1/3) and CLV3 INSENSITIVE KINASEs (CIK2/3/4/5/6), forming a signaling module that suppresses protophloem differentiation (Figure 3; Hu et al., 2022; Hardtke, 2023). This CLE25/26/45–BAM1/3–CIK2/3/4/5/6 signaling complex operates downstream of BREVIS RADIX (BRX) and OCTOPUS (OPS) to modulate protophloem development and prevent premature differentiation, as evidenced by the ability of *cle25*, *26*, *45*, and *cik2,3,4,5,6* mutations to rescue root defects in *brx* and *ops* mutants (Hu et al., 2022; Qian et al., 2022; Hardtke, 2023).

CLAVATA3/Embryo Surrounding Region peptides also regulate LR development, particularly in response to environmental signals such as nutrient availability (Araya et al., 2014; Fletcher, 2020). For instance, *CLE1*, *CLE3*, *CLE4*, and *CLE7* are upregulated under N-deficient conditions, where their overexpression inhibits LR primordium growth and elongation (Figure 3; Araya et al., 2014). These CLEs mediate N-responsive mechanisms through CLV1, enabling plants to adjust root architecture in response to fluctuating nutrient conditions (Araya et al., 2014). While CLEs play significant roles in root system development, especially under N-deficient conditions, the precise mechanisms by which SPs, including CLEs, regulate plant responses to nutrient stress will be explored in subsequent sections. This broader context will provide a more comprehensive understanding of how CLE signaling integrates environmental cues into root developmental processes.

Other peptides in root development

In addition to RGF, PSK, and CLE peptides, several other peptide families play crucial roles in regulating root development (Figure 3). For example, PSY peptides promote root elongation by binding to PSY receptors (PSYRs), thereby alleviating the inhibitory effects of these receptors on root growth (Amano et al., 2007; Ogawa-Ohnishi et al., 2022; Yimer et al., 2023). Conversely, CEP peptides interact with CEPR1 and CEPR2 receptors to regulate LR growth, particularly under conditions of N or sucrose deficiency (Tabata et al., 2014; Zhu et al., 2021b). These peptides inhibit LR elongation by reducing both cell size and cell number, and they contribute to the systemic response to N starvation. Furthermore, RALF peptides modulate root growth and differentiation via calcium signaling pathways (Gjetting et al., 2020), while CIF peptides are essential for Casparian strip formation, as well as LR initiation and root hair development (Nakayama et al., 2017; Zhang et al., 2024). Additionally, microRNA-encoded peptides, such as vvi-miPEP171d1 in grapevine (*Vitis vinifera*), regulate adventitious root formation by activating their corresponding microRNAs (Chen et al., 2020a). Collectively, these diverse peptide families regulate various aspects of root development through distinct mechanisms, thereby ensuring the flexibility and adaptability of root structure and function.

In conclusion, SPs are key regulators of plant root development, governing processes such as meristem maintenance, LR initiation, root elongation, and tissue differentiation. As discussed, peptide families such as RGF, PSK, and CLE play distinct yet interconnected roles within complex signaling networks. Notably, RGF and CLE peptides work together to balance stem cell maintenance and differentiation (Stahl et al., 2009, 2013; Matsuzaki et al., 2010; Meng et al., 2012; Shao et al., 2020; Zhu et al., 2021a), while PSK and CLE peptides integrate nutrient and hormonal signals to control root elongation and LR formation (Andrews and Rothnagel, 2014; Araya et al., 2014; Fletcher, 2020; Kaufmann et al., 2021). Importantly, these peptides do not function in isolation but operate through intertwined signaling pathways that coordinate plant development and environmental adaptation. By translating environmental cues, such as nutrient availability and stress, into precise developmental responses, these peptides fine-tune root growth and architecture. Therefore, further investigation into their interactions with hormone pathways, such as auxins and cytokinins, may provide deeper insights into plant developmental plasticity.

Small peptides regulate plant stomatal development

Given the crucial roles of SPs in root development, it is essential to investigate how these peptides, especially members of the EPF/EPFL, and CLE families, regulate stomatal development and environmental responses. Understanding these mechanisms further highlights their broad influence on plant growth and adaptability.

In *Arabidopsis*, the EPF/EPFL family comprises 11 members that play crucial roles in regulating stomatal development, patterning, and density (Figure 4; Hara et al., 2007, 2009; Hunt and Gray, 2009; Kondo et al., 2010; Sugano et al., 2010; Lee et al., 2015; Lin et al., 2017; Herrmann and Torii, 2021). These secreted CRPs contain conserved C-terminal domains with six to eight cysteine residues, which form intramolecular disulfide bonds essential for maintaining structural stability and biological function (Herrmann and Torii, 2021). EPF1 and EPF2 act as key negative regulators of stomatal development by inhibiting stomatal differentiation and ensuring appropriate spacing (Hara et al., 2007, 2009; Hunt and Gray, 2009; Wang et al., 2016a). EPF1 and EPF2 signal through receptor kinases of the ERECTA family (ERf), including ERECTA, ERECTA-LIKE1 (ERL1), and ERECTA-LIKE2 (ERL2). By binding to ERL1 and ERL2, EPF1 and EPF2 form receptor complexes with the co-receptor TOO MANY MOUTHS (TMM), creating a signaling platform essential for transmitting downstream signals (Figure 4; Hara et al., 2007, 2009; Kondo et al., 2010; Sugano et al., 2010; Lee et al., 2012; Lin et al., 2017). The EPF-ERECTA-TMM receptor complex initiates pathways that suppress the differentiation of stomatal precursor cells by inhibiting SPEECHLESS (SPCH), a basic helix-loop-helix (bHLH) TF crucial for initiating the stomatal lineage (Figure 4; Shpak et al., 2005; MacAlister et al., 2007; Pillitteri et al., 2007; Lampard et al., 2008; Lau et al., 2014; Horst et al., 2015; Putarjunan et al., 2019). Through ERf receptors, this pathway restricts SPCH activity, limiting both the proliferation and differentiation potential of meristemoid mother cells (MMCs) within the stomatal lineage (Lampard et al., 2008; Horst et al., 2015; Lee et al., 2015; Lin et al., 2017; Qi et al., 2017; Putarjunan et al., 2019). Such regulation by the ERf family is critical for balanced stomatal development and for maintaining precise stomatal patterning and spacing.

Conversely, EPFL9 (STOMAGEN), an antagonist of EPF peptides, functions as a positive regulator of stomatal development (Figure 4; Hunt et al., 2010; Kondo et al., 2010; Sugano et al., 2010; Lee et al., 2012, 2015). EPFL9 competes with EPF2 for binding to the ERECTA receptor, inhibiting

receptor activation and promoting stomatal formation (Lee et al., 2012, 2015). This competitive interaction fine-tunes stomatal density, ensuring that plants maintain the optimal number of stomata for efficient gas exchange and water conservation. The balance between EPF2's inhibitory action and STOMAGEN's stimulatory role ensures precise stomatal patterning. Beyond its role in stomatal development, EPFL9 also integrates environmental signals. For instance, light exposure enhances the accumulation of the bZIP TF ELONGATED HYPOCOTYL 5 (HY5), a key regulator of light signaling (Wang et al., 2021). HY5 binds to the EPFL9 promoter, upregulating its expression in mesophyll cells and promoting stomatal development in response to light (Wang et al., 2021). This mechanism demonstrates how light signaling enhances stomatal formation by increasing *EPFL9* transcription and counteracting the inhibitory effects of EPF2 (Figure 4). In contrast, auxin signaling mediated by the TF ARF5/MONOPTEROS (MP) represses *EPFL9* expression, illustrating how hormonal pathways further regulate stomatal development under varying environmental conditions (Zhang et al., 2014).

In addition, CLE peptides also contribute to stomatal development and environmental stress responses, working in concert with EPF/EPFL peptides to ensure precise stomatal density control (Qian et al., 2018; Takahashi et al., 2018; Vatén et al., 2018). For instance, CLE25, secreted by roots under drought conditions, is transported to the leaves, where it promotes stomatal closure via abscisic acid (ABA) accumulation (Figure 4; Takahashi et al., 2018). Additionally, CLE peptides such as CLE9/10 inhibit the formation of stomatal precursor MMCs, further emphasizing their role in stomatal development regulation (Figure 4; Qian et al., 2018; Vatén et al., 2018). While the broader EPF/EPFL and CLE signaling networks remain to be fully elucidated, EPF1, EPF2, and CLE peptides clearly play critical roles in modulating stomatal density, enabling plants to adapt to environmental conditions. The coordination between drought-induced CLE peptide movement and light-regulated EPFL9 expression highlights the complex layers of control within these regulatory networks, ensuring that stomatal development is finely tuned in response to both abiotic stress and environmental cues.

Small peptides regulate plant reproductive development

Small peptides play an essential role in regulating plant reproductive development, extending beyond their well-known functions in root and stomatal development (Tavormina et al., 2015; Ge et al., 2017; Kawamoto et al., 2020; Gao et al., 2023; Lan et al., 2023; Fang et al., 2024). These versatile signaling molecules influence critical processes such as fertilization, seed formation, and fruit development (Figure 5; Ge et al., 2017; Mecchia et al., 2017; Kawamoto et al., 2020; Gao et al., 2023; Lan et al., 2023; Fang et al., 2024). Among the SP families, the RALF family is a key regulator of reproductive development, particularly through its involvement in interactions between male and female gametophytes (Figure 5; Ge et al., 2017, 2019; Mecchia et al., 2017; Gao et al., 2023).

RAPID ALKALINIZATION FACTOR peptides, which are cysteine-rich and possess conserved structural motifs essential for receptor binding, act as essential regulators of pollen tube guidance and rupture (Murphy and De Smet, 2014; Ge et al., 2017; Gao et al., 2023). Specifically, RALF4 and RALF19 control pollen tube growth by interacting with BUPS1 and BUPS2 (Buddha's Paper Seal 1/2) receptors, which further associate with ANXUR1 and ANXUR2 receptors (Figure 5A; Ge et al., 2017, 2019; Mecchia et al., 2017). This receptor complex stabilizes the elongating pollen tube, preventing premature rupture as it grows toward the ovule. Upon arrival at the embryo sac, the female-derived ligand RALF34 competes with RALF4 and RALF19 for binding to the BUPS1/2–ANXUR1/2 receptor complex, triggering pollen tube rupture and sperm cell release to facilitate double fertilization (Figure 5B; Ge et al., 2017, 2019). Co-receptors such as LORELEI-like GPI-anchored proteins (LLG2/3) enhance the stability and functionality of this receptor complex (Figure 5B; Ge et al., 2017, 2019). Additionally, RALF peptides activate downstream signaling pathways, such as the MARIS pathway and MILDEW RESISTANCE LOCUS O (MLO) proteins (AtMLO1, 5, 9, and 15), which act as Ca^{2+} channels. This triggers a localized influx of Ca^{2+} at the pollen tube tip, a process essential for maintaining pollen tube integrity and directional growth (Figure 5A; Gao et al., 2023). Importantly, RALF peptides have also been shown to overcome reproductive barriers in intergeneric hybridization (Lan et al., 2023). Synthetic RALF peptides derived from pollen allow pollen tubes from distantly related *Brassicaceae* species to penetrate *Arabidopsis* stigmas, enabling hybrid embryo formation (Lan et al., 2023). This capability to bridge reproductive barriers highlights the potential of RALF peptides as tools for plant breeding and hybridization strategies.

In addition to RALF peptides, LURE peptides are critical regulators of pollen tube guidance and species-specific male–female communication in plants (Figure 5A; Okuda et al., 2009; Takeuchi and Higashiyama, 2012; Liu et al., 2013; Takeuchi and Higashiyama, 2016; Wang et al., 2016b; Yang et al., 2022). Secreted by the synergid cells of the ovule, LURE peptides form a gradient that directs pollen tubes toward the ovule micropyle (Okuda et al., 2009; Takeuchi and Higashiyama, 2012; Liu et al., 2013). The perception of LURE peptides is mediated by a receptor complex consisting of MDIS1 and MIK1/2, which are plasma membrane-localized receptor-like kinases characterized by extracellular leucine-rich repeat (LRR) domains (Figure 5A; Wang et al., 2016b). Binding of LURE1 peptides induces dimerization of MDIS1 and MIK1/2, thereby activating the kinase activity of MIK1 and initiating downstream signaling cascades (Wang et al., 2016b). Lost In Pollen tube guidance 1/2 (LIP1/2), essential receptor-like kinases, further stabilize the MDIS1–MIK1/2 receptor complex, ensuring efficient LURE signal perception and transmission required for micropylar pollen tube guidance (Figure 5A; Liu et al., 2013). Pollen-specific receptor kinase 3/6 (PRK3/6), pollen tube-specific receptor-like kinases, work in conjunction with the MDIS1–MIK receptor complex to

amplify LURE-mediated signaling (Figure 5A; Takeuchi and Higashiyama, 2016; Yang et al., 2022). PRK3/6 interact with ROPGEFs (Rho of plant guanine nucleotide-exchange factors), which activate ROP1, a critical Rho GTPase that orchestrates cytoskeletal remodeling at the pollen tube apex (Figure 5A; Takeuchi and Higashiyama, 2016). This signaling cascade drives the reorganization of actin filaments and microtubules, facilitating precise reorientation and navigation of the pollen tube along the LURE gradient. Furthermore, LURE signaling dynamically redistributes PRK3/6 and MDIS1 to the tip of the pollen tube, enhancing its sensitivity to the attractant and ensuring efficient male–female communication (Takeuchi and Higashiyama, 2016; Wang et al., 2016b; Yang et al., 2022). Collectively, these findings highlight the indispensable role of LURE peptides and their receptor complexes in regulating pollen tube growth, ovule targeting, and species-specific fertilization.

