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Identification of *Triticum aestivum* GTP-Binding Protein 1/ Elongation Factor 1 Alpha (GBP1/EF1α) that interacts to the Phosphoinositide-Specific Phospholipase C1 (PI-PLC1)

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The putative *Triticum aestivum* GTP-binding protein 1 type elongation factor 1 alpha, GBP1/EF1α, is a cold-induced gene that was found to be differentially expressed in comparisons between cold-tolerant winter wheat and cold-sensitive spring wheat. GBP1/EF1α was mapped on chromosome 2 from the A subgenome of the allohexaploid *Triticum aestivum*. Wheat *GBP1/EF1α* encodes a 533 aa protein with a molecular weight of 58.9 kDa. The phylogenetic analysis shows a close relationship between GBP1/EF1α and EF1 homologs in other plant species. The transit expression of wheat GBP1/EF1α in *Nicotiana benthamiana* leaf epidermal cells showed intercellular localization to the endoplasmic reticulum (ER). Using a bimolecular fluorescent complementation assay (BiFC), GBP1/EF1α was found to interact with the phosphoinositide-phospholipase C-1 (PI-PLC1) on the ER and plasma membrane. This investigation sheds light on the role of GBP1/EF1α (GTP-binding protein 1 type elongation factor 1 alpha) in the signal transduction pathway.

Keywords: GTP-binding protein 1*; Triticum aestivum; Phylogenetic tree; subcellular localization; BiFC assay.*

INTRODUCTION

Cold tolerance in plants has a wide range of genetic variation and inherited as is a mutagenic trait. It develops in wheat over a period of acclimation during which the expression levels of many genes are altered (Gulick et al. 2005; Monroy et al. 2007; Laudencia-Chingcuanco et al. 2011). Different environmental stress responses in plants share common signaling pathways that recruit stress gene expression regulators such as transcription factors, protein kinases and phosphatases, and RNA binding proteins (Shinozaki and Yamaguchi-Shinozaki, 2000; Shinozaki et al. 2003; Guo and Wang, 2012; Avramova, 2017). As signal transduction is the bridge between plant-sensors and gene expression, other effectors may induce the same regulation if perceived by the same or related sensors as cold, drought, and salt stress. Many reports emphasized that protein kinases and phospholipases are regulated by both cold, drought and salt stresses (Shinozaki and Yamaguchi-Shinozaki, 2000; Seki et al. 2002; Shinozaki et al. 2003).

GTP-binding proteins also play a critical role in signaling. GTP-binding proteins are classified into three main groups including alpha subunits of heterotrimeric GTP-binding proteins, the small monomeric GTP-biding proteins with a molecular mass of 20 to 30 KD, and the alpha subunit of polypeptide chain elongation factors (EF-1- α), which catalyze the binding of aminoacyl tRNAs to A site of the ribosome (Albert et al. 2002). The three groups share the consensus sequence of the

GTP-binding domain that contains three elements GXXXXGK, DXXG and NKXD (Saraste et al. 1990). The first and the third elements were known to form the P-loop which has been proposed for phosphate-binding sites. In plants, several unique types of GTP-binding proteins, called unconventional, or non-canonical, GTP-binding proteins, have been reported (Assmann, 2002). The non-canonical G proteins are diverse and there is high sequence similarity among them other than their GTP binding domains; examples of non-canonical G proteins in plants included the extra-large G-proteins (XLG), root hair defective proteins (RHD) and developmentally regulated G proteins (DRGs).

Wheat GTP-binding protein 1 type elongation factor 1 alpha, GBP1/EF1α, was among a number of genes with regulatory or signaling characteristics that were found to be highly induced during cold acclimation in wheat (Gulick et al. 2005). In addition to GBP1/EF1α, these included genes coding the GTP-binding proteins *Gα* and, two genes encoding different phospholipase C proteins, phosphoinositide-specific phospholipase C (PI-PLC) and phosphatidylglycerol specific phospholipase C (PG-PLC), as well as some members of the alpha-tubulin gene family, *αTub*s (Farajalla and Gulick, 2007). Liu, (2005) reported the transcriptional induction of both wheat *PI-PLC1* and GBP1/EF1α during the first 14 days of cold treatment.

