Computational analysis of miRNA-target community network reveals cross talk among different metabolisms

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To date, only a few conserved miRNAs have been predicted in hexaploid (AABBDD) bread wheat and till now community behavior among miRNA is still in dark. Analysis of publically available 1287279 ESTs from NCBI resulted 262 putative pre-miRNAs and 39 novel mature miRNAs. A total 22,468 targets were identified on 21 chromosomes. MiRNA target community was identified for genomes with different levels of cross talks. Gene ontology of these community targets suggests their differential involvement in different metabolisms along with common and stringent involvement in nitrogen metabolism.

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1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/nucest.

2. Introduction

MicroRNAs (miRNAs) play important roles in plant growth regulations, development and adaptation to abiotic stresses [1–3]. It is an important class of small RNAs, a non-protein coding segment of genome, originates from fold back precursors and functions as negative regulators of gene expression in plant [1,4,5]. A large number of miRNAs have been discovered and functionally identified in plant kingdom. Currently, 8496 mature miRNAs have been identified from Viridiplantae and deposited in a publicly available database (miRBase, Release 21, June 2014) [6]. In agriculturally important monocot species, miRNAs have been reported in large number for fully sequenced genome but fewer in partially or incomplete sequenced genomes. Though, wheat (Triticum aestivum) is one of the most extensively cultivated crop among monocots but only 115 mature miRNAs have been reported [7]. Whereas in other monocots viz. Oryza sativa, Zea mays, Sorghum bicolor and Hordeum vulgare, 713, 321, 241 and 71 mature miRNAs have been reported respectively. The regulatory role of many of these miRNAs under drought, salinity and low temperature stress has been demonstrated [8–10]. Moreover, the homeostasis of the nutrients such as sulfur, copper and phosphate, which are critical for growth and development, has been found to be regulated by miRNAs [11–13]. A number of putative miRNAs in wheat have been identified [14–17] yet the association of miRNA with different metabolic processes is still in dark. Therefore, identification of novel miRNAs as well as their association with metabolic pathways in wheat may facilitate its improvement and production.

The most challenging problem in understanding plant miRNAs is the identification of novel miRNAs. EST based computational analysis is a powerful technique for identifying conserved miRNAs, especially for plants with partially or un-sequenced genomes [18]. Thus in the present study, EST based computational technique was employed in search of potential miRNA in wheat. Available miRNAs in miRBase and downloaded wheat EST dataset were used for detection of potential miRNAs. Altogether 39 novel miRNAs were detected and 22,468 target genes were identified in wheat. Further, the mechanism of miRNA-target crosstalk was deciphered by employing a meta-analysis for
chromosomal mapping, gene ontology, and miRNA-target community network. It was observed that most of these miRNAs in wheat were primarily involved in regulating nitrogen metabolism that needs to be further validated.

3. Materials and methods

3.1. MiRNA reference set

For prediction of potential miRNAs, previously known miRNAs (8496) of whole Viridiplantae were obtained from miRNA Registry database (Release 21, June 2014). To avoid the overlap of miRNAs, the redundant miRNA sequences were removed using an in-house Perl script and the remaining sequences were used as a reference set.

3.2. Software used

Initially, software BLAST-2.2.14 was used from NCBI GenBank. CAP3 was used for assembly in the form of contigs and singlets. Triplet SVM classifier was used to predict potential miRNA precursor [19]. These precursor sequences were used for BLASTx analysis for removing the protein-coding sequences and retained only non-protein encoding sequences. RNAfold was used online to analyze secondary structure of RNAs. BLASTn from NCBI (http://www.ncbi.nlm.nih.gov) was used to
analyze the potential targets of miRNAs. psRNATarget web server (http://plantgrn.noble.org/psRNATarget/) was used for identification of the potential targets.

3.3. Prediction of miRNAs

The overall workflow followed in the present study is represented as schematic diagram (Fig. 1). Firstly, 1,287,279 ESTs of *T. aestivum* from dbEST (http://www.ncbi.nlm.nih.gov/nucest/) and 8496 known mature miRNAs of Viridiplantae along with their precursor sequences from mirBase (http://www.mirbase.org/cgi-bin/browse.pl) were downloaded. All, the downloaded EST sequences were trimmed using EST trimmer. The trimmed EST sequences were then assembled using CAP3 resulting in 1196 hairpin precursors. These precursors having similarity matches with the previously reported miRNAs in wheat and therefore these are manually curated and 39 novel miRNAs were obtained (Supplementary le 1).

3.4. Prediction of secondary structure

Potentially stable secondary structures of the precursors were generated by RNAfold algorithm. The parameters used for the secondary structure prediction using RNAfold were: minimum free energy and partition function; avoid isolated base pairs; dangling energy on both sides of the helix in any case; RNA parameters; rescale energy parameters at a given temperature 37 °C; interactive RNA secondary structure plots with reliability annotation; RNA parameters; ligation energy region of 50 nucleotides were selected using an in-house Perl script. As per the standard rule, a precursor must form a hairpin structure finally obtained.

3.5. Prediction of mRNA targets of miRNAs

The draft genome sequences of *T. aestivum* for all 21 (3 × 7) chromosomes were downloaded from URGi database (http://wheat-urgi.versailles.inra.fr/Seq-Repository). Chromosomal sequences were used for miRNA targets search using psRNATarget web server (http://plantgrn.noble.org/psRNATarget/) [23]. Parameters used were maximum expectation = 3.0, length for complementarity scoring (hpsize) = 20 bp, number of top target genes for each small RNA = 200, target accessibility-allowed maximum energy to unpair the target site (UPE) = 25, flanking length around target site for target accessibility analysis = 17 bp in upstream/13 bp in downstream and range of central mismatch leading to translational inhibition = 9–11 ntd.

