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The power of small signaling peptides in crop and horticultural plants

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ABSTRACT

Small signaling peptides, generally comprising fewer than 100 amino acids, act as crucial signaling molecules in cell-to-cell communications. Upon perception by their membrane-localized corresponding receptors or co-receptors, these peptide-receptor modules then (de)activate either long-distance or local signaling pathways, thereby orchestrating developmental and adaptive responses via (post)transcriptional, (post)translational, and epigenetic regulations. The physiological functions of small signaling peptides are implicated in a multitude of developmental processes and adaptive responses, including but not limited to, shoot and root morphogenesis, organ abscission, nodulation, Casparian strip formation, pollen development, taproot growth, and various abiotic stress responses such as aluminum, cadmium, drought, cold, and salinity. Additionally, they play a critical role in response to pathogenic invasions. These small signaling peptides also modulate significant agronomic and horticultural traits, such as fruit size, maize kernel development, fiber elongation, and rice awn formation. Here, we underscore the roles of several small signaling peptide families such as CLE, RALF, EPFL, miPEP, CEP, IDA/IDL, and PSK in regulating these biological processes. These novel insights will deepen our current understanding of small signaling peptides, and offer innovative strategies for genetic breeding stress-tolerant crops and horticultural plants, contributing to establish sustainable agricultural systems.

1. Introduction

Plants exhibit prodigious developmental plasticity to ensure optimal cellular and physiological outputs in response to a plethora of intrinsic and extrinsic signals. Developmental adaptations to fluctuating environmental or internal signals can be orchestrated by phytohormones or small signaling peptides, which act either locally or systemically across organs at the whole-plant level through long-distance transport systems [1–6]. Small signaling peptides are encoded in various regions of the plant genome and typically consist of less than 100 amino acids [7,8]. In general, plant small peptides are derived from precursor proteins or non-precursor proteins (Fig. 1A) [3,8–11]. Small signaling peptides can be processed from nonfunctional or functional precursor proteins [3,10,11]. Nonfunctional precursor-derived small peptides can be further subdivided into post-translationally modified (PTM) peptides, cysteine-rich peptides (CRPs), and peptides that lack PTMs or CRPs but contain specific amino acids for their biological activity (Fig. 1A) [3,10,11]. Members of the PTM and CRP peptide families generally contain an N-terminal signal sequence, a central variable region, and conserved motifs or cysteine-rich domains at or near the C-terminus. The PTM and CRP peptides are typically generated by enzyme-mediated processing or modifications of their precursor prepropeptides [3,11,12].

The small peptide encoding genes are initially translated into prepropeptides containing a signal sequence at the N-terminus, which directs them into the secretory pathway. Subsequently, these prepropeptides undergo proteolytic processing, the signal sequence at the N-terminus is cleaved by endoplasmic reticulum (ER)-localized signal peptidase, to yield propeptides, which are

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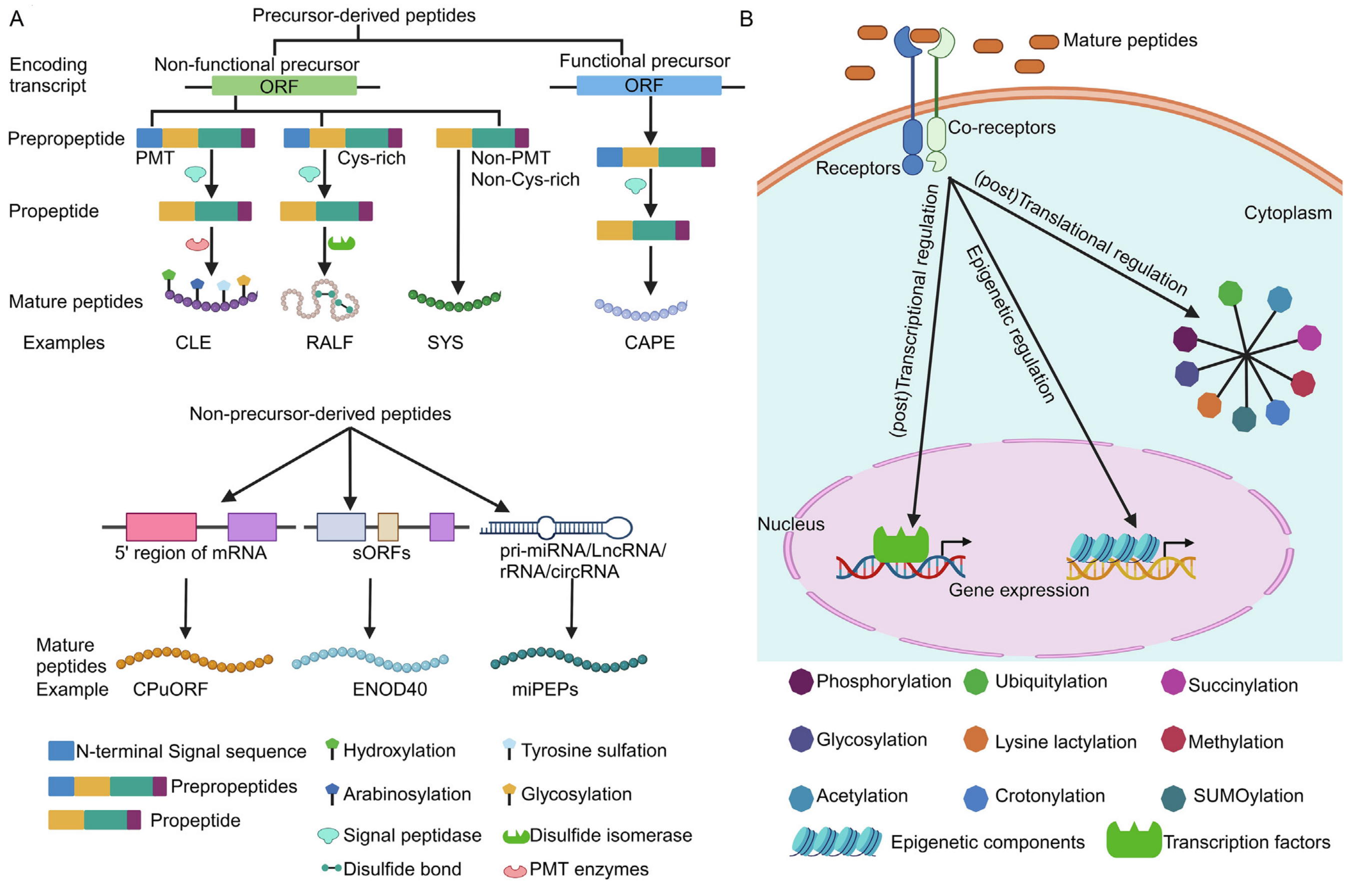

Fig. 1. The diverse small signaling peptides in plants. (A) The biogenesis and classification of small signaling peptides. Most peptides follow this classification, but some exceptions, like peptides lacking N-terminal sequence or multiple peptides being derived from a single precursor, may also exist. Many enzymes play important roles in production of bioactive small peptides, such as signal peptidase for N-terminal cleavage, TPST for tyrosine sulfation, P4H for hydroxylation, SBTs for processing, HPAT, XEG and RRA for arabinosylation. (B) Small peptides bind to receptors or co-receptors to trigger signal transduction. The figure was created with BioRender.com.

