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Research Article

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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 ^{o}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 bacteria 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-

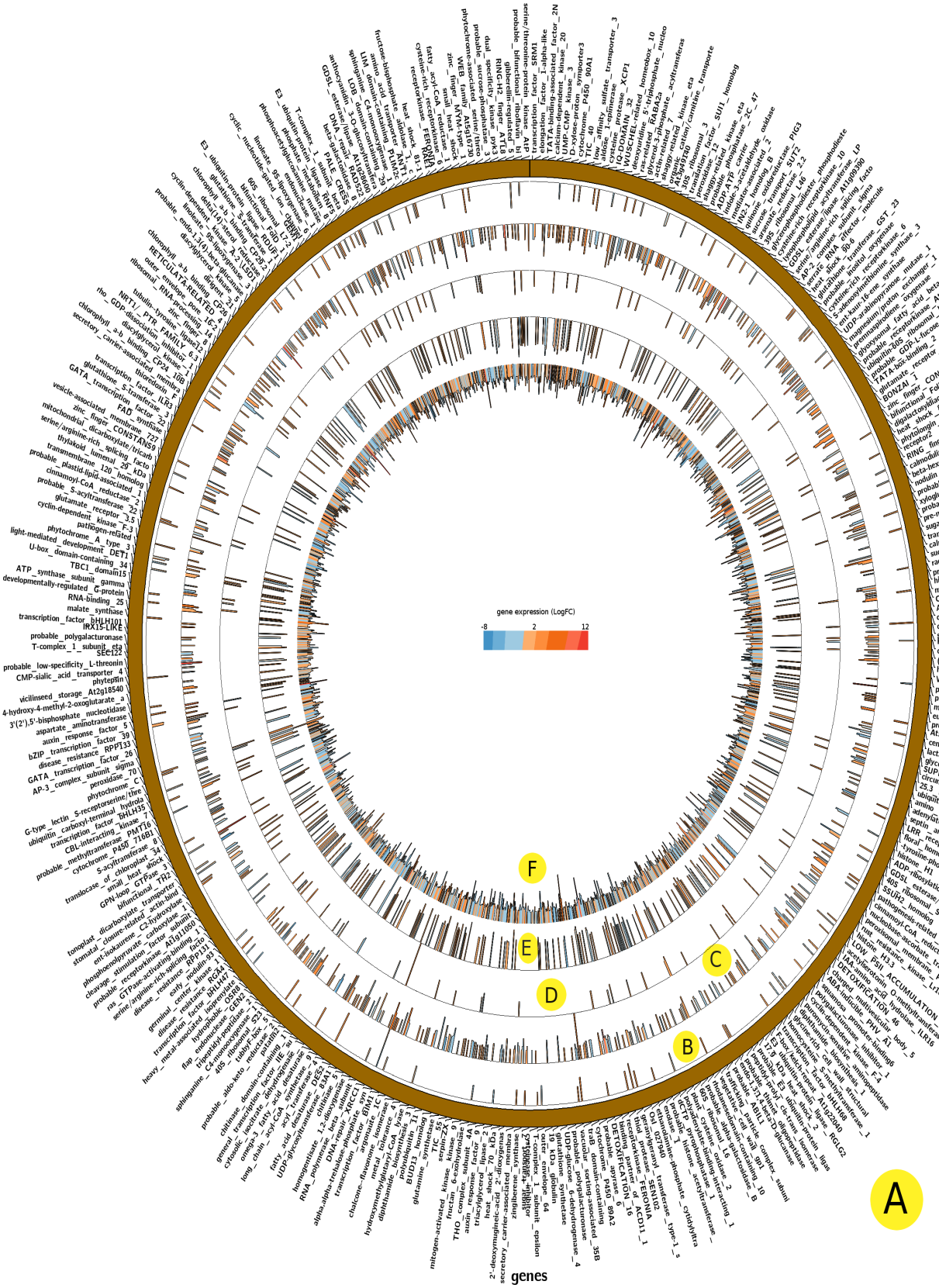

Figure 1. The gene expression and significance scores of DEGs of wheat genome. The gene expression (LogFC) and the significance (logpvalue) wheat genes (A) in D-ctrl (B), H-ctrl (C), HD-ctrl (D), HD-M (E), M-ctrl (F) gene profiles. The chart high and color stand for gene significance and expression, 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 synthase 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 down-regulated 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

