Wang C *et al.* (2022)
Notulae Botanicae Horti Agrobotanici Cluj-Napoca
Volume 50, Issue 3, Article number 12611
DOI:10.15835/nbha50312611
Research Article

Identification of the glycerol-3-phosphate dehydrogenase (GPDH) gene family in wheat and its expression profiling analysis under different stress treatments

Chao WANG[1a], Zixian ZHOU[2b], Shan JIANG[1], Qiang LI[1], Licao CUI[2*], Yong ZHOU[2*]

[1]*Huazhong Agricultural University, College of Plant Science and Technology, Wuhan 430070, China; jiangshan_0328@163.com; sergioramos@126.com; qli@mail.hzau.edu.cn*
[2]*Jiangxi Agricultural University, College of Bioscience and Bioengineering, Jiangxi Engineering Laboratory for the Development and Utilization of Agricultural Microbial Resources, Nanchang 330045, China; zixianzhou@163.com; cuilicao@jxau.edu.cn; yongzhou@jxau.edu.cn (*corresponding author)*
[a,b]*These authors contributed equally to the work*

Abstract

Glycerol-3-phosphate dehydrogenase (GPDH) catalyses the interconversion of glycerol-3-phosphate (G3P) and dihydroxyacetone phosphate (DHAP), and plays key roles in different developmental processes and stress responses. *GPDH* family genes have been previously investigated in various plant species, such as *Arabidopsis*, maize, and soybean. However, very little is known in *GPDH* family genes in wheat. In this study, a total of 17 *TaGPDH* genes were identified from the wheat genome, including eight cytosolic *GPDHs*, six chloroplastic *GPDHs* and three mitochondrial *GPDHs*. Gene duplication analysis showed that segmental duplications contributed to the expansion of this gene family. Phylogenetic results showed that TaGPDHs were clustered into three groups with the same subcellular localization and domain distribution, and similar conserved motif arrangement and gene structure. Expression analysis based on the RNA-seq data showed that *GPDH* genes exhibited preferential expression in different tissues, and several genes displayed altered expression under various abiotic stresses. These findings provide the foundation for further research of wheat *GPDH* genes in plant growth, development and stress responses.

Keywords: abiotic stress; expression profile; glycerol-3-phosphate dehydrogenase (GPDH); phylogeny; wheat

Introduction

Glycerol-3-phosphate dehydrogenase (GPDH) is an essential enzyme in glycerol-3-phosphate (G3P) biosynthesis and metabolism. G3P can be generated directly from dihydroxyacetone phosphate (DHAP) via NAD^+-dependent GPDH, which catalyses the conversion of DHAP to G3P (Vigeolas *et al.*, 2007; Xue *et al.*, 2017; He *et al.*, 2020). As an important intermediate, G3P plays crucial roles in several metabolic processes,

Received: 01 Jul 2022. Received in revised form: 12 Aug 2022. Accepted: 22 Aug 2022. Published online: 06 Sep 2022.
From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

such as glycolysis, glycerol and glycerolipid metabolism (Vigeolas and Geigenberger, 2004; Chanda *et al*., 2011; Driver *et al*., 2017; Zhao *et al*., 2021a). Importantly, G3P plays a key role in G3P shuttle which is a complex process that transfers electrons from cytosolic NADH to the mitochondrial electron transport chain (Mráček *et al*., 2013). In this pathway, a cytoplasmic NAD^+-dependent GPDH (EC 1.1.1.8) and a FAD-dependent mitochondrial GPDH (EC 1.1.5.3) were commonly components in G3P shuttle and take part in keeping balance of the NADH/NAD^+ ratio (Shen *et al*., 2003; Shen *et al*., 2006).

Two classes of GPDH enzymes were identified in various species depending on their associated cofactors, namely NAD^+-GPDH and FAD-GPDH (Wu *et al*., 2019). Different GPDH isoforms possess diverse protein structures and subcellular localizations. In general, NAD^+-GPDHs are located in the cytoplasm or chloroplast and harboured a NAD^+-binding domain (PF01210) and/or a NAD^+-GPD domain (PF07479), while FAD-GPDHs are mitochondrion-localized and possess FAD-dependent oxidoreductase domain (DAO, PF01266) and an alpha-glycerophosphate oxidase domain (DAO_C, PF16901) (Zhao *et al*., 2018; Wu *et al*., 2019).

So far, a number of *GPDH* genes have been well characterized, and some reports highlighted the key roles of them in intracellular glycerol production and lipid accumulation. For example, seed-specific overexpression of a yeast gene encoding the cytosolic GPDH (GPD1) in oil-seed rape (*Brassica napus* L.) led to a 40% increase in the final lipid content of the seed while the seed protein content was unchanged (Vigeolas *et al.,* 2007). Similarly, seed-specific overexpression of the yeast *GPD1* can also increase seed mass, seed size and seed yield in transgenic *Camelina sativa*, and co-expression of the yeast *ScGPD1* and *Arabidopsis diacylglycerol acyltransferase1* (*DGAT1*) genes in transgenic *C. sativa* plants resulted in obviously higher oil harvest index, seed and oil yields on a dry weight basis than the wild type or plants overexpressing *ScGPD1* and *DGAT1* alone (Chhikara *et al.,* 2018). When compared with the wild type, both of *Chlamydomonas reinhardtii CrGPDH2* and *CrGPDH3* knockdown lines displayed reduced lipid accumulation during nitrogen and phosphorus starvation, while only GPD3 overexpression lines showed reduced glycerol concentration and altered to lipid composition (Driver *et al.,* 2017). There are five GPDH isoforms in *Arabidopsis*, with two cytosolic NAD^+-GPDHs, two plastidic NAD^+-GPDHs and one mitochondria FAD-GPDH, respectively. A plastidic NAD^+-GPDH gene, *AtGPDHp2* (*SFD1*/*GLY1*, At2G40690) is required for glycerolipid synthesis in the chloroplasts, and it can increase plastidic lipid content when overexpression in transgenic rice plants (Nandi *et al.,* 2004; Lorenc-Kukula *et al.,* 2012; Singh *et al.,* 2016). A recent study revealed that transgenic soybean plants overexpressing of a chloroplast-localized NAD^+-GPDH gene, *GmGPDHp1*, which is a homolog of *AtGPDHp2*, displayed increased oil content in mature seeds (Zhao *et al.,* 2021a). In addition, the function of GPDH family members in stress responses has also been reported. For example, both *C. reinhardtii CrGPDH2* and *CrGPDH3* can restore glycerol production and rescue the salt tolerance to the salt-sensitive *gpd1Δgpd2Δ* double mutant in yeast (Casais-Molina *et al.,* 2016). Overexpression of *ZmGPDH1*, a maize cytosolic NAD^+-dependent GPDH gene, conferred salinity and osmotic stress tolerance in transgenic *Arabidopsis* plants (Zhao *et al.,* 2019a).

