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Database issue

AMIR: a multi-omics data platform for *Asteraceae* plants genetics and breeding research

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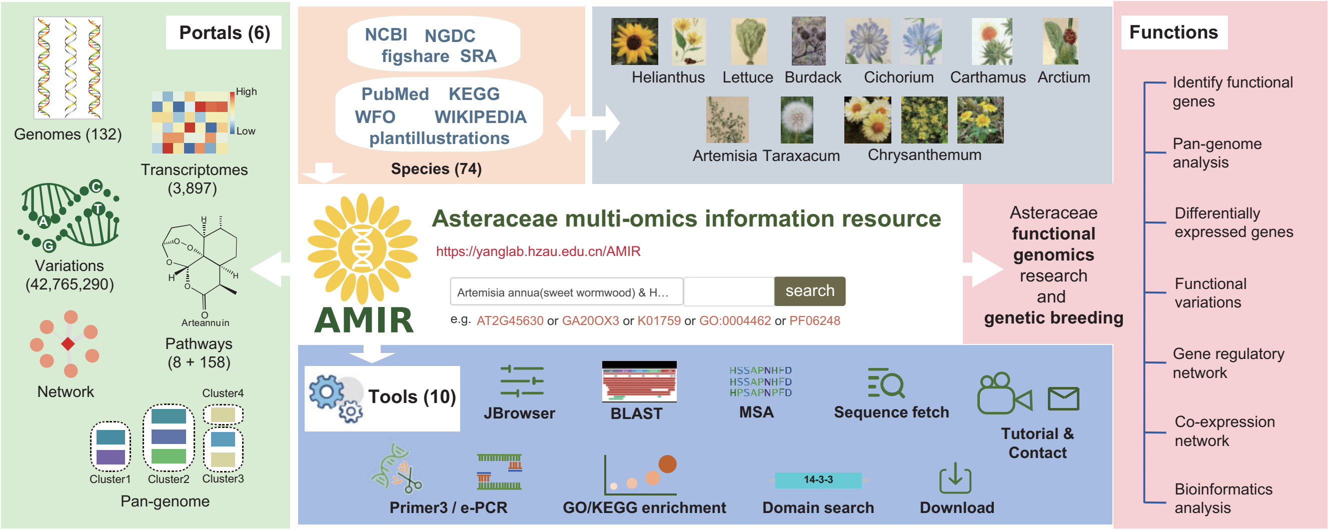
Abstract

As the largest family of dicotyledon, the *Asteraceae* family comprises a variety of economically important crops, ornamental plants and numerous medicinal herbs. Advancements in genomics and transcriptomic have revolutionized research in *Asteraceae* species, generating extensive omics data that necessitate an efficient platform for data integration and analysis. However, existing databases face challenges in mining genes with specific functions and supporting cross-species studies. To address these gaps, we introduce the Asteraceae Multi-omics Information Resource (AMIR; https://yanglab.hzau.edu.cn/AMIR/), a multi-omics hub for the *Asteraceae* plant community. AMIR integrates diverse omics data from 74 species, encompassing 132 genomes, 4 408 432 genes annotated across seven different perspectives, 3897 transcriptome sequencing samples spanning 131 organs, tissues and stimuli, 42 765 290 unique variants and 15 662 metabolites genes. Leveraging these data, AMIR establishes the first pan-genome, comparative genomics and transcriptome system for the *Asteraceae* family. Furthermore, AMIR offers user-friendly tools designed to facilitate extensive customized bioinformatics analyses. Two case studies demonstrate AMIR's capability to provide rapid, reproducible and reliable analysis results. In summary, by integrating multi-omics data of *Asteraceae* species and developing powerful analytical tools, AMIR significantly advances functional genomics research and contributes to breeding practices of *Asteraceae*.

Graphical abstract



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Introduction

The *Asteraceae* family, accounting for 7% of flowering plant diversity with approximately 1600–1700 genera and over 26 000 species (1–4), holds paramount economic and ecological significance. It includes well-known oilseed crops such as sunflowers, vegetables such as lettuce, chicory and artichoke, ornamental plants such as chrysanthemum, and various weeds and invasive species (1–3). Additionally, *Asteraceae* plants are widely used in traditional medicine and valued for their production of diverse secondary metabolites (5,6).

Recent advancements in genomics and transcriptomics have revolutionized research in *Asteraceae* species, facilitating the identification of functional genes, crop genetic breeding and pharmaceutical discovery (7–12). Several *Asteraceae* species, including sunflower, lettuce, cultivated chrysanthemum, chicory, endive, great burdock and yacon, have been decoded (7–9,12), providing valuable references for genetic information mining. However, genomic studies of *Asteraceae* plants still face challenges due to scarcity of publicly accessible gene annotations, lack of comparative genomics/transcriptomic platforms, and difficulties in managing and analyzing multi-omics data. Platforms such as LettuceGDB, SGD (https://sunflowergenome.org/), the burdock database and Chrysanthemum Genome Database (http://210.22.121.250:8880/) have been developed to integrate and utilize these data (13,14). For example, LettuceGDB integrates multi-omics data and germplasm resources, and provides several tools for lettuce (13). SGD contains tools such as JBrowse, BLAST and gene expression browser, and allows the users to download genomic, variations and transcriptomes data of sunflower. The burdock multi-omics database integrates mitochondrial, chloroplast, and nuclear genome, transcriptome and SRAP fingerprints of different varieties of burdock (14). The Chrysanthemum Genome Database was launched in April 2023, and contains genomes, transcriptomes, comparative genomics and various bioinformatics analysis tools. While these existing databases address specific species, they struggle with efficient gene mining and cross-species studies. Therefore, there is a critical need for an integrative *Asteraceae* omics database that offers comprehensive annotations, data integrity and user-friendly tools to support fundamental research and breeding practices across the *Asteraceae* family.

To address these challenges, we introduce the Asteraceae Multi-omics Information Resource (AMIR) platform. AMIR integrates various data types, including genomes, gene functional annotations, homolog relationships, transcriptome and variations for 74 *Asteraceae* species. It provides the first pan-genome and comparative genomics platform for *Asteraceae* species, along with a comprehensive transcriptome system comprising over 3897 RNA sequencing (RNA-seq) libraries. AMIR also features user-friendly tools, including JBrowser, BLAST, Multi-alignment viewer, Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (GO/KEGG) enrichment, Primer3 and e-PCR, enabling extensive customized bioinformatics analyses. By integrating multi-omics data of *Asteraceae* species and offering powerful analytical tools, AMIR aims to advance functional genomics research and enhance breeding practices of *Asteraceae*.

Materials and methods

Data collection

To encompass a broad range of *Asteraceae* species, we compiled 132 genome assembly datasets from 74 species sourced primarily from the National Center for Biotechnology Information (NCBI) (15), National Genomics Data Center (16) and European Bioinformatics Institute (17). These include well-known species such as sunflower (7), lettuce (9), cultivated *Chrysanthemum* (10) and *Artemisia annua* (18), with 76 genomes assembled at the chromosome level. Additionally, we collected 3897 RNA-seq libraries for 44 species from NCBI's SRA database, which were utilized for genetic variation analysis and co-expression network construction.

