# Characterization and Expression Analysis of *B12D-Like* Gene From Pearl Millet

Zainab M Almutairi

Department of Biology, College of Science and Humanities in Al-Kharj, Prince Sattam Bin Abdulaziz University, Al-kharj, Saudi Arabia.

Evolutionary Bioinformatics
Volume 18: 1–7

DOI: 10.1177/11769343221142285

**ABSTRACT:** *B12D-Like* is a member of the B12D domain-containing protein family, which includes several transmembrane proteins in plants. In this study, the cDNA of *PgB12D-Like* from *Pennisetum glaucum subsp. monodii* (Maire) Brunken was sequenced and characterized. The 446-bp cDNA for *PgB12D-Like* encodes for a deduced protein of 95 amino acids. The PgB12D-Like protein contains a B12D domain and a transmembrane helix embedded in the mitochondrial membrane. Cis-regulatory elements analysis reveals binding sites for various transcription factors involved in responses to stress, light, and plant hormones in the putative promoter sequence for *PgB12D-Like*. Several proteins involved in floral organ development were also found to have binding sites in the *PgB12D-Like* promoter, such as agamous-like proteins and squamosa promoter binding proteins. Real-time PCR reveals high expression of *PgB12D-Like* in flowers during heading, whereas its expression in a 4-day-old seedling shoot was the lowest. Moreover, cold, drought, and heat stress were found to upregulate *PgB12D-Like*, whereas gibberellic acid downregulated its expression in seedlings. The present study helps to uncover the function of the *B12D-Like* in response to plant hormones and abiotic stress during *P. glaucum* development.

**KEYWORDS:** B12D-Like, cDNA, cis-elements, gene expression, *Pennisetum glaucum*

**RECEIVED:** August 14, 2022. **ACCEPTED:** November 14, 2022.

**TYPE:** Original Research

**FUNDING:** The author disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The author extends their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia, for funding this research work through project number 2022/01/21762.

**DECLARATION OF CONFLICTING INTERESTS:** The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**CORRESPONDING AUTHOR:** Zainab M Almutairi, Associate Professor, Department of Biology, College of Science and Humanities in Al-Kharj, Prince Sattam bin Abdulaziz University, P.O. Box. 83, Al-kharj 11942, Saudi Arabia. Email: z.almutairi@psau.edu.sa

## Introduction

Genomic and transcriptomic studies of plants have revealed several small transmembrane proteins containing the B12D domain that are induced in plant organs at various stages.[1-3] Six B12D transmembrane proteins have been identified in the rice genome,[4] and more than 8 B12D proteins are thought to be involved in the early growth of barley.[3] Nevertheless, the specific function of each B12D protein in plants has not been clearly described, although several B12D proteins appear to be anchored in the mitochondrial inner membrane and involved in cell metabolism and transport.[4] One of the B12D transmembrane proteins that was first characterized in rice, B12D-Like protein Os07g17330, is highly expressed during rice developmental stages in various organs. The promotor for *OsB12D-Like* has various regulatory *cis*-elements including ABRE, G-box, GARE, GC-motif, and TCA-element, suggesting that *OsB12D-Like* is regulated by multiple transcription factors during stress responses and plant development.[4]

Previous studies have indicated that *B12D-Like* is regulated by multiple plant hormones and various biotic and abiotic stressors. *OsB12D-Like* is regulated by abscisic acid (ABA), gibberellic acid (GA), and auxin during rice grain development.[4,5] *OsB12D-Like* is induced by flooding, salt, and heat and cold stress during germination[4,6]. Additionally, B12D-Like is deferentially expressed between tolerant and resistant wheat lines under aluminum stress[7]; it is upregulated in rice roots under cadmium stress[8] and upregulated in barley by boron stress.[9] Likewise, *OsB12D-Like* is involved in the rice defense response to biotic stress induced by blast disease caused by *Magnaporthe oryzae*.[10] It is also downregulated upon infection with a mixture of fungal pathogens in the susceptible sorghum genotype.[11]

Pearl millet is a cereal crop that can grow in an arid environment.[12] It has several *B12Ds* that are regulated by abiotic stress and expressed in various plant tissues.[13,14] However, the molecular function of each of these B12Ds has not clearly described. The present study aims to sequence the cDNA of *B12D-Like* from pearl millet, characterize the deduced protein, analyze cis-regulatory elements in the putative promoter sequence, and profile the expression patterns at various stages and after exposure to plant hormones and abiotic stress. This study provides insight into the role of *B12D-Like* during pearl millet germination under stress and after treatment with plant hormones.

