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ORIGINAL ARTICLE

Identification and characterization of durum wheat microRNAs in leaf and root tissues

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Abstract MicroRNAs are a class of post-transcriptional regulators of plant developmental and physiological processes and responses to environmental stresses. Here, we present the study regarding the annotation and characterization of *MIR* genes conducted in durum wheat. We characterized the miRNAome of leaf and root tissues at tillering stage under two environmental conditions: irrigated with 100% (control) and 55% of evapotranspiration (early water stress). In total, 90 microRNAs were identified, of which 32 were classified as putative novel and species-specific miRNAs. In addition, seven microRNA homeologous groups were identified in each of the two genomes of the tetraploid durum wheat. Differential expression analysis highlighted a total of 45 microRNAs significantly differentially regulated in the pairwise comparisons leaf versus root. The miRNA families, miR530, miR395, miR393, miR5168, miR396 and miR166, miR171, miR319, and miR167, were the most expressed in leaves in comparison to roots. Putative microRNA targets were predicted for both five and three prime sequences derived from the stem-loop of the *MIR* gene. Gene ontology analysis showed significant overrepresented gene categories in microRNA targets belonging to transcription factors, phenylpropanoids, oxydases, and lipid binding-protein. This work represents one of the first genome wide characterization of *MIR* genes in durum wheat, identifying leaf and root tissue-specific microRNAs. This genomic identification of microRNAs together with the analysis of their expression profiles is a well-accepted starting point leading to a better comprehension of the role of *MIR* genes in the genus *Triticum*.

Keywords Durum wheat · Drought · microRNA · Leaf · Root · *Triticum turgidum*

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Introduction

Durum wheat (*Triticum durum Desf*) is a tetraploid wheat cultivated around the world. This cereal is characterized by a high resistance to semiarid climates and presents advantages compared to bread wheat under water-deficit conditions. As stated by the International Grains Council, the global durum wheat production is around 35 million metric tons per year. In developing countries, durum wheat is usually cultivated in challenging environments especially on poor soil and semiarid conditions. In these harsh environments, production is usually characterized by high variability due to fluctuations in annual rainfall. Tolerance to water stress is a key aspect of the improvement of durum wheat for Mediterranean environments, where a reduced water supply greatly limits the production

from a quantitative and qualitative point of view (Peng et al. 2011).

Since the discovery of small RNAs, increased attention has been given to the importance of their function in post-transcriptional gene regulation in response to environmental stresses. MiRNAs are a class of small noncoding RNAs that are between 21 and 24 nucleotides (nt) long. Mature miRNAs are produced from longer noncoding pre-miRNAs, and they are processed by multiple cleavage steps, involving a complex system of different enzymes (Kurihara and Watanabe 2004). They are mostly involved in silencing the expression of genes through hybridization with their targets, with which they share a complementary sequence (Jeong et al. 2001). They are present in all plant genomes in large families with 1–32 loci. Interestingly, among members of the same family, miRNAs potentially encode identical or nearly identical mature sequences. Approximately 20 families are present in the plant kingdom. Among them, some members have been shown to be present in primitive land plants while others seem to be linked with more recent evolutionary events (Sunkar et al. 2008). These small RNAs have been associated with responses to different environmental stresses including drought (Cheah et al. 2015; Shui et al. 2013). The identification of those involved in the regulation of key genes in secondary metabolism will be highly desirable (Martinelli and Tonutti 2012). The number of newly discovered miRNAs is continually increasing, especially in Arabidopsis (Kozomara and Griffiths-Jones 2014). Although many of the new microRNAs have not been analyzed in response to environmental changes, the role of some has been clarified. For example, miR169 is one of the most conserved miRNAs and it has been associated with plant responses to abiotic stress (Li et al. 2008; Zhao et al. 2011). Several cereal miRNAomes have been characterized, although knowledge of the complex networks in which these miRNAs are involved is far from completely understood (Budak et al. 2015).

Specifically in wheat, several studies have been conducted with the aim to characterize the miRNAome of wheat species and identify the key miRNA involved in tolerance and resistance to abiotic stresses (Pandey et al. 2014; Liu et al. 2015; Zhao et al. 2014; Ma et al. 2015; Akpinar et al. 2015; Alptekin and Budak 2016; Alptekin et al. 2016; Liu et al. 2016a; Giusti et al. 2016). However, no studies have been conducted which include the annotation and chromosome mapping of tissue-specific miRNAs. Here, we propose the first attempt to accurately annotate and map miRNA in relation to their expression in leaf and root tissues under the following two conditions: irrigated to 100% and 55% of evapotranspiration (early water stress).

Materials and methods

Plant material and experimental design

The experiment was conducted at Pietranera Farm (Sicily, Italy; 37° 30′ N, 13° 31′ E) using durum wheat (*Triticum durum Desf*) plants grown outdoors in pots under the shade. In order to characterize the miRNAome under two conditions (i.e., irrigated and water stress), a complete randomized factorial design replicated six times was adopted. Each pot (diameter 150 mm, height 130 mm) was filled with a vermiculite/soil mixture (1:1). Soil properties were as follows: 273 g kg^{-1} clay, 249 g kg^{-1} silt, and 488 g kg^{-1} sand; pH 8.0; 7.4 g kg^{-1} total C; and 0.86 g kg^{-1} total N. All pots were weighed, fully wetted, and, after allowing them to drain freely, weighed again to determine the soil water content (SWC) at the retention capacity.

Eighteen seeds of durum wheat (cv. Simeto), previously surface-sterilized with hydrogen peroxide at 4% for 3 min, were sown in each pot. Simeto, released in 1988, is the most widely grown variety in southern Italy; it is an early maturing, short, high-yielding cultivar with excellent grain quality, and it is sensitive to drought (Bresta et al. 2011). One week after emergence, plants were thinned to six seedlings per pot. From emergence to tillering stage, plants were grown under well-watered conditions. Starting from the advanced tillering stage (stages 22–24 of the Zadoks scale, Zadoks et al. 1974), plants were subjected to four different water regimes: Contr100 which consisted of total replenishment of lost water daily; STR55, STR70, and STR85 which consisted in replenishing of 55, 70, and 85% of the daily evapotranspiration, respectively, as measured on Contr100. The pots were weighed daily and the water amounts were regulated by weight. The SWC during the period of application of the different water regimes was calculated for each pot as difference between the pot weight at retention capacity and the pot weight measured daily. All the experimental treatments were watered at the same time as the Contr100 treatment.

All pots were harvested after 10 days from the start of water stress. Plant biomass was immediately separated into roots and shoots, and fresh weights were recorded. One pool of leaves and one of roots (primary and secondary), both equal to approximately one third of the respective total fresh weights, have been sampled, immediately frozen in liquid nitrogen (N), ground, and weighed out 1 g for the analysis. At the same time, a sample of fresh full expanded leaves (about 1 g) was taken from each pot to determine the leaf relative water content (RWC). The leaves were weighed immediately (to determine fresh mass, FM), cut into several sections, and soon soaked in deionized water for 12 h, and then weighed again to determine the fully turgid mass (TM). Finally, the leaves were dried at 65 °C to determine the dry mass (DM). The leaf

RWC was calculated as follows (Salisbury and Ross 1992):

$$RWC = (FM-DM)/(TM-DM)$$

For each pot, the remaining plant roots and shoots (both always greater than the 50% of the total fresh weight of each pot) were separately dried at 65 °C to determine the dry matter content and calculate the belowground and aboveground dry masses.

Among the three levels of water stress, we chose the extreme water stress treatment (STR55) and Contr100 for the characterization of the durum wheat miRNAome. Ten days after that stress was applied, leaf and root tissues were harvested followed by miRNA extraction and sequencing.

