The Role of EPFL Peptides in Plant Development and Stress Responses

A. D. Maiboroda[1], A. A. Makeeva[1], R. A. Azarkina[1], A. S. Barashkova[1,2], A. S. Mamaeva[1*]
[1]Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, 117997 Russia
[2]All-Russian Institute of Plant Protection, St. Petersburg, Pushkin, 196608 Russia
*E-mail: AnnetteSt@yandex.ru
Received April 18, 2025; in final form, July 01, 2025
DOI: 10.32607/actanaturae.27675
Copyright © 2025 National Research University Higher School of Economics. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT **Cysteine-rich peptides belonging to the EPF/EPFL (epidermal patterning factor/epidermal patterning factor-like) family are common in many plants, from mosses to angiosperms. EPF/EPFL play an important role in morphogenesis: they regulate stomatal patterning, the functioning of the shoot apical and lateral meristems, inflorescence architecture, vascular development, growth of leaf margin, as well as the development of flowers and fruits. Recent studies have indicated that EPFL may be involved in plant adaptation to biotic and abiotic stress. This review examines the structure, phylogenetic distribution, mechanisms of signal transduction, and functions of the EPF/EPFL peptide family.**
KEYWORDS **plant regulatory peptides; cysteine-rich peptides; EPF/EPFL.**
ABBREVIATIONS **EPF – epidermal patterning factor; EPFL – epidermal patterning factor-like; ABA – abscisic acid; MAPK – mitogen-activated protein kinase; MDA – malondialdehyde; MMC – megaspore mother cell.**

INTRODUCTION

As sessile organisms, plants adapt to environmental changes through a flexible system that regulates physiological processes. A crucial role in this adaptation is played by signal peptides, which control a broad range of responses, including growth and development, sexual reproduction, intercellular communication, senescence, symbiosis, as well as resistance to pathogens and abiotic stress [1, 2]. The first identified plant regulatory peptide, systemin, was isolated from tomato leaves in 1991 [3]. Numerous peptide families, originating either from the processing of precursor proteins or via translation of short open reading frames, have been described since then [1, 4].

Peptides derived from precursor proteins are classified into three functionally and structurally distinct groups: post-translationally modified peptides [5], cysteine-rich peptides, and unmodified peptides without cysteine residues [6, 7]. Cysteine-rich peptides carry an even number of cysteine residues that form disulfide bonds, a disposition that ensures the stability of their spatial structure. Antimicrobial peptides were the first members of this group to be discovered and described [8]. It was originally believed that the functions of cysteine-rich peptides were limited to defense against pathogens [4, 9]. However, subsequent research demonstrated that cysteine-rich peptides have a much broader range of functions, encompassing the regulation of stomatal initiation, symbiosis, reproductive processes, and stress responses [10–12].

The cysteine-rich peptides EPF/EPFL were first identified as key regulators of stomatal development in *Arabidopsis thaliana* (Arabidopsis) [10, 13–15]. Further research revealed that these peptides are involved in the regulation of the size of shoot apical meristem, inflorescence development, and stress adaptation. Although the body of experimental data on the subject continues to grow, there are currently no systematic reviews that summarize information about this family. Our study has endeavored to consolidate the data on EPF/EPFL peptides, including their structure, evolutionary diversity, and biological functions.

THE STRUCTURE AND SIGNAL TRANSDUCTION

Cysteine-rich plant peptides can be roughly divided into defensive (antimicrobial) and regulatory peptides and comprise several families, including the EPF/EPFL one [16]. The structure of defensive peptides has been the one studied most thoroughly: NMR analyses have been performed for many of these pep-

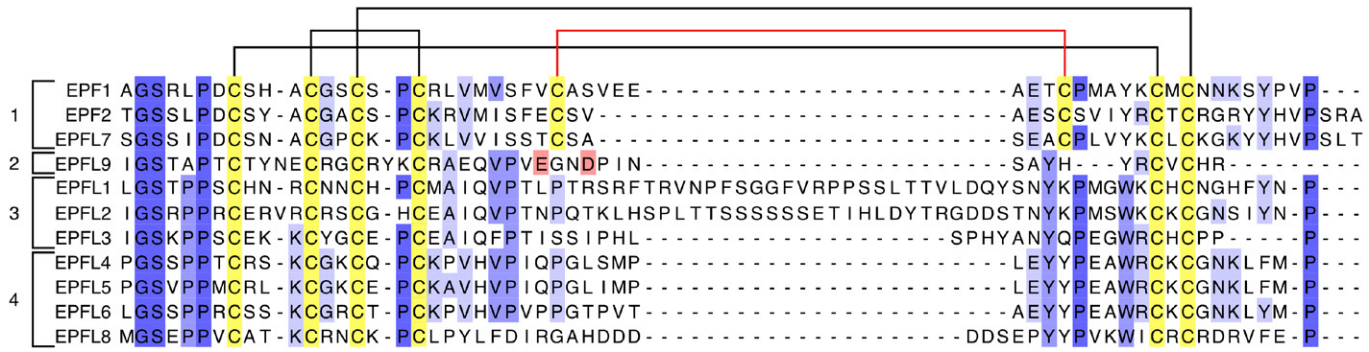

Fig. 1. Multiple alignment of mature peptides belonging to the EPF/EPFL family in *A. thaliana* conducted using the Muscle algorithm. (1–4) clades of peptides; cysteine amino acid residues are highlighted in yellow. Three conservative disulfide bonds are indicated with black brackets; the fourth disulfide bond, specific to the EPF1/EPF2/EPFL7 clade, is indicated with red bracket. Glu28 and Asp31 amino acid residues in EPFL9 are highlighted in pink. UniProt ID: EPF1: Q8S8I4; EPF2: Q8LC53; EPFL7: C4B8C5; EPFL9: Q9SV72; EPFL1: Q9LFT5; EPFL2: Q9T068; EPFL3: C4B8C4; EPFL4: Q2V3I3; EPFL5: Q9LUH9; EPFL6: Q1PEY6; EPFL8: Q1G3V9

tides, and the structural determinants of their antimicrobial activity have been identified [17, 18]. The structural features of cysteine-rich signal peptides in plants, including the EPF/EPFL family, have been investigated less thoroughly; however, the primary and spatial structures of the EPFL9 peptide isolated from the *A. thaliana* apoplast have been determined [19, 20]. Furthermore, structural data on peptide-receptor interactions for several peptides belonging to this family has been obtained [21]. Eleven peptides have been identified in *A. thaliana*, the classical model organism that is most commonly used to study this peptide family: EPF1–2 and EPFL1–9, including EPFL9/Stomagen [22]. EPF1 and EPF2 were the first to be characterized, followed by other EPF1 paralogs named EPFL [23]. The EPF/EPFL peptides were divided into four clades by phylogenetic analysis (*Fig. 1*). Members of two of these clades, EPF1–EPF2–EPFL7 and EPFL9, have been the most thoroughly studied.

Like most peptide hormones and antimicrobial peptides in plants, members of the EPF/EPFL family are synthesized as precursor proteins consisting of an N-terminal signal peptide, a prodomain, and a mature peptide (*Fig. 2A*) [24]. The signal peptide guides the precursor to the endoplasmic reticulum, where it is then cleaved off and degraded by peptidases. The prodomain is subsequently removed, and a mature peptide capable of interacting with receptor complexes is released [25].

The primary structure of EPF/EPFL peptides is rich in cysteine residues; six of them are conserved across the entire family, and two additional residues occur only in the EPF1/EPF2/EPFL7 clade (*Fig. 1*). All the peptides belonging to this family carry the Gly-Ser motif in the N-terminal region. This motif is known to be critical in peptides binding to their receptors [21]. A conserved Pro residue is also present in the N-terminal region. This residue probably helps maintain the spatial conformation of the peptide by bending the polypeptide chain.