## Materials and Methods

### *Primer design*

The *Hordeum vulgare* B12D-like protein MLOC_11524 from the STRING database (https://string-db.org/) was used to retrieve the sequence of *PgB12D-Like* in the *P. glaucum* genome via tBLASTn tblastn: search translated nucleotide databases using a protein query. (nih.gov). Primers were designed from the conserved sequence between the query protein and the similar *P. glaucum* genomic region, which is found on chromosome 7 (LKME02052033). The primer pair used to successfully amplify the cDNA of *PgB12D-Like* is F: 5′-GAG CCA CGA AAG AAA CAG ATC T-3′ and R: 5′-TCC GAC CAA ACC ATC CGA TC-3′.

### PgB12D-Like *cDNA sequencing and characterization*

The *P. glaucum* variety used in this study is available from the Saudi Centre of Genetic Resources under 1316. Total RNA was extracted from 4-day-old seedlings using TRI reagent from Sigma. The *PgB12D-Like* cDNA was generated using the Revert Aid First Strand cDNA Synthesis Kit from Thermo Scientific. To amplify *PgB12D-Like*, a PCR reaction was conducted with iProof High-fidelity master mix from BIO-RAD, according to the recommended protocol. PCR cycling was started at 98°C for 3 minutes; followed by 28 cycles each of 20 seconds at 98°C, 25 seconds at 58°C, and 40 seconds at 72°C; then 6 minutes at 72°C. Sequencing of the PCR product was conducted using the method described by Sanger[15] with a *BigDye* Terminator v3.1.

The resulting cDNA sequence was translated into an amino acid sequence via EMBOSS Transeq (https://www.ebi.ac.uk/Tools/st/emboss_transeq/). The domains in the deduced protein were predicted by the Batch CD-search tool (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi). The isoelectric point and molecular weight of *PgB12D-Like* protein were estimated by GeneScript (https://www.genscript.com/tools/). The secondary and tertiary structures of *PgB12D-Like* protein were predicted using the PSIPRED server (Nugent and Jones 2013).[16] The MOLE server was used to find and characterize transmembrane protein channels Mole (upol.cz).[17] Subcellular localization of the deduced protein was conducted using the LOCTree3 https://rostlab.org/services/loctree3/.[18] The consensus topology of transmembrane helices was predicted using the CCTOP server http://cctop.enzim.ttk.mta.hu/.

### *Promoter screening for* cis*-elements*

The promoter sequence for B12D-Like was screened for *cis*-elements by retrieving the sequence 1200 bp upstream from the start codon, which was on chromosome 7, (LKME02052033) from nucleotides 68 636 264 to 68 637 263. Two *in silico* tools were used for promoter screening: the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/),[19] and the PLACE database (https://www.dna.affrc.go.jp/PLACE/).[20]

### *Stress treatments and gene expression analysis*

The expression patterns of *PgB12D-Like* was analyzed in various plant tissues during germination and reproduction, using real-time PCR. The expression of *PgB12D-Like* was also examined during germination under 4 types of abiotic stress (heat, salt, cold, and drought), in addition to the plant hormones ABA and GA. The conditions of the stress treatments have been determined based on an initial gradient experiment for each stress type. The tested temperatures for heat stress were 40°C, 44°C, 48°C, and 52°C. The tested temperatures for cold stress were 2°C, 5°C, 8°C, and 12°C. The tested NaCl concentrations for salt stress were 100, 150, 200, and 250 mM. These gradient conditions were applied to 3-day-old seedlings for 24 hours. The stress conditions which can affect growth negatively below optimum levels were selected for plant treatments. Stressors were applied to 3-day-old seedlings as follows: heat stress was applied by incubating seedlings at 48°C for 2 hours; salt stress was applied via treatment with 200 mM NaCl for 2 hours; cold stress was applied by incubating seedlings at 8°C for 2 hours; drought was induced by exposing seedlings to an air stream for 10 minutes and leaving the plants in open dishes for 20 hours, with relative humidity of 15%.

To examine the expression of *PgB12D-Like* after treatment with the ABA and GA hormones, the seeds were soaked in distilled $H_2O$ for 24 hours and then soaked for 4 hours in 1 mg/ml of ABA or GA. To examine *PgB12D-Like* expression during the reproductive stage, plants were grown in the field to collect flowers during the heading stage and spikes during the early ripening stage. Three independent biological replicates from each treatment were subjected to gene expression analysis.

RNA was quantified using the Qubit® 2 fluorometer (Invitrogen) to equalize the concentrations of all cDNA samples used in real time-PCR. The *PgB12D-Like* expression primers used are F: 5′-CAG CCA GCA CGC GTT CCG CCG C-3′ and R: 5′- CTC GGA GAA GAA GCG GTT GAG G-3′. *Elongation factor-1 alpha* gene was used to standardize the *PgB12D-Like* expression in all samples using the primer pair 5′-GTT ACA ACC CAG ACA AGA TTG C-3′ and 5′-TGG ACC TCT CAA TCG TGT TG-3′. The real-time PCR reaction was performed with 1X of QuantiTect SYBR green PCR buffer from Qiagen, 1.5 µL of cDNA template, and 0.4 µM of each primer in a total volume of 50 µL. The cycling program began with 95°C for 15 minutes, followed by 50 cycles each of 15 seconds at 94°C, 30 seconds at 60°C, and 30 seconds at 72°C. The cycles to threshold were calculated according to Livak and Schmittgen[21] for expression assessment.