Because of protein interactions can occur in different subcellular locations, determination of the location of a protein complex *in vivo* can provide insight into its functional roles and regulation. Bi-fluorescence complementation (BiFC) can indicate the intracellular localization of the interaction between two proteins. In the BiFC assay, the two interacting partners are expressed as two fusions to two different halves of a yellow fluorescent protein (YFP) and their interaction facilitates the reconstitution of the YFP (Hu and Kerppola, 2005). The fluorescence is detected via confocal microscopy in a heterologous system such as *Nicotiana benthamiana*. This approach enables the visualization of subcellular locations of specific protein interactions in the leaf epidermal tissues in a normal intercellular environment. The interaction between wheat GBP1/EF1α and PI-PLC1 *in vivo* was investigated for further understanding the role of GBP1/EF1α in the signal transduction pathway.

MATERIALS AND METHODS

Bioinformatic analyses

Basic Local Alignment Search Tool (BLAST) algorithms provided by the National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/) were used in this investigation for comparison of nucleic acid and amino acid sequences. European Bioinformatics Institute (EBI, http://www.ebi.ac.uk/) ClustalW2 was used for multiple sequence alignment and analysis of sequence structure. The cDNA sequence was also mapped to the wheat genome provided by the international wheat genome sequence consortium (IWGSC, https://www.wheatgenome.org/).

For phylogenetic analysis, a tree-building algorithm for the minimum evolution method (Rzhetsky and Nei, 1992) provided by MEGA-X (Kumar et al. 2018) was applied. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method (Zuckerkandl and Pauling, 1965) and were in the units of the number of amino acid substitutions per site. The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985).

Plant growth conditions

Tobacco (*Nicotiana benthamiana)* seeds were germinated in 10 cm pots in the greenhouse with a supplemental light to extend the day length to 16h light / 8h dark and maintained at 20°C and grown for 2-4 weeks.

Generating entry clones

The coding region of the wheat GBP1/EF1α (1602 nt) was cloned into pDONR207 vector (GATEWAY™ Cloning Technology, Invitrogen, USA) using gene-specific primers with attB1 and attB2 extensions (For. 5' GGGGACAAGTTTGTACAAAAAAGCAGGCTTCA TGGAGCACGACGCGCCG 3'; Rev. 5' GGGGACCACTTTGTACAAGAAAGCTGGGTCA GCTGAAAACGTTGAC 3'). The PCR reaction was carried using PCR temperature cycling: 94°C for 3 minutes, followed by two different types of cycles, 10 cycles of 30 seconds at 94°C, 40 seconds at (53 to 58°C), 2 minutes at 72°C; these were followed by 40 cycles of 30 seconds at 94°C, 40 seconds at 69°C, 2 minutes at 72°C; then 10 minutes at 72°C. The PCR product was electrophoresed to confirm

the correct molecular sizes of the PCR products. The PCR fragment was then subcloned into pDONR 207 via BP reaction Gateway ® BP clonase II mix (Invitrogen, USA). The entry clone was verified by PCR, then confirmed by sequencing using the universal Invitrogen attL1 and attL2 sequencing primers.

Plant expression clones

Gateway LR reactions were used to transfer the insert of the entry clone of wheat GBP1/EF1α to the plant destination binary vector, PK7FWG2, to generate Enhanced Green Fluorescent Protein (EGFP) C-terminal fusions. The same entry clone was also transferred to Gateway Bimolecular Fluorescent Complementation (BiFC) binary Yellow Fluorescent Protein (YFP) vectors, BatL-B-sYFP-N and pBatL-B-sYFP-C, to study the possibility of its protein-protein interactions with *Ta*PI-PLC1. The BiFC vectors were obtained from Alan M. Jones, Departments of Biology and Pharmacology, University of North Carolina. Plasmid for the expression clones of each construct was purified using QIAGEN Spin Miniprep Kit. PCR reactions were carried out with the expression clones to verify the presence of the full length of the insert in the clones.