The NMR spectroscopy data garnered for EPFL9 suggest that the three-dimensional structure of EPF/EPFL peptides consists of two antiparallel β-sheets (a scaffold) connected by a loop region and stabilized by disulfide bonds (*Fig. 2B*). The loop region is more variable than the scaffold and plays a crucial role in the specificity of the binding to receptors [19]. The spatial structures of other family members have been determined via homology modeling.

Conserved cysteine residues are involved in the formation of disulfide bonds, whose number and arrangement affect the functional activity and conformation of the peptide. Thus, the ability to stimulate stomatal initiation was lost after cysteine residues had been replaced with serine in the EPFL9 molecule [19]. Conversely, variable regions can be responsible for the functional specificity of the peptides. Thus, EPF1/2 peptides act as negative regulators of stomatal development, whereas EPFL9 is a positive regulator [20]. The diversity in physiological responses are probably a result of structural differences in the loop region of these peptides [21].

Thus, replacing the EPF2 loop with the corresponding sequence from EPFL9 converted the peptide's function from inhibition to promotion of stomatal development. Meanwhile, a chimeric peptide carrying the EPF2 loop and the EPFL9 scaffold exhibited an inhibitory activity [19]. The ERECTA family (ERf) kinases, which belong to the leucine-rich repeat receptor-like kinases (LRR-RLK) clade XIII, act as receptors for EPFL peptides. In Arabidopsis, this family includes the ERECTA (ER), ERECTA-LIKE 1 (ERL1), and ERECTA-LIKE 2 (ERL2) proteins. The combined signaling pathway involves the MAPK (mitogen-activated protein kinase) cascade, which consists of MAPKKK YODA, MKK4/5, and the terminal kinases MPK3/6 in Arabidopsis [27]. The peptide–receptor interaction depends on whether the receptor is part of a complex with LRR–RLP (leucine-rich repeat receptor-like protein) TMM (Too Many Mouths). Interestingly, EPF1/2 bind only to the ERf–TMM complex, while EPFL4 interacts with each of three ERf in the absence of TMM [21].

PHYLOGENETIC DIVERSITY IN PLANTS

The EPF and EPFL peptides have been identified only in terrestrial plants, but they are not found in algae [28, 29]. This indicates that this peptide family evolved after plants had colonized the land and may have played an important role in their adaptation to terrestrial life. There is a hypothesis holding that the key genetic components ensuring the formation of the stomatal apparatus, including EPF/EPFL, originated at the early stages of the evolution of terrestrial plants [30].

Peptide sequences are conserved across different taxa: *Pp*EPF1, a homolog of *At*EPF1 and *At*EPF2, was identified in moss *Physcomitrium patens*. Phylogenetic analysis shows that *Pp*EPF1 is closer to *At*EPF1 and *At*EPF2 than *At*EPFL9 [28]. This is rather interesting, since the stomatal apparatus of mosses differs from that of angiosperms, and yet their developmental mechanisms seem to be similar [31, 32]. In addition to *Pp*EPF1, ten EPFL peptides have been identified in moss; their functions are still to be characterized [28]. In angiosperms, the genes encoding EPF/EPFL peptides are unevenly distributed across chromosomes, which may be a result of genetic duplication events [33, 34]

This peptide family in Arabidopsis is phylogenetically subdivided into four clades: EPF1–EPF2–EPFL7, EPFL9, EPFL1–3, and EPFL4–6–EPFL8 (*Fig. 1*) [28, 34]. These groups differ in both structure and putative functions. Thus, members of the EPF1–EPF2–EPFL7 clade carry four conserved disulfide bonds, one located in the loop region, whereas the peptides from the other clades carry three disulfide bonds. This feature affects the ability of the peptides to bind to receptor complexes [21, 28].

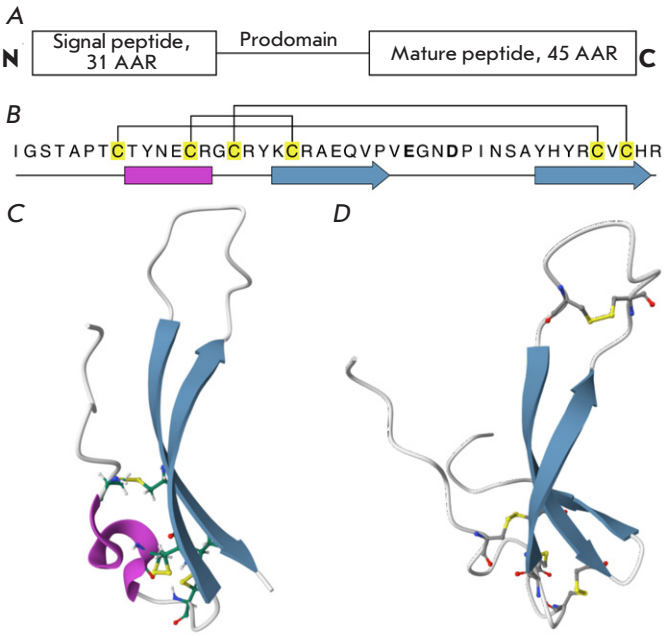

Fig. 2. The structure of EPFL9 peptide. (*A*) The structure of preproprotein [20]. (*B*) The primary structure of peptide [19]. β-sheets are shown with blue arrows; the 3_{10}-helix is shown with a pink rectangle; Cys residues are highlighted in yellow; disulfide bonds are shown with brackets. Negatively charged amino acid residues Glu28 and Asp31 in the loop region are highlighted in bold. (*C*) The spatial structure of EPFL9 (PDB ID: 2LIY). (*D*) The structural model of EPFL7 peptide in *A. thaliana*, generated using the AlphaFold3 algorithm [26]

The EPFL9 peptide was found in all the studied vascular plants, from lycophytes (*Selaginella moellendorffii*) and gymnosperms to angiosperms [28]. However, it was not identified in moss *P. patens*, although a EPF1/EPF2 homolog is present in that plant. Notably, the emergence of EPFL9, which activates stomatal development, coincides with an abrupt rise in stomatal density on leaf surfaces in the Late Devonian period, when megaphylls – large leaves with a well-developed vascular system – evolved [28, 35].

The number of sequenced plant genomes has recently increased, thus substantially facilitating the search for and subsequent validation of homologs. The genomes of a large number of agricultural flowering plants have been analyzed using bioinformat-