## Results

### PgB12D-Like *cDNA characterization*

The sequenced cDNA sequence of *PgB12D-Like* is 446 bp; it has an open reading frame of 285 bp. The cDNA and deduced protein sequences of the *PgB12D-Like* were deposited in the NCBI database under the numbers MN594511.1 and QLP90154, respectively. The deduced *PgB12D-Like* protein is 95 aa and contains a B12D domain Pfam06522, spanning from 11 to 79 aa. The molecular weight for the deduced protein is 10.79 KD, and the theoretical isoelectric point is 10.02. An NCBI BLASTn search against the *P. glaucum* genome revealed 2 identical genomic regions on chromosome 7 (LKME02052033) sharing 99% identity with the *PgB12D-Like* cDNA. These 2 regions span from nucleotides 68 637 190 to 68 637 907 and from nucleotides 68 649 674 to 68 649 904,

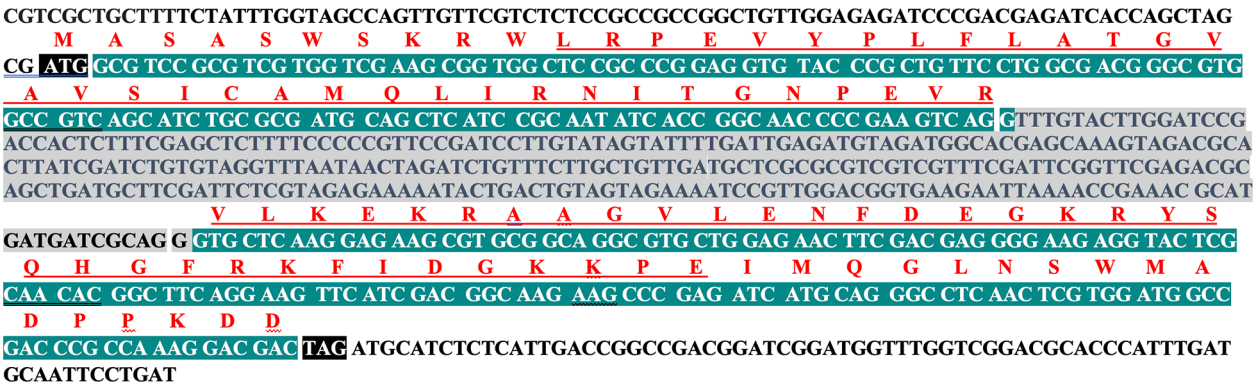


**Figure 1.** The exon/intron structure of *PgB12D-like* based on the alignment of cDNA sequence MN594511 with the genomic region of *P. glaucum* chromosome number 7 (LKME02052033) from nucleotide 68 637 190 to 68 637 907. Exons are highlighted with bluish green, the intron sequence is highlighted with gray, and the 3' and 5' untranslated regions are shown in black letters. The start and stop codons are shown in black boxes. The deduced protein sequence is shown in red above the nucleotide sequence of open reading frame, and the B12D domain sequence in the deduced proteins is underlined.

respectively. Alignment of the cDNA sequence with each of these genomic regions revealed 2 exons in *PgB12D-Like*. The length of the first exon is 216 bp, while the second exon is 229 bp; the intron is 285 bp (Figure 1).

### *PgB12D-Like secondary structure and transmembrane topology prediction*

The secondary and tertiary structures of the *PgB12D-Like* protein show 5 α-helices and a single β-strand (Figure 2). Subcellular localization reveals that the *PgB12D-Like* protein is integral to the mitochondrial inner membrane, with expected accuracy of 91% (Supplemental Figure S1). Transmembrane topology reveals a transmembrane helix in *PgB12D-Like* protein, spanning from 14 to 33 aa. The consensus topology predicted by CCTOP indicates that the N-terminus of *PgB12D-Like* protein is oriented inside the organelle, whereas the C-terminus is oriented outside the membrane (Supplemental Figure S2). Four channels were found in the *PgB12D-Like* protein: 2 pores and 2 tunnels. The tunnels are 11.5 and 16.7 Å in length; the radius of the bottlenecks are 1.2 Å for both tunnels. The pores are 8.7 and 11.4 Å in length; the radii of the pore bottlenecks are 2.2 and 1.5 Å. The properties of the predicted channels are displayed in Supplemental Figure S3 and in Table 1.

