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ORIGINAL ARTICLE

PtncRNAdb: plant transfer RNA-derived non-coding RNAs (tncRNAs) database

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Abstract

Specific endonucleolytic cleavage of tRNA molecules leads to the biogenesis of heterogeneously sized fragments called tRNA-derived non-coding RNAs (tncRNAs). The role of tncRNAs is well studied in human processes, and diseases including different types of cancers and other ailments. They are also generated under stress conditions in plants. Considering the potential role of tncRNAs in the plant system, we have developed a user-friendly, open-access web resource, PtncRNAdb (https://nipgr.ac.in/PtncRNAdb). PtncRNAdb consists of 4,809,503 tncRNA entries identified from ~ 2500 single-end small RNA-seq libraries from six plants, viz., *Arabidopsis thaliana*, *Cicer arietinum*, *Zea mays*, *Oryza sativa*, *Medicago truncatula*, *and Solanum lycopersicum*. It is provided with assorted options to search, browse, visualize, interpret, and download tncRNAs data. Users can perform query search using 'BLASTN' against PtncRNAdb entries. Highcharts have been included for better statistical PtncRNAdb data readability to the users. Additionally, PtncRNAdb includes 'DE tncRNAs' module for differentially expressed tncRNAs under various conditions. Their secondary structure, putative targets, interactive networks of target enrichment, and related publications are also incorporated for further interpretation of their biological functions. PtncRNAdb is an efficient, user-friendly, and exhaustive database, which will aid the ongoing research in plant tncRNAs as well as help in deciphering their role in gene regulation. We hope that it provides a promising platform for researchers to facilitate the understanding of tncRNAs, and their involvement in numerous pathways related to plant development and stress tolerance.

Keywords Database · NGS · Small RNAs · Regulatory RNAs · tncRNAs · tRNA

Introduction

With the advancements in high-throughput sequencing, there is a tremendous increase in non-coding RNAs (ncRNAs) research in the past decade. These ncRNAs constitute a significant section of the transcriptome (Hüttenhofer et al. 2005; Li and Liu 2019), and play numerous biological roles including transcriptional and post-transcriptional gene regulation, cellular homeostasis, epigenetic modifications, etc. (Vaucheret 2006; Zhang et al. 2019). Among these, small interfering RNAs (siRNAs) and microRNAs (miRNAs) have been a hotspot of research studies over the past decades, because of their contribution to gene regulation in many cellular processes as well as disease progression in prokaryotes and eukaryotes (Costa 2010). Understanding miRNA gene regulation mechanisms and their association with AGO protein complexes has enabled a whole new world of possibilities via miRNA-mediated gene silencing (Loss-Morais et al. 2013; Alves et al. 2017). Besides miRNAs, small ncRNAs of 14–50 nucleotide (nt) in size are formed by cleavage of tRNA molecules (Kumar et al. 2014). These are collectively known as tRNA-derived non-coding RNA molecules (tncRNAs) and are crucial to all life forms (Keam and Hutvagner 2015). Though the exact mechanism of biogenesis of tncRNAs is unclear, many studies suggest that their formation is induced by specific endonucleolytic cleavage of tRNA by angiogenin and other enzymes (Dicer, RNAase Z, etc.) (Haussecker et al. 2010; Kuscu et al. 2018; Megel et al. 2019). tncRNAs include 5′ tRH and 3 ′ tRH