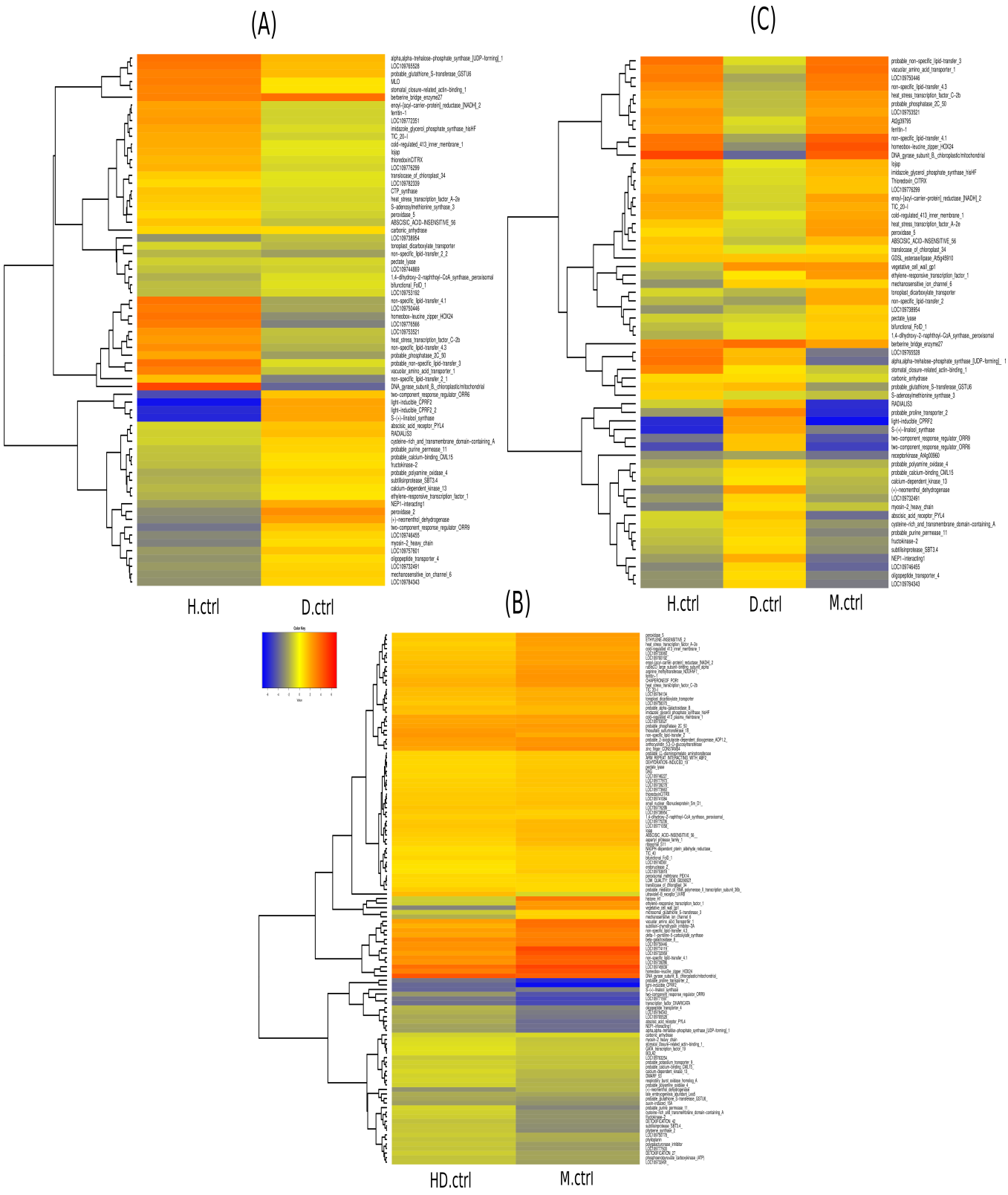

Figure 2. Heatmap and hierarchical clustering of differential gene expression. The gene expression of shared genes between (A) H.ctrl and D.ctrl, (B) HD.ctrl and M.ctrl, and (C) H.ctrl, D.ctrl and M.ctrl.

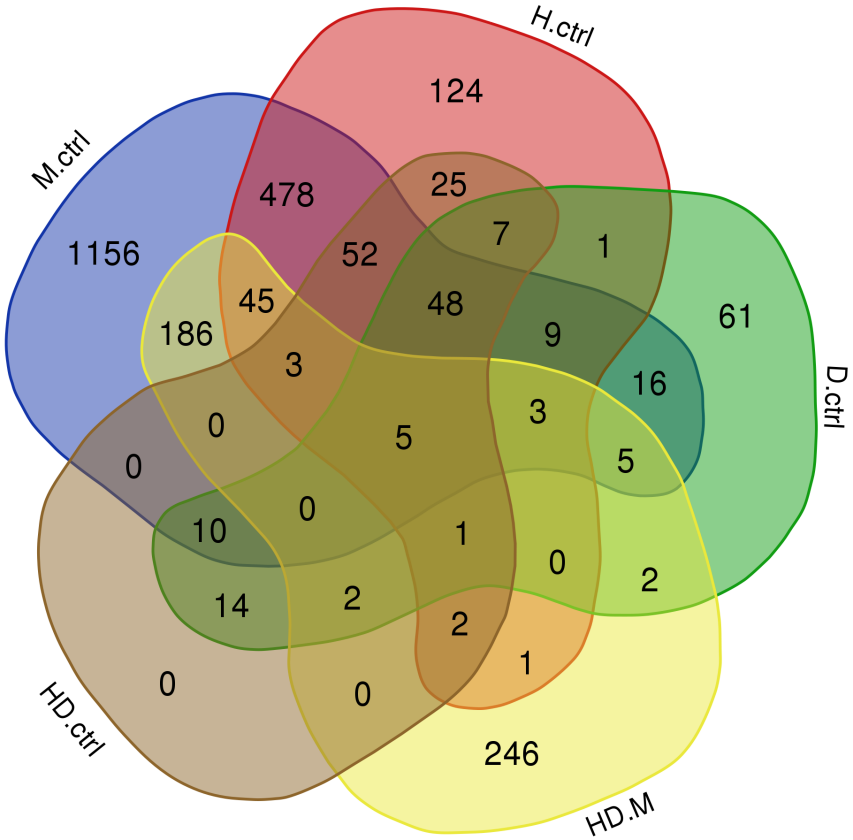

Figure 3. Venn diagram analysis between gene profiles of shared and unique genes.

