

Divergent Cell Wall Remodelling in Maize Under Cold or Drought

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ABSTRACT

The plant cell wall is the first line of defense against environmental changes. We previously described that the maize B73 is a stalk-lodging resistant inbred that undergoes extensive modifications in general physiology and cell wall composition under drought. To extend these findings, we investigated cell wall responses under cold, which represents another major abiotic stress. We found that cold stress does not alter total lignin or cellulose contents but induces qualitative changes in cell wall architecture, including shifts in lignin composition and modifications in hemicellulose and pectin organization. Transcriptomic analyses revealed that cell wall genes are predominantly induced by cold stress, contrasting with the general repression previously observed under drought. Integration of cold and drought transcriptomic datasets revealed six co-expression patterns for cell wall genes, including genes with specific and shared patterns of response to both stresses. We classified the cell wall genes from each pattern according to their biological roles, and our results suggest that cold-triggered inductions enhance cell wall remodeling, while drought-triggered repressions promote energy conservation and growth-limiting strategies. Transcription factor enrichment identified potential regulators of cell wall genes upon one or both stresses. By integrating these predicted transcriptional regulatory networks with the cell wall genes expression alteration patterns, we propose that the cold-induced remodeling of the cell wall is driven by broad regulatory networks, whereas drought-induced resource conservation is controlled by a small number of central regulators.

Introduction

Plants face and adapt to environmental changes throughout their life cycle. Understanding how plants integrate these signals is crucial to improve stress tolerance and ensure agricultural sustainability. Among abiotic stressors, drought and cold are major limiting factors that affect plant growth. These stresses trigger a wide range of morphological, physiological, biochemical, and molecular changes (Bohnert et al., 1995; Krasensky and Jonak, 2012; Calderone et al., 2024; Arce et al., 2026), ultimately compromising crop productivity and global food security (Devot et al., 2023). Maize (*Zea mays* L.), one of the most

economically important crops worldwide, is highly sensitive to both drought and cold stresses, which significantly reduce its yield (Chen et al., 2024). In this context, the cell wall plays a crucial role in maintaining mechanical support under stress conditions, and both its composition and structure can be deeply affected by cold and drought (Rajashekar and Lafta, 1996; Zenda et al., 2019; Takahashi et al., 2021).

The plant cell wall is a dynamic structure divided into two main layers: a primary cell wall surrounding growing cells, and a secondary cell wall that is formed when the cell has ceased to grow and thickens its walls (Keegstra, 2010). The primary cell wall is composed mainly of cellulose, hemicellulose, pectins and structural proteins (Carpita and

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Gibeaut, 1993; Cosgrove, 2005). As a commelinid plant species, the main hemicellulose of maize is arabinoxylan, which can be partially substituted with glucuronic acid, producing glucuronarabinoxylans. Different hemicellulose chains can be crosslinked by different types of diferulates to form the matrix-like polysaccharidic structure (Hatfield et al., 2017). The synthesis of these cell wall polysaccharides depends on sugar activation through production of nucleotide diphosphate-sugars (NDPs), which serve as substrates for glycosyltransferases (GTs) for their assembly (Scheller and Ulvskov, 2010; Kleczkowski and Decker, 2015; Temple et al., 2016). Several studies have demonstrated that the activity of these enzymes is altered in response to drought and/or cold, indicating that cell wall biosynthesis is tightly connected to environmental sensing and adaptation (Chen et al., 2005; Tenhaken, 2015).

Both structural and non-structural cell wall proteins, including arabinogalactan proteins, extensins, expansins, carbohydrate-remodelling enzymes, class III peroxidases, lacases, and others, play important roles in shaping cell wall architecture and mechanics. They are also involved in cell expansion and division, wall reinforcement, and resistance to various biotic and abiotic stresses (Cosgrove et al., 2002; Lamport et al., 2011; Ma and Johnson, 2023). Similarly, glycosylphosphatidylinositol-anchored proteins (GPIs) also contribute to cell wall organization by directing polysaccharides into their proper orientations and participate in stress signaling cascades (Yeats et al., 2018; Beihammer et al., 2020).

In addition, secondary cell walls contain lignin, a phenolic polymer synthesized through the phenylpropanoid pathway, which in grasses such as maize is closely associated with hydroxycinnamates and can be crosslinked to arabinoxylans through ferulates (Hatfield et al., 2017; Mnich et al., 2020; Peracchi et al., 2024). Lignin is composed of three main monolignols, *p*-hydroxyphenyl, guaiacyl and syringyl, that are transported to the cell wall and, by the action of peroxidases and lacases, are polymerized giving rise to the H, G and S subunits, respectively. These compounds determine the lignin structure, crosslinking capacity and cell wall properties (Gray et al., 2012). Thus, lignin enhances mechanical strength, stress resistance, and water transport efficiency (Boerjan et al., 2003; Weng and Chapple, 2010). Upstream intermediates of the phenylpropanoids pathway can also be directed into the synthesis of flavonoids, which are known to accumulate in response to both biotic and abiotic stresses, including water stress and low temperatures (Chalker-Scott, 1999; Ortiz and Sansinenea, 2023).

Several transcription factors (TFs) belonging to different families have been identified as key regulators of cold and drought responses. Among them, several dehydration-responsive element-binding protein/C-repeat binding factors (DREB1/CBF) genes and ethylene response factors (ERF) have been associated with cold and drought responses (Liu et al., 1998; Nguyen et al., 2009; Zhao et al., 2016). Moreover, TFs belonging to the basic Helix-Loop-Helix (bHLH), MYB and Cys2/His2 (C2H2) zinc finger families modulate CBF genes expression (Fedotova et al., 2017; Zhang et al., 2024). TFs from these families have been associated with the regulation of cell wall genes (CWGs) upon drought, suggesting that they contribute to the regulation of cell walls biogenesis under environmental changes (Cao et al., 2021b; Cao et al., 2021a; Calderone et al., 2024).

In a previous study, we identified the maize B73 as a stalk-lodging resistant inbred (Manga-Robles et al., 2021). Later, biochemical and transcriptomic studies revealed that B73 is sensitive to drought, suffering major changes in cell wall composition, transcriptional regulation and proteome alterations (Calderone et al., 2024; Arce et al.,

2026). In this work, we have analyzed the effect of cold in the B73 cell wall by integrating biochemical and transcriptomic approaches. In addition, we used weighted gene correlation network analysis to compare this cold transcriptome with our previously published drought transcriptome (Calderone et al., 2024). We identified four sets of cell wall genes (CWGs) whose expression is specifically affected only by one stress, as well as two sets of CWGs that are affected by both stresses in a similar manner. To further dissect the basis of these responses, we inferred behavior-specific transcriptional regulatory networks for the different sets of CWGs. Thus, we identified TFs potentially controlling CWGs expression upon cold and/or drought conditions. In sum, these results allow us to dissect the response of CWGs upon drought and cold, contributing to a better understanding of how cell walls face different environmental changes in maize.

Results

Effect of cold on cell wall remodeling in B73 maize plants

Maize B73 plants subjected to 16 °C are smaller than control plants in the same developmental stage (Figure 1A), and leaves present a red coloration, suggesting an accumulation of anthocyanins (Figure 1B). In line with the phenotypical observation, the content of both anthocyanins and flavanols is increased in the plants grown in cold conditions (Figure 1C). We determined the lignin and polysaccharides content to study the impact of cold on cell wall composition. These studies revealed that cold does not affect lignin content but changes its composition, reducing the H monomers and increasing the S monomers, resulting in an increased S/G ratio (Figure 2). Although the lignin content is not affected, the phenylpropanoid flux towards the production of flavonoids is enhanced by cold. In addition, cell walls of plants subjected to cold display higher levels of esterified ferulates (FA) compared to control plants, while the *p*-coumarates (pCA) levels remain unchanged with respect control plants (Figure 3). Therefore, the FA/pCA ratio is increased in plants subjected to cold. In addition, cold also increases the amount of total diferulates (DFA), including the cross-linked diferulates 5-5 DFA, 8-O-4 DFA and 8-5bf DFA.

Regarding cell wall polysaccharides, cellulose content was not significantly affected by cold (Figure 4A). Likewise, the monosaccharide composition of hemicelluloses was unchanged under cold conditions, with no significant differences detected in the levels of major monosaccharides xylose, arabinose, glucose and galactose (Figure 4A). Cell wall fractionation was also performed to determine whether cold affects polysaccharide distribution among the calcium-bridged pectins (CDTA fraction), loosely-crosslinked hemicelluloses (K-I fraction) and strongly-crosslinked hemicelluloses (K-II fraction) (Figure 4B). Although cold does not alter the total distribution of sugar on these three fractions, the amount of extractable loosely-crosslinked hemicelluloses in the K-I fraction is decreased upon cold. This stress also affects the uronic acids content and distribution, which are decreased in the CDTA fraction, and increased in the K-I and K-II fractions of the cell wall.

Finally, we characterized the fine-structural features such as methyl-esterification and substitution patterns by immunodetection assay (IDA) (Figure 5). In cold conditions, the cell walls of B73 have an increase in glucuronic acid-containing arabinogalactan proteins in the CDTA and K-II fractions (detected using the LM2 antibodies) (Figure 5). In addition, cold affects the degree of substitutions in xylan polysaccharides, especially in the CDTA and K-I fraction (LM10, LM11 and LM15 antibodies).

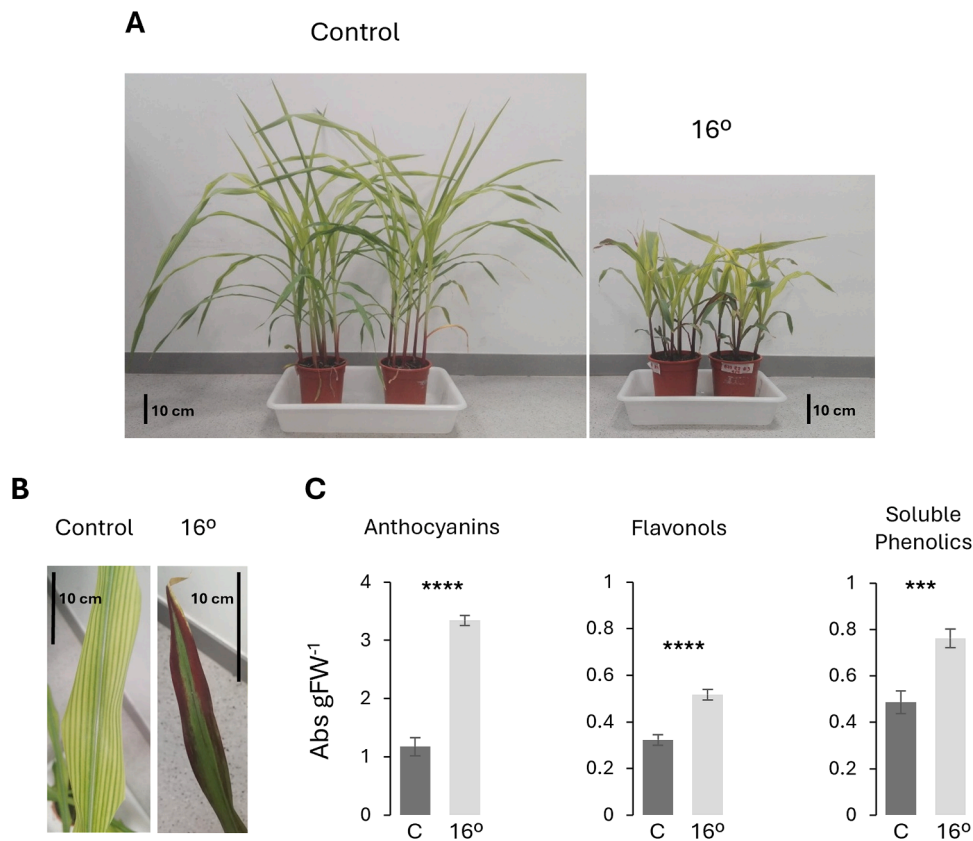


Figure 1. Macroscopic phenotype and phenolics contents of B73 grown in control and cold conditions. (A) 20 days-old maize plants grown in control (left) and cold (right) conditions. (B) First leaf of 20 days-old maize plants grown in control (left) and cold (right) conditions. (C) Relative quantification of anthocyanins, flavonols, and soluble phenolics. Total phenolics are presented as relative absorbance units normalized to fresh weight. “C” and “16°” refer to control and cold (16 °C) conditions, respectively. Samples were taken from 4 plants and data correspond to the mean of the values obtained $\pm$ SD. Asterisks refer to a significant difference of plants grown at 16 °C compared to control ones by Student t-test (**** $p \leq 0.0001$; *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$).

