Biol. Stud. 2023; 17(4): 3–14 • DOI: https://doi.org/10.30970/sbi.1704.748
www.http://publications.lnu.edu.ua/journals/index.php/biology

UDC: 577:632.7

SCOTS PINE DEFENSINS INHIBIT *IPS ACUMINATUS* α-AMYLASE ACTIVITY

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Yusypovych, Y., Kit, O., Kramarets, V., Shalovylo, Y., Korol, M., Zaika, V., Krynytskyy, H., & Kovaleva, V. (2023). Scots pine defensins inhibit *Ips acuminatus* α-amylase activity. *Studia Biologica*, 17(4), 3–14. doi:10.30970/sbi.1704.748

Background. Pine bark beetle *Ips acuminatus* (Gyllenhal, 1827) is one of the most harmful pests of pine trees as it affects the phloem of the upper part of the stem and branches, disrupting the flow of nutrients and water to the crown. *I. acuminatus* feeds by plant tissues rich in starch, so α-amylases must play a pivotal role in the carbohydrate metabolism of these insects. However, in conifer bark beetles, α-amylases remain poorly understood.

Materials and Methods. To detect the α-amylase activity in the digestive system of *I. acuminatus*, we obtained extracts from larvae, pupae, and adults that were collected from naturally infested Scots pine. The α-amylase activity of crude extracts from different stages and parts of the bark beetle's body was assessed using 1% starch agar plates. The quantitative evaluation of the α-amylase inhibitory activity of recombinant defensins *Ps*Def1, *Ps*Def2, and *Ps*Def5.1 was performed using the Bernfeld method. The docking models of Scots pine defensins and *Ips typographus* L. α-amylase (Amy*Ip*) complexes were predicted using the ClusPro 2.0 web server.

Results and Discussion. As a result, we found the presence of α-amylase activity in the digestive systems of both larvae and adults of *I. acuminatus*, but not in pupae. All tested defensins, *Ps*Def1, *Ps*Def2, and *Ps*Def5.1, exhibited inhibitory activity against insect α-amylase at micromolar concentrations. The IC_{50} values for these peptides were 4.9±0.6 μM, 4.6±0.8 μM, and 2.8±0.5 μM, respectively. In the *Ps*Defs-Amy*It* complexes, a network of hydrogen bonds, ionic bridges, and nonbonded contacts are formed between the enzyme and its inhibitor, which prevents the substrate from reaching the

catalytic site. The *Ps*Def5.1-Amy*It* complex has the largest interfacial contact area, 2328 Å^2, in comparison with two other defensins, which correlates well with the inhibitory activity of defensins in this study.

Conclusion. Thus, we have identified α-amylase activity in *I. acuminatus* and demonstrated the ability of Scots pine defensins to inhibit it, suggesting that they play a role in pine defenses against this pest.

Keywords: *Pinus sylvestris* L., pine bark beetle, plant defensin, α-amylase, molecular docking

INTRODUCTION

The pine bark beetle, *Ips acuminatus* (Gyllenhal) (Coleoptera: Curculionidae, Scolytinae), also known as the sharp-dentated engraver beetle, has recently been ranked among the ten most damaging wood boring insects in Europe (Colombari *et al.*, 2013). The adult beetles of *I. acuminatus* attack the upper part of the stem and branches of pine trees. They bore into the bark and chew galleries within the phloem, disrupting the flow of nutrients and water to the crown. This ultimately leads to the death of the tree (Meshkova *et al.*, 2022). Like most bark beetles, *I. acuminatus* primarily feeds on the phloem of pine trees. This tissue is rich in cellulose, hemicellulose, lignin, starch, and other carbohydrates that serve as vital nutrients (Noronha *et al.*, 2018). Because of this diet, α-amylases must play a pivotal role in the carbohydrate metabolism of these insects.

α-Amylases (EC 3.2.1.1) are enzymes that hydrolyze α-1,4 glycosidic bonds within α-linked polysaccharides, such as starch and glycogen, yielding maltose, maltotriose, and residual branched maltodextrins. While these enzymes have been extensively studied in many insects, including their structure, biochemical properties, phylogeny, localization, expression profiles, and regulatory mechanisms (Da Lage, 2018), α-amylases in conifer bark beetles remain poorly understood. I. Viktorinova *et al.* (2011) demonstrated the presence of two closely related α-amylase genes (*Amy*) in *Ips typographus*, and another bark beetle α-amylase was identified in *Dendroctonus rhizophagus* (Soto-Robles *et al.*, 2020).

