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Comprehensive analysis of the gene expression profile of wheat at the crossroads of heat, drought and combined stress

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Abstract

Heat and drought are among the leading environmental stresses which have a major impact on plant development. In our research, identification and characterization of differentially expressed genes (DEGs) regulating the response of wheat to drought, heat and combined stress was carried out. We analyzed data from the Gene Expression Omnibus database (GEO) microarrays containing 24 samples of wheat, which were categorized by different treatments (control: ctrl, drought: D, heat: H, and mixed: HD). Significant DEGs were examined for gene annotation, gene ontology, co-expression, protein-protein interaction (PPI) and their heterogeneity and consistency through drought, heat and combined stress was also studied. Genes such as *gyrB, C6orf132* homolog, *PYR1* were highly associated with wheat response to drought with P-value (-log10) of 9.3, 7.3, 6.4, and logFC of -3.9, 2.0, 1.6, respectively. DEGs associated with drought tolerance were highly related to the protein domains of lipid-transfer (LTP). Wheat response to heat stress was strongly associated with genes such as RuBisCO activase B, small heat shock, LTP3, YLS3, At2g33490, *PETH* with p-values (-log10) ranging from 9.3 to 12.3. In addition, a relatively high number of protein interactions involved the *SDH*, *PEPCK*, and *G6PD* genes under heat stress.

Keywords: Wheat, Drought, Heat, Combined stress, Gene expression.

Cereals are by far the most commonly cultivated crops in the globe and the foundation of food security stability. Maize, rice and wheat are the single most important food item in the human diet, accounting for approximately 42.5% of the global food calorie consumption [1]. Recent climate change models expect significant declines in worldwide crop production based on changing ecosystems [2]. The significant losses in wheat production are more driven by abiotic stresses including drought, heat and salinity. A reported meta-analysis forecasts a significant wheat yield decline in tropical regions with every 1 °C increase [3].

Drought and heat are among the leading environmental stresses which have a major impact on plant development and growth. Drought occurs in almost all climatic zones, causing losses in crop production that are classified as one of the largest losses in agriculture [4]. It dramatically limits crop production and the period of drought cycles is rising due to climate change, and water scarcity in most grain cropping regions in the world [5]. In addition, Trenberth *et al.* [6] recorded extreme precipitation events with changes in global temperatures. This would increase the demand for accessible land and water sources, leading to more severe droughts in a number of areas. In particular, high temperature stress cause permanent enzyme denaturation and membrane fluidity problems [7]. Also heat stress increases plant senescence and decreases the duration of growth, resulting in reduced yield [8]. Wheat was the first crop to be domesticated within these crops and is the main staple food in the world[9]. Wheat dominates the land area harvested (38.8%) and delivers considerably more protein per gram (12–15%) than rice or corn (2–3%) [10], it provides 19% of calories and 21% of proteins [11]. Moreover, with world population expected to grow to 9.6 billion by 2050, demand for wheat will expand [12].

During the wheat reproductive growth stage, flowering is highly subjected to heat stress, affecting daytime flowering and grain production [13, 14] . During heat stress photosynthesis is reduced due to decreased of chlorophyll content and increased oxidative stress [15]. Additionally, the combination of increased temperature and drought has a destructive, additive effect on crop physiology and phenology, i.e. chlorophyll content, photosynthesis of the leaf, growth, spikelet fertility, grain number, grain filling and therefore grain yield [16, 17]. Combined drought and heat stress, in particular, decreases the chlorophyll content of the leaf by 49%, whereas drought or heat alone reduces it by 9% or 27%, respectively [18].

Maintaining crop productivity levels is a major challenge in modern agriculture, and thus studying the effects of these stresses becomes essential for wheat enhancement that have primarily relied on the genetic changes found in the wheat genome throughout traditional breeding [10]. The transcriptome has been extensively studied after the development of next generation sequencing (NGS) technology, in order to understand the molecular mechanisms through which plant species evolve to their ecosystem. Transcriptome data analysis of plants are effectively carried out in different organisms under various conditions, including susceptibility to abiotic stresses [19]. Microarray technology is one of the major applications of transcriptome data analysis that has allowed the expression levels of thousands of genes to be tracked simultaneously. Such technologies have been successfully used in the study of crop response to drought, heat and combined stress in crops, and thousands of stress- responsive genes enriched with various biological functions, such as: Heat Shock Proteins (HSPs), Heat Shock Factors (HSFs), and Drought Responsive Element Binding Factors (DREBs) were described [20, 21].

Consequently, the production of wheat varieties with high and stable yields under these environmental stresses is one of the most important breeding objective [22]. The aim of our investigation is to study the wheat genetic profile response to drought (DS), heat stress (HS), and combined stress (heat and drought) (HD). We aim to use comprehensivly bioinformatics techniques tools to identify (a) differentially regulated genes commonly expressed in wheat response to DS, HS, and HD stress; (b) to study gene families, gene networks, ontologies, pathways, and protein-protein interactions controlling wheat tolerance to these stresses; (c) to determine whether there were significant interactions between the effects of DS, HS, and HD.

Materials and Methods

Dataset

The available GEO GSE45563 dataset was downloaded from the Gene Expression Omnibus database (GEO). This data is based on genome chip sets of Affymetrix wheat genome uncovering the gene expression profiles of two durum wheat varieties (Ofanto and Cappelli) (using 24 samples). Categorized by different water use efficiency (WUE), grown to a booting stage and exposed

to a mixture of drought and heat stress, a scenario similar to the experience of a crop grown in Mediterranean conditions and subjected to terminal drought / heat stress [23].

Differential gene expression analysis

The recognition of differentially expressed genes (DEGs) in the transcription profiles was analyzed through default parameters of GEO2R. The data on the gene expression profile consists of 24 different samples of wheat which have been classified by different treatments (control, drought, heat, and mixed). In order to create a more efficient analysis, these samples were compared as follow: (1) Drought samples were compared to control samples (Dctrl); (2) Heat samples were compared to control samples (Hctrl); (3) Mixed condition samples were compared to control samples (Mctrl); (4) combined data of heat and drought samples were compared to control (HDctrl); (5) combined data of heat and drought samples were compared to mixed samples (HD.M). The GEO2R analysis results for these groups were used for further analysis, where DEGs with significance p-value of < 0.001 were used.

Heatmap and hierarchical clustering analysis

The heatmap allows the general expression of the DEGs to be compared under abiotic stress conditions. Hierarchical clustering can be used to combine related elements into a binary tree, and is commonly used in microarray data analysis. R programming language was used to plot the heatmap and perform the hierarchical clustering analysis.

GO enrichment and PPI network analysis

The local BLAST tool [24] was used to identify gene annotation of unknown DEGs genes for durum wheat using the available*Aegilops tauschii* proteome on NCBI. The BLAST gene annotation and protein sequences have been used for further analysis. Evaluation of the network and gene ontology (GO) enrichment of protein-protein interaction (PPI) was conducted in accordance with the STRING repository framework [25]. Cytoscape program was used to simulate protein-protein networks [26]. Venn online software (http://bioinformatics.psb.ugent.be/webtools/Venn/) was utilized to obtain the intersection of Dctrl, Hctrl, Mctrl, HDctrl, HD.M profiles.