subsequently transported through the Golgi complex, followed by secretion to the extracellular space or the plasma membrane [3,12]. PTMs including proline hydroxylation, tyrosine sulfation, arabinosylation, and glycosylation, allow plants to produce bioactive mature PTM peptides [3,11,12]. Disulfide isomerase functions in the formation of disulfide bonds within the CRP peptides [3,11,12]. Various enzymes involved in the processing step, post-translationally modifications and disulfide bond formation, including TYROSYLPROTEIN SULFO TRANSFERASE (TPST), PROLYL-4 HYDROXYLASE (P4H), O-arabinosyltransferase (HPAT), SUBTILASEs (SBTs), XYLOGLUCANASE (XEG), REDUCED RESIDUALARABINOSE (RRA) have been discovered [3,11,12]. Small peptides can also be directly translated from short open reading frames (sORFs), which are embedded in the 5′-leader sequences of messenger RNAs, primary transcripts of microRNAs (miRNAs), long non-coding RNAs (lncRNAs), ribosomal RNAs (rRNAs), circular RNAs (circRNAs) or within transcripts coding for short proteins (Fig. 1A) [9–11].

With the development of advanced genomic sequencing techniques and bioinformatic tools, thousands of small signaling peptides encoding genes have been identified in diverse plant species [2]. Acting as either local or systemic signaling molecules, small signaling peptides are typically perceived by membrane-bound receptors or co-receptors, predominantly members of the receptor-like kinase (RLK) family [2,13]. The binding of these small signaling peptides to their specific receptor-coreceptor complexes modulates various intracellular signaling pathways, thereby orchestrating plant growth and environmental responses through (post)transcriptional, (post)translational, or epigenetic mechanisms (Fig. 1B) [3–6].

Plants are frequently exposed to adverse conditions, and both biotic and abiotic stresses repress their growth and reduce productivity [14–16]. To optimize development in fluctuating environments, plants have evolved multiple mechanisms to integrate environmental cues, coordinating cellular and physiological responses. Modulation of plant growth under stress conditions via the (de)activation of phytohormone signaling pathways represents an adaptive strategy [17]. Small peptides function as local or long-distance signals, orchestrating plant adaptations to both abiotic and biotic stresses by regulating diverse signaling pathways in a manner similar to phytohormones [2–6].

Computational bioinformatics approaches and mass spectrometry (MS) analyses have been employed to characterize distinct small peptide families in the genomes of agricultural and horticultural species, and their biological functions are being gradually uncovered (Table 1). This review describes small signaling peptide families that have been functionally characterized in the modulation of growth and development in crop and horticultural plants. We also address their roles in responses to various abiotic and biotic stresses.

2. Small signaling peptides are crucial regulators of growth and development

2.1. Shoot apical meristem (SAM) development

SAM development requires a precise balance between cell proliferation and differentiation, which is regulated by the CLAVATA3/

Table 1
Small peptides that have been functionally characterized in crops and horticultural plants.

Peptide family	Peptide name	Putative receptor	Biological function	Species	Reference
CLE	FON4/FON2	FON1	SAM and FM development	*Oryza sativa*	Chu et al. [19], Suzaki et al. [23], Xu et al. [24], Meng et al. [25], Ren et al. [26]
	FCP1/OsCLE402	Unknown	SAM development	*Oryza sativa*	Suzaki et al. [21], Kinoshita et al. [27]
	FOS1	Unknown	SAM development	*Oryza sativa*	Suzaki et al. [22]
	FCP1/FCP2	Unknown	RAM development	*Oryza sativa*	Suzaki et al. [21], Ohmori et al. [33], Chu et al. [34]
	ZmCLE7	Unknown	SAM and kernel development	*Zea mays*	Liu et al. [69]
	ZmFCP1	FEA3	SAM and kernel development	*Zea mays*	Je et al. [70]
	SlCLV3	SlCLV1/ SlCLV2	SAM, fruit size	*Solanum lycopersicum*	Xu et al. [28]
	SlCLV3	SlCLV1/ SlBAM1	Abscission	*Solanum lycopersicum*	Cheng et al. [44]
	SlCLE11	Unknown	Arbuscular mycorrhizal colonization	*Solanum lycopersicum*	Wulf et al. [61]
	GmRIC2/GmRIC1	GmNARK	Nodulation	*Glycine max*	Lim et al. [49], Reid et al. [50], Wang et al. [51]
	GmNIC1/GmNIC2	GmNARK	Nodulation	*Glycine max*	Lim et al. [52], Fu et al. [53]
	RsCLE22a	Unknown	Taproot growth	*Raphanus sativus*	Dong et al. [55]
	StCLE4	Unknown	Root and shoot development	*Solanum tuberosum*	Gancheva et al. [36]
	BnCLV3	BnCLV1, BnCLV2	Silique development	*Brassica napus*	Yang et al. [32]
RALF	OsRALF45/OsRALF46	OsMRLK63	Drought response	*Oryza sativa*	Jing et al. [73]
	OsRALF26	OsFLR1	Responses to pathogen	*Oryza sativa*	Kwon et al. [91]
	OsRALF17/OsRALF19	OsMTD2	Pollen tube development	*Oryza sativa*	Kim et al. [56]
	PvRALF1/PvRALF6	PvFER	Nodulation	*Phaseolus vulgaris*	Solís-Miranda et al. [54]
	GhRALF1	Unknown	Fiber elongation	*Gossypium hirsutum*	Wang et al. [63]
	SlRALF2	SlFER	Root development	*Solanum lycopersicum*	Fan et al. [37]
EPFL	GAD1/OsEPFL2/OsEPFL6/ OsEPFL7/OsEPFL9	OsER1	Rice awn and spikelet development,	*Oryza sativa*	Jin et al. [64], Xiong et al. [65], Guo et al. [66,67]
	Bna.EPF2	Unknown	Drought stress	*Brassica napus*	Jiao et al. [74]
	HvEPF1	Unknown	Drought stress	*Hordeum vulgare*	Hughes et al. [75]
miPEP	OsmiPEP156e	Unknown	Cadmium stress	*Oryza sativa*	Lu et al. [79]
	miPEP171d1	Unknown	Adventitious root development	*Vitis vinifera*	Chen et al. [39]
	miPEP172b/ miPEP3635b	Unknown	Cold response	*Vitis vinifera*	Chen et al. [77]
	miPEP156a	Unknown	Root development	*Brassicaceae*	Erokhina et al. [38]
Pep	OsPep3	OsPEPR1/ OsPEPR2	Responses to pathogen	*Oryza sativa*	Shen et al. [93]
	StPep1	Unknown	Responses to nematodes	*Solanum tuberosum*	Zhang et al. [94]
CIF	OsCIF1a/OsCIF1b/OsCIF2	OsSGN3a/ OsSGN3b	Casparian strip development	*Oryza sativa*	Wang et al. [30]
CEP	ZmCEP1	Unknown	Maize kernel development	*Zea mays*	Xu et al. [68]
	SlCEP2	SlCEPR1	Lateral root development	*Solanum lycopersicum*	Hsieh et al. [41]
IDA/IDL	SlIDL6	Unknown	Abscission	*Solanum lycopersicum*	Li et al. [43]
	SlIDA	Unknown	Pollen tubes elongation	*Solanum lycopersicum*	Wang et al. [57]
	GmIDL2a/GmIDL4a	Unknown	Lateral root development	*Glycine max*	Liu et al. [40]
IMA	OsIMA	Unknown	Iron uptake	*Oryza sativa*	Kobayashi et al. [81], Peng et al. [82]
	TaIMA3A	Unknown	Responses to Cd and Fe	*Triticum aestivum*	Zhu et al. [80]
CAPE	ZmCAPE	Unknown	Responses to herbivores and pathogens	*Zea mays*	Lin et al. [90]
	CAPE	Unknown	Responses to herbivores and pathogens	*Solanum lycopersicum*	Chen et al. [89]
SYS	SYS	SYR1	Responses to herbivores and pathogens	*Solanum lycopersicum*	Orozco-Cardenas et al. [84], Coppola et al. [85], Coppola et al. [87]
PSK	SlPSK	Unknown	Drought-induced abscission	*Solanum lycopersicum*	Reichardt et al. [45]
	GhPSK-α	Unknown	Fiber elongation	*Gossypium hirsutum*	Han et al. [62]
PIP	StPIP1	Unknown	Responses to potato virus Y (PVY)	*Solanum tuberosum*	Goyer et al. [96]
PIP	StPIP1	Unknown	Responses to potato virus Y (PVY)	*Solanum tuberosum*	Goyer et al. [96]

