Reviews

UDC 632.938.2:57.023:57.21:57.087.1

doi: 10.15389/agrobiology.2017.1.3rus

doi: 10.15389/agrobiology.2017.1.3eng

PLANT DEFENSINS: BIOLOGICAL FUNCTION, MECHANISMS OF ACTION AND METHODS OF ANALYSIS (review)

O.A. KULAEVA¹, M.S. KLIUKOVA¹, M.N. POVDYSH², I.A. TIKHONOVICH^{1, 3},
V.A. ZHUKOV¹

¹All-Russian Research Institute for Agricultural Microbiology, Federal Agency of Scientific Organizations, 3, sh. Podbel'skogo, St. Petersburg, 196608 Russia, e-mail zhukoff01@yahoo.com, VZhukov@ARRIAM.ru;

²Saint-Petersburg State Chemical-Pharmaceutical Academy, Ministry of Healthcare of the Russian Federation, 14, ul. Prof. Popova, St. Petersburg, 197376 Russia;

³Saint Petersburg State University, 7/9, Universitetskaya nab., St. Petersburg, 199034 Russia

ORCID:

Kulaeva O.A. orcid.org/0000-0003-2687-9693

Tikhonovich I.A. orcid.org/0000-0001-8968-854X

Kliukova M.S. orcid.org/0000-0003-1119-5512

Zhukov V.A. orcid.org/0000-0002-2411-9191

Povydysh M.N. orcid.org/0000-0002-7768-9059

The authors declare no conflict of interests

Acknowledgements:

Supported by Russian Foundation for Basic Research (grant № 16-34-60132 for O.A. Kulaeva), by grant of the President of the Russian Federation, project HHI-6759.2016.4, for M.S. Kliukova, by Russian Science Foundation (grant № 14-24-00135 for I.A. Tikhonovich and grant № 16-16-00118 for V.A. Zhukov)

Received December 7, 2016

Abstract

One of the actual problems of modern agriculture is crop loss due to various biotic and abiotic factors. In plants there is a multicomponent protection system, including the formation of protective barriers, activation the reaction of hypersensitivity and synthesis of antimicrobial peptides, which are low molecular weight compounds showing broad spectrum activity against fungi, bacteria and viruses. This group consists of several groups of peptides, including defensins, which are one of the most common classes of antimicrobial peptides and are detectable in all living organisms. Defensins are small (45-54 amino acids), cysteine-rich peptides involved in a different protective responses (B.P. Thomma et al., 2002). Genes which are coding plant defensins are expressed in different organs of plants, where their products are necessary for biotic and abiotic stresses. Thus, these peptides are extremely important in terms of getting crop lines that are resistant to pathogens and abiotic stresses. Defensins are characterized by a strong sequence variability that seems to correlate with a variety of mechanisms of action of these peptides that can induce pathogen's cell death by penetrating into a cell or by being on its surface (T.M. Shafee et al., 2016). Most of the plant defensins are characterized by the antifungal activity. Some defensins have antibacterial activity, which may be combined with activity against fungi. For a small number of plant defensins their participation in the processes of resistance to heavy metals, cold stress, drought, salinity, and in the development process is indicated. Modern approaches of molecular and computational biology allow an effective search for new forms of defensins activity by studying the wild, non-model plant objects. The development of next-generation sequencing methods («Next Generation Sequencing») make possible the intensive study of the transcriptomes of such objects. However, the correct annotation of the sequences of peptides, characterized by small size and high variability, can be done by usage the special programs, such as SPADA (Small Peptide Alignment Discovery Application) (P. Zhou et al., 2013). SPADA makes multiple sequence alignment of all known paralogous genes within a gene family and builds a predictive model for the search of new members of the same family. Prediction of newly identified active defensins and identification of conserved amino acids can also be performed by computational biology methods. An approach based on a multiple sequence alignment and subsequent cluster analysis allows dividing defensins into groups with similar functional activity (N.L. van der Weerden et al., 2013). Thus, the combination of modern methods of molecular and computational biology allows carrying out the study of this group of protective peptides with high efficiency.

Keywords: defensins, cysteine-rich peptides, defense reactions, biotic and abiotic stress, next-generation sequencing

Various damages caused by insects, fungi, viruses or physical factors lead to crop losses. To fight against negative environmental exposure, plants use a multicomponent system that includes a hypersensitivity response, consolidation of protective barriers using cell wall components, activation of the production of immune proteins and antimicrobial peptides [1]. Antimicrobial peptides are the low-molecular compounds (12-95 amino acids), which are synthesized in the cells of all living organisms. The structure and action mechanisms of antimicrobial peptides are much diversified. Plant antimicrobial peptides are divided into several families — cyclotides, defensins, thionines, lipid-carrying proteins, hevea-like proteins, etc. [1-3].