Plants exhibit some degree of resistance to insect pests, which is reflected in the ability of a number of insects to feed on a given plant. Evolutionarily, plants have developed several potent constitutive and inducible defenses against insect pests, including compounds that inhibit the activity of digestive enzymes, particularly α-amylases. Two classes of amylase inhibitors (α-AIs) have been identified in plants so far: proteinaceous and non-proteinaceous inhibitors (Li *et al.*, 2021). Seven types of natural proteinaceous α-amylase inhibitors have been identified, among which are the γ-thionin-like type inhibitors (or defensins). The α-amylase inhibitory activity has been described for plant defensins *Vr*D1 (Liu *et al.*, 2006), *Vu*D1 (Pelegrini *et al.*, 2008), SIa1, SIa2, and SIa3 (Bloch & Richardson,1991), 1-H thionin (Mendez *et al.* 1990), *Tv*D1 (Vijayan *et al.*, 2012), and *Zm*DEF1 from maize (Vi *et al.*, 2017). Structural analysis suggests that the inhibition of the α-amylase activity occurs due to the insertion of mobile sections of the defensin molecule (loops) into the catalytic active center of the enzyme, thereby blocking the formation of the substrate-enzyme interactions (Franco *et al.*, 2002).

Recently, we discovered that Scots pine defensins *Ps*Def1 and *Ps*Def2 can also inhibit the α-amylase activity of certain insect pests that impact important crops, utilizing

the same mechanism (Kovaleva *et al.*, 2020; Bukhteeva *et al.*, 2022). We hypothesized that defensins might also inhibit the bark beetle α-amylase activity and tested this hypothesis in the current study.

Therefore, the goals of this study were to identify the amylolytic activity in the pine bark beetle, characterize the inhibitory potential of pine defensins in relation to this activity, and elucidate the mechanism of inhibition of bark beetle α-amylase by defensins at the molecular level.

MATERIALS AND METHODS

Biological material. Naturally infested Scots pine (*Pinus sylvestris* L.) was collected from the 'Rava-Rus'ka Forestry' locality (N 50.234934°, E 23.660452°), Lviv Region, Ukraine, and transported to the laboratory to obtain larvae, pupae, and adults of *Ips acuminatus*. The head and abdominal segments of the larvae, as well as the head-pronotum and abdomen of the beetles, were separately placed into Eppendorf vials containing 20 mM Tris-HCL buffer, pH 7.0, with 1 mM $CaCl_2$, 50 mM NaCl, and 1 mM phenylmethanesulfonic acid. Samples were completely crushed using a pestle on ice and then centrifuged at 14,000 g for 15 min at 4 °C. Filter-sterilized supernatants (crude extracts) were used as a source of their amylase activity. Three biological replicates of each stage were processed.

Recombinant Scots pine defensins. Recombinant proteins *Ps*Def1, *Ps*Def2, and *Ps*Def5.1 were obtained as detailed in our previous reports (Kovaleva *et al.*, 2011; Hrunyk *et al.*, 2019; Shalovylo *et al.*, 2021).

Enzyme Assay. The α-amylase activity of *I. acuminatus* was assessed using 1 % starch agar plates. A hole was created on the starch agar plates using a Pasteur pipette, and then 10 µL of α-amylase crude extract was added. The plates were incubated at 30 °C for 20 minutes and subsequently stained using an iodine reagent solution (0.02 % I_2 and 0.2 % KI in 0.05 N HCl) to visualize the enzyme activity around the holes.

The inhibitory effect of defensins *Ps*Def1, *Ps*Def2, and *Ps*Def5.1 against beetle bark α-amylases was determined on 1 % starch agar plates. Recombinant peptides and amylase crude extracts were pre-incubated at 30 °C for 20 min before being added to the holes. The enzyme activity around the holes was documented as previously described.