Table 1 (*continued*)

Peptide family	Peptide name	Putative receptor	Biological function	Species	Reference
Unknown	GmENOD40	Unknown	Nodulation	*Glycine max*	Ferguson et al. [46], Wang et al. [47], Xu et al. [48]
	OsCDT3	Unknown	Aluminum stress	*Oryza sativa*	Xia et al. [78]
	OsDT11	Unknown	Drought response	*Oryza sativa*	Li et al. [71]
	OsDSSR1	Unknown	Drought response	*Oryza sativa*	Cui et al. [72]
	Ospep5	Unknown	Salt stress	*Oryza sativa*	Wang et al. [76]

EMBRYO SURROUNDING REGION-RELATED (CLE) peptides [18]. In rice, FLORAL ORGAN NUMBER 4 (FON4)/FON2, FON2-LIKE CLE PROTEIN1 (FCP1)/OsCLE402, and FON2 SPARE1 (FOS1) are close homologs of *Arabidopsis* CLAVATA3 (CLV3) peptide, and they play conserved roles in rice SAM regulation [19–22]. The SAM size is larger in the *fon4*/*fon2* mutant than in wild-type (WT) rice [19]. Overexpression of *FON4*/*FON2* or the exogenous application of synthetic FON4 peptide led to the inhibition of rice SAM [19–21]. *FON1* encodes a receptor-like kinase close to the *Arabidopsis* CLAVATA1 (CLV1) receptor, and overexpression of *FON4*/*FON2* does not induce significant SAM aberrations in the *fon1* rice mutant, implying that FON1 is likely a receptor for the FON2 peptide [23]. *FON4* modulates the activity of floral meristem (FM) through its interaction with floral homeotic genes, resulting in aberrant spikelet numbers, underscoring its pivotal role in increasing grain number and increasing rice yield [24–26]. FON2-LIKE CLE PROTEIN1 (FCP1)/OsCLE402 and FOS1 also regulates the SAM development [21,22,27]. Transgenic rice lines overexpressing *FCP1* or *FOS1* exhibit a flattened and diminished SAM size in both wild type and *fon1* rice mutant, suggesting that FCP1 and FOS1 peptides function independently of the FON1 receptor [21–23].

Tomato *CLV3* gene regulates fruit size. The tomato *fasciated* (*fas*) mutant, characterized by a partial loss of function due to an inversion disrupting the *SlCLV3* promoter, leads to branched inflorescences with fasciated flowers and increased fruit size. Alterations in the diverse *cis*-regulatory elements within the *SlCLV3* promoter region variably influence fruit size and SAM [28–31]. Mutations in the tomato homologs of CLV1 and CLV2 also resulted in larger fruit size, suggesting that SlCLV1 and SlCLV2 serve as receptors for the SlCLV3 peptide [28]. Mutation of *CLV3* gene and its receptors, *CLV1* and *CLV2* in *Brassica napus* results in aberrant SAM and multilocular siliques with markedly increased seed numbers, implying its potential utility in increasing seed yield [32].

2.2. Root development

The synthetic FCP1 peptide inhibits the elongation of rice roots. Simultaneous mutations in both *FCP1* and *FCP2* genes resulted in rootless phenotypes [33]. Exogenous application of the FCP2 peptide inhibits root growth and reduces RAM size [34]. However, overexpression of *FCP2* induces profound defects in RAM development. Treatment with the FCP2 peptide or overexpression of *FCP2* disrupts the formation of late metaxylem in the rice root procambium by repressing the expression of *QUIESCENT-CENTER-SPECIFIC HOMEOBOX* (*QHB*) [34]. In *Solanum tuberosum*, *StCLE4* is expressed mainly in the roots, and overexpression of *StCLE4* promotes root growth under nitrogen-deficient conditions by regulating the expression of genes in the auxin signaling pathway [35,36]. Overexpression of *StCLE4* also triggers SAM termination, but resumes leaf growth after the initial SAM arrest [36].

The tomato RAPID ALKALINISATION FACTOR 2 (SlRALF2) and its receptor FERONIA (SlFER) are crucial for root development [37]. The *slralf2* and *slfer* tomato mutants exhibit a shorter primary root compared to WT tomato plants. SlFER interacts with the transcription factor SlMYB63, facilitating its degradation via the 26S proteasome pathway in a manner dependent on phosphorylation. SlMYB63 then directly binds to *DIR19* promoter, activating its transcription, which in turn modulates lignin biosynthesis and root growth. Overall, SlRALF2 peptide modulates tomato root growth by regulating lignin biosynthesis.

In *Brassicaceae*, the miPEP156a peptide is absorbed by roots and accumulates at nucleus. Nuclear-localized miPEP156a then binds to the chromatin histones, thereby influencing the transcriptional activity of the target genes [38]. Consequently, the application of miPEP156a peptide promotes root growth. miPEP156a peptide is also translocated from roots to leaves, where they accumulate and may affect the morphological growth of *Brassicaceae* seedlings [38].

The formation of adventitious root is governed by a complex regulatory mechanism that involves the miPEP171d1 peptide in grapevines (*Vitis vinifera*) [39]. External application of the miPEP171d1 peptide greatly increases the formation of adventitious roots, while it simultaneously inhibits root growth.