RNA extraction, library preparation, and microRNA profiling

RNA was extracted using the Spectrum Plant Total RNA kit (Sigma-Aldrich) from a total of 12 samples, composed of 6 leaves and 6 roots, grown under water stress and control conditions. For each sample, a pool of leaves and roots were used for the analysis and three biological replicates were considered for both conditions. RNA quantity and quality was measured using the Nanodrop (Thermo Fisher Scientific, MA, USA) and the integrity was checked using electrophoresis by loading 1 μl of sample on 2% agarose gel. RNA samples were processed to generate small RNA-seq libraries containing short inserts according to the TruSeq Small RNA Library Preparation Kit (Illumina, San Diego, CA), following manufacturer's instruction.

Once quality control was passed, next-generation sequencing (NGS) was performed using an Illumina Hiseq2500 sequencer at IGA Technology Services (Udine, Italy).

Raw reads were uploaded to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) BioProject ID: PRJNA360997.

Small RNA annotation

Raw sequencing data were of good quality (mean sequence quality score: Phred >30) and no quality filters were applied. Three prime sequencing adapters were trimmed using the program Cutadapt (Martin 2011) version 1.8.3 with the settings: –trim-n –a TGGAATTCTCGGGTGCCAAGG –m 15 –M 35, resulting in trimmed reads ranged from 15 to 35 nucleotides in length.

For each sample, trimmed reads were mapped independently against the hexaploid *Triticum aestivum* cv. *Chinese Spring* reference genome version IWGSC2 downloaded from Ensembl Genomes (ftp://ftp.ensemblgenomes.org/pub/plants/release-26). Since the official tetraploid wheat genome is not yet available, only chromosomes belonging to genomes A and B were considered as reference sequences to create a synthetic *Triticum durum Desf* reference. Moreover, mitochondrial and plastid genome were not considered in this analysis.

Bowtie (Langmead et al. 2009) version 1.0.1 was used to align trimmed reads to the reference genome allowing two mismatches. The mapping results of each sample were analyzed with ShortStack (Axtell 2013) version v.2.0.9 with default settings, annotating a total of 66,795 clusters corresponding to significant genomic regions harboring small RNA accumulation. This initial annotation was restricted to 90 high confidence miRNA loci based on the current criteria for the annotation of plant mircoRNA (Meyers et al. 2008).

To identify conserved miRNA loci, BLASTn (McGinnis and Madden 2004) with *E* value <e-10 was applied using as subject all the hairpin sequences belonging to monocotyledonous species present in miRBase (Kozomara and Griffiths-Jones 2014) version 21.

Finally, homeologous miRNA loci were identified based on sequence similarity along the entire stem-loop sequence using the clustering program CD-HIT (Fu et al. 2012) with sequence identity of 0.95.

miRNA target identification and annotation

The non-redundant set of five and three prime miRNA sequences was used to predict targets in the full set of cDNA transcripts annotated in the *T. aestivum* cv. *Chinese Spring* version IWGSC2 downloaded from Ensembl Genomes (ftp://ftp.ensemblgenomes.org/pub/plants/release-26).

The program TargetFinder (Fahlgren et al. 2007) with default parameters was applied and only results with a score cutoff of 3 or less were considered as putative miRNA targets.

The resulted list of unique *T. durum* miRNA targets was annotated using TRAPID (Van Bel et al. 2013), an online tool for functional and comparative transcriptome analysis. Similarity searches were conducted against the model grass species *Brachypodium distachyion* based on the data source contained in the PLAZA 2.5 database (Proost et al. 2009).

Differential expression analysis

The detection of differentially expressed miRNAs was identified using the Bioconductor R package DESeq2 (Love et al. 2014) with Wald hypothesis test. For each miRNA locus, the total number of sequencing read counts mapped unambiguously to the hairpin sequence in each condition was used as the input to the expression analysis. No normalization methods were applied to the raw data before performing the test since DESeq2 uses an internal method to normalize the raw data (Love et al. 2014). MicroRNAs with FDR ≤0.05 were considered differentially expressed.

Statistical analysis

Data on soil water content, plant biomass, and leaf RWC were subjected to analysis of variance (ANOVA) according to the experimental design. Variables corresponding to proportions were arcsine transformed before analysis to assure a better fit with the Gaussian law distribution. Normality was confirmed using a Kolmogorov-Smirnov test. Treatment means were compared using Fisher's protected least significant differences test at the 5% probability level.

Results

Soil water content, plant biomass, and leaf RWC

In the Contr100 treatment, the soil water content never dropped below 23% during the experiment, whereas in all other treatments soil water content decreased more or less rapidly as a function of the level of the applied water stress (Fig. 1). At harvest, the soil water content ranged from 11.6% (STR55) to 23.5% (Contr100).

Water regime significantly affected the aboveground plant biomass and the leaf RWC but not the belowground plant biomass (Table 1). In particular, decreases in shoot biomass were observed when the intensity of water stress increased. Leaf RWC ranged from a high of approximately 90% for the well-watered treatment (Contr100) to a low of 82% for the most highly water-stressed treatment (STR55).

Overview of microRNA profiling data

In this study, the Illumina sequencing platform was used to identify and characterize at genomic level miRNAs in durum wheat, focusing on leaf and root tissues subjected to drought stress. In total, 12 small RNA (sRNA) libraries were constructed using total RNAs isolated from four conditions (namely, leaf-control (leaf-N), root-control (root-N), leaf-drought (leaf-S), root-drought (root-S)). sRNA sequencing yielded a total of 189,135,623 high-quality raw sequences with an average number of raw reads over the 3 replicates of at least 30 million (Table 2).

After removing the sequencing adapters, sRNA sequencing reads were mapped against the bread wheat reference genome produced by the International Wheat Genome Sequencing Consortium (2014) considering only chromosomes belonging to A and B genome to de novo identify *MIR* genes in *Triticum durum Desf*. Between 40% and 83% (Table 2) of the reads generated from root and leaf were mapped respectively to bread wheat genome allowed us to perform a miRNA identification at genome level using the pipeline ShortStack as proposed by Axtell (2013). This approach resulted in 66,795 small RNA clusters corresponding to significant genomic regions harboring small RNA sequences. Of these, 90 high confidence miRNA loci were retained since they passed all the current criteria for the plant microRNA annotation (Table 3) (Meyers et al. 2008). A Plot Diagram of the differential analysis between roots and leaves was performed (Fig. 2).

Identification of conserved and novel miRNAs in durum wheat

Among the 90 high confidence miRNA loci found, 59 were found to be conserved with a sequence identity greater than 82% with miRNAs representing 41 families (Table 3). In particular, the conserved miRNAs belonged to families identified in *Agilops tauschii* (33 miRNAs), *T. aestivum* (16 miRNAs), *Brachypodium distachyon* (7 miRNAs), *Hordeum vulgare* (2 miRNAs), and *Zea mays* (1 miRNAs) and in most of them the 5 and 3 prime sequences were found to be conserved with

