RESEARCH ARTICLE

Impact of arbuscular mycorrhizal fungi (AMF) on gene expression of some cell wall and membrane elements of wheat (*Triticum aestivum* L.) under water deficit using transcriptome analysis

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Abstract Mycorrhizal symbiotic relationship is one of the most common collaborations between plant roots and the arbuscular mycorrhizal fungi (AMF). The first barrier for establishing this symbiosis is plant cell wall which strongly provides protection against biotic and abiotic stresses. The aim of this study was to investigate the gene expression changes in cell wall of wheat root cv. Chamran after inoculation with AMF, *Funneliformis mosseae* under two different irrigation regimes. To carry out this investigation, total RNA was extracted from the roots of mycorrhizal and non-mycorrhizal plants, and analyzed using RNA-Seq in an Illumina Next-Seq 500 platform. The results showed that symbiotic association between wheat and AMF and irrigation not only affect transcription profile of the plant growth, but also cell wall and membrane components. Of the 114428 genes expressed in wheat roots, the most differentially expressed genes were related to symbiotic plants under water stress. The most differentially expressed genes were observed in carbohydrate metabolic process, lipid metabolic process, cellulose synthase activity, membrane transports, nitrogen compound metabolic process and chitinase activity related genes. Our results indicated alteration in cell wall and membrane composition due to mycorrhization and irrigation regimes might have a noteworthy effect on the plant tolerance to water deficit.

Keywords Arbuscular mycorrhizae · Plant cell wall · RNA-Seq · *Triticum aestivum* · Water deficit

Introduction

Bread wheat (*Triticum aestivum* L.) is one of the main crops containing high carbohydrate content that provides more than one-third of the world population's food (Gill et al. 2004). Water deficiency is considered as one of the most significant limiting factors of wheat crop production and plant growth parameters in Iran (Nezhadahmadi et al. 2013), and other arid and semi-arid areas all around the world (Li et al. 2019). One of the most beneficial cooperation in the environment that can decrease adverse effects of abiotic stresses is arbuscular mycorrhizal symbiosis, in which mutualistic confrontation is beneficial for both plant and fungi (Ye et al. 2019). To colonize and establish a relationship, root cell wall is the first and basic border that must be passed by AMF, and initiate an interaction and nutrient exchange (Rich et al. 2014). Cell wall as the outermost part of the plant cells plays crucial roles in growth, development, preservation of the cell shape, as well as protection against variety of external and internal factors for plant (Essahibi et al. 2018). As apoplastic compartments of plant cell, cell wall and membrane are directly involved in establishment and development of the mycorrhizal symbiosis. For constructing the symbiotic structure in roots of host plant, it needs to access cell wall and membrane materials to make a conservative envelope which keeps and improves symbiotic relationship, and also plant cells integrity (Essahibi et al. 2018). Guether et al. (2009) have reported the induced genes related to plasma membrane and cell wall activities affected by AMF as plant response to water stress. Nanjareddy et al. (2017)

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performed comparative transcriptome analysis of *Phaseolus vulgaris* symbiotic-roots with AMF and identified differentially expressed genes associated with defense responses and cell wall. Recently, next generation sequencing (NGS) based on transcriptome (TC) has provided rapid genome wide transcript profiling. Transcriptome based analysis of genes helps to better understand biological processes including development, organogenesis and responses to biotic and abiotic stresses (Zhu et al. 2012; Brenchley et al. 2012; Jayasena et al. 2014; Lucas et al. 2014). Dong et al. (2012) used Illumina (NGS) for studying gene expression changes in *Sinapis alba* under water stress. However, identification of all genes associated with AMF symbiosis demands extensive further research (Garcia et al. 2017; Sugimura and Saito 2017; An et al. 2018; Nanjareddy et al. 2017; Li et al. 2018; Jacott et al. 2017). The experimental hypothesis of the present study was that the mycorrhization could improve the wheat tolerance to water stress by changing cell wall and membrane genes expression pattern in addition to the other common factors that influence the plant tolerance. To test the hypothesis, the contribution of an AM fungal species (*Funneliformis mosseae*) to a drought semi-tolerant cultivar, “Chamran”, was studied in both normal irrigation and water deficit conditions. Moreover, gene expression changes in cell membrane and cell wall were investigated using transcriptome analysis. This can be the first study on bread wheat plants, in which we exposed plants to four different growth conditions using AMF alleviation and different irrigation regimes, and our concentration has been on some genes related to cell wall and membrane and their relation with enhancement of plant tolerance to water shortage.

Materials and methods

Plant material and growth conditions

The wheat cultivar Chamran, a drought semi-tolerant cultivar, was used in this study. Seeds were surface-sterilized in 70% ethanol for 2 min, rinsed in sterile distilled water for three times, and soaked in 40% sodium hypochlorite for 10 min. Finally, seeds were rinsed six times in sterile distilled water. To germinate seeds, they were transferred into sterile Petri dishes with wet filter papers at 26 °C. After 3 days, 10 germinated seeds were planted into each 0.5 L plastic autoclaved pots containing sterile field soil, sand and perlite (2:1:1, V:V:V). The air-dried soil samples which collected from Tehran, Iran, were passed through a 2 mm sieve, and diluted with sand and perlite, sterilized for 1 h using 100 °C steaming on 3 consecutive days. The pH of soil was 8; the texture of soil before mixing was

composed of 35.5% sand, 15.4% clay, 48.7% silt, and almost 0.5% organic matters.

AMF and water deficit treatments

In order to evaluate the effect of AMF and water deficit on transcript level of Chamran cultivar, four combinations of treatments were performed in a factorial experiment design in two levels for both AMF (inoculated and non-inoculated) and irrigation (normal and water deficit) with three replications and 10 seedlings per replicate. For AMF inoculation, *Funneliformis mosseae*, isolate *Gmb17*, was provided by Department of Plant Protection, Faculty of Agriculture, Rafsanjan University, Iran.

Inoculum was prepared in the pots containing 100–120 mycorrhizal infection unit/g of a mixture of soil and sand and the germinated seeds were planted.

The pots of each level of AMF treatment, inoculated and non-inoculated plants, were irrigated in two levels including normal irrigation (each pot three times weekly, 50 ml each time) and water deficit condition (each pot three times weekly irrigating, 25 ml per time) for 8 weeks.

Mycorrhizal colonization assessment

For colonization assessment, four 8-week old plants and 15 segments of roots of both AMF treatments were removed and washed in tap water. After washing, the roots were cleared in 10% KOH solution, and stained with 0.1% trypan blue according to Phillips and Hayman method (1970). For evaluating of colonization, roots were cut down into 1 cm fragments and located on microscopic slides in lactoglycerol. Analysis of colonization percentage was performed based on McGonigle et al. (1990) and under a light microscope.

Assessment of plant growth parameters

After 8 weeks, plants were harvested and some growth related parameters such as root and shoot lengths, root and shoot fresh weights, and spike fresh weight were measured.

RNA extraction and NGS sequencing

Eight weeks after transplanting germinated seeds into the pots, two biological replicates of root samples were prepared from each treatment, including mycorrhizal plants in normal irrigation and water deficit conditions and also non-mycorrhizal plants in normal irrigation and water deficit condition. Each biological replicate contained a pooled root sample of 10 seedlings. Then, total RNA was extracted from 30 mg of root tissues of each pooled sample using Qiagen kit according to the manufacturer's instruction and

immediately stored at -80°C . RNA integrity and concentration was measured using gel electrophoresis and Agilent 2100 Bio-analyzer. An Illumina TruSeq stranded total RNA HT sample preparation kit was used to convert total RNA into library based on manufacturer's instruction with an average library size of 360 bp. The reads were demultiplexed with one allowed mismatch and "no lane splitting". Libraries were sequenced using an Illumina Next Seq 500/High Output Chemistry with 150 bp paired-end reads. Estimated output was approximately 120 Gb and 800 Million reads, paired-end sequencing with 150 bp read length generated from eight samples.

Paired-end RNA-seq reads were mapped to wheat reference genome (http://plants.ensemble.org/Triticum_aestivum; Clavijo et al. 2017) using TopHat v2.1.1 (<http://ccb.jhu.edu/software/tophat/index.shtml>; Kim et al.; licensee BioMed Central Ltd. 2013) and Bowtie2 v2.2.9 (Langmead and Salzberg 2012). Assembling of transcripts and determining their expression level were performed using Cufflinks (v2.2.1) based on fragments per kb of exon per million mapped reads (FPKM). A q value of 0.01 and Log twofold change (LOG_2FC) ≥ 2 and ≤ -2 was considered as significant threshold for gene differential expression.

Quantitative real-time-PCR analysis

The quantitative RT-PCR was performed to validate the RNA-Seq data in four randomly selected transcripts. Gene specific primers were designed using Oligo 7 software. Total RNA from control and treatment of each condition was treated with 1.5 μl DNAaseI to remove DNA contamination. cDNA was synthesized by mixing 2 μg of total RNA, 0.2 μl specific primers, 0.5 μl Oligo dT and 10 μl DEPC water using Thermo-scientific kit according to manufactures' instructions. Then, the mixture was incubated at 65°C for 10 min; 10 μl of thermo-scientific master mix 2X was added, and the samples were incubated at 42°C for 90 min.

Real-time PCR was carried out using Step-one RT-PCR and 1 μl of cDNA template, 0.3 μl of primers, 3 μl SYBR green mix 5X Takara mix, 0.25 μl ROX and 15 μl DEPC water. The PCR cycling program included of: 94°C for 120 s, 94°C for 15 s, 60°C for 30 s and 72°C for 20 s, and melting curve point was tested in the range of 60 and 95°C with a heating rate of 5°C for every 15 s. Treatment and control samples were assessed in two independent biological replicates.

The relative expression was calculated using $2^{-\Delta\Delta\text{CT}}$ method (Rasmussen 2001). Elongation factor-1- α (EF-1 α) gene (AF475129), a housekeeping gene, was used as endogenous gene to normalize the expression level of the target genes (Balestrini and Lanfranco 2006).