Lateral root development is regulated by the INFLORESCENCE DEFICIENT IN ABSCISSION (IDA) and IDA-LIKE (IDL) peptides and CEP peptides. In *Glycine* max, *GmIDL2a* and *GmIDL4a* are active in the cell layers where the lateral root primordium (LRP) initiates, implicating them in lateral root (LR) ontogenesis [40]. Overexpression of *GmIDL2a* and *GmIDL4a* genes leads to increased LR densities, likely through the upregulation of cell wall remodeling (CWR) genes, including *EXPANSIN*s (*EXP*s), *XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDROLASE*s (*XTH*s), and *POLYGALACTURONASE*s (*PG*s) [40].

Tomato plants treated with arbuscular mycorrhizal (AM) fungi *R. irregularis* (Ri) spores exhibit an increased number and density of lateral roots and down-regulated *C-TERMINALLY ENCODED PEPTIDE2* (*CEP2*) expression. *CEP2*-knockdown tomato plants display increased lateral root number and density, while *CEP2*-overexpression plants show a reduced number and density of lateral roots and are insensitive to Ri. AM suppresses *CEP2* expression, and CEP2 peptide can be recognized by CEP RECEPTOR1 (CEPR1) receptor. The CEP2-CEPR1 module then releases the repression of auxin biosynthesis and polar transport. Auxin is transported to the lateral root, stimulating the transcription of lateral root-associated genes to promote lateral formation [41].

2.3. Organ abscission

Abscission of plant organs is crucial for both vegetative and reproductive development, which is regulated by the IDA/IDL peptide family [42]. In tomato, *SlIDL6* acts in low-light-induced flower abscission through both ethylene-dependent and independent pathways [43]. While low light triggers flower abscission, *SlIDL6* knockout plants show delayed flower abscission. In contrast, treatment with synthetic SlIDL6 peptide accelerates flower abscission. The SlWRKY17 transcription factor binds to the W-box in the *SlIDL6* promoter to increase its expression in low light, SlWRKY17-SlIDL6 regulatory module then increases the expression of genes related to cell wall remodeling and disassembly, influencing low-light-induced flower abscission [43].

The tomato SlCLV3 peptide also modulates low-light-induced flower abscission [44]. Under low-light conditions, *SlCLV3* expression is upregulated in the pedicel abscission zone. The SlCLV1 and BARELY ANY MERISTEM1 (SlBAM1) receptors then perceive SlCLV3 signal, thereby inhibiting the transcription of *WUSCHEL* (*SlWUS*). Upon activation of the SlCLV3-SlWUS signaling pathway, the expression of *KNOX-LIKE HOMEDOMAIN PROTEIN1* (*SlKD1*) and *FRUITFULL2* (*SlFUL2*) transcription factors is elevated. As a result, the auxin gradient and ethylene biosynthesis are disrupted in the abscission zone, leading to organ abscission. In the absence of SlCLV3, SlCLE2 can functionally substitute to regulate abscission [44].

The phytosulfokine (PSK) peptide also regulates the premature abscission of flowers and fruits in tomato under drought stress conditions [45]. The overexpression of the tomato *PHYTASPASE 2* (*SlPhyt2*) gene, which encodes a protease that processes peptide precursors, promotes flower drop under drought. *SlPhyt2*-silenced tomato plants exhibit delayed flower drop. PSK peptides are substrates for SlPhyt2. The synthetic PSK peptide increases the activity of cell wall hydrolases, thus accelerating the abscission process under drought conditions. *SlPhyt2* is upregulated in the proximal pedicel region, where it cleaves PSK peptides, which then serve as signals for drought-induced flower abscission.

2.4. Nodulation

The lncRNA-encoded peptide, ENOD40, promotes soybean nodulation through nitrogen availability [46]. In soybean roots, *ENOD40* expression is upregulated by *Bradyrhizobium japonicum* inoculation or nod factor treatments. Modulations in *ENOD40* expression affect nodule numbers, indicating the role of *ENOD40* in nodulation [46]. *miR172c*, a member of the *miR172* family, which is highly expressed in nodules, promotes the formation of nodule primordia when overexpressed [47]. *miR172c* suppresses the expression of *NODULE NUMBER CONTROL1* (*NNC1*), a transcription factor directly targeting the promoter of *ENOD40*. This inhibition of *NNC1* leads to increased *ENOD40* expression, thus facilitating nodule development [47]. *ENOD40* expression is also modulated by the miR169c-NFYA-C module, leading to nodule formation in response to nitrogen availability [48].

The *RHIZOBIA-INDUCED CLE1* (*GmRIC1*) and *GmRIC2*, belonging to *CLE* gene family, are essential for the regulation of soybean nodulation. Overexpression of *GmRIC1* and *GmRIC2* genes inhibit soybean nodulation in a NODULE AUTOREGULATION RECEPTOR KINASE (GmNARK) receptor dependent manner [49,50]. Rhizobia infection increases the expression of *GmNINa*, an ortholog of the *NODULE INCEPTION* (*NIN*) transcription factor. This upregulation of *NIN* subsequently increases *miR172c* transcription, which alleviates the transcriptional repression of *GmRIC1* and *GmRIC2* via the AP2 transcriptional repressor NNC1, thus facilitating nodule formation [51]. *GmNIC1* and *GmNIC2* also encode CLE peptides, and their overexpression reduces nodule number in wild-type soybean plants, but not in *GmNARK* loss-of-function mutants [52]. The soybean NIN-LIKE PROTEIN1 (GmNLP1) and GmNLP4 transcription factors can bind to the promoter region of *GmNIC1*, thus activating the expression of *GmNIC1*, ultimately inhibiting nodulation [53]. Collectively, soybean CLE peptides modulate nodulation through NARK receptors and downstream transcription factors.

In *Phaseolus vulgaris*, knocking down of *PvRALF6* leads to fewer nodules, while silencing of *PvRALF1* does not affect nodule formation. In contrast, overexpressing of *PvRALF1* increases nodule numbers, but overexpressing *PvRALF6* does not change nodulation compared to wild-type plants. Both PvRALF1 and PvRALF6 interact with the PvFER receptor. Reducing *PvFER* expression decreases nodule number, while its overexpression increases nodule formation, likely owing to the changed expression of genes related to the autoregulation of nodulation (AON) and nitrate-mediated nodulation regulation (NRN) [54].

2.5. Taproot growth

The supplement of the synthetic radish RsCLE22a peptide reduces taproot diameter, whereas the suppression of *RsCLE22a* expression increases it, likely owing to modifications in gene expression associated with meristematic activity and auxin signaling pathways [55]. RsCLE22a peptide likely regulates auxin distribution through the ARABIDOPSIS CRINKLY4 (ACR4) receptor. The accumulated auxin subsequently suppresses *RsWOX4* expression, thereby modulating stem cell proliferation within the vascular cambium, thus influencing taproot growth [55].

2.6. Pollen tube development

The rice *OsRALF17* and *OsRALF19* function in pollen development [56]. The pollen grains of *osralf17 osralf19* double mutant fail to produce intact pollen tubes. Interaction assays reveal that OsRALF17 and OsRALF19 interact with the OsMTD2 receptor. Upon activation by OsRALF17 and OsRALF19 ligands, OsMTD2 is internalized to prevent excessive reactive oxygen species (ROS) generation in the pollen tube. Thus, the OsRALF17/OsRALF19-OsMTD2 module regulates pollen tube growth by managing ROS levels.