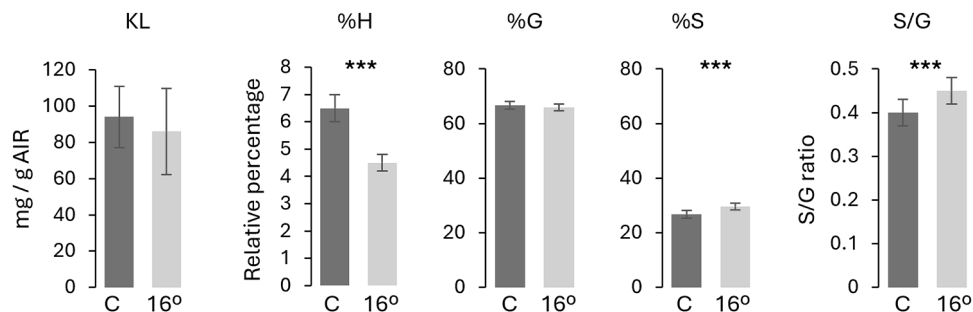


Figure 2. Lignin content and composition of B73 plants grown in control and cold (16 °C) conditions. Lignin content was determined by the Klason assay and the relative amounts of the three main subunits (H: p-Hydroxyphenyl, G: Guaiacyl, and S: Syringyl) was determined by thioacidolysis GC-MS. “KL” refers to Klason Lignin. “AIR” refers to Alcohol Insoluble Residue. “C” and “16°” refer to control and cold (16 °C) conditions, respectively. Data are presented as the mean $\pm$ SD of three biological replicates, each comprising the entire aerial part from 4-5 pooled plants, and three technical replicates. Asterisks refer to a significant difference of plants grown at 16 °C compared to control ones by Student t-test (*** $p \leq 0.001$).

Changes in cell wall genes expression in response to cold stress in B73

To extend the biochemical characterization of the cell wall, we investigated how cold stress affects the expression of the 1485 maize CWGs (Calderone et al., 2024). The transcriptomic analysis showed that the number of genes in the whole transcriptome affected by cold increases over time, 9% of them being induced and 7% repressed after 8 hours of exposure at 4 °C (Figure 6A top). The effect of cold on the induction of CWGs is similar to that observed in the full transcriptome, but the percentage of CWGs whose expression is repressed after 8 hours of cold is 4%, lower than the 7% observed in the full transcriptome. To gain insight into how the cell wall responds to cold stress, the 1485 CWGs

were grouped into four functional categories: (1) 540 CWGs involved in the synthesis of polysaccharides (SP), (2) 272 cell wall associated proteins (including those involved in suberin biosynthesis) (WP), (3) 341 CWGs coding for cell wall degrading enzymes (DE) and (4) 332 CWGs involved in the synthesis of lignin (SL). The percentage of CWGs whose expression is induced by cold ranged from 7% to 10% in the four categories, while those that are repressed ranged from 3% to 5% (Figure 6B).

To further investigate the behavior of the cell wall under cold stress, we classified the most affected gene families belonging to each functional category (Figure 6C). We found that 64 CWGs involved in the synthesis of polysaccharides (SP) (11.9%) were affected by cold. Among

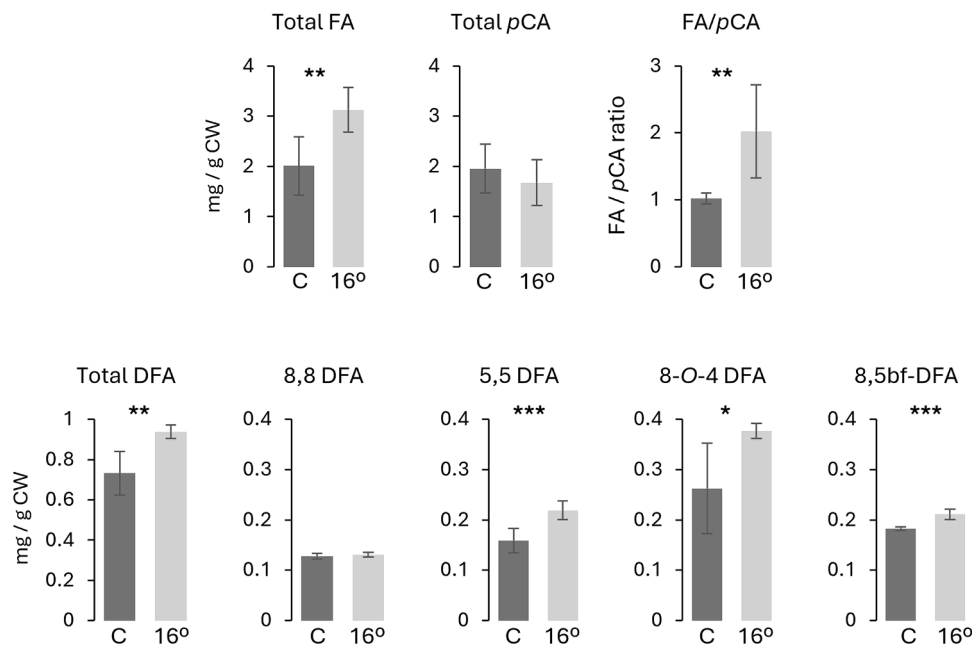


Figure 3. Cell wall hydroxycinnamate composition in B73 grown in control and cold conditions. The upper panel shows the determination of Ferulates (FA) and p-Coumarates (pCA), and the FA/pCA ratio in control and cold conditions. The bottom panel corresponds to the determination of total diferulates (DFA) in control and cold conditions, corresponding to 8,8 DFA, 5-5 DFA, 8-O-4 DFA and 8-5bf (benzofurans forms) DFA. “C” and “16°” refer to control and cold conditions, respectively. “CW” refers to Cell Wall. Data is presented as the mean $\pm$ SD of two biological replicates, each comprising the entire aerial part pooled from 5 plants, and three technical replicates. Asterisks refer to a significant difference of plants grown at 16 °C compared to control ones by Student t-test (***p $\leq$ 0.001; **p $\leq$ 0.01; *p $\leq$ 0.05).

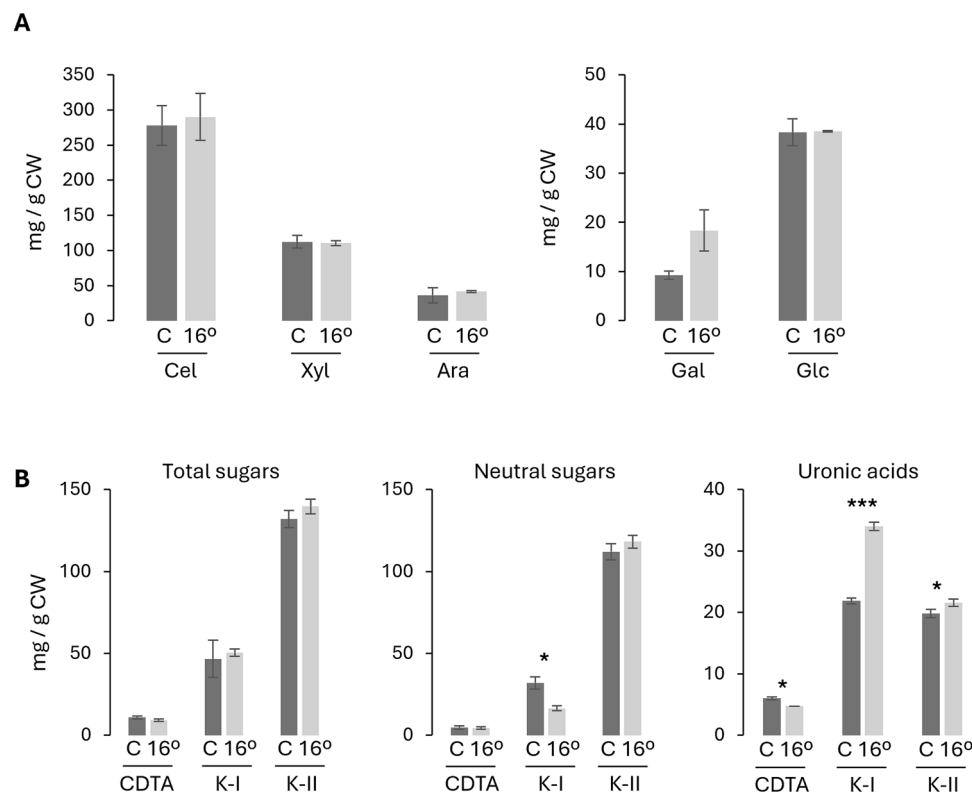


Figure 4. Cell wall polysaccharide determination in B73 grown in control and cold conditions. (A) Cell wall cellulose and hemicellulose composition of B73 grown in control (dark gray bars) and at 16 °C (light gray bars). “Cel” refers to Cellulose, “Xyl” refers to xylose, “Ara”, to arabinose, “Gal”, to galactose, “Glc”, to glucose. “CW” refers to Cell Wall. (B) Total sugar content in hemicellulose fractions from cell walls B73 grown in control and cold conditions. “CDTA” refers to Ca²⁺-bridged pectins extracted with CDTA. “K-I” refers to loosely-crosslinked hemicelluloses extracted with 0.1 N KOH-0.1 N. “K-II” refers to strongly-crosslinked hemicelluloses extracted with 4 N KOH. Data are presented as the mean $\pm$ SD of two biological replicates, each comprising the entire aerial part pooled from five plants, and three technical replicates. “C” and “16°” refer to control and cold conditions, respectively. Asterisks refer to a significant difference of plants grown at 16 °C compared to control ones by Student t-test (***p $\leq$ 0.001; **p $\leq$ 0.01; *p $\leq$ 0.05).

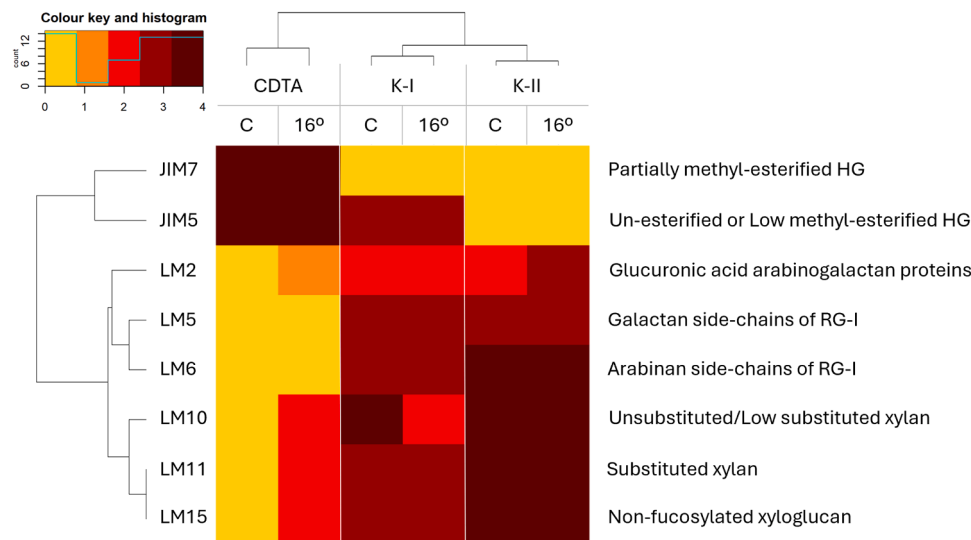


Figure 5. Heatmap of the immunodetection of cell wall epitopes. Analysis of the calcium-bridged pectins (CDTA), the loosely-crosslinked hemicelluloses (K-I), and the strongly-crosslinked hemicelluloses (K-II) cell wall fractions of B73 grown in control and cold conditions. “C” and “16°” refer to control and cold conditions, respectively. The commercial antibodies used are indicated on the left side of the heatmap. The epitope recognised by each antibody is shown on the right side of the heatmap. “HG” refers to homogalacturonan and “RG-I” refers to rhamnogalacturonan I. For details of antibodies used see [Calderone et al., 2024](#).

them, 56 CWGs are related to hemicellulose and pectins biosynthesis, including glycosyl transferases, cellulose synthase-like, acetyl-transferases and nucleotide-sugar interconversion and transporters proteins. In addition, the expression of 8 genes involved in the biosynthesis and organization of cellulose were also affected, corresponding to cellulose synthases and GPIs. The WP category has 35 CWGs (12.9%) affected by cold. In this category, several CWGs coding for protein glycosyl transferases were detected, as well as genes coding for membrane-bound and cell wall proteins, such as expansins, extensins, AGPs, receptor-like kinases (RLKs) and yieldins. Curiously, although cold predominantly induced CWGs, the family of extensins and RLKs contain more genes that are repressed than induced. The DE category has 45 CWGs (13.2%) that are affected by cold. Among them, 12 genes correspond to the glycosyl hydrolases, 8 genes to polygalacturonases and 7 genes to xyloglucan endotransglucosylase-hydrolases. Some genes coding for pectin methyl esterases, cysteine proteases and feruloyl esterases were also detected. Finally, the SL category has 51 CWGs (15.4%) affected by cold. Several genes involved in different steps of the phenylpropanoids pathway were affected, but the most abundant ones corresponded to the family of peroxidases: 8 genes corresponding to peroxidases involved in the radicalization of the sinapyl alcohol (S-peroxidases) and 9 genes to other types of peroxidases. Interestingly, these latest peroxidases group contain more genes repressed than induced, while all the S-peroxidases are induced by cold. This induction of all S-Peroxidases is in line with the increase in the lignin S subunit observed under long-term cold conditions ([Figure 2](#)). In addition to peroxidases, 9 genes coding for laccases were also detected.

