Biochemical and Biophysical Research Communications 444 (2014) 520–524

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Novel properties of antimicrobial peptide anoplin

Barbora Jindřichová [a,b], Lenka Burketová [a,*], Zuzana Novotná [b]

[a] Institute of Experimental Botany, Czech Academy of Science, Rozvojová 313, 165 02 Prague 6, Czech Republic
[b] Department of Biochemistry and Microbiology, Institute of Chemical Technology Prague, Technická 5, 165 21 Prague 6, Czech Republic

ARTICLE INFO

Article history:
Received 13 January 2014
Available online 25 January 2014

Keywords:
Anoplin
Antimicrobial peptide
Antifungal
Brassica napus
Leptosphaeria maculans

ABSTRACT

Antimicrobial decapeptide anoplin was tested for its antifungal activity against plant pathogen *Leptosphaeria maculans* and protection of *Brassica napus* plants from disease. To reveal the mode of action of the peptide, a natural form of anoplin amidated on C-terminus (ANP-NH_2), and its carboxylated analog (ANP-OH) were used in the study. We demonstrated strong antifungal activity of anoplin *in vitro* regardless C-terminus modification. In addition we show that both ANP-NH_2 and ANP-OH induce expression of defence genes in *B. napus* and protects plants from *L. maculans* infection. The results indicate that the amidation of anoplin is not essential for its antifungal and plant defence stimulating activities.

1. Introduction

Antimicrobial peptides (AMPs) are of interest to scientists not only as a promising alternative to antibiotics in human medicine but also as possible protective agents of plants from their diseases. Primary attention has been focused on the bactericide properties of these small molecules, but their activities against fungi, viruses and other parasites have also been confirmed. To date, more than 1700 peptides have been identified as important constituents of innate immunity found in diverse organisms including arthropods, plants and animals [1].

In addition to well-defined direct antimicrobial activity of these small peptides, there is increasing evidence that their other biological functions, e.g., plant defensins protecting germinating plants from soil-borne fungal pathogens mediate tolerance to abiotic stresses, and are also implicated in growth regulation, development and fertilisation [2]. The indirect role of several peptides as endogenous elicitors of a plant's immune system also has been described. These elicitors include both peptides derived from precursor proteins as systemins or Pep family peptides [3,4] and cryptic peptides like inceptins [5,6].

The antimicrobial activity of AMPs is complex. A frequent target is a cytoplasmic membrane but the peptides often enter into a cell that is facilitated by hydrophobic and cationic amino acid residues present in these peptides and their ability to fold into amphipathic or amphiphilic conformations, induced by their interaction with membranes [7]. The efficiency of AMPs' interaction with a membrane is determined by the cationic characteristic of the peptide and its post translational modifications, such as glycosylation, amidation, halogenation, methylation, etc. C-terminal amidation has been proposed as a mechanism by which peptide gains enhanced antibacterial activity associated with a more rigid and extended α-helical structure [8]. Slight modifications in the balance of hydrophobicity and charge can give rise to marked changes in antimicrobial selectivity/activity [9].

The preponderance of AMPs research has been devoted to their antibacterial effect since human bacterial infections have a drastic effect on human health. The mechanism of action against fungal pathogens is not well documented. In contrast to antibacterial peptides, the antifungal agents have evolved a number of different mechanisms, and single peptides often display several divergent modes of action. In spite of this, only very few peptides show both antibacterial and antifungal activity [1].

Till now our study has recognised unknown biological activities of peptide anoplin, a small peptide composed of 10 amino acid residues. On a plant pathosystem *Brassica napus–Leptosphaeria maculans*, we demonstrated the antifungal activity of amidated and non-amidated isoforms of decapeptide both *in vitro* and *in planta*, the role of C-terminal amidation in the antifungal efficiency, and anopliń s capability of inducing defence responses in plants.

2. Materials and methods

2.1. Plant and pathogen cultivation

Plants of *B. napus* cv. Columbus were grown hydroponically in perlite supplied with Steiner's nutrient solution [10] under defined

Abbreviations: AMPs, antimicrobial peptides; ANP-NH_2, amidated (natural) form of anoplin; ANP-OH, non-amidated form of anoplin; AOS, allene oxide synthase; HEL, hevein–like; JA, jasmonic acid; PR1, pathogenesis related 1; SA, salicylic acid.