3.6. Gene ontology analysis

To better understand the function of *T. aestivum* miRNAs, AgriGO tool (http://bioinfo.cau.edu.cn/agriGO/) [24] was employed to investigate the predicted target genes. For enrichment analysis, a hypergeometric distribution based statistical test (level of significance at 0.05%) was used to reject the chances of randomness in association to target genes with their corresponding ontology term. To moderate the false positives in multiple hypothesis testing procedure Benjamini and Hochberg false discovery rate (FDR) correction [25] was applied.

4. Results

4.1. Potential miRNAs in *T. aestivum*

Overall the current study is given in Fig. 1. Here, total 1,287,279 wheat ESTs were downloaded and pre-processed for poly-A/T tail removals and then assembled into 36,140 contigs and 414,041 singlets. These assembled sequences were used for homolog search against 3999 unique, Viridiplantae miRNAs (out of total 8496 downloaded miRNAs). 13,815 candidate precursors with the flanking region of 50 nucleotides were selected using an in-house Perl script. As per the standard rule, a precursor must form a hairpin structure subsequently all precursors were processed with Triplet-SVM classifier. Filtered precursors were subjected to BLASTx and Rfam search [20] and non-protein coding precursors were finally obtained.

5. Result and discussion

5.1. Chromosomal distribution of targets

In the current study using a bioinformatics approach, 39 novel miRNAs were identified from the EST libraries of *T. aestivum* and their genome wide (using draft genome sequence) target was searched using psRNATarget. None of the predicted miRNAs showed identity against 3999 unique, Viridiplantae miRNAs (out of total 8496 downloaded miRNAs). 13,815 candidate precursors with the flanking region of 50 nucleotides were selected using an in-house Perl script. As per the standard rule, a precursor must form a hairpin structure subsequently all precursors were processed with Triplet-SVM classifier. Filtered precursors were subjected to BLASTx and Rfam search [20] and non-protein coding precursors were finally obtained.

| Chr  | Target counts |
|------|---------------|
| Chr1  | 2783          |
| Chr2  | 3112          |
| Chr3  | 5947          |
| Chr4  | 2740          |
| Chr5  | 2808          |
| Chr6  | 3437          |
| Chr7  | 4753          |

Table 2: Chromosomal distribution of the miRNA targets in on chromosomes of *Triticum aestivum* (AABBDD).
5.2. MiRNA target community

Gene Regulatory Network (GRN) in the present study explores the dimension of miRNA and their target multiplicity and cooperative in the sense of their complex genome organization and duplication event. The miRNA target gene identified for three genomes was used for the network generation using Cytoscape 3.0.2 [28]. Here we have used the UPE energy information in between the miRNA and their targets for network generation. There were three different networks formed as depicted in Fig. 3. The GRN represented in blue color is from A genome for 39 miRNAs regulating 3203 mRNA targets (Fig. 3a). Moreover, GRN represented in red color is from B genome that contributes 5207 mRNA targets, captured by 39 miRNAs (Fig. 3b), whereas from D genome, the largest number i.e., 14,058 of miRNA targets was identified for 39 miRNAs (Fig. 3c). This network graph of miRNA and their targets thus explore the actual contribution from all three genomes (AABBDD).

Fig. 3. MiRNA target community within three genomes (AABBDD) of wheat revealing the within and in between crosstalks.

Fig. 4. Genome wise enriched biological activities in Triticum aestivum (AABBDD) on the basis of SEA analysis. Only top five GO terms has been plotted here.
5.3. *T. aestivum* miRNA targets and their functions

With the ongoing efforts in the field of wheat genomics it is logical to identify the contribution of three genomes governing polyploidy in wheat. Separately, genome contribution analysis for miRNA targeted shown that three genomes (AA), (BB) and (DD) contribute in different ratios. In result we got a total of 22,486 targets of 39 miRNAs in wheat genome. For the identification of biological process separately governed by three genomes (AABBDD), gene ontology was performed using agriGO tool with the parameter of p value of <0.05 (Supplementary file 3). Due to the huge number of targets we performed a comparative analysis of gene ontology, where only top five activities were shown in Fig. 4 and Supplementary file 1. A genome suggests their involvement in different metabolic and cellular processes along with nitrogen metabolic process. Whereas in case of B genome miRNA targets we found that membrane bound organelle and primary metabolic process, along with nitrogen metabolic process was found to be enriched. However with the case of D genome similar type of activities were observed along with highly enriched process related to chromatin remodeling complex.

6. Conclusion

However miRNA-related research is one of the most common re-search topics in biological areas but miRNA research on wheat is still very far from the other plant species. Till now, only 115 known mature miRNAs have been deposited in miRBASE for *T. aestivum*. In the present study, we have tried to identify potential miRNAs in this highly economical plant species and we are able to identify 39 potential miRNAs along with 22,468 potential target genes in three genomes (AABBDD). Our analysis therefore added novel as well as potential miRNAs into the existed database. These miRNA were shown to regulate their targets with different ratios in all three genomes (AABBDD). Further they have found to exist as a “miRNA community” with different levels of crosstalks. Interestingly we found “nitrogen metabolism” as a common activity within miRNA community of three genomes suggesting their strong as well as common regulation by them.

Competing interests

The authors have declared that no competing interests exist.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.gdata.2015.04.028.