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Functional Predictions of Long Noncoding RNAs by MiRNAs in *Populus Euphratica* Oliv.

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Abstract. Long noncoding RNAs (lncRNAs) are refer as noncoding transcript RNAs and longer than 200 nucleotides (nt) in length but shorter than 10,000 nt. LncRNAs are involved in a variety of biological processes, such as development, stress response. However, the functions of lncRNAs in plants are largely unclear. MicroRNAs (miRNAs) can regulate gene expression at transcriptional or post-transcriptional level in eukaryotes by sequence complementation. At present, the research on noncoding RNAs targeted by miRNAs is seldom explored in plants. *Populus euphratica* Oliv. (*P. euphratica*) is one of ideal model plants for studying development and stress resistance. To uncover the potential functions of lncRNAs in *P. euphratica*, the tender leaves of *P. euphratica* were analyzed using sequencing, and the data including lncRNAs and miRNAs were obtained, followed by bioinformatics methods to predict the potential binding sites of 258 mature miRNAs at 4460 lncRNAs in *P. euphratica*. It was found that 159 lncRNAs can act as the targets of 33 miRNAs. The corrections of miRNAs-mRNAs-lncRNAs were constructed and the functions of lncRNAs were predicted according to the roles of targets (mRNAs) of corresponding miRNAs. Our results indicated that lncRNAs of *P. euphratica* can regulate development, metabolism, and stress response and regulation process by decoying corresponding miRNAs.

Introduction

Long noncoding RNAs (lncRNAs) are long transcripts of more than 200 nucleotide (nt) but less than 10,000 nt that lack a coding sequence (CDS) [1, 2, 3]. LncRNAs can regulate gene expression at transcriptional and post-transcriptional levels and involving in the regulation of reproductive process, organ morphogenesis and stimulus response *et al* [4-8]. At present, the studies about lncRNAs major focus on animal, lesser on plant.

The poplar species *Populus euphratica* Oliv. (*P. euphratica*) grows almost exclusively in the desert, is the only tree species in the Taklimakan Desert (China) [9]. *P. euphratica* has a high tolerance for salinity, drought, cold, and wind, meanwhile also exhibits a typical heterophylly [9, 10]. All above characters of *P. euphratica* make it one of ideal model plants for study development and stress resistance. The types and functions of lncRNAs in *P. euphratica* are unknown still. In this study, to explore the functions of lncRNAs acting as miRNA targets or decoys, the noncoding RNA (ncRNA) sequencing with *P. euphratica* leaves were performed at first, then, a genome-scale analysis among miRNAs and lncRNAs was performed. The functions of lncRNAs were predicted and annotated according to the ceRNA hypothesis at last.

Materials and Methods

Plant Materials and NcRNA Sequencing

The tender leaves, including linear, lanceolate, oval and broad-oval leaves, were collected from *P. euphratica* in Alar, Xinjiang province, China. These samples were disposed immediately according to the methods of Zhao and Qin [10]. Total RNAs were extracted using the mirVana miRNA Isolation Kit (Ambion) following the manufacturer′s protocol. RNA integrity was evaluated using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The samples with RNA Integrity Number (RIN) ≥ 7 were subjected to the subsequent analysis. Strand-Specific RNA libraries were constructed using TruSeq Stranded Total RNA LT - (with Ribo-Zero Plant) according to the manufacturer’s instructions. Then these libraries were sequenced on the Illumina sequencing platform (HiSeqTM 2500 or other platform) and 150 bp/125bp paired-end reads were generated. Small RNA libraries and Sequencing were following the procedure described by Zhao and Qin [10].

The transcripts with splicing of each sample were combined and screened as lncRNAs with Cuffmerge, and then compared to the *P. euphratica* genome (ftp://ftp. ncbi.nlm.nih.gov/genomes/all/GCF_000495115.1_PopEup_1.0/) with Cuffcompare Software. For miRNAs, clean reads were screened the length of 21–22 nt as miRNAs, and located to refer sequence with bowtie. Combined with miREvo Softwareand mirdeep2 Software [11], the functions of novel miRNAs were analyzed. All sequencing programs were performed by OE Biotech (China, Shanghai).