* Corresponding author.
E-mail address: burketova@ueb.cas.cz (L. Burketová).

http://dx.doi.org/10.1016/j.bbrc.2014.01.097
0006-291X/

conditions (day/night: 16/8 h, 24/20 °C, photon flux density 150 μmol/m^2/s). Cotyledons of 11-day-old plants were used for anoplin treatment in an inoculation test; 14-day-old cotyledons were treated with anoplin for RNA isolation.

The fungus *L. maculans* (anamorph *Phoma lingam*), isolate JN2 [11] was cultured on V8 juice agar at 24 °C in dark. Sporulation cultures and conidia suspension were prepared according to Šašek et al. [12].

2.2. Anoplin treatment

Decapeptide amide, GLLKRIKTLLNH$_2$ and a non-amide form of decapeptide GLLKRIKTLL were used in this study. The C-terminus amidated form of decapeptide (ANP-NH$_2$) was synthesised by GeneCust (Dudelange, Luxembourg). A non-amide form of decapeptide (ANP-OH) was prepared by ChinaPeptides (Shanghai, China). For the antimicrobial test, anoplin (amide and non-amide form) was dissolved in 10 mM MES (pH 6.8); for plant treatment, anoplin (a form of amide and non-amide) was dissolved in distilled water and the solution was infiltrated by syringe without needle into cotyledons. In experiments 11- or 14-day-old plants were used for inoculation test or gene expression study, respectively.

2.3. Antifungal assay

Antifungal activity was measured using the spores of *L. maculans* isolate JN2 with a constitutive expression of GFP (JN2::GFP) prepared by Šašek et al. [13]. Spores of *L. maculans* JN2::GFP were suspended to the final concentration 5×10^4 spore/ml in a Gamborg B5 medium (Duchefa Biochemie BV, Haarlem Netherlands) supplemented with 0.3% saccharose and 10 mM MES (pH 6.8). 50 μl of the spore suspension (approximately 2500 spores) was pipetted into each well of a black 96-well plate. Peptides were dissolved in 10 mM MES (pH 6.8) and 50 μl of the solution were added in each well with spore suspension; 4 wells were used for each treatment. Growth control was performed in 50 μl 10 mM MES (pH 6.8) and 50 μl spore suspension. Final concentrations of anoplin ranged from 0.1 to 100 μg/ml. As a control treatment, tebuconazole (30 μM) was used instead of anoplin in the form of a commercial fungicide Horizon 250 EW (Bayer CropScience AG, Monheim, Germany). All the steps were performed under sterile conditions. Plates were covered with a lid and sealed with Micropore tape. Plates were incubated in darkness at 26 °C. Fluorescence was measured by a Tecan F200 fluorescence reader (Tecan Schweiz AG, Männedorf, Switzerland) with an excitation filter 485/20 nm and an emission filter 535/25 nm every 24 h for 4 days. A minimal inhibitory concentration (MIC) was determined as a minimum concentration of anoplin which caused 100% growth inhibition of *L. maculans*. All MIC determinations were performed in four parallels and are the average of three independent determinations.

2.4. Inoculation test

A peptide solution was infiltrated into cotyledons of 11-day-old plants using a syringe without needle at concentrations 25 or 50 μg/ml; control cotyledons were treated with distilled water. After 2 days the plants (14-day-old) were both equally inoculated by a conidia suspension of *L. maculans* JN2 (10^5 conidia/ml) until completely saturated.

Developed necroses of *L. maculans* on cotyledons were evaluated by means of image analysis APS Assess 2.0 software 10–12 days after inoculation. The relative area of the lesions was related to water-treated plants when the value for control plants was set at 100% with 24 cotyledons of each treatment used for analysis.