### Cis-*elements in the putative promoter of* PgB12D-Like

Screening for regulatory *cis*-elements reveals that the 1200 bp putative promoter for *PgB12D-Like* includes binding sites for various stress-responsive factors and plant hormones. Both retrieved databases (PLACE and PlantCARE) reveal binding sites for stress and hormone-responsive transcription factors, including dehydration response element-binding protein 1A (DREB1A), DREB2C, ABA-responsive element (AREB), ABA insensitive 3 (ABI3), ethylene response factors (ERFs), auxin-responsive factors (ARFs), ABF2, CBF1, BHLH34, MYC2, MYC3, FUS3, Arabidopsis response regulator 10 (ARR10), and TGA1. Additionally, several light-responsive transcription factors were found in the promoter: phytochrome-interacting transcription factor 4 (PIF4), TCP2, HYH, HY5, and GBF2. Binding sites are also present for calmodulin-binding transcription activator 3 (CMTA3), DOF3 (a regulator for seed storage proteins), and AHL25, which binds to *GA*-negative feedback element I (Supplemental Table S1, Figure 3). Supplemental Tables S2 and S3 show all motifs found by PlantCARE and PLACE, respectively. Many regulatory elements for floral development regulation proteins were revealed by PlantCARE only; these include MYB24, SEPALLATA 3 (SEP3), squamosa promoter binding protein 3 (SPL3), SPL8, ANAC050, SPATULA (SPT), the MADS box proteins, Agamous-like3 (AGL3), AGL42, and AGL55.

### PgB12D-Like *expression patterns*

Gene expression patterns in germinated *P. glaucum* seedlings subjected to the 4 abiotic stressors and the 2 plant hormones reveals that the *PgB12D-Like* was upregulated (>2 folds to control) by heat, cold, and drought; it was downregulated (<0.5-folds) only by GA treatment. However, the $2^{-ddCt}$ values for *PgB12D-Like* gene expression in response to salt and ABA were between 0.5 and 2 folds (Figure 4A). The *PgB12D-Like* gene was highly expressed in flowers during heading (36.4-folds), as compared to spikes and the 3 tissues of the germinated seedlings (root, shoot, and seed). The *PgB12D-Like* gene had the lowest level of expression in seedling shoots (0.08-folds), whereas its expression in the spikes and in seedling roots and seeds were 3.5, 2.9, and 3.2-folds, respectively (Figure 4B).

## Discussion

In this study, we characterized the *PgB12D-Like* gene, which encodes a transmembrane protein in *P. glaucum*. We found the sequence of *PgB12D-Like* in 2 duplicates in chromosome 7 of

