Identification of new stress-induced microRNA and their targets in wheat using computational approach

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Keywords: miRNA, computational prediction, wheat, target prediction, EST

Abbreviations: GSS, genomic survey sequences; EST, expressed sequenced tag; MFEI, minimal folding free energy index; DCL1, Dicer-like 1 enzyme; RISC, RNA-induced silencing complex; GSSs, genome survey sequences; HTGSs, high-throughput genomics sequences; NRs, non-redundant nucleotides

MicroRNAs (miRNAs) are a class of short endogenous non-coding small RNA molecules of about 18–22 nucleotides in length. Their main function is to downregulate gene expression in different manners like translational repression, mRNA cleavage and epigenetic modification. Computational predictions have raised the number of miRNAs in wheat significantly using an EST based approach. Hence, a combinatorial approach which is amalgamation of bioinformatics software and Perl script was used to identify new miRNA to add to the growing database of wheat miRNA. Identification of miRNAs was initiated by mining the EST (Expressed Sequence Tags) database available at National Center for Biotechnology Information. In this investigation, 4677 mature microRNA sequences belonging to 50 miRNA families from different plant species were used to predict miRNA in wheat. A total of five new abiostress-responsive miRNAs were predicted and named Ta-miR5653, Ta-miR855, Ta-miR819k, Ta-miR3708 and Ta-miR5156. In addition, four previously identified miRNA i.e., Ta-miR1122, miR1117, Ta-miR1134 and Ta-miR1133 were predicted in newly identified EST sequence and 14 potential target genes were subsequently predicted, most of which seems to encode ubiquitin carrier protein, serine/threonine protein kinase, 40S ribosomal protein, F-box/kelch-repeat protein, BTB/POZ domain-containing protein, transcription factors which are involved in growth, development, metabolism and stress response. Our result has increased the number of miRNAs in wheat, which should be useful for further investigation into the biological functions and evolution of miRNAs in wheat and other plant species.

Introduction

Wheat (Triticum aestivum L., AABBDD, 2n = 42) is one of the most extensively grown crops throughout the world, providing protein content, as well as basic caloric value.1 Until recently, wheat was the last major crop for which no genome sequencing effort was underway. However, recent technological advances such as new-generation sequencing platforms now offer large scale programs that can deliver needed genomic resources for wheat. The International Wheat Genome Sequencing Consortium’s (IWGSC) project studies are already revealing valuable information about wheat genome structure.2,3

MicroRNAs (miRNAs) are a class of endogenous, small, non-coding, single-stranded RNAs that act as post-transcriptional regulators in eukaryotes.4 It has been estimated that miRNAs account for ~1% of predicted genes in higher eukaryotic genomes and up to 10–30% of genes may be regulated by miRNAs.5 miRNAs regulate expression of functional genes involved in plant development and other physiological processes.6 The maturation of miRNAs in plants involves several steps requiring key enzymes such as Dicer-like 1 enzyme (DCL1) and HASTY.8-10 Mature miRNAs are incorporated into RNA-induced silencing complex (RISC) which is induced by miRNAs to target mRNAs causing the cleavage or repression of target genes.11,12

Plant miRNAs negatively regulate the corresponding transcripts levels of their target genes and play important roles in plant growth including leaf morphology and polarity, organ development, cell differentiation and proliferation, cell death, signal transduction stress response, lateral root formation, hormone signaling, transition from juvenile to adult vegetative phase,
Identification of miRNAs using EST. Plants are exposed to a wide array of environmental stresses leading to various functional and structural changes to cope with these stresses. Molecular characterization of transcriptional and biochemical alterations are crucial to dissect the underlying regulatory mechanism of these abiotic stress. In order to identify new miRNAs in wheat we have to rely on wheat EST sequences, since the sequence information of wheat genome sequence is restricted.28 To discover new miRNAs in wheat, we exploited known mature miRNAs already submitted in miRBase database from various plant species including plant species and can be used for identification of conserved miRNAs. GSS and HTGS of GeneBank represent only short stretches of genomic sequence but can still provide a broader sampling of unfinished genomes. The NR database contains finished genomic sequences and cDNAs. Previously Zhang et al.25 identified conserved miRNAs in plants using ESTs alone.

