Differential gene expression in wild emmer wheat genotypes contrasting in drought resistance

Krugman T[1], Chagué V[2], Peleg Z[3], Brodsky L[1], Balzergue S[2], Boudet N[2], Korol AB[1], Nevo E[1], Saranga Y[3], Chalhoub B[2] and Fahima T[1]

[1]Department of Evolutionary and Environmental Biology, Institute of Evolution, Faculty of Science and Science Education, University of Haifa, Mt. Carmel, Haifa 31905, Israel. [2]Unité de Recherché en Génomique Vegetal (URGV), 91057 Evry, France. [3]Inst. of Plant Science and Genetics in Agriculture, the Hebrew University of Jerusalem,   Rehovot, 76100, Israel

ABSTRACT

Plants adapt to drought stress at molecular, cellular and whole plant levels by a range of physiological and biochemical mechanisms, controlled by a network of genes which could be activated or repressed in response to drought stress. The aim of the current study was to identify candidate genes for drought resistance derived from wild emmer. Wild emmer wheat (*Triticum turgidum* ssp. *dicoccoides*), the progenitor of cultivated wheat, is a promising source for improvement of drought resistance. We describe here a comparison of global gene expression in drought resistant (Y12-3) and drought susceptible (A24-39) genotypes of wild emmer, under severe water stress *vs*. well-watered conditions, using Affymetrix GeneChip® technology. Analysis of variance (ANOVA) revealed 5,571 differentially expressed transcripts in at least one of the four genotype/treatment combinations. Further analysis showed that a group of 126 genotype-specific transcripts were highly expressed under drought stress in Y12-3, while 63 transcripts were highly expressed in A24-39. Gene annotation of the highly expressed transcripts in Y12-3 showed that 63% of the putative proteins were structural and functional and 8% were signalling and regulatory proteins, while 29% were not identified. The identified functional proteins can be classified based on their subcellular localization and/or biological pathways, such as: membrane proteins involved in metal ion binding, transport, and electron transfer; proteins involved in metabolism of carbohydrate, lipid and proteins; and abiotic stress and senescence proteins. Furthermore, some of these proteins are known to be involved in drought tolerance in other plant species. Therefore, these transcripts are considered as potential candidate genes for drought resistance. We show here that the wild emmer gene pool is a promising source of candidate genes for improvement of drought resistance in cultivated wheat.

INTRODUCTION

Drought is the most important environmental factor limiting plant development and crop productivity worldwide. Plants respond and adapt to drought at molecular, cellular and whole plant levels by activating a range of physiological and biochemical responses controlled by a network of genes[1]. The identification of the genetic components of drought resistance is a necessary requirement to ensure further progress in plant breeding for drought resistant crops. Transcriptome

analysis was demonstrated to be an efficient approach to reveal the components responsible for abiotic stress tolerance by comparing between tolerant and susceptible genotypes under stress *vs*. control conditions in several crop species, including wheat[2].

Wild emmer wheat [*Triticum turgidum* ssp. *dicoccoides* (körn.) Thell]*,* is an allotetraploid (2n=4x=28; genome BBAA) species considered to be the progenitor of cultivated wheat[3]. Wheat progenitors are indigenous to semi-arid zones of West and Central Asia and consequently, well adapted to biotic and abiotic stresses that are ubiquitous in the region. Wild emmer was shown to harbor wide genetic and phenotypic diversity in its adaptation to drought conditions, suggesting that it may offer a rich allelic repertoire required to improve drought resistance in cultivated wheat[3,4,5]. Transcriptome analysis was used in the current study to identify potential candidate genes for drought resistance within the gene pool of wild emmer by comparing between drought resistant and drought susceptible genotypes under severe water stress *vs*. well watered control conditions.

MATERIAL AND METHODS

Plant materials and stress treatment: Two wild emmer wheat genotypes were selected for the present study, based on results accumulated during three years of research described in Peleg et al. (2005, 2008): (1) a drought resistant genotype (Y12-3) from Yehudiyya (35°42' N; 32°56' E), showing high productivity under drought stress, high yield stability and high water use efficiency (WUE); and (2) a drought susceptible genotype (A24-39) from Amirim (35°27' N; 32°55' E), showing low productivity under stress and low yield stability, with high WUE.

Plants were grown in pots (5 liter) containing Quartz sand (80%) and peat (20%), under natural Mediterranean winter conditions for 10 weeks, then transferred to a controlled environment greenhouse for three weeks of acclimation (22/18°C; 12 h day/12 night), followed by drought stress treatment. Severe drought stress was applied at heading time ('terminal drought'), 3-5 days after emergence of first awn**,** by withholding water for eight days until reaching leaf relative water content (RWC) of ~50%. Harvested leaves were immediately frozen in liquid nitrogen and stored at -80ºC for RNA extraction.