Results and Discussion

We studied wheat response to drought (DS), heat (HS), and combined stress (HD) (heat and drought). The available GEO GSE45563 data set was used to investigate these stresses impact on wheat genomic content using bioinformatics analysis. Compared to the previous research used the same data [23], we performed gene annotation of DEGs, conducted a detailed PPI analysis and performed a more detailed gene enrichment analysis. In addition, we analyzed wheat response to drought, heat, and combined stress through further molecular analysis.

Differentially expressed genes among wheat samples

In order to conduct more comprehensive and productive analysis, the gene expression profiles of control, drought, heat and mixed treatments were compared. **Table 1** shows the number of DEGs revealed in every comparison group as conducted through GEO2R tool. Additionally, detailed information have been shown in **Figure 1** and **Table S1**.

Table 1. The DEGS analysis significance scores for the different analysis groups as revealed by GEO2R software.

Group	Count	HEAD	Min	Min. count	Max	Max. count
D.ctrl	292.00	logpvalue	9.30	1.00	0.00	1.00
		logFC	-3.94	1.00	3.77	1.00
H.ctrl	3443.00	logpvalue	12.30	1.00	0.00	12.00
		logFC	-8.93	1.00	10.57	1.00
M.ctrl	5483.00	logpvalue	13.41	1.00	0.00	9.00
		logFC	-10.98	1.00	11.67	1.00
HD.M	1005.00	logpvalue	6.51	1.00	0.00	2.00
		logFC	-5.64	1.00	5.70	1.00
HD.ctrl	568.00	logpvalue	9.63	1.00	0.00	1.00
		logFC	-4.09	1.00	4.44	1.00

Among the DEGs genes revealed in D.ctrl group, DNA gyrase subunit B (*gyrB*),*C6orf132*homolog, and abscisic acid receptor (*PYR1*) were the highest, with P-value (-log10) of 9.3, 7.3, 6.4, and logFC of -3.9, 2.0, 1.6, respectively (**Figure 1** and **Table S1**). The*gyrB*gene plays a crucial role in the partitioning of chloroplast nucleoid by regulating the topology of DNA [27].DNA gyrase is special among topoisomerases because it is the only enzyme that actively uses the energy of ATP hydrolysis to supercoil DNA [28].The association between topoisomerases proteins and the ability of plants to maintain plant biological system under drought stress was previously reported [29].The pyrabactin resistance 1 (*PYR1*) is essential for ABA-mediated responses including stomatal closure and inhibition of plant growth [30].ABA levels were shown to boost in the plant under abiotic stress, such as drought and high salinity, triggering adaptive responses featuring inhibition of type 2C protein phosphatases (*PP2C*), and stimulation of non-fermenting kinases 2 related protein sucrose (*SnRK2*) [31].

In this analysis, gene expression fold change was high in genes such as zinc finger MYM-type 1 (*ZMYM1*), ricin B lectin R40G3 (*R40G3*), berberine bridge enzyme 27 (BBE27), with approximately 3.5 logFC (**Figure 1** and **Table S1**). Significant expression of the*ZMYM1*,*R40G3*, and*BBE27*genes in plant response to drought could assign new functions to these genes and provide more insight into their role.Carbohydrate-binding proteins or lectins such as Ricin B lectin are a particular class of entomotoxic proteins that have an important role in plant direct defense responses, in addition such proteins regulation have been reported in plant response to cold stress [32] and drought [33]. Moreover, although a significant number of genes encoding BBE-like proteins have been detected in plants and bacte-

ria in recent years, the role of *BBE* homologs in plant growth remains mysterious. These molecules have been linked to the plant response to osmotic stress and the pathogen attack has been shown to induce up-regulation of some of these family members [34, 35]. Additionally, the overexpression of *ZMYM1* has been reported in various plant stress resistance researchers through gene expression analysis such as peanut [36] and wheat [37].

In the H.ctrl group, genes with a known role in plant heat tolerance and genes with a potential role in other biotic and abiotic stresses had the highest significance and logFC values. Genes such as ribulose bisphosphate carboxylase/oxygenase activase B (RuBisCO activase), small heat shock, non-specific lipid-transfer 3 (*LTP3*), *YLS3, At2g33490*, ferredoxin-NADP reductase (*PETH*) were among the highest significance DEGs with p-values (-log10) ranged from 9.3 to 12.3. Additionally, proteins labeled with "heat shock" have the highest p-values and logFC values indicating its priority in maintaining plant tolerance to heat stress (**Figure 1** and **Table S1**).

The association between RuBisCo activase and the plant response to heat could be due to the involvement of RuBisCo activase in the regulation of different cellular processes as a catalytic chaperone and its ability to modulate the activity of RuBisCo and to protect the nascent proteins from aggregation during heat stress. RuBisCo is deactivated through heat stress due to catalytic misfiring as well as a higher rate of dead-end product production, thus inhibiting the process of photosynthesis [38, 39]. Non-specific lipid transfer proteins (*nsLTPs*) comprise small, simple proteins with an eight-cysteine patterns. The biological roles of these proteins were stated to include plant reproduction and response to biotic or abiotic stress [40]. Additionally, it was speculated that*LTP3*play an important role in wheat tolerance to heat and drought [41, 42] . It was suggested that, due to heat stress, key proteins that regulate electron transport activity have a potential role to play in plant response, such observations have been observed in soybean [43]. This could explain the high-fold change in*PETH*and LHCII due to the fact that both proteins play a crucial role in the plant photosystem. PETH is the last enzyme in the transition of photosynthesis electrons from Photosystem I to NADPH in the Calvin cycle reactions, while*LHC*operates as a light receptor that encapsulates and provides excitation energy to photosystems I and II with which it is closely connected [43, 44].

