AlnC: An extensive database of long non-coding RNAs in Angiosperms

Ajeet Singh¹, AT Vivek¹, Shailesh Kumar^{1*}

*Corresponding author

Shailesh Kumar

<https://orcid.org/0000-0002-1872-9903>

¹Bioinformatics Lab, National Institute of Plant Genome Research (NIPGR), Aruna Asaf Ali Marg, New Delhi 110067, India. Tel: 91-11-26735217, Fax: 91-11-26741658, Email: shailesh@nipgr.ac.in

Abstract

Long non-coding RNAs (lncRNAs) play a major role in diverse biological processes that are contemplated to have diverse regulatory roles in plants. While lncRNAs have been described and annotated in major plant species, many of plant species lack annotated lncRNAs despite the availability of transcriptome data in public repositories. In this study, we have identified and annotated the lncRNAs in the transcriptomic data from the 1000 plant genome project (1KP), and developed a user-friendly, open-access database called AlnC. This can be used for the exploration of lncRNAs in 682 Angiosperm plants. The current version of AlnC offers 10,855,598 annotated lncRNA transcripts across 809 sample tissues of diverse flowering plants. To enhance the AlnC interface, we have provided the features for browsing, searching and downloading of lncRNA data, interactive graphics, and online BLAST service. In addition to these information, each lncRNA record is annotated with possible Short open reading frames (e.g. sORFs) to facilitate the study of peptides encoded within lncRNAs. With this user-friendly interface, we assume that AlnC will serve as a rich source of lncRNAs that will contribute to small-and large-scale studies in a wide range of flowering plants.

Keywords: Angiosperms; 1KP Project; lncRNAs; RNA-seq; sORFs

Database URL: <http://nipgr.ac.in/AlnC>

29 **Introduction**

30 Angiosperms (flowering plants) are land plants with more than 3,000,000 recorded species
 31 worldwide, comprising one of the most diverse group within the plant kingdom [1,2]. Most plants
 32 belonging to this group have been intensively studied in order to understand both the flowering and
 33 other major mechanisms. As a result, research in flowering plants has exploded with the advent of
 34 next generation sequencing leading to an improved picture of transcriptome, especially from the
 35 point of non-coding RNAs (ncRNAs). Of all recent studies in plant non-coding RNAs, long non-
 36 coding RNAs (lncRNAs) have been shown greater interest to study alongside miRNAs, which are
 37 typically more than 200 nucleotides with almost no protein coding capacity. There is also
 38 compelling evidence to lncRNAs functioning in multiple biochemical pathways of plants over the
 39 years [3–5]. Despite being in the spotlight, lncRNAs are yet to be annotated in a variety of plant
 40 species, despite the presence of data banks focused primarily on model plants and major crops. This
 41 research gap can be addressed by the use of RNA sequencing (RNA-seq) data as it provides
 42 enormous opportunities to understand transcriptome and classify potential lncRNAs
 43 [6,7]. Eventually, with the rise of transcriptome data in public repositories, thousands of plant
 44 lncRNAs were identified and maintained in a number of databases e.g. PLNlncRbase,
 45 CANTATAdb, GREENC, PLncDB v2.0, and lncRNAdb v2.0 [8–12]. The available lncRNA
 46 databases are limited to model plants and fewer other plants are another major concern, as
 47 information on the potential lncRNAs of many Angiosperms is still largely scarce and hinders the
 48 advancement of lncRNA research in several of these plants. Several plant species have been
 49 investigated for genome-wide lncRNAs using independent bioinformatics pipelines for annotation
 50 and archiving in databases, but several plants are still unexplored due to a complete lack of genome
 51 details. In order to address this problem in organisms with no genome data, various lncRNA
 52 methodologies are available, and are increasingly developed to improve lncRNA identification and
 53 annotation from *de novo* assembled transcripts [13,14].

