

Comprehensive Computational Analysis of *cis*-Regulatory Elements in 5' Regulatory Region of ADP Glucose Pyrophosphorylase in Different Plants

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Abstract- ADP-glucose pyrophosphorylase (AGPase) is a key regulatory enzyme for starch synthesis in plant. Starch is an important carbohydrate consisting of a large number of glucose units and the primary energy source in plants. The essential transcriptional gene regulatory units are *cis*-acting regulatory elements as they control various stress responses. Therefore, understanding of the transcriptional gene regulation necessitates functional analyses of *cis*-acting elements. In this study, *cis*-acting regulatory elements present with in the 5'_regulatory region of AGPase small and large subunit in rice, arabidopsis, wheat, barley, potato and maize were identified using bioinformatics approach. Prediction of folding state of AGPase in indicates that some of them also have disordered amino acids. Phylogenetic analysis revealed a high degree of homology to all available AGPase sequences using ClustalW2 and Neighbour-joining method. In addition to this, we also modeled the structure of C-terminal β -helix domain of AGPase of wheat LS which interacts with catalytic domain and is responsible for the functionality of enzyme. This study revealed the possible role of *cis*-acting regulatory elements in the expression and regulation of AGPase gene in *Triticum aestivum* and other plants during cellular development or other biological conditions.

Index Term- ADP Glucose Pyrophosphorylase, Cis regulatory elements, PLACE, Plant CARE, Fasta, Motif, Annotation, Subunits

1. Introduction

Starch is an important carbohydrate and the primary energy source in plants. It has several industrial applications as reviewed by Slattery et al. [1]. Starch biosynthesis occurs mainly by the participation of three enzymes; ADP-glucose pyrophosphorylase (AGPase), branching enzymes and starch synthase [2, 3]. The first enzyme in starch biosynthesis is the AGPase that catalyzes the conversion of Glc-1-P and ATP to ADP-glucose and pyrophosphate (PPi). ADP-glucose is then used by starch synthase for the synthesis of polyglucans. Many researchers have revealed that the AGPase catalyzes the rate limiting step in starch biosynthesis in higher plants [1, 2, 4]. AGPase from higher plants has a heterotetrameric structure ($\alpha 2\beta 2$) composed of pairs of SS and large LS subunits encoded by at least two different genes. The LS plays a major role in allosteric regulation through its interaction with the small catalytic subunit (SS). The LS is encoded by the shrunken-2 (Sh2) and the SS by brittle-2 (Bt2) [5]. Transcriptional gene regulation is important for the function and development of all organisms [6]. Transcription factors (TFs) are essential for the differential gene expression in higher organisms [7]. Identification and annotation of TF catalogues representing different plant species will provide an insight on organization and biological functionality of the TFs as well as their evolution. TFs play a key role in gene regulation. Computational analysis of TFs at genome scale is the first

step toward understanding the mechanism of gene expression and regulation. Mostly in plant, the regulatory portion of genes is located primarily in 1000 bp upstream of regulatory region [8]. The promoter region consists of specific DNA sequences and response elements that act in the recruitment of protein factors to facilitate transcription of the protein-coding region of the gene. These regulatory sequence elements located on the same strand as the coding region of the gene are called the *cis*-acting regulatory elements or the transcription factor binding sites. The natural environment for plants is poised of a complex set of abiotic stresses and biotic stresses. Plant responses to these stresses are also complex. Systems biology approaches assist a multi-targeted approach by allowing one to identify regulatory hubs in complex networks [9]. The specific interactions between TFs and their binding sites (regulatory sequences), plays a central role in the regulation of different biological processes which are responsible for functionality of gene. Thus, the identification of regulatory motifs and their organization is an important step to advance understanding of gene expression and regulation. So far, AGPase has been cloned from wheat, maize and potato [10-12] but no information on the *cis*-acting element that regulates the expression of AGPase during the stress condition is available. Therefore, the aim of this study was to predict available bioinformatics tools to reveal a comprehensive description of *cis*-acting regulatory elements that are present within the 5'_ regulatory region

of the DNA sequences of AGPase gene in wheat and other selected plants. Additionally in order to understand the sequence and structure relationship, analysis to infer theoretical pI, domain sequences, sub-cellular location and folding states was carried out. In order to see the evolutionary relationship amongst AGPase in plants, we performed a phylogenetic analysis based on protein sequences and developed 3-D model for C- terminal left handed β -helix domain.