Agrobacterium transformation

Electrocompetent *Agrobacterium tumefaciens* strain AGL1 was transformed with expression clones by electroporation and selected on LB agar plates containing 100 µg/ml of ampicillin and 50 µg/ml spectinomycin.

Agroinfiltration

A. tumefaciens transformed with GFP or YFP fluorescent protein fusion expression constructs were grown overnight in LB media at 29°C with the appropriate antibiotics. Bacteria were pelleted by centrifugation at 4000 xg for 15 min at 4°C and resuspended in 10 mM $MgCl_2$ and 150 µM Acetosyringone at an OD_{600} of 0.1. The cells were incubated in this solution for 2 hours at room temperature (Walter et al. 2004). Equal volumes three *Agrobacterium* culture suspensions containing expression vectors for the experimental sample, the mCherry fluorescent cellular marker proteins (Nelson et al. 2007), and expression of P19 of tomato bushy stunt virus, used to suppress gene silencing were mixed. The agroinfiltration solution of transformed *Agrobacterium* was co-infiltrated into the leaf abaxial air space of 2-4 week old *N. benthamina* plants. The plants were grown in a controlled environment chamber under long days (16h light / 8h dark) at 20ºC.

Confocal laser scanning microscopy

The epidermic tissues of tobacco leaves were examined two days after infiltration, using SD2 (Scanning Disck2) confocal microscope, at The Cell Imaging and Analysis Network (CIAN) laboratory, McGill University. GFP was excited at a wavelength of 491 nm by the diode laser and the emitted fluorescence was collected through a 520/535nm band-pass. YFP was excited at the same wavelength laser, and the emitted fluorescence was collected through a 543 nm long-pass filter, while mCherry Red Fluorescent Protein (RFP) was excited at a wavelength of 561 nm and the emitted fluorescence was collected through a 624/640 nm long-pass filter**.**

RESULTS AND DISCUSSION

The repertoire of heterotrimeric G-proteins in plants is limited, so plants may have additional GTP-binding proteins. This investigation studied the interaction of wheat GBP1/EF1α (GB: ABF4840) with PI-PLC1 (GB: ADR00313) proteins in *N. benthamina* epidermal leaves suggesting a possible role of GBP1/EF1α in the signal transduction pathway as a GTP-binding protein. Previously, characterizations showed that the Gα subunit of the heterotrimeric G protein complex from wheat, GA3, and phosphoinositide-specific phospholipase C, PI-PLC1 interacted both *in vivo* and *in vitro* (Khalil et al. 2011). Wheat GBP1/EF1α was identified as a cold acclimation induced gene (Gulick et al. 2005), and the expression of GBP1/EF1α was strongly induced during gene profile microarray analysis performed after cold treatment. The level of cold induction of GBP1/EF1α was different between winter and spring wheat. The early response of GBP1/EF1α indicates the possibility of its activation in the initial signaling events in response to cold.

A putative wheat GTP-binding protein 1 type elongation factor 1 alpha (GBP1/EF1α)

The full-length cDNA of wheat GBP1/EF1α was formerly cloned and has a GenBank accession number DQ489316. Wheat GBP1/EF1α full-length cDNA has an insert of 1800 bp including 60 nucleotides upstream of the ATG initiation codon, a 1602 nucleotide ORF, and 3'UTR sequence of 138 nt. The whole wheat genome sequence provided from IWGSC was employed to map GBP1/EF1α (DQ489316). The ORF sequence of GBP1/EF1α was 100% matched to a sequence on

chromosome 2A (TraesCS2A02G076700.1) indicating that GBP1/EF1α belonged to the subgenome A of the allohexaploid wheat.

