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Genome-wide in silico analysis of α-hairpinin in tepary bean (*Phaseolus acutifolius*)

Abstract

This study characterized antimicrobial peptides of the α-hairpinin family in the genome of tepary bean (*Phaseolus acutifolius*). Ten sequences with the α-hairpinin motif (PaHrp1-10) were identified. These motifs were found in proteins with Kunitz_legume, Peptidase_C1/Inhibitor_I29, and Root_cap domains. Most PaHrps exhibited a signal peptide in the N-terminal region, except for PaHrp1, PaHrp5, and PaHrp10. The α-hairpinin motifs varied in length from 19 to 34 amino acids, with molecular weights ranging from 2.08 kDa to 3.74 kDa, and isoelectric points ranging from 3.58 to 9.13. In silico analyses showed that PaHrp7 has antifungal, antibacterial, and antiviral activities, while PaHrp1 and PaHrp9 exhibited antifungal and antibacterial activities. PaHrp6 demonstrated antifungal and antiviral activities, and PaHrp8 showed antibacterial and antiviral activities. PaHrp2 and PaHrp4 exhibited only antiviral activity; PaHrp5 and PaHrp10 showed antifungal activity; and PaHrp3 exhibited only antibacterial activity. The 10 PaHrp genes were mapped to six of the 11 chromosomes of the species, with Chr11 anchoring five genes (PaHrp5-9). The PaHrp motifs exhibited four conserved cysteine residues, forming two disulfide bridges characteristic of α-hairpinins. This study expands knowledge about antimicrobial peptides in *P. acutifolius*, suggesting their potential for therapeutic and biotechnological applications in agriculture and human health.

Keywords: In silico analysis, α-hairpinin famil, genomic, antimicrobial peptides

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Introduction

The α-hairpinin family of antimicrobial peptides was recently identified in plants, possessing a specific signature motif (C1XXXC2-X(n)-C3XXXC4).[1–3] α-hairpinin proteins exhibit a secondary structure composed of two antiparallel α-helices connected by a loop (helix-loop-helix), stabilized in the tertiary structure by two disulfide bonds (Cys1-Cys4/Cys2-Cys3).[4–6] α-hairpinin peptides are sources of bioactive molecules with a wide structural diversity and exhibit various biological activities.[3] They demonstrate antifungal and antibacterial activity in several species, including barnyard grass (*Echinochloa crus-galli*),[7] chickweed (*Stellaria media*),[8] macadamia (*Macadamia integrifolia*),[9] and wheat (*Triticum kiharae*).[5] Among the α-hairpinin peptides with trypsin inhibitory activity are BWI-2a and BWI-2b from buckwheat (*Fagopyrum esculentum*),[4] C2 from pumpkin (*Cucurbita maxima*)[10] and VhTI from *Veronica hederifolia*.[11] Additionally, some α-hairpinins possess ribosome-inactivating activity, such as Luffin P1 from sponge gourd (*Luffa cylindrica*).[12] Due to these diverse biological activities, this family of short peptides, with potent antimicrobial activities and a relatively simple structure, is a promising candidate for biotechnological applications.[2] Tepary bean (*Phaseolus acutifolius*) is a sister species of common bean (*Phaseolus vulgaris* L.), highly adapted to heat and drought.[13–15] Native to the Sonoran Desert, this species possesses genetic characteristics that make it resilient to moderate thermal stress conditions, with a reduced genetic repertoire for disease resistance, consistent with its adaptation to arid and hot environments.[16,17]

The significant genetic similarity and shared content among Phaseolus species provide a valuable foundation for engineering climate adaptation in common beans, a critical crop for food security.[18,19] Additionally, tepary bean shows promise as an alternative protein source for regions facing hotter and drier conditions. However, its wider adoption remains limited due to challenges like insufficient marketing and less appealing culinary qualities.[18] The objective of this study was to characterize the antimicrobial peptides of the α-hairpinin family present in the genome of tepary bean, analyzing their physicochemical characteristics, in silico biological activities, and three-dimensional structure.