To date, the *GPDH* gene family has been investigated and characterized at the whole-genome level in several organism, such as *Chlamydomonas reinhardtii* (Herrera-Valencia *et al*., 2012), maize (*Zea mays*) (Zhao *et al*., 2018), *Dunaliella salina* (Wu *et al*., 2019), and soybean (Zhao *et al*., 2021b). However, there have been no studies on the *GPDH* gene family in wheat so far. In this study, we identified the *GPDH* family members in wheat, and analysed their evolutionary relationships, chromosomal distributions, protein and gene structures, as well as tissue-specific expression and expression patterns under stress conditions. Our findings have shed light on the functions of *GPDH* genes in wheat.

Materials and Methods

Screening and identification of GPDH genes in the wheat genome

To identify GPDH proteins in wheat, the Basic Local Alignment Search Tool algorithm program (BLASTP) was performed against the wheat reference sequence in the Ensembl Plants database (http://plants.ensembl.org/Triticum_aestivum) and Phytozome (https://phytozome-next.jgi.doe.gov/) with the published *Arabidopsis* and rice GPDH protein sequences as queries. After removing the repeated sequences, the remaining candidate sequences were verified for the presence of NAD^+-binding domain (PF01210) or GPDH-type DAO domain (PF01266) by using the Pfam database (https://pfam.xfam.org/) and SMART (http://smart.embl-heidelberg.de/).

The protein sequences of the wheat GPDH genes were obtained from Ensembl Plants database, and the physical parameters including molecular weight (MW), isoelectric point (pI) and grand average of hydropathicity (GRAVY) of each TaGPDH protein were calculated using the ProtParam of the ExPasy website (http://www.expasy.org/). We used the ProtComp 9.0 server (http://linux1.softberry.com/berry.phtml) to analyze the subcellular localization of TaGPDH proteins.

Chromosomal location, gene duplication, synteny and phylogenetic analyses of TaGPDHs in wheat

The chromosomal location information of each wheat GPDH gene was visualized with the MapChart tool (Voorrips, 2002). Gene duplication events and synteny analysis were determined following the methods as previously described (Chen *et al*., 2020; Jin *et al*., 2020). Multiple sequence alignments of the GPDH proteins were performed using MAFFT (https://www.ebi.ac.uk/Tools/msa/mafft/) with default parameters. The NJ (Neighbour-Joining) phylogenetic tree was created using MEGA7.0 software with 1000 replicated-bootstraps (Kumar *et al*., 2016).

Conserved motif and gene structure analyses of TaGPDH genes in wheat

The online MEME website (http://meme-suite.org/) was employed to find the conserved motifs of TaGPDH proteins. The parameters were set as follows: the maximum number of motifs 10, and the optimum motif width from 6 to 50. The sequences of CDS and gDNA of the wheat GPDH genes were obtained from Ensembl Plants database, and the GSDS server was used to conduct the structural analysis of the wheat *GPDH* genes (Hu *et al*., 2015).

In silico expression analysis of TaGPDH genes

The expression patterns of *TaGPDH* genes in different tissues were retrieved from publicly available RNA-seq data at the Wheat Expression Browser (www.wheat-expression.com) (Borrill *et al*., 2016). For in silico expression analysis of expression during abiotic stress, RNA-seq data of the 'TAM 107' cultivar grown under drought and heat were downloaded from the NCBI Short Read Archive (SRA) database under the number of PRJNA257938 (Liu *et al*., 2015). And the expression values of each *TaGPDH* gene were analysed and presented as TPM (transcripts per million) according to the previous studies (Gholizadeh and Mirzaghaderi, 2020; He *et al*., 2021). The log2(TPM+1) values were used to create the heat map with the TBtools software as previously described (Liao *et al*., 2021).

Results

Genome-wide identification of GPDH gene family in wheat

Wheat GPDH family members were identified by BLASTP analysis using AtGPDH and OsGPDH members as queries. In total, 17 TaGPDHs were identified in the wheat genome, and they were designated from TaGPDH1-1A to TaGPDH17-Un based on their positions on the chromosomes (Table 1).

The length of cDNA and gDNA of *GPDH* genes ranged from 846 bp (*TaGPDH14-4A*) to 2734 bp (*TaGPDH6-3A*) and from 2903 bp (*TaGPDH1-1A*) to 6285 bp (*TaGPDH4-2B*), respectively. Correspondingly, the protein sequences of TaGPDHs ranged from 272 aa (TaGPDH14-4A) to 635 aa (TaGPDH4-2B), and their relative MWs, pIs, and GRAVYs varied between 29.56 to 68.40 kDa, 5.58 to 10.16, and -0.161 to 0.114, respectively (Table 1). In addition, eight, six and three TaGPDH proteins were predicted to localize on the cytoplasm, chloroplast, and mitochondrion, respectively (Table 1).

Table 1. Identification and characterization of *GPDH* gene family in wheat

Gene	Locus	Chromosome	Chromosomal position	gDNA (bp)	cDNA length (bp)	Domain (aa)				Protein				
						NAD_Gly3P_dh_N	NAD_Gly3P_dh_C	DAO	DAO_C	Length (aa)	MW (KDa)	pI	Gravy	Subcellular prediction
TaGPDH1-1A	TraesCS1A02G312400	1A	504,313,051-504,315,953	2903	1767	134-253	272-427	/	/	466	51.42	8.77	-0.146	Cytoplasm
TaGPDH2-1D	TraesCS1D02G312600	1D	408,431,786-408,435,115	3330	2118	129-247	267-422	/	/	461	51.13	8.77	-0.161	Cytoplasm
TaGPDH3-2A	TraesCS2A02G070500	2A	31,083,834-31,089,923	6090	2312	/	/	75-443	465-601	631	68.28	8.35	-0.095	Mitochondrion
TaGPDH4-2B	TraesCS2B02G084200	2B	47,167,045-47,173,329	6285	2335	/	/	79-447	469-605	635	68.40	8.59	-0.057	Mitochondrion
TaGPDH5-2D	TraesCS2D02G069500	2D	29,164,465-29,170,337	5873	1899	/	/	42-402	424-560	632	64.12	8.20	-0.089	Mitochondrion
TaGPDH6-3A	TraesCS3A02G330600	3A	575,310,438-575,314,943	4506	2734	137-256	275-431	/	/	469	51.68	7.60	-0.145	Cytoplasm
TaGPDH7-3A	TraesCS3A02G478100	3A	710,273,906-710,278,618	4713	1999	130-250	268-424	/	/	456	50.77	7.63	-0.118	Cytoplasm
TaGPDH8-3A	TraesCS3A02G523900	3A	739,152,544-739,156,445	3902	1686	10-181	200-348	/	/	366	39.97	5.58	0.009	Chloroplast
TaGPDH9-3B	TraesCS3B02G360600	3B	572,484,474-572,488,820	4347	2592	137-256	275-431	/	/	469	51.74	8.02	-0.150	Cytoplasm
TaGPDH10-3B	TraesCS3B02G521400	3B	764,693,235-764,697,597	4363	1909	130-250	268-424	/	/	456	50.77	7.64	-0.099	Cytoplasm
TaGPDH11-3D	TraesCS3D02G324000	3D	437,214,820-437,218,809	3990	2232	137-256	275-431	/	/	469	51.72	7.61	-0.149	Cytoplasm
TaGPDH12-3D	TraesCS3D02G473600	3D	575,125,986-575,130,294	4309	1819	130-251	268-424	/	/	456	50.82	7.64	-0.100	Cytoplasm
TaGPDH13-3D	TraesCS3D02G529400	3D	606,889,871-606,893,796	3926	1645	10-181	200-339	/	/	357	39.08	5.79	-0.022	Chloroplast
TaGPDH14-4A	TraesCS4A02G017300	4A	11,671,283-11,674,540	3258	846	87-247	/	/	/	272	29.56	10.16	-0.121	Chloroplast
TaGPDH15-4B	TraesCS4B02G286700	4B	570,141,148-570,146,552	5405	1876	102-262	281-422	/	/	433	46.64	9.31	0.114	Chloroplast
TaGPDH16-4D	TraesCS4D02G285400	4D	456,049,680-456,054,895	5216	1617	122-282	301-442	/	/	453	48.44	9.25	0.040	Chloroplast
TaGPDH17-Un	TraesCSU02G054700	Un	42,598,577-42,603,775	3769	1781	10-181	200-339	/	/	357	39.08	5.68	-0.022	Chloroplast

