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Database issue

ncPlantDB: a plant ncRNA database with potential ncPEP information and cell type-specific interaction

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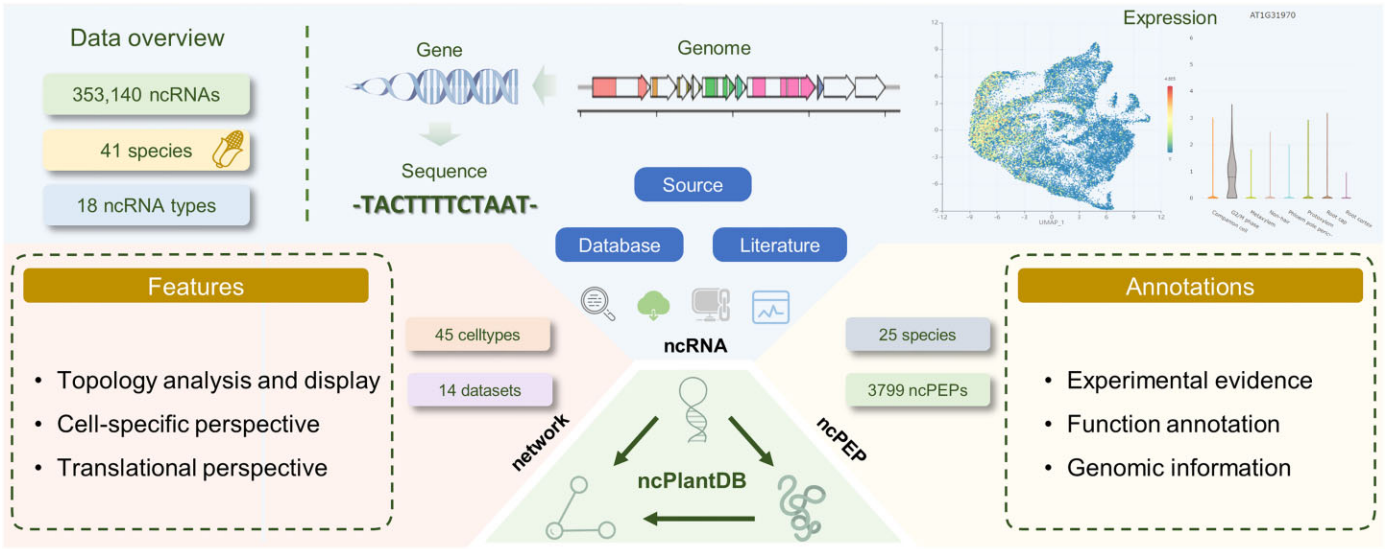
Abstract

The field of plant non-coding RNAs (ncRNAs) has seen significant advancements in recent years, with many ncRNAs recognized as important regulators of gene expression during plant development and stress responses. Moreover, the coding potential of these ncRNAs, giving rise to ncRNA-encoded peptides (ncPEPs), has emerged as an essential area of study. However, existing plant ncRNA databases lack comprehensive information on ncRNA-encoded peptides (ncPEPs) and cell type-specific interactions. To address this gap, we present ncPlantDB (https://bis.zju.edu.cn/ncPlantDB), a comprehensive database integrating ncRNA and ncPEP data across 43 plant species. ncPlantDB encompasses 353 140 ncRNAs, 3799 ncPEPs and 4 647 071 interactions, sourced from established databases and literature mining. The database offers unique features including translational potential data, cell-specific interaction networks derived from single-cell RNA sequencing and Ribo-seq analyses, and interactive visualization tools. ncPlantDB provides a user-friendly interface for exploring ncRNA expression patterns at the single-cell level, facilitating the discovery of tissue-specific ncRNAs and potential ncPEPs. By integrating diverse data types and offering advanced analytical tools, ncPlantDB serves as a valuable resource for researchers investigating plant ncRNA functions, interactions, and their potential coding capacity. This database significantly enhances our understanding of plant ncRNA biology and opens new avenues for exploring the complex regulatory networks in plant genomics.

Graphical abstract



Introduction

Non-coding RNAs (ncRNAs) play crucial regulatory roles in plant biology, influencing development, stress responses, and adaptation (1–4). Recent advances highlight the significance of ncRNAs in mediating these processes through intricate molecular mechanisms (3,5).

