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Integrative analysis of transcriptomic and physiological reveals drought adaption strategies in different maize genotypes

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Abstract

Background: Drought is an environmental stress that adversely affects maize productivity. However, drought adaption strategies of different maize varieties are not fully clear at the transcriptomic level. In the paper, drought-sensitive SD902 and -resistant SD609 varieties were analyzed to explore transcriptional and physiological alterations to drought stress.

Results: The higher SOD, CAT, GSH enzymatic antioxidants, stomatal conductance, transpiration, net photosynthesis rate suggested better performance of SD609 than SD902 variety under drought stress. In transcriptome profiling, a total of 8985 and 7305 difference expression genes (DEGs) were identified in SD902 and SD609 respectively. These genes were overall involved in antioxidation reduce, osmotic adjustment, protein modification (e.g. HSP and chaperone protein), photosynthesis, phytohormone (e.g. ABA, IAA, ethylene), transcription factors (TFs) (e.g. ERF, WRKY, NAC and bZIP) and MAPK (MAPK1/8, MKK4/9 and MKKK17) cascade. Among them, the upregulated genes significantly correlated with stress adjustment, HSPs and chaperone functions might better reduce drought-induced damage in both SD902 and especially SD609. The higher genes expression of IAA, ethylene and electron transfer in SD609 may be closely related to drought-tolerant performance than SD902 plants. Moreover, the misregulation of TFs, MAPK and ABA signaling would appear vital to explain the various sensitivity to drought in both varieties.

Conclusion: The more drought-tolerant SD609 presented a beneficial and significantly higher genes expression of stress protection, IAA transduction, photosynthesis compared with drought-sensitive SD902 variety. Our findings provide vital insights into the molecular signatures underpinning drought resistance in maize.

Keywords: Maize, Drought stress, Transcriptome, Adaption strategy, Tolerance mechanism

Background

Sufficient water supply is essential for land plants growth and reproduction in natural environment. However, increasing global temperature leads to more frequency drought risk in agricultural production [1–3]. Drought adversely affect photosynthesis and thereby cause excessive accumulation of reactive oxygen species (ROS) that damage the plant growth and survival by oxidation [4]. To cope with the damage under drought stressful conditions, plants have evolved multiple strategies to adaption to drought condition [5]. For example, plants appropriately close stomatal to reduce water loss; decrease photoinhibition to protect photosystem; improve antioxidant level to decrease oxidative damage; induce heat shock proteins (HSPs) and molecular chaperones to protect proteins [6, 7]. At the molecular levels, molecular sensors promote the signal transduction and thereby activate various transcriptional regulators. Ultimately, the upstream controls result in a great variety of activation of genes and proteins to achieve stress adjustment and growth [7]. Also, phytohormones [8], transcription factors (TFs) [9] and others drought-responsive factors also widely participate in the response to drought in plant. In general, drought adaption process involved in multiple metabolism pathways that cause complex regulation mechanism in plants.

With rapid development of high-throughput sequencing technology, transcriptomic has provided huge amounts of transcriptional evidences to systematically compare and analyze complex mechanism in response to drought stress in plants [10]. Currently, transcriptomic analyses has been widely used to reveal biological adaption to drought and other stresses in rice [11], sorghum [5], wheat [12], watermelon [13] and other plants. Nevertheless, despite major progress in crops, there are relatively few studies on drought adaptation at the transcriptome level in maize.

Maize (*Zea mays* L.), a most important food crop as it is economically valuable and nutritious in industry, agriculture and animal husbandry, is wildly grown in the word. Previous reports suggested that maize plants are more sensitive to drought environment that adversely affect maize growth and development in the seedling stage [3]. Until now, a number of studies associated with water-deficit resistance and water use efficiency have been performed, and some vital drought-induced genes were identified in maize [14–17]. Thus, it is necessary that drought adaption strategies in the regulation of plant tolerance to drought stress needs to be further clarified, especially in contrasting maize varieties.

In the present paper, an integrated transcriptome and physiological analysis were

performed to assay the drought response of SD902 and SD609. Combined with the RNA-seq data, we analyzed the physiological responses of maize plants subjected to drought stress, such as ROS level, antioxidants activity and photosynthesis. Our study provides the complete information about photosynthesis system, energy metabolism, protein protection, phytohormone transduction, and MAPK cascade and TFs in the maize leaves responding to drought stress. These data systematically reflect a regulation network to drought tolerance on maize and exist a modulation difference between SD902 and SD609 during drought stressful environment.

Results

Variety SD609 exhibit stronger tolerance than SD902 under drought stress

To obtain insight into the phenotypic and physiological features under drought stress condition, seedings of two maize hybrids were exposed to drought treatment. As shown in Fig.1A, drought stress result in leaves wilting, curl and chlorosis in SD609 and SD902 varieties, and the latter displayed a more obvious drought-susceptible symptom in morphologies. According to physiological indexes and photosynthesis parameters, we found significant differences in hydrogen peroxide (H_2O_2), oxygen radical (O_2^-) and malondialdehyde (MDA) content, antioxidant enzymatic activities and total photosynthesis rate in both the varieties. Compared with drought-tolerant SD609 variety, there are higher levels of H_2O_2, O_2^- and MDA in SD902 subjected to drought treatment (Fig.1B-D). The activity of antioxidant enzymes (SOD, POD, CAT APX, GSH and GR) was significantly improved in drought-treated maize, compared with control plants (Fig.1E-G, I-K). Apart from GR, others antioxidants had a significant difference in two varieties. Moreover, drought stress adversely reduced total chlorophyll level in two hybrids, inducing various significance in leaves color compared to well-watered condition (Fig.1H). The survey of gas exchange parameters found that drought significantly decreased stomatal conductance (G_s), intercellular CO_2 concentration (C_i) and transpiration rate (Tr) in SD902 and SD609 variety. Compared with SD902 plants, SD609 had a lower G_s and Tr under drought condition, which effectively prevent water loss and caused a higher net photosynthetic rate (P_n) (Fig.1L-O). In short, drought stress increases reactive oxygen species (ROS) accumulation, enhances antioxidants activity and decreases photosynthetic efficiency at the physiological level in maize plants, which ultimately might affect growth and development of maize plants.

Screening of transcripts abundance and different expression analysis to drought stress

Based on the RNA-seq, the transcription profiles of leaf samples from SD609 and SD902 varieties exposed to drought and normal treatment condition then were analyzed and compared systematically to study gene expression. Twelve cDNA libraries were prepared from collection samples and subjected to paired-end sequencing. The total number of raw reads in the libraries ranged from 22.71 to 39.12 million. After filtering out low quality reads, clean reads were between 22 and 38 million, with 97.17% clean reads and 93.46% Q30 rate. The clean reads were then mapped onto the maize reference genome (NCBI accession No. GCF_000005005.2) using HISAT (hierarchical indexing for spliced alignment of transcripts). The reads that could not be mapped to the maize genome were discarded, and only the mapped reads were further analyzed. FPKM values were calculated to measure the expression abundance of the transcripts. Finally, totals of 8985 DEGs (4826 up-regulated and 4159 down-regulated) in SD902 and 7305 DEGs (3892 up-regulated and 3413 down-regulated) in SD609 with the standards of fold changes > 1 and *P*-value < 0.05 were found respectively (Fig. 2A, Table S1). The Venn diagram discovered that 4707 DEGs were common to two drought stress treatment groups, of which, 2413 DEGs were increased by drought pressure (Fig. 2B). These DEGs may play the important roles in the adaption to drought stress.

Functional categorization and enrichment analysis of DEGs among SD902 and SD609

The Gene Ontology (GO) enrichment revealed 55 biological terms consisted of 21 cellular components (CC), 9 molecular functions (MF) and 25 biological processes (BP) in SD609 and SD902 varieties (Figure S1). The highly related to CC terms were thylakoid (GO:0009579) and photosystem (GO:0009521). For MF category, chlorophyll binding (GO:0016168) and tetrapyrrole binding (GO:0046906) obviously were involved in stress. The most significant enrichment of BP was photosynthesis (GO:0015979), light harvesting (GO:0009765), light reaction (GO:0019684) and protein-chromophore linkage (GO:0018298). Further analysis of BP found that drought induced more BP terms (1063 DEGs) in SD902, such as various amino acid catabolic (GO:1901606, GO:0009063, GO:0009074), benzene-containing compound metabolic (GO:0042537), monocarboxylic acid metabolic (GO:0072330, GO:0032787), phenylpropanoid metabolic (GO:0009698) and carbohydrate derivative catabolic

(GO:1901136). By contrast, generation of precursor metabolites and energy (GO:0006091) and chlorophyll biosynthetic (GO:0015995) were found only in SD609 variety (Fig. 2C). Moreover, KEGG enrichment found 51 and 26 metabolic pathways in SD902 and SD609, respectively (Table S2). The highest enrichment category was photosynthesis proteins (ko00194), and second highest enrichment category were photosynthesis (ko00195) and photosynthesis-antenna proteins (ko00196) in SD902 and SD609. The top 5 enrichment factors were involved in photosynthetic reaction (ko00194, ko00195, ko00196), carbon fixation (ko00710), starch and sucrose metabolism (ko00500), glutathione metabolism (Ko00799) and chaperones and folding catalysts (ko03110) (Figure S2). Ribosome (ko03011 and ko03010) pathways were significantly enriched only in SD902, while protein processing in endoplasmic reticulum (ko04141), carbohydrate metabolism (ko99981), fructose and mannose metabolism (ko00051), butanoate metabolism (zma:00650) were certainly triggered in SD609. These results suggested that photosynthesis, energy transformation, protein protection and cell detoxification processes may mainly response to drought stress in two maize varieties.

