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LUDMILA MARQUES DOS SANTOS VELOSO

ANÁLISE *IN SILICO* DA EVOLUÇÃO DE HEVEÍNAS NO REINO VEGETAL

PATOS DE MINAS – MG
JUNHO DE 2022

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Dissertação de Mestrado apresentada ao Programa de Pós-graduação em Biotecnologia como requisito parcial para obtenção do título de mestre em Biotecnologia.

Orientador: Prof. Dr. Aulus Estevão Anjos de Deus Barbosa

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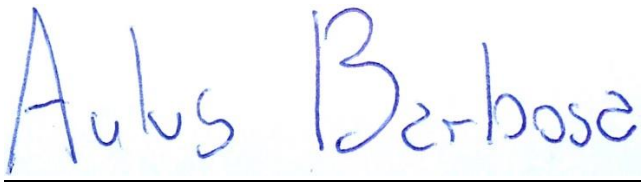

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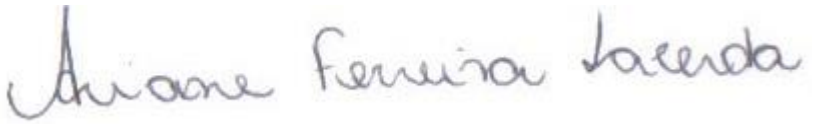

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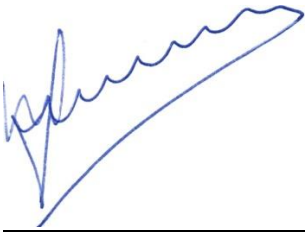

Dr. Matheus de Souza Gomes

PATOS DE MINAS

2022

DEDICATÓRIA

Dedico este trabalho aos meus pais, avos e minha madrinha pelo estímulo, carinho e compreensão.

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RESUMO

Ao longo do processo evolutivo, as plantas se tornaram uma fonte de moléculas biologicamente ativas com diversas propriedades. Entre essas moléculas encontramos várias famílias de peptídeos antimicrobianos (AMPs) e entre estes, podemos destacar a família das heveínas, que são ricas em cisteínas, glicinas e que têm a capacidade de se ligar à quitina, tornando-as moléculas com grande potencial biotecnológico. O trabalho verificou a presença de heveínas em espécies do reino Plantae, com o objetivo de verificar a origem evolutiva desses AMPs. Foi realizado uma busca na literatura por sequências de heveínas, seguido do levantamento das sequências codificadoras em bancos de dados específicos. As sequências foram submetidas ao algoritmo TBLASTN na base de dados "1K Plants" contra as linhagens de algas verdes, Briófitas, Pteridófitas e coníferas. A identificação das heveínas foi feita pela verificação da presença dos domínios conservados PFAM IPRO001002 e PFAM IPRO001153, utilizando a ferramenta INTERPROSCAN do programa BLAST2GO. Em seguida foi realizado alinhamento global dessas sequências utilizando a ferramenta MUSCLE. Após a confirmação da presença dos AMPs, foi realizado a construção da árvore filogenética e predição das estruturas tridimensionais. Ao final das análises foi possível identificar 5 sequências de heveínas, sendo que todas apresentam as propriedades físico-químicas e estruturas tridimensionais semelhantes a heveínas previamente descritas, e todos pertencem a classe 8C-Hev. Os resultados sugerem que as heveínas encontradas hoje nas plantas superiores surgiram no grupo de plantas Pteridófitas após eventos de mutação, como clivagem e/ou translocação, que resultaram na junção dos domínios PFAM IPRO001002 e PFAM IPRO001153. Nas algas e Briófitas esses domínios fazem parte de outras proteínas e não foram encontrados juntos. Portanto, podemos concluir que as heveínas surgiram nas Pteridófitas e todas as sequências identificadas pertencem a classe 8C-Hev das heveínas, as outras classes de heveínas (6C-Hev e 10C-Hev) devem ter surgido no grupo das plantas Angiospermas.

Palavras-chave: Algas verdes. Briófitas. Pteridófitas. Peptídeos antimicrobianos. Heveínas

ABSTRACT

Plants evolution suggests that Green Algae are the direct precursors of land plants, followed by Bryophytes and Pteridophytes. Throughout the evolutionary process, plants have become a source of biologically active molecules with diverse properties. Among these molecules we find several antimicrobial peptides (AMPs) families and among these, we can highlight hevein family, which are rich in cysteines, glycines and have the ability to bind chitin, making them a great potential biotechnological tool. This job verified hevein presence in Plantae kingdom species using bioinformatics tools. A literature search was performed for standard hevein sequences, followed by its sequences download. Sequences were submitted to the TBLASTN algorithm in the "1K Plants" database against the Green Algae, BBryophytes, PPteridophytes and CConifers transcriptomes. Conserved domains (PFAM IPRO001002 and PFAM IPRO001153) presence were verified using the INTERPROSCAN tool, followed by global alignment using MUSCLE tool. After confirming hevein presence, phylogenetic analysis and three-dimensional structures prediction were performed. It was possible to identify 5 hevein sequences, all of which have heveins basic physicochemical properties and three-dimensional structures and all belong to the 8C-Hev heveins class. The results suggest that the hevein found in higher plants today appeared in the Pteridophyte group after several mutation events, such as cleavage and translocation, from the Green Algae and bryophyte groups, which resulted in the hevein peptide. Therefore, we can conclude that heveis arose in PPteridophytes and the sequences identified belong to the 8C-Hev hevein class, the other hevein classes (6C-Hev and 10C-Hev) must have arisen in the Angiosperms group.

Keywords: Green Algae. Bryophytes. Pteridophytes. Antimicrobial Peptides. Hevein

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CAPÍTULO I – REFERENCIAL TEÓRICO

1 INTRODUÇÃO

O reino Plantae é constituído por mais de 400.000 espécies que são, em geral, autotróficas. As plantas terrestres (Embryophyta) apresentam um registro fóssil de cerca de 470 milhões de anos. O seu primeiro ancestral comum, identificado através da análise filogenômica, são algas Zygnematophyceae (Carófitas) (LIGRONE, 2019; MENDÃO, 2007).

Durante o processo evolutivo as plantas desenvolveram diversos compostos que permitiram a sua sobrevivência em diversos ambientes, e alguns destes compostos possuem aplicações tanto na medicina quanto na agricultura. Dentre estas moléculas biologicamente ativas encontramos os peptídeos antimicrobianos (AMPs) (BARASHKOVA; ROGOZHIN, 2020). O primeiro peptídeo antimicrobiano vegetal foi isolado em 1972, sendo ele uma purotionina encontrada da farinha de trigo (*Triticum aestivum*) (FERNANDEZ DE CALEYA et al., 1972). Os peptídeos têm sido isolados das mais diversas fontes, dentre raízes, sementes, flores, caules e folhas, e têm demonstrado atividades contra fitopatógenos, bem como contra bactérias patogênicas para humanos (BARASHKOVA; ROGOZHIN, 2020; BARBOSA PELEGRINI et al., 2011). Dentre as famílias dos peptídeos antimicrobianos vegetais encontre-se a família das heveínas (hevein-like), composta por três grupos (6C-Hev, 8C-Hev e 10C-Hev) e com alto potencial biotecnológico, devido sua função de se ligar a quitina presente em nematóides, na parede celular de fungos e no exoesqueleto de insetos (SLAVOKHOTOVA et al., 2017). No entanto, não há dados na literatura até o presente momento sobre a origem das heveínas no reino vegetal.

1.2 Hipótese

Os peptídeos heveínas vêm sendo identificados e descritos em várias plantas superiores, no entanto as pesquisas realizadas até o momento identificaram esses peptídeos nas linhagens de plantas ancestrais (Algas verdes, Briófitas e Pteridófitas). Temos como hipótese de que esses peptídeos não se originaram nas plantas superiores, devendo existir desde as Algas verdes.

1.3 Objetivos

1.3.1 Objetivo geral

O presente trabalho teve como objetivo verificar a origem de heveínas em espécies do reino *Plantae*, desde Algas verdes, BBriófitas, PPteridófitas e Coníferas e descrever a anotação destes peptídeos.

1.3.2 Objetivos específicos

- Analisar os transcriptomas de diferentes espécies de Algas, Briófitas, Pteridófitas e Coníferas para verificar a presença de peptídeos antimicrobianos do tipo heveínas;
- Avaliar os domínios conversados e pontos de clivagem das sequências encontradas;
- Realizar análise filogenética para classificar as heveínas encontradas nas classes 6C-Hev, 8C-Hev e 10C-Hev;
- Predizer as estruturas tridimensionais das heveínas encontradas.

1.4 Justificativa

Os peptídeos antimicrobianos são uma ferramenta poderosa para o desenvolvimento de produtos para o controle de pragas da agricultura, e também possuem um grande potencial para o desenvolvimento de novos antibióticos para o tratamento de diversas infecções humanas (BARBOSA PELEGRINI et al., 2011). No entanto, existem poucos estudos publicados sobre as heveínas, sendo o grupo de AMPs menos estudado, o que aumenta a importância deste trabalho. O uso da bioinformática tem sido imprescindível na identificação de peptídeos antimicrobianos, como também é uma importante ferramenta no auxílio da compreensão da evolução dos genes no reino vegetal, desta forma auxiliando no desenvolvimento de inúmeras aplicações biotecnológicas.

2 REFERENCIAL TEÓRICO

2.1 Reino vegetal

As plantas terrestres são o segundo maior grupo de eucariotos, com mais de 400.000 espécies descritas (LIGRONE, 2019). Os primeiros ancestrais das plantas terrestres surgiram a cerca de 500 a 470 milhões de anos atrás (WODNIOK et al., 2011). Diversas alterações ambientais provocaram o desenvolvimento de caracteres adaptativos às novas condições ambientais da mesma forma que, também, impossibilitaram o desenvolvimento de muitas plantas (MENDÃO, 2007). As alterações ambientais possibilitaram, por meio da seleção natural, o surgimento de três grandes grupos, sendo as Briófitas, compostas pelas plantas hepáticas, *antóceros* e musgos, as Pteridófitas, compostas pelas licófitas e monilófitas, e as espermatófitas (WODNIOK et al., 2011).

O ciclo de vida das plantas terrestres é constituído por uma alternância de gerações, o esporófito (diplóide) e gametófito (haplóide). Os táxons vegetais que precederam as plantas terrestres, as algas, têm um ciclo de vida diplôntico com meiose espórica. As algas verdes pertencem ao ramo das plantas verdes e os três tipos de algas (algas vermelhas, algas marrons e algas verdes) são apenas relacionados entre si fisiologicamente (BENNICI, 2008). As algas verdes são divididas em dois grupos: Chlorophyta e Streptophyta (UMEN, 2014).

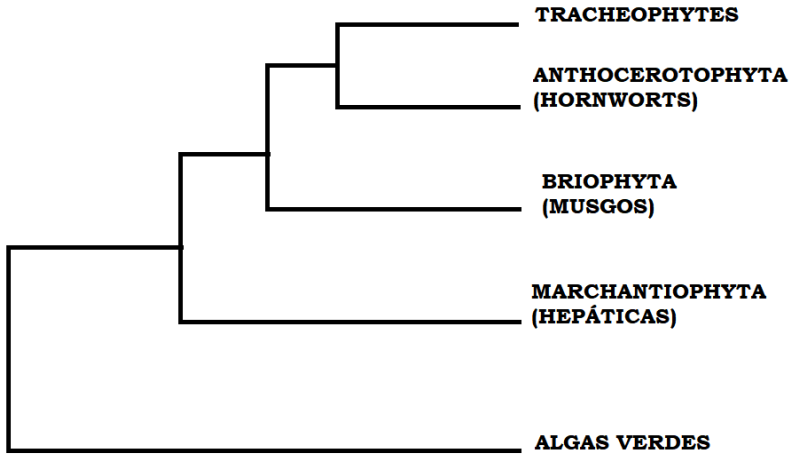
Observado o ponto de ramificação da *Nitella* na cadeia evolutiva, pode-se sustentar a ideia de que as algas verdes constituem o precursor direto das plantas terrestres. Sendo que as Briófitas e as Pteridófitas se separaram após a emergência das espermatófitas (HORI; LIM; OSAWA, 1985).