Comparison of CWGs expression alteration upon drought and cold in B73

Since previous work demonstrated that CWGs in B73 inbred are highly responsive to drought ([Calderone et al., 2024](#)), we analyzed whether these CWGs can be grouped according to their expression patterns across the progression of both stresses independently. Thus, we integrated the transcriptomic data of 3, 5 and 8 hours of cold treatment (this study) and of 7, 9 and 10 days of drought conditions ([Calderone et al., 2024](#)). Principal component analysis (PCA) of the combined dataset revealed that all untreated plants are grouped together, with PC1 grouping plants subjected to drought and PC2 grouping plants subjected

to cold (Supplementary Figure S1). We then studied CWGs expression correlation between the two stresses by performing a weighted gene correlation network analysis (WGCNA). Out of the initial list of 1485 CWGs, we identified 331 presented co-expression patterns and were significantly correlated with these stresses, and were defined as the CWGs hub ([Table 1](#), Supplementary Table S2). These CWGs were grouped into six modules based on their behavior in response to cold or drought, and classified them as follows: the “Similar effect” modules (Magenta and Red) containing CWGs that are induced or repressed by both stresses, respectively; the “Only drought effect” modules (Green and Black) containing CWGs that are only induced or repressed by drought, respectively; and the “Only cold effect” modules (Brown and Pink) containing CWGs that are only induced or repressed by cold, respectively ([Table 1](#); [Figure 7](#); Supplementary Figure S2).

We then compared the impact of drought and cold in the different CWGs families ([Figure 8](#)). Concerning the CWGs involved in the “Synthesis of Polysaccharides” ([Figure 8A](#)), our results show that all CWGs families are predominantly repressed only by drought (Black module). However, a subset of genes, such as polysaccharide synthases, glycosyltransferases (involved in the glycosylation of celluloses, hemicelluloses and pectins) and GPIs are induced only by cold (Brown module) ([Table 2](#)).

CWGs belonging to the “Cell wall Degrading Enzymes” category ([Figure 8B](#)) are mainly repressed only by drought (Black module). This is the only functional category lacking CWGs that are only repressed by cold (Pink module). While some pectin, cellulose and hemicellulose-degrading enzymes are repressed by drought (Black module) and by both stresses (Red module), a proportion of them are also induced only by cold (Brown module) ([Table 3](#)).

With respect to the genes related to the “Cell Wall Associated Proteins” ([Figure 8C](#)), most structural proteins genes and a large portion of RLKs are repressed only by drought (Black module). However, in the case of RLKs, a considerable number of genes is also repressed by both stresses (Red), indicating a mixed response of this gene family. Concerning the expansins family, they are classified as α - and β -expansins ([Cosgrove, 2015](#)). In our results, β -expansins are mainly repressed only by drought (Black module), although a third of them is induced only by drought (Green). On the other hand, α -expansins are mainly repressed by both stresses (Red module). Glycosyltransferase families associated

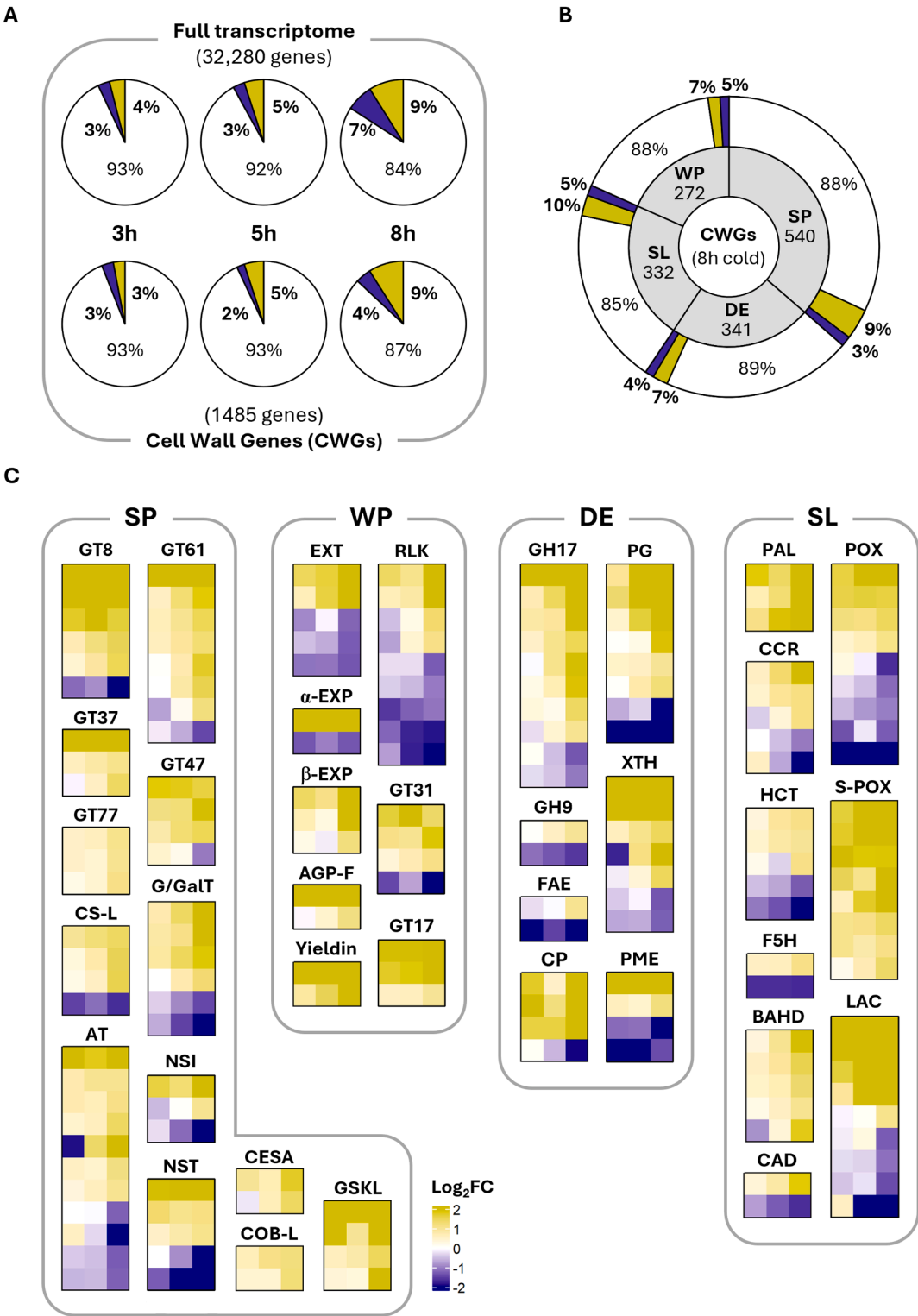


Figure 6. Transcriptomic response of maize B73 to cold (3, 5, and 8 h). (A) Upper pie charts correspond to the full transcriptome and bottom pie charts, to the cell wall genes (CWGs). Genes were considered induced when $\log_2FC \geq 1$ and repressed for $\log_2FC \leq -1$, with $p\text{-adj} < 0.05$. Inductions and repressions are represented in yellow and blue, respectively. White sections of the pie charts represent genes with unaltered expression (NA). The percentage for each case is shown in the pie chart. The full transcriptome consists of 32,280 genes, while the CWGs catalogue includes 1485 genes. (B) Sunburst chart representing the CWGs divided according to four functional categories: genes involved in the Synthesis of Polysaccharides (SP), genes related to the Cell Wall Associated Proteins (WP), genes coding for Cell Wall Degrading Enzymes (DE), and genes involved in the Synthesis of Lignin (SL). The number of CWGs of each category is shown under the name of the functional category, and the percentage of CWGs induced, repressed and not affected at 8 hours of cold in each functional category is also indicated. (C) Heatmap of CWGs of SP, SO, DE and SL functional categories at 3, 5 and 8 hours of cold stresses. Genes that are unique for a given family are not represented in the heatmap. The full names of the genes are shown in supplementary table S5. The RNA seq data containing the expression values is shown in Supplementary Table S1.

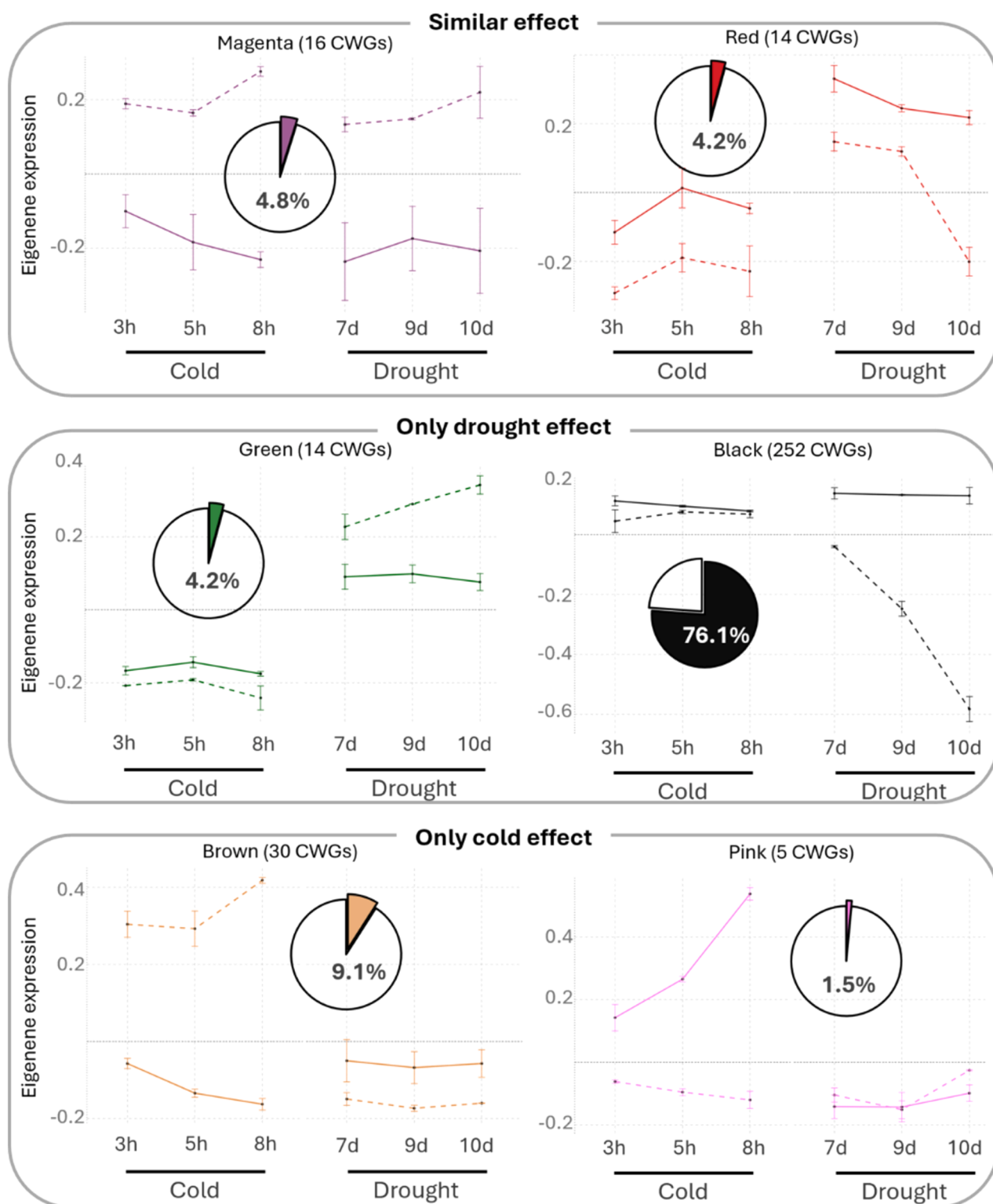


Figure 7. WGCNA modules for CWGs hub in cold and drought stresses. Differentially expressed CWGs (331 genes) were grouped into six different modules according to their expression patterns in response to cold and drought stresses. The six modules are divided into three conditions: “Similar effect” (top panel) corresponds to the Magenta and Red modules and contains CWGs that are induced (Magenta) or repressed (Red) by both stresses. “Only Drought effect” (middle panel) corresponds to the Green and Black modules and contains CWGs that are induced only upon drought (Green) or repressed only upon drought (Black). “Only cold effect” (bottom panel) corresponds to the Brown and Pink modules and includes CWGs that are induced only by cold (Brown) or repressed only by cold (Pink). The mean of eigengene expression (y axis) is plotted against the different stresses (in dashed lines) and controls (in solid lines) for the 3, 5 and 8 hours cold (left side), and 7, 9 and 10-days drought (right side). Error bars show the standard deviation between replicates for each condition and timepoint. The number of the CWGs that belong to each module is shown at the top of each graph. The pie chart of each module indicates the percentage of CWGs of the hub having the expression pattern of the module. Genes from each module are shown in Supplementary Table S2.

Table 1
Assignment of CWGs to WGCNA modules based on their co-expression patterns. The CWGs hub represents the 331 CWGs that have a module membership (MM) > 0.8 and gene significance (GS) > 0.2.

WGCNA CWGs co-expression modules					
Total list of CWGs					
1485					
CWGs with detectable gene expression					
1251					
CWGs hub assigned to WGCNA modules					
331					
CWGs with Similar effect		CWGs with Only drought effect		CWGs with Only cold effect	
Magenta	Red	Green	Black	Brown	Pink
16	14	14	252	30	5

with the glycosylation of cell wall structural proteins are divided into three main responses: only drought induced (Green module), only drought repressed (Black module) and only cold induced (Brown module); showing the most diverse behavior among all cell wall families (Table 4).