Drought affect light harvesting antenna system among SD902 and SD609

As shown in Table 1, seventeen genes concentrated in light harvesting antenna complex I (LHCI) and light harvesting antenna complex II (LHCII) were significantly downregulated during drought stress condition. Apart from LhcI3 belonged to LHCI, other sixteen decreased DEGs are part of LHCII system, such as CP30, CP29, CP25, CP24 and other members. The downregulated ratio of LhcI3 in SD902 and SD609 was 4.61 and 2.41, respectively. The other identified LHCII genes in SD902 were decreased by 1.83 to 11.16 times, while them were also decreased by 1.67 to 4.55 times in SD609. In short, numerous genes related to light harvesting antenna proteins were adversely downregulated during drought condition, which suggest that LHCII system is more sensitive to drought stress in SD609 and especially SD902 variety.

Drought decrease photosynthesis efficiency among SD609 and SD902

We found that 41 DEGs correlated with electron transport of photosystem were changed under drought condition (Table 2). In SD902 variety, in addition to a *OEE3* gene upregulated, other eight DEGs (LOC100272890, LOC100191684, LOC107648855, LOC100281199, LOC100273117, LOC103627333, PSBQ1 and LOC103647735)

related to oxygen-evolving enhancer proteins were also downregulated by 1.74 to 7.81 times, while these DEGs were downregulated by 1.68 to 3.33 in SD609. Moreover, a total of five DEGs (involved in *PsbY*, *PsbR*, *PsbS* and *PsbW*) encoding photosystem II reaction center subunits were significantly decreased by 1.26 to 4.65 times, while 14 DEGs (involved in *PsaD*, *PsaF*, *PsaE*, *PsaG*, *PsaL* and *PsaN*, *PsaH* and *PsaK*) related to photosystem I reaction center subunits were remarkably reduced by 1.97 to 6.10 times among SD902 and SD609. Drought stress also adversely affected expression of Cyt b6/f complex (*petB*), plastocyanin (PC) and ferredoxin (Fd). Apart from four Fd genes of SD902 and two Fds of SD609, other DEGs involved in electron transport elements were drastically decreased in SD902 and SD609 such as *petB* (cyt b6), *petE* (PC) and *petF* (Fd) genes. Accordingly, compared with SD902 variety, we also found that SD609 variety had a higher effective quantum yield (Y(I)), electron transport rate (ETR(I)), the quantum yield of regulatory energy dissipation (Y(NPQ)) and the quantum yield of non-regulatory energy dissipation (Y(NO)) to drought (Fig. 3A, B, G and H). Effective quantum yield (Y(II)) and the electron transport rate (ETR(II)) only displayed slightly difference in SD902 and SD609 (Fig. 3E, F). The Y(NA) of SD902 had a higher level than SD609, while Y(ND) was significantly decreased in two varieties (Fig. 3 C, D). In general, 88% and 94% genes involved in electron transport on photosynthesis in SD902 and SD609 were significantly downregulated compared to CK plants, and these genes caused a difference of photosynthetic efficiency in two varieties.

Drought change energy catabolism among SD902 and SD609

Upon drought stress treatment, as shown in Table 3, the expression abundance of genes encoding ATPase γ, ATPase δ and ATPase b in SD902 and SD609 were decreased by 1.49 to 3.08 times. A total of 15 DEGs related to CO_2 assimilation were significantly changed at the transcription level. Among them, two DEGs (*PEPC* and *NADP-ME*) in SD902 were increased by 1.87 and 6.08 times, respectively. The other 13 DEGs were significantly downregulated by 1.29 to 5.57 times, such as *phosphoenolpyruvate carboxylase* (*PEPC*), *sedoheptulose-1,7-bisphosphatase* (*SBPase*), *ribulose-bisphosphate carboxylase small chains* (*rbcS*), *pyruvate orthophosphate dikinases* (*PPDK*), *phosphoenolpyruvate carboxykinases* (*PEPCK*), *malatedehydrogenases* (*MDH*) and *NADP-malatedehydrogenases* (*NADP-ME*). By comparison, a *PEPC*, *PEPCK* and *NADP-ME* in SD609 were upregulated by 1.46, 1.44 and 3.29 times

respectively, and the other 12 DEGs were decreased by 1.17 to 2.71 times. The analysis of qRT-PCR and enzyme activity found that drought stress significantly decreased enzyme activity and gene transcription abundance for PPDK, PEPC, NADP-ME and Rubisco in SD902 and SD609 (Fig. 3I-P). The NADP-ME and Rubisco had an obvious difference in maize varieties after drought treatment, while genes expression level of *PEPC* and *rbcS* displayed significant difference. Moreover, we also found many DEGs were involved in glucose metabolism in SD902 and SD609 under drought-adaption process (Table 4). More than 76% DEGs were downregulated impacting starch and sucrose biosynthesis, such as starch synthase, granule-bound starch synthase (GBSS), hexokinase (HK), sucrose-6-phosphatase and sucrose-phosphate synthase (SPS). Except for one alpha-amylase gene (LOC103651265) was upregulated, and more than 83% DEGs related to starch and sucrose degradation were slightly downregulated including beta-amylase, beta-fructofuranosidase and sucrose synthase in SD902 and SD609. Furthermore, 53% and 33% beta-glucosidase genes involved in cellulose degradation were significantly upregulated in both SD902 and especially SD609. In general, drought stress affects the ATP and starch production by photosynthesis, sucrose and starch conversion, and the transcriptional difference of the related-genes cause various adaption to drought in SD92 and SD609 varieties.

Drought enhance anti-oxidation and protein protection among SD902 and SD609

After drought stress treatment, ten DEGs related to osmotic adjustment were identified in SD902 and SD609 varieties. As shown in Table S3, four DEGs (LOC541646, LOC103626390, LOC100384855 and LOC100136885) encoding superoxide dismutase in SD902 were decreased by 1.32 to 1.74 times, but two superoxide dismutase (LOC541646 and LOC542722) in SD609 were significantly increased by 1.63 and 1.78 times. Three peroxidases (LOC100384529, LOC100194355 and LOC103504713) in SD902 and SD609 were identified. Among them, apart from LOC100384529 upregulated in SD902 (6.26 times) and SD902 (5.44 times), other two peroxidases were also decreased respectively. The expression abundance of two catalase DEGs (LOC542369 and LOC542230) were significantly changed in two maize varieties by drought stress. Furthermore, a number of DEGs involved in GSH-AsA metabolism were widely changed, including glutamate cysteine ligase, glutathione dehydrogenase/transferase, glutathione peroxidase (GSH-Px), NADPH-glutathione reductase, glutathione S-transferase (GST) and L-ascorbate peroxidase (APX). The

upregulated glutamate cysteine ligase and downregulated glutathione dehydrogenase/transferase were specifically found only in SD902. GSH-Px had a downregulation trend in two plants, but NADPH-glutathione reductase was upregulated in SD902 (1.42 times) and SD609 (2.01 times) respectively. A total of 29 and 22 DEGs encoding GST in SD902 and SD609 were identified, of which, 38% genes (eleven GSTs) in SD902 were decreased by 1.02 to 4.46, and 23% genes (five GSTs) in SD609 were decreased by 1.49 to 2.96. Two out of nine APX increased were common to drought stress in SD902 and SD609, and all APX in SD902 have a higher ratio than SD609. In addition, numerous genes related to molecular chaperonin and heat shock protein (HSP) were found in SD902 and SD609 under drought condition, such as ATP-dependent Clp protease ATP-binding subunit (ClpB), calreticulin, FtsH, GroEL, GroES, DnaK, GrpE, HtpG, HscB, HSP20s, HSP70s and HSP90s (Table 5). The total number of chaperonin genes induced by drought stress were similar between SD902 and SD609, but there were obvious differences in transcripts types, such as ClpB and FtsH genes. Compared to SD902, more HSPs consisted of HSP90, HSP70 and HSP20 in SD609 were upregulated. In short, the DEGs of antioxidants and protein protection were significantly upregulated in SD902 and especially SD609, which cause the stronger resistance in SD609 to drought.

Differential modulation of TFs among SD902 and SD609

Except for structure genes, transcription factors (TFs) play the essential roles in regulation plant development and induction expression of downstream stress-related genes to adaption stress. Our RNA-seq data revealed 346 TFs (154 upregulated and 192 downregulated) belonged to 39 diverse families in SD902 and 279 TFs (126 upregulated and 153 downregulated) belonged to 44 diverse families in SD609, respectively (Table S4). We compared the top ten TF families in two maize varieties due to the TFs numbers (Fig. 4A), which suggested that bHLH, WRKY, ERF, MYB, NAC, bZIP, etc. TFs participated in the response and modulation to drought in two varieties. For example, the TFs *MYB166* and *WRKY29* were significantly upregulated, while *WRKY96*, *MYBR115*, *bZIP17*, *MYBR95*, *EREB68* and *bHLH36* were significantly downregulated both SD902 and SD609. In addition, the upregulated *bHLH104* (9.60-fold), *EREB34* (4.14-fold) and *HDZIV14* (6.10-fold) and downregulated *MYB64* (-6.15-fold) only exists in SD902, while *EREB179* (6.84-fold), *bZIP111* (5.60-fold) and *EREB27* (-4.06-fold), *MYB103* (-3.72-fold) only were promoted in SD609.