The effect of heat stress on gene expression fold change of the peroxidase gene is of interest in our analysis. Genes such as peroxidase 1 (*APX1*), cysteine-rich repeat secretory 55 (*CRRSP55*), *WIR1A*, chlorophyll a-b binding of *LHCII* type 1 have a high negative fold change ranged from -7 to -8 (**Figure 1** and **Table S1**). Peroxidases cause the transformation of hydrogen peroxide to water and oxygen are component of the enzymatic defense of plant cells. The relationship between peroxidase activities and heat tolerance have been reported in strawberry [45]. Additionally, studying the effect of response of *Arabidopsis thaliana* to stress combination of heat and drought re-
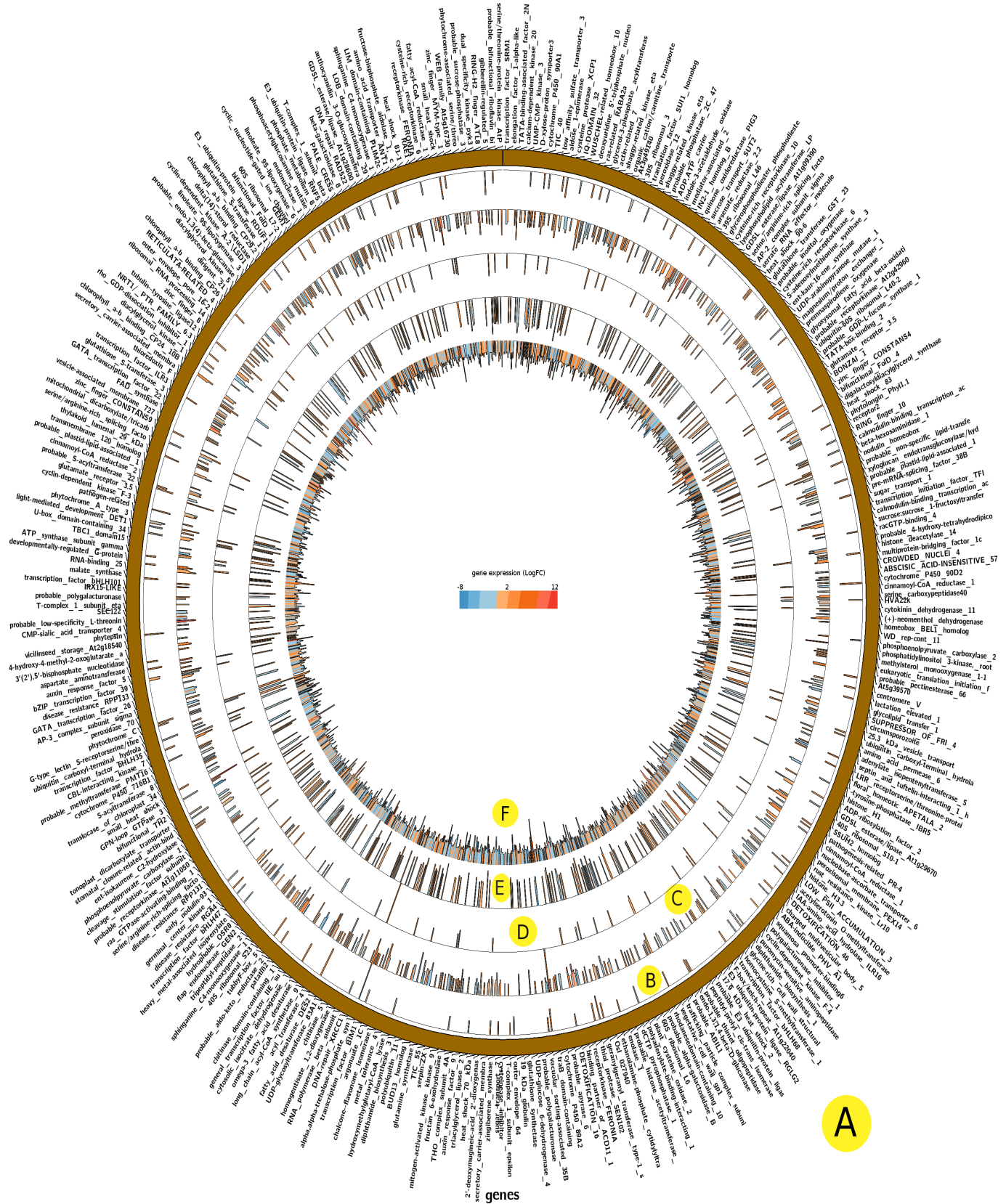
Figure 1. The gene expression and significance scores of DEGs of wheat genome. The gene expression (LogFC) and the significance (logpvalue) wheat genes (A) in D-ctrl (B), H-ctrl (C), HD-ctrl (D), HD-M (E), M-ctrl (F) gene profiles. The chart high and color stand for gene significance and expression, respectively.

vealed that, APX1-deficient mutant produced more hydrogen peroxide and was considerably more sensitive to the combination of stress than wild-type [46].

In our analysis gene expression profiles of M.ctrl provides more insight about the mixed stresses effect on wheat. The M.ctrl gene expression profile provided 2,236 genes with p-values (-log10) ranges from 3 (LOC109775146) to 12.3 (RuBisCO) and fold change (logFC) reaches -8.3 (*CRRSP55*) and 11 (16.9 kDa class I heat shock 1: HSP16.9A) (**Figure 1** and **Table S1**). Among these genes about 25 were labeled as heat shock proteins. The high number of significant genes could infer the high effect of mixed stress on the biological system of plants, which is rationally expected [47].

On the other hand, 174 genes were differentially expressed in HD.ctrl sample set, among which the p-values (-log10) ranges from 3 (LOC109732491) to 9.6 (*gyrB*). The fold change (logFC) reaches from -4 (S-linalool synthase:LIS) and 4.5 (*gyrB*) (**Figure 1** and **Table S1**). While comparing HD profile to M (HD.M) provided p-values (-log10) ranges from 3 (probable amino acid permease 7 *AAP7*) to 5.3 probable purine permease 11 (*PUP11*). The fold change (logFC) reaches from -5.6 (acyl transferase 15 *AT15*) and 4.3 (group 3 late embryogenesis abundant*LEA*). The *PUP11* overexpression in plant response to heat stress have been reported in wheat [48]. This gene belongs to the PUP gene family, which is responsible for the transportation of nucleoside-type Natural Cytokinins (CKs) in some plant species, thereby playing an important role in plant growth and development, including tolerance for drought [49].

Additionally, expression of LEA gene in barley has been reported to confer tolerance to water deficit and salt stress . This could be due to some LEA interacting with a receptor like cytoplasmic kinase and controlling the responses to environmental stress, in addition to initiating plant repair under various abiotic stress conditions [50, 51, 52].

We investigated the fold change difference in plant responses against drought, heat, and combination stresses. About 68, 117, and 57 and genes were shared between H.ctrl and D.ctrl, HD.ctrl and M.ctrl, H.ctrl , M.ctrl and D.ctrl across DEGs profiles (**Figure 2** and **Table S2**). Comparing DEGs profiles of H.ctrl to D.ctrl highlighted the extreme fold change in some DEGs belong to genes such as *LIS*, Light-inducible protein *CPRF2*, and two-component response regulator *ORR6* between H.ctrl (down regulated) and D.ctrl (up regulated) (**Figure 2A** and **Table S2**).

In addition, we can see that there is some contrast between heat and drought gene expression. In rice the *ILS* gene is responsible for linalool production, which control antibacterial activity and is essential for resistance to blight pathogen [53]. Interestingly, transcriptome and metabolite profiling have been reported to reveal that severe drought modulates the pathway of phenylpropanoid and terpenoid in white grapes, including the production of linalool [54].

The CPRF2 protein which is a member of bZIP TF family is related to the regulation system controlling chalcone syn-

thase through hormone control or light-induction. The overexpression of such gene under drought stress have been reported in Sesame [55], which could be due it is known relation in flower development [56]. The *ORR6* gene is related to cytokinin signaling, and recently it has been reported that it could be downregulated in some salt-tolerant maize inbred line [57]. We can also see a slight difference in the expression of the berberine bridge enzyme (*BBE*) between heat and drought. It was reported that *BBE* is highly important in plant adaptation to phosphorus deficiency [58], which is more related to drought [59].

Generally, according to shared DEGs, there is no noticeable difference between HD.ctrl and M.ctrl in gene expression, which could infer the collective impact of mixed drought and heat stress (**Figure 2B** and **Table S2**). In addition, the comparison between D.ctrl, H.ctrl and M.ctrl revealed a high similarity between M.ctrl and H.ctrl compared to D.ctrl, which could infer the prevalence impact of heat stress on the wheat biological system under mixed stress (**Figure 2C** and **Table S2**). Such a result supports previous reports that revealed that DS transcriptomes have a distinct connection to HS and HD, implying a significant shift in expression of genes in DS responsive transcriptome relative to HS and HD [21].