Plant defensins are small cysteine-rich peptides consisting of 45-54 amino acids. They are located in majority of the Plant Kingdom. Structurally and functionally, defensins of the plants, insects, mammals and fungi are similar [4-6]. Genes of plant defensins are expressed in different organs, e.g. seeds [7, 8], leaves [9], roots [10], flowers [11, 12] and symbiotic tubercles [13]. There is both constitutive and inducible expression of genes, encoding plant defensins [11, 14]. The main function of defensins is to inhibit fungal disease [8, 11, 15], but for some peptides there are antibacterial activity [16, 17], inhibition of trypsin [18], and participation in the formation of resistance to heavy metals [19], cold stress [20, 21], drought [22], salinity [23, 24] and in developmental processes [25].

Structure of plant defensins. Modern studies show that, based on the structural organization, defensins of animals, plants and fungi can be divided into two superfamilies (cis- and trans-defensins) with independent evolutionary descent [26]. In cis-defensins, two parallel disulfide bridges bind the α -spiral to β -bands, while in trans-defensins similar disulfide bridges are oppositely oriented and are connected with different structural elements. Plant defensins are the main members of cis-defensins family. Using nuclear-magnetic resonance spectroscopy, it was shown that in plant defensins the basis of the molecule is formed by a three-layer element consisting of one α -spiral connected with cysteine bridges to β -bands (Fig. 1) [7, 8, 27, 28].

Fig. 1. Amino acid sequence of mature defensin MtDef4. The lines connecting the cysteine residues are disulfide bridges. The spiral and arrows indicate the location of the α -spirals and β -bands, respectively. Conserved cysteine residues are in bold.

All plant defensins are formed during the processing of precursor proteins, which may not be the same in different species. Defensins with 8 or 10 conserved cysteine residues have been identified in plant organisms. It is supposed that such residues are necessary to maintain the conformation of the molecule, whereas the revealed high variability in functional regions of defensins ensures their specificity related to the targets and determines the diversity of functions of this protein family [26, 29].

Functional characteristics of some defensins. For most known defensins, antifungal and (or) bactericidal activity is specific. Defensin NaD1 was segregated from the outer cell layers of the decorative tobacco flower *Nicotiana glauca*, which correlates with its protective function in the reproductive organs. In vitro studies have shown that NaD1, presented as a dimer, suppresses the growth of *Botrytis cinerea* and *Fusarium oxysporus* [11]. ω -Chordothionine and γ -chordothionine, found in the endosperm of barley grains, inhibit translational activity in both eukaryotic cell-free mammalian systems (lysates of mouse liver cells and rabbit reticulocytes) and prokaryotic systems (*Escherichia coli*), but

do not exert the same effect in relation to translation processes in plant systems [30]. AlfAFP from the seeds of *Medicago sativa* is antifungal peptide and provides resistance to fungal pathogen *Verticillium dahlia* in transgenic potato plants [11, 31]. Defensins from *Raphanus sativus* (RsAFP) are accumulated as the seeds ripen and are released after the integrity of seed membranes is destroyed to create a microenvironment that suppresses the growth of fungi, with a decrement in the elongation of hyphae and their enhanced branching [8, 32]. MsDef1 from *M. sativa* seeds significantly inhibits the growth of *F. graminearum* in vitro [33]. These defensins are examples of peptides with 8 conserved cysteine residues. Another subclass (with 10 conserved cysteine residues) includes defensins PhD1 and PhD2 (*Petunia hybrida* defensin 1 and 2, respectively) isolated from petunia flowers. In their three-dimensional structure, there is an additional disulfide bond [11, 34], which does not change the conformation as compared to other defensins, but probably gives additional thermodynamic stability [34]. In vitro experiments, PhD1 and PhD2 blocked the growth of *F. oxysporum* and, in a lesser extent, *B. cinerea* [11].

In addition to antifungal activity, some plant defensins have antibacterial properties. Thus, Cp-thionine II, identified in seeds of *Vigna unguiculata*, acts against Gram-positive and Gram-negative bacteria, *Staphylococcus aureus*, *E. coli* and *Pseudomonas syringae* [16]. Fabatins isolated from *Vicia faba* beans also inhibit the growth of various bacteria, but are inactive against yeast *Saccharomyces cerevisiae* and *Candida albicans* [17]. PsD1 (from *Pisum sativum*) is an antibacterial peptide that is localized in nuclei of the treated *Neurospora crassa* cells and interacts with proteins involved in control and termination of cell cycle, for example, cyclin F [35].

It is shown that 8 genes of *M. truncatula* *MtDefNS* are specifically expressed in nodules formed in symbiosis with *Sinorhizobium meliloti*, and (with the exception of one gene) are in the chromosome 8, which suggests some specialization of these sequences. Also, most of them are clustered phylogenetically and form a separate clade in relation to other classical defensins [13]. However, the specific function of defensins in symbiotic relationships is unknown by now.