Large number of miRNA has been identified in many model crops but only few miRNA are reported in wheat till date which is very less compared with the other plant miRNAs. Steady significant increase in the wheat EST sequences in the database motivated us to predict additional miRNA in wheat. Computational approaches have been developed to identify miRNAs in wheat and their targets in publically available ESTs.26 Evidence suggesting that miRNAs play a role in plant stress responses arises from the discovery that miR398 targets genes with known roles in stress tolerance. In addition, the expression profiles of most miRNAs that are implicated in plant growth and development are significantly changed during stress. These later findings imply that attenuated plant growth and development under stress may be under the control of stress-responsive miRNAs. Here, in this study we examined all miRNAs deposited in the miRNA Registry Database publicly available at www.mirbase.org/ (Release 19, November 2012),27 to search against wheat EST sequences. We used newly identified miRNAs to predict their targets in wheat and found 14 target genes encoding transcription factors, enzymes implicated in metabolic processes and in stress responses. In this study, new miRNAs were mined for the purpose of understanding their roles in regulating growth, development, metabolism and other physiological processes in T. aestivum.
they qualify plant miRNA annotation criteria. The precursor were made as database and as a result we found 10 EST. The pre-
program. The EST extracted from abiotic stress libraries of wheat 
redundant miRNA data set which was made as query for BLAST 
miRNAs were omitted by perl script. As a result we got 4677 non 
wheat miRNA to avoid false-positive result. The other redundant 
was performed on this data set to remove previously reported 
were provided computational evidence that these 5 newly identi-

tions of newly identified wheat miRNAs are similar to their coun-

ters in other plant predicted miRNA. All mature miRNAs 
precursors were found to fold into near hairpin-structures (Fig. 
The statistics and characterized parameters of predicted T. aei-
tivum precursor's sequences such as mean, standard deviation are 

| miRNAs | Sequence | Homologous | Location |
|--------|----------|------------|----------|
| miR565 | GUU GAG UUG AGU UGA GUU | ath-miR565 | 3' |
| miR855 | AAA GCU AAG GAA AAG GAA | ath-miR855 | 5' |
| miR819k | CUC GUA AAA CUG CAA AAA | osa-miR819 | 3' |
| miR3708 | CAC ACA ACA UUU CUC GUU | pab-miR3708 | 3' |
| miR5156 | CUC GUA AAA CUG CAA AAA | osa-miR5156 | 3' |

Table 1. Sequence and location of new miRNAs identified in wheat

EST expression. To emphasize the mechanistic stage and/ or tissue dependent roles of newly identified wheat miRNAs, we examined in silico expression patterns of miRNAs in different tissues using expressed sequence tags (ESTs) from GenBank database related to abiotic stress cDNA libraries expressed in different tissue types and developmental stages of T. aestivum. Newly identified miRNA from T. aestivum were detected in the seedling, sheath, leaf, root tips and root (Table 5). In this study, Ta-miR3708, Ta-miR819k-3p and Ta- miR5156 which were iso-
lated from wheat drought stressed cDNA library, were found to 
be most abundant in leaf tissue, whereas miR3708 was detected in 
seedling. On the contrary, Ta-miR5653 and Ta-miR855 corre-
spond to wheat cold-stressed and salt stressed libraries were found in seedling and sheath tissues. Ta-miR1134 and Ta-miR1117 belonging to drought stressed cDNA library were abundant in leaf tissue. Ta-miR1133 implies to wheat salt-stressed library

Arabidopsis, rice and maize (Fig. 1). Multiple sequence alignment 
was performed on this data set to remove previously reported 
wheat miRNA to avoid false-positive result. The other redundant 
miRNAs were omitted by perl script. As a result we got 4677 non 
redundant miRNA data set which was made as query for BLAST 
program. The EST extracted from abiotic stress libraries of wheat 
were made as database and as a result we found 10 EST. The pre-
dicted EST was against set to various filters to make sure that 
they qualify plant miRNA annotation criteria. The precursor 
sequences were predicted with 250 ntd upstream and 250 down-
stream of the miRNA BLAST hit and used for the hairpin struc-
ture predictions. For ESTs with less than 400 ntd we used the 
entire available sequence as a miRNA precursor sequence. These 
precursor sequences then BLASTXed, to remove the protein cod-
ing sequences and retained precursor sequences underwent hairpin 
structure prediction by Vienna RNA Package. The putative 
miRNA precursor was also BLASTed against RNA database to 
discard other RNAs such as rRNA, tRNA, snRNA and so on. As a result, 5 new miRNAs, (Table 1) were found. Furthermore, we provide computational evidence that these 5 newly identi-

tions of newly identified wheat miRNAs are similar to their coun-

ters in other plant predicted miRNA. All mature miRNAs 
precursors were found to fold into near hairpin-structures (Fig. 
2). The statistics and characterized parameters of predicted T. aei-
tivum precursor's sequences such as mean, standard deviation are 
shown in Table 3. 

