Agricultural Biotechnology Journal
Shahid Bahonar University of Kerman
Iranian Biotechnology Society
Print ISSN: 2228-6705 Online ISSN: 2228-6500

Weighted gene co-expressed network analysis in barley and expression of hub genes involved at the germination stage

Zohreh Hajibarat
Ph.D. Student, Department of Plant Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran. E-mail address: zohreh.hajbarat@yahoo.com

Abbas Saidi
*Corresponding author. Professor, Department of Plant Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran. E-mail address: abbas.saidi@gmail.com

Mohammad Reza Ghaffari
Assistant Professor, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran. E-mail address: ghaffari@abrii.ac.ir

Mehrshad Zeinalabedini
Associated Professor, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran. E-mail address: mzeinolabedini@abrii.ac.ir

Zahra Hajibarat
Ph.D. Student, Department of Plant Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran. E-mail address: zahrahajibarat@yahoo.com

Abstract

Objective

Seed germination is an important process that determines the beginning of the seed plant life cycle. However, the mechanism underlying seed germination in barley remains unclear. To understand the molecular mechanism of seed germination in barley, WGCNA analysis was used to detect the hub and responsive genes and to reveal the expression of the genes on seed germination. WGCNA is a valuable tool for studying the correlation between genes, identifying modules with high correlation, and identifying Hub genes in different modules.

Materials and methods

Raw microarray data related to the germination stage were obtained from the GEO microarray database for 0h, 3h, 9h, 18h, 33h, and 71h after the germination stage. Then, weighted gene co-expression network analysis (WGCNA) was utilized for the detection of co-expressed network genes. In the present study, a barley cultivar, Mahtab, was utilized to show expression patterns after 9h, 18h, and 71h after the germination stage. A total number of 4137 differentially expressed

genes (DEG) were identified, with some genes showing higher expression in Mahtab and three genes verified by qRT-PCR.

Results

Most of these DEGs were involved in metabolic processes, cellular processes, glycolysis, and response to a stimulus. Hub gene in MEbrown was alpha/beta-Hydrolases superfamily protein, MEpurple was U-box domain-containing protein 4, and MEdarkgrey was B3 domain-containing transcription factor ABI3 which were positively correlated with germinated seeds. The results showed a microarray database and candidate genes for further study of barley at germination stages. In addition, DEGs were divided into three modules by WGCNA. In this study, gene modules associated with seed germination during barley seed germination were identified. Transcription factor and alpha/beta-hydrolase played an important role at the germination stage. Also, gene modules and hub genes at 9h, 18h, and 71h after germination were detected. As there is a lack of information on the seed germination requirements of barley, this research was conducted to study seed germination mechanisms as well as evaluation of hub genes to study molecular mechanism of seed germination in barley.

Conclusions

The results of the present study provide new insights into the molecular mechanism underlying barley seed germination. Based on the network analysis, transcription factors (TFs) and ubiquitin proteins were involved in germination. Most of the genes related to each module were related to proteins involved in carbohydrate metabolism, glycolysis, and protein degradation. Our gene expression results can serve as molecular markers in barley cultivars during seed germination. These findings can be suitable for molecular-assisted selection and breeding of fast-germinating barley genotypes.

Keywords: WGCNA, Transcription factors, Gene network, Germination stage.
Paper Type: Research Paper.

Citation: Hajibarat Z, Saidi A, Ghaffari MR, Zeinalabedini M (2022) Weighted gene co-expressed network analysis in barley and expression of hub genes involved at germination stage. *Agricultural Biotechnology Journal* 14 (4), 247-267.

Agricultural Biotechnology Journal 14 (4), 247-267. DOI: 10.22103/jab.2022.20183.1427
Received: October 23, 2022. Received in revised form: November 23, 2022.
Accepted: November 24, 2022. Published online: November 30, 2022
Publisher: Faculty of Agriculture and Technology Institute of Plant Production, Shahid Bahonar University of Kerman-Iranian Biotechnology Society.

Introduction

Seed germination is a complex process that involves some morphological and physiological changes (Rajjou et al. 2012). One of the most sensitive stages of plant growth is the germination stage. Seed germination is started with the initial water uptake followed by root growth from the seed coat (Kucera et al. 2005). Some transcription factors were induced more at the germination stage (time points) than seedling and reproductive stages (Jones et al. 2010). A previous study showed that signaling pathways and regulatory networks, including glycolysis and hormonal signaling, and transcription factors are involved in the germination stage of *Arabidopsis* seeds (Ruan 2014). The data set for the early stages of germination provides a broad view of gene expression networks and their role in controlling seed germination. Energy production plays an important role during seed germination. After sufficient water absorption, the amino acid metabolism, glycolysis, and the TCA cycle are activated in the seed (Nguyen et al. 2016).

During seed germination, a large number of differentially expressed TFs are up-regulated in this stage. Various hormones including gibberellins (GA), abscisic acid (ABA), brassinosteroids (BRs), ethylene, and auxins are involved in cell differentiation, germination, and cell wall breakdown. One study showed that seed germination was controlled by a complex network of signaling and regulation of gene expression (Li et al. 2022). Plants may share similar molecular mechanisms such as the behavior of plant hormones, transcription and activation of translation, and the process of rooting. However, different plant species have specific mechanisms, in particular for repositioning storage and activating metabolism (Jing and Lin 2020). Between model plants, only slight transcription data have been reported for barley seed germination (Asakura et al. 2012; Jones and Vodkin 2013). To date, "omics" methods have been utilized to provide information about changes in the levels of proteins and gene transcripts (Liew et al. 2020). Many of the genes involved in seed germination show different expression patterns during different hours of germination. According to a report, during seed growth in rice, endosperm also affects seed germination by affecting ABA signaling through sugar metabolism (Stotz et al. 2009). In tomatoes, weakening of the endosperm cap during uptake due to hydrolysis of galactomannans in cell walls is a prerequisite for ensuring the complete emergence of the root (Kader 1996). After germination, the stored reserves in the endosperm for growth are important during seedling emergence (Nguyen et al. 2016). Based on a study, microarray data for the detection of WGCNA modules were performed to identify hub genes associated with the glandless trait in cotton through transcription analysis. In addition, qRT-PCR analysis was used to confirm the results of gene expression (Qu et al. 2019). Weight Correlation Network Analysis (WGCNA) provided co-expressed regulatory gene networks to identify thousands of genes. The main purpose of this study was to investigate hub genes and their function during barley seed germination. This study

aimed to elucidate the molecular mechanisms involved in barley seed germination, which is an important complement to biological changes that happen during seed germination. In the current research, we compared the germination stages at 9, 18, and 71 hours after germination using qRT-PCR.

Material and methods

Raw microarray data related to germination data were obtained from the GEO microarray database (GSM578686, GSM578687, GSM578688, GSM578689, GSM578690, GSM578691, GSM578692, GSM578693, GSM578694, GSM578695, GSM578696, GSM578697, GSM578698, GSM578699, GSM578700) for 0h, 3h,9h, 18h, 33h, and 71h after germination stages. Converting the probe set to gene IDs was done using the DAVID site (https://david.ncifcrf.gov). Subsequently, WGCNA (Weighted gene co-expression network analysis) was performed to establish the barely DEGs co-expression network using the R package (flashClust). WGCNA was used to identify modules with highly expressed genes, and categorize them with module eigengene (ME). We performed WGCNA analysis in barley genotypes at germination stages. The adjacency matrix was utilized to analyze the topographical overlap matrix (TOM), which measures the number of neighbors having a pair of proteins in common. To find the hub genes for each module, the top 10% of genes with the highest scores were used by the igraph package. A significant module was presented using String (https://string-db.org/). Gene ontology for DEGs was performed for three significant modules at germination stages using the gprofiler web server at https://biit.cs.ut.ee/gprofiler/gost. The prediction of cis-elements of hub genes was performed using the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

Barley growth at germination stages: Mahtab cultivar was used as experimental material was provided by the Seed and Plant Improvement Institute (SPII), located in Karaj. The seeds of the Mahtab cultivar were cultivated in plastic bags (26 cm height, 25 cm diameter) filled with soil in the greenhouse at 16 hours light and 8 hours dark. Germinated seeds of Mahtab were evaluated for expression analysis at 9h, 18h, and 71h after germination.