PHYTOSULFOKINE peptides also play a vital role in plant reproductive development. PSK peptides significantly influence pollen germination and tube growth (Chen et al., 2000; Stührwohldt et al., 2015; Kou et al., 2020). PSK-α peptides enhance these processes in low-density culture conditions, demonstrating their role in promoting the pollen population effect (Chen et al., 2000; Stührwohldt et al., 2015). Emerging evidence suggests that PSK and RALF signaling pathways interact synergistically to optimize pollen tube growth and guidance, highlighting the coordination of multiple SP networks during reproductive development (Chen et al., 2000; Stührwohldt et al., 2015; Ge et al., 2017, 2019; Kou et al., 2020). This interaction is supported by observations that mutants with disrupted PSK signaling, such as *pskr1-3*, *pskr2-1*, and *tpst-1*, exhibit reduced fertility, underscoring the importance of PSK in reproductive success (Stührwohldt et al., 2015). Beyond its role in pollen development, PSK also plays a pivotal role in fruit development and quality formation (Figure 5B; Fang et al., 2024). Recent studies using tomato (*Solanum lycopersicum*) as a model system revealed that PSK promotes fruit ripening and enhances fruit quality, while *pskr1* mutants exhibit delayed ripening (Fang et al., 2024). Transcriptomic analysis indicates that PSKR1 influences key molecular pathways and metabolic processes involved in ripening (Fang et al., 2024). One critical component of this pathway is the TF DEHYDRATION-RESPONSIVE ELEMENT BINDING PROTEIN 2F (DREB2F), which interacts with PSKR1. Silencing DREB2F not only delays fruit ripening but also diminishes the promotive effects of PSK (Fang et al., 2024). Mechanistically, PSKR1 phosphorylates DREB2F at the tyrosine-30 site, enhancing its transcriptional activity and upregulating ripening-related target genes (Fang et al., 2024). Exogenous PSK application amplifies these effects, underscoring its role in regulating fruit ripening. Collectively, the PSK-PSKR1-DREB2F signaling pathway represents a promising target for genetic or chemical interventions aimed at improving fruit yield, accelerating ripening, and enhancing fruit quality traits.

The EPF family of SPs also plays a vital role in plant reproductive development, with EPFL2 and EPFL9 serving as prominent regulators of ovule initiation and fruit growth (Figure 5B; Kawamoto et al., 2020). EPFL2 is specifically expressed in the inter-ovule spaces of the carpel wall, where it interacts with ERECTA-family leucine-rich repeat receptor kinases (LRR-RKs), particularly ERL1 and ERL2. This interaction regulates ovule spacing and prevents ovule twinning, ensuring the regular positioning of ovule initials, which is essential for efficient seed development and optimal allocation of resources during fruit maturation (Kawamoto et al., 2020). Loss-of-function mutations in EPFL2 result in ovule clustering and twin ovule formation, which ultimately affects seed arrangement and fruit size (Kawamoto et al., 2020). The EPFL2-ERL1/ERL2 pathway underscores the critical role of inter-organ boundary signaling in fruit development (Figure 5B). In contrast, EPFL9 has a distinct role in fruit elongation. While EPFL9 acts antagonistically to other EPF/EPFL peptides in stomatal patterning (Lee et al., 2015), it functions independently to regulate fruit growth (Kawamoto et al., 2020). EPFL9 promotes fruit elongation by interacting with ER, ERL1, and ERL2 receptors. Notably, its function in fruit growth is independent of the MAPK signaling pathway, distinguishing it from its role in stomatal development (Lee et al., 2015; Kawamoto et al., 2020). Loss-of-function mutations or RNA interference (RNAi) knockdown of *EPFL9* reduce fruit length and increase seed density, while ectopic expression of *EPFL9* disrupts ovule initiation patterns, leading to twin ovule formation, a phenotype similar to that seen in EPFL2 mutants (Kawamoto et al., 2020). These findings demonstrate that EPFL2 and EPFL9 have distinct but complementary roles in coordinating ovule development and fruit growth (Figure 5B). The functional divergence of EPFL2 and EPFL9 highlights the sophisticated control of fruit development through receptor-mediated peptide signaling.

The regulatory network involving RALF, LURE, PSK, and EPF peptides highlights the complexity and precision of SP-mediated signaling in plant reproductive development (Figure 5). While the distinct roles of these peptides in regulating reproductive processes have been extensively studied, future research should focus on elucidating potential crosstalk between SP pathways, such as the integration of LURE signaling with RALF and PSK pathways, as well as their broader implications in overcoming reproductive barriers and enhancing crop resilience. Furthermore, leveraging advances in bioinformatics and single-cell sequencing may provide novel insights into the spatial and temporal dynamics of SP signaling *in planta* (Slavov, 2021; Ctortecka et al., 2024). This signaling network underscores the central role of SP signals in plant reproductive development, offering potential targets for crop improvement. Engineering SP-related pathways could provide novel strategies to enhance crop yield, improve fruit size, and overcome hybridization barriers in breeding programs. The coordinated actions of RALF, PSK, LURE, and EPF peptides in regulating pollen germination, pollen tube growth, ovule development, and fruit maturation

reveal their significance as master regulators of plant reproductive success. Their mechanistic insights deepen our understanding of plant reproductive biology and present new opportunities for agricultural innovation.

SMALL PEPTIDES MEDIATE PLANT STRESS RESPONSE

As sessile organisms, plants are frequently exposed to a wide range of environmental stresses, including abiotic factors such as drought, high salinity, temperature fluctuations, and soil nutrient deficiencies, as well as biotic factors like insect pests, fungi, bacteria, herbivores, and weeds. Small peptides serve as critical hormone-like signaling molecules that mediate various plant stress signaling pathways (Tavormina et al., 2015; Datta et al., 2024; Chang and Xiao, 2025). In the following sections, we summarize the functional roles of these peptides in plant stress responses (Table 2) and discuss recent findings on the signaling pathways they regulate in response to different stress factors.

Small peptides regulate the abiotic stress response

Dehydration stress

Small peptides have been implicated in plant responses to abiotic stress, particularly dehydration (Takahashi et al., 2018; Stührwohldt et al., 2021; Chang and Xiao, 2025). Notably, two members of the CLE peptide family, CLE25 and CLE9, play pivotal roles in mediating drought tolerance in *Arabidopsis* (Figure 6A). CLE25, produced in the roots, functions as a mobile signaling molecule that modulates ABA biosynthesis and triggers stomatal closure via long-distance root-to-shoot signaling in response to water deficit, with this process mediated by BAM receptors in the leaves (Figure 6A; Takahashi et al., 2018). Additionally, *cle25* knockout mutants exhibit increased sensitivity to dehydration compared to wild-type plants, underscoring the importance of CLE25 in dehydration stress response (Takahashi et al., 2018). In contrast, CLE9 is specifically expressed in guard cells and enhances drought tolerance through a localized signaling mechanism that regulates stomatal closure. This process involves the activation of MPK3 and MPK6 signaling pathways, further emphasizing CLE9's role in mediating guard cell responses to dehydration stress (Zhang et al., 2019).

Additionally, CEP peptides have been reported to play a role in regulating drought and osmotic tolerance. Notably, overexpression or exogenous application of the CEP5 peptide enhances recovery from drought after re-watering compared to wild-type plants, suggesting that CEP5 positively mediates drought stress tolerance (Figure 6A; Smith et al., 2020). The PSK peptide is also involved in the regulation of various plant stress responses. Studies have shown that the expression of PSK peptide precursor genes, including *PSK1*, *PSK3*, *PSK4*, and *PSK5*, along with three subtilisin-like serine protease genes (*SBT1.4*, *SBT3.7*, and *SBT3.8*) responsible for processing mature PSK, are significantly upregulated in response to osmotic stress in *Arabidopsis* (Figure 6A; Stührwohldt et al., 2021). Transgenic plants overexpressing the PSK precursor (proPSK1) and SBT3.8 exhibit increased fresh weight and enhanced osmotic stress tolerance compared to wild-type plants (Stührwohldt et al., 2021).These studies suggest that plants manage water deficit conditions by activating various SP signaling pathways. However, the potential cross-talk and synergistic interactions among these pathways, as well as the role of antagonistic SPs signals in modulating drought responses, remain critical areas for further investigation. Exploring these interactions could reveal the intricate layers of drought tolerance regulation, offering valuable insights into how multiple SP signals might cooperate or counteract to finely tune plant stress responses.

Salinity stress

Several SPs have been identified as critical regulators in plant responses to high salinity stress (Zhou et al., 2022; Chang and Xiao, 2025). For instance, CAP-derived peptide 1 (CAPE1) was initially discovered through a peptidomics approach in tomato leaves (Chen et al., 2014). CAPE1 functions as a negative regulator of salt stress tolerance in *Arabidopsis*. Application of synthetic AtCAPE1 peptide or overexpression of its precursor, PROAtCAPE1, restores the salt-sensitive phenotype in *proatcape1* mutants, underscoring its significant role in the salt stress response (Chien et al., 2015). Our research team recently emphasized the importance of PAMP-INDUCED SECRETED PEPTIDE 3 (PIP3) in maintaining sodium ion (Na^+) homeostasis under salt stress (Figure 6B; Zhou et al., 2022). Under salt stress conditions, PIP3 is secreted and binds to its receptor RLK7, forming an active ligand-receptor signaling cascade. This peptide–receptor cascade modulates plant salt tolerance by activating the MPK3/MPK6 pathway in *Arabidopsis*, demonstrating PIP3's crucial role in enhancing salt tolerance (Zhou et al., 2022).

RAPID ALKALINIZATION FACTOR peptides play a critical role in modulating plant responses to salt stress. Specifically, RALF1, in conjunction with its receptor FERONIA (FER), positively regulates salt tolerance (Feng et al., 2018). Notably, the *ralf1* mutant exhibits growth inhibition under salt stress that is similar to that of wild-type plants, underscoring RALF1's essential role in enhancing salt tolerance (Feng et al., 2018). RALF22 and RALF23 peptides enhance salt tolerance by interacting with LEUCINE-RICH REPEAT EXTENSINS (LRX) proteins and FER receptors (Figure 6B). Under normal conditions, LRX3 and LRX4 associate with RALF22 and RALF23, inhibiting their interaction with FER and preventing FER internalization (Figure 6B; Zhao et al., 2018). However, under salt stress, mature RALF22 and RALF23 are released from LRX proteins due to cell wall perturbations and S1P protease activity. This release facilitates the internalization of FER receptors through an endosomal pathway, leading to changes in cell wall integrity, inhibition of ABA signaling, and increased accumulation of ROS. This cascade of events significantly contributes to improved salt tolerance (Figure 6B; Zhao et al., 2018). Additionally, PLANT ELICITOR