The Venn analysis was used to infer shared and unique genes between different gene expression profiles (**Figure 3** and **Table S3**). The Venn analysis revealed only 5 genes shared between all profiles (H.ctrl, D.ctrl, HD.ctrl, M.ctrl, and HD.M) (**Figure 3** and **Table S3**). These genes include heat stress transcription factor A-2e (*HSFA2E*), *PUP11*, (+)-neomenthol dehydrogenase (SDR1), mechanosensitive ion channel 6, and Fructokinase-2 (*FRK2*). In this regard, correlation between plant response to heat stress and the expression of *FRK2* was reported in soybean [60]. The highest number of genes shared is between M.ctrl and H.ctrl (478 genes), which confirm the high prevalence of heat in combination stress. There are nine genes between D.ctrl, H.ctrl and M.ctrl that are shared and unique in comparison to other profiles. These genes include *LTP3, ORR6, BBE27,* ankyrin repeat domain-containing protein 2A (*AKR2A*), amino acid permease 6 (*AAP6*), S-adenosylmethionine synthase 3 (*SAM3*), organic cation/carnitine transporter 7 (*OCT7*) (**Figure 3** and **Table S3**). Interestingly, *AKR2A* displays chaperone activity towards mitochondrion outer membrane, endoplasmic reticulum membrane, chloroplast outer envelope membrane, and peroxisomal proteins.

Additionally, it is important for chloroplast biogenesis, and increases plant biological system capacity for abiotic stresses [61, 62]. On the other hand, *AAP6* is correlated with plant drought tolerance [63]. In human, the transcriptional activity of *OCT7* is correlated with osmotic stress in epididymal cells, which could explain its correlation with both drought and heat stresses in plant [64]. The intersections of M.ctrl and HD.ctrl, and HD.M profiles do not share unique genes, while M.ctrl and HD.M contain, respectively, 1,156 and 246 unique genes that are not shared with other profiles. We could assume that the
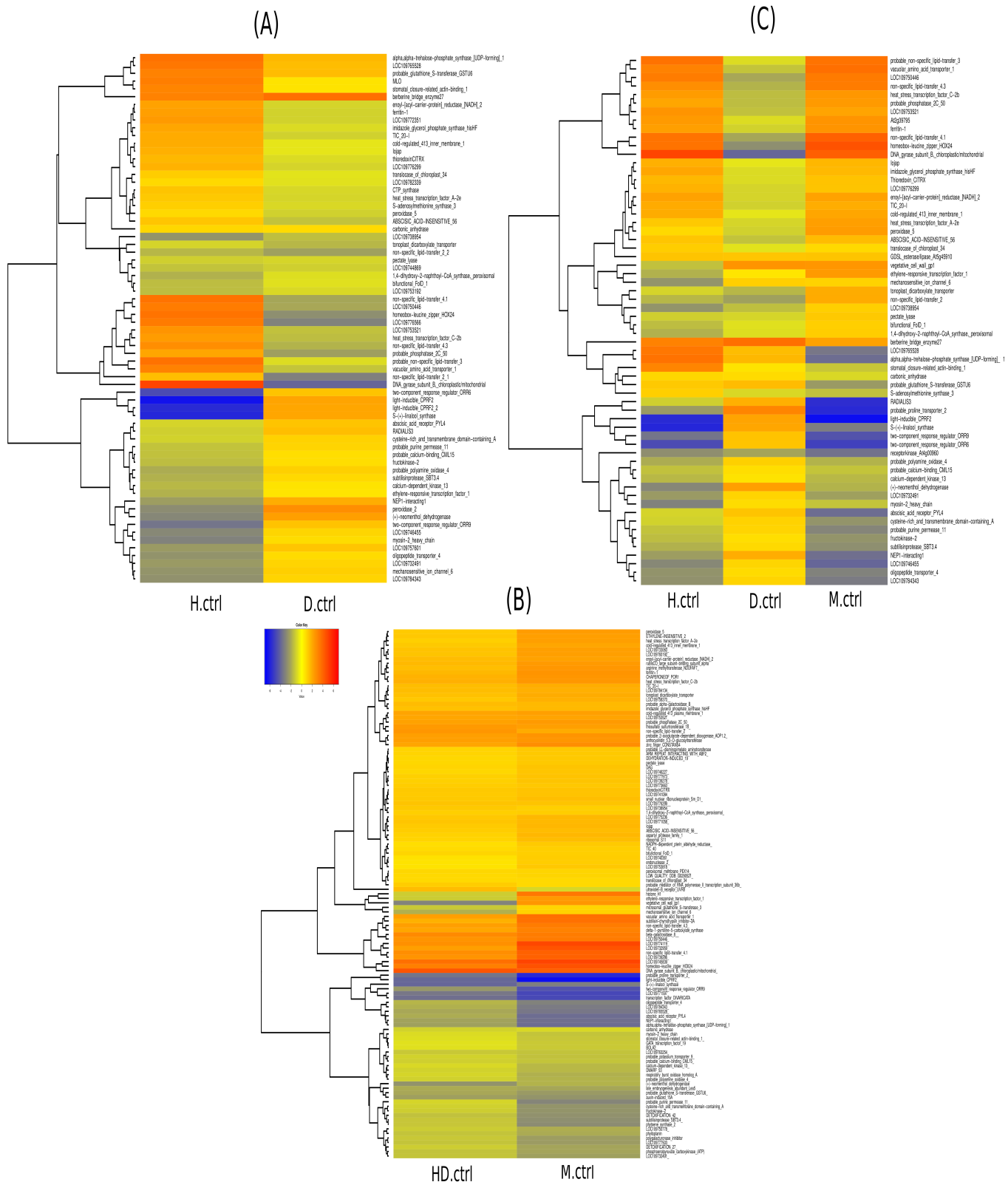
Figure 2. Heatmap and hierarchical clustering of differential gene expression. The gene expression of shared genes between (A) H.ctrl and D.ctrl, (B) HD.ctrl and M.ctrl, and (C) H.ctrl, D.ctrl and M.ctrl.
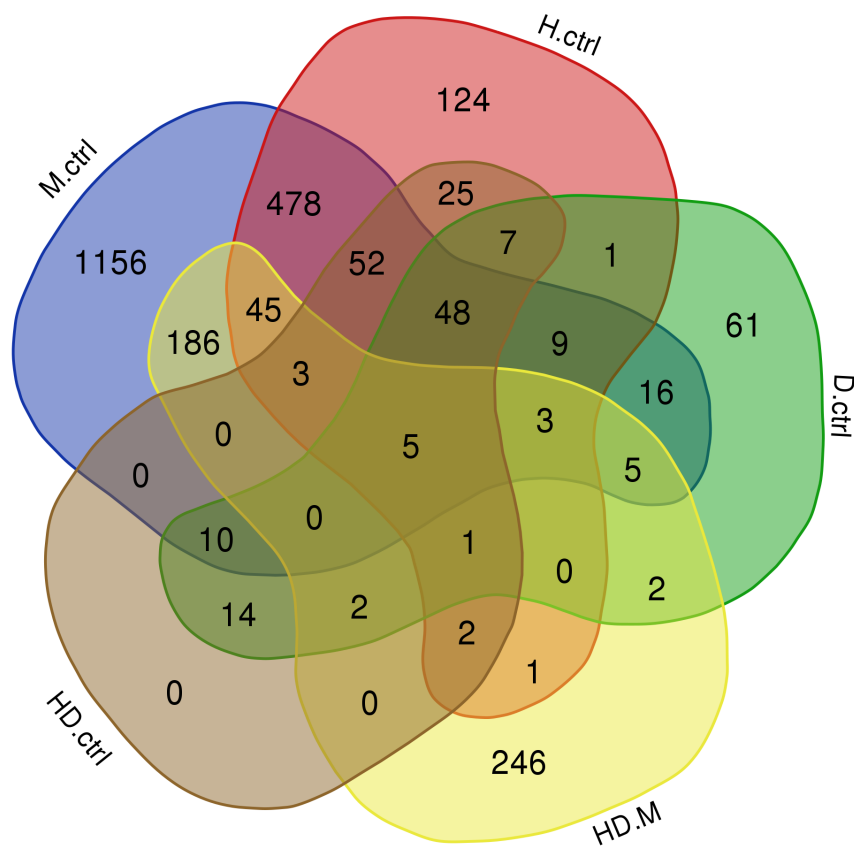
Figure 3. Venn diagram analysis between gene profiles of shared and unique genes.

