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Full Length Research Paper

Wheat cold and light stress analysis based on the Arabidopsis homology protein-protein interaction (PPI) network

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Light and cold temperature are as two pivotal factors for wheat flowering. To investigate the mechanisms of wheat equipped to reduce cold temperature and light stress, we take advantage of the developed microarray technology. Our analysis revealed that 74 wheat homology genes significantly response to the stress. These genes included; FTSH10, AtCHR12, NF-YB2, HAP3b, GLT1, VHA-A and MPK6, most of which were proved to be involved in the response to cold or light stress. In addition, we identified that DNA replication, spliceosome and mismatch repair pathways were associated with cold and light stress in wheat.

Key words: Cold and light stress, wheat, protein-protein interaction (PPI) network.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is normally a long-day plant, flowering in spring and early summer when days are lengthening. Wheat cultivars can be broadly divided into two categories, winter or spring varieties, according to whether they require an extended period of cold to become competent to flowering. For winter varieties, changing from vegetative to reproductive phase is promoted by exposure to low temperatures for weeks, namely verbalization. Therefore, light and cold temperature are two pivotal factors for wheat flowering (Winfield et al., 2009).

When plant is exposed to unfavorable environmental factors, such as freezing temperature and high/weak lighting stress, they immediately activate signaling machinery and changing their physiological status in a defense mechanism. A lot of wheat genes have been isolated and characterized under cold or light stress, such as Wcor14, wlt10, Wcor15, psbD/C and TaVRN1 (Wada et al., 1994; Tsvetanov et al., 2000; Ohno et al., 2001; Takumi et al., 2003; Xu et al., 2010).

The genome of *Arabidopsis thaliana* has been sequenced and it is the model plant for investigating the response mechanism of plants under cold and light stress. Many genes are proved to be related to cold and light response. For example, UDP-glucose pyrophosphorylase (UGPase) is a key enzyme producing UDP-glucose, which is involved in an array of metabolic pathways concerned with the synthesis of sucrose and cellulose. UGP, an *A. thaliana* UGPase-encoding gene, was profoundly up-regulated by an exposure of plants to low temperature (Ciereszko et al., 2001). Several C2H2 zinc finger transcription factors such as AZF2, ZAT10 and ZAT12 were induced expression during UV-B and cold stress, which may play central roles in reactive oxygen and abiotic stress signaling in Arabidopsis (Kilian et al., 2007). Study also showed that light had a profound effect in increasing the amount of transcripts from so-called cold-responsive genes. Cold/light up-regulate as twice genes as the Cold/dark treatment, including iron superoxide dismutase (FeSOD) and glutathione-dependent hydrogen peroxide peroxidases (GPX), phenylpropanoids and photosynthesis-related carotenoids (Soitamo et al., 2008).

High-throughput gene expression analysis has become a frequent and powerful research tool in biology (Zimmermann et al., 2004). To identify potential genes

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Table 1. Growth condition for the time-course (Winfield et al., 2009).

Duration (days)	Weeks after germination	Temperature (°C)		Day-length (h)	PAR
		Day	Night		
21	3	16	14	14	280
7	5	14	10	12	185
7	9	8	4	9	95

PAR = Photosynthetically active radiation.

important to cold tolerance, global expression profile analysis was performed on plants subjected to stress treatments (Kreps et al., 2002). Without adequate data under stress for wheat, we mapped the wheat genes to Arabidopsis, and used the homology genes to investigate the mechanism of wheat under cold and light stress.

In this work, we performed a similar strategy to identify different genes that expressed in wheat cold or light stress. Furthermore, the relevant target genes and pathways were analyzed to depict underlying interaction mechanisms response to the stress.

MATERIALS AND METHODS

Transcriptome data

The transcription profile of cold and light stress -- GSE11774 (Winfield et al., 2009) was obtained from a public functional genomics data repository GEO (http://www.ncbi.nlm.nih.gov/geo/), which is based on the Affymetrix wheat genome array. The 3 or 5 week germination data are compared to any of the other one. All of the DEGs were collected (Table 1).