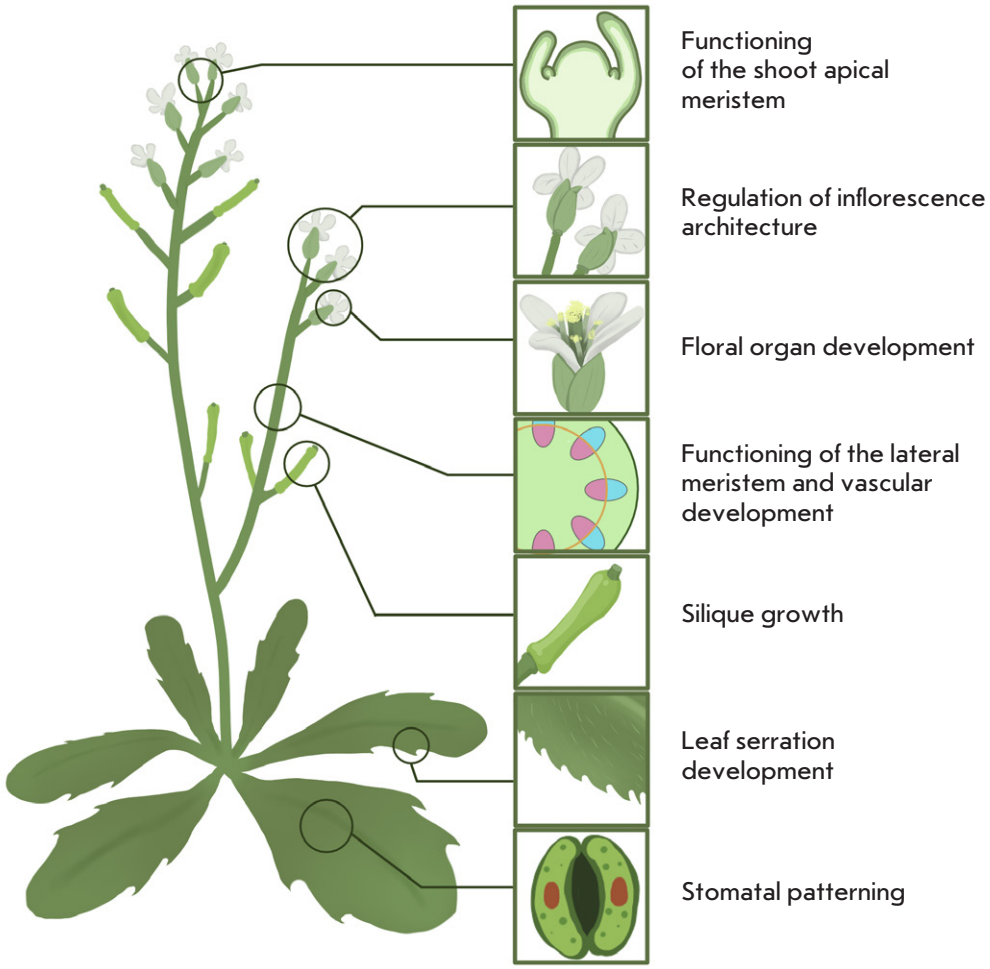

Fig. 3. Morphogenetic processes regulated by peptides of the EPF/EPFL family in *Arabidopsis thaliana*

ic tools. A total of 132 *EPF/EPFL* genes have been identified in the four cotton plant genomes: 20 and 24 genes in diploid species, and 44 genes in each of the tetraploid species [34]. Fourteen genes have been identified in potato plants [36]; and 27 genes, in rapeseed [37]. Fifteen *EPF/EPFL* genes have been identified in the black cottonwood *Populus trichocarpa* [38], while 14 genes have been in the Euphrates poplar *P. euphratica* [33]. *EPFL* genes were also discovered in monocots: 12 genes were identified in rice, sorghum, and rye [39–41]; 18 genes, in maize [42]; and 35 genes, in wheat [43]. The considerable abundance of the *EPF/EPFL* genes across different evolutionary lineages of angiosperms and other plants underscores their importance in adaptation to terrestrial environments, while the functions of many recently identified homologs remain unclear, requiring further experimental research.

STOMATAL INITIATION

EPF/EPFL peptides are known to orchestrate a broad spectrum of morphogenetic programs; regulation of stomatal patterning was the first function of these peptides to be discovered (*Fig. 3*, *Table 1*) [10].

In Arabidopsis, *EPF1* is expressed in young leaves; namely, in stomatal precursor cells. *EPF1* overexpression reduces the stomatal density, while *EPF1* knockout increases the stomatal density and clustering [10]. The EPF1 homolog, EPF2, also inhibits stomatal development: plants that overexpress the *EPF2* gene are characterized by a reduced stomatal density, whereas *EPF2*-knockout plants demonstrate an increased stomatal density but do not form clusters [13]. Both peptides enforce the “one-cell spacing rule” dictating that at least one intervening nonstomatal epidermal cell should separate two stomata [10, 13, 14]. *EPF2* is expressed in stomatal precursors earlier than *EPF1*.

Table 1. Functions of the EPF/EPFL peptides in *A. thaliana*

Peptide	Function	Reference
*At*EPF1/2	Inhibition of stomatal formation	[10, 13, 15, 23, 44]
*At*EPFL9 (STOMAGEN)	Stimulation of stomatal formation	[11, 15, 44, 45]
	Silique elongation	[46]
*At*EPFL2	Leaf serration development	[47]
	Regular ovule spacing and increased ovule number per silique	[46]
*At*EPFL1/2/4/6	Regulation of functioning of the apical meristem	[48–51]
	Enhancement of pathogen resistance	[52]
	Elongation of inflorescences and pedicels	[53]
	Formation of a single megaspore mother cell	[54]
*At*EPFL1–6	Envelopment of the nucellus by integuments	[55]
*At*EPFL4–6	Stamen filament elongation due to cell proliferation	[56, 57]

Thus, EPF2 regulates the initiation of stomatal differentiation, while EPF1 controls further development [10, 14, 15]. Contrariwise, the EPFL9 peptide promotes stomatal development: *EPFL9* overexpression increases stomatal density and causes clustering, whereas silencing of *EPFL9* inhibits stomatal development [45]. Both the EPF1–2 and EPFL9 peptides have been shown to bind to the ER receptor; EPFL9 competitively displaces EPF1–2 from this complex [44]. The EPF1/2 peptides are expressed in stomatal cells, bind to ER and ERL1, and inhibit stomatal differentiation, whereas EPFL9 is expressed in mesophyll cells, competes with EPF2 for binding to ER, and promotes stomatal formation [15, 44]. Hence, EPF1/2 and EPFL9 act as antagonists in the stomatal density control [44].

The role of EPF peptides are best studied in Arabidopsis; however, their involvement in the regulation of stomatal development has been demonstrated for other plants as well. For example, overexpression of poplar *Pe*EPF2, a homolog of *At*EPF2, in *At*EPF2 knockout Arabidopsis plants reduced the stomatal density on leaves and rescued the mutant phenotype [33]. Orthologs of *AtEPF2* and *AtEPFL9* involved in the regulation of stomatal development in Arabidopsis have been detected in the genomes of the monocots *Triticum aestivum* and *Brachypodium distachyon* [58]. These peptides also exert an opposing effect on stomatal development.

It has been demonstrated that the EPF/TMM/ERECTA module is a rather ancient regulator of stomatal development: its components control stomatal patterning in early terrestrial plants, in moss *P. patens* in particular [59]. In *P. patens*, stomata form on the sporophyte; *Pp*EPF1, a homolog of Arabidopsis *EPF1/2*, negatively regulates their development. However, *Pp*EPF1 overexpression cannot restore the normal stomatal density in the Arabidopsis mutant *epf2*. Meanwhile, *P. patens* lacks an *At*EPFL9 ortholog and *At*EPFL9 overexpression does not affect its stomatal density, an indication that competitive regulation of stomatal patterning emerged at later stages of terrestrial plant evolution [59].

Hence, EPFL peptides are conserved and ancient regulators of stomatal development in terrestrial plants.

FUNCTIONING OF THE SHOOT APICAL MERISTEM

The shoot apical meristem is a key structure that ensures the development of plant aerial organs. Its spatial organization, size, and activity are tightly regulated by a network of signaling cascades: EPFL peptides also participate in the process.

In *A. thaliana*, the EPFL1, EPFL2, EPFL4, and EPFL6 peptides are preferentially expressed in the periphery of the shoot apical meristem and within the boundary region between the meristem and leaf primordia [48]. Meanwhile, the ER, ERL1, and ERL2 receptors are active in the central zone of the meristem, suggesting that they are involved in the spatial regulation of meristem cell division and differentiation. The *EPFL1/2/4/6* and *ERf* knockout mutants share a phenotype: a larger meristem, fewer leaf primordia, and a reduced overall plant biomass [48]. These data support the hypothesis that EPFL peptides and ER receptors are functionally redundant when regulating the size of the shoot apical meristem and initiating leaf growth [60].

EPFL2 knockout mutants exhibit disrupted symmetry and irregular organ spacing, as well as changes in the auxin maxima number in the shoot apical meristem [49]. That is consistent with the results of another study that reported that *epfl2* mutants showed impaired shape of leaves and cotyledons due to change in auxin maxima number [50].

Furthermore, it has been demonstrated that treatment with synthetic EPFL4 and EPFL6 peptides ERf-dependently limits the lateral growth of the meristem by downregulating the expression of the key apical meristem regulators CLV3 (CLAVATA3) and WUS (WUSCHEL) [51]. The interaction between these pep-

tides and their receptors determines both the meristem size and its boundaries, thus contributing to the regulation of the number of initiated organs and ensuring normal plant development.