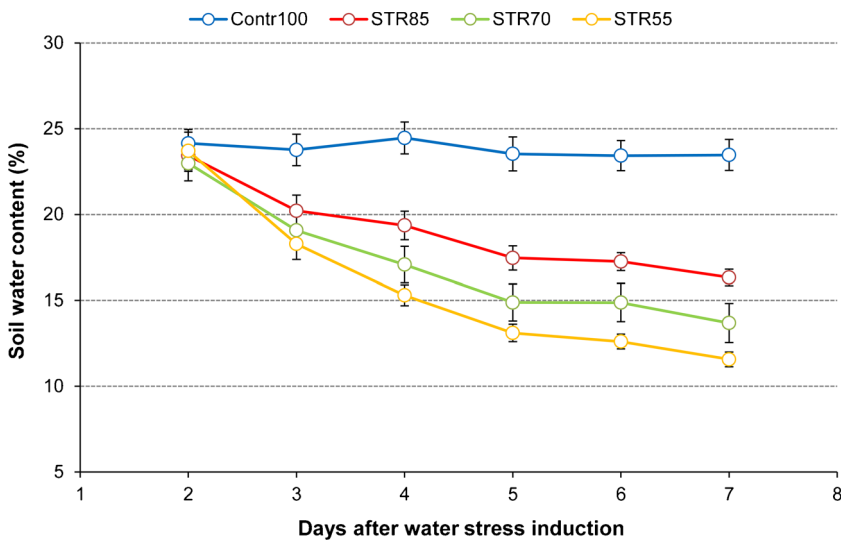

Fig. 1 Changes in soil water content (±S.D. $n = 6$) (%) in the days after the water stress induction. Contr100 = total replenishing of the amount of water lost daily from the pots; STR85, STR70, and STR55 = replenishing of 85, 70, and 55% of the daily evapotranspiration measured on Contr100

Table 1 Below and aboveground biomass (as grams of dry matter per pot) and leaf relative water content (leaf RWC) under different water regimes

Water regime	Belowground biomass	Aboveground biomass		Leaf RWC	
	g DM pot^{-1}	g DM pot^{-1}			
Contr100	2.13	5.07	a	0.92	a
STR85	2.05	4.91	a	0.89	a
STR70	2.09	4.51	ab	0.86	ab
STR55	2.14	4.12	b	0.82	b

Contr100 = total replenishing of the amount of water lost daily from the pots; STR85, STR70, and STR55 = replenishing of 85, 70, and 55% of the daily evapotranspiration measured on Contr100. Within each column, different letters denote significant differences at 5% probability level. Data are means of six replicates

100% identity. Well-represented miRNA families were *MIR156*, *MIR167*, and *MIR396*. About one third of the total *MIR* loci found were classified as novel miRNAs since no hit against the deposited miRNAs were found (Table 4). Unlike conserved miRNAs, which were absent in three chromosomes, the novel miRNAs were distributed among the seven chromosomes of durum wheat. BlastN results of known miRNA were shown in ESM 1.

Differential expression analysis

Differential miRNA expression profiles were observed between leaf and root tissue but not between drought and control. This was particularly due to the high variance observed between the three biological replicates where biological replications of control leaf were found more similar to the water-stressed treatment. Moreover, miRNA expression patterns in root samples were even more variable and this situation did not allow us to identify any significantly regulated miRNA in either leaf or root tissue in response to drought. Therefore, we focused our differential expression analysis on the comparison between the two tissues using the full set of six samples as biological replicates in each tissue, increasing the power of the statistical test. A total of 45 miRNAs were differentially expressed (DE) between root and leaf with FDR = 0.05 (Table 5). In particular, we noticed an equal distribution of the miRNAs' expression pattern in which 23 miRNAs were induced in the root tissue whereas 22 miRNAs were induced in leaf tissue. Interestingly, among the most DE, we found the homeologous miRNAs which showed a conservation also in the pattern of expression. miR530, miR1432, and miR5168 were drastically downregulated in root compared to miR171 and miR396 that vice versa were found upregulated in root (Fig. 3).

miRNA mapping and homeologous identification

To better investigate the genomic organization of MIR genes in durum wheat, we have performed a cluster analysis of the 90 hairpin miRNA structures from the current experiment, using a sequence identity cutoff of 95%. All the miRNAs (known and unknown) were mapped on wheat chromosomes (Fig. 4). This analysis allowed us to highlight for the first time seven miRNA syntenic groups within homeologus chromosomes. We defined paralog homeologous miRNAs all the pre-miRNA structures belonging to the same family which mapped on a couple of homeolog chromosomes (Table 6). Three MIR genes were found on chromosomes 2 (miR530, miR1432, miR171a), two (miR156, miR5168) on chromosome 5, and one (miR156c and miR396e) respectively on chromosomes 6 and 7. Interestingly, two inversions were found on chromosomes 2 and 5 respectively between miR530–miR1432 and miR156–miR5168. To better investigate the chromosomal miRNA distribution and organization, we plotted miRNAs based on their coordinates on the bread wheat genome (Fig. 5). Interestingly, miRNAs were found randomly distributed along the seven chromosomes and

Table 2 Data set summary of miRNA sequencing data. The average number of reads for the three biological replicates in each condition and tissue are shown

	Roots		Leaves	
	Control	Drought	Control	Drought
Total raw reads	58,677,892	50,893,655	30,665,332	48,898,744
Uniquely mapped reads	6,789,788	4,963,645	2,404,013	3,487,512
Multi mapped reads	20,549,748	16,102,052	21,173,031	37,276,683
Total mapped reads	27,339,536 (46%)	21,065,697 (41%)	23,577,044 (76%)	40,764,195 (83%)