Gene annotation pipeline

Gene annotation was performed for 24 *Asteraceae* species lacking gene annotation data using Braker3 (19). This tool integrates *ab initio* gene prediction, homology-based protein prediction using sequences from *Arabidopsis thaliana* (TAIR10) (https://www.arabidopsis.org/), sunflower (HanXRQr2.0-SUNRISE) (7), cultivated *Chrysanthemum* (Chrysanthemum_x_morifolium_Ramat_Zszgv0) (10) and lettuce (Lsat_Salinas_v11) (9), and RNA-seq data with a mapping rate exceeding 70% across diverse tissues and treatments (Supplementary Table S1) (20). The completeness of protein-coding annotations was evaluated using BUSCO v4.0.5 (21), and results are detailed in Supplementary Table S2. For ease of access, a uniform gene identifier (AMID) was assigned to genes in general feature format (GFF) files across 68 collected and predicted genomes.

Functional annotation of protein-coding genes

To enhance genome annotation completeness, we conducted unified functional annotation for each genome. Homologous genes from *Asteraceae* species were identified using a sequence similarity-based approach previously described (22). Specifically, protein sequences from genes in *Arabidopsis* and sunflower (7) were used as queries. BLASTP (2.10.0+) searches were performed against protein sequences of each *Asteraceae* species under the conditions of *E*-value <1e−5 and identity >50% (22). Subsequently, all protein sequences from *Asteraceae* species were reciprocally searched against protein sequences from *Arabidopsis* and sunflower. For protein sequences matching those from *Arabidopsis* and sunflower, only the best alignment sequence was kept. Based on the results from these searches, homologous genes in *Asteraceae* species were extracted.

Transcription factors (TFs) in 68 *Asteraceae* genomes were predicted using PlantTFDB v5.0 (23) and iTAK (24). Amino acid sequences were uploaded to PlantTFDB and iTAK database for analysis. Gene families in the *Asteraceae* genomes were identified based on corresponding gene family members in *Arabidopsis* (https://www.arabidopsis.org). To identify conserved motifs, protein sequences from *Asteraceae* species were subjected to HMMER models using HMMER v3.2.1 hmmsearch (25) with an *E*-value threshold of 1e−5. Protein sequences from each pair of genomes were compared using Diamond v.0.9.14.115 (26). Subsequently, gene collinearity was detected using the McScan (Python version) (27). Visualization of collinearity results for specific species and regions was achieved using ShinySyn (28) to facilitate user access.

Ortholog groups among the *Asteraceae* genomes

To analyze ortholog groups among *Asteraceae* genomes, we first constructed a distance-based phylogenetic tree using JolyTree with default parameters (29). The tree was saved in Newick format and visualized with the ggtree R package (30). We selected genomes from 43 *Asteraceae* species to establish a robust pan-genome based on two criteria: (i) BUSCO completeness scores >80% (31) and (ii) the inclusion of RNA-seq data for gene structure prediction. Drawing from pan-genome construction approaches in Bambusoideae (32), rice (20), wild grape (33) and poplar (34), we developed a gene-based pan-genome for the *Asteraceae* (33). Protein sequences from genes in 43 *Asteraceae* genomes were collected and analyzed using OrthoFinder v2.5.4 (35) with default parameters to identify ortholog groups, which are clusters of genes. These ortholog groups include three categories: core, dispensable and species-specific gene clusters. Core gene clusters are genes present in at least 39 genomes (>90% genomes) (36), species-specific gene clusters occur only in one species and the remaining gene clusters found in 2–38 species are classified as dispensable gene clusters.

RNA-seq analysis and gene regulatory network construction

After removing adapter sequences and low-quality reads using fastp (v.0.23.0) (37), clean reads were aligned to the reference genome of each species using Hisat2 (v.2.1.2) (38) with default parameters. Expression quantification was performed using StringTie (v.1.3.5) (39) with default settings, and gene expression levels were normalized using transcripts per kilobase million reads. Raw gene expression counts were generated using featureCounts (v1.6.4) (40). Differential gene expression analysis was conducted using the Bioconductor package DESeq2 (41) in R (v4.3.1). Genes with a $\log_2$-transformed fold change ≥ 1 or ≤ -1 and a false discovery rate ≤ 0.05 were considered differentially expressed genes (DEGs). Tissue-specific gene expression was assessed using transcriptomes from various developmental. Batch effects were removed using the ComBat (42) package in R (v4.3.1). To measure tissue specificity, expression specificity metric (Tau) (43) and specificity measure (SPM) (44) were calculated. A co-expression network was constructed by calculating the Pearson correlation coefficient (PCC) of pairwise gene expression levels with gene pairs having a PCC > 0.8 retained to form the network. Furthermore, TF regulatory networks for 54 species were predicted using the regulatory prediction function in PlantRegMap (45), based on the regulatory network of *Arabidopsis*.

Genome variation calling

Due to the complexity and large size of *Asteraceae* genomes, population-level resequencing studies are limited (10,46). Nonetheless, transcriptome-based variant identification has emerged as a cost-effective alternative and has been successfully applied in several species, such as lettuce, maize and rice (47–49). In this study, we adopted a workflow previously used for variant identification using RNA-seq data (47–49). Initially, high-quality clean reads were aligned to the reference genome using STAR with default parameters (50). The alignment outputs were subsequently converted and sorted using Samtools (v1.13) (51). The mapped reads were further processed for sorting, read group addition and duplicate marking with Sambamba (v0.8.2) (52). After that, GATK's SplitNCigarReads was utilized to split '*N*' cigar reads (53). GATK HaplotypeCaller was then employed to generate Genomic Variant Call Format (GVCF) files for each sample. These individual GVCF files were combined using CombineGVCFs and GenotypeGVCFs to produce a unified Variant Call Format (VCF) file. The raw VCF file was filtered using GATK's VariantFiltration to remove low-quality single-nucleotide polymorphisms (SNPs), applying criteria of 'QUAL < 30.0 || MQ < 50.0 || QD < 2'. SNPs and Insertions and Deletions (InDels) with minor allele frequencies <0.01 or missing rates >0.1 were discarded using VCFtools (v.0.1.16) (54). Finally, genetic variant annotation and effect prediction were carried out using SnpEff (5.0d) (55).

Database implementation

AMIR was implemented using ThinkPHP framework (v.5.0.24) (https://www.thinkphp.cn/) for its backend. The core JavaScript libraries utilized include Vue.js as the main framework (https://vuejs.org), vis.js for network visualization (https://visjs.org), plotly.js (https://plotly.com), echarts (https://echarts.apache.org), D3.js (https://d3js.org), igv.js (56) and BlasterJS (57) for interactive charts. The system operates on an Apache2 Web server (v.2.4.53) and employs MySQL (v.8.0.29) as its database engine. The database is publicly accessible online without requiring registration and has been optimized for Chrome (recommended), Opera, Firefox, Windows Edge and macOS Safari. Gene and protein sequence alignments were performed using NCBI BLAST (v.2.13.0+) (58) and MAFFT (v7.490) with the '–maxiterate 1000' parameter (59). Genome sequences, gene models, variations and gene expression levels were displayed using JBrowse 2 (60). Web BLAST functionality was powered by Sequenceserver (61), while primer design was facilitated by Primer3 (62). Gene set enrichment analysis was conducted using the R package fgsea (63).