Hence, EPFL peptides play a pivotal role in the spatiotemporal regulation of the activity of the shoot apical meristem.

REGULATION OF THE LATERAL MERISTEM AND VASCULAR DEVELOPMENT

Regulation of lateral meristems and vascular tissue initiation are the key processes responsible for the proper development of both vegetative and reproductive organs. The receptor kinases ER and ERL1 participate in the regulation of lateral meristems in the hypocotyls and inflorescence of Arabidopsis [61–63]. Thus, expression of the *ER* and *ERL1* genes – but not *ERL2* – was detected in the central cylinder of the hypocotyl [61]. In comparison with wild-type plants, *er erl1* double mutants have thickened hypocotyls caused by excessive xylem development; this xylem has a higher proportion of cells with lignified cell walls [61]. In other words, ER and ERL1 prevent excessive xylem development in hypocotyls.

The ER and ERL1 kinases also regulate procambium development in inflorescence stems [62, 63]. The vascular bundle structure was impaired in *er erl1* double mutants: the procambium layer was discontinuous, and direct contact between xylem and phloem frequently occurred. It has been demonstrated that *ER* and *ERL1* are expressed in the xylem and phloem, phloem-specific expression of the *ER* gene being crucial for the regulation of the anatomical structure of the inflorescence stem [62]. It is hypothesized that the EPFL4 and EPFL6 peptides, which are expressed in the endodermis and bind to ER, are involved in this process. However, the *epfl4 epfl6* double mutant does not seem to have a disrupted vascular bundle structure. Therefore, it still remains an open question which EPFL peptides are involved in the regulation of the lateral meristem function.

Hence, it has been demonstrated that the ER and ERL1 receptors – and presumably their ligands – participate in the regulation of the formation and function of lateral meristems, as well as vascular tissue initiation.

DEVELOPMENT OF THE SERRATED LEAF MARGIN

Another role of EPFL peptides is the development of leaf margin serration [47]. In Arabidopsis, this process is regulated by the EPFL2 peptide, together with the ER and ERL1/2 receptors. *EPFL2* knockout mutants, as well as ERf double mutants, have no serrated leaf margin. Moreover, the interaction between EPFL2 and each of the three ERf has been confirmed by co-immunoprecipitation [47]. The *EPFL2* gene is expressed in growing leaves, except for the serrated tips and developing veins [47]. Interestingly, the *ERL2* expression contrasts with that of *EPFL2*: it has been detected on the serrated tips and in the veins, while *ER* and *ERL1* are expressed in the entire leaf blade. Hence, the EPFL2–ERf regulatory module suppresses the auxin response, confining it to a few cells on the tip of the developing serration.

THE DEVELOPMENT OF REPRODUCTIVE ORGANS

Angiosperms have evolutionarily developed complex and diverse reproductive structures, with EPF/EPFL peptides playing a crucial role in the formation of these structures, from regulating the inflorescence architecture to seed formation.

EPFL4/6, and to a lesser extent EPFL1/2 together with ERf, stimulate the elongation of inflorescences and pedicels in *A. thaliana* [53]. EPFL4/6, which act as ER ligands, are expressed in endodermal cells, while the *ER* gene is expressed in the epidermis, phloem, and xylem. However, signal reception in the phloem is essential for the development of a normal inflorescence architecture, since *ER* expression under the phloem-specific *SUC1* promoter restores the phenotype of *er* mutants. This effect has not been observed for *ER* expression under promoters active in the xylem and epidermis [53]. Hence, EPFL4/6 peptides are expressed in endodermal cells in plant inflorescences and transported to the phloem, where they bind to the ER and stimulate the growth of the inflorescence stem and pedicels [53]. Transcriptomic data demonstrate that many differentially expressed genes in *A. thaliana er-2* and *epfl4/6* mutants are components of the auxin and gibberellin response pathways. In particular, the expression of ARGOS, which promotes the growth of aerial organs [64], is suppressed, as well as the expression of the transcription factor WRKY15 [53].

The role played by EPFL peptides in the regulation of the inflorescence architecture has also been demonstrated in rice. Thus, *Os*EPFL5–9 regulate the panicle architecture and grain size. *Os*EPFL6–9 decrease the number of spikelets per panicle, while *Os*EPFL5 increase it, acting as an antagonist [65]. Further signaling in *Os*EPFL6–9 proceeds via the *Os*ER1 receptor and the MAPK cascade comprising *Os*MKKK10–*Os*MKK4–*Os*MPK6 [65, 66].

Peptides belonging to the EPFL family control not only the overall development of inflorescences, but also the development of male and female reproductive organs and the resulting fruits. In Arabidopsis plants, EPFL4/5/6 promote stamen filament elongation

by regulating cell proliferation [56, 57]. Impaired self-pollination and male sterility are observed in *epfl4/5/6* triple mutants, since stamens become significantly shorter than the pistil [57]. At lower temperatures, self-pollination is already impaired for the mutant carrying a single *epfl6* mutation [56]. ER mediates the elongation of both the stamens and the pistil [56].

EPFL1 in *T. aestivum* and EPFL6 in *Brassica napus* also appear to regulate the morphology of floral organs. Their overexpression in *A. thaliana* plants reduces the number of stamens and the stamen-to-pistil length ratio [67, 68].

EPFL peptides can also regulate *A. thaliana* silique development. EPFL9 recognized by ER promotes silique elongation, whereas *EPFL2* expressed in inter-ovule spaces increases the number of ovules per silique and ensures regular ovule spacing by interacting with the ERL2 and ERL1 receptors [46]. Interestingly, EPFL9 and EPFL2 may act as antagonists, since the *EPFL9* expression under the *EPFL2* promoter produce a phenotype similar to that of the *epfl2* mutant [46].

EPFL1/2/4/6 also control the initial stages of female gametophyte development. These peptides are needed for differentiation of a single megaspore mother cell (MMC), preventing both the initiation of multiple MMCs and their absence [54].

At later stages of ovule development, EPFL1–6 ensure proper envelopment of the nucellus by integuments [55]. The EPFL1–6, ER, and ERL1/2 genes are expressed at different ovule developmental stages, while mutations in these genes disrupt integument formation. In this process, SERK1/2/3 function as coreceptors: the interaction between SERK and ERf kinases is enhanced in the presence of exogenous EPFL4/6 peptides [55].

EPF/EPFL peptides control the awn development, an important agricultural trait of rice. In wild rice (*Oryza rufipogon*) the *EPFL1* gene is actively expressed in developing inflorescences and ensures the formation of longer awns and fewer grains per panicle [69]. Mutations altering the number of cysteine residues in *OsEPFL1* were detected in most awnless cultivars of rice *O. sativa*, and introduction of the *EPFL1* allele from African rice cultivar (*O. glaberrima*) leads to awned seeds in *O. sativa ssp. japonica* [69]. In the *O. sativa ssp. aus* cv. Kasalath, other *EPF/EPFL* genes are responsible for the awned phenotype: the *osepfl1* single mutant retains awns, whereas the *osepfl2* mutant is awnless and displays shorter grains, lower grain weights, and a decreasing number of cells along the longitudinal axis. *OsEPF2*, *OsEPFL7*, *OsEPFL9*, and *OsEPFL10* also contribute to awn development. Both the *OsEPFL1/GAD1/RAE2* and *OsEPFL2/9/10* genes are believed to have undergone selection during rice domestication [39, 69].

The functions of EPFL peptides in the reproductive development of plants are extremely diverse. The members of this family orchestrate the inflorescence architecture, growth of floral organs, and proper formation of the female gametophyte.