To quantitatively assess the α-amylase inhibitory activity of recombinant Scots pine defensins, the Bernfeld method (Bernfeld, 1955) was employed. Different concentrations of Scots pine defensins (1.5; 3.0; 6.0 and 9.0 µM were pre-incubated with crude extract in 200 µL buffer (20 mM Tris-HCl, pH 7.0, 50 mM NaCl, and 1 mM $CaCl_2$) for 20 min at 30 °C. The enzymatic reaction was initiated by adding 250 µL of 0.5 % (w/v) starch (Sigma-Aldrich). After a 20-minute incubation at 30 °C, the solution was mixed with 300 µL of 1 % 3,5-dinitrosalicylic acid in 0.4 M NaOH, followed by heating at 100 °C for 5 min and subsequent cooling on ice. The enzyme activity was measured by monitoring the increase in absorbance at 530 nm. One α-amylase unit was defined as the quantity of enzyme that caused a 0.1 OD increase during a 20-minute assay. The α-amylase inhibitor activity was expressed as a percentage of inhibition, calculated by comparison with the control experiment. All experiments were performed in triplicate, with distilled water used as a negative control. The concentrations of the compound resulting in 50 % inhibition of the enzyme activity (IC_{50}) were determined graphically.

Molecular docking. The 3D structure of the α-amylase from *Ips typographus* (UniProt entry E7DYB1) was retrieved from the AlphaFold DB (https://alphafold.ebi.ac.uk/entry/E7DYB1). For docking, we utilized the solution NMR structures of *Ps*Def1 (PDB code: 5NCE), *Ps*Def2 (PDB code: 7LNS), and the structure of *Ps*Def5.1, which was modeled in a previous study (Shalovylo *et al.*, 2021). The 3D structures of Scots pine defensins were docked onto the α-amylase structure using the ClusPro 2.0 web server (Kozakov *et al.*, 2017), which employs rigid body docking, root-mean-square deviation-based clustering, and energy minimization of the final structures. The interactions between the defensins and the α-amylase were visualized using PyMol software, version 2.5.5. Intermolecular contacts were analyzed using the PDBsum server (Laskowski *et al.*, 2018), with a cutoff distance of 5 Å.

Statistical Analysis. Inhibition curves were generated by conducting a regression analysis on the average absorption values obtained from three replicates using Microsoft Excel 2016. The IC_{50} was subsequently calculated from these curves. The IC_{50} values are presented as the mean ± standard error derived from three independent experiments. Results were deemed statistically significant for P values less than or equal to 0.05.

RESULTS AND DISCUSSION

α-Amylase activity of *I. acuminatus* crude extracts. The pine bark beetle is one of the most threatening pests of pine trees causing mechanical damage to the phloem, which disrupts the flow of nutrients and also serves as a vector for transmitting spores of ophiostomatoid and other fungi that are responsible for wood discoloration, serious tree diseases and high rates of tree mortality (Davydenko *et al.*, 2017). Some tree-killing bark beetles are associated with symbiotic fungi and bacteria that may mobilize nutrients from the sapwood to the bark, where they can be utilized by beetles and developing larvae (Bleiker & Six, 2007). Therefore, the question of the ability of bark beetles to hydrolyze complex polysaccharides of the plant cell has remained controversial for a long time. Only a few α-amylases have been characterized in coniferous bark beetles, such as AmyA and AmyB from *I. typographus* (Viktorinova *et al.*, 2011), and AmyDr of D. *rhizophagus* (Soto-Robles *et al.*, 2020).

To identify the α-amylase activity in the digestive system of the pine bark beetle at different stages of its ontogenesis, we prepared crude extracts from pupae and the head and abdominal segments of the larvae, as well as the head-pronotum and abdomen of the adults of *I. acuminatus*. As shown in **Fig. 1**, the α-amylase activity was observed in larvae and adult pine bark beetles, but not in pupae, indicating that the expression of the *Amy* gene could be connected with feeding. A similar expression profile of amylase genes has been reported for other bark beetles (Soto-Robles *et al.*, 2020). Notably, α-amylase activity was observed in both parts of the body, for both larvae and adults, suggesting that starch hydrolysis begins in the oral cavity of *I. acuminatus.* Moreover, α-amylase activity in the abdomen segments of both stages of the pine bark beetle was higher than in its head-pronotum region. It is proposed that these differences in the activity may be due to several reasons. In particular, the lack of an optimal pH for the activity of α-amylases, the high sclerotization of the anterior part of the bark beetle digestive system (Díaz *et al.*, 2003), or the presence of inhibitors which are synthesized in the response to mechanical injury caused by insects as part of the tree defense system (Krokene, 2015).