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(tRNA halves, formed by cleavage in anticodon loop); tRF-5 and tRF-3 (cleavage in D and T loop respectively); tRF-1 (from trailer sequence of pre-tRNA); tRFs sourcing from leader region of pre-tRNA as well as other-tRFs coming from internal region of mature tRNA (Anderson and Ivanov 2014; Zahra et al. 2021). The generation of tncRNAs depends on the type of tRNA, cell, tissue, developmental stage, and stress (Raina and Ibba 2014; Soares and Santos 2017). tncRNAs are extensively studied in humans and are known to play role in diseases like cancer (Zhu et al. 2019), neurodegenerative diseases, viral infection, and metabolic disorders (Qureshi and Mehler 2012; Yang and Schimmel 2011; Schageman et al. 2013). Analysis of PAR-CLIP sequencing libraries showed that some tncRNAs bind to AGO proteins and enables miRNA-type gene silencing by employing different mechanisms (Kumar et al. 2014; Haussecker et al. 2010; Deng et al. 2015). Though the significance of tncRNAs is well studied and proven in human diseases, fewer studies are establishing their role in plants (Zhu et al. 2018). In plants, few studies have shed light on tncRNAs generation in response to oxidative stress in tissues like seedlings and flowers (Thompson et al. 2008), phosphate starvation in roots (Hsieh et al. 2009), under abiotic stresses like drought and salt stress (Alves et al. 2017), and also during plant–microbe interactions (Visser et al. 2014; Asha and Soniya 2016; Ren et al. 2019).

Considering the significance of tncRNAs in plants, and their potential to be used as a tool for gene silencing, there is a compelling need for developing databases and pipelines, to enable robust tncRNA studies in diverse plant species. Currently, there are only two plant-specific tRF databases, i.e., tRex (Thompson et al. 2018) and PtRFdb (Gupta et al. 2018), both of which lack information on complete tncRNAs, and only provide information on tRFs. Besides, the identification of tncRNAs is an erroneous process due to chemical modifications in tRNAs, conserved genomic loci, and the presence of CCA tail (Hoffmann et al. 2018). This leads to an increase in mismatches, thereby, lowering down the overall mapping score and escaping the score threshold filtering, giving false positives as well as loss of genuine tncRNAs. Recently, we have addressed these challenges and developed a software package viz. tncRNA toolkit (https://nipgr.ac.in/tncRNA) (Zahra et al. 2021). Further, we have analyzed a total of 2434 small RNA-seq samples from six angiosperms viz. *Arabidopsis thaliana* (model plant), *Solanum lycopersicum* (tomato), *Cicer arietinum* (chickpea), *Medicago truncatula* (model legume), *Oryza sativa* (rice), and *Zea mays* (maize), and developed this database viz. 'PtncRNAdb' (https://nipgr.ac.in/PtncRNAdb). At this web resource, different types of search and browse options are available. PtncRNAdb is also equipped with BLASTN facility to perform a similarity search for the user's submitted query sequence. Apart from basic tncRNA information, details like tRNA modification, tncRNA secondary structure, and its alignment on parental tRNA can also be obtained, thus making the database more informative. Dynamic graphs are provided to showcase various tncRNA distributions in a user-friendly way. Descriptive details related to the differentially expressed tncRNAs (DE tncRNAs) and their predicted targets are also integrated for the enhanced exploration of potential tncRNAs functions in plants. Functional interaction networks and enrichment of DE tncRNA targets can also be visualized. Also, to further consolidate DE tncRNAs targets study, literature associated with the tncRNA targets under various stress conditions is amalgamated with our database. The overall architecture and features of the PtncRNAdb are illustrated in Fig. 1.

Materials and methods

Data retrieval, tncRNA identification, and web-interface development

The tncRNA population identified in our previous study was compiled for the development of PtncRNAdb (Zahra et al. 2021). The complete methodology applied has been provided in the "Methods" section of the database (https://nipgr.ac.in/PtncRNAdb/method.php). RNAstructure package (Reuter and Mathews 2010) was utilized for tncRNA secondary structure prediction. Differentially expressed tncRNAs were identified using DESeq2 (Love et al. 2014) in our earlier study (Zahra et al. 2021). tncRNAs with a p value less than 0.05 were considered to be significant. Those with log2FC value greater than or equal to 1 (≥ 1) and less than or equal to -1 (≤ -1) were considered up- and down-regulated tncRNAs, respectively, and incorporated in the database as DE tncRNAs (Supplementary sheet S1). The front-end web interface for PtncRNAdb was designed using HTML, PHP, CSS, and JavaScript, on the Apache server. Back-end processing of the data was done by MySQL.

