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Application of the antimicrobial peptide ctx(ile^{21})-ha in peanut seeds to evaluate their germinative parameters

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All data will be shared if requested.

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Autor contribution
LEVNB: Conceptualization, experimental data collection, data storage, data analysis, literature review, manuscript writing, manuscript revision; VLAO: Experimental data collection, data storage, data analysis; LFM: Literature review, manuscript writing, manuscript revision; WQL: Experimental data collection, data storage; EFV: Manuscript revision, supervision, funding acquisition.

Abstract

Antimicrobial peptides have gained prominence in scientific research due to their biological potential and antifungal capacity. These peptides are small proteins intrinsic to the system of many living organisms and have activity against microorganisms, bacteria, fungi, viruses and helminths. Therefore, fungi of the genus *Aspergillus* spp are very common in nature, and affect the production of peanuts, almonds, chestnuts and others. Thus, the present work sought a strategy to reduce or mitigate the activity of this fungus in peanut seeds during germination by applying three different dosages (3.6, 4.8 and 7.2 mg/mL) of the antimicrobial peptide Ctx(Ile^{21})-Ha in ultrapure water, and had as objective to evaluate its effects on germination parameters in comparison with the use of the commercial fungicide (Mayran), widely used in peanut crops in the Tupã - São Paulo. The most promising results occurred with dosages of 3.6 mg/mL and 7.2 mg/mL of the peptide, and the Mayran fungicide also showed high efficiency rates for peanut germination, but it presents high toxicity for the producer who handles the fungicide.

Keywords

Germination; Production; Microorganisms; Peptide; Fungicide.

Introduction

Peanut (*Arachis hypogaea L.*) belongs to the *Fabaceae* family and is among the five most important oilseed species in the world (Stalker; Wilson, 2016). Peanuts are a food that can be transported and stored easily and consumed raw. Such aspects may have been substantial in the process of domestication of this species by natives in South America, which originated in a period estimated between 3,500 and 9,400 years ago, in the region of Northwest Argentina and Southwest Bolivia (EMBRAPA, 2023).

Less than ten years ago, peanut farming in Brazil was considered rustic and maintained an informal production chain (MAPA, 2024). However, over time, peanut production has increased regularly, supplying domestic market demand and exporting a surplus equivalent to 60% of the 794 thousand tons produced in the 2020–2021 harvest (EMBRAPA, 2023). Furthermore, the São Paulo state corresponded to 92% of the total peanut production in Brazil in 2023 (USDA, 2024).

In this sense, nowadays the demand for Brazilian exports and the international demand of the consumer market are growing, causing peanut producers to seek new technologies, including the use of quality seeds produced under the standards established by the Ministry of Agriculture, Livestock and Food Supply (MAPA, 2024). Therefore, the development of the peanut seed sector is essential, with the aim of improving the understanding of the acquisition of physiological seed quality and productivity (Okada *et al.*, 2021).

Furthermore, Brazilian research has focused efforts on promoting the safety of products grown in the country, but contamination by aflatoxins and contact with pesticides and their residues are the main risks to worker and consumer health (EMBRAPA, 2023). In this way, it is essential to promote more sustainable practices in the peanut production chain, from the use of certain seeds for better germination in crops, to the harvesting, storage, transportation, processing, consumption and use of organic waste, towards consolidating

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the Sustainable Development Goals (SDGs), from the 2030 Agenda, from the United Nations (UN). Therefore, the objective of this work is to apply the antimicrobial peptide Ctx(Ile21)-Ha in peanut seeds to evaluate their germination parameters.

Materials and methods

Synthesis of the peptide Ctx(Ile^{21})-Ha

The antimicrobial peptide Ctx(Ile^{21})-Ha was synthesized in the laboratory using the Solid Phase Peptide Synthesis (SPFS) technique proposed by Merrifield (1963). For the synthesis, Fmoc chemistry was used, which results in a growth of the peptide chain from the C-terminal to the N-terminal. Initially, a polymeric support, popularly known as "Rink Amide" type resin, was used to begin the couplings.

From this, the coupling of amino acids began using methods, namely: method 1: diisopropylcarbodiimide (DIC) / N-hydroxybenzotriazole (HOBT); method 2: N-ethyldiisopropylamine hexafluorophosphate (DIEA) / O-(benzotriazol-1-yl)-1,1,3,3 tetramethyluronic acid (HBTU) in case of recouplings and method 3: N-methylmorpholine (NMM)/hexafluorophosphate 2-(1-H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium (HATU) in case of recouplings. Methods 2 or 3 are used if 100% efficient couplings do not occur when using the first method. The excess of reagents used was three times. The reaction time for each of the methods used was two hours, one hour and half an hour, respectively.