Table 2. Typical SPs involved in plant abiotic and biotic stress responses

Peptide	Plant	Stress	Precursor	Key receptor	Function	References
CEP	*Arabidopsis*, *M. truncatula*	Drought N deficiency	proCEP	CEPR1 CEPR2	Enhances drought tolerance by downregulating auxin-related genes; upregulate nitrate transporter NRT2.1 to enhance nitrate uptake under low N.	Tabata et al. (2014); Ohkubo et al. (2017); Smith et al. (2020); Luo et al. (2022)
PSK	*Arabidopsis*, *S. lycopersicum*	Drought Immunity Heat stress	proPSK	PSKR1 PSKR2	Regulates drought-induced flower drop. Triggers calcium influx, activating auxin-mediated pathways to enhance immunity; helps plants tolerate heat stress.	Yamakawa et al. (1999); Zhang et al. (2018); Reichardt et al. (2020); Stührwohldt et al. (2021)
CAPE1	*Arabidopsis*, *S. lycopersicum*, *T. aestivum*	Salinity Immunity Insect pests	proCAPE1	Unknown	Negatively regulates salt tolerance by downregulating abscisic acid (ABA)-dependent and salinity-responsive genes; exhibits insect resistance; enhances plant immunity against pathogens.	Chen et al. (2014); Chien et al. (2015); Sung et al. (2021)
RALF	*Arabidopsis*	Salinity Immunity	proRALF	FER	Enhances salt tolerance by maintaining cell wall integrity and suppressing ABA signaling; regulates immunity by modulating receptor-mediated signaling.	Stegmann et al. (2017); Feng et al. (2018); Zhao et al. (2018); He et al. (2023b)
PEP	*Arabidopsis*	Salinity Immunity	proPEP	PEPR1	Activates immune responses under extracellular alkaline conditions; deprotonation enhances receptor binding and immune activation; links salt tolerance and immunity.	Nakaminami et al. (2018); He et al. (2023b); Tsai and Schmidt (2021); Liu et al. (2022)
PIP1 PIP3	*Arabidopsis*, *S. pimpinellifolium*	Salinity Immunity	proPIP1 proPIP3	RLK7	Regulates salt tolerance via activating the MPK3/6 pathway; regulates immunity via the salicylic acid (SA) and jasmonic acid (JA) signaling pathways.	Hou et al. (2014); Najafi et al. (2019); Zhou et al. (2022); Yang et al. (2023)
CLE25	*Arabidopsis*	Drought	proCLE25	BAM1 BAM3	Induces stomatal closure to prevent water loss under drought conditions, enabling dehydration resistance.	Takahashi et al. (2018)
CLE45	*Arabidopsis*	Temperature stress	proCLE45	SKM1 SKM2	Enhances pollen tube growth under heat stress, supporting reproductive success and seed production.	Endo et al. (2013)
CLE9 CLE10	*Arabidopsis*	Drought	proCLE9 proCLE10	HSL1 BAM1	Enhances drought tolerance by inducing stomatal closure to reduce water loss.	Qian et al. (2018); Zhang et al. (2019)
CLE1 CLE3 CLE4 CLE7	*Arabidopsis*	N deficiency	proCLE1 proCLE3 proCLE4 proCLE7	CLV1	Modulates root system architecture by inhibiting lateral root growth and promoting primary root elongation under N-deficient conditions.	Araya et al. (2014)
CLE14	*Arabidopsis*	Pi deficiency	proCLE14	CLV2 PEPR2	Induces RAM differentiation and inhibits root growth to optimize resource allocation under low Pi availability.	Gutiérrez-Alanís et al., 2017
RGF1 RGF2	*Arabidopsis*	Pi deficiency	proRGF1 proRGF2	RGFR1 RGFR2	Regulates root hair growth to increase Pi uptake and adapt to Pi deficiency.	Cederholm and Benfey (2015)

Continued

Table 2. Continued

Peptide	Plant	Stress	Precursor	Key receptor	Function	References
IMA	*Arabidopsis*, *T. aestivum*, *L. japonicus*	Fe, Cd, Cu, N homeostasis	proIMA	Unknown	Regulates Fe homeostasis by stabilizing bHLH transcription factors and inhibiting BTS E3 ligase; enhances Cd tolerance by activating Fe deficiency responses; maintains Cu homeostasis by interacting with CITF1; modulates nitrogen–Fe balance during symbiotic nitrogen fixation.	Grillet et al. (2018); Hirayama et al. (2018); Li et al. (2021); Meng et al. (2022); Ito et al. (2024)
Systemin	*S. lycopersicum*	Insect pests	proSystemin	SYR1	Enhances defense against pests and promotes resistance to pathogens through JA and ethylene pathways.	Aprile et al. (2022)
EPFL6	*Arabidopsis*	Temperature stress	proEPFL6	ER	Coordinates stamen elongation at low temperatures, enabling proper self-pollination for reproductive success.	Negoro et al. (2023)
IDA	*Arabidopsis*	Immunity	proIDA	HAE HSL2	Enhances immunity by inducing Ca^{2+} release, promoting reactive oxygen species (ROS) production, and upregulating immune-related genes to protect against pathogens.	Lalun et al. (2024)

Abbreviations: BAM, barely any meristem; BTS, BRUTUS (E3 ligase); CAPE1, CAP-Derived Peptide 1; CEP, C-terminally encoded peptide; CEPR1/2, CEP Receptors 1/2; CITF1, Cu-Deficiency Induced Transcription Factor 1; CLE, CLAVATA3/embryo surrounding region; CLV1, CLAVATA1; EPFL, epidermal patterning factor-like; ER, ERECTA; FER, FERONIA; HAE, HAESA; FRO2, Ferric Reduction Oxidase 2; HSL2, HAESA-Like 2; IMA, IRON MAN peptides; IRT1, Iron-Regulated Transporter 1; MPK3/6, Mitogen-Activated Protein Kinase 3/6; NRT1/2, Nitrate Transporters 1/2; PIP1/3, PAMP-Induced Secreted Peptide 1/3; PSK, phytosulfokine; PEPR1, PEP receptor 1; RAM, root apical meristem; SKM1/2, Sterility-Regulating Kinase Member 1/2; RGF, root meristem growth factor; RGFR, RGF receptor; RALF, rapid alkalinization factor; RLK7, Receptor-Like Kinase 7.

PEPTIDES (PEPs) modulate plant responses to salt stress. Among the eight *AtPROPEP* members in *Arabidopsis*, *AtPROPEP3* is significantly induced by high salinity. Knockdown of *AtPROPEP3* increases sensitivity to salt stress, whereas overexpression of *AtPROPEP3* or treatment with synthetic AtPEP3 enhances salt tolerance (Nakaminami et al., 2018). These findings highlight the crucial roles of signaling peptides in mediating plant responses to high salinity stress and illustrate their diverse mechanisms of action in enhancing tolerance under challenging environmental conditions.

Temperature stress

Plants frequently encounter fluctuating temperature conditions, and SPs are increasingly recognized as critical regulators of temperature stress responses. Recent studies have shown that two SPs derived from miRNAs, vvi-miPEP172b and vvi-miPEP3635b, significantly enhance cold tolerance in grapevine. When synthetic vvi-miPEP172b and vvi-miPEP3635b were applied to grapevine tissue culture plantlets, these plants demonstrated improved cold tolerance compared to controls (Chen et al., 2022). Additionally, the secreted peptide EPIDERMAL PATTERNING FACTOR-LIKE 6 (EPFL6) plays an essential role in coordinating reproductive organ development under low temperatures, thus supporting successful reproduction in *Arabidopsis* (Negoro et al., 2023). Specifically, under cool conditions, *EPFL6* expression in stamen filaments promotes elongation, aligning stamen and pistil lengths within a single flower and facilitating effective self-pollination (Negoro et al., 2023). Notably, this regulatory role of EPFL6 is absent at moderate temperatures, highlighting a temperature-specific function of EPFL6 in stress adaptation. The receptor ERECTA mediates this stamen-pistil growth coordination, thus underscoring the specialized roles of SPs in low-temperature responses.

Small peptides also contribute to high-temperature tolerance. For instance, the CLE45 peptide promotes pollen tube growth under high temperatures, thereby facilitating reproductive success in *Arabidopsis* (Figure 6C; Endo et al., 2013). At 22°C, CLE45 expression is localized to the stigma, but at 30°C, it extends along the transmitting tract, aligning with the path of pollen tubes (Figure 6C; Endo et al., 2013). CLE45 interacts with pollen-expressed receptors SKM1 and SKM2, and disrupting this signaling through RNAi suppression of CLE45 or by introducing a kinase-inactive version of SKM1 significantly reduces seed production under heat stress. This CLE45-SKM1/SKM2 pathway supports pollen viability and seed production under high temperatures by countering mitochondrial decay in pollen tubes (Figure 6C; Endo et al., 2013).

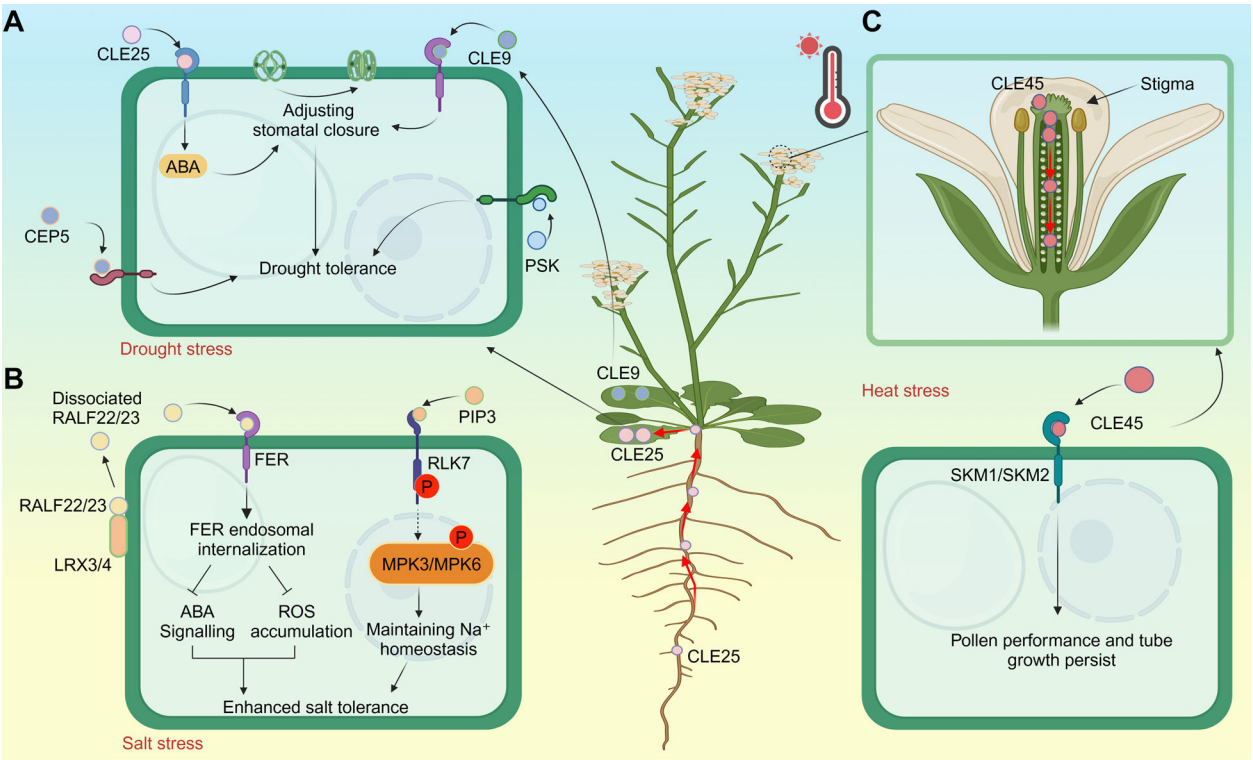

Figure 6. Plant small peptides (SPs) regulate abiotic stress responses

(A) Small peptides regulating drought stress responses. Root-derived CLE25 acts as a mobile signaling molecule that modulates abscisic acid (ABA) biosynthesis and promotes stomatal closure through long-distance root-to-shoot signaling in response to water deficit. CLE9, specifically expressed in guard cells, serves as a local signal that enhances drought resistance by regulating stomatal closure. Additionally, CEP5 positively regulates drought tolerance, while PSK enhances osmotic stress tolerance. **(B)** Salt tolerance mechanisms regulated by SPs. This section of the diagram illustrates the signaling pathways involving RALF22, RALF23, and PIP3 in modulating plant responses to salt stress. Under non-stress conditions, LRX3 and LRX4 bind to RALF22 and RALF23, inhibiting their interaction with the receptor FER and preventing FER internalization. Salt stress disrupts the cell wall and activates S1P protease activity, leading to the release of RALF22 and RALF23 from LRX proteins, thus allowing FER internalization through an endosomal pathway. This internalization alters cell wall integrity, inhibits ABA signaling, and inhibits the accumulation of reactive oxygen species (ROS), thereby enhancing salt tolerance. Furthermore, under salt stress, PIP3 binds to its receptor RLK7, initiating a ligand-receptor cascade that activates the MAPK3/MAPK6 pathway, which is crucial for maintaining Na^+ homeostasis and further supporting salt tolerance. **(C)** CLE45 peptide functionality under heat stress. The CLE45 peptide is recognized by the SKM1 and SKM2 receptors. Under heat stress, CLE45 expression expands from the stigma to the pistil tract, where pollen tubes develop. Pollen grains express receptors for CLE45, facilitating the pollen–pistil interaction. This interaction, mediated by the CLE45-SKM1/2 signaling pathway, enhances pollen viability and promotes pollen tube growth, thereby ensuring successful seed production even at elevated temperatures. CLE, CLAVATA3/ESR-related peptide; CEP, C-terminally encoded peptide; FER, FERONIA; LRX, leucine-rich repeat extensins; MAPK, mitogen-activated protein kinase; PIP, PAMP-induced secreted peptide; PSK, phytosulfokine; RLK7, Receptor-Like Kinase 7; SKM1/2, Sterility-Regulating Kinase Member 1/2.