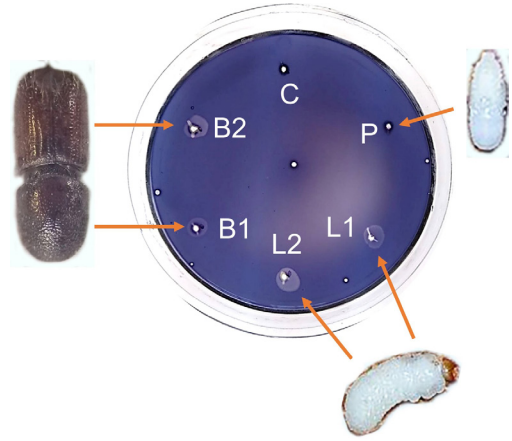

Fig. 1. The α-amylase activity of *I. acuminatus* crude extracts from pupae (P); head (L1) and abdominal segments (L2) of the larvae; head-pronotum (B1) and abdomen (B2) of adult beetles. Ten microliters of α-amylase crude extract was added to the hole at 1 % starch agar. After incubation for 20 minutes at 30 °C, the agar was treated with an iodine solution. The extraction buffer served as a control (C)

Inhibition of *I. acuminatus* α-amylase by Scots pine defensins. Plant responses to wounding are associated with the activation of the octadecanoid pathway leading to the biosynthesis of jasmonic acid (Koo & Howe, 2009). This hormone is thought to play a central role in wound signaling, allowing activation of plant defensin genes, including the conifer defensins *Pg*D1 from *Picea abies* and *Ps*Def1-4 from *Pinus sylvestris* (Pervieux *et al.*, 2004; Shalovylo *et al.*, 2015). Defensin genes are expressed in the xylem and phloem of plants of the genus *Pinus* under normal conditions, and due to the influence of biotic stressors and mechanical damage, as evidenced by the presence of transcripts of these genes in EST libraries from the xylem of *Pinus taeda* (LIBEST_002815; LIBEST_009983); transition latewood of *Pinus radiata* (LIBEST_022780); mechanically wounded xylem of *Pinus banksiana* (LIBEST_026166; LIBEST_026167) and RNA-seq libraries from phloem of *P. sylvestris* trees (Ojeda *et al.*, 2019).

Previously, we reported that recombinant *Ps*Def1 and *Ps*Def2 possessed inhibitory properties against insect α-amylases (Bukhteeva *et al.*, 2022). Since defensins are expressed in the phloem and xylem, where the pine bark beetle feeds, we hypothesized that they could affect the α-amylase activity of the pest. The results of the starch plate assay clearly indicate the inhibitory activity of all the tested *Ps*Def1, *Ps*Def2, and *Ps*Def5.1 against α-amylase from *I. acuminatus* (**Fig. 2*A***). The inhibitory effect is expressed by the reduction in starch hydrolysis zones and their darker color compared to the hydrolysis zone formed by α-amylase without defensin.

The quantitative evaluation of the effect of Scots pine defensins on α-amylase activity is based on the determination of reducing sugars. All tested peptides showed high inhibitory activity (**Fig. 2*B***). At a concentration of 9 μM, the highest concentration used in the experiment, *Ps*Def1 inhibited 73.7±3.0 % of *I. acuminatus* α-amylase activity, *Ps*Def2 – 78.6±4.9 %, and *Ps*Def5.1 – 94.4±5.2 %. The IC_{50} values for these peptides were 4.9±0.6 μM, 4.6±0.8 μM, and 2.8±0.5 μM, respectively.

These results, in conjunction with the findings from our previous studies (Bukhteeva *et al.*, 2022), indicate that Scots pine defensins exhibit significantly higher inhibitory potential against α-amylases of insects that are specialized in coniferous trees compared to those that are not their natural hosts. These findings indicate a co-evolutionary relationship between the digestive enzymes of pest and proteinaceous inhibitors of the plant host (Carmona *et al.*, 2015). However, the molecular mechanisms underlying the adaptation and specialization of insects to specific plants remain poorly understood.

Consequently, studying the complexes formed by insect amylases with their inhibitors can provide valuable insights into the mechanisms of molecular adaptation within the host-pest system.