During the coupling stages, pre-purifications are carried out through washing to eliminate possible residues that are not interesting for the process. These washes were carried out with dichloromethane (DCM) and dimethylformamide (DMF) between the process steps. Removal of the Fmoc group occurred through the addition of washing with 10% w/v piperazine in 9:1 DMF/ethanol for 20 minutes. Confirmation of the departure of free amino groups, after each coupling step, was done using the ninhydrin test. The bluish color indicated that there were free amino groups, and the transparent color indicated that there were none.

The separation of the polymeric support from the peptide chain, called cleavage, occurred after complete drying of the peptide with the aid of a desiccator. To do this, trifluoroacetic acid (TFA) and a scavengers solution containing ultra-pure water and ethanedithiol (EDT) were used. The use of extremely cold ether served to precipitate the scavengers, which were removed after centrifugation. The extraction of the molecule of interest was carried out by adding solutions of water + TFA and acetonitrile (ACN) + TFA.

The final cleavage product was lyophilized for 48 hours in a Liotop model K108 lyophilizer to obtain a white, flocculent powder, called crude peptide.

Purification of the peptide Ctx(Ile^{21})-Ha

For the purification stage, the High Efficiency Liquid Chromatography (HPLC) methodology was used, with a C18 reversed phase analytical column, Shimadzu, Prominence model. The purity of the peptide fractions was determined using solvents A and B, which contained 0.045% TFA and ultra-pure water and 0.036% TFA in ACN, respectively. The program used had a gradient from 5 to 95% of solution B in 30 minutes. The molecule obtained showed purity greater than 95% and, therefore, it was possible to use it. Otherwise, it would not be possible to apply the molecule to seeds.

Application of the peptide Ctx(Ile^{21})-Ha in peanut seeds

The methodology used followed the Rule for Seed Analysed (RSA) (Brasil, 1992). Sixty peanut seeds were evaluated for each treatment and controls, totaling 240 seeds. Solutions with different amounts of peptides were prepared, the smallest 3.6 mg/mL, 5.4 mg/mL and 7.20 mg/mL in distilled water. The experiment was carried out in triplicate.

Soon after the seeds were immersed in the treatment solutions, and then rested for one minute. They were placed on germination paper, rolled up and placed in closed plastic bags. The tests took place in a germination chamber programmed at a temperature of 25ºC and 12 hours of light. After seven days of incubation, the tests were analyzed, as well as the percentage of seeds that germinated. And, as a positive control, 0.00125 mg/mL was used of the fungicide "Mayran".

Results and discussion

Synthesis and purification of the peptide Ctx(Ile^{21})-Ha

The synthesis was carried out successfully without the need for many recoupling reactions. The initial mass was 1,368 mg and the final peptidyl resin mass was 3500 mg. At the end of the cleavage, a crude peptide mass of 2,700 mg was obtained due to losses occurring during the process, such as mass adhesion to the walls of the glassware and evaporation of compounds. After purifying the peptide, the chromatographic profile was generated with the help of the LabSolutions software coupled to the HPLC. In Figure 1, it is possible to observe that the peptide had a purity level greater than 95% and was therefore used for the experiment.

Application of the peptide Ctx(Ile^{21})-Ha in peanut seeds

After opening the germination papers, the germinated seeds were counted. Seeds that had root tips were considered as "germinated", otherwise they were counted as "not germinated". Table 1 represents the results of seed germination. Some seeds subjected to treatment with the peptide showed fungi in their structure, which resulted in their "non-germination". The seeds that germinated after undergoing peptide treatment probably behaved in accordance with studies by Roque-Borda and collaborators, which verified high biological activity and antifungal capacity of this molecule in question (Roque-Borda *et al*., 2021).

Conclusions

The use of the peptide for the germination of peanut seeds was most effective at dosages of 3.6 mg/mL and 7.2 mg/mL. The intermediate dosage did not show good germination parameters. The fungicide Mayran showed the best results in relation to germination, but it presents high toxicity for the producer who handles the fungicide, interfering with the health of the worker and the consumer. For the next steps, it is necessary to test a longer contact time of the seeds with the peptide solution and/or some material that facilitates direct contact between the molecule and the seed, promoting greater involvement of the peanut with the peptide.