For a small number of defensins, participation in plant adaptation to abiotic factors has been described. Thus, increased concentrations of NaCl influenced the change in expression of genes encoding defensins in *Arachis hypogaea* [23] and *Arabidopsis thaliana* [24]. The effect of water deficiency on changes in the expression of genes encoding defensins is shown in *Glycine max* [22, 36]. Cold also affects the expression of defensin genes [20, 21]. Winter wheat has *Tad1* gene, the expression of which is induced specifically under cold stress [21]. The hyper-accumulator of zinc and cadmium, *Arabidopsis halleri*, has gene, encoding defensin AhPDF1.1, which is actively studied [19, 37-40]. Expression of this gene in *A. thaliana* and *S. cerevisiae* leads to an increase in resistance to zinc, but not to cadmium, cobalt, iron or sodium. *A. halleri* shows a zinc dependence of the pool of defensins both at transcript and peptide levels. Compared to *A. thaliana*, *A. halleri* shows an increase in the defensin amount in seedlings [19].

Some defensins are involved in a response to several stressors. In *Brassica rapa*, the expression of the genes encoding defensins BrDLFP and BrBetvAFP was significantly altered at cold stress, drought and salinity [41]. When studying the effect of pre-treatment of arabidopsis plants with nontoxic concentrations of silicon and cadmium on *B. cinerea* infection, the enhancement of expression of the gene encoding defensin PDF1.2 [42] was shown. Expression of the defensin gene NbDef2.2 in *N. benthamiana* increases not only after infection of *Pseudomonas syringae* pv. *tabaci*, but also in wounding and ethylene treatment [43]. Thus, plant defensins can be components of overlapping re-

sponses to abiotic and biotic agents.

Action mechanisms of plant defensins. The strong variability of plant defensin sequences seems to correlate with the variety of their action mechanisms [26]. By now, the action mechanisms of defensins with antifungal activity have been thoroughly studied. Plant defensins can interact with specific binding sites (receptors) — components of the target cell membrane, for example, bacterial lipid II, sphingolipids [44] and phospholipids of fungi [45]. In this case, different plant defensins interact with different classes of sphingolipids [46-49]. Thus, RsAFP2 from the radish binds to the glycosphingolipids of *Pichia pastoris* and *C. albicans* [50]. Defensin NaD1 interacts with various phospholipids, but not with sphingolipids [45]. The presence of specific sphingolipids on the surface of the membrane is necessary to mediate the cell death of fungi, since yeast mutants defective in the genes, involved in the synthesis of sphingolipids, are resistant to the defensins that bind them [47, 48, 51]. After interaction with the receptors, the defensins either penetrate the interior of the fungal cell and interact with intracellular targets, or remain on the surface (for example, as MsDef1) and induce cell death by inducing the signal cascade (Fig. 2) [52]. An example of defensin penetrating cell is MtDef4 (see Fig. 2). In its sequence, the RGFRRR motif is identified, which apparently serves as a translocation signal necessary for penetration into the fungal cell. When replacing this motif with AAAARR or RGFRAA, MtDef4 loses its ability to penetrate the interior of the cell [53]. Nevertheless, there is no evidence that this sequence is in other plant defensins penetrating fungal cells [52].

Recently, more and more information has appeared on peptides that cause cell death, stimulating the production of reactive oxygen species (ROS) [54-56]. The formation of ROS and induction of oxidative stress were observed in target cells at treatment with defensins RsAFP2, DmAMP1 [54], HsAFP1 [57] and NaD1 [55].

Fig. 2. Suggested mechanisms of anti-fungal action on the example of defensins MsDef1 (A, does not penetrate the fungal cell) and MtDef4 (B, penetrates into the fungal cell): DF — defensin, CW — fungus cell wall, PP — periplasmic space, PM — plasma membrane, CZ — cytosol, MAPK — mitogen-activated protein kinase (cit. ex 52 with changes).

So far, molecular mechanisms of defensin action in abiotic stress remain unknown. This is due to a small number of studies (when compared to the role of defensins in biotic stress), the complexity of such experiments and the fact that defensins vary greatly in both structure and action mechanisms. The works are mostly devoted to the study of activation of certain genes encoding defensins and discussion on their putative role in the resistance to certain factors [39].