RNA extraction and quantitative real-time PCR (qRT-PCR) analysis: QRT-PCR analysis was used to confirm the *in-silico* results. RNA was extracted from germinated barley seeds using an Anacell RNA extraction kit from Anacell Co., Iran according to the instructions. For RNA preparation, germinated seed samples were collected separately. Samplings from germinated seeds were collected at 9, 18, and 71 hours after germination. The three sampling times had significant modules and the hub genes were selected from the significant modules (brown, purple, and dark grey). DNase I (Sinaclon Co, Iran) was used to remove genomic DNA

Agricultural Biotechnology Journal; Print ISSN: 2228-6705, Electronic ISSN: 2228-6500

contamination in RNA samples. RNA concentration was determined by nanodrop and its quality was evaluated using 1% agarose gel analysis. After RNA extraction, the synthesis of cDNA by a one-step reverse transcription kit (Easy cDNA Synthesis Kit, Iran) was performed. Three biological replications with three technical repetitions were performed to analyze each gene. The barley actin gene was used as a reference. The primers were designed using VectorNTI and are given in Table 1. Reaction (qRT-PCR) was performed with ABI 7500 using SYBR Green Supermix. Relative expression was determined via the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001) after normalization of the Ct value for selected genes versus Actin as the reference gene. RT-qPCR was conducted to determine the expression profile for the B3, U-box, ABH hydrolase genes, and actin genes using seed germination. The significant variations between means were compared at $p < 0.05$ (Duncan's test).

Table 1. Primer and sequences of primers used in this study

Primer Name	Sequence 5'→ 3'	Product size of each primer (bp)
B3 domain-containing protein F	TGCGCCGAGGCATACTTCC	180
b3 domain-containing protein R	GTATCGCCCGCTTGTAAGTGC	
U-boxF	TGCTAAGACAAGCATGGTCGG	162
U-boxR	ACTGTAAGTGTCCCAGGC	
ABH hydrolase F	TACAGGCTCGCGCCCGA	110
ABH hydrolase R	GCCGGATCACCGTGTGCAGC	
Actin F	GGTCCATCCTAGCCTCACTC	129
Actin R	GATAACAGCAGTGGAGCGCT	

Results and Discussion

Based on the WGCNA, we identified modules that were positively associated with the germination stage, indicating that modules consist of germination-regulated DEGs. Due to an increase in the total protein content of plants at the germination stage, the synthesis of many transcription factors proteins has probably increased as well. WGCNA identifies expressed gene modules and examines the relationships between gene networks and the intended target in the network. Analysis of gene enrichment in the target module can lead to accurate information in biological processes (Van Dam et al. 2018; Bakhtiarizadeh et al. 2018).

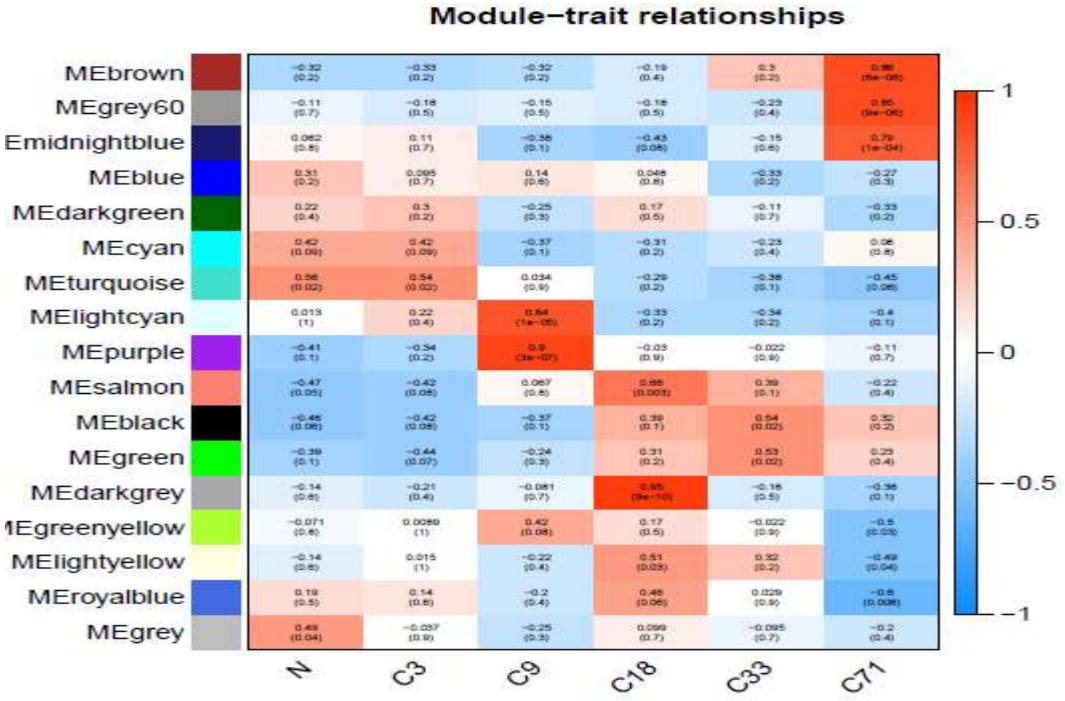
Raw files were downloaded from seed germination from the GEO database. A total of 23450 genes were obtained from samples. A gene regulatory network was utilized to identify highly co-

expressed gene clusters (modules). Expression networks were drawn based on the correlation of genes at different stages of germination. Our results showed that 17 modules were identified as shown in the dendrogram in Figure 1a. Of the 17 modules obtained, three modules showed a high correlation with germination stages. In these modules, some genes are expressed in a sample. We further applied the WGCNA approach to 4137 DEGs. In this study, DEGs were ultimately categorized into 17 modules with members ranging from 41 to 1117 DEGs (Figure 1). The soft threshold power of 18 ($\beta = 18$) was selected according to the preconditions of approximate scale-free topology. As shown in Figure 1, 41 DEGs to the MEbrown module, 1117 DEGs to the MEcyn module, 602 DEGs to the MEblue module, 110 DEGs to the MEdarkgrey module, 150 DEGs to the MEgreen, 177 DEGs to the MEdarkgreen, 679 DEGs to the MEgreenyellow, 60 DEGs to the MEgrey60, 84 DEGs to the MElightgreen module, 176 DEGs to the MEmidnightblue module, 63 DEGs to the MEblack module, 160 DEGs to the MEpurple module, 152 DEGs to the MEpurple module, 41 DEGs to the MElightyellow, 79 DEGs to the MEgrey60, 171 DEGs to the MEsalmone and 44 DEGs to the MEgreenyellow were identified. The DEGs in the gray module were removed during further analysis. The three functional modules along with their correlation and *p* values are depicted in Figure 1b. Among the surveyed modules, the highest number of co-expressed genes were related to MEcyn, MEblue, and MEgreenyellow, and the lowest number of co-expressed genes were related to MElightyellow. The comparative analysis of seed germination allowed for up and down-regulation of DEGs with differential germination stages. The hierarchical clustering of the meta-genes, from across the periods of seed germination using the topological overlap matrix (TOM), is presented in Figure 1a. Heatmaps showed the expression patterns of eigengenes in modules (Figure 1b). Out of 10 modules identified, MEpurple, MEdarkgrey, and MEbrown modules were involved in 9h, 18h, and 71h after seed germination (Figure 1c). Bar graphs revealed the expression levels of the module eigengenes (Figure 1d).

