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Identification of novel non-coding RNAs in *Rhizoctonia solani* through mining of transcriptomic data

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ABSTRACT

Rhizoctonia solani is a plant pathogenic fungus infecting a wide range of hosts including economically important crops such as wheat, rice, and vegetables, etc. and leads to a loss in agricultural production. Various chemicals and bio fungicides are used to control *R. solani*. Understanding of gene expression and its function during host interaction will be useful to identify potential targets in *R.solani* for its effective control. In recent studies, non-coding RNAs (ncRNAs) are found to have a role in regulating cellular functions. In the current study, we report 16 ncRNAs from *R. solani* identified using raw transcriptomic data from three different bio projects reported in NCBI's sequence read archive database. The ncRNAs from F001 to ncRNA F0011 was expressed with fragments per kilo million reads (FPKM) values ranging from 100 to 20,000. Out of these 11 ncRNAs, 7 ncRNAs has the same intron splicing sites in all three bio projects. The ncRNA F0012 to ncRNA F0016 was found to be expressed approximately 10–80 FPKM and are present in all three bio projects, out of these five ncRNAs, three are found to have similar splicing sites in all three bio projects. The high expression levels of the ncRNAs and their presence in the genome confirmed by different datasets point to the fact that they might have a major function in the organism and should be studied further to characterize it functionally and the current study might serve as the first step to achieve it.

1. INTRODUCTION

Rhizoctonia solani is a plant pathogenic fungus which belongs to phylum Basidiomycota, has a wide range of host and causes major loss in food crops. *Rhizoctonia solani* is divided into 14 anastomosis groups based on their anastomosis behavior (AG1-AG13 and AGBI) [1]. AG1 is again classified into six subgroups (IA, IB, IC, ID, IE, and IF) [2–4], Rice sheath blight is majorly caused by intraspecific group IA and studied a lot due to the economic importance of the host [5–7]. *Rhizoctonia solani* AG1-IA genome from NCBI (Accession No: GCA_000334115.1) is 36.94 Mb long and has 10489 protein sequences, and is widely used in transcriptome analysis for *R. solani* AG1-IA [8]. RNASeq study-based evidence suggests that some of the regions in this genome are highly expressed but are not annotated. This partial annotation might lead to the omission of certain important gene expression during the study of host infection. In the current study, the existing transcriptome data for *R. solani* for their host interaction were analyzed and found that certain non-coding RNAs (ncRNAs) gets expressed during pathogenesis fragments per kilo million reads (FPKM).

NcRNAs that do not code any proteins are widely studied recently [9]. The role of ncRNA in plants and animal were shown to be involved in their immunity during infection [10]. Some mobile ncRNA were reported in the fungal pathogen and their mechanism through entering into the host cell and suppress the genes related to immunity [11]. These ncRNAs are of two types, long non-coding RNAs (lncRNAs) which are above 200 nucleotides long, and small non-coding (sncRNAs) with less than 200 nucleotides long. These lncRNAs are transcribed by DNA polymerase 2 from the genome, similar to mRNA and also they undergo 5′ capping, splicing, and poly-A tailing [12]. Most of the lncRNAs transcribed from ORF had only a single peptide match, but very few lncRNAs contains cryptic ORF [13]. lncRNAs in yeast is found to have

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a functional role like regulating gene expression, response to environment and chromosome pairing, etc [14,15]. Swr1, Isw2, Rsc, and Ino80 are distinct chromatin remodeling complexes in *Saccharomyces cerevisiae* that are global repressors of lncRNA transcription [16].

Here, SRR files from NCBI were downloaded and the differentially expressed genes were analyzed to discover certain unclassified genes in the *R. solani* AG1-IA genome to be expressed significantly that has an important biological and molecular function during infection. Also, differentially expressed lncRNAs and sncRNAs were identified and studied. This finding can help in improving the study of lncRNA, function, and metabolism in plant photogenic fungi.