Wheat *GBP1/EF1α* encodes a 533 aa protein with a molecular weight of 58.9 kDa. Sequence comparison of GBP1/EF1α protein (ABF48401) by BLASTP revealed 100%, 99.44%, and 99.25% identity to wheat predicted protein TraesCS2A02G076700 PP, TraesCS2B02G091600 PP, and TraesCS2D02G075300, respectively, from IWGSC data set. From the GenBank sequences, GBP1/EF1α protein was also showed 99.44% and 99.25% identity to unnamed proteins (VAH41490 and SPT16037) from *T. turgidum* and *T. aestivum*, respectively. In addition, GBP1/EF1α protein was 99.06% identical to *Aegilops tauschii* eukaryotic peptide annotated as chain release factor GTP-binding subunit (ERF3A).

Wheat GBP1/EF1α protein was also found in other related plant species. Wheat GBP1/EF1α protein had 97% identity to *Hordeum vulgare* GTP-binding protein (KAE8777495), 94% identity to *Brachypodium distachyon* ERF3A (P_003573207), and 94 to unnamed protein KAF0892895 from *Oryza meyeriana.*

The evolutionary phylogenetic tree was generated using the Minimum Evolution method **(Figure 1)**. The analysis performed on the protein sequence of GBP1/EF1α (ABF48401) and the most related protein sequences in the IWGSC and GenBank NR databases (TraesCS2A02G076700, TraesCS2B02G091600, TraesCS2D02G075300, SPT16037, VAH41490, XP_020194267, KAE8777495, XP_003573207, KAF0892895, TVU13355, PUZ46297, and XP_002446212). The constructed tree revealed the distance between GBP1/EF1α (ABF48401) and other similar sequences indicating that this protein was encoded from a gene from subgenome A of the wheat genome.

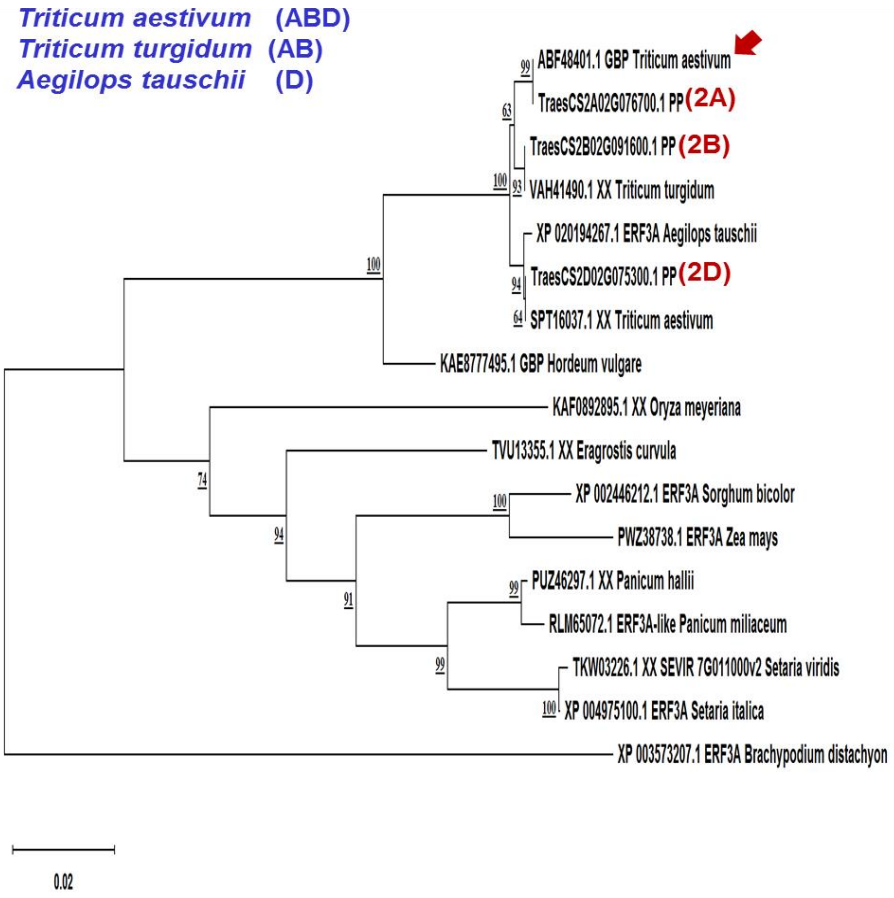

Figure 1: Phylogenetic tree of GBP1/EF1α along with plant homologs.