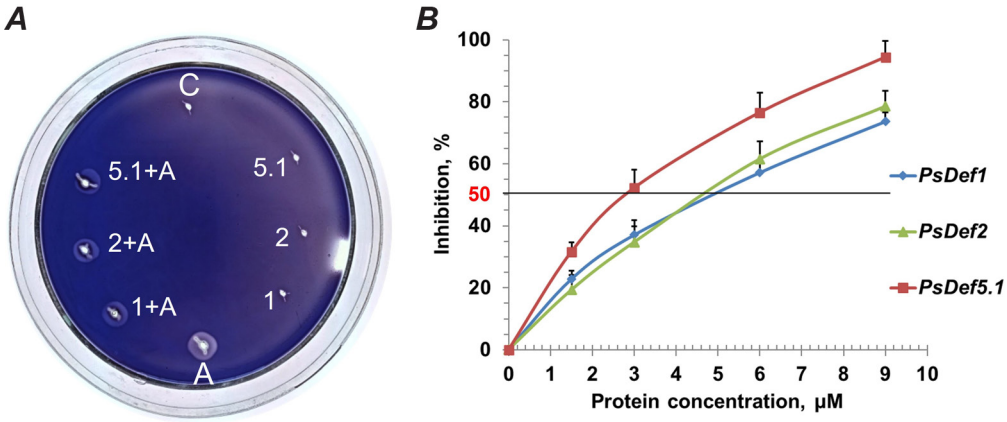

Fig. 2. Inhibitory activity of *Ps*Def1, *Ps*Def2 and *Ps*Def5.1 against α-amylase from the *I. acuminatus* digestive system. (***A***) A starch agar plate assay of the α-amylase inhibitory of the Scots pine defensins: 1, 2, 5.1 – aqueous solutions of *Ps*Def1, *Ps*Def2 and *Ps*Def5.1; 1+A, 2+A, 5.1+A – α-amylase extracts preincubated with defensins solution; A – α-amylase extracts preincubated with water; C – control (extraction buffer). (***B***) Quantitative determination of the inhibitory activity of Scots pine defensins against α-amylase. Vertical bars correspond to standard deviation. Each assay was performed in triplicate

Molecular docking of Scots pine defensins to bark beetle α-amylase. To understand the reason for the differences in the inhibitory activity of Scots pine defensins against bark beetle α-amylase at the molecular level, we conducted an *in silico* docking experiment using the ClusPro web server (Kozakov *et al.*, 2017). Due to the unavailability of the primary structure of *I. acuminatus*, we utilized the three-dimensional structure of α-amylase from the closely related *I. typographus* for the docking of pine defensins. Amy*It* shares approximately 55 % amino acid sequence identity with the α-amylase from *Tenebrio molitor* larvae (TMA), which is the most extensively characterized insect α-amylase (Strobl *et al.*, 1998). The active site of Amy*It* is formed by 21 amino acid residues among these three catalytic residues D200, E236, and D301 (TMA: D185, E222, and D287), a calcium-binding residue H204 (TMA: H189), and the other conserved residues for substrate recognition and orientation. Residues located in and around the Amy*It* active site create a surface with a strong negative potential that attracts positively charged molecules such as plant defensins (**Fig. 3*A***).

The docking models show that all defensins bind to the α-amylase active site, but their orientation relative to Amy*It* is different (**Fig. 3*A***). Approximately 40% of the amino acid residues of defensins are involved in interactions with residues of the substrate-binding cavity (**Fig. 4**, in red) of α-amylase and those located around it (**Fig. 4**, in purple). Interacting residues mainly belong to the mobile regions (loops) of the defensin molecule, specifically, loops L1 and L3. In all three models, loop 3 plays a key role in blocking the substrate binding site, consistently with previously reported models: *Vr*D1-TMA (Liu *et al.*, 2006), *Tv*D1-TMA (Pelegrini *et al.*, 2008), *Ps*Def1-TMA (Kovaleva, *et al.*, 2020), *Ps*Def1-CPBA (Bukhteeva *et al.*, 2022). All amino acid residues of the L3 loops of pine defensins (with the exception of S40 in *Ps*Def2) bind to residues of the active site of the enzyme (**Fig. 4**).