Material and methods

Genome retrieval and α-hairpinin identification

The genome of *P. acutifolius* was obtained from Phytozome (ID: 580, NCBI taxonomy ID: 33129).[20] The identification of sequences belonging to the α-hairpinin family was conducted using the Cysmotif Searcher[21] with a cysteine motif pattern (C1XXXC2-X(n)-C3XXXC4) characteristic of this family.[1,22]

Sequence characterization

Candidate sequences were subjected to InterProScan (https://www.ebi.ac.uk/interpro/search/sequence/) to identify characteristic conserved domains of the α-hairpinin class. The presence of signal peptides was predicted using SignalP 6.0,[23] while subcellular localization was predicted using the ProtComp 9.0 tool from Softberry Inc. (Mount Kisco, NY, USA). Subsequently, sequences were evaluated for molecular weight (MW), isoelectric point (pI), and grand average of hydropathicity (GRAVY) using ExPASy ProtParam software (http://web.expasy.org/protparam/). Conserved sequence patterns were analyzed through multiple sequence alignment using Clustal Omega.[24]

In silico biological activity analysis

Candidate sequences were evaluated for antimicrobial activity using CAMPR3, employing four different algorithms: Support Vector Machine (SVM), Random Forest (RF), Artificial Neural Network (ANN), and Discriminant Analysis (DA),[25] in addition to ClassAMP.[26]

Antifungal activity was analyzed using Antifp[27] and antiviral activity using Meta-iVP.[28] Activity was considered significant for predictions with a score greater than 0.5 (50%).

Results and discussion

After mining, 10 sequences containing the α-hairpinin motif from *P. acutifolius* (PaHrp1-10) were identified. This motif was found in proteins with Kunitz_legume (1), Peptidase_C1/Inhibitor_I29 (3), and Root_cap (6) domains (Table 1-S1), as observed in plant species such as the legume *Peltophorum dubium*.[29] Most PaHrps exhibited a signal peptide in the N-terminal region, except for PaHrp1, PaHrp5, and PaHrp10. The α-hairpinin motifs varied in length from 19 (PaHrp7) to 34 (PaHrp2-4) amino acids (aa), with an average length of 31 aa. The molecular weight of the proteins ranged from 2.08 kDa (PaHrp10) to 3.74 kDa (PaHrp9), and the isoelectric point (pI) ranged from 3.58 (PaHrp4) to 9.13 (PaHrp7 and PaHrp9) (Table 1). α-hairpinins are low molecular weight antimicrobial peptides (10 kDa), and so far, few α-hairpinins have been reported, including MBP-1, MiAMP2s, Ec-AMP1, Luffin P1, VhT1, BWI-2c, Tk-AMP-Xs, and Sm-AMP-X.[2,3] In lima bean (*Phaseolus lunatus*), PlHrp1 was present in 41 aminoacids, with a pI of 6.28 and molecular mass of 4.81.[6] The 10 PaHrp genes were mapped onto the chromosomes of *P. acutifolius* (Figure 1). Out of the species' 11 chromosomes, only 6 contain PaHrp genes. Chromosome 11 (Chr11) harbors 5 genes (PaHrp5-9), while the remaining chromosomes each contain only one gene: Chr01 (PaHrp4), Chr04 (PaHrp1), Chr06 (PaHrp10), Chr07 (PaHrp2), and Chr09 (PaHrp3).

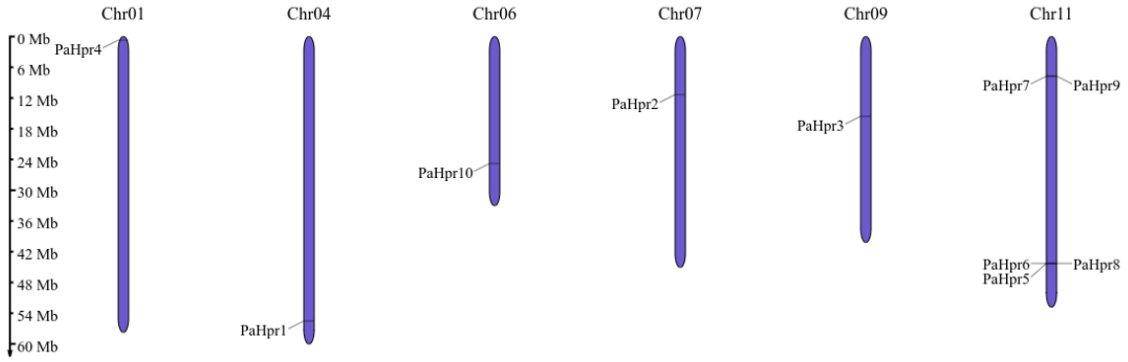

Figure 1 Distribution of PaHrp genes across chromosomes. The scale represents megabases (Mb). Chromosome numbers are indicated above each vertical bar.