2 Materials and methods

2.1 Source of data

The complete nucleotide sequence for both LS and SS of AGPase for wheat (DQ839506, AF244997), rice (AY028314, FJ750945), maize (NM_001112547, AY032604), potato (NM_001288466, AY186620), barley (KF442976, EU275212) and Arabidopsis (AY070429, U70616) were retrieved from the NCBI (National Center for Biotechnology Information) (<http://www.ncbi.nlm.nih.gov/>). Additional sequences of AGPase were collected by using the Basic Local Alignment Tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Complete nucleotide and protein sequences of large subunit of wheat AGPase were considered for BLAST homology searches against the plant species.

2.2 Identification of the 5' regulatory region

AGPase nucleotide sequences were scanned for the presence of putative *cis*-acting regulatory elements identical with or similar to the registered in Plant CARE (<http://bioinformatics.psb.ugent.be/webtools/plantcare/cgi-bin/CallMatIE55.html>), and PLACE (<http://www.dna.affrc.go.jp/PLACE/signalscan.html>) databases.

2.3 Bioinformatics analysis

Based on program of ExPASy, (http://web.expasy.org/compute_pi/) speculative pI was calculated for various plants under study. The folding states were predicted by FoldIndex program (<http://biportal.weizmann.ac.il/fldbin/findex>). Protein targeting analysis program iPSORT (<http://hc.ims.u-tokyo.ac.jp/iPSORT/>), and PREDOTAR (<http://urgi.versailles.inra.fr/predotar/>) were used to estimate the putative sub-cellular locations of the candidate proteins.

Gene ontology (GO) annotation was performed by using AmiGO (<http://www.geneontology.org/>) AGPase of

different plants to investigate their biological process, cellular component and molecular functions.

Phylogenic analysis was generated using homologous AGPase sequences using the neighbor-joining (NJ) distance method [13]. Phylogenetic tree was drawn with MEGA v5.0 software [14]. Evolutionary distances were calculated following the Poisson correction evolutionary model, and the excessive gaps/ missing data were handled using the pair-wise delete option.

2.4 3D model prediction and validation

The protein sequence of wheat AGPase small subunit was subjected to Swiss Modeller (<http://swissmodel.expasy.org/>) for 3D model prediction and the modeled structure was assessed using the protein structure and model assessment tools at the Swiss Model Server, which utilizes various local and global quality estimation parameters. Lastly the model was visualized using pymol [15].

3 Result and discussion

3.1 *cis*-acting regulatory elements analysis

Advancement of recent bioinformatics tools have opened up the door for studies on the regulation of expression of genes of interest. The presence of various *cis*-regulated in their regulatory regions inferred that they are well regulated by similar cellular or environmental factors. The complete nucleotide sequence and the coding domain sequence (CDS) of the AGPase gene in wheat and other crops (Table 1) were computationally examined to identify the *cis*- regulatory region. In large subunit of AGPase (TaAGPaseLS) core elements (Table 2): CANNTG, NGATT, RYCGAC, and YAACKG motifs which are nearby to the translational start site (ATG) were found to be very close to one another. However, CACCTG was found at the distal part of the 5' regulatory region in all of the genes. On the contrary in small subunit (TaAGPaseSS) GATAAG, GATA and CAAT motifs were closest to the translational site and found to be very close to one another in several of the genes. GATA-box and Sp1motif is well-known as light responsive *cis*-element (LREs) are found in the regulatory region of light-regulated genes, apparently essential for light-controlled transcriptional activity [16] The CuRE motif has been connected with genes that require copper for their expression, through copper-response elements coupled with them [17] ARR1AT family motif was found in the regulatory region of large subunit TaAGPase whereas

RAV1BAT family motif was found in the regulatory region of large subunit of both TaAGPase and HvAGPase. MYBCORE family motif was found in the regulatory region of large subunit of AtAGPase and also found in small subunit of OsAGPase. MYB-proteins play crucial roles in cell proliferation and differentiation [18]. CAATbox1 was found in large subunit of StAGPase and the small subunit of AtAGPase.

