Peptide regulation of plant cells differentiation and growth

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Abstract. The main task of agriculture is to increase the productivity of cultivated plants, and therefore the development of methods that regulate the growth and development of agricultural crops is becoming increasingly important. It is known that plant development in response to external stimuli is regulated by peptide phytohormones. In addition, peptides are considered as antimicrobial agents. The review examines peptides of the CLE, EPF, PSY, PSK, RGF, CIF families, dipeptide KE, tripeptide EDR and tetrapeptides AEDL, KEDG, AEDR, KEDP and their role in the regulation of plant differentiation and growth. The wide range of biological activity of peptides allows us to propose their use to increase the productivity of new generation plants.

1 Introduction

The increase in yields of major crops, achieved in the 20th century through the development of fertilizers and pesticides, has occurred less rapidly and effectively in the last two decades [1]. A modern method of increasing productivity is genetic engineering. The goal of this direction was to increase the expression of genes for plant growth and development. However, the development and implementation of this method takes a long time and is associated with a number of difficulties. A more effective way to increase productivity is the use of plant growth regulators - organic substances that have biological activity in low concentrations. These substances regulate seed germination, growth, differentiation of plant tissues and organs, flowering, and fruit ripening. One of the proposed mechanisms of their action is the regulation of the expression of genes associated with the proliferation and differentiation of plant cells. Such growth regulators as cytokinin, ethylene, jasmonic acid, and indole-acetic acid are known [2].

In recent years, peptides have become a popular subject of research in the field of plant growing as antimicrobial and immune inducers, plant growth regulators, insecticides and herbicides due to the availability of their synthesis and high biological activity [3]. For example, antimicrobial peptides have demonstrated the ability to destroy pathogenic fungi and bacteria in plants [4]. Di- and tetrapeptides regulate the expression of genes for proliferation and differentiation of Nicotiana Tabacum leaves in micro- and nanomolar concentrations [5]. It can be assumed that short peptides consisting of 2-4 amino acid residues can exhibit the properties of plant growth regulators.

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The purpose of the review is to characterize the peptide regulation of plant cell differentiation and growth and the prospects for the use of short peptides in plant growing.

2 Peptide regulation of plant cells differentiation

Along with endogenous plant hormones, such as auxin and cytokinin [6], intercellular signaling is carried out by plant peptides [7]. Despite the data that the number of genes encoding small plant signal peptides potentially exceeds 2.5 thousand, only a portion of them have currently been identified. Identification is also complicated by the fact that many peptides appear to function in plants at extremely low concentrations, which technically limits their detection [8]. The most currently studied families of peptide phytohormones and plant growth regulators are discussed below.

2.1 CLE family peptides

One of the main and most studied families of plant peptides is Clavata3/endosperm surrounding region (CLE). The CLE genes encode precursor peptides containing an N-terminal signal domain, a central variable domain, and a conserved CLE domain of 12-14 amino acid residues at the C-terminus. By proteolytic cleavage, the full-length peptide is processed, resulting in the formation of a mature CLE peptide, which can subsequently undergo post-translational modifications [9]. Mature CLE peptides bind to receptors on cell membranes, which transmit a signal into the cell, regulating the activity of downstream transcription factors and phytohormone signaling pathways. CLE genes are ubiquitously expressed in plants. For example, in the model plant Arabidopsis thaliana, each tissue type expresses at least one CLE gene [10].

The CLV3 gene is expressed exclusively in the sprouts of Arabidopsis thaliana, where its protein product, the CLV3 peptide, regulates the pool of stem cells and their differentiation through interaction with the transcription factor WUS. The CLV3-CLV1-WUS pathway is functionally conserved among a number of flowering plants, including Arabidopsis, rice Oryza sativa, tomato Solanum lycopersicum, maize and Brassica juncea. The CLE1/3/4/7 peptide interacts with the CLV1 receptor and slows down the growth of the lateral root of Arabidopsis thaliana. Peptides CLE17 and CLE19 ensure the maintenance of homeostasis of the root apical meristem, and peptide CLE41/44 ensures the development of the vascular cambium. In total, 32 genes encoding CLE peptides and 27 unique peptides have been identified in Arabidopsis thaliana [10]. Recently, the CLE33 gene was identified in Arabidopsis thaliana, which is involved in the formation of protophloem and is similar in structure and function to CLE45 [11].