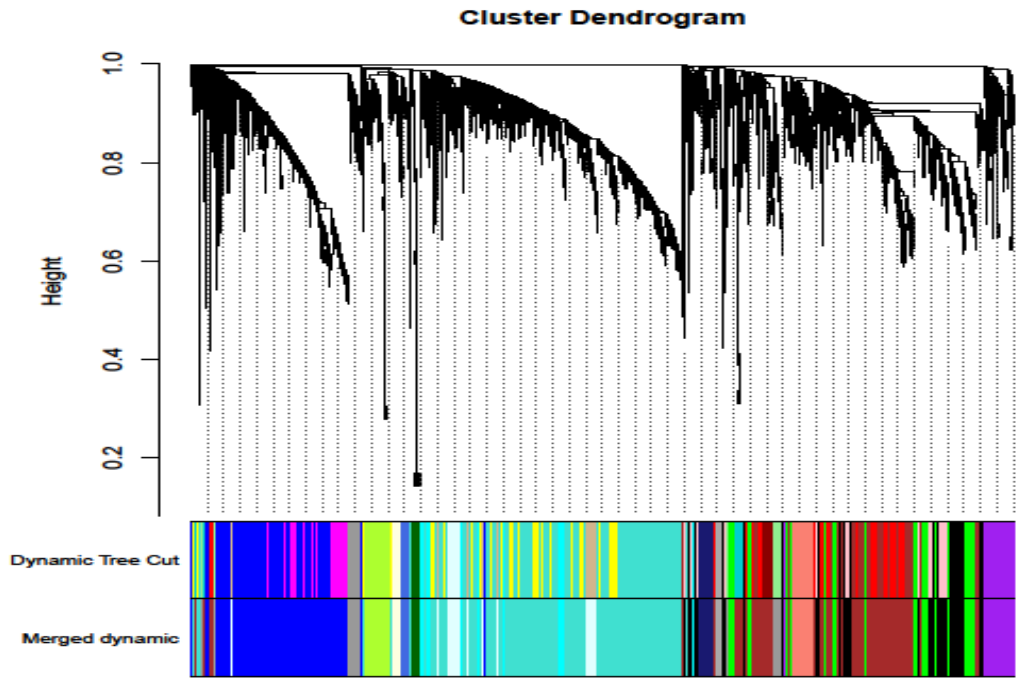
Functional gene ontology Analysis and Hub Gene Analysis of three Modules involved at germination stages: Gene ontology showed that molecular function in MEbrown is related to the cytoplasm (GO:005737), MEdarkgrey is related to protein binding (GO:0005515) and protein dimerization activity (GO:0046983), and MEpurple is related to tyrosine-tRNA ligase activity (GO: 0004831). In biological processes, MEdarkgrey is related to cellular component biogenesis (GO:0044085) and Golgi apparatus (GO:0005794), and MEpurple is related to tyrosyl-tRNA aminoacylation (GO:0006437). For cellular components, MEdarkgrey is related to the nucleolus (GO:0005730) and nucleus (GO:0005634), and MEpurple is related to Golgi cisterna (GO:0031985) and Golgi stack (GO:0005795). WGCNA analysis has been utilized for the detection of hub genes. Hub gene in MEbrown was B3 domain-containing transcription factor

Agricultural Biotechnology Journal; Print ISSN: 2228-6705, Electronic ISSN: 2228-6500

ABI3, in MEpurple was U-box domain-containing protein 4, and in MEdarkgrey was alpha/beta-Hydrolases superfamily protein, which was positively correlated with germination stages.

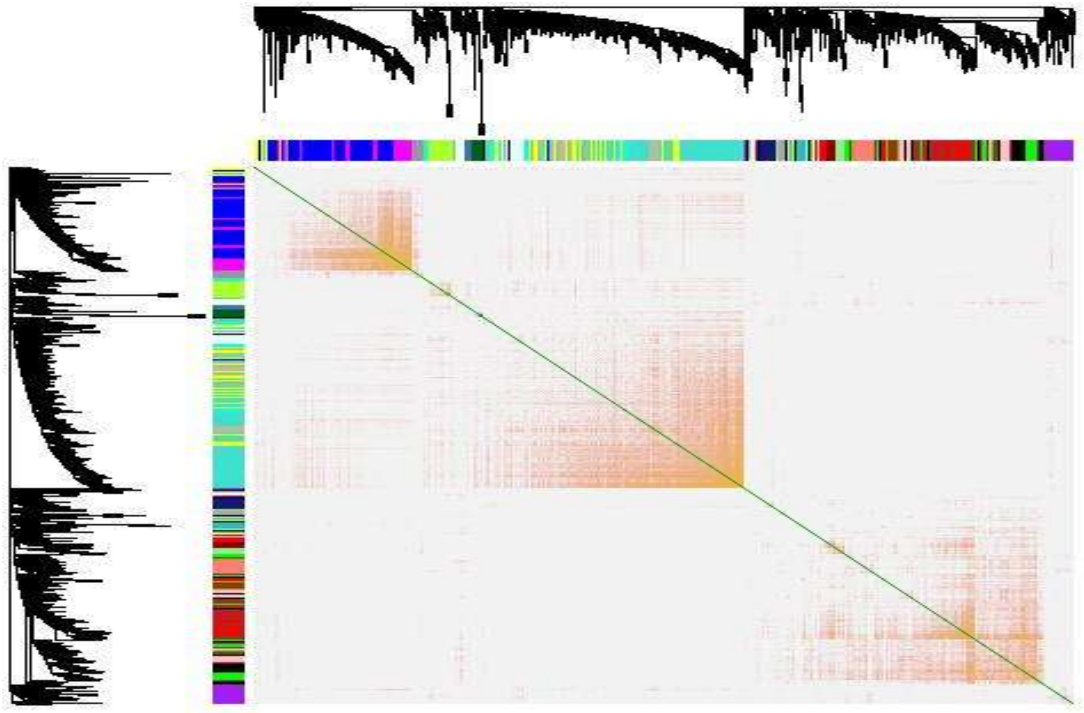


a

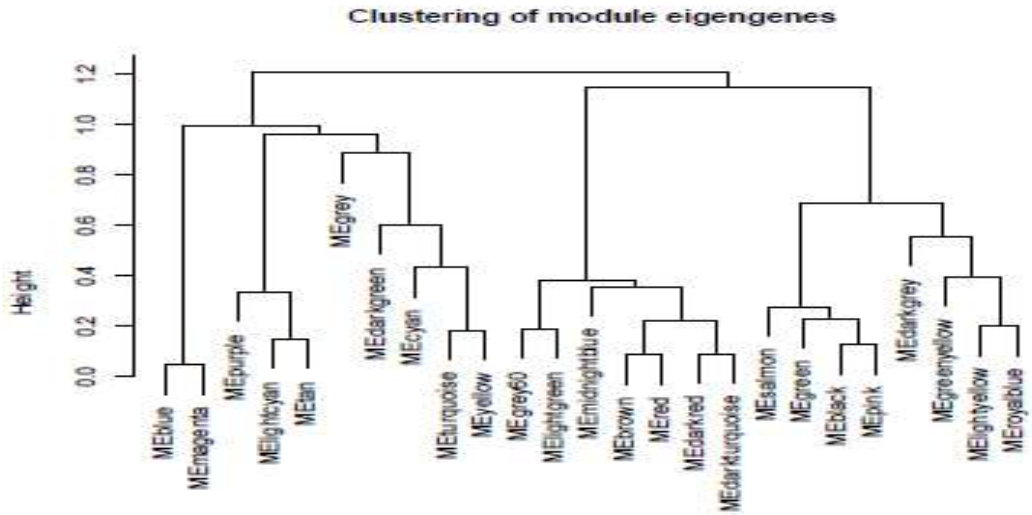


b

Agricultural Biotechnology Journal; Print ISSN: 2228-6705, Electronic ISSN: 2228-6500