Statistical analysis

Statistical analysis of data was carried out using SPSS program. The mean differences were estimated based on the Duncan test at a probability of 5%.

Result

Detection of mycorrhizal colonization percentage

Non-inoculated roots did not show any colonization with AMF in roots. The colonization rate in inoculated roots under water deficiency and normal irrigated plants was 55% and 72.5% respectively (Fig. 1).

Analysis of plant growth related traits

The individual application of AMF significantly increased root fresh weight by 23.8% when compared to the untreated control (Fig. 2a). Water deficit treatment led to the significant decrease in root fresh weight by 72.5% relative to the control. Interestingly, the mycorrhiza treatment mitigated the growth inhibition associated with water deficit by 15.5% (Fig. 2a). Showing similar trend, AMF inoculation enhanced shoot fresh weight by 15.6% than the control. Water deficit significantly reduced shoot fresh weight by 56.7% which was relieved by AMF supplementation and reached to 32% in the AMF + Water deficit treatment group (Fig. 2b). Similarly, the water deficit was associated with significant decrease in both root and shoot length (Fig. 2c, d). Moreover, water deficit-associated reduction in spike fresh weight was significantly alleviated by AMF utilization (Fig. 2e).

Fig. 1 Colonization rate of two inoculated treatments: D + M: mycorrhizal plants under water deficiency, N + M: non-mycorrhizal plants with normal irrigation

Fig. 2 Water deficit and/or mycorrhiza-mediated changes in growth-related parameters. **a** root fresh weight, **b** shoot fresh weight; **c** root length, **d** shoot length; **e** spike fresh weight. Bars indicate the standard errors

Bioinformatic analysis of NGS RNA-Seq data

To perform bioinformatics analysis, TopHat Alignment software v 2.1.1 was used, and reads trimmed to 125 bp; also, read mapping and FRKM estimation of reference genes and transcripts were performed using TopHat 2 aligner. Assembly of novel transcripts, and differential expression of novel and reference transcripts were implemented with Cufflinks v 2.2.1 software. The summary of

the bioinformatics data obtained in this study is shown in Table 1.

Paired-end RNA-Seq reads were obtained from each library aligned to *Triticum aestivum* genome sequence accessible at http://plants.ensembl.org/Triticum_aestivum for distinguishing per gene molecular function or biological processes. Log 2 fold change (LOG_2FC) ≥ 2 and ≤ -2 were considered for dividing genes to two up-regulated and down-regulated genes, subsequently.

Table 1 Read numbers achieved from bioinformatics analysis

Sample name	Sequenced reads	Overall read mapping rate (%)	Aligned pairs	Multiple alignments	Discordant alignments	Concordant alignment (%)
A (D – M)	59651235	56.1	31304199	2720387 (8.7%)	757957 (2.4%)	51.2
E (D – M)	63094284	55.1	32499992	3114223 (9.6%)	898573 (2.8%)	50.1
B (D + M)	68576329	51.2	32762588	5609760 (17.1%)	1951342 (6.0%)	44.9
D (D + M)	81053620	49.9	37989411	5144110 (13.5%)	1626126 (4.3%)	44.9
G (N – M)	72618555	44.6	29503597	2708038 (9.2%)	642998 (2.2%)	39.7
K (N – M)	63905579	38.7	22643132	4092368 (18.1%)	1217924 (5.4%)	33.5
H (N + M)	64067079	49.4	29488910	2548789 (8.6%)	656539 (2.2%)	45.0
I (N + M)	59966731	51.2	28647390	2643137 (9.2%)	742274 (2.6%)	46.5

D – M: non-mycorrhizal roots under water deficiency, N – M: non-mycorrhizal roots with normal irrigation, D + M: mycorrhizal roots under water deficiency, N + M: mycorrhizal roots with normal irrigation

Table 2 Summary of identified genes in four different libraries

Data	N – M	N + M	D – M	D + M
Total annotated genes	83691	84513	84418	85454
Up-regulated genes	10966	11825	10329	13405
Down-regulated genes	1709	2608	3226	2081
Significant genes based on expression difference	1759	4226	2701	4808
Genes with known function	1156	2535	1680	3049
Genes with unknown function	603	1691	1021	1759
ncRNA (in significant genes)	27	149	135	132

N – M: non-mycorrhizal roots with normal irrigation, N + M: mycorrhizal roots with normal irrigation, D – M: non-mycorrhizal roots under water stress D + M: mycorrhizal root under water stress

Table 3 Induced and suppressed transcripts identified in wheat cv. Chamran under water-deficit

Gene ID	Protein name	Value_1 (D-M)	Value_2 (N-M)	Accession ID in NCBI	Source organism
TRIAE_CS42_4BL_TGACv1_321131_AA1055910	Anti-sigma-I factor RsgI6-like	0	0.67093	XM_020311775.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4BL_TGACv1_320705_AA1046770	Xyloglucan endotransglucosylase/hydrolase protein 31-like	0	0.41745	XM_020338007.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_U_TGACv1_642890_AA2124500	Chi gene for endochitinase	1.97046	0	X76041.1	<i>T.aestivum</i> (Chinese spring)
TRIAE_CS42_7DS_TGACv1_622542_AA2041460	Patatin-like protein 2	3.80167	0	XM_020293126.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_6DL_TGACv1_526537_AA1686380	Putative laccase-9	19.6026	0	XM_020320013.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4DL_TGACv1_344866_AA1150230	Malate dehydrogenase [NADP] 1	12.0765	0	XM_020325953.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>

Table 4 DEGs identified in wheat cv. Chamran showing down-regulation and up-regulation under water deficit

Gene ID	Protein name	Log2 (fold change)	Accession ID in NCBI	Source organism
TRIAE_CS42_4BS_TGACv1_327875_AA1077360	Protein ZINC INDUCED FACILITATOR-LIKE 1-like	- 7.0352	XM_020334708.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2BL_TGACv1_129932_AA0399780	Beta-glucosidase BoGH3B-like	- 5.3941	XM_020296045.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2BL_TGACv1_133066_AA0441180	WT003_M11	- 3.9284	AK332352.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_3DL_TGACv1_250209_AA0864280	cht4 chitinase	- 3.6586	KR049250.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_4DS_TGACv1_361542_AA1169880	Putative clathrin assembly protein Atlg33340	- 3.1668	XM_020300262.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2AL_TGACv1_095187_AA0308020	Beta-glucosidase BoGH3B-like	- 3.1291	XM_020296045.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2BL_TGACv1_129576_AA0389360	Putative xyloglucan endotransglucosylase/hydrolase protein 13	- 2.8189	XM_020307537.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2BL_TGACv1_130022_AA0401820	WT003_N09	- 2.6079	AK332377.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_2AL_TGACv1_093126_AA0272780	Fructan 6-exohydrolase	- 2.5755	AB196524.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_U_TGACv1_643084_AA2127090	Lipase-like	- 2.5461	XM_020344369.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2DL_TGACv1_158029_AA0506490	Xyloglucan endotransglucosylase/hydrolase protein 24-like	- 2.2207	XM_020307539.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4BS_TGACv1_328000_AA1080810	HIPL1 protein-like	- 2.1827	XM_020300864.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4BS_TGACv1_328633_AA1091330	sn1-specific diacylglycerol lipase beta	- 2.0071	XM_020326437.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_7DL_TGACv1_604135_AA1994270	Patatin-like protein 2	2.01008	XM_020306914.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2BL_TGACv1_132929_AA0440510	Beta-glucosidase	2.02	AB236423.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_1BS_TGACv1_049914_AA0164140	tp1b0043f07	2.04793	AK450976.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_4BL_TGACv1_320333_AA1035610	B12 alpha expansin 6 (EXPA6)	2.12293	KC441067.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_1BS_TGACv1_049965_AA0164870	Phenylalanine ammonia-lyase-like	2.18288	XM_020317649.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2BL_TGACv1_129561_AA0388630	Beta-glucosidase	2.25564	AB100035.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_4DL_TGACv1_343409_AA1134090	Omega-3 fatty acid desaturase, endoplasmic reticulum-like	2.38774	XM_020337300.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4BS_TGACv1_328921_AA1095710	Beta-tubulin (TUB)	2.71602	MG852134.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_1BS_TGACv1_050729_AA0174830	Phenylalanine ammonia-lyase-like	2.81184	XM_020319266.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_3B_TGACv1_246242_AA0834280	Expansin-A24-like	3.38153	XM_020322455.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2AL_TGACv1_094425_AA0297430	Beta-glucosidase	5.74049	AB548284.1	<i>Triticum aestivum</i> L.

Fig. 3 Log2 (fold change) of down and up-regulated genes under water stress. 1: transmembrane transport, 2, 3, 6, 8, 9, 12, 15, 19: carbohydrate metabolic process, 4: chitinase activity, 5: clathrin coat assembly, 7: xyloglucan metabolic process, 10, 13, 20: lipid

metabolic process, 11: cellular glucan metabolic process, 14: lipid catabolic process, 16, 18, 22: L-phenylalanine catabolic process, 17, 23: plant-type cell wall organization, 21: microtubule-based process, 24: beta-glucosidase activity

Differentially expressed genes

In the present study, we generated four libraries including non-mycorrhizal roots with normal irrigation (N – M), mycorrhizal roots with normal irrigation (N + M), non-mycorrhizal roots under water deficiency (D – M) and mycorrhizal root under water deficiency (D + M).

While the published “*Triticum aestivum* L.” transcriptome includes 174639 gene transcripts, from among, 104390 are coding genes and 18087 are non-coding genes (http://plants.ensemble.org/Triticum_aestivum), we totally found 114428 genes expressed in wheat roots from four libraries of which, 12774 transcript were differentially expressed, 8754 transcripts had known biological process and molecular function; 1022 transcripts were non-coding and others were still unknown for their biological and molecular functions.