Minimum evolution phylogenetic tree of GBP1/EF1α and the most similar plant protein homologs was generated. There were a total of 551 positions in the final dataset. Evolutionary analyses were conducted in MEGA –X. GBP: GTP-binding protein; PP: Predicted Protein; XX: Unnamed protein; ERF3A: Eukaryotic peptide chain release factor GTP-binding subunit.

Subcellular localization of wheat GBP1/EF1α

Experimental subcellular localization of wheat GBP1/EF1α-GFP fusion expressed in leaf epidermal cells of *N. benthamiana* revealed the localization of GBP1/EF1α on the endoplasmic reticulum, ER **(Figure 2A)**. The results showed the subcellular localization of GBP1/EF1α on ER where it co-localized with the ER mCherry marker ER-rk CD3-959 that contains the signal peptide of Arabidopsis wall-associated kinase2 (AtWAK2) at the N-terminus of protein, and ER retention signal, HisAsp -Glu-leu, at the C- terminus **(Figure 2A)**. Previously, we assigned the subcellular localization of *Ta*PI-PLC1 on the ER and plasma membrane (PM) when fused to eGFP, and co-localized with both ER and PM fluorescent markers (Khalil et al. 2011).

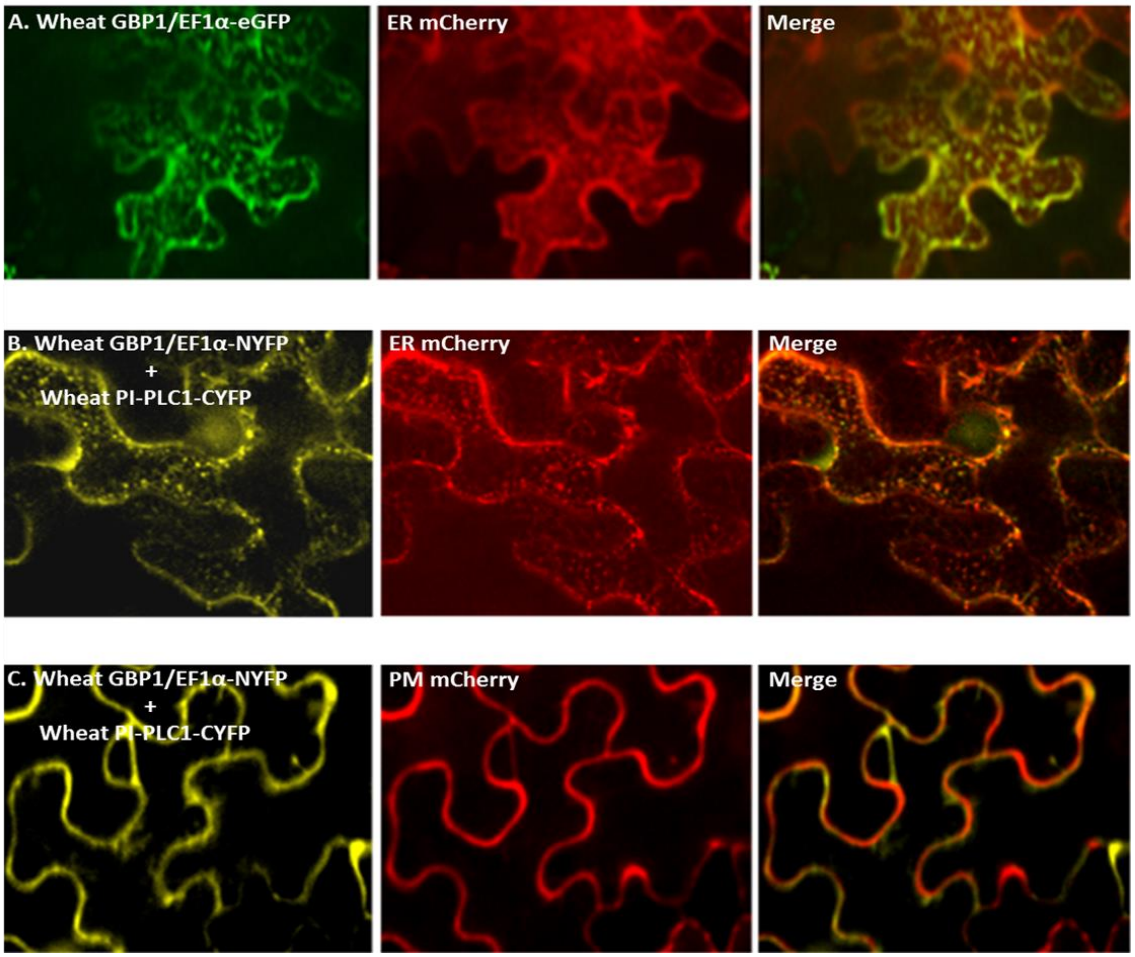

Figure 2: Subcellular localization of GBP1/EF1α fused to GFP and its interaction to PI-PLC in epidermic tobacco tissues.

A. The subcellular localization of GBP1/EF1α-eGFP to ER mCherry marker (ER-rk CD3-959). **B.** BiFC visualization of wheat GBP1/EF1α-NYFP and PI-PLC1-CYFP colocalized to ER (Endoplasmic Reticulum) mCherry marker and **C.** BiFC visualization of wheat GBP1/EF1α-NYFP and PI-PLC1-CYFP colocalized to PM (Plasma Membrane) mCherry; Scale bar = 24 µM.

