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Review Article

Plant Antimicrobial Peptides: Bioinformatics Approaches for Discovery, Characterization, and Functional Insights

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Abstract

Plant antimicrobial peptides (AMPs) are small, naturally occurring biomolecules that play crucial roles in the plant innate immune system. Their structural diversity, cationic nature, and stability under adverse conditions enable them to act effectively against a broad spectrum of pathogens, including fungi, bacteria, and viruses. Beyond biotic defense, AMPs also contribute to tolerance against abiotic stresses such as drought, salinity, and ultraviolet radiation. This review integrates findings from peer-reviewed studies and biological databases focusing on plant AMPs, with an emphasis on bioinformatics tools and computational approaches. Both conventional sequence-based analyses (e.g., BLAST, HMMER) and recent advances in machine learning and structural modeling (e.g., Deep-AmPEP, AlphaFold) were assessed to illustrate their role in AMP discovery and functional characterization. Comparative analyses reveal the evolution of bioinformatics methodologies and their successful application in crop species for large-scale AMP identification and *in silico* characterization. Case studies demonstrate how integrative computational pipelines can accelerate AMP discovery and provide practical insights for agricultural improvement. Additionally, the review highlights the potential for developing a comprehensive Iranian plant AMP database and species-specific predictive models to facilitate regional research. Plant AMPs represent promising molecules for biotechnology, agriculture, and pharmaceuticals. The integration of computational prediction, genetic engineering, and applied research will accelerate their use in crop improvement and therapeutic development. Future studies focusing on database expansion and machine learning applications are expected to further enhance our understanding and utilization of plant-derived AMPs.

Keywords: Antimicrobial Peptides, Bioinformatics, Machine Learning, *In Silico* Prediction, Bioinformatics Pipelines, Database Development

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Introduction

In recent decades, the increasing resistance of microorganisms to conventional antibiotics has emerged as a serious global health concern, affecting humans, animals, and plants alike. This alarming trend has created an urgent demand for novel antimicrobial agents with distinct mechanisms of action. Among the alternatives under investigation, antimicrobial peptides (AMPs) have gained significant attention due to their broad-spectrum activity against bacteria, fungi, and viruses, as well as their multifunctional roles in immunity and stress responses.[1,2] Plants constitute a rich and promising reservoir of AMPs. Functioning as crucial elements of the plant innate immune system, these peptides play dual roles: they not only defend against a wide range of pathogens but also contribute to plant adaptation under abiotic stresses, including drought, salinity, and extreme temperatures.[3] Their remarkable structural diversity, strong stability ensured by multiple disulfide bonds, and generally low toxicity highlight their suitability as candidates for applications in pharmaceuticals, sustainable agriculture, and food preservation. Moreover, compared to animal-derived AMPs, plant peptides usually cause fewer adverse effects, which further underscores their potential for biotechnological exploitation.[4] Despite these advantages, the discovery and characterization of plant AMPs face several challenges, including their low abundance, sequence diversity, and structural variability.[5] Recent advances in bioinformatics have greatly improved the ability to predict, classify, and analyze AMPs through sequence-based searches, structural modeling, and machine learning approaches.[6-8] However, major gaps remain, such as the lack of comprehensive plant-specific databases and limited experimental validation of computational findings.[9] This review aims to address these issues by summarizing current knowledge of plant AMPs, evaluating bioinformatics tools used in their study, presenting case studies in crop species, and discussing future perspectives with an emphasis on database development and predictive model design. This study also introduces a novel conceptual framework proposing the development of a region-specific database for Iranian plant AMPs and the design of plant-oriented predictive models, an approach not yet reported in the current literature.

Materials and Methods

This review collected relevant literature on plant antimicrobial peptides (AMPs) and bioinformatics tools from PubMed, Scopus, Web of Science, and Google Scholar (2000–2025, English articles). The following search query was used: ("plant antimicrobial peptide" OR "plant AMPs" OR "defensin" OR "thionin" OR "cyclotide" OR "snaking" OR "hevein-like peptide") AND ("bioinformatics" OR "*in silico*" OR "sequence analysis" OR "AMP prediction" OR "machine learning" OR "computational approach"). Peer-reviewed original papers, reviews, and case studies on plant-derived AMPs were included; animal or microbial AMP studies were excluded. Bibliometric analysis was performed using VOSviewer to identify research trends. Data extraction and synthesis followed a narrative review approach, focusing on structural and functional classification, databases, prediction algorithms, and future perspectives.

Types of Plant AMPs and Their Characteristics

Plant antimicrobial peptides (AMPs) can be seen as the natural guardians of plants, small but powerful molecules that help plants fight off microbial invaders. They are produced in different plant tissues and act as a living shield against harmful organisms. Because of their unique structures and biological roles, plant AMPs are not only central to innate immunity but also hold promise as future biopharma- ceuticals, natural antimicrobial agents, biopesticides, and even carriers for therapeutic delivery.[10] One of the striking features of plant AMPs is their diversity. They differ widely in amino acid sequence, three-dimensional structure, and mechanism of action. This diversity has led to their classification into several families, each with specific structural traits and biological functions.[11] Plant AMPs are classified into several families based on structure and function.[12] Table 1 summarizes the main families, their key structural motifs, biological mechanisms, and representative roles (Table 1).

Table 1. Classification of Plant Antimicrobial Peptide (AMP) Families, Highlighting Their Major Structural and Functional Attributes

Family	Structure	Mechanism of action	Function / Notes	Reference
Defensins	β-sheets, 4–5 disulfide bonds	Membrane disruption, ion imbalance	Broad-spectrum antifungal and antibacterial	[36, 37]
Thionins	Small, cationic peptides	Pore formation in membranes	Antibacterial, some anticancer	[38]
Hevein-like peptides	Chitin-binding domain, multiple disulfide bonds	Bind fungal cell walls	Antifungal, antiviral	[39, 40]
Lipid Transfer Proteins (LTPs)	Compact, α-helical, disulfide-stabilized	Lipid transport, stress response	Antimicrobial, signaling role	[28, 39-41]
Cyclotides	Cyclic cystine-knot motif	Membrane perturbation	Antimicrobial, antitumor	[42]
Snakins	Cysteine-rich, α-helix and β-sheet	Broad membrane disruption	Antifungal and antibacterial	[10, 43]