Furthermore, the interaction analysis of TFs in two varieties discovered that SD902 variety has a more complex biological relationship involved in more TF genes than SD609 (Fig. 4B), which may imply a more efficient response prosses to drought by the regulation of TFs.

Various modulation of MAPK and phytohormone signaling

MAPK is an important signals transmitter from cell to nucleus. Here, as shown in Table 6, nine MAPK family genes were identified involved in MAPKs (MAPK1 and MAPK8), MKKs (MKK4 and MK9) and MKKKs (four MKKK17s). Compare with control plants, these MAPK genes involved in nine MAPKs in SD902 and two MAPKs in SD609 were significantly decreased at the transcription level, which may show a more drastically MAPK cascade in response to drought in SD902 variety. Moreover, we found a number of plant hormones in response to drought stress in two varieties, such as abscisic acid (ABA), ethylene (ET), jasmonic acid (JA), cytokinin and auxin (IAA). Compared to two downregulated PYR/PYL elements of ABA signal in SD609, SD902 had more ABA response genes including five PYR/PYLs and four PP2Cs. For ethylene signaling, two varieties displayed a response different that ethylene-responsive transcription factor in SD902 was decreased by 1.63 times and ethylene-insensitive protein 3 was increased in SD609 by 4.04 times. The identified JA-acid amino signaling in two varieties was also downregulated. Two cytokinin receptor kinases, HK1b2 and hk3 were reduced in SD609 by 1.57 and 1.61 times, and HK1b2 was decreased in SD902 by 2.43 times. In addition, drought induced a number of auxin response genes, such as SAUR and auxin members. By comparison, more genes (four genes) related to auxin signaling pathway in SD609 were upregulated than of SD902 (one gene), which may lead to a batter cell growth in SD609 variety under drought stress environment

Discussion

Drought event is one of the environment stresses treating the growth, yield and quality of crop plants. In plants, the responses to drought are generally regulated based on multiple metabolic pathways that may form complex interaction each other, through the promotion of TFs and kinases signals as essential core regulators. Therefore, understanding genes transcription strategies to drought stress are vital. In the present study, we used RNA-seq profiling to characterize abundance of transcripts from two

distinct drought-sensitive maize varieties under drought stress condition. Our findings reveal a stronger resistance to drought stress in SD609 than SD902 based on the biological regulations involved in photosynthesis, energy metabolism, osmotic regulation and protein protection.

SD902 and SD609 exert difference in response to drought stress

After drought stress treatment, plants phenotype from two maize hybrids displayed a significant difference in leaves color and shape compared with well-watered treatment. The drought-sensitive SD902 have a weaker drought-tolerant performance (Fig. 1A). ROS accumulation along with oxidized plasma membrane are vital challenge for growth of maize plants under drought condition [22, 23]. Herein, we also found significant accumulation of H_2O_2, O_2^- and MDA after drought treatment (Fig. 1B-D). Antioxidant enzymes play the essential role in improving drought resistance [24]. According to activity measure, we suggest that SOD, POD and CAT enzymes participate in drought-resistant adjustment at the physiological level, especially SOD and CAT (Fig. 1E-G). Moreover, GSH-AsA system contains important reducing substances in plants, which have the role in maintaining the stability of proteins, the structural integrity of the bio-membrane system and the defense against membrane lipid peroxidation [25]. Our results confirm that these substances (APX, GSH and GR) played an important function in decreasing oxidative damage from drought stress (Fig. 1I-K). RNA-seq data provide important transcriptomic evidences at the molecular level. The higher upregulation and stronger antioxidant enzymes by DEGs showed a correlation between physiology response and molecular regulation to enhance drought-tolerant ability in maize varieties and cause batter drought resistance in SD609 varieties compared with SD902.

Responses of photosynthetic system involved in drought stress

Photosynthesis, a most basic physiological process, provide vital energy for various biological activities. Previous reports showed that drought can inhibit photosynthetic efficiency on maize [26, 27], rice [28] , wheat [29] and other photosynthetic plants. In this study, a major number of genes related to Chl metabolism, light energy transfer and carbon fixation were significantly affected at the transcription level. We identified 13 DEGs decreased involved in chlorophyll biosynthesis in SD902 and SD609 (Table S4).

For example, three glutamyl tRNA reductases (GluTRs) genes as rate-limiting enzyme in tetrapyrrole control the Chl synthesis rate [30]. Eight DEGs downregulated including porphobilinogen synthase (PBGS), protoporphyrinogen oxidase (PPO), Mg chelatase subunit I/H/D (CHLI/H/D), Mg-protoporphyrin IX monomethyl ester (oxidative) cyclase (CRDI) and divinyl chlorophyllide a 8-vinyl-reductase (DVR) respectively participate in the formation porphobilinogen, protoporphyrin IX, Mg-protoporphyrin IX, Mg-protoporphyrin IX monomethyl ester and protochlorophyllide [31–33]. Two chlorophyllide a oxygenase (CAO) genes catalyze the conversion of Chl a to Chl b [34]. Moreover, four DEGs (encoding chlorophyll b reductase, chlorophyllase (Chlase) and Mg siumdechelatase) were upregulated involved in chlorophyll degradation [35, 36].

The antenna system mainly responsible for light harvest and electron transfer, but there are sensitive to stresses factor, especially drought environment [37]. Therefore, we found numerous chlorophyll a-b binding elements (CP24, CP25, CP29, CP30 and other members), PSI reaction center subunits (PsaD, PsaF, PsaE, PsaG, PsaH, PsaK, PsaL and PsaN) and PSII reaction center subunits (PsbY, PsbW, PsbS and PsbR) were significantly decreased. In addition, more over 77% genes of electron transfer were also decreased, such as oxygen-evolving enhancer protein, cyt b6, PC and Fd. Compare with SD609 plants, these DEGs have a higher downregulation ratio at the transcriptional level in SD902 variety (Table 1, 2). Accordingly, SD609 variety display a higher Y(I), ETR (I) and Y (NPQ) than SD902, ultimate causing batter photosynthetic efficiency to adaption drought (Fig. 1, 3). ATPase as main component on photosynthesis system drive Calvin cycle to produce sucrose [38]. Here, four genes encoding ATPase subunits (γ, δ and b) were downregulated after drought stress. The key enzymes of carbon assimilation is an underlying inhibition mechanism on plant photosynthesis by drought [39]. In our drought experiment, rbcS, PPDK, PEPC, PEPCK, MDH, NADP-ME and SBPase were significantly downregulated (Fig. 3, Table 3). The enzymes activity of carbon fixation in C4 plant photosynthesis determine the utilization rate of CO_2 in the intercellular space [28]. These evidences show that key genes involved in photosynthesis were affected by drought stress limiting energy production in both SD609 and especially SD902.

Osmotic adjustment and protein protection relate to drought resistance

Osmotic adjustment is widely regarded as significant role in regulation plant adaption and survival under stress conditions based on protecting cellular functions and maintaining turgor. Antioxidant mechanism is an important part of osmotic response

[4]. Herein, we have confirmed effective ROS-removing by various antioxidants. Importantly, transcriptome data found significantly up-regulated polysaccharide degradation genes, such as α-amylase, beta-fructofuranosidase (Table 4). Soluble sugars are vital compounds related to energy production and protein biosynthesis, but also as indispensable osmoprotectants that participate in plant biological resistance to osmotic stress [40, 41]. Accompanied with the transcriptional difference of DEGs related mainly to starch biosynthesis and degradation, sucrose biosynthesis and degradation and cellulose degradation, the levels of soluble sugar based on several monosaccharide may be increased maximum in both SD902 and especially SD609 under drought treatment. These alterations are also part of the drought tolerance of maize.

In addition to antioxidation and osmotic adjustment, molecular chaperones and heat shock proteins (HSPs), as essential modification tools in organisms, are responsible for protein assembly, folding, translocation and degradation in normal cellular processes, and can help to stabilize proteins and membranes as well as promote protein refolding under numerous stress conditions [42]. According to our results, more DEGs involved to HSP members consisted of HSP90, HSP70 and HSP20 were significantly upregulated in both SD902 and especially SD609 (Table 5). Furthermore, drought induced more upregulated DEGs encoding ClpB, calreticulin, FtsH, GroES, DnaK, GrpE and HtpG in SD609 plants, about which, play the vital role in keeping proteins biological function and decreasing damage from stress environment [43–46]. In general, the higher regulation from antioxidation, osmotic adjustment and protein protect may result in the stronger tolerance in both SD902 and especially SD609 variety.