In this current genomic era, it is essential to make lncRNA annotation models for diverse plant species in terms of improving our understanding of lncRNA biology. In parallel, the creation and maintenance of a stable lncRNA data repository is equally necessary if lncRNA biology is to be understood. [15,16]. In this research work, we have used large-scale transcriptome data to identify potential lncRNAs with three major goals. First, we anticipate to provide information on most potential lncRNAs of plant species with no available genome sequence. The second goal is to mediate the importance of unused large-scale datasets as an annotation source for thousands of lncRNAs. Lastly, to develop a database to act as a catalyst to promote lncRNA investigations in Angiosperms, and to provide a one-stop data access platform for lncRNA researchers. We therefore capitalised on large-scale transcriptomic data of 682 angiosperms from 1 Kp as this enabled us to annotate 10,855,598 lncRNAs. The results of the study have been organised and deposited in a user-friendly web-interface, the Angiosperm LncRNA Catalog (AlnC) with a plan to update periodically on the basis of new knowledge and an expansion in the number of species of Angiosperm in the future.

Material and Methods

Data collection and systematic lncRNA identification

We have downloaded the *de novo* assembled transcripts of 682 plant species belonging to Angiosperms from 1kp project (http://www.onekp.com/public_data.html), and identified the potential lncRNAs [17]. For each species, lncRNAs were identified from each library relying on a bioinformatics pipeline previously exploited by Singh et al., 2017, and transcripts longer than 200 bp were retained that are not overlapping with protein-coding gene models (Fig 1(A))[18]. First, we excluded potential coding transcripts (translated proteins of matched orthogroups derived from annotated plant genomes) from assembled transcripts set for that species [19]. Further, protein-coding transcripts were discarded by using PLncPRO (python prediction.py -p plncpro-result-file -i sequence.fa-m models/<monocot or dicot>.model -o plncpro-out -d lib/blastdb/swiss-protDB -t 15 -r) on the basis of a BLAST approach with the curated list of Swiss-Prot proteins [20]. In the final

filtering step, high-confident lncRNA transcripts were extracted by setting a minimum length threshold of 200 nt, and a non-coding probability score of 0.8 (python predstoseq.py -f sequence.fa -o output-file -p plncpro-result-file -l 0 -s 0.8 --min 200).

Fig 1(A) Systematic workflow adopted to annotate potential lncRNAs of all flowering plants available from the 1KP project.

Database construction and implementation

After the compilation of all the information regarding lncRNAs, AlnC web interface was developed using Hypertext Mark-up Language (HTML), Cascading Style Sheets (CSS), Structured Query Language, Java scripting language, PERL and Hypertext Preprocessor on Apache Hypertext Transfer Protocol server. All lncRNAs available at AlnC, and other related annotations have been handled by a relational database set up with MySQL. A stand-alone BLAST (v2.11.0) tool is installed for online similarity search feature. ViennaRNA (v2.4.16) and ORFfinder (v0.4.3) tools are used for the secondary structure visualization, and identification of Small open reading frames (sORFs) respectively [21–23].

Results and Discussion

Data content in AlnC

We have identified a total of 10,855,598 lncRNAs from 809 RNA-seq samples of Angiosperms, available at 1kp project webpage (http://www.onekp.com/public_data.html). All the related numbers of total transcripts, filtered transcripts, lncRNAs, and filtered lncRNAs identified in each plant species are mentioned at the ‘Statistics’ section (<http://nipgr.ac.in/AlnC/stat-data.php>) of AlnC webpage. At AlnC, we have organised and compiled this catalogue of 10,855,598 lncRNAs from 809 samples available in the 1 KP project, which functions as a comprehensive catalogue of potential lncRNAs in 682 flowering plants. No other data repositories on lncRNAs on this scale exist, and most lncRNAs of the species included in AlnC belong to poorly studied taxa, rendering AlnC of wide interest among plant researchers (Fig 1(B & C)). At AlnC, the length of newly identified lncRNAs of flowering plants across clades have the size ranges from 200 to 7633 nt with an average length of 405 nt (Fig 1(C)). The median length of the identified lncRNAs is smaller than