Nas Briófitas, as primeiras plantas terrestres eucarióticas, o esporófito permitem uma forte dispersão aérea dos seus esporos e, portanto, têm um maior potencial de variação genética e podem ocupar mais ambientes. Existe uma grande lacuna evolutiva entre as algas verdes e as Briófitas, não somente no ciclo de vida, mas no nível do desenvolvimento estrutural (BENNICI, 2008). As Briófitas são divididas em musgos, hepáticas e antóceros. Esse grupo de plantas contém cerca de 15.000 a 20.000 espécies. Elas apresentam um ciclo de vida no qual o gametófito é perene e dominante (tamanho e longevidade) e o esporófito não se apresenta ramificado, monosporangiado e o seu desenvolvimento completo está ligado ao gametófito materno (JONATHAN SHAW; SZÖVÉNYI; SHAW, 2011).

Para a maioria dos botânicos, as Briófitas e as Pteridófitas basais são os mais antigos remanescentes vivos das plantas eucarióticas que colonizaram a terra (RENZAGLIA et al.,

2000). Suas características, gametófitos e esporófitos, permitiram a adaptação e existência terrestre dessas espécies e, em particular, no desenvolvimento da sua capacidade reprodutiva por dispersão de esporos (BENNICI, 2008). Na imagem abaixo (Figura 1) podemos verificar a evolução dos grupos ancestrais terrestres.

Figura 1 – Evolução das plantas ancestrais terrestres



Fonte: adaptado de JONATHAN SHAW; SZÖVÉNYI; SHAW, 2011.

As Pteridófitas são consideradas as primeiras plantas terrestres verdadeiras, a sua origem ocorreu a cerca de 400 milhões de anos atrás e elas são consideradas o segundo maior grupo de plantas vasculares. Elas são plantas vasculares sem a presença de sementes e a sua reprodução ocorre por meio de esporos, consideras homosporos (reproduz um único tipo de esporo) ou heterosporos (reproduz dois tipos de esporos). A divisão das Pteridófitas são compostas principalmente por plantas herbáceas e arbustivas, e sua maior diversidade está presente na região dos trópicos (KREFT et al., 2010; PRAVEEN; PANDEY, 2020).

As Coníferas são uma linhagem de plantas rica em espécies com uma distribuição natural entre o hemisfério norte e tropical. Nessa linhagem encontra-se a família Pinaceae que apresenta de 10 a 11 gêneros existentes (mais de 230 espécies), sendo a família mais basal das coníferas. Essa família domina as florestas temperadas e boreais no hemisfério norte (GERNANDT et al., 2018; LIN et al., 2010). As Pinaceae pertencem ao grupo das gimnospermas e fornecem informações importantes sobre a evolução das coníferas no reino Plantae (LIN et al., 2010).

Esta grande diversidade de plantas verdes permite realizar estudos sobre os mecanismos moleculares subjacentes à sua evolução, bem como o histórico dos genes, incluindo a duplicação de genes, perdas de genes, transferência horizontal e rearranjos de genes (KOONIN, 2005).

2.2 Bioinformática

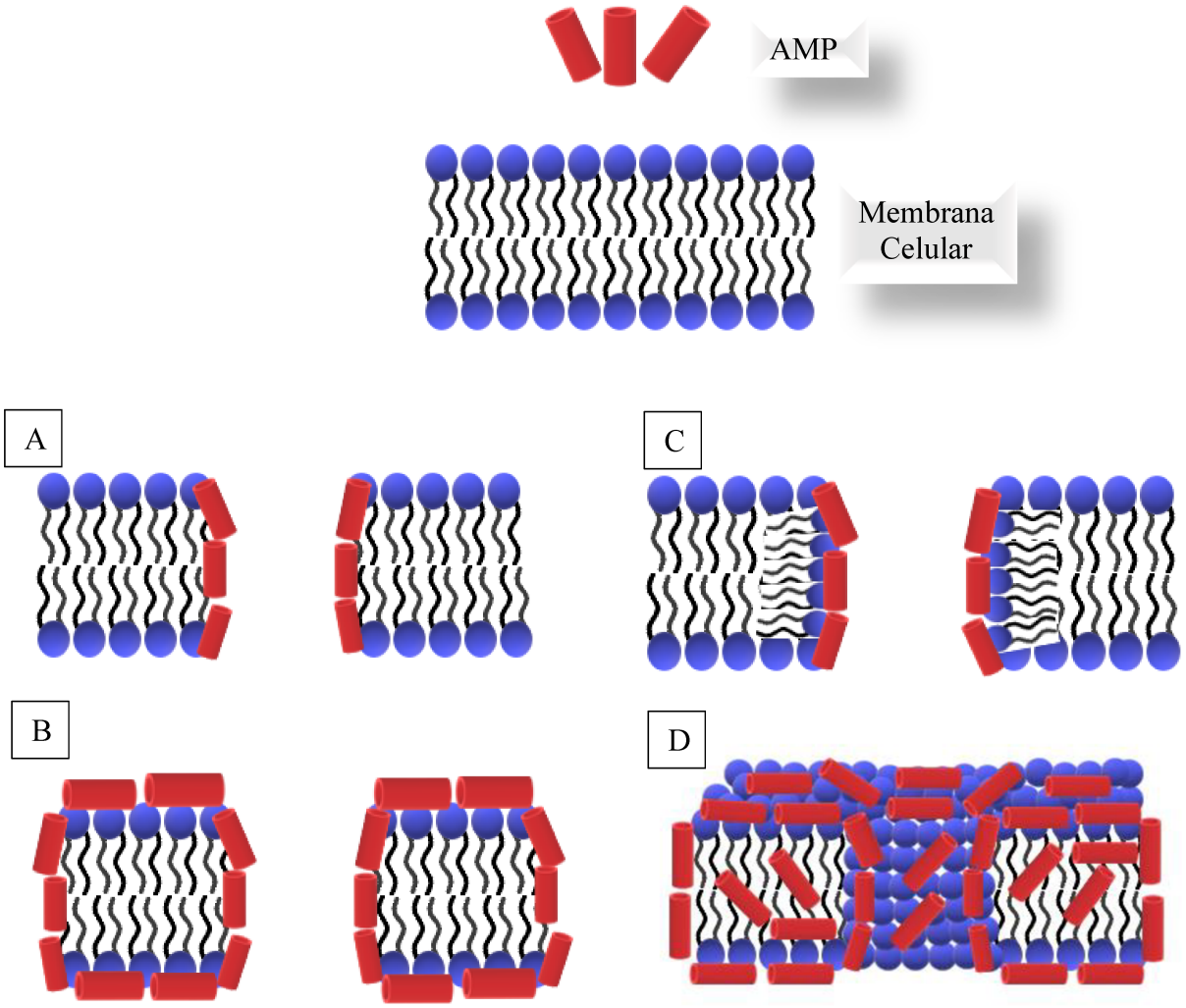
Nas abordagens *in silico* os processos biológicos e fisiológicos são simulados em computador, permitindo que o pesquisador realize vários estudos utilizando ferramentas de bioinformática. O acesso público em vários bancos de dados tem possibilitado vários estudos de integração de dados derivados de genômica, transcriptoma, proteômica e entre outras plataformas "ômicas". A utilização desses bancos de dados é imprescindível na aplicação de abordagens de bioinformática em análises *in silico* de conjuntos de dados genômicos, transcriptomas, proteômicos e metabolômicos. Tais abordagens preliminares têm sido comumente associadas a técnicas moleculares subsequentes para identificar e caracterizar genes codificadores, produtos e regulação, além de validar aspectos funcionais putativos (PESTANA-CALSA MC, RIBEIRO IL, CALSA T JR, 2010).

A utilização da bioinformática é imprescindível na identificação de peptídeos antimicrobianos, bem como outras moléculas. As abordagens in silico para identificação de peptídeos antimicrobianos e seus respectivos genes codificadores contribuem na determinação da maneira pela qual diferentes sequências podem afetar patógenos específicos.

2.3 Peptídeos antimicrobianos

Os peptídeos antimicrobianos (AMPs) são moléculas de defesa contra patógenos encontrados em diversas espécies (ZHAO et al., 2017). São peptídeos pequenos de aproximadamente 2 a 9 kDa, com cerca de 10 a 50 resíduos de aminoácidos e a maioria deles contêm resíduos hidrofóbicos e catiônicos. Seu mecanismo de ação envolve a formação de danos a membrana celular do patógeno (Figura 2), principalmente levando a formação de poros na membrana por meio da ação de forças eletrostáticas, e que provocam morte celular (BARBOSA PELEGRINI et al., 2011; ZHAO et al., 2017). Existe quatro modelos propostos da interação de um AMP com a membrana celular do patógeno: poro no formato de barril (Figura 2-a), mecanismo de carpete (Figura 2-b), poro toroidal (Figura 2-c) e poro toroidal desordenado (agregado) (Figura 2-d) (LI et al., 2021).

Figura 2 – Representação dos quatro mecanismos de ação dos AMPs: (A) os AMPs são inseridos verticalmente na membrana formando poros transmembranas (formato de barril), (B) os AMPs são adsorvidos paralelamente a membrana cobrindo-a (mecanismo de tapete), (C) mecanismo intermediário entre a formação de barril e mecanismo tapete (poro toroidal) e (D) formação de poros aleatórios.



Fonte: adaptado de LI et al., 2021.

AMPs possuem carga positiva e natureza anfipática, características que favorecem à sua atividade de interação com membranas, ligando-se aos os microrganismos por meio de forças eletrostáticas entre os resíduos de aminoácidos positivos e cargas negativas expostas na superfície celular (GUILHELMELLI et al., 2013; NAWROT et al., 2014). Eles são ricos em cisteínas, possuem cerca de 4 a 12 resíduos cisteína, cujo motivo resulta na formação de 2 a 6 pontes dissulfeto – contribuindo desta forma na compactação da estrutura, além de conferir alta estabilidade molecular e resistência à degradação química e proteolítica (TAM et al., 2015).

Os principais parâmetros físico-químico e estruturais que afetam a atividade de AMPs de plantas são seus resíduos de aminoácidos, carga líquida, hidrofobicidade, natureza anfipática

e características estruturais. Além dos fatores externos, como pH, temperatura e íons metálicos (BHATTACHARJYA; RAMAMOORTHY, 2009; LI et al., 2021)

A classificação dos AMPs em diferentes famílias tem como base a sua sequência de aminoácidos, número e espaçamento dos resíduos de cisteína e as posições das pontes dissulfeto (LAY; ANDERSON, 2005; TAM et al., 2015). As principais famílias de AMPs encontradas nos vegetais são as famílias de ciclotídeos, defensinas, heveínas, knotinas, proteínas de transferência de lipídeos (LTPs), snakinas e tioninas (LI et al., 2021).

2.3.1 Heveínas

Descrita pela primeira vez em 1960 (ARCHER, 1960), o nome heveína foi atribuído devido aos componentes básicos das estruturas vacuolares do látex da seringueira (*Hevea brasiliensis* Moll. Arg.) e sua sequência de aminoácidos foi determinada pela primeira em 1975 (WALUJONO et al., 1975).