Responses of TFs, MAPK and plant hormone involved in drought stress

TFs as regulatory molecules that play the key role in controlling genes transcription and adapting to variety stress environment [9]. There are 346 TFs belonging to 39 families in SD902 and 279 TFs belonging to 44 families in SD609, respectively (Table S3). The major TFs members including *ERF*, *WRKY, NAC* and *bZIP* were significantly increased, whereas the *MYB* and *bHLH* were decreased in drought stress condition (Fig. 4). Previous research shown that overexpression of *ERF* genes in *Arabidopsis*, rice, tomato and tobacco were able to enhance tolerance capability under diverse biotic and abiotic stresses [47, 48]. Here, more than 60% ERF TFs in SD902 and SD609 were upregulated responding to drought stress, such as *ERF1*, *ERF34* and *ERF65*. *WRKY*s were identified

in response to numerous stress factors and involved in regulating transcription reprogramming under stress condition [49]. More than 70% *WRKY*s were upregulated in SD609 variety. Overexpression of *NAC* in rice increased resistance to drought and heat stresses via resulting in stress-inducible gene [50]. Here, 11 out of 25 and 13 out of 15 *NAC*s were upregulated in SD902 and SD609, which may play the important roles in enhancing drought tolerance. The biological functions of *bHLH*s are gradually being identified in regulating drought and other stresses (Li et al., 2016; Z. Li et al., 2019; Yao et al., 2018). Numerous *bHLH*s (more than 73%) were changed at the transcription level in SD902 and SD609, such as *bHLH36*, *bHLH145*, *bHLH104*. Among them, *bHLH104* played a vital role in improving plant iron tolerance [52, 53, 55]. *MfbHLH38* identified effectively improves the drought resistance of *Arabidopsis* [56]. In general, biological function of these TFs are yet to be discovered on maize, especially in drought adaption process.

Signal transmission is a crucial part of cell growth and response to environment. MAPK cascade, as one of the important signal pathways, is widely involved in plant developmental regulation and stress response [57]. Here, we identified three types MAPKs (MAPK, MKK and MKKK) (Table 6). Compared with well-watered treatment plants, these KAPKs were significantly decreased in drought-treated SD902 and SD609 plants. Previous study found that many MAPK members negatively participate in maintaining ROS homeostasis and controlling other life events [58, 59]. The results in our experiment may result from co-regulation with other signal networks and/or negative feedback regulation. Furthermore, plant hormones are essential way in plants avoiding to stresses damage [8]. Our research also found five kinds of phytohormones that together cope with drought stress (Table 6). These plant hormones displayed a response difference in quantity and type in two maize varieties exposed to drought stress, especially ABA, IAA and ethylene. Through coping strategies of the phytohormones involved in drought resistance in plants has done a lot of research [8], the complex networks still require more studies to identify their biological mechanism.

Conclusion

In this paper, two maize varieties, drought-tolerant SD609 and -sensitive SD902, were detailed investigated. We found that drought results in significant ROS accumulation and limits photosynthesis, which decreases performance of maize to drought condition in both SD609 especially SD902 variety. The expression of genes related to

antioxidants, osmotic adjustment, HSPs and chaperone protein enhance maize tolerance to drought via the regulation of ABA, MAPK cascade and TFs. Compared to drought-sensitive SD902 variety, the higher stress adjustment (enzymatic antioxidants, HSPs and chaperone proteins), IAA signaling response, photosynthesis efficiency and drought-induced factors regulation to cope better drought stress in SD609. Accordingly, we proposed a molecular adaption network to drought based on two contrasting maize (Fig. 5). The study is helpful to further explore drought-tolerant mechanisms and develop cultivars withstand.

Materials and methods

Plant material and treatment design

Two maize (*Zea mays* L.) cultivars Shaandan609 (SD609) and Shaandan902 (SD902) obtained from Shaanxi Dadi Seed Company, were identified as drought-resistant and -sensitive genotypes in our previous work [18]. Here, drought-tolerance SD609 and drought-sensitive SD902 were used as experimental material. Uniformly germinated seeds directly were sown into plastic pots (diameter 30 cm, deep 45 cm) filled with a mixture consisted of 0.064% total nitrogen and 1.62% organic matter. All treatment seedings were sustained in a green-house at 28/20℃ (day/night) with relative humidity of 60% and nature light at Northwest A&F University, Yangling (34°283′N, 108°067′E), China. Before the treatment experiments, all plants were normally watered every day, maintaining soil water content (SWC) at 80 ± 5%. At five-leaf stage, half of SD902 and SD609 seedings were exposed to drought stress condition (50 ± 5% SWC) for five days by controlled water measure. Finally, the collected leaves samples with three biological replicates for each treatment were frozen immediately in liquid nitrogen and stored at -80℃.

Chl concentration and gas exchange measurement

The chlorophyll (Chl) concentration was measured using SPAD meter (SPAD-502, Konica-Minolta, Japan) [19]. The gas exchange parameters including net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transpiration rate (Tr) were determined by a portable photosynthesis system instrument (LI-6400XT; LI-COR Biosciences, United States) at a light intensity of 1000 μmol/(m^2 s) [20]. Each experiment was repeated three time, and the measurements were taken for six plants.

Energy conversion efficiency measurement

According to the previous methods [21], The quantum yields of photosystem I (PSI) and photosystem II (PSII) with the upper second fully expanded maize leaves were measured by a pulse amplitude-modulated system (Dual-PAM-100, Heinz Walz, Germany). The measured fluorescence parameters of PSII were involved in the effective quantum yield (Y(II)), the quantum yield of non-regulatory energy dissipation (Y(NO)), the quantum yield of regulatory energy dissipation (Y(NPQ)) and the electron transport rate (ETR(II)). The measured fluorescence parameters of PSI were involved in the effective quantum yield (Y(I)), the quantum yield of non-photochemical energy dissipation due to donor side limitation (Y(ND)), the quantum yield of non-photochemical energy dissipation due to accept or side limitation (Y(NA)) and the electron transport rate (ETR(I)).

Chemical measurement and enzyme activity assay

According to thiobarbituric (TBA) method, MDA content was measured using MDA Kit (MDA-2-Y) to reply to lipid peroxidation in maize leaves. The contents of H_2O_2 and O_2^- were measured using H_2O_2 Kit (H_2O_2-2-Y) and O_2^- Kit (SAQ-2-G), respectively. The enzyme activity of SOD, POD, CAT, APX, GSH and GR were measured based on manufacturer instructions of Kit including SOD Kit (SOD-2-Y), POD Kit (POD-2-Y), CAT Kit (CAT-2-Y), APX Kit (APX-2-W), GSH Kit (GSH-2-W), GR Kit (GR-2-W), respectively. All Kits were obtained from Suzhou Comin Biotechnology Co., Ltd., China. The activity of photosynthesis enzymes was measured by enzyme-linked immunosorbent assay (ELISA) Kit (JINGKANG, Shanghai) based on operation instruction.

RNA extraction, library preparation and mRNA sequencing

Total RNA was extracted from 0.2 g leaf samples using the RNeasy Plant Mini Kit (Qiagen) following the manufacturer instructions. RNA quality and quantity were determined by the NanoDrop (Thermo Fisher Scientific). For each sample, 0.5 μg of high-quality total RNA with RNA Integrity Number > 8.8 were used for cDNA library preparation by the TruSeq RNA Sample Preparation Kit (Illumina Inc.). Sequence determination of 150 bp pair-end reads were performed on an Illumina HiSeq2500 platform. For each treatment, three biological replicates were constructed libraries and sequenced independently, and each replicate pooled from three individuals. The

sequencing of cDNA was carried by Novogene Bioinformatics Technology Co., Ltd. (Beijing, China) using Illumina HiSeq 2500 platform.

Bioinformatics analysis

Gene Ontology (GO) enrichment was conducted based annotation (http://geneontology.org/). The pathway enrichment was carried out with the Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg). GO and KEGG enrichment analysis were performed via clusterProfile R package. The ggplot2 and pathview R package were used for plotting. Transcription factors (TFs) were predicted using PlantTFDB (http://planttfdb.cbi.pku.edu.cn). STRING online database (https://string-db.org/cgi/input.pl) and cytoscape software were used to visualize interaction relationship.

qRT-PCR analysis

Total RNA from leaf samples (1.0 g) ground into liquid nitrogen was extracted using RNA extraction kit (TIANGEN, China). The 20 μL reverse transcription system was performed by manufacturer instructions (Fast Quant RT Kit, TIANGEN, China). The gene specific primers were designed using Primer Premier 3.0 (Table S6). The qRT-PCR program was performed based on manufacturer instructions (SYBR Green, TIANGEN) with three biological replicates. The maize gene *ZmGADPH* (gene ID: 542367) was used to achieve data proof. The correlative expression levels were calculated using $2^{-\Delta\Delta CT}$ method.

Statistical analyses

The results were means ± standard deviation (SD) with three independent biological replicates. Statistical was analyzed using the SPSS 17.0 software based on Duncan's multiple range test ($P < 0.05$). The SigmaPlot 14.0 software was used to achieve data visualization.