In tomato, the mutation of *SlIDA* results in compromised male gametogenesis, diminished pollen germination rates, and defective pollen tube growth. RNA sequencing reveals that numerous genes associated with reactive ROS homeostasis and programmed cell death (PCD) are perturbed in the *slida* mutant. Treatment with the SlIDA peptide promotes pollen tube elongation, anther dehiscence, and ROS production. The disruptions in pollen development induced by SlIDA deficiency may lead to reduced fruit set and seed yield, underscoring the essential role of the SlIDA peptide in tomato fertilization [57].

2.7. Casparian strip development

The CASPARIAN STRIP INTEGRITY FACTOR (CIF) peptides are regulators of Casparian strip (CS) development [58,59]. In rice, knockout mutants of *OsCIF1a OsCIF1b OsCIF2* discontinuous CS and reduced endodermis [60]. Overexpression of *OsCIF1*s or *OsCIF2* induces CS formation and excessive lignification and suberization in cortical cell layers adjacent to the endodermis. Knockout of OsSGN3a and OsSGN3b, the putative receptor of CIF peptides [59,60], also triggers a discontinuous CS formation, indicating that OsSGN3a and OsSGN3b function with OsCIF peptides in regulating CS development [60].

2.8. CLE peptide suppresses arbuscular mycorrhizal (AM) colonization

Four tomato CLE genes (*SlCLE5*, *SlCLE11*, *SlCLE13*, and *SlCLE14*) are upregulated upon mycorrhizal colonization, with *SlCLE11* showing the most pronounced expression levels. Mutation of *SlCLE11* in tomato facilitates AM colonization, whereas overexpression of *SlCLE11* inhibits it. *SlCLE10* also functions redundantly with *SlCLE11*. The SlCLE11-mediated repression of AM colonization does not require the involvement of the SlFAB (a CLAVATA1 homolog) or SlCLV2 receptors, but rather depends on the FIN-mediated arabinosylation of SlCLE11 peptides [61].

3. Small signaling peptides define agronomic and horticultural traits

3.1. Cotton fiber elongation

Two peptide family members, GhPhytosulfokine-α (GhPSK-α) and GhRALF1, are pivotal in regulating fiber elongation dynamics [62,63].

Exogenous application of even very low concentration (0.05 μmol L^{-1}) of synthetic PSK-α peptide increases fiber length [62]. Transgenic cotton overexpressing *GhPSK-α* shows an increase in fiber length relative to WT plants, whereas the *GhPSK-α* RNAi lines show no increase in fiber length [62]. GhPSK-α peptide might regulate electron transport and ROS production in rapidly elongating fiber cells, probably by modulating the transcription of NADH-ubiquinone oxidoreductase, class Ⅲ peroxidase and glutathione S-transferase.

The GhRALF1 and GhRALF2 peptides regulate fiber cell elongation [63]. The synthetic GhRALF1 peptide inhibits fiber cell elongation in a dose-dependent manner, whereas the GhRALF2 peptide exerts minimal influence on fiber elongation at comparable concentrations. GhRALF1 peptide modulates fiber growth in a circadian rhythmic-dependent manner, likely by modulation of auxin signaling and H^+-ATPase activity [63].

3.2. Rice awn and spikelet development

The EPIDERMAL PATTERNING FACTOR-LIKE (EPFL) family functions in rice grain and awn development and panicle structure [64–67]. Reducing the levels of *GRAIN NUMBER, GRAIN LENGTH AND AWN DEVELOPMENT1* (*GAD1*), an *EPFL* member, leads to more grains per panicle, shorter grains, and reduced or absent awns [64]. Disruption of *OsEPFL2* also results in shorter or absent awns and smaller grains [65]. Other *EPFL* members can also affect spikelet numbers per panicle [66]. The *osepfl6 osepfl7 osepfl9* triple mutant increases grain yield without reducing spikelet fertility. These EPFL peptides are recognized by the receptor OsER1, which activates the OsMKKK10-OsMKK4-OsMPK6 signaling pathway to impair panicle architecture without affecting spikelet fertility [67].

3.3. Maize kernel development

Maize kernel development is regulated by CEP1 peptide [68]. Disruption of the *ZmCEP1* gene in maize leads to increased plant and ear height. There is a marked increase in kernel length and width, accompanied by a rise in 100-kernel weight and a kernel mass per ear. Overexpression of *ZmCEP1* in maize suppresses kernel development and mass. *ZmCEP1* likely modulates the expression of genes associated with auxin and nitrogen pathways, thereby negatively regulating maize kernel development.

CRISPR-Cas9-mediated promoter editing of *CLE* genes ($CLE^{CR\text{-}pro}$) leads to modifications in several grain-yield-related traits in maize [69]. The $ZmCLE7^{CR\text{-}pro}$ transgenic maize exhibits increased ear diameter, cob diameter, kernel row number, kernel depth, ear weight, and grain yield. Mutation of the *ZmCLE1E5* gene increases the *zmcle7* mutant phenotype, while the *zmcle15* mutant shows no effect on agronomic traits. Mutation of *ZmFCP1*, a member of *CLE* family, results in similar developmental defects observed in the *zmcle7* mutant [70].

4. Small signaling peptides-mediate diverse abiotic stress responses

4.1. Drought response

The rice *OsDT11* gene encodes a predicted 88-amino acid protein featuring a signal peptide and eight cysteine residues at the C-terminus [71]. Transgenic rice plants overexpressing *OsDT11* exhibit reduced wilting compared to wild-type plants during drought stress. By contrast, *OsDT11* knockdown lines show increased drought sensitivity. *OsDT11* likely modulates the expression of ABA biosynthesis and drought-responsive genes, thereby enhancing increasing stomatal density and improving drought tolerance in rice.

The *OsDSSR1* gene in rice encodes a 75-amino acid polypeptide that is localized in both the nucleus and cytoplasm [72]. Elevated expression of *OsDSSR1* in rice results in increased concentrations of free proline, abscisic acid (ABA), and soluble saccharides. It upregulates the expression of antioxidant-related genes and increases the enzymatic activities of superoxide dismutase (SOD) and ascorbate peroxidase (APX). Transgenic rice overexpressing *OsDSSR1* shows increased drought resilience, attributed to elevated levels of stress-inducible genes and ABA content.

In rice, application of synthetic OsRALF45 and OsRALF46 peptides promotes drought tolerance [73]. OsRALF45 and OsRALF46 physically interact with MALECTIN/MALECTIN-LIKE DOMAIN-CONTAINING RECEPTOR-LIKE KINASE63 (MRLK63). The OsRALF45/OsRALF46MRLK63 complex then elicits ROS production by phosphorylating Ser26 in the N-terminal of RESPIRATORY BURST OXIDASE HOMOLOGUE A (OsRBOHA), ultimately promoting drought tolerance. Rice synthesizes three distinct small peptides to facilitate adaptations to drought stress [71–73]. It appears that these three drought-responsive peptides converge on ABA and ROS signaling pathways to increase drought tolerance.