Os peptídeos da família heveínas são peptídeos pequenos, contendo de 29 a 45 resíduos de aminoácidos, ricos em cisteínas, glicinas e alguns aminoácidos aromáticos. Seu domínio conservado pode variar de acordo com a quantidade de cisteínas, sendo de 6 a 10 resíduos, e possuem de 3-5 ligações dissulfeto (JIMÉNEZ-BARBERO et al., 2006; SLAVOKHOTOVA et al., 2017). A sua estrutura terciária é composta pelo motivo estrutural α- hélice–β1–β2–α-hélice–β3, na qual as folhas-beta são antiparalelas e contém variações baseadas na presença de curvas curtas nas α-hélices e folha β3. As folhas-betas antiparalelas são responsáveis pela formação da folha-β central do motivo, com as duas α-hélices longas situadas em cada lado e estabilizada por pontes de dissulfeto (JIMÉNEZ-BARBERO et al., 2006). As heveínas são classificadas de acordo com a quantidade de cisteínas presentes, sendo 6C-Hev, 8C-Hev e 10C-Hev, sendo que as heveínas mais comuns contém 8 resíduos de cisteína (SLAVOKHOTOVA et al., 2017).

Seis resíduos de cisteínas assumem posições bastante conservadas na molécula do peptídeo e formam um motivo de cisteína expresso pela fórmula $C^1X_{4-5}C^2X_4C^3C^4X_5C^5X_6C^6$, sendo C representando cisteína e X qualquer outro resíduo, e a formação da ligação dissulfeto ocorre entre os resíduos de cisteína C1-C4, C2-C5 e C3-C6. Os peptídeos semelhantes à heveína apresentam como característica principal um sítio conservado de ligação da quitina com a sequência de aminoácidos SXFGY/SXYGY, sendo que o local de ligação da quitina também é semelhante para outras proteínas capazes de se ligar à quitina, na qual ela é um polímero de N-acetilglucosamina. Sendo assim, a sua capacidade de se ligar à quitina é de grande interesse

biotecnológico, devido a quitina está presente em nematóides, na parede celular de fungos e no exoesqueleto de insetos, podendo assim ser utilizada no desenvolvimento de plantas resistentes a essas pragas (SLAVOKHOTOVA et al., 2017). A capacidade de se ligar a quitina, como já mencionado anteriormente, ocorre devido a ligação do polímero beta-1,4 de N-acetilglucosamina e polissacarídeos relacionados contendo N-acetilglucosamina ou mesmo ácido N-acetilneuramínico. Esse domínio de ligação à quitina é responsável pela ligação ao carboidrato e o significado funcional dos resíduos de cisteína adicionais das heveínas ainda precisam ser esclarecidos (ODINTSOVA et al., 2020).

O peptídeo heveína apresenta-se na forma de proteína pro-heveína (Figura 3), que é processada para então gerar o peptídeo maduro, e durante esse processo de clivagem o peptídeo de 4-6 resíduos iniciais e o domínio C-terminal são perdidos (Figura 4) (BERTHELOT; PERUCH; LECOMTE, 2016).

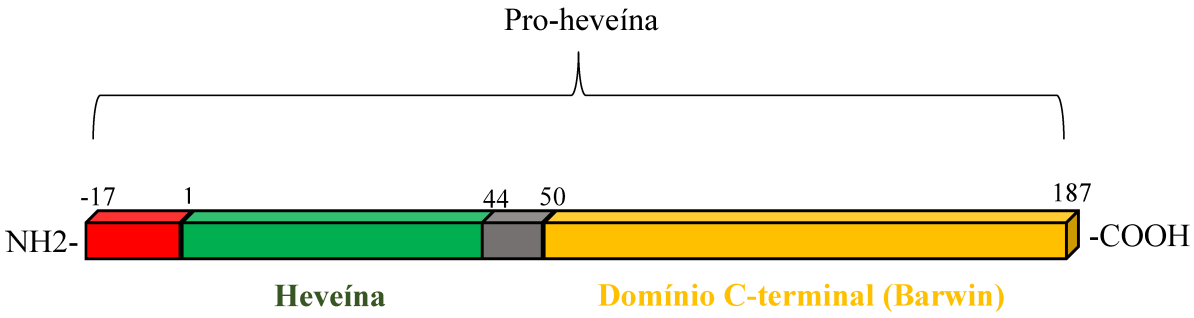
O domínio C-terminal (domínio BARWIN) está associado ao domínio semelhante à heveína, também conhecido como domínio de ligação à quitina. Estudos tem demonstrado que o domínio BARWIN apresenta atividade RNAse (BAI et al., 2013; BERTINI et al., 2009; FRANCO et al., 2014; GUEVARA-MORATO et al., 2010; HUET et al., 2013; KIM; HWANG, 2015; PEREIRA MENEZES et al., 2014) e a atividade está relacionada a dois importantes resíduos de histidina necessários, uma na posição 11 e outro na posição 113, numerados de acordo com a sequência de BARWIN madura (MAIA et al., 2021).

O domínio BARWIN também está relacionado as proteínas PR-4 (família da proteína-4 relacionada à patogênese), onde elas podem ser divididas em duas classes. A classe I apresenta o domínio N-terminal rico em cisteína (classe essa conhecida como proteína semelhante à heveína) e a classe II que não possui o domínio N-terminal rico em cisteína (BAI et al., 2013).

2.3.1.1 Peptídeos heveína com seis resíduos de cisteínas

Heveínas com seis cisteínas são as moléculas mais curtas com atividade antifúngica, antibacteriana e capacidade de se ligar à quitina. Eles apresentam três ligações dissulfeto e um comprimento total de 29 a 30 aminoácidos. A maioria dos peptídeos foram isolados de plantas da família Amaranthaceae; entretanto, alguns foram obtidos de plantas da família Caryophyllaceae (SLAVOKHOTOVA et al., 2017). Os primeiros peptídeos (Ac-AMP1, Ac-AMP2) foram isolados em 1992 das sementes de *Amaranthus caudatus L.*(BROEKAERT et al., 1992). Na Figura 5 podemos visualizar a estrutura 3D deste grupo.

Figura 3 – Representação da Pro-heveína composta pelo N-terminal, domínio heveína e C-terminal e a sequência correspondente.

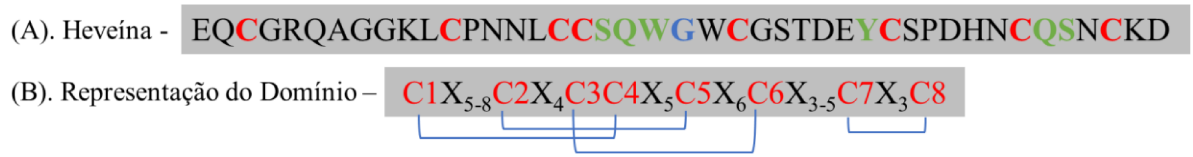


Sequência de aminoácidos:

MNIFIVVLLCLTGVAIAEQCGRQAGGKLCPNNLCCSQWGWCGSTDEYCSPDHNCQSNCKDSG
EGVGGGSASNVLATYHLYNSQDHGWDLNAASAYCSTWDANKPYSWRSKYGWTAFCGPVGAHG
QSSCGKCLSVTNTGTGAKTTVRIVDQCSNGGLDLDVNVFRQLDTDGKGYERGHITVNYQFVD
CGDSFNPLFSVMKSSVIN

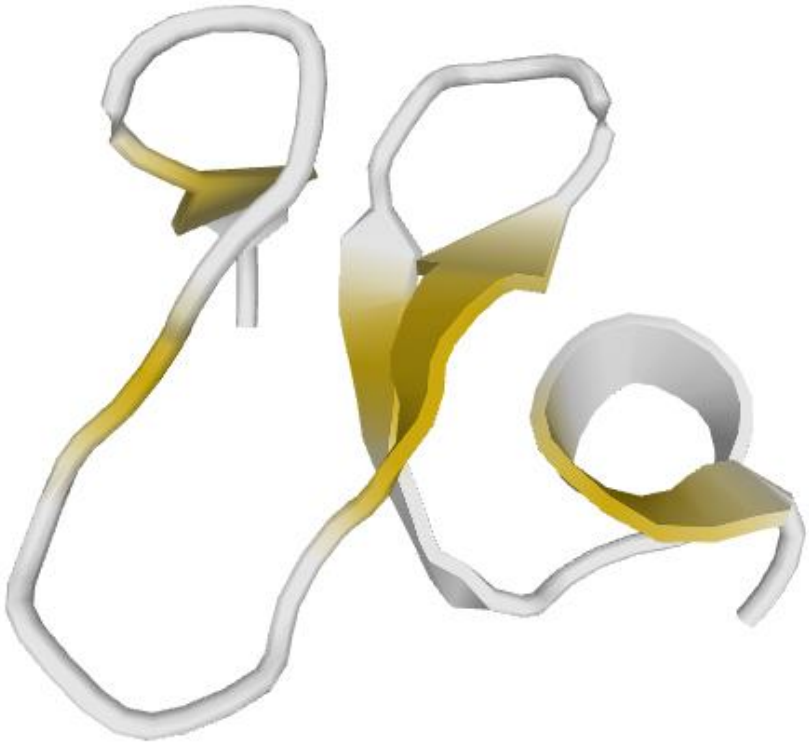
Fonte: Adaptado de BERTHELOT; PERUCH; LECOMTE, 2016.

Figura 4 – Domínio conservado heveína. A figura A representa a sequência da heveína HEVE_HEVBR (UniProt: P02877) composta por 8 cisteínas e os aminoácidos nas cores verde e azul representam os aminoácidos conservados na maioria das sequências de heveína. A figura B representa o domínio de heveína e suas respectivas pontes de dissulfeto.



Fonte: Própria autora.

Figura 5 – Estrutura tridimensional do grupo de peptídeo 6C-heveína. Estrutura do peptídeo AMP_AMACA (P27275) representante da classe 6C-heveína. A cor amarela indica a posição das 6 cisteínas presentes nessa classe.



Fonte: Estrutura gerada na plataforma SWISS-MODEL

2.3.1.2 Peptídeos heveína com oitos resíduos de cisteínas)

Heveínas com oito resíduos de cisteína foram isoladas de diferentes plantas das famílias Poaceae, Convolvulaceae e Polygonaceae. Essa subclasse de heveína apresenta atividade antifúngica contra fungos contendo ou não quitina na parede celular, antibacteriana e contra leveduras (SLAVOKHOTOVA et al., 2017). Eles são capazes de penetrar as hifas fúngicas rapidamente, causando despolarização da actina, ruptura da membrana e paralisação da integridade do citoplasma. Como exemplo temos, os peptídeos Pn-AMP1 e Pn-AMP2 isolados das sementes de Ipomeia (*Pharbitis nil*) pertencentes à família Convolvulaceae (KOO et al., 1998; SLAVOKHOTOVA et al., 2017). Na Figura 6 podemos visualizar um exemplo da estrutura 3D do grupo.

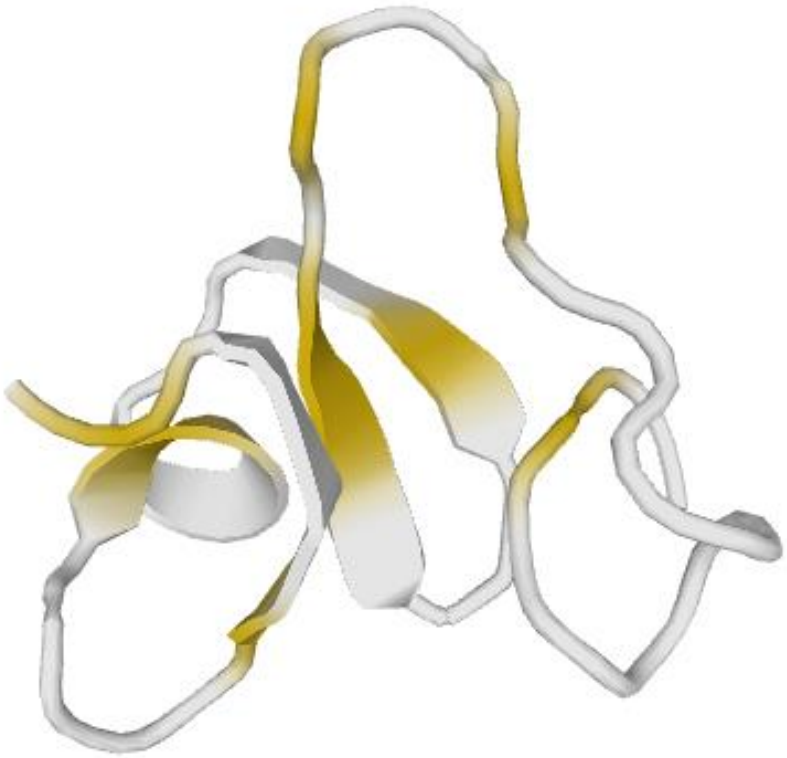
Figura 6 – Estrutura tridimensional do grupo de peptídeo 8C-heveína. Estrutura do peptídeo AMP_IPONI (P81591) representante da classe 8C-heveína. A marcação amarela indica as posições das 8 cisteínas presentes nessa classe.