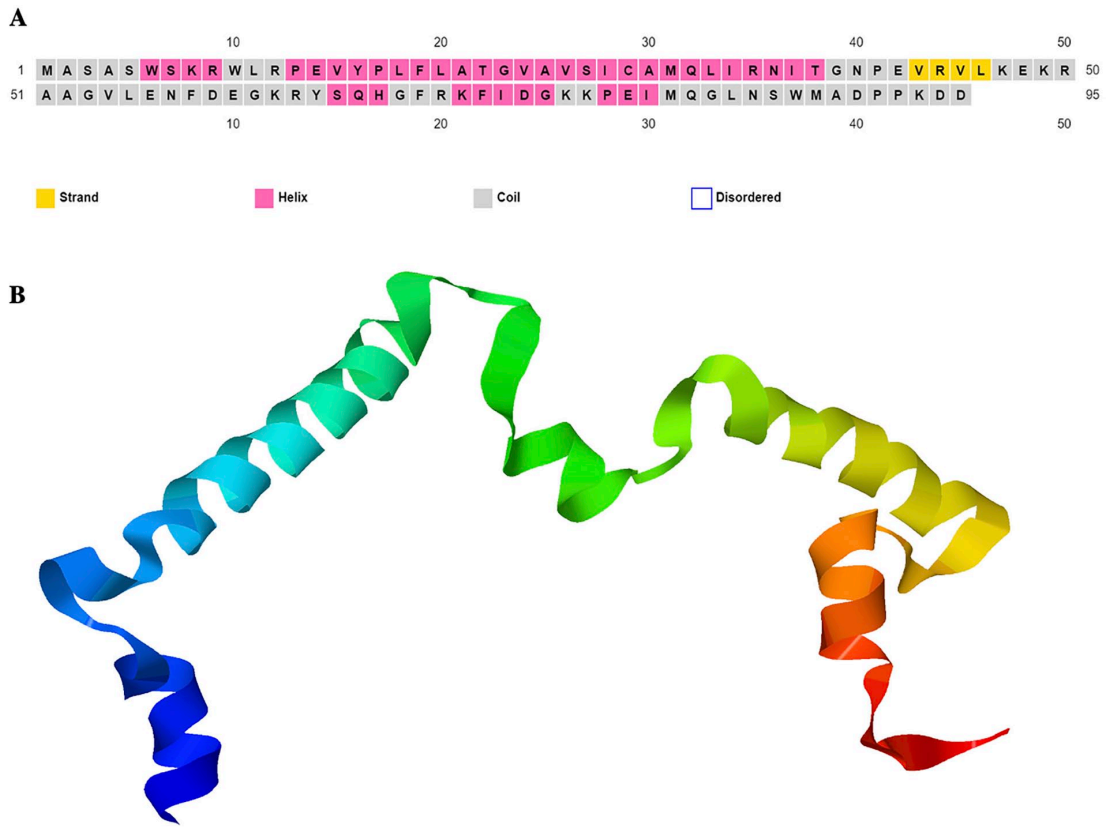


**Figure 2.** Secondary and tertiary structures of the deduced *PgB12D-like* protein generated by PSIPRED: (A) the secondary of structure *PgB12D-like* protein illustrated in the amino acids sequence. Pink and yellow colors indicate for α-helices and β-strand, respectively and (B) the tertiary structure of the PgB12Dg protein is generated by DMPfold tool in PSIPRED website. The N-terminal is appeared in blue and the C-terminal appeared in red.

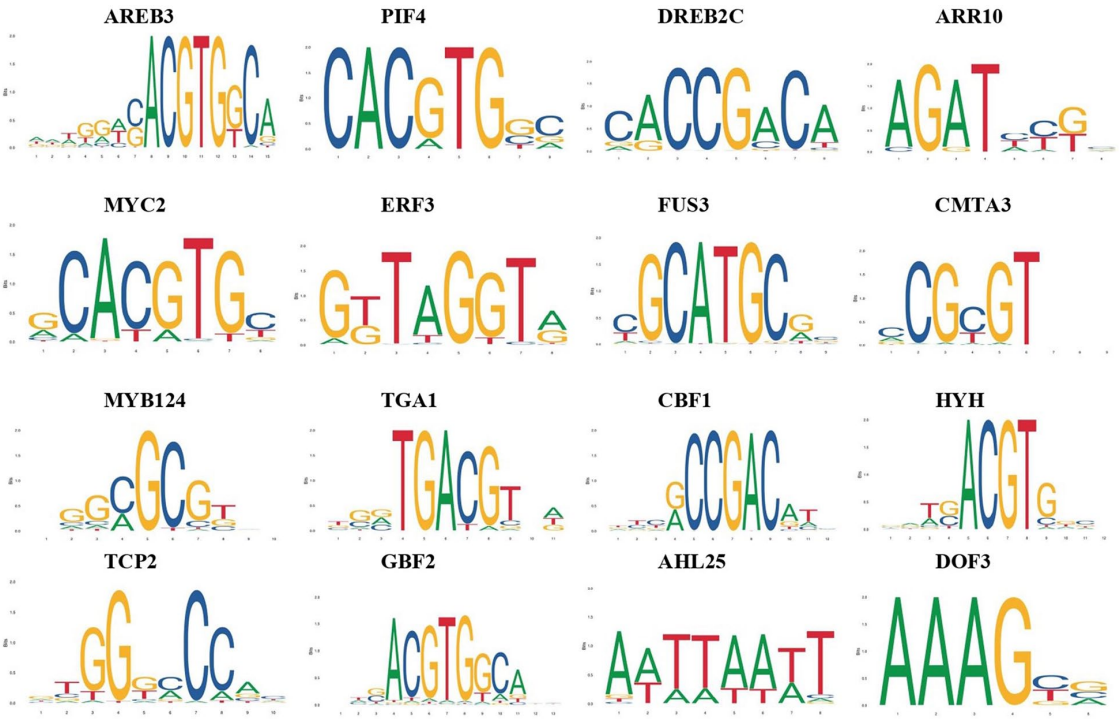


**Figure 3.** Cis-regulatory elements in the putative promoter for *PgB12D-like*, predicted by the PLACE and PlantCARE databases. Only motifs confirmed by both prediction tools (PLACE and PlantCARE) are shown. The retrieved promoter sequence is 1200 bp upstream from the start codon. The names of transcription factors and the profiles of motifs come from the JASPAR 2020 *Arabidopsis thaliana* core database http://jaspar.genereg.net/. The function, sequence, and position of the *cis*-elements found in *PgB12D-like* promoter are illustrated in Supplemental Table 1.

**Table 1.** The physicochemical characteristics of the 4 predicted channels in the deduced *PgB12D-like* protein. The 4 channels and their characteristics were predicted using the MOLE server.