***In vivo* interaction between wheat GBP1/EF1α and PI-PLC1 using BiFC**

To study the involvement of GBP1/EF1α in signal transduction in plant cells, two BiFC constructs (C and N terminus) containing the GBP1/EF1α were generated and transformed into *N. benthamiana* epidermal cells. BiFC assay showed a positive interaction between wheat PI-PLC1 and GBP1/EF1α which was localized on the ER and PM. The interaction between the proteins reconstituted the YFP that was detected in transformed *N. benthamiana* leaf cells. The two interacting pairs were co-localized with the ER and

PM mCherry markers **(Figure 2B and 2C)**. Positive controls included AtRGS1 N-terminal-YFP, Arabidopsis regulator of G-protein signaling protein 1, and AtGPA1 C-terminal-YFP, Arabidopsis Gα, which showed clear interaction on the PM (Grigston et al. 2008). With the negative control pair, AtPIS-C-terminal, Arabidopsis Ptdlns synthase, and AtHVA22d-N-terminnal-YFPl, an Arabidopsis ABA-responsive protein there was no interaction observed.

CONCLUSION

There are many studies of the heterotrimeric G proteins in plants, but few studies of the other types of GTP-binding proteins. This study focused on the GTP-binding protein type elongation factor 1 Alpha. Wheat GBP1/EF1α was mapped on chromosome 2A of *Triticum aestivum.* This investigation indicated the interaction of the wheat GBP1/EF1α and PI-PLC1 in ER and PM. Further studies are essential to investigate the physiological role of GBP1/EF1α protein during cold stress.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Hala Badr Khalil designed and performleed the experiments and also wrote the manuscript.

REFERENCES

Albert PR, Robillard L. 2002. G protein specificity: Traffic direction required. Cell Signal, 14:407–418.

Assmann SM. 2002. Heterotrimeric and Unconventional GTP Binding Proteins in Plant Cell Signaling. Plant Cell. 14: S355-S373.