Target prediction of newly identified miRNAs functional 
annotation. Previous studied on miRNA target identification 
has shown that most plant miRNAs bind to the protein-coding 
region of their miRNA targets with complementarity and inhibit 

the translation mechanism. sRNA ToolKit was used to predict potential target of newly indentified miRNAs by searching against wheat miRNAs. Wheat miRNAs preferred to target the Ubiquitin carrier protein, serine/threonine protein kinase, transcriptional activator Myb, 40S ribosomal protein, F-box/kelch-repeat protein, BTB/POZ domain-containing protein involved in wheat development (Table 4). We observed that one miRNA family can have more than one targets. In contrast, miR5156 has one target gene. An additional target gene family was found to be 
involved during stress responses which greatly influence the wheat 
production. Identification and validation of miRNA targets is a 
landmark step to unravel the central role of miRNA in regulatory 
network of abiotic stress tolerance. EST based search in various 
databases played a vital role for the discovery of miRNA targets 
in plants based on the homology between miRNA and its target 
sequences. Our prediction of target genes for the 10 miRNAs 
(including new and previously reported) also supported that, there 
could be more than one potential target for each miRNA (Table 
4). In the functional annotation performed under gene ontology 
revealed that each miRNA has specific target gene, for example 
miR855 are transcriptional activator and transporter activity, 
miR5653 and miR819k are involved in ubiquitin protein ligase, 
miR5156 and miR3708 are involved in translation and transcrip-
tion, respectively. The pathway analysis of predicted target genes 
showed that miRNAs5653 was associated with sulfur metabolism 
and signaling pathway, miRNAs855 with transporters, miR819k 
with Chemokine signaling pathway and miR5156 with ribosome 
biogenesis pathway respectively. All the predicted targets share 
high homology with Arabidopsis, Oryza and Zea mays. Most of the 
predicted targets of newly identified miRNA may have potential 
role in plant growth and development.
and found abundantly in root. On the other hand, Ta-miR1122 belonging to cold-stressed and aluminum-stressed library was found in seedling and root tip. These newly detected miRNAs are potentially interesting, but require experimental verification by an independent technique. Using experimental approach to understand expression profile of identified miRNA in wheat will help to unravel a new dimension of regulatory network of miRNAs during abiotic stress.

**Sequence alignment and phylogenetic analysis of the new miRNAs.** Primary and mature plant miRNAs are highly conserved among distantly related plant species.\(^3\) Comparison of the precursor sequences of the predicted miRNAs with other members in the same family showed that most members could be found to have a high degree of sequence similarity with others. The precursor sequence identity between miR819k-3p and miR5156 members was 100%, followed by that between miR3708 and miR5156, was over 46% (Table 6). Least identity was shown between miR855 and miR1122. Based on the pre-miRNA sequence comparisons, the evolutionary relationships of *T. aestivum* miRNAs with other members from the same families were analyzed using Mega 4. Phylogenetic analysis of identified miRNA along with previously identified miRNA in wheat revealed that the miR3708 and miR444a were closely related, miR5156 clustered with miR167 family i.e., 167a, 167b and 167c, while miR855 showing its relatedness with miR156 family with three class; 156a, 156b and 156c. MiR5653 showed evolutionary relatedness with miR156 family i.e., 167a, 176b and 167c, while miR855 showing its relatedness with miR156 family with three class; 156a, 156b and 156c. MiR5653 showed evolutionary relatedness with miR1134 whereas miR819k-3p was related to miR159b, miR319B, miR172 and miR398 (Fig. 3).

**Discussion**

The current literature suggests that plant genes are involved in response to abiotic stresses such as drought and heat which may be regulated at the post-transcriptional level by miRNAs. These plant miRNAs are involved in regulation of numerous cellular events under various stress responses.\(^6\) Computational identification of miRNA form wheat has been done earlier by using express sequence tag.\(^2\) Till now, only 270 known mature miRNA have been reported in wheat (https://pag.confex.com/pag/xxii/webprogram/Paper6355.html). This suggests that miRNA prediction and their validation in wheat requires more concerted efforts. In the present study, using wheat EST database, we have identified 5 new abiotic stress-responsive miRNAs along with their potential target genes (Table 4). These newly identified miRNAs belong to drought, cold and salt specific stress condition which is potentially interesting.