ABIOTIC STRESS

In recent years, multiple studies have concentrated on the identification of EPF/EPFL genes in various crop species. The promoter regions of these genes have been often found to contain the *cis*-regulatory elements associated with responses to stress factors and phytohormones [33, 34, 36, 40, 41]. Moreover, it has been experimentally verified that these factors regulate the expression of individual EPF/EPFL genes. This suggests that EPF/EPFL peptides may contribute to plant tolerance to environmental stress.

For example, *EPFL8* expression is upregulated after treatment of maize plants with abscisic acid (ABA), methyl jasmonate, and salicylic acid, while expression of a number of other *EPFL* genes is downregulated under the same conditions [34]. Furthermore, water deficit can simultaneously alter the expression of several *EPFL* genes, indirectly demonstrating that they are possibly involved in the regulation of the drought response [34, 70]. Rye has both osmotic stress-induced and osmotic stress-repressed *EPFL* genes, as well as two heat-inducible *EPFL* genes [40]. A significant decline in the expression of seven *EPF* genes in rapeseed in response to salt stress was demonstrated in [37]. *EPFL* genes differentially expressed in response to osmotic stress have also been identified in sorghum, potato, poplar, and apple [33, 36, 41, 71].

EPF1/2 are known to inhibit stomatal formation in *A. thaliana*, while *EPFL9* promotes it [10, 13, 45]. Stomatal density and transpiration intensity are responsible for the drought resistance of a plant. Comparison of the expression of *EPF/EPFL* genes in drought-tolerant and drought-sensitive apple (*Malus domestica*) cultivars has demonstrated that the expression of *MdEPF2*, an *AtEPF2* ortholog, is more strongly induced by drought in the leaves of tolerant cultivar [71]. Treatment with abscisic acid (ABA), a key regulator of the osmotic stress response, also induces *MdEPF2* expression. Tomato plants overexpressing *MdEPF2* were shown to exhibit enhanced tolerance to osmotic stress. Under drought conditions, these plants were characterized by greater biomass, higher photosynthetic rates and relative water content, lower levels of malondialdehyde (MDA, a marker of oxidative stress) and hydrogen peroxide, as well as higher activity of antioxidant enzymes compared to

that in wild-type plants [71]. The primary morphological effect of *MdEPF2* overexpression consisted in a decline in stomatal density, which can be considered a key reason behind the greater osmotic stress tolerance observed in these plants.

The physiological role of the *AtEPF2* ortholog, PdEPF2, identified in the poplar genome was studied previously [72]. Expression of *PdEPF2* is induced by drought and ABA. Arabidopsis plants overexpressing *PdEPF2* showed enhanced drought tolerance: their proline level and photosynthetic intensity were increased under osmotic stress conditions.

Four *EPF/EPFL* genes respond to drought in potato: *EPF4* is downregulated, while the other three genes are upregulated [36]. Plants with either *EPF4* knockdown or *EPF4* overexpression were generated. Knockdown of this gene increased drought tolerance. Under drought stress these plants had a higher relative water content, proline level, and displayed activity of antioxidant enzymes (SOD, POD, and CAT), along with a lower MDA level than in wild-type plants. Conversely, the opposite effects were observed under drought conditions in plants overexpressing *EPF4* [36]. Altered *EPF4* expression affected the stomatal density, which was lower in *EPF4* knockdown plants and higher in plants overexpressing *EPF4*. The negative role of EPF4 in the regulation of the osmotic stress response can possibly be associated with its effect on stomatal formation.

Taken together genomic and physiological data obtained for various agricultural crops, it can be concluded that *EPF/EPFL* peptides are potentially involved in plant responses to abiotic stresses, primarily to drought. Regulation of stomatal density and transpiration are the most frequently proposed mechanisms of action for these peptides; however, other mechanisms cannot be ruled out. Different members of this family can play both a positive and negative regulatory role, which underscores the functional diversity of EPF/EPFL peptides and suggests that further research into their specific functions across different physiological contexts is needed.

BIOTIC STRESS

Differential expression of various EPF/EPFL members was shown in several plant species upon infection by phytopathogenic fungi. Thus, infection of moss *P. patens* with the pathogenic fungus *Botrytis cinerea* significantly downregulates expression of the six genes encoding the predicted EPFL peptides [73]. It was demonstrated that the expression of the *EPFL1–6* and *EPFL9* genes in *A. thaliana* increases after inoculation with *Sclerotinia sclerotiorum*, while the expression of other members of the *EPF/EPFL* family remains unaltered [52]. Meanwhile, biotic stress appeared to have different effects on the expression of the *EPF/EPFL* genes in tomato *Solanum lycopersicum* plants. Thus, infection with the phytopathogen *Fusarium oxysporum* f. sp. *lycopersici* induces the expression of *SlEPF7* and decreases the expression of *SlEPF1/5*. Treatment with elicitors from a non-pathogenic for tomato *F. sambicinum* strain increases *SlEPF6/7* expression and decreases that of *SlEPF3/5* [74].

Simultaneous changes in the expression level of several *EPF/EPFL* genes upon interaction with phytopathogens suggest that peptides belonging to this family can coordinately regulate plant defense mechanisms. Thus, the growth of *S. sclerotiorum* and H_2O_2 generation were shown to increase significantly in Arabidopsis *epfl1,2,4,6* multiple mutants, whereas single mutants did not differ from wild-type plants [52]. Furthermore, pathogen-induced expression of the genes belonging to the *YODA DOWNSTREAM (YDD)* group was significantly reduced in the *epfl1,2,4,6* mutants. *YDD* is a group of genes positively regulated in constitutively active *YODA* mutants [52]. On the other hand, inducible *EPF1/2* expression in *A. thaliana* did not enhance plant resistance to the necrotrophic fungus *Plectosphaerella cucumerina* [75]. Many pathogens are known to penetrate into plant tissues through stomata; therefore, the weakened resistance of *ERf* mutants can plausibly be attributed to the increased stomatal density. Thus, treatment with EPFL9 increases the stomatal density and exacerbates the symptoms of infection [76].

Hence, data on the involvement of *EPF/EPFL* peptides in the regulation of the biotic stress response are extremely sparse. Meanwhile, it has been repeatedly demonstrated that receptors and components of the *EPF/EPFL* peptide signaling pathway are involved in ensuring phytopathogen resistance. Thus, *er* mutants were characterized by reduced resistance to the bacterium *Ralstonia solanacearum* [77], oomycete *Pythium irregulare* [78], as well as the pathogenic fungi *Verticillium longisporum* [79], *S. sclerotiorum* [80], and *P. cucumerina* [81, 82]. Additional knockout of the *ERL1/2* and *TMM* genes exacerbated infection symptoms [75, 80].

However, the reduced resistance to *R. solanacearum* after inoculation through damaged roots [77] indicates that the susceptibility of *er* mutants may be caused not only by the increased stomatal density but also by an impaired defense response. This is further supported by the downregulated expression of the pathogen-inducible genes *WRKY33*, *WRKY53*, *CYP79B2*, and *CYP81F2* in *er*, *bak1*, and *er bak1* mutants [75].

Meanwhile, the activity of ER was shown to have no effect on the expression of the genes induced by flg22, a 22-amino acid flagellin-derived epitope [75]. Furthermore, *er* mutants were no less resistant to infection by *B. cinerea*, *F. oxysporum* f. sp. *conglutinans*, and *Peronospora parasitica* than wild-type plants [81]. Therefore, ER is not always required for pathogen resistance. This can be associated with the functional redundancy of EPFL receptors.

ER regulates the Arabidopsis response to *S. sclerotiorum* infection via affecting binding between the WRKY33 transcription factor and promoters of the *YDD* genes [80]. This process involves the chromatin remodeling complex SWR1 and the ER–MPK6–WRKY33 regulatory module. SWR1 promotes the binding of the W-box transcription factor WRKY33 to promoters and activates expression of the YDD genes, which are necessary for resistance to *S. sclerotiorum* infection [80, 83].