Table 3 List of conserved miRNA in durum wheat tissues

Cluster name	Known pre-miRNA	%	5P cluster sequence	Known name	3P cluster sequence	Known name
Cluster_513	tae-MIR5384	100.00	UUCGCCGGUCGCGCGUUCCCC	-	UGAGCGCGCCGCCGUCGAAUG	tae-miR5384-3p
Cluster_1282	tae-MIR9664	93.86	ACGGCAUAGAGGCACUGCAAA	-	UGCAGUCCUCGAUGUCGUAG	tae-miR9664-3p
Cluster_6065	ata-MIR9863b	95.12	UGAGAAGGUAGAUCAUAAUAGC	ata-miR9863b-3p	UGUUAUGAUCUGCUUCUCAUU	ata-miR9863b-5p
Cluster_6404	tae-MIR9664	98.31	ACGGCAUAGAGGCACUGCAAA	-	UGCAGUCCUCGAUGUCGUAG	tae-miR9664-3p
Cluster_8073	tae-MIR399	84.33	GGGCGCUUCUCCCUUGGCACG	ata-miR399a-5p	UGCCAAAGGAGAAUUGCCCUG	ata-miR399a-3p
Cluster_10104	tae-MIR530	98.76	CUGCAUUUGCACCUGCACCUA	osa-miR530-5p	GGUGCAGUGGCAUAUGCAACU	tae-miR530
Cluster_10719	ata-MIR156c	99.09	UGACAGAAGAGAGUGAGCAC	ata-miR156c-5p	GCUCACUGCUCUAUCUGUCACC	ata-miR156c-3p
Cluster_11634	ata-MIR1432	90.65	CUCAGGAGAGAUGACACCGAC	ata-miR1432-5p	UGGUGUCACCUCGCCUGAACA	ata-miR1432-3p
Cluster_11636	ata-MIR1432	97.27	AUCAGGAGAGAUGACACCGAC	-	UGGUGUCACCUCGCCUGAACA	ata-miR1432-3p
Cluster_12802	bdi-MIR827	95.24	UUUUGCUGGUUGUCAUCUAACC	bdi-miR827-5p	UUAGAUGACCAUCAGCAAACA	bdi-miR827-3p
Cluster_13147	ata-MIR9776	86.90	UGGACGAGGAUGUGCAGCUGC	ata-miR9776-5p	AGCUGCACAUCCACUUCCAAG	ata-miR9776-3p
Cluster_13262	ata-MIR395a	95.24	AGUUCCCUUCAAGCACUUCAGG	ata-miR395a-5p	UGAAGUGUUUGGGGAACUCU	ata-miR395a-3p
Cluster_14263	ata-MIR166c	89.77	GGAACGUUGGCUGGCUCGAGG	ata-miR166c-5p	UCGGACCAUGCUUCAUUCCUC	ata-miR166c-3p
Cluster_14872	ata-MIR164c	94.78	UGGAGAAGCAGGGCACGUGCA	ata-miR164c-5p	CACGUGUUCUUCUCCUCCAUC	ata-miR164c-3p
Cluster_16065	ata-MIR1432	99.09	AUCAGGAGAGAUGACACCGA	ata-miR1432-5p	GGUGUCACCUCGCCUGAACA	ata-miR1432-3p
Cluster_18140	tae-MIR530	95.12	CUGCAUUUGCACCUGCACCUA	-	GGUGCAGUGGCAUAUGCAACU	tae-miR530
Cluster_18283	bdi-MIR827	97.83	UUUUGUUGGUUGUCAUCUAACC	bdi-miR827-5p	UUAGAUGACCAUCAGCAAACA	bdi-miR827-3p
Cluster_18506	ata-MIR156c	94.55	UGACAGAAGAGAGUGAGCAC	ata-miR156c-5p	GCUCACUGCUCUAUCUGUCACC	ata-miR156c-3p
Cluster_18775	ata-MIR171a	98.86	UGGUAUUGUUUCGGCUCAUA	ata-miR171a-5p	UGAGCCGAACCAAUAUCACU	ata-miR171a-3p
Cluster_20349	ata-MIR396d	87.50	UCCACAGGCUUUCUUGAACU	ata-miR396d-5p	UUCAAGAAAGCCCAUGGAAA	ata-miR396d-3p
Cluster_20589	ata-MIR393	89.13	UUCCAAAGGGAUCGCAUUGAU	ata-miR393-5p	CAGUGCAAUCCCUCUGGAAUU	ata-miR393-3p
Cluster_20808	hvu-MIR159a	94.94	GAGCUCCUAUCAUUCCAAUGA	hvu-miR159a	UUUGGAUUGAAGGGAGCUCUG	hvu-miR159a
Cluster_25388	bdi-MIR156f	93.94	UGACAGAAGAGAGUGAGCAC	bdi-miR156f-5p	GCUCACUGCUCUAUCUGUCAGC	bdi-miR156f-3p
Cluster_26451	tae-MIR9676	100.00	UGGAUGUCAUCGUGGCCGUACA	tae-miR9676-5p	UACGGCCUGAUGACAUCCACG	-
Cluster_28135	zma-MIR319b	87.70	AGAGCGUCCUUCAGUCCACUC	zma-miR319b-5p	UUGGACUGAAGGGUGCUCCCU	zma-miR319b-3p
Cluster_33098	ata-MIR167d	88.24	UGAAGCUGCCAGCAUGAUCUGA	ata-miR167d-5p	AGGUCAUGUGGCAGCUUCAUU	ata-miR167d-3p
Cluster_41670	ata-MIR167d	90.44	UGAAGCUGCCAGCAUGAUCUGA	ata-miR167d-5p	AGGUCAUGUGGCAGCUUCAUU	ata-miR167d-3p
Cluster_41943	ata-MIR166e	89.91	GGAAUGUUGUCUGGUUGGAGA	ata-miR166e-5p	UCGGACCAGGCUUCAUUCCCC	ata-miR166e-3p
Cluster_42520	tae-MIR9674a	93.10	AUAGCAUCAUCCAUUCUACCA	ata-miR9674a-5p	GUAGGAUGGCUGGUGCUAUGG	ata-miR9674a-3p
Cluster_43339	tae-MIR9666b	98.98	GCCAUCAUACGUCCAACCGU	tae-miR9666b-5p	GGUUGGGCUGUAUGAUGGCGA	tae-miR9666b-3p
Cluster_43357	tae-MIR156	99.15	UGACAGAAGAGAGUGAGCAC	bdi-miR156d-5p	GCUCACCCUCUCUCUGUCAGC	-
Cluster_43534	ata-MIR5168	97.58	GGGUUGUUGUCUGGUUCAAG	ata-miR5168-5p	CGGACCAGGCUUCAAUCCCU	ata-miR5168-3p
Cluster_44265	ata-MIR167a	83.67	UGAAGCUGCCAGCAUGAUCUA	ata-miR167a-5p	GAUCGUGCUGUGACAGUUUCACU	ata-miR167a-3p
Cluster_47002	ata-MIR167c	98.96	UGAAGCUGCCAGCAUGAUCUA	ata-miR167c-5p	GAUCAUGACUGACAGCCUCAUU	ata-miR167c-3p
Cluster_47003	ata-MIR167b	97.94	UGAAGCUGCCAGCAUGAUCUGA	ata-miR167b-5p	AGGUCAUGCUGGAGUUUCAUC	ata-miR167b-3p

Table 3 (continued)

Cluster name	Known pre-miRNA	%	5P cluster sequence	Known name	3P cluster sequence	Known name
Cluster_48327	ata-MIR5168	96.77	GGGUUGUUGUCUGGUUCAAG	ata-miR5168-5p	CGGACCAGGCUUCAAUCCCU	ata-miR5168-3p
Cluster_48425	tae-MIR156	98.29	UGACAGAAGAGAGUGAGCAC	bdi-miR156d-5p	GCUCACCCUCUCUCUGUCAGC	-
Cluster_49696	ata-MIR166b	99.00	GAAUGACGCCGGGUCCGAAAG	ata-miR166b-5p	UUCGGACCAGGCUUCAUUCCC	ata-miR166b-3p
Cluster_52794	tae-MIR9670	97.47	UUCUUCAAGUACUCCACUUUU	-	AGGUGGAAUACUUGAAGAAGA	tae-miR9670-3p
Cluster_53245	bdi-MIR156c	92.70	UUGACAGAAGAGAGUGAGCAC	bdi-miR156c	GCUCACUCCUCUUUCUGUCAGCC	-
Cluster_53705	tae-MIR397	95.74	UUGAGUGCAGCGUUGAUGAAC	-	UCACCGGCGCUGCACACAAUG	tae-miR397-5p
Cluster_55297	bdi-MIR396c	100.00	UUCCACAGCUUUCUUGAACUG	bdi-miR396c-5p	GUUCAAUAAAGCUGUGGGAAG	bdi-miR396c-3p
Cluster_55342	ata-MIR396c	96.57	UUCCACAGCUUUCUUGAACUU	ata-miR396c-5p	GGUCAAGAAAGCUGUGGGAAG	ata-miR396c-3p
Cluster_55558	ata-MIR172b	96.33	GCAGCACCACCAAGAUUCACA	ata-miR172b-5p	AGAAUCUUGAUGAUGCUGCAU	ata-miR172b-3p
Cluster_55636	ata-MIR396b	83.91	UCCACAGGCUUUCUUGAACUG	ata-miR396b-5p	GUUCAAGAAAGUCCUUGGAAA	ata-miR396b-3p
Cluster_57238	bdi-MIR156c	94.16	UUGACAGAAGAGAGUGAGCAC	bdi-miR156c	GCUCACUCCUCUUUCUGUCAGCC	-
Cluster_57508	tae-MIR167c	91.10	UGAAGCUGCCAGCAUGAUCUGC	tae-miR167c-5p	AGAUCAUGCUGCAGCUUCAUU	-
Cluster_58599	tae-MIR9660	100.00	UUGCGAGCAACGGAUGAAUCAGCC	tae-miR9660-5p	CUGAUUUCUCCUUUGCUCGAGUAGA	-
Cluster_58886	ata-MIR396b	86.89	UCCACAGGCUUUCUUGAACUG	ata-miR396b-5p	GUUCAAGAAAGUCCUUGGAAA	ata-miR396b-3p
Cluster_59780	ata-MIR396e	97.17	UUCCACAGCUUUCUUGAACUG	ata-miR396e-5p	GUUCAAUAAAGCUGUGGGAAA	ata-miR396e-3p
Cluster_63022	tae-MIR5200	100.00	AAGCCUUAGUGAAUAUCUACA	tae-miR5200	UAGAUACUCCCUAAGGCUUGG	tae-miR5200-3p
Cluster_63134	ata-MIR396e	97.17	UUCCACAGCUUUCUUGAACUG	ata-miR396e-5p	GUUCAAUAAAGCUGUGGGAAA	ata-miR396e-3p
Cluster_65040	bdi-MIR399a	93.75	GUGCAGUUCUCCUCUGGCAUG	-	UGCCAAAGGAGAAUUGCCCUG	bdi-miR399a-3p
Cluster_65295	ata-MIR166d	95.45	GGAAUGUUGUCUGGCUCGGGG	ata-miR166d-5p	UCGGACCAGGCUUCAUUCCCC	ata-miR166d-3p
Cluster_65537	hvu-MIR5048a	82.59	UAUAUUUGCAGGUUUUAGGUCU	hvu-miR5048a	ACCUAGACAUGCAAGUAUAUU	-
Cluster_12754	ata-MIR171a	100.00	-	-	UGAGCCGAACCAAUAUCACUC	ata-miR171a-3p
Cluster_16064	ata-MIR1432	90.91	AUCAGGAGAGAUGACACCGAC	ata-miR1432-5p	-	-
Cluster_42519	ata-MIR9674c	89.81	UGAAUUUGUCCAUAGCAUCAG	ata-miR9674c-5p	-	-
Cluster_59780	ata-MIR396e	94.38	UUCCACAGCUUUCUUGAACUG	ata-miR396e-5p	GUUCAAUAAAGCUGUGGGAAA	ata-miR396e-3p