Furthermore, PSK-α has been shown to enhance heat tolerance by maintaining both growth and chlorophyll content in *Arabidopsis* seedlings exposed to elevated night-time temperatures (Yamakawa et al., 1999), thereby underscoring its specific role in temperature stress resilience.

However, it is important to note that while a few SPs are known to be involved in temperature stress responses, our understanding of the molecular mechanisms by which these peptides operate remains limited. Furthermore, the current repertoire of temperature-responsive SPs is inadequate, emphasizing the necessity of identifying and characterizing additional peptides. Therefore, further research into the molecular pathways and interactions involving SPs is essential for advancing our understanding and enhancing agricultural productivity under fluctuating temperature conditions.

Nutritional deficiency response

Small peptides play a crucial role in regulating plant responses to nutrient deficiency stress, particularly in N, inorganic phosphate (Pi), and metal ion signaling pathways. CEPs, CLEs, and RGFs are essential for N and Pi acquisition (Figure 7), while the IRON MAN (IMA) family uniquely regulates metal ion homeostasis, including iron (Fe), cadmium (Cd), and copper (Cu). These SPs collectively integrate nutrient signaling with plant development and stress adaptation, enabling plants to optimize nutrient use under challenging environmental conditions.

Nitrogen and phosphorus signaling

In *Arabidopsis*, CEPs function as long-distance root-to-shoot signals, playing a crucial role in enhancing compensatory N acquisition across different root zones, thereby ensuring

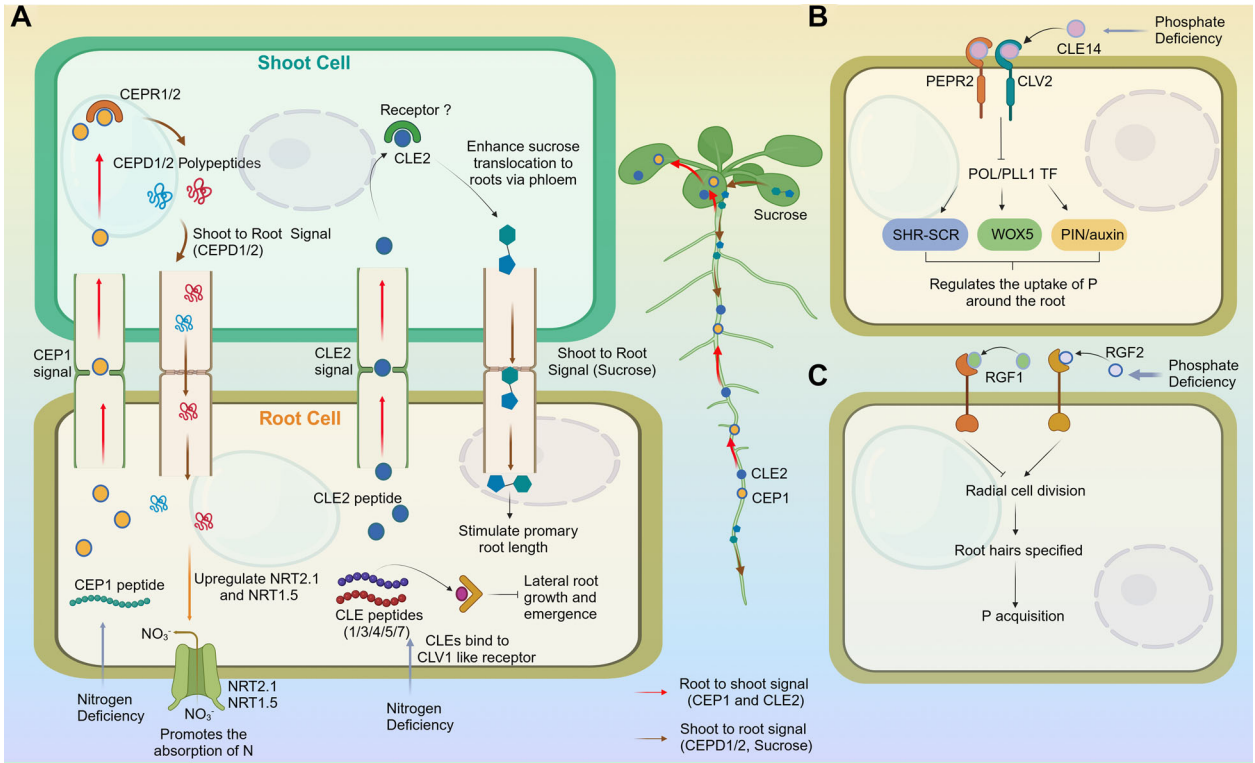

Figure 7. Plant small peptides (SPs) mediate nutritional deficiency responses

(A) Signaling pathways mediated by CEP and CLE peptides in response to N deficiency. Under N-deficient conditions, root-derived CEP1 peptides are transported via the xylem to shoots, where they bind to XIP1/CEPR1 and CEPR2 receptors, initiating a descending signal that induces CEPD1 and CEPD2 polypeptides (glutaredoxin family). These CEPD peptides travel through the phloem back to roots, upregulating NRT2.1 and NRT1.5 to enhance nitrogen uptake. Concurrently, N starvation upregulates CLE peptides (CLE1/3/4/5/7) in root pericycle cells. These CLE peptides bind to CLAVATA1-like receptors, inhibiting LR development. Additionally, N deficiency induces *CLE2* expression in roots, with CLE2 peptides translocating to leaves to facilitate sucrose transport to roots, promoting primary root growth and further N uptake. Together, these pathways modulate N acquisition and root architecture for adaptation to N-deficient conditions. (B-C) Signaling pathways mediated by CLE and RGF peptides in response to Pi deficiency. CLE14 binds to CLV2 and PEPR2 receptors, suppressing transcription factors such as SCR, SHR, and WOX5, which subsequently affects the PIN/auxin signaling pathway, thereby aiding plant adaptation to low Pi conditions **(B)**. RGF1 and RGF2 play crucial roles in regulating root hair growth under Pi deficiency. Upon perception by LRR-RLK receptors, RGF2 promotes both vertical and radial root growth, while RGF1 inhibits radial growth, enhancing inorganic Pi absorption **(C)**. CEPD, CEP downstream polypeptides; NRT, nitrate transporter; XIP1, Xylem Intermixed with Phloem 1; CEPR1/2, CEP Receptor 1/2; SCR, SCARECROW; SHR, SHORT-ROOT; WOX5, WUSCHEL-RELATED HOMEOBOX 5; PIN, PIN-FORMED proteins (auxin transporters).

efficient N uptake (Tabata et al., 2014; Ohkubo et al., 2017). Specifically, under low N conditions, CEP1 peptide is synthesized in roots and transported to the shoots via the xylem (Figure 7A; Tabata et al., 2014; Ohkubo et al., 2017). In the leaves, these root-derived CEP peptides are recognized by the CEPR1/2 receptors, which then modulate the transcription of nitrate transporters such as *NRT2.1*, facilitating enhanced N acquisition (Figure 7A; Tabata et al., 2014; Ohkubo et al., 2017). Additionally, the CEP-CEPR signaling pathway induces the production of CEPD polypeptides, members of the glutaredoxin family, which serve as descending signals via the phloem to the roots under N-limited conditions (Figure 7A). Here, they upregulate the expression of *NRT1.5* and *NRT2.1*, further promoting N uptake (Figure 7A; Tabata et al., 2014; Ohkubo et al., 2017; Ota et al., 2020; Luo et al., 2022).

CLAVATA3/Embryo Surrounding Region peptides play a crucial role in modulating plant responses to N deficiency, specifically by influencing root system architecture (Araya et al., 2014; Ma et al., 2020). Studies have shown that under N-deficient conditions, the expressions of *CLE1*, *CLE3*, *CLE4*, and *CLE7* are primarily induced in the pericycle cells of roots in *Arabidopsis* (Araya et al., 2014). These peptides suppress the development of LR primordia, thereby inhibiting their emergence from the primary root. However, in *clv1* mutants this inhibition is relieved, leading to accelerated outgrowth of lateral roots under low N conditions. CLAVATA1 (CLV1), a leucine-rich repeat receptor-like kinase expressed in the phloem companion cells, binds to CLE peptides, including CLE3, to form a regulatory signaling module that restricts LR expansion in response to N deficiency, thereby enabling plants to efficiently adapt to low N environments (Figure 7A; Araya et al., 2014). Beyond N deficiency, CLE peptides exhibit functional versatility by responding to various environmental cues. For example, CLE2 expression is upregulated not only in response to N availability but also by dark conditions and sugar starvation. In transgenic lines where *CLE2* expression is induced, CLE2 triggers the expression of various genes in

roots, with downstream activation of genes involved in light-dependent carbohydrate metabolism in shoots. This systemic signaling role of CLE2 highlights its cross-organ function, suggesting that CLE2 acts as a key signal integrator, coordinating light and nutrient responses that regulate metabolic processes between roots and shoots (Ma et al., 2020). Furthermore, GLV/RGF peptides also play a significant role in regulating N starvation signaling and root development. Recent studies have shown that GLV peptides in *M. truncatula* are significantly upregulated during nodule formation and exhibit differential responses to N deficiency and auxin treatment (Roy et al., 2024). Overexpression of these nodule-induced *GLV* genes in the hairy roots of *M. truncatula*, along with the application of their synthetic peptide analogs, results in a 25–50% reduction in nodule count (Roy et al., 2024). In summary, these signaling peptides are crucial regulators of N signaling, influencing nutrient uptake and modulating plant development in response to environmental changes.

Small peptides are crucial for maintaining Pi homeostasis, especially under low Pi conditions. In response to Pi deficiency, plants alter their root architecture to increase soil contact and enhance Pi acquisition (López-Bucio et al., 2002; Sánchez-Calderón et al., 2005). Among these SPs, CLE14 serves as a key mediator of low Pi signaling in *Arabidopsis*, promoting cell differentiation in the RAM (Figure 7B; Gutiérrez-Alanís et al., 2017). Under low Pi stress, CLE14 accumulates in the RAM and interacts with CLAVATA2 (CLV2) and PEPR2 receptors, activating a signaling pathway that restricts primary root growth by downregulating critical regulators of RAM maintenance and differentiation, including SCARECROW (SCR), SHORT-ROOT (SHR), and components of the PIN/auxin signaling pathway (Figure 7B; Gutiérrez-Alanís et al., 2017). Evidence indicates that the CLE14-CLV2/PEPR2 pathway may function through the POLTERGEIST (POLL) and POLTERGEIST-LIKE 1 (PLL1) pathways, with POLL and PLL1 acting as upstream negative regulators of SHR and SCR expression, thus linking CLE14 perception to the suppression of these key root regulators under low Pi conditions (Figure 7B; Gutiérrez-Alanís et al., 2017). Additionally, RGFs play a vital role in mediating Pi deprivation signaling by regulating specific aspects of root development (Cederholm and Benfey, 2015). Notably, under Pi deprivation, RGF1 restricts radial cell division in the root meristem, while RGF2 promotes longitudinal growth of the primary root and also enhances radial cell division (Figure 7C; Cederholm and Benfey, 2015). This dual function of RGF2 contributes to root hair specification, thereby improving Pi acquisition. In summary, SPs are integral to the plant's response to nutritional deficiencies, particularly by modulating root growth and development under P-limited conditions.

Metal ion regulation

Building upon the broader contributions of SPs in N and Pi deficiency responses, the IMA family of peptides has emerged as a key regulator of metal ion homeostasis, particularly for Fe, Cd, and Cu (Grillet et al., 2018; Okada et al., 2022; Hirayama et al., 2018; Meng et al., 2022; Wang et al., 2023b; Cai et al., 2023). IMA peptides contribute to Fe homeostasis by stabilizing subgroup IVc bHLH TFs, which are essential for the expression of Fe-uptake genes such as IRT1 and FRO2 (Grillet et al., 2018; Hirayama et al., 2018; Li et al., 2021). This stabilization occurs through the inhibition of the BRUTUS (BTS) E3 ligase, a negative regulator of iron acquisition that promotes the degradation of IVc bHLH TFs (Grillet et al., 2018; Hirayama et al., 2018; Li et al., 2021). Under Fe-deficient conditions, IMA peptides inhibit BTS, preventing the degradation of these TFs and facilitating enhanced expression of Fe-uptake genes, which in turn promotes iron acquisition (Grillet et al., 2018; Hirayama et al., 2018; Li et al., 2021; Wang et al., 2023b).