2.2 EPF family peptides

Another family, EPF (epidermal patterning factor), includes cysteine-rich peptides that regulate the development of plant stomata. This family is much smaller than the CLE family and in Arabidopsis thaliana includes 11 members: EPF1, EPF2 and EPFL1-9 [12]. The products of EPF genes are precursor peptides with a length of 102 amino acid residues. Mature EPF peptides consist only of a C-terminal domain containing 6–8 cysteine residues in strictly defined positions, between which 3–4 disulfide bridges are formed, which are structurally necessary for the binding of EPF peptides to their receptors [13].

Different EPF peptides regulate different stages of stomatal cell differentiation. Expression of the EPF2 gene was detected at the early stage of stomatal formation, as well as at the stage of early meristemoids. One of the functions of the EPF2 peptide is to
suppress the differentiation of parastomatal cells. Expression of the EPFL1 gene was detected in meristemoids at later stages. The EPF peptide regulates the orientation of meristemoid divisions and determines the distance between stomata [14]. Treatment of plants with exogenous peptides EPF1 and EPF2 causes defects in stomatal development, indicating their role as negative regulators of stomatal differentiation [15]. The EPFL9/STOMAGEN gene, on the contrary, is expressed in leaf mesophyll cells; its protein product diffuses into the epidermis and positively regulates stomatal development [16].

2.3 Tyrosine-sulfated peptides

Tyrosine sulfation is a post-translational modification of peptides. In several classes of plant peptide hormones, tyrosine sulfation occurs during their maturation. These peptides include peptides of the classes PSY (plant peptides containing tyrosine sulfation), PSK (phytosulfokines) and RGF/GLV/CLEL (root growth factor), CIF (casparian strip integrity factor). Tyrosine sulfation has been shown to be necessary for the maturation of such peptides. Plants lacking the TPST enzyme, which ensures tyrosine sulfation, are characterized by serious developmental defects, which are eliminated by treatment with exogenous peptides PSY1, PSK and RGF1. Probably, sulfate ensures the stability of the peptide during secretion into the protoplast, and also increases the binding affinity to its receptor [17].

PSK phytosulfokines are short peptides of 4-5 amino acid residues, formed from longer protein precursors of 80-120 amino acid residues. The pentapeptide PSK-α and the tetrapeptide PSK-β, formed from PSK-α as a result of the removal of the C-terminal glutamine residue, take special place among them. The PSK gene, encoding phytosulfokines, was found in the cultivated rice Oryza sativa [18]. It is known that the main effect of phytosulfokines is to increase proliferation. Even at a low concentration of plant cells in culture, the addition of PSK to the medium causes them to divide. For the stimulating effect of PSK, the presence of the phytohormones auxin and cytokinin in the environment is necessary [19]. It has been shown that the addition of PSK at a concentration of 1 nM to the culture medium accelerates root growth in Arabidopsis thaliana seedlings, and overexpression of one of the genes encoding PSK, AtPSK4, leads to elongation of plant roots and the formation of larger leaves [20].

In the family of plant peptides containing tyrosine sulfation (PSY), only PSY1 has been characterized as an 18 amino acid residue peptide containing one sulfated tyrosine and two hydroxylated prolines in the active domain, and involved in cell elongation. The functional activity of PSY1 is similar to that of phytosulfokines. Addition of PSY1 to plant suspension cultures also stimulates cell division and growth. Genes encoding PSY1 precursor peptides are expressed in various tissues of Arabidopsis thaliana, but their expression is most pronounced in the shoot apical meristem and root elongation zone [21]. PSK and PSY1 also appear to regulate plant-pathogen interactions. When infected with the model phytopathogen Pseudomonas syringae pv. Tomato, induction of expression of genes encoding PSK and PSY1 precursors and their receptors occurs: PSKR1, PSK1, 2, 4 and PSY1 [22].