Since EPF/EPFL peptides are primarily known as regulators of stomatal development, their role in stress adaptation is often attributed to their impact on stomatal density. However, the role of this peptide family under stress conditions appears to be broader and needs further investigation.

CONCLUSIONS

Despite significant progress in understanding EPF/EPFL peptides functions, knowledge gaps still remain. Thus, the vast majority of functional studies on EPF/EPFL have been conducted on the model plant *A. thaliana*. Furthermore, although homologs of the *EPF/EPFL* genes have been identified across different groups of angiosperms, their function need more comprehensive investigation. This issue is particularly relevant in the context of the plant phylogenetic diversity, since the results obtained for Arabidopsis may not fully represent the range of biological functions of EPFL peptides in other plant species.

Additional challenges arise from the functional redundancy of these peptides: multiple EPF/EPFL family members can partially compensate for each other, thus complicating the assessment of individual contributions. So, much of the research analyzes receptor mutants, which are also partially redundant, but their number is significantly smaller.

It has been demonstrated so far that *EPFL* expression can be altered in response to biotic and abiotic stresses; however, the association between peptide-mediated regulation and plant adaptive responses still needs to be fully elucidated.

Modulating the activity of EPFL peptides and their receptors may be used to optimize morphogenesis, enhance stress tolerance, and, therefore, improve cultivated crops. ●

This work was supported by the Russian Science Foundation (project No. 23-74-10048).

REFERENCES

1. Fukuda H, Hardtke CS. Peptide Signaling Pathways in Vascular Differentiation. *Plant Physiol.* 2020;182(4):1636–1644. doi: 10.1104/pp.19.01259
2. Mamaeva A, Makeeva A, Ganaeva D. The Small Key to the Treasure Chest: Endogenous Plant Peptides Involved in Symbiotic Interactions. *Plants (Basel).* 2025;14(3):378. doi: 10.3390/plants14030378
3. Pearce G, Strydom D, Johnson S, Ryan CA. A Polypeptide from Tomato Leaves Induces Wound-Inducible Proteinase Inhibitor Proteins. *Science.* 1991;253(5022):895–897. doi: 10.1126/science.253.5022.895
4. Tavormina P, De Coninck B, Nikonorova N, De Smet I, Cammue BP. The Plant Peptidome: An Expanding Repertoire of Structural Features and Biological Functions. *Plant Cell.* 2015;27(8):2095–2118. doi: 10.1105/tpc.15.00440
5. Stintzi A, Schaller A. Biogenesis of post-translationally modified peptide signals for plant reproductive development. *Curr Opin Plant Biol.* 2022;69:102274. doi: 10.1016/j.pbi.2022.102274
6. Feng YZ, Zhu QF, Xue J, Chen P, Yu Y. Shining in the dark: the big world of small peptides in plants. *aBIOTECH.* 2023;4(3):238–256. doi: 10.1007/s42994-023-00100-0
7. Gancheva MS, Malovichko YV, Poliushkevich LO, Dodueva IE, Lutova LA. Plant Peptide Hormones. *Russ J Plant Physiol.* 2019;66:171–189. doi: 10.1134/S1021443719010072
8. Okada T, Yoshizumi H, Terashima Y. A Lethal Toxic Substance for Brewing Yeast in Wheat and Barley: Part I. Assay of Toxicity on Various Grains, and Sensitivity of Various Yeast StrainsPart II. Isolation and Some Properties of Toxic Principle. *J Agric Biol Chem.* 1970;34(7):1084–1094. doi: 10.1080/00021369.1970.10859736
9. van der Weerden NL, Bleackley MR, Anderson MA. Properties and mechanisms of action of naturally occurring antifungal peptides. *Cell Mol Life Sci.* 2013;70(19):3545–3570. doi: 10.1007/s00018-013-1260-1
10. Hara K, Kajita R, Torii KU, Bergmann DC, Kakimoto T. The secretory peptide gene EPF1 enforces the stomatal one-cell-spacing rule. *Genes Dev.* 2007;21(14):1720–1725. doi: 10.1101/gad.1550707
11. Sugano SS, Shimada T, Imai Y, et al. Stomagen positively regulates stomatal density in Arabidopsis. *Nature.* 2010;463(7278):241–244. doi: 10.1038/nature08682