Fonte: Estrutura gerada na plataforma SWISS-MODEL

2.3.1.3 Peptídeos heveína com dez resíduos de cisteínas

Heveínas com 10 resíduos de cisteína apresentam cinco ligações dissulfeto. Os primeiros peptídeos desta classe (EAFP1 e EAFP2) foram isolados em 2002 de *Eucommia ulmoides*, da família Eucommiaceae (HUANG et al., 2002). Os peptídeos apresentam atividade antifúngica contra oito fungos patogênicos principais que atacam algodão, trigo, batata, tomate e tabaco. E a atividade inibitória foi observada contra fungos contendo ou não quitina (SLAVOKHOTOVA et al., 2017). Na Figura 7 podemos visualizar um exemplo da estrutura 3D do grupo.

Figura 7 – Estrutura tridimensional do grupo de peptídeo 10C-heveína. Estrutura do peptídeo AMP_TRIKH (P85966.2) representante da classe 10C-heveína. A marcação amarela indica as posições das 10 cisteínas presentes nessa classe.



Fonte: Estrutura gerada na plataforma SWISS-MODEL

CAPITULO II – ARTIGO CIENTÍFICO

In silico analysis of heveins evolution in the plant kingdom

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Abstract

Plants evolution suggests that green Algae are the direct precursors of land plants, followed by Bryophytes and Pteridophytes. Throughout the evolutionary process, plants have become a source of biologically active molecules with diverse properties. Among these molecules we find several families of antimicrobial peptides (AMPs) and among these, we can highlight heveins family, which are rich in cysteines, glycines and have the ability to bind chitin, making them a great potential biotechnological tool. This job verified hevein presence in Plantae kingdom species using bioinformatics tools. A literature search was performed for standard hevein sequences, followed by it sequences download. Sequences were submitted to the TBLASTN algorithm in the "1K Plants" database against the green Algae, Bryophytes, Pteridophytes and Conifers transcriptomes. Conserved domain (PFAM IPRO001002 and PFAM IPRO001153) presence were verified using the INTERPROSCAN tool, followed by global alignment using MUSCLE tool. After confirming hevein presence, phylogenetic analysis and three-dimensional structure prediction was performed. It was possible to identify 5 hevein

sequences, all of which have the basic physicochemical properties of the class, similar three-dimensional structures and all belong to the 8C-Hev hevein class. The results suggest that the heveins found in higher plants today appeared in the Pteridophyte group after several mutation events, such as cleavage and translocation, from the Green Algae and Bryophyte groups, which resulted in the hevein peptide. Therefore, we can conclude that hevein arose in Pteridophytes and the sequences identified belong to the 8C-Hev hevein class.

Keywords: Green Algae. Bryophytes. Pteridophytes. Hevein. Antimicrobial peptides. Bioinformatics.

1. Introduction

The Plantae kingdom consists of more than 400,000 species that are, in general, autotrophic (Ligrone, 2019). Environmental changes allowed the emergence of three large groups, namely the Bryophytes, composed of liver plants, hornworts and mosses, the Pteridophytes, composed of lycophytes and monilophytes, and the spermatophytes (Wodniok *et al.*, 2011). For most botanists, Bryophytes and basal Pteridophytes are the oldest living remnants of eukaryotic plants that colonized the land (Renzaglia *et al.*, 2000).

During the evolutionary process, plants have developed several biologically active substances with various properties and some of them can be applied in both medicine and agriculture. Among these sources of biologically active substances, we find antimicrobial peptides (AMPs) (Barashkova and Rogozhin, 2020). These peptides have been isolated from roots, seeds, flowers, stems and leaves, and have demonstrated activities against phytopathogens as well as bacteria pathogenic to humans (Barbosa Pelegrini *et al.*, 2011; Barashkova and Rogozhin, 2020).

AMPs are defense molecules against pathogens found in several species (Zhao *et al.*, 2017). They are small peptides with 2 to 9 kDa, with about 10 to 50 amino acid residues and most of them contain hydrophobic and cationic residues. Its action mechanism promotes damage to pathogen's cell membrane, inducing pores formation through electrostatic forces, ultimately leading to cell death (Barbosa Pelegrini *et al.*, 2011; Zhao *et al.*, 2017).

AMPs have a positive charge and amphipathic nature, characteristics that are related to their membrane-interacting activity, interacting with microorganisms through electrostatic forces between positive and negatively charged amino acid residues exposed on the cell surface (Guilhelmelli *et al.*, 2013; Nawrot *et al.*, 2014). The main physical-chemical and structural parameters that affect plant AMPs activity are their amino acid residues, net charge, hydrophobicity, amphipathic and structural characteristics. In addition to external factors such as pH, temperature and metal ions (Bhattacharjya and Ramamoorthy, 2009; Li *et al.*, 2021).

AMPs Classification into different families is based on their amino acid sequence, number and spacing of cysteine residues, and disulfide bond positions (Lay and Anderson, 2005; Tam *et al.*, 2015). The main AMPs families found in plants are cyclotides, defensins, heveins, knotins, lipid transfer proteins (LTPs), snakines and thionines. Hevein family (hevein-like) has high biotechnological potential, due to its binding chitin function, polysaccharide found in nematodes, in the cell wall of fungi and in the insect exoskeleton. In this family there are in three groups: 6C-hev, 8C-hev and 10C-hev (Slavokhotova *et al.*, 2017).

Heveins are small peptides, containing 29 to 45 amino acid residues, rich in cysteines, glycines and some aromatic amino acids. Its conserved domain can vary according to cysteines number, being from 6 to 10 residues, and having 3-5 disulfide bonds (Jiménez-Barbero *et al.*, 2006; Slavokhotova *et al.*, 2017).

Initially, hevein peptide is presented in the form of pro-hevein protein which is then processed to generate the final peptide and during this cleavage process the C-terminal domain

is lost and the hevein domain is kept (Berthelot, Peruch and Lecomte, 2016). The C-terminal domain (BARWIN domain) is associated with the hevein-like domain, also known as the chitin-binding domain. Studies have shown that the BARWIN domain has RNAse activity (Bertini *et al.*, 2009; Guevara-Morato *et al.*, 2010; Bai *et al.*, 2013; Huet *et al.*, 2013; Franco *et al.*, 2014; Pereira Menezes *et al.*, 2014; Kim and Hwang, 2015) and the activity is related to two important required histidine residues, one at position 11 and the other at position 113, numbered according to the mature BARWIN sequence (Maia *et al.*, 2021). The BARWIN domain is also related to PR-4 proteins (pathogenesis-related protein-4 family), where they can be divided into two classes. Class I has the N-terminal cysteine-rich domain (this class is known as hevein-like protein) and class II does not have the N-terminal cysteine-rich domain (Bai *et al.*, 2013).

Antimicrobial peptides are a powerful tool for the development of new techniques to control crop losses and, they also represent great potential in the new antibiotic development for human infections treatment (Barbosa Pelegrini *et al.*, 2011). There are few studies on hevein found in the literature, which increases the importance of this study.

The present work aimed to identify heveins presence in Green Algae, Bryophytes, Pteridophytes and Conifers species, and to describe these peptides. Peptides were analyzed for the presence of conserved domains and cleavage sites, phylogenetic analysis and three-dimensional structure.

2. Materials and Methods

2.1 Data collection

Data collection was carried out to identify reference hevein antimicrobial peptide sequences in the literature and then search for these sequences in the UniProt

(http://www.uniprot.org/) and NCBI (http://www.uniprot.org/) databases. www.ncbi.nlm.nih.gov/). Hevein peptide FASTA sequences were downloaded and used as query sequences and in another analysis, according to Berthelot, Peruch and Lecomte, 2016. The sequences used are listed in Table 1.

Table 1. Query Hevein protein sequences

Species	UniProt code
Hevea brasiliensis	Q6JYR0 (Q6JYR0_HEVBR)
Theobroma cacao	A0A061F1Z3 (A0A061F1Z3_THECC)
Capsicum annuum	Q9SEM3 (Q9SEM3_CAPAN)
Nicotiana tabacum	Q41231 (Q41231_TOBAC)
Phaseolus vulgaris	T2DPY4 (T2DPY4_PHAVU)
Arabidopsis thaliana	P43082 (HEVL_ARATH)

2.2 Hevein sequences search

Hevein peptide sequences were submitted to TBLASTN algorithm in the 1k Plant database (https://db.cngb.org/onekp/). Standard sequence used in the work to perform local alignments was *Hevea brasiliensis* sequence (UniProt Code: Q6JYR0).

Local alignments were performed against each available transcriptome in the following plant lineages: Green Algae, Bryophytes, Pteridophytes and Conifers (Table 2). Putative hevein found were organized in FASTA files and used in subsequent analyses.

Table 2. Plant species used for local alignment in the 1k Plant database

Plant Group	Division
Green Algae	Chlorophyta
Bryophytes	Hornworts -Anthocerotophyta
	Bryophyta (mosses)
	Marchantiophyta (liverworts)
Pteridophytes	Equissetales
	Osmundales
	Salviniales
Conifers	Pinaceae

2.3 Conserved domains analysis

Hevein conserved domain analysis was verified through an alignment by BLAST2GO program (Conesa *et al.*, 2005). Then, the result was evaluated by the INTERPROSCAN tool using the PFAM database. Sequences that did not show both hevein conserved domains (PFAM IPRO001002) and BARWIN (PFAM IPRO001153) were excluded from further analyses.

2.4 Sequences translation

Sequences translation that was identified in the previous item were performed using the OrfFinder platform (https://www.ncbi.nlm.nih.gov/orffinder/).

2.5 Physicochemical properties

To evaluate the physicochemical properties of each sequence found in item 2.3, the ProtParam platform (https://web.expasy.org/protparam/) was used. The platform calculates various physicochemical properties that can be deduced from a protein sequence, such as molecular weight, isoelectric point (pI), amino acid composition, atomic composition, and many others (Gasteiger *et al.*, 2005).

2.6 Sequences alignment

To confirm whether the peptides analyzed in item 2.3 were really heveins, a global alignment was performed using the MUSCLE tool of the MEGA X program (version 10.2.6) (Kumar *et al.*, 2018).

2.7 Phylogenetic analysis

After confirming heveins presence in the different lineages of Algae, BBryophytes, PPteridophytes and CConifers phylogenetic analysis was carried out using the MEGA X program (version 10.2.6) (Kumar *et al.*, 2018), using Neighbor-joining method (Saitou and Nei, 1987) and the evolutionary distances were calculated using the Poisson correction method (Zuckerkandl and Pauling, 1965).

2.8 Prediction of the three-dimensional structure

Three-dimensional (3D) structures for all hevein antimicrobial peptides found will be obtained by the SWISS-MODEL platform (https://swissmodel.expasy.org/), a server for comparative homology modeling of three-dimensional protein structures (Waterhouse *et al.*, 2018).