Database content and usage

Overview of AMIR

AMIR is dedicated to developing a comprehensive and user-friendly multi-omics data platform for the *Asteraceae* family. To this end, we have constructed eight specialized omics modules focusing on genes, including species, genomes, transcriptomes, variome, networks, pathways, tools, downloads and help (Figure 1). In the genomics module, AMIR encompasses 132 genome assembly data for 74 *Asteraceae* species to date (Supplementary Table S2). To address the scarcity of publicly available gene structure annotations, we predicted gene structure for 24 species using genomes at or above the scaffold level (Supplementary Table S2). We then performed comprehensive and integrated functional annotation on 4 408 432 protein-coding genes, identifying 2 892 067 GO/KEGG terms (64), 2 726 916 Pfam domains (65), 158 KEGG pathways (66), 173 009 TFs and 703 271 gene families (Figure 1). For construction of the *Asteraceae* pan-genome, the most frequently used reference genome from each species was selected, resulting in 95 770 gene clusters. AMIR incorporates 3897 RNA-seq datasets covering 44 species, 55 tissues and 76 stress treatments (Supplementary Table S1). Genetics vari-

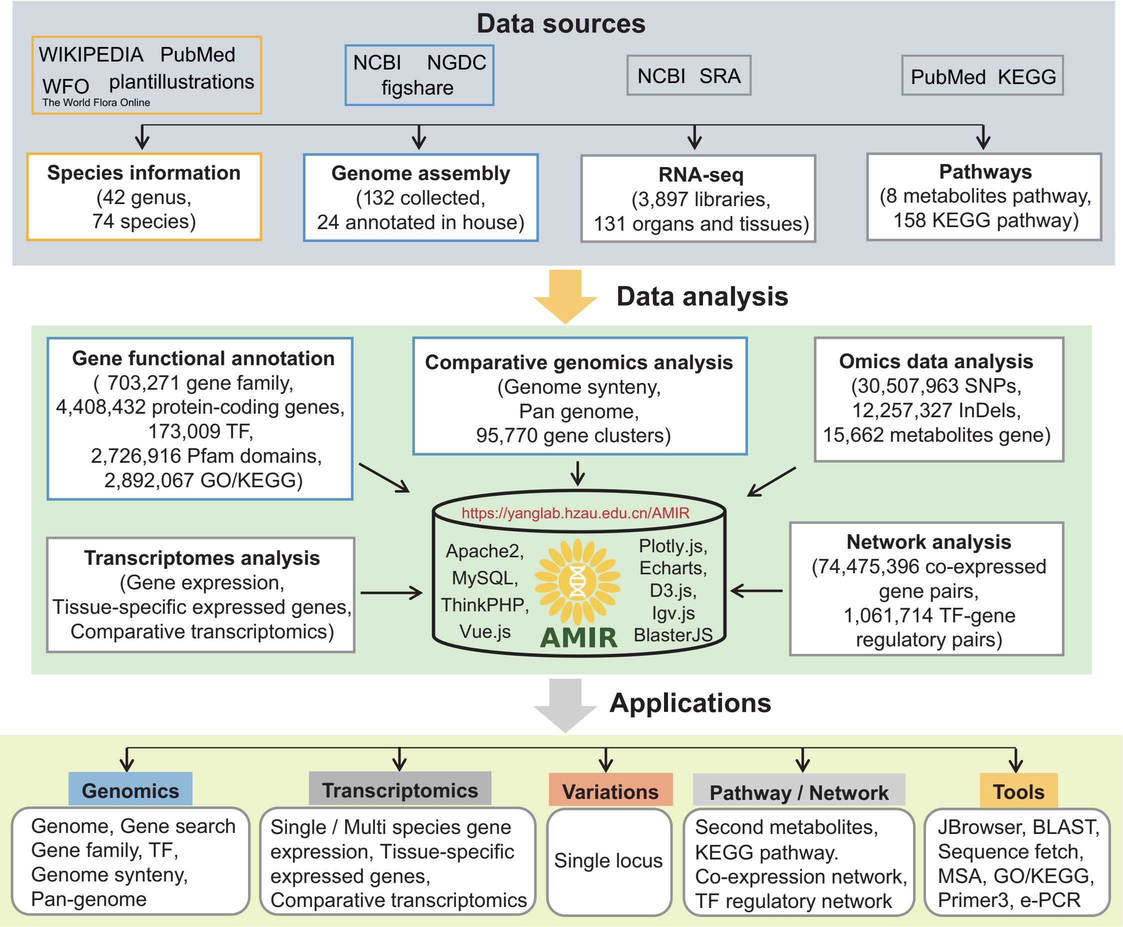


Figure 1. Construction pipelines of AMIR, depicting data collection, data processing and database implementation. The upper section represents data sources. The middle section depicts the data analysis outcomes. The lower section illustrates data storage and the corresponding functional modules accessible in AMIR.

ations were identified from transcriptome data, revealing a total of 30 507 963 SNPs and 12 257 327 InDels.

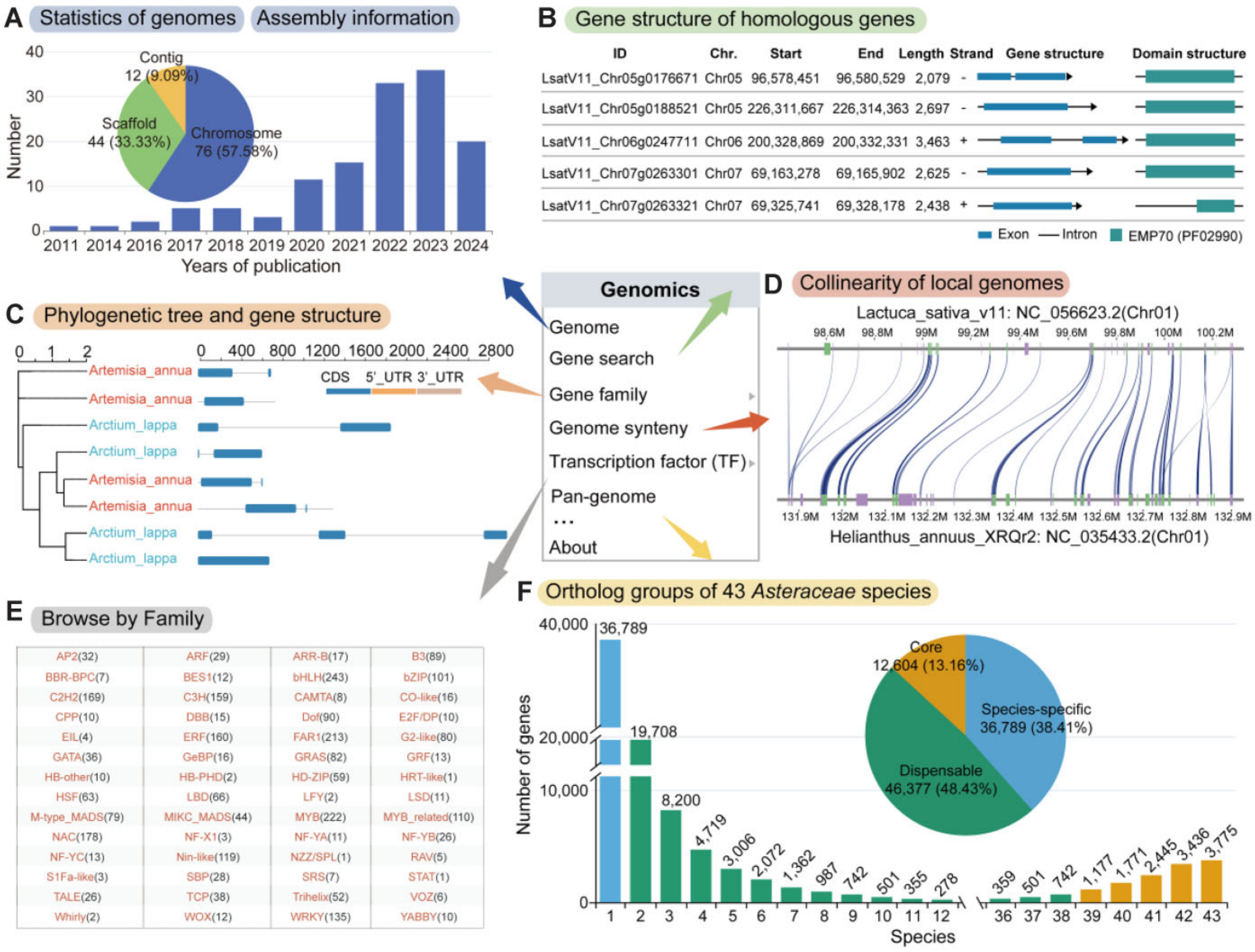
Gene search with unified identifier (AMID)