CWGs belonging to the “Synthesis of Lignin” category (Figure 8D) have diverse responses under drought and cold stresses, although a trend of progressive repression through the pathway can be seen in response to each stress. Enzymes from the early steps of the pathway (PAL, C4H and 4CL) are either repressed only by drought (Black module) or induced by both stresses (Magenta module). Genes involved in the synthesis of H, G and S subunits (CCR and CAD) are either repressed only by cold (Pink module) or induced by both stresses (Magenta module). Genes involved in the synthesis of G and S subunits (C3’H, HCT, CCoAOMT), and genes involved in the synthesis of S subunits (F5H and COMT) are mainly repressed only by drought (Black) or by both stresses (Red module). Lignin acyltransferases (BAHD) are exclusively repressed by drought (Black module), and the genes involved in lignin polymerization, such as peroxidases and laccases, are mainly repressed only by drought (Black module), although some are induced by both stresses (Magenta module). Interestingly, all peroxidases involved in the oxidation of sinapyl alcohol (S-peroxidases) that are affected by cold are induced, in agreement with the observed increase in S/G ratio upon cold (Table 5).

Identification of regulatory networks associated with the CWGs WGCNA modules

The different behavior of CWGs in response to drought or cold prompted us to search for transcription factors (TFs) that might be responsible for these expression differences. We obtained 12 clusters that included 43 TFs and 160 CWGs; however, cluster 5 was excluded due to its few interactions (2 TFs and 1 CWG). Therefore, we analyzed the 41 most significant TFs overrepresented 158 out of the 331 CWGs, distributed among 11 clusters (Figure 9x; Supplementary Tables 3.1 and 3.2). We have found that 7 of the 11 clusters grouped TFs with CWGs belonging to a same WGCNA module. Thus, clusters C-1 and C-2 grouped CWGs with a similar effect, clusters C-4, C-9, C-11, and C-12 grouped CWGs that are affected only by drought, and cluster C-6 grouped CWGs that are affected (induced) only by cold. Cluster C-8 also contains CWGs that are affected only by cold, but some of them are repressed and one is induced. Finally, clusters C-3, C-7, and C-11 grouped CWGs that are mainly affected (repressed) by drought, but with at least one CWG belonging to another WGCNA module.

We observed that the CWGs that are only affected (repressed) by drought form regulatory networks with a high CWGs-to-TFs ratio (from 5 to 32), with 13 TFs connecting 112 CWGs in 6 clusters (C-3, C-7, C-9, C-10, C-11 and C-12). These large clusters have major representation of MYB and EREB family members, together with NLP13, ABI19, VP1, bZIP23, THX25, and P1 (the full name of the TFs is described in

Supplementary Table S5). The CWGs belonging to the remaining five WGCNA modules form regulatory networks with a low CWGs-to-TFs ratio (around 2). In these cases, the TFs related to CWGs that are only induced by drought (C-4) include members of the bZIP, MADS-box, and MYB families, as well as OBF4 and ZAG5. The TFs related to CWGs that are affected only by cold (C-6 and C-8) include members of the AP2/ERF and TCP families and KN1, while the TFs related to CWGs affected by both stresses (C-1 and C-2) also include members of the AP2/ERF family, as well as members of the bHLH and WOX families (Table 6). A summary of the TFs forming regulatory networks with more than 10 cell wall genes, together with previous reports on their role is shown in Table 7.

Discussion

In this study, we examined the cell wall dynamics of maize under cold stress. This stress condition caused a reduction in H subunits of lignin, and an increase of the S/G ratio, indicating shifts in lignin monomer composition (Figure 2). In addition, we observed that cold stress caused an accumulation of ferulates (Figure 3). As ferulates act as anchorage points for lignin cross-linking (Hadj Bachir et al., 2022), our results suggest that the polysaccharide–lignin network is reinforced under cold stress. An increase in the S/G ratio together with an increase in ferulates has been observed in maize upon salt stress (Oliveira et al., 2020). Our results also showed that cold triggers higher accumulation of diferulates, which are well known to mediate the cross-linking among polysaccharide chains in grass cell walls (De and Buanafina, 2009). Thus, this result supports the idea that cold triggers the reinforcement of the cell wall polymers network.

We detected a cold-associated increase in uronic acids in the alkali-extractable K-I and K-II fractions of B73 cell walls (see Figure 4). This pattern suggests a redistribution or tighter association of acidic cell wall polymers under cold conditions. Consistently, the IDA heatmap revealed an increased presence of LM2-labelled AGPs epitopes, particularly in the CDTA and K-II fractions (Figure 5). AGPs are located at the plasma membrane–cell wall interface and have been implicated in plant responses to abiotic stress (Ayorinde et al., 2026). Our data agrees with previous results showing that cold induces the upregulation of specific AGPs, potentially due to a more suitable environment for cell–cell interaction and/or for local strengthening of the cell wall (Mareri et al., 2019). Additionally, the LM10 signal, which is associated with unsubstituted or low-substituted xylan, decreased in the K-I fraction under cold conditions, while substituted xylan epitopes remained abundant. This may indicate altered xylan accessibility, extractability or substitution status; however, increased glucuronoxylan substitution cannot be inferred directly from these data alone. As glucuronoxylan structure is known to influence cellulose microfibril organization and secondary wall architecture (Crowe et al., 2021), these results support the idea that cold stress promotes remodeling of acidic and hemicellulosic wall components that may contribute to cell wall reinforcement. Cold stress does not appear to trigger a broad quantitative change in cell wall polysaccharide composition, but rather a qualitative remodeling of polymer accessibility, extractability, and association among cell wall domains.

Although the characterization of cell wall changes requires prolonged exposure to cold conditions (10 days at 16°C), we analyzed the expression of cell wall genes following a short-term cold treatment (3-8 h at 4°C) to identify the early transcriptional responses triggered by this stress. The transcriptional state of plants exposed to 16°C for 10 days is not directly equivalent to that of plants subjected to 4°C for a few hours. However, our results allowed us to infer the initial molecular pathways and cell wall genes regulatory mechanisms activated upon cold, which may contribute to the long-term cell wall remodeling observed during cold acclimation. Cold mainly induced the expression of CWGs across all functional categories. This suggests a broad activation of cell wall remodeling mechanisms in response to cold (Figure 6B). This effect agrees with previous studies showing that cold responses often require a

Table 2

Distribution of CWGs involved in the Synthesis of Polysaccharides in the WGCNA modules. “C/HC” means “Cellulose and hemicellulose”, “GTs” means “Glycosyltransferases”, “ATs” means “Acetyltransferases”, and “GPIs” means “Glycosylphosphatidylinositol anchored proteins”. Magenta genes are induced by both stresses, red genes repressed by both stresses, green genes are induced only by drought, black genes are repressed only by drought, brown genes are induced only by cold, pink genes are repressed only by cold.

	Synthesis of Polysaccharides							Total
	C/HC GTs	Pectin GTs	Xylan ATs	Polysaccharide Synthases	Sugars Synthases	Sugars Transporters	GPIs	
Magenta	2	-	1	-	1	1	1	6
Red	1	-	-	-	-	1	-	2
Green	2	-	-	1	-	-	-	3
Black	14	18	12	18	14	11	5	92
Brown	4	5	-	3	-	1	3	16
Pink	1	-	-	-	-	-	-	1
Total	24	23	13	22	15	14	9	120

Table 3

Distribution of CWGs involved in Cell Wall Degrading in the WGCNA modules. “C/HC” means “Cellulose and hemicellulose”. Magenta genes are induced by both stresses, red genes repressed by both stresses, green genes are induced only by drought, black genes are repressed only by drought, brown genes are induced only by cold.

	Cell Wall Degrading Enzymes				
	C/HC Degrading	Pectin Degrading	Proteases	Suberin Degrading	Total
Magenta	1	-	-	-	1
Red	1	2	-	-	3
Green	-	-	1	-	1
Black	26	25	5	4	60
Brown	4	2	-	-	6
Total	32	29	6	4	71

more rapid activation of protective genes, particularly those involved in membrane and cell wall modifications, to counteract temperature-induced cellular damage (Krasensky and Jonak, 2012). In a previous

transcriptomic study, we showed that drought induces widespread gene expression changes in the B73 (Calderone et al., 2024). These changes included differential regulations of CWGs, which play a critical role in structural remodeling and adaptation to stress. To explore the singularities and commonalities of maize B73 response to either drought or cold, we combined the RNA-seq dataset from B73 maize plants exposed to cold stress with previously published transcriptomic data of B73 under drought (Calderone et al., 2024). Our new combined data revealed that 331 CWGs can be grouped into six co-expression modules based on their response to cold and/or drought (Figure 7, Supplementary Figure S2). The co-expression analysis demonstrates that drought and cold trigger distinct transcriptional programs targeting the cell wall. The dominance of drought-specific modules, mainly enriched in repressed CWGs, suggests that drought drives a more extensive shutdown of CWGs activity, likely as part of a deeper and broader growth-restrictive response. Although these patterns suggest stress-specific CWG regulation, differences in the duration or severity of the treatment may also contribute to the stronger repression observed under drought. In contrast, the less abundant cold-specific modules (mainly composed of induced CWGs) suggest a more selective, targeted

Table 4

Distribution of Cell Wall Associated Proteins in the WGCNA modules. “GTs” means “Glycosyltransferases”, “Exp” means “Expansins”, and “RLKs” means “Receptor-like kinases”. Magenta genes are induced by both stresses, red genes repressed by both stresses, green genes are induced only by drought, black genes are repressed only by drought, brown genes are induced only by cold, pink genes are repressed only by cold.

	Cell Wall Associated Proteins						Total
	Protein GTs	α-Exp	β-Exp	RLKs	Structural Proteins	Suberin Synthases	
Magenta	1	-	-	-	1	-	2
Red	-	2	1	3	1	-	7
Green	4	-	2	1	-	-	7
Black	6	1	7	11	25	3	53
Brown	3	-	-	1	2	-	6
Pink	1	-	-	1	-	-	2
Total	15	3	10	17	29	3	77

Table 5

Distribution of CWGs involved in the Synthesis of Lignin in the WGCNA modules. “H”, “G”, and “S” represent the three lignin subunits, “Lac” means “Laccases”, “Pox” means “Peroxidases”, “S-Pox” mean “Syringyl peroxidases”. Magenta genes are induced by both stresses, red genes repressed by both stresses, green genes are induced only by drought, black genes are repressed only by drought, brown genes are induced only by cold, pink genes are repressed only by cold.

	Synthesis of Lignin								Total
	Early Enzymes	H, G & S	G & S	S	Lac	Pox	S-Pox	BAHD	
Magenta	2	1	-	-	2	-	2	-	7
Red	-	-	1	1	-	-	-	-	2
Green	-	-	1	-	-	1	-	-	2
Black	3	-	7	1	9	5	13	8	46
Brown	-	-	-	-	1	-	1	-	2
Pink	-	1	1	-	-	-	-	-	2
Total	5	2	10	2	12	6	16	8	61