In *Brassica napus*, overexpression of *Bna.EPF2* reduces stomatal density and pore size, thereby decreasing transpiration and increasing water-use efficiency and drought tolerance [74]. Similarly, overexpression of the barley *HvEPF1* gene reduces stomatal density and increases drought tolerance [75]. Overexpression of *Bna.EPF2* or *HvEPF1* does not affect yield traits, suggesting that EPF peptides can modulate stomatal development to increase drought tolerance without compromising crop yield.

4.2. Salt stress

Rice Ospep5 peptide is an important player in response to salt stress [76]. Application of the synthetic Ospep5 peptide or overexpressing *Ospep5* trigger a reduced Na^+ accumulation and a lower Na^+/K^+ ratio in both shoots and roots by up-regulating the expression of ion transporter genes, thereby increasing salinity tolerance. *Ospep5* knockout rice shows increased sensitivity to salinity, characterized by a higher Na^+/K^+ ratio and down-regulated ion transporter gene expression.

4.3. Cold stress

Under low temperature (4 °C) conditions, grapevine *pri-miRNAs* exhibit differential expression levels, with *miPEP172b* and *miPEP3635b* showing the highest expression levels [77]. The application of synthetic miPEP172b and miPEP3635b peptides promote cold tolerance by regulating the expression of *NAC2* and *WRKY40*, which in turn adjusts ROS levels by modulating the activities of superoxide dismutase (SOD) and peroxidase (POD), ultimately increasing cold resistance.

4.4. Heavy metals

Two small rice peptides, OsCDT3 and OsmiPEP156e, modulate responses to aluminum (Al) and cadmium (Cd), respectively [78,79]. The *OsCDT3* gene encodes a 53-amino acid peptide with 14 cysteine residues. OsCDT3 localizes to the plasma membrane, and its expression is specifically up-regulated by Al exposure in roots. Silencing of *OsCDT3* leads to reduced Al tolerance. OsCDT3 does not transport Al, while it binds to Al, preventing Al entry into root cells and increasing Al tolerance in rice [78].

Cd exposure alters the expression patterns of *MIR156* family members in rice roots, and *pre-miR156e* shows the highest expression [79]. Owing to a higher accumulation of Cd and ROS, the CRISPR-Cas9 generated *miPEP156e* rice mutant is more sensitive to Cd stress. In contrast, overexpression of *miPEP156e* or external application of synthetic miPEP156e could reduce Cd uptake and accumulation as well as ROS levels by modulating the transcription of Cd transporter and ROS scavenging genes.

The wheat IRONMAN (IMA) peptide also increases Cd tolerance [80]. Induction of *TaIMA3A* expression induces a decreased Cd accumulation by up-regulating *TaNAS4D* and *TaNRAMP5* genes expression. Treatment with wheat TaIMA3A peptide also up-regulates genes involved in Cd sequestration and down-regulates Cd transporter genes, promoting Cd tolerance.

4.5. Iron uptake

Two *OsIMA* genes, *OsIMA1* and *OsIMA2*, regulate Fe deficiency in rice [81,82]. Overexpression of *OsIMA1* and *OsIMA2* in rice increases tolerance to Fe deficiency and increases Fe accumulation in leaves and seeds. Knockdown of these genes has minimal effects on Fe deficiency tolerance and Fe accumulation [81]. Fe sensors MOTIF-CONTAINING REALLY INTERESTING NEW GENE (RING) AND ZINC-FINGER PROTEIN 1 (OsHRZ1) and OsHRZ2 promote the degradation of OsIMA1 and OsIMA2 peptide, reducing Fe uptake [82]. Thus, OsIMAs and OsHRZs have antagonistic roles in the regulation of Fe deficiency responses in rice.

5. Small signaling peptides regulate biotic stress responses

5.1. Systemin (SYS) peptides

Systemin (SYS), an 18-amino acid peptide, mediates defense against an array of biotic stressors, including herbivores and pathogens. SYS interacts with the membrane-bound SYSTEMIN RECEPTOR1 (SYR1), coordinating signals such as ROS, Ca^{2+}, jasmonic acid, the pro-systemin mRNA, and volatile organic compounds into a cohesive defense response [83]. In tomato, silencing of the *SYS precursor gene* (*ProSys*) reduces resistance to *Manduca sexta*, whereas overexpression increases resistance against aphids and *Spodoptera littoralis* larvae [84,85]. Exogenous application of SYS peptide prompts production of protease inhibitors in tomato plants, hindering *Spodoptera littoralis* larvae growth and decreasing *Botrytis cinerea* leaf colonization [86,87]. SYS peptide influences posttranslational protein regulation and promotes the accumulation of specific secondary metabolites, strengthening defenses against necrotrophic fungi [88].

5.2. CAP-DERIVED PEPTIDE (CAPE) peptides

A mass spectrometry-based peptidomics approach identified CAPE1 as a defense peptide in tomato leaves, activated by wounding and methyl jasmonate (MeJA) treatment [89]. Tomato plants treated with synthetic CAPE1 peptide resist to *Spodoptera litura* larvae and show no infection or hypersensitive responses when exposed to *Pseudomonas syringae* pv. tomato DC3000 (Pst DC3000). In maize plants, the ZmCatB3 enzyme cleaves the CNYD motif of the *Ustilago maydis* PR-1La protein, releasing the UmCAPE-La peptide. UmCAPE-La presumably competes with ZmCAPE peptide for binding to the unidentified receptors, thus regulating the *pathogenesis-related* (*PR*) genes expression and increasing *Ustilago maydis* virulence [90]. CAPE peptide appears to serve as a novel damage-associated molecular pattern (DAMP) signal, eliciting immunity against pathogenesis.

5.3. RALF peptides

The rice peptide OsRALF26 confers resistance to the bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) [91]. OsRALF26 is found in the apoplastic space and its expression is significantly increased by *Xoo* through the rice immune receptor XA21. Recombinant OsRALF26 protein induces the expression of immune-related genes and ROS production. Transgenic rice overexpressing *OsRALF26* exhibits significantly increased resistance to *Xoo*. The rice receptor OsFLR1 perceives the OsRALF26 signal via the conserved YISY motif, leading to ROS production and callose deposition, which regulate immune responses [91,92].

5.4. Plant elicitor peptide (Pep) peptide

Plant elicitor peptide (Pep) and its receptor PEP RECEPTOR (PEPR) are critical for enhancing tolerance to *Nilaparvata lugens*, the brown planthopper (BPH) [93]. Among seven OsPep peptides, OsPep3 peptide triggers immune responses in rice by causing a strong ROS burst. Applying synthetic OsPep3 peptide increases BPH resistance in wild-type rice, but not in the *ospepr1 ospepr2* double mutant, indicating that the OsPep3-OsPEPR pathway functions in BPH resistance. Transcriptomic and metabolomic analyses show that OsPep3 peptide treatment increases the expression of genes involved in jasmonic acid (JA) biosynthesis, resulting in JA accumulation and BPH resistance.