CAAT boxes as well as other potentially important *cis*-acting regulatory elements that are required for minimal promoter activity in plants. ABRE is also an example of an extended *cis*-element that contains the G-box core sequence [(C/A) ACG (T/C) G (T/C/G) [19].

3.2 Functional characterization of AGPase

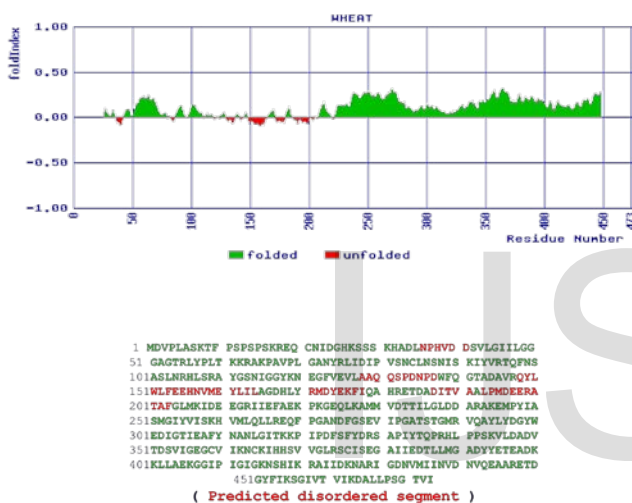


Fig 2. Foldindex plotted with window size 51 for AGPase, the plot showed that in small subunit of AGPase various little disordered regions are present. Positive and negative numbers represent ordered and non ordered protein, respectively. Amino acids suggested as being ordered is shown in green and unordered in red, respectively.

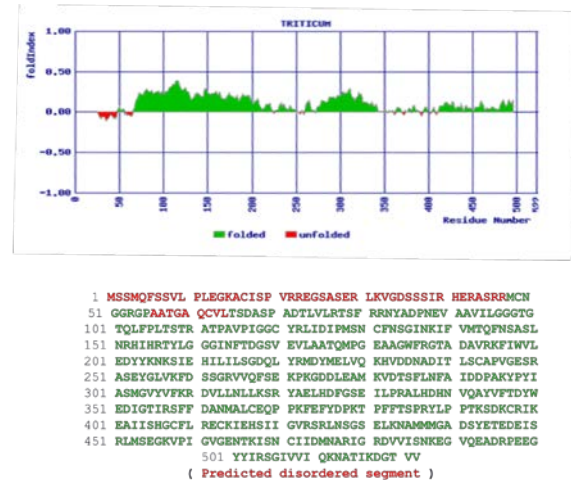


Fig 3. The plot showed that in large subunit of AGPase contains only two disordered regions which are of different length. Positive and negative numbers represent ordered and non ordered protein, respectively. Ordered amino acids are shown in green and unordered in red colour.

3.3 Gene ontology

Gene ontology (GO) annotation was performed using the AmiGO annotation tool and AGPase sequences were classified (Table 3) into three categories, namely, biological process, cellular component and molecular function. Molecular function assignment revealed members of broad range of category i.e. ATP binding glucose-1-phosphateadenyl transferase activity. All three categories named biological process; cellular component and molecular function in are given Table 3.

3.4 Structural analysis and phylogenetic tree construction

To study the polygenetic analysis, tree was generated for all the sequences of AGPase, the multiple sequence alignment (MSA) of *T. aestivum*, *O. sativa*, *Z. mays*, *S. tuberosis*, *H. vulgare* and *A. thaliana* (Fig 1) was carried out, MSA revealed a large number of conserved residues in the sequences of all the plant species. C-terminal beta helix domain is composed of residue from 341-466 that are highlighted (Fig 5) pink in the sequence of small subunit of wheat AGPase.



