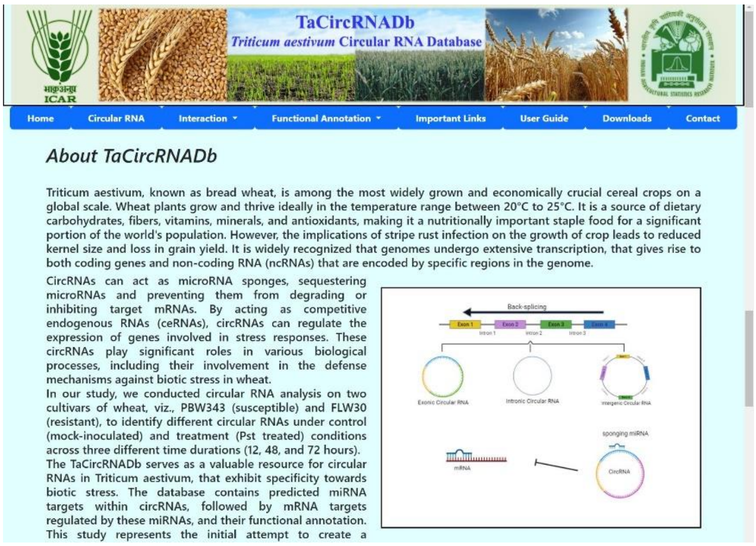
User Guide: Triticum aestivum circular RNA database (TaCircRNAdb)

TaCircRNAdb includes eight different tabs: Home, Circular RNA, Interaction, Functional Annotation, Important Links, User Guide, Downloads, Team (**Figure 1**).

1. **Home:** The main page is strategically designed to offer visitors with a detailed presentation of TaCircRNAdb, clarifying both its nature and significance. This facilitates deeper understanding of fundamental objectives of the database, its range, and the extensive array of information. Essentially, the homepage not only presents TaCircRNAdb but also explains the reasons behind its significance to researchers, scientists, and stakeholders in agriculture. It emphasizes its crucial contribution to the progress of wheat crop research and cultivation.



About TaCircRNADb

Triticum aestivum, known as bread wheat, is among the most widely grown and economically crucial cereal crops on a global scale. Wheat plants grow and thrive ideally in the temperature range between 20°C to 25°C. It is a source of dietary carbohydrates, fibers, vitamins, minerals, and antioxidants, making it a nutritionally important staple food for a significant portion of the world's population. However, the implications of stripe rust infection on the growth of crop leads to reduced kernel size and loss in grain yield. It is widely recognized that genomes undergo extensive transcription, that gives rise to both coding genes and non-coding RNA (ncRNAs) that are encoded by specific regions in the genome.

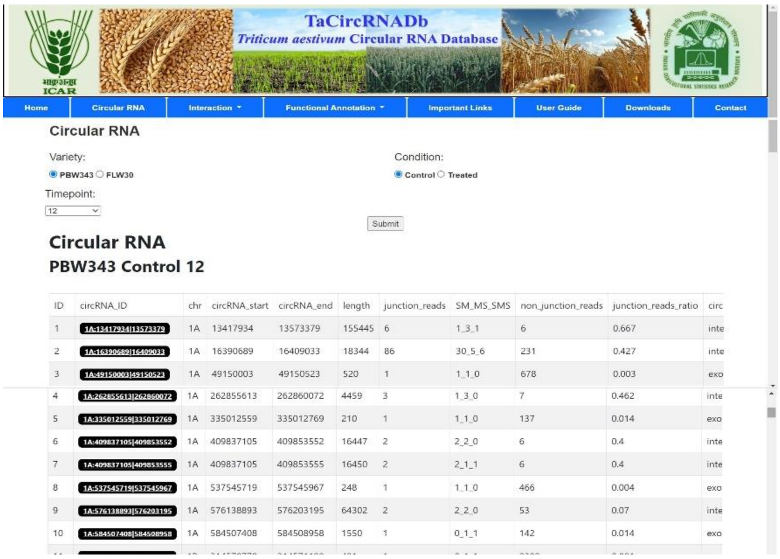
CircRNAs can act as microRNA sponges, sequestering microRNAs and preventing them from degrading or inhibiting target mRNAs. By acting as competitive endogenous RNAs (ceRNAs), circRNAs can regulate the expression of genes involved in stress responses. These circRNAs play significant roles in various biological processes, including their involvement in the defense mechanisms against biotic stress in wheat.

In our study, we conducted circular RNA analysis on two cultivars of wheat, viz., PBW343 (susceptible) and FLW30 (resistant), to identify different circular RNAs under control (mock-inoculated) and treatment (Pst treated) conditions across three different time durations (12, 48, and 72 hours).

