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Molecular mechanism of drought stress tolerance in barley (*Hordeum vulgare* L.) via a combined analysis of the transcriptome data

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Abstract: One of the main issues addressed by phytology in recent years has been plant tolerance mechanisms for abiotic stress. No combined analysis has been made to identify the genes involved in drought stress tolerance. The meta-analysis of microarray data related to drought stress was analysed by the R software packages and showed 3 029 upregulated genes and 3 017 downregulated genes. The upregulated genes were mostly related to the drought tolerance protein, abiotic stress response, and the Cys2His2 Zinc Finger Transcription Factor (*C2H2 zinc finger* TF). The downregulated genes were mainly related to the late embryogenesis abundant protein, abiotic stress response, and the basic leucine zipper (*bZIP*) TF. The common gene ontology (GO) terms in the upregulated and downregulated genes were mainly related to the metabolic process, response to stimulus, cellular metabolic process, and photorespiration. The up and down meta-differential expressed genes (meta-DEGs) mainly belonged to the those following Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways including: the biosynthesis of secondary metabolites, plant hormone signal transduction, mitogen-activated protein kinase (MAPK) signalling pathway, and RNA degradation. Moreover, in the upregulated and downregulated genes, the TFs with a high percentage mainly belonged to the Teosinte branched1/Cincinnata/proliferating cell factor (*TCP*), basic helix loop-helix (*bHLH*) and *bZIP*. Next, the hub upregulated genes were mainly related to the thiamine biosynthesis protein thiC, 4-hydroxyphenylpyruvate dioxygenase, ribose-5-phosphate isomerase precursor and heat shock protein. The hub downregulated genes were mainly associated with the elongation factor Ts, aldehyde dehydrogenase, and trigger factor. Finally, the data from the present meta-analysis were compared with previous studies on the qRT-PCR results and their up and down expressions were confirmed. Based on the findings of the current study, novel insights into the drought stress molecular response can be provided and various candidate genes can be introduced for barley drought stress tolerance breeding.

Keywords: bioinformatics; biological process; drought stress; hub genes; meta-analysis; transcription factor

Abbreviations: thiC – thiamine biosynthesis protein; TFs – transcription factors ; GO – gene ontology; MAPK – mitogen activated protein kinase; KEGG – Kyoto Encyclopedia of Genes and Genomes; DEGs – differential expressed genes); AP2/ERF – APETALA 2/ethylene-responsive element binding factor; HvDRF1 – dehydration-responsive factor 1; HVA1 – *Hordeum vulgare* aleurone 1; Dhns – dehydrins; ABA – abscisic acid; SnRK2 – SNF1-related kinase 2; PP2C – protein phosphatases 2C; PPI – protein-protein interaction; MCC – maximal clique centrality; JA – jasmonic acid; ET-Ts – elongation factor Ts; bHLH – basic helix loop-helix; EBF1 – EIN3-binding F-box protein 1; LEA – late embryogenesis abundant protein; bZIP – basic leucine zipper; JAZ – jasmonate ZIM-domain; PYR/PYL – pyrabactin resistance/pyrabactin resistance-like; PABP1 – polyadenylate-binding protein 1; *Dof* – NA binding with one finger; TCP – Teosinte branched1/Cincinnata/proliferating cell factor; SBP – squamosa promoter binding protein

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Drought as a critical global warming-related stress factor is considered as a threat for plant biodiversity that affects crop growth (Bailey-Serres et al. 2019). Worldwide climatic changes along with temperature increases and rainfall decreases necessitate the need for the development of stress-tolerant cultivars, especially for main crop species (Mir et al. 2012). Barley (*Hordeum vulgare* L.) is the fourth most cultivated cereal crop and it is distributed under different climates and is known to keep its growth at higher concentrations of NaCl (200–300 mM) for a minimum of 3 weeks (Roy 2016). Most genome-wide transcriptional profiling studies on drought stress in barley have focused on the response of vegetative organs. The response of the spike transcriptome to drought stress has been largely overlooked even though drought at the reproductive stage causes the greatest yield loss (Janiak et al. 2019).

Meta-analysis approaches have recently started to be applied to genetic expression studies in the medical, plant and other areas of research, which were shown to consider the sampling variation, and detect candidate genes that are uniformly differentially expressed in studies (Tseng et al. 2012). Microarray technology and transcriptional profiling has caused a dramatic advance in understanding the plant molecular response under abiotic stress conditions with regard to gene expression (Daryani et al. 2022). For interpreting the genome functional elements, understanding the transcriptome is important, which is mainly aimed at determining the gene transcriptional structure, cataloguing all the species of the transcripts and quantifying the expression levels of each transcript under different conditions (Benny et al. 2019). The first microarray study of the barley drought stress response at the transcriptome level was reported on the Totkak cultivar (Ozturk et al. 2002). The authors found that the differential upregulation of several stress-responsive genes were related to the leaf and root tissues, including the auxin-induced protein, jasmonate-induced proteins, metallothionein-like proteins, actin binding protein, protein phosphatases, dehydrins and late embryogenesis abundant protein, and the downregulated genes are mostly related to the photosynthesis metabolism including the rubisco small-chain precursor, chlorophyll a/b-binding proteins and ribulose-biphosphate carboxylase activase.

Transcription factors (TFs) control the expression of genes and help plants cope better with environmental stress (Cominelli et al. 2013). The APETALA 2/ethylene-responsive factor is an element binding factor (*AP2/ERF*) that controls the gene expression when abiotic stress reactions occur and is an important family of TFs (Mizoi et al. 2012). In barley, the increase in the *AP2/ERF* gene, dehydration-responsive factor 1 (*HvDRF1*) by the drought stress activated *Hordeum vulgare* aleurone 1 (*HVA1*) gene which is a responsive gene to drought stress (Xue & Loveridge 2004). Dehydrins (*Dhns*) are a large group of genes associated with drought stress where *Dhns* including 1-11 and *Dhn13* in barley were found to be upregulated by drought stress (Rodriguez et al. 2005). Likewise, Tommasini et al. (2008) proved the upregulation of the group of dehydrins including *Dhn1*, *Dhn2*, *Dhn3*, *Dhn4*, *Dhn7*, *Dhn9* and *Dhn10* by drought stress in barley.

The gene ontology (GO) analysis indicates that mitogen activated protein kinase (MAPK) module-regulated genes are enriched for abiotic stress responses, especially drought stress (Baldoni et al. 2021). MAPKs take part in different cellular processes including the development, growth and abiotic stress responses and they are present in the cytoplasm and nucleus (Khan et al. 2017). The minimum abscisic acid (ABA) signalling pathway includes the members of the regulatory component of the ABA receptor family (RCAR/PYR/PYL), the members of the SNF1-related kinase 2 (*SnRK2*) family and type 2C protein phosphatases (*PP2C*) (Sheard & Zheng 2009).

Due to it considering a wide range of experimental conditions, a meta-analysis can add a new perspective to the gene expression data analysis. Accordingly, for reporting precise results on drought tolerance mechanisms in barley, considering the results of various individual experiments is of importance. To the best of our knowledge, no meta-analysis has been conducted addressing barley microarray data under drought stress. In fact, the integration of various findings in individual experiments can lead to the identification of the key genes associated with the mechanisms of barley drought tolerance. The purpose of this study was to identify the drought stress-related hub genes (the most connected genes in the biological network), biological pathways, and TFs under drought stress through a meta-analysis of microarray data.

MATERIAL AND METHODS

The Gene Expression Omnibus website (www.ncbi.nlm.nih.gov/geo/) was used to download the microarray datasets (GPL14927, and GPL1340) at the leaf tissue under drought stress in 2021. Out of 12 series,

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Table 1. Microarray experiments used for the meta-analysis of *H. vulgare* L under drought stress

Platform	Accession	Genotype	Part of plant (barley)	Sample normal : stress
GPL14927	GSE128048	CamB	leaf	3 : 3
	GSE128048	Maresi	leaf	3 : 3
GPL14927	GSE103278	CamB	leaf	3 : 3
	GSE103278	Maresi	leaf	3 : 3
GPL1340	GSE56437	Georgia	leaf	6 : 6
	GSE56437	Sebastian	leaf	6 : 6
GPL1340	GSE17669	Barley spike	lemma	3 : 3
	GSE17669	Barley spike	palea	3 : 3
GPL1340	GSE6990	barley cv. Morex	leaf	3 : 3
GPL1340	GSE3170	Oregon Wolfe Barley (OWB) dominant	seedling	3 : 3
GPL1340	GSE3170	Oregon Wolfe Barley (OWB) recessive	seedling	3 : 3
GPL14927	GSE73789	Karat	leaf	3 : 3

The data of Table 1 that support this study are available in the NCBI GEO site at https://www.ncbi.nlm.nih.gov/geo/

7 series including 14 experiments with good quality were selected for the drought stress in the leaf tissue (Table 1). The main workflow microarray meta-analysis is depicted in Figure 1.