To facilitate cross-species research, AMIR has implemented a standardized naming system (AMID) for 68 genomes. AMID provides details on species, genome versions, chromosomes and gene order (Supplementary Figure S1A). Within AMIR, researchers can search for genes using multiple methods, such as AMID, homologous in *Arabidopsis* and sunflower, gene symbols, GO, KEGG, EC and PFAM identifier. For example, by selecting *Artemisia argyi* and entering the gene name ‘MEE23’, ‘AT2G34790’, ‘AargV1_Chr01g0000021’ or KEGG number ‘K22395’, users can access the same gene’s information (Supplementary Figure S1B). Additionally, initiating a search from the homepage or the top right corner of the navigation bar directs users to a global search page, where users can easily access detailed results for the target gene across relevant functional modules by clicking corresponding buttons (Supplementary Figure S1C).

Genome module

The genomics module is structured into eight key sections: genome, gene search, gene family, genome synteny, TFs, pan-genome and genome variations. The genome page displays assembly information and data sources for 132 *Asteraceae* genomes (Figure 2A). In the gene search section, users can select a species and search for genes by ID and position (Figure 2B), with results providing comprehensive annotations, including homologous in *Arabidopsis*/sunflower, gene position and functional annotations. Moreover, the page integrates a phylogenetic tree and visual representations of gene structures and protein domains based on homologous genes in *Arabidopsis* (Figure 2B). The gene family module is divided into gene family overview and phylogenetic sections. The overview presents detailed information on 181 gene families across 54 species, while the phylogenetic section illustrates evolutionary relationships within each gene family across different species (Figure 2C). Users can customize phylogenetic trees by selecting a varying number of species. Genome synteny section allows pairwise comparison of genomes from 53 species (Figure 2D), enabling users to explore collinearity relationships across chromosomes, identifying conserved regions and functional genes. The homolog search allows for the quick identification of homologous genes using gene IDs (Supplementary Figure S2A). It also provides flexibility in selecting genomes from different species, effectively identifying homologs and paralogs in the chosen genomes (Supplementary Figure S2B).

TFs play a pivotal role as key regulatory elements in various biological processes, including plant growth, development and responses to external environments (67). The TF module utilizes iTAK (24) and PlantTFDB (23) online tools to



ID	Chr.	Start	End	Length	Strand	Gene structure	Domain structure
LsatV11_Chr05g0176671	Chr05	96,578,451	96,580,529	2,079	-		
LsatV11_Chr05g0188521	Chr05	226,311,667	226,314,363	2,697	-		
LsatV11_Chr06g0247711	Chr06	200,328,869	200,332,331	3,463	+		
LsatV11_Chr07g0263301	Chr07	69,163,278	69,165,902	2,625	-		
LsatV11_Chr07g0263321	Chr07	69,325,741	69,328,178	2,438	+		

AP2(32)	ARF(29)	ARR-B(17)	B3(89)
BBR-BPC(7)	BES1(12)	bHLH(243)	bZIP(101)
C2H2(169)	C3H(159)	CAMTA(8)	CO-like(16)
CPP(10)	DBB(15)	Dof(90)	E2F/DP(10)
EIL(4)	ERF(160)	FAR1(213)	G2-like(80)
GATA(36)	GeBP(16)	GRAS(82)	GRF(13)
HB-other(10)	HB-PHD(2)	HD-ZIP(59)	HRT-like(1)
HSF(63)	LBD(66)	LFY(2)	LSD(11)
M-type_MADS(79)	MIKC_MADS(44)	MYB(222)	MYB_related(110)
NAC(178)	NF-X1(3)	NF-YA(11)	NF-YB(26)
NF-YC(13)	Nin-like(119)	NZZ/SPL(1)	RAV(5)
S1Fa-like(3)	SBP(28)	SRS(7)	STAT(1)
TALE(26)	TCP(38)	Trihelix(52)	VOZ(6)
Whirly(2)	WOX(12)	WRKY(135)	YABBY(10)

Figure 2. Functionality and utilization of the 'Genomics' section in AMIR. (**A**) Assembly information for 132 genomes within the *Asteraceae* family. (**B**) Gene search results, including genomic location, gene structure, functional annotation and homologous genes in *Arabidopsis* and sunflower. (**C**) Gene family phylogenetic analysis across different species. (**D**) Genome synteny pages displaying collinearity of chromosomes in target species. (**E**) TF page providing annotated information on TFs from various *Asteraceae* species. (**F**) Ortholog groups page presenting homologous gene clusters among 43 species, enabling exploration of gene presence or absence across species.

identify TFs in 54 species. iTAK detected a total of 70 TF families and 176 072 TF genes, while PlantTFDB identified 58 TF families and 173 009 TF genes. Users have the option to select a specific TF family or input an AMID to access TF information for a particular species (Figure 2E). The super-pangenome offers a comprehensive view of genomic variation within a genus, facilitating the identification of conserved and adaptive genes, and presenting significant opportunities for crop improvement (68). Drawing from pan-genome constructions in Bambusoideae (32), rice (20), wild grape (33), *Brassica oleracea* (69) and poplar (34), we developed the gene-based pan-genome of *Asteraceae* (33). Ortholog groups were identified using OrthoFinder (35), resulting in 95 770 gene clusters. Among these clusters, 12 604 (13.16%) are core gene clusters present in at least 39 genomes, 46 377 (48.43%) are dispensable gene clusters found in 2–38 species, and the remaining 36 789 (38.41%) represent species-specific gene clusters (Figure 2F). Within the pan-genome page, users can explore the presence/absence patterns of each gene across 43 species and examine gene conservation categories (core, dispensable and species-specific) (Figure 2F).