The TaCircRNADb serves as a valuable resource for circular RNAs in Triticum aestivum, that exhibit specificity towards biotic stress. The database contains predicted miRNA targets within circRNAs, followed by mRNA targets regulated by these miRNAs, and their functional annotation. This study represents the initial attempt to create a

Figure 1. The home page of *Triticum aestivum* circular RNA database (TaCircRNAdb)

2. **Circular RNA**: The page includes different types of circular RNA (Exonic, Intergenic and Intronic) along with their positions. The circular RNA of two varieties *viz.* PBW343 and FLW30 are present at different time point under control and treated conditions. The dropdown menu consists of circular RNA present at different time points such as 12, 48 and 72 hr (**Figure 2**).



Circular RNA

Variety: ● PBW343 ○ FLW30

Condition: ● Control ○ Treated

Timepoint: 12

Circular RNA

PBW343 Control 12

ID	circRNA_ID	chr	circRNA_start	circRNA_end	length	junction_reads	SM_MS_SMS	non_junction_reads	junction_reads_ratio	circ
1	1A:13417934|13573379	1A	13417934	13573379	155445	6	1_3_1	6	0.667	inte
2	1A:16390689|16409033	1A	16390689	16409033	18344	86	30_5_6	231	0.427	inte
3	1A:49150003|49150523	1A	49150003	49150523	520	1	1_1_0	678	0.003	exo
4	1A:262855613|262860072	1A	262855613	262860072	4459	3	1_3_0	7	0.462	inte
5	1A:335012559|335012769	1A	335012559	335012769	210	1	1_1_0	137	0.014	exo
6	1A:409837105|409853552	1A	409837105	409853552	16447	2	2_2_0	6	0.4	inte
7	1A:409837105|409853555	1A	409837105	409853555	16450	2	2_1_1	6	0.4	inte
8	1A:537545719|537545967	1A	537545719	537545967	248	1	1_1_0	466	0.004	exo
9	1A:576138893|576203195	1A	576138893	576203195	64302	2	2_2_0	53	0.07	inte
10	1A:584507408|584508958	1A	584507408	584508958	1550	1	0_1_1	142	0.014	exo

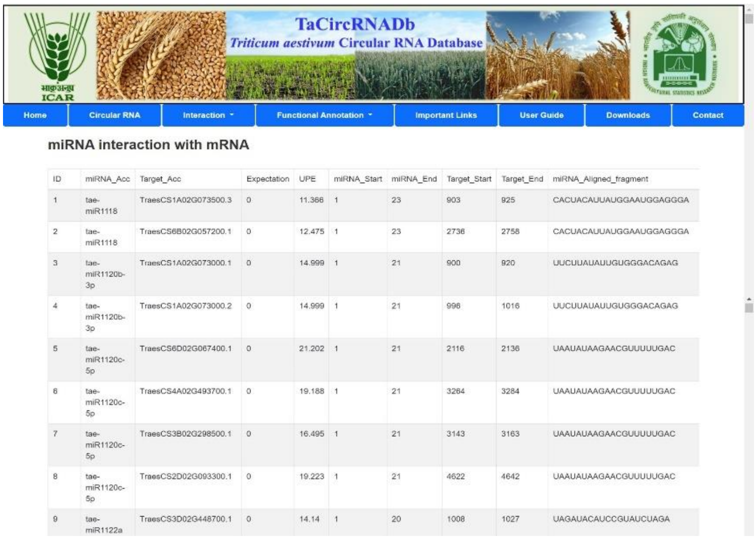
Figure 2. The circular RNA ID's opens to a pop up which contains sequence information of that circular RNA.

3. **Interaction:** It includes a dropdown menu consisting of interaction of Circular RNA with miRNA and interaction of miRNA with mRNA. The miRNA page in a database is a specialized section that focuses on miRNAs. Within this section, users can access comprehensive details about specific miRNAs, including their sequences and associated target genes (**Figure 3 and 4**). It allows users to delve into specific miRNA data, offering a deeper understanding of their regulatory functions, interactions with target genes, and potential impacts on diverse biological processes.