high number of unique DEG genes belonging to the M.ctrl and HD.M profiles could infer the high effect of mixed stress on the wheat biological system. The pathway analysis of such genes revealed high abundance of metabolic pathways in M.ctrl and carbon metabolism in HD.M (**Figure S4**).

Gene enrichment ontology analysis

We used gene ontology enrichment analysis to study the targeted biological pathways of DEGs (Figure 4). The DEGs genes of D.ctrl were highly related to the protein domains of lipid-transfer (LTP) (Figure 4A). In this regard, several lipid-transfer proteins have been reported to modulate plant response to biotic stress, where loss-of-function mutant LTPs may have a high sensitivity to drought stress [65]. Additionally, metabolic pathways and biosynthesis of secondary metabolites were highly correlated with H.ctrl, M.ctrl and HD.ctrl (**Figure 4B, 4D and 4E**). Furthermore, M.ctrl revealed high number of glycosyl hydrolases family 17 proteins (Figure 4D). The association between such family and heat stress have been reported in switchgrass [66], and rice [67]. The analysis of HD.M gene profile displayed high number of PCI (proteasome/CSN/eIF3) domains. The translation regulation plays an important role in plant species at all development stages and during the response to stresses.

The significance of the eIF3 complex lies not only in the global initiation event stage but also in the precise translation regulation of unique transcripts, thus maintaining the ability of plants to resolve environmental stresses [68].

Protein-protein interaction analysis

The STRING database is a highly respected tool for protein-protein interaction networks and could provide very comprehensive and fruitful information that provides a valuable result (**Figures 5, 6, 7, and 8**). Both delta-1-pyrroline-5-carboxylat (*P5C*) and putative aconitate hydratase show high number of interaction activity in D.ctrl profile (**Figure 6A**). The over-expression of *P5C* improves the production of proline and provides osmotolerance in transgenic plants and catalyzes the biosynthesis of proline in plants [69]. High number of H.ctrl interactions involve Succinate dehydrogenase (*SDH*), *Phosphoenolpyruvate carboxykinase* (*PEPCK*), Glucose-6-phosphate dehydrogenase (*G6PD*) (**Figure 7**). The gene of *PEPCK* have an essential role in organic acid metabolism. The overexpression of *PEPCK* in heat, drought, and salinity have been reported in some plants [70, 71]. Similar to D.ctrl, the *P5C* have the highest number of protein interactions followed by ATP synthase gamma subunit in M.ctrl and (**Figures 6B and 8**). The ATPase activity has a very es-

(A)

number of nodes: 66	expected number of edges: 4
number of edges: 14	PPI enrichment p-value: 0.00014
average node degree: 0.424	
avg. local clustering coefficient: 0.184	

PFAM Protein Domains

domain	description	count in gene set	false discovery rate
PF14368	Probable lipid transfer	5 of 71	3.89e-05
PF00234	Protease inhibitor/seed storage/LTP family	5 of 85	4.53e-05

SMART Protein Domains

domain	description	count in gene set	false discovery rate
SM00499	Plant lipid transfer protein / seed storage protein / trypsin-alph…	6 of 130	6.57e-06

(B)

number of nodes: 237	expected number of edges: 158
number of edges: 212	PPI enrichment p-value: 2.73e-05
average node degree: 1.79	
avg. local clustering coefficient: 0.312	

KEGG Pathways

pathway	description	count in gene set	false discovery rate
ats01100	Metabolic pathways	53 of 1598	3.24e-19
ats01110	Biosynthesis of secondary metabolites	22 of 1011	0.00013
ats00520	Amino sugar and nucleotide sugar metabolism	7 of 106	0.00036
ats00280	Valine, leucine and isoleucine degradation	4 of 26	0.00099
ats00053	Ascorbate and aldarate metabolism	4 of 32	0.0017
ats00480	Glutathione metabolism	6 of 109	0.0019
ats01230	Biosynthesis of amino acids	7 of 165	0.0022
ats04626	Plant-pathogen interaction	7 of 176	0.0028
ats01200	Carbon metabolism	7 of 185	0.0034
ats00330	Arginine and proline metabolism	4 of 50	0.0040
ats00010	Glycolysis / Gluconeogenesis	5 of 102	0.0062
ats04146	Peroxisome	4 of 67	0.0094

(C)

number of nodes: 140	expected number of edges: 81
number of edges: 111	PPI enrichment p-value: 0.000804
average node degree: 1.59	
avg. local clustering coefficient: 0.318	

KEGG Pathways

pathway	description	count in gene set	false discovery rate
ats01200	Carbon metabolism	9 of 185	5.90e-06
ats01100	Metabolic pathways	23 of 1598	5.90e-06
ats00520	Amino sugar and nucleotide sugar metabolism	6 of 106	0.00012
ats01230	Biosynthesis of amino acids	6 of 165	0.00097

PFAM Protein Domains

domain	description	count in gene set	false discovery rate
PF01399	PCI domain	3 of 24	0.0353
PF12515	Ca2+-ATPase N terminal autoinhibitory domain	2 of 6	0.0441
PF05770	Inositol 1, 3, 4-trisphosphate 5/6-kinase	2 of 6	0.0441
PF01590	GAF domain	2 of 8	0.0441
PF00512	His Kinase A (phospho-acceptor) domain	2 of 11	0.0485

SMART Protein Domains

domain	description	count in gene set	false discovery rate
SM00088	motif in proteasome subunits, Int-6, Nip-1 and TRIP-15	3 of 22	0.0072

(D)

number of nodes: 555	expected number of edges: 1001
number of edges: 1320	PPI enrichment p-value: < 1.0e-16
average node degree: 4.76	
avg. local clustering coefficient: 0.313	