Differential expressed genes (DEGs) and meta-analysis. The microarray expression data were pre-analysed as a separate dataset using the R language package. The data quality was controlled using Cor-

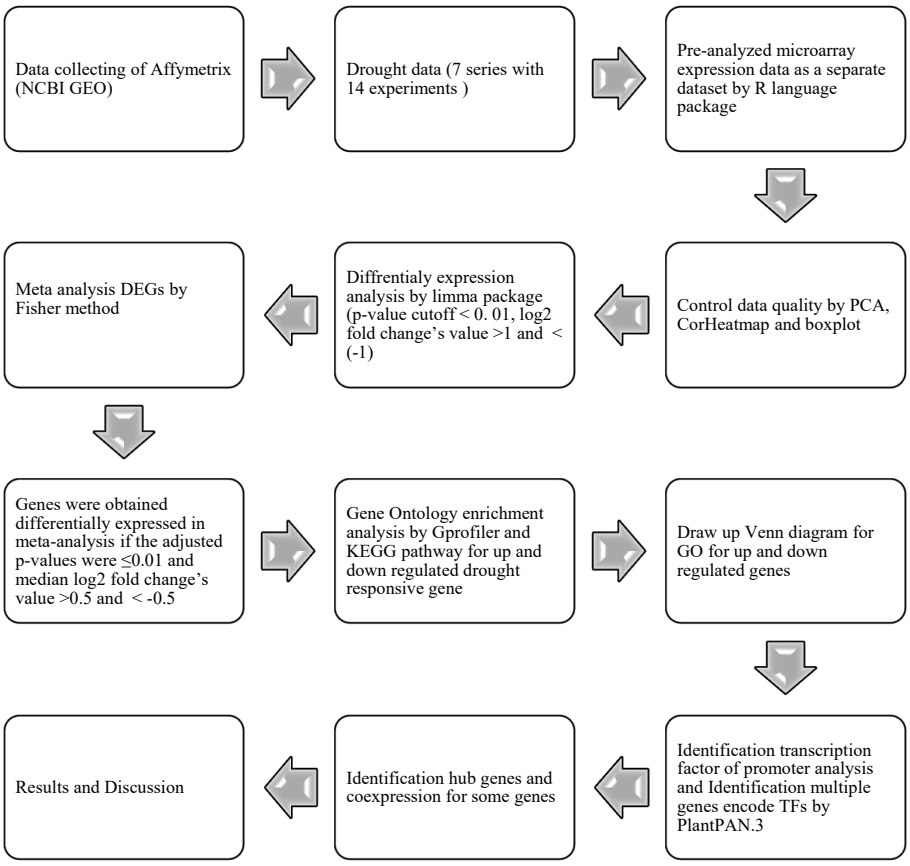

Figure 1. Microarray dataset meta-analysis workflow

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Heatmap, a boxplot, and a principal component analysis (PCA) plot. Based on the PCA, the control and treated data under drought stress were categorised into different groups. The background correction and quantile normalisation were performed on non-normalised data; subsequently, good RNA quality data were selected by the AgiMicroRna package for further analysis. By employing the Limma package (http://www.bioconductor.org/), a difference analysis of the selected data was performed. The obtained data were fitted to a linear model and by employing a simple Bayesian experimental model, and the standard errors were corrected. For each contrast/gene, the log2 fold change (LFC) and moderated t-statistic were calculated. The genes which showed significant *P*-values with a false discovery rate (FDR ≤ 0.01) were obtained and further analysis was considered for the DEG and LFC change values of each dataset (de Abreu Neto & Frei 2016). In various datasets, the relative expression values of the barley drought stress gene expression were performed to evaluate the overall simple gene response. For merging *P*-values that are unadjusted, the sum logs of Fisher method were undertaken. For setting the combined *P*-values for the datasets, the p. adjust function in metaRNASeq was applied by employing the 'fdr' method. In the case when the *P*-values were ≤ 0.01 and the median LFC values were >1 and < –1, the genes were considered as differentially expressed (Supplementary File 1 in Electronic Supplementary Material).

Venn diagram. The GO upregulated and downregulated meta-DEGs were subjected to the web tool Venny v2.1 and the common GO upregulated and downregulated meta-DEGs were identified.

Gene set enrichment analysis and pathway analysis of the meta-DEGs. The gene set enrichment analysis was performed by g.profiler for the core drought-responsive upregulated and downregulated genes (https://biit.cs.ut.ee/gprofiler/gost). The biological process categories of the GO annotation were characterised under drought stress and the functional annotation of upregulated and downregulated genes were confirmed the Kyoto Encyclopedia of Genes and Genomes (KEGG) website (https://www.kegg.jp/kegg/pathway.html).

Identification of the transcription factor in the promoters of the meta-DEGs. The genomic sequences (1 500 bp upstream) of the upregulated and downregulated genes were downloaded from the Ensembl Plants website (http://plants.ensembl.org/biomart/martview/). This set of sequences was analysed for conserved promoter elements, including TFs by the PlantPAN 3.0 website (http://plantpan.itps.ncku.edu.tw/) (Bhargava et al. 2013). The TFs were identified and the frequency of their occurrence in the promoters of the up- and downregulated genes was divided by all the transcription factors frequency in the promoters. Then, the percentage of transcription factors of the up- to downregulated genes was calculated and a related diagram was drawn for better visualisation.

Identification of the hub genes. The protein-protein interaction (PPI) network was drawn with the Cytoscape software and the information was derived from the STRING website. The hub genes were also characterised using the CytoHubba plugin in Cytoscape 3.6.1 with the maximal clique centrality (MCC) method (Chin et al. 2014).

Examination of the identified hub gene with twelve methods in CytoHubba. The identified hub genes by the MCC method were tested using the CytoHubba plugin in Cytoscape 3.6.1 with different ranking methods (including Edge Percolated Component (EPC), BottleNeck, Closeness, Degree, Density of Maximum Neighbourhood Component (DMNC), EcCentricity, Maximum Neighbourhood Component (MNC), Betweenness, Stress, ClusteringCoefficient, Radiality and Maximal clique centrality (MCC)) to obtain the importance of the nodes in a biological network.

Validation of upregulated and downregulated hub genes by other studies. Among the above-discussed genes belonging to the upregulated and downregulated hub genes, some genes were selected for gene expression verification. For validation, eight selected hub genes from the upregulated and downregulated genes were compared with the real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) data from other studies.

RESULTS

Our results showed 3 029 upregulated genes and 3 017 downregulated genes related to drought stress which were significant at a 1% probability level with log2-fold change values between >1 and < –1. The top 15 up and downregulated genes are presented in Table 2. The upregulated genes are mostly related to the drought tolerance protein, abiotic stress response, C2H2 zinc finger TF, and heat shock protein. On other hand, the downregulated genes are mainly related to the late embryogenesis abundant (*LEA*)

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Table 2. The top 15 up- and downregulated genes identified from the microarray meta-analysis of the drought stress of *Hordeum vulgare* L.

Gene stable ID	LFC	Meta *P*-value	UniProtKB/TrEMBL ID	Description
Upregulated genes				
HORVU.MOREX.r3.4HG0386900	6.74	0	Q10N71	similar to the plant-specific domain TIGR01589 family protein
HORVU.MOREX.r3.1HG0073130	4.83	0	F2DD06	similar to the plant-specific domain TIGR01589 family protein
HORVU.MOREX.r3.3HG0244320	2.42	0	A0A287KIG6	polyketide cyclase/dehydrase domain containing protein
HORVU.MOREX.r3.5HG0430940	2.30	0	A0A287QDF2	glycoside hydrolase, family 5 protein
HORVU.MOREX.r3.5HG0426450	2.20	1.01E-08	A0A0P0YD58	similar to the drought tolerance protein
HORVU.MOREX.r3.4HG0394630	1.91	0	M0YTF0	similar to the branched-chain-amino-acid aminotransferase 3, chloroplast precursor
HORVU.MOREX.r3.1HG0056640	1.9	0	A0A287FN76	hypothetical conserved gene
HORVU.MOREX.r3.5HG0476020	1.81	1.72E-11	F2DMW4	abiotic stress response
HORVU.MOREX.r3.3HG0237670	1.69	0	E5D3J5	class II small heat shock protein and heat tolerance
HORVU.MOREX.r3.4HG0380970	1.62	0	Q10MB0	similar to galactinol synthase
HORVU.MOREX.r3.5HG0516880	1.55	2.34E-08	A0A287SJD8	C2H2 zinc finger transcription factor, drought tolerance, and stomatal aperture control
HORVU.MOREX.r3.2HG0099870	1.54	2.22E-16	A0A0P0W604	similar to the heat shock protein 82
HORVU.MOREX.r3.3HG0302120	1.52	0	F2D2M4	similar to the regulator of chromosome condensation (RCC1) family protein
HORVU.MOREX.r3.7HG0699010	1.50	0	F2DAQ2	MYB transcription factor
HORVU.MOREX.r3.1HG0074910	1.23	9.6E-05	F2DVN6	R-R-type MYB-like transcription factor, and response to drought stress during reproductive development
Downregulated genes				
HORVU.MOREX.r3.7HG0637810	–7.95	0	C9ELM9	late embryogenesis abundant (LEA) group 1 family protein
HORVU.MOREX.r3.1HG0041720	–7.67	0	A0A287F819	protein with high similarity to proteins involved in wax production, and cuticular wax biosynthesis
HORVU.MOREX.r3.7HG0702620	–7.24	0	M0Z3G6	similar to the OSIGBa0142C11.2 protein
HORVU.MOREX.r3.7HG0658900	–7.14	0	A0A287VX26	similar to the MtN3 protein precursor
HORVU.MOREX.r3.1HG0002450	–6.46	0	M0XCI1	stress response
HORVU.MOREX.r3.5HG0473680	–4.619	0	-	abiotic stress response, and early panicle development
HORVU.MOREX.r3.5HG0477620	–2.75	0	M0Z0I7	similar to the ubiquitin-protein ligase

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Table 2 to be continued

Gene stable ID	LFC	Meta *P*-value	UniProtKB/TrEMBL ID	Description
Downregulated genes				
HORVU.MOREX.r3.6HG0613690	–2.41	1.82E-13	Q6Z6A7	calcium-binding protein, and drought stress tolerance
HORVU.MOREX.r3.6HG0604390	–2.18	2.13E-11	F2DAQ1	RING-type E3 ligase, and drought stress response
HORVU.MOREX.r3.5HG0459670	–2.05	1.02E-13	F2EIY5	stress responsive alpha-beta barrel domain containing protein
HORVU.MOREX.r3.7HG0665050	–1.89	3.33E-16	F2E7Z5	similar to the germin-like protein 1 precursor
HORVU.MOREX.r3.1HG0083950	–1.70	5.61E-08	F2D3K2	heat stress transcription factor
HORVU.MOREX.r3.6HG0604730	–1.22	2.22E-15	A0A287UH91	MYB transcription factor, and transcriptional activator in mediating stress and rhythm responsive gene expression
HORVU.MOREX.r3.5HG0480470	–1.02	1.84E-09	Q69P88	control of root system architecture, and drought avoidance
HORVU.MOREX.r3.6HG0619650	–1.01	1.12E-10	M0WPT5	bZIP transcription factor, regulation of ABA signalling, and drought resistance

LFC – log2 fold change

protein, abiotic stress response, calcium-binding protein, drought stress response, and basic leucine zipper (*bZIP*) TF.