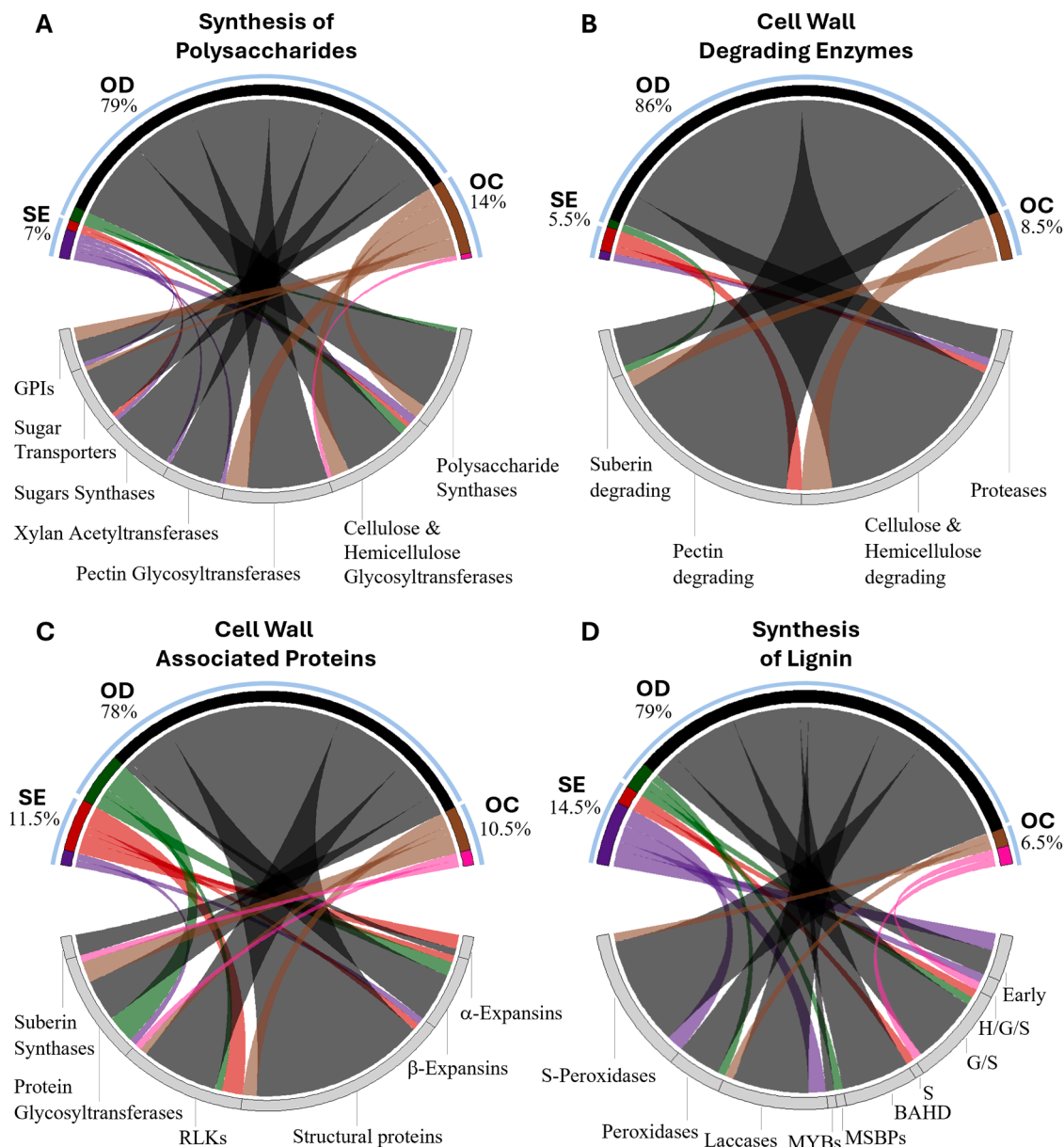


Figure 8. Classification of the 331 hub CWGs from WGCNA modules based on their functional categories. Bipartite chord diagrams depicting the CWGs from each regulatory module (top semicircles in their respective colors) into their corresponding gene families (bottom semicircle, in gray). CWGs are presented in four categories: (A) Synthesis of Polysaccharides, (B) Cell Wall Degrading Enzymes, (C) Cell Wall Associated Proteins, and (D) Synthesis of Lignin. CWGs with similar effects correspond to the Magenta (inductions) and Red (repressions) modules, those responding only to drought correspond to the Green (inductions) and Black (repressions) modules, and those responding only to cold correspond to the Brown (inductions) and Pink (repressions) modules. Lines (with the color of each behavior) connect CWG behavior with its corresponding gene family. The width of each line is proportional to the number of CWGs. “SE” means “Similar effect”, “OD” means “Only drought effect”, and “OC” means “Only cold effect”. In the synthesis of lignin, “S” means genes involved in the synthesis of S subunits, “G/S” means genes involved in the synthesis of G and S subunits, “H/G/S” means genes involved in the synthesis of the of H, G and S subunits, and “Early” means genes involved in the early steps of lignin biosynthesis. The list of genes and their assigned modules are shown in Supplementary Table S2.

activation of genes involved in structural adaptation. The small proportion of CWGs having shared regulation under both stresses implies that, while certain elements of the cell wall are commonly regulated, most of the cell wall response is stress-specific.

The analysis of individual CWG families revealed the specific enzymes driving the contrasting and shared regulatory patterns observed between cold and drought responses (Figure 8). The broad, cold-triggered induction of genes encoding both polysaccharide synthesases and glycosyltransferases, as well as those encoding polysaccharide-degrading enzymes, suggests that these compounds undergo active remodeling in the cell wall. This may indicate enhanced polysaccharide turnover or remodeling in response to cold stress. These transcriptional

changes may underly the biochemical remodeling observed under cold, particularly the increase in alkali-extractable uronic acids and the altered detection of AGP- and xylan-related epitopes, suggesting changes in polymer accessibility and wall matrix organization rather than a simple increase in total cell wall polysaccharide content. On the other hand, the repression of genes involved in both the synthesis and degradation of cell wall polysaccharides in response to drought suggests prioritization of energy conservation rather than active remodeling.

Our data also suggests that the cell wall is potentially reinforced upon cold treatment through the induction of structural proteins, such as AGPs and extensins (Figure 8), as these proteins have been related to the structural reinforcement of the cell wall in banana and pea under cold

Table 6
Distribution of CWGs from the WGCNA modules in regulatory networks. The number of CWGs is shown next to the number of TFs they interact with, as well as the ratio of CWGs and TFs. Magenta genes are induced by both stresses, red genes repressed by both stresses, green genes are induced only by drought, black genes are repressed only by drought, brown genes are induced only by cold, pink genes are repressed only by cold.

CWGs hub regulatory networks				
	Clusters	Number of CWGs	Number of TFs	CWG-to-TF ratio
Magenta	C-2, C-3	10	5	2
Red	C-1	9	5	1.8
Green	C-4	5	8	0.6
Black	C-3, C-7, C-9, C-10, C-11, C-12	112	13	8.6
Brown	C-6, C-7, C-8	18	10	1.8
Pink	C-8, C-10	4	4	1

stress (Weiser et al., 1990; Liu et al., 2025). Concerning expansins, it has been shown that α -expansins are involved in growth promotion by acting on the junctions between cellulose microfibrils, while β -expansins act on hemicelluloses (Cosgrove, 2024). In our results, the expression of several α -expansins is repressed by drought and cold, suggesting a common mechanism to limit growth to counter the impact of both stresses, as this type of proteins have been reported to play an important role in cell wall loosening and expansion in Arabidopsis and wheat (Kwon et al., 2008; Zhao et al., 2012). In contrast, the expression of specific β -expansins is induced only by drought. This result is in agreement with the fact that these proteins have been associated with an increase in water retention in wheat under drought conditions (Yang et al., 2020). Indeed, the overexpression of the maize β -expansin gene *ZmEXPB7* in Arabidopsis enhanced drought tolerance, improved root growth, reduced water loss, and increased survival under both osmotic and soil drought stress (Xiong et al., 2025). Thus, our results suggest that both stresses trigger a similar response to limit growth (implying the repression of some α -expansins), while only drought promotes changes in the hemicellulose network (implying the induction of some β -expansins).

Genes involved in lignin biosynthesis represent the functional category least affected exclusively by cold (Figure 8). Given that lignin is a non-recyclable polymer, this limited transcriptional response is consistent with a remodeling strategy that relies primarily on adjusting other, more dynamic cell wall components. In fact, most of the lignin genes that are induced by cold are also induced by drought. This includes genes from the early stages of the pathway (C4H and 4CL). We have determined that flavonoids are induced in cold (Figure 1). Thus, as early phenylpropanoid genes are not only committed to the synthesis of lignin, these results suggest that the metabolic flow of the phenylpropanoid pathway is redirected to the synthesis of these flavonoids. In fact, the overaccumulation of flavonoids, with known antioxidant activity, has been related to the response to these stresses (Li et al., 2021; Li et al., 2025). In addition, the observed induction of specific S-peroxidases by cold and by drought is in line with our observed increase in S subunits in the lignin polymer upon cold (Figure 2). These results are also in line with the observed increase in S subunits upon drought in

maize (Calderone et al., 2024). In fact, an increase in the S subunits promotes the formation of a more linear lignin polymer (Ralph et al., 2019), that has been associated with an improvement in water retention (Zhu et al., 2025).

We also identified 41 TFs that form regulatory networks with the affected CWGs (Figure 9). Interestingly, 35 of them form regulatory networks containing CWGs that belong to specific WGCNA modules, suggesting that CWGs with a similar alteration upon one or both stresses are commonly regulated. The CWG-to-TF ratio of each regulatory network suggests that different expression patterns are governed by distinct regulatory strategies, ranging from a broad and centralized control (high CWG-to-TF ratio) to a more specific and fine-tuned regulation (low CWG-to-TF ratio). Our results revealed that the proposed broad and centralized regulation is associated with CWGs that are only repressed by drought, while the proposed fine-tuned regulation is associated with the remaining affected CWGs. In fact, this low CWG-to-TF ratio was observed for some β -expansins and for genes involved in synthesis and degradation of polysaccharides and synthesis of structural proteins. This data suggests that genes involved in cell wall remodeling require a fine-tuned regulatory control, while growth suppression and energy conservation under drought stress is achieved by the broad and centralized repression of CWGs.

AP2/ERF, including members of the EREB, ABI, and VP1 transcription factor groups interact with 95 cell wall genes that respond to one of the two stresses or to both, suggesting that they play a central regulatory hub integrating maize responses to multiple environmental stresses. This interpretation is consistent with previous studies that have linked AP2/ERF family members to both drought and cold responses (Trzcinska-Danielewicz et al., 2009; Maghraby and Alzalaty, 2024). In contrast, several MYBs and bZIPs, together with NPL13, generate regulatory networks with 136 cell wall genes that are exclusively responsive to drought. Our results agree with the fact that several MYB and bZIP transcription factors have been associated with drought adaptation in maize (Chen et al., 2018; Cao et al., 2019). Members of the NLP family, however, have been mainly associated with nitrate signaling in maize (Wang et al., 2018) and only a potential role of NPL in drought responses has been suggested in poplar (Long et al., 2026). Thus, the

Table 7
Transcription factor families generating regulatory networks with more than ten CWGs. Number of transcription factors (TFs) from that family interacting with cell wall genes that respond only to cold, only to drought or to both stresses, and the total number of interactions with cell wall genes. The right column summarizes reported involvement of the TF families with cold and/or drought stresses in maize or other species. [1] (Maghraby and Alzalaty, 2024), [2] (Trzcinska-Danielewicz et al., 2009), [3] (Chen et al., 2018), [4] (Wang et al., 2018), [5] (Long et al., 2026), [6] (Viola et al., 2023), [7] (Cao et al., 2019), [8] (Sun et al., 2023).

TF families generating regulatory networks with more than ten CWGs					
TF family	Only cold	Only drought	Both stresses	CWGs interactions	References
AP2/ERF	4	4	4	95	Drought [1] and cold [2] responsive in maize.
MYB	-	7	-	74	Responsive to several abiotic stresses in maize, including drought [3]
NLP	-	1	-	48	Responsive to nitrate signaling [4], and drought response in poplar [5]
TCP	4	-	1	15	Temperature responsive [6] in maize
bZIP/OBF	-	5	-	14	Drought responsive in maize [7]
KN	1	-	-	11	Drought responsive in cotton [8]

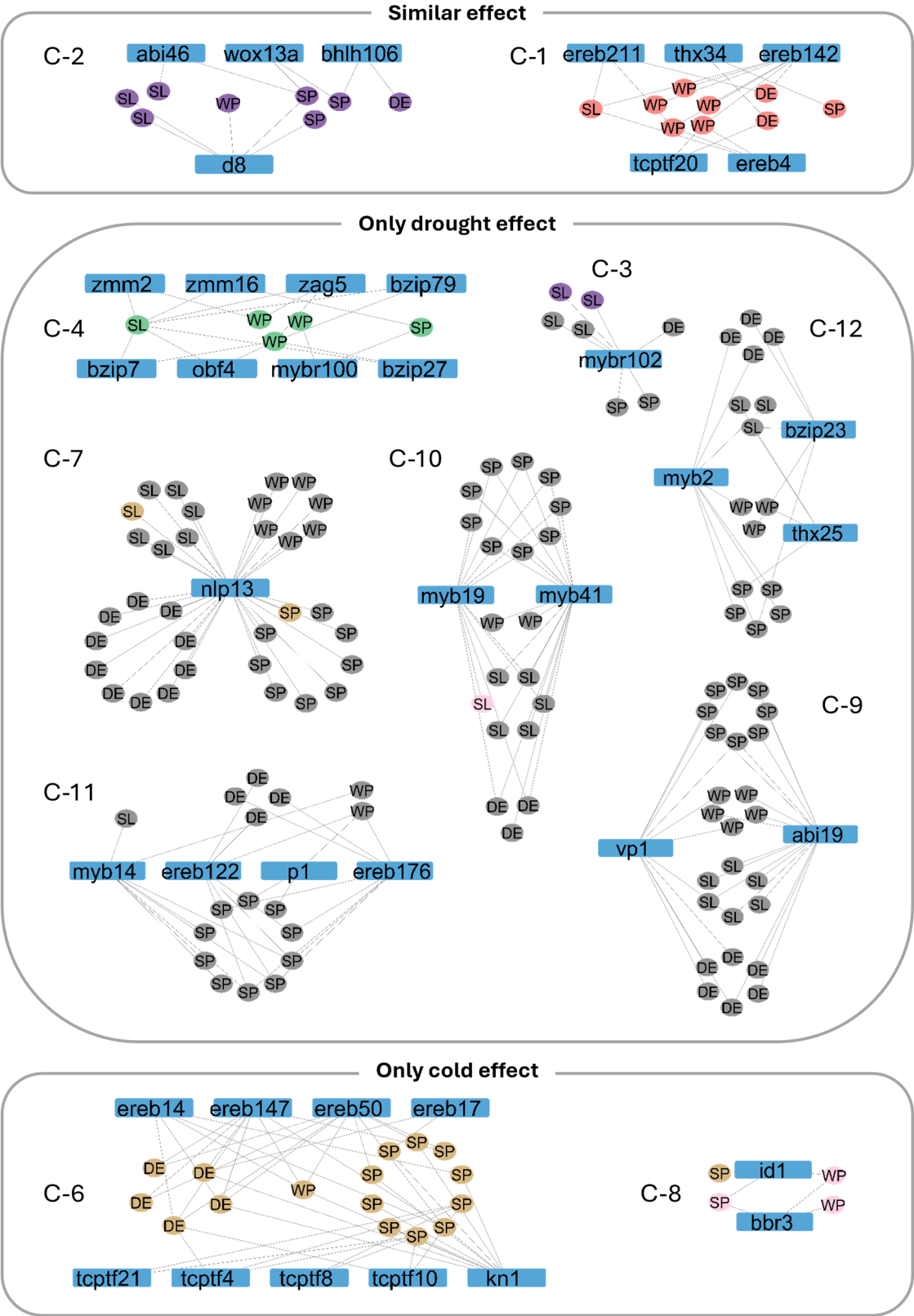


Figure 9. Regulatory networks of transcription factors generated with genes of the WGCNA CWGs hub in maize B73. CWGs are distributed in twelve clusters (C-number) generated by the GLayer tool in Cytoscape. The clusters are grouped according to expression behavior of their CWGs: “Similar effect (SE)” (Magenta and Red modules), “Only drought effect (OD)” (Green and Black modules), and “Only cold effect (OC)” (Brown and Pink modules). Balls represent CWGs and have the same color as their corresponding WGCNA modules. Each CWG is labeled with an abbreviation indicating its functional category: SP, “Synthesis of Polysaccharides”; WP, “Cell Wall Associated Proteins”; DE, “Cell Wall Degrading Enzymes”; and SL, “Synthesis of Lignin”. Transcription factors are shown as blue rectangles containing their names inside. The full name of the TFs is shown in Supplementary Table S5.