Transcriptome module

The 'Transcriptomics' module, compiled from 3897 transcriptome datasets across 44 species, is divided into four sections: 'Single-species gene expression profiles'; 'Multi-species gene expression profiles'; 'Tissue-specific expressed genes'; and 'Differential expression analysis'. In the 'Single-species gene expression profile' section, users can explore the expression profiles of genes or gene sets of a specific species across diverse samples (Figure 3A). Leveraging RNA-seq data from the same tissues and treatments across different species (Supplementary Table S3), we have developed multi-species gene expression profiles, enabling users to compare gene expression patterns across different species (Figure 3B). Tissue-specific expressed genes, responsible for conferring distinct morphological structures or physiological functions to various tissue or cell types (44), are identified and presented on a dedicated page using statistical measures like Tau and SPM (Supplementary Table S4). Users can search for tissue-specific expressed genes across various species on this page (Figure 3C). Additionally, by clicking the 'Expression Profile' button, users can navigate to the single-species gene expression profile

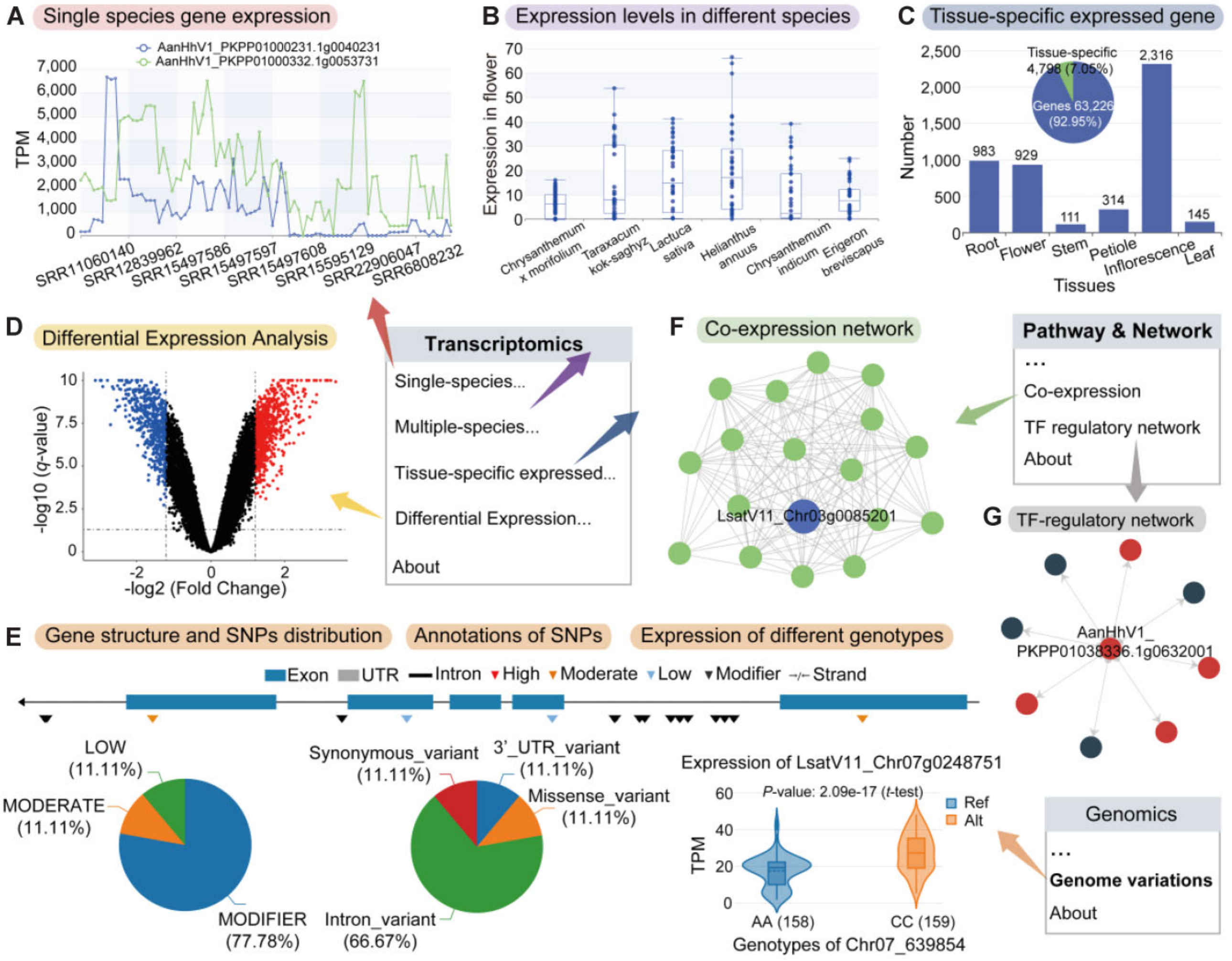


Figure 3. Functionality and utilization of the 'Transcriptomic', 'Genome variation' and 'Pathway & Network' sections in AMIR. (**A**) Single-species expression module providing gene or gene set expression profiles for a specific species. (**B**) Multi-species expression profile page displaying comparisons of expression levels in the same tissue across different species. (**C**) Tissue-specific expression page allowing users to query and retrieve information on genes specifically expressed in tissues across multiple species. (**D**) Differential expression analysis page offering data on DEGs under various conditions, with links to the GO/KEGG enrichment analysis. (**E**) Genome variations module allows users to search for SNP distributions and functional annotations on genes and facilitates comparisons of expression levels between different genotypes. (**F**) Co-expression network visualizes queried genes and co-expressed genes. (**G**) TF regulatory network features the queried TF gene as the central dot, with surrounding dots representing genes regulated by the TF, and directional arrows indicating the regulation.

page to explore the expression patterns of chosen genes. The 'Differential expression analysis' section compiles data from 53 transcriptome projects spanning 23 species and covering 25 different treatment conditions (Supplementary Table S5). On this page, users can search for DEGs under various conditions (Figure 3D and Supplementary Table S6). By selecting specific DEGs, users can transition to other functional pages, such as conducting GO/KEGG enrichment analysis or querying expression profiles for these genes, thereby enhancing the platform's exploratory capabilities.

Genome variation module

The identification of SNPs is essential for unraveling the regulatory mechanisms underlying phenotypic or expression variation. These SNPs are predominantly derived from whole-genome resequencing data. However, population resequencing can be expensive, particularly for the intricate and large-size genomes in *Asteraceae* (10,46). Alternatively, transcriptome sequencing presents a more cost-effective option for those genomes, enabling the identification of SNPs within transcribed regions (70). In some species, transcriptome-based variant identification has led to the identification of expression quantitative trait locus (eQTLs) and, subsequently, the revelation of genes associated with key traits (47–49).

To understand the impact of genetic variation on gene expression in *Asteraceae* species, we selected 2392 high-quality RNA-seq datasets for variant identification (Supplementary Table S7). Across 12 species, a total of 30 507 963 SNPs and 12 257 327 InDels were identified. These genetic variations formed the basis of the genome variations module. In the SNP mode of the single-locus page, distinct genetic variant loci are visually displayed below the gene structure diagram, allowing users to select various loci for detailed information and assess whether the locus is significantly associated with gene expression (Figure 3E). Furthermore, to facilitate user exploration of the relationship between haplotypes composed of multiple genetic variants and gene expression, we developed the 'Haplotype' mode. Users can select different haplotypes to obtain correlations with gene expression.