The core drought-responsive up and down meta-DEGs were analysed to recognise the significantly enriched GO terms in the biological process in the drought response with $P < 0.05$ (Table 3). Fifty (50) and 69 terms were enriched in up- and downregulated genes, respectively (Figure 2). The GO terms in the upregulated genes are mainly related to the metabolic process and response to stimulus and the downregulated genes are mainly related to the response to drought stress. The common GO terms in the up- and downregulated genes include GO:0004553 (organic substance metabolic process), GO:0005975 (metabolic process), GO:0016567 (primary metabolic process), GO:0005634 (response to stimulus), GO:0006807 (nitrogen compound metabolic process), GO:0016021 (response to abiotic stimulus), GO:0102250 (response to temperature stimulus), GO:0005506 (cellular metabolic process), GO:0009507 (photorespiration), GO:0016301 (organic substance metabolic process) and GO:0006032 (carbohydrate metabolic process).

To understand the biological pathways in the up and down meta-DEGs under drought stress, an analysis was performed using KEGG (https://www.kegg.jp/kegg/mapper/color.html). The up and down meta DEGs mainly belonged to the KEGG pathways including the metabolic pathways, biosynthesis of secondary metabolites, plant hormone signal transduction, phenylpropanoid biosynthesis, carbon metabolism, MAPK signalling pathway, glycolysis/gluconeogenesis, biosynthesis of amino acids, starch and sucrose metabolism, nitrogen metabolism, RNA degradation, zeatin biosynthesis, ribosome biogenesis, ABC transporters, mRNA surveillance pathway, DNA replication, and flavonoid biosynthesis. To explore the drought responsible genes, we focused on the analysis of the

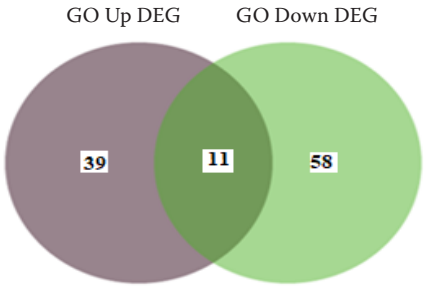

Figure 2. The common genes from the datasets under drought stress of several groups of differential expressed genes (DEGs)

The comparison of a number of gene ontology (GO) up- and downregulated DEGs of *H. vulgare* L. show 11 common GO terms

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plant hormone signalling pathway, MAPK signalling pathway, RNA degradation and DNA replication.

In the functions that belong to gibberellin and jasmonic acid (JA), significant changes were shown in the plant hormone signalling pathway (Figure 3A). The Jasmonate ZIM-domain (*JAZ)* interacts with *MYC2*, which can accordingly affect the abiotic stress response. In the functions that belong to ethylene, JA and ABA had significant changes in the MAPK signalling pathway (Figure 3B). The ABA signalling pathway consists of three core components, including Pyrabactin Resistance/Pyrabactin resistance-like (*PYR/PYL*), *PP2C* and *SnRK2* that can affect the adaption stress. In the functions that belong to Ccr-4-Not Complex, *Caf16* and Polyadenylate-binding protein 1 (*PABP1*) had significant changes in the RNA degradation (Figure 3C), and in the functions that belong to DNA polymerase α-primase complex, *α1* showed a significant change in the DNA replication pathway (Figure 3D).

Table 3. The top 15 biological process categories of the gene ontology (GO) annotation of the core drought-responsive regulated genes of *Hordeum vulgare* L.

GO	Gene Stable ID	UniProtKB/ TrEMBL ID	Description	Adjusted *P*-value
Upregulated genes				
GO:0004553	HORVU.MOREX.r3.5HG0430940	A0A287QDF2	organic substance metabolic process	1.24E-09
GO:0000166	HORVU.MOREX.r3.6HG0568470	F2D195	response to heat	4.88E-09
GO:0005975	HORVU.MOREX.r3.4HG0348850	M0ZB44	metabolic process	5.40E-09
GO:0005737	HORVU.MOREX.r3.6HG0567160	A5CFY5	microtubule cytoskeleton organisation	2.64E-07
GO:0016567	HORVU.MOREX.r3.1HG0039160	F2DJR8	primary metabolic process	5.35E-07
GO:0016020	HORVU.MOREX.r3.3HG0304970	F2DLX4	transmembrane transport	6.26E-07
GO:0005737	HORVU.MOREX.r3.4HG0387870	M0W8S8	cellular response to heat	1.29E-06
GO:0005634	HORVU.MOREX.r3.3HG0276810	M0W799	response to stimulus	3.59E-06
GO:0006807	HORVU.MOREX.r3.7HG0662970	A0A0P0WU26	nitrogen compound metabolic process	9.24E-06
GO:0006355	HORVU.MOREX.r3.5HG0474130	B2KJ70	response to stimulus	1.429E-05
GO:0016887	HORVU.MOREX.r3.5HG0491130	F2DB02	protein folding	1.49E-05
GO:0016021	HORVU.MOREX.r3.4HG0356420	A0A287NMD4	signal transduction	4.05E-05
GO:0005506	HORVU.MOREX.r3.6HG0592460	A0A287U8S1	cellular metabolic process	4.80E-05
GO:0009507	HORVU.MOREX.r3.2HG0104780	A0A287H239	photorespiration	7.781E-05
GO:0016301	HORVU.MOREX.r3.3HG0306200	F2E397	organic substance metabolic process	9.81E-05
Downregulated genes				
GO:0005975	HORVU.MOREX.r3.4HG0333780	A0A287MY79	metabolic process	3.60E-23
GO:0004553	HORVU.MOREX.r3.3HG0319100	O64938	cellular process	1.12E-16
GO:0005524	HORVU.MOREX.r3.4HG0346620	M0WQF9	organic substance metabolic process	1.71E-14
GO:0005975	HORVU.MOREX.r3.5HG0491740	A0A287RT76	carbohydrate metabolic process	1.08E-13
GO:0008935	HORVU.MOREX.r3.3HG0277690	Q8LR33	small molecule metabolic process	7.32E-10
GO:0016021	HORVU.MOREX.r3.7HG0687790	A0A287WLW9	primary metabolic process	8.13E-10
GO:0005634	HORVU.MOREX.r3.6HG0568570	F2D8Q7	response to fungus	1.27E-09
GO:0003993	HORVU.MOREX.r3.2HG0165920	M0YS06	response to stress	8.96E-09
GO:0005524	HORVU.MOREX.r3.6HG0568470	F2D195	response to heat	3.219E-08
GO:0009628	HORVU.MOREX.r3.1HG0041710	F2DYR4	response to abiotic stimulus	4.23E-06
GO:0005634	HORVU.MOREX.r3.4HG0380510	F2D639	response to osmotic stress	8.26E-05
GO:0004601	HORVU.MOREX.r3.4HG0351880	M0Y1R4	response to oxidative stress	5.94 E-04
GO:0009507	HORVU.MOREX.r3.2HG0104740	F2CT51	photosynthesis	5.64 E-03
GO:0005634	HORVU.MOREX.r3.6HG0619650	M0WPT5	response to water deprivation	9.56 E-03
GO:0016021	HORVU.MOREX.r3.3HG0225930	F2E8B5	response to abiotic stimulus	1.35 E-02