Beyond Fe regulation, IMA peptides have been shown to enhance tolerance to Cd stress by activating Fe deficiency responses (Meng et al., 2022; Wang et al., 2023b). This mechanism increases Fe accumulation while reducing Cd uptake via shared transporters, mitigating Cd toxicity (Meng et al., 2022; Wang et al., 2023b). Notably, overexpression of IMA1 and IMA3 in *Arabidopsis* and wheat (*Triticum aestivum*) improves root elongation and biomass under Cd exposure, highlighting the potential of IMA peptides in mitigating heavy metal toxicity in agricultural systems (Meng et al., 2022; Wang et al., 2023b). Furthermore, IMA peptides contribute to Cu homeostasis by interacting with the Cu-DEFICIENCY INDUCED TRANSCRIPTION FACTOR 1 (CITF1), inhibiting the transcription of Cu-uptake genes (Cai et al., 2024). This dual role in Fe and Cu homeostasis emphasizes the integrative function of IMA peptides in regulating nutrient balance under varied environmental conditions (Li et al., 2021; Cai et al., 2024). IMA peptide expression is also regulated by phytohormones such as jasmonic acid (JA), brassinosteroids, and auxins, linking their activity to broader nutrient and stress signaling networks (Kobayashi et al., 2020; Wang et al., 2023b). Additionally, IMA peptides have been implicated in the cross-talk between Fe deficiency and pathogen-triggered immune signaling, positioning them as pivotal regulators that synchronize nutrient acquisition with environmental adaptation (Cao et al., 2024; Vélez-Bermúdez and Schmidt, 2024).

In addition to metal ion homeostasis, IMA peptides also play a key role in nitrogen homeostasis by dynamically regulating the balance between nitrogen and Fe availability (Li et al., 2021; Ito et al., 2024). Specifically, in the model legume *Lotus japonicus*, IMA peptides such as LjIMA1 and LjIMA2 have been shown to regulate the allocation of Fe to the nodules during symbiotic nitrogen fixation, a process critical for nitrogen assimilation in legumes (Ito et al., 2024). This regulation of Fe transport to the nodules is responsive to internal nitrogen status, suggesting that IMA peptides function as integrators of metal ion homeostasis and nitrogen metabolism. In *Arabidopsis*, AtIMA peptides exhibit similar responses to nitrogen availability, modulating Fe acquisition under nitrogen-rich conditions, further underscoring the conserved role of IMA peptides in maintaining nutrient homeostasis across different plant species (Ito et al., 2024). The ability of IMA peptides to prevent excessive nitrate

accumulation during nitrogen fixation, thereby safeguarding optimal nodule function, is a testament to their integrative role in maintaining the nitrogen–Fe balance (Ito et al., 2024). By adjusting this balance, IMA peptides ensure that the plant can efficiently assimilate nitrogen while maintaining sufficient Fe levels for essential biochemical processes. Notably, the expression of LjIMA1/2 is tightly regulated by both nitrogen and Fe levels (Ito et al., 2024). When excessive nitrate interferes with normal plant growth, Fe supplementation can restore this balance (Ito et al., 2024), providing further evidence for the crucial role of IMA peptides in coordinating nutrient acquisition and maintaining physiological equilibrium.

Despite the significant progress made, several critical gaps remain in understanding the full scope of IMA peptides' roles. Future research should focus on elucidating the precise molecular mechanisms by which IMA peptides coordinate Fe, Cd, and Cu pathways, and their cross-talk with global signaling networks such as ROS, calcium, and phytohormones (Gratz et al., 2021; Wang et al., 2024). Additionally, exploring whether IMA peptides exhibit tissue-specific functions across organs will provide insight into their role in resource allocation and stress responses. Investigating their spatiotemporal roles in metal ion homeostasis and their evolutionary conservation could uncover universal strategies for enhancing nutrient efficiency and stress resilience.

Small peptides in extracellular pH sensing

Environmental pH plays a critical role in plant growth, development, and stress responses (Tsai and Schmidt, 2021). Small peptides, including RGFs, PEPs, and CLE peptides, act as key mediators of pH sensing, integrating these signals into broader developmental and stress-adaptive pathways (Liu et al., 2022; Diaz-Ardila et al., 2023). Through receptor interactions and PTMs, these peptides regulate growth and stress signaling, enabling plants to adapt to extracellular pH fluctuations (Figure 8; Liu et al., 2022; Diaz-Ardila et al., 2023).

ROOT MERISTEM GROWTH FACTORs, sulfated peptides essential for RAM activity (Meng et al., 2012; Shao et al., 2020), are highly responsive to pH changes (Liu et al., 2022). Under acidic conditions, RGF1 binds with greater affinity to its receptor kinases (RGFRs), promoting RAM cell division and root elongation (Figure 8; Meng et al., 2012; Shao et al., 2020; Liu et al., 2022). This pH-dependent interaction is mediated by sulfotyrosine residues, which serve as extracellular pH sensors

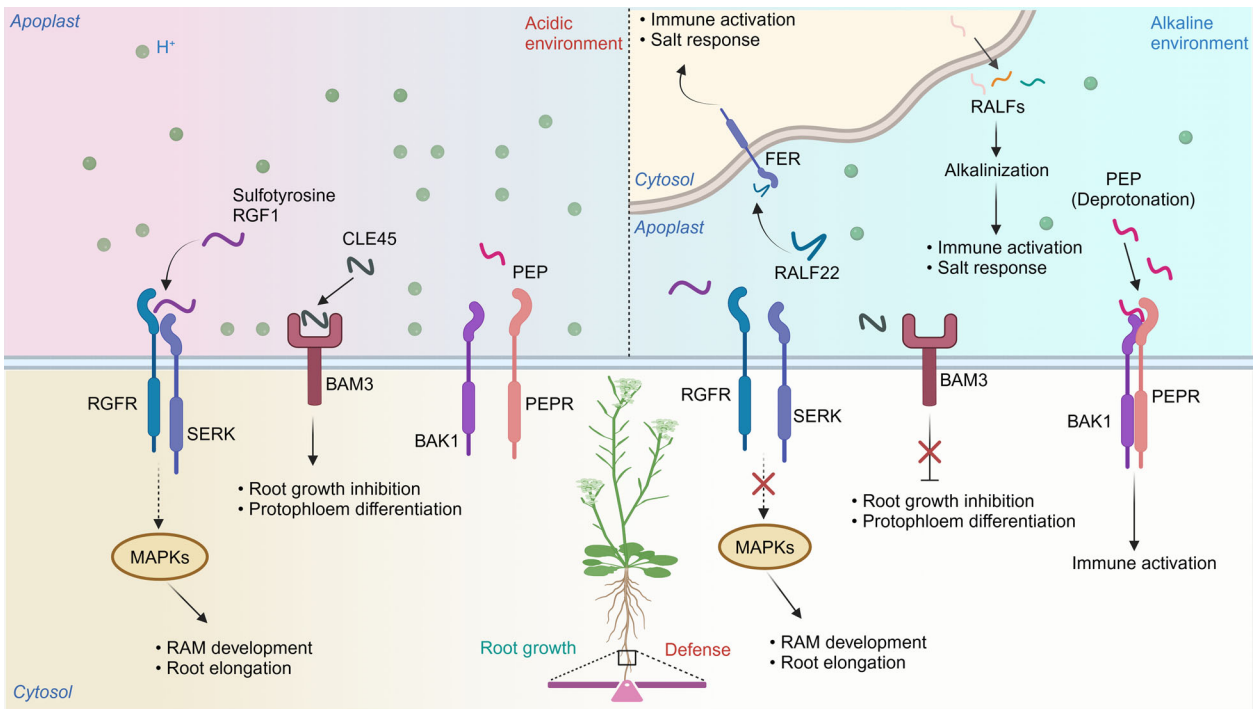

Figure 8. Extracellular pH sensing mechanisms mediated by small peptides (SPs) in plants

This figure illustrates pH-dependent signaling pathways mediated by SPs that regulate root growth and immune responses in plants. In extracellular acidic environments, CLE45 interacts with BAM3, inhibiting root growth while promoting protophloem differentiation. This interaction is pH-sensitive, with specific residues, such as an arginine at position 4, playing a crucial role in recognition by BAM3. RGF1 peptides, which are essential for RAM activity, show high responsiveness to pH changes. Under acidic conditions, sulfotyrosine modifications enhance RGF1 binding to RGFR receptors, promoting root elongation and cell division in the RAM. In contrast, alkaline conditions weaken this binding, reducing RAM activity and shifting plant responses toward defense, including immune activation. In alkaline environments, PEP1 undergoes deprotonation, increasing its affinity for PEPR and thereby activating immune responses. RALF peptides induce extracellular alkalinization, enhance salt tolerance by maintaining cell wall integrity, and promote immune responses, such as those induced by RALF22. This figure underscores the pivotal role of pH-sensitive SPs in balancing growth and stress responses in plants. MAPKs, mitogen-activated protein kinases; RGF, root growth factor; CLE, CLAVATA3/ESR-related peptide; PEPR, PEP receptor; RALF, rapid alkalinization factor; BAM3, Barely Any Meristem 3; FER, feronia; RAM, root apical meristem; PEPs, plant elicitor peptides.

(Liu et al., 2022). Acidification induces conformational changes in these residues, stabilizing the RGF1–RGFR complex, enhancing receptor–ligand interactions, and activating MAPK cascades critical for root development (Figure 8; Lu et al., 2020; Shao et al., 2020; Liu et al., 2022). Conversely, under alkaline conditions, disruption of this pH-dependent binding reduces RAM activity, redirecting plant responses toward stress processes like ion homeostasis and immune activation (Figure 8; Liu et al., 2022). This underscores the flexibility of peptide–receptor complexes in coordinating growth and stress responses under varying pH conditions.

In addition to RGFs, PEPs link extracellular pH changes to immune activation (Liu et al., 2022). Under alkaline conditions, deprotonation of specific amino acid residues in PEP1 enhances its binding affinity for the PEPRs receptor, stabilizing the peptide–receptor complex and triggering immune responses (Figure 8; Liu et al., 2022). This emphasizes the role of pH sensing in immune regulation, where extracellular pH changes directly influence immune activation (Tsai and Schmidt, 2021; Liu et al., 2022). Notably, PEPs such as PEP3 enhance both immunity and salt tolerance, revealing a close link between plant responses to salt stress and immune activation (Nakaminami et al., 2018; He et al., 2023b), suggesting that both pathways may be modulated by extracellular pH changes. RALF peptides also regulate salt tolerance and immune responses (Stegmann et al., 2017; Feng et al., 2018; Zhao et al., 2018; He et al., 2023b). For instance, RALF22 enhances salt tolerance by maintaining cell wall integrity and enhances immunity through interaction with FER (Feng et al., 2018; Zhao et al., 2018; He et al., 2023b). As alkalinization factors, RALF peptides induce rapid extracellular alkalinization, potentially regulating both salt tolerance and immunity (Figure 8; Haruta et al., 2014; Stegmann et al., 2017; Zhao et al., 2018; He et al., 2023b). The interplay between RALF-induced pH changes, salt stress, and immune activation further emphasizes the importance of pH sensing in coordinating plant responses to biotic and abiotic stresses.

CLAVATA3/Embryo Surrounding Region peptides, especially CLE45, are also integral to environmental pH sensing (Diaz-Ardila et al., 2023). Studies show that CLE45 perception is pH-dependent, regulating processes like protophloem differentiation in *Arabidopsis* roots (Diaz-Ardila et al., 2023). Under acidic conditions, CLE45 binds to its receptor, BAM3, promoting growth inhibition and protophloem differentiation (Figure 8; Diaz-Ardila et al., 2023). This interaction weakens under alkaline conditions, indicating pH-sensitive receptor binding. The CLE45–BAM3 interaction is mediated by specific residues, such as an arginine at position 4, essential for pH-dependent recognition (Diaz-Ardila et al., 2023). This demonstrates how CLE peptides fine-tune plant responses to pH fluctuations, promoting growth under acidic conditions while reducing growth-inhibitory signals under alkaline conditions. However, not all CLE peptides exhibit pH-dependent interactions. For example, CLE25 and CLE26, involved in vascular development, function independently of pH fluctuations, influencing neighboring cells irrespective of environmental pH changes (Diaz-Ardila et al., 2023).