RGF/GLV/CLEL peptides (hereinafter RGF) are 13–18 amino acid residues long and contain hydroxylated proline and sulfated tyrosine. These peptides were first discovered in A. thaliana as regulators of root growth and were subsequently identified in other plants [23]. In A. thaliana, the RGF family of peptides includes 11 members. Mature RGF peptides are formed by proteolytic processing from a precursor peptide of 86–163 amino acid residues in length [21]. The RGF signaling cascade - the RGF receptor regulates the formation of a gradient of PLETHORA transcription factors (PLT), which is necessary to maintain the root stem cell pool. Exogenous treatment with the RGF1 peptide causes an
increase in the root meristem and the expression levels of the PLT1 and PLT2 genes encoding the PLETHORA transcription factors [24]. Another function of RGF peptides is associated with the control of polar auxin transport and root gravitropism. Overexpression of the GLV3, GLV6, GLV9 genes leads to the growth of wavy roots. Treatment of roots with the RGF3 peptide causes the accumulation of the PIN2 protein, which regulates polar auxin transport in the plasma membrane and vesicles of epidermal cells of the root tip [25]. By regulating polar auxin transport, RGF peptides may be involved in the control of other auxin-dependent processes, such as root hair growth and lateral root development.

CIF (casparian strip integrity factor) are secreted peptides of 21-24 amino acid residues, formed from protein precursors encoded by five CIF genes. It is assumed that the main function of CIF peptides is to participate in the creation of extracellular barriers in plants, which serve to limit diffusion between tissues. In the differentiation zone, CIF peptides are involved in the initiation of lateral roots and the growth of root hairs [26].

2.4 Short peptides

Short peptides, in particular di- and tetrapeptides, demonstrate the ability to regulate the expression of proliferation and differentiation genes in various organisms. Thus, tetrapeptides AEDG and AEDL and dipeptide KE at a concentration of 10-8 M stimulated the growth, development and differentiation of callus cultures of tobacco Nicotiana tabacum. The regulatory function of these peptides is apparently realized through their influence on the genes of the CLE, GRF (growth factors) and KNOX1 (transcription factors) families. The KE dipeptide increased the expression of the CLE6 gene by 2 times and did not affect other genes of this family, while the tetrapeptides AEDG and AEDL stimulated the expression of the CLE2, CLE5 and CLE6 genes. The AEDG peptide reduced the expression of the CLE4 gene, and the AEDL peptide increased it almost 2-fold. The effect of the studied peptides on the KNOX1 and GRF genes was also gene specific. The expression of the KNAT1 and KNAT2 genes did not change under the influence of short peptides. The expression of the KNAT3 and KNAT6 genes was significantly increased under the influence of all studied peptides, to a greater extent AEDL and KE (six times compared to the control). The expression of the LET6 and LET12 genes increased under the influence of the AEDL peptide and remained virtually unchanged under the influence of the AEDG and KE peptides. The AEDG peptide increased the expression of the GRF1 gene by more than 2 times, while other peptides decreased it. The AEDL peptide also stimulated GRF3 expression, but AEDG and KE had no effect on it. At the same time, the peptides AEDL and KE did not affect the expression of GRF2, but AEDG increased it 10-fold. Thus, the effect of peptides on genes encoding proteins that regulate plant growth depends on the primary structure of the peptide [5].

In addition, the effect of short peptides AEDG, EDR, AEDL, KEDG, AEDR, KEDP on the hydrolysis of lambda phage DNA carried out by endonucleases WEN1 and WEN2 of wheat coleoptiles, in the presence and absence of histone H1, was studied. This study demonstrated for the first time the modulating effect of short peptides on the activity of wheat endonucleases, which occurs due to site-specific binding of peptides to DNA [27].

Pre-treatment of soybean seeds 1 month before planting with di- and tetrapeptides at concentrations of 0.01 g/l or 0.001 g/l had a positive effect on soybean growth. Treatment of seeds with a dipeptide led to an increase in soybean yield by 59-81%, and with a tetrapeptide – by 62-83% [28]. The data obtained indicate the possibility of using short peptides as regulators of plant differentiation and growth.
3 Conclusion

Short peptides and peptide hormones represent a large group of regulators of plant differentiation and growth. They regulate the maintenance of the pool of meristem cells, tissue differentiation, the formation of lateral organs, as well as the interaction of plants and microbes (Fig. 1). Peptide hormones are important systemic coordinators of plant growth in response to biotic and abiotic signals, since they are able to be transported over long distances through vascular tissues. Moreover, new groups of peptide hormones and their previously unknown functions are described in plants. Thus, short peptides can be considered as substances with prospects for use in increasing plant productivity.

![Diagram of plant functions regulated by peptide hormones](image)

**Fig. 1.** Main functions of peptide hormones in plants.

References