Common Features of Plant AMPs

Although plant AMPs differ widely in structure, they share several fundamental traits that explain their strong defensive abilities. First, their cationic nature, an overall positive charge, allows them to interact electrostatically with negatively charged microbial membranes, particularly those of bacteria and fungi, facilitating attachment and penetration.[13] Second, their small size (typically fewer than 50 amino acids) enables rapid diffusion across membranes and quick action, which is essential for effective immune responses.[14,15] Third, cysteine richness is a defining feature: multiple disulfide bonds formed by cysteine residues stabilize the three-dimensional structure and confer resistance to enzymatic degradation.[16] Finally, plant AMPs exhibit remarkable stability, remaining active under extreme conditions such as high temperature, acidic or alkaline pH, and proteolytic enzymes, thereby ensuring functionality across diverse biological environments (Table 2, Figure 1).[17]

Table 2. Common Characteristics of Plant Antimicrobial Peptides (AMPs), with Emphasis on Their Structure, Stability, and Mechanisms of Action

Common characteristic	Scientific explanation
Cationic nature	Facilitates strong electrostatic interactions with negatively charged bacterial and fungal membranes.
Small size	Typically composed of fewer than 50 amino acids, enabling high permeability and rapid action.
Cysteine richness	Presence of multiple disulfide bridges ensures high structural stability.
Resistance to harsh conditions	Stable under heat, proteolytic enzymes, and fluctuating pH levels.
Diverse mechanisms of action	Includes membrane disruption, inhibition of DNA synthesis, and suppression of pathogenic enzymatic activities.

Advanced Bioinformatics Approaches for the Identification, Analysis, and Design of Plant AMPs

With the advancement of computational technologies and the emergence of comprehensive genomic and proteomic databases, bioinformatics methods have become indispensable tools in the discovery and characterization of antimicrobial peptides (AMPs). These approaches allow researchers to identify, predict, and evaluate AMP functions without relying solely on time-consuming and costly experimental procedures. A typical bioinformatics pipeline for plant AMPs involves several key steps: (i) retrieving candidate sequences from public databases, (ii) performing homology searches to assign peptides to known AMP families, (iii) predicting antimicrobial activity using machine learning algorithms, (iv) analyzing physicochemical and structural properties critical for bioactivity, and (v) comparatively evaluating different computational platforms to select the most accurate prediction tools. Below, we present the most

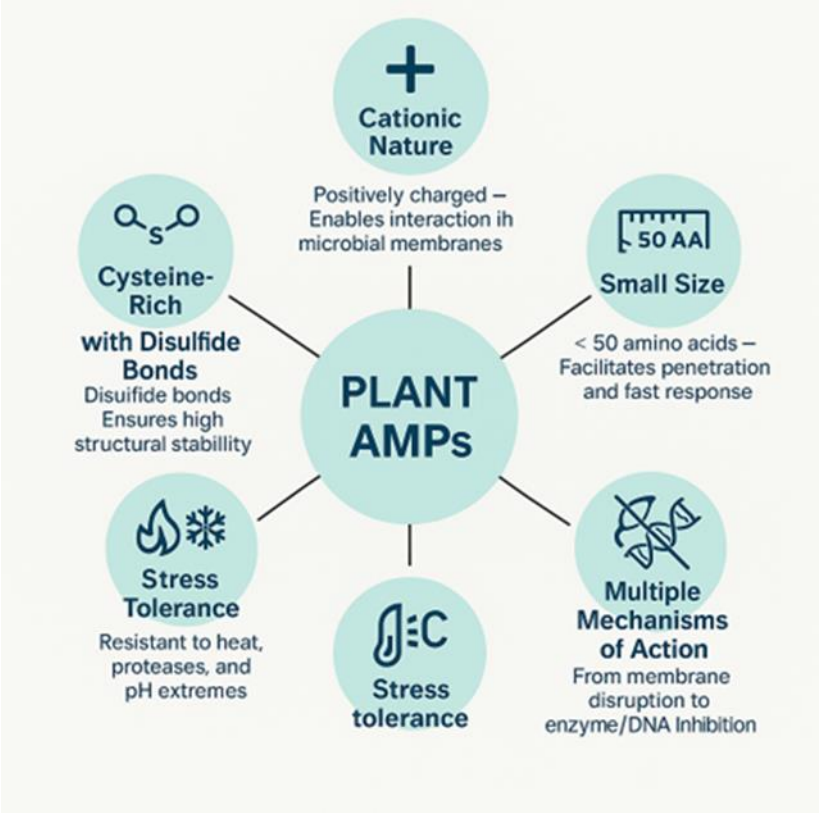

Figure 1. A Schematic Representation of the Structural, Physicochemical, and Functional Features of Plant Antimicrobial Peptides (AMPs), Including Cationic Charge, Small Size, Disulfide Bonds, and Diverse Modes of Action.

prominent bioinformatics tools and strategies used in plant AMP research, following this logical workflow.[18,19]

Sequence Retrieval from Databases

The initial step in any bioinformatics pipeline is accessing candidate gene or protein sequences. Public repositories such as NCBI GenBank, UniProtKB/Swiss-Prot, and Ensembl Plants provide reliable resources for extracting peptide sequences from various plant species. Researchers can search using keywords such as "antimicrobial peptide" or "defensin," or extract novel sequences from RNA-Seq datasets.[18]

Homology Search and AMP Family Identification

To detect novel AMPs, comparative algorithms such as BLASTp, PSI-BLAST, tBLASTn, and HMMER are employed. In particular, hidden Markov models (HMMs) are highly effective in identifying specific AMP families such as defensins or cyclotides. Databases like Pfam, InterPro, and CDD provide curated information on functional peptide domains.[19]

Sequence-Based Antimicrobial Activity Prediction

Machine learning algorithms have revolutionized the prediction of AMPs based solely on amino acid sequences. Several computational tools have been developed to classify peptide types, predict active regions, and design synthetic variants. The most relevant platforms are summarized in Table 3.

Analysis of Physicochemical and Structural Properties

Key physicochemical and structural characteristics, such as charge, hydrophobicity, cysteine content, instability index, and secondary structure, play essential roles in AMP bioactivity. Commonly used analysis tools are presented in Table 4.

