In silico Analysis of P-Type ATPases in Selected Members of Poaceae Family

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Plant Biotechnology

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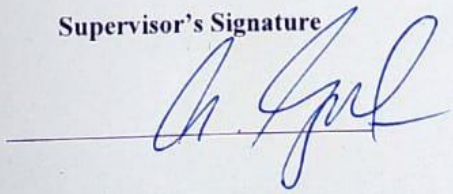

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MS THESIS WORK

We hereby recommend that the dissertation prepared under our supervision by **MAHNOOR ILYAS & 318579**
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Acknowledgment

I am blessed by Whom, who has created all that exists, who helped me to get through all the way, gave me the potential to do so gave me strength and guidance the one and only Allah Almighty. Countless salutations on the noble personage of Hazrat Muhammad صلى الله عليه وسلم ,and peace and mercy upon His noble companions'eminent members of family and true followers till the day of judgement. My parents and friends are my inspiration who never let me give up at any cost and support me to their fullest.

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Dedication

“My dissertation is dedicated to my Parents and my Best friend”

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Abstract

The members of Poaceae/grass family consists of crops having economical and agricultural significance such as wheat, rice, maize and sorghum that can adapt to any terrestrial environment therefore wild progenitors of these crops are great genetic source of beneficial alleles that can contribute to the vigorous plant growth and biotic and abiotic stress tolerance/resistance. Hexaploid wheat (*Triticum aestivum*) has supreme importance as major crop worldwide providing nutritive food to humans and fodder to animals. Plants counteract many environmental stresses that pose serious effects on their growth and development that is why an insight to the computational analysis can pave the way for understanding of advantageous gene functions which can be a valuable addition to crop improvement studies. Plant P-class pumps are divided into five sub-families i.e., P1B, P2, P3A, P4 and P5A type ATPases which play an important role in transporting cations/lipid complexes across the cell membranes while maintaining cellular homeostasis. In this research, P-type ATPases from 8 selected members of Poaceae family were employed for phylogenetic tree construction to demonstrate their evolutionary relationship. *Triticum aestivum* P-type ATPases were analyzed for identification of conserved motifs, gene structure (for intron/exon positions) and subcellular localization.

Keywords: Poaceae family, wheat, P-type ATPases, computational analysis.

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List of Abbreviations

TGN	Trans-Golgi network
SERCA	Sarco/endoplasmic reticulum Ca^{+2} ATPase
HMA	Heavy metal ATPase
PAA1	P-type ATP-Ase 1
ACA	Autoinhibited calcium ATPase
ECA	Endoplasmic reticulum calcium ATPase
AHA	Plasma membrane Proton ATPase
ALA	Aminophospholipid ATPase
ALIS	ALA interacting subunit
GTP	Guanosine-5'-triphosphate
CDS	Coding sequence
CaM	Calmodulin
FLS2	Flagellin sensing 2
Flg22	Flagellin 22
CAX1	Calcium exchanger 1
IWGSC	International wheat genome sequencing consortium
dsRNA	Double stranded RNA
CopA/CadA	Copper/Cadmium ATPase
MBD	Metal binding domain
PAMP	Pathogen associated molecular patterns
QTL	Quatitative trait loci
P14P	Phosphatidylinositol 4-phosphate
CMV	Cucumber mosaic virus
TX-100	Triton X-100
MEGA	Molecular evolutionary genetics analysis
GSDS	Gene structure display server

Chapter 1

1. Introduction

The world crop production has undergone significant changes after the "Green Revolution", occurred in the 1960s and 1970s, playing a critical role in the development of cereal crops through genetic breeding in Mexico and Philippines that provided the people of Pakistan, India, and Latin America with hybrid crop plants having high yield varieties and better qualities(Den Herder *et al.*, 2010; Ortiz, 2011). Proteomics is a systems biology approach that moves from genome to phenome helping the researchers to understand the basic phenomenon of metabolic and signaling pathways as a result of plant response to changing environmental stimuli(Großkinsky *et al.*, 2015; Slingo *et al.*, 2005; Tanou *et al.*, 2012). This chapter includes the introduction of the Poaceae family plants, etymology, taxonomic hierarchy, morphology, and P-type ATPases that are necessary for cellular transport and prevent plants from biotic and abiotic stress conditions.

1.1. Overview of Poaceae Family

Cereal grains, originated from the grass family of flowering monocots which is called Poaceae (formerly known as Gramineae), are still contributing to the world's greatest need for food through starchy caryopsis; fruit composed of the endosperm, bran, and germ abundant in starch, omega 3 fatty acids, and vitamin E, respectively, since their first agricultural venture by the ancient man, the evolutionary tree of monocots distinguishing Poaceae family is described in the Figure.1.1(Lásztity, 1998; Sarwar *et al.*, 2013). These plants can grow in almost every habitat but can't flourish in the ocean due to unfavorable vegetative conditions(Chapman *et al.*, 1990). These whole-grain cereals are not only an ample source of nutrition but also cover a wide range of phenolic compounds, tocols, β-glucans, amino acids, minerals, and vitamins, revitalizing the human immune system with the least likelihood of heart disease and cancer(Benincasa *et al.*, 2019; Mohanraj, 2020).

The world's grain consumption comprises more than 50% of the calories taken up in a day, emphasizing the increase in its demand and production to meet the need of

ever-increasing consumers and it has been estimated according to the Food and Agriculture Organization that by the end of seasons in 2021, world's grain stocks will be 890 million tons, which is 1.9% above the opening estimate as compared to 0.7% down from the September gauge showing a favorable high record(Awika, 2011; FAO, 2020).

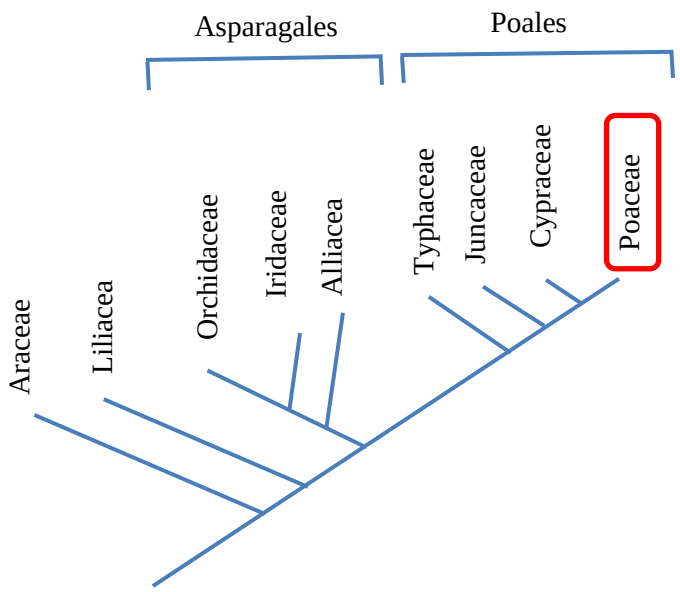


Figure. 1.1. Monocotyledonous Phylogenetic tree

The grass family has a significant contribution to the lives of human beings supporting them by agronomical and economic means, but the climate change is so abrupt that before adapting to the environmental conditions, most of the crops don't survive or continue to exist with low yield(Eckardt, 2008; K. M. K. Huda *et al.*, 2013).

1.1.1.Etymology and Taxonomic History

The name Poaceae comes from a Greek word “poa” meaning “fodder”, named by John Hendley Barnhart in 1895 based on tribe(subfamily) presented by Robert Brown in 1814 who scientifically subdivided monocot family into two subfamilies; Poaceae and Paniceae, for the first time before that Carl Linnaeus in 1753 assigned them as genus(Barnhart, 1895; Clark, 2004).

Table 1.1. Taxonomic Hierarchy of the Poaceae Family

Kingdom	*Plantae*
Clade	*Tracheophytes*
Clade	*Angiosperms*
Clade	*Monocots*
Clade	*Commelinids*
Order	*Poales*
Clade	*Graminid clade*
Family	*Poaceae*

Grasses have evolved 66 million years ago in the Cretaceous era as these were present in the fossilized dung of dinosaurs (phytoliths) which is a strong evidence of their presence during that period and microfossils from Pilauco were also found to have C_3 grass eaten by fauna living there (Álvarez-Barra, 2020; Piperno *et al.*, 2005). Monocot phylogeny and taxonomic pyramid of Poaceae has been shown in table 1.1.1.

1.1.2. Morphological Characteristics

The Poaceae family possesses distinct vegetative (root, stem, and leaves) and floral (seed, inflorescence, and fruit) traits based on which it is distinguished from other plant families and characterized under the angiosperm clade. The morphological features of the plants of the grass family are represented in table 1.1.2.

Table 1.1.2. Morphological Characteristics of Poaceae

Sr.no.	Vegetative Feature	Description	Reference
1.	Habit	Annuals or perennials, sometimes tree-like	(Kellogg, 2015) (Reinheimer *et al.*, 2005)
2.	Roots	Rhizomatous, fibrous, adventitious	
3.	Stem	Cylindrical Culms (upper stalks), hollow or solid, lignified, erect	
4.	Leaves	Alternate, distichous, exstipulate, ligules present, parallel-veined, and two-ranked blades with psudopetioles	
	Floral Features		
1.	Inflorescence	Compound spike, sessile/stalked	(Kubitzki *et al.*, 1990) (Peterson, 2001) (Kyozuka, 2014)
2.	Flower	Hypogynous, hermaphrodite, Small petals reduced to lodicules, bisexual subtended by two bracts: palea and lemma	
3.	Perianth	represented by 2 lodicules (membranous scales)	
4.	Androecium	Stamens 1-6, long filaments, dry pollen grains	
5.	Gynoecium	2 plumose stigma, ovary superior	
6.	Fruit	Caryopsis, pericarp, and testa fused	
7.	Seed	Endospermic, single cotyledon called scutellum	

1.1.3. Important Plants of the Poaceae Family

The Poaceae family includes cereal crops of economic importance that are used as food by human beings and livestock fodder i.e., wheat, rice, and maize are of supreme significance whose demand will increase to 33% by the year 2050 and following the key plants of the grass family, the table 1.1.3. gives a summary of their common and scientific names and genome size (Reeves *et al.*, 2016).