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-

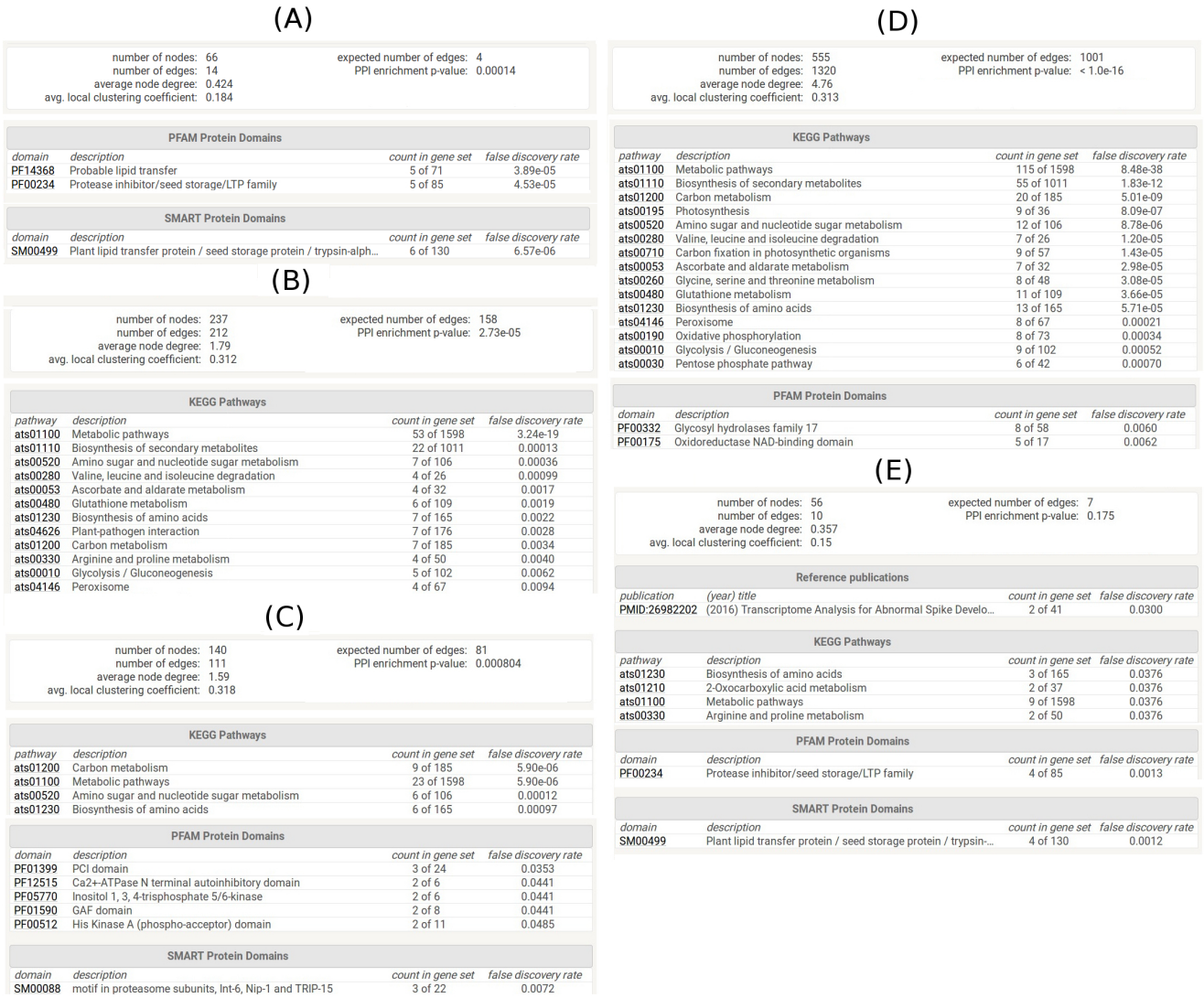

Figure 4. The gene ontology enrichment analysis for DEGs genes of (A) D.ctrl , (B) H.ctrl, (C) HD.M, (D) M.ctrl, and (E) HD.ctrl profiles.

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.