Phylogenetic analysis of GPDH gene family in wheat

To investigate the evolutionary history of the *GPDH* gene family, a NJ phylogenetic tree was created using five, six, and 17 GPDH protein sequences from *Arabidopsis*, rice, and wheat, respectively. The result showed that these GPDHs can be divided into three groups (Group A-C) (Figure 1). Amongst them, the

Group A contains the largest numbers of TaGPDHs (eight members), followed by Group B and C, which contains six and three TaGPDHs, respectively (Figure 1).

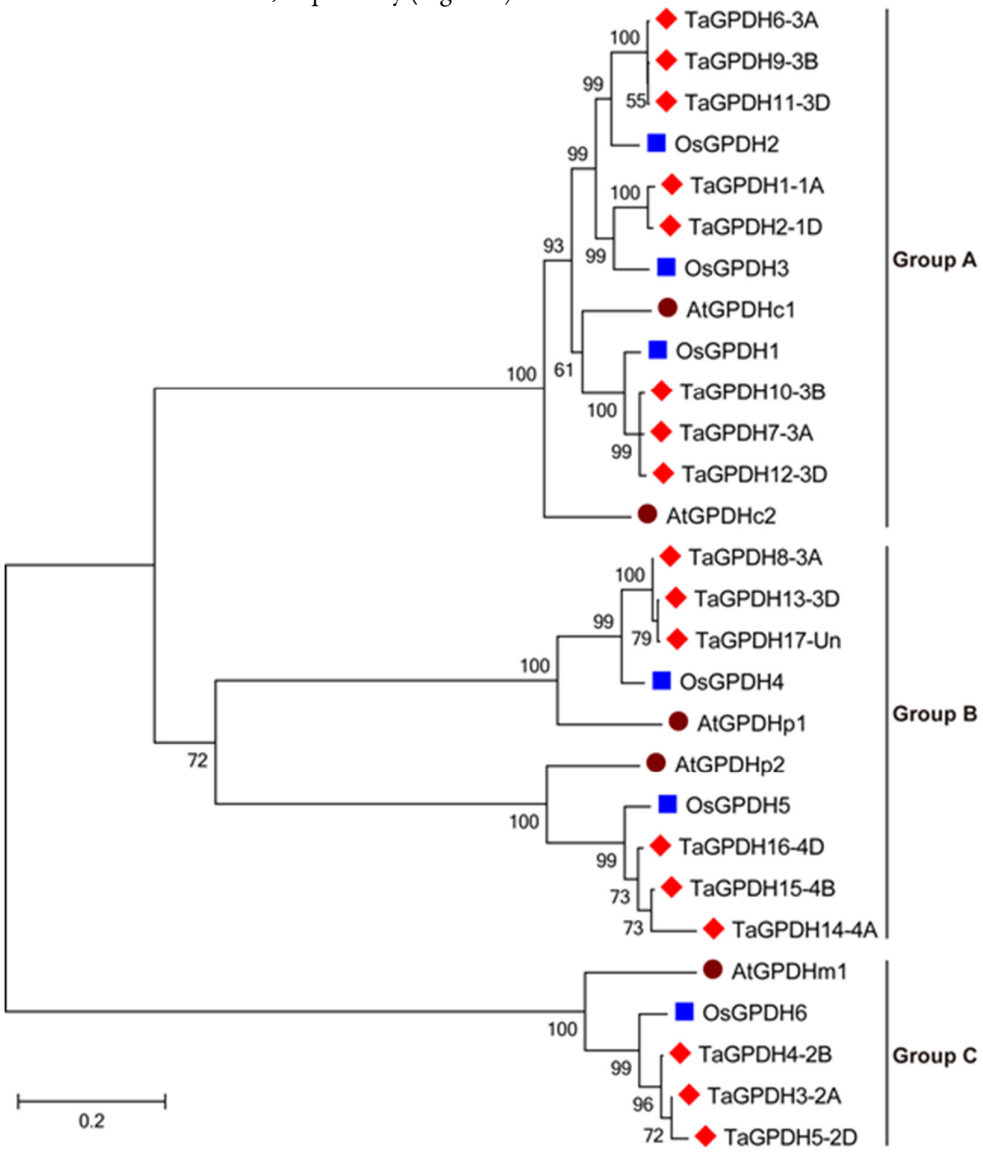

Figure 1. Phylogenetic tree of GPDH proteins of *Arabidopsis*, rice, and wheat. The full-length GPDH protein sequences of *Arabidopsis*, rice, and wheat were aligned with MAFFT and the NJ phylogenetic tree was constructed in MEGA 7.0 with 1000 bootstrap replications. The complete detail of GPDH proteins is provided in Table S1

Comparison of conserved motifs of TaGPDH proteins in wheat

Multiple sequence alignment results of the TaGPDH proteins were used to create a NJ phylogenetic tree and investigate the comparison of conserved motifs. Nearly all TaGPDHs clustered in Groups A and B contained a N-terminal NAD^+-binding domain (PF01210) and a C-terminal NAD^+-GPD domain (PF07479), with the exception of TaGPDH14-4A (Figure 2A, 2B). The remaining three TaGPDH proteins (TaGPDH3-2A, TaGPDH4-2B and TaGPDH5-2D) clustered in Group C contained a N-terminal FAD-dependent oxidoreductase domain (DAO, PF01266) and a C-terminal alpha-glycerophosphate oxidase domain (DAO_C, PF16901) (Figure 2A, 2B).

To further study the structural diversity of TaGPDH proteins, MEME analysis was carried out to predict the motif distribution and composition. As a result, a total of ten conserved motifs, designated as motifs 1–10, were identified (Figure 2C; Table S2). Of which, motif 3 was shared by all TaGPDH proteins. All TaGPDH proteins from Group A in the phylogenetic tree contained motifs 1–9 but lacked motif 10, while TaGPDH14-4A, TaGPDH15-4B, and TaGPDH16-4D from Group B harbored only motifs 7, 9 and 10 (Figures 2A, 2C). It is noteworthy that three TaGPDHs from Group B (TaGPDH8-3A, TaGPDH13-3D, and TaGPDH17-Un) and all TaGPDH proteins from Group C (TaGPDH3-2A, TaGPDH4-2B, and TaGPDH5-2D) possessed only motif 3 and motif 10, while TaGPDH3-2A, TaGPDH4-2B, and TaGPDH5-2D had double motif 3 in their C-terminal regions (Figures 2A, 2C).