The number of identified genes in each library and treatment is summarized in Table 2.

Transcriptome in each condition was screened for differentially expressed genes (DEGs) associated with cell components namely cell wall and plasma membrane. Some genes were only induced or suppressed in one special treatment and considered as specific responsive genes.

DEGs associated with cell wall and plasma membrane under water deficiency (D – M vs N – M)

Considering the cutoff threshold of log2 (≥ 2 and ≤ -2) and q value of ≤ 0.01 , a total number of 30 genes, associated with cell wall and plasma membrane showed significantly differential expression under water deficit. Among the up-regulated genes, chitinase activity, lipid

metabolic process, lignin catabolic process and malate dehydrogenase activity genes were only induced in plants under water deficit condition but not in normal irrigation regime suggesting water deficit responsive genes (see Tables 3, 4 and Fig. 3). Compared to normal irrigation in our experiments, water deficit could also up-regulate the expression of genes related to carbohydrate metabolic process, lipid catabolic process, oxidation/reduction process, hydrolase activity and plant-type cell wall organization, and down-regulate the expression of genes associated with carbohydrate metabolic process, lipid metabolic process, transmembrane transport and cell wall macromolecule catabolic process (13 genes).

DEGs associated with cell wall and plasma membrane in AMF-colonized wheat root (N – M vs N + M)

This experiment was performed to identify the expressed genes in the symbiosis between mycorrhiza and wheat in normal irrigation. Generally, 23 and 24 up- as well as down-regulated genes related to cell wall and plasma membrane were identified, respectively (Table 5, Fig. 4).

Among DEGs, 13 significant genes classified as a group of carbohydrate metabolic process; 7 showed up-regulation, and 6 were down-regulated due to mycorrhizal symbiosis. Genes related to hexose metabolic process, UDP-arabinopyranose mutase activity and alpha mannosidase activity were up-regulated. 5 genes were associated with lipid metabolic process; 2 appeared as up-regulated, and three were down-regulated. 2 cellulose biosynthetic process genes indicated increase in their expression influenced by mycorrhization. Among down-regulated genes, 3 of them were related to plant-type cell wall organization, 2

Table 5 DEGs identified in wheat cv. Chamran showing down-regulation and up-regulation in AMF colonized roots under normal irrigation

Gene ID	Protein name	Log2 (fold change)	Accession ID in NCBI	Source organism
TRIAE_CS42_2AL_TGACv1_094425_AA0297430	Beta-glucosidase	– 5.0471	AB548284.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_2BL_TGACv1_129561_AA0388630	Beta-glucosidase	– 3.7674	AB100035.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_2AL_TGACv1_093096_AA0271920	Probable xyloglucan endotransglucosylase/hydrolase protein 12	– 3.7085	XM_020317472.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_7AS_TGACv1_569995_AA1828380	Expansin-A17-like	– 3.3671	XM_020293675.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_3AS_TGACv1_212195_AA0698660	Xyloglucan endotransglucosylase/hydrolase protein 8-like	– 3.3353	XM_020316160.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4AS_TGACv1_306275_AA1005550	Phytosulfokines 4-like	– 3.3329	XM_020303724.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2BL_TGACv1_131489_AA0428390	Xyloglucan endotransglucosylase/hydrolase protein 12	– 3.1424	XM_020317472.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4BS_TGACv1_329606_AA1102700	Xylanase inhibitor	– 3.0417	AB302973.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_7DS_TGACv1_622353_AA2038220	Expansin-A17-like	– 2.9	XM_020293675.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4DL_TGACv1_342392_AA1112380	Phytosulfokines 4-like	– 2.6674	XM_020303724.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_1DL_TGACv1_062431_AA0214390	Patatin-like protein 1	– 2.4622	XM_020344309.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_1BL_TGACv1_033545_AA0140280	Patatin-like protein 1	– 2.4214	XM_020344309.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4BS_TGACv1_328921_AA1095710	Beta-tubulin (TUB)	– 2.3301	MG852134.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_4BS_TGACv1_327950_AA1079560	WT005_P03	– 2.299	AK333221.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_5AL_TGACv1_374195_AA1193240	Beta-glucosidase 30-like	– 2.2883	XM_020332410.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_7BL_TGACv1_578733_AA1899350	Agglutinin isolectin 3-like	– 2.2651	XM_020325269.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_3DS_TGACv1_271881_AA0910150	Xyloglucan endotransglucosylase/hydrolase protein 8-like	– 2.2407	XM_020316162.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4BL_TGACv1_320919_AA1051690	Isoform GSlr1 (GS)	– 2.2254	AY491968.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_4DL_TGACv1_345130_AA1151850	Laccase-3-like	– 2.149	XM_020292685.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4DS_TGACv1_361309_AA1165460	Beta-3 chain	– 2.1292	XM_020308075.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_1BS_TGACv1_051414_AA0179560	tp1b0012118	– 2.0773	AK456462.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_4DS_TGACv1_363201_AA1183500	tp1b0005g02	– 2.0566	AK454358.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_4BL_TGACv1_320618_AA1044770	Laccase-3-like	– 2.0072	XM_020292685.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_3DL_TGACv1_250306_AA0865810	Expansin-A8-like	– 2.0057	XM_020317488.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4BL_TGACv1_321498_AA1061390	Beta-glucosidase 6	2.05694	XM_020290811.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_1BS_TGACv1_049914_AA0164150	Phenylalanine ammonia-lyase-like	2.08107	XM_020317638.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2DS_TGACv1_177268_AA0571460	Uncharacterized	2.12878	XM_020322743.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2DL_TGACv1_158049_AA0507280	Aldose 1-epimerase-like	2.13367	XM_020290960.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2AS_TGACv1_114151_AA0364450	Gamma gliadin-A1	2.30073	MG560140.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_2AS_TGACv1_113897_AA0362110	Putative amidase C869.01	2.309	XM_020311367.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_1AL_TGACv1_001256_AA0027880	Endo-1,3-beta-glucosidase 13-like	2.43686	XM_020325014.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_1BL_TGACv1_033482_AA0139800	Glucan endo-1,3-beta-glucosidase 13-like	2.44328	XM_020325006.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4DS_TGACv1_360999_AA1158040	SET4_M23	2.52346	AK330584.1	<i>Triticum aestivum</i> L.

Table 5 continued

Gene ID	Protein name	Log2 (fold change)	Accession ID in NCBI	Source organism
TRIAE_CS42_1BL_TGACv1_030467_AA0091630	ABC transporter B family member 11-like	2.60003	XM_009391731.2	<i>Musa acuminata</i> subsp. malaccensis
TRIAE_CS42_1DS_TGACv1_080643_AA0251500	Laccase-25-like	2.68528	XM_020300631.1	<i>Aegilops tauschii</i> subsp. tauschii
TRIAE_CS42_4AL_TGACv1_290204_AA0982950	tp1b0045110	2.78117	AK451279.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_2BL_TGACv1_129780_AA0395640	Triacylglycerol lipase 2-like	2.87066	XM_020303075.1	<i>Aegilops tauschii</i> subsp. tauschii
TRIAE_CS42_2BS_TGACv1_147826_AA0487990	GT75-3	3.1223	KM670460.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_2BS_TGACv1_146914_AA0475330		3.24964		
TRIAE_CS42_2DS_TGACv1_180213_AA0610440	UDP-arabinopyranose mutase 3	3.27702	XM_020345004.1	<i>Aegilops tauschii</i> subsp. tauschii
TRIAE_CS42_2AS_TGACv1_113522_AA0357250	UDP-arabinopyranose mutase 3	3.42686	XM_020345004.1	<i>Aegilops tauschii</i> subsp. tauschii
TRIAE_CS42_2BS_TGACv1_145994_AA0452200	WT004_F09	3.68528	AK332578.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_2DL_TGACv1_157981_AA0504650	Beta-glucosidase 12-like	3.70993	XM_020329910.1	<i>Aegilops tauschii</i> subsp. tauschii
TRIAE_CS42_2AS_TGACv1_113828_AA0361200	Syntaxin-132-like	3.71212	XM_020290887.1	<i>Aegilops tauschii</i> subsp. tauschii
TRIAE_CS42_4BS_TGACv1_331402_AA1109940	Heparan-alpha-glucosaminide N-acetyltransferase-like	4.87088	XM_020314296.1	<i>Aegilops tauschii</i> subsp. tauschii
TRIAE_CS42_4BS_TGACv1_327875_AA1077360	Protein ZINC INDUCED FACILITATOR-LIKE 1-like	5.5069	XM_020334708.1	<i>Aegilops tauschii</i> subsp. tauschii
TRIAE_CS42_1BS_TGACv1_051503_AA0180180	Alpha-mannosidase-like	6.50912	XM_020334225.1	<i>Aegilops tauschii</i> subsp. tauschii

were linked to cell proliferation, 2 genes were involved in microtubule based process; 4 genes were annotated as transmembrane transports.

Apart from normal irrigation condition in this comparison, AMF led to the expression of 19 genes that were not expressed in control plants (Table 6). The most of these induced genes were associated with transporter activity and cell growth, and this can be attributed to the effect of AMF on improving plant behavior.

DEGs associated with cell wall and plasma membrane in AMF- roots under water deficit (D – M vs D + M)

Colonization of wheat roots with AMF changed the transcriptome profile of water-deficit plants. Compared to non-AMF roots, we observed 6 and 28 down-regulated and up-regulated genes in AMF-roots, respectively, which were associated with cell wall and plasma membrane (Table 7).

The interaction between mycorrhiza and water-deficit, caused up-regulation of 28 genes related to carbohydrate metabolic process, fatty acid metabolic process, lipid metabolic process, transmembrane transport activity, hydrolase activity, cellulose biosynthetic process, mannose metabolic process and ligase activity. 11 of these genes (see Table 7 and Fig. 5) were classified as a group of carbohydrate metabolic process of which some of them had role in cell wall biogenesis, consisting of genes related to mannosidase activity, galactoside-2-alpha-L-fucosyltransferase activity, hexose metabolic process, β -glucosidase activity, UDP-arabinopyranose mutase and hydrolase activity. Two other genes were associated with cellulose biosynthetic process (KM670460.1, XM_020345004.1). In AMF plants, ten membrane-related genes as transporters and compartments were up-regulated in wheat plants under water deficit.