Predictions of MiRNA Targets and the Corrections between LncRNAs and MRNAs

The miRNA targets of lncRNAs or mRNAs were predicted using miRanda Software, the lncRNAs or mRNAs were selected when the value of score ≥ 140. Then the corrections between lncRNAs and mRNAs were constructed basing on their interaction with miRNAs.

Functional Predictions and Annotation of LncRNAs

The sequences of all of the listed mRNAs connected with lncRNAs by miRNAs were compared to the transcriptome of Arabidopsis thaliana (GCF_ 000001735.3 _TAIR10_ rna.fna.gz, https://www.ncbi.nlm.nih.gov/genome/?term=rabidopsis+thaliana), then the homologous genes of these mRNAs in Arabidopsis thaliana were obtained, at last the gene symbols of these homologous genes were submitted to GO analysis. The overrepresented GO terms in the “biological process” category was obtained using Fisher’s exact test and the Bonferroni multiples test (P-value < 0.05) [11].

Results

The Expression of LncRNAs and MiRNAs in *P. euphratica*

The tender samples of linear, lanceolate, oval and broad-oval leaves were analyzed using a sequencing technique at germination stage. It was found that 4460 lncRNAs (including 442 novel lncRNAs and 4018 annotated lncRNAs) and 258 miRNAs (including 91 novel miRNAs and 167 annotated miRNAs) were expressed in *P. euphratica* (Fig. 1).

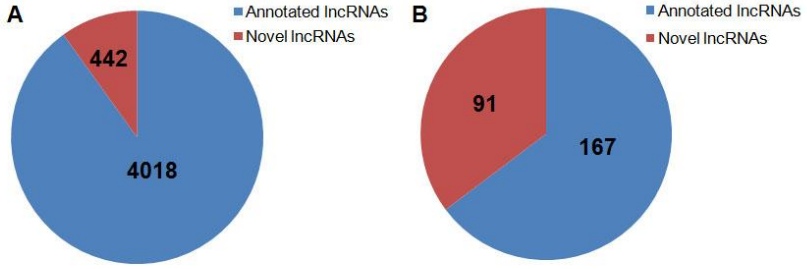

Fig. 1 The number of lncRNAs (A) and miRNAs (B) which expressed in *P. euphratica*.

The Corrections between MiRNAs and LncRNAs in *P. euphratica*

All of 4460 lncRNAs and 258 miRNAs of *P. euphratica* were analyzed with miRanda Software. It was found that 159 lncRNAs can act as the targets of 33 miRNAs. To more easily describe the corrections between miRNAs and lncRNAs, these 33 miRNAs were named with symbol numbers (SNs) (Table 1).

Table 1. The symbol numbers of miRNAs in this paper

miRNAs	SN	miRNAs	SN	miRNAs	SN
NW_011499846.1_1010	1	ptc-miR156a	12	ptc-miR396f	23
NW_011499846.1_779	2	ptc-miR156g	13	ptc-miR398a	24
NW_011499850.1_2817	3	ptc-miR160e-3p	14	ptc-miR403c-5p	25
NW_011499851.1_3625	4	ptc-miR160e-5p	15	ptc-miR408-3p	26
NW_011499858.1_5634	5	ptc-miR162a	16	ptc-miR473a-3p	27
NW_011499986.1_17983	6	ptc-miR169n-3p	17	ptc-miR477a-3p	28
NW_011500067.1_22368	7	ptc-miR3627b	18	ptc-miR477a-5p	29
NW_011500122.1_25017	8	ptc-miR394a-5p	19	ptc-miR6424	30
NW_011500297.1_28413	9	ptc-miR395a	20	ptc-miR6427-3p	31
NW_011500297.1_28413*	10	ptc-miR395b	21	ptc-miR6427-5p	32
NW_011500300.1_28478	11	ptc-miR396c	22	ptc-miR6462c-5p	33

The corrections between lncRNAs and miRNAs were displayed in Table 2. It was showed that some lncRNAs can only act as targets of one miRNA, such as TCONS_36417, it is only the target of NW_011500297.1_28413, but some lncRNAs were targets of multiple miRNAs, such as XR_843263.1, it is the targets of 18 miRNAs (Table 2).