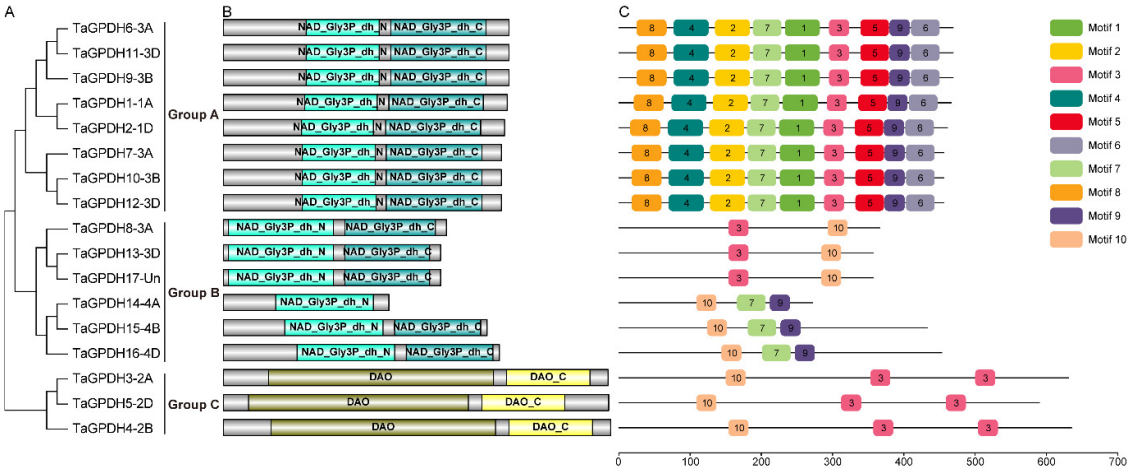

Figure 2. Phylogenetic relationships (A), schematic representation of conserved domains (B) and conserved motif arrangements (C) of TaGPDH proteins in wheat

Gene structure of TaGPDH genes in wheat

To study the structural characteristics of *TaGPDH* genes, we examined the gene structure of *TaGPDH* genes by using the GSDS tool, and found that all *TaGPDH* genes contain introns (Figure 3). The number of introns in the *TaGPDH* genes ranged from 4 to 10, with the greatest number of introns in the *TaGPDH8-3A*, *TaGPDH13-3D*, and *TaGPDH17-Un*. Gene structure analysis revealed that genes from Group A and Group C possessed have a similar structure and contained 4 and 5 introns, respectively, while genes from Group B a higher variation in the number of introns, ranged from 5 to 10 (Figure 3).

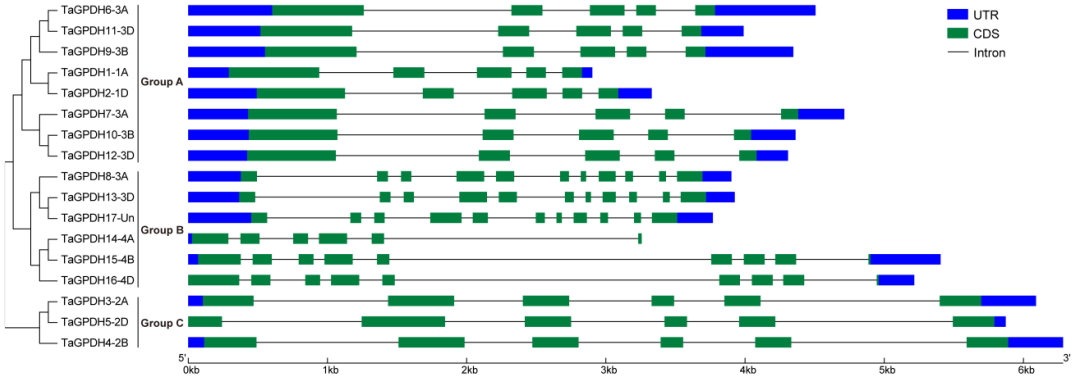

Figure 3. Gene structure analysis of the *TaGPDH* genes in wheat according to the phylogenetic relationship. The untranslated regions (UTRs), CDSs, and introns are indicated by blue frames, tawny frames, and gray lines, respectively

Chromosomal distribution and duplication analyses of TaGPDH genes

A total of 16 of 17 *TaGPDH* genes were unevenly distributed on 11 wheat chromosomes, and the information for each *TaGPDH* gene was visualized by using MapChart. There are three *TaGPDH* genes on chromosomes 3A and 3D, which contained the most *TaGPDH* genes, and two *TaGPDH* genes were located on chromosome 3B. In contrast, chromosomes 1A, 1D, 2A, 2B, 2D, 4A, 4B, and 4D each contain one *TaGPDH* gene (Figure 4).

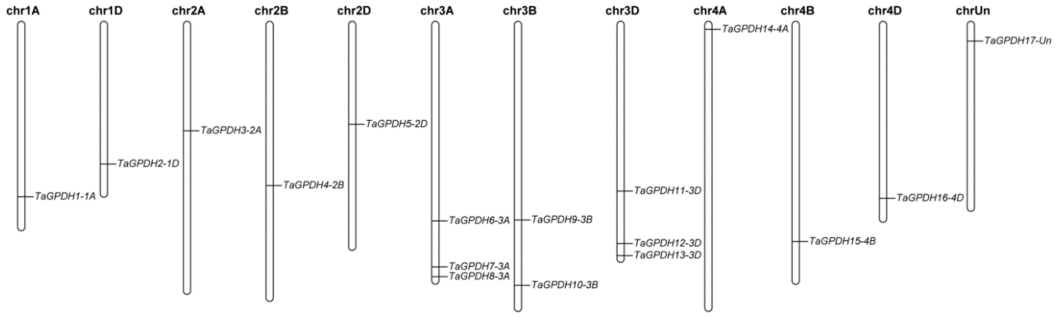

Figure 4. Chromosomal localization of the *TaGPDH* genes in the wheat genome

TBtools was used to investigate the duplication events of *TaGPDH* genes by using the full-length TaGPDH protein sequences. As a result, 16 *TaGPDH* genes were arranged in WGD/segmental duplication, and these genes made up 19 pairs of duplication events (Figure 5).