DE tncRNAs target prediction and enrichment analysis

psRNATarget software package was used (Dai et al. 2018) (2017 release, targets with the evalue less than or equal to 3.5 (≤ 3.5) were considered to be the putative targets) for the tncRNAs (17–25 nt) target prediction. The binding region of target transcripts was found using GFF file information processed by in-house scripts for each species. For the enrichment analysis and network visualization, STRING API (Szklarczyk et al. 2018) program has been incorporated. Publications related to each target for each stress studied were first extracted from UniProt, and then, manually curated after an exhaustive search at NCBI PubMed (https://pubmed.ncbi.nlm.nih.gov/). Moreover,

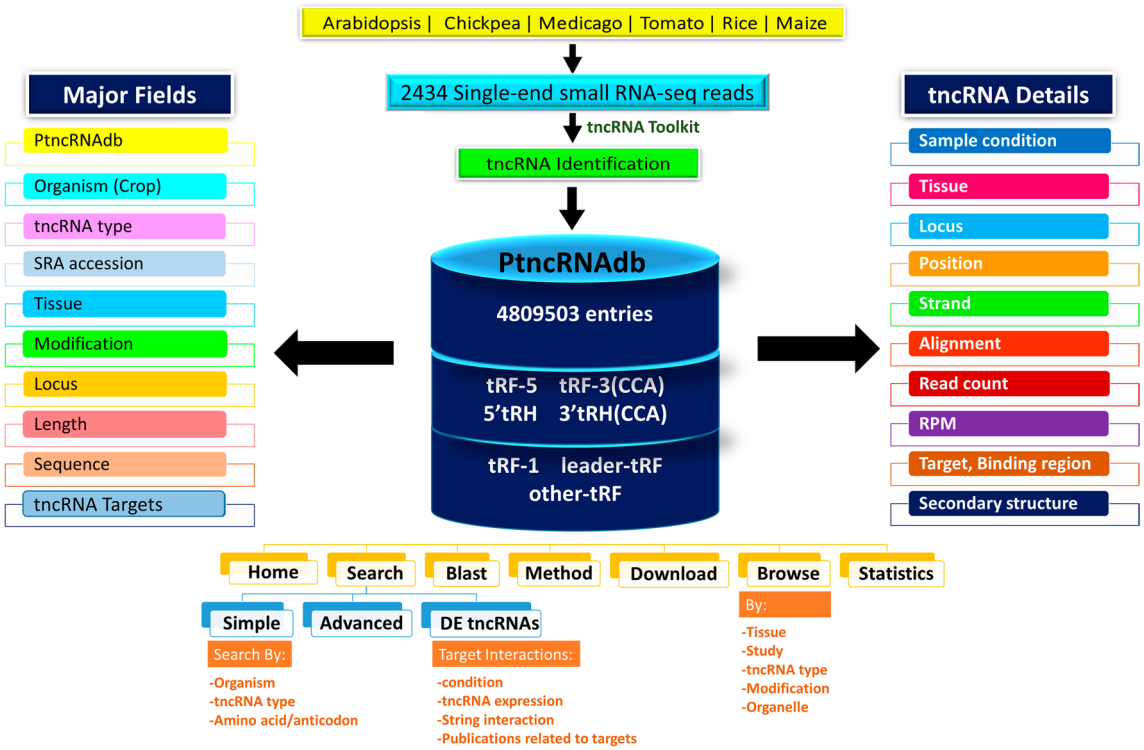

Fig. 1 Overall architecture, schematic representation, and various features exhibited by PncRNAdb

eggNOG-mapper v2 (Cantalapiedra et al. 2021) was utilized for the analysis of the functional annotation of DE tncRNA targets. For *Arabidopsis*, common targets for 6 h heat, drought, and NaCl excess were sorted. For maize, since the abiotic stress studies were numerous, common targets were screened from all unique genes of heat, drought, salt, and drought–salt combined stress (Supplementary sheet S2). Functional classification of all targets was done for tomato with tomato mosaic virus infection while for rice, common targets predicted during *Magnaporthe oryzae* infection were studied (Supplementary sheet S2). Annotated genes were manually screened for important candidate genes in *Arabidopsis* and rice stress studies (Supplementary sheets S3 and S4). Functions of screened genes in their respective plants were studied using UniProt (Bateman et al. 2021), and InterPro databases (Hunter et al. 2009).