Table 1 Gene identification

		Protein				Motifs		
Gene	**Gene-ID**	**Domain**	**Signal peptide**	**pI**	**MW**	**Length**	**pI**	**MW**
PaHrp1	Phacu.CVR.004G172300.1	Kunitz_legume	Not	8.85	23.01	31	4.86	3.48
PaHrp2	Phacu.CVR.007G109100.1	Peptidase_C1/ Inhibitor_I29	Yes	8.95	50.54	34	3.66	3.69
PaHrp3	Phacu.CVR.009G138200.1	Peptidase_C1/ Inhibitor_I29	Yes	6.05	40.09	34	3.8	3.59
PaHrp4	Phacu.CVR.001G009700.1	Peptidase_C1/ Inhibitor_I29	Yes	6.16	39.75	34	3.58	3.63
PaHrp5	Phacu.CVR.011G192600.1	Root_cap	Not	7.84	37.76	32	6.21	3.31
PaHrp6	Phacu.CVR.011G192300.1	Root_cap	Yes	8.24	40.61	33	6.17	3.5
PaHrp7	Phacu.CVR.011G088000.1	Root_cap	Yes	8.82	43.87	19	9.31	3.63
PaHrp8	Phacu.CVR.011G192500.1	Root_cap	Yes	7.84	37.76	32	6.21	3.59
PaHrp9	Phacu.CVR.011G088000.2	Root_cap	Yes	8.82	43.87	30	9.31	3.74
PaHrp10	Phacu.CVR.006G153300.1	Root_cap	Not	5.75	29.2	32	4.65	2.08

aa, motif length; pI, isoelectric point; MW, molecular weight; GRAVY, average hydropathy index

Table S1 Identification of complete sequences with the α-hairpinin motif in yellow, length, Pfam e domain.

Gene	Sequence	Size	Pfam
PaHrp1	MLHSETMKDSLLPFSILSFAFTIQLFIGIAVAAPEPVVDTSGQKLRTGVKYYILPVFRGKGGGLTVSSSG NNTCPLFVVQEKLEVLNGTPVTFTPYNAKSGVILTSTDLNIKSYGTTTSCDKPPVWKLLKVLTGVW FLSTGGVEGNPGIDTIVNWFKIEKAEKDYVISFCPSVCKCQTLCRELGLYVGDDGNKHLSLSDKVPS FRVMFKRA	398	pfam00197
PaHrp2	MLNLTAACCHLSMRLQFTLLPLHYTLMYSPAPPFQGYNCNYIIHEENPIKRRRRRRRRRKTKLVAEQAE EARTNMVAKRGHALSCFARISLVLFALTLSSARQTTVHDIAKKLKLEDNQLLRTEKKFNVFMENYGKK YSTREEYLQRLEIFAGNMLRAAENQALDPTAIHGVTQFSDLTEDEFQRHYTGVNGGFPWNNGVRD VAPPLKVDGLPEDFDWREKGAVTEVKMQGKCGSCWAFSTTGSIEGANFIATGKLLNLSEQQLVDC DNQCDITESTTCDNGCMGGLMTNAYKYLLQSGGLEEESSYPYTGAKGECKFDPGKVAVRITNFTNI PVDENQIAAYLVKHGPLAIGLNAIFMQTYIGGVSCPLICSKKWLNHGVLLVGYRAKGFSILRLGNKPY WIIKNSWGKRWGVDGYYKLCRGHGMCGMNTMVSAAMVTQTQTPSHNYASY	398	smart00848

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Table S1 Continued...