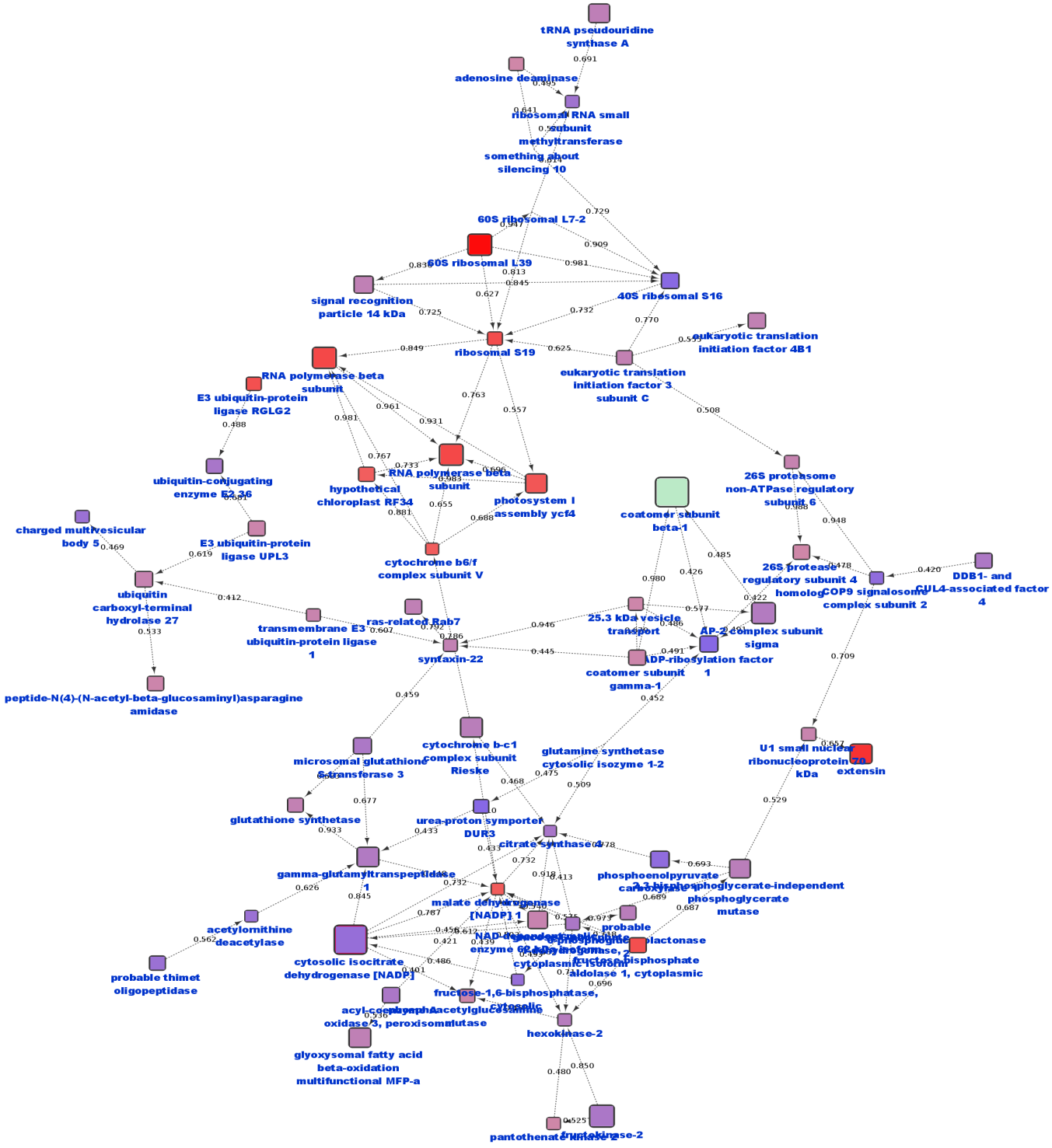

Figure 5. The protein-protein interaction networks of DEGs genes of HD.M gene profile. The node size and color stand for gene significance (log p value) and gene expression (LogFC) .

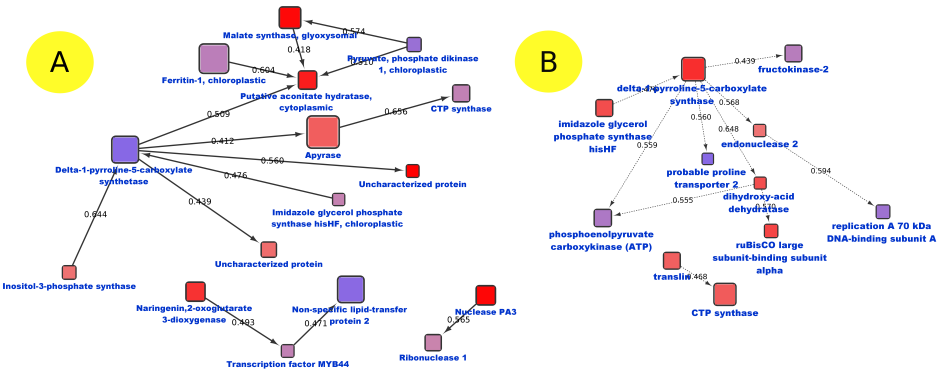

Figure 6. The protein-protein interaction networks of DEGs genes of D.ctrl (A) and HD.ctrl(B) gene profile. The node size and color stand for gene significance (logpvalue) and gene expression (LogFC) .