Sections and snippets

Search/browse modules

Detailed information for each tncRNA is provided in the database, which can be extracted using two user-friendly modules: 'Simple Search', and 'Advanced Search'. The Simple search allows the user to extract the information by selecting organism, tncRNA type, and amino acid/anticodon. Users can select the fields to be displayed using checkboxes, and up to ten fields can be displayed in PtncRNAdb. For a more sophisticated search, users may opt any four out of seven fields of choice, i.e. SRR accession, tissue, organism, modification, tncRNA type, condition, and length using Advanced search, and can make complex queries using conditional ('=', 'Like) and logical operators ('OR' and 'AND'). The 'Browse' section allows users to extract tncRNAs information through five different browsing categories viz. tissue, condition, organelle, tncRNA type, and tncRNA modification. Each query search opens a page having a list of all annotated tncRNAs and their respective details viz. PtncRNA ID, organism, tissue, SRR accession, tncRNA type, tRNA information, number of targets, length, tRNA modification, and tncRNA sequence. Each PtncRNA ID is hyperlinked to a webpage displaying detailed information on cognate tncRNA including its alignment on parental tRNA, locus, strand, raw read count, RPM, list of tncRNA targets, and binding region of tncRNA:target pair, as well as tncRNA's secondary structure. Further, by clicking any individual tncRNA target will redirect to a webpage showing details of respective tncRNA:target interaction including expectation cutoff value (Evalue), start and end positions of tncRNA as well as target transcript, target sequence, detailed description of target including its genomic locus, gene symbol, GO terms, PFAM details, gene function, and UniProt accession.

Differentially expressed tncRNAs (DE tncRNAs)

Study of stress-specific DE tncRNAs as well their corresponding target genes can explicate the putative active networks or key proteins functional in response to the stress. In PtncRNAdb, 'DE tncRNAs' is present explicitly as a search option. These tncRNAs can be searched by selecting the plant of choice, stress condition as well as expression type (down-regulated/up-regulated/all). The result page gives a list of differentially expressed tncRNAs for the selected stress condition along with relevant information including tissue, SRR study, tncRNA type, length, and sequence. It also displays the log2FC value, which specifies that whether the tncRNA is up- or down-regulated. This page also gives information about the number of targets associated with each tncRNA. Also, it provides an option to visualize the STRING network of all target proteins for each stress condition. The protein–protein interaction networks can be further directed to the STRING database (Szklarczyk et al. 2018) for extended exploration. Additionally, published pieces of literature associated with tncRNA target genes and their description are also displayed on this page.

Other PtncRNAdb features

Besides key features of the database, there are additional modules/features added to make the database more informative and user-interactive. These include, 'Method', describing the detailed methodology of tncRNAs identification and secondary structure prediction, 'Statistics', containing dynamic graphs displaying various kinds of tncRNAs distribution in PtncRNAdb, and 'Help', to aid the user to navigate the database. Moreover, complete tncRNAs data can be downloaded according to plant species or study, from the 'Download' section (https://nipgr.ac.in/PtncRNAdb/download.php).

Results and discussion

Database framework and statistics

PtncRNAdb retains data on transfer RNA-derived non-coding RNAs (tncRNAs) from six major angiosperms. It also provides information on differentially expressed (DE) tncRNAs under various conditions, their target transcripts, and their interaction networks. PtncRNAdb retains the tncRNAs identified and classified from a total of 2434 small RNA sequencing libraries from *A. thaliana* (1676), *S. lycopersicum* (142), *C. arietinum* (25), *M. truncatula* (127), *O. sativa* (243), and *Z. mays* (221) using tncRNA toolkit (14). The PtncRNAdb's interactive user interface is freely accessible at https://nipgr.ac.in/PtncRNAdb/. The distribution of total tncRNAs, unique tncRNAs (the identical tncRNA sequences were grouped and termed as unique), and total counts of different tncRNA classes viz. tRF-5, tRF-3, tRF-1, leader tRF, 5′tRH, 3′tRH, and other tRF for individual plants is illustrated in Table 1.