In addition to their regulatory functions, many ncRNAs have the ability to encode small peptides through

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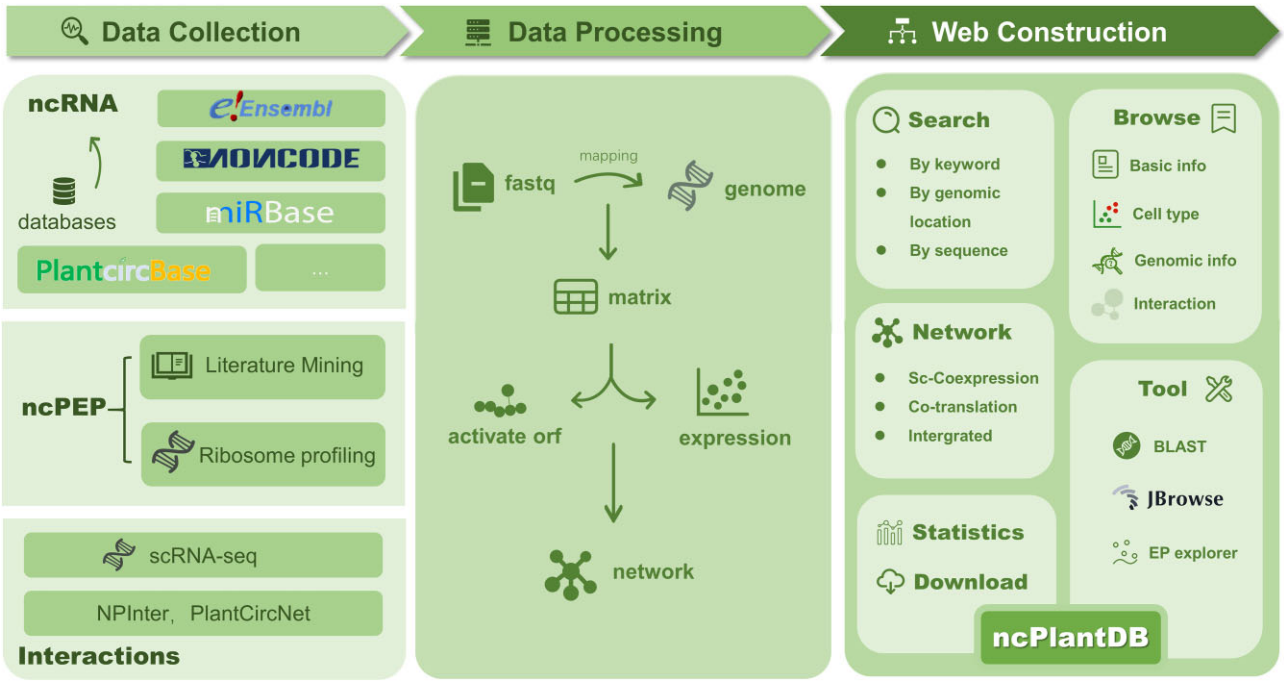

Figure 1. The pipeline of ncPlantDB, contains data collection, data processing and web construction.

their small open reading frames (sORFs). These peptides, known as ncRNA-encoded peptides (ncPEPs), are composed of 5–60 amino acids and provide an unexpected layer of gene regulation and protein function (6). Since Lauressergues *et al.* discovered miRNA-encoded regulatory peptides miPEP171b and miPEP165a, this concept has recently been extended to other miRNAs and additional ncRNA types (7). miPEPs, encoded by the first open reading frame (miORF) in the 5′ region of pri-miRNA, enhance the transcription of their originating pri-miRNAs (8,9). Additionally, these ncPEPs have been progressively demonstrated to possess potential for sustainable agriculture (6). Hence, the inclusion of ncPEPs provides valuable insights and expands our understanding of the multifaceted roles of ncRNAs in plant biology.

The advent of single-cell technologies has revolutionized our understanding of ncRNAs by enabling the detailed exploration of their expression and regulatory networks at the single-cell level. This allows for the dissection of cell-specific ncRNA functions and their contributions to cellular heterogeneity, essential for understanding complex biological processes in plants (5). Single-cell network analysis enhances our ability to delineate the precise regulatory roles of ncRNAs across different cell types and developmental stages, providing critical insights for both basic plant biology and agricultural applications (10,11).