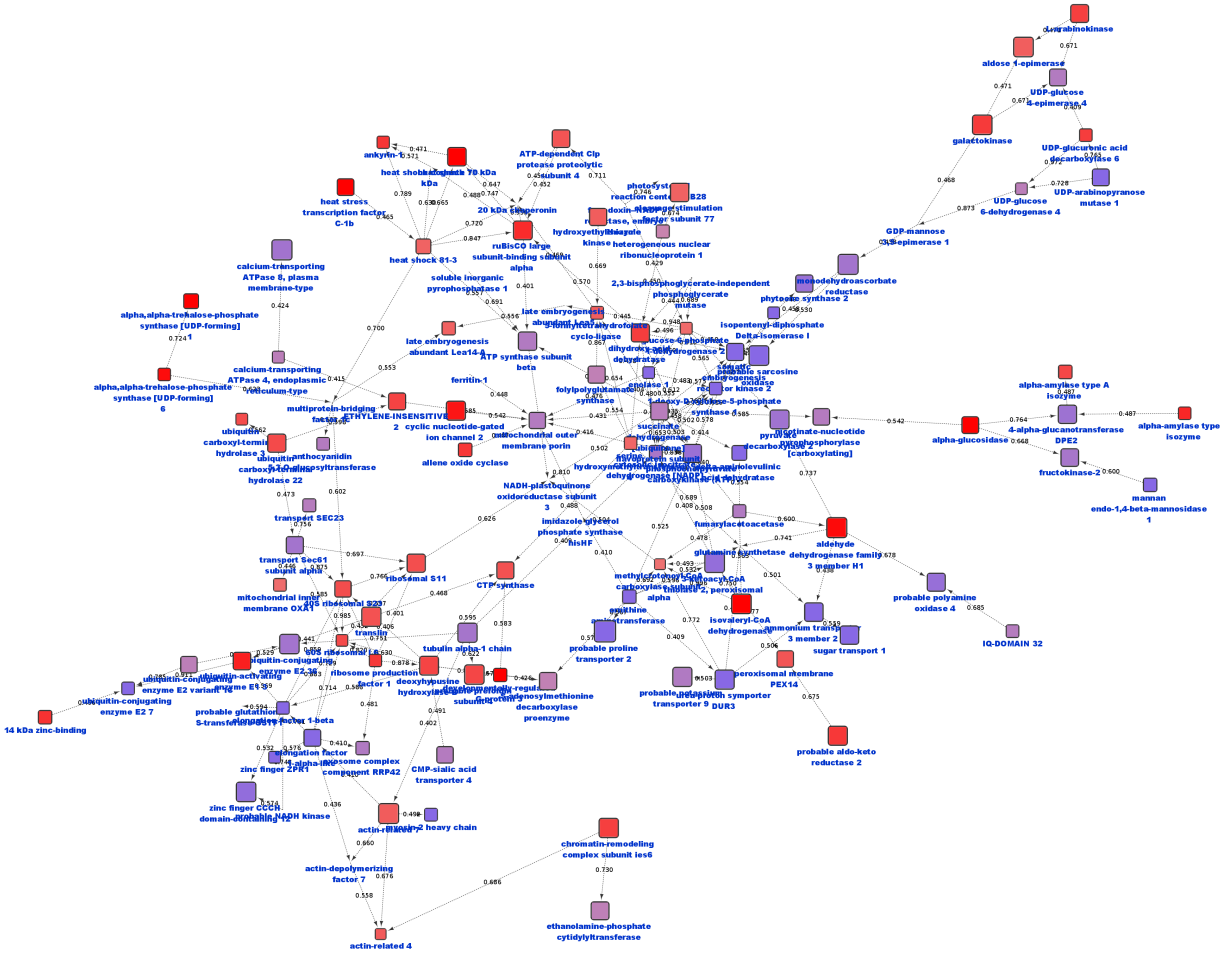

Figure 7. The protein-protein interaction networks of DEGs genes of H.ctrl gene profile. The node size and color stand for gene significance (logpvalue) and gene expression (LogFC) .

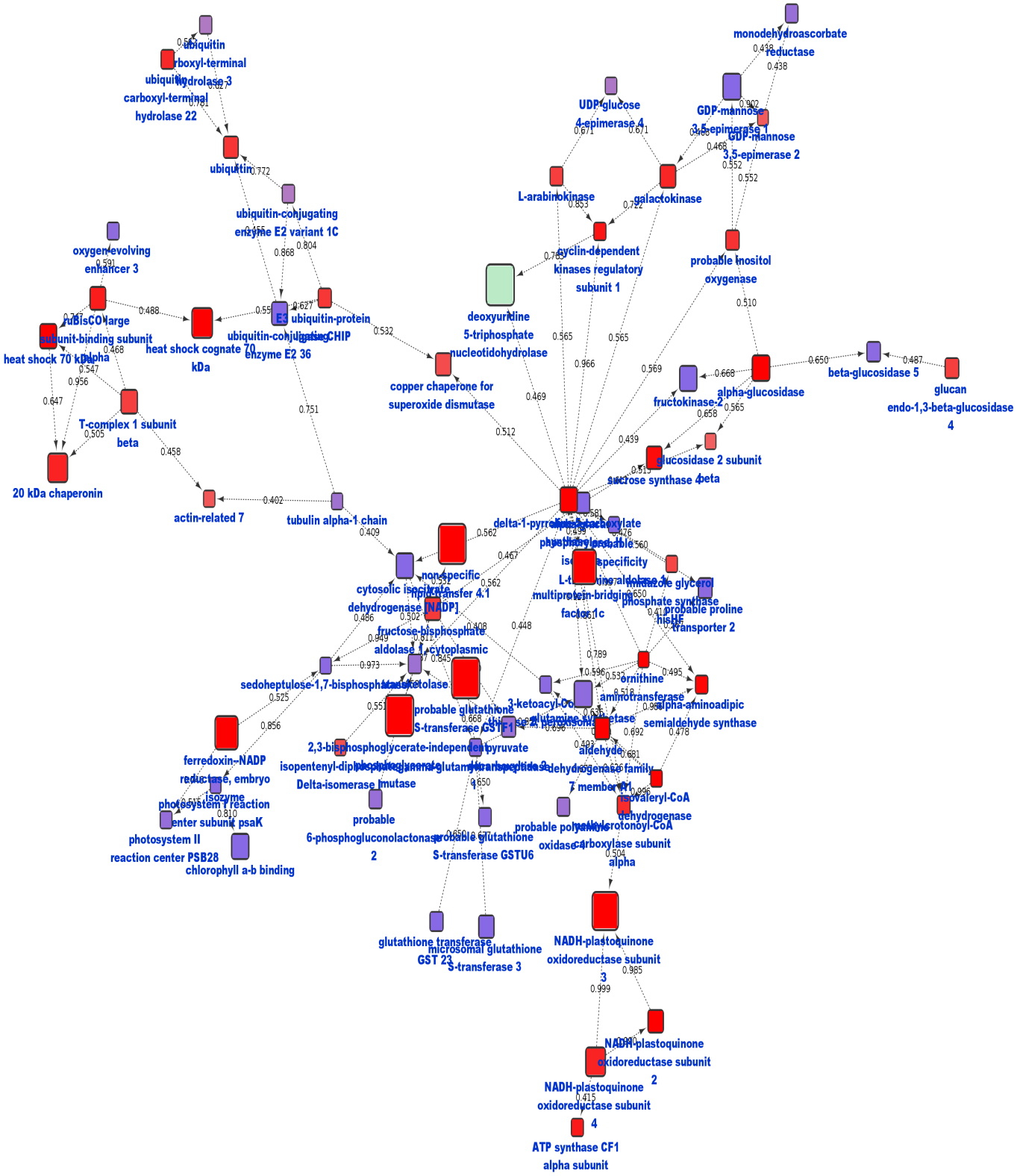

Figure 8. The protein-protein interaction networks of DEGs genes of M.ctrl gene profile. The node size and color stand for gene significance (logpvalue) and gene expression (LogFC) .