c

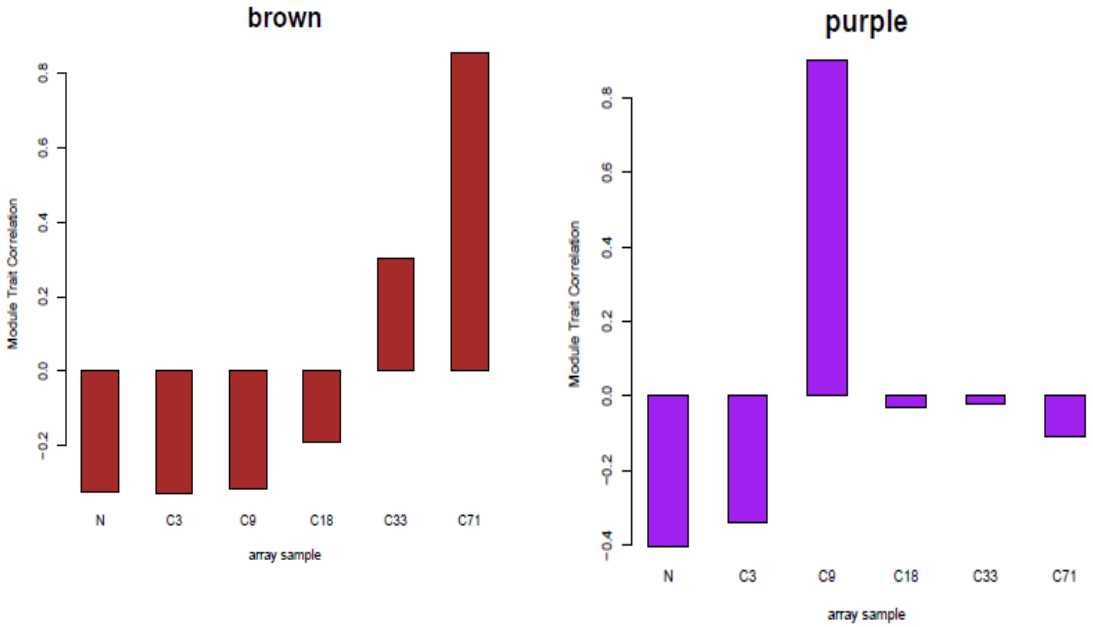


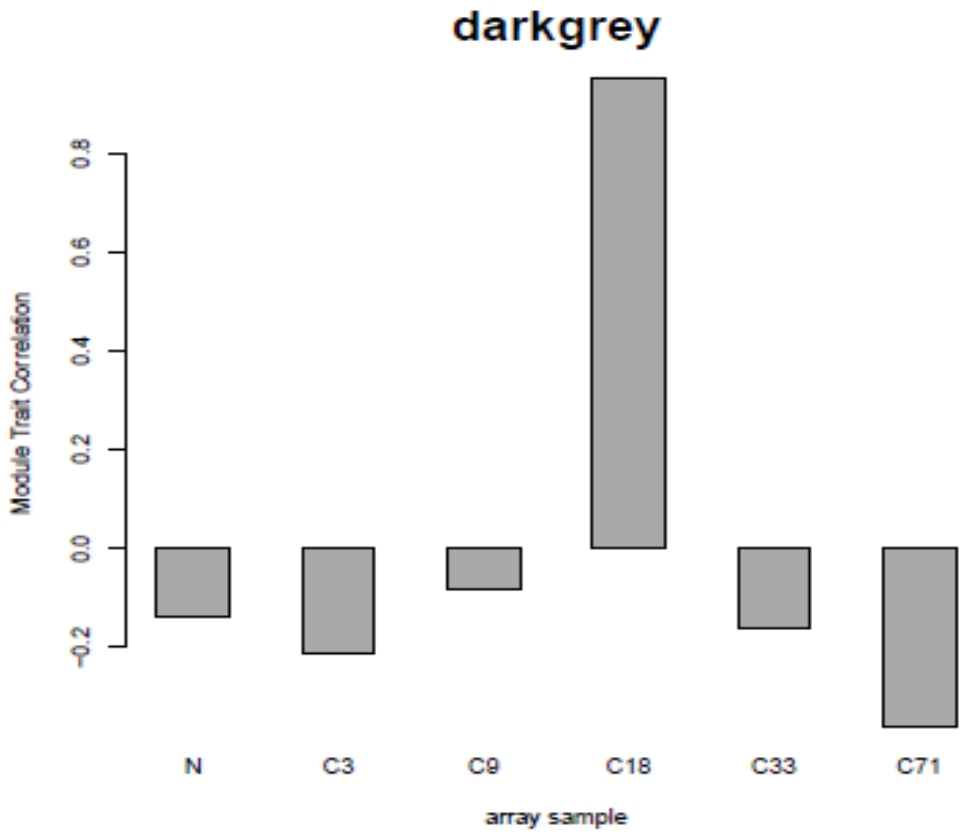
d

Figure 1. WGCNA of common DEGs in barley at germination stage. Module–sample association (a). The correlation between 17 modules and the germination stage (b). The hierarchical cluster tree shows 17 co-expression modules labeled by different colors based on the gene expression clustering results. Each row related to a module, is presented with a color as in (c). Clustering dendrograms of module eigengenes for identifying modules (d)

The gprofiler database was utilized to identify genes that control the transcription of module genes. Also, DEGs were divided into three significant modules by WGCNA analysis. Hub gene in MEgreenyellow was B3 domain-containing transcription factor ABI3, in MEred was U-box domain-containing protein 4, and in MEsalmone was alpha/beta-Hydrolases superfamily protein, which was positively correlated with germination stages. For seed germination, hormones such as gibberellin were activated which in turn activate the genes involved in seed germination. One such gene includes the Alpha/beta hydrolase (ABH) enzyme, a broad and functional group of proteins, involved in seed germination and plant growth due to their diverse biochemical activities (Davière and Achard 2013). ABH enzymes have catalytic activity in plants through selective changes in active site architecture and chemistry (Mindrebo et al. 2016).

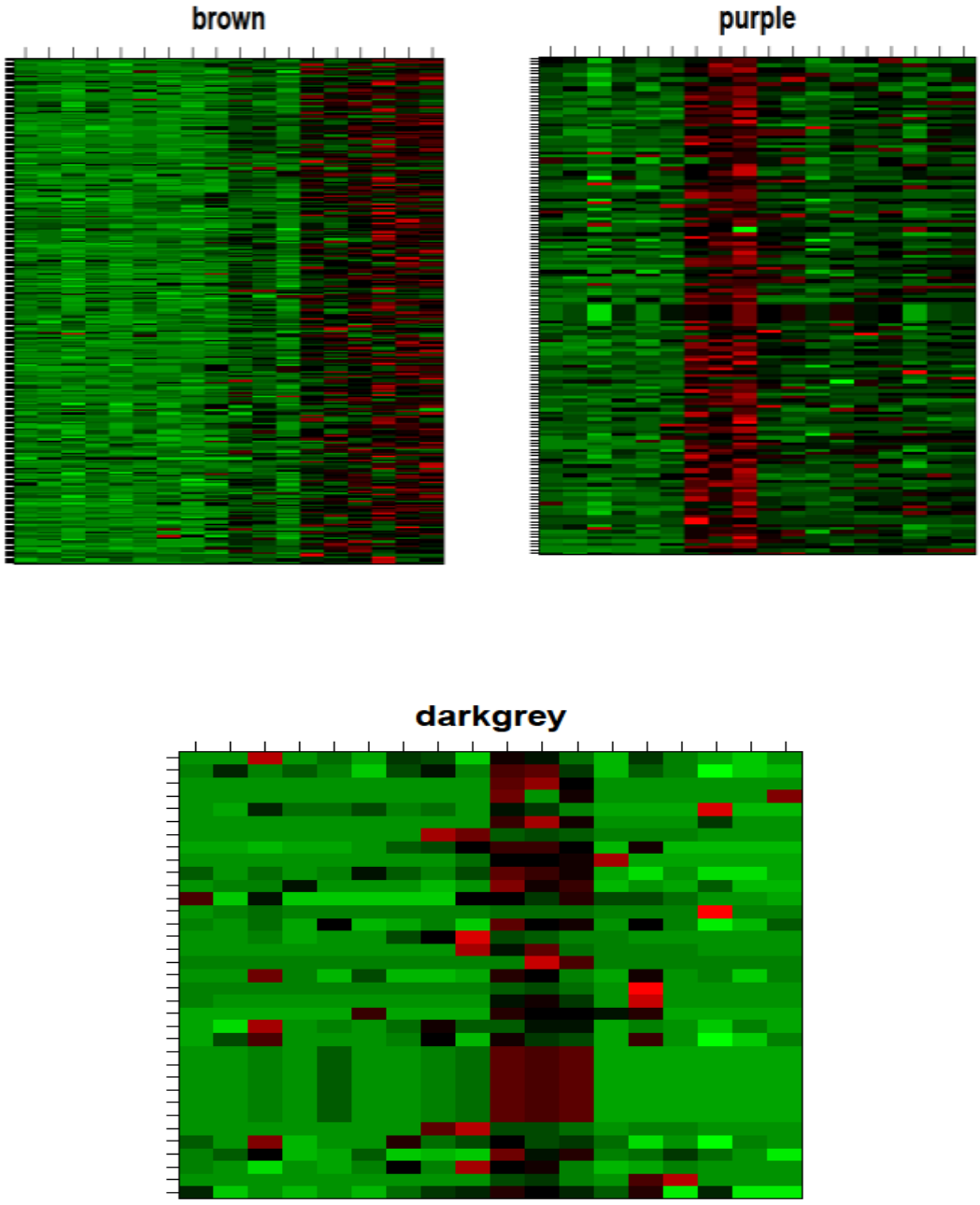
Protein-protein interaction (PPI) for germination stage: We analyzed the protein-protein interaction (PPI) network for genes involved in seed germination. Most of the genes were transcription factors (TFs), genes involved in glycolysis and starch degradation, signaling proteins (Ser/Thr protein kinase family), ATP-dependent DNA helicase, RING-type E3 ubiquitin transferase, and fructose-2,6-bisphosphate (Fru-2,6-bisP) (Figure 3). These results show that the degradation of starch is gradually reduced. As a result, the rapid decomposition of starch occurs mainly in the early stages of seed germination. Based on PPI, most the genes are involved in carbohydrate metabolism and glycolysis. A study showed that fructose-2,6-bisphosphate (Fru-2,6-bisP) is a marker in the germination stage of rice (*Oryza sativa*) before breaking the seed coat. During seed germination, fructose accumulation increased at the seed germination stage, when fructose was up-regulated significantly (Ling et al. 2005). Genes involved in the gene networks include ATP-dependent DNA helicase and glycosyltransferase 8. Glycosyltransferase has an important function in carbohydrate metabolism and important control point of photosynthate allocation (Saeedipour and Moradi 2011; Stein and Granot 2019; Wang et al. 2017). For the most part, eIF4A was not phosphorylated throughout most of the seed development and germination (Lee and Kang 2016). The RNA helicase is a diverse family in plants and its expression could be regulated in response to changes in specific environmental conditions such as oxygen levels, light, temperature, and salt stresses. During abiotic stress, OsRH58 can play a role in growth/development and plant vigor. In addition, it is essential to increase seed germination and plant growth under salt or drought stresses (Chi et al. 2012).