Table 3. Comparison of Machine Learning–Based Tools for AMP Prediction

Tool	Algorithm / Model	Main function	Key features	Reference
iAMP-2L	SVM	Classifies peptides by antimicrobial type	Dual-layer classifier; high accuracy	[44]
AMPA	Statistical Scoring	Identifies antimicrobial regions	Detects local activity motifs	[45]
CAMPR3	SVM, Random Forest, ANN	Predicts and classifies AMPs	Integrates multiple ML algorithms	[46]
AMPScanner V2	Deep Neural Network	Classifies peptides with improved sensitivity	Learns nonlinear sequence relationships	[46]
Deep-AmPEP30	CNN–RNN hybrid	Predicts AMPs targeting pathogens	Handles complex sequence patterns	[20]

Table 4. Bioinformatic tools for physicochemical and structural analysis of AMPs

Tool	Purpose / Function	Parameters or features analyzed	Output / Application	Reference
ProtParam	Physicochemical profiling	pI, MW, GRAVY, instability	Basic peptide properties	[47]
PepCalc	Peptide composition analysis	Molecular weight, net charge, pI	Physicochemical data	[48]
PSIPRED / JPred	Secondary structure prediction	α-helix, β-sheet, coil	Fold pattern estimation	[5]
I-TASSER / AlphaFold2	3D structure modeling	Template-based prediction	Tertiary model generation	[7]
Disulfide by Design / CYS_REC	Disulfide bridge prediction	Cysteine pair identification	Structural stability inference	[8]

Comparative Overview of Computational Platforms for AMP Prediction

With the increasing application of artificial intelligence in peptide research, various computational platforms have been developed to evaluate and predict antimicrobial activity with higher accuracy. Early tools primarily relied on traditional machine learning algorithms such as support vector machines (SVMs) and random forests, which performed reasonably well on benchmark datasets but often struggled with complex, nonlinear sequence patterns. In contrast, recent deep learning-based platforms, including convolutional neural networks (CNNs), recurrent neural networks (RNNs), and transformer-based models, have significantly improved prediction accuracy by capturing hidden sequence features and contextual relationships. Table 5 summarizes the comparative performance of major AMP prediction tools reported in benchmark studies, illustrating the steady advancement from conventional SVM-based classifiers to deep learning frameworks. Such comparative evaluations are essential for researchers to select the most appropriate tool based on their specific needs, whether prioritizing high sensitivity, specificity, or computational efficiency.

Subcellular Localization and Biological Targeting Prediction

Predicting the subcellular localization of an AMP, whether it resides in the cytoplasm, mitochondria, or other organelles, is essential for understanding its biological function and mechanism of action. Moreover, knowing the specific target (e.g., bacterial membrane, fungal cell wall, or viral envelope) helps prioritize peptides for downstream experimental validation. Several computational tools have been developed for this purpose, including SignalP and TargetP for signal peptide prediction, WoLF PSORT and SUBA4 for general subcellular localization, and Plant-mPLoc, which is specifically optimized for plant proteins. Table 6 lists commonly used tools for subcellular localization and biological targeting prediction, along with their input requirements and output formats.

Table 5. Comparative Evaluation of Major AMP Prediction Platforms Based on Reported Performance Metrics in Benchmark Studies

Tool	Algorithm / Model	Dataset type	Reported accuracy (%)	Highlights	Reference
iAMP-2L	Multi-label SVM	Benchmark AMP dataset (APD, UniProt)	~88–89	Reliable for broad AMP type classification (antibacterial, antifungal, antiviral)	[49]
CAMPR3	Random Forest / SVM ensemble	AMP DB + SwissProt	~83–85	Integrates several classifiers; suitable for initial screening	[50]
AMPScanner V2	Deep Neural Network (CNN + Dense Layers)	APD3, UniProt (AMP/Non-AMP)	~91–93	High accuracy and recall; handles nonlinear features effectively	[51]
Deep-AmPEP30	Bi-LSTM Deep Learning	Curated AMP datasets (APD3)	~94–95	Best performance; captures deep contextual peptide features	[52]

Table 6. Tools for Subcellular Localization and Biological Targeting Prediction

Tool	Function	Target / Localization type	Output utility	Reference
SignalP / TargetP	Predict signal peptides	Secretory and transit peptides	Protein export prediction	[6-9]
SUBA4 / WoLF PSORT	Predict subcellular localization	Mitochondrial, cytoplasmic, extracellular	Organellar prediction	[9, 53, 54]
Plant-mPLoc	Multi-location prediction	Plant-specific organelle localization	Plant-specific annotation	[55]
PeptideRanker	Activity ranking	Peptide bioactivity score	Estimation of activity probability	[56]

Molecular Docking and Interaction Simulation

To explore AMP function at the molecular level, docking simulations are widely employed to study interactions between AMPs and microbial membranes or specific enzyme targets. These simulations provide valuable insights into binding affinity, interaction residues, and the energetic landscape of AMP-target complexes, which can guide the rational design of more potent derivatives. Tools such as AutoDock, HADDOCK, ClusPro, and SwissDock allow flexible or rigid docking, with some platforms capable of simulating protein-peptide or peptide-lipid interactions. Figure 2 illustrates a typical docking workflow, while Table 6 includes relevant docking tools and their key features. Together, these approaches bridge the gap between sequence-based prediction and mechanistic understanding.

Functional Network and Pathway Analysis

Functional network analysis helps interpret AMPs within broader cellular systems and metabolic contexts. Rather than viewing AMPs as isolated effectors, network-based approaches

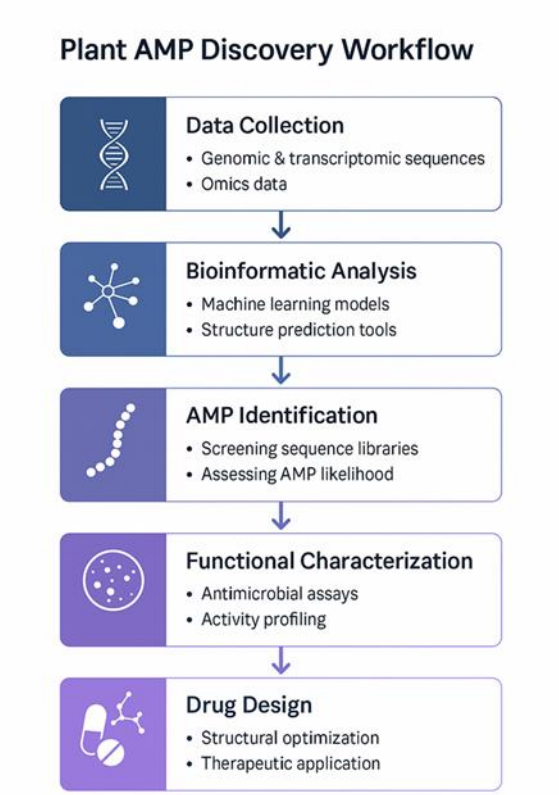

Figure 2. An Overview of the Plant AMP Discovery Workflow Using Bioinformatics Approaches, from Data Retrieval to Functional Prediction and Drug Design.