2.5. Gene expression analysis

Plant tissue discs were cut from 12 cotyledons and immediately frozen in liquid nitrogen. The tissue was homogenized in tubes with 1 g of 1.3 mm silica beads and the total RNA was isolated using a Spectrum Plant Total RNA kit (Sigma–Aldrich corp., St. Louis, MO, USA) and treated with a DNA*free* Kit (Ambion Inc., Austin, TX, USA). Subsequently, 1 μg of RNA was converted to cDNA with a M-MLV RNase H–Point Mutant reverse transcriptase (Promega Corporation, Prague, Czech Republic) and oligo dT$_{21}$ primer (Metabion International AG, Martinsried, Germany). An equivalent of 6.25 ng RNA was loaded into a 10 μl reaction with the Eva-Line (E1LC, GeneON, Ludwigshafen am Rhein, Germany). All reactions were carried out in polycarbonate capillaries (Genaxxon bioscience GmbH, Ulm, Germany) and Light-Cycler 1.5 (Roche Diagnostics World AG, Risch, Switzerland). A PCR condition was used at 95 °C for 10 min, followed by 45 cycles at 95 °C for 10 s, 55 °C for 10 s and 72 °C for 10 s, followed by melting curve analysis. Threshold cycles and melting curves were calculated using LightCycler Software 4.1 (Roche). The relative expression was calculated with efficiency correction and normalisation to Actin. Primers were designed using PerlPrimer v1.1.17 [14]. *B. napus* primers were used: *Act*, AF111812, FP: 5′-CTG GAA TTG CTG ACC GTA TGA G-3′, RP: 5′-TGT TGG AAA GTG CTG AGG GA-3′; *PR1*, BNU21849, FP: 5′-CAT CCC TCG AAA GCT CAA GAC-3′, RP: 5′-CCA CTG CAC GGG ACC TAC-3′; *AOS*, EV124323, FP: 5′-CGC CAC CAA AAC AAC AAA G-3′, RP: 5′-GGG AGG AAG GAG AGA GGT TG-3′; *HEL*, FG577475, FP: 5′-GGA ACA CAA GGA CTA ATG C-3′, RP: 5′-TTT CGA TAG CCA TCA CCA-3′.

2.6. Statistical analysis

At least three independent biological experiments were performed in each test. All statistical analyses were completed using *T*-test by Microsoft Excel 2010. Differences were considered to be significant at $P < 0.05$.

3. Results

3.1. Direct antifungal activity of an amidated and non-amidated forms of anoplin

Anoplin is a decapeptide with an antimicrobial activity against gram-negative and gram-positive bacteria. It was demonstrated

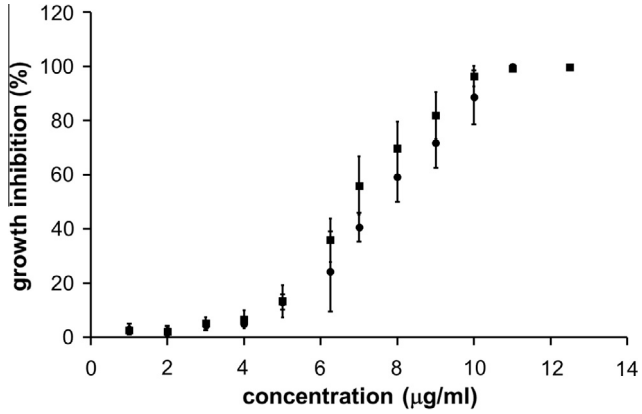

Fig. 1. Antifungal activity of ANP-NH$_2$ and ANP-OH determined by microplate assay. Spores of *L. maculans* tagged with GFP were cultivated with different concentrations of a peptide for 96 h in a microtitre plate. The growth of mycelium was quantified as an increase in GFP fluorescence. The data represent mean values of three independent experiments. Error bars represent mean ± SE values from three independent experiments; ■ treatment with ANP-NH$_2$; ● treatment with ANP-OH.

earlier that the deamidation resulted in a drastic reduction of antimicrobial activity when the MIC increased above 100 μg/ml [15]. A non-amidated form of anoplin (ANP-OH) has no hemolytic and mast cell degranulation activities. At this point, we tested the antifungal activity of amidated and non-amidated forms of anoplin against plant fungal pathogen *L. maculans* (Fig. 1). In experiments, the *L. maculans* isolate JN2 tagged GFP was used [13].