12. Maróti G, Downie JA, Kondorosi É. Plant cysteine-rich peptides that inhibit pathogen growth and control rhizobial differentiation in legume nodules. *Curr Opin Plant Biol.* 2015;26:57–63. doi: 10.1016/j.pbi.2015.05.031
13. Hunt L, Gray JE. The Signaling Peptide EPF2 Controls Asymmetric Cell Divisions during Stomatal Development. *Curr Biol.* 2009;19(10):864–869. doi: 10.1016/j.cub.2009.03.069
14. Richardson LGL, Torii KU. Take a deep breath: peptide signalling in stomatal patterning and differentiation. *J Exp Bot.* 2013;64(17):5243–5251. doi: 10.1093/jxb/ert246
15. Lee JS, Kuroha T, Hnilova M, et al. Direct interaction of ligand–receptor pairs specifying stomatal patterning. *Genes Dev.* 2012;26(2):126–136. doi: 10.1101/gad.179895.111
16. Silverstein KAT, Moskal WA Jr, Wu HC, et al. Small cysteine-rich peptides resembling antimicrobial peptides have been under-predicted in plants. *Plant J.* 2007;51(2):262–280. doi: 10.1111/j.1365-313X.2007.03136.x
17. Finkina EI, Melnikova DN, Bogdanov IV, Ovchinnikova TV. Peptides of the innate immune system of plants. Part I. Structure, biological activity and mechanisms of action. *Rus J Bioorgan Chem.* 2019;45(1):3–16. doi: 10.1134/S013234231901007X
18. Finkina EI, Melnikova DN, Bogdanov IV, Ovchinnikova TV. Peptides of the innate immune system of plants. Part II. Biosynthesis, biological functions, and possible practical applications. *Rus J Bioorgan Chem.* 2019;45(2):55–65. doi: 10.1134/S1068162019020043
19. Ohki S, Takeuchi M, Mori M. The NMR structure of stomagen reveals the basis of stomatal density regulation by plant peptide hormones. *Nat Commun.* 2011;2:512. doi: 10.1038/ncomms1520
20. Kondo T, Kajita R, Miyazaki A, et al. Stomatal Density is Controlled by a Mesophyll-Derived Signaling Molecule. *Plant Cell Physiol.* 2010;51(1):1–8. doi: 10.1093/pcp/pcp180
21. Lin G, Zhang L, Han Z, et al. A receptor-like protein acts as a specificity switch for the regulation of stomatal development. *Genes Dev.* 2017;31(9):927–938. doi: 10.1101/gad.297580.117
22. Rowe MH, Bergmann DC. Complex signals for simple cells: the expanding ranks of signals and receptors guiding stomatal development. *Curr Opin Plant Biol.* 2010;13(5):548–555. doi: 10.1016/j.pbi.2010.06.002
23. Hara K, Yokoo T, Kajita R, et al. Epidermal cell density is autoregulated via a secretory peptide, EPIDERMAL PATTERNING FACTOR 2 in Arabidopsis leaves. *Plant Cell Physiol.* 2009;50(6):1019–1031. doi: 10.1093/pcp/pcp068
24. Tabata R, Sawa S. Maturation processes and structures of small secreted peptides in plants. *Front Plant Sci.* 2014;5:311. doi: 10.3389/fpls.2014.00311
25. Olsson V, Joos L, Zhu S, Gevaert K, Butenko MA, De Smet I. Look Closely, the Beautiful May Be Small: Precursor-Derived Peptides in Plants. *Annu Rev Plant Biol.* 2019;70:153–186. doi: 10.1146/annurev-arplant-042817-040413
26. Abramson J, Adler J, Dunger J, et al. Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature.* 2024;630(8016):493–500. doi: 10.1038/s41586-024-07487-w
27. Wang H, Ngwenyama N, Liu Y, Walker JC, Zhang S. Stomatal Development and Patterning Are Regulated by Environmentally Responsive Mitogen-Activated Protein Kinases in Arabidopsis. *Plant Cell.* 2007;19(1):63–73. doi: 10.1105/tpc.106.048298
28. Takata N, Yokota K, Ohki S, Mori M, Taniguchi T, Kurita M. Evolutionary relationship and structural characterization of the EPF/EPFL gene family. *PLoS One.* 2013;8(6):e65183. doi: 10.1371/journal.pone.0065183
29. Bowman JL, Kohchi T, Yamato KT, et al. Insights into Land Plant Evolution Garnered from the Marchantia polymorpha Genome. *Cell.* 2017;171(2):287–304.e15. doi: 10.1016/j.cell.2017.09.030
30. Rychel AL, Peterson KM, Torii KU. Plant twitter: ligands under 140 amino acids enforcing stomatal patterning. *J Plant Res.* 2010;123(3):275–280. doi: 10.1007/s10265-010-0330-9
31. Caine RS, Chater CC, Kamisugi Y, et al. An ancestral stomatal patterning module revealed in the non-vascular land plant Physcomitrella patens. *Development.* 2016;143(18):3306–3314. doi: 10.1242/dev.135038
32. Caine RS, Chater CCC, Fleming AJ, Gray JE. Stomata and Sporophytes of the Model Moss Physcomitrium patens. *Front Plant Sci.* 2020;11:643. doi: 10.3389/fpls.2020.00643
33. Jia M, Wang Y, Jin H, et al. Comparative Genomics Analysis of the Populus Epidermal Pattern Factor (EPF) Family Revealed Their Regulatory Effects in Populus euphratica Stomatal Development. *Int J Mol Sci.* 2024;25(18):10052. doi: 10.3390/ijms251810052
34. Li P, Zhao Z, Wang W, et al. Genome-wide analyses of member identification, expression pattern, and protein–protein interaction of EPF/EPFL gene family in Gossypium. *BMC Plant Biol.* 2024;24(1):554. doi: 10.1186/s12870-024-05262-7
35. McElwain JC, Chaloner WG. Stomatal Density and Index of Fossil Plants Track Atmospheric Carbon Dioxide in the Palaeozoic. *Annals Bot.* 1995;76(4):389–395. doi: 10.1006/anbo.1995.1112
36. Qiao R, Yang J, Deng Y, et al. Genome-Wide Identification of Epidermal Pattern Factor (EPF) Gene Family in Potato and Functional Characterization of StEPF4 in Regulating Drought Stress. *Agronomy.* 2024;14(12):2948. doi: 10.3390/agronomy14122948
37. Wang S, Wang W, Chen J, et al. Comprehensive Identification and Expression Profiling of Epidermal Pattern Factor (EPF) Gene Family in Oilseed Rape (Brassica napus L.) under Salt Stress. *Genes.* 2024;15(7):912. doi: 10.3390/genes15070912
38. Liu S, Chen T, Li X, Cui J, Tian Y. Genome-wide identification and expression analysis of EPF/EPFL gene family in Populus trichocarpa. *Front Genet.* 2024;15:1432376. doi: 10.3389/fgene.2024.1432376
39. Xiong L, Huang Y, Liu Z, et al. Small EPIDERMAL PATTERNING FACTOR-LIKE2 peptides regulate awn development in rice. *Plant Physiol.* 2022;190(1):516–531. doi: 10.1093/plphys/kiac278
40. Zhiling L, Wenhua D, Fangyuan Z. Genome-wide identification and phylogenetic and expression pattern analyses of EPF/EPFL family genes in the Rye (Secale cereale L.). *BMC Genomics.* 2024;25(1):532. doi: 10.1186/s12864-024-10425-9
41. Jiao Z, Wang J, Shi Y, et al. Genome-Wide Identification and Analysis of the EPF Gene Family in Sorghum bicolor (L.) Moench. *Plants (Basel).* 2023;12(22):3912. doi: 10.3390/plants12223912
42. Liu R, Xu K, Li Y, et al. Investigation on the Potential Functions of ZmEPF/EPFL Family Members in Response to Abiotic Stress in Maize. *Int J Mol Sci.* 2024;25(13):7196. doi: 10.3390/ijms25137196
43. Wei D, Chang P, Liu JY, et al. Genome-wide identification of EPF/EPFL gene family in wheat (Triticum