Cluster name, known pre-miRNA, percentage of identity with the known pre-miRNA sequence, 5P cluster sequence, 5P known name, 3P cluster sequence, and 3P known name are indicated. Hyphen indicates that the sequence found differs from miRBase or was not found in our data.

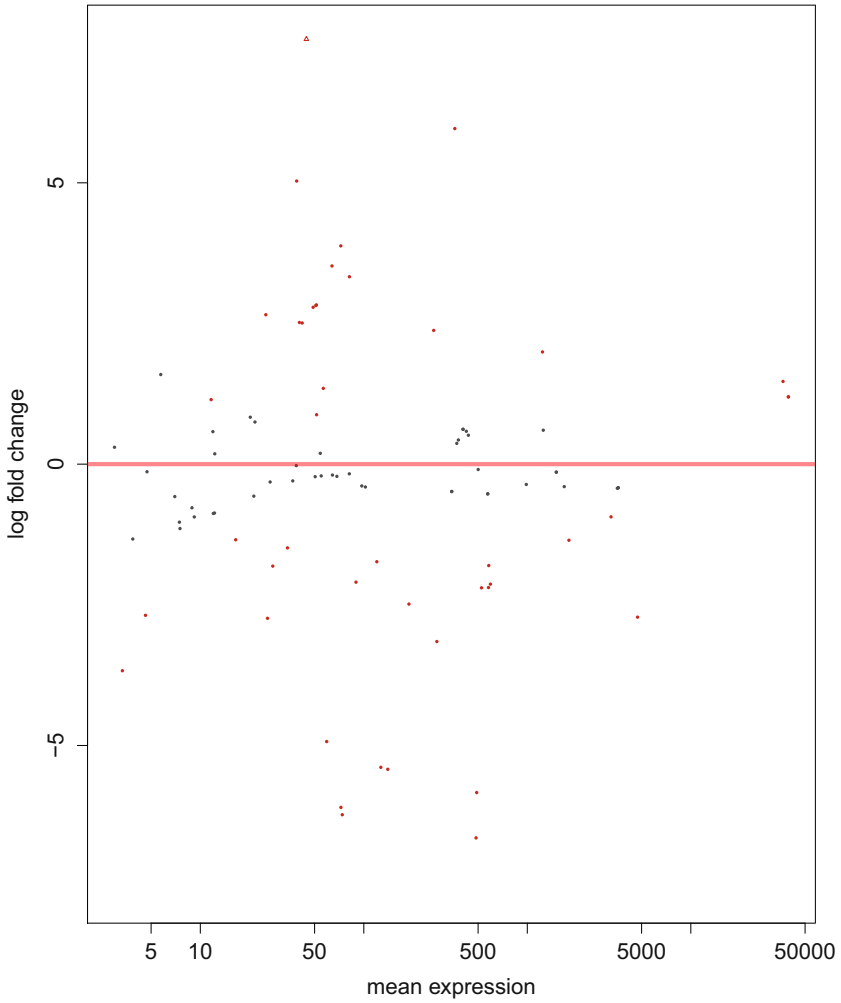

Fig. 2 MA-Plot diagram of the differential expression anlysis between roots and leaves. In red miRNAs differentially expressed with padj <0.05; triangle indicates that the microRNA has a fold change value exceeding the scale in the Y

accumulation of miRNAs appear on chromosomes 2, 5, 6, and 7 while some chromosomes such as 3A had very few miRNAs.

Potential targets of conserved and novel miRNAs

To understand better the biological functions of both known and novel miRNAs in durum wheat, putative miRNA targets were identified according to the computational tool TargetFinder using as reference transcript database the cDNA transcripts annotated in the Triticum aestivum (version IWGSC2). Five and three prime sequences of both known and novel miRNAs were used as query sequences database to search for targets. Secondary structures of all the MIR loci identified in this work were deposited on figshare (https://doi.org/10.6084/m9.figshare.4127190.v1). The putative miRNA target genes with score cut-off not higher than 3 were identified (Table ESM1 and ESM2). Their GO terms, interpro description and gene families were identified (Table ESM4–ESM7).Interestingly the 5 homeologous miRNAs differentially expressed between root/leaf were found to have conserved putative targets. This feature together with the conservation of the miRNA expression pattern could be related to a mechanism associated to miRNA biology in polyploidy species. Moreover, the GO enrichment analysis showed a high number of target genes annotated as nucleoside phosphate, purine nucleotide, ion and carbohydrate binding. A clear enrichment of gene target ontologies was related to lignin metabolism, phenylpropanoids, and oxidoreductases.

Moreover, the GO enrichment analysis showed a high number of target genes annotated as having the function of nucleoside phosphate, purine nucleotide, ion, and carbohydrate binding. A clear enrichment of gene target ontologies was related to lignin metabolism, phenylpropanoids, and oxidoreductases.