In addition to up-regulated transcripts associated with cell wall and membrane, 25 genes were recognized only in AMF inoculated roots but not in the control samples suggesting AMF specific genes under water deficit.

However, only 1 gene was suppressed in the treated samples (Table 8).

DEGs related to cell wall and plasma membrane in AMF roots under water deficit and normal irrigation

In this experiment, the transcripts of AMF colonized roots under water deficit were compared to normal irrigation.

By evaluating the difference between their gene expression patterns, we identified 25 significantly DEGs associated with cell wall and plasma membrane. Among these, 12 and 13 genes down-regulated as well as up-regulated in D + M (Table 9 and Fig. 6). Regarding the slight difference between these two treatments, it could be

Fig. 4 Log2 (fold change) of down- and up-regulated genes in symbiotic normal-irrigated plants. 1: scopolin beta-glucosidase activity, 2, 8, 14, 15, 22, 25, 31, 32, 43: carbohydrate metabolic process, 3: xyloglucan metabolic process, 4, 9, 24, 33: plant-type cell wall organization, 5, 7, 17: cellular glucan metabolic process, 6, 10: growth factor activity, 11, 12, 21, 37: lipid metabolic process, 13, 20: microtubule-based process, 16: chitin binding, 18: nitrogen compound

metabolic process, 19, 23, 35: lignin catabolic process, 26: L-phenylalanine catabolic process, 27, 29, 39: D-alanine ligase activity, 28: hexose metabolic process, 30: carbon–nitrogen ligase activity, 34: transmembrane transport, 36: long-chain fatty acid-CoA ligase activity, 38, 41: cellulose biosynthetic process, 40: UDP-arabinopyranose mutase activity, 42, 44: SNAP receptor activity, 45, 46: integral component of membrane, 47: mannose metabolic process

concluded that the effect of mycorrhiza on the amount of expression was greater than that of irrigation regimes. 10 significantly expressed genes were categorized as a group of carbohydrate metabolic process of which half were down-regulated and the others were up-regulated. 2 up-regulated genes were involved in lipid metabolic process, and the remaining two had role in cell division and plant-type cell wall organization. Considering the specific genes, three were expressed only in D + M samples. Cellulose synthase activity, plant-type cell wall organization and carbohydrate metabolic process genes were found specific related to N + M, and the genes associated with lipid metabolic process, chitin binding and plant-type hypersensitive response only expressed in D + M (Table 10).

Validation of DEGs by q-RT PCR

To confirm the accuracy of the result of transcriptome analysis, the expression of three randomly selected DEGs, in RNA-Seq analysis were examined by qRT-PCR (Fig. 7).

The genes examined with q-RT PCR represented the close expression level similar to RNA-Seq data. The selected genes were associated to cellulose biosynthetic process (XM_020345004.1), beta-glucosidase BoGH3B-like (XM_020296045.1), and chitinase activity (AY973229.1), respectively.

Discussion

Since severe soil drought significantly decrease root AMF colonization and hyphal growth in the soil, we applied a mild water stress in our experiments. The beneficial role of the symbiosis relationship concerning the tolerance to abiotic stresses is clearly revealed (Bernardo et al. 2019;

Ren et al. 2019; Ouledali et al. 2019). AMF inoculation not only enhanced plant growth and yield, but also improved plant resistance against water deficit. In line with our findings, AMF improved plant growth and productivity under water stress situation (Li et al. 2019). The improvement of plant growth can be related to root system development and recuperating of water and minerals accessibility. This conclusion is in corroboration with previous findings (Nezhadahmadi et al. 2013; Li et al. 2019; Ye et al. 2019).

To discover novel genes involved in plant-AMF interaction in different conditions, whole-genome Transcriptome analysis technique was used. Moreover, applying two biological replicates per condition with 10 pooled roots per replicate for sequencing can strengthen our results. We have also considered precise criteria in order to separate differential expressed genes which have resulted in the omission of genes with low expression differences.

Interaction with arbuscular mycorrhizal fungus *Funneliformis mosseae*, cell wall of plant root is the first line of defense which can highly protect the plant. On the other hand, in mycorrhizal symbiosis, plant root cell wall opens a way for symbiotic establishment, development and nutrient exchange (Rich et al. 2014). In the present study, we tried to connect some cell wall and membrane genes generated from RNA-Seq data set to plants growth and tolerance against water deficit in the presence or absence of AMF; since the most up- and down- regulated genes related to transferase activity and biosynthetic processes are cell membrane proteins (An et al. 2018; Vangelisti et al. 2018). Here we focused on several DEGs that were directly or indirectly involved in cell wall and membrane modulation and synthesis, and tried to make a connection between these DEGs and plant tolerance. It seems that the plant cell wall plays a significant role in stress perception through

Table 6 Induced transcripts identified in wheat cv. Chamran AMF colonized roots in normal irrigation

Gene ID	Protein name	Value 1 (N – M)	Value 2 (N + M)	Accession ID in NCBI	Source organism
TRIAE_CS42_6DL_TGACv1_527547_AA1705600	Laccase-21-like	0	0.54396	XM_020336472.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_1AL_TGACv1_000750_AA0018290	Glucan endo-1,3-beta-glucosidase 13-like	0	1.56382	XM_020325012.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2BL_TGACv1_132538_AA0438240	Acidic endochitinase-like	0	19.0282	XM_020321162.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2BL_TGACv1_130318_AA0408760	Acidic endochitinase-like	0	19.645	XM_020321162.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_7DL_TGACv1_603253_AA1979180	Putative cellulose synthase A catalytic subunit 11	0	1.02689	XM_020331230.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_1AL_TGACv1_001368_AA0029420	S-type anion channel SLAH2-like	0	0.38224	XM_020295652.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_1AS_TGACv1_021078_AA0080890	CASP-like protein 4D1	0	2.29829	XM_020308584.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2BL_TGACv1_130367_AA0409580	WAT1-related protein Atlg68170-like	0	1.79156	XM_020322988.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_3AS_TGACv1_211406_AA0689890	Phenylalanine ammonia-lyase-like	0	0.66478	XM_020341608.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_3B_TGACv1_226641_AA0818320	Ammonium transporter 3 member 1-like	0	6.40827	XM_020310974.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_3DL_TGACv1_249337_AA0845800	Sugar transport protein 1-like	0	1.14417	XM_020334105.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4AS_TGACv1_306829_AA1013910	WAT1-related protein Atlg68170-like	0	0.7281	XM_020338332.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4BL_TGACv1_321771_AA1065240	Heptahelical transmembrane protein 4-like	0	0.85227	XM_020290761.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4DL_TGACv1_344866_AA1150230	Malate dehydrogenase [NADP] 1	0	5.60577	XM_020325953.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_1BL_TGACv1_031679_AA0118650	Beta-glucosidase 22-like	0	6.93309	XM_020330200.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2BL_TGACv1_129438_AA0383660	COBRA-like protein 7	0	0.4556	XM_020312442.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2BL_TGACv1_130858_AA0418950	Beta-glucosidase 12-like	0	8.15626	XM_020329910.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2DS_TGACv1_180706_AA0611240	COBRA-like protein 7	0	0.72135	XM_020322746.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2AS_TGACv1_112645_AA0342840	Copper transporter 4-like	0	0.59084	XM_020325425.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>

Table 7 DEGs identified in wheat cv. Chamran showing down-regulation and up-regulation in AMF colonized roots under water deficiency

Gene Id	Protein name	Log2(fold change)	Accession ID in NCBI	Source organism
TRIAE_CS42_3B_TGACv1_224369_AA0795760	HIPL1 protein-like	− 3.9997	XM_020331354.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4BL_TGACv1_320919_AA1051690	Isoform GSR1 (GS)	− 2.5829	AY491968.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_4AS_TGACv1_307728_AA1023060	Cereale cytosolic glutamine synthetase isoform (GS1-4)	− 2.2636	JN188394.1	<i>Triticum turgidum</i> subsp. <i>durum</i>
TRIAE_CS42_2DL_TGACv1_158029_AA0506490	Xyloglucan endotransglucosylase/hydrolase protein 24-like	− 2.2546	XM_020307539.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4DL_TGACv1_343321_AA1133210	Glutamine synthetase isoform GSR2 (GS) gene	− 2.1786	AY491969.1	<i>Triticum aestivum</i>
TRIAE_CS42_2AL_TGACv1_093096_AA0271920	Probable xyloglucan endotransglucosylase/hydrolase protein 12	− 2.1437	XM_020317472.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_1AL_TGACv1_002286_AA0040530	Fatty acid amide hydrolase-like	2.0061	XM_020323605.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2AS_TGACv1_112829_AA0345860	Plastid acetyl-CoA carboxylase (Acc-1) gene	2.02936	EU660900.1	<i>Triticum aestivum</i>
TRIAE_CS42_2AL_TGACv1_093900_AA0288950	Beta-glucosidase 16-like	2.07524	XM_020327283.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_5BS_TGACv1_423370_AA1375340	PI-PLC X domain-containing protein At5g67130-like	2.16637	XM_020312303.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2DL_TGACv1_157992_AA0505150	Beta-glucosidase 16-like	2.17812	XM_020327283.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4BL_TGACv1_321498_AA1061390	Beta-glucosidase 6	2.28425	XM_020290811.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_1BL_TGACv1_030467_AA0091630	ABC transporter B family member 11-like	2.31482	XM_009391731.2	<i>Musa acuminata</i> subsp. <i>malaccensis</i>
TRIAE_CS42_1BL_TGACv1_032327_AA0128440	WAT1-related protein At5g07050-like	2.509	XM_020338654.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2DL_TGACv1_158049_AA0507280	Aldose 1-epimerase-like	2.51875	XM_020290960.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2BS_TGACv1_146482_AA0466120	ABC transporter B family member 4-like	2.56886	XM_020293040.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2BL_TGACv1_131851_AA0432880	Aldose 1-epimerase-like	2.60357	XM_020290960.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4DL_TGACv1_343670_AA1138050	Protein transport protein Sec61 subunit alpha-like	2.61792	XM_020290809.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2AS_TGACv1_112362_AA0336420	ABC transporter B family member 4-like	2.69261	XM_020293040.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2BL_TGACv1_129780_AA0395640	Triacylglycerol lipase 2-like	3.34377	XM_020303075.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2AS_TGACv1_113028_AA0350040	ABC transporter C family member 14-like	3.34819	XM_020332672.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_6AL_TGACv1_474105_AA1534120	2-Alpha-L-fucosyltransferase-like	3.42443	XM_020309974.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4DS_TGACv1_360999_AA1158040	SET4_M23	3.58926	AK330584.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_4AL_TGACv1_290204_AA0982950	tpb0045110	3.70217	AK451279.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_2AL_TGACv1_094425_AA0297430	Beta-glucosidase	3.80462	AB548284.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_2DL_TGACv1_157981_AA0504650	Beta-glucosidase 12-like	3.89686	XM_020329910.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2AS_TGACv1_113828_AA0361200	Syntaxin-132-like	3.91589	XM_020290887.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2BS_TGACv1_145994_AA0452200	WT004_F09	4.11817	AK332578.1	<i>Triticum aestivum</i> L.