2. METHODS

2.1. Sequence Read Archive (SRA) Files Selected for the Study

The NCBI SRA tool kit was used to download the RNA Seq raw data from SRA database and under bio project numbers PRJNA298635, PRJNA369092, and PRJNA377841. A total of 11 conditions were selected from these three bio projects, 1_C1 (*R. solani* AG1-IA infected on Tetep, 1DPI), 1_C2 (*R. solani* AG1-IA infected on TP309, 1DPI), 1_C3 (*R. solani* AG1-IA infected on PB1, 1DPI), 1_C4 (*R. solani* AG1-IA infected on Tetep, 3DPI), 1_C5 (*R. solani* AG1-IA infected on TP309, 3DPI), 1_C6 (*R. solani* AG1-IA infected on PB1, 3DPI), 2_C1 *(R. solani* AG1-IA infected on soya leaf, the onset of necrosis), 2_C2 (*R. solani* AG1-IA infected on soya leaf, post-onset of necrosis), 3_C1 (*R. solani* AG1-IA infected on soya leaf), 3_C2 (*R. solani* AG1-IA infected on maize leaves) and 3_C3 (*R. solani* AG1-IA infected on rice leaf) [17–19].

2.2. Mining of Transcriptomic Data for Non-Coding RNA

The raw reads were analyzed for quality and adapter contamination using FastQC (version-0.11.9) [20]. The low-quality reads and adapters were trimmed using trimmomatic (version-0.39) [21] and reads with length below 50 nucleotides were dropped. The filtered reads were then mapped to *R. solani* AG1-IA genome (GCA_000334115.1) using tophat (version-2.1.1) [22]. The mapped reads were then processed with cufflinks (version-2.2.1), cufflinks (version-1.0.0) and cuffdiff (version-2.2.1) [23]. These genes were traced back to the genome and the sequence was retrieved using bed tools (version-2.27.1) [24]. The obtained sequences were locally blasted against the NR database using blastx (version-2.9.0+) [25].

2.3. Identification of ncRNAs

The unannotated genes were further filtered to remove transcriptional noise by avoiding single-exon genes and genes with FPKM value <0.5. The differently expressed genes with p value less than 0.5 were taken for identification of long non-coding. The sequences above 200 nucleotides were selected for identifying lncRNAs. The coding potential was calculated using CPC2, CNIT, and PLEK tools [26–28]. The sequences were considered as non-coding if all the three tools predicted it as non-coding [29]. These genes were further filtered by the presence of one gene in more than eight conditions or FPKM above 100, which is present in at least four conditions and more than one bio project transcriptomic data.

3. RESULTS AND DISCUSSION

3.1. Mining of Transcriptomic Data for Non-Coding RNA

LncRNA have vital role in regulation of gene expression and associated with many diseases such as cancer [30]. It also plays a crucial role in developing immunity against various pathogen in plants and animals [31]. In the present study, a total of 26 transcriptome data for *R. solani* interaction with host were downloaded from all three bio projects and the quality trimed reads were mapped to the genome. The raw read counts after trimming and the percentage of reads mapped to the genome are listed in Table 1. The percentage of the reads mapped to the genome varies drastically between the SRA files as per Table 1. The reason could be that the SRA data is mostly mixed with the host transcriptomic data, which accounts for the major portion of these files. The RNA Seq analysis using tophat, cufflinks, and cuffdiff revealed 2,448 unclassified genes in the reference genome and the sequences were retrieved from the genome using bed tools. Among the non-coding transcripts, the one with more than 200 nt are classified as long ncRNAs and are considered for further analysis [32]. The annotation of these sequences using blastx against nr database annotated 1,868 sequences, and the remaining 580 sequences had no blast hits. These 580 sequences either have no predecessor sequences in the NR database or they might be potential non-coding sequences.

3.2. Identification of ncRNAs

The 580 genes with no blast hits were further filtered and analyzed for their coding potential using CPC2, CNIT, and PLEK. The tools predicted 536, 556, and 569 non-coding sequences with CPC2, CNIT, and PLEK, respectively. The 511 sequences predicted as non-coding by all the three tools as depicted in Figure 1 were considered for further analysis. The predicted potential non-coding sequences were further screened based on their FPKM values and the number of conditions they were present in and narrowed it down to 16 sequences. These 16 sequences were finalized as ncRNA. Out of this, 5-ncRNA was differently expressed in ≥ 8 conditions with FPKM less than 100, 11ncRNA was found to be expressed with FPKM values more than 100 ranging up to 20,000 and are present in more than one bio project transcriptomic data. Ten ncRNA retains similar splice sites in all three bioprojects and presented in Table 4. These results were supported by Nitsche et al. [33] who reported that there is a conserved splice site among the ncRNAs and lead to track the evolutionary changes. The details of the number of conditions the ncRNAs were expressed, the number of bio projects, and the type of ncRNA for the ncRNAs having FPKM values above 100 and below 100 are given in Tables 2 and 3, respectively. There are numerous reports stating lncRNAs have a role in regulatory mechanism in gene expression level and the same were reviewed recently [34]. Wang et al. [35] reported that 161 long non coding RNAs were differentially expressed in rice responsive to Blast fungal infection. This also supports the