Table 7. Molecular Docking and Interaction Tools for AMP Studies

Tool	Application	Key features	Reference
AutoDock / HADDOCK	Docking AMPs with microbial proteins	Free-energy calculation, flexible docking	[57]
ClusPro / SwissDock	Protein–protein docking	Visualizes AMP–target interactions	[58]

map protein-protein interactions (PPIs), co-expression patterns, and shared signaling pathways. This can reveal whether an AMP participates not only in direct pathogen defense but also in stress signaling, hormone crosstalk, or developmental processes. Tools such as STRING and STITCH predict functional associations from curated databases and high-throughput experiments, while KEGG Mapper and BioCyc place AMPs into known metabolic or regulatory pathways. Table 7 summarizes the main tools used for functional network and pathway analysis. Integrating these analyses with localization and docking results provides a holistic view of AMP function within the plant immune system.

Deep Learning Models and Modern Data Mining

Emerging deep learning platforms such as AMPNet, DeepAMP, and AI4AMP leverage hidden sequence features, beyond simple amino acid composition, to discover or even design novel AMPs. Unlike traditional machine learning models that rely on handcrafted features, deep learning architectures, including convolutional neural networks (CNNs), recurrent neural networks (RNNs), long short-term memory (LSTM) networks, and transformer-based models, automatically learn hierarchical representations from raw peptide sequences. These models capture long-range dependencies, motif patterns, and contextual relationships that are often invisible to conventional algorithms. In addition to prediction, modern data mining techniques, such as clustering, association rule mining, and anomaly detection, are increasingly used to extract meaningful patterns from large-scale peptidomics and transcriptomic datasets. These models have proven effective in creating targeted, stable, and low-toxicity peptides. For example, the Deep-AmPEP30 model has demonstrated high accuracy in predicting AMPs targeting human pathogens.[20,21] Table 8 and Figure 3 provide an overview of representative deep learning tools and the evolutionary timeline of AMP prediction approaches. Together, these advanced bioinformatics frameworks, spanning sequence-based classifiers, structural modeling, subcellular localization, network analyses, and deep learning, are transforming the landscape of AMP research. By integrating multiple layers of computational evidence, they establish a coherent and predictive pipeline for the discovery, design, and functional characterization of plant AMPs. Continued progress in these computational strategies will not only enhance our understanding of plant

Table 8. Network and Pathway-Based Tools for Functional AMP Analysis

Tool	Function	Type of analysis	Application	Reference
STRING / STITCH	Protein–protein interaction mapping	Detects functional partners and signaling pathways	Integration of AMP-related networks	[59]
KEGG Mapper / BioCyc	Pathway mapping	Links AMPs to metabolic pathways	Visualization of molecular pathways	[60]

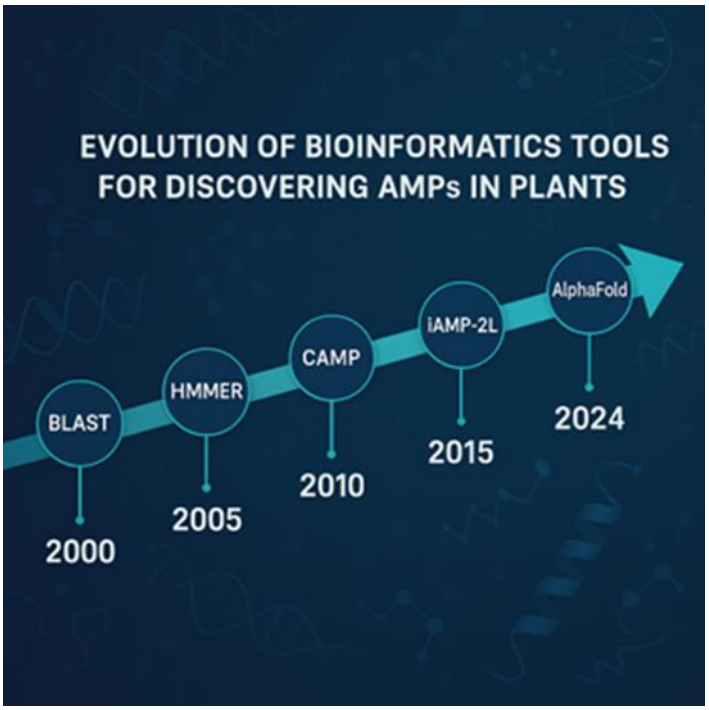

Figure 3. Timeline Illustrating the Evolution of Bioinformatics Tools for Discovering Plant Antimicrobial Peptides (AMPs) from 2000 to 2024.

defense mechanisms but also bridge the gap between *in silico* predictions and real-world applications, fostering sustainable innovations in agriculture, medicine, and biotechnology.

Case Studies: Bioinformatics Approaches in the Discovery of Plant AMPs

In recent years, the use of advanced bioinformatics tools for identifying and characterizing plant AMPs has gained significant momentum. These studies leverage a wide array of computational strategies, including machine learning algorithms, structural modeling, transcriptomic mining, and AMP-specific databases, enabling rapid and accurate discovery of bioactive peptides without the need for laborious and time-consuming wet-lab procedures. For instance, deep learning models have been successfully applied to predict defensins in black pepper (*Piper nigrum*), while homology-based searches and motif analysis have uncovered novel snakin peptides in pepper (*Capsicum annuum*) and hevein-like peptides in rubber tree (*Hevea brasiliensis*). Additionally, integrative pipelines combining transcriptome mining with AMP databases have identified thionins in tobacco (*Nicotiana tabacum*) and lipid transfer proteins in rice (*Oryza sativa*). Table 9 summarizes selected case studies involving different plant species and bioinformatic strategies used to uncover novel AMPs. As shown, various AMP families, including defensins, cyclotides, lipid transfer proteins (LTPs), snakins, and hevein-like peptides, have been discovered across a wide range of plant species, from model systems such as *Arabidopsis* and *Medicago truncatula* to wild medicinal plants. The bioinformatic approaches employed range from simple homology-based searches and structural modeling (e.g., using AlphaFold or I-TASSER) to curated AMP databases (e.g., PhytAMP, CAMP) and advanced machine learning classifiers (e.g., random forests, deep neural networks). These case studies collectively demonstrate the versatility, scalability, and efficacy of computational strategies in accelerating AMP discovery. Moreover, they highlight how *in silico* predictions can guide subsequent experimental validation, thereby reducing costs and increasing the success rate of identifying functional plant AMPs for agricultural and pharmaceutical applications.