Table 1.1.3. List of Selected Plants and their Genomic Size

Sr. no.	Common name	Scientific name	Genome	References
1.	Common bread wheat	*Triticum aestivum*	~17 Gb (2n=42)	(Han *et al.*, 2019)
2.	Red wild einkorn wheat	*Triticum urartu*	4.94 Gb (2n=14)	(Ling *et al.*, 2013)
3.	Asian rice	*Oryza sativa*	~420 Mb (2n=24)	(Goff *et al.*, 2002b)
4.	Annual wild rice of Africa	*Oryza barthii*	~411 Mb (2n=24)	(Jacquemin *et al.*, 2013b)
5.	Wild rice	*Oryza brachyantha*	~261 Mb (2n=24)	(J. Chen *et al.*, 2013)
6.	Purple false brome or stiff brome	*Brachypodium distachyon*	~355 Mb (2n = 10)	(Initiative, 2010)
7.	Great millet/ Jowar	*Sorghum bicolor*	~730 Mb (2n=20)	(J.-S. Kim *et al.*, 2005)
8.	Corn	*Zea mays*	~2.3 Gb (2n=20)	(Schnable *et al.*, 2009)

In a study conducted to quantify the phenolic compounds, two commercial samples of hard and soft wheat (*Triticum aestivum*) were used for antioxidant assays and bound phenolic content in the bran tissues show significantly higher antioxidant activity than the esterified and free phenolics(Liyana-Pathirana *et al.*, 2006). Amplified restriction length polymorphism (AFLP) was utilized to evaluate genetic diversity in *Triticum uartu* (wild einkorn wheat) whose genetic makeup has been utilized to improve disease resistance in *Triticum aestivum* against Ug99 viral strain, leaf rust disease, and powdery mildew and extremely diverse genetic regions were observed(Baum *et al.*, 2013).

Phytochemistry of *Oryza sativa* shows bioactive compounds (BACs) that have certain biological activities such as anti-oxidants, anti-inflammation, and anti-cancer components and divulgence of this knowledge can create awareness for industrial use in nutraceuticals and food as well(Verma *et al.*, 2020).

The accumulation of reactive oxygen species (ROS) to a toxic level in plant cells is due to environmental stress such as drought or salinity therefore two rice cultivars were checked for a correlation between antioxidant transcriptional genes and ROS enzymes by giving them NaCl stress Cyt-GR1, Cyt-APX, CAT A and genes of proline metabolism (Çelik et al., 2019). *Oryza barthii,* which is the wild progenitor of African domesticated rice was analyzed to have more effective starch synthase (*SSIIIa*) or less effective starch branching enzymes (*SBEIIb*) that is valuable gelatinization property of rice(Wang *et al.*, 2015). Stomata play a crucial role in conducting air and water in plants and large stomatal pores were observed in the wild rice, but speciation has turned stomata of domesticated Asian rice smaller but with increased density, and *Oryza barthii* being a wild progenitor can be used for the breeding of leading cultivars of rice(Chatterjee *et al.*, 2020). Molecular and phylogenetic comparison between *Oryza brachyantha*; diverged approx. 15 million years ago from today cultivated rice, and *Oryza sativa* strikingly shows syntenic gene loss adjacent to the centromere and chromatin dynamics were determined in chromosome number 1,7 and 9 due to pericentric inversions(Liao *et al.*, 2018). *Brachypodium distachyon* is known as the monocotyledonous model plant for studying functional genomics of grass family, besides having the simplest genome of five distinguishable chromosomes its

embryogenic callus can be transformed by particle bombardment method showing resistance to yellow stripe rust, powdery mildew, rice blast, and cereal brown rust diseases and also an interesting fact is its large seed size despite its short stature that can be utilized to improve grain filling property of cultivated cereals(Andersen *et al.*, 2016; Draper *et al.*, 2001).

Sorghum is ranked at 5th position among the top important cereal crops which is a C_4 plant having certain genes that can endure adverse arid African climate, diverged from *Zea mays* 15 million years ago and *Agrobacterium*-mediated transformation can be performed(Doggett, 1991; Mullet *et al.*, 2002). The phytochemicals such as tannins, anthocyanins, phenols, and phytosterols present in sorghum are potentially helpful in the reduction of obesity, cancer, and cardiovascular diseases, respectively(Awika *et al.*, 2004). *Zea mays* is the 3rd leading crop on the Earth after wheat and rice and it is consumed as a staple food in many countries such as the United States, Europe, and Canada having health benefits in the form of phytochemicals reducing the probability of obesity, cecal cancer, atherosclerosis and stomach ailments(R. H. J. T. J. o. n. Liu, 2004; Shah *et al.*, 2016).

1.2. P-type ATPases

ATPases are the class of enzymes majorly divided into P-type ATPases; phosphorylate ATP to transport ions across the cell membrane, F/V-type ATPases; F-type use proton gradient to synthesize ATP while V-type only pump protons into lysosome and vacuoles and ABC superfamily; translocate small molecules, out of which plant P-type ATPases are important in plant growth, development of cell signaling and opening and closing of stomata(Kühlbrandt *et al.*, 2016; Pedersen *et al.*, 1987b; Young *et al.*, 1999).

All P-type ATPases are structurally and functionally related to four highly conserved protein domain-containing proteins where 10 α-helices of membrane domain provide the site for ions transport that interact with 3 cytoplasmic domains to undergo ATP hydrolysis(Kühlbrandt, 2004).

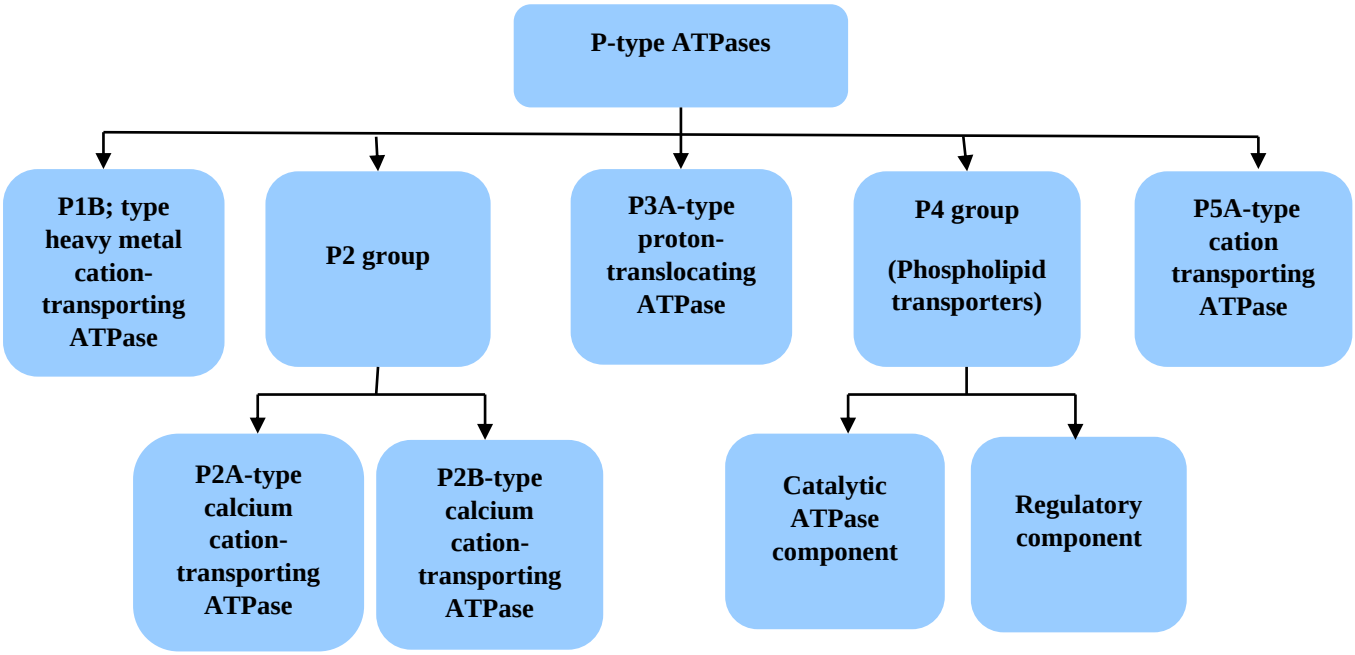


Figure. 1.2. Classification of Plant P-type ATPases

The ultimate function of P-type ATPases is to maintain an electrochemical gradient across the cell membranes and remove toxic ions from the cell to create a homeostatic environment and their regulatory domains carry out regulation of these metabolic processes(M. G. Palmgren *et al.*, 2011b). *Arabidopsis* genome contains 45 genes encoding proteins of P-type ATPases primarily involved in cellular transport and grouped into five subfamilies (Figure 1.2) based on the type of ions they transport viz., P1B; translocate heavy metal ions, P2A/P2B; Ca^{2+} transporter pumps, P3A; Proton pumps of the plasma membrane, P4; phospholipid transporters, P5A; transport cations but are least characterized(Axelsen *et al.*, 2001; Chan *et al.*, 2010). P1B-type ATPases are heavy metal (transition metal) transporters in plants translocating zinc, cadmium, lead, cobalt, and copper from the cytoplasm to vacuole in a controlled manner, playing a vital role in phytoremediation by detoxifying toxic metals(Arguello *et al.*, 2007; Williams *et al.*, 2005b). ER-type Ca^{2+}-ATPases (P2A/*ECAs*) and autoinhibited Ca^{2+}-ATPases (P2B/*ACAs*) are two subtypes of P2-type ATPases that are key regulators of

cytosolic Ca2+ efflux that is the crucial secondary messenger in cell signaling during stress conditions(García Bossi *et al.*, 2020).