| NAME | LENGTH OF THE CHANNEL | RADIUS OF THE CHANNEL BOTTLENECK | AVERAGE OF HYDROPATHY INDEX | CHARGE | AVERAGE OF LINING AMINO ACID POLARITIES | AVERAGE OF RELATIVE MUTABILITY INDEX | LIPOPHILICITY LOGP | LIPOPHILICITY LOGD | SOLUBILITY | IONIZABLE RESIDUES |
|---|---|---|---|---|---|---|---|---|---|---|
| Tunnel 1 | 11.5 Å | 1.2 Å | 0.96 | 1 | 4.6 | 66 | 0.41 | 0.27 | −0.15 | 1 |
| Tunnel 2 | 16.7 Å | 1.2 Å | −0.98 | 0 | 19.97 | 79 | 0.17 | −0.38 | 0.33 | 2 |
| Pore 1 | 8.7 Å | 2.2 Å | 1.77 | 0 | 1.54 | 65 | 1.09 | 1.09 | −0.78 | N/A |
| Pore 2 | 11.4 Å | 1.5 Å | −1.66 | 1 | 22.3 | 88 | −0.21 | −0.51 | 0.36 | 1 |

*P. glaucum*. *PgB12D-Like* protein shares 92% similarity with its ortholog from *O. sativa* (Os07t0274700 and Os07g17330 in the rice annotation project database; https://rapdb.dna.affrc.go.jp/). However, the genomic structure reveals 2 exons in *PgB12D-Like* while its ortholog from rice has 3 exons.[4] This difference in exon numbers among these orthologs may result from the multiple evolution events of *B12Ds* in Poaceae species. *OsB12D-Like* has a tandem duplicate with very low expression levels compared to *OsB12D-Like*, suggesting that some B12D duplicate genes may be silenced during plant evolution.[4] High conservation was shown between *PgB12D-Like* and its paralog B12Dg (QQO98583), with 64% identity.

*Cis*-elements screening reveals various types of regulatory elements in the putative promoter for *PgB12D-Like*, including the responsive elements for ABA, GA, salicylic acid, jasmonic acid, cytokinin, auxin, ethylene, dehydration, salt, heat, cold, and light, indicating that *PgB12D-Like* plays a role in plant development and in responses to various stressors. Two GA-regulatory motifs—GA-downregulated (ACGTGTC) and CARE (CAACTC)—were found by the PLACE server but not PlantCARE. The GA-downregulated motif is known to exist in the promoters of genes downregulated by GA,[22] while CARE is known to cooperate with other GA-regulatory motifs, GAREs, and multiple transcription factors to induce GA.[23] However, the screening of regulatory elements has not revealed any GAREs (TAACAGA and TAACGTA) in the putative promoter for *PgB12D-Like*. This finding is consistent with the downregulation of *PgB12D-Like* by GA in this study. In contrast, the ortholog of rice (*OsB12D-Like*) is upregulated by GA, and its promoter contains the GARE motif,[4] suggesting different responses to GA between *B12D-Like* from pearl millet and rice. Three regulatory elements—ABA, GC-motif, and G-box—that were previously found in *OsB12D-Like* by He et al[4] were consistently found in the *PgB12D-Like* promoter.

The high expression of *PgB12D-Like* in flowers during heading is consistent with the regulatory elements found in the *PgB12D-Like* putative promoter that are involved in floral development, such as MYB24, AGLs, and SPLs. While *PgB12D-Like* was downregulated by GA, it was upregulated by drought and both low and high temperatures, but not by salt or ABA. Similarly, the homolog of B12D-like from apple is downregulated by GA.[24] This result aligns with the expression patterns of the ortholog *OsB12D-Like* Os07t0274700, which is highly expressed in rice anther and ovary, according to the Rice Expression Profile Database (https://ricexpro.dna.affrc.go.jp/; Sato et al.[25]). Similar to our result, *OsB12D-Like*is upregulated by cold and drought, as evidenced by the transcriptome encyclopedia of rice (https://tenor.dna.affrc.go.jp/; Kawahara et al.[26]). On the other hand, *OsB12D-Like* is downregulated by salt and upregulated by ABA,[4] while our results do not show up- or downregulation of *PgB12D-Like* by salt or ABA treatment.