Protein interaction data

The Arabidopsis protein-protein interaction (PPI) data were collected from TAIR (Swarbreck et al., 2008), including 72267 pairs of interactions.

Microarray analysis

For the GSE11774 datasets, the limma method (Smyth, 2004) was used to identify differential expressed genes (DEGs). The original expression datasets from all conditions were processed into expression estimates using the RMA method with the default settings implemented in Bioconductor. The DEGs with the absolute fold change value larger than 2 and p-value less than 0.05 were selected.

Mapping the homology genes to arabidopsis

The wheat DEGs were mapped to Arabidopsis using BLASTn (Altschul et al., 1997) with e-value ≤ 1e-5.

GO and domain enrichment analysis

Gene ontology (GO) data and functional domain data were extracted using the DAVID (Huang et al., 2009). GO terms and domains with less than 2 genes were discarded. Over-represented groups of GO terms and functional domains were identified using a hypergeometric test, with a threshold of p-value ≤0.01. IntroPro domains analysis (Hunter et al., 2009) with the p-value ≤0.05 was to find the significant domains.

PPI network analysis

Using the PPI data collected from TAIR database, we matched the interactions between two homology genes. If the Pearson correlation coefficient (PCC) of any two genes were larger than 0.7, the two genes were kept for potential interactionship.

Pathway enrichment analysis

Many pathway analysis methods (Shen and Tseng, 2010) have been developed to identify enriched pathways. We used the DAVID to find the highly related pathways.

RESULTS

Microarray analysis

To identify genes under cold and stress in wheat, we compared 3 microarray groups each other with the different temperature and day-length. Total 1569 probes were detected differentially expressed. Then mapping these to the fasta sequences, total 513 unique sequences were selected. For further analysis the wheat genes, we used the BLAST to get the homology genes to Arabidopsis. At last, 74 homology genes were got.

GO enrichment analysis

Several GO categories were enriched in the PPI network, including response to temperature stimulus, heat and abiotic stimulus (Table 2 and Figure 1).

Domain enrichment analysis

For the Domain analysis with the p-value ≤0.05, most of genes enriched in SPX, Peroxidase, Dehydrin and so on (Figure 1).

Table 2. GO enrichment.

Category	Term	Count	P-value	FDR
BP	GO:0009266~response to temperature stimulus	12	1.51E-07	2.07E-04
BP	GO:0009408~response to heat	8	7.77E-07	0.001065
BP	GO:0009628~response to abiotic stimulus	17	1.47E-05	0.020184
BP	GO:0042542~response to hydrogen peroxide	6	1.11E-04	0.151649
BP	GO:0000302~response to reactive oxygen species	6	2.57E-04	0.351185
BP	GO:0010035~response to inorganic substance	10	3.05E-04	0.416636
BP	GO:0006979~response to oxidative stress	7	0.001064	1.446917
BP	GO:0009409~response to cold	6	0.002535	3.416081

BP: Biological process.