P3A-type ATPases are plasma membrane H+ pumps having roles in maintaining electrochemical gradient by hydrolyzing ATP to pump protons out of the cell, with a similar structure as that of other P-type ATPases except for an enlarged C-terminal present for enzyme regulation(Duby *et al.*, 2009b). P4-type ATPases transport phospholipids by vesicle budding and maintain an asymmetry of membrane lipids which is an important feature for cell signaling and a confocal microscopy-based study showed that *ALA3*; AMINOPHOSPHOLIPID ATPase 3, forming a trans-Golgi network (TGN) is required for appropriate initiation of defense response on the host-pathogen interface(Andersen et al., 2016; Underwood *et al.*, 2017). P5-type ATPases are grouped into P5A and P5B ATPases and only one P5A-ATPase is present in *Arabidopsis* which is evolutionarily related with P4-type ATPases acting as lipid flippases involved in Golgi-to-ER transport(López-Marqués *et al.*, 2020).

1.3. Significance of Studies

Globally there is an increasing demand for staple food crops with an ever-increasing rate of population. Adverse climatic conditions result in the susceptibility of crop plants to several biotic and abiotic factors. To date, no report shows gene structure and subcellular localization of all five subfamilies of P-type ATPases of the Poaceae family utilizing *in silico* analysis. Gene structural prediction and 2-dimensional illustrations are potential targets to give an insight into plant functional genomics that help in the evolutionary study of genomes.

1.4. Aims and Objectives

This research work has the following aims and objectives:

1) To identify P-type ATPases among different members of the Poaceae family
2) To conduct a phylogenetic analysis of different species of Poaceae based on P-type ATPases
3) To identify conserved motifs in P-type ATPases of *Triticum aestivum*
4) To predict gene structure and subcellular localization of P-type ATPases of *Triticum aestivum*
5) To determine the localization signals in cellular compartments of *Triticum aestivum*

Chapter 2

2. Literature Review

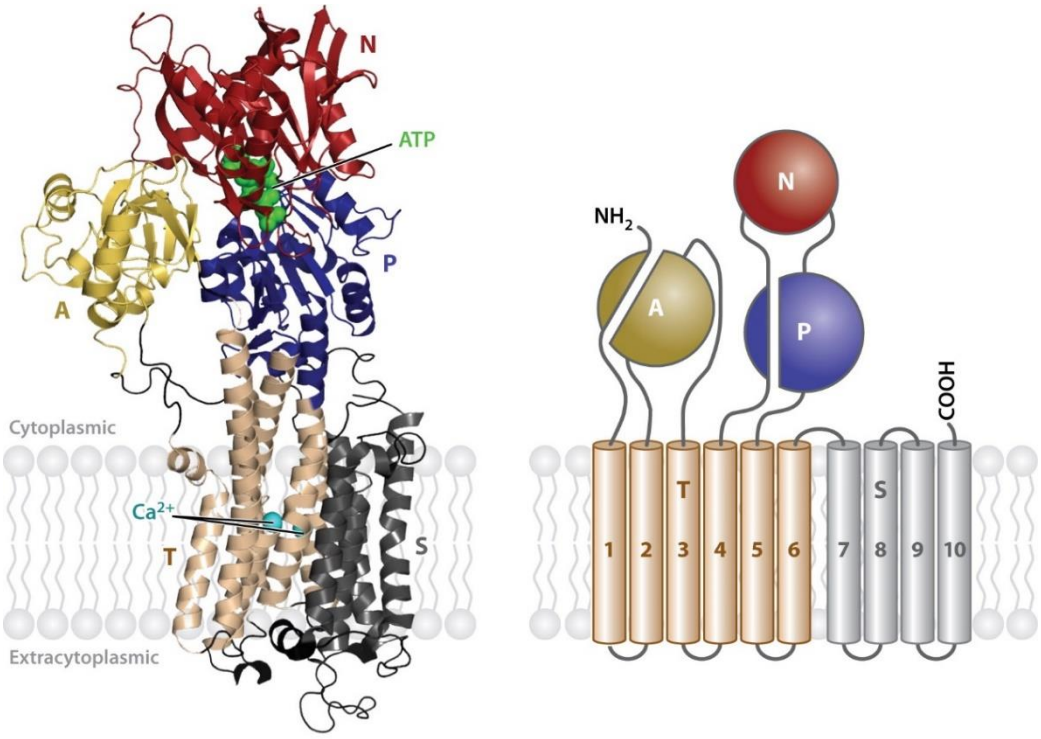
The selective breeding of plants for crop improvement both by nature and human intervention has provided the natural variants originated from their wild progenitors, and the genetic basis of these morphological and physiological variations can be studied employing high throughput sequencing but it is also a fact that wild relatives of a domesticated crop have more diversified genome, providing a valuable genetic and ecological resource for future research while introgression and genetic drift are two important factors in maintaining the beneficial ancestral genes in today's cultivated crops(Feuillet *et al.*, 2011; Huang *et al.*, 2014). The information of complete DNA (deoxyribonucleic acid) sequence is essential to decoding the functional and structural features of plants, their evolutionary relatedness, and the science behind regulatory pathways undergoing in the cell's osmotic environment as the predicted protein sequence of rice was 90% coherent with that of *Arabidopsis* showing homology at the proteome level(Bevan *et al.*, 2013; RGSP International, 2005).

The origin of P-type ATPase research was from an experiment on the leg nerve membranes of *Carcinus maenas* (shore crab) conducted by Skou to analyze ATP hydrolysis and it was observed that sodium ions were involved in the active extrusion of Na^+ from nerves, afterward that sodium/potassium was found to be a part of transmembrane proteins known as P-type ATPases(Pedersen *et al.*, 1987a; Skou, 1957). Metal transporting P-type ATPases play an important part in maintaining the cellular cation homeostasis not only in eukaryotes but also in prokaryotes as a metal-binding consensus sequence of GMxCxxC (dithiol motif) was found in copper and cadmium ATPases (CopA and CadA) of *Enterococcus hirae* and *Staphylococcus aureus*, respectively(Silver *et al.*, 1996). These are multisubunit transmembrane proteins with varying molecular weights of 72 kDa to 200 kDa for bacterial and plasmodium ATPase 1 and catalytic subunits also have homology in conserved motifs; MXGDGXNDXP (ion binding site), DKTGS/T (phosphorylation domain), and TGDN (ATP-binding site) along with the TGES/A motif that is present in a flexible spiral of ATPase confirmation(Krishna *et al.*, 1993; Lutsenko *et al.*, 1995; Nucifora *et al.*, 1989).

P-type ATPases are involved in plant physiological events and regulatory mechanisms and to check their presence in higher plants, several amino acid sequences from known metal transporting ATPases were compared to form degenerate primers for PCR amplification of *Arabidopsis* cDNA library and cloning after that larger amplified segment of 1000 bp was assumed to be the first P-type ATPase (PAA1) in *Arabidopsis* having all the known motifs for ion transport (CPC), phosphorylation, ATP-binding, metal binding and phosphatase domain(J. V. Møller *et al.*, 1996; Solioz *et al.*, 1996; Tabata *et al.*, 1997). The first P-type ATPase with determined crystal structure at 2.6 Å resolution was Ca^{+2} ATPase of rabbit skeletal muscles known as *SERCA1a* with 2 calcium ions consisting of ten α-helices in the transmembrane domain(Toyoshima *et al.*, 2000). There are five distinct subfamilies of P-type ATPases; P1A-K^+ pumps in bacteria, P1B- heavy metal transporters, P2A/P2B- Ca^+ pumps, P2C-$H^{+/}K^+$ and $Na^{+/}K^+$ pumps in animals, P2D-fungal Na^+ pumps, P3A- plasma membrane H^+ pumps, P4-lipid flippase complex and P5 ATPases with unknown transporting ligand(Bublitz *et al.*, 2011).