**Abiotic stress in wheat is a major problem limiting wheat production.** In the recent past, several attempts have been made to explore the active role of miRNAs to regulate various developmental stages under different abiotic stress condition. In this study, we found that Ta-miR855 target MYB transcription factor which primarily regulates leaf development and might also be involved in regulating genes of other organ development. This is in agreement with functionality of miR159.\(^3\) Various kinds of proteins such as ubiquitin carrier protein and Serine/threonine protein kinase were predicted to be the target of miR5653. Previous finding suggests that ubiquitin carrier protein play major role in regulating diverse cellular process such as control of cell cycle, activation of various transcription factors, recycling of abnormal proteins and metabolic regulation.\(^4\) Serine/threonine protein kinase has significant roles in controlling different signal transduction pathways leading to plant defense under both, biotic and abiotic stress.\(^4\) Earlier studied have documented that most of the miRNAs largely target transcription factors, metabolic transporters and signal transduction factors.\(^14\) Palatnik et al.\(^19\) and Aukerman et al.\(^38\), has confirmed the role of miRNA targets are involve in organ development, as floral organ identity, leaf morphogenesis, root development, various stress responses in model plant, *Arabidopsis*. Ta-miR5156 was identified to target 40S ribosomal proteins structural constituent of ribosome. Similarly, Ta-miR3708 was known to target F-box proteins which mediates the process of polyubiquitination, transcription elongation, centromere binding and translation repression.\(^4\) In wheat,

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**Table 2.** New wheat miRNA families homologous to known miRNAs from other plant species

| miRNAs   | MFE(ΔG, kcal/mol) | MFEI | LP (nt) | (G+C)% | (A+U)% | A%  | C%  | G%  | U%  | A/U ratio | C/G ratio |
|----------|------------------|------|--------|--------|--------|-----|-----|-----|-----|----------|----------|
| miR5653  | 27.7             | 0.83 | 80     | 40     | 60     | 22.78| 17.72| 22.78| 37.97| 0.59     | 0.77     |
| miR855   | 33.5             | 0.64 | 79     | 65.8   | 34.2   | 21.51| 30.37| 35.44| 13.92| 1.54     | 0.85     |
| miR819k  | 20.1             | 0.65 | 90     | 34.4   | 65.6   | 30   | 14.4 | 20   | 35.5  | 0.84     | 0.72     |
| miR3708  | 20.5             | 0.66 | 100    | 31     | 69     | 37   | 11   | 20   | 32   | 1.15     | 0.55     |
| miR5156  | 20.1             | 0.65 | 90     | 34.4   | 65.6   | 30   | 14.4 | 20   | 35.5  | 0.84     | 0.72     |

LP, length of pre-cursors; MFE, minimal folding free energy; MFEI, minimal folding free energy indexes.

**Table 3.** Statistics and characterized parameters of predicted *T. aestivum* precursors

| Parameters         | Mean | Standard deviation | Minimal | Maximal |
|--------------------|------|--------------------|---------|---------|
| MFE(ΔG, kcal/mol)  | 24.38| 6.04               | 20.1    | 33.5    |
| MFE                | 0.69 | 0.08               | 0.65    | 0.83    |
| Precursor Length(nt)| 87.8 | 8.61              | 79      | 100     |
| (G+C)%            | 41.12| 14.17             | 31      | 65.8    |
| (A+U)%            | 58.88| 14.17             | 34.2    | 69      |
| A%                | 28.25| 6.29              | 21.51   | 37      |
| C%                | 17.57| 7.54              | 11      | 30.37   |
| G%                | 23.64| 6.70              | 20      | 35.44   |
| U%                | 30.98| 9.77              | 13.92   | 37.97   |
| A/U ratio        | 0.99 | 0.37             | 0.59    | 1.15    |
| C/G ratio        | 0.72 | 0.11             | 0.55    | 85      |
Figure 2. The predicted secondary step-loop structures of newly identified wheat miRNAs. (A) miR855 (B) MiR819k (C) miR3708 (D) miR5156 (E) miR5653.
Materials and Methods

Sequences and software. To search potential new miRNA in *T. aestivum* the sequences of previously known mature miRNA sequences from *Arabidopsis*, *Brassica*, *Hordeum*, *Populus*, *Glycine*, *Saccharum*, *Sorghum*, *Vitis*, *Solanum*, *Oryza*, *Triticum* and remaining from other plant species, were downloaded from the miRNA registry database (www.mirbase.org/; Release 19: November 2012). This data set contains contained, 6220 mature miRNA sequences from 43 plants belonging to 50 miRNA families. The data set was screened with the help of in house perl script (www.perl.org) and the redundant miRNA sequences were removed. We retrieved 4677 non-redundant miRNA sequences to be used as reference set. Wheat ESTs from abiotic stress-treated cDNA libraries were obtained from GenBank nucleotide database available at NCBI. This sequence information contained 3, 74, 608 ESTs (Till November 2012). Blast -2.2.25 was downloaded from NCBI and set up locally.