Abbreviations

ROS: reactive oxygen species; HSPs: heat shock proteins; TF: transcription factor; H_2O_2: hydrogen peroxide; O_2^-: oxygen radical; MDA: malondialdehyde; SOD: superoxide dismutase; POD: peroxidase; CAT: catalase; APX: aseorbateperoxidase; GSH: glutathione; GR: glutathione reductase; Gs: stomatal conductance; Ci:

intercellular CO_2 concentration; Tr: transpiration rate; Pn: net photosynthetic rate; LHCI: light harvesting antenna complex I; LHCII: light harvesting antenna complex II; PEPC: phosphoenolpyruvate carboxylase; SBPase: sedoheptulose-1,7-bisphosphatase; rbcS: ribulose-bisphosphate carboxylase small chains; PPDK: pyruvate orthophosphate dikinases; PEPCK: phosphoenolpyruvate carboxykinases; MDH: malatedehydrogenases; NADP-ME: NADP-malatedehydrogenases; Chl a: chlorophyll a; Chl b: chlorophyll b; GluTRs: glutamyl tRNA reductases; PBGS: porphobilinogen synthase; PPO: protoporphyrinogen oxidase; CHLI/H/D: Mg chelatase subunit I/H/D; CRDI: Mg-protoporphyrin IX monomethyl ester (oxidative) cyclase; DVR: divinyl chlorophyllide a 8-vinyl-reductase; CAO: chlorophyllide a oxygenase; Y(II): effective quantum yield of PSII; Y(NO): the quantum yield of non-regulatory energy dissipation of PSII; Y(NPQ): the quantum yield of regulatory energy dissipation of PSII; ETR(II): the electron transport rate of PSII; Y(I): effective quantum yield of PSI; Y(ND): the quantum yield of non-photochemical energy dissipation due to donor side limitation of PSI; Y(NA): the quantum yield of PSI; non-photochemical energy dissipation due to accept or side limitation of PSI; ETR(I): electron transport rate of PSI.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data generated and/or analyzed during this study are including in this published article and its supplementary information files. The sequencing data are available in NCBI SRA database under accession number PRJNA765291 (https://www.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA765291).

Competing interest

The authors declare that they have no competing interests.

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Authors contributions

YFW designed the experiment, analyzed the data and wrote the manuscript. XW did the experiment and gave some good suggestions on the manuscript. HJL and MYH did a part of experiment, RHZ conceived the experiment, revised the manuscript and provided the funding. All authors have read and approved the manuscript.

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791 Table legends

792 Table 1. Effect of drought stress on the expression of chlorophyll a-b binding protein in

793 maize leaves.

Gene name	Gene ID	Description	log2 (Fold change)	
			SD902	SD609
LhcII1	LOC103651578	chlorophyll a-b binding protein	-11.16	-4.25
LhcII1	LOC103651577	chlorophyll a-b binding protein	-10.65	-4.55
LhcII2	LOC100282054	chlorophyll a-b binding protein	-9.73	-3.04
LhcII2	LOC100274453	chlorophyll a-b binding protein	-9.62	-3.57
LhcII3	LOC100284154	chlorophyll a-b binding protein	-8.65	-3.24
LhcII9	LOC103643653	chlorophyll a-b binding protein	-7.55	-3.33
LhcII2	LOC100281879	chlorophyll a-b binding protein	-7.39	-3.34
LhcIId (CP25)	LOC542321	chlorophyll a-b binding protein	-6.99	-3.45
LhcII3	LOC542530	chlorophyll a-b binding protein	-6.26	-2.65
LhcII5	LOC542716	chlorophyll a-b binding protein	-5.98	-3.12
LhcII6	LOC100283212	chlorophyll a-b binding protein	-5.66	-2.28
LhcIIa (CP29)	LOC542478	photosystem II subunit 29	-5.62	-2.96
LhcIIa (CP30)	LOC100216729	photosystem II subunit 29	-5.24	-3.07
LhcIId (CP24)	LOC100273752	photosystem II subunit 24	-4.82	-2.58
LhcII7	LOC100193833	chlorophyll a-b binding protein	-4.81	-2.36
LhcI3	LOC100282214	chlorophyll a-b binding protein	-4.61	-2.41
LhcII4	LOC100281795	chlorophyll a-b binding protein	-1.83	-1.67

794

795 Table 2. Effect of drought stress on the expression of the photosynthetic electron
796 transport related genes in maize leaves.

Gene name	Gene ID	Description	log2 (Fold change)	
			SD902	SD609
PsbO1 (OEE1)	LOC100272890	oxygen-evolving enhancer protein 1	-3.71	-2.56
PsbO2 (OEE2)	LOC100191684	oxygen-evolving enhancer protein 1.2	-3.77	-2.88
PsbP1 (MSP1)	LOC107648855	oxygen-evolving enhancer protein 2.1	-3.29	-2.29
PsbP2 (MSP2)	LOC100281199	oxygen-evolving enhancer protein 2.2	-3.31	-2.11
PsbP3 (MSP3)	LOC100273117	oxygen-evolving enhancer protein 2.3	-5.04	-3.07
OEE3	LOC103653672	oxygen-evolving enhancer protein 3	1.35	–
OEE3-2	LOC103627333	oxygen-evolving enhancer protein 3.2	-1.74	-1.68
OEE3-3	PSBQ1	oxygen-evolving enhancer protein 3.3	-4.09	-2.65
OEE3-4	LOC103647735	oxygen-evolving enhancer protein 3.4	-7.81	-3.33
PsbY	LOC100280994	photosystem II reaction center Y protein	-4.15	-3.29
PsbW	cl5838_2	photosystem II reaction center PsbW protein	-2.59	-1.83
PsbW	pco070877	photosystem II reaction center PsbW protein	-3.63	-2.47
PsbR	LOC100281646	photosystem II 10 kDa protein	-1.95	–
PsbS	psbs1	Photosystem II 22 kDa protein	-2.01	-1.26
PsaD	LOC100191984	photosystem I reaction center subunit II	-4.31	-2.29
PsaD	LOC541791	photosystem I reaction center subunit II	-4.88	-1.97
PsaF	LOC100282027	photosystem I reaction center subunit III	-3.33	-2.11
PsaF	LOC103625835	photosystem I reaction center subunit III	-4.41	-2.49
PsaE	LOC100283327	photosystem I reaction center subunit IV	-3.78	-2.28
PsaE	gpm930	photosystem I reaction center subunit IV	-3.88	-2.29
PsaG	LOC100285458	Photosystem I reaction center subunit V	-3.96	-2.28
PsaG	LOC100284847	Photosystem I reaction center subunit V	-4.16	-2.44
PsaH	psah1	photosystem I reaction center subunit VI	-4.22	-2.18
PsaK	psa6	photosystem I reaction center subunit X	-6.10	-2.84
PsaL	LOC100281679	photosystem I reaction center subunit XI	-2.83	-1.36
PsaL	umc1974	photosystem I reaction center subunit XI	-4.25	-2.28
PsaN	LOC103640891	photosystem I reaction center subunit PsaN	-3.57	-2.46
PsaN	LOC542605	photosystem I reaction center subunit PsaN	-4.05	-2.43
petB (cyt b6)	LOC100273026	cytochrome b6	-2.69	-1.19
petB (cyt b6)	ris2	cytochrome b6	-2.82	-1.52
petE (PC)	LOC103629356	plastocyanin	-2.75	-1.51
petE (PC)	LOC100192779	plastocyanin	-5.04	-2.50
petF (Fd)	LOC100382495	ferredoxin	7.79	10.94
petF (Fd)	LOC100281226	ferredoxin	3.01	–
petF (Fd)	pco072676(750)	ferredoxin	1.97	–
petF (Fd)	fdx3	ferredoxin	1.52	–
petF (Fd)	LOC100284745	ferredoxin	-1.79	-1.53
petF (Fd)	fdx5	ferredoxin	-2.41	-1.56
petF (Fd)	fdx2	ferredoxin	-2.83	-1.49
petF (Fd)	FDX1	ferredoxin	-2.98	-2.11
petF (Fd)	LOC100283643	ferredoxin	–	1.27

797

798 Table 3. Effect of drought stress on the expression of the energy metabolism related
799 genes in maize leaves.

Gene name	Gene ID	Description	log2 (Fold change)	
			DS902	DS609
ATPase γ	LOC100284505	ATP synthase gamma chain	-2.83	-2.28
ATPase δ	LOC100281924	ATP synthase delta chain	-2.93	-1.89
ATPase δ	LOC103627748	ATP synthase delta chain	-3.08	-2.15
ATPase b	LOC100282566	ATP synthase subunit b	-2.63	-1.49
rbcS	LOC542212	ribulose-bisphosphate carboxylase small chain 1	-2.86	-2.46
rbcS	LOC100279574	ribulose-bisphosphate carboxylase small chain 2	-2.84	-1.80
PPDK	LOC542759	pyruvate orthophosphate dikinase	-3.86	-1.83
PPDK	LOC103635678	pyruvate orthophosphate dikinase	-5.57	-2.69
PEPC	LOC542372	phosphoenolpyruvate carboxylase	-4.37	-2.71
PEPC	LOC542479	phosphoenolpyruvate carboxylase	1.87	1.46
PEPC	LOC103642664	phosphoenolpyruvate carboxylase	-4.32	-3.01
PEPCK	LOC541622	phosphoenolpyruvate carboxykinase	-3.69	-2.44
PEPCK	LOC100279748	phosphoenolpyruvate carboxykinase	–	1.44
MDH	LOC100282134	malatedehydrogenase	-1.29	–
MDH	mdh5	malatedehydrogenase	-2.49	-1.57
MDH	LOC107305678	malatedehydrogenase	-5.38	-3.31
MDH	LOC103648465	malatedehydrogenase	–	3.29
NADP-ME	me4	malatedehydrogenase (NADP+)	6.08	–
NADP-ME	me3	malatedehydrogenase (NADP+)	-2.92	-2.25
NADP-ME	mdh6	malatedehydrogenase (NADP+)	-1.49	-1.17
SBPase	shbp1	sedoheptulose-1,7-bisphosphatase	-2.03	-1.50