The MIC was determined using serially diluted anoplin forms. The concentrations needed to inhibit the 100% growth (MIC) of *L. maculans* started from 10 or 11 μg/ml of ANP-NH2 and ANP-OH, respectively (Fig. 1). A 50% growth inhibition was detected in anoplin concentration 7 or 8 μg/ml of ANP-NH_2 and ANP-OH, respectively. Practically no activity was seen at concentrations below 4 μg/ml in both peptides. The difference in antifungal activity between ANP-OH and ANP-NH_2 was not statistically significant. Thus these findings show that C-terminal amidation of anoplin does not play a critical role in its fungicide activity.

3.2. Amidated and non-amidated forms of anoplin protect B. napus plants from infection by L. maculans

To evaluate the impact of anoplin in natural surroundings *in planta*, we treated *B. napus* cotyledons with both forms of anoplin 72 h before inoculation with *L. maculans*. Anoplin treatment causes suppression of pathogen development in plants. Progress of lesions caused by *L. maculans* was documented (Fig. 2) 12 days after inoculation and evaluated using an image analysis. Both peptides decreased lesion development, however, the protecting activity of ANP-NH_2 started at concentration of 25 μg/ml, and a significant decrease in lesion extent was found only at 50 μg/ml of ANP-OH, that were much higher than in antifungal activity *in vitro*. ANP-NH_2 and ANP-OH at a concentration 25 μg/ml reduced the area of lesion by about 25% or 11% and at a concentration 50 μg/ml by about 37% or 33%, respectively. Both peptides in concentrations 12.5 μg/ml did not influence lesion development compared to water-treated control plants (data not shown).

3.3. Activation of plant defence reaction in B. napus

To clarify the mechanism by which both forms of anoplin protect *B. napus* plants from infection by *L. maculans*, we ascertained whether the treatment of plants with the peptides activates defence responses in tissues. Salicylic acid, jasmonic acid and ethylene are typical signalling molecules involved in plant-pathogen interactions. In our previous work [12], we determined marker genes of these main signalling pathways in *B. napus*. The following marker genes were chosen in present study: *pathogenesis related 1* (*PR1*) gene for the SA pathway, *allene oxide synthase* (*AOS*) gene for JA pathway and *hevein-like* (*HEL*) gene for ethylene pathway. Relative gene expression was measured 24 h after the anoplin treatment. Fig. 3A shows that both peptides (amidated and non-amidated) induced a slight expression of the *PR1* gene in *B. napus* cotyledons 24 h after treatment. The relative gene expression was 6–8 times higher than the expression in control plants and no significant difference between both peptides concentrations was found. *HEL* gene expression was significantly increased in anoplin treated *B. napus* cotyledons (Fig. 3C). Treatment of the cotyledons with amidated and non-amidated peptide at concentration of