Fig 1. Multiple sequence alignment using amino acid sequence of different cereal crop. Conserved amino acids are shown in green boxes. Protein structural features are indicated above the alignment. α -helix and β -strands are elements of secondary structure represented as rods and arrow

This domain is length of approximately 125 residues. Multiple sequence alignment using amino acid sequence of different cereal crop revealed that conserved amino acids are shown in green boxes. Protein structural features are indicated above the alignment. Alpha helix and beta strands are elements of secondary structure represented as rods and arrow. Phylogenetic analysis of all AGPase sequences formed two distinct clusters. Phylogenetic analysis showed (Figure4) two large clusters Cluster I containing the approximately 52 sequences and cluster II contain 48 sequences; total sequences used for tree construction were 100.

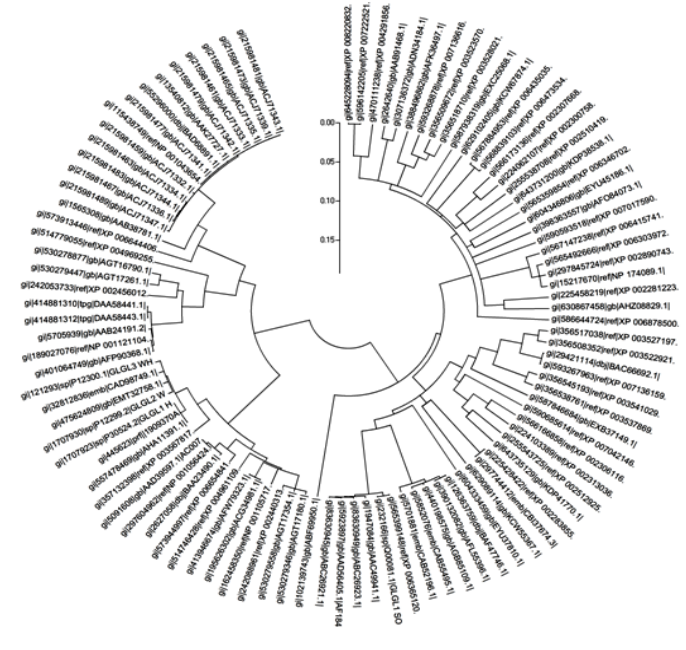


Fig 4. Phylogenetic tree of AGPase sequences study in this work. This analysis showed two large clusters Cluster I and cluster II, both these are of approximately equal length. Phylogenetic analysis of all AGPase sequences from different plant species constructed by the Neighbor-joining method using the MEGA 5 program with bootstrap values are indicated against each branch.

Phylogenetic analysis of all AGPase sequences from different species constructed by the Neighbour-joining method using the MEGA 5 program with bootstrap values are indicated against each branch. In cluster I we observed that the Accession no ACJ71342 i.e. large subunit of *Oryza sativa* and ACG34981 *Zea mays* large subunit were distantly present. On the contrary accession no DAA58441.1 and AAB24191.2 were present nearest to each other.

3.5 Analysis of 3D-Structure of left handed beta helix domain

The 3D- structure of left handed beta domain of small subunit of wheat AGPase was predicted by homology Modelling using Swiss Model [21]. (Figure5). Target sequence showed 49% identity with template PDB code 1Y2 (Crystal structure of Small subunit of Potato AGPase). Sequence alignment between query and template indicated that catalytic residues and left handed domain residues are well conserved between both the sequences (Figure 6A).