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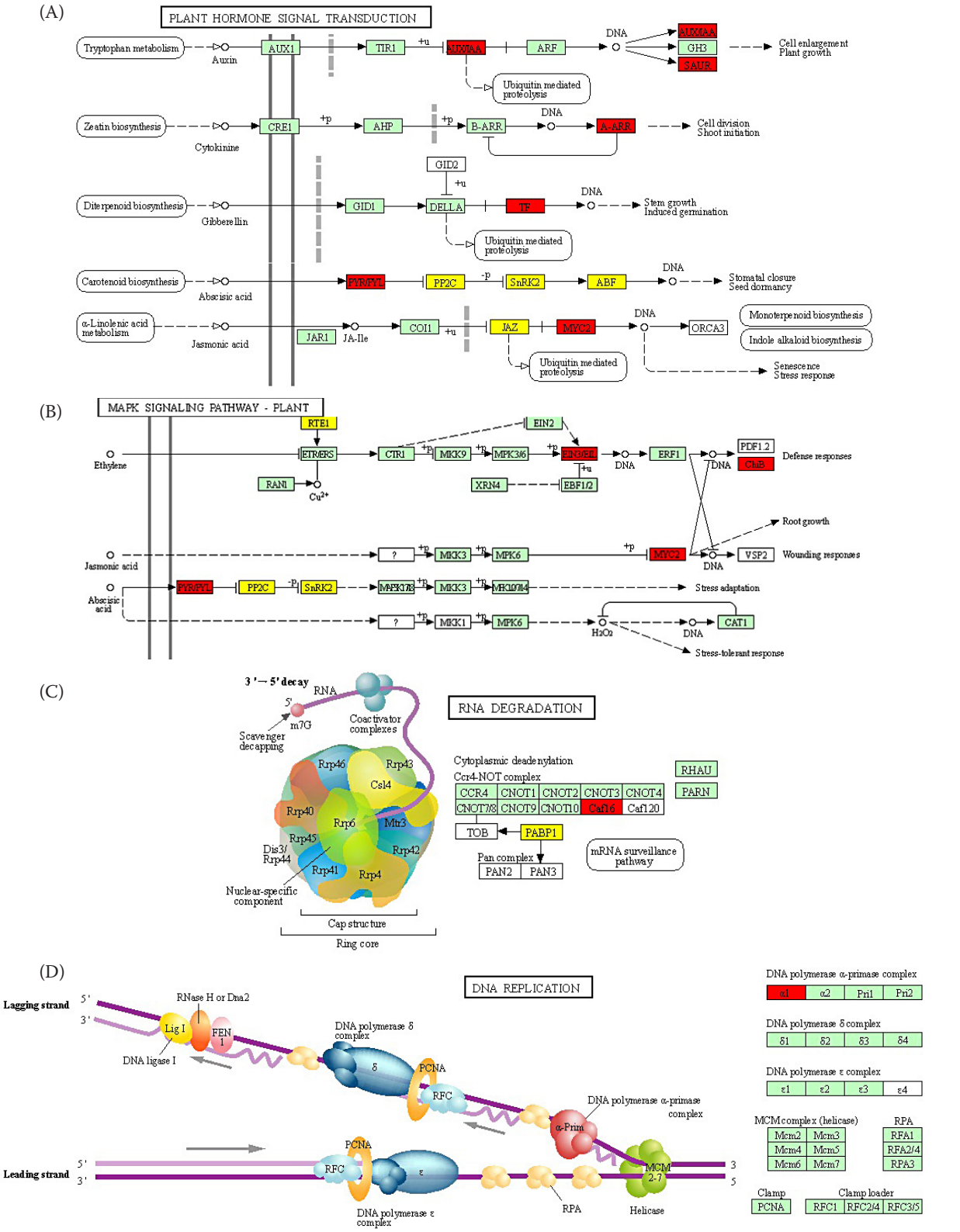

Figure 3. Up- and downregulation meta differential expressed gene (DEG) in the plant hormone signalling pathway (A), mitogen activated protein kinase (MAPK) signalling pathway (B), RNA degradation (C) and DNA replication (D) of *Hordeum vulgare* L. under drought stress

The red box represents the upregulated genes and yellow box shows the downregulated genes

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The promoter sequence (1 500 bp) of the meta-DEGs was downloaded from the genome of *H. vulgare* L. from Biomart and Transcription Factor Binding Site (TFBS) scanned in the promoter sequence (PlantPAN 3.0). The fifty TF families were categorised, 57.12% of the upregulated TFs and 60.07% of the downregulated TFs belonged to NA binding with one finger (*Dof*), Teosinte branched1/Cincinnata/proliferating cell factor (*TCP*), basic helix loop-helix (*bHLH*), *B3*, *bZIP* and squamosa promoter binding proteins (*SBPs*) (Figure 4A). *TCP*, *bHLH* and *bZIP* were higher in the upregulated and downregulated meta-DEG. Examination of the frequency of the TFs up- to downregulated showed that the *TCP* under drought stress activates 1475% more upregulated genes than downregulated genes and *LEA_5* activates 261% more downregulated genes than upregulated genes (Figure 4B). These results display that a peculiar set of TFs regulated the expression of genes in response to drought stress.

To identify the biological relationships between the genes, the PPI network was drawn using Cytoscape (Ver. 3.6.1) and the STRING website (https://string-db.org/) for the down- and upregulated meta-DEG under drought stress separately (Figures 5 and 6). Among the 30 top commonly upregulated DEGs hub genes, five similar to heat shock protein 70 (*HORVU. MOREX.r3.4HG0396100*, *HORVU. MOREX.r3.5HG0491130*, *HORVU. MOREX.r3.1HG0028320*, *HORVU. MOREX.r3.4HG0387870* and *HORVU. MOREX.r3.4HG0416940*); DNA-directed RNA polymerase (*HORVU. MOREX.r3.3HG0295570*) similar to the low temperature-responsive RNA-binding protein (*HORVU. MOREX.r3.7HG0720730*); conserved hypothetical protein (*HORVU. MOREX.r3.6HG0552580*) and MYB TF (*HORVU. MOREX.r3.7HG0699010*) are shown in Table 4. The upregulated meta-DEGs hub genes obtained by the MCC method in CytoHubba are mainly related to the heat shock protein, and DNA-directed RNA polymerase.

Among the top 30 commonly downregulated meta-DEGs hub genes, *HORVU. MOREX.r3.5HG0444900* (similar to the elongation factor Ts), *HORVU. MOREX.r3.4HG0333190* (plastid RNA-binding protein, regulation of chloroplast RNA metabolism), *HORVU. MOREX.r3.2HG0102500* (plastid chaperonin 60 alpha subunit, and seedling development), *HORVU. MOREX.r3.2HG0122100* (nucleotide-binding, alpha-beta plait domain containing protein), *HORVU. MOREX.r3.4HG0352600* (Similar to elongation factor P (EF-P)), *HORVU. MOREX.r3.1HG0089000* (PRC-barrel domain containing protein), *HORVU. MOREX.r3.5HG0471050* (similar to plastid-specific 30S ribosomal protein 2, and chloroplast precursor (PSRP-2), *HORVU. MOREX.r3.6HG0613690* (calcium-binding protein, and drought stress tolerance),

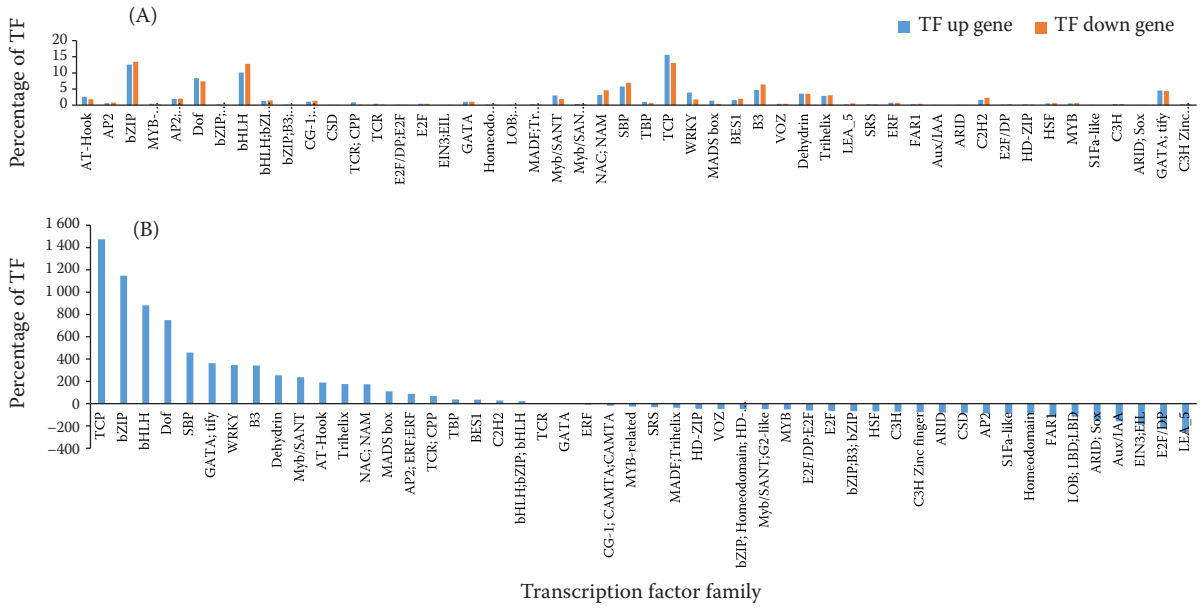

Figure 4. Diagram of the transcription factor of upregulated and downregulated drought-responsive differential expressed genes (DEGs) based on PlantPAN 3.0 in *Hordeum vulgare* L.: transcription factor (TF) of up- and downregulated genes under drought stress (A), percentage of TF upregulated to downregulated genes under drought stress (B)

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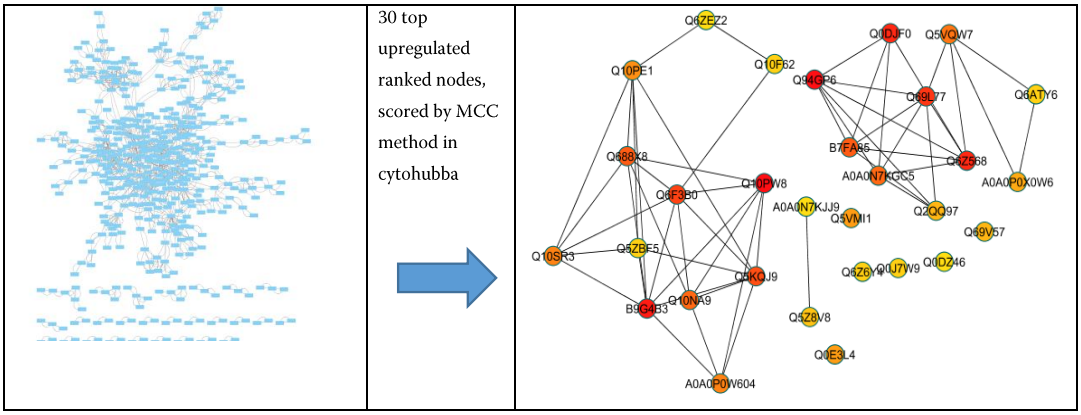