Gene	Sequence	Size	Pfam
PaHrp3	MATPSLFFLFSLLLFSATLVIANRIDGEDDLLIRQVVPDAEEHLLNAEHHFSAFKTKFGRTYATKE EHDYRFRIFKNNLLRAKSHQKLDPSAVHGVTKFSDLTPAEFSRQFLGLKPLRLPSDAQKAPILP TSDLPSDFDWRDHGAVTGVKNQGSCGSCWSFSAVGALEGAHFLSTGELVSLSEQQLVDCD HECDPEERGACDAGCGGGLMTNAFEYSLKAGGLMRENDYPYIGRDRGPCKFDKSRIAASVA NFSVVSLDEEQIAANLVKNGPLAVGINAIFMQTYIGGVSCPYICGKHLDHGVLLVGYGAGAYA PIRFKEKPFWIIKNSWGESWGENGYYKICRGHNVCGVDSMVSTVAAIHTSSH	263	smart00848
PaHrp4	MARYTLCALLLFAAVAAAAAGASTDADDILIRQVVPEGEVEDHLLNAEHHFSTFKVKFGKTYA TKEEHDHRFGVFKSNMRRARLHAQLDPSAVHGVTKFSDLTPAEFHRQFLGLKPLRLPAHAQK APILPTNNLPKDFDWRDKGAVTNVKDQGSCGSCWSFSTTGALEGAHFLATGELVSLSEQQL VDCDHVCDPEEYGACDAGCNGGLMNNAFEYIIGSGGVQREKDYPYTGRDGTCKFDKSKIA ASVSNYSVVSLDEEQIAANLVKNGPLAVAINAVYMQTYVGGVSCPYICGKHLDHGVLLVGYGE GAYAPIRFKEKPYWIIKNSWGENWGENGYYKICRGRNVCGVDSMVSTVGAIHASTQ	341	smart00848
PaHrp5	MEITKGSSIIIAVLLFVSCLSLQANAYYYRQCSTKGTRCYGKYIRCPNECPSSESTDPKAKVCQID CDKPICRAVCRSRKPNCNAPGSGCYDPRFIGGDGRVFYFHGKTNEHFALVSDSSLQINARFIG HRPAGRARDYTWIQALGVLFNSKTLSLEAPKTSQWNEDVDHLKFTYNGNHLLLPQGPLST WHSPQKDVKVERVAARNSVIVTLEDVAEILVNVVPVTKEDDAVHNYQVPQDDCFAHLEVQF RFFGLSPKVDGVLGRTYREDFENPAKVGVAMPVVGGEDKYRTTSLLSPNCASCVFSPPSSHHI EATEVSAELMGTLDCSKFSYGLGIVCKK	341	pfam06830
PaHrp6	MSFSINSDLLVFTSNWYLLVYKRKAMSVSSRNSLIYLLLLFAFCEMQIIAGKDTQTCISRKSPCFG KKVPCPDECPQKSPSDPKAKVCYLDCDSPICQAQCKTRKPNCNDRGSACLDPRFVGADGI VFYFHGRRNEHFALVSDVNLQINARFIGLRPATRTRDYTWIQALGLLFGSHKFTIEAIPAASWN DEVDHLKFSHNGKELAIPNGYLSTWQCPQNQLRIERTSSKNSVTITLPEVAEIFVNVVPVTNE DSRIHNYQIPKEDCFAHLEVQFKFHGLSSKVEGILGRTYQPDFQNPAKLGVAMAVVGGEDKY RTTSLVSADCGVCLFDGGKESSEKINSVSEYGLLDCTAAANSGNGIVCRR	365	pfam06830
PaHrp7	MGGKKWSILVAIFILLIAMEAAIAQGQGNGNDKGKGNENGNGNGKGKGSDNGKGKGEG SDDGKGKKNKKDDGKEKKPKEKKPKKQRDEASDYDKLSALPSGQERGFCRTNTTCEFKTI VCPSECAERKPKKNKKKKACFIDCSSSTCEATCKVRKANCDGYGSLCYDPRFVGGDGVMF YFHGAKGGNFAIVSDEEFQINAHFIGSRPQGRTRDYTWVQALGVMFDSHTLVIAANRVSHW NDKVDSLTVKWDGEVINVPTDGEAEWRANGDEREVVVERTDETNSVRVMVSGLVEMDISVK PIGEQENKVHNYQLPQNDAFAHLETQFRFKKSTDNFEGVLGQTYRPGYVSPVKRGVPMPMM GGENKYQTLSLFSTSCKRCMFQRPSSIASTEGLVAQY	211	pfam06830
PaHrp8	MEITKGSSIIIAVLLFVSCLSLQANAYYYRQCSTKGTRCYGKYIRCPNECPSSESTDPKAKVCQID CDKPICRAVCRSRKPNCNAPGSGCYDPRFIGGDGRVFYFHGKTNEHFALVSDSSLQINARFIG HRPAGRARDYTWIQALGVLFNSKTLSLEAPKTSQWNEDVDHLKFTYNGNHLLLPQGPLSTW HSPQKDVKVERVAARNSVIVTLEDVAEILVNVVPVTKEDDAVHNYQVPQDDCFAHLEVQFRFF GLSPKVDGVLGRTYREDFENPAKVGVAMPVVGGEDKYRTTSLLSPNCASCVFSPPSSHHIEATE VSAELMGTLDCSKFSYGLGIVCKK	450	pfam06830
PaHrp9	MGGKKWSILVAIFILLIAMEAAIAQGQGNGNDKGKGNENGNGNGKGKGSDNGKGKGEG SDDGKGKKNKKDDGKEKKPKEKKPKKQRDEASDYDKLSALPSGQERGFCRTNTTCEFKTIV CPSECAERKPKKNKKKKACFIDCSSSTCEATCKVRKANCDGYGSLCYDPRFVGGDGVMFYF HGAKGGNFAIVSDEEFQINAHFIGSRPQGRTRDYTWVQALGVMFDSHTLVIAANRVSHWND KVDSLTVKWDGEVINVPTDGEAEWRANGDEREVVVERTDETNSVRVMVSGLVEMDISVKPIGE QENKVHNYQLPQNDAFAHLETQFRFKKSTDNFEGVLGQTYRPGYVSPVKRGVPMPMMGGE NKYQTLSLFSTSCKRCMFQRPSSIASTEGLVAQY	366	pfam06830
PaHrp10	MEHVCPNACPRGCEVDCITCKPVCKCDRPGAVCQDPRFIGGDGITFYFHGKKDRNFCLVSD PNLHINAHFIGRRNNNMKRDFTWVQSIAILFDNHQLFVGALRTPTWEDHIDRLALTFDGQPL TLYESEGATWTSSTVPNVSIVRTTSTNSVLVEVEGRLRVTAKVVPITEEDSRIHDYGITKEDCFAH LDLGFKFFTLSNEVSGVLGQTYKASYVSRVNVGANMPVMGGGKEFETTSLFSPDCSVARFIGK NELTEGDAFVS	365	pfam06830