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>gi|232172|sp|P30523.1|GLGS_WHEAT RecName: Full=Glucose-1-phosphate
adenyltransferase small subunit, chloroplastic/amyloplastic; AltName: Full=ADP-
glucose pyrophosphorylase; AltName: Full=ADP-glucose synthase; AltName: Full=AGPase
B; AltName: Full=Alpha-D-glucose-1-phosphate adenylyl transferase; Flags: Precursor
MDVFLASRTFFSPSPKREQCNI DGHKSSSKHADLNPHVDSDVLGII LGGAGTRLYPLTKKRAKPAVPL
GANYRLIDIPVSNCLNSNISKIYVRTQFNASLNRHLSRAYSGNIGGYKNEGFVEVLAQQSPONPDWQ
GTADAVRQYLWLFEEHNVMEYLILAGDHLRYMDYKFTQAHRETADITVAALPMDEERATAFGLMKIDE
EGRIIEFAEKPKGQLKAMVVDITLGLDARAKEMFYIASMGIIYISKHVMQLLREQFPGANDFGSEV
IPGATSTGMRVQAYLYDGYWEDIGTIEAFYNANLGITTKKPIPFSPFYDRSAPIYTPRHLPPSRVLDADV
TDSVIGEGGVINKCKIHRHSVGLRSCISEGAIIEOTLLMGADYYETEADKLLAEKGGIGTIGIKNSHIK
RAITDKNARIGDNVMIINVDNVQEAARETDGYFTKSGIVTVIKDALLPSGTVI
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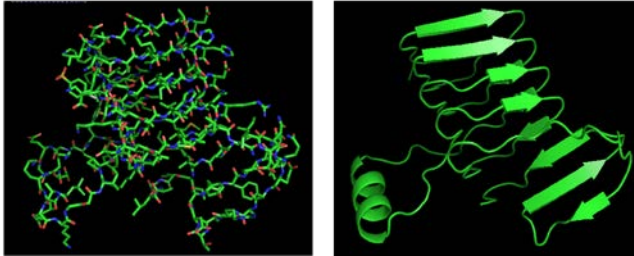


Fig 5. C-terminal β -helix domain. A composed of residue from 341-466 highlighted pink in the above sequence B the stick representation of C-terminal beta helix domain C shows the cartoon representation of this domain. The catalytic domain is connected to the C-terminal β -helix domain by a long loop. This loop makes numerous interactions with the equivalent region of another subunit.

The structure generated was assessed using the Protein Structure and Model Assessment Tools at the Swiss Model Server, which include Z score [22], QMEAN6 [23]. The predicted Z-score was -0.210, QMEAN6 score was 0.738. Negative energy values (in green in Fig.6 B) represent a favourable energy environment whereas positive values (in red) depict unfavourable energy environment for a given amino acid.

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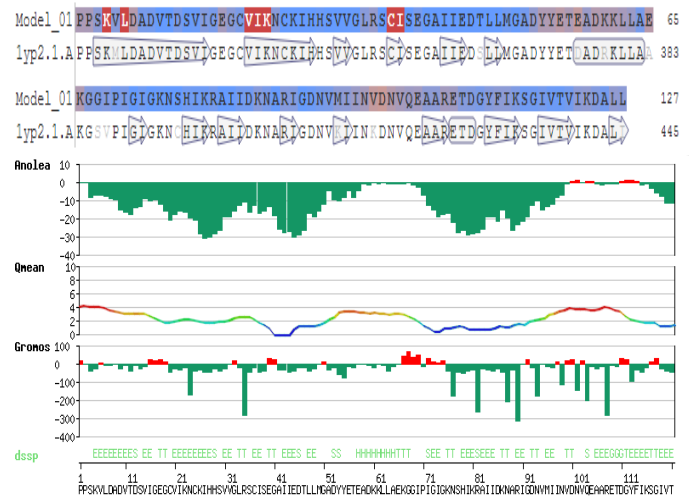


Fig 6. (A) Sequence alignment between query (wheat AGPase) and template (1YP2) showed the significant no of residues were conserved in both the sequences. (B) Assessment for Anolea, Qmean and Gromos force fields of left handed domain of small subunit of wheat AGPase. Green favourable energy environment, red unfavorable energy environment.