Several integrative ncRNA databases for plants, such as GreeNC (12), PLncDB (13) and PNRD (14), have been established to support research in this burgeoning field. However, these databases lack a focus on the potential coding capacity of ncRNAs and do not incorporate single-cell data for expression and interaction analysis. This gap hinders comprehensive understanding and exploration of ncRNA functionalities at the cellular and molecular levels.

To address these limitations, we developed ncPlantDB, integrating comprehensive datasets, providing translational potential information, and incorporating single-cell data analysis. ncPlantDB is an indispensable resource for researchers, facilitating in-depth exploration of ncRNA and ncPEP expression, interactions, and functions in plant genomics. We anticipate that ncPlantDB will significantly contribute to the field and look forward to its adoption by the scientific community.

Materials and methods

Data sources

Currently, ncPlantDB includes 353 140 ncRNA entries from 41 plant species, spanning from green algae to angiosperms (Supplementary Table S1). Data were collected from authoritative ncRNA databases such as NONCODE (15) and miRBase (16), as well as model plant websites and literature (Figures 1 and 2A). For ncPEPs, we manually reviewed over 300 publications to identify 91 experimentally validated or predicted ncPEPs, providing respective references (Figures 1 and 2A). Information on coding potential for circRNAs was sourced from PlantcircBase (17) (Figures 1 and 2A).

To further evaluate ncRNAs' coding potential, we collected Ribo-seq data from the NCBI SRA database (https://www.ncbi.nlm.nih.gov/sra/) for *Arabidopsis thaliana*, *Oryza sativa*, *Solanum lycopersicum*, *Zea mays* and *Triticum aestivum* (Supplementary Table S2). Using our standardized pipeline, we identified ORFs with coding potential from these datasets (Figure 2B).

Additionally, to construct comprehensive cell-specific co-expression networks and identify their regulatory roles of plant ncRNAs, 14 single-cell datasets were obtained from NCBI SRA, and GSA database, including *A. thaliana*, *O. sativa*, *T. aestivum*, *Z. mays* and *Medicago truncatula* (Figure 2C, Supplementary Table S3 and S4).