Use of sequencing and bioinformatics methods for the study of defensins. The protective properties manifested by defensins make them helpful in crop selection for resistance to various pathogens. Seeking for new

forms of activity of defensins in wild plants that do not belong to model ones is also prospective. With the development of NGS (next generation sequencing), it became possible to quickly identify genomes and/or transcripts sequences of many plant species, including non-model ones [58, 59]. At the same time, the problem of the annotation and functional characteristics of sequences obtained becomes urgent [60]. Due to their high divergence in defensins of one group and a limited number of experimentally detected peptides, the commonly used tools for searching similar sequences and gene detection do not mostly provide reliable identification of such short peptides [61]. For reliable detection of genes encoding short peptides, special algorithms are developed. These include SPADA (Small Peptide Alignment Discovery Application). The SPADA algorithm allows finding all related paralogic genes within a gene family, multiple alignments of sequences and construction of a predictive model for searching. In SPADA, several approaches to searching for similar sequences are used, i.e. BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and HMMER (<http://hmmer.org/>), as well as tools for predicting genes, for example Augustus (<http://bioinf.uni-greifswald.de/augustus/>), which significantly increases the efficiency of annotation of gene families with a large number of members. It is shown that almost all representatives of gene families encoding short cysteine-rich proteins in *A. thaliana* and *M. truncatula* are annotated more effectively in SPADA, than in other programs [61]. Thus, SPADA can be successfully used to identify genes encoding defensins, including those of non-model objects.

With the advent of the ability to quickly obtain information about the genome and/or transcriptome of the studied object, and due to the development of algorithms for proper annotation, the number of identified defensin-related sequences is steadily growing. Within specific groups of peptides, it is necessary to study their biological activity, but a full analysis of the activity of defensins detected by traditional methods has almost never been carried out. Only certain aspects of the action against certain fungi or bacteria have been found out. When analyzing a large number of defensin genes, the methods of computational biology can be used to predict the activity of newly detected peptides and to detect conserved amino acids. Thus, the approach based on multiple sequence alignment and subsequent cluster analysis allowed to separate the defensins of different species, presented in the NCBI database (National Center for Biotechnology Information, USA) into 18 groups [29]. It turned out that defensins from one group possess similar functional activity. It is worth noting that, due to low similarity of the sequences, the comparative analysis of defensins carried out by classical direct sequence comparison may have extremely low efficacy. To align more correctly such complex sets of sequences, special methods based on the barcoding of conserved sections of cysteine sequences [62] should be used. This allows comparative analysis of even very different sequences, including defensins.

The presentation of the results of different research teams in databases with free access increases the effectiveness of research. Thus, using the expression atlases, it becomes possible to identify the expression profile of certain groups of genes. This allows us to estimate the organ or tissue specificity of the expression of a particular gene and its involvement in certain processes [63, 64].

Thus, plant defensins (a group of peptides with antifungal and bactericidal activity, which also participate various responses to abiotic effects) are extremely important in view of obtaining resistant crop varieties. Representatives of this peptide group are characterized by an extremely low similarity of the primary structure (with the exception of conservatively located cysteine residues), however modern methods of computational biology in combination

with sequencing technologies accelerate the identification and analysis of sequences encoding defensins, including those of non-model plants.