Table 4 Complete list of miRNA identified in durum wheat

Cluster name	Chromosome	Coordinates (start-end)	Strand
Cluster_513	1A	15,633,872–15,633,951	−
Cluster_1282	1A	79,918,253–79,918,380	−
Cluster_6065	1B	112,783,112–112,783,726	−
Cluster_6206	1B	122,612,157–122,612,269	−
Cluster_6404	1B	141,665,387–141,665,516	−
Cluster_6749	1B	170,059,132–170,059,244	−
Cluster_8073	1B	260,670,499–260,670,644	+
Cluster_10104	2A	45,376,842–45,377,015	+
Cluster_10719	2A	84,081,359–84,081,605	−
Cluster_11634	2A	143,411,184–143,411,299	−
Cluster_11636	2A	143,414,592–143,414,715	−
Cluster_12802	2A	213,324,183–213,324,354	−
Cluster_13143	2A	226,019,236–226,019,319	−
Cluster_13147	2A	226,139,343–226,139,454	−
Cluster_13262	2A	230,308,678–230,308,772	−
Cluster_13639	2A	240,081,115–240,081,289	−
Cluster_14263	2B	2,222,883–2,222,985	−
Cluster_14872	2B	23,833,127–23,833,288	+
Cluster_15646	2B	58,576,789–58,577,076	+
Cluster_15688	2B	60,519,240–60,519,467	+
Cluster_16065	2B	84,759,598–84,759,722	−
Cluster_18140	2B	217,777,285–217,777,488	+
Cluster_18283	2B	227,895,401–227,895,646	+
Cluster_18506	2B	243,201,566–243,201,809	−
Cluster_18775	2B	259,602,537–259,602,631	+
Cluster_20349	2B	335,036,178–335,036,300	−
Cluster_20589	2B	342,233,257–342,233,339	+
Cluster_20808	3A	2,080,433–2,080,633	−
Cluster_25388	3B	110,274,755–110,274,947	+
Cluster_25487	3B	119,184,659–119,184,775	+
Cluster_26451	3B	200,808,871–200,809,000	+
Cluster_27040	3B	269,731,006–269,731,167	−
Cluster_28135	3B	397,138,896–397,139,082	+
Cluster_33098	4A	2,957,490–2,957,630	−
Cluster_33442	4A	20,971,211–20,971,293	−
Cluster_34476	4A	78,276,500–78,276,742	+
Cluster_34518	4A	80,581,584–80,581,770	−
Cluster_37179	4A	201,734,016–201,734,186	+
Cluster_38047	4B	14,197,219–14,197,444	+
Cluster_39268	4B	111,421,293–111,421,546	+
Cluster_41670	4B	277,748,474–277,748,655	−
Cluster_41943	4B	289,870,488–289,870,595	+
Cluster_42520	4B	313,527,214–313,527,308	+
Cluster_43339	5A	59,439,291–59,439,409	+
Cluster_43357	5A	61,589,422–61,589,539	+
Cluster_43534	5A	73,069,415–73,069,551	−
Cluster_43663	5A	80,552,389–80,552,564	+
Cluster_44265	5A	109,129,156–109,129,252	+
Cluster_44683	5A	124,245,876–124,246,107	+
Cluster_46325	5B	53,725,031–53,725,280	+
Cluster_47002	5B	89,505,892–89,506,004	+
Cluster_47003	5B	89,508,484–89,508,593	+
Cluster_47293	5B	105,883,615–105,883,794	+
Cluster_48327	5B	163,330,209–163,330,340	+
Cluster_48425	5B	168,674,312–168,674,427	−
Cluster_49696	5B	217,147,284–217,147,387	+
Cluster_50473	5B	243,482,443–243,482,634	−
Cluster_50615	5B	248,077,388–248,077,474	+
Cluster_52794	6A	54,567,623–54,567,714	+
Cluster_53245	6A	87,949,906–87,950,062	+
Cluster_53705	6A	120,354,259–120,354,366	−
Cluster_55297	6A	198,934,069–198,934,223	+
Cluster_55342	6A	199,645,078–199,645,263	−
Cluster_55558	6A	203,905,899–203,906,020	+

Table 4 (continued)

Cluster name	Chromosome	Coordinates (start-end)	Strand
Cluster_55636	6A	205,704,194–205,704,363	−
Cluster_57238	6B	96,378,404–96,378,579	+
Cluster_57508	6B	117,919,946–117,920,091	−
Cluster_58599	6B	190,032,396–190,032,500	−
Cluster_58886	6B	201,394,961–201,395,149	−
Cluster_59228	7A	5,577,146–5,577,252	+
Cluster_59780	7A	20,932,743–20,932,904	+
Cluster_60668	7A	67,722,401–67,722,565	+
Cluster_62182	7A	158,762,241–158,762,338	−
Cluster_63022	7B	3,426,764–3,426,925	+
Cluster_63023	7B	3,427,326–3,427,561	+
Cluster_63134	7B	8,739,076–8,739,320	−
Cluster_64950	7B	152,744,447–152,744,540	+
Cluster_65040	7B	162,293,419–162,293,567	−
Cluster_65295	7B	177,711,980–177,712,189	−
Cluster_65537	7B	193,135,466–193,135,860	−
Cluster_66590	7B	244,136,062–244,136,291	−
Cluster_9890	2A	30,340,652–30,340,876	−
Cluster_11382	2A	128,544,212–128,544,409	−
Cluster_12754	2A	210,529,750–210,529,991	−
Cluster_16064	2B	84,756,262–84,756,383	−
Cluster_35124	4A	121,123,343–121,123,572	+
Cluster_42519	4B	313,526,045–313,526,385	+
Cluster_45124	5A	144,036,512–144,036,609	−
Cluster_53303	6A	92,088,615–92,088,860	−
Cluster_66669	7B	247,337,007–247,337,127	−

Cluster name, chromosome, coordinates and strand are indicated

Discussion

Durum wheat is an important cereal widely grown in the Mediterranean basin and is an ancestral progenitor of bread wheat, contributing genomes A and B to the bread wheat genome. Its tetraploid nature offers a valid alternative to hexaploid bread wheat as a model for investigation of the role of miRNA in the regulation of key phenomenon in cereal physiology. With the purpose of clarifying the roles of miRNAs in durum wheat physiology and development, we present the annotation and mapping of tissue-specific miRNAs in durum wheat as well as the analysis of miRNA expression in leaf and root tissues. We identified 90 known and 32 novel miRNAs and additionally, we predicted all the potential targets for both novel and known miRNAs.

Previous studies with the aim of identifying miRNAs and their target genes have already been conducted in different species including bread wheat (Xin et al. 2010; Tang et al. 2012; Wang et al. 2013). Several studies have been conducted to analyze tissue-specific expression of miRNAs in wheat (Agharbaoui et al. 2015; Liu et al. 2015; Ma et al. 2015). Previous articles have dealt with the identification of the cis-element RHE in the promoters of genes (Won et al. 2009; Bruex et al. 2012). Other important leaf cis-acting regulatory elements (motifs) were found in the promoters of genes that were predominantly expressed in leaves (Xu et al. 2011; Zhang et al. 2012). An interesting work has been conducted by Lucas and Budak (2012) on *T. aestivum* chromosome 1AL. They demonstrated that a number of miRNA sequences were closely related to transposable elements and they proposed a strategy for the annotation to minimize the risk of misidentifying TE sequences as miRNAs. The polyploid nature of *Triticum* species renders the correct discrimination of homologous copies of miRNA more difficult for both in silico and experimental validation. Recently, two new scripts, "SUmirPredictor" and "SUmirLocator," have been developed to improve previous methods of miRNA prediction and identification, especially for species with highly repetitive genomic sequences (Alptekin et al. 2017). These freely available tools furnish comprehensive understanding of how miRNA precursors are located in the genome and transcriptome and their association with transposons.