2.1. Structural Domains and Catalytic Activity

There are generally five functional domains in P-type ATPase pumps in which T and S are two transmembrane domains for transport and class-specific support while N, A, and P are three cytoplasmic domains for nucleotide binding, actuator, and phosphorylation, respectively(J. V. Møller *et al.*, 2010; M. G. Palmgren *et al.*, 2011a). The N-domain act as an intrinsic protein kinase and phosphorylates the P-domain then A-domain dephosphorylates P-domain as a built-in protein phosphatase which is a conversion from E1-P to E2-P energy state simultaneously with E2 to E1 transition and the cycle continues while transporting ions (Ca^{+2} ions in *SERCa2* ATPase) across the membrane as the cations are bound to the center of T-domain shown in Figure. 2.1(Albers, 1967; Karlish *et al.*, 1978; Post *et al.*, 1967). P-domain has the highest degree of conservation and similar catalytic mechanism as that of phosphatases of haloacid dehalogenase-like hydrolase family from *Pseudomonas sp.* which was investigated for crystal structure by multiple isomorphous replacement method and homodimeric enzyme had an R factor of 19.5% refined at 2.5 Å crystallographic resolution(Aravind *et al.*, 1998; Hisano *et al.*, 1996).



Palmgren MG, Nissen P. 2011.
Annu. Rev. Biophys. 40:243–66

Figure. 2.1. Schematic diagram of structural domains of P-type ATPases

The nucleotide binding domain; the most variable of cytoplasmic domains, inserted into P-domain by a narrow joint that binds ATP for phosphorylation, phosphorylation domain; highly conserved domain containing the phosphorylation site DKTG (Asp-Lys-Thr-Gly) along with GDGXND and TGDN motifs for magnesium ion transport, actuator domain; acts like a protein phosphatase linked to the membrane spanning segments, M1, M2 and M3 by 2 or 3 linker sequences having a conserved TGE (Thr-Gly-Glu) motif, transport domain; contains the ion binding site which comprises six transmembrane segments that moves during catalysis having a conserved sequence motif PEGL present in all P-type ATPases, support domain; provides structural and functional coordination for ion binding regions and located at varying positions such as in P1B-ATPases it is present at N-terminal, in P2-, P3-, and P4-ATPases it is situated at C-terminal and in P5-ATPases it is situated in both sites representing lower conservation of sequence motifs(Dmitriev et al., 2006; Hilge et al., 2003; Malmström et al., 1997; Portillo et al., 1989; Ridder et al., 1999; T. L.-M. Sørensen et al., 2004; Tak*AHAs*hi et al., 2007).

Table 2.1. Characteristic features of P-type ATPase family members

Plant P-type ATPase Family Members	Characteristic Domain	Conserved Motif	Transporting Ion	Function
P1B	Heavy metal-binding domain	CPx/SPC, HP locus, TGES/DGET (common to all)	Cu^{+}, Cu^{2+}, Cd2+, Zn^{2+}, Pb^{2+}, Co^{2+}, Ag^{+}	Phytoremediation of metal ions, Homeostasis
P2A(*ECAs*)	Ca^{+2} bing domains (M5, M6 and M8)	ER retention motif KxKxx (*ECA1*, *ECA2*, *ECA4*), C-terminal Golgi signal KDRRDK (*ECA3*) KGAxE	Ca^{2+}, Mn^{2+}, Zn^{+2}	Cell signaling, Trace element homeostasis
P2B(*ACAs*)	N-terminal autoinhibitory domain (CaM binding)	KGAPE	Ca^{2+}	Calcium ions transport in the plasma membrane, Signaling
P3A	C-terminal autoinhibitory domain (14-3-3 protein binding)	TGES, N-Terminal E/DXXXXLL, Thr-924, Thr-947, Ser-899	H+	Involved in Plasma membrane potential, pH homeostasis
P4	Autophosphorylation at a conserved aspartate residue	PISL, Asn^{181} residue,	Phospholipids	Lipid transport and lipid bi-layer asymmetry
P5A	Ma and Mb catalytic domains	TSVI, KGA, PPxxP, CFDKTGTL	Unknown	Fertilization and pollen development

2.2. P1B-type ATPase pumps (*HMAs*)

The P1B subfamily of P-type ATPases is involved in the transduction of heavy metal ions of zinc, copper, cobalt, lead, and cadmium thereby maintaining a homeostatic environment in the cell which is possible due to its metal-binding domain in C- or N- terminal along with a conserved sequence of CPx/SPC located in 6^{th} out of 8 membrane-spanning domain and *Arabidopsis* plant contains eight types of *HMAs* having distinguishing characteristics(Hall *et al.*, 2003; Hussain *et al.*, 2004; Williams *et al.*, 2005a). HP locus is conserved only in plant *HMAs* and absent in two divalent cation transporters of algae i.e., *CrHMA1* and *CmHMA2*, that along with a nearby glutamate help in nucleotide coordination and also in catalytic activity as suggested by an experiment done on *E. coli* Znta (*EcZntA*) mutants(Hanikenne *et al.*, 2005; Okkeri *et al.*, 2002). *Arabidopsis HMA1* localized in green tissues (chloroplast envelope) is involved in the transport of copper ions and it is analyzed by experiment on the deletion mutants of *HMA1* that absence of N-terminal histidine-domain somewhat affects this transport and these mutants are also characterized to have lower Cu^{+2} ion concentration and complete blockage of superoxide dismutase activity in the chloroplast(Seigneurin-Berny *et al.*, 2006). *HMA6/PAA1* also has the same catalytic activity as that of *HMA1* and mediates the import of copper ions in chloroplast envelope which are essential micronutrients as well as activate cell-damaging free radicals hence playing an important functional role in the diverse pathway of Cu^{+2} homeostasis and *paa1* mutants recovered their phenotypes when provided with copper supplements(Boutigny *et al.*, 2014).

HMA2 maintains the heavy metal content in plants mainly Zn^{+2} translocation and metal-binding domain (MBD) located at C-terminal of *HMA2* rich in cysteine-histidine residues when modified after removing 244 amino acids lowered its enzymatic activity by 43% and diethylpyrocarbonate activity disrupted zinc binding to histidine residues showing a crucial part of His-residues in metal binding(Eren *et al.*, 2006). *HMA4* involved in the maintenance of Zn and Cu concentration, when investigated for heavy metal homeostasis grouped in *HMAs* (transporting Cu/Zn/Cd/Pb/Co) subclass of P-type ATPases in the phylogenetic analysis and its cDNA sequencing also showed the same conserved motifs that are the characteristic of this sub-class. Yeast expression

system also represented Cd resistance in the cells having *HMA4* ATPase while increased levels of Manganese and Zinc in roots elevated the expression of *AtHMA4* with the highest expression in roots as compared to other organs(Mills *et al.*, 2003).

OsHMA3 (rice *HMA3*) was found to control Cd transport from root to shoot analyzed by positional cloning and localized in the vacuolar membrane by green fluorescent protein (GFP) while *OsHMA3mc* is a defective allele that hyper accumulates Cd for translocating it to xylem cells as a result of this shortcoming Cd is transported to shoot instead of its sequestration in the vacuole(Miyadate *et al.*, 2011). A heavy metal such as Cu, Mn, Fe, Ni, Zn, and Co are essential micronutrients for plant growth and development but an excess of these elements along with non-essential elements like Cd, Ag, Se, Pb, and Hg can cause phytotoxicity which can be the consequence of blockage or displacement of crucial biological molecules or enzymes that regulate the metabolic pathways, explained in Figure 2.2(Chaudhary *et al.*, 2016; Park *et al.*, 2014).

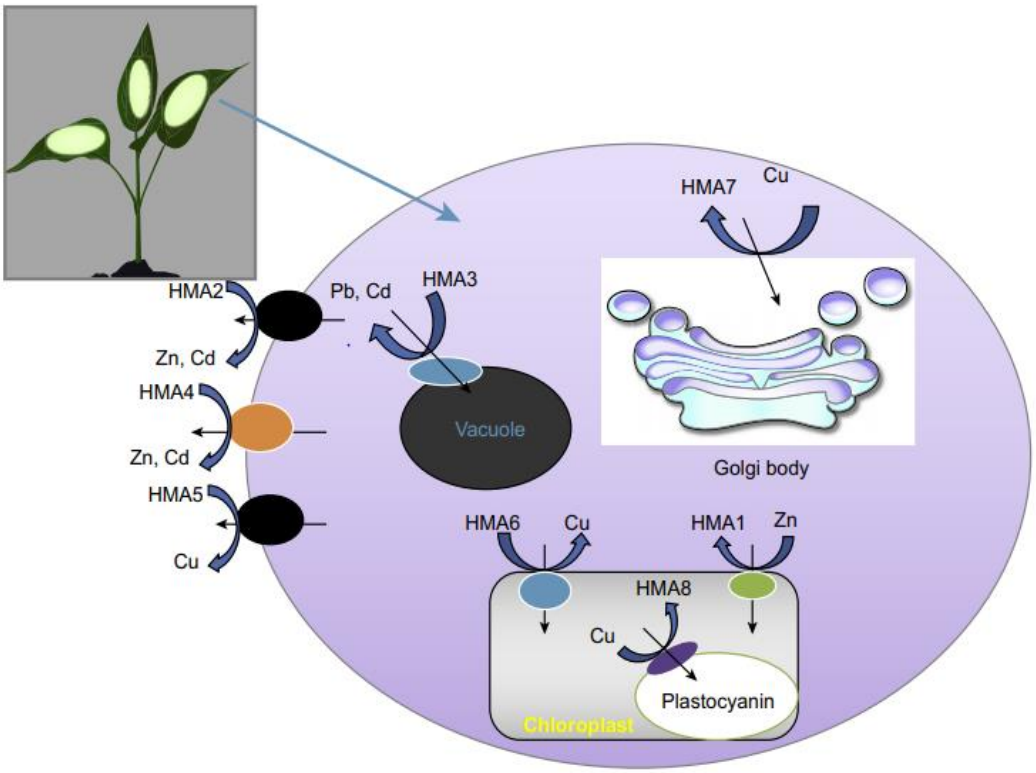


Figure. 2.2. Hyperaccumulation of heavy metals by plant *HMAs*

(Chaudhary *et al.*, 2016)

HMA2 gene of *Triticum aestivum* (*TaHMA2)* when overexpressed in rice plants, showed an improved level of Zn and Cd translocation(Tan *et al.*, 2013). *Zea mays* cultivar also hyper accumulates chromium from the industrial wastewater into roots and young leaves when toxicity tests were conducted for tannery leftover water(Calheiros *et al.*, 2008). *AtHMA5* has been identified as a transporter of Cu that delivers it in the secretory pathway with the help of ATX1-like metallochaperone and its mutants after T-DNA insertion are hypersensitive to copper but not to other metals defining their regulatory role in copper accumulation in roots and this regulation is due to binding of chaperons with strictly conserved amino acid residues (Andrés-Colás *et al.*, 2006; Kobayashi *et al.*, 2008). *OsHMA9* is involved in zinc transport as a metal ion efflux ATPase and can be utilized to increase levels of essential micronutrients such as Zn and Fe as a medium for biofortification in wheat and rice by QTL (Quantitative Trait Loci) mapping and high throughput genotyping and in this way candidate genes can be identified and allelic variations can be studied(S. Lee *et al.*, 2007; Tong *et al.*, 2020).