REFERENCES

1. Goyal R.K., Mattoo A.K. Multitasking antimicrobial peptides in plant development and host defense against biotic/abiotic stress. *Plant Sci.*, 2014, 228: 135-149 (doi: 10.1016/j.plantsci.2014.05.012).
2. Tam J.P., Wang S., Wong K.H., Tan W.L. Antimicrobial peptides from plants. *Pharmaceuticals*, 2015, 8(4): 711-757 (doi: 10.3390/ph8040711).
3. Egorov T.A., Odintsova T.I. Defense peptides of plant immune system. *Russian Journal of Bioorganic Chemistry*, 2012, 38(1): 1-9 (doi: 10.1134/S1068162012010062).
4. Thomma B.P., Cammue B.P., Thevissen K. Plant defensins. *Planta*, 2002, 216(2): 193-202 (doi: 10.1007/s00425-002-0902-6).
5. Stotz H.U., Thomson J., Wang Y. Plant defensins: defense, development and application. *Plant Signal. Behav.*, 2009, 4(11): 1010-1012 (doi: 10.4161/psb.4.11.9755).
6. Komaletdinova F.M. *Sel'skokhozyaistvennaya biologiya [Agricultural Biology]*, 2009, 5: 8-16 (in Russ.).
7. Bruix M., Jimenez M.A., Santoro J., Gonzalez C., Colilla F.J., Mendez E., Rico M. Solution structure of gamma 1-H and gamma 1-P thionins from barley and wheat endosperm determined by proton NMR: a structural motif common to toxic arthropod proteins. *Biochemistry*, 1993, 32(2): 715-724 (doi: 10.1021/bi00053a041).
8. Fant F., Vranken W., Broekaert W., Borremans F. Determination of the three-dimensional solution structure of *Raphanus sativus* antifungal protein 1 by 1 H NMR. *J. Mol. Biol.*, 1998, 279(1): 257-270 (doi: 10.1010/pdb1ayj.pdb).
9. Do H.M., Lee S.C., Jung H.W., Sohn K.H., Hwang B.K. Differential expression and in situ localization of a pepper defensin (CADEF1) gene in response to pathogen infection, abiotic elicitors and environmental stresses in *Capsicum annuum*. *Plant Sci.*, 2004, 166(5): 1297-1305 (doi: 10.1016/j.plantsci.2004.01.008).
10. Huang G.-J., Lai H.-C., Chang Y.-S., Sheu M.-J., Lu T.-L., Huang S.-S., Lin Y.-H. Antimicrobial, dehydroascorbate reductase, and monodehydroascorbate reductase activities of defensin from sweet potato [*Ipomoea batatas* (L.) Lam."tainong 57"] storage roots. *J. Agric. Food Chem.*, 2008, 56(9): 2989-2995 (doi: 10.1021/jf072994j).
11. Lay F.T., Brugliera F., Anderson M.A. Isolation and properties of floral defensins from ornamental tobacco and petunia. *Plant Physiol.*, 2003, 131(3): 1283-1293 (doi: 10.1104/pp.102.016626).
12. Urdangarin M.C., Norero N.S., Broekaert W.F., de la Canal L. A defensin gene expressed in sunflower inflorescence. *Plant Physiol. Biochem.*, 2000, 38(3): 253-258 (doi: 10.1016/S0981-9428(00)00737-3).
13. Maryti G., Downie J.A., 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).
14. Chiang C.C., Hadwiger L.A. The *Fusarium solani*-Induced expression of a pea gene family encoding high cysteine content proteins. *Mol. Plant-Microbe Interact.*, 1991, 4(4): 324-331 (doi: 10.1094/MPMI-4-324).
15. Lacerda A., Vasconcelos É.A.R., Pelegrini P.B., Grossi-de-Sa M.F. Antifungal defensins and their role in plant defense. *Front. Microbiol.*, 2014, 5: 116 (doi: 10.3389/fmicb.2014.00116).
16. Franco O.L., Murad A.M., Leite J.R., Mendes P.A., Prates M.V., Bloch C. Identification of a cowpea γ -thionin with bactericidal activity. *FEBS J.*, 2006, 273(15): 3489-3497 (doi: 10.1111/j.1742-4658.2006.05349.x).
17. Zhang Y., Lewis K. Fabinins: new antimicrobial plant peptides. *FEMS Microbiol. Lett.*, 1997, 149(1): 59-64 (doi: 10.1111/j.1574-6968.1997.tb10308.x).
18. Melo F.R., Rigden D.J., Franco O.L., Mello L.V., Ary M.B., Grossi de Sá M.F., Bloch C. Inhibition of trypsin by cowpea thionin: Characterization, molecular modeling, and docking. *Proteins: Struct., Funct., Bioinf.*, 2002, 48(2): 311-319 (doi: 10.1002/prot.10142).
19. Mirouze M., Sels J., Richard O., Czernic P., Loubet S., Jacquier A., François I.E.J.A., Cammue B.P.A., Lebrun M., Berthomieu P., Marquis L. A putative novel role for plant defensins: a defensin from the zinc hyper-accumulating plant, *Arabidopsis halleri*, confers zinc tolerance. *Plant J.*, 2006, 47(3): 329-342 (doi: 10.1111/j.1365-3113.2006.02788.x).
20. Gaudet D.A., Laroche A., Frick M., Huel R., Puchalski B. Cold induced expression of plant defensin and lipid transfer protein transcripts in winter wheat. *Physiologia Plantarum*, 2003, 117(2): 195-205 (doi: 10.1034/j.1399-3054.2003.00041.x).
21. Koike M., Okamoto T., Tsuda S., Imai R. A novel plant defensin-like gene of