Challenges in the Bioinformatics Identification of Plant AMPs

With the expansion of bioinformatics tools, the identification and analysis of plant AMPs have entered a new era of precision and scalability. These technologies enable the discovery, functional prediction, and rational design of AMPs

Table 9. Selected Case Studies on Bioinformatic Discovery of Plant AMPs

Plant species	Computational tool/Method	AMP type/Family	Methodology & key findings	Reference
Moringa oleifera	LC–MS/MS integrated with bioinformatics hydrolysate analysis	Novel AMPs	Seed protein hydrolysates screened by LC–MS/MS and functional predictions showed β-sheet structure and potent antimicrobial activity against S. aureus; peptides stable under salt and high temperature stress	[61]
Piper nigrum L	Deep learning model ensemble (LSTM, attention)	Defensins	Sequence data processed by LSTM and attention mechanism networks identifying candidates with molecular docking confirming specificity; validated key AMP candidates	[62]
Capsicum annuum	Motif search combined with PhytAMP database querying	Snakins	Motif-based scanning against the PhytAMP database predicted snakin peptides, with *in silico* structural and functional annotation	[61]
Solanum lycopersicum	Machine learning pipeline with empirical filtering and ranking	Defensins	Sequential empirical selection and classification filters identified novel hexapeptides from sequence libraries tested *in vitro*	[63]
Hevea brasiliensis	Defensins	Hevein-like peptides	Novel peptides isolated by mass spectrometry From latex analyzed by AMP databases showed predicted antifungal activity	[64]
Nicotiana tabacum	Integrative bioinformatics using AMP databases (APD, DBAASP) and motif retrieval	Thionins	Mining transcriptomic data with AMP databases identified novel thionin family peptides, with predicted structures and antimicrobial assays suggested	[65,66]
Oryza sativa	PhytAMP database and homology modeling	Lipid Transfer Proteins	*In silico* homology models of rice lipid transfer proteins revealed conserved AMP motifs; their defense role predicted by machine learning classifiers	[65]
Medicago truncatula	Transcriptome mining + deep learning AMP classifiers (iAMP-DL)	Defensins	Transcriptome sequences screened by iAMP-DL deep learning pipeline to find defensin peptides with predicted antimicrobial function	[67]
Capsicum chinense	BLASTp and motif search within plant peptide databases	Snakin peptides	Using sequence similarity search against snakin references, novel AMPs identified and structurally characterized *in silico*	[68]
Phaseolus vulgaris	Genomic mining + MS-guided identification	Cyclotides	Genome and proteome data mined for cyclotide precursors; peptides isolated and structurally validated by mass spectrometry	[17,69]
Allium sativum	Deep learning model AMPlify with multi-attentional mechanisms	Novel AMPs	Using attentional Bi-LSTM model on garlic peptides, several novel sequences predicted with broad-spectrum antimicrobial potential	[23]
Vitis vinifera	Machine learning pipeline combining random forests and peptide property analysis	Hevein-like, Defensins	Random forest-based Macrel pipeline used for AMP prediction from grape transcriptomes; peptides distinctively exhibit antifungal properties	[23]

without the need for time-consuming and costly laboratory procedures. However, to fully exploit the potential of bioinformatics in this field, several technical and data-related barriers must be addressed.[22] The main challenges are outlined below:

Lack of Comprehensive and Plant-Specific AMP Databases

Most existing AMP repositories, such as APD (Antimicrobial Peptide Database) and CAMP, focus primarily on animal or microbial peptides, with plant-derived sequences often treated as an afterthought. Consequently, data related to plant AMPs, including full-length sequences, experimentally determined structures, biological functions, and expression patterns under stress, remain fragmented across the literature, limited in coverage, and poorly standardized. This fragmentation hampers large-scale comparative studies and the training of plant-specific machine learning models.[23]

Low Accuracy of Traditional Tools in Predicting Novel or Atypical Peptides

Conventional algorithms, including BLAST-based homology searches and simple SVM classifiers, often rely on linear sequence features such as amino acid composition or dipeptide frequency. While these methods perform reasonably well for known AMP families, they struggle to detect non-canonical motifs, atypical cysteine patterns, or structurally diverse peptides that deviate from training sets. As a result, many potentially novel plant AMPs, particularly those from non-model species, remain unidentified.[23]

Scarcity of Structural Data (Tertiary and Quaternary Structures)

The biological activity of many AMPs depends critically on their three-dimensional conformation, including disulfide bonding patterns, alpha-helical or beta-sheet arrangements, and in some cases, oligomerization. However, most predictive tools are still based on linear sequences, neglecting the role of spatial folding. Experimental structural determination (e.g., NMR, X-ray crystallography) is resource-intensive, and high-quality 3D models are available for only a small fraction of known plant AMPs. This scarcity limits our mechanistic understanding and the rational design of

improved variants.[23]

Gap Between In Vitro Findings and in Planta Performance

Most functional validations of AMPs are conducted under artificial conditions, using synthetic peptides, purified microbial cultures, and buffered solutions, which do not replicate the complex environment of plant tissues. Factors such as protease activity, pH variations, cell wall components, and interactions with plant metabolites can dramatically alter AMP stability and efficacy. Consequently, there is limited insight into how these peptides behave within the actual physiological context of the plant, leading to frequent discrepancies between promising *in vitro* results and disappointing in planta performance.[24]

Lack of Multifunctional Analysis Tools for Pleiotropic AMP Roles

Emerging evidence indicates that some plant AMPs are not merely antimicrobial agents but also participate in growth regulation, hormonal signaling (e.g., auxin, gibberellin), and responses to abiotic stresses such as drought, salinity, and cold. However, current bioinformatics tools are largely designed for single-function prediction (e.g., antimicrobial activity only) and rarely allow integrated analysis of such multifunctionality. There is a pressing need for platforms that can correlate sequence features with multiple biological roles, bridging the gap between defense and development.[24]

Golden Opportunities in the Bioinformatics Discovery of Plant AMPs

The rapid advancement of computational technologies and molecular modeling tools has opened new horizons for the discovery and functional analysis of plant AMPs. What once required extensive wet-lab screening can now be accelerated through high-throughput *in silico* pipelines, reducing both time and cost. Moreover, the increasing availability of plant genomic and transcriptomic data, coupled with the growing power of artificial intelligence, has created a fertile ground for identifying previously unknown AMP families and predicting their mechanisms of action with remarkable accuracy. These developments not only complement experimental approaches but also enable rational design of synthetic peptides with optimized activity and stability. Below are the key opportunities and promising directions in this emerging field:

Advances in Machine Learning and Deep Learning Algorithms

The emergence of tools such as iAMPpred, Deep-AmPEP, and AlphaFold has significantly enhanced the accuracy of activity prediction and 3D structure modeling of AMPs. Unlike traditional methods that rely on handcrafted features, these deep learning models automatically extract relevant patterns from large datasets, capturing subtle sequence-structure relationships. They leverage both sequence and structural features to improve the precision of AMP discovery, enabling researchers to prioritize the most promising candidates for experimental validation. Furthermore, the integration of protein language models (e.g., ProtBERT) is expected to push the boundaries of prediction accuracy even further.[25]