The Functions of LncRNAs Predicted by MiRNAs

According to the GO, the targets (mRNAs) of one miRNA involved in dozens to hundreds of biologic processes, it is difficult to determine what the major function of a miRNA is. So that, for a miRNA, the ratio of target counts which involved in a biologic process with the counts of all Arabidopsis thaliana genes which involved in this biologic process was obtained, then the top 5 biologic processes were used to character this miRNA. The top GO terms of these 33 miRNAs involved in add up to 89; these 89-biologic processes can be categorized as development, metabolism, stress response and regulation. The types of these 33 miRNAs involved in were displayed in Fig. 2. Additionally, the top 1 biologic process of miRNAs which involved in was list in Table 3.

Combined Table 2 with Fig. 2, it was showed that 127 lncRNAs participate in jointly the biologic process regulation of *P. euphratica* by decoying miRNA 6, 8,

Table 2. The corrections between lncRNAs and miRNAs

lncRNA	miR	lncRNAs	miR	lncRNA	miR	lncRNA	miR	lncRNA	miR	lncRNA	miR
TCONS_36417	9	TCONS_36043	2,20	TCONS_13682	9,15,21	TCONS_04524	6,9,11-13	TCONS_13683	2,4,9,15,21,24	TCONS_05067	4,9-13,17,33
TCONS_38473	13	TCONS_37246	1,9	TCONS_19115	7,12,26	TCONS_05248	4,7,13,20,33	TCONS_48508	4,9,20,21,31,32	TCONS_15764	2,12,13,17,18,20,22,23,27
TCONS_52792	26	TCONS_41310	4,19	TCONS_20392	9,12,24	TCONS_14004	9,13,15,26	TCONS_50801	2,14,19,21,25,31	TCONS_17878	5,10-13,17,20,28,33
TCONS_71621	13	TCONS_52797	7,9,16	TCONS_37144	17,22,28	TCONS_15766	10,14,17,23	TCONS_66459	9,12,13,31,33	TCONS_19119	4,7,9-13,20,24,26,28,32,33
XR_839143.1	31	TCONS_55145	9,20	TCONS_50009	5,7,31	TCONS_28715	6,9,10,11,17	XR_838919.1	5,9,10,14,15	TCONS_22457	2,6,9,11,14,20,22,24,27,29
XR_839182.1	11	TCONS_55151	9,29	TCONS_63334	9,26,30	TCONS_31028	5,9,11,24,31	XR_838948.1	4,5,9,10,12,13,29	TCONS_66043	2,9,10,16,20,21,26,30,33
XR_839515.1	17	TCONS_56312	28	TCONS_64440	9,11,29	TCONS_52953	11,13,21,30,31	XR_838977.1	2,10,17,26,31,33	TCONS_69356	2,4,5,11,17,19,26,31
XR_840568.1	9	TCONS_59459	9	TCONS_70591	5,9,21	TCONS_61722	3,11,14,18,21	XR_839038.1	9,10,16,17,19,22,25	TCONS_69358	2,4,5,11,17,19,26,31
XR_840706.1	9	TCONS_69002	17,33	XR_838920.1	5,9,14,15,	TCONS_62629	2,9,10,25,30	XR_839681.1	12,13,19,20,29,31	TCONS_69472	1,9,10,12,24,25,31,33
XR_840946.1	25	TCONS_69982	28	XR_839316.1	1,17,31	XR_838841.1	11,20,31,32	XR_839697.1	2,9,10,19,20,22,23	XR_839669.1	3,10,11,14,15,17,24,27,28