References

1. Faostat F. Statistical databases. Food and Agriculture Organization of the United Nations. 2017;.
2. Carraro C, Edenhofer O, Flachsland C, Kolstad C, Stavins R, Stowe R. The IPCC at a crossroads: Opportunities for reform. Science. 2015;350(6256):34–35.
3. Lobell DB, Howden SM, Smith DR, Chhetri N. A meta-analysis of crop yield under climate change and adaptation, Nat. Clim Change. 2014;4(4):287–291.
4. Daryanto S, Wang L, Jacinthe PA. Global synthesis of drought effects on maize and wheat production. PloS one. 2016;11(5).
5. Lobell DB, Gourdji SM. The influence of climate change on global crop productivity. Plant physiology. 2012;160(4):1686–1697.
6. Trenberth KE, Dai A, Van Der Schrier G, Jones PD, Barichivich J, Briffa KR, et al. Global warming and changes in drought. Nature Climate Change. 2014;4(1):17–22.
7. Hakeem KR, Chandna R, Ahmad P, Iqbal M, Ozturk M. Relevance of proteomic investigations in plant abiotic stress physiology. Omics: a journal of integrative biology. 2012;16(11):621–635.
8. Al-Khatib K, Paulsen GM. Mode of high temperature injury to wheat during grain development. Physiologia plantarum. 1984;61(3) :363–368.
9. Tack J, Barkley A, Nalley LL. Effect of warming temperatures on US wheat yields. Proceedings of the National Academy of Sciences. 2015;112(22):6931–6936.
10. Abhinandan K, Skori L, Stanic M, Hickerson N, Jamshed M, Samuel MA. Abiotic stress signaling in wheat–an inclusive overview of hormonal interactions during abiotic stress responses in wheat. Frontiers in plant science. 2018;9:734.
11. Braun HJ, Atlin G, Payne T, et al. Multi-location testing as a tool to identify plant response to global climate change. Climate change and crop production. 2010;1:115–138.
12. DeSA UN, Others. World population prospects: the 2012 revision. Population division of the department of economic and social affairs of the United Nations Secretariat, New York. 2013;18.
13. Aiqing S, Somayanda I, Sebastian SV, Singh K, Gill K, Prasad PVV, et al. Heat stress during flowering affects time of day of flowering, seed set, and grain quality in spring wheat. Crop Science. 2018;58(1):380–392.
14. Farooq M, Bramley H, Palta JA, Siddique KHM. Heat stress in wheat during reproductive and grain-filling phases. Critical Reviews in Plant Sciences. 2011;30(6):491–507.
15. Sairam RK, Srivastava GC, Saxena DC. Increased antioxidant activity under elevated temperatures: a mechanism of heat stress tolerance in wheat genotypes. Biologia Plantarum. 2000;43(2):245–251.
16. Perdomo JA, Capó-Bauçà S, Carmo-Silva E, Galmés J. Rubisco and rubisco activase play an important role in the biochemical limitations of photosynthesis in rice, wheat, and maize under high temperature and water deficit. Frontiers in plant science. 2017;8:490.
17. Prasad PVV, Pisipati SR, Momčilović I, Ristic Z. Independent and combined effects of high temperature and drought stress during grain filling on plant yield and chloroplast EF-Tu expression in spring wheat. Journal of Agronomy and Crop Science. 2011;197(6):430–441.
18. Awasthi R, Kaushal N, Vadez V, Turner NC, Berger J, Siddique KHM, et al. Individual and combined effects of transient drought and heat stress on carbon assimilation and seed filling in chickpea. Functional Plant Biology. 2014;41(11):1148–1167.
19. Kang WH, Sim YM, Koo N, Nam JY, Lee J, Kim N, et al. Transcriptome profiling of abiotic responses to heat, cold, salt, and osmotic stress of Capsicum annuum L. Scientific Data. 2020;7(1):1–7.
20. Johnson SM, Lim FL, Finkler A, Fromm H, Slabas AR, Knight MR. Transcriptomic analysis of Sorghum bicolor responding to combined heat and drought stress. BMC genomics. 2014;15(1):456.
21. Liu Z, Xin M, Qin J, Peng H, Ni Z, Yao Y, et al. Temporal transcriptome profiling reveals expression partitioning of homeologous genes contributing to heat and drought acclimation in wheat (Triticum aestivum L.). BMC plant biology. 2015;15(1):152.
22. Tilman D, Balzer C, Hill J, Befort BL. Global food demand and the sustainable intensification of agriculture. Proceedings of the national academy of sciences. 2011;108(50):20260–20264.
23. Aprile A, Havlickova L, Panna R, Marè C, Borrelli GM, Marone D, et al. Different stress responsive strategies to drought and heat in two durum wheat cultivars with contrasting water use efficiency. BMC genomics. 2013;14(1):821.
24. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic acids research. 1997;25(17):3389–3402.
25. Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, et al. The STRING database in 2017: quality-controlled protein–protein association networks, made broadly accessible. Nucleic acids research. 2016;p. gkw937.
26. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome research. 2003;13(11):2498–2504.
27. Cho HS, Lee SS, Kim KD, Hwang I, Lim JS, Park YI, et al. DNA gyrase is involved in chloroplast nucleoid partitioning. The Plant Cell. 2004;16(10):2665–2682.
28. Evans-Roberts KM, Breuer C, Wall MK, Sugimoto-Shirasu K, Maxwell A. Arabidopsis thaliana GYRB3 does not encode a DNA gyrase subunit. PloS one. 2010;5(3).
29. Manjulatha M, Sreevathsa R, Kumar AM, Sudhakar C, Prasad TG, Tuteja N, et al. Overexpression of a pea DNA helicase (PDH45) in peanut (Arachis hypogaea L.) confers improvement of cellular level tolerance and productivity under drought stress. Molecular biotechnology. 2014;56(2):111–125.
30. Park SY, Fung P, Nishimura N, Jensen DR, Fujii H, Zhao Y, et al. Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. science. 2009;324(5930):1068–1071.