the median length of the coding sequences (Fig 1(B) and 3(E)). Moreover, most lncRNAs (66%) were less than 400 bp in length whereas only 2.9% lncRNAs were more than 1000 bp in length. Our attempts to identify ortholog relationships using BLASTN search reported large hits, however, we could only identify a moderate number of annotated lncRNAs at AlnC, identical to those stored in other databases reasoning to less conservation and apparent differences in the number of lncRNAs, but it also suggest certain significant sequence similarities across species. We have included the significant hits to NONCODE lncRNAs (384) and experimentally validated lncRNAs available in Plncdb (6) in Table S1 [11,24]. Further, the AU content of lncRNAs, available at AlnC, varied from 50-90% with an average of 75% in comparison to the coding transcripts which ranged 30-80% with an average of 60% (Fig 1(D)). Most lncRNAs contained more than 75% AU content, and the analysis implies the richness of AU than that in coding sequences [25–27]. A brief description of the lncRNA entries available at AlnC is represented in the Fig 1 and Table 1. The relationship analysis of annotated lncRNA with assembled transcripts and protein-coding transcripts yielded two distinct clusters of monocot and dicots, respectively (Fig 2). These clusters indicates the proportional discovery rate of lncRNAs with respect to the 1kp assembled transcripts, and also suggests the ratio of total transcripts to coding transcripts is smaller in dicot species whereas the reverse in case of monocots.

Fig 1. (B) Length-wise distribution of lncRNAs across clades, (C) Pie chart represents the percentage of lncRNA entries in AlnC, (D) Percentage composition of AU content in protein-coding transcripts and lncRNAs in AlnC.

Fig 2. Ternary plot of AlnC lncRNAs, protein-coding and 1Kp assembled transcripts across clades. The bubble size represents the size of 1Kp assembled transcripts, protein-coding and AlnC lncRNAs in (A), (B) and (C), respectively.

Modules available at AlnC

Search options

Current release of AlnC provides two search interfaces; (a) Simple Search, and (b) Advanced Search (Fig 3(A)). ‘Simple Search’ allows the user to quickly search the lncRNAs on the basis of taxonomic rank (Clade, Order, Family, Species), and non-coding probability score (min: 0.8; max: 1.0), while ‘Advanced Search’ provides enhanced query functionality using logical operators

(AND/OR/>=<=). Moreover, the ‘Advanced Search’ feature helps the users to search and apply filters to query a wide range of features and metadata fields e.g. Species, Tissue, lncRNA length, AlnC ID, and Non-coding probability. Consequently, a list of potential lncRNAs can be displayed at the result page according to the user defined queries, with the options to download and save the results. The search results shows the entries of the chosen species, covering the basic meta details of the 1kp sample code with links describing the sample information, including sample preparation, sample supplier, sample extractor, tissue type NCBI ID showing experimental sample library and run data, source transcript ID from which lncRNA is annotated, lncRNA length, and non-coding probability score (Fig 3(B)). The users can further explore the AlnC ID link to view and navigate at the detailed record of the lncRNA with primary lncRNA features, secondary structure, and other useful information e.g. ‘lncRNA Information’.

Fig 3 (A) Interface of simple search and advanced search modules, (B) Results page showing a table view of all lncRNA entries of the plant species *Aextoxicon punctatum*.

Each ‘lncRNA Information’ page of AlnC enables the user to access to basic information of selected lncRNA with the information of structure (Fig 3(C)). This detail page is divided into two parts; the first section contains basic details of the lncRNA sequence (including the species name of the annotated lncRNA, the source transcript ID, length, GC content and coding/non-probability), and details of the secondary structure in dot-bracket notation with a provision for the user to view and download the structures in PNG or PS format. At the second section, user can also explore the ORFs and conceptual translation products of lncRNA sequence. All the associated annotations of the concerned lncRNA entry can be downloaded in a tabular form.

Fig 3. (C) Sequence features including primary sequence information, secondary structure and possible sORFs as well peptides are displayed for lncRNA entry AlnC_6143890.

BLAST module

BLAST feature is very helpful to find regions of similarity between the user provided input sequence and lncRNAs available at AlnC by using BLASTN with the option to change Expect value (E value). User can perform BLAST search against the database of a particular Clade, Order,

167 Family, and Species. We have categorise the lncRNAs accordingly to avoid the memory intensive
168 search against the full dataset available at AlnC. The BLAST search output includes pairwise
169 alignment and a report with BLAST hits based upon alignment scores and other measures of
170 statistical significance. After the BLAST run, user can directly go at the entry of any particular
171 lncRNA showing similarity against the query sequence by a click on the AlnC ID present in the
172 BLAST results.