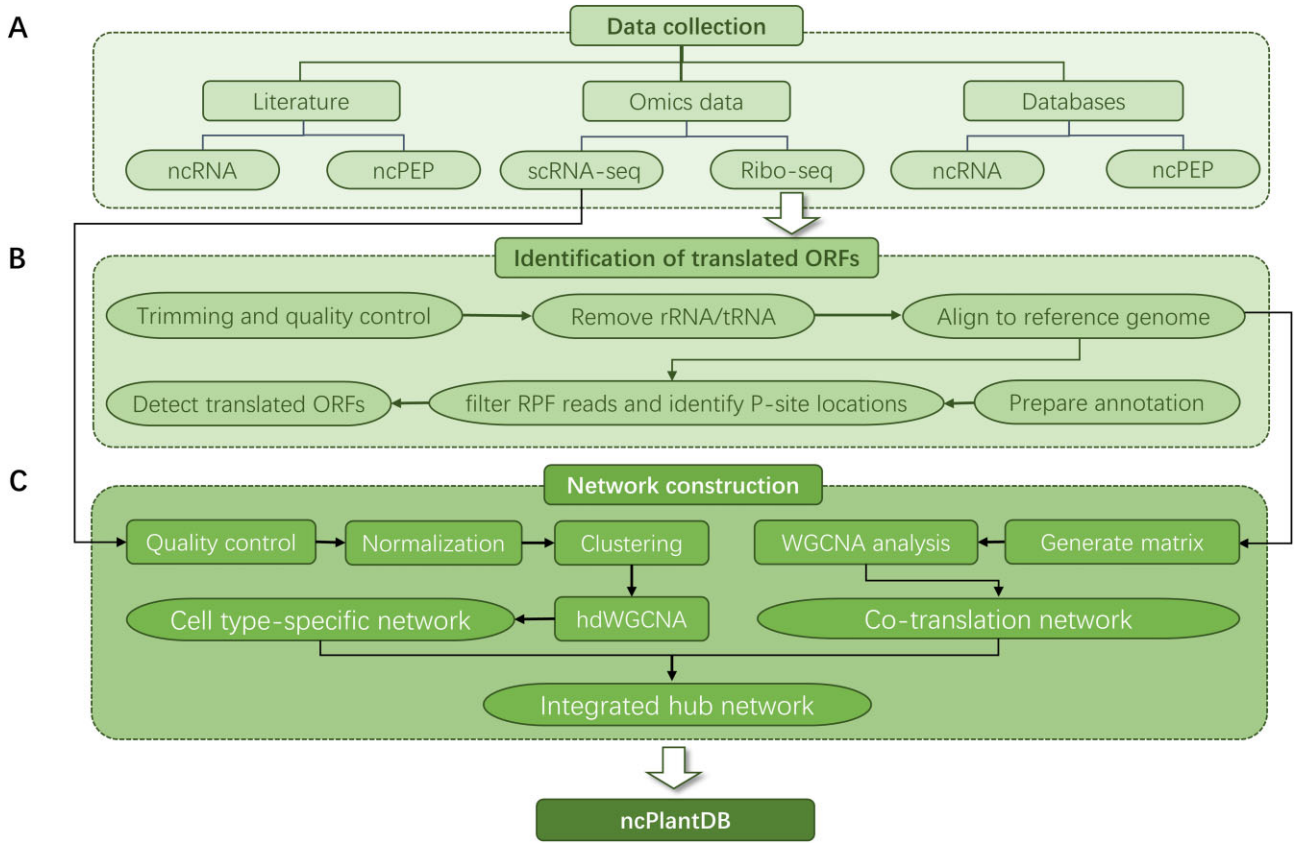

Figure 2. Data processing and network construction workflow. (**A**) Data collection. (**B**) Identification of translated ORFs from Ribo-seq data. (**C**) Network construction, leading to integration into the ncPlantDB database.

Data pre-processing

For Ribo-seq data, raw reads underwent quality control, trimming, and filtering using Trim Galore (v0.6.10) with parameters '-q 20 -length 20 –max-length 35′. To extract meaningful information from ribosome footprints, it was essential to remove reads originating from rRNA and tRNA (18). Reference sequences for rRNA and tRNA for each species were obtained from the Rfam (19) database. We used Bowtie2 (v2.5.2) to align the reads to these reference sequences (20) and subsequently removed any reads that aligned to rRNA or tRNA. Cleaned reads were then aligned to the reference genomes of the respective species using STAR (v2.7.11) (21).

For scRNA-seq data in SRA format were converted to FASTQ format using sratoolkit (v2.10.7). Barcode assignment and UMI quantification were performed using specialized tools optimized for each dataset: Cell Ranger (v5.0.1) for 10x Genomics data and the WTA Local bioinformatics pipeline for BD Rhapsody data. The resulting count matrices were subsequently analyzed using Seurat (v4.0.0) (22). Cells with fewer than 200 detected genes or with mitochondrial gene expression exceeding 10% were excluded from further analysis. Cutoff values for nFeature_RNA was determined using the interquartile range (IQR) method. The 3000 most variable genes were identified through the 'vst' method within the 'FindVariableFeatures' function. The data were then scaled with regression on mitochondrial UMIs. Principal component analysis (PCA) was performed using 'RunPCA', followed by cluster visualization with UMAP. Clustering was conducted using a shared nearest neighbor (SNN) graph and the Louvain method, with a resolution parameter set to 0.8.

Translation potential identification

Ribocode (v1.2.13) was employed with default parameters to analyze the rRNA-depleted Ribo-seq data (23). This tool identifies actively translating open reading frames (ORFs) in non-coding RNAs (ncRNAs) by leveraging periodicity and length specificity, pinpointing both known and novel ORFs in active translation. The distribution of ribosome-protected fragments (RPFs) was analyzed using the metaplot function within the RiboCode software. Transcriptional annotations were processed and converted with the prepare_transcripts tool. Based on the distribution of read counts for each read length, the ribosome protection range and the corresponding P-site positions were determined for various read lengths. Subsequently, the core RiboCode tool was applied to predict small open reading frames (sORFs) using the parameters '-l no -g' (Figure 2B). ORFs in the translation process identified using the RiboCode were classified into seven types, 'annotated', 'uORF', 'dORF', 'Overlap_uORF', 'Overlap_dORF', 'internal' and 'novel'. Active sORFs shorter than 300 nucleotides classified as 'novel' were derived from non-coding genes or non-coding transcripts of the coding genes, thus being considered to encode ncPEPs potentially.