XR_840947.1	25	XR_838878.1	2,5,6	XR_839470.1	10,20,31	XR_839441.1	9,10,16,29	XR_839997.1	2,10,12,28,32,33	XR_839864.1	1,2,5,9,14,17,19,20,21,25,28,33
XR_841203.1	11	XR_838945.1	12,21	XR_840290.1	20,27,28	XR_840494.1	2,4,8,9,11,28	XR_840123.1	1,7,8,10,15,17,21,29	XR_839868.1	1,2,4,8-10,12,13,17,20,23,26-28,31,32
XR_841212.1	11	XR_839090.1	1,18	XR_840598.1	8-10	XR_840845.1	4,9,12,22,23	XR_840367.1	11,12,13,15,20,27,31	XR_840010.1	9,11,14,17,18,21,31,32
XR_841230.1	4	XR_839858.1	9,22	XR_840662.1	1,25,31	XR_840943.1	1,5,7,21-23	XR_840592.1	9,11,14,19,28,29,31	XR_842022.1	1,10-14,16,17,22,32
XR_841288.1	17	XR_839880.1	9,10	XR_841316.1	11,22,31	XR_841301.1	9,26-28	XR_840659.1	9,19,31-33	XR_842144.1	2,9-11,16,17,20,22,26,28,29
XR_841304.1	17	XR_840560.1	20,31	XR_841096.1	12,13,22	XR_845396.1	5,9,10,17	XR_840952.1	4,5,6,9,11,13,28	XR_842262.1	11-13,17,18,24,27,33
XR_841650.1	9	XR_845498.1	10,14	XR_842452.1	3,9,11	XR_841520.1	1,4,5,11,13	XR_841522.1	2,9,11,22,23,32	XR_842577.1	1,5,9,11-14,27
XR_842363.1	22	XR_842170.1	8-10	XR_842547.1	9,20,32	XR_842748.1	5,9,23,29,33	XR_842118.1	12,17,20,24,33	XR_843262.1	2,4,6,9,11-13,15,17,21,22,25-28,33
XR_842650.1	2	XR_841107.1	10,14	XR_842615.1	9,10,14	XR_842974.1	12,13,19,21	XR_842556.1	5,9,10,12,13,16,21,26	XR_843263.1	2,4,6,9,11-13,15,17,21,22,25-28,31,33
XR_842653.1	21	XR_841147.1	5,11	XR_843201.1	13,16,17	XR_843408.1	9,10,17,22	XR_842659.1	9,13,14,20-22	XR_843267.1	2,4,6,9,11-13,17,21,22,25,26,28,33
XR_842709.1	8	XR_841204.1	9,24	XR_844951.1	9,22,32	XR_844334.1	12,13,16,29	XR_843136.1	2,9,17,20,21,27,32	XR_843929.1	2,4,9-13,21,29
XR_842721.1	1	XR_841299.1	7,21	XR_844953.1	4,30,31	XR_844684.1	9,10,11,28	XR_843592.1	14,17,20,21,32,33	XR_845057.1	2,4,5,9,11-14,16,17,19,21,24,27,33
XR_842753.1	20	XR_841839.1	11,20	XR_845174.1	8,10,27	XR_845125.1	11,20,22,33	XR_843968.1	2,9-11,21	XR_845110.1	9,11-13,16,17,19-21,24,29,31
XR_843156.1	31,	XR_842919.1	12,27	XR_845298.1	21,22,28	XR_845133.1	11,20,22,33	XR_844047.1	4,10-12,19,31	XR_845111.1	9,11-13,16,17,1-21,29,31
XR_844426.1	14	XR_843576.1	2,9,26	XR_845356.1	9,11,16	XR_845232.1	4,6,9,13,17	XR_844531.1	8,11,21,22,28	XR_845221.1	5,10,12,13,18,20
XR_845145.1	5	XR_843606.1	8,10,	XR_841312.1	13,19,24	XR_845338.1	9,15,19,31	XR_844658.1	8-10,22,29	XR_845292.1	4,9-11,14,17,25,33
XR_845339.1	23	XR_843736.1	9,24	XR_840986.1	12,13,17						