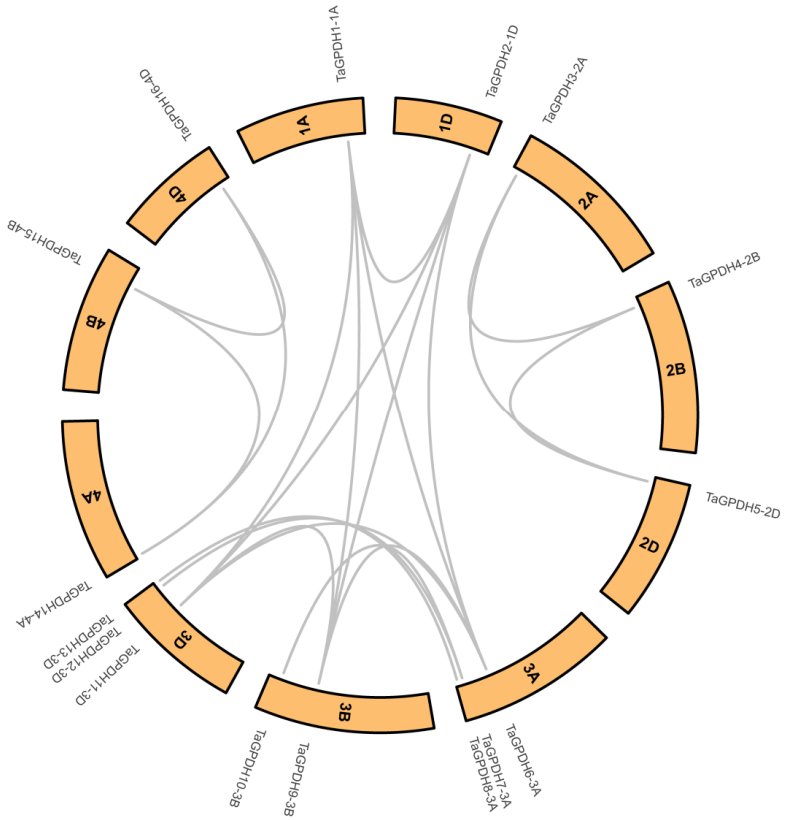

Figure 5. Duplication analysis of the *TaGPDH* genes in the wheat genome. The WGD/segmentally duplicated *TaGPDH* genes are connected with lines

Syntenic relationship analysis of GPDH genes

To study the phylogenetic mechanisms behind the extension of *GPDH* genes, synteny analysis of *GPDH* genes was conducted by MCScanX among wheat and its corresponding progenitor species, including *Aegilops tauschii* (DD), *T. urartu* (AA, green box), *T. turgidum* (AABB), and *T. dicoccoides* (AABB). The results showed that a total of 2, 10, 10, and 5 *GPDH* genes had a syntenic relationship between wheat and *T. urartu*, *T. turgidum*, *T. dicoccoides*, and *Ae. tauschii*, respectively (Figure 6). The results indicated that the two rounds of allopolyploidization made great contribution for the expansion of *GPDH* genes in wheat.

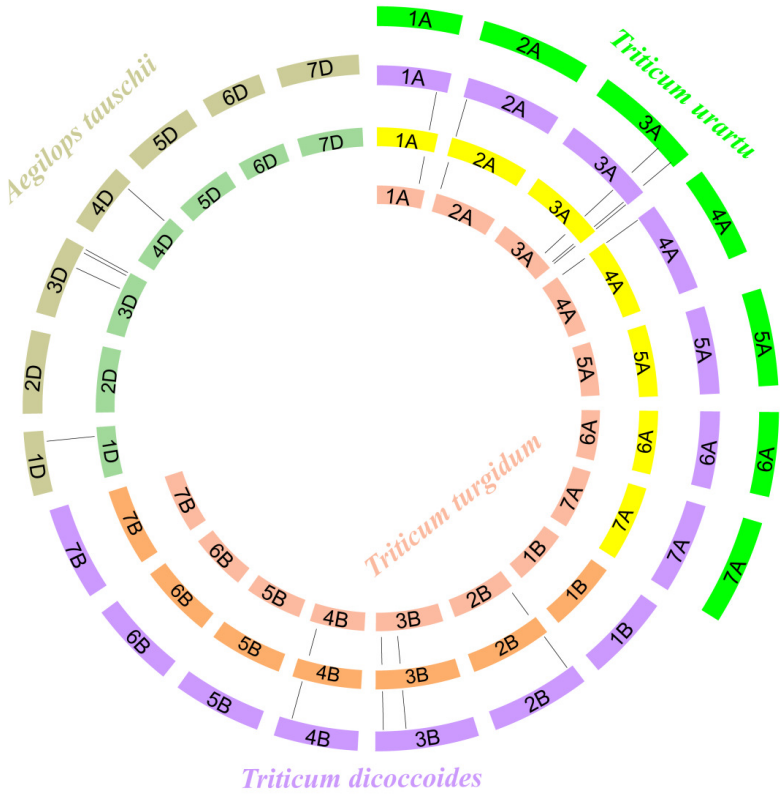

Figure 6. Syntenic relationship analysis of *GPDH* genes from wheat (*Triticum aestivum*, AABBDD) and its corresponding progenitor species *Aegilops tauschii* (DD), *T. urartu* (AA, green box), *T. turgidum* (AABB), and *T. dicoccoides* (AABB). The collinearity among *GPDH* genes is connected by the lines between orthologue gene pairs

Expression pattern of TaGPDH genes in different tissues

To investigate the potential function of *TaGPDH* genes in wheat growth and development, tissue expression profiling of the 17 *TaGPDH* genes were determined based on public RNA-seq data. As a result, all 17 *TaGPDH* genes were expressed in at least one tissue (TPM value > 1) (Figure 7). Of the Group A *TaGPDH* genes, *TaGPDH1-1A*, *TaGPDH2-1D*, *TaGPDH6-3A*, *TaGPDH9-3B*, and *TaGPDH11-3D* showed significant higher expression in anther, while *TaGPDH7-3A*, *TaGPDH10-3B*, and *TaGPDH12-3D* displayed the highest expression in spike (Figure 7). Of the Group B *TaGPDH* genes, *TaGPDH8-3A*, *TaGPDH13-3D*, and *TaGPDH17-Un* exhibited the highest transcript abundances in anthers but no detectable expression or low expression (TPM value < 1) in flag leaf blade, leaf ligule, internode, awn, glume, lemma, and endosperm (Figure 7). Instead, *TaGPDH14-4A*, *TaGPDH15-4B*, and *TaGPDH16-4D* exhibited roughly steady level of

expression in most of the tissues. Noticeably, all *TaGPDH* genes from Group C *(TaGPDH3-2A*, *TaGPDH4-2B*, and *TaGPDH5-2D*) exhibited constitutive expression in all detected tissues (TPM value > 1) (Figure 7).