Prediction of *T. aestivum* miRNAs. The sequences of previously known plant miRNAs were used as query sequences for

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Table 4. Major potential target genes for newly identified miRNAs in wheat

| miRNA family | Targeted proteins | Targeted genes | Function annotation | KEGG pathway |
|--------------|------------------|----------------|---------------------|--------------|
| miR5653      | Wheat ubiquitin carrier protein | Ta.56283 | nucleotide binding, ubiquitin-protein ligase activity, ATP binding, ligase activity, acid-amino acid ligase activity | Sulfur relay system |
|              | serine/threonine protein kinase (PK4) | Ta.13351 | protein kinase activity, ATP binding, transferase activity, transferring phosphorus-containing groups, kinase activity | Signaling pathway |
| miR855       | transcriptional activator Myb | Ta.56324 | transcription factor | No Hits found |
|              | transmembrane protein | Ta.26126 | transporter activity | transporters |
| miR819k      | Rho GTPase | Ta.38900 | activation protein | Chemokine signaling pathway |
| miR5156      | 40S ribosomal protein | Ta.62246 | translation | Ribosome biogenesis in eukaryotes |
| miR3708      | F-box/kelch-repeat protein | Ta.34556 | regulation of transcription, DNA-dependent | No Hits found |
| miR1122      | TP53 regulating kinase | Ta.58315 | regulation | No Hits found |
|              | Maf-like protein | Ta.43051 | attachment factor 1 | No Hits found |
| miR1117      | receptor-like protein kinase | Ta.51694 | regulation pathway | No Hits found |
| miR1134      | - | - | no target found | - |
| miR1133      | FCA-like protein | TC368568 | nucleotide binding | No Hits found |

The miRNAs newly identified in wheat are shown in bold.
Table 5. Identified miRNAs in wheat

| miRNA     | EST     | Length | cDNA library                  | Tissue                             |
|-----------|---------|--------|-------------------------------|------------------------------------|
| miR5653   | BQ161232| 504    | Wheat cold-stressed seedling   |                                    |
| miR855    | BG313724| 190    | Wheat salt-stressed sheath     |                                    |
| miR819k   | BQ172254| 481    | Chinese Spring wheat drought stressed leaf |                                    |
| miR3708   | BE470984| 250    | drought-stressed seedling      |                                    |
| miR5156   | BQ172254| 481    | Chinese Spring wheat drought stressed leaf |                                    |
| miR1122   | BQ483040, BU101273| 669, 589| Wheat cold-stressed, Chinese Spring aluminum-stressed seedling, root tip |                                    |
| miR1117   | BQ172250| 469    | Chinese Spring wheat drought stressed leaf |                                    |
| miR1134   | BQ168491| 472    | Wheat drought stressed leaf    |                                    |
| miR1133   | BQ744063| 715    | Wheat salt-stressed root       |                                    |

Table 6. The ClustalW multiple sequence alignment of precursor sequences of miRNA

| miRNA     | miR5653 | miR855 | miR819k | miR3708 | miR5156 | miR1122 | miR1117 | miR1134 | miR1133 |
|-----------|---------|--------|---------|---------|---------|---------|---------|---------|---------|
| miR5653   | -       | 32.10  | 32.22   | 38      | 32.22   | 27.72   | 40.48   | 40.96   | 27.20   |
| miR855    | 32.10   | -      | 27.47   | 26.00   | 27.47   | 23.76   | 37.35   | 33.73   | 23.33   |
| miR819k   | 32.22   | 27.47  | -       | 43.69   | 100     | 41.18   | 37.63   | 33.33   | 31.97   |
| miR3708   | 38.00   | 26.00  | 43.69   | -       | 43.69   | 41.35   | 38.24   | 30.69   | 33.6    |
| miR5156   | 32.22   | 27.47  | 100     | 44.66   | -       | 41.18   | 37.63   | 33.33   | 31.97   |
| miR1122   | 27.72   | 23.76  | 41.18   | 41.35   | 41.18   | -       | 35.64   | 40.00   | 45.00   |
| miR1117   | 40.48   | 37.35  | 37.63   | 38.24   | 37.63   | 35.64   | -       | 44.68   | 33.33   |
| miR1134   | 40.96   | 33.73  | 33.33   | 30.69   | 33.33   | 40.00   | 44.68   | -       | 33.06   |
| miR1133   | 27.20   | 23.33  | 31.97   | 33.6    | 31.97   | 45.00   | 33.33   | 33.06   | -       |

The miRNAs newly identified in wheat are shown in bold.