The statistics of tncRNAs in the corresponding transcriptome depend on a lot of technical factors, like small RNA sequencing protocol, depth, read quality, etc., as well as additional factors like tissue type, stress conditions, etc. hence, cannot be used to draw comparisons among different plant species/samples. A total of 4,809,503 tncRNA entries are stored in PtncRNAdb, out of which 137,611 are unique. Unique tncRNAs derived from the pool of total tncRNAs for a single genome can give insight into the tncRNAs biology. Out of 33,62,128 tncRNA sequences detected in *Arabidopsis thaliana,* only, 50,619 of them were unique. This indicates that specific tncRNA fragments are generated for regulating multiple processes under varied conditions. Interestingly, although only 25 libraries were available for *Cicer arietinum*, 10,713 tncRNAs were detected unique from a total of 54,537 tncRNAs, making its unique tncRNAs percentage the highest.

Table 1 Plant-wise comparison of sequencing libraries utilized, total, unique tncRNAs, and total count of each tncRNA type studied in PtncRNAdb

	A. thaliana	*C. arietinum*	*M. truncatula*	*O. sativa*	*S. lycopersicum*	*Z. mays*
Libraries	1676	25	127	243	142	221
Total tncRNAs	3,362,128	54,537	406,496	3,068,336	251,313	428,193
Unique tncRNAs	50,619	10,713	23,117	11,971	22,887	18,304
tRF-5	760,155	8837	63,157	100,828	56,176	95,748
tRF-3	36,104	715	4291	4641	4547	3810
tRF-1	22,505	77	779	1553	1867	1959
5′tRH	164,074	2151	15,232	19,070	18,216	37,768
3′tRH	3499	39	69	419	385	695
leader tRF	2359	26	137	53	130	164
other tRF	1,601,478	30,901	140,897	184,308	112,736	185,587

PtncRNAdb also provides details of all 48,09,503 tncRNAs entries. Their class-wise distribution for each plant and anticodon can be visualized in the 'Statistics' section (https://nipgr.ac.in/PtncRNAdb/stat-data.php). Among major tncRNA categories, the most abundant class of tncRNAs is tRF-5 in all plant species. Many studies report the production of tRF-5 in stress conditions and hence, corroborates this result (Loss-Morais et al. 2013; Alves et al. 2017). Leader tRFs were found to be the least abundant in all plants. Surprisingly, tncRNAs obtained from internal cleavage of tRNA (i.e. other-tRFs) were observed in huge abundance. However, the functional significance of these fragments in plants is unknown.

Organelle-specific tncRNAs pool

We observed that many identical tncRNAs mapped on tRNA from multiple sources and it must be due to the high sequence conservation among the tRNA molecules irrespective of their organelle of origin. In contrary to those tncRNAs, we also observed a pool of tncRNAs that mapped exclusively only to a single source, i.e. spawning from either nuclear, mitochondrial, or plastidial tRNAs. Their lengthwise distribution showed that the majority of such tncRNAs were 30–35 nt long (Supplementary figure S1) in all plant species. *Arabidopsis* also showed a high number of nuclear tncRNAs of size 15–16 nt and 40–41 nt. Similarly, *Zea mays* showed a high number of mitochondrial tncRNAs for the 40–41 nt length range. Looking at the anticodon contribution, we observed that tRNA isoacceptors AlaAGC and GluTTC, SerGCT, and GluTTC majorly gave rise to nuclear, mitochondrial, and plastidial tncRNAs in different plant species respectively (Supplementary figure S2). It can be anticipated that these tncRNAs might have organelle-specific roles in the plant cell, especially the plastidial tncRNAs might regulate photosynthesis and storage functions in the cell. The molecular role of single-origin tncRNAs coming from specific anticodons needs to be investigated in plants. Organelle-exclusive tncRNAs for individual plant species can be retrieved from the 'Download' page.