2.3. P2A/P2B-type ATPase Pumps (Ca^{+2} ATPases)

Plant calcium ATPases pumping calcium ions out of the cytoplasm, maintaining redox balance, regulating growth and development are divided into two subgroups i.e., P2A and P2B type ATPases both present in the plasma membrane and endomembrane in which the latter is indirectly involved in the calcium homeostasis by binding calmodulin thereby activating Ca^{+2} pump(Geisler *et al.*, 2000; Plieth, 2001). A study conducted to compare calcium ATPases of *Arabidopsis thaliana* with *Oryza sativa* for multiple alignment, phylogenetic analysis, homology percentage, conserved motifs, and cis-regulatory domains disclosed that P2A-type ATPases of rice were forming clusters with *Arabidopsis* but P2B-type ATPases showed variability while biotic and abiotic stress signaling appeared to be a major function of rice calcium ATPases(Huda *et al.*, 2013). All P-type ATPases have 9 conserved motifs located in particular positions of 8 transmembrane domains for nucleotide binding, catalysis, phosphorylation, ATP binding, and conformational changes in the protein transition state(Fusca *et al.*, 2009; J. V. Møller et al., 1996; Thever *et al.*, 2009). The changes in cytosolic Ca^{+2} ion concentration are involved in several physiological processes such as gravitropism,

hormonal activity, mitosis, stomatal opening and closing, and phytochrome related mechanisms(Bush, 1995).

Arabidopsis ECA1 showed a higher similarity of 53% with Sarco/endoplasmic reticulum membrane than with 32% of plasma membrane calcium ATPases when analyzed by heterologous yeast expression system restoring the multiplication of mutant *pmr1* on manganese ion-containing medium and Mn^{+2} dependent phosphoprotein also formed suggesting its role in Mn^{+2} homeostasis(Liang *et al.*, 1997; Wu *et al.*, 2002). Root hydrotropism (directional growth of roots towards high moisture gradient) in *Arabidopsis* was studied isolating the ahydrotropic mutant *miz1* (*mizu-kussei1*) whose natural variant *MIZ1* directly binds to *ECA1* for its negative regulation which is a regulatory process underlying root hydrotropic behavior(Miyazawa *et al.*, 2020; Shkolnik *et al.*, 2018). *ECAs* are specifically bound to calcium ions while *ACAs* can bind to manganese and zinc along with calcium ions, similarly the former preferentially hydrolyses ATP while the latter can hydrolyze GTP also which means that these two types of calcium ATPases can co-exist in plants with distinguishing characteristics(Bonza *et al.*, 2011; Hwang *et al.*, 1997). Ca^{+2} ions are required for the secretory pathways of eukaryotic cells and *AtECA1*, *AtECA2* and *AtECA3* are endomembrane ATPases transporting calcium and manganese in the compartments of a cell to increase the proliferation of yeast in the expression analysis which deciphers their role in Ca^{+2}/Mn^{+2} tolerance/de-toxification and their mutants such as *ECA3* decreased root growth(X. Li *et al.*, 2008). Root epidermal cells showed high expression patterns of *ECAs* while the root elongation stage emphasized the higher expression levels of *ECA3* and ECA4 proteins and their presence was detected during pollen tube development(Pereira, 2018).

A cauliflower gene *BCA1* encodes a vacuolar Calcium ATPase to which Calmodulin directly binds to regulate calcium ion transport and also has sequence similarity with Arabidopsis *ACA1* located in the chloroplast envelope but its CaM-binding domain is present in the N-terminal in contrast to that of *ACA1* Calmodulin-binding domain situated in the C-terminal which means that amino-terminal also has regulatory features(Malmström et al., 1997; Sze *et al.*, 2000).

It has been proved by expression analysis of Arabidopsis *ACA2* in tobacco plants that a point mutation can cause truncation in the original *ACA2* vacuolar pump due to which its activity increases by 10 folds that result in the vigorous growth of agriculturally important plants and cation exchanger 1 (CAX1) antiporter is also upregulated by calcium availabilities in transgenic crops(Hirschi, 2001; Thompson, 2012). *OsLCT1* gene encodes a low-affinity calcium transporter whose level was upregulated by *ACA3* and *ACA13* genes by the application of calcium acetate in rice crop under cadmium stress due to which Cd levels tend to decrease in the affected plant grains and roots without affecting its yield and quality(Treesubsuntorn *et al.*, 2019). The mRNA expression profiles of 6 *OsCAX* genes and 4 *ACAs* (*OsACA4-7*) were compared in salt-tolerant Pokkali and salinity sensitive IR29 rice varieties in which only Os*ACA4* transcript levels were first decreased on the action of high salt concentration and then an increased expression was observed showing its role in the salinity stress(M. Geisler *et al.*, 2000; Sharma *et al.*, 2021; Yamada *et al.*, 2014).

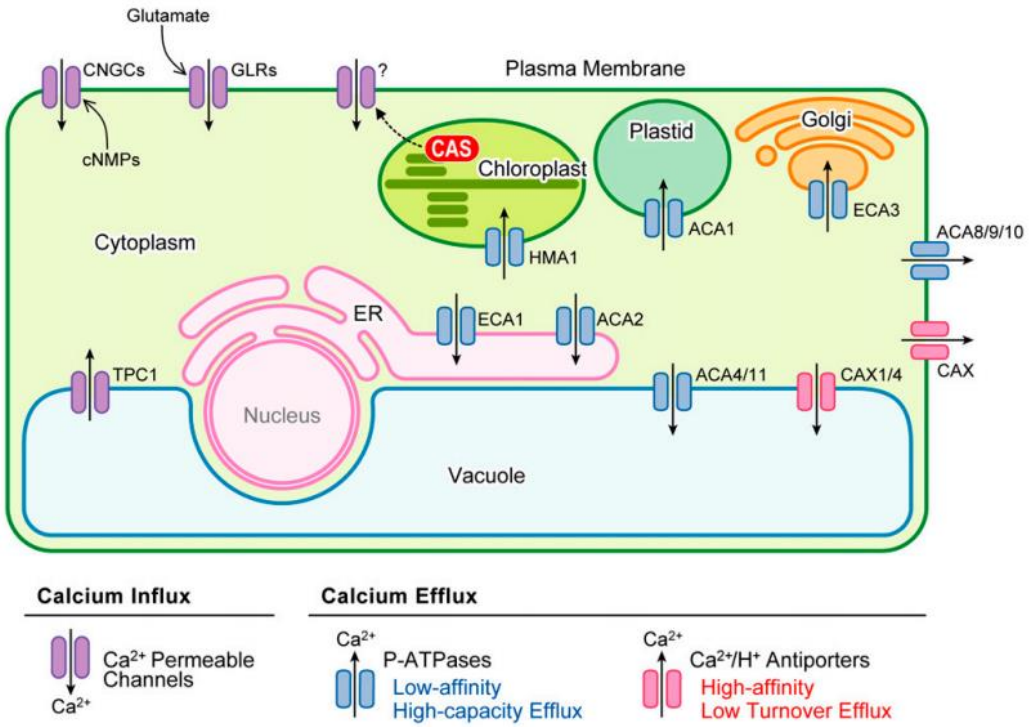


Figure. 2.3. Transporting Calcium ions in *Arabidopsis* cell (Kudla *et al.*, 2010)

Transgenic tobacco lines were used to determine the role of *OsACA6* under high levels of abscisic acid, salinity, heat, and desiccation and as a result of these adverse conditions plants showed overexpression of *OsACA6* gene encoding proteins and several physiological changes such as plasma membrane stability, increased sequestration of proline, higher levels of chlorophyll and reduced accumulation of reactive oxygen species (ROS) to undergo stress tolerance(K. M. Huda *et al.*, 2013).

ACA9 and *ACA10* were mutated to study the effect of loss-of-function that showed the importance of normal transmembrane protein pump for typical fertilization process and pollen tube growth and development in *Arabidopsis thaliana* revealing the crucial biological role of this calcium ATPase(George *et al.*, 2008; Schiøtt *et al.*, 2004). *AtACA11* was observed to be present in the vacuolar membrane which was analyzed by GFP tag in the transgenic plants while a CaM-binding domain located within the first 37 motifs of the amino-terminal autoinhibitory region that regulates calcium transport which can be determined by loss of function mutation(Lee *et al.*, 2007).