800

801 Table 4. Effect of drought stress on the expression of the sugar metabolism related genes
802 in maize leaves.

Gene name	Gene ID	Description	log2 (Fold change)	
			DS902	DS609
Starch degradation				
α-amylase	LOC103651265	alpha-amylase	5.52	1.93
α-amylase	LOC100383492	alpha-amylase	-2.70	–
β-amylase	LOC100192000	beta-amylase	-1.03	–
β-amylase	LOC100194176	beta-amylase	-1.23	-1.66
β-amylase	pco104637	beta-amylase	-2.12	-1.97
β-amylase	LOC103637673	beta-amylase	-3.29	-1.64
β-amylase	LOC100502394	beta-amylase	-3.40	-1.35
β-amylase	LOC100382206	beta-amylase	-6.86	-3.54
Starch biosynthesis				
SS	du1	starch synthase	4.34	8.13
SS	LOC542481	starch synthase	2.57	–
SS	LOC100170236	starch synthase	-1.24	-1.07
SS	LOC100101526	starch synthase	-1.66	-1.19
SS	LOC100136828	starch synthase	-1.76	-2.55
SS	gss1	starch synthase	-2.23	-2.14
SS	LOC100101527	starch synthase	-3.37	-2.06
SS	LOC100101528	starch synthase	-3.70	-1.88
SS	ss1	starch synthase	–	1.13
GBSS	LOC111589219	granule-boundstarchsynthase	6.34	–
GBSS	GBSSIIa	granule-boundstarchsynthase	-5.44	-2.79
HK	LOC100279587	hexokinase	1.36	–
HK	LOC100283735	hexokinase	–	1.69
HK	LOC103651223	hexokinase	–	-1.20
Sucrose degradation				
–	IVR1	beta-fructofuranosidase	-1.25	-1.55
–	LOC109943123	beta-fructofuranosidase	-2.26	–
–	sus1	sucrose synthase	-2.53	-1.07

Gene name	Gene ID	Description	log2 (Fold change)	
			DS902	DS609
Cellulose degradation				
–	glu1	beta-glucosidase	7.39	3.83
–	TIDP3759	beta-glucosidase	4.98	–
–	pco088410	beta-glucosidase	4.87	2.02
–	LOC103634742	beta-glucosidase	2.28	–
–	LOC111591355	beta-glucosidase	1.87	2.24
–	glu2	beta-glucosidase	1.85	–
–	gpm205	beta-glucosidase	1.64	–
–	LOC100192939	beta-glucosidase	1.09	–
–	LOC100279909	beta-glucosidase	-1.12	–
–	si486037a06	beta-glucosidase	-1.15	-1.37
–	LOC100279348	beta-glucosidase	-1.35	-1.52
–	umc1721	beta-glucosidase	-2.66	–
–	LOC100501315	beta-glucosidase	-3.07	-2.84
–	LOC103650610	beta-glucosidase	-7.43	-3.28
–	LOC100174972	beta-glucosidase	-7.86	-3.22
–	LOC100502335	beta-glucosidase	–	1.30
–	cl1052_1	beta-glucosidase	–	-1.21
–	LOC103646225	beta-glucosidase	–	-1.60
–	LOC100272792	beta-glucosidase	–	-2.19
Sucrose biosynthesis				
–	LOC542661	sucrose-6-phosphatase	-1.04	–
SPS	LOC103650849	sucrose-phosphate synthase	-1.13	-1.05
SPS	LOC100501516	sucrose-phosphate synthase	-1.52	-1.18
SPS	LOC100275248	sucrose-phosphate synthase	-1.58	-1.37
SPS	LOC103626734	sucrose-phosphate synthase	-2.07	-1.05
SPS	sps1	sucrose-phosphate synthase	-3.69	-2.51
SPS	LOC100384650	sucrose-phosphate synthase	–	1.12
SPS	LOC100278478	sucrose-phosphate synthase	-1.36	–

803

804 Table 5. Effect of drought stress on the expression of the Molecular chaperones and
805 heat shock proteins related genes in maize leaves.

Gene ID	Description	log2 (Fold change)	
		SD902	SD609
hsp101	ATP-dependent Clpprotease ATP-binding subunit ClpB	–	4.05
LOC100217072	ATP-dependent Clpprotease ATP-binding subunit ClpB	2.03	3.30
pco093519b	ATP-dependent Clpprotease ATP-binding subunit ClpB	–	1.85
uaz98	ATP-dependent Clpprotease ATP-binding subunit ClpB	–	1.18
LOC103647314	calreticulin	1.17	4.26
crt2	calreticulin	1.22	2.66
LOC100273288	calreticulin	-1.04	–
LOC100191560	cell division protease FtsH	–	2.60
LOC100502422	cell division protease FtsH	–	1.43
LOC100502182	cell division protease FtsH	-1.25	–
pco103778a	cell division protease FtsH	-1.30	–
LOC103633537	chaperonin GroEL	8.41	3.97
CPNA	chaperonin GroEL	2.83	3.00
CPN60II	chaperonin GroEL	1.42	2.46
LOC100280498	chaperonin GroEL	2.05	1.78
LOC100272259	chaperonin GroEL	1.60	1.71
LOC100280498	chaperonin GroEL	2.05	–
LOC103629384	chaperonin GroEL	1.98	–
LOC542185	chaperonin GroEL	1.29	–
LOC100285797	chaperonin GroES	3.14	4.55
LOC103647892	chaperonin GroES	2.65	4.34
pco135715c	chaperonin GroES	3.65	3.95
LOC100282681	chaperonin GroES	4.85	3.69
LOC103636392	chaperonin GroES	–	2.70
LOC100193174	chaperonin GroES	–	2.56
cl21464_1	chaperonin GroES	1.34	1.07
LOC100383971	chaperonin GroES	2.89	–
LOC100285374	molecular chaperone DnaK	1.07	1.66
LOC100286241	molecular chaperone DnaK	-1.31	–
pco137067a	molecular chaperone GrpE	–	1.52
pco153543(105)	molecular chaperone HtpG	–	4.58
hsp82	molecular chaperone HtpG	3.38	4.23
pco150589a	molecular chaperone HtpG	1.70	3.37
LOC103651583	molecular chaperone HscB	1.31	–
LOC100285108	heat shock protein 90kDa	–	2.99
LOC103629136	heat shock protein 90kDa	2.26	2.94
LOC100286003	heat shock protein 90kDa	1.83	2.86
LOC100279992	heat shock protein 90kDa	3.25	2.50
shpl1	heat shock protein 90kDa	–	2.02
LOC100383793	heat shock protein 90kDa	3.25	1.47
pco094428	heat shock protein 70kDa	4.85	4.96
LOC100501536	heat shock protein 70kDa	2.58	3.55
LOC103651028	heat shock protein 70kDa	–	2.65
LOC103635762	heat shock protein 70kDa	-1.22	2.43
pco083553	heat shock protein 70kDa	–	2.16
LOC103652717	heat shock protein 70kDa	–	1.36
LOC100280354	heat shock protein 70kDa	–	1.30
pco134267	heat shock protein 70kDa	3.56	3.57
LOC103630125	heat shock protein 70kDa	–	2.61
LOC100285213	heat shock protein 70kDa	–	1.27
LOC100281415	heat shock protein 70kDa	–	1.24
LOC103637376	heat shock protein 70kDa	–	5.91
bip2	heat shock protein 70kDa	–	2.66
bip1	heat shock protein 70kDa	–	4.60
LOC103625886	heat shock protein 71kDa	-1.41	–
LOC100286168	heat shock protein 72kDa	1.31	–
pco083553	heat shock protein 73kDa	-2.33	–
LOC100275241	hsp70-interacting protein	1.14	2.22
LOC100279983	hsp71-interacting protein	–	2.12
LOC100383105	heat shock protein 20kDa	–	9.03
LOC100191552	heat shock protein 20kDa	6.12	8.45
hsp18c	heat shock protein 20kDa	7.23	8.35
TIDP2749	heat shock protein 20kDa	2.83	7.98
hsp26	heat shock protein 20kDa	7.88	7.67
LOC103639183	heat shock protein 20kDa	–	7.66
hsp22	heat shock protein 20kDa	3.50	7.61
hsp18f	heat shock protein 20kDa	7.71	6.75
hsp18a	heat shock protein 20kDa	–	6.61
LOC100282088	heat shock protein 20kDa	7.03	6.09
LOC100286044	heat shock protein 20kDa	8.94	6.00
LOC100280576	heat shock protein 20kDa	7.46	5.91
LOC100284772	heat shock protein 20kDa	7.43	5.70
LOC100281798	heat shock protein 20kDa	–	5.09
LOC103634220	heat shock protein 20kDa	–	4.01
LOC100282956	heat shock protein 20kDa	3.00	3.87
IDPC175	heat shock protein 20kDa	–	3.74
LOC100283239	heat shock protein 20kDa	1.29	3.49
LOC100191598	heat shock protein 20kDa	–	2.80
LOC100276133	heat shock protein 20kDa	–	2.56
LOC103634805	heat shock protein 20kDa	2.42	2.19
LOC103633231	heat shock protein 20kDa	2.71	1.92
LOC103654949	heat shock protein 20kDa	-4.34	-1.34
LOC100285576	heat shock protein 20kDa	–	-1.64
LOC100283886	heat shock protein 21kDa	1.70	–