In *Solanum tuberosum*, the StPep1 peptide modulates resistance against the root-knot nematode *Meloidogyne chitwoodi* [94]. After *Meloidogyne chitwoodi* infection, there is no difference in the juvenile nematode number in roots of StPep1 peptide-treated or control treated potato seedlings, suggesting that StPep1 peptide does not affect nematode ingress into the roots. But StPep1-treated potato plants show fewer galls and egg masses, and their biomass remains unchanged, suggesting that StPep1 increases plant defense against nematodes. StPep1 peptide activates defense genes via the jasmonic acid receptor, boosting potato resistance to root-knot nematodes. Notably, *Bacillus subtilis* can synthesize bioactive StPep1 peptide. Potato plants treated with *Bacillus subtilis* that secrete StPep1 also show resistance to *Meloidogyne chitwoodi*.

5.5. PAMP-INDUCED PEPTIDE (PIP) peptide

Potato virus Y (PVY), an RNA plant virus, inhibits the development of potato plants and reduces yield. The PAMP-INDUCED PEPTIDE1 (PIP1) has been identified as a regulator of PVY infection [95]. Application of exogenously synthesized StPIP1 peptide triggers various defense responses, including Ca^{2+} influx, ROS accumulation, and the activation of immunity genes associated with both pattern-triggered immunity (PTI) and effector-triggered immunity (ETI). Transgenic potato plants overexpressing *StPIP1* show reduced sensitivity to PVY infection, likely due to increased callose deposition in foliar tissues [96]. StSERK3A/B and RLK7 receptors may perceive StPIP signal to mediate the response to *Phytophthora infestans* infection, however, the involvement of these receptors in StPIP1-dependent PVY response is unclear [97].

6. Future perspectives

The biological roles of small signaling peptides are progressively being elucidated, and the utilization of these synthesized small peptides can influence the development and growth of crop and horticultural plants, as well as their responses to both biotic and abiotic stresses. Future studies should address the following questions.

6.1. Identification of small peptides by advanced MS and sequencing techniques

MS is a credible method to identify and verify the majority of most peptide members in plants. Several adjustments including sample preparation, protease digestion, data acquisition, and data analysis have been suggested to improve MS-based detection of small coding proteins [98]. However, MS is limited in detecting low-abundance peptides in plant tissues. The advanced label-free MS imaging (MSI) techniques including matrix-assisted laser desorption/ionization (MALDI), desorption electrospray ionization (DESI), laser desorption ionization (LDI) and laser ablation electrospray ionization (LAESI) have facilitated the mapping of small peptides or proteins in mammalian cells [99–101]. MSI is an advanced technique for mapping endogenous small peptides spatially due to its highly informative nature, superior sensitivity, and high spatial resolution, allowing detection of low-abundance small peptides at nano concentration levels. Improved spatial resolution of MSI allows for small peptide analysis at the single-cell level. MSI can illustrate the spatial distribution of small peptides in various tissues at multiple developmental stages or in response to biotic and abiotic stresses in both 2D and 3D dimensions. To apply MSI to plant species, existing protocols for mammalian cells can be adapted [100,101]. Plant tissues such as roots and leaves can be collected at multiple developmental stages or subjected to specific biotic or abiotic stresses. After fixation, the tissues should be sliced into uniform sections of appropriate thickness using a cryostat and placed on compatible MSI target plates. Following digestion, on-tissue analysis of endogenous small peptides can be performed using instruments like MALDI quadrupole TOF, MALDI quadrupole ion trap TOF, and MALDI Fourier transform mass spectrometry (FTMS) [100].

Genomic sequencing methods such as Hi-C, PacBio HiFi, nanopore ultralong sequencing, and non-coding RNA have facilitated the production of high-quality and non-gap telomere-to-telomere genome assemblies [102]. The improved genomes have facilitated the genome-wide discovery of novel genes encoding small signaling peptides in various plant species [9]. Ribosome profiling has uncovered numerous noncoding RNAs, small open reading frames (ORFs), and upstream ORFs that encode new small peptides in previously unannotated genomic areas [103]. Computational tools such as RiboTaper, RiboToolkit, RiboFlow, ANNOgesic, MiPepid, sORF finder, PhyloCSF, and uPEPperoni have been used to identify unannotated small signaling peptides across different plant species [9,76]. These advanced MS and sequencing technologies will enable the identification of additional small signaling peptide members, particularly rRNA or circRNA-encoded peptides that are not currently reported in crops and horticultural plants [9] (Fig. 2A).

6.2. What are the physiological roles of small peptides?

Due to the large number of identified peptide members, the biological functions of most small peptides remain unclear. CRISPR-generated single or multiple mutants [104], along with overexpression transgenic lines, present an opportunity to their roles in development and stress responses (Fig. 2B). Conjugated small peptides with fluorescent probes and photo-caging groups can be employed to investigate the subcellular dynamics, long-distance or local transport mechanisms of small signaling peptides, as well as their receptor binding affinities *in vivo* [105,106]. Identification of the enzymes involved in small peptide processing and modifications will elucidate their biosynthesis [12].