2.3. P3A-type ATPase pumps (Plasma membrane ATPases)

P3A- type ATPases are plasma membrane proton ATPase pumps present in protists, fungi, archaea, and plants but absent in animals and their first clone was from plants and yeast *S. cerevisiae* and higher plants have 9-12 gene members of this ATPase family (Arango *et al.*, 2003; Boutry *et al.*, 1989; Serrano *et al.*, 1986). *AHA1* (predominant in shoots) and *AHA2* (predominant in roots) are two important members of P3A-type ATPases virtually expressed in all tissues of the plants which seem to play as house-keeping genes for proton homeostasis; *AHA3* present in companion cells of leaf phloem; *AHA4* shows higher expression in the root endodermis when analyzed by reporter gene application; *AHA5* show least expression patterns all over the plant tissues; *AHA6* and *AHA9* mRNA transcripts expressed in anthers; *AHA7*/8 appeared in pollens; *AHA10* in emerging seed coat endothelium, and *ACA11* observed to appear throughout the plant due to which it has been proved that their expression in particular regions shows specialized functions associated with each P3A-type ATPase(Alsterfjord *et al.*, 2004; Baxter *et al.*, 2005; DeWitt *et al.*, 1991; Houlne *et al.*, 1994; Vitart *et al.*, 2001).

Plasma membrane H+ ATPases have characteristic features that make them unique as compared to other P-type ATPases i.e., to neutralize the effect of proton transport out of the membrane they don't counter-transport any other ions, only one proton (H^+) is exported with each ATP breakdown and its C-terminal binds a group of conserved proteins (14-3-3 protein) instead of CaM to their autoinhibitory domain(Gaxiola *et al.*, 2007; M. G. Palmgren & P. Nissen, 2011a). Two aspartate and arginine residues (Asp684 and Arg655) are conserved charged residues present around the lumen of the central cavity present in the T-domain of *AHA2* that are essential for proton transport(Buch-Pedersen *et al.*, 2003).

It was observed through site-directed mutagenesis that along with 946YpTV motif (a specific phosphothreonine motif) of *AHA2*, Thr-924 motif is also important for phosphorylation-independent binding of a conserved 14-3-3 protein, and 34 C-terminal residues are involved in this non-phosphorylated peptide-binding which is an exception for 14-3-3 proteins that always have interaction with phosphorylated binding residues in plant cells(Fuglsang *et al.*, 2003).

The plant plasma membrane is a basic medium of interaction between microorganisms and plant cell and several stimuli by microbes are first encountered by ion pumps and enzymes situated in the plasma membrane and as a response of molecular effectors, these enzymes help to carry out defense mechanism following the membrane transport processes(Boller *et al.*, 2009; Elmore *et al.*, 2011). *AHA1* and *AHA2* with single knockout show no major effects as other isoforms compensate for the lethal effect but loss of function mutation at two sites appeared to have destructive effects on the ion transport pathway (Haruta *et al.*, 2010; J. Liu *et al.*, 2009). PM H^+ ATPases are essential for voltage-gated channel activity as membrane potential can be altered by activation or inhibition of these pumps because they utilize energy coming from ATP hydrolysis to transport protons from cytoplasm to outside the cell in extracellular space(Sondergaard *et al.*, 2004; Ward *et al.*, 2009). PAMP (Pathogen-associated Molecular Pattern) triggered immunity i.e., PTI is the earliest immune response of a cell on pathogen attack in which high proton influx results in membrane de-polymerization and cell culture medium rapidly becomes alkaline as observed in mesophyll cells of *Arabidopsis* when a pathogen elicitors flg22 is recognized by FLS2(Benschop *et al.*, 2007; Jeworutzki *et al.*, 2010).

It has been investigated that *AtAHA1-AtAHA4* are localized to DRM (detergent-resistant membrane) which is defined based on insolubility in a cold non-ionic detergent such as TX-100 having the ability to make microdomains in living organism and this sterol-enriched environment can regulate *AHAs* activity via post-translational modifications(Keinath *et al.*, 2010; Morel *et al.*, 2006). Activation of *AHAs* also results in acidification of the apoplast to respond defensively as their accumulation can trigger the stimulus for sequestration of salicylic acid (SA) and transcriptional activation of pathogenesis-related (PR) genes during effector-triggered immunity(Elmore & Coaker, 2011; Schaller *et al.*, 1999). Constitutive overexpression of PMA4 (deletion of inhibitory C-terminal domain in *AHA4*) transcripts in tobacco plants activated the H^+ pumps and plants became salinity tolerant(Gévaudant *et al.*, 2007; Wani *et al.*, 2020).

The simultaneous suppression of both endogenous and transgenic PMA4 proton ATPase results in the inactivity of sucrose transport to the sink tissues, guard cell opening, and photosynthetic process in mature leaves(Duby *et al.*, 2009a; Zhao *et al.*, 2000). It has been investigated that *AHA7* pump autoinhibition is regulated by extracellular proton gradient and developed by residue located between 7 and 8 transmembrane domains and root hair elongation strictly depends on *AHA2* and *AHA7* activity(Hoffmann *et al.*, 2019). Three members of *A. thaliana AHAs* namely, *AHA6*, *AHA8*, and *AHA9* are involved in the polar growth of pollen tube that is necessary for the transport of sperm cells during angiosperm fertilization(W. Chen *et al.*, 2020). The interaction of *AtCBL10* (calcium-binding protein gene) with C-terminal of *AtAHA11* (PM H^+ ATPase) gene by yeast two-hybrid expression analysis suggested that study of ATPase regulatory sites can be done to search for the underlying mechanisms(Yang *et al.*, 2016).

A higher concentration of Aluminium (Al) in soil tends to increase acidity due to which activation of *AHAs* by the application of indole acetic acid or magnesium can maintain cytosolic pH and alleviates the toxic level of Al by making cultivars tolerant to such toxicity which would badly affect the nutrient uptake and development of plants(Zhang *et al.*, 2017). Two conserved motifs present in most P-type ATPases i.e., GDGV and CSDK were used for degenerate primer construction for PCR amplification then a new isoform of proton ATPases was cloned and partially sequenced which proved to be the most divergent ATPases; *AHA10* of *Arabidopsis* and GUS reporter

analysis showed its physiological role in seed development(Harper *et al.,* 1994). The roots of *Sorghum bicolor* release biological nitrification inhibitors under the stimulus of ammonium cation (NH_4^+) concentration (≤1.0 mM) present in the rhizosphere that which functionally linked with *AHAs* transcriptional activity(Zeng *et al.,* 2016).

2.4. P4-type ATPase pumps (Lipid flippases)

P4-type ATPases are also known as lipid flippases, involved in the transportation of phospholipids as they function to flip lipids from the extracellular side of the eukaryotic membrane to cytosolic sides and also in vesicle formation maintaining lipid composition in the cell(Tang *et al.*, 1996; Zhou *et al.*, 2009). Yeast has 5 P4-type ATPase pumps while there are 14 lipid flippases in humans which show distinct subcellular localization, tissue-specific expression patterns and specificity to a particular substrate, and a human flippase, ATP8A1–CDC50a has shown a homology with the yeast Drs2–Cdc50 investigated through their structural analysis(Best *et al.*, 2019; Hiraizumi *et al.*, 2019; M. Palmgren *et al.*, 2019). The structures of two lipid flippases from yeast i.e., Dnf1–Lem3 and Dnf2–Lem3, have been identified showing the unexpected function of Lem3 in binding the substrate phosphatidylcholine molecules that have similar structures in both exoplasm and cytosol and these lipid flippases also have conserved helix-turn-helix insertion in the P-domain that must be evolved to support substrate transportation process(Bai *et al.,* 2020). The structural analysis of Drs2p–Cdc50p (binds with phosphatidylserine and phosphatidylethanolamine) by electron microscope showed a conserved PISL motif in 2,4 and 5 transmembrane domains specifically in the central position of the lipid bilayer(Timcenko *et al.*, 2019).

The *Arabidopsis ALA1* flippase lies in the cytoplasm (needs a β-subunit) for proper functioning and retains in the endoplasmic reticulum when not connected with ALIS protein(Lopez-Marques *et al.*, 2012). *AtALA2* is present in the pre-vacuolar compartment and the determinants/signals that are specific for lipids are located in the ALA α-subunit(López-Marqués *et al.*, 2010). In *Arabidopsis, AtALA3* translocate phosphatidylserine, phosphatidylcholine, and phosphatidylethanolamine residing in the trans-Golgi membrane (TGM) of root cells helping in vesicle budding and mutation in

AtALA3 results in diminished growth of roots and shoots while a β-subunit (*ALIS1* protein) is essential for *ALA3* functioning for secretory pathway and plant growth(Poulsen *et al.*, 2008). A central cavity in transmembrane 4 containing Y374 and F375 residues, is involved in the lipid transport specificity of *AtALA10* and its mutant *ala10* appeared to lose the ability to transport lipids(Jensen *et al.*, 2017; López-Marqués et al., 2010).