806

807 Table 6. Effect of drought stress on the expression of the MAPK and phytohormone
808 related genes in maize leaves.

Gene name	Gene ID	Descrition	Log2 (Fold change)	
			SD902	SD609
MAPK signling pathway				
MAPK1	LOC100281832	MAP kinase 1	-1.42	–
MAPK8	cl4121_1	Mitogen-activated protein kinase 8	-3.32	-2.48
MKK4	LOC109946070	Mitogen-activated protein kinase kinase 4	-1.57	–
MKK9	LOC107326007	Mitogen-activated protein kinase kinase 9	-2.62	-1.85
MKKK17	LOC103636208	Mitogen-activated protein kinase kinase kinase 17	-3.44	–
MKKK17	LOC103652527	Mitogen-activated protein kinase kinase kinase 17	-3.24	–
MKKK17	LOC103630679	Mitogen-activated protein kinase kinase kinase 17	-1.64	–
MKKK17	LOC107403149	Mitogen-activated protein kinase kinase kinase 17	-2.00	–
MKKK17	LOC103637414	Mitogen-activated protein kinase kinase kinase 17	-2.41	–
ABA signaling pathway				
PYR/PYL	LOC100216590	abscisic acid receptor PYR/PYL family	5.55	–
PYR/PYL	LOC103634514	abscisic acid receptor PYR/PYL family	-2.15	–
PYR/PYL5	PYL5	abscisic acid receptor PYR/PYL family	-3.16	–
PYR/PYL	LOC100383950	abscisic acid receptor PYR/PYL family	-2.45	-1.39
PYR/PYL	LOC100274679	abscisic acid receptor PYR/PYL family	-2.45	-1.55
PP2C	LOC100279578	protein phosphatase 2C	4.20	–
PP2C	LOC103646787	protein phosphatase 2C	6.78	–
PP2C12	PP2C12	protein phosphatase 2C	5.19	–
PP2C	LOC100273512	protein phosphatase 2C	8.26	–
Ethylene signaling pathway				
–	LOC100191919	Ethylene-responsive transcription factor	-1.63	–
–	LOC103647946	Ethylene-insensitive protein 3	–	4.04
JA signaling pathway				
JAR1	cl2682_1	Jasmonic acid-smonic synthetase	-2.44	-1.79
Cytokinin signaling pathway				
HPK	HK1b2	Histidine protein kinase 2 (cytokinin receptor)	-2.43	-1.61
HPK	hk3	Histidine protein kinase 2 (cytokinin receptor)	–	-1.57

Gene name	Gene ID	Descrition	Log2 (Fold change)	
			SD902	SD609
Auxin signaling pathway				
SAUR	LOC103632809	SAUR family protein	6.76	–
SAUR	LOC100191931	SAUR family protein	-1.54	–
SAUR	umc1194	SAUR family protein	-1.79	–
SAUR	LOC100281845	SAUR family protein	-2.44	-1.58
SAUR	LOC103627198	SAUR family protein	-2.04	–
SAUR	LOC103627479	SAUR family protein	-1.05	–
SAUR	LOC100282558	SAUR family protein	–	5.60
SAUR	LOC103644435	SAUR family protein	–	4.98
SAUR	LOC103643117	SAUR family protein	–	4.12
SAUR	LOC103627798	SAUR family protein	–	-1.17
SAUR	LOC103654922	SAUR family protein	–	-2.00
IAA	umc1527	auxin-responsive protein IAA	-3.56	–
IAA	AUX15	auxin-responsive protein IAA	-1.09	–
IAA	umc1460	auxin-responsive protein IAA	-1.73	-2.40
IAA	pco137466(715)	auxin-responsive protein IAA	-1.53	–
IAA	TIDP3717	auxin-responsive protein IAA	-2.56	–
IAA	LOC100284398	auxin-responsive protein IAA	-4.98	-2.39
IAA	LOC100191581	auxin-responsive protein IAA	-1.68	-1.08
IAA	LOC100281448	auxin-responsive protein IAA	-2.05	–
IAA	AUX3	auxin-responsive protein IAA	-2.20	-1.41
IAA	LOC103642379	auxin-responsive protein IAA	–	6.85
IAA	LOC100283579	auxin-responsive protein IAA	–	-1.80
ARF	pco123854	auxin response factor	-1.09	–
ARF	LOC103646418	auxin response factor	-1.01	-1.02

809

Figure legends

Fig.1 Effects of drought stress on maize (A) phenotypes, (B-G, I-K) physiological index, (H) chlorophyll concentration and (L-O) gas exchange parameters. The data shown are the means of three replicates (± SD) based on Duncan's multiple range test. Means denoted with the same letter did not significantly differ at $P < 0.05$. DS, drought stress treatment; CK, well-watered treatment.

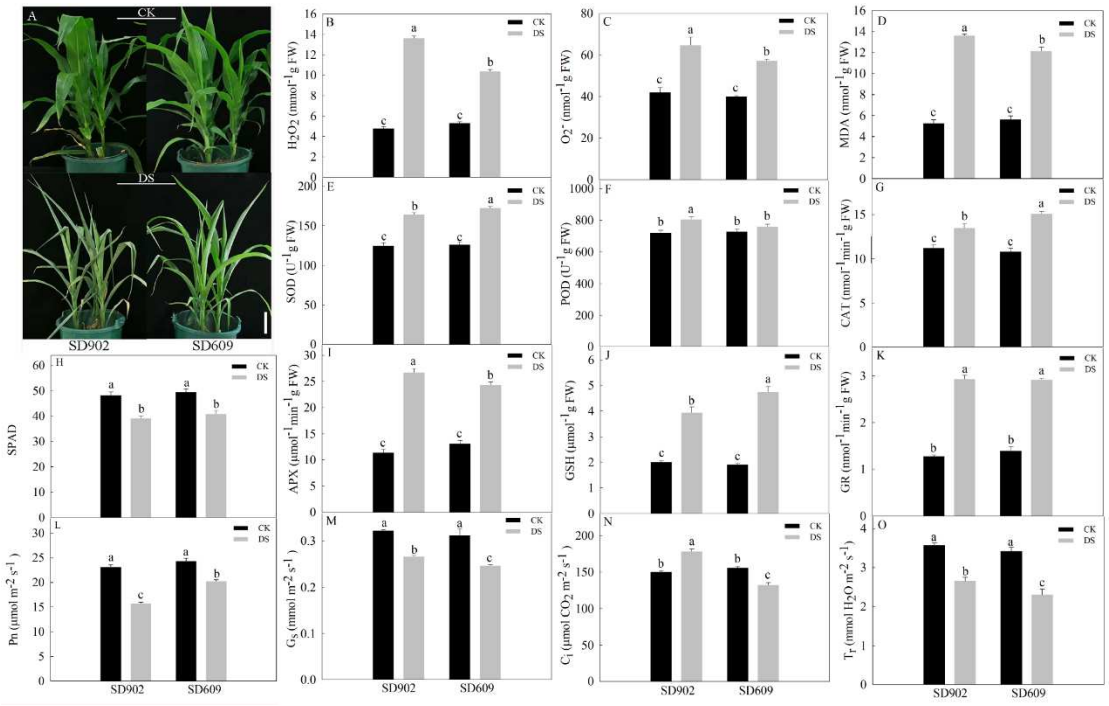


Fig.2 Effects of drought stress on the genome-wide expression profiles of SD902 and SD609 based on RNA-seq. (A) The bar graph shows the up-regulated and down-regulated DEGs in two maize materials. (B) Venn diagram shows the numbers of DEGs. (C) The enrichment analysis of biology process of GO in SD902 and SD609.