Figure 5. Protein-protein interaction (PPI) network of the meta differentially expressed genes (DEGs) for upregulated genes under drought stress; a cyto-Hubba analysis result using the meta DEG upregulated genes in the Cytoscape package; after the calculation finished, the top 30 essential nodes ranked by maximal clique centrality (MCC) scores were selected from the Barley network

The hub nodes are shown with a colour scheme from red (highly essential) to yellow (essential)

HORVU. MOREX.r3.5HG0491740 (membrane-associated chitinase-like protein) and *HORVU. MOREX.r3.7HG0701900* (conserved hypothetical protein) are shown in Table 5. The downregulated meta-DEGs hub genes obtained by the MCC method in CytoHubba are mainly related to the glycolysis processes and abiotic stress related factors.

The hub genes were tested using the CytoHubba plugin in Cytoscape 3.6.1 with the MCC method (Figures 5 and 6). As the genomic information of barley has not yet been fully reported on, rice orthologues were used from Ensembl Plants (https://plants.ensembl.org). Then, the hub genes were validated by another twelve ranking methods. *HORVU. MOREX.r3.4HG0334210* (*Q10F62*) in eight methods; *HORVU. MOREX.r3.6HG0567270* (*Q0E3L4*) in seven methods; *HORVU. MOREX.r3.5HG0510120* (*Q94GP6*) and *HORVU. MOREX.r3.6HG0548700* (*Q6ZEZ2*) in six methods and *HORVU. MOREX.r3.4HG0396100* (*Q10PW8*) in five methods were presented as the candidate hub genes upregulated meta-DEG (Figure 7). Moreover, *HORVU. MOREX.r3.2HG0122100* (*Q8LHN4*) in eight methods; *HORVU. MOREX.r3.5HG0444900* (*Q2QP54*) in seven methods; *HORVU. MOREX.r3.7HG0674730 (Q5Z4M6), HORVU. MOREX.r3.5HG0480430*

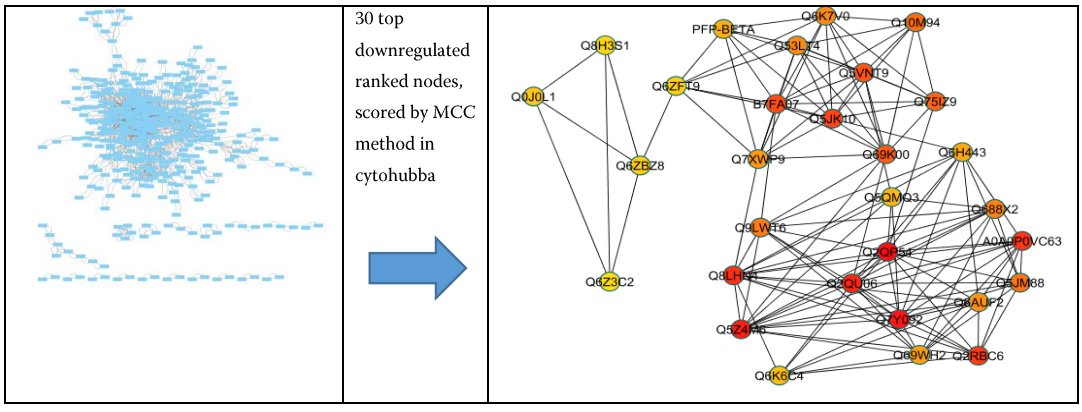

Figure 6. Protein-protein interaction (PPI) network of the meta differentially expressed genes (DEGs) for downregulated under drought stress; a CytoHubba analysis result using the meta DEG downregulated genes in the Cytoscape package; after the calculation finished, the top 30 essential nodes ranked by the maximal clique centrality (MCC) scores were selected from the Barley network

Hub nodes are shown with a colour scheme from red (highly essential) to yellow (essential)

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Table 4. List of the top 30 upregulated meta-differential expressed genes (meta-DEGs) ranked nodes, scored by the maximal clique centrality (MCC) method in CytoHubba under drought stress in *Hordeum vulgare* L.

Rank	Name	Gene stable ID	Score	Description
1	Q94GP6	HORVU.MOREX.r3.5HG0510120	49	WD-40 repeat containing protein
1	Q10PW8	HORVU.MOREX.r3.4HG0396100	49	similar to the 70kD heat shock protein
3	B9G4B3	HORVU.MOREX.r3.5HG0491130	46	similar to the heat shock protein 70
4	Q0DJF0	HORVU.MOREX.r3.5HG0530580	38	hypothetical conserved gene
4	Q6Z568	HORVU.MOREX.r3.6HG0540760	38	nucleotide-binding, alpha-beta plait domain containing protein
6	Q69L77	HORVU.MOREX.r3.6HG0589890	32	brix domain containing protein
7	Q6F3B0	HORVU.MOREX.r3.5HG0517090	31	similar to the DnaJ homologue
8	Q5KQJ9	HORVU.MOREX.r3.1HG0028320	30	heat shock protein Hsp70 family protein
9	Q688X8	HORVU.MOREX.r3.1HG0087430	27	similar to the DnaJ-like protein
9	B7FA85	HORVU.MOREX.r3.7HG0738150	27	similar to fibrillarin-2
11	A0A0N7KGC5	HORVU.MOREX.r3.7HG0738150	26	similar to fibrillarin-2
11	Q10NA9	HORVU.MOREX.r3.4HG0387870	26	similar to the heat shock protein 70
13	Q5VQW7	HORVU.MOREX.r3.3HG0295570	24	DNA-directed RNA polymerase
14	A0A0P0W604	HORVU.MOREX.r3.2HG0099870	23	similar to the heat shock protein 82
14	Q10SR3	HORVU.MOREX.r3.4HG0416940	23	similar to the heat shock 70 kDa protein, mitochondrial precursor
16	Q10PE1	HORVU.MOREX.r3.4HG0393540	21	similar to the predicted protein
17	Q5VMI1	HORVU.MOREX.r3.7HG0672740	20	similar to the receptor-like protein kinase
17	Q0E3L4	HORVU.MOREX.r3.6HG0567270	20	similar to 4-hydroxyphenylpyruvate dioxygenase
19	A0A0P0X0W6	HORVU.MOREX.r3.2HG0113180	16	similar to DNA-directed RNA polymerases I, II, and III
20	Q2QQ97	HORVU.MOREX.r3.3HG0310510	14	similar to the low temperature-responsive RNA-binding protein
20	Q69V57	HORVU.MOREX.r3.7HG0720730	14	similar to Fructose-bisphosphate aldolase, cytoplasmic isozyme
22	Q5Z8V8	HORVU.MOREX.r3.7HG0726610	13	NAD(P)-binding domain containing protein
23	Q10F62	HORVU.MOREX.r3.4HG0334210	12	similar to the Thiamine biosynthesis protein thiC
23	Q6Z6Y4	HORVU.MOREX.r3.6HG0552580	12	conserved hypothetical protein
23	Q0J7W9	HORVU.MOREX.r3.7HG0699010	12	MYB transcription factor, Circadian clock
23	Q6ATY6	HORVU.MOREX.r3.1HG0063540	12	similar to the DNA-directed RNA polymerase II 19 kDa polypeptide
23	Q5ZBF5	HORVU.MOREX.r3.3HG0273010	12	WD40 repeat-like domain containing protein
28	A0A0N7KJJ9	HORVU.MOREX.r3.2HG0186470	11	similar to the H0404F02.9 protein
28	Q6ZEZ2	HORVU.MOREX.r3.6HG0548700	11	similar to the ribose-5-phosphate isomerase precursor
28	Q0DZ46	HORVU.MOREX.r3.6HG0599310	11	similar to fatty aldehyde dehydrogenase 1

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Table 5. List of the top 30 downregulated meta-differential expressed gene (meta-DEG) ranked nodes, scored by the maximal clique centrality (MCC) method in CytoHubba under drought stress in *Hordeum vulgare* L.