Table 1: List of SRA files included in the study.

S. no.	Bio project	SRR accession No.	Total reads before trimming	Total reads after trimming	Reads mapped to genome (%)
1	Bio project 1	SRR2854160	28,511,520	28,511,520	61.1%
2		SRR2859034	31,832,558	31,832,558	37.9%
3		SRR2859785	35,111,684	35,111,684	58.8%
4		SRR2873752	28,967,576	28,967,576	12.6%
5		SRR2906372	38,624,268	38,624,268	17.4%
6		SRR2906726	40,052,168	40,052,168	2.5%
7		SRR9044112	38,404,774	38,404,774	29.0%
8		SRR9044119	43,550,864	43,550,864	5.4%
9	Bio project 2	SRR5209772	35,087,068	31,203,980	76.0%
10		SRR5209773	31,206,092	26,117,842	75.4%
11		SRR5209774	28,346,372	25,616,466	76.3%
12		SRR5209775	22,617,670	20,136,584	73.9%
13		SRR5209776	29,720,586	27,091,046	73.9%
14		SRR5209777	26,818,988	23,161,090	73.6%
15		SRR5209778	24,634,370	22,206,974	69.5%
16		SRR5209779	28,176,672	23,151,690	68.1%
17		SRR5209780	27,320,880	24,483,114	70.2%
18		SRR5209781	27,030,046	24,526,572	70.7%
19		SRR5209782	30,295,896	27,285,188	70.9%
20		SRR5209783	24,009,354	22,436,774	71.3%
21	Bio project 3	SRR5500529	26,587,034	25,944,658	68.1%
22		SRR5500530	28,289,406	27,655,028	67.9%
23		SRR5500532	26,958,568	26,296,358	73.1%
24		SRR5500533	24,732,110	24,217,014	73.4%
25		SRR5500534	23,405,190	22,787,166	78.0%
26		SRR5500535	29,428,220	28,834,604	78.2%

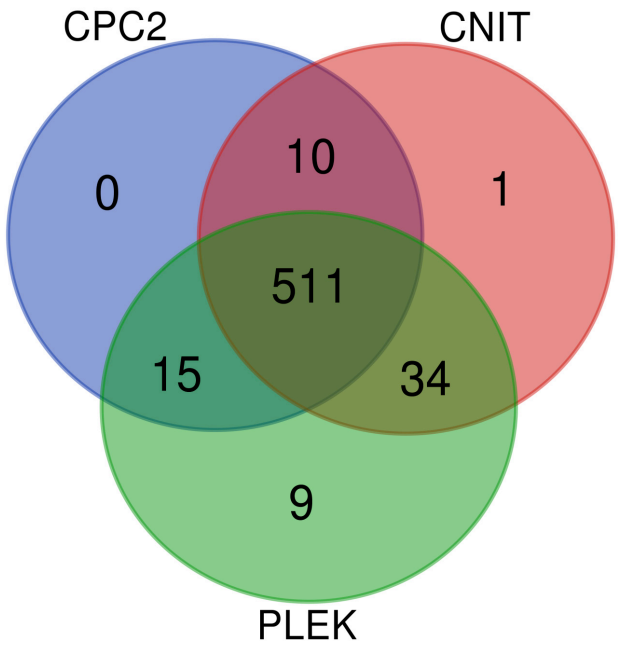

Figure 1: Coding potential calculation using CPC2, CNIT, and PLEK.

Table 2: List of predicted ncRNA expressed with FPKM more than 100.