Pathway and gene regulatory network

The discovery of new functional genes through cross-species homologs has been widely applied in several important species, such as *A. thaliana*, rice and sorghum (71–73). To identify candidate genes associated with the synthesis of secondary metabolites in the *Asteraceae*, we compiled eight validated pathways. These pathways include the synthesis of terpenoids (74–76), costunolide (77), artemisinin (78,79), chicoric acids (80,81) and cyanidin (82). These secondary metabolites are either unique to the *Asteraceae* or distinguished by their high content and widespread distribution. Key genes from multiple species, such as *A. thaliana*, *A. annua*, sunflower and chicory, contribute to these pathways. Candidate genes from other species were identified through homologous relationships, and eight interactive scalable vector graphics were designed to investigate these candidate genes (Supplementary Figure S3). Users can hover over key enzyme nodes in the metabolic pathways to reveals basic information and click for detailed information about the candidate genes in each species. Additionally, we collected and expanded 158 pathways from the KEGG database (66), resulting in the creation of the KEGG pathway page. Users can access detailed pathways for their species of interest by selecting the pathway map ID (83). Yellow nodes within these maps can be hovered over to reveal associated functional genes. Furthermore, the KEGG Pathway tool includes an AMID-based search function, facilitating the discovery of pathways containing target genes. Users can select the species of interest and then explore pathway details by clicking on the respective map ID. Yellow nodes can also be hovered over to identify functional gene locations. Users can also directly query the pathways involving specific genes using the search function.

We constructed a co-expression module using 74 475 396 co-expression pairs from 31 species. This module allows users to input genes or gene sets to generate a gene co-expression network. Moreover, users can adjust parameters such as search depth and threshold to access varying levels of the co-expression network (Figure 3F). Additionally, we established a transcriptional regulatory network for 54 *Asteraceae* species based on binding motifs from PlantTFDB. Users have the flexibility to select from three modes to retrieve regulations according to their requirements. In the TF-regulation network, the 'TF' mode facilitates the retrieval of downstream targets of input TFs, the 'Target' mode retrieves upstream regulators of input genes and the 'Gene' mode uncovers internal regulations among input genes (Figure 3G). Overall, the 'Network' module provides researchers with a comprehensive platform to explore gene functions and potential regulatory patterns.

Tools and download

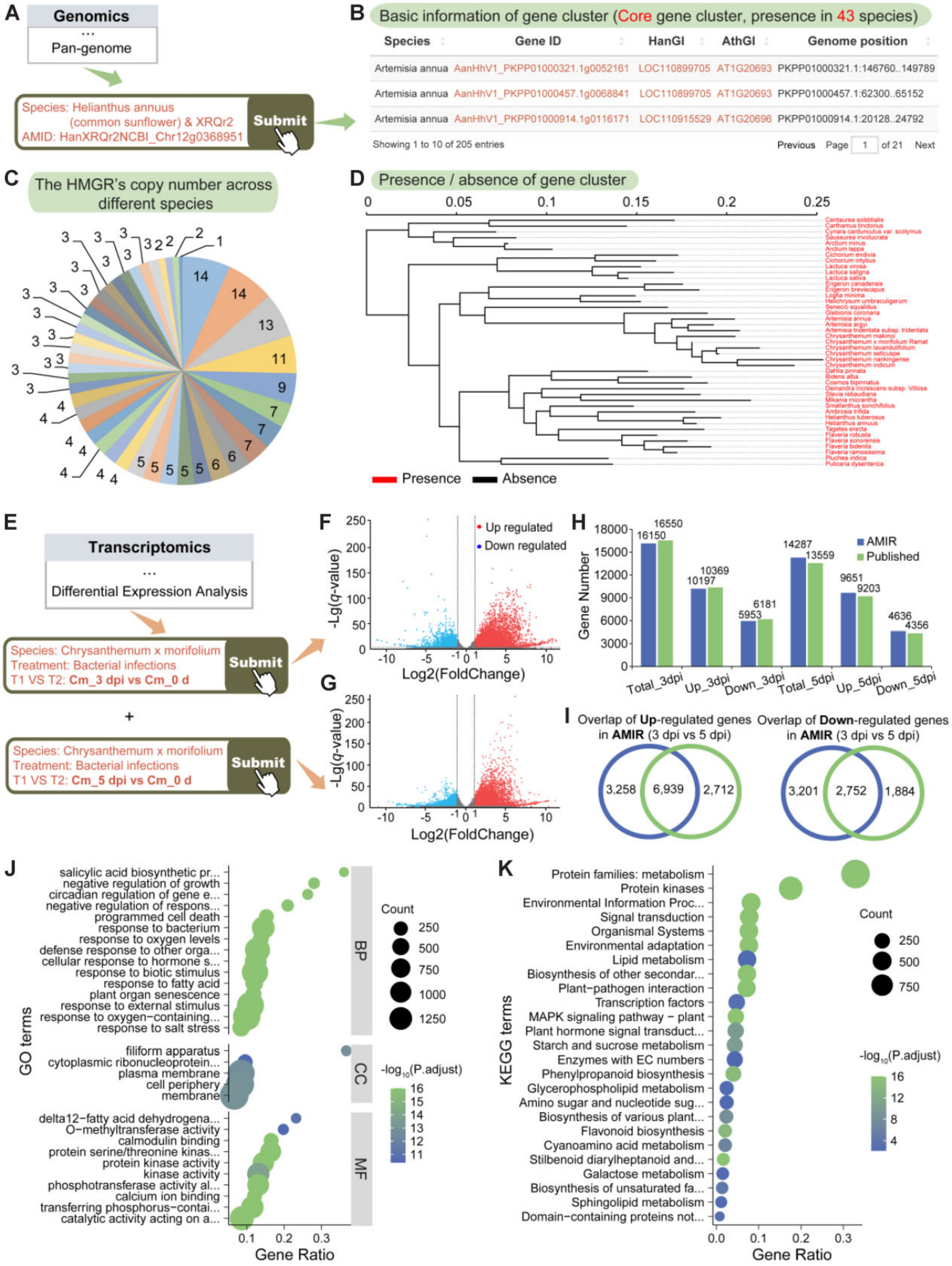
To improve user convenience and accessibility, we integrated nine bioinformatics tools into the Tools portal in AMIR (Supplementary Figure S4A). These tools encompass a range of functions, including JBrowser, BLAST, Multi-alignment viewer, Sequence fetch, GO and KEGG enrichment, Primer3, e-PCR and Domain search. Notably, the 'JBrowser' tool incorporates the 68 *Asteraceae* genome and GFF3 annotations, enabling users to visually explore genomic sequence and gene information within specific regions (Supplementary Figure S4B). With the 'BLAST tool, users can input query nucleotide or protein sequences for homo comparisons within or across species, facilitating functional analysis (Supplementary Figure S4C). The 'Multi Alignment Viewer' allows users to perform multiple sequence alignments to identify conserved regions across sequences (Supplementary Figure S4D). Moreover, the 'Sequence fetch' tool enables users to retrieve reference sequences, including genome sequence, coding sequence (CDS) and protein sequence, for customized analysis (Supplementary Figure S4E). For functional prediction of gene sets, the 'GO/KEGG enrichment' tools offer enrichment analysis capabilities with downloadable results and visualizing of enrichment pathway bubble diagrams (Supplementary Figure S4F). Modules such as 'Primer3' and 'e-PCR' aid users in quickly selecting optimal primer pairs for genes or nucleotide sequences to facilitate downstream experiments (Supplementary Figure S4G and H). Lastly, the 'Domain search' tool facilitates the identification of protein domains, providing insights into their functional roles. In summary, the development of the Tools in AMIR supports functional gene discovery, comparative genomic studies and molecular experiments within *Asteraceae* species.