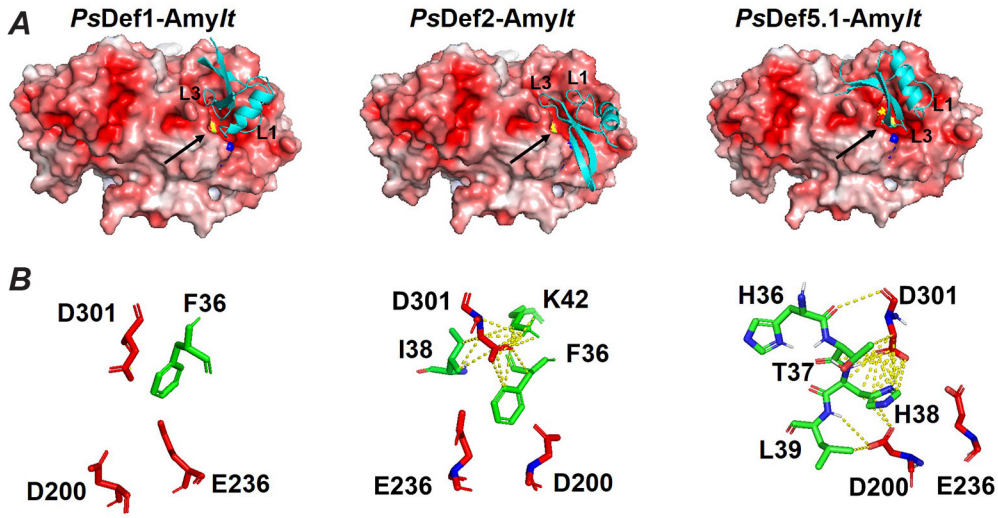

Fig. 3. Molecular docking of Scots pine defensins to *Ips typographus* α-amylase (Amy*It*). (***A***) *Ps*Defs-Amy*Ip* complexes. The electrostatic potential is shown on the surface of Amy*Ip*. The blue color represents a positive charge, and the red color represents a negative charge. The active site of Amy*Ip* is highly negatively charged (indicated by the arrow). Yellow spots represent the predicted catalytic residues of Amy*It*. (***B***) Interactions of Scots pine defensin residues with the catalytic residues of Amy*It* (D200, E236, and D301). Yellow dotted lines show any contacts within 5 Å

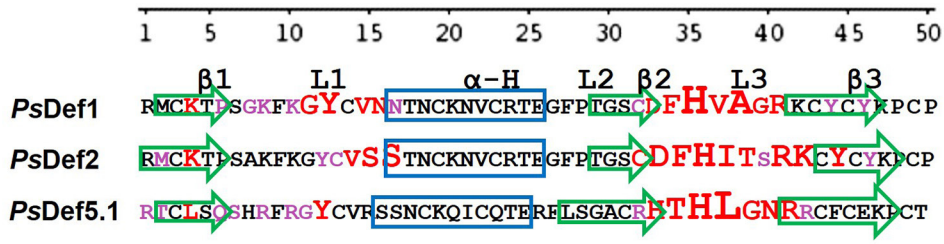

Fig. 4. Scots pine defensin residues involved in any contacts within (red) and around (purple) the active site of the enzyme. The height of the letters (red) is proportional to the number of any contacts formed by the residue with enzyme residues within 5 Å. Arrows indicate β-strands, rectangles – α-helixes

Among the triad of catalytic residues, only D301 was found within 5 Å in all three complexes. In the *Ps*Def1-Amy*It* complex, a residue F36 forms nonbonded contacts with this catalytic residue (**Fig. 3*B***). Stronger interactions in the catalytic pocket are observed in the other two complexes, where, in addition to nonbonded contacts, salt bridges and hydrogen bonds are formed. In the *Ps*Def2-Amy*It* complex, residues F36, K42, and I38 of the inhibitor form a network from ionic and hydrogen bonds, along with nonbonded contacts with only a catalytic residue D301 of Amy*It*. In the *Ps*Def5.1-Amy*It* complex, H38 forms salt bridges and hydrogen bonds with catalytic residue D301, and nonbonded contacts with another catalytic residue, D200. Additionally, the three residues H36, T37, and L39 of loop 3 enhance the binding strength of the inhibitor to the enzyme by forming contacts with D200 and D301 residues. None of the complexes of pine defensins with Amy*It* showed any contacts of the catalytic residue E236 with amino acid residues of defensins within 5 Å. Our results showed that all three defensins block the active