NC-RNA	Locus	Number of conditions has expression	Bioprojects containing the expression	NC-RNA type
NC-RNA F001	KB317696.1:824260-827451	8	3	LNC-RNA
NC-RNA F002	KB317698.1:97800-98802	8	3	LNC-RNA
NC-RNA F003	KB317700.1:980840-981146	7	2	SNC-RNA
NC-RNA F004	KB317701.1:439656-439959	3	2	SNC-RNA
NC-RNA F005	KB317701.1:483929-485975	8	3	LNC-RNA
NC-RNA F006	KB317705.1:471573-471886	10	3	LNC-RNA
NC-RNA F007	KB317706.1:637144-637947	8	3	LNC-RNA
NC-RNA F008	KB317710.1:116289-116584	8	3	LNC-RNA
NC-RNA F009	KB317726.1:101314-107653	7	3	LNC-RNA
NC-RNA F0010	KB317748.1:130750-131698	10	3	LNC-RNA
NC-RNA F0011	KB320145.1:0-237	4	2	SNC-RNA

Table 3: List of predicted ncRNA that are expressed in more than or equal to eight conditions with FPKM less than 100.

NC-RNA	Locus	Number of conditions has expression	Bioprojects containing the expression	NC-RNA type
NC-RNA F0012	KB317707.1:280114-281077	10	3	LNC-RNA
NC-RNA F0013	KB317709.1:358322-358980	8	2	LNC-RNA
NC-RNA F0014	KB317711.1:412940-413725	9	3	LNC-RNA
NC-RNA F0015	KB317725.1:115863-118971	8	3	LNC-RNA
NC-RNA F0016	KB317744.1:164906-167799	9	3	LNC-RNA

Five ncRNA was identified, out of that one was found to be expressed in ten conditions from three bio projects, two ncRNA was expressed in nine conditions from three bio projects and two was expressed in eight conditions.

Table 4: The splicing sites for 10 ncRNAs are exactly same in all three bio projects that is the intron region is common for these ncRNAs that are listed with the exon regions.

Sl. no.	NC-RNA	Accession	Exon		NC-RNA type
			Start	End	
1	NC-RNA F001	KB317696.1	824,261	824,407	LNC-RNA
			824,458	824,644	
2	NC-RNA F002	KB317698.1	97,811	98,013	LNC-RNA
			98,072	98,163	
			98,304	98,487	
3	NC-RNA F005	KB317701.1	483,930	484,058	LNC-RNA
			484,110	484,342	
4	NC-RNA F006	KB317705.1	471,574	471,729	LNC-RNA
			471,781	471,887	
5	NC-RNA F008	KB317710.1	116,290	116,376	LNC-RNA
			116,436	116,585	
6	NC-RNA F009	KB317726.1	107,184	107,387	LNC-RNA
			107,444	107,654	
7	NC-RNA F0011	KB320145.1	1	42	SNC-RNA
			111	218	
8	NC-RNA F0013	KB317709.1	358,323	358,556	LNC-RNA
			358,621	358,707	
			358,762	358,847	
9	NC-RNA F0014	KB317711.1	412,941	413,436	LNC-RNA
			413,488	413,560	
10	NC-RNA F0015	KB317725.1	115,864	115,916	LNC-RNA
			116,105	116268	

regulatory functions of identified lncRNAs in *R. solani* during its interaction with host plants.

4. CONCLUSION

The SRA database of NCBI contains an immense amount of raw data with untapped potential, much of the data has been generated by taking a single aspect of an experimental setup. We have successfully utilized some of the RNASeq based raw data available for identifying certain Novel lncRNA's that are significantly expressed by *R. solani*. We have identified 11 ncRNAs with FPKM values ranging from 100 to 2,000 and 5 ncRNAs with FPKM values between 10 and 80. However the functional characterization has not been conducted for these lncRNAs, their prediction using the raw data might trigger the interest of the scientific community to utilize the available data to identify some of the previously unannotated genes or ncRNAs. Some of these ncRNAs have very high expression rate and their prediction based on data generated from different groups itself serves as a validation of their presence in the genome. The role and mechanism of the non-coding RNA were reviewed recently and explained its role in plant immunity and pathogenesis [36]. Extending the studies on the function and mechanism of these lncRNA will helps to understand more mechanism underlying the pathogenesis and will create a novel methods for its control.

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6. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

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