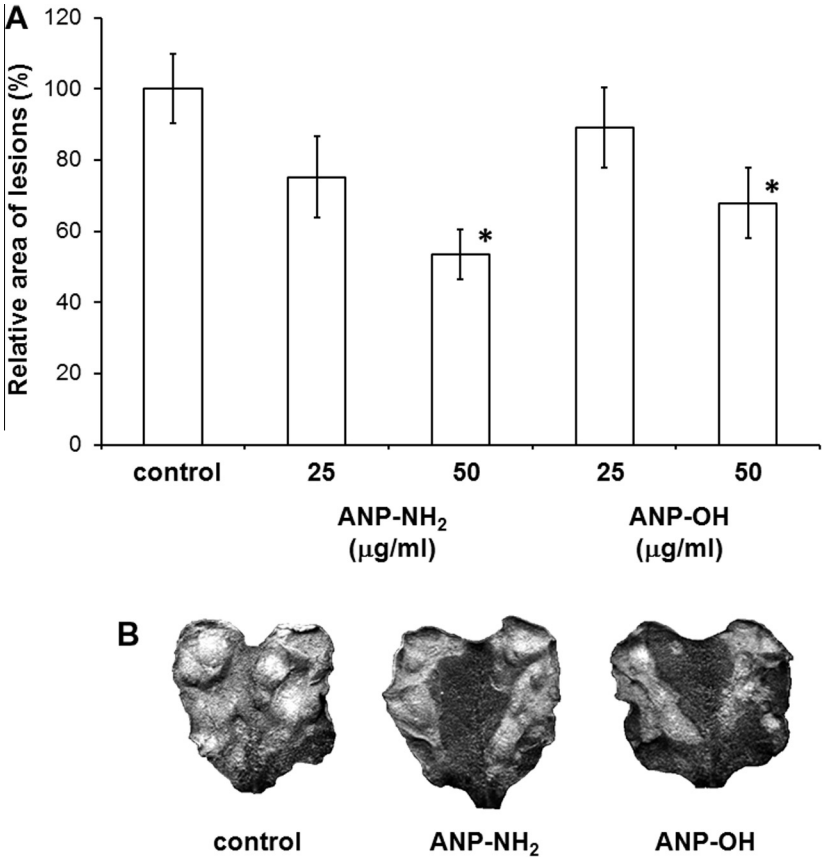

Fig. 2. Effect of ANP-NH_2 and ANP-OH on the development of *Leptosphaeria maculans* infection symptoms. *Brassica napus* cotyledons (12-day old) were infiltrated with different concentrations (25 and 50 μg/ml) of the tested peptides 2 days before *L. maculans* inoculation. Disease symptoms were evaluated 11 days after inoculation. (A) Quantification of disease symptoms was done by image analysis. Lesion area was quantified using APS Assess 2.0 software. Bars represent mean ± SE values from three independent experiments. (B) Typical leaves showing the dose-dependent protecting effect of ANP-NH_2 and ANP-OH (50 μg/ml).

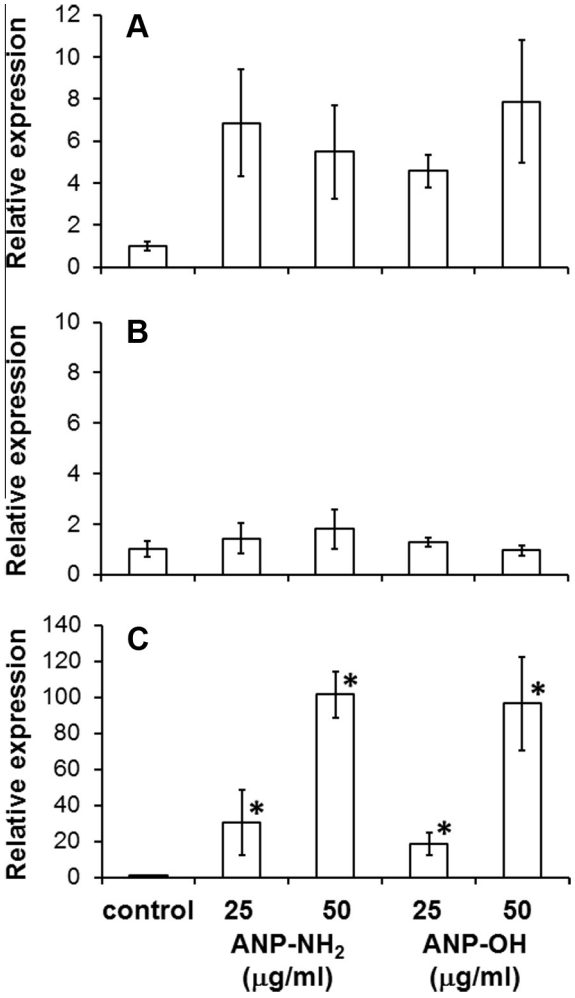

Fig. 3. Effect of ANP-NH_2 and ANP-OH on activation of plant defence pathways in *Brassica napus* cotyledons. Expression of salicylic acid, jasmonic acid and ethylene pathways marker genes in *Brassica napus* cotyledons (14-days old) infiltrated with ANP-NH_2 and ANP-OH was detected 24 h after treatment by compounds. Control plants were infiltrated with distilled water. (A) Gene expression of *PR1* (*pathogenesis related 1*), (B) *AOS* (*allene oxide synthase*), and (C) *HEL* (*hevein-like*). Bars represent mean ± SE values from three independent experiments.