Lipid flippases are involved in transport-dependent functions such as lipid scavenging, vesicle budding, and membrane bending and transport-independent function i.e., cell signaling and regulation of cytoskeleton (Figure. 2.5). *AtALA1* has shown to encode aminophospholipid translocase that compensates the cold sensitivity of *drs2* mutant in yeast and its upregulation results in translocation of lipids in the membrane vesicles of yeast which means that it plays an important role in cold-stress tolerance preparing the plant against impaired growth, loss of coloration and premature cell death along with transporting lipid molecules(Gomès *et al.*, 2000; Sanghera *et al.*, 2011).

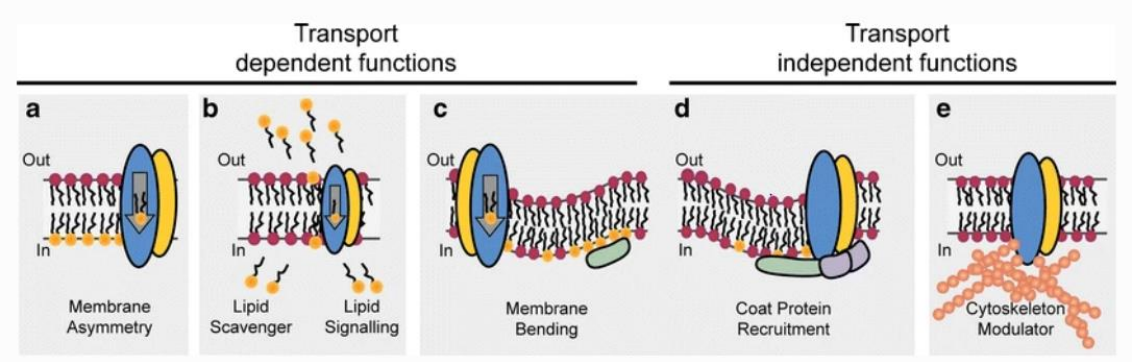


Figure. 2.4. Functions of P4-type ATPases(Lopez-Marques *et al.*, 2014)

P4 flippases form heterodimers with the cell division control 50 (Cdc50) protein family having a non-selective interaction approach as some of them interact with β-subunit and others bind with different isoforms of ALA-interacting subunit (ALIS) therefore a mutagenesis approach was used to check the functioning of ectodomain in *ALIS5* β-subunit in which 2 N-glycosylated residues Asn^{181} and Asn^{231} were determined with former having least effect on function and latter conserved residue

function was disrupted(Costa *et al.*, 2016). *AtALA1* and *AtALA2* are involved in antiviral gene silencing and their mutants (*ala1/ala2*) after substitution of the single amino acid were inactivated and disease susceptibility was increased in cucumber for cucumber mosaic virus (CMV) as these *ALAs* normally act to form dsRNA by RNA-dependent RNA polymerase 1 and 2 in a eukaryotic cell(Guo *et al.*, 2017). *ALA4* and *ALA5* from *Arabidopsis thaliana* play a significant role in glycerolipid and sphingolipid maintenance and homeostasis and their knockout mutants (*ala4*/*5*) showed growth retardation in the vegetative tissues while *ALA6/7* mutants divulged defects in the pollen fertility(Davis *et al.*, 2020). P4-type ATPases function in vesicle budding in the endocytic pathway and their transporting molecules are lipid complexes that are much bigger than cations transported by other P-type ATPases owing to the name "the giant substrate problem" needs a proper β-subunit for regulatory activity and lipid translocation from Endoplasmic reticulum(Nintemann *et al.*, 2019).

2.5. P5A-type ATPase pump

Arabidopsis contain ony one member from P5A-type ATPase subfamily known as MIA because of Male Gametogenesis Impaired Anthers phenotype of mutan gene while it is naturally present in higher concentration in developing pollen grains and Endoplasmic Reticulum (ER) having showing 41% identity with Spf1p from yeast and 44% identity with ATY2 present in humans(Axelsen *et al.*, 1998). An experiment was performed by making knoeck out mutant of *AtP5* gene which showed disruption in secretory function of gene and also the naturally present MIA gene complemented the effect of mutated *Spf1p*, which is one member of the P5-ATPase subfamily in *S. cereviseae* which proved its importance in secretion of vesicles to cell membrane(Jakobsen *et al.*, 2005). When *Spf1p* gene was deleted then retarded glycosylation mechanism was observed as well as mevalonate biosynthetic pathway also showed deficiency in ubiquitin-dependent degradation of a particular enzyme functioning in this pathway but the latter effect was partially reversed after the addition of calcium ions leading to a hypothesis that P5 ATPase could be the Ca^{+2} transporter ATPase(Cronin *et al.*, 2000; Suzuki *et al.*, 1999).

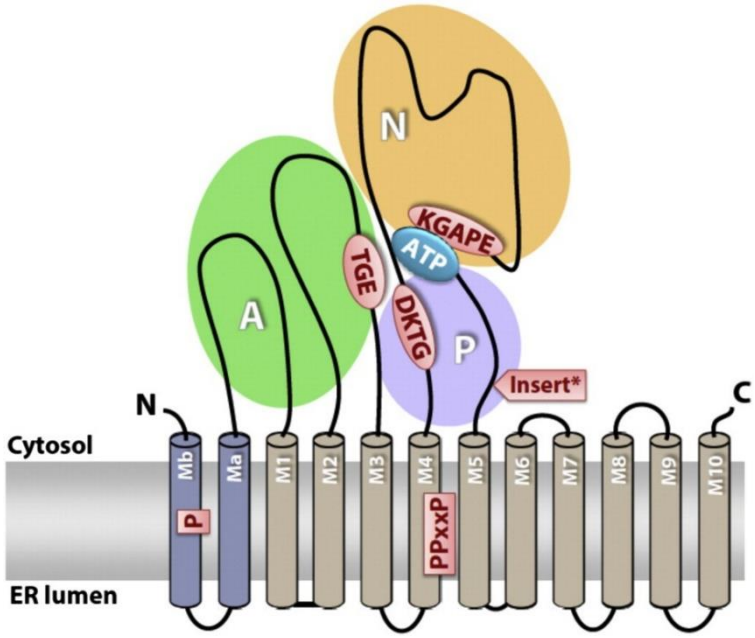


Figure. 2.5. Primary structure of P5A-type ATPase(Sørensen *et al.*, 2015)

In silico analysis performed to distinguish between P5A and P5B ATPases from different organisms including *Arabidopsis* and yeast showed that both carboxyl- and amino-terminal ends of P5A were found inside the cytoplasm as in case of *S. cerevisiae* Spf1p global topology map was predicted and two extra membrane helices, Ma and Mb were identified before M1 domain of calcium and sodium/ potassium ion ATPase but P5B contains only Ma doamin(H. Kim *et al.*, 2006; Sørensen *et al.*, 2010).

P5A-type ATPases are also known as 'orphan pumps' as their biochemical function is still ambiguous. In a study conducted on HvP5A1 gene from barley showed that it compensated the knockout effect of yeast and *Arbidopsis* P5A gene mutants and rescued their functional entity by forming a reaction cycle intermediate at Asp-488 residue and it was also observed that only calcium ions induced de-phosphoylation independent from the P5A pump phosphatase motif as well as M4 transmembrane binding site which depicts that Ca^{+2} is not a transporting ligand of P5A but allow affinity binding side is present that might help in the regulation of transporting ions(Sørensen *et al.*, 2012). P5A ATPases are differentiated from other P-type ATPases on the basis of conserved PPXXP motif present in the TM domain M4 for substrate

binding(Møller *et al.,* 2008). The deletion mutation of *SPF1* induces stress on Endopalsmic Reticulum and *Ypk9* is its nearest homologous gene that functions for manganese ion homeostasis thereby deletion of *SPF1* reduces the Mn^{+2} activity in ER but a parallel increase was observed in cytosolic Mn^{+2} transport which demonstrate the role of *Spf1p* in manganese ion translocation(Cohen *et al.,* 2013).

Spf1p can be a Mg^{+2} ion transporter as magnesium ions stimulate ATP hydrolysis at P-domain and also involved in the formation of phosphoenzyme but no recent data is available on if Mg^{+2} is a P5A transporting ligand(Corradi *et al.,* 2012). It ahs been investigated that *SPF1* exhibits negative effect on *SAC1* gene involved in vesicular transport that encodes phosphatidylinositol 4-phosphate (PI4P) and also on *OSH1-OSH6* genes that code for Osh proteins which after induction by PI4P, drive lipids and sterols from the Endoplasmic Reticulum thereby helping in sterol homeostasis(Sørensen *et al.,* 2019).

Chapter 3

3. Methodology

1.1. Sequence Retrieval and Phylogenetic Tree Construction

Protein sequences of five subfamilies of P-type ATPases from eight monocotyledonous plants were retrieved from different databases (Appendix I) using *A. thaliana, O. sativa,* and *B. distachyon* as model plants(Aslam *et al.,* 2017). *Arabidopsis thaliana* is a model plant having the smallest genome of ~135 Mb(Meinke *et al.,* 1998) that is sequenced, and most of its genes are identified therefore its P-type ATPase sequences were retrieved from the Aramemnon database, which is plant membrane protein database(Schwacke *et al.,* 2018). *Arabidopsis* ATPase sequences were used to perform BLAST searches in UniProtKB and Ensembl Plants to retrieve selected plant species(Bolser *et al.,* 2017; Boutet *et al.,* 2007). The amino acid sequences of *Brachypodium distachyon, Oryza sativa, and Zea mays* were also retrieved from the Aramemnon database and cross-verified from the sequences obtained from BLAST searches of *Arabidopsis* P-type ATPases.