Alignment results revealed that the PaHrp motifs exhibited four conserved cysteine (C) residues, forming two disulfide bonds with the characteristic pattern of α-hairpinins (Figure 2). α-hairpinins have a structure characterized by a helix-loop-helix motif, with four cysteine residues forming two disulfide bonds, thereby conferring stability to the structure.[2,30] Despite showing high similarity in spatial structure, α-hairpinins exhibit low sequence homology and high functional diversity.[3,30,31] These findings suggest that, despite the structural conservation of cysteines, variability in amino acid sequences may be related to the functional adaptation of these proteins, reflecting a wide range of specific biological activities.[6] Analyses of in silico biological activities demonstrated that only PaHrp7 exhibited antifungal, antibacterial, and antiviral activities. PaHrp1 and PaHrp9 showed antifungal and antibacterial activities, PaHrp6 exhibited antifungal and antiviral activities, and PaHrp8 showed antibacterial and antiviral activities. PaHrp2 and PaHrp4 displayed only antiviral activity, while PaHrp5 and PaHrp10 showed antifungal activity, and PaHrp3 exhibited antibacterial activity (Table 2). In vitro antimicrobial

assays demonstrated that maize α-hairpinin (MBP-1) contributed to grain resistance against infection caused by pathogenic fungi and bacteria such as *Fusarium moniliforme* and *F. graminearum*, as well as action against *Escherichia coli* and *Clavibacter michiganense*.[32] Suppression of growth in filamentous fungi and bacteria was also observed in α-hairpinins from *Stellaria media*,[8] *Triticum kiharae*,[5] and *Echinochloa crus-galli*.[33]

Table 2 Antimicrobial and antiviral potential of PaHrps.