While significant progress has been made in understanding how peptides like RGFs, PEPs, and CLEs regulate pH sensing, future research should focus on elucidating the molecular mechanisms of pH perception at the receptor level, especially the dynamic regulation of PTMs, such as sulfation in RGFs and deprotonation in PEPs, under different environmental conditions (Tsai and Schmidt, 2021; Liu et al., 2022). Additionally, understanding how extracellular pH sensing mediated by SPs integrates with other signaling networks, particularly calcium signaling, ROS, and plant hormones, is crucial for uncovering how pH cues coordinate growth and stress responses. Evolutionary studies of pH-responsive peptides across diverse plant species, especially those adapted to extreme environments, could provide insights into the diversity of pH sensing mechanisms and inform strategies for improving crop stress tolerance through genetic engineering or biotechnology.

Small peptides mediate the biotic stress response

Small peptides are central to plant responses to biotic stress (Figure 9). Systemin, the first SP identified in plants, consists of 18 amino acids and plays a pivotal role in regulating plant wound response signaling (Pearce et al., 1991). Upon herbivore or pathogen attack, Systemin interacts with its receptor SYR1, initiating defense responses such as the production of protease inhibitors and the biosynthesis of JA and ethylene (Figure 9A; Wang et al., 2018). The combined treatment of Systemin with the beneficial fungus *Trichoderma afroharzianum* T22 enhances protection against the insect pest *Tuta absoluta* by modulating JA and salicylic acid (SA) signaling pathways (Figure 9A; Aprile et al., 2022). Additionally, the CAPE (CAP-derived) peptide, derived from pathogenesis-related protein 1 (PR-1), inhibits insect growth and activates defense mechanisms against pathogens (Chen et al., 2014). Feeding *Spodoptera litura* larvae with CAPE1-treated tomato leaves suppresses larval growth, and CAPE1 enhances plant immunity against pathogens such as *Pseudomonas syringae* pv. tomato DC3000 (*Pst* DC3000) in tomato and *Parastagonospora nodorum* in wheat (*Triticum aestivum*) (Figure 9B; Chen et al., 2014; Sung et al., 2021).

Pathogen attacks are among the most severe biotic stresses that plants face. Small peptides play a crucial role in amplifying and fine-tuning plant immune responses to these challenges. Peptide elicitors such as PEPs, PIPs, and serine-rich endogenous peptides (SCOOPs) have been frequently reported to activate immune signaling pathways and enhance resistance to pathogens (Figure 9C, D; Hou et al., 2014; Gully et al., 2019; He et al., 2023b). For example, the exogenous application of mature Pep3, PIP1, and SCOOP12 has been shown to increase resistance against *Pst* DC3000 in *Arabidopsis*, and against *Phytophthora infestans* in tomato (*Solanum pimpinellifolium*) (Figure 9D; Hou et al., 2014; Gully et al., 2019; Yang et al., 2023; He et al., 2023b). Several RALF peptides play crucial roles in plant immunity. RALF23 and RALF33 negatively regulate responses to hemibiotrophic bacterial and fungal pathogens by modulating immune

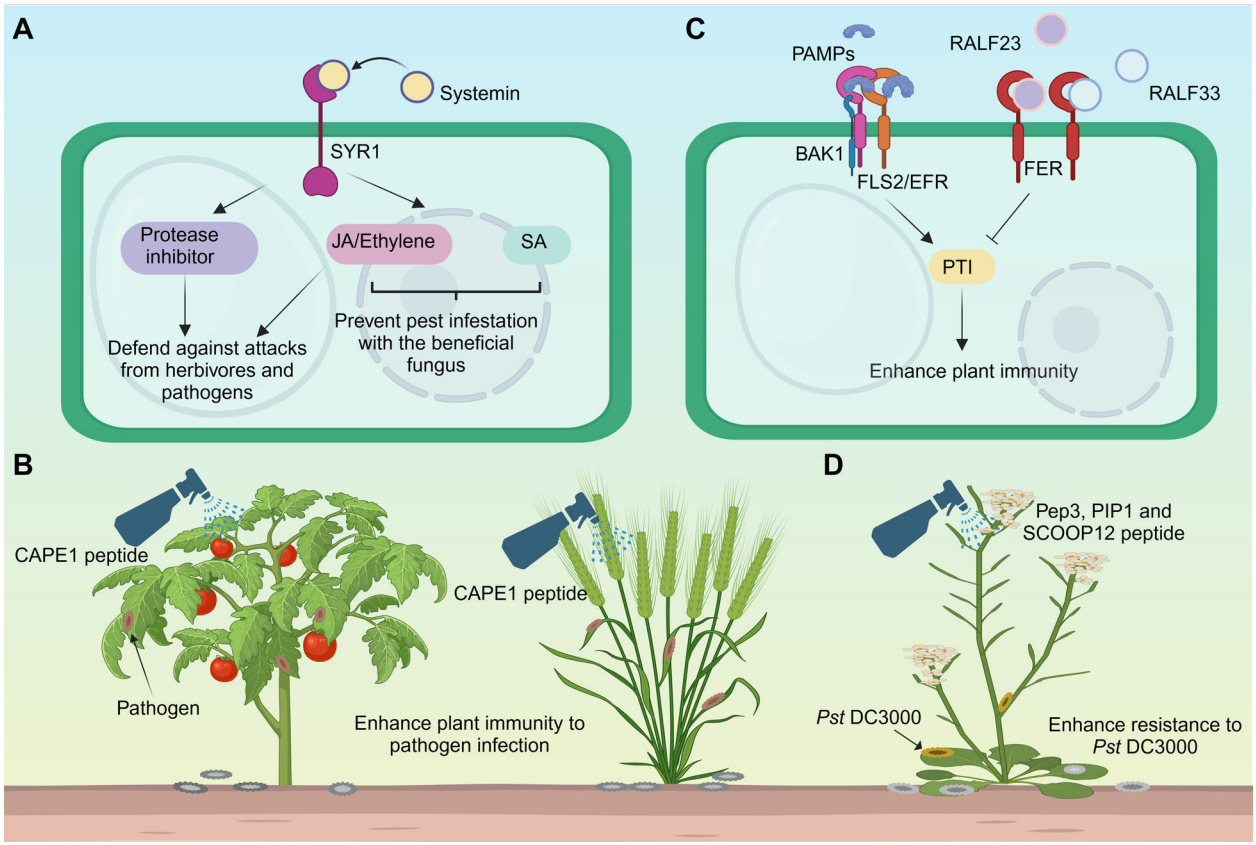

Figure 9. Plant small peptides (SPs) mediate biotic stress responses

(A) Systemin interacts with its receptor SYR1 to trigger the production of proteinase inhibitors and the biosynthesis of jasmonic acid (JA) and ethylene as defense signals against herbivores or pathogens. Systemin and the beneficial fungus *Trichoderma afroharzianum* T22 confer resistance against the pest *Tuta absoluta* by modulating the JA and salicylic acid (SA) signaling pathways. **(B)** CAPE1 enhances plant immunity against pathogens such as *Pst* DC3000 in tomato and *Pyrenophora nodorum* in wheat. **(C)** RALF23 and RALF33 negatively regulate plant immune responses by interacting with the receptor-like kinase FER. This interaction promotes the formation of receptor complexes with immune kinases EFR and FLS2, as well as the co-receptor BAK1, thereby attenuating immune signaling pathways and suppressing PTI. **(D)** Exogenous application of mature Pep3, PIP1, and SCOOP12 enhances resistance to *Pst* DC3000 in *Arabidopsis*. CAPE, CAP-derived peptide; PTI, pattern-triggered immunity; FER, FERONIA; FLS2, Flagellin-Sensing 2; EFR, EF-Tu receptor; PEP, plant elicitor peptide; PIP, PAMP-induced secreted peptide; SCOOP12, Serine-Rich Endogenous Peptide 12.

signaling pathways (Figure 9C; Stegmann et al., 2017). They achieve this through their interaction with the FER, which facilitates the assembly of receptor complexes that include the immune receptor kinases EF-TU RECEPTOR (EFR) and FLAGELLIN-SENSING 2 (FLS2), along with their co-receptor BAK1 (Figure 9C; Stegmann et al., 2017). In contrast, RALF22 enhances immune signaling by amplifying the responses induced by Pep3. Co-infiltration of RALF22 and Pep3 significantly enhances resistance to the necrotrophic fungus *Sclerotinia sclerotiorum* in *Brassica* crops (He et al., 2023b). This finding suggests that RALF22 may function through similar receptor complexes as RALF23 and RALF33, utilizing FER as a scaffold to strengthen plant defense. Furthermore, PSK has been shown to regulate immune responses in tomato via the PSKR1 receptor (Zhang et al., 2018). PSK perception elevates cytosolic [Ca^{2+}] levels, activating auxin-dependent defense pathways and enhancing resistance to *Botrytis cinerea* (Zhang et al., 2018). Recent research has also identified that the IDA peptide, initially recognized for its role in organ separation (Kumpf et al., 2013), enhances immune responses by inducing cytosolic Ca^{2+} release, promoting ROS production, and upregulating immune-related genes, providing additional protection against pathogens (Lalun et al., 2024). These findings emphasize the pivotal role of secreted plant peptides in coordinating developmental and immune signaling pathways.

Additionally, plant-derived AMPs have gained significant attention for their role in mediating innate defense mechanisms and acting as immune inducers against a wide range of pathogens and pests (Erdem Büyükkiraz and Kesmen, 2022). Approximately 4,000 AMPs have been cataloged in the Antimicrobial Peptide Database (https://aps.unmc.edu/), originating from plants, animals, and microorganisms. Plant-derived AMPs are classified into several families based on their sequences and structures, including thionins, defensins, hevein-like peptides, knottins, stable-like peptides, lipid transfer proteins, snakins, and cyclotides (Erdem Büyükkiraz and Kesmen, 2022). These peptides exhibit broad-spectrum

inhibitory effects against various pathogens, including bacteria, viruses, fungi, and parasites.

Defensins and hevein-like peptides, in particular, play pivotal roles in modulating plant immune responses to biotic stress (Gao et al., 2000; Shukurov et al., 2012). These peptides inhibit pathogen growth by disrupting microbial membranes and work synergistically with other SPs to enhance immune responses. For instance, the alfalfa antifungal peptide (alfAFP), a defensin isolated from *Medicago sativa* seeds, demonstrates potent antifungal activity against *Verticillium dahliae*. When expressed in transgenic potato plants, alfAFP confers significant resistance to fungal pathogens such as *Phytophthora infestans* and *Fusarium solani* (Gao et al., 2000). This resistance, observed in both greenhouse and field conditions, provides protection comparable to current fumigant treatments. Additionally, synthetic short peptides derived from OsAFP1, a defensin from rice, exhibit strong antifungal activity against the rice blast pathogen *Pyricularia oryzae* (Sagehashi et al., 2017), further underscoring the potential of AMPs in enhancing plant immunity. Hevein-like peptides, which target chitin in fungal cell walls, play a crucial role in disrupting pathogen structures and reinforcing plant immune defenses. For example, two novel antifungal hevein-like peptides, SmAMP1.1a and SmAMP2.2a, have been identified in *Stellaria media* (Shukurov et al., 2012). The precursor genes for these peptides, pro-SmAMP1 and pro-SmAMP2, exhibit tissue-specific and fungal-inducible expression (Shukurov et al., 2012). Transgenic *Arabidopsis* and tobacco plants expressing pro-SmAMP1 demonstrate enhanced resistance to fungal pathogens such as *Bipolaris sorokiniana* and *Thielaviopsis basicola*, suggesting these genes could be valuable tools for improving plant resistance to fungal diseases.

Taken together, SPs play a pivotal role in coordinating plant immune responses to biotic stress by regulating key signaling pathways, such as those involving JA and SA, which are essential for defense against fungi, bacteria, and herbivorous insects (Wang et al., 2018; Aprile et al., 2022). By interacting with other signaling molecules, SPs fine-tune these responses, ensuring robust defense mechanisms against a wide array of pathogens and pests. The roles of SPs, as exemplified by peptides such as RALF and IDA, illustrate their capacity to bridge developmental regulation and immune responses, thereby enhancing plant adaptability and resilience to diverse environmental stresses (He et al., 2023b; Lalun et al., 2024). Future research should aim to unravel the molecular interactions between SPs and pathogen- or pest-derived effector proteins, which are crucial in enabling these biotic agents to evade plant immune defenses. Elucidating how SPs neutralize these effectors could uncover new strategies to combat pathogen adaptation and pest resistance, providing potential breakthroughs in disease and pest management. Moreover, understanding how SPs integrate with hormonal signaling pathways will be critical for breeding crops with enhanced and flexible immune systems. These studies will not only deepen our understanding of SPs in innate immunity but also pave the way for biotechnological innovations to develop crops with sustainable, eco-friendly resistance to biotic stresses—an essential step in ensuring resilient and productive agricultural systems in the face of evolving environmental challenges.