Rank	Name	Gene Stable ID	Score	Description
1	Q2QP54	HORVU.MOREX.r3.5HG0444900	4 443	similar to the elongation factor Ts
2	Q7Y092	HORVU.MOREX.r3.4HG0333190	4 338	plastid RNA-binding protein, and Regulation of chloroplast RNA metabolism
3	Q5Z4M6	HORVU.MOREX.r3.7HG0674730	3 813	similar to the trigger factor-like protein
4	Q2QU06	HORVU.MOREX.r3.2HG0102500	3 451	plastid chaperonin 60 alpha subunit, and seedling development
5	Q8LHN4	HORVU.MOREX.r3.2HG0122100	3 400	nucleotide-binding, alpha-beta plait domain containing protein
6	A0A0P0VC63	HORVU.MOREX.r3.3HG0310400	2 730	methyltransferase small domain containing protein
7	Q2RBC6	HORVU.MOREX.r3.4HG0352600	2 505	similar to elongation factor P (EF-P)
8	Q5JK10	HORVU.MOREX.r3.3HG0312010	2 205	similar to trans aldolase
9	B7FA07	HORVU.MOREX.r3.5HG0477350	2 178	enolase
10	Q5VNT9	HORVU.MOREX.r3.7HG0647190	1 938	similar to enolase 1
11	Q69K00	HORVU.MOREX.r3.5HG0499580	1 743	similar to triosephosphate isomerase
12	Q75IZ9	HORVU.MOREX.r3.6HG0568480	1 498	similar to the glucose-6-phosphate dehydrogenase precursor
13	Q10M94	HORVU.MOREX.r3.4HG0380840	1 473	chloroplast precursor
14	Q5JM88	HORVU.MOREX.r3.3HG0329300	1 274	similar to the radical SAM domain-containing protein
15	Q9LWT6	HORVU.MOREX.r3.7HG0639380	1 228	hypothetical gene
16	Q688X2	HORVU.MOREX.r3.1HG0089000	1 224	PRC-barrel domain containing protein
17	Q6K7V0	HORVU.MOREX.r3.4HG0346620	1 087	similar to the uridine kinase-like protein
17	Q53LT4	HORVU.MOREX.r3.4HG0346620	1 087	uridine kinase family protein
19	Q6AUF2	HORVU.MOREX.r3.1HG0088680	1 061	protein of unknown function DUF561 family protein
20	Q7XWP9	HORVU.MOREX.r3.2HG0106830	741	transketolase C-terminal-like domain containing protein
21	Q69WH2	HORVU.MOREX.r3.7HG0711020	599	similar to the bundle sheath defective protein 2
22	Q6H443	HORVU.MOREX.r3.5HG0471050	556	similar to the plastid-specific 30S ribosomal protein 2, and chloroplast precursor (PSRP- 2)
23	PFP-BETA	HORVU.MOREX.r3.6HG0613690	494	calcium-binding protein, and drought stress tolerance
24	Q5QMQ3	HORVU.MOREX.r3.3HG0279360	419	similar to the ATP synthase protein I -related
25	Q6K6C4	HORVU.MOREX.r3.6HG0628900	369	similar to the 29 kDa ribonucleoprotein
26	Q0J0L1	HORVU.MOREX.r3.5HG0491740	266	membrane-associated chitinase-like protein
27	Q6ZFT9	HORVU.MOREX.r3.6HG0609360	250	similar to the pyrophosphate--fructose 6-phosphate 1-phosphotransferase alpha subunit
27	Q6ZBZ8	HORVU.MOREX.r3.7HG0701900	250	conserved hypothetical protein
29	Q8H3S1	HORVU.MOREX.r3.5HG0493200	249	FAS1 domain containing protein
30	Q6Z3C2	HORVU.MOREX.r3.3HG0308570	248	protein of unknown function DUF1218 family protein

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(*Q69P84*), *HORVU. MOREX.r3.5HG0499580* (*Q69K00*) in six methods and *HORVU. MOREX. r3.5HG0514980* (*Q10CU7*) in five methods were presented as the candidate hub genes downregulated meta-DEG (Figure 8). The list of the top ten gene upregulated and downregulated by the twelve methods is provided in Tables 6 and 7. Screening the genes into five to ten methods confirmed the high importance of these genes in the drought tolerance.

To confirm the results of this study, our results were compared with the other researcher's results. The upregulated hub genes of this study including HORVU. MOREX.r3.4HG0396100 (Os03g0218500), HORVU. MOREX.r3.6HG0548700 (Os07g0176900), HORVU. MOREX.r3.2HG0099870 (Os04g0107900) and HORVU. MOREX.r3.7HG0672740 (Os06g0288100) and the downregulated hub genes including HORVU. MOREX.r3.5HG0444900 (Os12g0541500), HORVU. MOREX.r3.5HG0499580 (Os09g0535000), HORVU. MOREX.r3.5HG0514980 (Os03g0780500) and HORVU. MOREX.r3.7HG0637280 (Os12g0448900) agree with those obtained from other qRT-PCR data studies (Figure 9).

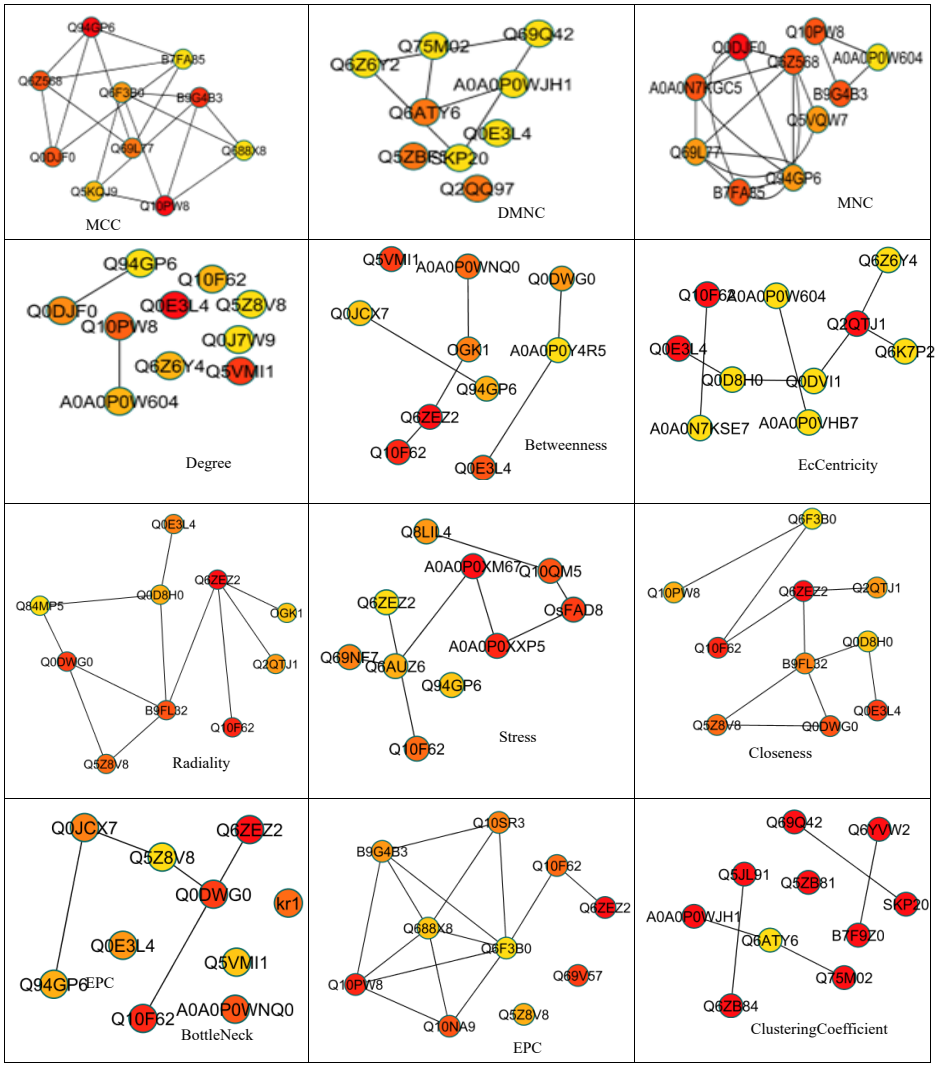

Figure 7. Construction of the protein-protein interaction (PPI) network and identification of the upregulated meta differentially expressed gene (DEG) candidate hub genes with twelve methods of Cytohubba by Cytoscape 3.6.1 (Edge Percolated Component (EPC), Bottle Neck, Closeness, Degree, Density of Maximum Neighbourhood Component (DMNC), EcCentricity, Maximum Neighbourhood Component (MNC), Betweenness, Stress, ClusteringCoefficient, Radiality and Maximal clique centrality (MCC))
The top ten nodes are presented with a colour scheme from red (highly essential) to yellow (essential)