Integration of Multi-Omics Data for Functional Insights

The convergence of genomic, transcriptomic, proteomic, and metabolomic data provides a systems-level approach to uncover complex biological interactions and regulatory pathways involving AMPs. This holistic strategy facilitates the identification of multifunctional roles and network-based functions of AMPs that would remain hidden in single-omics studies. For example, co-expression analysis of transcriptomic data can reveal which AMPs are induced under specific stress conditions, while proteomic and metabolomic data can confirm their actual production and activity. Such integrative approaches are essential for moving from simple sequence prediction to understanding real biological function.[26]

Development of Regional and Species-Specific Databases

Building dedicated databases for medicinal and native plant species, particularly those from biodiversity-rich regions such as Iran, can reveal novel and unexplored AMP candidates. These repositories should include sequence information, structural data, physicochemical properties, subcellular localization, and expression profiles under various stress conditions.[27] A well-designed regional database would not only preserve endemic genetic resources but also provide high-quality training data for plant-specific machine learning models. This is especially valuable because global databases are often biased toward model organisms and economically major crops, leaving many unique AMPs from native plants undiscovered.

Utilization of Molecular Docking and Interaction Simulations

Tools such as AutoDock, ClusPro, and SwissDock allow detailed simulation of AMP-target interactions at the molecular level. These platforms play a crucial role in the rational design of targeted AMPs and optimization of their functional properties. By predicting binding affinity, interaction residues, and the energetic landscape of peptide-membrane or peptide-protein complexes, researchers can modify sequences to enhance specificity, reduce toxicity, or broaden antimicrobial spectra. Moreover, docking simulations can be combined with molecular dynamics (MD) to assess stability over time, offering a virtual laboratory for AMP engineering before costly wet-lab testing.[28]

Expanding Functional Applications of Plant AMPs

Plant-derived AMPs are not only effective in defending against pathogens but also play roles in enhancing resistance to abiotic stresses such as drought, salinity, heat, and UV exposure. These multifunctional properties position AMPs as valuable candidates for developing climate-resilient and eco-friendly agricultural solutions. For instance, AMPs that activate stress signaling pathways could be used as biostimulants, while those with dual antimicrobial and stress-protective functions may be engineered into crops to tackle multiple challenges simultaneously. Beyond agriculture, their stability and low toxicity make them attractive for food preservation, biodegradable packaging, and even cosmetic formulations.[29]

Finally, bioinformatics offers a dynamic, interdisciplinary platform for the exploration and application of plant AMPs across therapeutics, sustainable agriculture, and biotechnology. Realizing this potential requires investment in data infrastructure, predictive tool development, and in planta functional validation to bridge the gap between computational insights and biological outcomes. Collaborative efforts between bioinformaticians, plant biologists, and agricultural scientists are essential to translate *in silico* discoveries into field-ready solutions. With continued innovation in AI, multi-omics integration, and database development, plant AMPs are poised to become key players in next-generation crop protection and biomedicine.

Advanced Recommendations for Future Research in Plant AMPs

With the rapid advancement of biological and computational technologies, the field of plant AMPs is undergoing a transformative shift. What was once limited by low-throughput experimental screening and small sequence datasets can now be addressed through genome-scale mining, deep learning prediction, and high-resolution structural modeling. These technological breakthroughs have not only accelerated the rate of AMP discovery but have also opened the door to rational design and targeted engineering of peptides with desired properties. However, to fully harness this momentum, strategic planning and coordinated efforts are needed to overcome remaining gaps in data availability, tool accuracy, and experimental validation. Below are key proposals for the strategic development of future research in this area:

Development of a Comprehensive, Region-Specific AMP Database

The absence of a dedicated database for plant AMPs, especially those from medicinal or endemic species such as Iranian crops, remains a critical gap. Most existing databases are heavily biased toward well-studied model plants and economically major crops, leaving AMPs from native and underutilized species largely unexplored. Creating a database containing sequence data, physicochemical properties, 3D structural models, stress-induced gene expression, and user-friendly interfaces can accelerate both research and practical applications. Such a regional database would not only preserve genetic resources but also serve as a high-quality training ground for plant-specific machine learning models. Moreover, incorporating features like BLAST search, annotation tools, and download options would make it accessible to a wide range of users, from molecular biologists to bioinformaticians.[30]

Design of Plant-Specific Machine Learning Models

Tailored ML models trained on transcriptomic data (e.g., RNA-Seq), structural attributes, and stress-responsive gene expression can significantly enhance the prediction of novel AMPs. General-purpose AMP prediction tools are often trained on mixed datasets (animal, microbial, and plant), which may not capture plant-specific sequence features such as cysteine motifs or signal peptides. Advanced algorithms such as LSTM (Long Short-Term Memory), Transformers, and Graph Neural Networks (GNNs) can uncover hidden, nonlinear patterns within peptide sequences that traditional models miss. For example, GNNs can explicitly model the structural graph of a peptide, while Transformers capture long-range dependencies. Developing open-source, plant-trained versions of these models would be a major step forward.[30]

Timeline Analysis of Bioinformatics Tool Development

Mapping the evolution of AMP identification tools, from traditional methods like BLAST and CAMP to modern platforms like Deep-AmPEP and AlphaFold, offers valuable insight into the trajectory of methodological progress in this domain. A systematic timeline analysis would reveal key inflection points, such as the introduction of SVM-based classifiers in the early 2000s, the shift to random forests in the 2010s, and the current dominance of deep learning architectures. Such an analysis can help researchers understand which methods are best suited for different tasks (e.g., high-throughput screening vs. accurate 3D modeling) and identify remaining methodological gaps. Furthermore, it can guide funding agencies and research groups toward underdeveloped areas, such as plant-specific structure prediction.[13]

Strategic Value of Plant AMPs in Future Biotechnology

Emerging evidence suggests that plant AMPs are not only active against biotic stressors but also play roles in abiotic stress responses such as drought, salinity, UV radiation, and cold. These peptides may contribute to the development of climate-resilient crops. Their potential extends far beyond disease resistance to antimicrobial food packaging, natural

preservatives, biofertilizers, and even bio-based ingredients in cosmetic and pharmaceutical industries, making them key players in sustainable biotechnology.[10] For instance, AMP-impregnated biodegradable films could extend the shelf life of fresh produce, while AMP-based biofertilizers could reduce the need for chemical pesticides. The multi-functionality of plant AMPs aligns perfectly with the principles of the circular bioeconomy.[10]