site, but in different ways. *Ps*Def2 and *Ps*Def5.1 inhibit the enzyme by insertion of L3 into the α-amylase active site, thereby establishing a network of hydrogen bonds with catalytic and substrate-binding residues. This mechanism of action was described for plant defensins *Vr*D1 (Liu *et al.*, 2006) and *Tv*D1 (Vijayan *et al.*, 2012). *Ps*Def1 does not interact directly with any catalytic residues of the enzyme, but amino acid residues of its loops L1 and L3 interact strongly with substrate-binding residues and a calcium-binding residue (H204) near the catalytic site through the formation of hydrogen bonds, salt bridges and nonbonded contacts, and thereby prevent substrate access. That mode of inhibition was previously described for the inhibitor BASI (Vallée *et al.*, 1998). Thus, the analysis of interactions in the catalytic site of α-amylase indicates a higher blocking potential of defensin 5.1 compared to the other two pine defensins.

In the docked models, *Ps*Def1, *Ps*Def2 and *Ps*Def5.1 form networks of 10, 12, and 15 hydrogen bonds with Amy*It*, respectively (**Table**). In addition, the *Ps*Def5.1- Amy*It*

Characteristics of *Ps*Defs-Amy*It* complexes

Complex	*Ps*Def1-Amy*It*		*Ps*Def2-Amy*It*		*Ps*Def5.1-Amy*It*	
Protein	*Ps*Def1	Amy*It*	*Ps*Def2	Amy*It*	*Ps*Def5.1	Amy*It*
No. of interface residues	21	25	19	24	18	22
Interface area (Å^2)	1106	1001	1125	1021	1209	1119
No. of salt bridges	3		2		6	
No. of hydrogen bonds	10		12		18	
No. of nonbonded contacts	172		173		178	

has the largest interfacial contact area, 2328 Å^2, whereas those for the complexes *Ps*Def1-Amy*It* and *Ps*Def2- Amy*It* are 2107 Å2 and 2146 Å^2, respectively. These values are close to the value 2240 Å^2 for the experimentally determined interfacial surface area between an α-amylase inhibitor (AAI) isolated from *Amaranthus hypochondriacus* and TMA (Lu *et al.*, 1999). Several studies have highlighted the importance of positively charged amino acids present along the sequence for the *α*-amylase inhibitory activity (Liu *et al.*, 2006; Pelegrini *et al.*, 2008). The data from this study also support this, as *Ps*Def5.1, which exhibited a higher inhibitory activity against α-amylase from *I. acuminatus* than the other two peptides, is characterized by a higher positive charge, resulting in the formation of six salt bridges in the *Ps*Def5.1-Amy*It* complex, compared to three for *Ps*Def1-Amy*It* and two for *Ps*Def2-Amy*It*. In addition, earlier mutagenesis showed a negative effect of acidic amino acids localized in loop 3 on the inhibitory properties of defensin (Lin *et al.*, 2007). The presence of a negatively charged D35 residue in the blocking loop of defensins 1 and 2 can also affect the inhibitory potential of these peptides. It is worth noting that the sequence of *Ps*Def5.1 is only 50 percent identical to that of *Ps*Def1 or *Ps*Def2, which differ from each other by only six amino acids and showed a similar activity against α-amylase from *I. acuminatus.* The most significant differences in Scots pine defensin sequences are observed in the N-terminal region and loop 3, with these residues being involved in the formation of complexes with the enzyme (**Fig. 4**). We believe that these differences contribute to the superior docking of *Ps*Def5.1 with Amy*It* and its interaction with catalytic residues resulting in its high inhibitory activity against α-amylase from pine bark beetle.

CONCLUSION

In conclusion, this study has demonstrated the presence of amylolytic activity in the digestive systems of both larvae and adults of *I. acuminatus,* a harmful and destructive

pest of Scots pine stands. Additionally, we have shown a high inhibitory potential of Scots pine defensins against α-amylase from the pine bark beetle. Based on these findings, we suggest that pine defensins may play an important protective role during the initial stages of colonization of pine trees by the pine bark beetle. Since pioneer beetles evaluate the tree's protective potential and nutritional value during this stage, inhibiting the digestive enzyme with defensins may affect the ability of these first attackers to produce pheromones, and thus prevent a massive bark beetle attack on the tree, ultimately leading to its demise. Therefore, it may be promising to consider using defensin genes in genetic breeding programs aimed at enhancing Scots pine resistance to bark beetles.