KEGG Pathways

pathway	description	count in gene set	false discovery rate
ats01100	Metabolic pathways	115 of 1598	8.48e-38
ats01110	Biosynthesis of secondary metabolites	55 of 1011	1.83e-12
ats01200	Carbon metabolism	20 of 185	5.01e-09
ats00195	Photosynthesis	9 of 36	8.09e-07
ats00520	Amino sugar and nucleotide sugar metabolism	12 of 106	8.78e-06
ats00280	Valine, leucine and isoleucine degradation	7 of 26	1.20e-05
ats00710	Carbon fixation in photosynthetic organisms	9 of 57	1.43e-05
ats00053	Ascorbate and aldarate metabolism	7 of 32	2.98e-05
ats00260	Glycine, serine and threonine metabolism	8 of 48	3.08e-05
ats00480	Glutathione metabolism	11 of 109	3.66e-05
ats01230	Biosynthesis of amino acids	13 of 165	5.71e-05
ats04146	Peroxisome	8 of 67	0.00021
ats00190	Oxidative phosphorylation	8 of 73	0.00034
ats00010	Glycolysis / Gluconeogenesis	9 of 102	0.00052
ats00030	Pentose phosphate pathway	6 of 42	0.00070

PFAM Protein Domains

domain	description	count in gene set	false discovery rate
PF00332	Glycosyl hydrolases family 17	8 of 58	0.0060
PF00175	Oxidoreductase NAD-binding domain	5 of 17	0.0062

(E)

number of nodes: 56	expected number of edges: 7
number of edges: 10	PPI enrichment p-value: 0.175
average node degree: 0.357	
avg. local clustering coefficient: 0.15	

Reference publications

publication	(year) title	count in gene set	false discovery rate
PMID:26982202	(2016) Transcriptome Analysis for Abnormal Spike Develo…	2 of 41	0.0300

KEGG Pathways

pathway	description	count in gene set	false discovery rate
ats01230	Biosynthesis of amino acids	3 of 165	0.0376
ats01210	2-Oxocarboxylic acid metabolism	2 of 37	0.0376
ats01100	Metabolic pathways	9 of 1598	0.0376
ats00330	Arginine and proline metabolism	2 of 50	0.0376

PFAM Protein Domains

domain	description	count in gene set	false discovery rate
PF00234	Protease inhibitor/seed storage/LTP family	4 of 85	0.0013

SMART Protein Domains

domain	description	count in gene set	false discovery rate
SM00499	Plant lipid transfer protein / seed storage protein / trypsin-…	4 of 130	0.0012

Figure 4. The gene ontology enrichment analysis for DEGs genes of (A) D.ctrl , (B) H.ctrl, (C) HD.M, (D) M.ctrl, and (E) HD.ctrl profiles.

sential role in maintaining plant biological performance under some abiotic stresses [72]. Additionally, in HD.M the protein of malate dehydrogenase (NADP+) have the highest number of interactions. Malate dehydrogenase is critical throughout exposure to abiotic stresses which at cellular level are known to induce oxidative stress such as salt stress [73].

Conclusions

In conclusion we found that *in silico* analysis of GEO GSE45 5 63 data, reveal differences in gene expression profile under normal and stressed conditions. Compared to previous reports, which studied the interaction of drought and heat stress in the plant biological system, we compared the expression of plant genes by multiple stages of abiotic stress and added a more comprehensive analysis. We studied the heat and drought regulation gene PPI, providing a blueprint for how these genes influence the ability of wheat to control drought and/or heat tolerance.

We found specific genes differentially expressed under different treatment. Thus the use of *in silico* gene mining strategies provides an excellent framework for the initial identification of key genes whose expression is altered under abiotic stress. The data generated in these study provide a starting point for investigations aimed to elucidate the molecular basis of abiotic stress tolerance in wheat. and can be useful for breeding and and crop improvement.