CircRNA interaction with miRNA

ID	miRNA_Acc	Target_Acc	Expectation	UPE	miRNA_Start	miRNA_End	Target_Start	Target_End	miRNA_Aligned_fragment
1	tae-miR1127b-3p	5B:478758295-478918980	1	23.908	1	21	58271	58291	ACAAGUAUUUCUGGACGGAGG
2	tae-miR1128	5B:478758295-478918980	2.5	19.602	1	21	57359	57379	UACUACUCCCUCCGUCCGAAA
3	tae-miR1130a	1B:689504569-689555554	2.5	18.556	1	23	49192	49214	CCUCCGUCUCGUAAUGUAAGACG
4	tae-miR1133	5B:478758295-478918980	2.5	23.887	1	22	157975	157996	CAUAUACUCCCUCCGUCCGAAA
5	tae-miR7757-5p	5B:478758295-478918980	2.5	20.773	1	22	159151	159172	AUAAAACCUUCAGCUAUCCAUC
6	tae-miR1128	5B:478758295-478918980	3	20.453	1	21	58375	58395	UACUACUCCCUCCGUCCGAAA
7	tae-miR1128	5B:478758295-478918980	3	18.979	1	21	103531	103551	UACUACUCCCUCCGUCCGAAA
8	tae-miR1131	3D:610139253-610190476	3	15.683	1	22	37860	37881	UAGUACCGGUUCGUGGCUAACC
9	tae-miR1133	5B:478758295-478918980	3	19.753	1	22	58375	58396	CAUAUACUCCCUCCGUCCGAAA

Figure 3. Depicting the circular RNA interaction with miRNA along with their sequence information and positions. This page establishes links to the miRBase database by clicking on miRNA accession.

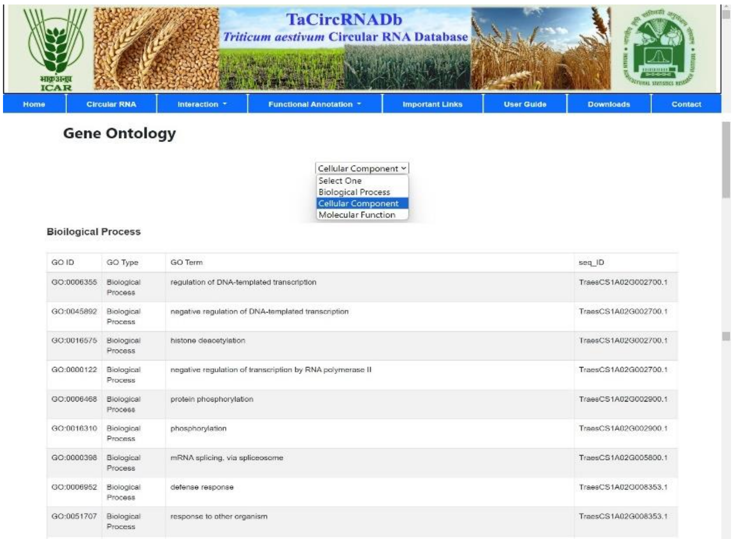


miRNA interaction with mRNA

ID	miRNA_Acc	Target_Acc	Expectation	UPE	miRNA_Start	miRNA_End	Target_Start	Target_End	miRNA_Aligned_fragment
1	tae-miR1118	TraesCS1A02G073500.3	0	11.366	1	23	903	925	CACUACAUUAUGGAAUGGAGGGA
2	tae-miR1118	TraesCS6B02G057200.1	0	12.475	1	23	2736	2758	CACUACAUUAUGGAAUGGAGGGA
3	tae-miR1120b-3p	TraesCS1A02G073000.1	0	14.999	1	21	900	920	UUCUUAUAUUGUGGGACAGAG
4	tae-miR1120b-3p	TraesCS1A02G073000.2	0	14.999	1	21	996	1016	UUCUUAUAUUGUGGGACAGAG
5	tae-miR1120c-5p	TraesCS6D02G067400.1	0	21.202	1	21	2116	2136	UAAUAUAAGAACGUUUUUGAC
6	tae-miR1120c-5p	TraesCS4A02G493700.1	0	19.188	1	21	3264	3284	UAAUAUAAGAACGUUUUUGAC
7	tae-miR1120c-5p	TraesCS3B02G298500.1	0	16.495	1	21	3143	3163	UAAUAUAAGAACGUUUUUGAC
8	tae-miR1120c-5p	TraesCS2D02G093300.1	0	19.223	1	21	4622	4642	UAAUAUAAGAACGUUUUUGAC
9	tae-miR1122a	TraesCS3D02G448700.1	0	14.14	1	20	1008	1027	UAGAUACAUCCGUAUCUAGA

Figure 4. Showing the list of miRNAs along with their respective targets. Users can access the information about TamiRNA from miRBase website and the link has been provided in Important links tab.

4. **Functional Annotation:** The dropdown menu includes Gene Ontology (GO) and Pathways associated with genes involved in rust infection. The information in GO categories encompasses biological processes, molecular functions, and cellular components (**Figure 5**). The hyperlink to the respective databases on the specific ID is provided for further details. The tab Pathways in drop down menu represents KEGG pathways associated with each gene.