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7,

19, 22, 26, 28, 29 and 33; 109 lncRNAs can decoy miRNA 1, 3, 7, 10, 12, 15, 16, 25, 27, 28, 30 and 32, and participate in jointly the stress response of *P. euphratica*; 151 lncRNAs can decoy miRNA 1, 3-10, 12-17, 19-24, 26, 27, 29 and 31-33 respectively, and play a role in *P. euphratica* metabolism together.

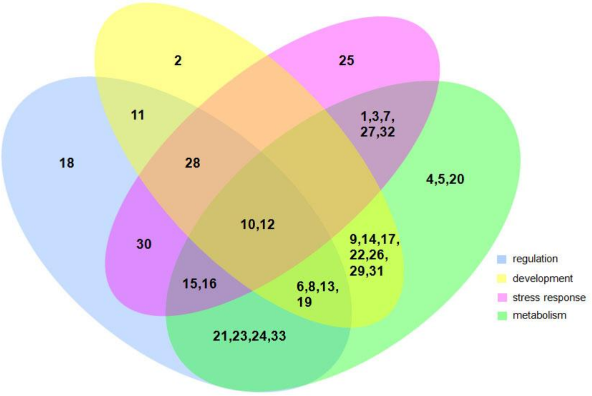

Fig. 2. The types of 33 miRNAs involved in biologic processes of *P. euphratica*. The different colors show the different types of biological processes. The symbol of a miRNA in a color showed the biologic process which participating in only belongs to this type. If a miRNA in the overlap areas of multiple colors, it is indicated that this miRNA can participate in multiple biological processes simultaneously.

Table 3. The top 1 biologic process of miRNAs which involved in

miR	**GO accession**	**Ratio**	***P* value**	**miR**	**GO accession**	**Ratio**	***P* value**	**miR**	**GO accession**	**Ratio**	***P* value**
1	0009809	0.10	0.0373	12	0060429	0.28	0.0465	23	0006066	0.10	0.0382
2	0030048	0.32	0.0031	13	0048532	0.17	0.0057	24	0051093	0.10	0.0030
3	0019438	0.01	0.0002	14	0060429	0.28	0.0193	25	1901615	0.05	0.0278
4	0098660	0.08	0.0008	15	0046274	0.24	0.0142	26	0048440	0.11	0.0419
5	0006816	0.21	0.0090	16	0010105	0.25	0.0325	27	0009808	0.12	0.0061
6	0006928	0.15	0.0182	17	0070588	0.32	0.0089	28	0044242	0.13	0.0118
7	0006955	0.03	0.0220	18	0009890	0.04	0.0092	29	0009913	0.33	0.0171
8	0009790	0.03	0.0067	19	0009658	0.11	0.0032	30	0010646	0.05	0.0076
9	0060429	0.44	0.0042	20	0009687	0.27	0.0212	31	0048646	0.09	0.0037
10	0030048	0.32	0.0147	21	0070588	0.32	0.0040	32	0071395	0.10	0.0243
11	0030048	0.37	0.0006	22	0030048	0.32	0.0043	33	0034220	0.08	0.0010

Note: Ratio means the ratio of target counts which involved in a biologic process with the counts of all Arabidopsis thaliana genes which involved in this biologic process. *P* value means the *P* value of corresponding GO term.

Discussion

With their importance in regulating gene expression, lncRNAs have garnered significant attention in life science field [3]. Although increasing lncRNAs have been predicted and identified in plants [3], the amounts and functions of lncRNAs in *P. euphratica* are unknown until today. In this study, 4460 lncRNAs of *P. euphratica* were obtained by sequencing technique, and then the functions of 159 lncRNAs were predicted by ceRNA hypothesis.

According to the GO enrichment with the targets of miRNAs and the major lncRNAs involved in metabolism (Fig. 2), it was showed that metabolism not only provided materials for life, but also was a basis of many biologic processes. For example, Kalve *et al*. found that the cytoplasmic growth is mainly based on macromolecular synthesis and therefore consumes amount of energy, so that metabolism can affect cell growth [12]. *P. euphratica* has typical heterophyllly [9, 10], 140 lncRNAs involved in development process, this result indicated that these lncRNAs maybe play a key role in the *P. euphratica* leaf development. 109 lncRNAs involved in stress response, it was indicated that these lncRNAs involved in regulation resistance of salt, temperature and drought stresses, enable *P. euphratica* to live in the desert [10]. 127 lncRNAs involved in regulation process, illustrating that lncRNAs can also affect the expression of numerous genes by decoying limited miRNAs.

Conclusions

In this study, 4460 lncRNAs of *P. euphratica* were obtained by sequencing technique, and then the functions of 159 lncRNAs were predicted by ceRNA hypothesis. It be suggested that 151 lncRNAs could regulate metabolic process by decoying 27 miRNAs respectively; 149 lncRNAs could regulate development by decoying 16 miRNAs respectively; 127 lncRNAs were involved in regulation process by decoying 16 miRNAs respectively; 109 lncRNAs were involved in stress response by decoying 12 miRNAs respectively. All above results indicated that lncRNAs of *P. euphratica* play vital roles in metabolism, leaf development, stress response *et al*., and enable *P. euphratica* to live in desert.

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