Cotranslation network construction

Transcript expression levels were estimated by RSEM (v1.3.3) using the default parameters (24), and the output matrixes were then used to construct a translatome coexpression network (Figure 2C). The coexpression networks were con-

Table 1. The number of different categories of ncRNAs and ncPEPs for some widely studied plant species

Species	Number of ncRNAs					Number of ncPEPs
	lncRNA	miRNA	circRNA	Others	Total	
Arabidopsis thaliana	22 692	325	52 393	17 067	92 477	574
Oryza sativa	1190	694	43 889	3285	49 058	1403
Medicago truncatula	6132	1049	0	1846	9027	14
Triticum aestivum	12 427	190	162	12 793	25 572	11
Zea mays	29 434	347	1517	8470	39 768	123
Brassica rapa	6457	185	591	1079	8312	47
Cucumis sativus	2550	97	4832	585	8064	306
Glycine max	2242	763	9734	1171	13 910	417
Gossypium hirsutum	0	78	3944	0	4022	30
Gossypium raimondii	1247	296	1478	0	3021	165
Physcomitrella patens	471	247	0	440	1158	4
Populus trichocarpa	2248	352	4408	0	7008	56
Solanum lycopersicum	3822	168	3796	1111	8897	322
Vitis vinifera	3351	163	0	0	3514	4
Other species (28)	47 668	2673	17 244	11 747	79 332	323
All species	141 931	7627	143 988	59 594	353 140	3799

structed using WGCNA (v1.70–3) with the default parameters (25).

Cell type-specific network construction

Cell-type annotation

We get cell-type annotation from scPlantDB (26). Cells were annotated using experimentally verified marker genes, based on a standardized taxonomy from the Plant Ontology database.

Co-expression network analysis

The hdWGCNA package integrates the entire workflow for constructing networks using high-resolution omics data (25,27). The pipeline includes normalizing gene expression data, performing hierarchical clustering, determining soft thresholding power, constructing a weighted gene co-expression network, and identifying gene modules. For constructing cell type-specific networks, we utilize the hdWGCNA package (v0.2.24). To retain as many ncRNAs as possible, 'gene_select' was set to 'all' during the 'SetupForWGCNA' step. When constructing metacells in each group, we select 'ident.group' as the cell type and use the default parameters '$k = 25$, max_shared $= 10$'. Subsequently, we select each cell type for the construction of co-expression networks (Figure 2C).

Integrated network construction

We integrated cell type-specific co-expression networks derived from single-cell data with potentially translated ncPEP genes identified through our pipeline to construct an integrated cell type-specific network containing potential translation nodes (Figure 2C). Given the typically large size of cell type-specific co-expression networks, characterized by a high number of nodes and edges, we applied the MCC algorithm to extract hub networks, focusing on the top 500 genes based on MCC rank. All edges associated with ncPEP genes were then incorporated into the hub network (Figure 2C). Additionally, each ncPEP gene node was assigned a label to be displayed in our visualization interface.

Database development

ncPlantDB was constructed using robust technologies to ensure high performance, efficient data handling, and user-friendly interaction. MySQL was used as its storage engine for reliable and efficient datasets management. The backend web framework was developed with Django, facilitating the rapid development of a secure and maintainable web application.

The user interface was created using Bootstrap, enhancing usability. For data visualization, advanced libraries such as Echarts, Plotly.js, and D3 were integrated, offering interactive capabilities for data exploration and analysis. Network visualization was achieved using Echarts and sigma.js, enabling users to visualize complex data relationships and uncover insights that might not be immediately apparent from raw data alone.

Results

Database content

ncPlantDB integrates a comprehensive collection of ncRNA annotations from multiple reputable sources (15–17,28,29), as well as from the literature. This integration has resulted in a repository of 353 140 ncRNAs encompassing 18 types across 41 plant species (Table 1). This extensive dataset ensures that ncPlantDB remains a leading resource in plant ncRNA research.

A distinguishing feature of ncPlantDB is its inclusion of non-coding peptides (ncPEPs). Data on ncPEPs are derived from literature, Ribo-seq analyses, and databases like PlantCircBase (17). Specifically, 91 ncPEPs were identified from literature (51 experimentally validated), 743 from Ribo-seq and 2 965 from PlantCircBase (17). This makes ncPlantDB the first database to provide such extensive coverage of ncPEPs, annotating those with translational potential across 25 species.