Supplementary

Table S1: The information of DEGs genes through studied profiles. Table S2: The information of DEGs genes shared between (A) D.ctrl and H.ctrl,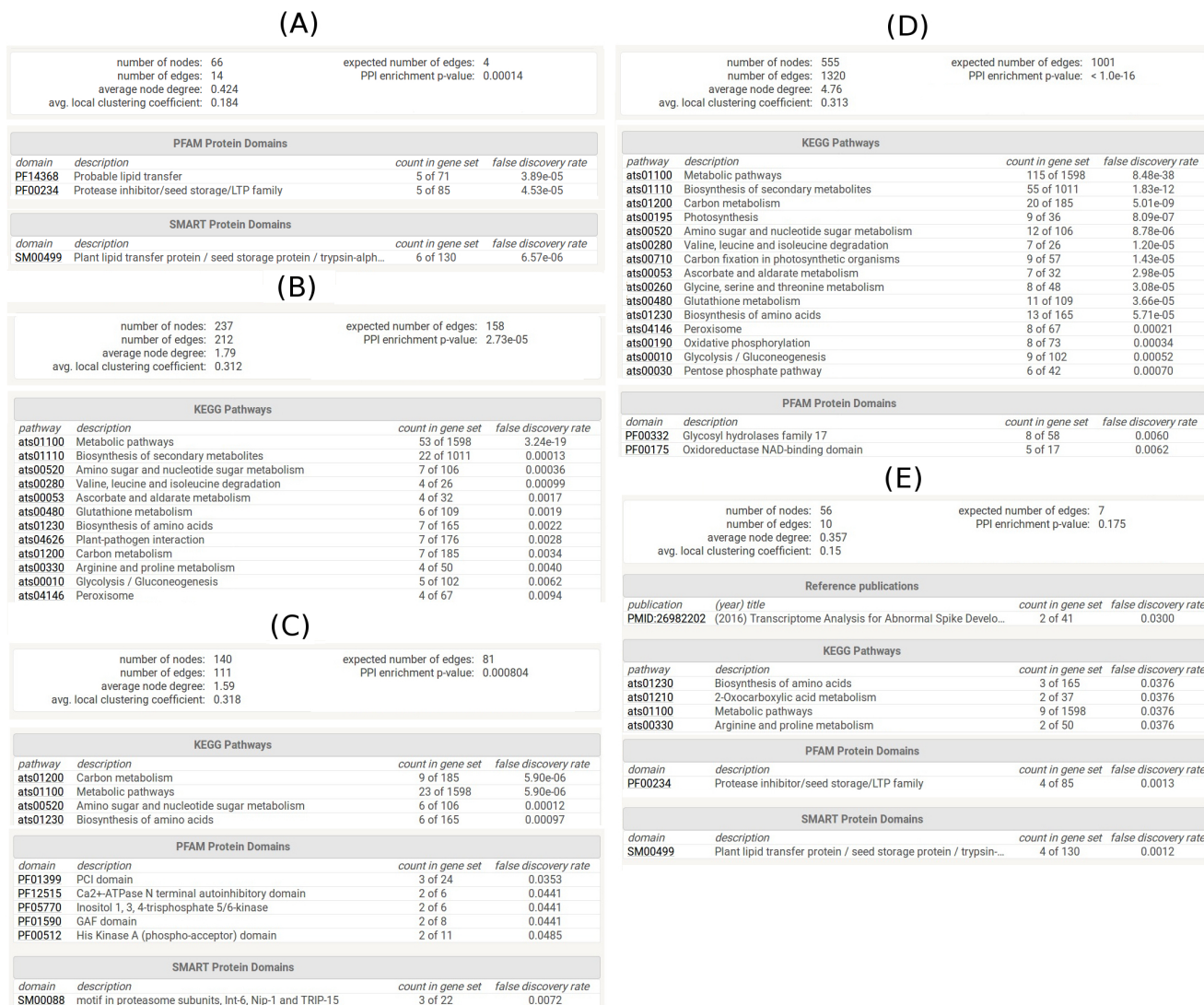 (B) H.ctrl, D.ctrl, and M.ctrl, and (C) HD.ctrl and M.ctrl. Table S3: The information of DEGs genes that are shared and unique among gene profiles according to Venn diagram analysis. Figure S4: the gene enrichment analysis of unique DEGs genes in M.ctrl and HD.M profiles.
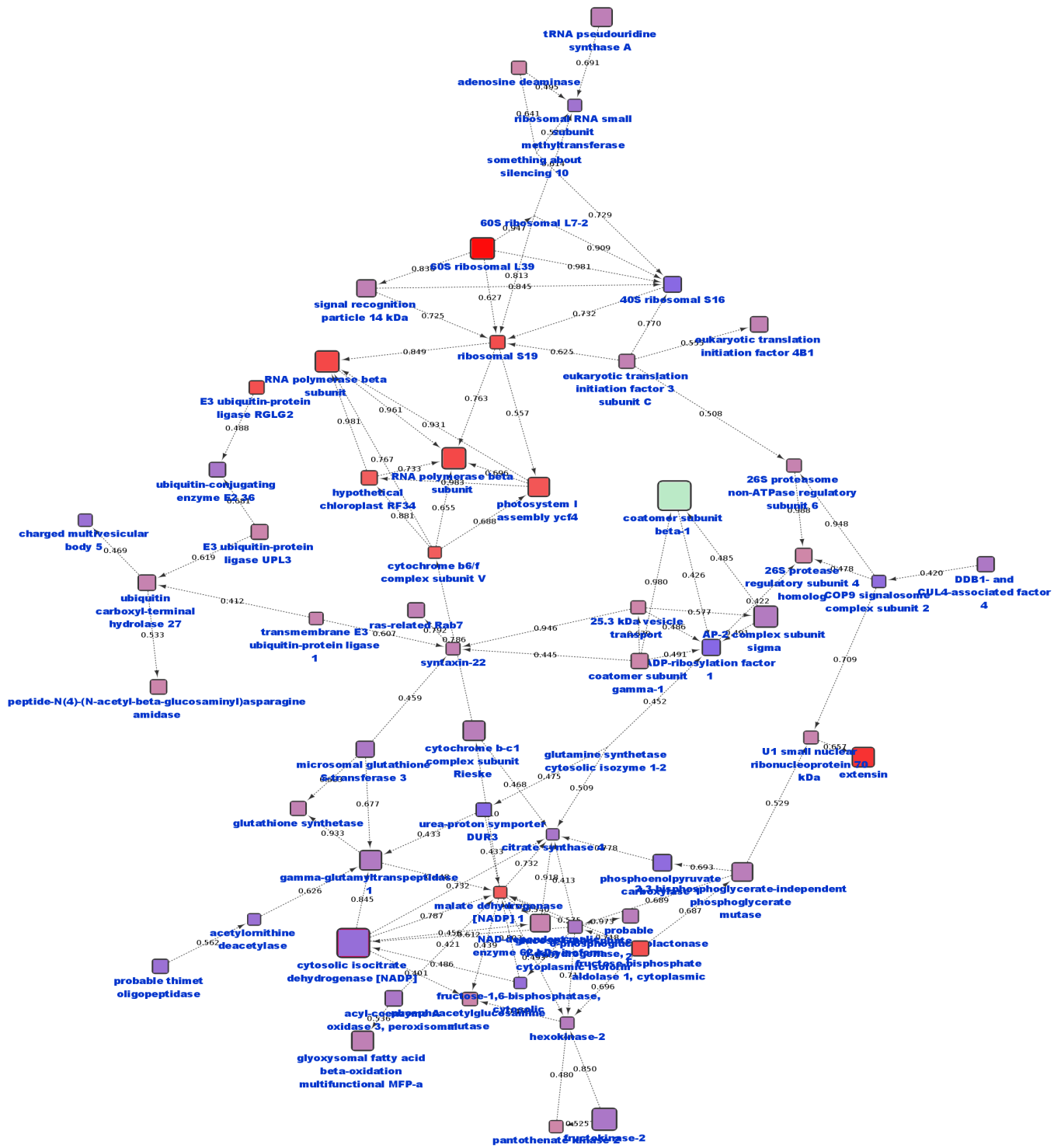
Figure 5. The protein-protein interaction networks of DEGs genes of HD.M gene profile. The node size and color stand for gene significance (log p value) and gene expression (LogFC) .
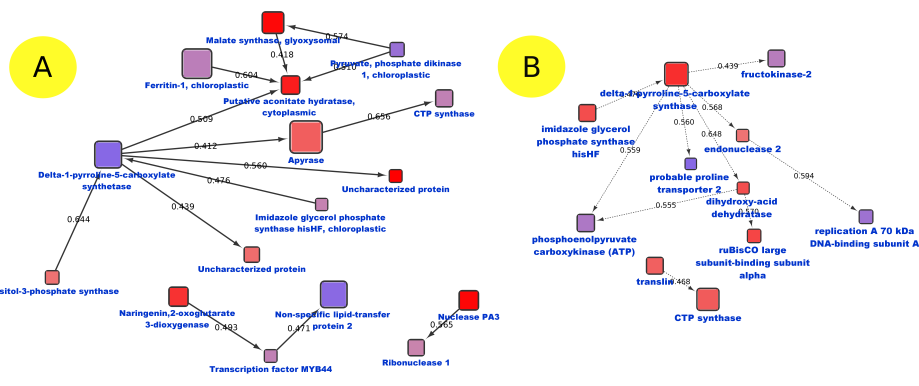