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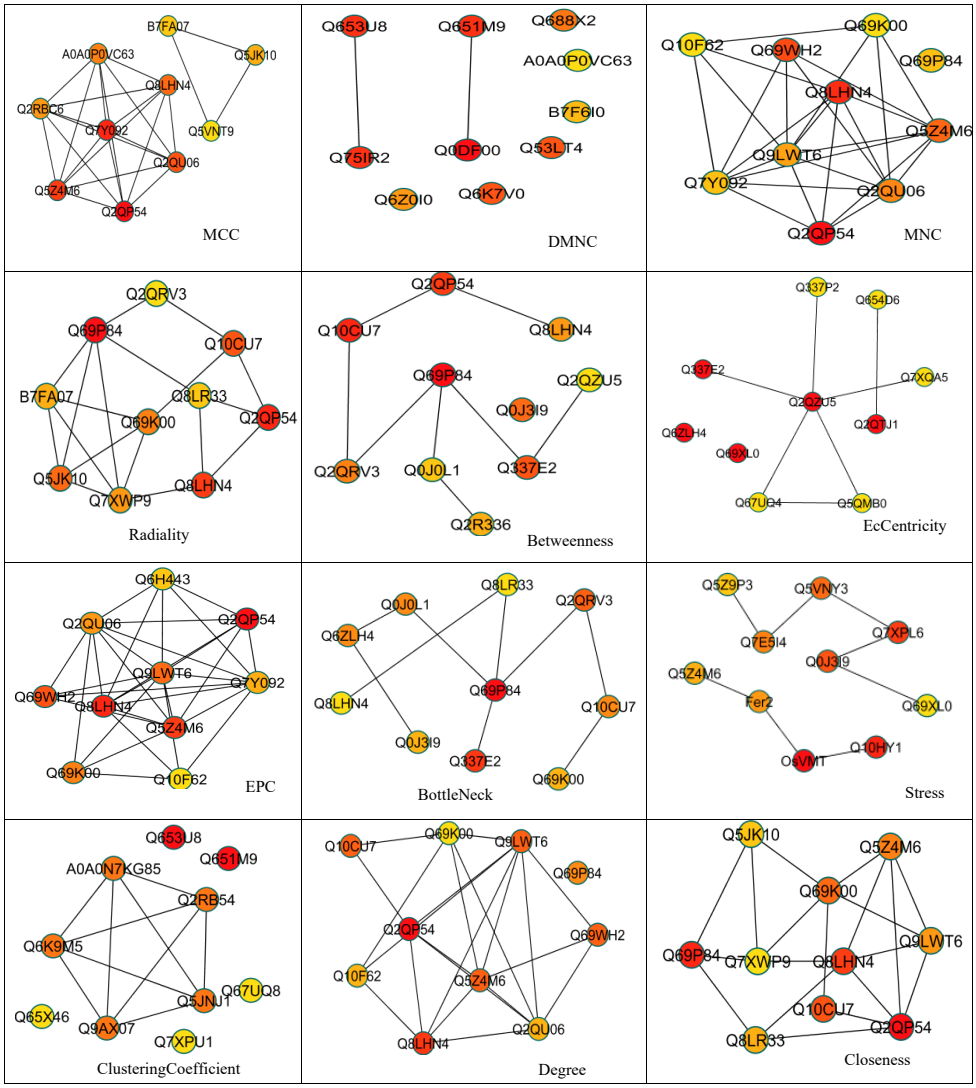

Figure 8. Construction of the protein-protein interaction (PPI) network and identification of the downregulated meta differentially expressed gene (DEG) candidate hub genes with twelve methods of Cytohubba by Cytoscape 3.6.1 (Edge Percolated Component (EPC), Bottle Neck, Closeness, Degree, Density of Maximum Neighbourhood Component (DMNC), EcCentricity, Maximum Neighbourhood Component (MNC), Betweenness, Stress, ClusteringCoefficient, Radiality and Maximal clique centrality (MCC))

The top ten nodes are presented with a colour scheme from red (highly essential) to yellow (essential)

DISCUSSION

In this study, a large-scale comparative analysis of the transcriptome was performed through a meta-analysis using gene expression data from microarrays to estimate the differential expression of genes between normal and drought stress in barley. The plants show different physiological responses to different environments which can be determined by differentially expressed genes (DEGs). In this research, the GO terms in the upregulated genes are mainly related to the metabolic process and response to stimulus and are mainly associated with response to abiotic stresses in the downregulated genes (Table 3). The drought stress tolerance-related genes can control the stomata closure via carbon metabolism, glycine-betaine synthesis and reactive oxygen scavenging (Guo et al. 2009). Secondary metabolites play an important role in overcoming environmental pressures and are engaged in protection functions in response to abiotic stress. One effective mechanism to reduce damage from drought stress is the accumulation of high

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Table 6. List of the top 10 upregulated meta-differential expressed gene (meta-DEG) ranked nodes, scored by twelve topological algorithms in CytoHubba under drought stress in *Hordeum vulgare* L.

Method	Gene name
MCC	Q94GP6, Q10PW8, B9G4B3, Q0DJF0, Q6Z568, Q69L77, Q6F3B0, Q5KQJ9, Q688X8, B7FA85
MNC	Q0DJF0, A0A0N7KGC5, Q6Z568, Q10PW8, B9G4B3, B7FA85, Q69L77, Q94GP6, Q5VQW7, A0A0P0W604
Degree	Q0E3L4, Q5VMI1, Q10PW8, Q0DJF0, Q10F62, Q6Z6Y4, A0A0P0W604, Q94GP6, Q5Z8V8, Q0J7W9
Closeness	Q6ZEZ2, Q10F62, Q0E3L4, Q0DWG0, Q5Z8V8, B9FL32, Q2QTJ1, Q10PW8, Q0D8H0, Q6F3B0
BottleNeck	Q6ZEZ2, Q10F62, Q0DWG0, A0A0P0WNQ0, kr1, Q0JCX7, Q0E3L4, Q94GP6, Q5VMI1, Q5Z8V8
Stress	A0A0P0XM67, A0A0P0XXP5, OsFAD8, Q10QM5, Q10F62, Q69NF7, Q8LIL4, Q6AUZ6, Q94GP6, Q6ZEZ2
DMNC	Q6F3B0, Q2QQ97, Q6ATY6, Q5ZBF5, A0A0P0WJH1, Q0E3L4, SKP20, Q69Q42, Q6Z6Y2, Q75M02
EcCentricity	Q10F62, Q0E3L4, Q2QTJ1, Q6K7P2, Q0D8H0, Q0DVI1, A0A0N7KSE7, Q6Z6Y4, A0A0P0VHB7, A0A0P0W604
EPC	Q6ZEZ2, Q10PW8, B9G4B3, Q10NA9, Q6F3B0, Q69V57, Q688X8, Q10PE1, Q10F62, A0A0P0W604
Radiality	Q6ZEZ2, Q10F62, Q0DWG0, B9FL32, Q5Z8V8, Q0E3L4, Q2QTJ1, Q0D8H0, OGK1, Q84MP5
Betweenness	Q6ZEZ2, Q10F62, Q5VMI1, Q0E3L4, A0A0P0WNQ0, OGK1, Q0DWG0, Q94GP6, Q0JCX7, A0A0P0Y4R5
ClusteringCoefficient	A0A0P0WJH1, SKP20, Q69Q42, Q75M02, Q6ZB84, Q5JL91, B7F9Z0, Q6YVW2, Q5ZB81, Q6ATY6

MCC – Maximal clique centrality; MNC – Maximum Neighbourhood Component; DMNC – Density of Maximum Neighbourhood Component; EPC – Edge Percolated Component

intracellular levels of osmoprotectant compounds such as proline (Rontein et al. 2002).

In this study, based on the TF frequency, Dof, *TCP*, *bHLH*, *B3*, *bZIP*, *SBP*, *Dhns* and *NAC*; *NAM* showed the highest percentage of TFs related to the upregulated and downregulated meta-DEGs (Figure 4A). TFs of *NAC* have been shown to work not only with plant development, but also with responses to abiotic

Table 7. List of the top 10 downregulated meta-differential expressed gene (meta-DEG) ranked nodes, scored by twelve topological algorithms in CytoHubba under drought stress in *Hordeum vulgare* L.

Method	Gene name
MCC	Q2QP54, Q7Y092, Q5Z4M6, Q2QU06, Q8LHN4, A0A0P0VC63, Q2RBC6, Q5JK10, B7FA07, Q5VNT9
MNC	Q2QP54, Q8LHN4, Q69WH2, Q5Z4M6, Q2QU06, Q9LWT6, Q7Y092, Q69P84, Q10F62, Q69K00
Degree	Q2QP54, Q8LHN4, Q10CU7, Q5Z4M6, Q9LWT6, Q69WH2, Q69P84, Q2QU06, Q10F62, Q69K00
Closeness	Q2QP54, Q69P84, Q8LHN4, Q10CU7, Q69K00, Q5Z4M6, Q9LWT6, Q8LR33, Q5JK10, Q7XWP9
BottleNeck	Q69P84, Q337E2, Q2QRV3, Q10CU7, Q0J0L1, Q6ZLH4, Q0J3I9, Q69K00, Q8LHN4, Q8LR33
Stress	OsVMT, Q10HY1, Q7XPL6, Q0J3I9, Q5VNY3, Q7E5I4, Fer2, Q5Z4M6, Q5Z9P3, Q69XL0
DMNC	Q0DF00, Q75IR2, Q653U8, Q651M9, Q6K7V0, Q53LT4, Q688X2, Q6Z0I0, B7F6I0, A0A0P0VC63
EcCentricity	Q69XL0, Q2QZU5, Q2QTJ1, Q337E2, Q6ZLH4, Q654D6, Q5QMB0, Q7XQA5, Q67UQ4, Q337P2
EPC	Q2QP54, Q8LHN4, Q5Z4M6, Q69WH2, Q9LWT6, Q69K00, Q2QU06, Q10F62, Q7Y092, Q6KA61
Radiality	Q69P84, Q2QP54, Q8LHN4, Q10CU7, Q5JK10, Q69K00, Q7XWP9, B7FA07, Q8LR33, Q2QRV3
Betweenness	Q69P84, Q10CU7, Q2QP54, Q337E2, Q0J3I9, Q2QRV3, Q8LHN4, Q2R336, Q0J0L1, Q2QZU5
ClusteringCoefficient	Q653U8, Q651M9, A0A0N7KG85, Q2RB54, Q9AX07, Q5JNJ1, Q6K9M5, Q67UQ8, Q7XPU1, Q65X46

MCC – Maximal clique centrality; MNC – Maximum Neighbourhood Component; DMNC – Density of Maximum Neighbourhood Component; EPC – Edge Percolated Component

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stress (Zhou et al. 2009). A gene called *HvSNAC1* from the *NAC* TF family has been isolated from drought-stressed barley plants and the gene under the control of the maize ubiquitin promoter that was introduced to barley (cv. Golden promise) and transgenic plants constitutively expressed the *HvSNAC1* gene were reported to have higher drought tolerance along with a productivity increase via the expression of several stress-inducible genes (Al Abdallat et al. 2014). *bHLH* proteins play a crucial role in the secondary metabolism and ABA-mediated signal transduction pathway, and they have the capacity of regulating the adaptive response of plants to abiotic stresses (Nakashima et al. 2012). *Dhns* are one of the main groups of genes that are induced by drought stress. A wide range of studies have addressed the changes in dehydrin gene expression in response to drought stress. Ten of the barley *Dhns* gene family (*Dhn1-11*) were reported to be upregulated by the ABA treatment and dehydration (Choi & Close 2000). A significant increase was found in the expression of *Dhn13* by drought in the cv. Morex and Tibetan hull-less barley (Rodriguez et al. 2005; Qian et al. 2008).