3. Results and discussion

3.1 Local alignment and conserved domain

In the four major groups of terrestrial plants, a total of 536 sequences were identified with some similarity to the standard hevein sequence of the analyzes (P02877). Of these, 91 belong to the Green Algae group (16.98% of the total), 257 belong to the Bryophytes group (47.95% of the total), 88 belong to the Pteridophytes (16.42% of the total) and 100 sequences belong to the Conifer group (18.65% of the total) (Table 3) (Supplementary table 1). However, the presence of the hevein and BARWIN domains together were observed in a few sequences,

from the Pteridophytes group. This may indicate that translocation or deletion events may have joined these two domains first in the Pteridophytes plants.

Table 3. Blats results on the 1k Plants platform.

Kind	Number of samples found	Presence of the Hevein Domain	Presence of the BARWIN Domain	Presence of the 2 domains together	No presence of Hevein-related domain
Green Algae (Chlorophyta)	91	32	1	0	58
Bryophytes - Bryophyta	100	79	0	0	21
- Anthocerotophyta	57	15	7	0	35
- Marchantiophyta	100	69	18	0	13
Pteridophytes - Equisetales	23	9	1	1	10
- Osmundales	43	15	4	3	21
- Salviniales	22	7	0	0	15
Conifers (Pinaceae)	100	34	29	1	36
TOTAL	**536**	**260**	**61**	**5**	**210**

Through the BLASTx alignment in the BLAST2GO program (Conesa *et al.*, 2005), followed by the INTERPROSCAN tool, only Pteridophytes and Conifers presented sequences with both domains together. After identifying these hevein-like peptides, the presence of the start and stop codon of each translated amino acid sample was verified using the ORFfinder tool (https://www.ncbi.nlm.nih.gov/orffinder/). From these, all samples that did not have the codons were excluded, keeping only four samples in the work. Results were shown in table 4.

Table 4. Results found using the BLAST2GO program. Description: Number of amino acids is related to pro hevein. Interpro Ids: hevein domain (IPR001002) or the BARWIN domain (IPR001153).

SEQUENCE	DESCRIPTION	AMOUNT AA	E-VALUE	IDENTITY (%)	INTERPRO IDS (PFAM)
gnl|onekp|CAPN_scaffold_2001180	Pro-hevein, partial [Mucuna pruriens]	192	4,55 e-45	76,79	IPR001153; IPR001002
gnl|onekp|VIBO_scaffold_2074299	pathogenesis-related protein PR-4 [Tripterygium wilfordii]	204	1,03 e-64	79,87	IPR001153; IPR001002
gnl|onekp|RFMZ_scaffold_2007325	pathogenesis-related protein PR-4 [Tripterygium wilfordii]	204	1,16 e-68	73,03	IPR001153; IPR001002
gnl|onekp|UOMY_scaffold_2076116	pathogenesis-related protein PR-4 [Tripterygium wilfordii]	205	1,28 e-75	74,64	IPR001153; IPR001002
gnl|onekp|NPRL_scaffold_2121795	hevein-like preproprotein [Durio zibethinus]	210	2,90 e-81	82,27	IPR001153; IPR001002

BLAST2GO results showed samples similarity with the pathogenesis-related protein-4 (PR-4) family, this family being a proteins group composed by the BARWIN domain. BARWIN domain is associated with chitin binding typical of lectins and PR-4 proteins are grouped based on the presence or absence of this chitin binding domain, characteristic of Hevein peptides (Maia *et al.*, 2021).

A total of 536 samples were identified, and most had only one of the domains discussed, the hevein domain (IPR001002) or the BARWIN domain (IPR001153). IPR009009 domain was also identified, which is the RlpA-like protein domain, double psi beta barrel domain (RlpA-like_DPBB), which is similar to the BARWIN domain (Castillo *et al.*, 1999). This domain was only found in the Bryophyta and Marchantiophyta divisions, which may suggest that this domain was a primary domain found in the hevein of lower plants, in which it underwent amino acid deletions and translocations.

3.2 Physicochemical properties

Using the ProtParam program, it was possible to determine the isoelectric point (p.I.), the molecular weight (Da) and the total number of negatively and positively charged residues of each sample found (Table 5). The physicochemical properties are fundamental for the activity of AMPs against pathogens membrane (LI *et al.*, 2021) and the results found show a large number of positive residues (cationic), rich in arginine or lisin, and negative residues (anionic), rich in aspartic acid or glutamic acid, with an average of 15 positive residues and 16 negative residues. Amino acid sequence has a direct influence on heveins structure and function, and changes in both the amino acid sequence, length and net charge affect the hydrophobicity of the short amphiphilic peptide and directly affect its antibacterial activity and cytotoxicity (Sprules *et al.*, 2004; Gong *et al.*, 2019; Li *et al.*, 2021).

Table 5. Molecular characteristics of found hevein

SAMPLE	AA	p.I	Weight	Waste + (Arg + Lys)	waste - (Asp + Glu)
gnl|onekp|CAPN scaffold 2001180	192	8,58	20355,05	17	11
gnl|onekp|VIBO scaffold 2074299	204	6,84	21673,47	16	16
gnl|onekp|RFMZ scaffold 2007325	204	5,42	21606,14	12	17
gnl|onekp|UOMY scaffold 2076116	205	6,04	21787,57	14	16
gnl|onekp|NPRL_scaffold_2121795	210	5,54	22238,81	15	18
Average	**201**			**15**	**16**

3.3 Global Alignment

Sequences global alignment demonstrated amino acids conservation of the two domains, and the samples of the work present identity with the standard sample of higher plant hevein (Figure 1).

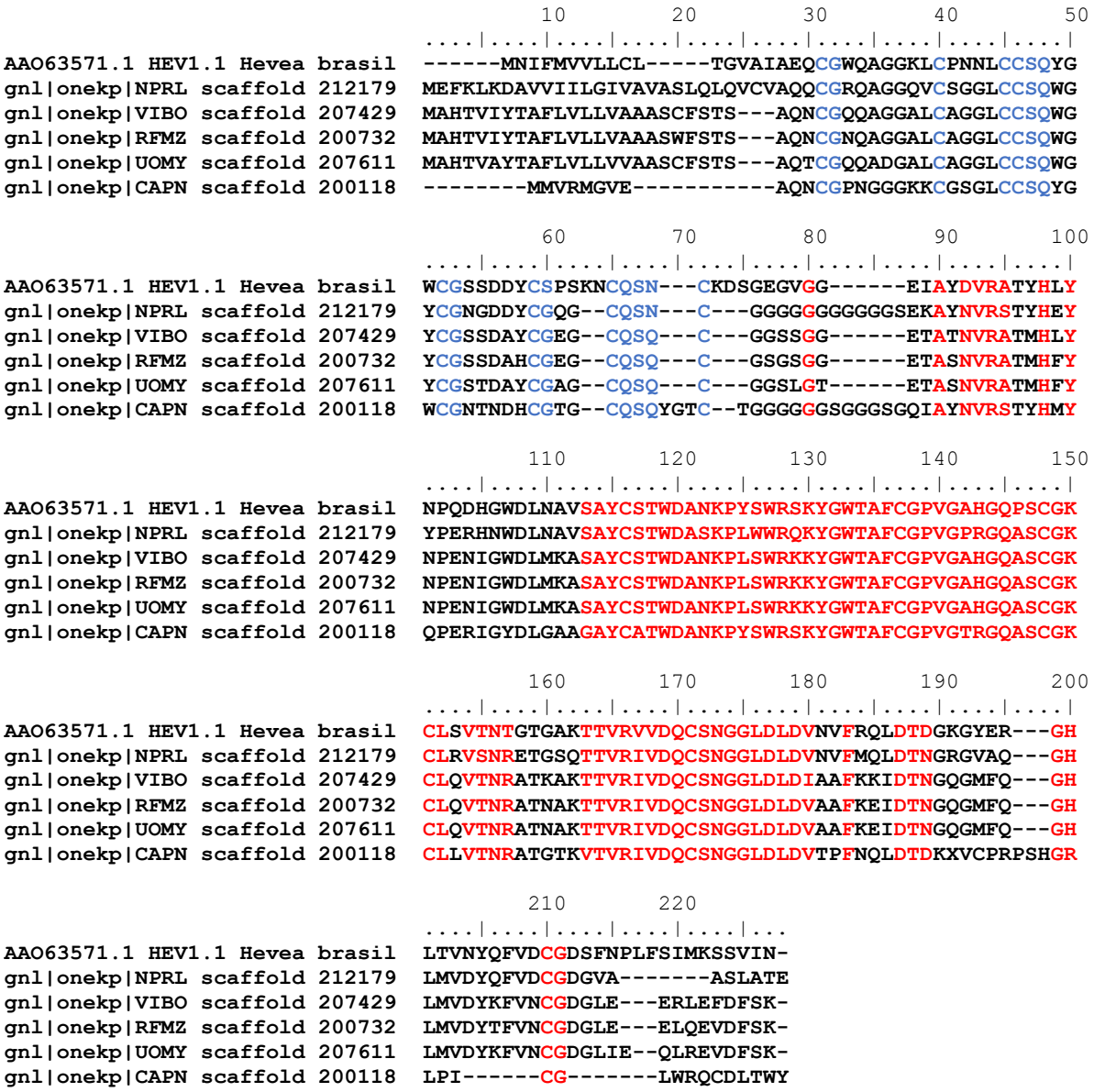

Fig.1. Hevein sequences global alignment. Hevein domain conserved amino acids are in blue and BARWIN domain conserved amino acids are in red.

Regarding hevein domain present in the samples, four disulfide bonds were identified, considering the Pteridophytes group and the Equisetales division, the species presented bonds in positions Cys12-Cys27, Cys21-Cys33, Cys26-Cys40 and Cys44-Cys51, for the species from the Osmundales division all had disulfide bonds in the same positions, being Cys28-Cys43, Cys37-Cys49, Cys42-Cys56 and Cys60-Cys64. In Conifers group of the Pinaceae division the disulfide bonds are in positions Cys31-Cys46, Cys40- Cys52, Cys45-Cys59 and Cys63-Cys67.

In relation to the BARWIN domain, all identified sequences showed the three disulfide bonds characteristic of the domain, with the Pteridophytes group and the Equisetales division the bonds being at positions Cys93-Cys128, Cys114-Cys148 and Cys125-Cys181, for the species of the division Osmundales Cys99-Cys134, Cys120-Cys154 and Cys131-Cys190, and for the Conifer group of the division Pinaceae Cys108-Cys143, Cys129-Cys163 and Cys140-Cys199.

3.3 Phylogenetic analysis

In figure 2 we can see the two orders of the Pteridophytes group and the order of the Conifers group that presented the appropriate result separately. In the following figure (Figure 3) we can see the phylogenetic tree of the sequences of Pteridophytes and the Conifers group with the three standard sequences of the 6C-Hev, 8C-Hev and 10C-Hev groups.