Table 7 continued

Gene Id	Protein name	Log2(fold change)	Accession ID in NCBI	Source organism
TRIAE_CS42_2AS_TGACv1_113522_AA0357250	UDP-arabinopyranose mutase 3	4.12231	XM_020345004.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_3B_TGACv1_221856_AA0751810	Peptide-N4-(N-acetyl-beta-glucosaminyl)asparagine amidase A-like	4.43885	XM_020315658.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2DS_TGACv1_180213_AA0610440	UDP-arabinopyranose mutase 3	4.50941	XM_020345004.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2BS_TGACv1_147826_AA0487990	GT75-3	4.56522	KM670460.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_4BS_TGACv1_331402_AA1109940	Heparan-alpha-glucosaminide N-acetyltransferase-like	5.66096	XM_020314296.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_1BS_TGACv1_051503_AA0180180	Alpha-mannosidase-like	7.44879	XM_020334225.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>

changing and remodeling the growth strategies in response to stress (Kesten et al. 2017). Furthermore, different molecules of cell wall can play a role as signaling factors to warn the plant immune system under adverse situations (Malinovskiy et al. 2014). Cellulose as the major component of cell wall has considerably shown up-regulation in mycorrhizal plants. Comparing D – M/D + M, we found three genes (XM_020312442.1, XM_020331230.1, XM_020312442.1) to be only expressed in mycorrhizal treatment as mycorrhizal-induced gene (Table 8); analogously, there were three specific induced cellulose-related genes (Table 6) in N – M/N + M comparison (XM_020331230.1, XM_020312442.1, XM_020322746.1). Considering these data, it seems that mycorrhizal symbiosis can effectively increase cellulose biosynthetic process and cell growth. Interestingly, cellulose synthases and also linked microtubules can perceive stress signals directly or indirectly to trigger reproducing and remodeling the cellulose microfibrils and proteins like expansin as the best response (Kesten et al. 2017). Wang et al. (2016) have reported that cellulose deficient mutant plants are more sensitive to abiotic stress than wild-type ones (Wang et al. 2016); therefore, a stress-response related role can be considered for cellulose, since cellulose microfibrils and the other factors that lead the direction of cell growth can be regulated by water availability (Wang et al. 2016). Based on our results, increased cellulose synthesis occurs in plants in normal irrigation, and this data is content with plant growth analysis (Fig. 2); and as we observed a gene (XM_020308861.1) only expressed in N + M treatment compared to D + M (Table 10), it can be perceived that probably the better irrigation condition leads to more cellulose microfibril biosynthesis in plant cell wall, and consequently, cell growth and symbiosis establishment; thus, we can introduce this gene (XM_020308861.1) as a N + M-specific gene. Therefore, cellulose biosynthesis related genes recognized as significant expressed in mycorrhizal-alleviated plants. Our results suggest that a defense-related role can be considered for cellulose in plant cell wall, since cellulose is the most abundant component of that, and cell wall appears as a very complex network for providing protection and perception to confront critical situations (Kesten et al. 2017; Vangelisti et al. 2018; Ren et al. 2019). A possible explanation for increasing of cellulose biosynthesis might be a relevance between cellulose content increase toward raising alleviated wheat plants tolerance; referring previous studies (Balestrini and Bonfante 2014; Kesten et al. 2017; Wang et al. 2016; Zhang et al. 2014), disordering the cellulose microfibrils organization occurred by biotic or abiotic factors, water deficit or AMF in this case, can trigger defense response in plants; Although discovering the exact mechanism requires more investigation.

Fig. 5 Log2 (fold change) of down and up-regulated genes under mycorrhizal water-stress plants. 1, 9, 11, 12, 26: carbohydrate metabolic process, 2, 3, 5: nitrogen compound metabolic process, 4: cellular glucan metabolic process, 6: xyloglucan metabolic process, 7: carbon–nitrogen ligase activity, 8: fatty acid biosynthetic process, 10, 20: lipid metabolic process, 13, 14, 16, 19, 21, 30: transmembrane

transport, 15, 17: hexose metabolic process, 18, 33: integral component of membrane, 22: galactoside 2- α -L-fucosyltransferase activity, 23: plant-type cell wall, 24: long-chain fatty acid-CoA ligase activity, 25: beta-glucosidase activity, 27, 28: SNAP receptor activity, 29, 32: cellulose biosynthetic process, 31: UDP-arabinopyranose mutase activity, 34: mannose metabolic process

Deducing from our results, lipid metabolic process associated genes such as fatty acid biosynthetic process, were up-regulated in AMF plants in comparison to non-mycorrhizal plants, especially under water deficit; comparing D – M/D + M conditions, there were three up-regulated genes related to lipid biosynthesis, and two up-regulated (FN564431.1, XM_020337300.1) and one specific gene (XM_020293126.1) related to D + M treatment in comparison with N + M. Since lipids are very important component of cell membrane, water deficiency may affect the composition and amount of lipids due to stress (Ivanov and Harrison. 2018). Increasing the lipid metabolic process due to AMF and well watering may suggest the role of lipids in maintaining the normal structure and function of cell membrane (Ivanov et al. 2019); to this aim, since AMF increases plant defense in stress situation, specially water deficit condition, it endeavors to enhance the cell membrane thickness by increased biosynthesis of lipid compartments either for forming arbuscular structures or counteracting abiotic stresses. Considering the increased lipid-related gene expression in D + M plants, we propose the role of AMF to increase the plant tolerance by targeting the cell membrane composition, which is consistent with previous reports (López-Ráez et al. 2010; Zhang et al. 2014).

In addition, we identified genes related to microfibril organization and cytoskeleton structure as mycorrhizal-responsive genes. As the cytoskeletal restructuring is related to cell membrane integrity, different stresses such as water shortage can change this arrangement, membrane fluidity and transmembrane transporters activity (Vangelisti et al. 2018). It could be suggested that up-regulation of the cytoskeletal compartments related genes in symbiotic plants protect the membrane integrity and transports in

response to water stress; as it has been suggested that depolymerization and re-organization of the cytoskeletal microtubules can be important for plant tolerance to salt stress as an abiotic stress (Wang et al. 2013). It is demonstrated that mycorrhizal symbiosis can enhance the absorption of minerals in host plant (Smith et al. 2011). In agreement with these results, we found the up-regulation of genes involved in membrane transferase compartments because of mycorrhizal relationship. Genes such as ammonium transporters and integral components of membrane are presented as mycorrhizal-responsive genes (Tables 6, 8). Up-regulation of ammonium transporters in AMF roots of host plants has already been reported in some plant species (Breuillin-Sessoms et al. 2015; Hong et al. 2012; Pérez-Tienda et al. 2014). It is proposed that the presence of transporters in pre-arbuscular membrane can help arbusculated cells survival and maintenance of symbiotic relationship in root cells (Breuillin-Sessoms et al. 2015).