Gene	ClassAMP				ANTIFP		Meta-iAVP	
	SVM	Score	RF	Score	Prediction	Score	Prediction	Score
PaHrp1	Antifungal	0.977	Antibacterial	0.550	Antifungal	0,200	Non-AVP	0.406
PaHrp2	Antiviral	0.778	Antiviral	0.398	Antifungal	0,039	AVP	0.928
PaHrp3	Antibacterial	0.711	Antibacterial	0.408	Antifungal	0,003	Non-AVP	0
PaHrp4	Antiviral	0.810	Antibacterial	0.426	Antifungal	0,115	AVP	0.876
PaHrp5	Antifungal	0.900	Antibacterial	0.394	Antifungal	0,285	Non-AVP	0.294
PaHrp6	Antifungal	0.939	Antibacterial	0.484	Antifungal	0,179	AVP	0.524
PaHrp7	Antifungal	0.905	Antibacterial	0.612	Antifungal	0,635	AVP	0.962
PaHrp8	Antibacterial	0.940	Antibacterial	0.436	Non-Antifungal	-0,095	AVP	0.630
PaHrp9	Antifungal	0.894	Antibacterial	0.610	Antifungal	0,713	Non-AVP	0.316
PaHrp10	Antifungal	0.934	Antibacterial	0.454	Antifungal	0,641	Non-AVP	0.016

SVM, support vector machine; RFC, random forest classifier, DAC, discriminant analysis classifier

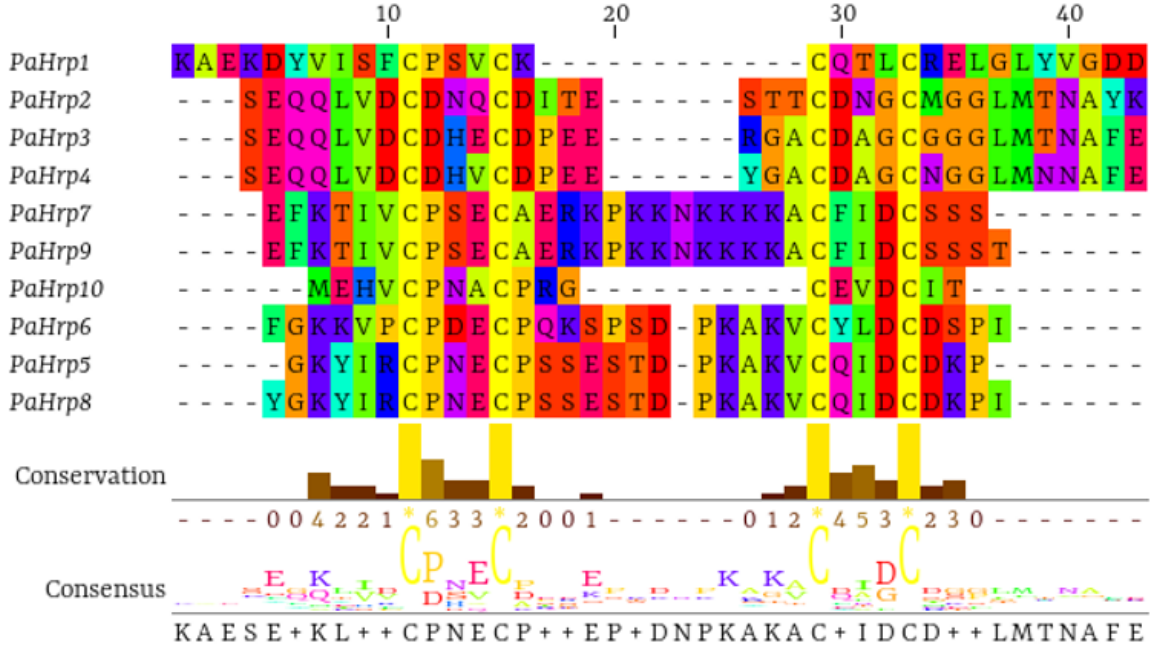

Figure 2 Multiple alignment of PaHrps demonstrating low sequence conservation, but revealing the conserved cysteines characteristic of α-hairpinins.

Conclusion

This study significantly expands the understanding of antimicrobial peptides in *P. acutifolius*, particularly by characterizing α-hairpinin motifs across 10 identified PaHrp sequences. The structural versatility, despite low sequence homology, suggests an adaptive functional role that could be harnessed in various applications. The unique distribution of PaHrp genes across multiple chromosomes and the ability of certain peptides, like PaHrp7, to exhibit multi-target antimicrobial activity further underscore the potential of *P. acutifolius* peptides as valuable resources for developing therapeutic agents and enhancing crop protection strategies. These findings support future research into the biotechnological applications of PaHrps in agriculture and human health, paving the way for new avenues in natural antimicrobial development.

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Conflicts of interest

The authors declared that there are no conflicts of interest.

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