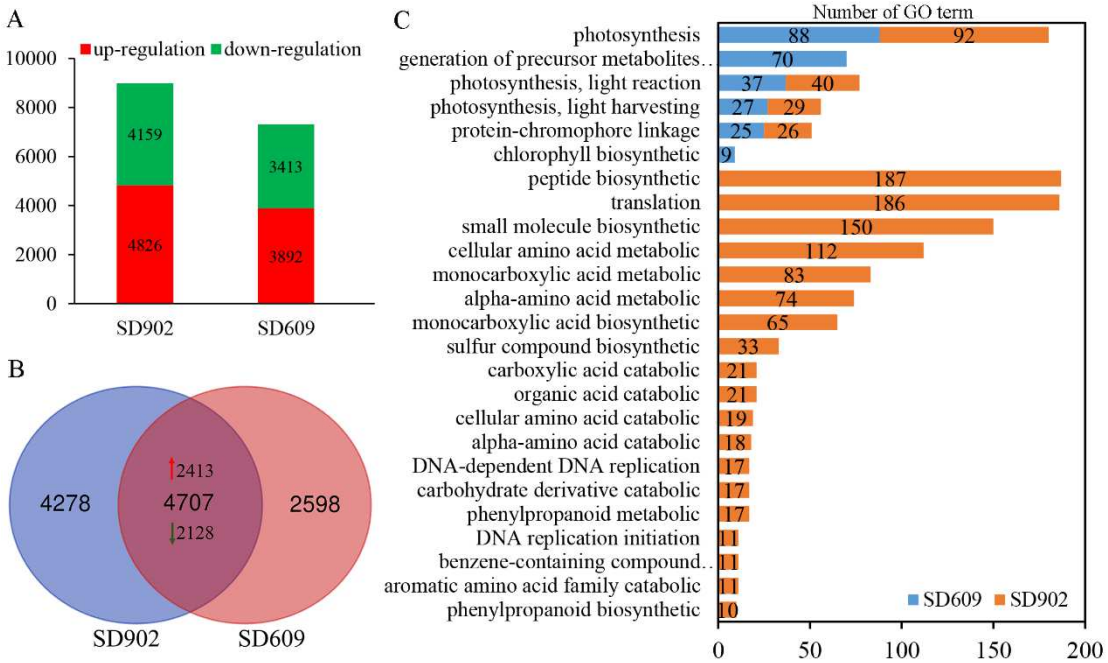


Fig.3 Effects of drought stress on the (A-H) photosynthesis feature and (I-P) carbon assimilation in SD902 and SD609. The data shown are the means of three replicates (± SD). Means denoted with the same letter did not significantly differ at $P < 0.05$. DS, drought stress treatment; CK, well-watered treatment. (I-L) The correlation analysis of enzyme activity was obtained from the enzyme-linked immunosorbent assay (ELISA) Kit. (M-P) The expression level of genes was obtained from qRT-PCR.

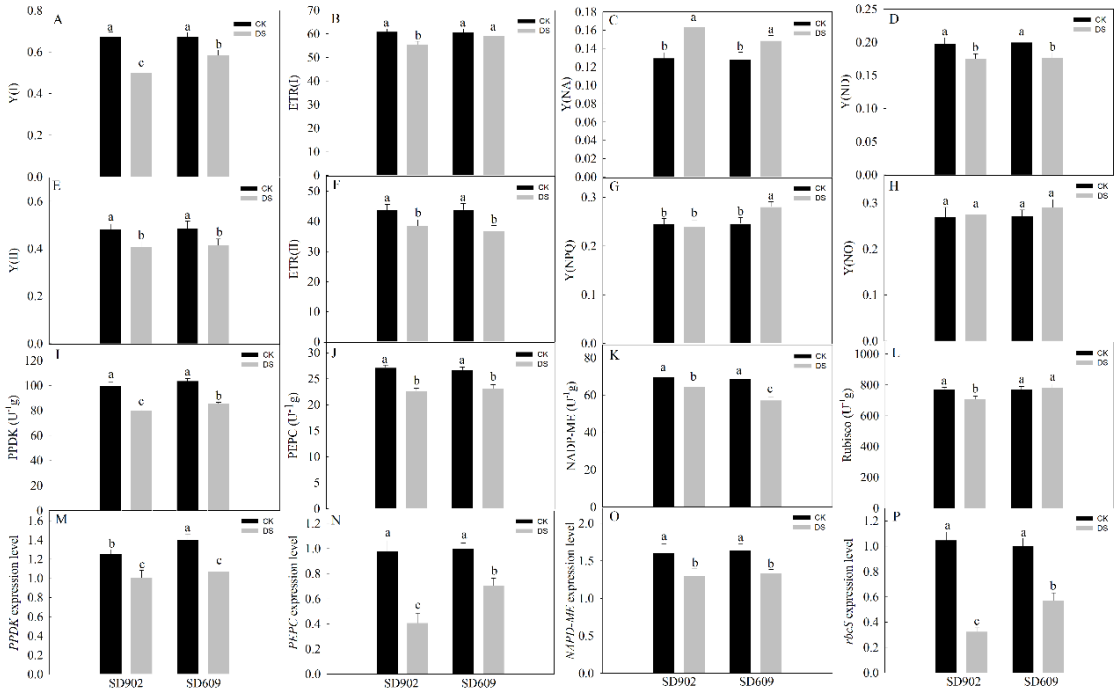


Fig.4 Effects of drought stress on the transcription factors regulation network in SD902 and SD609. (A) The transcription factors were obtained by RNA-seq. (B) The visual regulation network from transcription factors was obtained by online data STRING and cytoscape software.

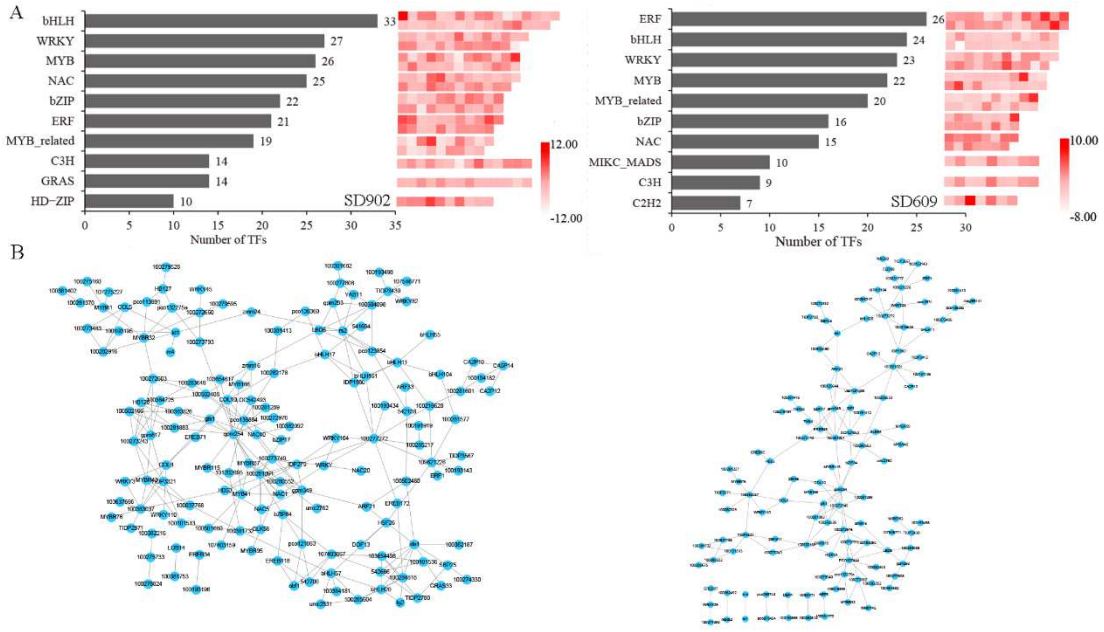
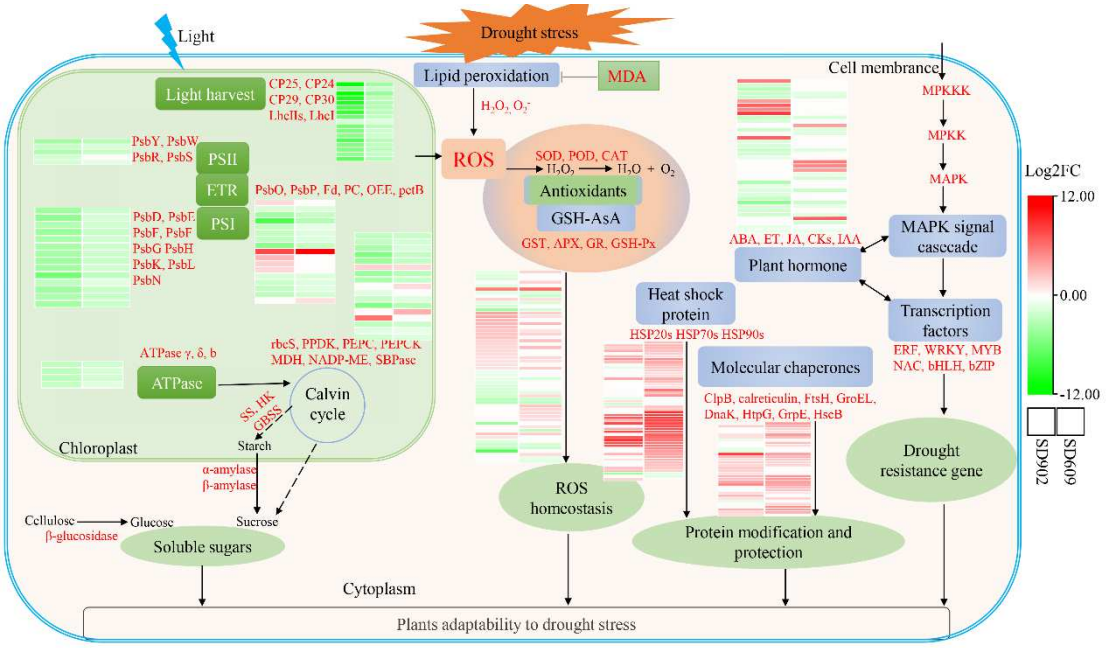


Fig.5 Drought adaption strategy at the molecular level in SD902 and SD609 plants exposed to drought stress condition. Heat maps were summarized by Log2 (FC) of mRNA levels. Red words represent DEGs in two maize seedings.



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