Moreover, several carbohydrate metabolic process related genes were identified as significant differentially expressed genes in this study and mainly related to root cell wall degradation and remodeling (Vangelisti et al. 2018); which some such as mannosidase, hexose metabolic process and β -glucosidase activity with roles in cell wall biogenesis. In comparison between D – M/N – M, there were two carbohydrate-metabolic-process related genes (Table 3) whose expression level were completely induced in control plants, and it can be associated with normal irrigation condition. Since four induced genes (XM_020321162.1, XM_020321162.1, XM_020329909.1, XM_020310973.1) related to carbohydrate metabolic process, hydrolase activity and xylan biosynthetic process were found in AMF plants (Table 8), it can be deduced that

Table 8 Induced and suppressed transcripts identified in wheat cv. Chamran in AMF roots under water deficiency

Gene ID	Protein name	Value 1 (D – M)	Value 2 (D + M)	Accession ID in NCBI	Source organism
TRIAE_CS42_6DL_TGACv1_527547_AA1705600	Laccase-21-like	0	0.94653	XM_020336472.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2BL_TGACv1_132538_AA0438240	Acidic endochitinase-like	0	34.2003	XM_020321162.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2BL_TGACv1_130318_AA0408760	Acidic endochitinase-like	0	37.2347	XM_020321162.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2AL_TGACv1_096263_AA0318280	COBRA-like protein 7	0	0.57753	XM_020312442.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_7DL_TGACv1_603253_AA1979180	Putative cellulose synthase A catalytic subunit 11	0	1.39848	XM_020331230.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_6AL_TGACv1_472962_AA1527570	Chitinase 6-like	0	3.36273	XM_020317430.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_1AL_TGACv1_001368_AA0029420	S-type anion channel SLAH2-like	0	1.48604	XM_020295652.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_1AS_TGACv1_021078_AA0080890	CASP-like protein 4D1	0	2.3401	XM_020308584.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2BL_TGACv1_130367_AA0409580	WAT1-related protein Atlg68170-like	0	7.59737	XM_020322988.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2BL_TGACv1_131444_AA0427790	Probable purine permease 11	0	1.81115	XM_020344559.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_3AS_TGACv1_210868_AA0680480	Protein NRT1/PTR FAMILY 8.2-like	0	1.02073	XM_020335824.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_3AS_TGACv1_211406_AA0689890	Phenylalanine ammonia-lyase-like	0	1.25925	XM_020341608.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_3B_TGACv1_221895_AA0752610	protein NRT1/PTR FAMILY 4.5-like	0	36.8762	XM_020341045.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_3B_TGACv1_226641_AA0818320	Ammonium transporter 3 member 1-like	0	6.44725	XM_020310974.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_3B_TGACv1_227304_AA0822670	Protein NRT1/PTR FAMILY 1.2-like	0	3.71422	XM_020312497.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_3DL_TGACv1_249337_AA0845800	Sugar transport protein 1-like	0	0.63211	XM_020334105.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4AS_TGACv1_306829_AA1013910	WAT1-related protein Atlg68170-like	0	2.20539	XM_020338332.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4BL_TGACv1_321310_AA1058840	Nitrate transporter (TaNP6.1 gene)	0	1.14233	HF544988.1	<i>Triticum aestivum</i> cv. Paragon
TRIAE_CS42_4BL_TGACv1_321771_AA1065240	Heptahelical transmembrane protein 4-like	0	1.55394	XM_020290761.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_5AL_TGACv1_373992_AA1186890	Nitrate transporter (TaNP6.1 gene)	0	1.86885	HF544988.1	<i>Triticum aestivum</i> cv. Paragon
TRIAE_CS42_2AL_TGACv1_094209_AA0294360	Beta-glucosidase 12-like	0	0.61419	XM_020329909.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2DL_TGACv1_161025_AA0556070	COBRA-like protein 7	0	0.69365	XM_020312442.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_7AL_TGACv1_559082_AA1798000	Class II chitinase gene	0	1.06682	AY973229.1	<i>Triticum aestivum</i> cultivar Gamenya
TRIAE_CS42_2AL_TGACv1_093541_AA0282250	HKT1	0	0.41727	KF443078.1	<i>Triticum durum</i>
TRIAE_CS42_3DL_TGACv1_251611_AA0883070	UDP-glucuronate:xylan alpha-glucuronosyltransferase 1-like	0	0.49347	XM_020310973.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_6DL_TGACv1_526537_AA1686380	Putative laccase-9	19.5213	0	XM_020320013.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>

Table 9 DEGs identified in wheat cv. Chamran showing down-regulation and up-regulation in AMF colonized roots under water deficit and normal irrigation

Gene Id	Protein name	Log2(fold change)	Accession ID in NCBI	Source organism
TRIAE_CS42_4BS_TGACv1_328907_AA1095330	Cytosolic glutamine synthetase (GSe-B4) gene	– 4.6836	KF2425113.1	<i>Triticum turgidum</i>
TRIAE_CS42_4DS_TGACv1_361179_AA1162880	Glutamine synthetase cytosolic isozyme 1-3	– 3.4703	XM_020323172.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2BL_TGACv1_129932_AA0399780	Beta-glucosidase BoGH3B-like	– 3.3528	XM_020296045.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_7BL_TGACv1_578733_AA1899350	Agglutinin isolectin 3-like	– 3.1321	XM_020325269.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2AL_TGACv1_094425_AA0297430	Beta-glucosidase	– 3.1248	AB548284.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_5AL_TGACv1_374243_AA1194960	Beta-glucosidase BoGH3B-like	– 2.6959	XM_020293489.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4DL_TGACv1_343897_AA1141200	Glucan endo-1,3-beta-glucosidase 1-like	– 2.5333	XM_020343229.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_7DL_TGACv1_602790_AA1968680	Agglutinin isolectin 3-like	– 2.4325	XM_020325269.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4AL_TGACv1_288277_AA0943450	Cytosolic glutamine synthetase (GSe-A4) gene	– 2.3568	KF2425112.1	<i>Triticum turgidum</i>
TRIAE_CS42_2AL_TGACv1_095187_AA0308020	Beta-glucosidase BoGH3B-like	– 2.288	XM_020296045.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2BL_TGACv1_129561_AA0388630	Beta-glucosidase	– 2.1882	AB100035.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_2BL_TGACv1_130367_AA0409580	WAT1-related protein Atlg68170-like	– 2.0993	XM_020322988.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_1DS_TGACv1_080861_AA0254810	Phenylalanine ammonia-lyase-like	2.00132	XM_020319266.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_2AS_TGACv1_113168_AA0352430	Phenylalanine ammonia-lyase-like	2.05164	XM_020310911.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_3B_TGACv1_224369_AA0795760	HIPL1 protein-like	2.11947	XM_020331354.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_1BS_TGACv1_050729_AA0174830	Phenylalanine ammonia-lyase-like	2.24533	XM_020319266.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_5AS_TGACv1_392939_AA1266520	–	2.27591	CT009586.1	<i>Triticum aestivum</i>
TRIAE_CS42_1AL_TGACv1_001387_AA0029810	Chromosome 3B-specific BAC library	2.29746	FN564431.1	<i>Triticum aestivum</i>
TRIAE_CS42_1AL_TGACv1_001256_AA0027880	Endo-1,3-beta-glucosidase 13-like	2.30594	XM_020325014.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4DL_TGACv1_343409_AA1134090	Omega-3 fatty acid desaturase, endoplasmic reticulum-like	2.37468	XM_020337300.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_4AS_TGACv1_308797_AA1029560	Expansin-A12-like	2.50807	XM_020306411.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_1BS_TGACv1_049914_AA0164140	tp1b0043f07	2.57319	AK450976.1	<i>Triticum aestivum</i> L.
TRIAE_CS42_1DL_TGACv1_061987_AA0206950	Glucan endo-1,3-beta-glucosidase 13-like	2.63057	XM_020325014.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_5AL_TGACv1_374914_AA1211820	Uncharacterized LOC109761629	2.67513	XM_020320447.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>
TRIAE_CS42_1DL_TGACv1_061159_AA0187430	Glucan endo-1,3-beta-glucosidase 13-like	3.07913	XM_020325013.1	<i>Aegilops tauschii</i> subsp. <i>tauschii</i>

Fig. 6 Log₂ (fold change) of down- and up-regulated genes in symbiotic normal and low-irrigated plants. 1, 2, 9: nitrogen compound metabolic process, 3, 6, 7, 10, 11, 15, 19, 23, 24, 25: carbohydrate metabolic process, 4, 8: chitin binding, 5: scopolin beta-glucosidase

activity, 12: transmembrane transporter activity, 13, 14, 16: L-phenylalanine catabolic process, 17: cell division, 18, 20: lipid metabolic process, 21, 22: plant-type cell wall organization

Table 10 Induced transcripts identified in wheat cv. Chamran AMF colonized roots in normal and deficit conditions

Gene ID	Protein name	Value_1 (D + M)	Value_2 (N + M)	Accession ID in NCBI	Source organism
TRIAE_CS42_7AL_TGACv1_557532_AA1782680	Mixed-linked glucan synthase 2-like	0	0.38386	XM_020308861.1	<i>Aegilops tauschii</i> subsp. tauschii
TRIAE_CS42_1DL_TGACv1_061987_AA0206890	Glucan endo-1,3-beta-glucosidase 13-like	0	0.70549	XM_020325007.1	<i>Aegilops tauschii</i> subsp. tauschii
TRIAE_CS42_5BL_TGACv1_405270_AA1323650	Expansin-A31-like	0	0.41256	XM_020334430.1	<i>Aegilops tauschii</i> subsp. tauschii
TRIAE_CS42_7DS_TGACv1_622542_AA02041460	Patatin-like protein 2	3.75019	0	XM_020293126.1	<i>Aegilops tauschii</i> subsp. tauschii
TRIAE_CS42_1AL_TGACv1_003048_AA0047290	Germ agglutinin isolectin A	1.21392	0	M25536.1	Wheat (<i>T.aestivum</i>)
TRIAE_CS42_3DL_TGACv1_251714_AA0884080	WPR4b gene	0.545005	0	AJ006099.1	<i>Triticum aestivum</i>

Fig. 7 Log₂ (fold change) of three selected genes, Z1: cellulose biosynthetic process, Z2: beta-glucosidase BoGH3B-like Z3: chitinase activity, analyzed by q-RT PCR

AMF could effectively improve cell behavior by changing its gene expression pattern. Amongst different roles of carbohydrates in plant cells, such as mechanical support imparted by xylan, mannan and xyloglucan in cell wall can

be considered as one of their important roles (Houston et al. 2016). It seems that some carbohydrates related to the host plant are allocated to AM development, and accumulation of some carbohydrates, like monosaccharides, can improve the osmotic adjustment and water preservation of the host plant (Wu et al. 2013). It has been shown that carbohydrates play an important role in plant defense response and immunity (Lastdrager et al. 2014). Some participate as elicitors, whilst, others can imply such as phyto-hormones and signaling molecules (Trouvelot et al. 2014), or elicitors which are derived from plant cell wall during plant's interaction with microorganisms (Boudart et al. 2003). Since water stress can directly affect the cell membrane construction, it is suggested that carbohydrates play a key role in integral redox network and mitigate the destructive effects of drought stress (Keunen et al. 2013). Chen et al. (2014) has previously reported increased the expression of monosaccharide genes like mannose and glucose in symbiotic plants. Hemicellulose, as a complex and important part of the plant cell wall is composed of

mannose, glucose or xylose and reinforces the cell wall through interaction with cellulose microfibrils and lignin (Malinovsky et al. 2014). Here we can suggest a mycorrhizal-responsive role for abovementioned monosaccharide related genes which appeared as upregulated DEGs in colonized plants (Tables 5, 7). Also, AMF positively affect the plant immune system by increasing the expression of genes annotated as hemicellulose compartments, such as mannose and xylose, and fortifying the cell wall as a first barrier of plant cell.