identification of the maize NPL13 in our regulatory networks suggests that this family of transcription factors can be also involved in drought response regulation in maize. In fact, the 48 interactions identified of NPL13 highlight its potential role as a key regulator of cell wall genes upon drought in maize. Finally, the TCP and KN interact with 26 cell wall genes that are affected only by cold stress. Previous works have associated TCPs with temperature-dependent responses in maize (Viola et al., 2023). In contrast, although members of the KN family have been linked to drought responses in cotton (Sun et al., 2023), their involvement in cold stress has not previously been reported in maize. Thus, our results suggest that cold-responsive cell wall remodeling may involve not only TCPs, but also KN1, emerging as a new candidate regulator of cold-stress response in maize.

In sum, our study reveals the alteration of the maize cell wall upon cold, as well as the commonalities and singularities of maize CWGs response to drought and cold. The identification of the specific gene families that respond according to the different expression patterns, as well as the identified transcription factors that generate regulatory networks with CWGs, provides new insights into the response of the maize cell wall to one or both stresses.

Materials and Methods

Plant material and growth conditions

Maize is particularly sensitive to low temperatures, exhibiting strong retardation of growth and development below 17 °C, severe physiological damage below 8 °C, and lethality at temperatures around 4 °C (Sobkowiak et al., 2016). Based on this sensitivity, we selected 16 °C for the biochemical analyses as a mild cold condition that allows the study of cold-induced responses avoiding cellular damage or tissue injury. In addition, for gene expression we performed a short-term 4 °C cold shock (3–8 h). B73 *Zea mays* L. plants were grown in a growth chamber (16/8 h day/night, 28/22 °C) until the third leaf was fully developed (14 days). For long-term treatment (cell wall biochemistry), plants were transferred to 16 °C and grown for 10 days, leaving the other conditions unaltered. In control plants, the temperature was not changed. At the end of the 10-days of growth, the whole aerial parts of four control and four cold-treated plants were taken and immediately frozen at -80 °C. Three independent biological replicates were performed. For short-term treatment (cell wall genes expression), plants were transferred to 4 °C. This temperature was chosen to produce a sharp response in the transcript population, to detect transcripts that allow rapid adaptation to the cold condition. The whole aerial part of four plants was harvested after 3, 5 and 8 h and immediately frozen at -80 °C. ERF3 gene was used as a control for transcriptomic change under cold (Nguyen et al., 2009, Supplementary Figure S3). Two independent biological replicates were performed.

Quantification of soluble phenolics

Total phenolics were determined using a modified Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965). Samples (100 mg FW) were extracted in 1 ml ethanol (80%), incubated 2 h at 4 °C in the dark and then centrifuged to remove cell debris. Distilled water was added to aliquots of supernatant to reach a final volume of 3 ml. Then 0.5 ml Folin-Ciocalteu reagent (1:1 with water) and 2 ml of Na₂CO₃ (20%) were added. The solution was warmed 15 min at 45 °C, cooled to room temperature and the absorbance was measured at 650 nm. Total flavonols were determined according to (Chang et al., 2002). Samples were extracted in 80% ethanol as described above. After centrifugation, methanol was added to aliquots of supernatant to reach a final volume of 2 ml and sequentially mixed with 0.1 ml aluminium chloride (10% water solution), 0.1 ml K-acetate 1 M and 2.8 ml distilled water. After 30 min incubation at room temperature, absorbance at 415 nm was recorded. Total anthocyanins were determined according to (Laitinen et al., 2008).

Samples (100 mg FW) were extracted with 1 ml of extraction solvent (methanol, water, hydrochloric acid, 7:2:1) at 4 °C for 20 h and centrifuged (20 min, 10000 rpm, 4 °C). To correct for the effect of chlorophyll we used the following equation: AA = (A530 - 0.25 A657) M⁻¹, being AA the corrected anthocyanins absorbance and M the FW of the tissue extracted (Mancinelli and Rabino, 1984).

Cell wall analyses

Samples from control and cold-stressed plants were ground to a fine powder, extracted with 20 volumes of methanol, and filtered through Whatman GF/A microfiber filters. This alcohol insoluble residue (AIR) was dried at 60 °C for 4 days. AIR was then de-starched, treated with acidified phenol and washed with organic solvents to obtain the cell wall residue, as previously described (Mélida et al., 2010).

Total lignin content was gravimetrically determined by the Klason procedure, whereas the lignin composition was determined by thioacidolysis (followed by GC-MS), as already reported (Fornalé et al., 2015). The assays were conducted on three biological replicates, each comprising the entire aerial part pooled from five plants for both treatment and control conditions. The experimental procedure was repeated with three technical replicates for each biological replicate.

Neutral sugar analysis was performed according to Albersheim et al., (1967). Dried cell walls were hydrolyzed with 2M TFA (trifluoroacetic acid) for 1 h at 121 °C and the resulting sugars were derivatized to alditol acetates and finally analyzed by gas chromatography (GC). Uronic acid contents were determined by the m-hydroxybiphenyl method (Blumenkrantz and Asboe-Hansen, 1973), using galacturonic acid as a standard. Cellulose was quantified in crude cell walls by the Updegraff method (Updegraff, 1969) with the hydrolytic conditions already described (Saeman et al., 1963) and the quantification of the glucose released was performed by the anthrone method (Dische, 1962) using glucose as a standard.

For cell wall fractionation, dried cell walls were extracted (1 mg/2.5 mL) with 50 mM cyclohexane-trans-1,2-diamine-N,N',N'-tetraacetic acid sodium salt (CDTA) at pH 6.5 for 8 hours and washed with boiling distilled water. The residue was incubated with 0.1N KOH⁺ 20mM NaBH₄ (KOH I) for 8 hours and washed with distilled water. Then, 4N KOH⁺ 20mM NaBH₄ (KOH II) was added to the residue for 8 hours and washed again with distilled water. The extracts and their washings were combined and acidified to pH 5.0 with glacial acetic acid, representing CDTA, K-I and K-II fractions, respectively. Ester-linked ferulate and p-coumarate monomers, as well as ferulate dimers (diferulates) were extracted from the AIR with 2M NaOH at room temperature. The extract was clarified by centrifugation and the supernatant was fractionated against ethyl acetate at pH 5 (x2). The organic fraction was collected, dried and analysed by HPLC following the procedure previously described (Martínez-Rubio et al., 2020). The retention times and peak spectra (220–340 nm) were compared with standard solutions of ferulate, p-coumarate, 8,5-open (noncyclic), 8,5-benzofuran (cyclic), 8-O-4', and 5-5' diferulates. Quantification was carried out using vanillic acid as an internal standard (10 µg/mL per sample). Total diferulates were calculated as the sum of four identified and quantified dimers.

Cell wall immune-dot assays

Immuno-dot assays (IDAs) were performed as described by (García-Angulo et al., 2006). A 1 µL aliquot from each cell wall fraction was spotted onto nitrocellulose-membranes (Scheicher & Schull, Dassel, Germany) as replicated 1/5 dilution series of the preceding one. After drying, the nitrocellulose membranes were blocked with 4% (m/v) fat-free milk powder in phosphate-buffered saline (M-PBS: 0.14 M NaCl, 2.7 mM KCl, 7.8 mM Na₂HPO₄ 12 H₂O, 1.5 mM KH₂PO₄, pH 7.2) and then incubated with a 1/5 dilution of primary antibodies (Plant Probes, Leeds, UK) in M-PBS for 2 h at room temperature. The membranes were further washed under running tap water followed by PBS and incubated

with the secondary antibody (anti-rat horseradish peroxidase conjugate, Sigma®) diluted to 1/1000 in M-PBS for 2 h at room temperature. The membranes were washed as described above prior to color development in the revealing solution (for membrane: 5 mL distilled H₂O + 1 mL of methanol with 5 mg/mL 4-chloro-1-naphthol + 6 µL 30% (w/v) H₂O₂). Color development was stopped by washing the membranes with running tap water.

Immunolabelling semi-quantification for each fraction was performed by scoring the number of dilutions labelled. Thus, a value ranging from 0 to 4 was assigned according to the number of colored spots appeared after the color development. Finally, the IDA results were used to generate a heatmap that was carried out by RStudio (v.4.0.2) (R Core Team, 2013) using factoextra (Kassambara and Mundt, 2020), FactoMineR (Lê et al., 2008) and gplots (Warnes et al., 2015) packages. The assays were conducted on two biological replicates, each comprising the entire aerial part pooled from five plants for both treatment and control conditions. The experimental procedure was repeated with three technical replicates for each biological replicate.

Transcriptomic analysis

Total RNA was extracted from control and short-term cold-stressed B73 maize plants using the Maxwell® RSC RNA Purification Kit (Promega, Madison, USA) following the manufacturer's instructions. Downstream analysis was conducted as detailed by Calderone et al. (2024). RNA sequencing was performed at the “Centro Nacional de Análisis Genómico” (CNAG-CRG, Barcelona, Spain) where RNA-Seq reads were pre-processed, mapped, assembled, and the differential expression analysis were done by the CNAG-CRG's bioinformatics unit. Two biological replicates per condition were used.

Differential expression analysis was performed using the DESeq2 package in R (v 3.19) (Love et al., 2014) with additional functions from tidyverse (Wickham et al., 2019). Differential expression values were calculated for each pair of conditions (treatment against control), and only pairs with log₂FC higher than 1 or lower than -1 with an adjusted p-value lower than 0.05 were considered differentially expressed (Supplementary Table S1).

Real-time quantitative reverse transcription PCR (qRT-PCR) assays were conducted to assess the expression of several cell wall genes to validate RNA-seq data using gene-specific primers designed by the “Primer Blast” program from the NCBI webpage. Three technical replicates were conducted for each of the two biological replicates, and qPCR reactions were run on a Light Cycler 480 (Roche, Basel, Switzerland) using SYBR Green dye (Roche). The maize Elongating factor 1 gene (GRMZM2G153541) was used for data normalization, as it was previously described as a suitable housekeeping gene for cold-stress assays (Lin et al., 2014). The qRT-PCR conditions comprised an initial denaturation step at 95°C for 10 min, followed by 45 cycles consisting of a denaturation step at 95°C for 10 s, an annealing step at 60°C for 30 s and an extension step at 72°C for 30 s. The primer list and results are shown in Supplementary Table S4. We compared log₂ fold-changes obtained by RNA-seq with qRT-PCR results for eight representative genes. The two datasets showed a very strong positive correlation (Pearson $r = 0.913$), confirming the reliability of the RNA-seq measurements.

For the cold and drought comparison, the selected time-points represent comparable stages of stress progression for each condition. In the case of cold stress, the gradual and consistent induction of the cold-responsive marker gene *ZmERF3* between 1 and 8 hours indicated the establishment and intensification of the cold response over this time period (Nguyen et al., 2009). Likewise, in the drought experiments, drought-responsive marker gene *ZmRab17* expression showed strong induction after 7, 9 and 10 days of water deprivation (Calderone et al., 2024), indicating a progressive increase in water stress.