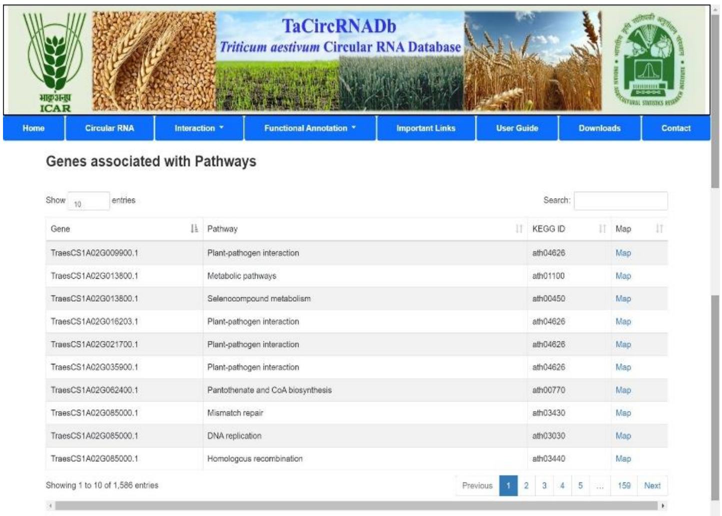


Gene Ontology

Bioilogical Process

GO ID	GO Type	GO Term	seq_ID
GO:0006355	Biological Process	regulation of DNA-templated transcription	TraesCS1A02G002700.1
GO:0045892	Biological Process	negative regulation of DNA-templated transcription	TraesCS1A02G002700.1
GO:0016575	Biological Process	histone deacetylation	TraesCS1A02G002700.1
GO:0000122	Biological Process	negative regulation of transcription by RNA polymerase II	TraesCS1A02G002700.1
GO:0006468	Biological Process	protein phosphorylation	TraesCS1A02G002900.1
GO:0016310	Biological Process	phosphorylation	TraesCS1A02G002900.1
GO:0000398	Biological Process	mRNA splicing, via spliceosome	TraesCS1A02G005800.1
GO:0006952	Biological Process	defense response	TraesCS1A02G008353.1
GO:0051707	Biological Process	response to other organism	TraesCS1A02G008353.1

Figure 5. Users have the option to select the different gene ontology term from the dropdown menu.



Genes associated with Pathways

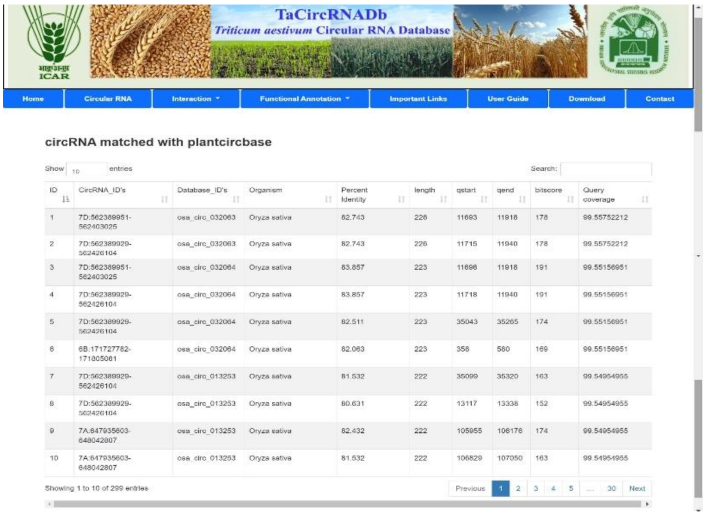
Gene	Pathway	KEGG ID	Map
TraesCS1A02G009900.1	Plant-pathogen interaction	ath04626	Map
TraesCS1A02G013800.1	Metabolic pathways	ath01100	Map
TraesCS1A02G013800.1	Selenocompound metabolism	ath00450	Map
TraesCS1A02G016203.1	Plant-pathogen interaction	ath04626	Map
TraesCS1A02G021700.1	Plant-pathogen interaction	ath04626	Map
TraesCS1A02G035900.1	Plant-pathogen interaction	ath04626	Map
TraesCS1A02G062400.1	Pantothenate and CoA biosynthesis	ath00770	Map
TraesCS1A02G085000.1	Mismatch repair	ath03430	Map
TraesCS1A02G085000.1	DNA replication	ath03030	Map
TraesCS1A02G085000.1	Homologous recombination	ath03440	Map

Showing 1 to 10 of 1,586 entries

Figure 6: Showing the diverse pathways associated with each gene. This page establishes links to the KEGG database to access pathway maps through pathway IDs.