Integration of AMP Discovery with Genome Editing and AI

The convergence of CRISPR/Cas9 gene editing, synthetic peptide design, and artificial intelligence will enable the generation of crops that stably express beneficial AMPs. This integration may revolutionize plant immunity and stress resilience.[30] For example, AI could first predict a potent AMP from a wild relative of a crop plant, then CRISPR could be used to knock in the corresponding gene or to edit regulatory elements for constitutive or inducible expression. Moreover, synthetic biology approaches could produce AMPs in heterologous systems (e.g., yeast or algae) for topical agricultural applications. The combination of these technologies holds promise for developing durable, broad-spectrum resistance while minimizing off-target effects.[30]

The future of plant AMP research depends on inter-disciplinary integration of molecular biology, data science, and advanced bioinformatics. Researchers who embrace this convergence will be well-positioned to develop innovative, sustainable solutions to global challenges in health, agriculture, and the environment. Achieving this vision will require not only technical advances but also collaborative frameworks that bridge academic laboratories, industry partners, and governmental agencies. Open data policies, reproducible workflows, and cross-disciplinary training programs will be essential to nurture a new generation of scientists fluent in both plant biology and computational methods. With strategic investments in databases, AI models, and validation pipelines, plant AMPs can move from promising leads to real-world solutions in the coming decade.[30]

Cluster Analysis of Research Trends in Plant AMPs

Using VOSviewer software, a co-occurrence analysis of keywords from scientific publications related to plant AMPs was conducted. The resulting conceptual map (Figure 4) visualizes the clustering pattern of frequently co-occurring terms, where node size reflects keyword frequency and color indicates conceptual groupings. The analysis revealed four major research clusters, each representing a dominant trend in the field:

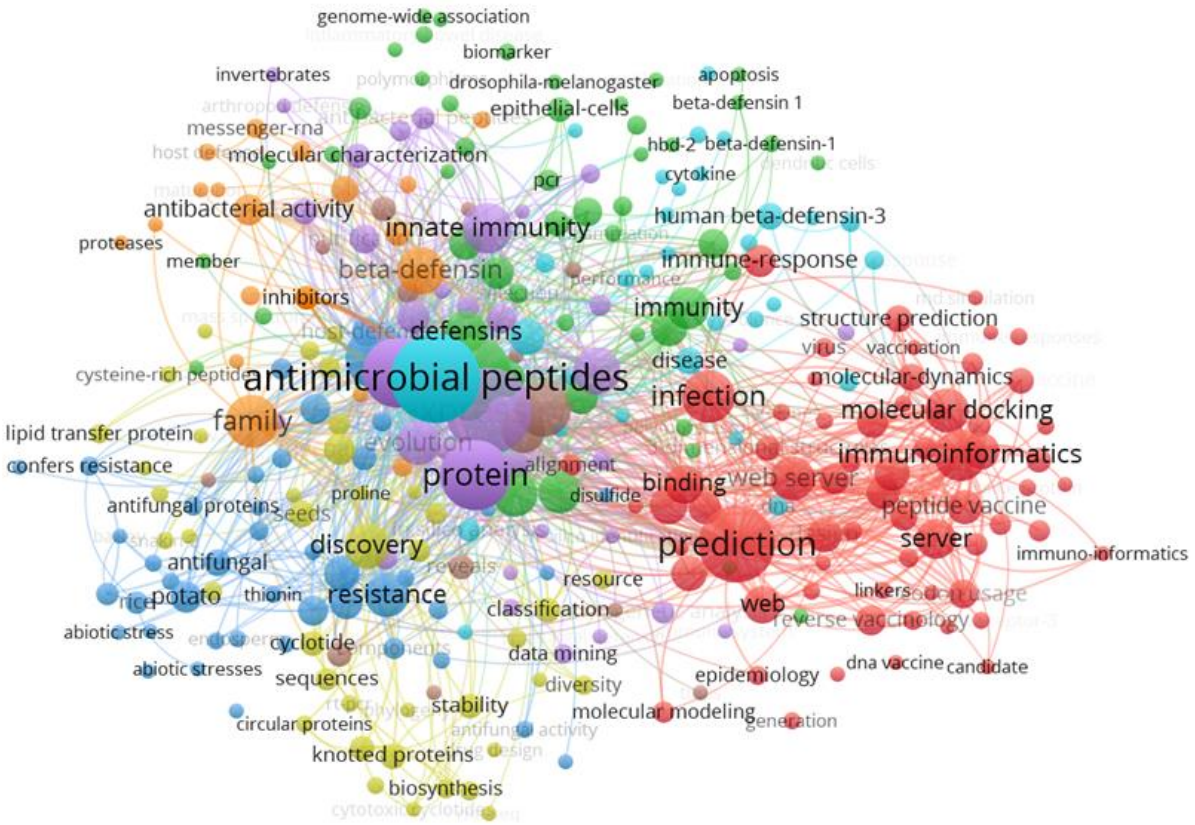

Figure 4. Bibliometric Cluster Map Illustrating Keyword Co-Occurrence in the Scientific Literature on Plant Antimicrobial peptides (AMPs). Cluster 1: Computational Modeling and Design (red color); Cluster 2: Peptide Structure and Chemistry (green color); Cluster 3: Biological Applications and Stress Responses (blue color); Cluster 4: General Bioinformatics and Sequence Analysis (yellow color).

- **Cluster 1– Computational Modeling and Design:** This group (Figure 4, red color) includes keywords such as prediction, immunoinformatics, web server, molecular docking, and peptide vaccine. These terms highlight the growing focus on machine learning, *in silico* screening, and web-based tools for the identification, classification, and optimization of AMPs.
- **Cluster 2– Peptide Structure and Chemistry:** This group (Figure 4, green color) includes keywords like cyclotide, beta-defensin, disulfide, and cysteine-rich are prominent in this cluster, representing studies related to the structural features, disulfide bonding patterns, and molecular stability of plant AMPs.
- **Cluster 3– Biological Applications and Stress Responses:** This cluster (Figure 4, blue color) centers on terms such as abiotic stress, antifungal, potato, resistance, and biosynthesis, reflecting a focus on the roles of AMPs in plant defense mechanisms under both biotic and abiotic stress conditions, especially in agricultural contexts.
- **Cluster 4– General Bioinformatics and Sequence Analysis:** This group (Figure 4, yellow color) Comprising keywords like motif, alignment, classification, and diversity, this group captures the foundational bio-informatics tools used for identifying, annotating, and classifying AMP sequences.

Analysis of the "average publication year" and keyword centrality shows a recent shift toward computational peptide design, structure-based prediction, and functional application of AMPs in sustainable agriculture and food security, highlighting the maturation of this interdisciplinary field. This bibliometric cluster analysis offers a comprehensive overview of current research directions in the AMP domain. It serves as a roadmap for organizing thematic trends, identifying research gaps, and guiding early-career scientists toward high-impact areas of study in plant AMPs (Figure 4).