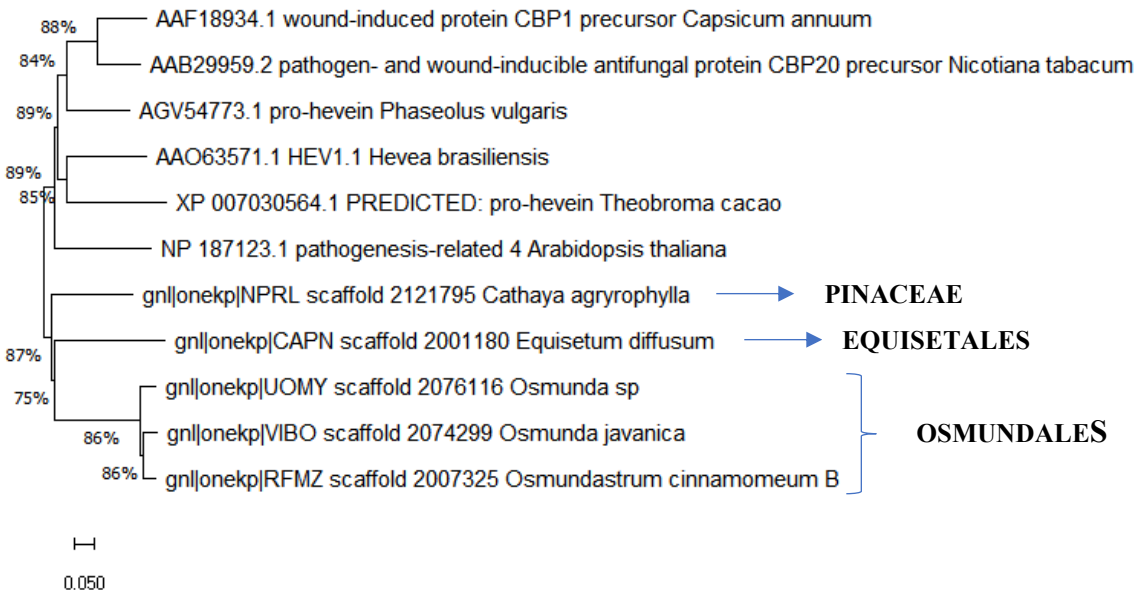

Fig. 2. Phylogenetic analysis from the Pteridophytes and Conifers sequences.

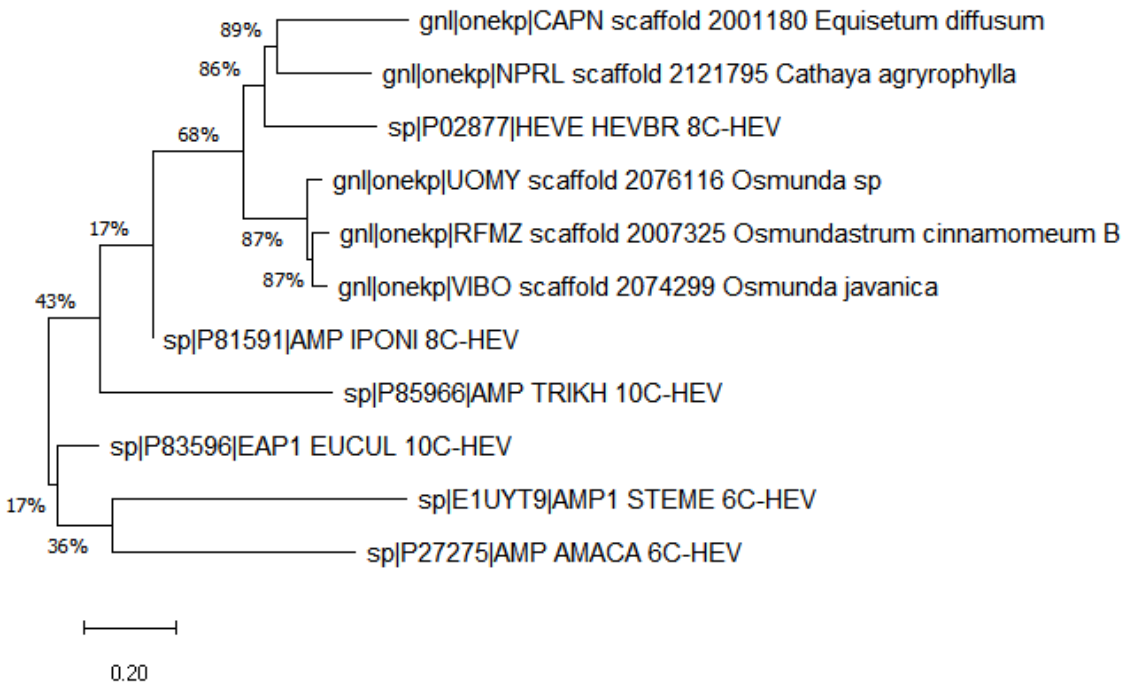

Fig.3. Phylogenetic analysys of Pteridophytes and Conifers with groups 6C-hev, 8C-hev and 10C-hev. The percentage of replicated trees in which the associated taxa clustered in the bootstrap test (1000 replicates) are shown next to the branches.

The results also demonstrate that the hevein peptides identified in higher plants evolved from the Pteridophytes group. It can be predicted that hevein peptides of Pteridophytes, when evolving to higher plants, suffered some cleavage or deletion processes of some amino acids, and even so, they kept the two domains in conservation in most of the hevein sequences identified so far. And in the phylogenetic tree (Figure 2), a strong positive correlation can be observed between the sequences identified in the Pteridophytes and Conifers group with the standard hevein sequences.

3.4 Three-dimensional structure

Hevein three-dimensional structures prediction was performed by homology with SWISS-MODEL online program (Waterhouse *et al.*, 2018) and the BARWIN domain was evaluated, in which it was excluded during the cleavage process of the pro-hevein protein. (Berthelot, Peruch and Lecomte, 2016). In the Pteridophytes Equisetales class it was possible to identify a gene referring to the BARWIN domain with 60.83% identity, code PDB 4jp7.2.A (High resolution structure of a barwin papaya-like protein) and a gene referring to the Hevein domain with 60.00% identity, PDB code 4mpi.1.A (Crystal structure of the chitin-binding module (CBM18) of a chitinase-like protein from *Hevea brasiliensis*) (Figure 4). In the Osmundales class, for the species Osmunda javanica (VIBO_scaffold_2074299) it was possible to identify a gene related to the Hevein domain with 68.29% identity, code PDB 4mpi.1.A (Crystal structure of the chitin binding module (CBM18) of a chitinase-like protein from Hevea brasiliensis) and a gene related to the BARWIN domain with 70.49% identity, code PDB 4jp7.2.A (High resolution structure of a barwin papaya-like protein (crystal form 2) (Figure 5-A). For the species *Osmundastrum cinnamomeum* B it was possible to identify a gene related to the BARWIN domain with 71.31% of identity, code PDB 4jp7.2.A (High resolution structure of a protein similar to papaya barwin (crystal form 2)) and a gene related to the Hevein domain with 60.00% identity, code PDB 1ulm.1.A (Lectin-D2 crystal structure of Pokeweed Lectin-D2 complexed with tri-N-acetylchitotriose) (Figure 5-B). As for the species Osmunda sp. it was possible to identify a gene related to the BARWIN domain with 71.31% identity, code PDB 4jp7.2.A (High resolution structure of a barwin papaya-like protein (crystal form 2)) and a gene related to the Hevein domain with 68.29% identity, PDB code 4mpi.1.A (Crystal structure of the chitin-binding module (CBM18) of a chitinase-like protein from *Hevea brasiliensis*) (Figure 5-C).

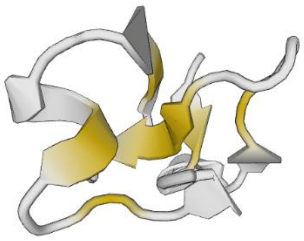

Fig. 4. Three-dimensional structure code 2001180

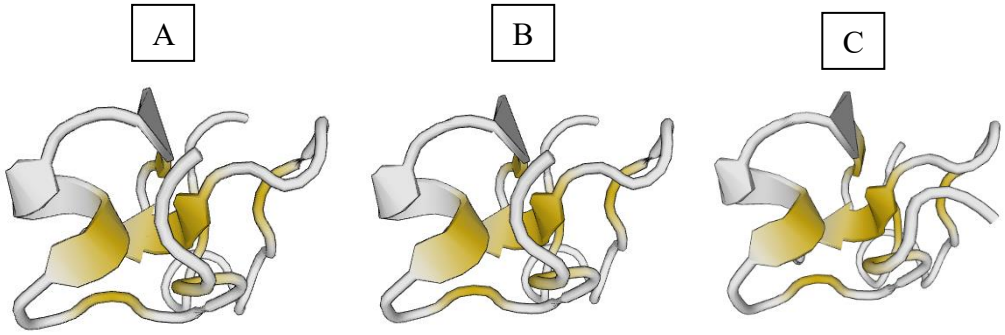

Fig. 5. Three-dimensional structure of the Osmundales class: A (code 2074299), B (code 2007325) and C (code 2076116).

For the Conifer class Pinaceae, a gene related to the BARWIN domain was identified with 67.21% identity, code 4jp7.2.A (High resolution structure of a barwin papaya-like protein (crystal form 2)) and two genes related to the Hevein domain with 43.28% and 40.00% identity , PDB codes 6lnr.1.A (Intact chitinase structure with hevein domain from the plant Simarouba glauca, known for its traditional anti-inflammatory efficacy) and 1ulk. 1.B (Crystal structure of Pokeweed Lectin-C), respectively (Figure 6).

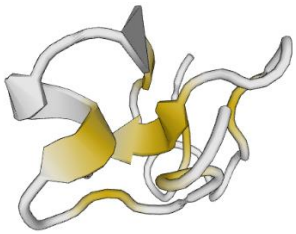

Fig. 6. Three-dimensional structure of the class Pinaceae code 2121795.

4. Conclusion

The present study identified that hevein peptides of higher plants arose from the Pteridophytes class, with 4 sequences being identified in this class and 1 in the Conifer class. Hevein domains were also identified in Green Algae and Bryophytes, but they were being part of other proteins, mainly chitinases. All peptides identified belong to the 8C-Hev class and present the basic hevein physicochemical properties and tridimensional structures.

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References

Bai, S. *et al.* (2013) 'A PR-4 gene identified from Malus domestica is involved in the defense responses against Botryosphaeria dothidea', *Plant Physiology and Biochemistry*, 62, pp. 23–32. doi: 10.1016/j.plaphy.2012.10.016.

Barashkova, A. S. and Rogozhin, E. A. (2020) 'Isolation of antimicrobial peptides from

different plant sources: Does a general extraction method exist?', *Plant Methods*, 16(1), pp. 1–10. doi: 10.1186/s13007-020-00687-1.

Barbosa Pelegrini, P. *et al.* (2011) 'Antibacterial peptides from plants: What they are and how they probably work', *Biochemistry Research International*, 2011. doi: 10.1155/2011/250349.

Berthelot, K., Peruch, F. and Lecomte, S. (2016) 'Highlights on Hevea brasiliensis (pro)hevein proteins', *Biochimie*, 127, pp. 258–270. doi: 10.1016/j.biochi.2016.06.006.

Bertini, L. *et al.* (2009) 'Structural basis of the antifungal activity of wheat PR4 proteins', *FEBS Letters*, 583(17), pp. 2865–2871. doi: 10.1016/j.febslet.2009.07.045.

Bhattacharjya, S. and Ramamoorthy, A. (2009) 'Multifunctional host defense peptides: Functional and mechanistic insights from NMR structures of potent antimicrobial peptides', *FEBS Journal*, 276(22), pp. 6465–6473. doi: 10.1111/j.1742-4658.2009.07357.x.

Castillo, R. M. *et al.* (1999) 'A six-stranded double-psi β barrel is shared by several protein superfamilies', *Structure*, 7(2), pp. 227–236. doi: 10.1016/S0969-2126(99)80028-8.

Conesa, A. *et al.* (2005) 'Blast2GO: A universal tool for annotation, visualization and analysis in functional genomics research', *Bioinformatics*, 21(18), pp. 3674–3676. doi: 10.1093/bioinformatics/bti610.

Franco, F. P. *et al.* (2014) 'The sugarcane defense protein SUGARWIN2 causes cell death in Colletotrichum falcatum but not in non-pathogenic fungi', *PLoS ONE*, 9(3), pp. 1–8. doi: 10.1371/journal.pone.0091159.