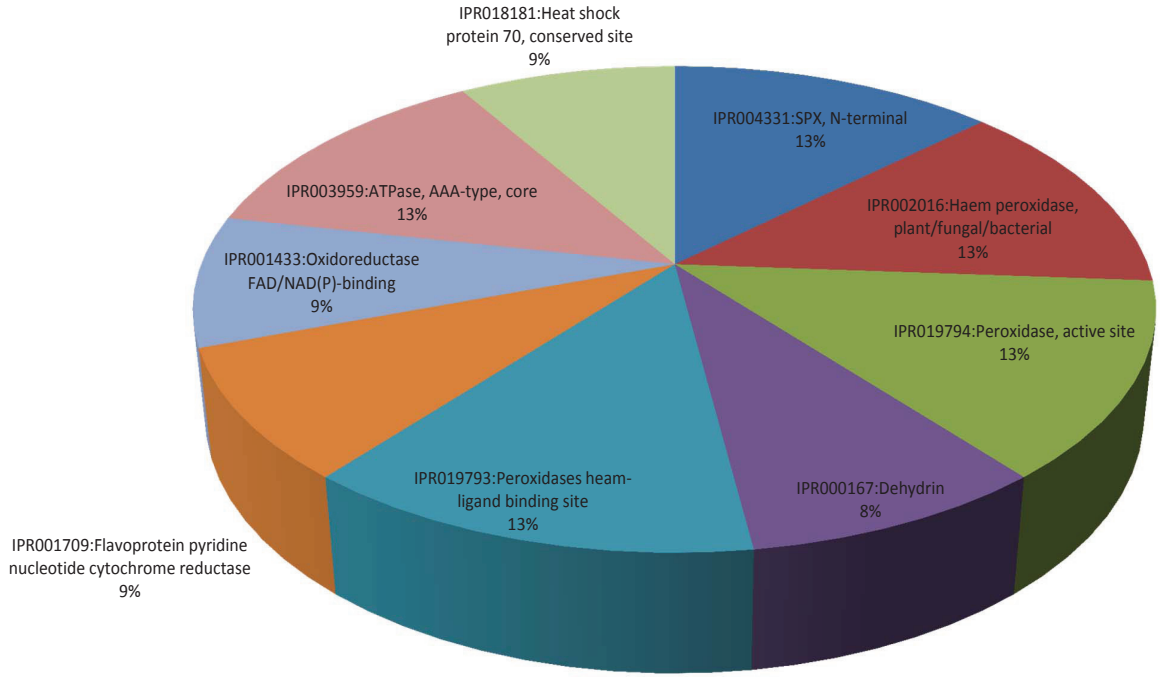

Figure 1. Domain enrichment of 74 target genes.

PPI network

PPI network (Figure 2) was constructed with the co-expressed value (PCC) ≥0.7 as the threshold. Finally, we got 38 PPI relationships between 39 DEGs and 10 related genes. FTSH10, AtCHR12, NF-YB2, GLT1, VHA-A, PIP28, CDKA1 and MPK6 are related to cold and light stress, directly or indirectly.

Pathway enrichment analysis

Mapping total 49 genes in the PPI network to pathway with the p-value less than 0.05, we found 3 pathways, including spliceosome, mismatch repair and DNA replication (Table 3).

DISCUSSION

We found that many targets and pathways have been linked closely related with cold or light stress. ATCHR12, FTSH10 and NF-YB2 are hub nodes in our PPIb network and they could interact with target genes to influence stress in direct or indirect manner.

FTSH10, a metalloprotease, belongs to the FtsH family. FtsH10 and FtsH3 form homo- and hetero-oligomeric m-AAA protease complexes assembling with prohibitins in the mitochondrial matrix, similar to their yeast counterparts. Most Arabidopsis thaliana plants of the