ACKNOWLEDGMENTS AND FUNDING SOURCES

This article is based on work supported by a grant from the National Research Foundation of Ukraine (project 2021.01/0184). This work was partially supported by a grant from the Simons Foundation ([award 1030281], [VAK]).

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, [V.K.; Y.Y.]; methodology, [V.K.; Y.Y.; O.K.; V.K.]; validation, [H.K.]; formal analysis, [O.K.; M.K.; Y.S.]; investigation, [Y.Y.; O.K.; V.K.]; resources, [V.Z.; H.K.; V.K.]; data curation, [V.K.; O.K.; M.K.]; writing – original draft preparation, [V.K.]; writing – review and editing, [V.K.; Y.S.]; visualization, [Y.Y.; O.K.]; supervision, [V.K.]; project administration, [V.K.; H.K.]; funding acquisition, [Y.Y.; O.K.; V.K. Y.S.; M.K.; H.K.; V.K.].

All authors have read and agreed to the published version of the manuscript.

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ДЕФЕНЗИНИ СОСНИ ЗВИЧАЙНОЇ ІНГІБУЮТЬ АКТИВНІСТЬ α-АМІЛАЗИ *IPS ACCUMINATUS*

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Вступ. *Ips acuminatus* є одним із найсерйозніших шкідників сосни звичайної, оскільки він заселяє верхні частини стовбура та гілки, порушуючи надходження поживних речовин і води до крони. Верхівковий короїд живиться рослинними тканинами, багатими на крохмаль, тому α-амілази мають відігравати ключову роль у вуглеводному обміні цих комах. Однак α-амілази у короїдів хвойних рослин залишаються недостатньо вивченими.

Матеріали та методи. Для виявлення α-амілазної активності у травній системі *I. acuminatus* ми отримали екстракти з личинок, лялечок і дорослих особин, які були зібрані з природно заселеної сосни звичайної (*Pinus sylvestris* L.). Активність α-амілази екстрактів із різних частин тіла короїда на різних стадіях його онтогенезу оцінювали з використанням 1 % крохмального агару. Кількісну оцінку інгібіторної активності рекомбінантних дефензинів *Ps*Def1, *Ps*Def2 та *Ps*Def5.1 проти α-амілази проводили за методом Бернфельда. Моделі комплексів дефензинів сосни звичайної з α-амілазою *Ips typographus* (Amy*Ip*) були побудовані за допомогою веб-сервера ClusPro 2.0.

Результати. У результаті ми виявили α-амілазну активність у травній системі личинок та імаго верхівкового короїда, але не у лялечок. Усі протестовані дефензини, *Ps*Def1, *Ps*Def2 і *Ps*Def5.1, виявили інгібіторну активність проти α-амілази комах у мікромолярних концентраціях. Значення IC_{50} для цих пептидів становили 4,9±0,6 мкМ, 4,6±0,8 мкМ і 2,8±0,5 мкМ, відповідно. У комплексах *Ps*Defs-Amy*It* утворюється мережа водневих зв'язків, іонних містків і гідрофобних взаємодій між ферментом і його інгібітором, що перешкоджає субстратові досягти каталітичного центру. Комплекс *Ps*Def5.1-Amy*It* має найбільшу площу поверхневого контакту, 2328 Å^2 порівняно з двома іншими дефензинами, що добре корелює з інгібіторною активністю дефензинів у цьому дослідженні.

Висновки. Отже, ми вперше ідентифікували активність α-амілази у *I. acuminatus* і продемонстрували здатність дефензинів сосни пригнічувати її, а це свідчить про те, що дефензини беруть участь у захисті сосни від верхівкового короїда.

Ключові слова: *Pinus sylvestris* L., верхівковий короїд, дефензин рослин, α-амілаза, молекулярний докінг

Received / Одержано	Revision / Доопрацьовано	Accepted / Прийнято	Published / Опубліковано
24 September, 2023	10 October, 2023	15 December, 2023	19 December, 2023