25 μg/ml caused the expression to be 30 or 18 times higher and at concentration 50 μg/ml 100 or 95 times higher, respectively, comparing to mock-treated control. Expression of the *AOS* gene was not elevated (Fig. 3B).

4. Discussion

Anoplin, a cationic decapeptide amide with 4 lysine residues, (GLLKRIKTLL-NH_2), has been described as an antimicrobial peptide from the venom of a solitary wasp *Anoplius samariensis* [16]. With its 10 amino acid residues, it is one of the shortest AMPs ever discovered. It is active against Gram-positive and Gram-negative bacteria and is not hemolytic towards human erythrocytes. Anoplin forms a helical structure when interacting with membranes but is probably not able to form transmembrane helices [17]. An extremely small size of this peptide enables to synthesize anoplin derivatives and test their biological activity. Studies on analogues containing substitutions in amino acid positions have revealed that increasing the charge and/or hydrophobicity improved antimicrobial activity [17]. On the other hand, the deamidation of the C-terminus of anoplin caused the loss of its biological activity because the deamidation introduced a negative charge to a positively charged peptide, causing a loss of amphipathicity. This slight modification in a peptide's primary structure disturbed the interaction with bilayers and biological membranes [15].

Antifungal activity of anoplin has not yet been reported. We focused on a phytopathogenic filamentous fungus *L. maculans* from the class Dothideomycetes, which is a serious pathogen of *Brassica* crops, mainly oilseed rape. In our study, we found very strong fungicide activity (MIC about 10 μg/ml) of natural (amidated) anoplin *in vitro*. The anoplin halted the growth of the fungus in early stage development as well as *in vivo*. When infiltrated into plant cotyledons, anoplin reduced the spread of *L. maculans* in tissues. To better understand the mode of action of this peptide, we focused on C-terminal amidation, since it has been attributed to both antibacterial and antifungal activities due to efficient permeabilisation of cell membranes [15,18]. In contrary to bacteria, the deamidation of anoplin did not significantly change antifungal activity against *L. maculans* in our experiments. This result indicates that C-terminal amidation of anoplin is dispensable for its antifungal activity. Similar results were reported regarding temporins, which increased resistance to plant pathogens *Phytophthora infestans* and *Rhizoctonia solani* in a transgenic potato. These short (10–14 amino acids) AMPs which, in addition to several frog species, were also isolated from wasp venom, and also resemble anoplin by an α-helical structure and amidation on their C-termini [19]. However, their antimicrobobial activity could hardly be explained by cell wall permeation itself since the permeabilisation activity of temporins is weak only because they are not as basic as are other cationic peptides. Data dealing with antifungal activities of AMPs are still scarce and indicate that permeabilisation alone may not be sufficient for fungus cell death [20]. Thus, the peptides are more likely to exert activity through intracellular targets resulting in ROS accumulation, interference with MAPK signalling, activation of the cell wall integrity pathway, etc. [1,18,21].

Considering the possible utilisation of AMPs for plant protection, their impact on plants should also be taken into account for their possible phytotoxicity or, in contrary, their stimulating activity. The latter effect was demonstrated on cyclic lipopeptides such as surfactins, fengycins and massetolide A by the plant biocontrol bacteria [22,23], and on short cationic synthetic lipopeptides as well [24]. These lipopeptides induced a systemic defence response in treated plants and resistance to infection with pathogens. Stimulation of plants by molecules derived from biocontrol agents is not so surprising since these peptides activate the plant's immune system and induce systemic resistance (ISR) [25] normally under natural conditions. Here we demonstrated that anoplin, the peptide without any clear relation to plant immunity, activated expression of defence genes in *B. napus*, namely *PR1* and *HEL*, regulated by salicylic acid and ethylene signalling pathways, respectively. As we have shown earlier [12], both pathways are also involved in the resistance of *B. napus* to *L. maculans*. Hence we speculate that anoplin, in addition to antifungal activity, may act also as a resistance inducer to *L. maculans* in *B. napus*. However, in contrast to lipopeptides, finding an explanation for anoplin elicitation activity is not easy with respect to its origin in wasp venom. One possibility is that it is recognised by a plant as a foreign molecule similarly to the "pathogen- or microbe-associated molecular patters" (PAMPs or MAMPs) [26], which are present on the pathogen's surface. Numerous MAMPs have been characterised, e.g., flagellin protein forming bacterial flagellum, bacterial extracellular lipopolysaccharides, peptidoglycans, β-glucans, chitin, etc. An intriguing similarity could be found in peptide endogenous elicitors of plant immune responses, which are basically of the plant's origin [27].