31. Dorosh L, Kharenko OA, Rajagopalan N, Loewen MC, Stepanova M. Molecular mechanisms in the activation of abscisic acid receptor PYR1. PLoS computational biology. 2013;9(6).
32. Hlaváčková I, V\'\itámvás P, Šantr\uuček J, Kosová K, Zelenková S, Prášil IT, et al. Proteins involved in distinct phases of cold hardening process in frost resistant winter barley (Hordeum vulgare L.) cv Luxor. International journal of molecular sciences. 2013;14(4):8000–8024.
33. V\'\itámvás P, Urban MO, Škodáček Z, Kosová K, Pitelková I, V\'\itámvás J, et al. Quantitative analysis of proteome extracted from barley crowns grown under different drought conditions. Frontiers in plant science. 2015;6:479.
34. González-Candelas L, Alamar S, Sánchez-Torres P, Zacar\'\ias L, Marcos JF. A transcriptomic approach highlights induction of secondary metabolism in citrus fruit in response to Penicillium digitatuminfection. BMC plant biology. 2010;10(1):194.
35. Attila C, Ueda A, Cirillo SLG, Cirillo JD, Chen W, Wood TK. Pseudomonas aeruginosa PAO1 virulence factors and poplar tree response in the rhizosphere. Microbial biotechnology. 2008;1(1):17–29.
36. Sui J, Li G, Chen G, Zhao C, Kong X, Hou X, et al. RNA-seq analysis reveals the role of a small GTP-binding protein, Rab7, in regulating clathrin-mediated endocytosis and salinity-stress resistance in peanut. Plant Biotechnology Reports. 2017;11(1):43–52.
37. Gupta S, Singh Y, Kumar H, Raj U, Rao AR, Varadwaj PK. Identification of novel abiotic stress proteins in Triticum aestivum through functional annotation of hypothetical proteins. Interdisciplinary Sciences: Computational Life Sciences. 2018;10(1):205–220.
38. Kumar RR, Goswami S, Dubey K, Singh K, Singh JP, Kumar A, et al. RuBisCo activaseâ[80?][94?]a catalytic chaperone involved in modulating the RuBisCo activity and heat stress-tolerance in wheat. Journal of plant biochemistry and biotechnology.2019; 28 (1):63–75.
39. Qu M, Bunce JA, Sicher RC, Zhu X, Gao B, Chen G. An attempt to interpret a biochemical mechanism of C4 photosynthetic thermo-tolerance under sudden heat shock on detached leaf in elevated CO2 grown maize. PloS one. 2017;12(12).
40. Zhang M, Kim Y, Zong J, Lin H, Dievart A, Li H, et al. Genome-wide analysis of the barley non-specific lipid transfer protein gene family. The Crop Journal. 2019;7(1):65–76.
41. Wang F, Zang Xs, Kabir MR, Liu Kl, Liu Zs, Ni Zf, et al. A wheat lipid transfer protein 3 could enhance the basal thermotolerance and oxidative stress resistance of Arabidopsis. Gene. 2014;550(1):18–26.
42. Altenbach SB, Kothari KM, Tanaka CK, Hurkman WJ. Expression of 9-kDa non-specific lipid transfer protein genes in developing wheat grain is enhanced by high temperatures but not by post-anthesis fertilizer. Journal of cereal science. 2008;47(2):201–213.
43. Das A, Eldakak M, Paudel B, Kim DW, Hemmati H, Basu C, et al. Leaf proteome analysis reveals prospective drought and heat stress response mechanisms in soybean. BioMed research international. 2016;2016.
44. Werwie M, Dworak L, Bottin A, Mayer L, Basché T, Wachtveitl J, et al. Light-harvesting chlorophyll protein (LHCII) drives electron transfer in semiconductor nanocrystals. Biochimica et Biophysica Acta (BBA)-Bioenergetics. 2018;1859(3):174–181.
45. Panchuk II, Volkov RA, Schöffl F. Heat stress-and heat shock transcription factor-dependent expression and activity of ascorbate peroxidase in Arabidopsis. Plant physiology. 2002;129(2) :838–853.
46. Koussevitzky S, Suzuki N, Huntington S, Armijo L, Sha W, Cortes D, et al. Ascorbate peroxidase 1 plays a key role in the response of Arabidopsis thaliana to stress combination. Journal of Biological Chemistry. 2008;283(49):34197–34203.
47. Wang W, Vinocur B, Shoseyov O, Altman A. Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends in plant science. 2004;9(5):244–252.
48. Kumar RR, Goswami S, Sharma SK, Kala YK, Rai GK, Mishra DC, et al. Harnessing next generation sequencing in climate change: RNA-Seq analysis of heat stress-responsive genes in wheat (Triticum aestivum L.). Omics: a journal of integrative biology. 2015;19(10):632–647.
49. Qi Z, Xiong L. Characterization of a Purine Permease Family Gene Os PUP 7 Involved in Growth and Development Control in Rice. Journal of integrative plant biology. 2013;55(11):1119–1135.
50. Sun M, Shen Y, Yin K, Guo Y, Cai X, Yang J, et al. A late embryogenesis abundant protein GsPM30 interacts with a receptor like cytoplasmic kinase GsCBRLK and regulates environmental stress responses. Plant Science. 2019;283:70–82.
51. Xu D, Duan X, Wang B, Hong B, Ho THD, Wu R. Expression of a late embryogenesis abundant protein gene, HVA1, from barley confers tolerance to water deficit and salt stress in transgenic rice. Plant physiology. 1996;110(1):249–257.
52. Nagaraju M, Kumar SA, Reddy PS, Kumar A, Rao DM, Kishor PBK. Genome-scale identification, classification, and tissue specific expression analysis of late embryogenesis abundant (LEA) genes under abiotic stress conditions in Sorghum bicolor L. PloS one. 2019;14(1).
53. Taniguchi S, HOSOKAWA-SHINONAGA Y, Tamaoki D, Yamada S, Akimitsu K, Gomi K. Jasmonate induction of the monoterpene linalool confers resistance to rice bacterial blight and its biosynthesis is regulated by JAZ protein in rice. Plant, cell & environment. 2014;37(2):451–461.
54. Savoi S, Wong DCJ, Arapitsas P, Miculan M, Bucchetti B, Peterlunger E, et al. Transcriptome and metabolite profiling reveals that prolonged drought modulates the phenylpropanoid and terpenoid pathway in white grapes (Vitis vinifera L.). BMC plant biology. 2016;16(1):67.
55. Baghery MA, Kamal S, Kazemitabar AD, Mehrabanjoubani P, Mehdi M, Naghizadeh AMN. Transcription Factors and micro RNA Genes Regulatory Network Construction Under Drought Stress in Sesame (*Sesamum indicum* L.). Research Square; 2020. DOI: 10.21203/rs.2.21135/v1.
56. Strathmann A, Kuhlmann M, Heinekamp T, Dröge-Laser W. BZI-1 specifically heterodimerises with the tobacco bZIP transcription factors BZI-2, BZI-3/TBZF and BZI-4, and is functionally involved in flower development. The Plant Journal. 2001; 28(4): 397–408.