173 *LncRNA vs mRNA module*

174 This module of AlnC database allows the users to visualize and compare length-wise distribution,
175 and AU% in annotated lncRNA transcripts and protein-coding transcripts in multiple samples of
176 each species available at this database (Fig 3(D) and 3(E)). This page also enables the user to
177 download the images of this comparative analysis in multiple file formats.

178 **Fig 3.** (D) Percentage composition of AU content lncRNAs relative to mRNAs, (E) Pie chart
179 representation of length-wise distribution of *Aextoxicon punctatum* lncRNAs and mRNAs.

180

181 *Download AlnC data*

182 At this module, user can select the lncRNAs sequences for a particular Clade, Order, Family and
183 Species, and download the complete sets from the AlnC archive. Complete AlnC collection can be
184 found on the download module. Here users have access to both the hierarchical bulk download and
185 the species-wise download in the FASTA file format.

186 **Submit Data**

187 As there are sizeable researchers working on several flowering plants, we created a user form to
188 submit any information or data with regard to lncRNAs of angiosperm species. This will allow us to
189 develop, upgrade and manage AlnC on a regular basis. The related lncRNA data, research results
190 and publications can be submitted using the form provided on the 'Submit data' page
191 (http://nipgr.ac.in/AlnC/submit_data.php) of AlnC. If the submitted data found to be relevant, the
192 received data will be curated manually and appended to AlnC. All submitted lncRNA data will be
193 processed in compliance with the AlnC standard pipeline mentioned in Fig 1(A) and manually

curated. We encourage submissions to AInC curators as this will drive our plans to include additional species in the future.

Conclusion and future prospects

In this research work, we have used the transcriptome data of 682 flowering plants, most of which had no genomic information and/or no documented lncRNA studies prior to this work. In our opinion, this is a key feature of AInC, which was constructed with the primary objective of promoting the investigation of lncRNA in various Angiosperm species. The analysis workflow used in this study was optimized for plants and could be used for RNA-seq-based lncRNA identification for any other plant species as well. The workflow includes a ML based bioinformatics pipeline to identify high confident lncRNAs across distinct Angiosperm species, which significantly differs from other annotation approaches in lncRNA databases such CATATAdb [9]. Our approach yielded a large number of putative lncRNA transcripts, and all the annotated lncRNA transcripts were organised and catalogued in the AInC web interface. It stores information of 10,855,598 lncRNA loci derived from 809 samples and provides a user-friendly platform for browsing, searching and accessing all annotated lncRNAs through simple and interactive web pages. AInC includes lncRNAs with evidence of non-coding RNA probability score, and allows further exploration of sORFs alongside other primary lncRNA features, thus providing researchers with functional capability to leverage on AInC data, and information on their individual projects using our web interface. With this research work, we have attempted to develop a first-ever database covering the largest number of confident lncRNA entries of wide-range plant species, including those with no information on lncRNA of any sort. Although, it is clear that several plants belonging to Angiosperms are still to be discovered and transcriptomes are waiting to be studied by individual research groups, AInC will be continuously updated. At the same time, AInC will strive to periodically search freely accessible databases, and other forms of documentation to collect useful information for annotated lncRNAs, and add additional functionality to enhance user engagement. It is our intention that AInC will move forward to provide new databases representing additional

species as well as to fine-tune, and optimise the currently available annotations. We will also aim to focus on the new pipeline to develop lncRNA annotations as the plant lncRNA biology research progresses. All in all, AlnC will strive to continue to be in line with the lncRNA community, and remain to serve useful lncRNA data in future.

Conflict of interest

The authors declare that there is no conflict of interest.

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Author Contributions

A.S. and AT V. analysed the data and developed the web interface of this database. A.S., AT V., and SK wrote the manuscript. S.K. conceived the idea and coordinated the project. S.K. agrees to serve as the author responsible for contact and ensures communication.

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Figure Legends

Fig 1. Overview of the lncRNAs in AlnC. (A) Systematic workflow adopted to annotate potential lncRNAs of all flowering plants available from the 1KP project, (B) Length-wise distribution of lncRNAs across clades, (C) Pie chart represents the percentage of lncRNA entries in AlnC, (D) Percentage composition of AU content in protein-coding transcripts and lncRNAs in AlnC.