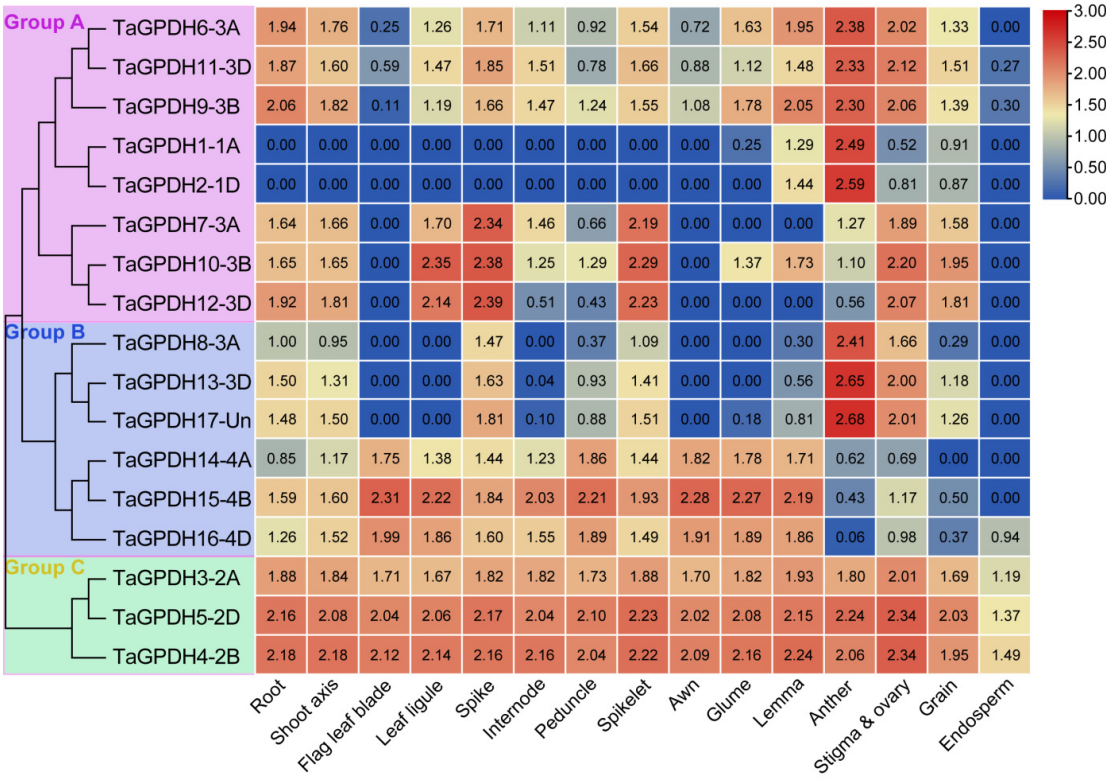

Figure 7. Expression pattern analysis of *TaGPDH* genes in different tissues. The expression data were presented as TPM values with log2 normalization

Expression pattern of TaGPDH genes under different stresses

To explore the possible roles of *TaGPDH* genes associated with plant abiotic stress responses, the transcript levels of *TaGPDH* genes under drought and heat stresses were examined based on the transcriptome data. Generally, several *TaGPDH* genes displayed observably induced/repressed expression under different stress treatments. Under drought stress, *TaGPDH2-1D* showed up-regulated expression at 1 h, while *TaGPDH15-4B* showed down-regulated expression at 6 h, when compared to the control (Figure 8). Under heat stress treatment, *TaGPDH2-1D* and *TaGPDH5-2D* were significantly induced at 1 h and 6 h, respectively, while *TaGPDH14-4A*, *TaGPDH15-4B*, and *TaGPDH16-4D* were notably down-regulated at 1 h (Figure 8). Under drought and heat combined stress, only *TaGPDH15-4B* and *TaGPDH16-4D* showed significantly down-regulated expression at 1 h compared to the control (Figure 8).

Discussion

In this study, 17 members of the GPDH family were identified from the wheat genome, including eight cytosolic GPDHs, six chloroplastic GPDHs and three mitochondrial GPDHs (Table 1). And the number of TaGPDHs identified in wheat was apparently higher than that in other plants, such as *Arabidopsis* (five AtGPDHs) (Wei *et al.,* 2001; Shen *et al.,* 2006; Zhao *et al.,* 2019b), maize (six ZmGPDHs) (Zhao *et al*., 2018),

and soybean (13 GmGPDHs) (Zhao *et al.* 2021b). The intra- and inter-species syntenic analysis showed that WGD/segmental duplication and polyploidization should be the mainly driving force for the expansion of GPDH gene family in wheat, resulting high numbers of TaGPDH genes (Figures 5 and 6).

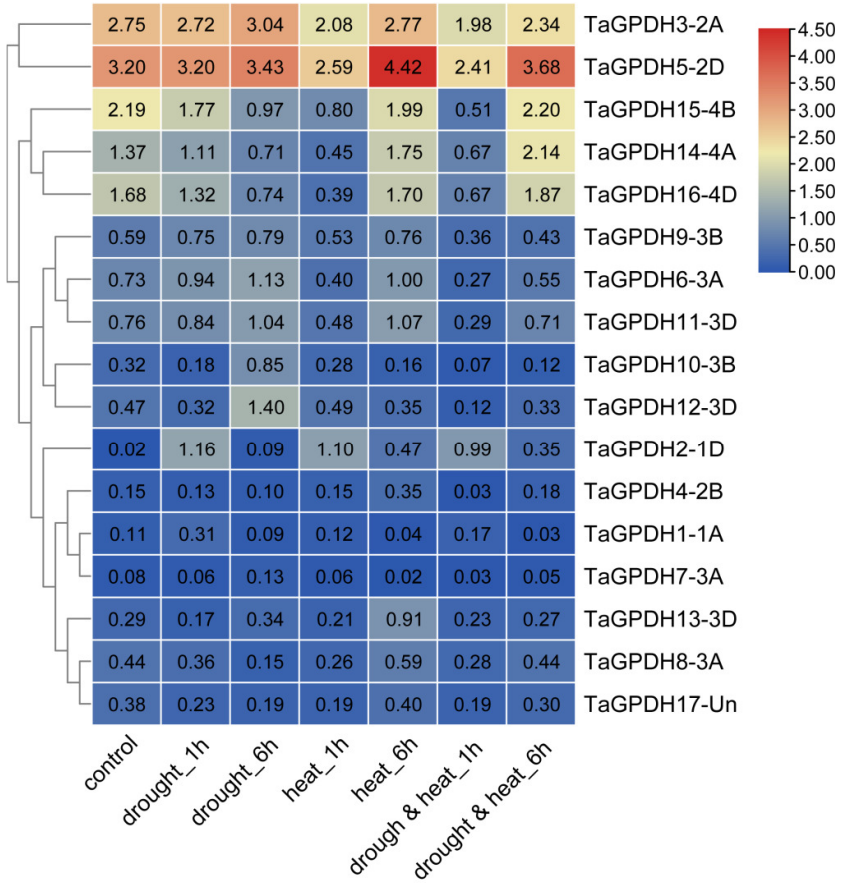

Figure 8. Expression patterns of *TaGPDH* genes under drought and heat combined stress for 1 h and 6 h (cultivar: TAM 107). The expression data were presented as TPM values with log2 normalization

As the phylogenetic tree of 28 GPDH protein sequences from the examined plants (*Arabidopsis*, rice, and wheat) showed that these GPDHs were divided into three groups (Group A-C), and the results of subcellular localization and domain distribution of GPDHs also supported the phylogenetic classification (Figure 1; Table 1). For example, members of Group A and Group B are NAD^+-GPDHs, and they were predicted to be located in the cytoplasm and chloroplast, respectively. However, members of Group C are mitochondrial FAD-GPDHs and contained a N-terminal FAD-dependent oxidoreductase domain and a C-terminal alpha-glycerophosphate oxidase domain (Figure 2; Table 1). Besides, the three groups contained different numbers of TaGPDHs, AtGPDHs and OsGPDHs, and GPDHs from wheat had closer evolutionary relationship with those from rice than with those from *Arabidopsis* (Figure 1), which is consistent with the results in maize, soybean, and other plants (Zhao *et al.,* 2018; Zhao *et al.,* 2021b). These results indicated the large extent of conservation among *GPDH* genes, and the *GPDH* gene family might be diverged after the separation of monocots and dicots. In addition, 10 conserved motifs were identified among TaGPDHs and their distributions displayed obvious evolutionary conservation (Figure 2), implying the functional divergence of TaGPDHs of different groups. We also determined the gene structure of *TaGPDH* genes, the exon-intron structures of TaGPDH genes from Group A and Group C were quite conservative, while Group B genes have

a variation with relatively higher number of introns (Figure 3), suggesting that intron gain may have occurred in these genes during evolution.