- winter wheat is specifically induced during cold acclimation. *BBRC*, 2002, 298(1): 46-53 (doi: 10.1016/S0006-291X(02)02391-4).
22. Stolf-Moreira R., Medri M.E., Neumaier N., Lemos N.G., Brogin R.L., Marcelino F.C., De Oliveira M.C.N., Farias J.R.B., Abdelnoor R.V., Nepomuceno A.L. Cloning and quantitative expression analysis of drought-induced genes in soybean. *Genet. Mol. Res.*, 2010, 9(2): 858-867 (doi: 10.4238/vol9-2gmr701).
 23. Sui J., Jiang D., Zhang D., Song X., Wang J., Zhao M., Qiao L. The salinity responsive mechanism of a hydroxyproline-tolerant mutant of peanut based on digital gene expression profiling analysis. *PLoS ONE*, 2016, 11(9): e0162556 (doi: 10.1371/journal.pone.0162556).
 24. Nishiyama R., Le D.T., Watanabe Y., Matsui A., Tanaka M., Seki M., Yamaguchi-Shinozaki K., Shinozaki K., Tran L.S.P. Transcriptome analyses of a salt-tolerant cytokinin-deficient mutant reveal differential regulation of salt stress response by cytokinin deficiency. *PLoS ONE*, 2012, 7(2): e32124 (doi: 10.1371/journal.pone.0032124).
 25. Okuda S., Tsutsui H., Shiina K., Sprunck S., Takeuchi H., Yui R., Kasahara R.D., Hamamura Y., Mizukami A., Susaki D., Kawano N., Sakakibara T., Namoki S., Itoh K., Otsuka K., Matsuzaki M., Nozaki H., Kuroiwa T., Nakano A., Kanaoka M.M., Dresselhaus T., Sasaki N., Higashiyama T. Defensin-like polypeptide LUREs are pollen tube attractants secreted from synergid cells. *Nature*, 2009, 458(7236): 357-361 (doi: 10.1038/nature07882).
 26. Shafee T.M., Lay F.T., Hulett M.D., Anderson M.A. The defensins consist of two independent, convergent protein superfamilies. *Mol. Biol. Evol.*, 2016, 33(9): 2345-2356 (doi: 10.1093/molbev/msw106).
 27. Almeida M.S., Cabral K.M., Kurtenbach E., Almeida F.C., Valente A.P. Solution structure of *Pisum sativum* defensin 1 by high resolution NMR: plant defensins, identical backbone with different mechanisms of action. *J. Mol. Biol.*, 2002, 315(4): 749-757 (doi: 10.1006/jmbi.2001.5252).
 28. Broekaert W.F., Terras F.R., Cammue B.P., Osborn R.W. Plant defensins: novel antimicrobial peptides as components of the host defense system. *Plant Physiol.*, 1995, 108(4): 1353-1358 (doi: 10.1104/pp.108.4.1353).
 29. van der Weerden N.L., Anderson M.A. Plant defensins: common fold, multiple functions. *Fungal Biol. Rev.*, 2013, 26(4): 121-131 (doi: 10.1016/j.fbr.2012.08.004).
 30. Méndez E., Rocher A., Calero M., Girbés T., Citores L., Soriano F. Primary structure of omega-hordothionin, a member of a novel family of thionins from barley endosperm, and its inhibition of protein synthesis in eukaryotic and prokaryotic cell-free systems. *Eur. J. Biochem.*, 1996, 239(1): 67-73 (doi: 10.1111/j.1432-1033.1996.0067u.x).
 31. Gao A.-G., Hakimi S.M., Mittanck C.A., Wu Y., Woerner B.M., Stark D.M., Shah D.M., Liang J., Rommens C.M. Fungal pathogen protection in potato by expression of a plant defensin peptide. *Nat. Biotechnol.*, 2000, 18(12): 1307-1310 (doi: 10.1038/82436).
 32. Terras F.R., Eggermont K., Kovaleva V., Raikhel N.V., Osborn R.W., Kester A., Rees S.B., Torrekens S., Van Leuven F., Vanderleyden J. Small cysteine-rich antifungal proteins from radish: their role in host defense. *Plant Cell*, 1995, 7(5): 573-588 (doi: 10.1105/tpc.7.5.573).
 33. Spelbrink R.G., Dilmac N., Allen A., Smith T.J., Shah D.M., Hockerman G.H. Differential antifungal and calcium channel-blocking activity among structurally related plant defensins. *Plant Physiol.*, 2004, 135(4): 2055-2067 (doi: 10.1104/pp.104.040873).
 34. Janssen B.J., Schirra H.J., Lay F.T., Anderson M.A., Craik D.J. Structure of *Petunia hybrida* defensin 1, a novel plant defensin with five disulfide bonds. *Biochemistry*, 2003, 42(27): 8214-8222 (doi: 10.1021/bi034379o).
 35. Lobo D.S., Pereira I.B., Frágel-Madeira L., Medeiros L.N., Cabral L.M., Faria J., Bellio M., Campos R.C., Linden R., Kurtenbach E. Antifungal *Pisum sativum* defensin 1 interacts with *Neurospora crassa* cyclin F related to the cell cycle. *Biochemistry*, 2007, 46(4): 987-996 (doi: 10.1021/bi061441j).
 36. Stolf-Moreira R., Medri M.E., Neumaier N., Lemos N.G., Pimenta J.A., Tobita S., Brogin R.L., Marcelino-Guimarães F.C., Oliveira M.C.N., Farias J.R.B., Abdelnoor R.V., Nepomuceno A.L. Soybean physiology and gene expression during drought. *Genet. Mol. Res.*, 2010, 9(4): 1946-1956 (doi: 10.4238/vol9-4gmr851).
 37. Mith O., Benhamdi A., Castillo T., Berge M., MacDiarmid C.W., Steffen J., Eide D.J., Perrier V., Subileau M., Gosti F., Berthomieu P., Marques L. The antifungal plant defensin AhPDF1.1b is a beneficial factor involved in adaptive response to zinc overload when it is expressed in yeast cells. *MicrobiologyOpen*, 2015, 4(3): 409-422 (doi: 10.1002/mbo3.248).
 38. Oomen R.J., Séveno-Carpentier E., Ricodeau N., Bournaud C., Conéjéro G., Paris N., Berthomieu P., Marquès L. Plant defensin AhPDF1.1 is not secreted in leaves but it accumulates in intracellular compartments. *New Phytol.*, 2011, 192(1): 140-150 (doi: 10.1111/j.1469-8137.2011.03792.x).
 39. Shahzad Z., Ranwez V., Fizames C., Marquès L., Martret B., Alasimone J., Godé C., Lacombe E., Castillo T., Saumitou-Laprade P.,