In this study, in the MAPK signalling pathway, the *RTE1* gene as the downregulated gene and *ChiB* and *EIN3/EIL* genes as the upregulated genes affect the defensive response in ethylene. The *MYC2* gene as the upregulated gene affects the growth root in the JA. Next, *PYR/PYL* as the upregulated gene, and *PP2C* and *SnRK2* as the downregulated genes affect the adaption stress in the ABA (Figure 3B). MAPK cascades are activated by drought, salinity, cold, and hormone stimuli, as well as by developmental processes, such as proper cell division and differentiation or abscission (Colcombet & Hirt 2008). The ethylene signalling pathway was represented in the study of Janiak et al. (2018) by observing a higher initial expression of the gene encoding EIN3-binding F-box protein 1 (*EBF1*) in CamB genotype roots of barley. In the plant hormone signal transduction pathway, the *JAZ* gene as the downregulated gene interacts with *MYC2* as the upregulated gene, which can affect the abiotic stress response in the JA. In the ABA biosynthesis, the *PYR/PYL* receptor as the upregulated gene; *PP2C*, *SnRK2* and the ABRE-binding factors (*ABFs*) as the downregulated genes affect the stomatal closure. The genes including AUX/IAA and SAURS in Auxin biosynthesis (which affect the cell elongation and plant growth) as well as the A-ARR gene in cytokinin with cell division properties were

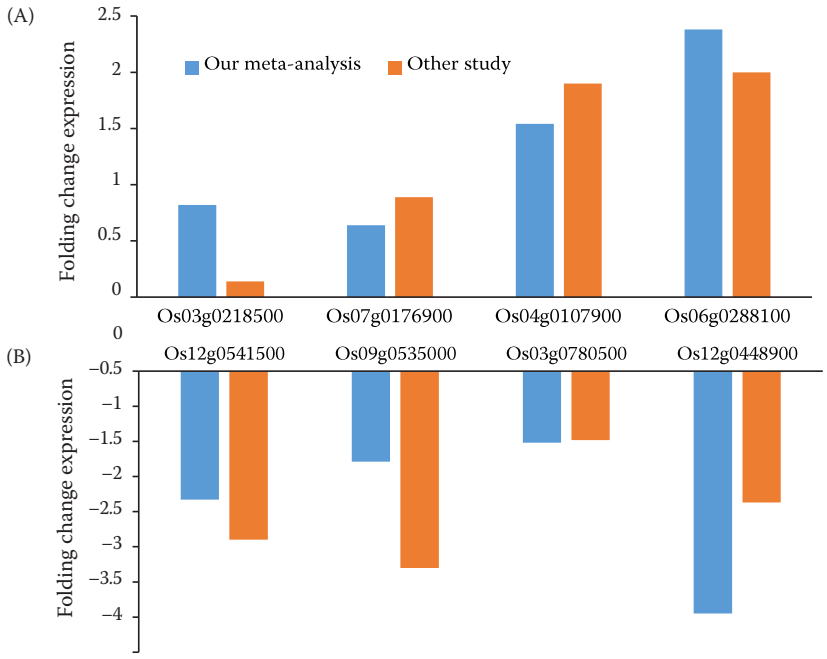

Figure 9. Validation of the upregulated and downregulated hub genes by other studies; the expression fold changes from (A): upregulated hub genes (Os03g0218500 (Ueda et al. 2012), Os07g0176900 (Aslam et al. 2019), Os04g0107900 (Rerksiri et al. 2013) and Os06g0288100 (Kan et al. 2017)) and (B): downregulated hub genes (Os12g0541500 (Shimono et al. 2016), Os09g0535000 (Park et al. 2012), Os03g0780500 (Jeong et al. 2017), and Os12g0448900 (Lee et al. 2016)) are strongly in agreement with those obtained from other qRT-PCR result studies

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considered as upregulated genes (Figure 3A). JAs are lipid-derived compounds with signal functions that mediate plant stress responses and development processes (Wasternack & Kombrink 2010). In *Arabidopsis*, induction of the *AtMPK1/2* kinase activity is observed in the leaves 1 h after JA treatment, which is involved in the stomatal closure (Lee et al. 2016). *ABFs* are among the *bZIP*-type TFs that have the responsibility of controlling the gene expression in an ABA-dependent manner and, according to a study conducted by Liu et al. (2017), the serine/threonine kinase family responds to cold stresses and is involved in the ABA signalling pathway.

In the RNA degradation pathway, the genes *Caf16* and *PABP1* in Ccr4-Not Complex were identified as up- and downregulated genes, respectively (Figure 3C). On the other hand, in the DNA replication pathway, the *α1* gene in the DNA polymerase α -primase complex was identified as an upregulated gene (Figure 3D). CAF-1 chaperone is bound to Proliferating Cell Nuclear Antigen (PCNA) at the replication fork. CAF-1 is recruited by PCNA to the replication fork and is involved in supplying *de novo* synthesised H3–H4 dimers to the DNA replication machinery (Foltman et al. 2013).

To determine the genes with a prominent role in the network, the genes with high connectivity known as hub genes were identified. In this study, upregulated DEGs hub genes are mainly related to the thiamine biosynthesis protein (*thiC*), 4-hydroxyphenylpyruvate dioxygenase, ribose-5-phosphate isomerase precursor and heat shock protein (*Hsp70*) (Table 4 and Figure 7). Tunc-Ozdemir et al. (2009) reported the role of thiamine pyrophosphate (TPP) as an important stress response molecule as it was demonstrated that thiamine biosynthesis is induced in *Arabidopsis* in response to different abiotic stresses as an upregulated gene. *Hsps* chaperones are responsible for protein folding, assembly, translocation and degradation in many normal cellular processes, stabilised proteins and membranes, and can assist in protein refolding under abiotic stress conditions (Lin et al. 2001). Moreover, the downregulated DEGs hub genes are mainly related to the nucleotide-binding, alpha-beta plait domain containing protein, EF-Ts, aldehyde dehydrogenase and seed maturation, trigger factor-like protein and inosine-5-monophosphate dehydrogenase 2 (Table 5 and Figure 8). The protein synthesis elongation factor Tu (EF-Tu) plays an important role in the elongation phase of protein synthesis in organelles such as plastids and mitochondria (Allen et al. 2011). The cytosolic homologue of EF-Tu in plants is EF-1α. EF-Ts can facilitate the exchange of EF-Tu-bound GDP for GTP and can be considered as a key factor in the ribosomal biosynthesis of proteins in cells. Soybean EF-1α might be an important factor in translation regulation during abiotic stresses (Chung et al. 2009). Chloroplasts are the only subcellular compartments in eukaryotes that seem to have molecular chaperones of the trigger factor family (Allen et al. 2011). Trigger factor binds the ribosome-D1 complex prior to the association with the thylakoid targeted Signal Recognition Particle (*cpSRP54*), a chaperone-like moiety and has an important role in the translation regulation in abiotic stresses. In plants, aldehyde dehydrogenases (*ALDHs*) play the role of aldehyde scavengers since they remove reactive aldehydes, accordingly affecting the abiotic stress responses. The *ALDH 7* gene of the soybean has been reported to tolerate drought in *Arabidopsis thaliana* and the overexpression of the *CsALDH* gene in alfalfa has been promoted to cause resistance to abiotic stress (Duan et al. 2014).

To confirm the accuracy of the meta-analysis results, we compared the expression fold changes of four selected genes from the upregulated hub genes with those obtained from other qRT-PCR studies (*Os03g0218500* (Ueda et al. 2012), *Os07g0176900* (Aslam et al. 2019), *Os04g0107900* (Rerksiri et al. 2013) and *Os06g0288100* (Kan et al. 2017)), and also from the downregulated hub genes (*Os12g0541500* (Shimono et al. 2016), *Os09g0535000* (Park et al. 2012), *Os03g0780500* (Jeong et al. 2017) and *Os12g0448900* (Lee et al. 2016)) (Figure 9).

CONCLUSION

Researchers can study the expression of thousands of genes and their co-expression partners under different stresses at the same time using advances in genome-scale transcriptome analysis methods. DEGs represent the genes in different signalling pathways and networks and this provides an opportunity to find out which genes play the major role in various stresses. On bias study of the references, this is the first study addressing barley as the target plant to detect genes involved in drought stress-tolerance responses. In this study, we performed a meta-analysis and computational system biology analysis for the genome-wide expression data to identify the responsive genes to understand how

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H. vulgare L. responds to drought stress. Most of the DEGs were involved in the DNA replication pathway, MAPK signalling pathway, pathways of biosynthesis of secondary metabolites, RNA degradation and plant hormone signal transduction. Identification of the hub genes by several methods could be effectual in getting an overview of the hub genes that play a crucial role in drought stress response in barley. This research promoted a new line of approach addressing the expression profiles of different genes under drought conditions, identified the main genes in response to drought stress, and introduced them as candidate genes with the aim of improving plant tolerance by employing genetic engineering approaches.

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