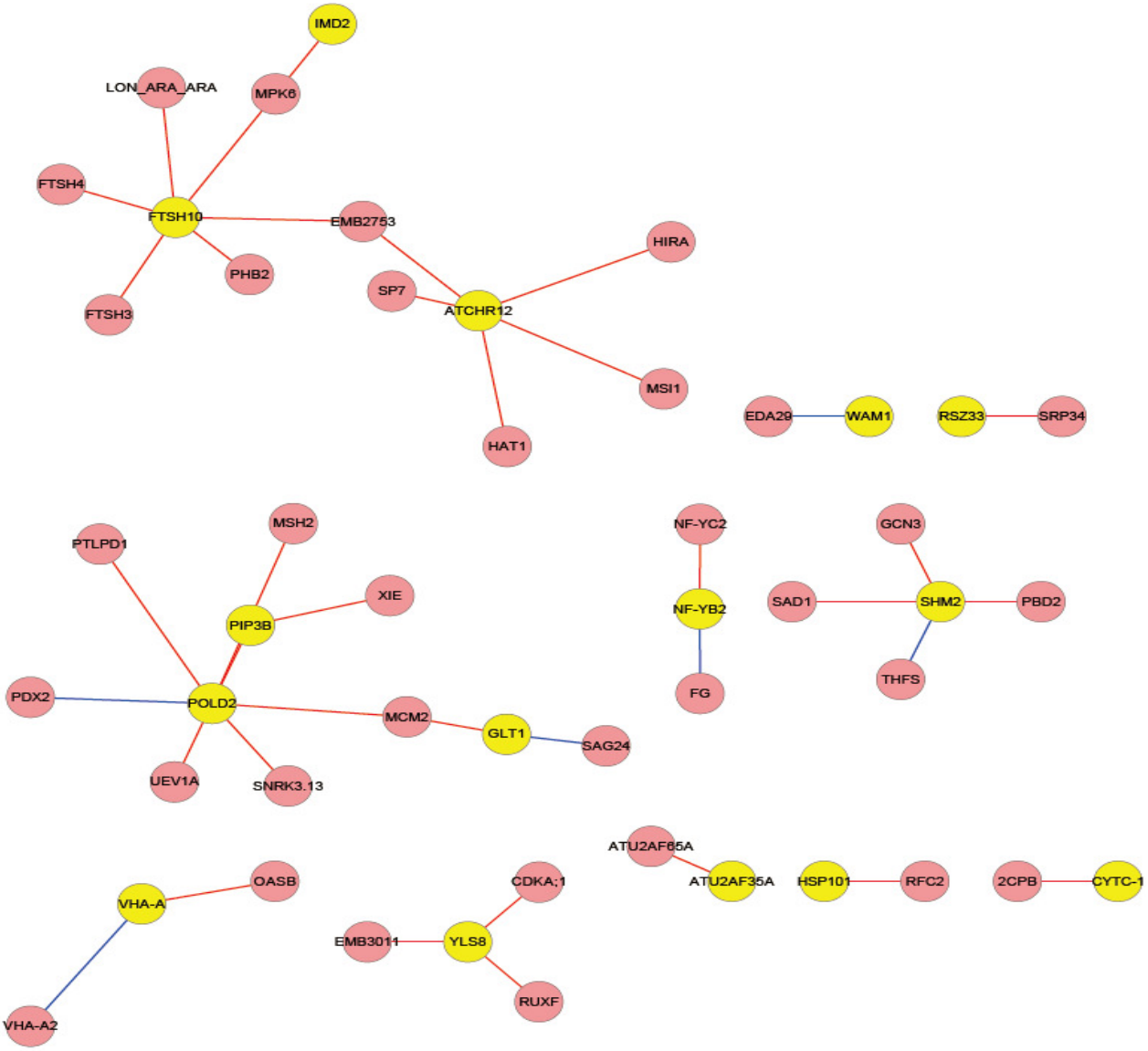

Figure 2. PPI network of wheat homology genes.

Table 3. Pathway enrichment.

Term	Count	P-value	FDR
ath03040:Spliceosome	5	0.009588	8.179763
ath03430:Mismatch repair	3	0.019549	16.04374
ath03030:DNA replication	3	0.03712	28.47003

homozygote knock-down mutant ΔFtsH10 displayed much stronger phenotypic divergence than control, when placed early into the field with higher intensity, with small rosettes and reduced seed production (Wagner et al., 2011).

AtCHR12 encodes a SNF2/Brahma-type ATPase. It can mediate temporary growth arrest in Arabidopsis uponperceiving environmental stress. High expression of AtDRM1-type genes is associated with dormant buds up-regulated by cold stress and phosphate starvation. Their expression in the atchr12 knockout was significantly reduced, suggesting that AtCHR12 may contribute to the

establishment of dormancy-like growth arrest in cold and light stress through regulating the expression of AtDRM1(Mlynarova et al., 2007).

NF-YB2 is a subunit of NF-Y complex, which is a cis-acting regulatory element found in all eukaryotic species and is present in the promoter regions of approximately 30% of genes. NF-YB2 is essential for the normal induction of flowering by long-days and act through regulation of the expression of flowering locus T (FT), suggesting NF-YB2 would decrease expression when in weak light stress (Kumimoto et al., 2008). In addition, plants with HAP3b over-expressed showed decreased survival rates while plants homozygous for the null allele hap3b showed an improved freezing tolerance compared to wild-type plants. This result demonstrated that HAP3b is a negative regulator in controlling response to cold stress (Liang, 2010).