a

Agricultural Biotechnology Journal; Print ISSN: 2228-6705, Electronic ISSN: 2228-6500



b

Figure 2. (a) Relationship between the WGCNA modules (brown, purple, and dark grey) expression of the consistent eigengene across the samples in the modules. The heatmap and barplot of eigengene expression have the same samples. (b) Rows of the heatmap related to genes, columns to samples; red in the color key indicates overexpression, and the green represents under expression

Further, *AtRH57* was involved in ABA-dependent inhibition of germination and seedling development (Hsu et al. 2014). Another RNA helicase, *RH17*, plays an important role in plant tolerance under NaCl treatment during plant germination and at the seedling stage. ABHs are commonly associated with household gene maps that are involved in cellular protein breakdown and foreign biotoxicity (Canonne et al. 2011; Gershater and Edwards 2007). Phospholipases, many of which belong to the ABH plant family, are key to the production of chemical signals in cell membranes. Phospholipases are involved in chemical signals in cell membranes. Plants are also dependent on lipases as catalytic hubs in the biosynthesis of volatile jasmonic acid and phytohormones (Canonne et al. 2011).

Ubiquitin E3 ligases are of particular importance because they produce a substrate property that catalyzes the binding of ubiquitin to protein targets and can be classified into distinct families (RING, HECT, or U-box domains) (Dangl and Jones 2001; Thordal-Christensen 2003; Bonas and Lahaye 2012; Hajibarat et al. 2022; Nürnberger et al. 2004). There is a large extension of the U-box gene family in plants that may be attributed to biological processes related to plant life cycle and germination. Plant U-box (PUB) proteins have been reported to be largely involved in abiotic and biological stress responses (Durrant and Dong 2004). To survive in stressful conditions, plants have used many defense strategies, often involving the rapid accumulation of abscisic acid (ABA), an important plant hormone that controls seed dormancy and guides seed maturation for optimal growth conditions.

During plant growth and maturation, ABA accumulation can protect plants from stress-induced damage (Finkelstein et al. 2002). Even though some aspects of ABA signaling are clear, the involvement of ubiquitin E3 ligands, particularly the U-box, helps us gain more insight into the entire ABA signal transmission network. AtPUB9 regulates the transcription factor ABI3 and increases ABA susceptibility during seed germination (Liu and Stone 2011). By using a hierarchical dendrogram, WGCNA showed that all after seed germination expressed genes in the three modules at 9h, 18h, and 71h samples are given in Figure 2. Bar graphs revealed the expression levels of the module eigengenes in various samples. The samples were taken under the normal conditions with three biological replicates. Using WGCNA analysis, three modules (MEdarkgrey, MEpurple, and MEbrown) had a positive correlation with the germination stage. The modules were significantly enriched in carbon metabolism, glycolysis, and gluconeogenesis pathways. The correlation between module eigengene and seed germination was performed using Module-trait associations. Network imaging for modules was drawn using the string. Auxin was also involved in seed germination. One study showed that auxin was linked to a NAC-mediated signaling cascade during seed germination via the IAA30 gene (Park et al. 2011; Saidi et al. 2021).

Agricultural Biotechnology Journal; Print ISSN: 2228-6705, Electronic ISSN: 2228-6500

The results of a study indicated that ClNAC68 positively controls seed germination and root elongation by increasing the free IAA content (Opassiri et al. 2006; Wang et al. 2021). The glycolysis energy pathway is essential for the supply of ATP and NADH in plant cells, but also has a wide range of other physiological functions (Zhang et al. 2005). In seeds, extensive studies have been performed on regulatory networks involved in seed germination, with genes of the B3 TF family being identified as the main regulators at this stage (Carbonero et al. 2017).

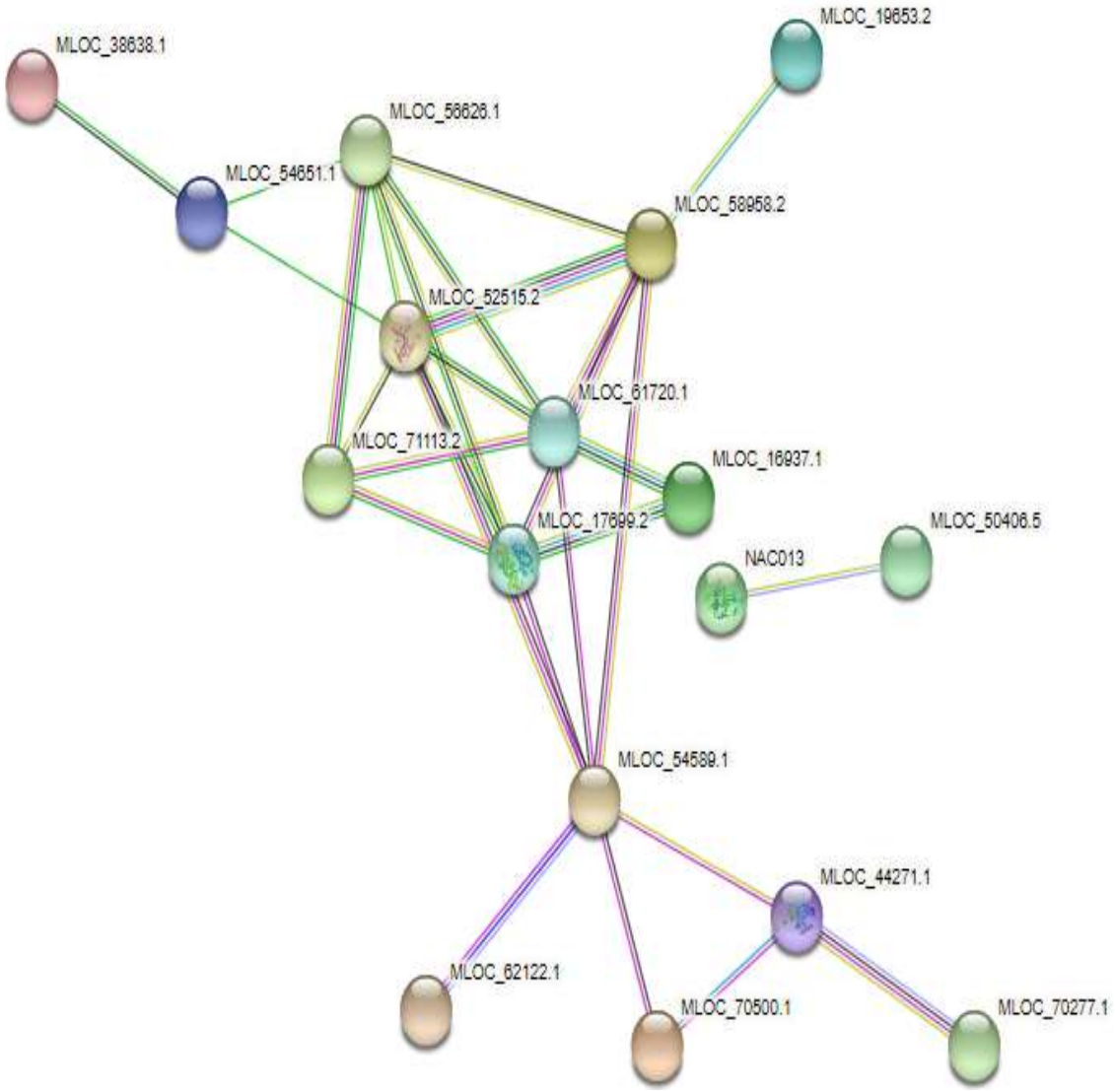


Figure 3. The protein-protein interaction (PPI) network in barley was constructed using the STRING database