Mapping miRNA in durum wheat

Our analysis was focused on mapping the identified tissue-specific miRNA and we delivered the first map of miRNA distribution in the two genomes of durum wheat. Although the genome sequence for *Triticum durum Desf* has not yet been completed, we mapped the miRNA to the durum wheat chromosomes that are present in the bread wheat genome. Mapping the miRNAs will allow the targeting of them through genetic improvement schemes assisted by molecular markers. In addition, our work can further the development of new molecular markers of important agronomic traits linked

Table 5 MicroRNAs differentially expressed between roots and leaves

Cluster name	Base mean	$\log_2$ Fold change	*p*-value	Known name
Cluster_10104	73.842	−6.231	5.91E-40	tae-MIR530
Cluster_11382	44.531	8.683	3.77E-14	Unknown
Cluster_11634	89.553	−2.097	1.20E-10	ata-MIR1432
Cluster_11636	578.675	−2.193	1.91E-08	ata-MIR1432
Cluster_12754	41.992	2.509	1.27E-10	ata-MIR171a
Cluster_13147	267.452	2.377	1.23E-18	ata-MIR9776
Cluster_13262	3.337	−3.671	1.00E-03	ata-MIR395a
Cluster_14263	63.869	3.522	4.31E-05	ata-MIR166c
Cluster_16064	524.537	−2.197	4.62E-08	ata-MIR1432
Cluster_16065	594.784	−2.133	1.51E-08	ata-MIR1432
Cluster_18140	72.432	−6.100	1.68E-39	tae-MIR530
Cluster_18775	40.373	2.518	2.64E-10	ata-MIR171a
Cluster_20589	279.681	−3.153	7.94E-16	ata-MIR393
Cluster_20808	3250.971	−0.937	1.22E-03	hvu-MIR159a
Cluster_26451	27.742	−1.812	1.00E-04	tae-MIR9676
Cluster_28135	360.330	5.964	6.42E-62	zma-MIR319b
Cluster_33442	56.529	1.347	2.03E-03	Unknown
Cluster_34518	59.294	−4.930	4.99E-28	Unknown
Cluster_35124	72.293	3.879	4.88E-14	Unknown
Cluster_38047	11.644	1.147	1.98E-02	Unknown
Cluster_41943	39,522.440	1.196	9.35E-06	ata-MIR166e
Cluster_42519	120.106	−1.734	2.36E-06	ata-MIR9674c
Cluster_43534	485.938	−6.642	6.78E-80	ata-MIR5168
Cluster_44265	25.156	2.656	6.93E-10	ata-MIR167a
Cluster_45124	51.401	0.878	9.26E-03	Unknown
Cluster_48327	490.112	−5.837	2.81E-56	ata-MIR5168
Cluster_49696	39,546.873	1.196	9.18E-06	ata-MIR166b
Cluster_50615	1238.103	1.994	8.41E-07	Unknown
Cluster_53303	4721.973	−2.719	1.71E-04	Unknown
Cluster_55297	48.980	2.787	7.26E-12	bdi-MIR396c
Cluster_55342	188.782	−2.487	9.44E-07	ata-MIR396c
Cluster_57508	580.931	−1.802	5.62E-11	tae-MIR167c
Cluster_59228	81.647	3.331	2.22E-12	Unknown
Cluster_59780	51.272	2.830	5.45E-13	ata-MIR396e
Cluster_59780	51.272	2.830	5.45E-13	ata-MIR396e
Cluster_62182	25.762	−2.739	1.88E-08	Unknown
Cluster_63022	140.125	−5.423	4.68E-33	tae-MIR5200
Cluster_63023	127.093	−5.388	5.96E-31	Unknown
Cluster_63134	50.893	2.820	8.48E-13	ata-MIR396e
Cluster_65295	36,643.371	1.470	8.16E-08	ata-MIR166d
Cluster_65537	1796.238	−1.353	4.27E-08	hvu-MIR5048a
Cluster_66590	34.121	−1.488	3.18E-04	Unknown
Cluster_66669	38.841	5.031	1.42E-21	Unknown
Cluster_6749	4.620	−2.687	7.24E-04	Unknown
Cluster_9890	16.453	−1.344	3.71E-03	Unknown

Cluster name, base mean, log2 fold change, *p* value and known name are indicated

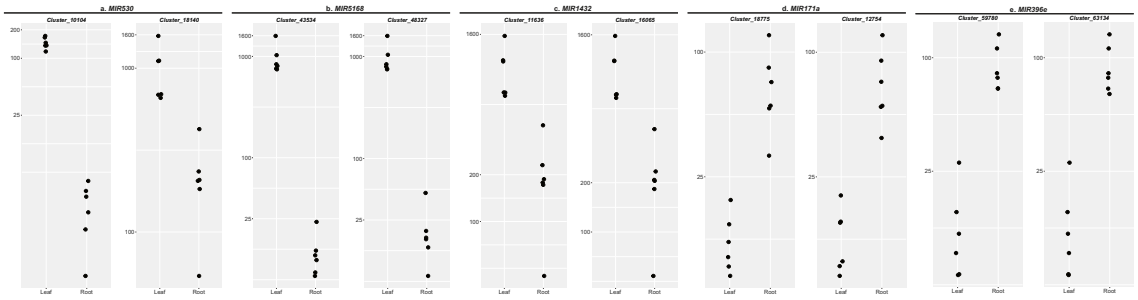

Fig. 3 MiRNA homeologous genes differentially expressed between roots and leaves. **a** miRNA530, **b** miRNA5168, **c** miRNA1432, **d** miRNA171, **e** miRNA396. X axis shows counts, Y axis shows plant tissues.