Fig 2. Ternary plot of AlnC lncRNAs, protein-coding and 1Kp assembled transcripts across clades. The bubble size represents the size of 1Kp assembled transcripts, protein-coding and AlnC lncRNAs in (A), (B) and (C), respectively.

Fig 3. Screenshots of AlnC web interface. (A) Interface of simple search and advanced search modules, (B) Results page showing a table view of all lncRNA entries of the plant species *Aextoxicon punctatum*, (C) Sequence features including primary sequence information, secondary structure and possible sORFs as well peptides are displayed for lncRNA entry AlnC_6143890, (D) Percentage composition of AU content lncRNAs relative to mRNAs, (E) Pie chart representation of length-wise distribution of *Aextoxicon punctatum* lncRNAs and mRNAs.

Tables

Table 1: Summary of annotated lncRNAs across higher-level clades in AlnC database.

Clade	No. of Species	Number of Samples	Number of lncRNAs
Basal Eudicots	33	55	835185
Basalmost angiosperms	8	8	281514
Chloranthales	2	2	46074
Core Eudicots	95	116	1828555
Core Eudicots/Asterids	217	242	3656812
Core Eudicots/Rosids	201	250	2627218

Magnoliids	26	27	383010
Monocots	61	64	829121
Monocots/Commelinids	39	45	568109
Total	682	809	10855598

352

353 Supporting information

354

355 **Table S1:** List of significant hits of lncRNAs at AlnC, to lncRNAs available at NONCODE and
356 PlncDB databases.

357

A

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B **C** **D**

Fig 1

Fig 2

A

Simple search

Select Clade:	Basal Eudicots
Select Order:	Berberidopsidales
Select Family:	Aextoniaceae
Select Species:	Aexticon punctatum
Non-coding Probability:	0.8 to 0.9 (min: 0.8; max: 1.0)

Advanced search

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C

[illegible]

lncRNA structure

CDS

[illegible]

Peptides

[illegible]

B

AlnC-ID	Species	Tissue	Sample code	NCBI link	Source transcript ID	Length	Non-coding probability
AlnC_6143890	<i>Aeetoxicon punctatum</i>	leaves	QUTB	ERS1829250	scaffold-QUTB-2000004-Aeetoxicon_punctatum	534	0.811
AlnC_6143892	<i>Aeetoxicon punctatum</i>	leaves	QUTB	ERS1829250	scaffold-QUTB-2000010-Aeetoxicon_punctatum	309	0.866
AlnC_6143893	<i>Aeetoxicon punctatum</i>	leaves	QUTB	ERS1829250	scaffold-QUTB-2000011-Aeetoxicon_punctatum	412	0.885
AlnC_6143897	<i>Aeetoxicon punctatum</i>	leaves	QUTB	ERS1829250	scaffold-QUTB-2000021-Aeetoxicon_punctatum	277	0.838
AlnC_6143898	<i>Aeetoxicon punctatum</i>	leaves	QUTB	ERS1829250	scaffold-QUTB-2000022-Aeetoxicon_punctatum	711	0.892
AlnC_6143899	<i>Aeetoxicon punctatum</i>	leaves	QUTB	ERS1829250	scaffold-QUTB-2000032-Aeetoxicon_punctatum	229	0.818
AlnC_6143901	<i>Aeetoxicon punctatum</i>	leaves	QUTB	ERS1829250	scaffold-QUTB-2000034-Aeetoxicon_punctatum	338	0.845
AlnC_6143902	<i>Aeetoxicon punctatum</i>	leaves	QUTB	ERS1829250	scaffold-QUTB-2000040-Aeetoxicon_punctatum	269	0.859
AlnC_6143905	<i>Aeetoxicon punctatum</i>	leaves	QUTB	ERS1829250	scaffold-QUTB-2000050-Aeetoxicon_punctatum	326	0.844
AlnC_6143905	<i>Aeetoxicon punctatum</i>	leaves	QUTB	ERS1829250	scaffold-QUTB-2000077-Aeetoxicon_punctatum	227	0.891

Page 1 of 869 1 2 3 4 5 6 7 ... 868 869 Next Last

D

AU Content Distribution

E

IncrRNA Length Distribution

mRNA Length Distribution

Fig 3