5. **Important Links:** The links in the page provides information to different databases and websites related to wheat and other plant species.

6. **Downloads:** This tab contains the links to the files which contains information of matched CircRNA Id's from our dataset with PlantCircBase. The second link provides annotation of mRNA targets with their NCBI Id's and their description about the functions of each gene.



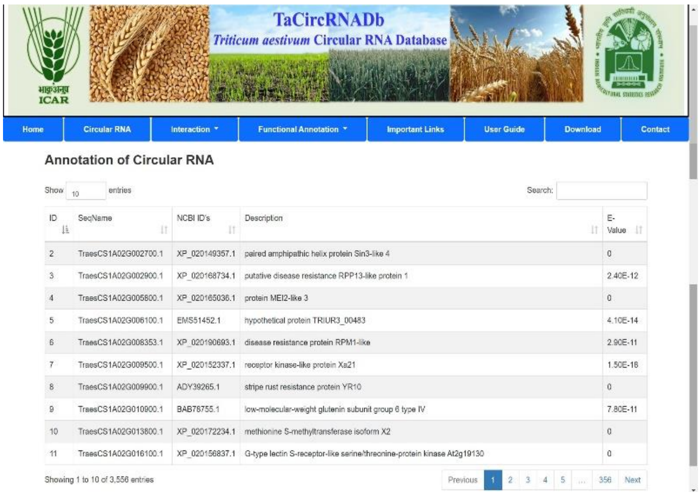
circRNA matched with plantcircbase

Show 10 entries Search:

ID	CircRNA_ID's	Database_ID's	Organism	Percent Identity	length	qstart	qend	bitscore	Query coverage
1	7D:562389951-562403025	osa_circ_032063	Oryza sativa	82.743	226	11693	11918	178	99.55752212
2	7D:562389929-562426104	osa_circ_032063	Oryza sativa	82.743	226	11715	11940	178	99.55752212
3	7D:562389951-562403025	osa_circ_032064	Oryza sativa	83.857	223	11696	11918	191	99.55156951
4	7D:562389929-562426104	osa_circ_032064	Oryza sativa	83.857	223	11718	11940	191	99.55156951
5	7D:562389929-562426104	osa_circ_032064	Oryza sativa	82.511	223	35043	35265	174	99.55156951
6	6B:171727782-171805061	osa_circ_032064	Oryza sativa	82.063	223	358	580	169	99.55156951
7	7D:562389929-562426104	osa_circ_013253	Oryza sativa	81.532	222	35099	35320	163	99.54954955
8	7D:562389929-562426104	osa_circ_013253	Oryza sativa	80.631	222	13117	13338	152	99.54954955
9	7A:647935603-648042807	osa_circ_013253	Oryza sativa	82.432	222	105955	106176	174	99.54954955
10	7A:647935603-648042807	osa_circ_013253	Oryza sativa	81.532	222	106829	107050	163	99.54954955

Showing 1 to 10 of 299 entries

Figure 7. Conservation analysis of identified circRNAs from our dataset. The third column contains circular RNA Ids of different species.



Annotation of Circular RNA

Show 10 entries Search:

ID	SeqName	NCBI ID's	Description	E-Value
2	TraesCS1A02G002700.1	XP_020149357.1	paired amphipathic helix protein Sin3-like 4	0
3	TraesCS1A02G002900.1	XP_020168734.1	putative disease resistance RPP13-like protein 1	2.40E-12
4	TraesCS1A02G005800.1	XP_020165036.1	protein MEI2-like 3	0
5	TraesCS1A02G006100.1	EMS51452.1	hypothetical protein TRIUR3_00483	4.10E-14
6	TraesCS1A02G008353.1	XP_020190693.1	disease resistance protein RPM1-like	2.90E-11
7	TraesCS1A02G009500.1	XP_020152337.1	receptor kinase-like protein Xa21	1.50E-18
8	TraesCS1A02G009900.1	ADY39265.1	stripe rust resistance protein YR10	0
9	TraesCS1A02G010900.1	BAB78755.1	low-molecular-weight glutenin subunit group 6 type IV	7.80E-11
10	TraesCS1A02G013800.1	XP_020172234.1	methionine S-methyltransferase isoform X2	0
11	TraesCS1A02G016100.1	XP_020156837.1	G-type lectin S-receptor-like serine/threonine-protein kinase At2g19130	0

Showing 1 to 10 of 3,556 entries

Figure 8: Results from blast analysis