Figure 6. The protein-protein interaction networks of DEGs genes of D.ctrl (A) and HD.ctrl(B) gene profile. The node size and color stand for gene significance (logpvalue) and gene expression (LogFC) .
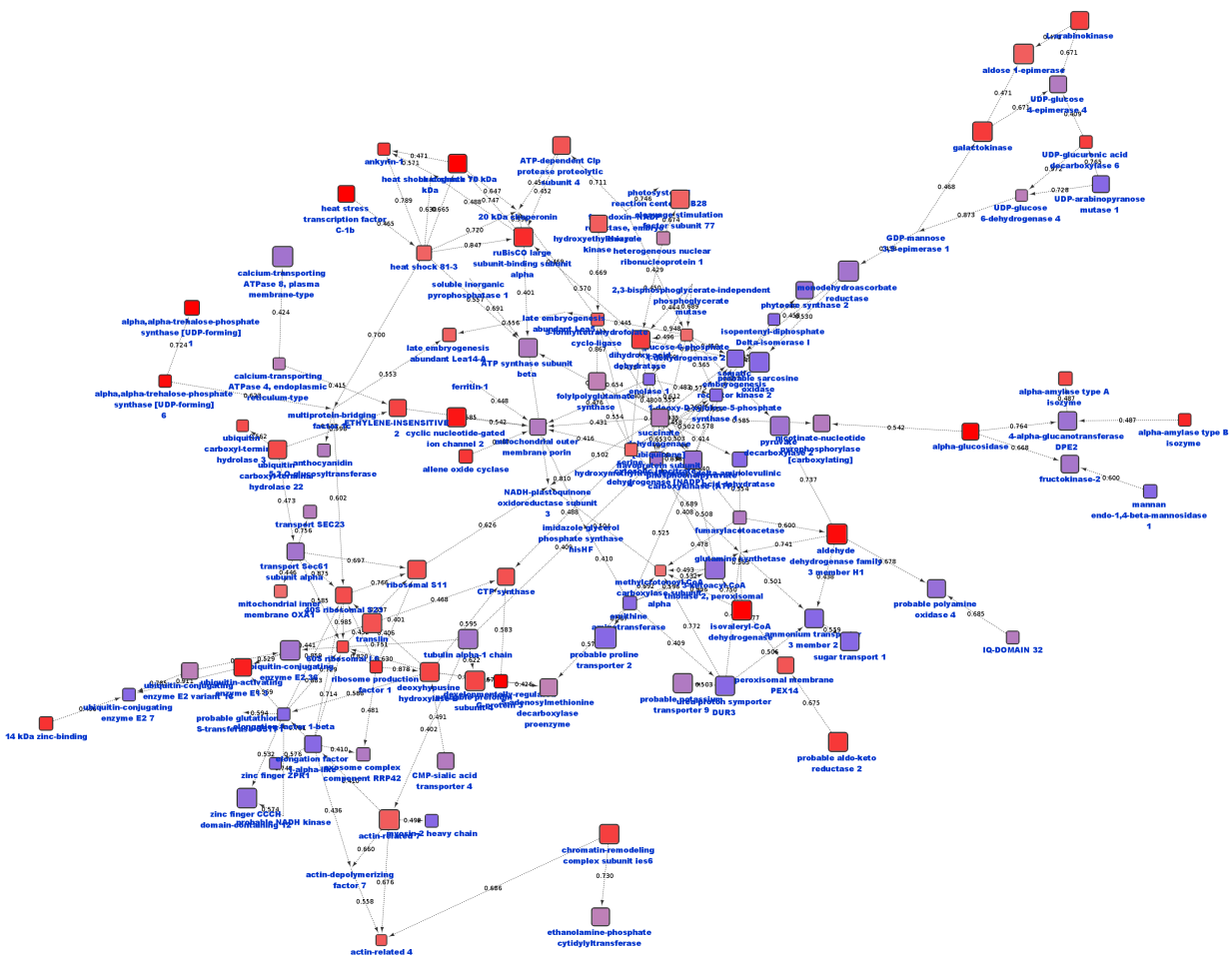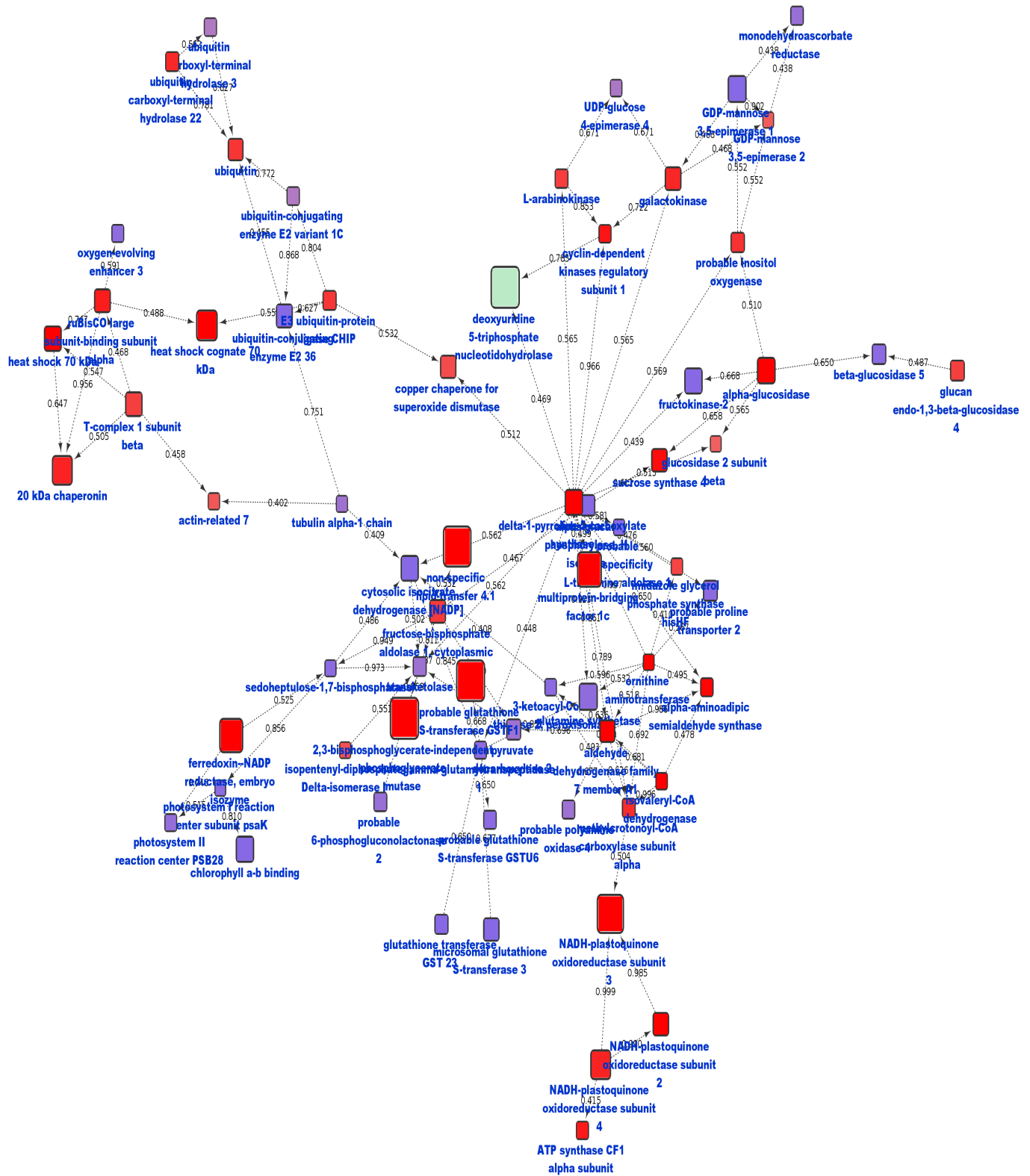

Figure 7. The protein-protein interaction networks of DEGs genes of H.ctrl gene profile. The node size and color stand for gene significance (logpvalue) and gene expression (LogFC) .

Figure 8. The protein-protein interaction networks of DEGs genes of M.ctrl gene profile. The node size and color stand for gene significance (logpvalue) and gene expression (LogFC) .

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