BLASTN search (parameters for BLAST alignment was Expect: 0.01; Word Size: 11) against the wheat EST database.\(^{45,46}\) miRNA sequences matching at least 18 ntd and <3 ntd mismatch with all known plant mature miRNA were selected for further analysis. Wherever available, precursor sequence of 250 nt base pair upstream and downstream to the BLAST hits were extracted and used for hairpin structure prediction. To predict real miRNA precursor, triplet-SVM classifier program\(^{47}\) which is based on support vector machine was used. This software needs other packages namely RNAfold, LibSVM. The predicted precursor sequences were used against BLASTX program; protein coding precursor sequences were removed and non-coding were retained. BLASTN search was performed against Rfam 11.0 (rfam.sanger.ac.uk/) to distinguish between miRNA and other RNA families such as rRNA, snRNA, tRNA. The work was performed by in house script developed using ASP.NET technology\(^{48}\) and C# as scripting language for retrieving matching and non-matching sequences in BLAST result.

Prediction of secondary structure. The precursor sequences formed hairpin structure through Vienna RNA Package.\(^{30}\) Certain criteria mentioned below were chosen for the confirmation of miRNA homologs: (1) appropriate formation of stem-loop hairpin secondary structure; (2) presence of less than 3 nt substitutions in predicted mature miRNAs as compared with the known miRNAs; (3) miRNA sequences without any loop and break; (4) MFE index (MFEI). The MFEI was calculated using the following equation:

\[
\text{MFEI} = \left(\frac{\text{MFE}}{\text{length of the RNA sequence}}\right) \times 100\% / (G+C) \%
\]

Whereas MFE denotes the negative folding free energies (ΔG).

Prediction of miRNA targets genes. The putative target sites of identified miRNAs were predicted using Plant Target Prediction Tool available on UEA sRNA ToolKit (srna-tools.cmp.uea.ac.uk/plant/cgi-bin/srna-tools.cgi). miRNA binds to the targets with perfect or nearly-perfect complementarily and influence transcript regulation. Gaps and more than 4 mismatches between mature miRNAs and their potential target mRNA were not acceptable.

EST expression and phylogenetic analysis of the identified miRNAs. The expression analysis of predicted miRNA was performed using unigene (www.ncbi.nlm.nih.gov/UniGene/). The precursor sequences of the identified and the well known wheat miRNAs were aligned and phylogenetically analyzed to investigate their evolutionary relationships (www.clustal.org/). Evolutionary distances were calculated neighbor-joining (NJ) method\(^{49}\) following 1000 bootstrapped replicates. All the analyses were performed using the MEGA v4.0 software.\(^{50}\)

Functional analysis of target genes. The functional annotation of predicted targets genes of 5 miRNAs were carried under
the gene ontology system by AmiGO program (amigo.genontology.org) for consistent descriptions of biological process. The pathways and the network of molecular interaction of the predicted target genes were studied by KEGG (www.genome.jp/kegg).

Conclusions

In this paper with a bioinformatics approach, five new miRNAs were identified from the ESTs of abiotic stress treated libraries of T. aestivum. None of the predicted miRNAs showed identity with the previously reported miRNAs in wheat and these are addition into wheat miRNA data set. In addition, five new ESTs identified as a miRNA and 14 potential targets of them were predicted, which appear to be related to the development, growth, metabolism and other physiological processes under stress response. Identification of new miRNAs and their target genes will provide the future path leading to the understanding of the core regulatory interactions during abiotic stress in wheat. Researcher can further verify these predicted miRNA experimentally by high throughput sequencing of small RNA libraries.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Authors thankfully acknowledge the financial support for Indian Council of Agricultural (ICAR), Ministry of Agriculture, Government of India, New Delhi for Grant-in-Aid project No. DWR/RP/10-5.3 and grateful to anonymous reviewers for their helpful suggestions.

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