- Berthomieu P., Gosti F. Plant Defensin type 1 (PDF1): protein promiscuity and expression variation within the *Arabidopsis* genus shed light on zinc tolerance acquisition in *Arabidopsis halleri*. *New Phytol.*, 2013, 200(3): 820-833 (doi: 10.1111/nph.12396).
40. Nguyen N.N., Ranwez V., Vile D., Soulié M.-C., Dellagi A., Expert D., Gosti F. Evolutionary tinkering of the expression of PDFIs suggests their joint effect on zinc tolerance and the response to pathogen attack. *Front. Plant Sci.*, 2014, 5: 70 (doi: 10.3389/fpls.2014.00070).
41. Ahmed N.U., Park J.-I., Jung H.-J., Seo M.-S., Kumar T.S., Lee I.-H., Nou I.-S. Identification and characterization of stress resistance related genes of *Brassica rapa*. *Biotechnol. Lett.*, 2012, 34(5): 979-987 (doi: 10.1007/s10529-012-0860-4).
42. Cabot C., Gallego B., Martos S., Barceley J., Poschenrieder C. Signal cross talk in *Arabidopsis* exposed to cadmium, silicon, and *Botrytis cinerea*. *Planta*, 2013, 237(1): 337-349 (doi: 10.1007/s00425-012-1779-7).
43. Bahramnejad B., Erickson L.R., Chuthamat A., Goodwin P.H. Differential expression of eight defensin genes of *N. benthamiana* following biotic stress, wounding, ethylene, and benzothiadiazole treatments. *Plant Cell Rep.*, 2009, 28(4): 703-717 (doi: 10.1007/s00299-009-0672-8).
44. Wilmes M., Cammue B.P., Sahl H.-G., Thevissen K. Antibiotic activities of host defense peptides: more to it than lipid bilayer perturbation. *Nat. Prod. Rep.*, 2011, 28(8): 1350-1358 (doi: 10.1039/c1np00022e).
45. Poon I.K., Baxter A.A., Lay F.T., Mills G.D., Adda C.G., Payne J.A., Phan T.K., Ryan G.F., White J.A., Veneer P.K., van der Weerden N., Anderson M.A., Kvensakul M., Hulett M.D. Phosphoinositide-mediated oligomerization of a defensin induces cell lysis. *Elife*, 2014, 3: e01808 (doi: 10.7554/eLife.01808).
46. Terras F.R., Schoofs H.M., De Bolle M.F., Van Leuven F., Rees S.B., Vanderleyden J., Cammue B.P., Broekaert W.F. Analysis of two novel classes of plant antifungal proteins from radish (*Raphanus sativus* L.) seeds. *J. Biol. Chem.*, 1992, 267(22): 15301-15309.
47. Thevissen K., Cammue B.P., Lemaire K., Winderickx J., Dickson R.C., Lester R.L., Ferket K.K., Van Even F., Parret A.H., Broekaert W.F. A gene encoding a sphingolipid biosynthesis enzyme determines the sensitivity of *Saccharomyces cerevisiae* to an antifungal plant defensin from dahlia (*Dahlia merckii*). *PNAS*, 2000, 97(17): 9531-9536 (doi: 10.1073/pnas.160077797).
48. Thevissen K., Warnecke D.C., François I.E., Leipelt M., Heinz E., Ott C., Zähringer U., Thomma B.P., Ferket K.K., Cammue B.P. Defensins from insects and plants interact with fungal glucosylceramides. *J. Biol. Chem.*, 2004, 279(6): 3900-3905 (doi: 10.1074/jbc.M311165200).
49. Thevissen K., Ferket K.K., François I.E., Cammue B.P. Interactions of antifungal plant defensins with fungal membrane components. *Peptides*, 2003, 24(11): 1705-1712 (doi: 10.1016/j.peptides.2003.09.014).
50. Thevissen K., de Mello Tavares P., Xu D., Blankenship J., Vandenbosch D., Idkowiak-Baldys J., Govaert G., Bink A., Rozentel S., de Groot P.W.J., Davis T.R., Kumamoto C.A., Vargas G., Nimrichter L., Coenye T., Mitchell A., Roemer T., Hannun Y.A., Cammue B.P.A. The plant defensin RsAFP2 induces cell wall stress, septin mislocalization and accumulation of ceramides in *Candida albicans*. *Mol. Microbiol.*, 2012, 84(1): 166-180 (doi: 10.1111/j.1365-2958.2012.08017.x).
51. Ramamoorthy V., Cahoon E.B., Li J., Thokala M., Minto R.E., Shah D.M. Glucosylceramide synthase is essential for alfalfa defensin-mediated growth inhibition but not for pathogenicity of *Fusarium graminearum*. *Mol. Microbiol.*, 2007, 66(3): 771-786 (doi: 10.1111/j.1365-2958.2007.05955.x).
52. Vriens K., Cammue B., Thevissen K. Antifungal plant defensins: mechanisms of action and production. *Molecules*, 2014, 19(8): 12280-12303 (doi: 10.3390/molecules190812280).
53. Sagaram U.S., El-Mounadi K., Buchko G.W., Berg H.R., Kaur J., Pandurangi R.S., Smith T.J., Shah D.M. Structural and functional studies of a phosphatidic acid-binding antifungal plant defensin MtDef4: identification of an RGFRRR motif governing fungal cell entry. *PLoS ONE*, 2013, 8(12): e82485 (doi: 10.1371/journal.pone.0082485).
54. Aerts A.M., François I.E., Bammens L., Cammue B., Smets B., Winderickx J., Accardo S., De Vos D.E., Thevissen K. Level of M (IP) 2C sphingolipid affects plant defensin sensitivity, oxidative stress resistance and chronological life-span in yeast. *FEBS Lett.*, 2006, 580(7): 1903-1907 (doi: 10.1016/j.febslet.2006.02.061).
55. van der Weerden N.L., Lay F.T., Anderson M.A. The plant defensin, NaD1, enters the cytoplasm of *Fusarium oxysporum* hyphae. *J. Biol. Chem.*, 2008, 283(21): 14445-14452 (doi: 10.1074/jbc.M709867200).
56. Mello E.O., Ribeiro S.F., Carvalho A.O., Santos I.S., Da Cunha M., Santa-Catarina C., Gomes V.M. Antifungal activity of PvD1 defensin involves plasma membrane permeabilization, inhibition of medium acidification, and induction of ROS in fungi cells. *Curr. Microbiol.*, 2011, 62(4): 1209-1217 (doi: 10.1007/s00284-010-9847-3).