GLT1 gene encodes a glucose translocator to mediate the export of glucose from the chloroplasts in starch breakdown process. Transcript profiles for key enzymes in the starch-degradation pathway in response to cold shock were determined in Arabidopsis leaves. The results showed that transcript levels of GLT1 showed statistically lower than control levels during 96 h of cold shock, which led to starch-dependent maltose accumulation, in turn protecting photosynthetic electron transport chain during freezing stress (Kaplan and Guy, 2005).

VHA-A gene encodes catalytic subunit A of the vacuolar ATP synthase. The vacuolar-type ATPase (V-ATPase) and the vacuolar H+ pyrophosphatase are electrogenic proton pumps at plant endomembrane that create the proton motive force required for secondary activated transport and metabolite accumulation during plant development and adaptation to a variety of adverse growth conditions, such as heavy metal exposure, cold, drought and salinity. The application of short-term cold stress induced a strong decrease in the amount of VHA transcripts in leaves and roots (Kluge et al., 2003).

PIP2-8, a plasma membrane intrinsic protein, belongs to one member of PIP2s water channel protein family. PIP2-8 was identified expression in cold stress, but PIP2-8 maintained the original expression level during the first 12 h or 1 day of cold treatment and their expression decreased to lower level afterward (Jang et al., 2004).

CDKA1 gene encodes A-type cyclin-dependent kinase. A-type CDKs are most closely related to the mammalian CDK1 and CDK2, contain the characteristic PSTAIRE amino acid sequence in their cyclin-binding domain, and play a role at both the G1-to-S and G2-to-M transition points. Study showed that Orica EL2 remarkably strongly induced in cold stress to bind with CDKA1 to arrest the cell cycle (Peres et al., 2007).

MPK6 encodes a MAP kinase and can be up-regulated by mitogen-activated protein kinase, such as MKK2, MKK3, MKK4, and MKK5 to involve in signaling pathway in response to different stress. One of them, MKK2-MPK6 cascades was indicated in cold stress signaling. Protein level of MPK6 remained constant throughout cold treatment, confirming that activation of MPK6 occurs primarily by posttranslational modification (Teige et al., 2004; Takahashi et al., 2007).

Post-transcriptional regulatory mechanism, such as pre-mRNA splicing, mRNA export and small RNA-directed mRNA degradation, play an important role in cold stress responses. The serine/arginine-rich (SR) proteins are part of the spliceosome and act as splicing regulators in eukaryotes. In Arabidopsis, cold stresses regulate the alternative splicing of pre-mRNAs of many SR genes, which might produce different isoforms of SR proteins with altered splicing functions under stress conditions (Chinnusamy et al., 2007).

There is evidence that cell cycle regulation play an important role in cold responses. Several members of the cyclin and CDK families, as well as members of the E2F/retinoblastoma-related (RBR) pathway, were down-regulated in cold stress. Specifically, an A-type cyclin (CYCA31) showed the largest down- regulation upon treatment, followed by CDKA11, CDKD1, DEL1, RBR22, and an E2F homolog. Additionally, two KRPs were strongly up-regulated in the leaf meristem, on average 8- and 14-fold, respectively. Taken together, these genes down-regulated expression inhibited cell cycle progression, namely influencing DNA replication in the leaf meristem at low night temperature (Rymen et al., 2007).

In conclusion, microarray analysis could be used as an effective avenue to explore the pathobiology mechanism of cold and light stress, based on the assumption that the cold and light stress is a contextual attribute of distinct patterns of interactions between multiple genes. The salient results of our study included many core target genes such as ATCHR12, FTSH10 and NF-YB2. These genes all were demonstrated associated with cold stress in previous reports. However, further detail experiments are still indispensable to confirm our conclusion.

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