Gasteiger, E. *et al.* (2005) 'The Proteomics Protocols Handbook', *The Proteomics Protocols Handbook*, pp. 571–608. doi: 10.1385/1592598900.

Gong, H. *et al.* (2019) 'Hydrophobic Control of the Bioactivity and Cytotoxicity of de Novo-Designed Antimicrobial Peptides', *ACS Applied Materials and Interfaces*, 11(38), pp. 34609–34620. doi: 10.1021/acsami.9b10028.

Guevara-Morato, M. Á. *et al.* (2010) 'Characterization of a pathogenesis-related protein 4

(PR-4) induced in Capsicum chinense L3 plants with dual RNase and DNase activities', *Journal of Experimental Botany*, 61(12), pp. 3259–3271. doi: 10.1093/jxb/erq148.

Guilhelmelli, F. *et al.* (2013) 'Antibiotic development challenges: The various mechanisms of action of antimicrobial peptides and of bacterial resistance', *Frontiers in Microbiology*, 4(DEC), pp. 1–12. doi: 10.3389/fmicb.2013.00353.

Huet, J. *et al.* (2013) 'High-resolution structure of a papaya plant-defence barwin-like protein solved by in-house sulfur-SAD phasing', *Acta Crystallographica Section D: Biological Crystallography*, 69(10), pp. 2017–2026. doi: 10.1107/S0907444913018015.

Jiménez-Barbero, J. *et al.* (2006) *Hevein Domains: An Attractive Model to Study Carbohydrate-Protein Interactions at Atomic Resolution*, *Advances in Carbohydrate Chemistry and Biochemistry*. doi: 10.1016/S0065-2318(06)60007-3.

Kim, N. H. and Hwang, B. K. (2015) 'Pepper pathogenesis-related protein 4c is a plasma membrane-localized cysteine protease inhibitor that is required for plant cell death and defense signaling', *Plant Journal*, 81(1), pp. 81–94. doi: 10.1111/tpj.12709.

Kumar, S. *et al.* (2018) 'MEGA X: Molecular evolutionary genetics analysis across computing platforms', *Molecular Biology and Evolution*, 35(6), pp. 1547–1549. doi: 10.1093/molbev/msy096.

Lay, F. and Anderson, M. (2005) 'Defensins - Components of the Innate Immune System in Plants', *Current Protein & Peptide Science*, 6(1), pp. 85–101. doi: 10.2174/1389203053027575.

Li, J. *et al.* (2021) 'Plant antimicrobial peptides: structures, functions, and applications', *Botanical Studies*, 62(1). doi: 10.1186/s40529-021-00312-x.

Ligrone, R. (2019) *Biological Innovations that Built the World*, *Biological Innovations that Built the World*. doi: 10.1007/978-3-030-16057-9.

Maia, L. B. L. *et al.* (2021) 'Structural and Evolutionary Analyses of PR-4 SUGARWINs

Points to a Different Pattern of Protein Function', *Frontiers in Plant Science*, 12(September), pp. 1–13. doi: 10.3389/fpls.2021.734248.

Nawrot, R. *et al.* (2014) 'Plant antimicrobial peptides', *Folia Microbiologica*, 59(3), pp. 181–196. doi: 10.1007/s12223-013-0280-4.

Pereira Menezes, S. *et al.* (2014) 'The pathogenesis-related protein PR-4b from Theobroma cacao presents RNase activity, Ca2+ and Mg2+ dependent-DNase activity and antifungal action on Moniliophthora perniciosa', *BMC Plant Biology*, 14(1), pp. 1–21. doi: 10.1186/1471-2229-14-161.

Renzaglia, K. S. *et al.* (2000) 'Vegetative and reproductive innovations of early land plants: Implications for a unified phylogeny', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 355(1398), pp. 769–793. doi: 10.1098/rstb.2000.0615.

Saitou, N. and Nei, M. (1987) 'The neighbor-joining method: a new method for reconstructing phylogenetic trees.', *Molecular biology and evolution*, 4(4), pp. 406–425. doi: 10.1093/oxfordjournals.molbev.a040454.

Slavokhotova, A. A. *et al.* (2017) 'Hevein-like antimicrobial peptides of plants', *Biochemistry (Moscow)*, 82(13), pp. 1659–1674. doi: 10.1134/S0006297917130065.

Sprules, T. *et al.* (2004) 'NMR solution structure of the precursor for carnobacteriocin B2, an antimicrobial peptide from Carnobacterium piscicola: Implications of the α-helical leader section for export and inhibition of type IIa bacteriocin activity', *European Journal of Biochemistry*, 271(9), pp. 1748–1756. doi: 10.1111/j.1432-1033.2004.04085.x.

Tam, J. P. *et al.* (2015) 'Antimicrobial peptides from plants', *Pharmaceuticals*, 8(4), pp. 711–757. doi: 10.3390/ph8040711.

Waterhouse, A. *et al.* (2018) 'SWISS-MODEL: Homology modelling of protein structures and complexes', *Nucleic Acids Research*, 46(W1), pp. W296–W303. doi: 10.1093/nar/gky427.

Wodniok, S. *et al.* (2011) 'Origin of land plants: Do conjugating Green Algae hold the key?', *BMC Evolutionary Biology*, 11(1), p. 104. doi: 10.1186/1471-2148-11-104.

Zhao, D. *et al.* (2017) 'Analysis of ribosome-associated mRNAs in rice reveals the importance of transcript size and GC content in translation', *G3: Genes, Genomes, Genetics*, 7(1), pp. 203–219. doi: 10.1534/g3.116.036020.

Zuckerkandl, E. and Pauling, L. (1965) 'Evolutionary Divergence and Convergence in Proteins', *Evolving Genes and Proteins*, pp. 97–166. doi: 10.1016/b978-1-4832-2734-4.50017-6.

Supplementary table 1. Identifies species with hevein and/or barwin domain by TBLASTN and number of sequences.

Division	Species	Number of sequences
Chorophytas	*Aphanochaete repens*	1
	Asteromonas gracilis	2
	Carteria crucifera	4
	Carteria obtuse	1
	Chlamydomonas	10
	Chloromonas oogama	1
	Chloromonas perforate	2
	Chloromonas reticulata	1
	Chloromonas rosae	1
	Chloromonas subdivisa	1
	Cylindrocapsa geminella	3
	Dunaliella primolecta	1
	Dunaliella salina	2
	Dunaliella tertiolecta	1
	Eremosphaera viridis	2
	Ettlia oleoabundans	2
	Fritschiella tuberosa	3
	Hafniomonas reticulata	1
	Leptosira obovate	1
	Mantoniella squamata	1
	Microthamnion kuetzingianum	2
	N/A	2
	Neodesmus pupukensis	1
	Oedogonium cardiacum	2
	Oedogonium foveolatum	1
	Oltmannsiellopsis viridis	1
	Parachlorella kessleri	1
	Pediastrum duplex	11
	Phacotus lenticularis	4
	Picochlorum atomus	1
	Pleurastrum insigne	2
	Pseudoscourfieldia marina	1
	Pteromonas angulosa	1
	Stigeoclonium helveticum	1
	Tetradesmus dimorphus	2
	Uronema belkae	26
Anthocerotophyta	*Anthoceros agrestis*	8
	Leiosporoceros dussii	5
	Megaceros flagellaris	4
	Nothoceros vincentianus	2
	Paraphymatoceros hallii	4
	Phaeoceros carolinianus	39
	Phaeomegaceros coriaceus	1
Bryophyta	*Andreaea rupestris*	3
	Atrichum angustatum	3
	Aulacomnium heterostichum	1
	Bryum argenteum	3
	Buxbaumia aphylla	4
	Ceratodon purpureus	1
	Climacium dendroides	2

	Dicranum scoparium	2
	Diphyscium foliosum	2
	Encalypta streptocarpa	5
	Fontinalis antipyretica	2
	Frisvollia varia	2
	Hedwigia ciliate	1
	Leucobryum albidum	1
	Leucobryum glaucum	1
	Leucodon julaceus	4
	Loeskeobryum brevirostre	3
	N/A	5
	Neckera douglasii	7
	Niphotrichum elongatum	2
	Philonotis fontana	7
	Plagiomnium insigne	1
	Polytrichum commune	2
	Pseudotaxiphyllum elegans	3
	Pulvigera lyellii	2
	Rhynchostegium serrulatum	1
	Rosulabryum capillare	2
	Scouleria aquatica	2
	Sphagnum lescurii	9
	Sphagnum palustre	4
	Sphagnum recurvum	4
	Stereodon subimponens	5
	Syntrichia princeps	3
	Takakia lepidozioides	2
	Tetraphis pellucida	6
	Timmia austriaca	2
Marchantiophyta	*Barbilophozia barbata*	1
	Bazzania trilobata	7
	Calypogeia fissa	4
	Conocephalum conicum	2
	Lunularia cruciate	6
	Marchantia emarginata	9
	Marchantia paleacea	15
	Marchantia polymorpha	5
	Monoclea gottschei	3
	N/A	8
	Noteroclada confluens	1
	Odontoschisma prostratum	2
	Pallavicinia lyellii	3
	Pellia epiphylla	5
	Pellia neesiana	6
	Porella navicularis	4
	Porella pinnata	8
	Ptilidium pulcherrimum	3
	Radula lindenbergiana	4
	Ricciocarpos natans	7
	Scapania nemorea	3
	Sphaerocarpos texanus	4
Equisetales	*Equisetum diffusum*	16
	Equisetum hyemale	9
Osmundales	N/A	18
	Osmundastrum cinnamomeum	13
	Plenasium javanicum	14

Salviniales	*Azolla caroliniana*	9
	Pilularia globulifera	14
Pinaceae	*Abies lasiocarpa*	5
	Cathaya argyrophylla	9
	Cedrus libani	10
	Keteleeria evelyniana	7
	Larix griffithii var. speciosa	8
	Nothotsuga longibracteata	11
	Picea engelmannii	5
	Pinus jeffreyi	4
	Pinus parviflora	8
	Pinus ponderosa	4
	Pinus radiata	4
	Pseudolarix amabilis	10
	Pseudotsuga sinensis var. wilsoniana	7
	Tsuga heterophylla	10

4 CONCLUSÃO

O presente estudo possibilitou identificar que os peptídeos Heveínas das plantas superiores surgiram da classe das Pteridófitas, sendo identificado 4 sequências nessa classe e 1 na classe das Coníferas. Também foi possível identificar sequências de peptídeos Heveínas nas demais classes, Algas verdes e Briófitas, mas esses não são considerados um heveinas completas pois não apresentavam os dois domínios essências de uma heveína, domínio Heveína e BARWIN. Os domínios heveina presentes nas algas e Briófitas fazem parte de outras proteínas, principalmente quitinases.

Por meio da árvore filogenética foi determinado que as sequências identificadas no estudo pertencem a classe 8C-Hev e essas sequências também apresentam as propriedades físico-químicas e estrutura tridimensional básicas das heveínas.