All in all, we have shown that antimicrobial peptide anoplin (its C-terminus amidated form and carboxylated C-terminus form) can directly inhibit growth of a filamentous fungus or induce a defence

response in *B. napus*. These findings support a recently proposed impact of AMPs as protecting agents in plants from pathogens and indicate their possible future utilization in agriculture.

Acknowledgment

This research was supported by a Grant from the Czech Science Foundation (GAČR), No. 522/09/1693.

References

[1] N.L. van der Weerden, M.R. Bleackley, A. Marilyn, M.A. Anderson, Properties and mechanisms of action of naturally occurring antifungal peptides, Cell. Mol. Life Sci. 70 (2013) 3545–3570.
[2] A.D. Carvalho, V.M. Gomes, Plant defensins – prospects for the biological functions and biotechnological properties, Peptides 30 (2009) 1007–1020.
[3] G. Pearce, D. Strydom, S. Johnson, C.A. Ryan, A polypeptide from tomato leaves induces wound-inducible proteinase-inhibitor proteins, Science 253 (1991) 895–898.
[4] A. Huffaker, G. Pearce, C.A. Ryan, An endogenous peptide signal in Arabidopsis activates components of the innate immune response, Proc. Natl. Acad. Sci. USA 103 (2006) 10098–10103.
[5] E.A. Schmelz, M.J. Carroll, S. LeClere, S.M. Phipps, J. Meredith, P.S. Chourey, H.T. Alborn, P.E.A. Teal, Fragments of ATP synthase mediate plant perception of insect attack, Proc. Natl. Acad. Sci. USA 103 (2006) 8894–8899.
[6] E.A. Schmelz, S. LeClere, M.J. Carroll, H.T. Alborn, P.E.A. Teal, Cowpea chloroplastic ATP synthase is the source of multiple plant defense elicitors during insect herbivory, Plant Physiol. 144 (2007) 793–805.
[7] J.P. Powers, R.E. Hancock, The relationship between peptide structure and antibacterial activity, Peptides 24 (2003) 1681–1691.
[8] J.Y. Kim, S.C. Park, M.Y. Yoon, K.S. Hahm, Y. Park, C-terminal amidation of PMAP-23: translocation to the inner membrane of Gram-negative bacteria, Amino Acids 40 (2011) 183–195.
[9] G. Laverty, S.P. Gorman, B.F. Gilmore, The potential of antimicrobial peptides as biocides, Int. J. Mol. Sci. 12 (2011) 6566–6596.
[10] A.A. Steiner, The universal nutrient solution in Proceedings of the Sixth International Congress on "Soilless Culture", Pudoc, Wageningen, (1984) 633–650.
[11] M.H. Balesdent, A. Attard, D. Ansan-Melayah, R. Delourme, M. Renard, T. Rouxel, Genetic control and host range of avirulence toward *Brassica napus* cultivars Quinta and Jet Neuf in *Leptosphaeria maculans*, Phytopathology 91 (2001) 70–76.
[12] V. Sasek, M. Novakova, B. Jindrichova, K. Boka, O. Valentova, L. Burketova, Recognition of avirulence gene *AvrLm1* from hemibiotrophic ascomycete *Leptosphaeria maculans* triggers salicylic acid and ethylene signaling in *Brassica napus*, Mol. Plant-Microbe Interact. 25 (2012) 1238–1250.
[13] V. Sasek, M. Novakova, P.I. Dobrev, O. Valentova, L. Burketova, Beta-aminobutyric acid protects *Brassica napus* plants from infection by *Leptosphaeria maculans* Resistance induction or a direct antifungal effect?, Eur J. Plant Pathol. 133 (2012) 279–289.