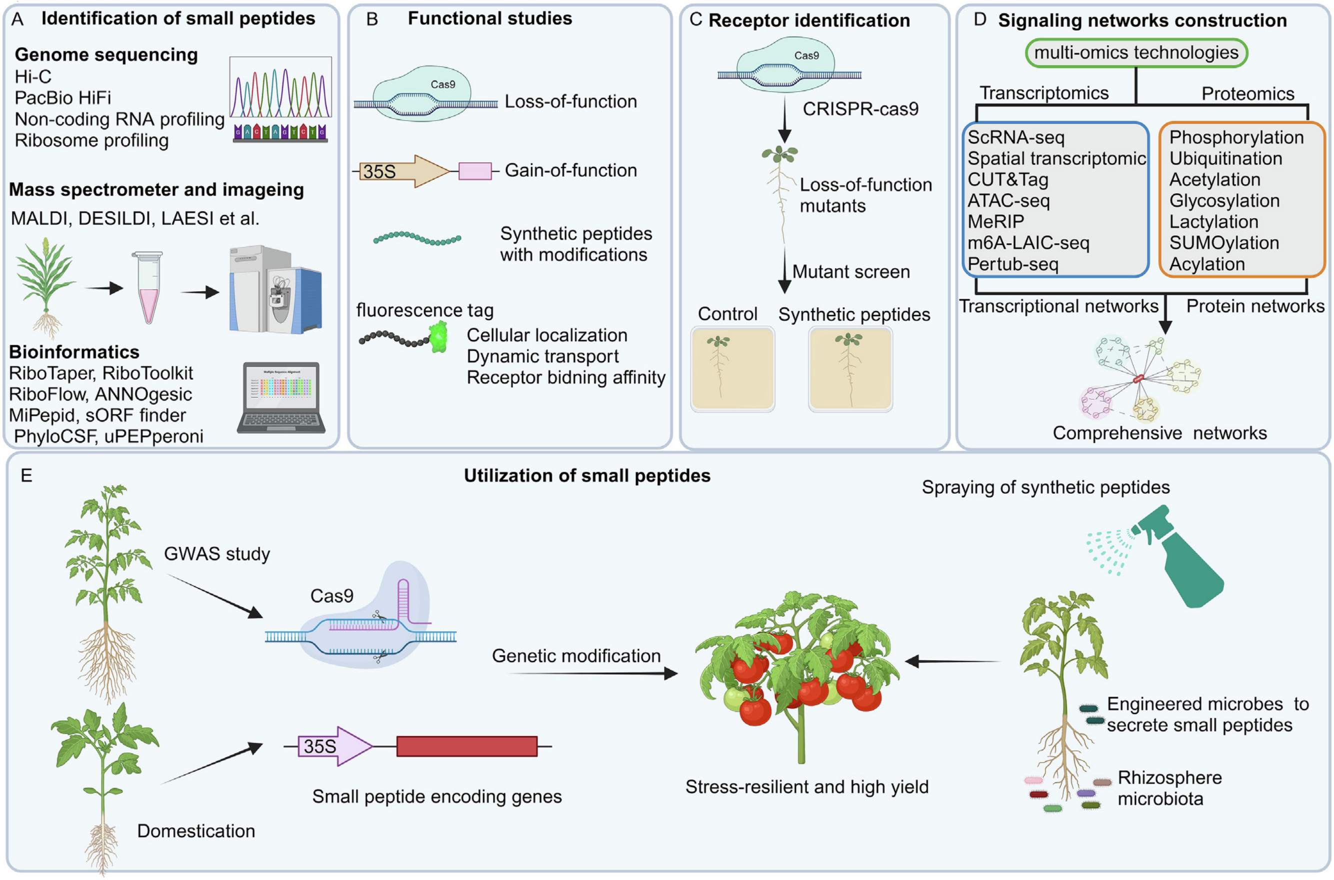

Fig. 2. Exploration and application of small signaling peptides in crop and horticultural plants. (A) Discovery of small peptides. (B) Study of the biological roles and subcellular localization of small peptides. (C) Identification of the receptors or co-receptors. (D) Construction of regulatory networks. (E) Application of synthetic small peptides in agriculture and genetic breeding. The figure was created via BioRender.com.

In addition to these canonical peptide families, the advanced sequencing technologies have revealed a prevalence of noncanonical peptides (NCPs) derived from previously annotated non-coding regions, such as intergenic regions, 5′ untranslated regions (5′ UTRs), 3′ UTRs, intronic regions, and various genomic junctions [107–109]. Increasing evidence indicates the participation of NCPs in a myriad of biological processes, such as soybean nodulation [47,48] and pathogen responses in maize [110]. Using established methodologies [108,109], it is now possible to identify NCPs across various crop and horticultural species and to further elucidate their undefined roles in developmental and adaptive processes.

6.3. How to maintain the homeostasis of small signaling peptides?

Plants produce diverse small signaling peptides (Table 1), the unanswered key question is that why crop and horticultural plants produce such a diverse array of small signaling peptides. Because these signaling peptides may act synergistically or antagonistically, it is desirable to understand how plants equilibrate the levels of these small signaling peptides to achieve optimal cellular responses and overall growth. Plant phytohormones such as auxin, ethylene and cytokinin regulate POLARIS (PLS) peptide function and *CLE* gene expression level [111,112], however, mechanisms underlying their interactions in crop and horticultural plants await identification.

6.4. Identification of receptors for small signaling peptides

Plasma membrane (PM)-localized RLK receptors are key hubs to perceive diverse small signaling ligands to regulate plant development and environmental responses [13,113,114]. Although numerous RLKs have been identified, many unknown receptors remain to be characterized. 4-azidosalicylic acid ([125] ASA)-labeled peptides could be used to identify undefined RLK receptors [115]. CRISPR-based genetic screening systems allow generating single or multiple mutants of RLKs from the same family simultaneously [116]. This approach could be employed to discover uncharacterized receptors for small peptides with high specificity and throughput (Fig. 2C). In addition, the precise mechanisms of recognition between small peptides and their corresponding receptors in crops and horticultural plants remain to be elucidated [117,118].

6.5. Exploration of molecular mechanisms

In comparison to the model plant *Arabidopsis thaliana*, the mechanisms underlying the signal transduction of small signaling peptides in crops and horticultural plants, however, remain largely unknown. The integration of single cell RNA-seq (scRNA-seq) technique with spatial transcriptome will reveal transcriptional variations in diverse cell types and capture the dynamic changes in transcript abundance at single-cell resolution [119,120]. CRISPR-based gene expression regulation tools such as CRISPR interference (CRISPRi), CRISPR activation (CRISPRa), CRISPRoff, CROP-seq, CRISP-seq, the CRISPR epigenetic system, and Pertub-seq [104], can be employed to investigate how small signaling peptides influence growth, agronomic/horticultural traits and stress responses, as well as to construct unprecedented transcriptional networks driven by these small signaling peptides. Epigenetic regulation is also involved in small peptide signaling [121], several RNA and DNA modification sequencing methods such as MeRIP, m6A-LAIC-seq, CUT&Tag and ATAC-seq have been used to elucidate the epigenetic mechanisms [122,123]. These methods can reveal novel epigenetic mechanisms regulated by small signaling peptides. The newly developed mass spectrometer will facilitate high-throughput exploration of 4D proteomics, characterized by high robustness, sensitivity, and specificity [124]. This approach will reveal protein abundance, stability and PTMs, thereby contributing to the establishment of protein networks orchestrated by small signaling peptides. These advanced techniques will facilitate the construction of comprehensive and robust regulatory networks that are mediated by small signaling peptides in various developmental and adaptive processes (Fig. 2D).

6.6. How to utilize synthetic biology to produce small peptides?

High-performance liquid chromatography (HPLC) is commonly employed for the synthesis of small signaling peptides, although the production of post-translational modified small signaling peptides by this method is costly. Alternatively, microbial systems can be engineered for the biosynthesis of plant-derived small peptides. The StPep1 peptide can be produced from *Bacillus subtilis* with bioactivity [94]. Microorganisms present a viable option for *in vitro* production of various small peptides. Synthetic biology offers the potential to engineer controllable biosynthetic pathways, facilitating production of diverse small peptides with lower cost while keeping their bioactivity [125]. Deploying these microbes to release plant small peptides into the soil could increase plant growth and development under fluctuating environmental conditions (Fig. 2E).

6.7. The application of small signaling peptides in agriculture and genetic breeding

Due to their low molecular weight and small size, and they are environmentally safe and non-polluting. Small signaling peptides are easily synthesized and serve as ideal agents for exogenous application in agriculture to promote plant growth, yield, and pathogen resistance (Fig. 2E). Genome-wide association studies (GWAS) and domestication analyses can pinpoint genes encoding small signaling peptides that govern specific agronomic or horticultural traits. Gene editing and overexpression can further elucidate their biological functions, which will facilitate precise targeted genetic modifications of agronomic or horticultural traits.

CRediT authorship contribution statement

Chao Ji: Writing – original draft. **Hui Li:** Writing – original draft. **Zilin Zhang:** Writing – original draft. **Shuaiying Peng:** Writing – review & editing, Funding acquisition. **Jianping Liu:** Writing – review & editing, Funding acquisition. **Yong Zhou:** Writing – review & editing, Funding acquisition, Conceptualization. **Youxin Yang:** Writing – review & editing, Funding acquisition, Conceptualization. **Huibin Han:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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