The expression data about chitin binding and chitinase activity genes, revealed increasing pattern in presence of AMF, especially under water deficit condition. This result is consistent with Behringer et al. (2015) observation in *Picea abies*, and other reports demonstrating the role of chitinase enzymes in increasing plant tolerance (Couto et al. 2013; Dana et al. 2006; Hermosa et al. 2012; Lucas et al. 2014).

As fundamental molecule of fungus cell wall, chitin and chitin-derived molecules play a key role as signals to initiate defense responses in plant cells in symbiosis relationship (Shimizu et al. 2010; Hayafune et al. 2014). It could be suggested that chitin-related genes such as chitinase detect chitin molecules of fungus as a signal to trigger a proper defense response, and also increase the plant tolerance.

Cufflink assembly data indicated higher expression of lignin catabolic processes related genes. Gutjahr et al. (2015) have reported plant secondary cell wall genes such as lignin, down-regulated during symbiosis, and it might facilitate mycorrhizal fungus penetration and development through plant cell wall, and this is in agreement with our results.

Conclusion

Our analysis of significantly expressed genes in AMF roots of wheat cv. “Chamran” proposes that a primary defense response related to cell wall and membrane and also some membrane located molecules such as carbohydrates as a very significant group of molecules could be accomplished in mycorrhizal plants. These two important external parts of plant cells could initiate the first step of defense response against water deficiency through signaling processes in which different molecules of cell wall and also membrane transporters and compartments are involved. For the first time, in the present research it has been suggested that modifications and changes in cell wall components and their activities and cell-membrane-located molecules induced by AMF as an invader in the first place, may provide a tolerance-specified system in which structural part, such as cellulose and hemicellulose compartments and lipid molecules, and non-structural molecules, such as

many carbohydrates, cooperate toward improving biosynthetic processes and physically strengthening of the outermost parts of the host plant which lead to plant growth improvement as well. Since there are great number of genes addressed to cell wall and membrane, understanding the exact genes and their roles, obviously needs more investigations in the future. Our results represent that symbiotic plants displayed differentially amount of expressed genes, most related to restructuring and strengthening of cell wall and membrane, and also their organization and remodeling to protect the host plant, and this tolerance response can provide more durability and better growth status for the symbiotic plants.

Finally, despite several investigations on cell wall and membrane changes in symbiotic plants and the relationship with plant tolerance to achieve a precise conclusion, this study has tried to focus on some significant genes and introduce some specific genes associated with mycorrhiza under two different irrigation regimes. Notwithstanding the delicate and extended range of water stress condition as one of the most important abiotic stresses, the relationship between cell wall and membrane genes expression pattern and the intensity and duration of water deficit can be very impressive; so the studies domain can be really vast and detailed in this case. Probably, our outcomes can highlight the advantage of transcriptome profiling to identify additional genes and the mycorrhiza-associated genes that have been found, provide a field for further investigations into cell wall-related genes and their relationship with plant tolerance.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- An J, Sun M, Velzen R, Ji C, Zheng Z, Limpens E, Bisseling T, Deng X, Xiao S, Pan Z (2018) Comparative transcriptome analysis of *Poncirus trifoliata* identifies a core set of genes involved in arbuscular mycorrhizal symbiosis. *J Exp Bot* 69(21):5255–5264. <https://doi.org/10.1093/jxb/ery283>
- Balestrini R, Bonfante P (2014) Cell wall remodeling in mycorrhizal symbiosis: a way towards biotrophism. *Front Plant Sci* 5:237. <https://doi.org/10.3389/fpls.2014.00237>
- Balestrini R, Lanfranco L (2006) Fungal and plant gene expression in arbuscular mycorrhizal symbiosis. *Mycorrhiza* 16:509–524
- Behringer D, Zimmermann H, Ziegenhagen B, Liepelt S (2015) Differential gene expression reveals candidate genes for drought stress response in *Abies alba* (Pinaceae). *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0124564>
- Bernardo L, Carletti P, Badeck FW, Rizza F, Morcia C, Ghizzoni R, Ruphael Y, Colla G, Terzi V, Lucini L (2019) Metabolomic responses triggered by arbuscular mycorrhiza enhance tolerance

- to water stress in wheat cultivars. *Plant Physiol Biochem* 137:203–212. <https://doi.org/10.1016/j.plaphy>
- Boudart G, Charpentier M, Lafitte C, Martinez Y, Jauneau A, Gaulin E, Esquerre-Tugayé MT, Dumas B (2003) Elicitor activity of a fungal endopolygalacturonase in tobacco requires a functional catalytic site and cell wall localization. *Plant Physiol* 131(1):93–101. <https://doi.org/10.1104/pp.011585>
- Brenchley R, Spannagl M, Pfeifer M, Barker GL, D'Amore R, Allen AM, McKenzie N, Kramer M, Kerhormou A, Bolser D, Kay S, Waite D, Trick M, Bancroft I, Gu Y, Huo N, Luo MC, Sehgal S, Gill B, Kianian S, Anderson O, Kersey P, Dvorak J, McCombie WR, Hall A, Mayer KF, Edwards KJ, Bevan MW, Hall N (2012) Analysis of the bread wheat genome using whole-genome shotgun sequencing. *Nature* 491(7426):705–710. <https://doi.org/10.1038/nature11650>
- Breuillin-Sessoms F, Floss DS, Gomez SK, Pumphlin N, Ding Y, Levesque-Tremblay V, Noar RD, Daniels DA, Bravo A, Eaglesham JB, Benedito VA, Udvardi MK, Harrison MJ (2015) Suppression of arbuscule degeneration in *Medicago truncatula phosphate transporter4* mutants is dependent on the ammonium transporter 2 family protein AMT2;3. *Plant Cell* 27(4):1352–1366. <https://doi.org/10.1105/tpc.114.131144>
- Chen X, Song F, Liu F, Tian C, Liu S, Xu H, Zhu X (2014) Effect of different Arbuscular Mycorrhizal fungi on growth and physiology of maize at ambient and low temperature regimes. *Sci World J Article ID* 956141, 7. <https://doi.org/10.1155/2014/956141>
- Clavijo BJ, Venturini L, Schudoma C, Accinelli GG, Kaithakottil G, Wright J et al (2017) An improved assembly and annotation of the allohexaploid wheat genome identifies complete families of agronomic genes and provides genomic evidence for chromosomal translocations. *Genome Res* 27:885–896. <https://doi.org/10.1101/gr.217117.116>
- Couto M, Lovato P, Wipf D, Dumas-Gaudot E (2013) Proteomic studies of arbuscular mycorrhizal associations. *Adv Biol Chem* 3:48–58. <https://doi.org/10.4236/abc.2013.31007>
- Dana MM, Pintor-Toro JA, Cubero B (2006) Transgenic tobacco plants overexpressing chitinases of fungal origin show enhanced resistance to biotic and abiotic stress agents. *Plant Physiol* 142(2):722–730. <https://doi.org/10.1104/pp.106.086140>
- Dong CH, Li C, Yan XH, Huang SM, Huang JY, Wang LJ, Guo RX, Lu GY, Zhang XK, Fang XP, Wei WH (2012) Gene expression profiling of *Sinapis alba* leaves under drought stress and rewetting growth conditions with Illumina deep sequencing. *Mol Biol Rep* 39(5):5851–5857. <https://doi.org/10.1007/s11033-011-1395>
- Essahibi A, Benhiba L, Babram MA, Ghoulam C, Qaddoury A (2018) Influence of arbuscular mycorrhizal fungi on the functional mechanisms associated with drought tolerance in carob (*Ceratonia siliqua* L.). *Trees* 32:87–97. <https://doi.org/10.1007/s00468-017-1613-84>
- Garcia K, Chasman D, Roy S, Ané JM (2017) Physiological responses and gene co-expression network of mycorrhizal roots under K + deprivation. *Plant Physiol* 173:1811–1823
- Gill BS, Appels R, Botha-Oberholster AM, Buell CR, Bennetzen JL, Chalhoub B, Chumley F, Dvorák J, Iwanaga M, Keller B, Li W, McCombie WR, Ogihara Y, Quetier F, Sasaki T (2004) A workshop report on wheat genome sequencing: international genome research on wheat consortium. *Genetics* 168(2):1087–1096. <https://doi.org/10.1534/genetics.104.034769>
- Guether M, Balestrini R, Hannah M, He J, Udvardi MK, Bonfante P (2009) Genome-wide reprogramming of regulatory networks, transport, cell wall and membrane biogenesis during arbuscular mycorrhizal symbiosis in *Lotus japonicus*. *New Phytol* 182(1):200–212. <https://doi.org/10.1111/j.1469-8137.2008.02725>
- Gutjahr C, Sawers RJH, Marti G, Andrés-Hernández L, Yang SY, Casieri L et al (2015) Transcriptome diversity among rice root types during asymbiosis and interaction with arbuscular mycorrhizal fungi. *Proc Natl Acad Sci USA* 112:6754–6759. <https://doi.org/10.1073/pnas.1504142112>
- Hayafune M, Berisio R, Marchetti R, Silipo A, Kayama M, Desaki Y, Arima S, Squeglia F, Ruggiero A, Tokuyasu K, Molinaro A, Kaku H, Shibuya N (2014) Chitin-induced activation of immune signaling by the rice receptor CEBiP relies on a unique sandwich-type dimerization. *Proc Natl Acad Sci USA* 111(3):404–413. <https://doi.org/10.1073/pnas.1312099111>
- Hermosa R, Viterbo A, Chet I, Monte E (2012) Plant-beneficial effects of *Trichoderma* and of its genes. *Microbiology* 158(1):17–25. <https://doi.org/10.1099/mic.0.052274-0>
- Hong JJ, Park YS, Bravo A, Bhattarai KK, Daniels DA, Harrison MJ (2012) Diversity of morphology and function in arbuscular mycorrhizal symbioses in *Brachypodium distachyon*. *Planta* 236:851–865. <https://doi.org/10.1007/s00425-012-1677>
- Houston K, Tucker MR, Chowdhury J, Shirley N, Little A (2016) The plant cell wall: a complex and dynamic structure as revealed by the responses of genes under stress conditions. *Front Plant Sci* 7:984. <https://doi.org/10.3389/fpls.2016.00984>
- Ivanov S, Harrison MJ (2018) Accumulation of phosphoinositides in distinct regions of the periarbuscular membrane. *New Phytol* 221(4):2213–2227. <https://doi.org/10.1111/nph.15553>
- Ivanov S, Austin J, Berg RH, Harrison MJ (2019) Extensive membrane systems at the host-arbuscular mycorrhizal fungus interface. *Nat Plants* 5:194–203. <https://doi.org/10.1038/s41477-019-0364-5>
- Jacott CN, Murray JD, Ridout CJ (2017) Trade-offs in arbuscular mycorrhizal symbiosis: disease resistance, growth responses and perspectives for crop breeding. *Agronomy* 7(4):75. <https://doi.org/10.3390/agronomy7040075>
- Jayasena AS, Secco D, Bernath-Levin K, Berkowitz O, Whelan J, Mylne JS (2014) Next generation sequencing and de novo transcriptomics to study gene evolution. *Plant Methods* 10(1):34. <https://doi.org/10.1186/1746-4811-10-34>
- Kesten C, Menna A, Sánchez-Rodríguez C (2017) Regulation of cellulose synthesis in response to stress. *Curr Opin Plant Biol* 40:106–113. <https://doi.org/10.1016/j.pbi.2017.08.010>
- Keunen E, Peshev D, Vangronsveld J, Van Dem Ende W, Cuypers A (2013) Plant sugars are crucial players in the oxidative challenge during abiotic stress: extending the traditional concept. *Plant, Cell Environ* 36:1242–1255. <https://doi.org/10.1111/pce.12061>
- Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg SL (2013) TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol* 14:R36. <https://doi.org/10.1186/gb-2013-14-4-r36>
- Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. *Nat Methods* 9(4):357–359. <https://doi.org/10.1038/nmeth>
- Lastdrager J, Hanson J, Smeekens S (2014) Sugar signals and the control of plant growth and development. *J Exp Bot* 65(3):799–807. <https://doi.org/10.1093/jxb/ert474>
- Li M, Wang R, Tian H, Gao Y (2018) Transcriptome responses in wheat roots to colonization by the arbuscular mycorrhizal fungus *Rhizophagus irregularis*. *Mycorrhiza* 28:747. <https://doi.org/10.1007/s00572-018-0868-2>
- Li J, Meng B, Chai H, Yang X, Song W, Li S, Lu A, Zhang T, Sun W (2019) Arbuscular Mycorrhizal Fungi alleviate drought stress in C3 (*Leymus chinensis*) and C4 (*Hemarthria altissima*) grasses via altering antioxidant enzyme activities and photosynthesis. *Front Plant Sci*. <https://doi.org/10.3389/fpls.2019.00499>
- López-Ráez JA, Verhage A, Fernández I, García JM, Azcón-Aguilar C, Flors V, Pozo MJ (2010) Hormonal and transcriptional profiles highlight common and differential host responses to