Avramova Z, 2017. The jasmonic acid-signalling and abscisic acid-signalling pathways cross talk during one, but not repeated, dehydration stress: a non-specific 'panicky' or a meaningful response? Plant Cell Environ. 40: 1704–1710.

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.

Grigston JC, Osuna D, Scheible WR, Liu C, Stitt M, Jones AM. 2008. D-Glucose sensing by a plasma membrane regulator of G signaling protein, AtRGS1.FEBS Lett. 582: 3577-3584.

Gulick PJ, Drouin S, Yu Z, Danyluk J, Poisson G, Monroy AF, Sarhan F. 2005. Transcriptome comparison of winter and spring wheat responding to low temperature. Genome. 48: 913-923.

Guo L, Wang X. 2012. Crosstalk between Phospholipase D and Sphingosine Kinase in Plant Stress Signaling. Front. Plant Sci. 3.

Hu CD and Kerppola TK. 2005. Direct visualization of protein interactions in living cells using bimolecular fluorescence complementation analysis. Protein-Protein Interactions (ed. P. Adams and E. Golemis). Cold Spring Harbor Laboratory Press 34: 1-20.

Khalil HB, Wang Z, Wright JA, Ralevski A, Donayo AO, Gulick PJ. 2011. Heterotrimeric Gα subunit from wheat (Triticum aestivum), GA3, interacts with the calcium-binding protein, Clo3, and the phosphoinositide-specific phospholipase C, PI-PLC1. Plant Mol Biol, 77: 145-158.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution, 35:1547-1549.

Laudencia-Chingcuanco D, Ganenshan S, You F, Fowler B, Chibber R, Anderson O (2011). Genome-wide gene expression analysis supports a developmental model of low temperature tolerance gene regulation in wheat. BMC Genomics, 12: 299.

Liu W. 2005. Characterization of J822: a novel cold induced GTP-binding protein gene in wheat (*Triticum aestivum*). Masters thesis, Concordia University.

Nelson KB, Xue C, Andreas N. 2007. A multicolored set of in vivo organelle markers for co-localization studied in Arabidopsis and other plants. Plant J., 51:1126–1136.

Farajalla MR, Gulick PJ (2007). The alpha-tubulin gene family in wheat and dfferential gene expression during cold accumulation. Genome, 50: 502-510.

Rzhetsky A, Nei M. 1992. A simple method for estimating and testing minimum evolution trees. Molecular Biology and Evolution, 9:945-967.

Saraste M, Sibbald PR, Wittinghofer A. 1990. The P-loop - a common motif in ATP- and GTP-binding proteins. Trends in Biochemical Sciences, 15: 430–434.

Seki M, Narusaka M, Ishida J, Nanjo T, Fujita M, Oono Y, Kamiya A, Nakajima M, Enju A, Sakurai T et al. 2002. Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold, and high-salinity stresses using a full-length cDNA microarray. Plant J. 31: 279-292.

Shinozaki K, Yamaguchi-Shinozaki K. 2000. Molecular response to dehydration and low temperature: Differences and cross-talk between two stress signaling pathways. Curr. Opin. Plant Biol. 3: 217–223.

Shinozaki K, Yamaguchi-Shinozakiy K, Sekiz M. 2003. Regulatory network of gene expression in the drought and cold stress responses. Curr Opin Plant Biol. 6: 410-417.

Walter M, Chaban C, Schutze K, Batistic O, Weckermann K, Nake C, Blazevic D, Grefen C, Schumacher K, Oecking C, Hater K, Kudla J. 2004. Visualization of protein interaction in living plant cells using bimolecular fluorescence complementation. Plant J, 40:428-438.

Zuckerkandl E, Pauling L. 1965. Evolutionary divergence and convergence in proteins. Edited in Evolving Genes and Proteins by V. Bryson and H.J. Vogel, pp. 97-166. Academic Press, New York.