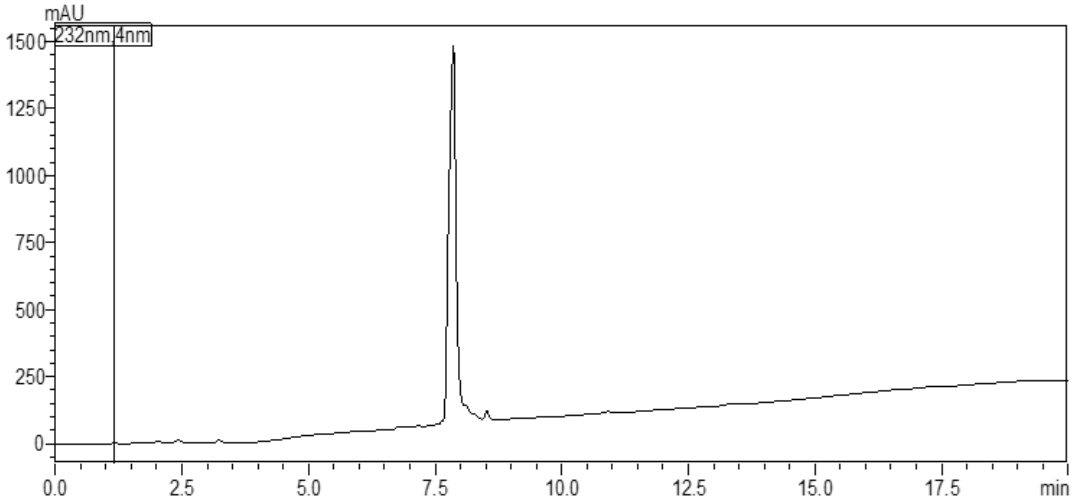

Figure 1. Chromatographic profile of the Ctx(Ile^{21})-Ha peptide.

Table 1. Germinated seeds according to treatment and dosage

Treatments	Dosage	Germinated seeds
Ctx(Ile^{21})-Ha Peptide	3,60 mg	35/60
Ctx(Ile^{21})-Ha Peptide	5,40 mg	5/60
Ctx(Ile^{21})-Ha Peptide	7,20 mg	25/60
Fungicide Mayran	1,25 mg	57/60

References

Brasil. Ministério da Agricultura, do Abastecimento e da Reforma Agrária. Secretaria Nacional de Defesa Agropecuária. (1992). Regras para análise de sementes. Brasília, DF.

Embrapa. (2023). Brasil em 50 alimentos. Recuperado em 19 de agosto de 2024, de https://www.embrapa.br/busca-de-publicacoes/-/publicacao/1153294/brasil-em-50-alimentos

MAPA. (2024). Cultura do amendoim vive 'evolução gigantesca' com sementes certificadas. Recuperado em 17 de agosto de 2024, de https://www.gov.br/agricultura/pt-br/assuntos/noticias/cultura-do-amendoim-vive-2018evolucao-gigantesca2019-com-sementes-certificadas

Merrifield, R. B. (1963). Solid phase peptide synthesis. I. The synthesis of a tetrapeptide. Journal of the American Chemical Society, 85(14), 2149–2154.

Okada, M. H., et al. (2021). Acquisition of the physiological quality of peanut (Arachis hypogaea L.) seeds during maturation under the influence of the maternal environment. PLoS One, 16(5), 1–15. https://doi.org/10.1371/journal.pone.0250293

Roque-Borda, C. A., et al. (2021). Alginate-based microparticles coated with HPMCP/AS cellulose-derivatives enable the Ctx(Ile21)-Ha antimicrobial peptide application as a feed additive. International Journal of Biological Macromolecules, 183, 1236–1247. https://doi.org/10.1016/j.ijbiomac.2021.05.011

Stalker, T., & Wilson, R. F. (2016). Peanuts: Genetics, processing and utilization (1st ed.). Academic Press and AOCS Press, Elsevier.

USDA. (2024). Peanut 2023: World production. Recuperado em 18 de agosto de 2024, de https://ipad.fas.usda.gov/cropexplorer/cropview/commodityView.aspx?startrow=11&cropid=2221000&sel_year=2023&rankby=Production

Von Muhlen, C., & Lanças, F. M. (2004). Cromatografia unificada. Química Nova, 27(5), 747–753.