[14] O.J. Marshall, PerlPrimer: cross-platform, graphical primer design for standard, bisulphite and real-time PCR, Bioinformatics 20 (2004) 2471–2472.
[15] M.P.D.S. Cabrera, M. Arcisio-Mirandaa, S.T.B. Costa, K. Konno, J.R. Ruggiero, J. Procopio, J.R. Neto, Study of the mechanism of action of anoplin, a helical antimicrobial decapeptide with ion channel-like activity, and the role of the amidated C-terminus, J. Pept. Sci. 14 (2008) 661–669.
[16] K. Konno, M. Hisada, R. Fontana, C.C.B. Lorenzi, H. Naoki, Y. Itagaki, A. Miwa, N. Kawai, Y. Nakata, T. Yasuhara, J.R. Neto, W.F. de Azevedo, M.S. Palma, T. Nakajima, Anoplin, a novel antimicrobial peptide from the venom of the solitary wasp *Anoplius samariensis*, Biochim. Biophys. Acta-Protein Struct. Mol. Enzymol. 1550 (2001) 70–80.
[17] A. Won, S. Pripotnev, A. Ruscito, A. Ianoul, Effect of point mutations on the secondary structure and membrane interaction of antimicrobial peptide anoplin, J. Phys. Chem. B 115 (2011) 2371–2379.
[18] N. Hegedus, F. Marx, Antifungial proteins: more than antimicrobials?, Fungal Biol Rev. 26 (2013) 132–145.
[19] L.A. Rollins-Smith, C. Carey, J.M. Conlon, L.K. Reinert, J.K. Doersam, T. Bergman, J. Silberring, H. Lankinen, D. Wade, Activities of temporin family peptides against the chytrid fungus (*Batrachochytrium dendro batidis*) associated with global amphibian declines, Antimicrob. Agents Chemother. 47 (2003) 1157–1160.
[20] M.L. Mangoni, Temporins, anti-infective peptides with expanding properties, Cell. Mol. Life Sci. 63 (2006) 1060–1069.
[21] P. Larranaga, P. Díaz-Dellavalle, A. Cabrera, D. Alem, C. Leoni, A.L. Almeida-Souza, S. Giovanni-De-Simone, M. Dalla-Rizza, Activity of naturally derived antimicrobial peptides against filamentous fungi relevant for agriculture, Sus. Agric. Res. 1 (2012) 211–221.
[22] M. Ongena, P. Jacques, *Bacillus* lipopeptides: versatile weapons for plant disease biocontrol, Trends Microbiol. 16 (2008) 115–125.
[23] H. Tran, A. Ficke, T. Asiimawe, M. Hofte, J.M. Raaijmakers, Role of the cyclic lipopeptide massetolide A in biological control of *Phytophthora infestans* and in colonization of tomato plants by *Pseudomonas fluorescens*, New Phytol. 175 (2007) 731–742.
[24] Y. Brotman, A. Makovitzki, Y. Shai, I. Chet, A. Viterbo, Synthetic ultrashort cationic lipopeptides induce systemic plant defense responses against bacterial and fungal pathogens, Appl. Environ. Microbiol. 75 (2009) 5373–5379.
[25] C.M.J. Pieterse, L.C. van Loon, Signalling cascades involved in induced resistance, in: D. Walters, A. Newton, G. Lyon (Eds.), Induced Resistance for Plant Defence: A Sustainable Approach to Crop Protection, Blackwell, 2007, pp. 68–88.
[26] J.D. Jones, J.L. Dangl, The plant immune system, Nature 444 (2006) 323–329.
[27] Y. Yamaguchi, A. Huffaker, Endogenous peptide elicitors in higher plants, Curr. Opin. Plant Biol. 14 (2011) 351–357.