References

- [1] C.J. Slattery, I.H. Kavakli and T.W. Okita, "Engineering starch for increased quantity and quality." *Trends in Plant Science* vol. 5, pp. 291–298, 2000.
- [2] C. Martin and A. M. Smith, "Starch biosynthesis," *Plant Cell*, vol. 7, no. 7, pp. 971–985, 1995.
- [3] P. R. Salamone, T. W. Greene, I. H. Kavakli and T.W. Okita, "Isolation and characterization of a higher plant ADP-glucose pyrophosphorylase small subunit homotetramer," *FEBS Letters*, vol. 482, pp. 113-118, 2000.
- [4] A. Tuncel, I.H. Kavakli, and O. Keskin, "Insights into subunit interactions in the heterotetrameric structure of potato ADP-Glucose Pyrophosphorylase", *Biophysical J.* vol. 95: pp.3628-3639, 2008.
- [5] M. R. Bhave, S. Lawrence, C. Barton and L. C. Hannah, "Identification and molecular characterization of shrunken-2 cDNA clones of maize" *Plant Cell*, vol. 2, pp. 581-588.1990.
- [6] W.W. Wyeth, S. Albin, "Applied bioinformatics for the identification of regulatory elements." *Nat. Rev. Genet.* Vol.5, pp. 276–287, 2004.

[7] G. Lloyd, P. Landini and S. Bushby, "Activation and repression of transcription initiation in bacteria." *Essays Biochem*, vol. 37, pp. 17–31, 2001.

[8] C. Dean, R. Schmidt, "Plant genomes: a current description". *Annu. Rev. Plant Physiol Plant Mol. Biol.*, vol. 46, pp. 395–418, 1995.

[9] G. R. Cramer, K. Urano, S. Delrot, M. Pezzotti and K. Shinzaki, "Effects of abiotic stress on plants: a systems biology perspective." *BMC Plant Biology*, vol. 11, pp. 163, 2011.

[10] M.R. Olive, R. J. Ellis, W.W. Schuch "Isolation and nucleotide sequences of cDNA clones encoding ADP-glucose pyrophosphorylase polypeptides from wheat leaf and endosperm." *Plant Mol Biol*, vol.12, pp. 525–38, 1989.

[11] J.L. Prioul, E. Jeannette, A. Reyss, N.Grégory, M.Giroux, L.C Hannah, and M Causse" Expression of ADP-glucose pyrophosphorylase in maize (*Zea mays* L.) grain and source leaf during grain filling." *Plant Physiol*, vol.104, pp179–187, 1994.

[12] U. L. Cognata, L. Willmitzer, B.M. Röber "Molecular cloning and characterization of novel isoforms of potato ADP-glucose pyrophosphorylase." *Molecular and General Genetics*, vol. 246, pp 538–48 1995;

[13] N. Saitou, M. Nei, "The neighbor-joining method: a new method for reconstruction of phylogenetic trees." *Mol Biol Evol*, vol. 4, pp. 406–425, 1987.

[14] K. Tamura, J. Dudley, M. Nei, S. Kumar, "MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0." *Mol Biol Evol*, vol. 24, pp. 1596–1599, 2007.

[15] W.L. DeLano, "The PyMOL Molecular Graphics System. San Carlos, CA: DeLano Scientific, 2002.

[16] E. Lam, N. H Chua, "ASF-2: a factor that binds to the cauliflower mosaic virus 35S promoter and a conserved GATA motif in Cab promoters." *Plant Cell* vol. pp. 1147–1156, 1989.

[17] J.M. Quinn, P. Barraco, M. Eriksson, S. Merchant. "Coordinate copper- and oxygen-responsive Cyc6 and Cpx1." *J Biol Chem*. Vol. 275, pp. 6080–6089, 2000.

[18] B. Luscher, R.N. Eisenman, "New light on Myc and Myb. Part II. Myb." *Genes Dev*, vol. 4, pp.2235–2241, 1990.