SMALL PEPTIDES REGULATE PLANT–MICROBIAL SYMBIOSES

Plants rely on symbiotic relationships with microbes to thrive in harsh, nutrient-poor environments by adapting their root system architecture and establishing crucial partnerships. Small peptides are instrumental in mediating these plant–microbe symbiotic interactions (Müller et al., 2019; Roy et al., 2024). The most extensively studied plant endosymbiotic relationships primarily involve N-fixing bacteria, known as rhizobia, and arbuscular mycorrhizal fungi (AMF) (Müller et al., 2019; Roy et al., 2024). In leguminous plants, roots engage with rhizobia to convert atmospheric nitrogen (N_2) into ammonia (NH_3), a form usable by plants. In return for this fixed N, host plants provide rhizobia with essential nutrients and develop specialized root nodules to support these beneficial microorganisms.

Small peptides regulate these symbiotic relationships by controlling the number of root nodules per root system (Figure 10A). For instance, MtCEP peptides redundantly promote nodule formation on *M. truncatula* roots (Zhu et al., 2021b). Overexpression of *MtCEP1* and the application of synthetic MtCEP1 have been shown to increase nodule number, size, and nitrogen fixation efficiency (Tabata et al., 2014; Zhu et al., 2021b). Notably, the induction of the symbiosis-specific CEP gene *MtCEP7* by rhizobia and Nod Factors enhances nodulation, while its downregulation reduces nodule numbers (Laffont et al., 2020). Both MtCEP1 and MtCEP7 regulate nodulation through a systemic signaling pathway mediated by the shoot-localized CRA2 receptor (Figure 10A; Laffont et al., 2020). Similarly, a legume-specific PSK, PSK-δ, and its precursor proteins (MtPSKδ, LjPSKδ, and GmPSKδ1), have been found to enhance symbiotic nodulation by improving nodule organogenesis (Yu et al., 2022). However, even when N needs are met, excessive nodule production can negatively impact plant growth. To maintain symbiotic balance, legumes have developed autoregulation of nodulation (AON), a mechanism that inhibits the formation of new nodules when they are no longer needed (Krusell et al., 2002).

Root-derived CLE peptides, along with their cognate shoot-expressed receptors, are essential in mediating the AON (Figure 10A; Krusell et al., 2002). Specific *CLE* genes associated with rhizobium-plant symbiosis include *MtCLE12*, *MtCLE13*, *MtCLE34*, and *MtCLE35* in *M. truncatula* (Mortier et al., 2010; Mens et al., 2021), *LjCLE-RS1*, *LjCLE-RS2*, *LjCLE-RS3*, and *LjCLE40* in *Lotus japonicus* (Okamoto et al., 2009; Nishida et al., 2016), *GmRIC1* and *GmRIC2* in *Glycine max* (Reid et al., 2011), and *PvRIC1* and

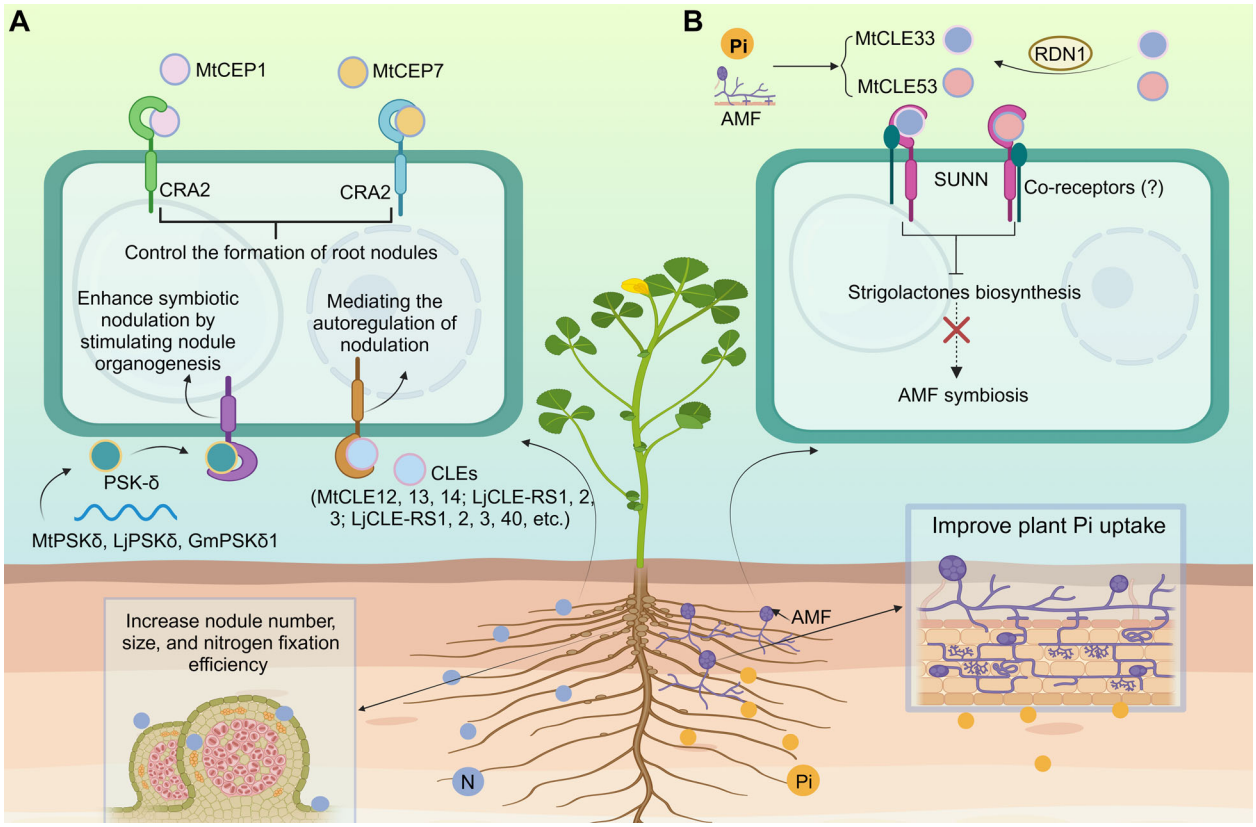

Figure 10. Regulation of plant–microbial symbioses by signaling peptides in legumes

(A) Systemic signaling pathways modulating symbiotic nodulation. Small peptides such as MtCEP1 and MtCEP7 regulate nodulation in legumes through a systemic pathway mediated by the shoot-localized CRA2 receptor. Treatment with synthetic MtCEP1 enhances nodulation by increasing nodule number, size, and nitrogen fixation efficiency. The legume-specific phytosulfokine PSK-δ variants (MtPSKδ, LjPSKδ, and GmPSKδ1) and their precursor proteins promote symbiotic nodulation by stimulating nodule organogenesis. In contrast, CLE peptide–receptor modules act as negative regulators of symbiosis, adjusting nodule numbers to align with the plant's nutrient requirements. **(B)** Mechanism of CLE peptide-mediated modulation of AMF symbiosis. Schematic representation of the mechanism by which MtCLE33 and MtCLE53 regulate AMF symbiosis in *Medicago truncatula* under elevated Pi conditions. High Pi levels and AMF colonization signals induce *MtCLE33* and *MtCLE53* expression in plant roots. These CLE precursor peptides are processed into active forms by RDN1, and the mature MtCLE33 and MtCLE53 peptides subsequently interact with the SUNN receptor, potentially involving an unidentified co-receptor. This CLE-SUNN signaling cascade downregulates strigolactone biosynthesis, reducing AMF colonization. This feedback mechanism enables plants to fine-tune AMF symbiosis in response to increased Pi availability, thereby optimizing nutrient allocation. MtCEP, C-terminally encoded peptide in *M. truncatula*; CRA2, Compact Root Architecture 2; PSK, phytosulfokine; CLEs, CLAVATA3/endosperm surrounding region-related peptides; SUNN, super numeric nodules; Pi, inorganic phosphate; AMF, arbuscular mycorrhizal fungi.

PvRIC2 in *Phaseolus vulgaris* (Ferguson et al., 2014). The expressions of *LjCLE-RS1*, *MtCLE12*, *MtCLE13*, *GmRIC1*, and *GmRIC2* are specifically upregulated by rhizobial infection in roots compared to mock-treated controls (Mortier et al., 2010; Reid et al., 2011; Nishida et al., 2016). Receptor kinase mutants that fail to perceive these peptides result in excessive nodule formation (Krusell et al., 2002). Moreover, overexpression of *MtCLE35*, a nitrate-responsive gene in *M. truncatula*, reduces root nodule numbers through a SUPER NUMERIC NODULES (SUNN)-dependent mechanism (Mens et al., 2021). These findings suggest that CLE peptide–receptor modules act as negative regulators of symbiosis, precisely controlling nodule numbers to align with plant needs and nutrient availability.

Another symbiotic relationship beneficial to land plants involves AMF, which enhance Pi uptake. High levels of exogenous Pi suppress the expression of root symbiotic genes and reduce AMF colonization (Breuillin et al., 2010). In addition to plant hormones such as strigolactones and gibberellic acid, SPs play a crucial role in modulating the phosphate-suppressive effect on AMF symbiosis (Figure 10B; Müller et al., 2019; Karlo et al., 2020). Similar to the regulation of nodulation symbiosis, CLE peptides also significantly contribute to the regulation of AMF symbiosis. In *M. truncatula*, the expression levels of *MtCLE16*, *MtCLE45*, and *MtCLE53* are significantly elevated in roots colonized by the AM fungus *Rhizophagus irregularis* compared to mock-inoculated controls (Müller et al., 2019). Furthermore, transgenic *M. truncatula* roots that ectopically overexpress *MtCLE33* or *MtCLE53* exhibit reduced AMF colonization, indicating that these CLE peptides negatively regulate AMF symbiosis (Figure 10B; Karlo et al., 2020). This negative regulation is mediated through a mechanism involving the receptor-like kinase SUNN and the post-translational peptide modifier ROOT DETERMINED

NODULATION1 (RDN1) (Figure 10B; Müller et al., 2019; Karlo et al., 2020). Notably, mutants of *rdn1* and *sunn* show increased AMF colonization compared to wild-type plants, further emphasizing the role of CLE peptides in regulating AMF symbiosis and their impact on Pi uptake in plant–microbe interactions.

In conclusion, SPs are crucial for regulating symbiosis with rhizobia and interactions with AMF. While progress has been made in understanding the roles of SPs, particularly CLE peptides, in nodulation, their roles in AMF interactions and Pi uptake remain underexplored. Given phosphorus's essential role in plant growth, it is vital to investigate how SPs facilitate AMF colonization and influence Pi dynamics within the plant–microbe symbiotic framework. Future research should focus on the specific functions of SPs in AMF symbiosis, especially regarding environmental conditions like drought and soil type, as well as nutrient availability, including N and potassium (K). These insights could be key to optimizing plant–microbe interactions and enhancing sustainable agriculture.