RNA preparation for microarray hybridization: RNAs of 12 samples (three replications of each of the four genotype/treatment combinations) were isolated and processed according to Affymetrix instructions. Arrays were washed using the GeneChip® Fluidics Station 450 (Affymetrix) and scanned using GeneChip® Scanner 3000 7G piloted by the GeneChip® Operating Software (GCOS).

Statistical analysis of microarray: Intensity values of perfect match (PM) and mismatch (MM) probe sets were transformed and normalized by quantile normalization. ANOVA model was used to test for the influence of two factors (treatment and genotype) on the probe expression log-signal[6].

Gene annotation: Annotation analysis was performed using WheatPLEX, a plant ontology database (http://www.plexdb.org/) and HarvEST:WheatChip (http://harvest.ucr.edu/). Functional classification was based on shared putative function, and/or common structural motifs, and/or common subcellular localization.
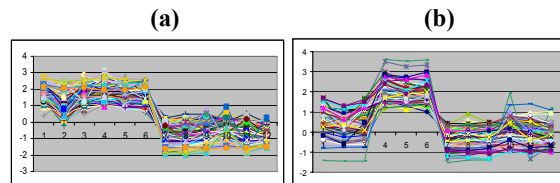
Quantitative PCR Analysis: Real-time PCR (qPCR) was used to validate the gene expression results of 12 transcripts, representing five different regulation patterns obtained by microarray analysis. Specific primers were designed within the region spanning the 11 Affymetrix probe set of each target sequence (http://www.affymetrix.com/analysis/index.affx).

RESULTS AND DISCUSSION

Expression patterns of Y12-3 and A24-39 under contrasting water availabilities: The main objective of the current study was to identify candidate genes associated with increased drought resistance. Therefore, we have compared gene expression profiles of drought resistant and drought susceptible genotypes of wild emmer wheat subjected to severe drought stress. ANOVA revealed that out of the 61,127 probe sets printed on the Affymetrix GeneChip® Wheat Genome Arrays, 5,571 transcripts were differentially expressed in at least one of the four genotype/treatment combinations, showing one or more of three significant effects: (*i*) stress (S); (*ii*) genotype (G); (*iii*) stress × genotype (S×G) interaction. Of the 5,571 transcripts, 3,723 (66%) transcripts were up-regulated and 1,846 (33%) transcripts were down-regulated, under stress. The relatively larger number of up-regulated transcripts shows that the response to severe drought stress involves a burst of gene activity. Of the differentially expressed transcripts, 2803 were up-regulated (Fig.1a) and 1,191 were down-regulated under stress in both genotypes. Further analysis was focused on differently regulated transcripts between the two genotypes under drought stress, showing all three significant effects (S, G, and S×G). This group included 126 transcripts that showed significantly higher up-regulation in Y12-3 than in A24-39 (Fig. 1b) and 63 transcripts that showed significantly higher up regulation in A24-39 than in Y12-3. An opposite trend was observed between genotypes in the proportion of the down-regulated transcripts: 58 transcripts showed significant down-regulation in Y12-3, and 96 transcripts down-regulation in A24-39.

Figure 1. **Regulation patterns of up-regulated transcripts under drought stress in wild emmer wheat.** (a) Transcripts showing common expression pattern between genotypes; (b) differentially expressed transcripts. Samples 1-6 are from stress treatment, while 7-12 are from control. Susceptible genotype is represented by samples 1-3 and 7-9, while resistant genotype by samples 4-5 and 10-12.

(a)   (b)

Quantitative real-time PCR (qPCR): Spearman correlation between the centralized Log_2 of expression values of 12 selected transcripts obtained by qPCR and intensity values obtained by microarray analysis was r=0.954 (*p*<0.001; n=48). These results indicate that the overall expression patterns of all the 12 transcripts were highly similar between the two platforms, confirming a high degree of reproducibility.