Expression profiles analysis of Hub genes at germination stages: In Mahtab cultivar, the expression levels of *B3, AHB hydrolase, and U-box* genes were determined at 9h, 18h, and 71 hours after germination, most showing a wide range of expression. To evaluate the expression pattern of hub genes under the germination stage, reverse transcription-PCR (qRT-PCR) analysis was used. The alpha/beta-Hydrolases superfamily protein, *U-box* domain-containing protein 4, and B3 domain-containing transcription factor ABI3 hub genes were expressed at the germination stage. The *B3* gene had low expression at 9 and 18 h, but was up-regulated at 71h after seed germination. The *U-box* gene had a low expression at 9 and 71h whereas, it was up-regulated at 18h after seed germination. Further, the *ABH hydrolase* gene had a high expression at 9h after seed germination, but it showed low expression at 18 and 71h after seed germination (Figure 4a-c).

Based on qRT-PCR, the hub genes were expressed at 9h, 18h, and 71h after germination. Most of the genes involved in co-expression network analysis included transcription factors and signaling proteins, involved in carbohydrate metabolism and glycolysis. The *U-box, B3,* and *ABH hydrolase* genes were increased during seed germination. Therefore, we can propose that the *U-box, B3,* and *ABH hydrolase* genes can be used as molecular markers in barley germination. The two *U-box* proteins, which are regulated by the phytohormone ABA, contain *AtPUB18* and *AtPUB19.* Both *AtPUB18* and *19* are homologous and modulate ABA signaling in harmony during germination (Bergler and Hoth 2011). The expression of *U-boxes* is reduced during seed germination at high ABA concentrations and also negatively regulates cell death in seed germination processes (Thordal-Christensen 2003). The seed endosperm, made available to the fetus through the activity of certain hydrolase enzymes, is made up of the starchy part of the seed and the surrounding aleurone. The Gibberellins stimulate the synthesis and production of hydrolase, resulting in seed germination (Yamaguchi 2008).

Prediction of cis-elements for hub genes: The PlantCARE database was used to examine the responsive *cis*-elements of the hub genes at the germination stage. The outcomes revealed that there are different *cis*-elements in the promoter regions of the three genes, involved in response to environmental and hormonal conditions (Figure 5). Most of the cis-elements in the promoter region of the *B3* gene were involved in response to various hormones such as salicylic acid, auxin, and jasmonic acid. The *cis*-elements in the promoter regions of the u-box genes were associated with light, drought, and abscisic acid. Also in the hydrolase gene, most of the *cis*-elements were related to dryness, light, as well as stress-related *cis*-elements.

Agricultural Biotechnology Journal; Print ISSN: 2228-6705, Electronic ISSN: 2228-6500

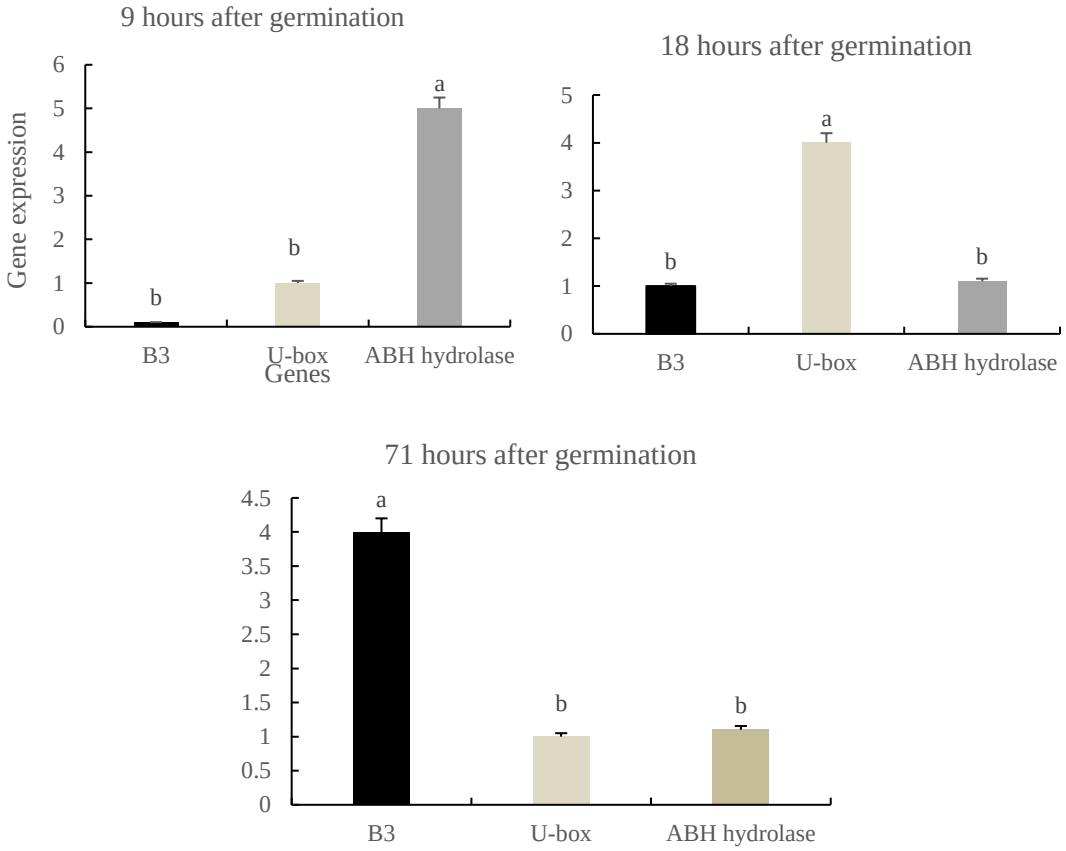


Figure 4. The qRT-PCR expression of Mahtab barley B3 domain-containing transcription factor ABI3, U-box domain-containing protein 4, and alpha/beta-Hydrolases superfamily protein genes of Mahtab cultivar for germination stage in 9h (a), 18h (b) and 71h (c) after germination. Bars with different lowercase letters are significantly different at $p < 0.05$. The significant variations between means were compared at $p < 0.05$ (Duncan's test)

Our findings indicated that the MYB elements can play a significant role in seed germination. This is probably due to the presence of cis-elements in the promoter regions of these genes. The MYBs play an important role in various processes such as hormonal signaling, metabolism, cell morphogenesis, and meristem formation. Next, we further analyzed the hormone-responsive elements in barley promoters. Our findings indicated that the *U-box* genes are regulated at seed germination as well as in many other important physiological processes in plants including reproductive development and stress response. Also, hydrolase plays a key role during the germination stage.