57. Aerts A.M., Bammens L., Govaert G., Carmona-Gutierrez D., Madeo F., Cammue B., Thevissen K. The antifungal plant defensin HsAFP1 from *Heuchera sanguinea* induces apoptosis in *Candida albicans*. *Front. Microbiol.*, 2011, 2: 47 (doi: 10.3389/fmicb.2011.00047).
58. Zhukov V.A., Kulaeva O.A., Zhernakov A.I., Tikhonovich I.A. «Next generation sequencing» for studying transcriptome profiles of tissues and organs of garden pea (*Pisum sativum* L.) (review). *Agricultural Biology*, 2015, 50(3): 278-287 (doi: 10.15389/agrobiol.2015.3.278eng) (in Engl.).
59. Zhukov V.A., Zhernakov A.I., Kulaeva O.A., Ershov N.I., Borisov A.Y., Tikhonovich I.A. De novo assembly of the pea (*Pisum sativum* L.) nodule transcriptome. *Int. J. Genomics*, 2015, 2015: 695947 (doi: 10.1155/2015/695947).
60. Conesa A., Madrigal P., Tarazona S., Gomez-Cabrero D., Cervera A., McPherson A., Szczesniak M.W., Gaffney D.J., Elo L.L., Zhang X., Mortazavi A. A survey of best practices for RNA-seq data analysis. *Genome Biology*, 2016, 17: 13 (doi: 10.1186/s13059-016-0881-8).
61. Zhou P., Silverstein K.A., Gao L., Walton J.D., Nallu S., Guhlin J., Young N.D. Detecting small plant peptides using SPADA (small peptide alignment discovery application). *BMC Bioinformatics*, 2013, 14: 335 (doi: 10.1186/1471-2105-14-335).
62. Shafee T.M., Robinson A.J., Weerden N., Anderson M.A. Structural homology guided alignment of cysteine rich proteins. *SpringerPlus*, 2016, 5: 27 (doi: 10.1186/s40064-015-1609-z).
63. Alves-Carvalho S., Aubert G., Carrere S., Cruaud C., Brochot A.-L., Jacquelin F., Klein A., Martin C., Boucherot K., Kreplak J., da Silva C., Moreau S., Gamas P., Wincker P., Gouzy J., Burstin J. Full-length de novo assembly of RNA-seq data in pea (*Pisum sativum* L.) provides a gene expression atlas and gives insights into root nodulation in this species. *Plant J.*, 2015, 84(1): 1-19 (doi: 10.1111/tpj.12967).
64. He J., Benedito V.A., Wang M., Murray J.D., Zhao P.X., Tang Y., Udvardi M.K. The *Medicago truncatula* gene expression atlas web server. *BMC Bioinformatics*, 2009, 10: 441 (doi: 10.1186/1471-2105-10-441).