Gene annotation and classification to biological function: The differentially expressed transcripts showing a significant up-regulation under stress in Y12-3 (126 transcripts) and A24-39 (63 transcripts), were subjected to annotation analysis and classified according to their common biological functions. The 189 differentially expressed transcripts included 17 cases of multiple (2-3) representations of transcripts per unigene. Therefore, the current annotation analysis included 112 unigenes in Y12-3 and 56 unigenes in A24-39. Biological function was assigned to 71% of the up-regulated unigenes in Y12-3 and to 59% of the up-regulated unigenes in A24-39. Functional classification revealed that in both genotypes, about 10% were proteins involved in signaling and transcription and more than 50% were structural and functional proteins. Major differences were found between Y12-3 and A24-39, within each functional group in the following quantitative and qualitative parameters: (*i*) the number (or %) of detected genes; (*ii*) the mean fold change (FC) (expression under stress vs. expression under control); and (*iii*) the assigned biological or molecular function of the proteins (Table 1).

Few examples demonstrating the main differences identified between Y12-3 and A24-39 are described below. The up-regulated proteins in Y12-3 included 10 proteins involved in ion binding and transport, some of them are membrane proteins (e.g., water transport, auxin carrier); eight highly regulated membrane proteins involved in electron transfer and energy conservation (e.g., pheophorbide-a oxygenase and three putative cytochrome P450 proteins) and four other membrane associated proteins with unknown function. In A24-39

only two membrane proteins were up-regulated: a metal ion binding (metallothionein, class II) and a defense related protein. About 30% of the up-regulated proteins in both genotypes are involved in metabolism. However in Y12-3, eight proteins involved in lipid metabolism (e.g., beta-ketoacyl reductase and epoxide hydrolase) were up-regulated and only two in A24-39 (phospholipid hydroperoxide glutathione peroxidase (PHGPX). Some of these lipid metabolism proteins are involved in detoxification under oxidative stress; however, few are involved in cutin synthesis. Nine up-regulated proteins in Y12-3 are involved in carbohydrate metabolism (e.g., xyloglucan endotransglycosylase, and beta-phosphoglucomutase) and nine protein are involved in other metabolic processes (e.g., cis,cis-muconate cycloisomerase).

We have identified ten up-regulated proteins in Y12-3 that are required for biogenesis or maintaining and regulating the structure and function of plastids (e.g., plastid starch synthase I precursor and iojap protein), whereas none were identified as up-regulated in A24-39. Our gene expression analysis indicated that many transcripts related to photosynthesis were reduced in both genotypes (not shown). The differential gene expression between genotypes demonstrated that although photosynthesis was severely affected in both genotypes, the chloroplast activity in Y12-3 was higher than in A24-39.

The signaling and regulation proteins identified in Y12-3 included transcription factors, cell progression cycle; regulators of protein synthesis; membrane trafficking that may serve as an important determinant of organelle identity and a calcium-dependent protein kinase CPK1 that is involved in signaling. A putative NAM protein that may have a role in the response to stress stimuli, such as wounding, drought[7] and/or senescence[8] was up-regulated in Y12-3. Major differences were also found between up-regulation of abiotic stresses related proteins and senescence related proteins.

CONCLUSIONS

The results obtained in the current study suggest that genes that were up-regulated in response to drought stress in Y12-3 are involved in multiple mechanisms that may contribute to drought resistance[9]. Some of the over expressed genes in Y12-3 under drought can be considered as potential candidates genes for improving drought resistance in wheat. Further studies are designed to explore their contribution to drought resistance by molecular genomic approaches and by testing their co-localization with drought related QTLs. The resistant genotype tested here, originated from a natural wild wheat population combining high productivity under water-limited conditions with high yield stability[4]. The results obtained in the current study further demonstrate that wild emmer wheat genepool is a promising source for candidate genes for improvement of drought resistance in cultivated wheat.

Table 1. Functional classification of up-regulated proteins under drought stress, in drought resistant (Y12-3) and drought susceptible (A24-39) wild emmer wheat.

Functional Classification	A24-39 No. of genes	A24-39 FC*	Y12-3 No. of genes	Y12-3 FC*
Metal ion binding and transport	1	13	10	5.3±0.9
Electron transfer	0	0	8	15.7±5.7
Membrane	0	0	3	8.8±5.6
Abiotic stress	2	27.25±19.1	6	6.9±3.5
Senescence	0		3	18±7.05
Metabolism: carbohydrate	6	8.11±4.30	9	9.3±2.82
Metabolism: proteins	5	11.30±8.80	9	6.5±1.42
Metabolism: lipid	2	2.45±0.50	8	5.9±2.44
Metabolic processes	6	14.95±10.3	7	10.4±4.25
Regulation	5	5.96±1.70	9	3.5±0.55
Unclassified	5	3.20±0.74	8	5.4±0.93
Unknown	24	4.62±0.74	32	5.6±1.29
Total	56		112	

*Fold change (FC) was calculated as the ratio between the expression under stress *vs*. expression under control**.

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