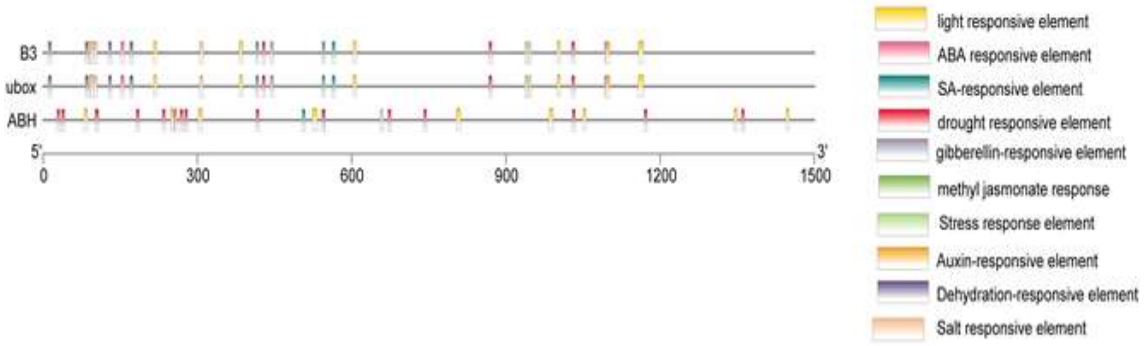


Figure 5. Cis-elements detected in the upstream of promoter regions and their frequencies in hub genes

Conclusion: Seed germination is highly related not only to the seedling's survival rate but also to its subsequent vegetative growth. To improve strong plant cultivars with rapid and uniform field emergence, it is important to know the genetic factors that contribute to proper germination and seedling growth performance. Because seed germination is a very complex trait, it is difficult to identify contributing genetic factors by conventional genetic or physiological analysis. We used weighted gene co-expression network analysis (WGCNA) to detect hub genes and network modules. In this research, we studied co-expressed network genes in barley at the germination stage. The genes encoding B3 domain-containing transcription factor ABI3, U-box domain-containing protein 4, and alpha/beta-Hydrolases superfamily protein were positively correlated with the germination stage. Signaling proteins such as protein kinases and ABHs play key roles in the regulation of hormone signaling, seed development, and seed size. Among surveyed DEGs, transcription factors such as B3 have important roles in the germination stage and growth and development. Protein-protein interaction (PPI) data is a direct source of information on the structure of signaling pathways during the growth period of the plants such as seed germination. The Ser/Thr protein kinase family and Glyceraldehyde-3-phosphate dehydrogenase, fructose-bisphosphate aldolase family, S-adenosylmethionine synthase 3, RING-type E3 ubiquitin transferase, peptidyl-prolyl cis-trans isomerase, and NAC transcription factors were shown to be involved in network analysis and co-expressed at seed germination. The results provide broad information on the genes involved at different germination stages.

Acknowledgments: The authors are thankful to the Department of Plant Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran for receiving the necessary help for this project work.

References

Asakura T, Tamura T, Terauchi K, et al. (2012) Global gene expression profiles in developing soybean seeds. PPB 52,147–153.

Bakhtiarizadeh MR, Hosseinpour B, Shahhoseini M, et al. (2018) Weighted gene co-expression network analysis of endometriosis and identification of functional modules associated with its main hallmarks. Front Genet 9, 453.

Bergler J, Hoth S (2011) Plant U-box armadillo repeat proteins AtPUB18 and AtPUB19 are involved in salt inhibition of germination in Arabidopsis. Plant Biol 13(5), 725-30.

Bonas U, Lahaye T (2002) Plant disease resistance triggered by pathogen-derived molecules: refined models of specific recognition. Curr Opin Microbiol 5(1), 44–50.

Canonne J, Froidure-Nicolas S, Rivas S (2011) Phospholipases in action during plant defense signaling. Plant Signal Behav 6(1), 13–18.

Carbonero P, Iglesias-Fernández R, Vicente-Carbajosa J (2017) The AFL subfamily of B3 transcription factors: evolution and function in angiosperm seeds. JXB 68(4), 871-80.

Chi W, He B, Mao, J et al. (2012) The function of RH22, a DEAD RNA helicase, in the biogenesis of the 50S ribosomal subunits of *Arabidopsis* chloroplasts. Plant Physiol 158(2), 693–707.

Dangl JL, Jones DGJ (2001) Plant pathogens and integrated defense responses to infection. Nature 411(6839), 826–833.

Davière J-M, Achard P (2013) Gibberellin signaling in plants. Development 140(6), 1147–1151.

Durrant WE, Dong X (2004) Systemic acquired resistance. Annu Rev Phytopathol 42(1),185–209.

Finkelstein R, Gampala S, Rock C (2002) Abscisic acid signaling in seeds and seedlings. Plant Cell 141(1), S15–S45

Gershater MC, Edwards R (2007) Regulating biological activity in plants with carboxylesterases. Plant Sci 173(6), 579–588.

Hajibarat Z, Saidi A, Zeinalabedini M, et al. (2022) Genome-wide identification of StU-box gene family and assessment of their expression in developmental stages of *Solanum tuberosum*. JGEB 20(1), 1-21.

Hsu YF, Chen YC, Hsiao YC, et al. (2014) A t RH 57, a DEAD-box RNA helicase, is involved in feedback inhibition of glucose-mediated abscisic acid accumulation during seedling development and additively affects pre-ribosomal RNA processing with high glucose. Plant J 77(1), 119-135.

Jing Y, Lin R (2020) Transcriptional regulatory network of the light signaling pathways. New Phytol 227(3), 683-97.

Jones SI, Gonzalez DO, Vodkin LO (2010) Flux of transcript patterns during soybean seed development. BMC Genomics 11(1),136.

Jones SI, Vodkin LO (2013) Using RNA-Seq to profile soybean seed development from fertilization to maturity. PLoS One 8(3).e59270.

Kader JC (1996) Lipid-transfer proteins in plants. Ann Rev Plant Physiol Plant Mol Biol. 47, 627–654.

Kucera B, Cohn MA, Leubner-Metzger G (2005) Plant hormone interactions during seed dormancy release and germination. Seed Sci Res 15(4), 281–307.

Lee K, Kang H (2016) Emerging roles of RNA-binding proteins in plant growth, development, and stress responses. Mol Cells 39(3), 179–185. doi: 10.14348/molcells.2016.2359.

Li H, Li X, Wang G, et al. (2022) Analysis of gene expression in early seed germination of rice: landscape and genetic regulation. BMC plant Boil 22(1), 1-14.

Liew LC, Narsai R, Wang Y, et al. (2020) Temporal tissue-specific regulation of transcriptomes during barley (Hordeum vulgare) seed germination. Plant J 101(3),700-715.

Ling TF, Xuan W, Fan YR, et al. (2005) The effect of exogenous glucose, fructose, and NO donor sodium nitroprusside (SNP) on rice seed germination under salt stress. Zhi wu Sheng li yu fen zi Sheng wu xue xue bao= Physiol Mol Biol Plants 31(2), 205-12.

Liu H, Stone SL (2011) E3 ubiquitin ligases and abscisic acid signaling. Plant Signal. Behav 6, 344-348.

Livak K.J, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25(4), 402-408.

Mindrebo JT, Nartey CM, Seto Y, et al. (2016) Unveiling the functional diversity of the alpha/beta hydrolase superfamily in the plant kingdom. COSB 41, 233-246.

Nguyen HT, Silva JE, Podicheti R, et al. (2013) Camelina seed transcriptome: a tool for meal and oil improvement and translational research. Plant Biotechnol J 11(6),759–769.

Nürnberger T, Brunner F, Kemmerling B, Piater L (2004) Innate immunity in plants and animals: striking similarities and obvious differences. Immunol Rev 198(1), 249–266.

Opassiri R, Pomthong B, Onkoksoong T, et al. (2006) Analysis of rice glycosyl hydrolase family 1 and expression of Os4bglu12 β-glucosidase. BMC Plant Biol 6(1), 33–40.

Park J, Kim YS, Kim SG, et al. (2011) Integration of auxin and salt signals by the NAC transcription factor NTM2 during seed germination in Arabidopsis. Plant Physiol 156(2), 537-549.

Qu C, Zuo Z, Cao L, et al. (2019) Comprehensive dissection of transcript and metabolite shifts during seed germination and post-germination stages in poplar. BMC plant Boil 19(1), 1-5.

Rajjou L, Duval M, Gallardo K, et al. (2012) Seed germination and vigor. Annu Rev Plant Biol 63(507), 2012

Ruan YL (2014) Sucrose metabolism: gateway to diverse carbon use and sugar signaling. Annual Review of Plant Biol 65(1), 33–67

Saeedipour S, Moradi F (2011) Comparison of the drought stress responses of tolerant and sensitive wheat cultivars during grain filling: impact of invertase activity on carbon metabolism during kernel development. J Agric Sci 3(2), 32.