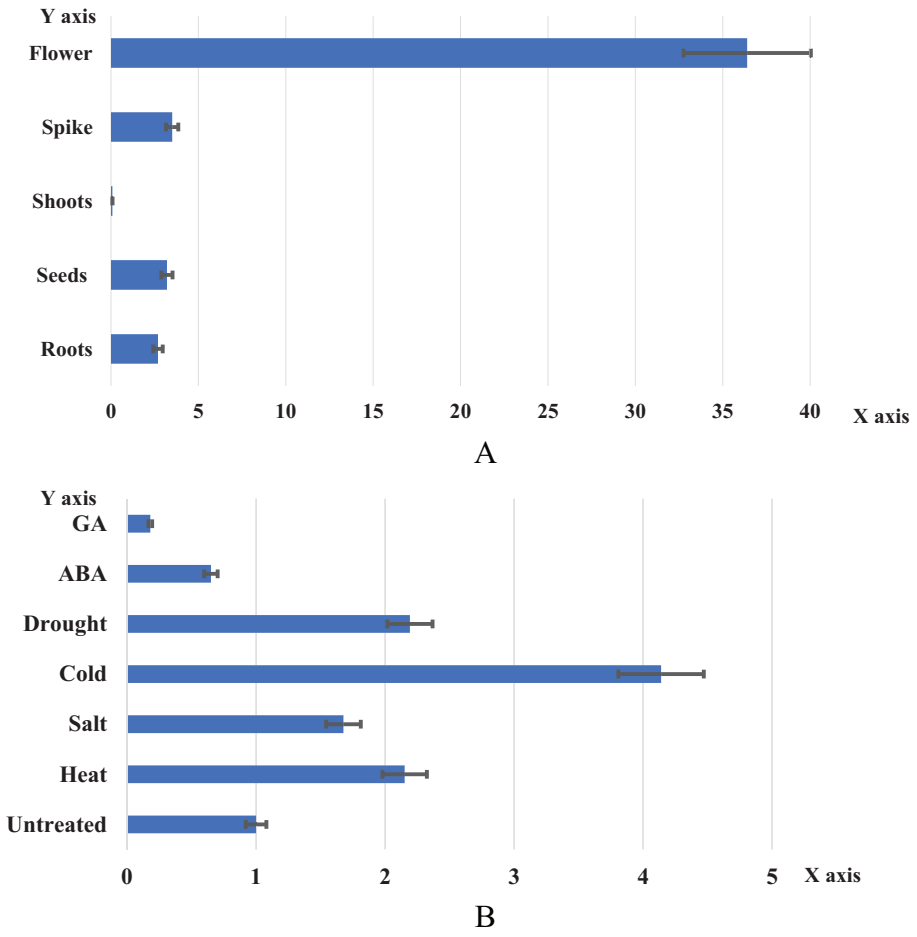


**Figure 4.** The expression of *PgB12D-like* in *P. glaucum* in various tissues and stressed seedlings, examined by real-time PCR: (A) the expression of *PgB12D-like* in flowers during heading, spikes during the early ripening stage, and the roots, seeds, and shoots of 4-day-old seedlings and (B) the expression of *PgB12D-like* after GA, ABA, and 4 types of abiotic stress treatment in addition to untreated plants. For GA and ABA treatment, 24 hours-old, soaked seeds were treated with GA and ABA for 2 hours. For stress treatment, the expression of *PgB12D-like* in the roots, shoots, and seeds of the 3-day-old germinated seedlings treated with drought, cold, salt, and heat was assessed. The RNA was extracted after 20 hours of water withholding (drought) and after 2 hours of treatment by cold, heat, or salt. *Elongation factor-1 alpha* was used the reference gene in real-time PCR analysis. The expression level is indicated by the $2^{-ddCt}$ value in the horizontal axis (x axis). The error bars indicate the standard error of the mean.

Similar expression patterns have been shown for another member of B12D family—MLRQ in *P. glaucum*—revealing high expression in flowers compared with seedling tissues or spikes before ripening. The roots of stressed seedlings show upregulation of MLRQ by salt and short exposure to low temperatures, while the shoots and seeds of these seedlings show upregulation by drought.[13] However, another member of B12D family, *B12Dg* of *P. glaucum*, shows opposite expression patterns with treatment by plant hormones: upregulation by GA and downregulation by ABA.[14] These varying expression patterns of paralogous *B12Ds* indicates a different role for each member of the B12D family during plant growth and stress responses. This study's findings are useful for further investigation of the function of B12D proteins in plants.

## Conclusion

*B12D-Like* is a member of the B12D family, which includes several transmembrane proteins in plants. In this study, we identified *B12D-Like* from *P. glaucum* and examined its expression under abiotic stress and in response to plant hormones during germination. In silico analysis revealed that the deduced PgB12Dg protein has a transmembrane helix embedded in the inner mitochondrial membrane. Expression analysis revealed that *PgB12D-Like* is highly expressed in flowers during heading. *PgB12D-Like* is also upregulated by cold, drought, and heat stress and downregulated by gibberellic acid in the seedlings. The finding of this study contributes to knowledge about the role of the *PgB12D-Like* during plant growth and response to plant hormones and stress.

## ORCID iD

Zainab M Almutairi https://orcid.org/0000-0002-7293-5767

## Data Availability

The cDNA and deduced protein sequences of the *PgB12D-Like* generated during the current study are available in the NCBI database under the numbers MN594511.1 and QLP90155, respectively.

## Supplemental Material

Supplemental material for this article is available online.