aestivum) and analysis of TaEPF1-2B associated with stomatal traits. *J Triticeae Crop.* 2021;41(11):1317–1329. doi: 10.7606/j.issn.1009-1041.2021.11.01
44. Lee JS, Hnilova M, Maes M, et al. Competitive binding of antagonistic peptides fine-tunes stomatal patterning. *Nature.* 2015;522(7557):439–443. doi: 10.1038/nature14561
45. Hunt L, Bailey KJ, Gray JE. The signalling peptide EPFL9 is a positive regulator of stomatal development. *New Phytol.* 2010;186(3):609–614. doi: 10.1111/j.1469-8137.2010.03200.x
46. Kawamoto N, Carpio DPD, Hofmann A, et al. A Peptide Pair Coordinates Regular Ovule Initiation Patterns with Seed Number and Fruit Size. *Curr Biol.* 2020;30(22):4352–4361.e4. doi: 10.1016/j.cub.2020.08.050
47. Tameshige T, Ikematsu S, Torii KU, Uchida N. Stem development through vascular tissues: EPFL–ERECTA family signaling that bounces in and out of phloem. *J Exp Bot.* 2017;68(1):45–53. doi: 10.1093/jxb/erw447
48. Kosentka PZ, Overholt A, Maradiaga R, Mitoubsi O, Shpak ED. EPFL Signals in the Boundary Region of the SAM Restrict Its Size and Promote Leaf Initiation. *Plant Physiol.* 2019;179(1):265–279. doi: 10.1104/pp.18.00714
49. Fujihara R, Uchida N, Tameshige T, et al. The boundary-expressed EPIDERMAL PATTERNING FACTOR-LIKE2 gene encoding a signaling peptide promotes cotyledon growth during Arabidopsis thaliana embryogenesis. *Plant Biotechnol (Tokyo).* 2021;38(3):317–322. doi: 10.5511/plantbiotechnology.21.0508a
50. Kimura Y, Tasaka M, Torii KU, Uchida N. ERECTA-family genes coordinate stem cell functions between the epidermal and internal layers of the shoot apical meristem. *Development.* 2018;145(1):dev156380. doi: 10.1242/dev.156380
51. Zhang L, DeGennaro D, Lin G, Chai J, Shpak ED. ERECTA family signaling constrains CLAVATA3 and WUSCHEL to the center of the shoot apical meristem. *Development.* 2021;148(5):dev189753. doi: 10.1242/dev.189753
52. Huang Y, Chai M, Xi X, et al. Functional analysis of EPF/EPFL genes in Arabidopsis resistance to Sclerotinia sclerotiorum. *J Fujian Agric For Univ (Nat Sci Ed).* 2022;51(4):486–492.
53. Uchida N, Lee JS, Horst RJ, et al. Regulation of inflorescence architecture by intertissue layer ligand–receptor communication between endodermis and phloem. *Proc Natl Acad Sci U S A.* 2012;109(16):6337–6342. doi: 10.1073/pnas.1117537109
54. Cai H, Huang Y, Liu L, et al. Signaling by the EPFL-ERECTA family coordinates female germline specification through the BZR1 family in Arabidopsis. *Plant Cell.* 2023;35(5):1455–1473. doi: 10.1093/plcell/koad032
55. Li M, Lv M, Wang X, et al. The EPFL–ERf–SERK signaling controls integument development in Arabidopsis. *New Phytol.* 2023;238(1):186–201. doi: 10.1111/nph.18701
56. Negoro S, Hirabayashi T, Iwasaki R, Torii KU, Uchida N. EPFL peptide signalling ensures robust self-pollination success under cool temperature stress by aligning the length of the stamen and pistil. *Plant Cell Environ.* 2023;46(2):451–463. doi: 10.1111/pce.14498
57. He Y, He X, Wang X, et al. An EPFL peptide signaling pathway promotes stamen elongation via enhancing filament cell proliferation to ensure successful self-pollination in Arabidopsis thaliana. *New Phytol.* 2023;238(3):1045–1058. doi: 10.1111/nph.18806
58. Jangra R, Brunetti SC, Wang X, et al. Duplicated antagonistic EPF peptides optimize grass stomatal initiation. *Development.* 2021;148(16):dev199780. doi: 10.1242/dev.199780
59. Caine RS, Chater CC, Kamisugi Y, et al. An ancestral stomatal patterning module revealed in the non-vascular land plant Physcomitrella patens. *Development.* 2016;143(18):3306–3314. doi: 10.1242/dev.135038
60. Uzair M, Urquidi Camacho RA, Liu Z, et al. An updated model of shoot apical meristem regulation by ERECTA family and CLAVATA3 signaling pathways in Arabidopsis. *Development.* 2024;151(12):dev202870. doi: 10.1242/dev.202870
61. Ikematsu S, Tasaka M, Torii KU, Uchida N. ERECTA-family receptor kinase genes redundantly prevent premature progression of secondary growth in the Arabidopsis hypocotyl. *New Phytol.* 2017;213(4):1697–1709. doi: 10.1111/nph.14335
62. Uchida N, Tasaka M. Regulation of plant vascular stem cells by endodermis-derived EPFL-family peptide hormones and phloem-expressed ERECTA-family receptor kinases. *J Exp Bot.* 2013;64(17):5335–5343. doi: 10.1093/jxb/ert196
63. Yuan B, Wang H. Peptide Signaling Pathways Regulate Plant Vascular Development. *Front Plant Sci.* 2021;12:719606. doi: 10.3389/fpls.2021.719606
64. Hu Y, Xie Q, Chua NH. The Arabidopsis Auxin-Inducible Gene ARGOS Controls Lateral Organ Size. *Plant Cell.* 2003;15(9):1951–1961. doi: 10.1105/tpc.013557
65. Guo T, Lu ZQ, Xiong Y, et al. Optimization of rice panicle architecture by specifically suppressing ligand–receptor pairs. *Nat Commun.* 2023;14(1):1640. doi: 10.1038/s41467-023-37326-x
66. Guo T, Lu ZQ, Shan JX, Ye WW, Dong NQ, Lin HX. ERECTA1 Acts Upstream of the OsMKKK10-OsMKK4-OsMPK6 Cascade to Control Spikelet Number by Regulating Cytokinin Metabolism in Rice. *Plant Cell.* 2020;32(9):2763–2779. doi: 10.1105/tpc.20.00351
67. Sun Q, Qu J, Yu Y, et al. TaEPFL1, an EPIDERMAL PATTERNING FACTOR-LIKE (EPFL) secreted peptide gene, is required for stamen development in wheat. *Genetica.* 2019;147(2):121–130. doi: 10.1007/s10709-019-00061-7
68. Huang Y, Tao Z, Liu Q, et al. BnEPFL6, an EPIDERMAL PATTERNING FACTOR-LIKE (EPFL) secreted peptide gene, is required for filament elongation in Brassica napus. *Plant Mol Biol.* 2014;85(4–5):505–517. doi: 10.1007/s11103-014-0200-2
69. Jin J, Hua L, Zhu Z, et al. GAD1 Encodes a Secreted Peptide That Regulates Grain Number, Grain Length, and Awn Development in Rice Domestication. *Plant Cell.* 2016;28(10):2453–2463. doi: 10.1105/tpc.16.00379
70. Xia H, Wang Q, Chen Z, et al. Identification and Functional Analysis of the EPF/EPFL Gene Family in Maize (Zea mays L.): Implications for Drought Stress Response. *Agronomy.* 2024;14(8):1734. doi: 10.3390/agronomy14081734
71. Jiang Q, Yang J, Wang Q, Zhou K, Mao K, Ma F. Overexpression of MdEPF2 improves water use efficiency and reduces oxidative stress in tomato. *Environ Exp Bot.* 2019;162:321–332. doi: 10.1016/j.envexpbot.2019.03.009
72. Liu S, Wang C, Jia F, et al. Secretory peptide PdEPF2 enhances drought tolerance by modulating stomatal density and regulates ABA response in transgenic Arabidopsis thaliana. *Plant Cell Tissue Organ Cult.* 2016;125(3):419–431. doi: 10.1007/s11240-016-0957-x
73. Lyapina I, Ganaeva D, Rogozhin EA, et al. Comparative analysis of small secreted peptide signaling during defense response: insights from vascular and non-vas-

cular plants. *Physiol Plant*. 2025;177(2):e70147. doi: 10.1111/ppl.70147
74. Slezina MP, Istomina EA, Korostyleva TV, et al. Molecular Insights into the Role of Cysteine-Rich Peptides in Induced Resistance to Fusarium oxysporum Infection in Tomato Based on Transcriptome Profiling. *Int J Mol Sci*. 2021;22(11):5741. doi: 10.3390/ijms22115741
75. Jordá L, Sopeña-Torres S, Escudero V, et al. ERECTA and BAK1 Receptor Like Kinases Interact to Regulate Immune Responses in Arabidopsis. *Front Plant Sci*. 2016;7:897. doi: 10.3389/fpls.2016.00897
76. Tateda C, Obara K, Abe Y, et al. The Host Stomatal Density Determines Resistance to Septoria gentianae in Japanese Gentian. *Mol Plant Microbe Interact*. 2019;32(4):428–436. doi: 10.1094/MPMI-05-18-0114-R
77. Godiard L, Sauviac L, Torii KU, et al. ERECTA, an LRR receptor-like kinase protein controlling development pleiotropically affects resistance to bacterial wilt. *Plant J*. 2003;36(3):353–365. doi: 10.1046/j.1365-313X.2003.01877.x
78. Adie BAT, Pérez-Pérez J, Pérez-Pérez MM, et al. ABA Is an Essential Signal for Plant Resistance to Pathogens Affecting JA Biosynthesis and the Activation of Defenses in Arabidopsis. *Plant Cell*. 2007;19(5):1665–1681. doi: 10.1105/tpc.106.048041
79. Häffner E, Karlovsky P, Splivallo R, Traczewska A, Diederichsen E. ERECTA, salicylic acid, abscisic acid, and jasmonic acid modulate quantitative disease resistance of Arabidopsis thaliana to Verticillium longisporum. *BMC Plant Biol*. 2014;14:85. doi: 10.1186/1471-2229-14-85
80. Cai H, Huang Y, Chen F, et al. ERECTA signaling regulates plant immune responses via chromatin-mediated promotion of WRKY33 binding to target genes. *New Phytol*. 2021;230(2):737–756. doi: 10.1111/nph.17200
81. Llorente F, Alonso-Blanco C, Sánchez-Rodriguez C, Jorda L, Molina A. ERECTA receptor-like kinase and heterotrimeric G protein from Arabidopsis are required for resistance to the necrotrophic fungus Plectosphaerella cucumerina. *Plant J*. 2005;43(2):165–180. doi: 10.1111/j.1365-313X.2005.02440.x
82. Sánchez-Rodríguez C, Estévez JM, Llorente F, et al. The ERECTA Receptor-Like Kinase Regulates Cell Wall–Mediated Resistance to Pathogens in Arabidopsis thaliana. *Mol Plant Microbe Interact*. 2009;22(8):953–963. doi: 10.1094/MPMI-22-8-0953
83. Sopeña-Torres S, Jordá L, Sánchez-Rodríguez C, et al. YODA MAP3K kinase regulates plant immune responses conferring broad-spectrum disease resistance. *New Phytol*. 2018;218(2):661–680. doi: 10.1111/nph.15007