Weighted gene co-expression network analysis

Weighted Gene Co-expression Network Analysis (WGCNA) was conducted using WGCNA package in R (v 1.72-5) (Langfelder and Horvath, 2008) to identify co-expression modules and assess their correlation with traits. Raw transcript counts were imported from Salmon quantification files using tximport function from DESeq2 package in R (Love et al., 2014). A transcript-to-gene mapping file and sample metadata were used to prepare the data. The metadata included treatment, duration, and replication information. Genes with less than 10 counts in all samples were removed. The DESeq2 package (Love et al., 2014) was used to create a DESeqDataSet, which was then normalized using the regularized log transformation (rlog). The expression data was subsetted to include only 1485 cell wall genes (Calderone et al., 2024). Soft-thresholding power was determined by analyzing scale-free topology fit indices and mean connectivity, selecting a power of 8 (Supplementary Figure S4). The topological overlap matrix (TOM) was calculated, and gene modules were identified using hierarchical clustering and dynamic tree cutting. Modules were assigned colors and further merged based on eigengene similarity, using a cut height of 0.15 (Supplementary Figure S5). Module-trait correlations were computed using categorical traits data (Peter Langfelder, 2018), and the significance of these correlations was assessed using a Student's t-test. Significant module-trait relationships were visualized with a heatmap (Supplementary Figure S6). Only modules with a significant correlation with the studied traits in at least one of the stresses were selected as highly correlated modules. Module membership and gene significance were calculated for highly correlated modules. For each module, genes having a gene significance higher than 0.2 and module membership higher than 0.8 were selected as hub genes for their respective modules and saved for subsequent analysis. (Supplementary Figure S7). The selected hub genes were further filtered to retain only biologically relevant CWGs as defined by having a differential expression of at least 2 folds (Log₂FC > 1 or < -1 and p-value < 0.05) in at least one sample (Supplementary Table S2).

Bipartite chord diagrams were produced using the circus plot function in R (Zhang et al., 2013) to correlate the CWGs from each module with the specific family they belong to.

Transcriptional regulatory network of CWGs

The genes of the CWGs hub, grouped by WGCNA modules, were submitted to the “TF Enrichment” tool available at the Plant Transcriptional Regulatory Map website (Tian et al., 2019) (<http://plantregmap.gao-lab.org/>) to infer the transcription factors with significantly overrepresented targets among their promoter regions defined as 1kb upstream their transcription starting site (adjusted p-value < 0.01). To preserve the specificity of the regulatory elements that could be associated with the distinct expression patterns of the CWGs in each WGCNA module, we performed the enrichment analysis with the CWGs of each module separately. The inferred transcriptional regulatory networks were then merged to retain shared TFs between modules and visualized using Cytoscape (Shannon et al., 2003), enabling an integrative view of the regulatory networks.

Graphical analysis

Principal Component Analyses, heatmaps, gene significance versus module membership dot blots were generated in R language (<http://www.r-project.org/>) using the ggplot2 library (Wickham, 2009) and some bar graphs using Microsoft Excel. Colors were chosen to ensure accessibility for individuals with color vision deficiencies and to avoid coincidence with the module colors assigned by WGCNA.

CRedit authorship contribution statement

Albert Muñoz-Blaxter: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rocio-Cecilia Arce:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Silvia Fornalé:** Writing – review & editing, Methodology, Formal analysis. **María-Luz Centeno:** Writing – review & editing, Methodology, Formal analysis. **Penelope García-Angulo:** Writing – review & editing, Methodology, Funding acquisition, Formal analysis. **Antonio Encina:** Writing – review & editing, Methodology, Funding acquisition, Formal analysis. **Silvia Calderone:** Writing – review & editing, Methodology, Formal analysis. **Mélanie Ormancey:** Writing – review & editing, Methodology. **Cameron Furrle:** Writing – review & editing, Software, Methodology. **Berta Arenyes-Cunill:** Writing – review & editing, Software, Methodology. **Julia I. Qüesta:** Writing – review & editing, Methodology, Funding acquisition. **Narciso Campos:** Writing – review & editing, Methodology. **David Caparrós-Ruiz:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.stress.2026.101534](https://doi.org/10.1016/j.stress.2026.101534).

Data availability

The transcriptomic data obtained in this study have been deposited in the European Nucleotide Archive (ENA) under the study accession number PRJEB90993.

References

- Albersheim, P., Nevins, D.J., English, P.D., Karr, A., 1967. A method for the analysis of sugars in plant cell-wall polysaccharides by gas-liquid chromatography. *Carbohydr Res.* [https://doi.org/10.1016/S0008-6215\(00\)80510-8](https://doi.org/10.1016/S0008-6215(00)80510-8).
- Arce, R.C., Muñoz-Blaxter, A., Sánchez-Retuerta, C., Mauri, N., Caparrós-Ruiz, D., 2026. Unravelling distinct proteome changes in two maize inbreds with contrasting stalk-lodging resistance upon drought. *Front Plant Sci.* <https://doi.org/10.3389/fpls.2026.1830066>.
- Ayorinde, D.A., Olanrewaju, G.O., Showalter, A.M., 2026. Transcriptomic insights into arabinogalactan protein mechanism of action in galactosyltransferase octuple mutants. *Front Plant Sci.* <https://doi.org/10.3389/fpls.2025.1706954>.
- Beihammer, G., Maresch, D., Altmann, F., Strasser, R., 2020. Glycosylphosphatidylinositol-Anchored Synthesis in Plants: A Glycobiology Perspective. *Front Plant Sci.* <https://doi.org/10.3389/fpls.2020.611188>.
- Blumenkrantz, N., Asboe-Hansen, G., 1973. New method for quantitative determination of uronic acids. *Anal Biochem.* [https://doi.org/10.1016/0003-2697\(73\)90377-1](https://doi.org/10.1016/0003-2697(73)90377-1).
- Boerjan, W., Ralph, J., Baucher, M., 2003. Lignin Biosynthesis. *Annu Rev Plant Biol* 54, 519–546.
- Bohnert, H.J., Nelson, D.E., Jensen, R.G., 1995. Adaptations to environmental stresses. *Plant Cell.* <https://doi.org/10.2307/3870060>.
- Calderone, S., Mauri, N., Manga-Robles, A., Fornalé, S., García-Mir, L., Centeno, M.L., Sánchez-Retuerta, C., Ursache, R., Acebes, J.L., Campos, N., et al., 2024. Diverging cell wall strategies for drought adaptation in two maize inbreds with contrasting lodging resistance. *Plant Cell Environ.* <https://doi.org/10.1111/pce.14822>.
- Cao, L., Lu, X., Wang, G., Zhang, P., Fu, J., Wang, Z., Wei, L., Wang, T., 2021a. Transcriptional regulatory networks in response to drought stress and rewetting in maize (*Zea mays* L.). *Molecular Genetics and Genomics.* <https://doi.org/10.1007/s00438-021-01820-y>.
- Cao, L., Lu, X., Wang, G., Zhang, Q., Zhang, X., Fan, Z., Cao, Y., Wei, L., Wang, T., Wang, Z., 2021b. Maize ZmbZIP33 Is Involved in Drought Resistance and Recovery Ability Through an Abscisic Acid-Dependent Signaling Pathway. *Front Plant Sci.* <https://doi.org/10.3389/fpls.2021.629903>.
- Cao, L., Lu, X., Zhang, P., Wang, G., Wei, L., Wang, T., 2019. Systematic analysis of differentially expressed maize ZmbZIP genes between drought and rewetting transcriptome reveals bZIP family members involved in abiotic stress responses. *Int J Mol Sci.* <https://doi.org/10.3390/ijms20174103>.
- Carpita, N.C., Gibeau, D.M., 1993. Structural models of primary cell walls in flowering plants: Consistency of molecular structure with the physical properties of the walls during growth. *Plant Journal.* <https://doi.org/10.1111/j.1365-313X.1993.tb00007.x>.
- Chalker-Scott, L., 1999. Environmental significance of anthocyanins in plant stress responses. *Photochem Photobiol.* <https://doi.org/10.1111/j.1751-1097.1999.tb01944.x>.
- Chang, C.C., Yang, M.H., Wen, H.M., Chern, J.C., 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal.* <https://doi.org/10.38212/2224-6614.2748>.
- Chen, D., Guo, Y., Zhao, Y., Zhang, J., Liu, X., Tong, Z., Zhao, C., 2024. Dynamic evolution characteristics and hazard assessment of compound drought/waterlogging and low temperature events for maize. *Science of The Total Environment* 946, 174427.
- Chen, Y.H., Cao, Y.Y., Wang, L.J., Li, L.M., Yang, J., Zou, M.X., 2018. Identification of MYB transcription factor genes and their expression during abiotic stresses in maize. *Biol Plant.* <https://doi.org/10.1007/s10535-017-0756-1>.
- Chen, Z., Hong, X., Zhang, H., Wang, Y., Li, X., Zhu, J.K., Gong, Z., 2005. Disruption of the cellulose synthase gene, *AtCesA8/IRX1*, enhances drought and osmotic stress tolerance in *Arabidopsis*. *Plant Journal* 43, 273–283.
- Cosgrove, D.J., 2005. Growth of the plant cell wall. *Nat Rev Mol Cell Biol* 6, 850–861.
- Cosgrove, D.J., 2015. Plant expansins: diversity and interactions with plant cell walls. *Curr Opin Plant Biol* 25, 162–172.
- Cosgrove, D.J., 2024. Plant Cell Wall Loosening by Expansins. *Annu Rev Cell Dev Biol* 40, 329–352.
- Cosgrove, D.J., Li, L.C., Cho, H-T, Hoffmann-Benning, S., Moore, R.C., Blecker, D., 2002. The Growing World of Expansins. *Plant Cell Physiol* 43, 1436–1444.
- Crowe, J.D., Hao, P., Pattathil, S., Pan, H., Ding, S.Y., Hodge, D.B., Jensen, J.K., 2021. Xylan Is Critical for Proper Bundling and Alignment of Cellulose Microfibrils in Plant Secondary Cell Walls. *Front Plant Sci.* <https://doi.org/10.3389/fpls.2021.737690>.
- De, M.M., Buanafina, O., 2009. Feruloylation in Grasses: Current and Future Perspectives. *Mol Plant* 2, 861–872.