The database also provides an extensive catalog of ncRNA interactions, derived from both single-cell RNA sequencing (scRNA-seq) and Ribo-seq data. The database includes 4 647 071 cell type-specific co-expression interactions, covering five plant species and 48 cell types. This wealth of interaction data allows for an in-depth exploration of ncRNA relationships and functionalities at the cellular level. The interaction data are presented with detailed topological informa-

Figure 3. Some modules of ncPlantDB. (**A**) Browse. (**B**) Detailed page of ncRNA. (**C**) Search. (**D**) Network. (**E**) One of the tools named Expression Pattern Explorer.

tion, enhanced by advanced filtering and search functionalities through visually appealing network visualizations using sigma.js.

To ensure that ncPlantDB remains a cutting-edge resource, the database is regularly updated with new datasets as they become available. This commitment to continuous improvement guarantees that researchers have access to the most current and comprehensive data available.

User interface of ncPlantDB

Browse

The ncPlantDB database provides a streamlined browsing interface that facilitates efficient data discovery and exploration. Users can access three primary content types: ncRNA, ncPEP and interaction data. Each content type offers specific filters to help users narrow down their searches effectively.

For ncRNA, users can filter results by species and ncRNA types, ensuring that they can quickly find the information they need (Figure 3A). Similarly, the ncPEP content type allows users to filter by species, host ncRNA types, and sequence length, which is particularly useful for those focusing on shorter sequences for chemical production. The interaction data can be filtered by species, cell types and ncRNA types, making it easy to locate relevant interaction information.

Once a specific entry is selected, for ncRNA and ncPEP, users are presented with a detailed information page divided into four sections: basic information, cell type-specific expression data from single-cell studies, genomic mapping information, and co-expression network data (Figure 3B). This comprehensive layout provides a holistic view of each entry, and users can easily switch between related ncPEP and ncRNA entries to explore translational potential. For the detailed page of the interaction network, it encompasses basic information about the start and end nodes, including their interaction types and cell types. Additionally, this page provides genomic and sequence data for the interaction molecules, as well as a module dedicated to the interaction network.

Search

To cater to different search needs, the ncPlantDB database offers both simple and advanced search functionalities. The simple search option is available at the top of the browse page for each content type, allowing users to perform quick keyword searches (Figure 3A). For more refined searches, the advanced search option can be accessed via the navigation bar on every page. This feature includes keyword searches, genomic location searches, and BLAST for sequence alignment, ensuring users can perform detailed and specific queries (Figure 3C).

Network

The network feature in ncPlantDB leverages tools including Gephi (30) and sigma.js to provide an in-depth exploration of network topologies. Users can examine the detailed topology information of each node by a simple click, with every node linked to a detailed page for further investigation (Figure 3D).

This network analysis is divided into three main sections. The Integrated Hub Network focuses on extracting hub genes from the co-expression network and includes information about ncRNAs that encode ncPEP. The ncRNA-Coexpression Network, derived from single-cell expression data, allows users to explore co-expression patterns of ncRNAs at a highly detailed cellular resolution. Lastly, the ncPEP-Cotranslation Network, based on Ribo-seq expression matrices, highlights co-expression relationships during the translation process, providing insights into protein synthesis and post-transcriptional regulation (31).

Tools

ncPlantDB includes several tools designed to enhance data analysis capabilities. The BLAST tool allows users to set advanced parameters and download results for sequence alignment (32). JBrowse supports keyword searches limited to transcript names, offering a visual representation of genomic data. The Expression Pattern (EP) Explorer tool is specifically designed for analyzing cell type-specific expression patterns from single-cell datasets. Users can input a list of gene names,

select a dataset (tissue), and submit their query to compare expression levels across different cell types, with results displayed in heatmap and table formats (Figure 3E).

Download

The download section of ncPlantDB is organized to provide easy access to data, divided into three parts: ncRNA, ncPEP and interaction data. All information is categorized by species and available in various file formats. For instance, ncRNA data can be downloaded as comprehensive CSV files (containing all relevant information), GFF annotation files, and FA sequence files. This structured approach ensures users can access and utilize the data efficiently for their research.