Fig. 4 Chromosome distribution of all identified miRNAs

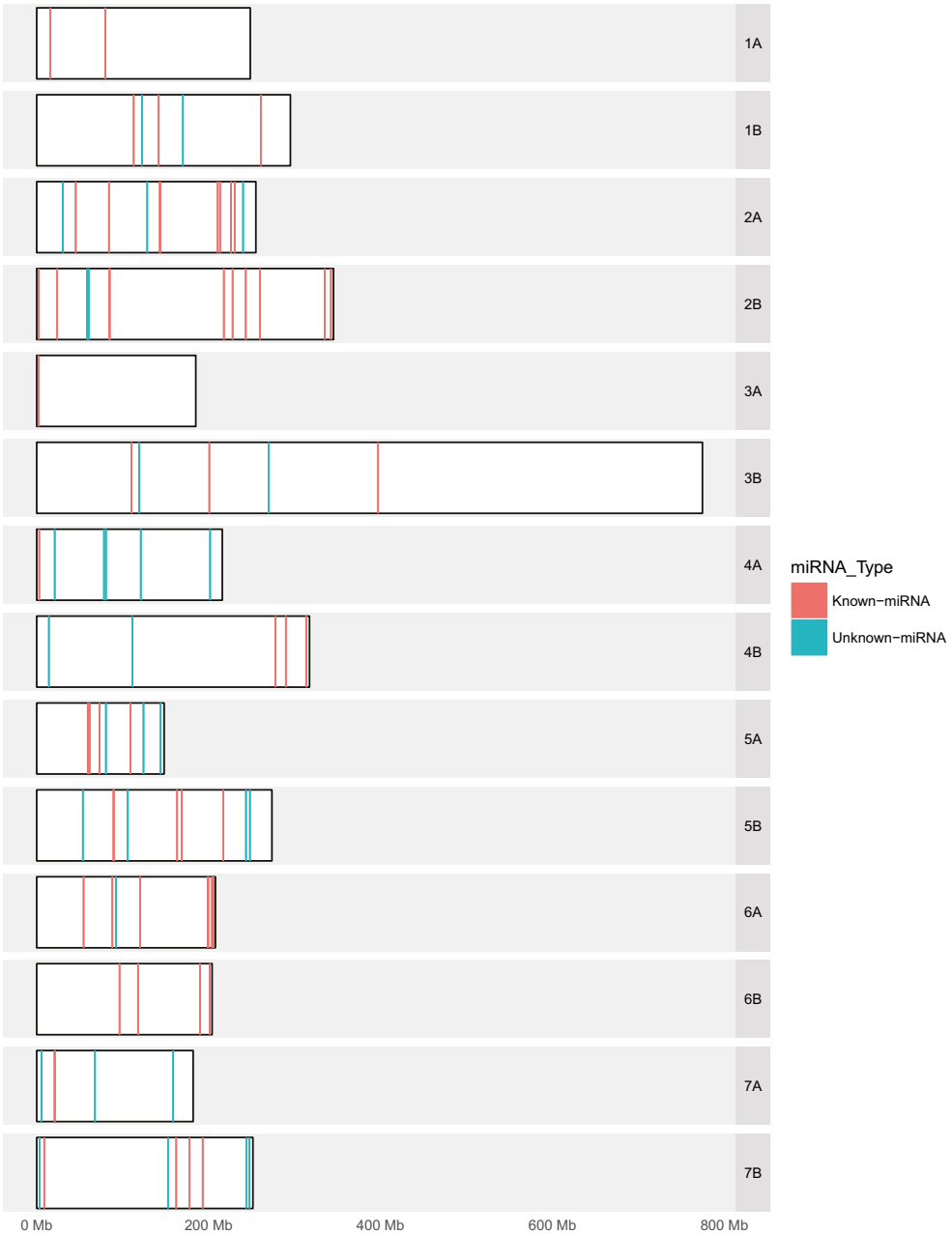

Table 6 List of homeologous known miRNAs in durum wheat

Cluster name	Chromosome	Coordinates(start-end)	Strand	Known name
Cluster_59780	7A	20,932,743–20,932,904	+	ata-MIR396e
Cluster_63134	7B	8,739,076–8,739,320	−	ata-MIR396e
Cluster_18775	2B	259,602,537–259,602,631	+	ata-MIR171a
Cluster_12754	2A	210,529,750–210,529,991	−	ata-MIR171a
Cluster_10104	2A	45,376,842–45,377,015	+	tae-MIR530
Cluster_18140	2B	217,777,285–217,777,488	+	tae-MIR530
Cluster_53245	6A	87,949,906–87,950,062	+	bdi-MIR156c
Cluster_57238	6B	96,378,404–96,378,579	+	bdi-MIR156c
Cluster_43534	5A	73,069,415–73,069,551	−	ata-MIR5168
Cluster_48327	5B	163,330,209–163,330,340	+	ata-MIR5168
Cluster_11636	2A	143,414,592–143,414,715	−	ata-MIR1432
Cluster_16065	2B	84,759,598–84,759,722	−	ata-MIR1432
Cluster_43357	5A	61,589,422–61,589,539	+	tae-MIR156
Cluster_48425	5B	168,674,312–168,674,427	−	tae-MIR156

Cluster name, chromosomal location, coordinates, strand and known name are indicated

with key miRNA actions. For example, 13 conserved miRNA (including miR166, miR172, and miR393) have been associated with genotypic diversity in relation to drought tolerance (Li et al. 2013). The mapping of these key miRNAs will be extremely important in the development of molecular markers useful for breeding purposes. We mapped miR156 in the wheat genome. Previous findings showed that miR156 members were modulated by drought in both wild and domesticated wheat, implying that this miRNA family is highly conserved between cereals. The function of this miRNA is to modulate the expression of SQUAMOSA promoter-binding like proteins. However, clear differences have been observed between the patterns of expression of members of this family, implying that they may have contributed to evolution of wheat species (Kantar et al. 2011, 2012; Kurtoglu et al. 2013, 2014). Previous studies have shown that the effect on drought tolerance might be dose-dependent, and their regulation might be due to the regulation of target genes using post-transcriptional or translation repression mechanisms. Seven conserved miRNAs and three novel miRNAs were analyzed in flag leaf and developing head tissues of different durum genotypes (Liu et al. 2015). These authors inferred the role of these miRNAs in water stress tolerance arising from the physiological modulation triggered by their target genes. These miRNAs are close relatives of wheat miRNAs mapped in the present work.

Leaves vs. roots

In the present work, we identified 58 miRNAs that were expressed in both leaves and root tissues and mapped to the A and B genomes. Of these, seven were homeologous gene pairs, present in both of the two genomes. Differential expression analysis highlighted 45 miRNAs significantly regulated in the leaf/root pairwise comparisons. miR530, miR395, miR393, miR5168, and miR396 were more expressed in leaves while miR166, miR171, miR319, and miR167 had higher expression in roots than in leaves.

Ma et al. (2015) characterized the miRNA in two different wheat genotypes. Three hundred and sixty seven differentially expressed miRNAs were reported and among them, there were members of miR156, miR166, miR167, miR168, and miR444. We observed significantly higher expression of miR166 in roots while miR167c was more expressed in leaves. Interestingly, miR166 and miR396 had opposite trends of expression patterns between tolerant and susceptible wheat genotypes. It is noteworthy that these two genes were also differentially regulated between leaf and root tissues. Both miRNAs were upregulated in root tissues compared to leaves, implying that they may have a key role in root physiological processes related to water stress conditions. miR166h targets HD-ZIP4 while miR396 targets GRFs (Ma et al. 2015). Indeed, these transcription factors might be involved in the modulation of genes involved in root architecture, development, and growth in response to drought.

Kantar et al. (2011) identified other miRNAs that were repressed in response to drought that showed to have a clear differential tissue-specific expression such as miR396 and miR166. MiR159a that targets a MYB3 transcription factor might play a role in cold-stress responses (Liu et al. 2013). Interestingly, we showed that this miRNA was more expressed in leaves than in roots. MYB genes have also been shown to be involved in plant tolerance to abiotic stresses through their action in hormone-related signaling networks (Phillips et al. 2007).

Fig. 5 Chromosome distribution of homeologous miRNAs

Functional analysis of miRNA targets

A high number of targets have been shown to have nucleic acid binding activities encoding proteins involved in signaling and defense responses. Several auxin-specific transcription factors and auxin-related genes have been identified as potential targets of abiotic stress-related miRNA (Liu et al. 2016b; Sun et al. 2016). It has been hypothesized that a miRNA-driven modulation of auxin genes might affect the lateral root development due to an altered auxin/cytokinin ratio (Su et al. 2011). Genes encoding lipid transfer proteins have been shown to be targeted by miRNA regulated under drought conditions (Liu et al. 2015). The regulation of these genes has been linked with genotypic differences in maintaining osmotic pressure. Lipid transfer proteins (LTPs) aid to prevent or adjust stress-induced damage in membranes related to changes in lipid composition (Jung et al. 2003). Interestingly, our data showed that a significant higher number of potential targets were related to lipid binding. We identified members of the miR164 family that target NAC transcription factors, which are known to have roles in various abiotic stress responses (Nakashima et al. 2012). Liu et al. (2015) found that several miRNA were drought responsive such as miR1136, miR1432, miR5048, miR5054, miR5071, miR5200, and miR6300. Most of them were successfully mapped to diverse chromosomes in the genomes A and B in wheat.

Conclusions

We have analyzed the miRNAome of these two tissues under two conditions, watered and drought, to determine if some of these tissue-specific miRNAs might be regulated under drought conditions. This work confirms the difficulties of analyzing the miRNAome under field conditions and suggests a need to perform studies under artificial conditions such as hydroponics. We have mapped and assigned key miRNAs involved in the response to each wheat chromosome, which will allow targeting of the miRNAs in genetic improvement schemes assisted by molecular markers. The specific expression of them in leaves or roots will also help to define their role in important plant developmental and physiological processes. The identification of different homeologous and the analysis of their expression trends are also essential to clarify the role of these miRNAs in the evolution of cereal species as well as their key agronomic aspects. The determination of expression patterns in different tissues is essential for the clarification of the role of key miRNAs in affecting the phenotype of important agronomic traits.

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Author's contributions FM conceived and designed the experiments. Experimental work was mostly performed by VF. EB designed and carried out all the computational analyses. Plant and soil measurements and analyses were performed by PR and DG. CC contributed to the discussion of experimental data. All authors contributed to write the manuscript.

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