To enhance user-customized analysis and facilitate data mining, we organized and categorized the data in AMIR, offering convenient downloads for four types of omics data. The genomic data include comprehensive details such as version information, assembly quality metrics and gene counts for 132 *Asteraceae* genomes; the transcriptomic data offer information on sources and sampling details for 3897 transcript datasets from 44 species; the variation data encompass variant information across 12 species, providing access to downloading VCF files of variants on different chromosomes; and metabolomics data feature 15 662 genes associated with the synthesis of various secondary metabolites.

Case study illustrating the utility of AMIR

To illustrate the capabilities of AMIR, we present two case studies that confirm the conservation of the HMGR gene and explore DEGs induced by *Alternaria* sp. infection. Mevalonic acid (MVA) is the most critical precursor in the biosynthetic pathway of terpenoids in plants (84), and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), the key rate-limiting enzyme in the MVA pathway, is widely present in *Asteraceae* (18,85). Using the 'Pan-genome' module, we verified the conservation of the HMGR gene in *Asteraceae*. Specifically, LOC110894660 (https://www.ncbi.nlm.nih.gov/gene/110894660) corresponds to the HMG1/2-like protein in sunflower, with AMIR search ID HanXRQr2NCBI_Chr12g0368951. By selecting *Helianthus annuus* (common sunflower) and XRQr2 in the species selection box and entering HanXRQr2NCBI_Chr12g0368951 in AMIR's search box (Figure 4A), we obtained results including homologous genes, conservation analysis and a phylogenetic tree of HMGR homologs within *Asteraceae* (Figure 4B–D). These findings confirm the presence of HMGR gene across all 43 species in the pan-genome construction, indicating its status as a core gene in *Asteraceae* (Figure 4D). Additionally, the gene's copy number varies from 1 to 14 across different species (Figure 4C), underscoring its conservation and highlighting the utility of the 'Pan-genome' module.

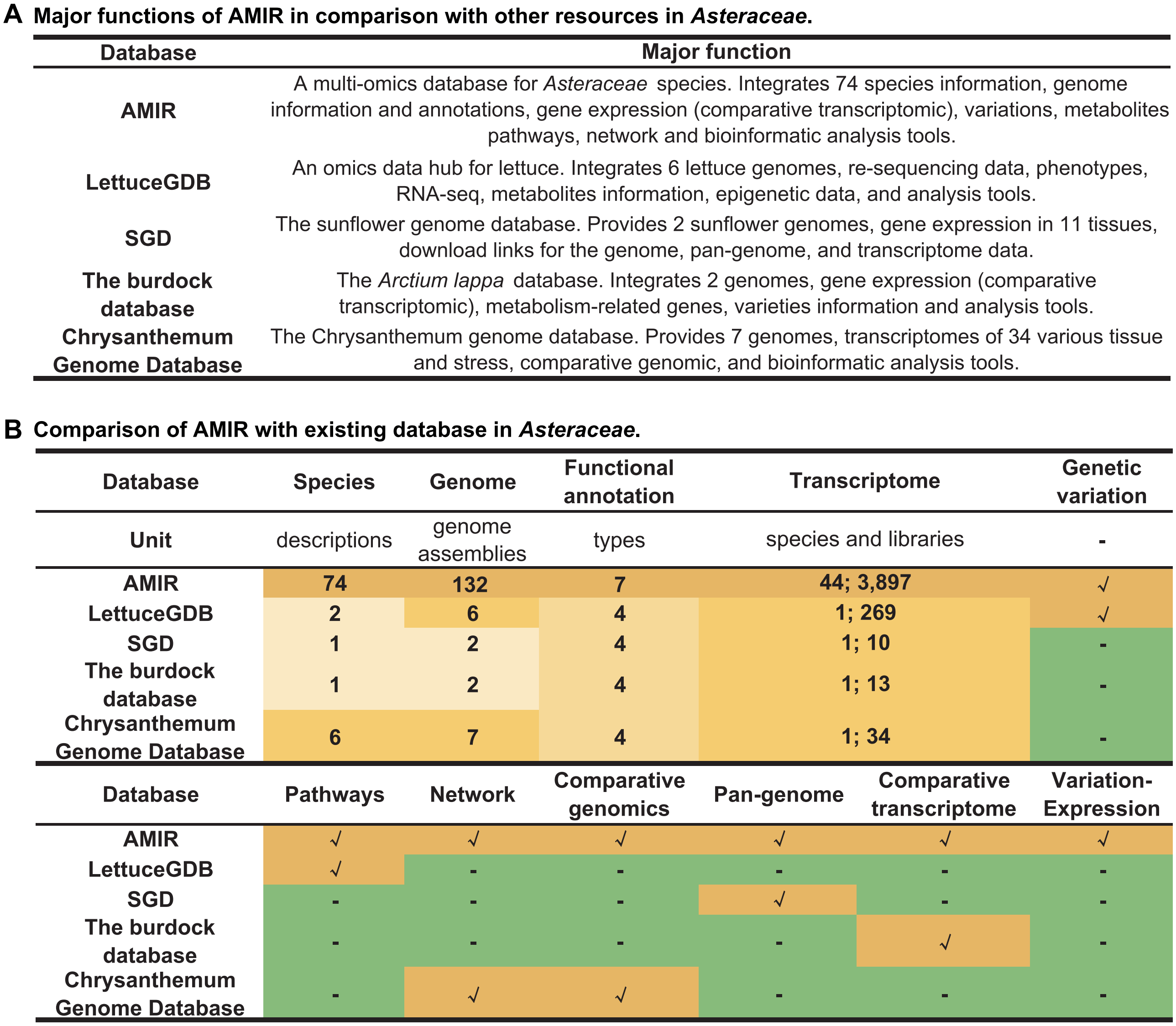
AMIR's 'Differential expression analysis' module includes data from project PRJNA448499, which involves transcriptomes of *C. morifolium* leaves at various time points inoculated with *Alternaria* sp. (86). Here, we will conduct a



Species	Gene ID	HanGI	AthGI	Genome position
Artemisia annua	AanHhV1_PKPP01000321.1g0052161	LOC110899705	AT1G20693	PKPP01000321.1:146760..149789
Artemisia annua	AanHhV1_PKPP01000457.1g0068841	LOC110899705	AT1G20693	PKPP01000457.1:62300..65152
Artemisia annua	AanHhV1_PKPP01000914.1g0116171	LOC110915529	AT1G20696	PKPP01000914.1:20128..24792

Showing 1 to 10 of 205 entries Previous Page 1 of 21 Next

Figure 4. Case study illustrating the utility of AMIR. (**A**) Process of searching for HMGR genes in the 'Pan-genome' module. (**B**) Pan-genome search results, including conservation of the queried gene, its distribution across different *Asteraceae* species and detailed gene information. (**C**) Number of homologous genes in each species. (**D**) Presence or absence of the queried gene in *Asteraceae* species. (**E**) Process of searching for DEGs in the 'Differential expression analysis' module. (**F**) Volcano plot of DEGs in *Chrysanthemum morifolium* leaves after 3 and 5 days of *Alternaria* sp. infection. (**G**) Comparison of the number of DEGs in AMIR with those reported in the original study. (**H**) Continuously upregulated genes after 3 and 5 days of *Alternaria* sp. infection. (**I**) Continuously downregulated genes after 3 and 5 days of *Alternaria* sp. infection. (**J**) GO enrichment analysis of continuously upregulated genes after 3 and 5 days of *Alternaria* sp. infection. (**K**) KEGG enrichment analysis of continuously upregulated genes after 3 and 5 days of *Alternaria* sp. infection.