Application case

CCT2 (AT4G15130), a gene encoding phosphorylcholine cytidylyltransferase 2 in *Arabidopsis thaliana*, is known to play a significant role in plant immunity (33). Using the search function in ncPlantDB, we queried the gene AT4G15130 (Supplementary Figure S1). The database provided detailed information about its genomic location, cell-type expression, and its co-expressed ncRNAs. Additionally, it highlighted the non-translating CDS may encoded a peptide AthNCP0021 located within the same genomic region. Utilizing its expression profiles, we visualized the expression patterns of CCT2. The violin plots revealed tissue-specific expression in immune-response tissues, such as leaf epidermis (34) and phloem parenchyma (35), supporting the hypothesis that CCT2 plays a role in immune-related lipid metabolism. Similarly, we can also find the interaction information of CCT2 in leaf epidermis and phloem parenchyma cells through the cell type-specific network in the database. In addition, we can view the interactions of CCT2 in other tissue types as well. To further verify the translational level function of CCT2, we searched the gene in co-translation network and viewed function and pathway enrichment result of its module. Results from GO enrichment indicated that CCT2 and its co-expressed genes are involved in processes such as 'response to stimulus', 'membrane biogenesis', and 'lipid metabolism'. These functions reinforce CCT2's role in pathogen defense by maintaining membrane integrity and promoting immune signaling. KEGG enrichment pinpointed specific pathways like 'plant-pathogen interaction' and 'phospholipid biosynthesis', further establishing CCT2's involvement in immune responses and cellular signaling. Notably, these pathways are linked to Effector-Triggered Immunity (ETI) and Pattern-Triggered Immunity (PTI), confirming CCT2's regulatory role in Arabidopsis immune mechanisms (33).

Discussion

The rapid advancement in biotechnology has led to the discovery of numerous plant-specific ncRNAs, crucial for various regulatory networks and agricultural applications (4). Currently, existing databases such as PlncDB (13), GreenNC (12), and CANTADB (36) focus on plant long non-coding RNAs (lncRNAs), while PMRD (37) and PmiREN (38) are dedicated to microRNAs (miRNAs). Despite their contributions, comprehensive ncRNA databases for plants are scarce, with PNRD (14) from 2015 being the only one. To address this gap, we developed ncPlantDB, an integrated plant ncRNA database.

An outstanding feature of ncPlantDB is the inclusion of translation information of ncRNAs. Although the potential of ncPEPs (non-coding RNA-encoded peptides) is increasingly recognized, it remains underexplored. These peptides hold promise in production applications (6,39). ncPlantDB is the first database to include translation-related information and visualization at the translational level, enhancing usability (31).

ncPlantDB also innovatively conducts co-expression network analysis on a large volume of plant single-cell RNA sequencing (scRNA-seq) data and integrates these analyses into the database. Advances in single-cell sequencing technology allow for the high-resolution exploration of regulatory network differences among cell types in specific plant tissues (40). While other databases may contain network-related information, they lack this level of detailed analysis(12–14,16–17,36–38).

Additionally, ncPlantDB offers a user-friendly interface with seamless browsing, a powerful search engine, and download portals. Its advanced visualization capabilities, especially for network data and single-cell expression data, provide customization options for user-defined visualizations. This feature sets ncPlantDB apart from other similar databases, making it more accessible and practical for researchers to interpret and share their findings.

We are committed to regularly updating ncPlantDB. Small updates are scheduled approximately every 6 to 12 months, focusing on integrating new data from recent literature, including non-coding RNA (ncRNA) and ncRNA-encoded peptide (ncPEP) data. Periodic larger updates will add new datasets, features, and species, ensuring the database stays current with scientific and technological advances. Through these efforts, we aim to maintain ncPlantDB as a reliable, up-to-date resource that contributes to the advancement of plant research.

By integrating diverse ncRNA information and innovative data analysis methods, ncPlantDB aims to become an essential resource for plant biologists, providing comprehensive insights and facilitating further research in the field.

Data availability

ncPlantDB can be accessed at https://bis.zju.edu.cn/ncPlantDB.

Supplementary data

Supplementary Data are available at NAR Online.

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Author contributions: M.C, L.L. and E.L. conceived the study. L.L. and E.L. designed and constructed the database jointly, with most work conducted by L.L. E.L. designed and built the pages and web functions related to networks. S.L. assisted with the database deployment. Data visualization was performed by L.L. and E.L. Data collection and analysis were carried out by L.L. and E.L. with the assistance of Y.S., L.W., S.Z., Y.H., Y.C. and L.X. The manuscript was written by L.L and revised and reviewed by E.L., M.C., Y.H., H.C. and Y.Z. All authors reviewed and approved the final manuscript.

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Conflict of interest statement

None declared.

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