REFERÊNCIAS

ARCHER, B. L. The proteins of Hevea brasiliensis Latex. 4. Isolation and characterization of crystalline hevein. **The Biochemical journal**, v. 75, p. 236–240, 1960. https://doi.org/10.1042/bj0750236

BAI, S. et al. A PR-4 gene identified from Malus domestica is involved in the defense responses against Botryosphaeria dothidea. **Plant Physiology and Biochemistry**, v. 62, p. 23–32, 2013. https://doi.org/10.1016/j.plaphy.2012.10.016

BARASHKOVA, A. S.; ROGOZHIN, E. A. Isolation of antimicrobial peptides from different plant sources: Does a general extraction method exist? **Plant Methods**, v. 16, n. 1, p. 1–10, 2020. https://doi.org/10.1186/s13007-020-00687-1

BARBOSA PELEGRINI, P. et al. Antibacterial peptides from plants: What they are and how they probably work. **Biochemistry Research International**, v. 2011, 2011. https://doi.org/10.1155/2011/250349

BENNICI, A. Origin and early evolution of land plants: Problems and considerations. **Communicative & integrative biology**, v. 1, n. 2, p. 212–8, 2008. https://doi.org/10.4161/cib.1.2.6987

BERTHELOT, K.; PERUCH, F.; LECOMTE, S. Highlights on Hevea brasiliensis (pro)hevein proteins. **Biochimie**, v. 127, p. 258–270, 2016. https://doi.org/10.1016/j.biochi.2016.06.006

BERTINI, L. et al. Structural basis of the antifungal activity of wheat PR4 proteins. **FEBS Letters**, v. 583, n. 17, p. 2865–2871, 2009. https://doi.org/10.1016/j.febslet.2009.07.045

BHATTACHARJYA, S.; RAMAMOORTHY, A. Multifunctional host defense peptides: Functional and mechanistic insights from NMR structures of potent antimicrobial peptides. **FEBS Journal**, v. 276, n. 22, p. 6465–6473, 2009. https://doi.org/10.1111/j.1742-4658.2009.07357.x

BROEKAERT, W. F. et al. Antimicrobial Peptides from Amaranthus Caudatus Seeds with Sequence Homology to the Cysteine/Glycine-Rich Domain of Chitin-Binding Proteins. **Biochemistry**, v. 31, n. 17, p. 4308–4314, 1992. https://doi.org/10.1021/bi00132a023

FERNANDEZ DE CALEYA, R. et al. Susceptibility of phytopathogenic bacteria to wheat purothionins in vitro. **Applied microbiology**, v. 23, n. 5, p. 998–1000, 1972. https://doi.org/10.1128/am.23.5.998-1000.1972

FRANCO, F. P. et al. The sugarcane defense protein SUGARWIN2 causes cell death in Colletotrichum falcatum but not in non-pathogenic fungi. **PLoS ONE**, v. 9, n. 3, p. 1–8, 2014. https://doi.org/10.1371/journal.pone.0091159

GERNANDT, D. S. et al. Incorporating fossils into the Pinaceae tree of life. **American Journal of Botany**, v. 105, n. 8, p. 1329–1344, 2018. https://doi.org/10.1002/ajb2.1139

GUEVARA-MORATO, M. Á. et al. Characterization of a pathogenesis-related protein 4 (PR-4) induced in Capsicum chinense L3 plants with dual RNase and DNase activities. **Journal of Experimental Botany**, v. 61, n. 12, p. 3259–3271, 2010. https://doi.org/10.1093/jxb/erq148

GUILHELMELLI, F. et al. Antibiotic development challenges: The various mechanisms of action of antimicrobial peptides and of bacterial resistance. **Frontiers in Microbiology**, v. 4, n. DEC, p. 1–12, 2013. https://doi.org/10.3389/fmicb.2013.00353

HORI, H.; LIM, B.-L.; OSAWA, S. Evolution of green plants as deduced from 5S rRNA sequences. **Proceedings of the National Academy of Sciences**, v. 82, n. 3, p. 820–823, 1985. https://doi.org/10.1073/pnas.82.3.820

HUANG, R. H. et al. Two novel antifungal peptides distinct with a five-disulfide motif from the bark of Eucommia ulmoides Oliv. **FEBS Letters**, v. 521, n. 1–3, p. 87–90, 2002. https://doi.org/10.1016/S0014-5793(02)02829-6

HUET, J. et al. High-resolution structure of a papaya plant-defence barwin-like protein solved by in-house sulfur-SAD phasing. **Acta Crystallographica Section D: Biological Crystallography**, v. 69, n. 10, p. 2017–2026, 2013. https://doi.org/10.1107/S0907444913018015

JIMÉNEZ-BARBERO, J. et al. Hevein Domains: An Attractive Model to Study Carbohydrate-Protein Interactions at Atomic Resolution. **Advances in carbohydrate chemistry and biochemistry**, v. 60, p. 303-354, 2006. https://doi.org/10.1016/S0065-2318(06)60007-3

JONATHAN SHAW, A.; SZÖVÉNYI, P.; SHAW, B. Bryophyte diversity and evolution: Windows into the early evolution of land plants. **American Journal of Botany**, v. 98, n. 3, p. 352–369, 2011. https://doi.org/10.3732/ajb.1000316

KIM, N. H.; HWANG, B. K. Pepper pathogenesis-related protein 4c is a plasma membrane-localized cysteine protease inhibitor that is required for plant cell death and defense signaling. **Plant Journal**, v. 81, n. 1, p. 81–94, 2015. https://doi.org/10.1111/tpj.12709

KOO, J. C. et al. Two hevein homologs isolated from the seed of Pharbitis nil L. exhibit potent antifungal activity. **Biochimica et Biophysica Acta - Protein Structure and Molecular Enzymology**, v. 1382, n. 1, p. 80–90, 1998. https://doi.org/10.1016/S0167-4838(97)00148-9

KOONIN, E. V. Orthologs, paralogs, and evolutionary genomics. **Annual Review of Genetics**, v. 39, p. 309–338, 2005. https://doi.org/10.1146/annurev.genet.39.073003.114725

KREFT, H. et al. Contrasting environmental and regional effects on global pteridophyte and seed plant diversity. **Ecography**, v. 33, n. 2, p. 408–419, 2010. https://doi.org/10.1111/j.1600-0587.2010.06434.x

LAY, F.; ANDERSON, M. Defensins - Components of the Innate Immune System in Plants. **Current Protein & Peptide Science**, v. 6, n. 1, p. 85–101, 2005. https://doi.org/10.2174/1389203053027575

LI, J. et al. Plant antimicrobial peptides: structures, functions, and applications. **Botanical Studies**, v. 62, n. 1, 2021. https://doi.org/10.1186/s40529-021-00312-x

LIGRONE, R. Land Plants. In: Biological Innovations that Built the World. **Springer International Publishing**, p. 347-397, 2019. https://doi.org/10.1007/978-3-030-16057-9

LIN, C. P. et al. Comparative chloroplast genomics reveals the evolution of Pinaceae genera and subfamilies. **Genome Biology and Evolution**, v. 2, n. 1, p. 504–517, 2010. https://doi.org/10.1093/gbe/evq036

MAIA, L. B. L. et al. Structural and Evolutionary Analyses of PR-4 SUGARWINs Points to a Different Pattern of Protein Function. **Frontiers in Plant Science**, v. 12, n. September, p. 1–13, 2021. https://doi.org/10.3389/fpls.2021.734248

MENDÃO, A. S. N. As grandes transformações das plantas ao longo da história da Terra. **Faculdade de Ciências e Tecnologia Departamento de Ciências da Terra**, p. 338, 2007.

NAWROT, R. et al. Plant antimicrobial peptides. **Folia Microbiologica**, v. 59, n. 3, p. 181–196, 2014. https://doi.org/10.1042/bj0750236

ODINTSOVA, T. et al. Hevein-like antimicrobial peptides wamps: Structure-function relationship in antifungal activity and sensitization of plant pathogenic fungi to tebuconazole by WAMP-2-derived peptides. **International Journal of Molecular Sciences**, v. 21, n. 21, p. 1–26, 2020. https://doi.org/10.3390/ijms21217912

PEREIRA MENEZES, S. et al. The pathogenesis-related protein PR-4b from Theobroma cacao presents RNase activity, Ca2+ and Mg2+ dependent-DNase activity and antifungal action on Moniliophthora perniciosa. **BMC Plant Biology**, v. 14, n. 1, p. 1–21, 2014. https://doi.org/10.1186/1471-2229-14-161

PESTANA-CALSA MC, RIBEIRO IL, CALSA T JR. Bioinformatics-coupled molecular approaches for unravelling potential antimicrobial peptides coding genes in Brazilian native and crop plant species. **Current protein & peptide science**, v. 11, n. 3, p 199-209, 2010. https://doi.org/10.2174/138920310791112138

PRAVEEN, A.; PANDEY, V. C. Pteridophytes in phytoremediation. **Environmental Geochemistry and Health**, v. 42, n. 8, p. 2399–2411, 2020. https://doi.org/10.1007/s10653-019-00425-0

RENZAGLIA, K. S. et al. Vegetative and reproductive innovations of early land plants: Implications for a unified phylogeny. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 355, n. 1398, p. 769–793, 2000. https://doi.org/10.1098/rstb.2000.0615

SLAVOKHOTOVA, A. A. et al. Hevein-like antimicrobial peptides of plants. **Biochemistry (Moscow)**, v. 82, n. 13, p. 1659–1674, 2017. https://doi.org/10.1134/S0006297917130065

TAM, J. P. et al. Antimicrobial peptides from plants. **Pharmaceuticals**, v. 8, n. 4, p. 711–757, 2015. https://doi.org/10.3390/ph8040711

UMEN, J. G. Green Algae and the Origins of Multicellularity in the Plant Kingdom. **Cold Spring Harbor Perspectives in Biology**, v. 6, n. 11, p. 1–27, 2014. https://doi.org/10.1101/cshperspect.a016170

WALUJONO, K. et al. Amino acid sequence of hevein BT - Int. Rubber Conference, Kuala Lumpur, vol II. In: Kuala Lumpur: Rubber Res. Inst. Malaysia, 1975.

WODNIOK, S. et al. Origin of land plants: Do conjugating Green Algae hold the key? **BMC Evolutionary Biology**, v. 11, n. 1, p. 104, 2011. https://doi.org/10.1186/1471-2148-11-104

ZHAO, D. et al. Analysis of ribosome-associated mRNAs in rice reveals the importance of transcript size and GC content in translation. **G3: Genes, Genomes, Genetics**, v. 7, n. 1, p. 203–219, 2017. https://doi.org/10.1534/g3.116.036020

APÊNDICE 1

Tabela 4 – Resultado Blast2GO

SEQUÊNCIA	DESCRIÇÃO	QUAN. AA	E-VALUE	IDENTIDADE (%)	INTERPRO IDS (PFAM)
gnl|onekp|CAPN_scaffold_2001180	Pro-hevein, partial [Mucuna pruriens]	192	4,55 e-^{45}	76,79	IPR001153; IPR001002
gnl|onekp|VIBO_scaffold_2074299	pathogenesis-related protein PR-4 [Tripterygium wilfordii]	204	1,03 e-^{64}	79,87	IPR001153; IPR001002
gnl|onekp|RFMZ_scaffold_2007325	pathogenesis-related protein PR-4 [Tripterygium wilfordii]	204	1,16 e-^{68}	73,03	IPR001153; IPR001002
gnl|onekp|UOMY_scaffold_2076116	pathogenesis-related protein PR-4 [Tripterygium wilfordii]	205	1,28 e-^{75}	74,64	IPR001153; IPR001002
gnl|onekp|NPRL_scaffold_2121795	hevein-like preproprotein [Durio zibethinus]	210	2,90 e-^{81}	82,27	IPR001153; IPR001002

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Reference to a journal publication with an article number:

Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2018. The art of writing a scientific article. Heliyon. 19, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.

Reference to a book:

Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.

Reference to a chapter in an edited book:

Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.

Reference to a website:

Cancer Research UK, 1975. Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/ (accessed 13 March 2003).

Reference to a dataset:

[dataset] Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T., 2015. Mortality data for Japanese oak wilt disease and surrounding forest compositions. Mendeley Data, v1. https://doi.org/10.17632/xwj98nb39r.1.

Reference to software:

Coon, E., Berndt, M., Jan, A., Svyatsky, D., Atchley, A., Kikinzon, E., Harp, D., Manzini, G., Shelef, E., Lipnikov, K., Garimella, R., Xu, C., Moulton, D., Karra, S., Painter, S., Jafarov, E., & Molins, S., 2020. Advanced Terrestrial Simulator (ATS) v0.88 (Version 0.88). Zenodo. https://doi.org/10.5281/zenodo.3727209.

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