- arbuscular mycorrhizal fungi and the regulation of the oxylipin pathway. *J Exp Bot* 61(10):2589–2601. <https://doi.org/10.1093/jxb/erq089>
- Lucas SJ, Akpinar BA, Šimková H, Kubaláková M, Doležal J, Budak H (2014) Next-generation sequencing of flow-sorted wheat chromosome 5D reveals lineage-specific translocations and widespread gene duplications. *BMC Genom* 15:1080. <https://doi.org/10.1186/1471-2164-15-1080>
- Malinovskiy FG, Fangel JU, Willats WG (2014) The role of the cell wall in plant immunity. *Front Plant Sci* 5:178. <https://doi.org/10.3389/fpls.2014.00178>
- McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular—arbuscular mycorrhizal fungi. *New Phytol* 115(3):495–501. <https://doi.org/10.1111/j.1469-8137.1990.tb00476.x>
- Nanjareddy K, Arthikala MK, Gómez BM, Blanco L, Lara M (2017) Differentially expressed genes in mycorrhized and nodulated roots of common bean are associated with defense, cell wall architecture, N metabolism, and P metabolism. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0182328>
- Nezhadahmadi A, Hossain PZ, Faruq G (2013) Drought tolerance in wheat. *Sci World J*. <https://doi.org/10.1155/2013/610721>
- Ouledali S, Ennajeh M, Ferrandino A, Khemira H, Schubert A, Secchi F (2019) Influence of arbuscular mycorrhizal fungi inoculation on the control of stomata functioning by abscisic acid (ABA) in drought-stressed olive plants. *S Afr J Bot* 121:152–158. <https://doi.org/10.1016/j.sajb.2018.10.024>
- Pérez-Tienda J, Corrêa A, Azcón-Aguilar C, Ferrol N (2014) Transcriptional regulation of host NH_4^+ transporters and GS/GOGAT pathway in arbuscular mycorrhizal rice roots. *Plant Physiol Biochem* 75:1–8. <https://doi.org/10.1016/j.plaphy.2013.11.029>
- Rasmussen R (2001) Quantification on the light cycler. In: Meurer S, Wittwer C, Nakagawara K (eds) *Rapid cycle real-time PCR, methods and applications*. Springer Press, Heidelberg, pp 21–34
- Ren CG, Kong CC, Yan K, Xie ZH (2019) transcriptome analysis reveals the impact of arbuscular mycorrhizal symbiosis on *Sesbania cannabina* expose to high salinity. *Sci Rep* 9:2780. <https://doi.org/10.1038/s41598-019-39463-0>
- Rich MK, Schorderet M, Reinhardt D (2014) The role of the cell wall compartment in mutualistic symbioses of plants. *Front Plant Sci* 5:238. <https://doi.org/10.3389/fpls.2014.00238>
- Shimizu T, Nakano T, Takamizawa D, Desaki Y, Ishii-Minami N, Nishizawa Y, Minami E, Okada K, Yamane H, Kaku H, Shibuya N (2010) Two LysM receptor molecules, CEBiP and OsCERK1, cooperatively regulate chitin elicitor signaling in rice. *Plant J* 64(2):204–214. <https://doi.org/10.1111/j.1365-3113X.2010.04324>
- Smith SE, Jakobsen I, Gronlund M, Smith FA (2011) Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. *Plant Physiol* 156(3):1050–1057. <https://doi.org/10.1104/pp.111.174581>
- Sugimura Y, Saito K (2017) Transcriptional profiling of arbuscular mycorrhizal roots exposed to high levels of phosphate reveals the repression of cell cycle-related genes and secreted protein genes in *Rhizophagus irregularis*. *Mycorrhiza* 27(2):139–146. <https://doi.org/10.1007/s00572-016-0735-y>
- Trouvelot S, Héloir MC, Poinssot B, Gauthier A, Paris F, Guillier C, Combiér M, Trdá L, Daire X, Adrian M (2014) Carbohydrates in plant immunity and plant protection: roles and potential application as foliar sprays. *Crop Sci Hortic* 5:592. <https://doi.org/10.3389/fpls.2014.00592>
- Vangelisti A, Natali L, Bernardi R, Sbrana C, Turrini A, Hassani-Pak K, Hughes D, Cavallini A, Giovannetti M, Giordani T (2018) Transcriptome changes induced by arbuscular mycorrhizal fungi in sunflower (*Helianthus annuus* L.) roots. *Sci Rep* 8(1):4. <https://doi.org/10.1038/s41598-017-18445-0>
- Wang Y, Yang L, Zheng Z, Grumet R, Loescher W, Zhu JK, Yang P, Hu Y, Chan Z (2013) Transcriptomic and physiological variations of three Arabidopsis ecotypes in response to salt stress. *PLoS ONE* 8(7):e69036. <https://doi.org/10.1371/journal.pone.0069036>
- Wang T, McFarlane HE, Persson S (2016) The impact of abiotic factors on cellulose synthesis. *J Exp Bot* 67(2):543–552. <https://doi.org/10.1093/jxb/erv488>
- Wu QS, Rivastava AK, Zou YN (2013) AMF-induced tolerance to drought stress in citrus: a review. *Sci Hortic* 164:77–87. <https://doi.org/10.1016/j.scienta.2013.09.010>
- Ye L, Zhao X, Bao E, Cao K, Zou Z (2019) Effects of Arbuscular Mycorrhizal Fungi on watermelon growth, elemental uptake, antioxidant, and photosystem II activities and stress-response gene expressions under salinity-alkalinity stresses. *Front Plant Sci*. <https://doi.org/10.3389/fpls.2019.00863>
- Zhang L, Peng J, Chen TT, Zhao XH, Zhang SP, Liu SD, Dong HL, Feng L, Yu SX (2014) Effect of drought stress on lipid peroxidation and proline content in cotton roots. *J Anim Plant Sci* 24(6):1729–1736
- Zhu Y, Mang HG, Sun Q, Qian J, Hipps A, Hua J (2012) Gene discovery using mutagen-induced polymorphisms and deep sequencing: application to plant disease resistance. *Genetics* 192(1):139–146. <https://doi.org/10.1534/genetics>

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