DE tncRNAs majorly bind to the CDS region of target mRNAs

DE tncRNAs were found to target multiple transcripts and their number varies significantly with tissue, stress conditions, and time duration of induced stress (Supplementary sheet S1). E.g., only a few DE tncRNAs were observed in the roots as compared to other tissues. The majority of tncRNAs (49–85%) bind to target transcripts in their coding sequence (CDS) while only a few tncRNAs binds to the 5' or 3' untranslated regions (UTRs) (Supplementary sheet S1). We also observed that there can be multiple target sites in the CDS for a single tncRNA/target transcript pair. The binding of tncRNA to multiple regions may direct efficient cleavage in the predicted target transcript.

Functional classification of DE tncRNA targets

Identifying specific candidate genes or COG (Cluster of orthologous groups) categories targeted by tncRNAs as a response to stress conditions will help us understand tncRNAs mediated gene regulation in plants. Hence, DE tncRNA targets for two crops under abiotic stresses were observed, i.e., both *Arabidopsis* and maize were analyzed for abiotic stresses viz. heat, drought, and salt (NaCl) stress. Moreover, rice and tomato targets were studied for biotic stress where they were infected with *M. oryzae* and tomato mosaic virus (TMV) respectively. It was observed that the high number of target genes for all stress conditions in all crops includes 'signal transduction mechanism genes' and 'post-translational modifications, protein turnover, chaperones' (Supplementary sheet S2). Interestingly, very few genes could be observed for defense mechanisms, hinting at the involvement of indirect networks for stress tolerance.

Some of the candidate target genes, significant for gene regulation were found for abiotic stress in *Arabidopsis* and biotic stress in rice (Supplementary sheets S3 and S4 respectively). Interestingly, some of the genes involved in signal transduction mechanisms, i.e., 'disease resistance protein' and 'protein kinase domain protein' as well as 'vacuolar protein sorting associated protein' were found to be common in both crops exhibiting two very different kinds of stress. These proteins are involved in cell signaling, plant growth, and development by both direct and indirect means (Supplementary sheets S3 and S4). The presence of histone deacetylase/acetyltransferases in both *Arabidopsis* and rice tncRNAs targets in stress conditions indicates that these tncRNAs play a major role in modulating chromatin structure and thus, controlling gene expression (Supplementary sheets S3 and S4). Post-translational modifications and ubiquitin-mediated protein degradation are the other biological processes targeted by DE tncRNAs in both crops (Supplementary sheets S3 and S4). The role of ubiquitin in selective proteolysis is via the ubiquitin–proteasome system (UPS) which is a fundamental player during the plant response to various abiotic and biotic stresses (Belknap and Garbarino 1996; Dreher and Callis 2007). Rice gene targets also include VWA domain-containing CoxE-like protein, which interestingly, is not studied in plants and is a bacterial protein. This hints that the tncRNAs in plants may also have inter-kingdom target genes apart from key players in gene regulatory networks and hence can be associated with inter-kingdom gene regulation and signaling (Supplementary sheet S4).

Conclusion

Eukaryotic tncRNAs are proving to be a major role player in the regulation of numerous cellular processes including growth, development, tolerance to abiotic stresses, induction of immune response to pathogen attacks as well as influence cross-kingdom interactions like symbiosis (Keam and Hutvagner 2015; Raina and Ibba 2014). There is a need for continuous growth in tncRNA identification pipelines, databases, and target prediction tools to keep pace with the evolution of sequencing technologies. In this study, we have extended tncRNAs research in plants and utilized the sequencing data generated by NGS technologies to develop a web resource for the exploration of tncRNAs in six major angiosperms, their putative targets, and interaction networks. Hence, the comprehensive, and efficient database available to the research community will be beneficial to boost tncRNAs research in planta. Experimental validation of tncRNAs, as well as their binding to probable targets, should be carried out for their functional characterization. Attempts will be made to update this database regularly by the addition of more and more tncRNAs and their targets as well. In the future, extended analysis of more and more small RNA-seq datasets for other plants may lead to enrichment of the tncRNAs repertoire in diverse species.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13205-022-03174-7.

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Author contributions SZ and RB processed and analyzed the data. RB developed the web interface and implemented other modules in the database. AS provided the raw data for the analysis. SZ, SS, and SK wrote the manuscript. SK conceived the study and coordinated the project.

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Declarations

Conflict of interest None declared.

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