A **Major functions of AMIR in comparison with other resources in *Asteraceae*.**

Database	Major function
AMIR	A multi-omics database for *Asteraceae* species. Integrates 74 species information, genome information and annotations, gene expression (comparative transcriptomic), variations, metabolites pathways, network and bioinformatic analysis tools.
LettuceGDB	An omics data hub for lettuce. Integrates 6 lettuce genomes, re-sequencing data, phenotypes, RNA-seq, metabolites information, epigenetic data, and analysis tools.
SGD	The sunflower genome database. Provides 2 sunflower genomes, gene expression in 11 tissues, download links for the genome, pan-genome, and transcriptome data.
The burdock database	The *Arctium lappa* database. Integrates 2 genomes, gene expression (comparative transcriptomic), metabolism-related genes, varieties information and analysis tools.
Chrysanthemum Genome Database	The Chrysanthemum genome database. Provides 7 genomes, transcriptomes of 34 various tissue and stress, comparative genomic, and bioinformatic analysis tools.

B **Comparison of AMIR with existing database in *Asteraceae*.**

Database	**Species**	**Genome**	**Functional annotation**	**Transcriptome**	**Genetic variation**
Unit	descriptions	genome assemblies	types	species and libraries	-
AMIR	**74**	**132**	**7**	**44; 3,897**	√
LettuceGDB	**2**	**6**	**4**	**1; 269**	√
SGD	**1**	**2**	**4**	**1; 10**	-
The burdock database	**1**	**2**	**4**	**1; 13**	-
Chrysanthemum Genome Database	**6**	**7**	**4**	**1; 34**	-

Database	**Pathways**	**Network**	**Comparative genomics**	**Pan-genome**	**Comparative transcriptome**	**Variation-Expression**
AMIR	√	√	√	√	√	√
LettuceGDB	√	-	-	-	-	-
SGD	-	-	-	√	-	-
The burdock database	-	-	-	-	√	-
Chrysanthemum Genome Database	-	√	√	-	-	-

Figure 5. Comparison of AMIR with existing database in *Asteraceae*. (**A**) Major functions of AMIR in comparison with other resources in *Asteraceae*. (**B**) Comparison of main functions and data volumes between AMIR and other databases, the values representing data volume and tick marks indicating the presence of specific functional modules.

differential expression analysis to demonstrate AMIR's robust data analysis capabilities. First, in the species and treatment selection menu of the module, *Chrysanthemum* × *morifolium* and Zszgv0 and Bacterial infections are chosen. Then, Cm_3 dpi versus Cm_0d and Cm_5 dpi versus Cm_0d are selected in the comparison box (Figure 4E). The search results include volcano plots of DEGs, detailed information on these genes and buttons for GO/KEGG enrichment analysis (Figure 4F and G). The results show that the number of DEGs observed after 3 and 5 days of *Alternaria* sp. infection are 16 150 and 14 287, respectively, closely aligning with the original study's reported figures of 16 550 and 13 559 (86) (Figure 4H). Moreover, persistent upregulated and downregulated gene counts at Cm_3 dpi and Cm_5 dpi are 6939 and 2752, respectively, slightly surpassing previously reported numbers of 5952 and 2435 (Figure 4I). Clicking the GO enrichment button reveals predominant enrichment in terms such as 'salicylic acid biosynthetic process', 'response to bacterium' and 'protein kinase activity' (Figure 4J). Furthermore, KEGG analysis highlights significant enrichment in the 'signal transduction', 'plant–pathogen interaction', 'phenylpropanoid biosynthesis' and 'flavonoid biosynthesis' categories (Figure 4K), consistent with the original study's findings (86). Additionally, comparing KEGG enriched terms based on gene hits between AMIR and the original study shows high consistency (Kendall's coefficient of concordance = 0.81, P-value = 9.06e−09). These results underscore AMIR's capability to quickly deliver reproducible and reliable analysis outcomes, including the identification of conserved genes, DEGs and differential pathways, without necessitating raw data downloads or complex bioinformatics procedures.

Discussion and future perspectives

With the rapid development of sequencing technology, numerous species in *Asteraceae* have been sequenced. However, publicly available gene annotations for most *Asteraceae* genomes have been limited, impeding the discovery of functional genes. Addressing this gap, we collected and annotated genomes from 74 species, totaling 132. Using 11 475 543 annotation data, we established the AMIR. AMIR stands as the first platform to systematically integrate, analyze and store multi-omics data for the *Asteraceae*, offering the most comprehensive collections of genomes, transcriptomes and variations available to date. This resource empowers users to conduct comparative, evolutionary and functional genomic studies effectively.

Compared to existing data resources in the *Asteraceae* such as LettuceGDB, SGD, the burdock database and Chrysanthemum Genome Database, AMIR provides enhanced features (Figure 5A): (i) it allows retrieval of annotation data for individual genes or gene sets by using gene IDs, GO/KEGG/Pfam identifiers or TF names, significantly enhancing the discovery of functional genes; (ii) it introduces the first super-pangenome and comparative genomics platform for the *Asteraceae*, enabling rapid exploration of gene conservation and function; (iii) it includes a comparative transcriptomics system to identify genes expressed specifically in certain tissues and to mine DEGs related to key traits or biological processes; and (iv) AMIR enables retrieval of SNPs/InDels in important genes or genomic regions, along with their frequency, haplotypes and correlation with gene expression, thereby uncovering variations and genes linked to important traits (Figure 5B).

Moreover, AMIR integrates nine commonly used bioinformatics analysis tools for constructing gene regulatory network, performing functional enrichment analysis and identifying potential functional genes and metabolic pathways. We validate AMIR's capabilities through two case studies: one confirming the conservation of the HMGR gene and the other exploring the DEGs induced by *Alternaria* sp. infection. These studies illustrate AMIR's integration of extensive, high-quality data and robust analytical workflows. These resources and tools will facilitate the discovery of evolutionarily conserved genes and pathways specific to certain species or associated with distinct environments and essential traits. In summary, this multi-omics database marks a significant advancement in *Asteraceae* genomic research, providing a foundational resource for molecular breeding and drug discovery efforts in *Asteraceae* plants.

Currently, AMIR houses a variety of data types, including species information, genomes and transcriptomes, as well as variations and co-expression data derived from transcriptome processing and analysis. As genomic research on *Asteraceae* progresses and the volume of whole genome sequencing data and epigenetic data (such as methylation, histone modifications, Assay for Targeting Accessible-Chromatin with high-throughout sequencing (ATAC-seq) and Chromatin Immunoprecipitation sequencing (ChIP-seq)) expands, we are committed to continuously enriching AMIR with new types of omics information. Through regular updates and maintenance, we hope that AMIR can serve as the central hub for functional research in the *Asteraceae* family, driving advancements in molecular breeding, drug discovery and landscaping.

Data availability

AMIR is accessible for free at https://yanglab.hzau.edu.cn/AMIR. All associated datasets can be downloaded via the 'Download' module of the database.

Supplementary data

Supplementary Data are available at NAR Online.

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Conflict of interest statement

None declared.

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