[19] P.K Busk and M. Pages, "Regulation of abscisic acid induced transcription." *Plant Mol Biol* vol.37, pp. 425–435, 1998.

[20] J. Prilusky, C. E Felder, T. Z Ben-Mordehai, E.H Rydberg, O. Man, J.S Beckmann, I. Silman and J. L Sussman, "FoldIndex: a simple tool to predict whether a given protein sequence is intrinsically unfolded". *Bioinformatic* vol. 21, pp. 3435–3438, 2005.

[21] N. Guex and M.C. Peitsch, "SWISS-MODEL and the Swiss-Pdb Viewer: an environment for comparative protein modeling." *Electrophoresis*, vol. 18, pp. 2714–2723, 1997.

[22] P. Benkert, M. Biasini and T. Schwede, "Toward the estimation of the absolute quality of individual protein structure models." *Bioinformatics*, vol. 27, pp.343–350, 2011.

[23] P. Benkert, S.C.E. Tosatto and D. Schomburg QMEAN: a comprehensive scoring function for model quality assessment." *Proteins Struct Funct Bioinformatics*, vol. 71, pp. 261–277, 2008.

Conclusions

ADP-glucose pyrophosphorylase, a key enzyme involved in higher plant starch biosynthesis, is composed of pair of large (LS) and small subunits (SS). Ample evidence has shown that the AGPase catalyzes the rate limiting step in starch biosynthesis in higher plants. Cis regulatory elements are found in the vicinity of the gene, or genes, they regulate. CREs typically regulates gene transcription by functioning as binding sites for transcription factors. Identification of *cis*-regulatory elements in gene promoters is an extremely challenging research issue in computational molecular biology. This computational study focuses on non-coding regions of AGPase genes and numbers of putative motifs at the 5' regulatory regions were identified. But the biological importance of these sequences would needs to be further investigated by using bioinformatics with experimental expression analysis. In addition to this, we also compiled detailed comparative information about ADP-glucose pyrophosphorylase in selected plants by analyzing their structural features e.g. secondary structural features, folding state, gene ontology and phylogenetic analysis. We used homology modeling to solve the structure of left handed beta helix domain of small subunit of AGPase. Present study will provide an insight for the biologists working with ADP-glucose pyrophosphorylase in order to understand the functionality of AGPase.

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Supplementary material

Supplementary Table 1. The complete nucleotide sequence and the coding domain sequence (CDS) of the AGPase gene in various plants.

Gene	Accession no	No of base pair	CDS	5'UTR beforeATG5'
TaAGPase LS	DQ839506	1947 bp	117..1685	1-116
TaAGPase SS	AF244997	1673 bp	16..1437	1-15
OsAGPase LS	AY028314	1620 bp	52..1608	1-51
OsAGPase SS	FJ750945	1880 bp	24..1526	1-23
ZmAGPase LS	NM_001112547	1616 bp	69..1586	1-68
ZmAGPase SS	AY032604	1927 bp	141..1673	1-140
HvAGPase LS	KF442976	1786 bp	97..1668	1-96
HvAGPase SS	EU275212	1741 bp	9..1550	1-8
StAGPase LS	NM_001288466	1708 bp	64..1515	1-63
StAGPase SS	AY186620	1845 bp	64..1629	1-63
AtAGPas LS	AY070429	1994 bp	127..1698	1-126
AtAGPaseSS	U70616	1763 bp	55..1617	1-54

Table 2. Cis regulatory element in 5' regulatory region of the AGPase in various crops