- Devot A, Royer L, Caron Giauffret E, Ayrat V, Deryng D, Arvis B, Giraud L, Institute E, Rouillard J (2023) Research for AGRI committee: The impact of extreme climate events on agricultural production in the EU.
- Dische, Z., 1962. General color reactions. *Methods Carbohydrate Chemistry* 1, 477–479.
- Fedotova, A.A., Bonchuk, A.N., Mogila, V.A., Georgiev, P.G., 2017. C2H2 zinc finger proteins: The largest but poorly explored family of higher eukaryotic transcription factors. *Acta Naturae*. <https://doi.org/10.32607/20758251-2017-9-2-47-58>.
- Fornalé, S., Rencoret, J., García-Calvo, L., Capellades, M., Encina, A., Santiago, R., Rigau, J., Gutiérrez, A., del Río, J.C., Caparros-Ruiz, D., 2015. Cell wall modifications triggered by the down-regulation of Coumarate 3-hydroxylase-1 in maize. *Plant Science*. <https://doi.org/10.1016/j.plantsci.2015.04.007>.
- García-Angulo, P., Willats, W.G.T., Encina, A.E., Alonso-Simón, A., Álvarez, J.M., Acebes, J.L., 2006. Immunocytochemical characterization of the cell walls of bean cell suspensions during habituation and dehabituation to dichlobenil. *Physiol Plant* 127, 87–99.
- Gray J, Caparros-Ruiz D, Grotewold E (2012) Grass phenylpropanoids: Regulate before using! *Plant Science*. doi: 10.1016/j.plantsci.2011.12.008.
- Hadj Bachir, I., Ployet, R., Teulieres, C., Cassan-Wang, H., Mounet, F., Grima-Pettenati, J., 2022. Regulation of secondary cell wall lignification by abiotic and biotic constraints. *Adv Bot Res* 104, 363–392.
- Hatfield, R.D., Rancour, D.M., Marita, J.M., 2017a. Grass Cell Walls: A Story of Cross-Linking. *Front Plant Sci* 7, 2056.
- Kassambara, A., Mundt, F., 2020. factoextra: extract and visualize the results of multivariate data analyses. R package version 1.0.7. <https://CRAN.R-project.org/package=factoextra>. Extract and visualize the results of multivariate data analyses.
- Keegstra, K., 2010. Plant Cell Walls. *Plant Physiol* 154, 483–486.
- Kleczkowski, L.A., Decker, D., 2015. Sugar Activation for Production of Nucleotide Sugars as Substrates for Glycosyltransferases in Plants. *J Appl Glycosci* (1999). <https://doi.org/10.5458/jag.jag.2015.003>.
- Krasensky, J., Jonak, C., 2012. Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. *J Exp Bot*. <https://doi.org/10.1093/jxb/err460>.
- Kwon, Y.R., Lee, H.J., Kim, K.H., Hong, S.W., Lee, S.J., Lee, H., 2008. Ectopic expression of Expansin3 or Expansin31 causes enhanced hormone and salt stress sensitivity in Arabidopsis. *Biotechnol Lett*. <https://doi.org/10.1007/s10529-008-9678-5>.
- Laitinen, R.A.E., Ainasoja, M., Broholm, S.K., Teeri, T.H., Elomaa, P., 2008. Identification of target genes for a MYB-type anthocyanin regulator in Gerbera hybrida. *J Exp Bot*. <https://doi.org/10.1093/jxb/ern216>.
- Lamport, D.T.A., Kieliszewski, M.J., Chen, Y., Cannon, M.C., 2011. Role of the Extensin Superfamily in Primary Cell Wall Architecture. *Plant Physiol* 156, 11–19.
- Langfelder, P., Horvath, S., 2008. WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics*. <https://doi.org/10.1186/1471-2105-9-559>.
- Lê, S., Josse, J., Housson, F., 2008. FactoMineR: An R Package for Multivariate Analysis. *J Stat Softw* 25, 1–18.
- Li, B., Fan, R., Sun, G., Sun, T., Fan, Y., Bai, S., Guo, S., Huang, S., Liu, J., Zhang, H., et al., 2021. Flavonoids improve drought tolerance of maize seedlings by regulating the homeostasis of reactive oxygen species. *Plant and Soil* 461, 389–405, 20211 461.
- Li, J., Yu, Q., Liu, C., Zhang, N., Xu, W., 2025. Flavonoids as key players in cold tolerance: molecular insights and applications in horticultural crops. *Hortic Res*. <https://doi.org/10.1093/HR/UHAE366>.
- Lin, Y., Zhang, C., Lan, H., Gao, S., Liu, H., Liu, J., Cao, M., Pan, G., Rong, T., Zhang, S., 2014. Validation of potential reference genes for qPCR in maize across abiotic stresses, hormone treatments, and tissue types. *PLoS One*. <https://doi.org/10.1371/journal.pone.0095445>.
- Liu, J., Chen, C., Chen, L., Sharif, R., Meng, J., Gulzar, S., Yi, Z., Chen, S., Zhan, H., Liu, H., et al., 2025. The banana MFLA227 confers cold tolerance partially through modulating cell wall remodeling. *Int J Biol Macromol* 290, 138748.
- Liu, Q., Kasuga, M., Sakuma, Y., Abe, H., Miura, S., Yamaguchi-Shinozaki, K., Shinozaki, K., 1998. Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in Arabidopsis. *Plant Cell*. <https://doi.org/10.1105/tpc.10.8.1391>.
- Long, X., Qiu, C., Sun, J., Song, T., Li, J., Jin, H., Miao, D., Han, X., Li, Z., Gai, Z., 2026. Identification of the NLP Gene Family in Populus euphratica and Its Expression Analysis Under Drought Stress. *Int J Mol Sci* 27, 3071.
- Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. <https://doi.org/10.1186/s13059-014-0550-8>.
- Ma, Y., Johnson, K., 2023. Arabinogalactan proteins – Multifunctional glycoproteins of the plant cell wall. *The Cell Surface* 9, 100102.
- Maghraby, A., Alzalaty, M., 2024. Genome-wide identification and evolutionary analysis of the AP2/EREBP, COX and LTP genes in Zea mays L. under drought stress. *Sci Rep* 14, 7610.
- Mancinelli, A.L., Rabino, I., 1984. Photoregulation of anthocyanin synthesis X. Dependence on photosynthesis of high irradiance response anthocyanin synthesis in brassica oleracea leaf disks and spirodela polyrrhiza. *Plant Cell Physiol* 25.
- Manga-Robles, A., Santiago, R., Malvar, R.A., Moreno-González, V., Fornalé, S., López, I., Centeno, M.L., Acebes, J.L., Álvarez, J.M., Caparros-Ruiz, D., et al., 2021. Elucidating compositional factors of maize cell walls contributing to stalk strength and lodging resistance. *Plant Science*. <https://doi.org/10.1016/j.plantsci.2021.110882>.
- Mareri, L., Romi, M., Cai, G., 2019. Arabinogalactan proteins: actors or spectators during abiotic and biotic stress in plants? *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology* 153, 173–185.
- Martínez-Rubio, R., Centeno, M.L., García-Angulo, P., Álvarez, J.M., Acebes, J.L., Encina, A., 2020. The role of cell wall phenolics during the early remodelling of cellulose-deficient maize cells. *Phytochemistry* 170, 112219.
- Mélida, H., García-Angulo, P., Alonso-Simón, A., Álvarez, J.M., Acebes, J.L., Encina, A., 2010. The phenolic profile of maize primary cell wall changes in cellulose-deficient cell cultures. *Phytochemistry*. <https://doi.org/10.1016/j.phytochem.2010.06.013>.
- Mnich, E., Bjarnholt, N., Eudes, A., Harholt, J., Holland, C., Jørgensen, B., Larsen, F.H., Liu, M., Manat, R., Meyer, A.S., et al., 2020. Phenolic cross-links: Building and deconstructing the plant cell wall. *Nat Prod Rep*. <https://doi.org/10.1039/c9np00028c>.
- Nguyen, H.T., Leipner, J., Stamp, P., Guerra-Peraza, O., 2009. Low temperature stress in maize (Zea mays L.) induces genes involved in photosynthesis and signal transduction as studied by suppression subtractive hybridization. *Plant Physiology and Biochemistry* 47, 116–122.
- Oliveira, D.M., Mota, T.R., Salatta, F.V., Sinzker, R.C., Končičková, R., Kopečný, D., Simister, R., Silva, M., Goeminne, G., Morreel, K., et al., 2020. Cell wall remodeling under salt stress: Insights into changes in polysaccharides, feruloylation, lignification, and phenolic metabolism in maize. *Plant Cell Environ* 43, 2172–2191.
- Ortiz, A., Sansinenea, E., 2023. Phenylpropanoid Derivatives and Their Role in Plants' Health and as antimicrobials. *Curr Microbiol*. <https://doi.org/10.1007/s00284-023-03502-x>.
- Peracchi, L.M., Panahabadi, R., Barros-Rios, J., Bartley, L.E., Sanguinet, K.A., 2024. Grass lignin: biosynthesis, biological roles, and industrial applications. *Front Plant Sci* 15, 1343097.
- Langfelder, Peter, 2018. Working with categorical variables. Insights from a billion correlations. <https://peterlangfelder.com/2018/11/25/working-with-categorical-variables/>.
- R Core Team, 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rajashekar, C.B., Lafta, A., 1996. Cell-wall changes and cell tension in response to cold acclimation and exogenous abscisic acid in leaves and cell cultures. *Plant Physiol*. <https://doi.org/10.1104/pp.111.2.605>.
- Ralph, J., Lapierre, C., Boerjan, W., 2019. Lignin structure and its engineering. *Curr Opin Biotechnol* 56, 240–249.
- Saeman, J.F., Moore, W., Millett, M.A., 1963. Sugar Units Present. *Cellulose*, W.R.L. Ed. Academic Press, New York, pp. 54–69.
- Scheller, H.V., Ulvskov, P., 2010. Hemicelluloses. *Annu Rev Plant Biol* 61, 263–289.
- Shannon, P., Markiel, A., Ozier, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: A software Environment for integrated models of biomolecular interaction networks. *Genome Res*. <https://doi.org/10.1101/gr.1239303>.
- Singleton, V.L., Rossi, J.A., 1965. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am J Enol Vitic*. <https://doi.org/10.5344/ajev.1965.16.3.144>.
- Sobkowiak, A., Jończyk, M., Adamczyk, J., Szczepanik, J., Solecka, D., Kuciara, I., Hetmańczyk, K., Trzcinska-Danielewicz, J., Grzybowski, M., Skoneczny, M., et al., 2016. Molecular foundations of chilling-tolerance of modern maize. *BMC Genomics*. <https://doi.org/10.1186/s12864-016-2453-4>.
- Sun, R., Qin, T., Wall, S.B., Wang, Y., Guo, X., Sun, J., Liu, Y., Wang, Q., Zhang, B., 2023. Genome-wide identification of KNOX transcription factors in cotton and the role of GhKNOX4-A and GhKNOX22-D in response to salt and drought stress. *Int J Biol Macromol*. <https://doi.org/10.1016/j.ijbiomac.2022.11.238>.
- Takahashi, D., Willick, I.R., Kasuga, J., Livingston, D.P., 2021. Responses of the Plant Cell Wall to Sub-Zero Temperatures: A Brief Update. *Plant Cell Physiol*. <https://doi.org/10.1093/pcp/pcab103>.
- Temple, H., Saez-Aguayo, S., Reyes, F.C., Orellana, A., 2016. The inside and outside: Topological issues in plant cell wall biosynthesis and the roles of nucleotide sugar transporters. *Glycobiology*. <https://doi.org/10.1093/glycob/cww054>.
- Tenhaken, R., 2015. Cell wall remodeling under abiotic stress. *Front Plant Sci* 5, 118089.
- Tian, F., Yang, D.-C., Meng, Y.-Q., Jin, J., Gao, J., 2019. PlantRegMap: charting functional regulatory maps in plants. *Nucleic Acids Res*. <https://doi.org/10.1093/nar/gkz1020>.
- Trzcinska-Danielewicz, J., Bilska, A., Fronk, J., Zielenkiewicz, P., Jarochowska, E., Roszczyk, M., Jończyk, M., Axentowicz, E., Skoneczny, M., Sowiński, P., 2009. Global analysis of gene expression in maize leaves treated with low temperature. I. Moderate chilling (14°C). *Plant Science*. <https://doi.org/10.1016/j.plantsci.2009.09.001>.
- Updegraff, D.M., 1969. Semimicro determination of cellulose in biological materials. *Anal Biochem*. [https://doi.org/10.1016/S0003-2697\(69\)80009-6](https://doi.org/10.1016/S0003-2697(69)80009-6).
- Viola, I.L., Alem, A.L., Jure, R.M., Gonzalez, D.H., 2023. Physiological Roles and Mechanisms of Action of Class I TCP Transcription Factors. *Int J Mol Sci*. <https://doi.org/10.3390/ijms24065437>.
- Wang, Z., Zhang, L., Sun, C., Gu, R., Mi, G., Yuan, L., 2018. Phylogenetic, expression and functional characterizations of the maize NLP transcription factor family reveal a role in nitrate assimilation and signaling. *Physiol Plant*. <https://doi.org/10.1111/pp.12696>.
- Warnes, G.R., Bolker, B., Bonebakker, L., Gentleman, R., Huber, W., Liaw, A., Lumley, T., Maechler, M., Magnusson, A., Moeller, S., 2015. Venables B. gplots: Various R programming tools for plotting data. R package version 3.
- Weiser, R.L., Wallner, S.J., Waddell, J.W., 1990. Cell wall and extensin mRNA changes during cold acclimation of pea seedlings. *Plant Physiol*. <https://doi.org/10.1104/pp.93.3.1021>.
- Weng, J.K., Chapple, C., 2010. The origin and evolution of lignin biosynthesis. *New Phytologist*. <https://doi.org/10.1111/j.1469-8137.2010.03327.x>.
- Wickham, H., 2009. Ggplot2: elegant graphics for data analysis. *J. Stat. Softw.* 35.

- Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grommond, G., Hayes, A., Henry, L., Hester, J., et al., 2019. Welcome to the Tidyverse. *J Open Source Softw.* <https://doi.org/10.21105/joss.01686>.
- Xiong, J., Wang, X., Ye, X., Liu, C., Han, J., 2025. ZmEXPB7, a β -expansin gene, contributes to drought tolerance in *Arabidopsis*. *Front Genet* 16, 1688658.
- Yang, J., Zhang, G., An, J., Li, Q., Chen, Y., Zhao, X., Wu, J., Wang, Y., Hao, Q., Wang, W., et al., 2020. Expansin gene TaEXPA2 positively regulates drought tolerance in transgenic wheat (*Triticum aestivum* L.). *Plant Science*. <https://doi.org/10.1016/j.plantsci.2020.110596>.
- Yeats, T.H., Bacic, A., Johnson, K.L., 2018. Plant glycosylphosphatidylinositol anchored proteins at the plasma membrane-cell wall nexus. *J Integr Plant Biol.* <https://doi.org/10.1111/jipb.12659>.
- Zenda, T., Liu, S., Wang, X., Liu, G., Jin, H., Dong, A., Yang, Y., Duan, H., 2019. Key maize drought-responsive genes and pathways revealed by comparative transcriptome and physiological analyses of contrasting inbred lines. *Int J Mol Sci.* <https://doi.org/10.3390/ijms20061268>.
- Zhang, H., Meltzer, P., Davis, S., 2013. RCircos: an R package for Circos 2D track plots. *BMC Bioinformatics* 14, 244.
- Zhang, Y., Xiao, W., Wang, M., Khan, M., Liu, J.H., 2024. A C2H2-type zinc finger protein ZAT12 of *Poncirus trifoliata* acts downstream of CBF1 to regulate cold tolerance. *Plant Journal*. <https://doi.org/10.1111/tpj.16562>.
- Zhao, C., Zhang, Z., Xie, S., Si, T., Li, Y., Zhu, J.K., 2016b. Mutational evidence for the critical role of CBF transcription factors in cold acclimation in *Arabidopsis*. *Plant Physiol.* <https://doi.org/10.1104/pp.16.00533>.
- Rong, Zhao M, yang, Han Y, nan, Feng Y, Li, F., Wang, W., 2012. Expansins are involved in cell growth mediated by abscisic acid and indole-3-acetic acid under drought stress in wheat. *Plant Cell Rep.* <https://doi.org/10.1007/s00299-011-1185-9>.
- Zhu, W., Zhang, J., Prabhakar, P.K., Chan, Y.O., Weber, R.A., Shu, M., Panzade, G., Cooper, C.J., Davidson, R.B., Parks, J.M., et al., 2025. Factors underlying a latitudinal gradient in the S/G lignin monomer ratio in natural poplar variants. *Proc Natl Acad Sci U S A* 122, e2503491122.