Gene expression profiling can provide insights into revealing of the biological roles of genes in plant growth and development. In the current study, many *TaGPDH* genes were found to express in specific tissues. For example, most *TaGPDH* genes from Group A and Group B displayed the highest expression in anther or spike/spikelet but rarely expressed in flag leaf blade and endosperm (Figure 7), suggesting their important roles of them in development of these tissues. There is evidence showing that maize GPDH genes exhibited preferential expression in the test tissues (Zhao *et al.,* 2018). The transcripts of plastidic *ZmGPDH4* and *ZmGPDH5* genes were mainly observed in pollen and anther, implying their roles in regulation of G3P adjustment during plant reproductive growth stages (Zhao *et al.,* 2018). However, the transcriptional levels of Group C *TaGPDH* genes were found in all the test tissues (Figure 7). And this phenomenon was also found in *GPDH* genes from maize (Zhao *et al.,* 2018), and *Arabidopsis* (Wei *et al.,* 2001; Shen *et al.,* 2006).

Previous reports have also shown that many GPDHs play key roles in various abiotic stresses (Chen *et al.,* 2011; Zhao *et al.,* 2019a; Zhao *et al.,* 2021b). For example, the expression of all *ZmGPDH* genes were notably altered under NaCl, $NaHCO_3$, PEG, 4°C treatments (Zhao *et al.,* 2018). Studies on the *Arabidopsis AtGPDHm1* showed that its expression is coupled to oxygen consumption and changed by ABA and stress conditions (Shen *et al.,* 2003). *TaGPDH5-2D* was homologous to *AtGPDHm1* (Figure 1), and it also exhibited obvious up-regulated expression under heat stress treatment, as well as drought and heat combined stress (Figure 8). G3P biosynthesis and metabolism can resulted in the accumulation of intracellular glycerol, which provides osmotic adjustment to environmental stresses in organisms. In a previous study, *ZmGPDH1* can confer salinity and osmotic stress tolerance in transgenic Arabidopsis plants by regulating glycerol production, redox homeostasis and ROS antioxidant defense (Zhao *et al.,* 2019a). Similarly, *AtGPDHc2* is also essential for regulating cellular redox homeostasis of *Arabidopsis* in response to salinity stress (Zhao *et al.,* 2019b). As the homologous genes of *AtGPDHm1*, *TaGPDH2-1D* and *TaGPDH12-3D* were induced by drought and/or heat stress (Figure 8), suggesting that they played possible roles in the transcriptional response during drought or heat stress adaptions.

Conclusions

In this study, we systemically identified 17 *GPDH* gene family members in wheat and examined the expression of them in various tissues and in response to drought and heat stresses. Our findings provided a better understanding for further functional exploration on the *TaGPDH* genes in developmental and stress-associated processes of wheat.

Authors' Contributions

Conceptualization: QL, LC and YZ; Data curation: SJ, YC, LC and YZ; Formal analysis: SJ, CW, ZZ, QL, LC and YZ; Funding acquisition: YZ; Investigation: SJ, CW, LC and YZ; Methodology: QL, LC and YZ; Validation: LC and YZ; Writing - original draft: LC and YZ; Writing - review and editing: QL, LC and YZ. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This work was supported by the Natural Science Foundation of Jiangxi Province, China, grant number 20202BABL205002.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Borrill P, Ramirez-Gonzalez R, Uauy C (2016). expVIP: a customizable RNA-seq data analysis and visualization platform. Plant Physiology 170(4):2172-2186. *https://doi.org/10.1104/pp.15.01667*

Casais-Molina ML, Peraza-Echeverria S, Echevarría-Machado I, Herrera-Valencia VA (2016). Expression of *Chlamydomonas reinhardtii CrGPDH2* and *CrGPDH3* cDNAs in yeast reveals that they encode functional glycerol-3-phosphate dehydrogenases involved in glycerol production and osmotic stress tolerance. Journal of Applied Phycology 28(1):219-226. *https://doi.org/10.1007/s10811-015-0588-3*

Chanda B, Xia Y, Mandal MK, Yu K, Sekine KT, Gao QM, ... Kachroo P (2011). Glycerol-3-phosphate is a critical mobile inducer of systemic immunity in plants. Nature Genetics 43(5):421-427. *https://doi.org/10.1038/ng.798*

Chen H, Lao YM, Jiang JG (2011). Effects of salinities on the gene expression of a NAD^+-dependent glycerol-3-phosphate dehydrogenase in *Dunaliella salina*. Science of the Total Environment 409(7):1291-1297. *https://doi.org/10.1016/j.scitotenv.2010.12.038*

Chen Z, Shen Z, Zhao D, Xu L, Zhang L, Zou Q (2020). Genome-wide analysis of LysM-containing gene family in wheat: Structural and phylogenetic analysis during development and defense. Genes (Basel) 12(1):31. *https://doi.org/10.3390/genes12010031*

Chhikara S, Abdullah HM, Akbari P, Schnell D, Dhankher OP (2018). Engineering *Camelina sativa* (L.) Crantz for enhanced oil and seed yields by combining diacylglycerol acyltransferase1 and glycerol-3-phosphate dehydrogenase expression. Plant Biotechnology Journal 16(5):1034-1045. *https://doi.org/10.1111/pbi.12847*

Driver T, Trivedi DK, McIntosh OA, Dean AP, Goodacre R, Pittman JK (2017). Two glycerol-3-phosphate dehydrogenases from *Chlamydomonas* have distinct roles in lipid metabolism. Plant Physiology 174(4):2083-2097. *https://doi.org/10.1104/pp.17.00491*

Gholizadeh F, Mirzaghaderi G (2020). Genome-wide analysis of the polyamine oxidase gene family in wheat (*Triticum aestivum* L.) reveals involvement in temperature stress response. PLoS One 15(8):e0236226. *https://doi.org/10.1371/journal.pone.0236226*

He Q, Toh JD, Ero R, Qiao Z, Kumar V, Serra A, ... Gao YG (2020). The unusual di-domain structure of *Dunaliella salina* glycerol-3-phosphate dehydrogenase enables direct conversion of dihydroxyacetone phosphate to glycerol. The Plant Journal 102(1):153-164. *https://doi.org/10.1111/tpj.14619*

He X, Liu M, Fang Z, Ma D, Zhou Y, Yin JL (2021). Genome-wide analysis of a plant at-rich sequence and zinc-binding protein (PLATZ) in *Triticum aestivum*. Phyton-Int Journal of Experimental Botany 90:971-986. *https://doi.org/10.32604/phyton.2021.012726*