Gene	Accession no	Factor name	Position	pattern	Function
TaAGPase LS	DQ839506	MYCCONSENSUSA T	12	CANNT G	Function as transcriptional activators response
		ARR1AT	16	NGATT	Regulators operate as transcriptional activators.
		CBFHV	45	RYCGAC	Function as dehydration- responsive element
		MYB2CONSENSUSA T	9	YAACK G	MYB recognition site found in the promoters of the dehydration-responsive gene
		RAV1BAT	103	CACCTG	RAV1 protein contain AP2- like and B3-like domains
TaAGPase SS	AF244997	IBOX	2	GATAA G	Helps in light regulation
		GATABOX	4	GATA	Required for high level, light regulated, and tissue specific expression
		CAATBOX1	14	CAAT	Common cis-acting element in promoter and enhancer regions
OsAGPase LS	AY028314	RHERPATEXPA7	2	KCACG W	Root Hair-specific cis- Elements.
		EBOXBNNAPA	17	CANNT G	This sequence is also known as RRE (R response element
		DOFCOREZM	40	AAAG	Required for expression of multiple genes involved in carbon metabolism
		CURECORECR	46	GTAC	GTAC is the core of a CuRE (copper-response element)
OsAGPase SS	FJ750945	MYBCORE	11	CNGTTR	involved in regulation of genes that are responsive to water stress

SbAGPase LS	NM_00111257	QELEMENTZMZM1 3	15	AGGTCA	Involved in expression enhancing activity
		LTRECOREATCOR1 5	21	CCGAC	Core of low temperature responsive element
		WRKY71OS	40	TGAC	a transcriptional repressor of the gibberellins signaling pathway
		CGACGOSAMY3	64	CGACG	May function as a coupling element for the G box element;
SbAGPase SS	AY032604	CACTFTPPCA1	55	YACT	phosphoenolpyruvate carboxylase (ppcA1) of the C4 dicot
		GTGANTG10	8	GTGA	Not specified
		MYBCOREATCYCB1	108	AACGG	able to activate reporter gene without leading to M- phase specific expression
HvAGPase LS	KF442976	ARR1AT	7	NGATT	ARR1 is a response regulator
		CGACGOSAMY3	49	CGACG	May function as a coupling element for the G box element;
		RAV1BAT	83	CACCTG	RAV1protein contain AP2- like and B3-like domains; The AP2-like andB3-like domains recognize the CAACA and CACCTG motifs, respectively;
HvAGPase SS	EU275212	CAATBOX1	7	CAAT	Common cis-acting element in promoter and enhancer regions
StAGPase LS	NM_001288466	WRKY71OS	17	TGAC	a transcriptional repressor of the DE gibberellins signaling pathway
		ANAERO4CONSEN SUS	45	GTTTHG CA	Not specified
		CAATBOX1	51	CAAT	Common cis-acting element in promoter and enhancer

					regions
StAGPase SS	AY186620	CACTFTPPCA1	8	YACT	Tetranucleotide (CACT) is a key component of Mem1 (mesophyll expression module 1) found in the cis-regulatory element in the distal region of the phosphoenolpyruvate carboxylase (ppcA1) of the C4 dicot
		MYCCONSENSUS	39	CANNT G	MYC recognition site found in the promoters region
		AT GAREAT	54	TAACA AR	dehydration-responsive gene
AtAGPase LS	AY070429	MYBCORE	9	CNGTTR	involved in regulation of genes that are responsive to water stress
		INRNTPSADB	44	YTCANT YY	Light-responsive transcription activator Required for high level, light regulated, and tissue specific expression
		GATABOX	80	GATA	Tetranucleotide (CACT) is a key component of Mem1 (mesophyll expression module 1) found in the cis-regulatory element in the distal region of the phosphoenolpyruvate carboxylase (ppcA1) of the C4 dicot
		CACTFTPPCA1	103	YACT	Not specified

AtAGPase SS	U70616	INRNTPSADB	8	YTCAN TYT	light-responsive transcription regulator
		CAATBOX1	24	CAAT	Common cis-acting element in promoter and enhancer regions
		CACTFTPPCA1	48	YACT	distal region of the phosphoenolpyruvate carboxylase (ppcA1) of DE the C4 dicot

Table3.Gene Ontology

AGPase	Cellular component	Biological process	Molecular function
Features	Amyloplast, Chloroplast	Glycogen biosynthetic process starch biosynthetic process	ATP binding glucose-1-phosphate adenylyltransferase activity