Saidi A, Hajibarat Z, Hajibarat Z (2021) Phylogeny, gene structure and GATA genes expression in different tissues of Solanaceae species. Biocatal Agric Biotechnol *35*,102015.

Stein O, Granot D (2019) An overview of sucrose synthases in plants. Front Plant Sci 8, 10:95.

Stotz HU, Thomson J, Wang Y (2009) Plant defensins. Plant Signal Behav 4(11), 1010–1012.

Thordal-Christensen H (2003) Fresh insights into processes of nonhost resistance. Curr Opin Plant Biol 6(4), 351–357.

Van Dam S, Vosa U, van der Graaf A, et al. (2018) Gene co-expression analysis for functional classification and gene-disease predictions. Briefings in bioinformatics. 19(4), 575-592.

Wang J, Liu S, Li C, et al. (2017) PnLRR-RLK27, a novel leucine-rich repeats receptor-like protein kinase from the Antarctic moss Pohlia nutans, positively regulates salinity and oxidation-stress tolerance. PLoS One 12(2), e0172869.

Wang J, Wang Y, Zhang J, et al. (2021) The NAC transcription factor ClNAC68 positively regulates sugar content and seed development in watermelon by repressing ClINV and ClGH3. 6. Hortic Res 8(1), 1-1.

Yamaguchi S (2008) Gibberellin metabolism and its regulation. Ann Rev Plant Biol 59, 225-251.

Zhang X, Garreton V, Chua NH (2005) The AIP2 E3 ligase acts as a novel negative regulator of ABA signaling by promoting ABI3 degradation. Genes Dev 19(13), 1532–1543.

مجله بیوتکنولوژی کشاورزی
شاپا چاپی: ۲۲۲۸-۶۷۰۵ شاپا الکترونیکی: ۲۲۲۸-۶۵۰۰

آنالیز شبکه هم‌بیان ژن وزنی (WGCNA) در جو و بیان ژن های هاب درگیر در مرحله جوانه‌زنی

زهره حاجی برات

دانشجوی دکتری، گروه علوم و زیستفناوری گیاهی، دانشکده علوم و فناوری زیستی ، دانشگاه شهید بهشتی، تهران، ایران. رایانامه: zohreh.hajbarat@yahoo.com

عباس سعیدی

*نویسنده مسئول: استاد، گروه علوم و زیستفناوری گیاهی، دانشکده علوم و فناوری زیستی ، دانشگاه شهید بهشتی، تهران، ایران. رایانامه: abbas.saidi@gmail.com

محمدرضا غفاری

استادیار، پژوهشگاه بیوتکنولوژی کشاورزی (ABRII) ،سازمان تحقیقات، آموزش و ترویج کشاورزی، کرج، ایران. رایانامه: ghaffari@abrii.ac.ir

مهرشاد زین العابدینی

استادیار، پژوهشگاه بیوتکنولوژی کشاورزی (ABRII) ،سازمان تحقیقات، آموزش و ترویج کشاورزی، کرج، ایران. رایانامه: mzeinolabedini@abrii.ac.ir

زهرا حاجی برات

دانشجوی دکتری، گروه علوم و زیستفناوری گیاهی، دانشکده علوم و فناوری زیستی ، دانشگاه شهید بهشتی، تهران، ایران. رایانامه: zahrahajibarat@yahoo.com

تاریخ دریافت: ۱۴۰۰/۱۱/۱۹ تاریخ دریافت فایل اصلاح شده نهایی: ۱۴۰۰/۱۲/۱۶ تاریخ پذیرش: ۱۴۰۰/۱۲/۱۷

چکیده

هدف: جوانه زدن بذر فرآیند مهمی است که شروع چرخه زندگی گیاه بذر را تعیین می کند. با این حال، مکانیسم مولکولی جوانه زنی بذر در جو نامشخص است. برای درک مکانیسم مولکولی جوانه زنی بذر در جو، از آنالیز WGCNA برای شناسایی هاب و ژن های پاسخگو و آشکارسازی بیان ژن ها در جوانه زنی بذر استفاده شد. WGCNA، ابزار ارزشمندی برای مطالعه همبستگی بین ژن ها، شناسایی ماژول های با همبستگی بالا و شناسایی ژن‌های Hub در ماژول‌های مختلف است.

مواد و روش‌ها: داده‌های خام ریزآرایه مربوط به مرحله جوانه زنی از پایگاه داده ریزآرایه GEO برای ۰ ساعت، ۳ ساعت، ۹ ساعت، ۱۸ ساعت، ۳۳ ساعت و ۷۱ ساعت پس از مرحله جوانه زنی به دست آمد. سپس از آنالیز شبکه هم‌بیان ژن وزنی (WGCNA) برای تشخیص ژن‌های شبکه هم‌بیان شده استفاده شد. در تحقیق حاضر، از رقم جو مهتاب برای نشان دادن الگوی بیان پس از ۹ ساعت، ۱۸ و ۷۱ ساعت پس از مرحله جوانه‌زنی استفاده شد. تعداد کل ۴۱۳۷ ژن با بیان متفاوت (DEG) شناسایی شد که برخی از ژن‌ها بیان بیشتری را در مهتاب نشان دادند و سه ژن با qRT-PCR تأیید شدند.

نتایج: بیشتر این DEGها در فرآیندهای متابولیک، فرآیندهای سلولی، گلیکولیز و پاسخ به محرک نقش داشتند. ژن هاب در MEbrown پروتئین ابرخانواده آلفا/بتا هیدرولاز، MEارغوانی پروتئین ۴ حاوی دامنه U-box و MEdarkgrey فاکتور رونویسی حاوی دامنه B3 ABI3 بود که با بذرهای جوانه زده همبستگی مثبت داشت. نتایج یک پایگاه داده ریزآرایه و ژن‌های کاندید برای مطالعه بیشتر جو در مراحل جوانه زنی را نشان داد. علاوه بر این، DEGها توسط WGCNA به سه ماژول تقسیم شدند. در این مطالعه، ماژول‌های ژنی مرتبط با جوانه‌زنی بذر در طول جوانه‌زنی بذر جو شناسایی شدند. فاکتور رونویسی و آلفا/بتا هیدرولاز نقش مهمی در مرحله جوانه زنی داشتند. همچنین، ماژول‌های ژنی و ژن‌های هاب در ۹ ساعت، ۱۸ و ۷۱ ساعت پس از جوانه زنی شناسایی شدند. با توجه به کمبود اطلاعات در مورد نیازهای جوانه زنی بذر جو، این تحقیق به منظور بررسی مکانیسم‌های جوانه زنی بذر و همچنین ارزیابی ژن‌های هاب جهت بررسی مکانیسم مولکولی جوانه زنی بذر در جو انجام شد.

نتیجه‌گیری: نتایج مطالعه حا ضر بینش جدیدی را در مورد مکانی سم مولکولی زیربنایی جوانه زنی بذر جو ارائه می‌دهد. بر ا ساس تجزیه و تحلیل شبکه، فاکتورهای رونوی سی و پروتئین‌های یوبیکوئیتین در جوانه زنی نقش دا شتند. بی شتر ژن‌های مربوط به هر ماژول مربوط به پروتئین‌های درگیر در متابولیسم کربوهیدرات، گلیکولیز و تجزیه پروتئین بودند. نتایج بیان ژن ما می‌تواند به عنوان نشانگر مولکولی در ارقام جو در طول جوانه زنی بذر عمل کند. این یافته ها می‌تواند برای انتخاب کمک مولکولی و ا صلاح ژنوتیپ های جو سریع جوانه زن مناسب باشد.

کلیدواژه‌ها: WGCNA، فاکتورهای رونویسی، شبکه ژنی، مرحله جوانه‌زنی

نوع مقاله: پژوهشی.

استناد: حاجی برات زهره، سعیدی عباس، غفاری محمدرضا، زین العابدینی مهرشاد، حاجی برات زهرا (۱۴۰۱) آنالیز شبکه هم‌بیان ژن وزنی (WGCNA) در جو و بیان ژن های هاب درگیر در مرحله جوانه زنی. *مجله بیوتکنولوژی کشاورزی*، ۱۴(۴)، ۲۶۷-۲۴۷.

Publisher: Faculty of Agriculture and Technology Institute of Plant Production, Shahid Bahonar University of Kerman-Iranian Biotechnology Society.