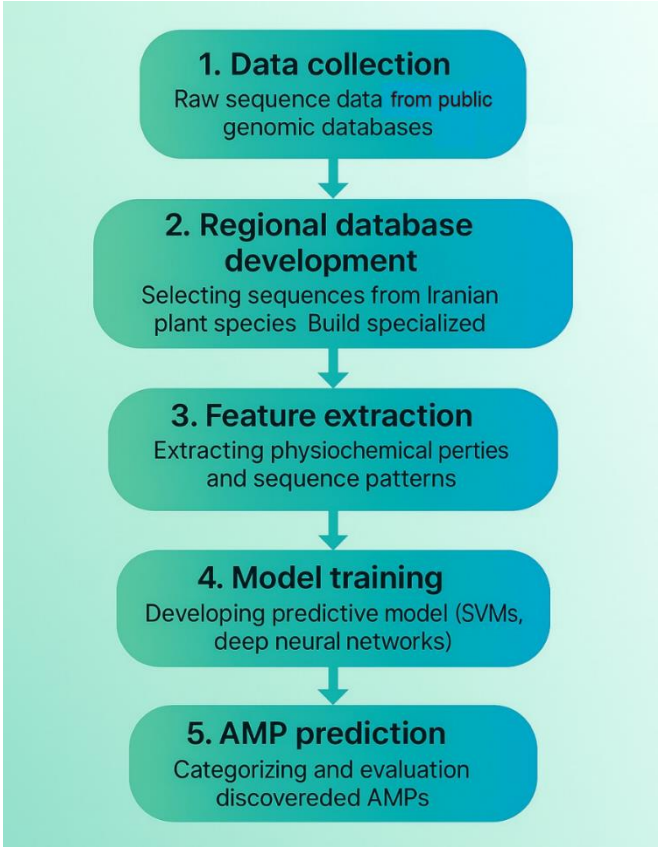

Figure 5. Conceptual Framework for Bioinformatics-Based Discovery and Characterization of Plant Antimicrobial Peptides (AMPs). The diagram illustrates the sequential computational workflow used in AMP discovery, from genomic and transcriptomic data retrieval, bioinformatic identification, and physicochemical/structural analyses, to functional annotation, validation, and proposed future directions involving the development of a regional AMP database and plant-specific machine learning models.

Limitations and Future Perspectives

Despite significant progress in computational AMP discovery, current studies face limitations such as small training datasets, limited experimental validation, and lack of standardized benchmarking across tools. The integration of *in silico* and *in vivo* validation pipelines will be crucial to overcome these issues, and expanding plant-specific datasets will enhance the accuracy and applicability of predictive

models in real agricultural systems.[31] With the rapid advancement of biotechnology and the growing integration of computational approaches in molecular biology, the role of plant AMPs is expected to expand far beyond their traditional applications. In the near future, several transformative directions are anticipated, including the integration with genome-editing technologies, where CRISPR/Cas9 will enable the design of plants that constitutively express specific AMPs, providing durable resistance against both biotic and abiotic stresses;[17] AI-driven synthetic peptide design, in which transcriptomic, proteomic, and three-dimensional structural data are merged within artificial intelligence frameworks to create synthetic peptides with enhanced stability, specificity, and multifunctionality;[32] and a wide range of industrial applications, as plant-derived AMPs hold great promise as natural and safe alternatives to antibiotics, biodegradable antimicrobial coatings, food preservatives, and active components in cosmetic formulations, while their activation in response to abiotic stresses such as drought and salinity offers potential for smart biofertilizers and growth stimulants in sustainable agriculture.[33] Realizing this vision will also require collaborative data-driven research, including establishing data-focused consortia, strengthening collaborations between bioinformatics and agricultural research laboratories, and developing comprehensive, open-access databases dedicated to plant AMPs.[32] Ultimately, the future will belong to researchers who transcend disciplinary boundaries, merging biology, data science, and innovation to create sustainable solutions for global challenges in health, agriculture, and the environment.

Future Work

Building upon the insights provided in this review, future research should focus on developing region-specific databases and predictive models tailored for plant AMPs. A dedicated Iranian Plant AMP Database (IPAD) could serve as a centralized repository that integrates sequence information, physicochemical properties, structural models, and expression profiles under various stress conditions.[34] Moreover, incorporating machine learning and deep learning frameworks (e.g., GNN, Transformer, and LSTM architectures) trained on plant-specific datasets could significantly improve AMP prediction accuracy. Such models would not only accelerate *in silico* screening but also bridge computational findings with experimental validation.[35] Establishing collaborative consortia between bioinformatics laboratories and agricultural biotechnology centers will be essential for data standardization, model benchmarking, and eventual integration of AMP discovery pipelines into crop improvement programs.[34,35] This multidisciplinary approach could transform computational AMP research into practical applications for sustainable agriculture and bioindustry (Figure 5).

Conclusion

Plant antimicrobial peptides (AMPs) are small but powerful biomolecules that play central roles in plant defense and stress adaptation. They protect plants from a wide range of pathogens, bacteria, fungi, and viruses, while also helping them cope with drought, salinity, and temperature stress. This review brought together current knowledge about plant AMPs, describing their main families, bioinformatics tools, and databases used for their discovery and functional analysis. The comparative *in silico* studies and bibliometric assessments presented here demonstrate how bioinformatics pipelines and machine learning models have accelerated AMP research, providing a clearer view of their structure, diversity, and function across different plant species. Even with these advances, challenges remain. Plant-specific AMP databases are still scarce, predictive accuracy for novel sequences is limited, and the gap between computational predictions and experimental validation needs to be bridged. Overcoming these challenges will require close collaboration among plant biologists, bioinformaticians, and data scientists. In the coming years, the combination of artificial intelligence, multi-omics technologies, and laboratory validation will play a decisive role in uncovering new plant AMPs and improving prediction models. Building specialized databases for AMP families such as plant defensins will help unify global data and promote accessibility. More importantly, translating computational discoveries into real-world applications can open new frontiers in sustainable agriculture and biotechnology. Plant AMPs have the potential to become natural alternatives to chemical pesticides, eco-friendly preservatives, smart fertilizers, and even active ingredients in pharmaceuticals and cosmetics. Strategic investment in this field will not only advance crop protection and food security but also strengthen the bridge between bioinformatics innovation and sustainable development.

Authors' Contributions

All authors contributed equally to the conception, design, data collection, analysis, and preparation of the manuscript. All authors have read and approved the final version of the manuscript.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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