Herrera-Valencia VA, Macario-González LA, Casais-Molina ML, Beltran-Aguilar AG, Peraza-Echeverría S (2012). In silico cloning and characterization of the glycerol-3-phosphate dehydrogenase (GPDH) gene family in the green

microalga *Chlamydomonas reinhardtii*. Current Microbiology 64(5):477-485. *https://doi.org/10.1007/s00284-012-0095-6*

Hu B, Jin J, Guo AY, Zhang H, Luo J, Gao G (2015). GSDS 2.0: an upgraded gene feature visualization server. Bioinformatics 31(8):1296-1297. *https://doi.org/10.1093/bioinformatics/btu817*

Jin P, Gao S, He L, Xu M, Zhang T, Zhang F, ... Chen J (2020). Genome-wide identification and expression analysis of the histone deacetylase gene family in wheat (*Triticum aestivum* L.). Plants (Basel) 10(1):19. *https://doi.org/10.3390/plants10010019*

Kumar S, Stecher G, Tamura K (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7):1870-1874. *https://doi.org/10.1093/molbev/msw054*

Liao L, Hu Z, Liu S, Yang Y, Zhou Y (2021). Characterization of germin-like proteins (GLPs) and their expression in response to abiotic and biotic stresses in cucumber. Horticulturae 7(10):412

Liu Z, Xin M, Qin J, Peng H, Ni Z, Yao Y, Sun Q (2015). Temporal transcriptome profiling reveals expression partitioning of homeologous genes contributing to heat and drought acclimation in wheat (*Triticum aestivum* L.). BMC Plant Biology 15:152. *https://doi.org/10.1186/s12870-015-0511-8*

Lorenc-Kukula K, Chaturvedi R, Roth M, Welti R, Shah J (2012). Biochemical and molecular-genetic characterization of SFD1's involvement in lipid metabolism and defense signaling. Frontiers in Plant Science 3:26. *https://doi.org/10.3389/fpls.2012.00026*

Mráček T, Drahota Z, Houštěk J (2013). The function and the role of the mitochondrial glycerol-3-phosphate dehydrogenase in mammalian tissues. Biochimica et Biophysica Acta 1827(3):401-410. *https://doi.org/10.1016/j.bbabio.2012.11.014*

Nandi A, Welti R, Shah J (2004). The *Arabidopsis thaliana* dihydroxyacetone phosphate reductase gene *SUPPRESSSOR OF FATTY ACID DESATURASE DEFICIENCY1* is required for glycerolipid metabolism and for the activation of systemic acquired resistance. Plant Cell 16(2):465-477. *https://doi.org/10.1105/tpc.016907*

Shen W, Wei Y, Dauk M, Tan Y, Taylor DC, Selvaraj G, Zou J (2006). Involvement of a glycerol-3-phosphate dehydrogenase in modulating the NADH/NAD^+ ratio provides evidence of a mitochondrial glycerol-3-phosphate shuttle in *Arabidopsis*. Plant Cell 18(2):422-441. *https://doi.org/10.1105/tpc.105.039750*

Shen W, Wei Y, Dauk M, Zheng Z, Zou J (2003). Identification of a mitochondrial glycerol-3-phosphate dehydrogenase from *Arabidopsis thaliana*: evidence for a mitochondrial glycerol-3-phosphate shuttle in plants. FEBS Letters 536(1-3):92-96. *https://doi.org/10.1016/s0014-5793(03)00033-4*

Singh V, Singh PK, Siddiqui A, Singh S, Banday ZZ, Nandi AK (2016). Over-expression of *Arabidopsis thaliana SFD1/GLY1*, the gene encoding plastid localized glycerol-3-phosphate dehydrogenase, increases plastidic lipid content in transgenic rice plants. Journal of Plant Research 129(2):285-293. *https://doi.org/10.1007/s10265-015-0781-0*

Vigeolas H, Geigenberger P (2004). Increased levels of glycerol-3-phosphate lead to a stimulation of flux into triacylglycerol synthesis after supplying glycerol to developing seeds of *Brassica napus* L. in planta. Planta 219(5):827-835. *https://doi.org/10.1007/s00425-004-1273-y*

Vigeolas H, Waldeck P, Zank T, Geigenberger P (2007). Increasing seed oil content in oil-seed rape (*Brassica napus* L.) by over-expression of a yeast glycerol-3-phosphate dehydrogenase under the control of a seed-specific promoter. Plant Biotechnology Journal 5(3):431-441. *https://doi.org/10.1111/j.1467-7652.2007.00252.x*

Voorrips RE. (2002). MapChart: software for the graphical presentation of linkage maps and QTLs. Journal of Heredity 93(1):77-78. *https://doi.org/10.1093/jhered/93.1.77*

Wei Y, Periappuram C, Datla R, Selvaraj G, Zou J (2001). Molecular and biochemical characterizations of a plastidic glycerol-3-phosphate dehydrogenase from *Arabidopsis*. Plant Physiology and Biochemistry 39(10):841-848. *https://doi.org/https://doi.org/10.1016/S0981-9428(01)01308-0*

Wu Q, Lan Y, Cao X, Yao H, Qiao D, Xu H, Cao Y (2019). Characterization and diverse evolution patterns of glycerol-3-phosphate dehydrogenase family genes in *Dunaliella salina*. Gene 710:161-169. *https://doi.org/10.1016/j.gene.2019.05.056*

Xue LL, Chen HH, Jiang JG (2017). Implications of glycerol metabolism for lipid production. Progress in Lipid Research 68:12-25. *https://doi.org/10.1016/j.plipres.2017.07.002*

Zhao Y, Cao P, Cui Y, Liu D, Li J, Zhao Y, ... Chen Q (2021a). Enhanced production of seed oil with improved fatty acid composition by overexpressing NAD⁺-dependent glycerol-3-phosphate dehydrogenase in soybean. J Journal of Integrative Plant Biology 63(6):1036-1053. *https://doi.org/10.1111/jipb.13094*

Zhao Y, Li X, Wang F, Zhao X, Gao Y, Zhao C, ... Xu J (2018). Glycerol-3-phosphate dehydrogenase (GPDH) gene family in *Zea mays* L.: Identification, subcellular localization, and transcriptional responses to abiotic stresses. PLoS One 13(7):e0200357. *https://doi.org/10.1371/journal.pone.0200357*

Zhao Y, Li X, Zhang Z, Pan W, Li S, Xing Y, ... Chen Q (2021b). *GmGPDH12*, a mitochondrial FAD-GPDH from soybean, increases salt and osmotic stress resistance by modulating redox state and respiration. Crop Journal 9(1):79-94. *https://doi.org/https://doi.org/10.1016/j.cj.2020.05.008*

Zhao Y, Liu M, He L, Li X, Wang F, Yan B, ... Xu J. (2019a). A cytosolic NAD⁺-dependent GPDH from maize (*ZmGPDH1*) is involved in conferring salt and osmotic stress tolerance. BMC Plant Biology 19(1):16. *https://doi.org/10.1186/s12870-018-1597-6*

Zhao Y, Liu M, Wang F, Ding D, Zhao CJ, He L, ... Xu JY. (2019b). The role of *AtGPDHc2* in regulating cellular redox homeostasis of *Arabidopsis* under salt stress. Journal of Integrative Agriculture 18(6):1266-1279. *https://doi.org/https://doi.org/10.1016/S2095-3119(18)62082-9*