APPLICATIONS OF SPs IN ENVIRONMENTAL STRESS ADAPTATION

Boosting plant immunity

In recent years, SPs have emerged as critical tools for sustainable agriculture, offering environmentally friendly strategies to enhance plant resilience against environmental stresses (Figure 11A). These peptides serve as promising alternatives to chemical treatments by strengthening plant immunity. One example is Stable Antimicrobial Peptides (SAMPs), developed by Invaio Sciences, which show high efficacy in managing citrus Huanglongbing (HLB) caused by *Candidatus Liberibacter asiaticus* (CLas) (Huang et al., 2021). Stable antimicrobial peptides, derived from *Microcitrus australasica*, is thermally stable and rapidly suppresses CLas infections when applied as a foliar spray. It operates through two mechanisms (Figure 11A; Huang et al., 2021). (i) The first is bactericidal action by disrupting bacterial membranes, particularly targeting Gram-negative α-proteobacteria like

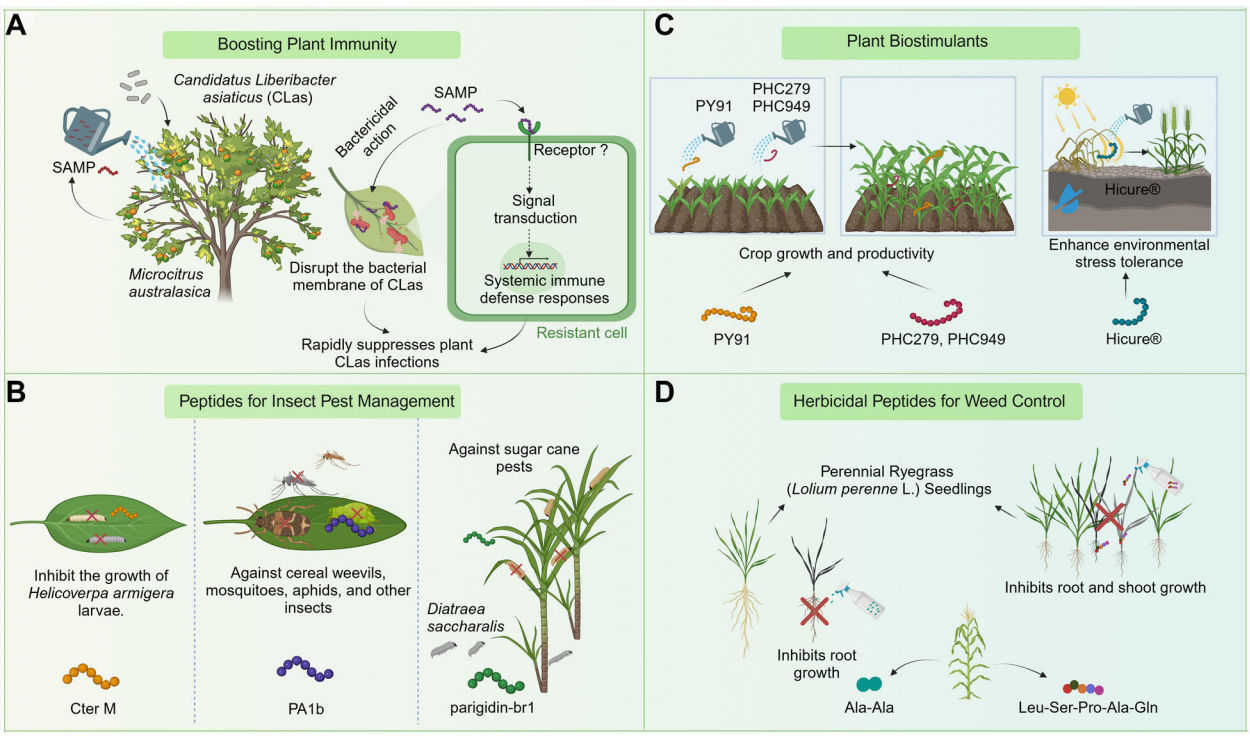

Figure 11. Applications of peptide-based approaches in enhancing environmental stress adaptation

(A) Enhancing plant immunity. The peptide Stable Antimicrobial Peptides (SAMP), derived from *Microcitrus australasica*, plays a crucial role in suppressing *Candidatus Liberibacter asiaticus* (CLas) infection. SAMP suppresses CLas through two primary mechanisms. First, it disrupts the bacterial membrane by forming pore-like structures, leading to bacterial lysis. Second, SAMP binds to an unidentified receptor, activating immune signaling pathways that strengthen plant defense responses. This dual action enables SAMP to effectively inhibit CLas, offering a sustainable alternative to chemical treatments. **(B)** Precision insect pest management with peptides. Peptides such as Cter M from *Clitoria ternatea* inhibit the growth of *Helicoverpa armigera* larvae. The PA1b knottin peptide targets cereal weevils, mosquitoes, and aphids, while parigidin-br1 from Rubiaceae targets neonate larvae of the *Lepidoptera Diatraea saccharalis* in sugarcane. **(C)** Peptides as biostimulants in crop management. PY91 promotes crop growth and productivity, while Hicure® enhances plant resilience to environmental stress. Additionally, PHC279 and PHC949 improve crop productivity by protecting against pathogens. **(D)** Herbicidal peptides for weed control. The pentapeptide Leu-Ser-Pro-Ala-Gln from corn gluten hydrolysate (CGH) inhibits overall growth in perennial ryegrass (*Lolium perenne* L.), while the dipeptide alaninylalanine (Ala-Ala) from corn gluten meal (CGM) specifically targets root growth, reducing root length and causing root tip damage at higher concentrations.

CLas. SAMP's amphipathic α-helix2 domain forms pore-like structures, leading to bacterial lysis. This mechanism reduces the risk of resistant strains emerging. (ii) The second is immune system priming, triggering key regulatory proteins involved in systemic acquired resistance (SAR) and effector-triggered immunity (ETI). While SAMP's receptor remains unidentified, its interaction with immune pathways implies it acts as a ligand for receptor-like proteins or kinases, enhancing defense responses. This dual function of bactericidal action and immune priming positions SAMP as a highly promising tool for modern plant protection, providing a sustainable alternative to chemical methods.

In addition to SAMP, recent studies have explored combining AMPs with elicitor peptides to create bifunctional peptide conjugates that further boost plant immunity (Oliveras et al., 2022). For instance, conjugates incorporating Pep13 have demonstrated significant antibacterial activity against *Xanthomonas* species and *Pseudomonas*, effectively reducing the severity of bacterial spot in tomato plants. This strategic combination not only optimizes antibacterial activity but also minimizes toxicity to the plants (Oliveras et al., 2022). Technologies such as PREtec (developed by Plant Health Care) exploit the synergy of immunostimulatory peptides (https://www.planthealthcare.com/new-technology/pretec), enhancing disease resistance and promoting growth while leaving no harmful residues. This technology highlights the importance of peptide-based solutions in improving plant resilience, especially amid climate change.

While SPs hold immense potential, more research is required to fully understand their mechanisms under diverse environmental conditions. Major challenges include improving SP stability (e.g., UV exposure, soil interactions), optimizing delivery systems, and reducing production costs. Advances in synthetic biology could enable scalable SP production, while enhancing synergy with defense activators can maximize resistance with minimal toxicity. These integrated strategies will offer sustainable agricultural solutions to address climate change and emerging pathogens.

Peptides for insect pest management

Small peptides have emerged as promising, eco-friendly alternatives for managing agricultural insect pests (Figure 11B). Among these, plant-derived peptides, particularly cyclotides, are notable for their stability and potent insecticidal effects (Gressent et al., 2011). Cyclotides, small circular peptides with a cystine knot structure, disrupt insect cell membranes, leading to growth inhibition and mortality in various pest species. A well-known example is PA1b, a knottin peptide from pea seeds, which shows strong insecticidal activity against *Sitophilus* spp., *Culex pipiens*, *Aedes aegypti*, and aphids by disrupting the digestive system (Gressent et al., 2011). Cyclotides from *Palicourea rigida* (*Rubiaceae*) and *Clitoria ternatea* (*C. ternatea*) also show potential. For instance, parigidin-br1 from *Rubiaceae* targets neonate larvae of *Lepidoptera* (*Diatraea saccharalis*) in sugarcane through membrane disruption (Pinto et al., 2012), while Cter M from *C. ternatea* inhibits the growth of *Helicoverpa armigera* larvae (Poth et al., 2011). This led to the development of Sero-X, an organic insecticide now registered in Australia. Notably, Sero-X has minimal toxicity to beneficial insects and mammals, making it an environmentally safer option.

Plant-derived peptides provide a sustainable pest control solution, utilizing multiple mechanisms like membrane disruption, which reduces the risk of resistance. Their natural origins and diverse action modes align with the global demand for eco-friendly agricultural practices. Future research should focus on optimizing application methods and advancing biotechnological innovations to enable crops to develop inherent pest resistance, providing a long-term solution for sustainable pest management.

Peptides as plant biostimulants

Small peptides have been effectively utilized as commercial plant biostimulants to enhance crop health and yield (Figure 11C). For instance, TBIO Crop Science introduced PY91, a functional polypeptide, in 2021 to modulate crop growth (https://biostimulants.eu /members/tbio-crop-science/). Similarly, Syngenta's Hicure® employs amino acids and peptides to improve plant performance and bolster resistance to environmental stress, particularly under abiotic conditions (https://www.syngenta. com/en). These products can be applied either foliarly or to the soil. Additionally, PHC279 and PHC949, developed by Plant Health Care (PHC), have demonstrated significant efficacy as plant vaccines across various crops, protecting against fungal and bacterial pathogens while enhancing crop vigor, resilience, and productivity (https://www.planthealthcare.com/). These examples underscore the multifaceted roles of SPs as plant growth stimulants. They facilitate nutrient uptake, activate growth signaling pathways, and enhance plant stress resistance.

Herbicidal peptides for weed control

Weeds are significant contributors to crop production losses, often surpassing the impact of insect pests and pathogens. Traditional herbicides can pose various issues, including crops and environmental residues, as well as the emergence of herbicide resistance. To address these concerns, both naturally occurring and synthetic peptides have emerged as promising alternatives for weed control in agriculture (Figure 11D). For example, a pentapeptide, Leu-Ser-Pro-Ala-Gln, isolated from corn gluten hydrolysate (CGH), significantly inhibits root and shoot growth in germinating perennial ryegrass (*Lolium perenne* L.) seedlings (Liu and Christians, 1996). Similarly, corn gluten meal (CGM), a by-product of corn wet-milling, contains natural dipeptides, including alaninylalanine (Ala-Ala), which also inhibits root growth in perennial ryegrass (Unruh et al., 1997). Treatment with Ala-Ala reduced root length by at least 42%, causing root tip damage and epidermal necrosis at higher concentrations (Unruh et al., 1997). These peptide-based approaches represent innovative strategies for plant protection and show substantial promise for developing

environmentally friendly agrochemicals. However, research on plant-derived polypeptide herbicides remains extremely limited. Future studies should focus on screening a broader range of these peptides, clarifying their herbicidal mechanisms, and optimizing their efficacy across diverse crops and environmental conditions to maximize their potential as sustainable weed control solutions.

CONCLUDING REMARKS AND PERSPECTIVES

Small peptides play a crucial role in plant growth, development, stress responses, and interactions with microbes. As emerging eco-friendly alternatives for plant protection, they serve as growth regulators and green pesticides against pathogens, pests, and weeds. Their potential as essential tools in sustainable agriculture are increasingly recognized. This review provides a systematic overview of plant SPs, focusing on their characteristics and identification methods. We discuss recent advancements in understanding their biological functions and signaling pathways, which regulate plant growth, plant–microbial symbioses, and resistance to various stresses. By highlighting these aspects, we underscore the integrative approach adopted in this review to advance the field of SPs research. Despite these advancements, several critical issues remain in both fundamental research and the practical application of SPs.

1. Issues in fundamental research on SPs

(i) Identifying SPs presents challenges due to their small size and diverse structures. Traditional methods often fall short in accurately detecting these peptides, especially those with unconventional structures (Andrews and Rothnagel, 2014; Yin et al., 2019; Wang et al., 2020a). To improve detection and analysis, advanced techniques that incorporate computational tools, bioinformatics, and omics technologies are necessary.

(ii) Our understanding of SPs' roles in transcriptional regulation, processing, modification, and receptor recognition remains incomplete. Additionally, the mechanisms governing their transport and functional targeting within plants are not fully elucidated. Further research is required to clarify the distribution and functions of SPs in plant cells and tissues, which will enhance our understanding of these regulatory mechanisms.

(iii) Our understanding of the interactions between SP-mediated molecular signaling and other signaling pathways, particularly those mediated by hormones, is still incomplete. Moving forward, future research should prioritize investigating how SPs detect extracellular pH fluctuations (Tsai and Schmidt, 2021). A critical aspect of this work should be exploring how pH sensing by SPs interacts with key signaling networks, including those involving calcium, ROS, and plant hormones, to balance plant growth and stress resistance.

(iv) Current research has predominantly focused on model plants, with limited studies on a broader range of species, especially those from extreme environments. Expanding such research is essential for understanding the evolutionary mechanisms of SPs and their adaptability, which will further elucidate how SPs contribute to plant adaptation under various environmental stresses.

2. Application issues for SPs

(v) The synthesis and purification technologies for SPs are currently underdeveloped, limiting their production in sufficient quantities and with high purity (Fabre et al., 2021). This limitation affects production costs and economic feasibility, constraining large-scale applications. Additionally, there is a lack of effective formulation technologies and promotion strategies. Future research should focus on advancing these areas through synthetic biology and genetic engineering to develop more cost-effective production methods.

(vi) The influence of environmental factors on peptide expression and function remains poorly understood, which restricts their practical applications. Incorporating environmental considerations into peptide research will be essential for optimizing application strategies and enhancing effectiveness across diverse conditions.

(vii) SPs exhibit a range of regulatory roles, and developing multifunctional peptides that integrate disease and pest resistance with growth-promoting properties could greatly enhance their value in integrated management strategies (Oliveras et al., 2022; He et al., 2023b). Additionally, investigating their interactions with traditional pesticides and other plant protection agents is crucial for optimizing their effectiveness within integrated pest management systems.

Addressing these issues is crucial for unlocking the full potential of SPs and advancing their application in agriculture, environmental protection, and biotechnology.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

F.X. conceived the project, including the overall concept and framework design, drafted the manuscript, and created the illustrations. F.X. and H.Z. contributed to the discussion of the article's framework. H.L. reviewed and edited the manuscript. All authors approved the final version.

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