## REFERENCES


1. Aalenf RB, Opsahl-Ferstad HG, Linnestad C, Olsen OA. Transcripts encoding an oleosin and a dormancy-related protein are present in both the aleurone layer and the embryo of developing barley (*Hordeum vulgare* L.) seeds. *Plant J*. 1994; 5:385-396.
2. Aalen RB, Salehian Z, Steinum TM. Stability of barley aleurone transcripts: dependence on protein synthesis, influence of the starchy endosperm and destabilization by GA3. *Physiol Plant*. 2001;112:403-413.
3. Steinum TM, Berner HS, Stacy RAP, Salehian Z, Aalen RB. Differential regulation of the barley (*Hordeum vulgare* ) transcripts B22E and B12D in mature aleurone layers. *Physiol Plant*. 1998;102:337-345.
4. He D, Zhang H, Yang P. The mitochondrion-located protein OsB12D1 enhances flooding tolerance during seed germination and early seedling growth in rice. *Int J Mol Sci*. 2014;15:13461-13481.
5. Hu Z, Lu SJ, Wang MJ, et al. A novel QTL qTGW3 encodes the GSK3/SHAGGY-Like kinase OsGSK5/OsSK41 that interacts with OsARF4 to negatively regulate grain size and weight in rice. *Mol Plant*. 2018;11:736-749.
6. Yun KY, Park MR, Mohanty B, et al. Transcriptional regulatory network triggered by oxidative signals configures the early response mechanisms of japonica rice to chilling stress. *BMC Plant Biol*. 2010;10:16.
7. Guo P, Bai G, Carver B, Li R, Bernardo A, Baum M. Transcriptional analysis between two wheat near-isogenic lines contrasting in aluminum tolerance under aluminum stress. *Mol Genet Genomics*. 2007;277:1-12.
8. Oono Y, Yazawa T, Kawahara Y, et al. Genome-wide transcriptome analysis reveals that cadmium stress signaling controls the expression of genes in drought stress signal pathways in rice. *PLoS One*. 2014;9:e96946.
9. Öz MT, Yilmaz R, Eyïdoğan F, Graaff LD, Yücel M, Öktem HA. Microarray analysis of late response to boron toxicity in barley (*Hordeum vulgare L.*) leaves. *Turk J Agric For*. 2009;33:191-202.
10. Bevitori R, Sircar S, de Mello RN, et al. Research article identification of co-expression gene networks controlling rice blast disease during an incompatible reaction. *Genet Mol Res*. 2020;19:1-21.
11. Nida H, Lee S, Li Y, Mengiste T. Transcriptome analysis of early stages of sorghum grain mold disease reveals defense regulators and metabolic pathways associated with resistance. *BMC Genomics*. 2021;22:295-317.
12. Vadez V, Hash T, Bidinger RF, Kholova J. II.1.5 Phenotyping pearl millet for adaptation to drought. *Front Physiol*. Published online October 19, 2012. doi:10.3389/fphys.2012.00386
13. Almutairi ZM. Molecular characterization and expression profiling of NADH-ubiquinone reductase complex 1 MLRQ subunit in *Pennisetum glaucum*. *J Plant Biochem Biotechnol*. 2022;31:361-369.
14. Almutairi ZM. Expression Profiling and Characterization of a G-Box Binding Protein, B12Dg, from Pearl Millet. *J King Saud Univ Sci*. 2023;35(1):102448.
15. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci*. 1977;74:5463-5467.
16. Nugent T, Jones DT. Membrane protein orientation and refinement using a knowledge-based statistical potential. *BMC Bioinformatics*. 2013;14:276. https://doi.org/10.1186/1471-2105-14-276
17. Pravda L, Sehnal D, Toušek D, et al. MOLEonline: a web-based tool for analyzing channels, tunnels and pores (2018 update). *Nucleic Acids Res*. 2018;46:W368-W373.
18. Yachdav G, Kloppmann E, Kajan L, et al. LocTree3 prediction of localization. *Nucleic Acids Res*. 2014;42:W350-W355.
19. Lescot M, Déhais P, Thijs G, et al. PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acids Res*. 2002;30:325-327.
20. Higo K, Ugawa Y, Iwamoto M, Korenaga T. Plant cis-acting regulatory DNA elements (PLACE) database: 1999. *Nucleic Acids Res*. 1999;27:297-300.
21. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods*. 2001;25:402-408.
22. Ogawa M, Hanada A, Yamauchi Y, Kuwahara A, Kamiya Y, Yamaguchi S. Gibberellin biosynthesis and response during Arabidopsis seed germination. *Plant Cell*. 2003;15:1591-1604.
23. Sutoh K, Yamauchi D. Two cis-acting elements necessary and sufficient for gibberellin-upregulated proteinase expression in rice seeds. *Plant J*. 2003;34: 635-645.
24. Zhang S, Gottschalk C, van Nocker S. Genetic mechanisms in the repression of flowering by gibberellins in apple (Malus x domestica Borkh.). *BMC Genomics*. 2019;20:747.
25. Sato Y, Takehisa H, Kamatsuki K, et al. RiceXPro version 3.0: expanding the informatics resource for rice transcriptome. *Nucleic Acids Res*. 2013;41:D1206-D1213.
26. Kawahara Y, Oono Y, Wakimoto H, et al. TENOR: database for comprehensive mRNA-Seq experiments in rice. *Plant Cell Physiol*. 2016;57:e7.