57. Wang M, Wang Y, Zhang Y, Li C, Gong S, Yan S, et al. Comparative transcriptome analysis of salt-sensitive and salt-tolerant maize reveals potential mechanisms to enhance salt resistance. Genes & genomics. 2019;41(7):781–801.
58. Wu W, Zhu S, Chen Q, Lin Y, Tian J, Liang C. Cell Wall Proteins Play Critical Roles in Plant Adaptation to Phosphorus Deficiency. International journal of molecular sciences.2019; 20(21) :5259.
59. Atkinson D, Davison AW. The effects of phosphorus deficiency on water content and response to drought. New Phytologist. 1973;72(2):307–313.
60. Arce-Paredes P, Mora-Escobedo R, Luna-Arias JP, Mendoza - Hernández G, Rojas-Espinosa O. Heat, salinity, and acidity, commonly upregulate A1aB1b proglycinin in soybean embryonic axes. Soybean -Biochemistry, Chemistry and Physiology. 2011;p. 402–422.
61. Ruibal C, Castro A, Carballo V, Szabados L, Vidal S. Recovery from heat, salt and osmotic stress in Physcomitrella patens requires a functional small heat shock protein PpHsp16. 4. BMC plant biology. 2013;13(1):174.
62. Chen L, Hou Y, Hu W, Qiu X, Lu H, Wei J, et al. The molecular chaperon AKR2A increases the mulberry chilling-tolerant capacity by maintaining SOD activity and unsaturated fatty acids composition. Scientific reports. 2018;8(1):1–11.
63. Pritchard J, Griffiths B, Hunt EJ. Can the plant-mediated impacts on aphids of elevated CO2 and drought be predicted? Global Change Biology. 2007;13(8):1616–1629.
64. Cotton LM, Rodriguez CM, Suzuki K, Orgebin-Crist MC, Hinton BT. Organic cation/carnitine transporter, OCTN2, transcriptional activity is regulated by osmotic stress in epididymal cells. Molecular Reproduction and Development: Incorporating Gamete Research. 2010;77(2):114–125.
65. Guo L, Yang H, Zhang X, Yang S. Lipid transfer protein 3 as a target of MYB96 mediates freezing and drought stress in Arabidopsis. Journal of experimental botany. 2013;64(6):1755–1767.
66. Li YF, Wang Y, Tang Y, Kakani VG, Mahalingam R. Transcriptome analysis of heat stress response in switchgrass (Panicum virgatumL.). BMC Plant Biology. 2013;13(1):153.
67. Sarkar NK, Kim YK, Grover A. Coexpression network analysis associated with call of rice seedlings for encountering heat stress. Plant molecular biology. 2014;84(1-2):125–143.
68. Raabe K, Honys D, Michailidis C. The role of eukaryotic initiation factor 3 in plant translation regulation. Plant Physiology and Biochemistry. 2019;.
69. Hu CA, Delauney AJ, Verma DP. A bifunctional enzyme (delta 1-pyrroline-5-carboxylate synthetase) catalyzes the first two steps in proline biosynthesis in plants. Proceedings of the National Academy of Sciences. 1992;89(19):9354–9358.
70. Sidari M, Mallamaci C, Muscolo A. Drought, salinity and heat differently affect seed germination of Pinuspinea. Journal of forest research. 2008;13(5):326–330.
71. Hu X, Wu L, Zhao F, Zhang D, Li N, Zhu G, et al. Phosphoproteomic analysis of the response of maize leaves to drought, heat and their combination stress. Frontiers in plant science. 2015;6:298.
72. Janicka-Russak M. Plant plasma membrane H+-ATPase in adaptation of plants to abiotic stresses. Abiotic stress response in plants-physiological, biochemical and genetic perspectives. 2011; 1:197–218.
73. Wang QJ, Sun H, Dong QL, Sun TY, Jin ZX, Hao YJ, et al. The enhancement of tolerance to salt and cold stresses by modifying the redox state and salicylic acid content via the cytosolic malate dehydrogenase gene in transgenic apple plants. Plant biotechnology journal. 2016;14(10):1986–1997.