Oryza sativa P-type ATPases were also verified with the locus IDs present on Michigan State University Rice Genome Annotation Project and the Rice Annotation Project (RAP) database (Kawahara *et al.,* 2013; Sakai *et al.,* 2013). The amino acid sequences of *Oryza barthii, Sorghum bicolor,* and *Triticum urartu* were retrieved from the query BLAST of *A.thaliana* from UniProtKB database and those of *Triticum aestivum* from *Brachypodium distachyon* BLAST in Ensembl Plants that utilizes data from "International Wheat Genome Sequencing Consortium (IWGSC)" and Plant Genome and Systems Biology that uses "*Triticum aestivum* IWGSCv2.2 PEP" database(Spannagl *et al.,* 2016). Rice is considered as a model plant for monocots and P-type ATPases of rice are reported in the database with designated names, so it was first used to search *Brachypodium* sequences that were cross-validated from the Aramemnon database and then those sequences were used as query to search for *T. aestivum* sequences through BLAST. The sequence alignment of retrieved P-type ATPases was performed in the alignment tool of Molecular Evolutionary Genetics

Analysis (MEGA X) software, which is a user-friendly software for complex evolutionary analysis such that P1B-type ATPases from all eight plant species were aligned and so on, then alignment file was selected for Phylogenetic tree construction through Maximum likelihood method using standard parameters(Hasegawa *et al.*, 1991; Kumar *et al.*, 2018).

MEGA X (version 10.2.1) reads input file in Newick format for graphical tree formation which is widely accepted notation for tree construction and the basic function of the Maximum Likelihood Method is employing aligned gene/protein sequences from many species and reconstructing the phylogenetic tree that depicts their evolutionary history based on JTT model named after Jones-Taylor-Thornton(Cardona *et al.*, 2008; Jones *et al.*, 1992). This resulted in five evolutionary trees of P1B, P2, P3A, P4, and P5A ATPases were colored and named according to the gene families they belong to. When the notepad file of sequences is uploaded in MEGA X then the following parameters were shown before selection of the alignment method.

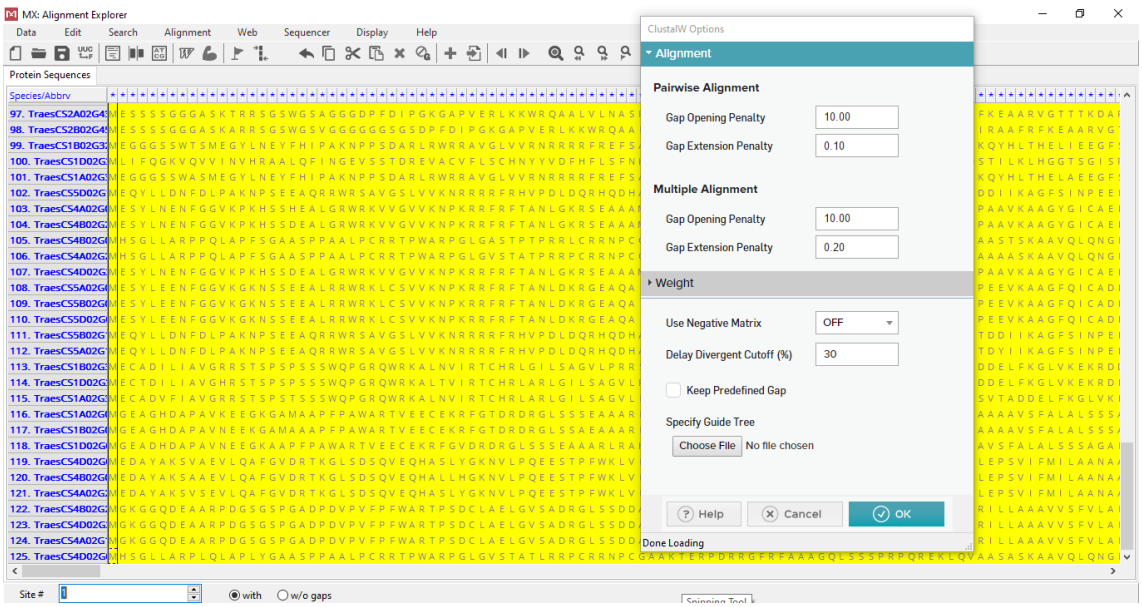


Figure. 3.1. MEGA X alignment

Then alignment file was generated showing conserved domains in designated colors using the ClustlW tool in the software.

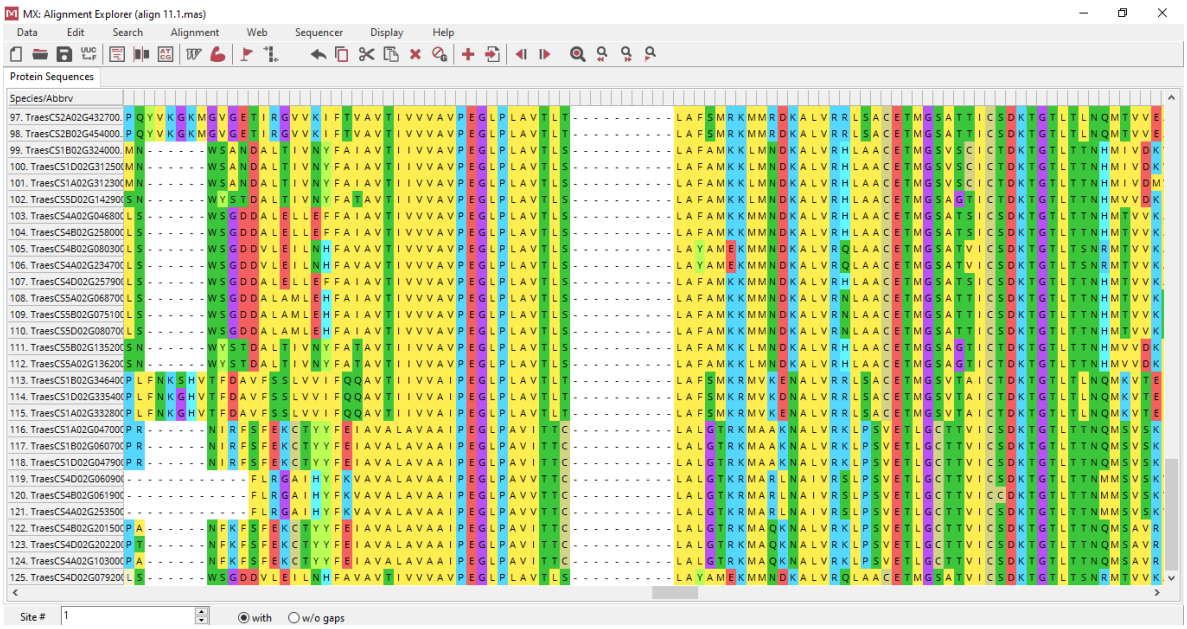


Figure. 3.2. Alignment output file

Following were the parameters shown when the alignment file was uploaded to construct the phylogenetic tree.

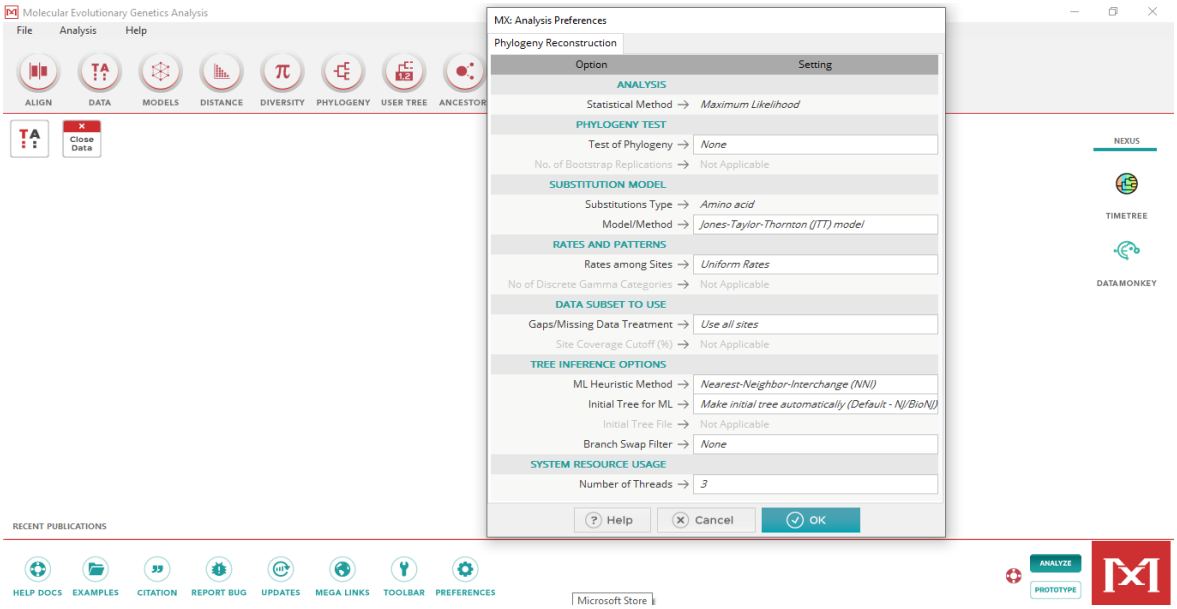


Figure. 3.3. Parameters for tree construction

This tree was constructed after processing of P-type ATPase alignment file that was later shaded and named.

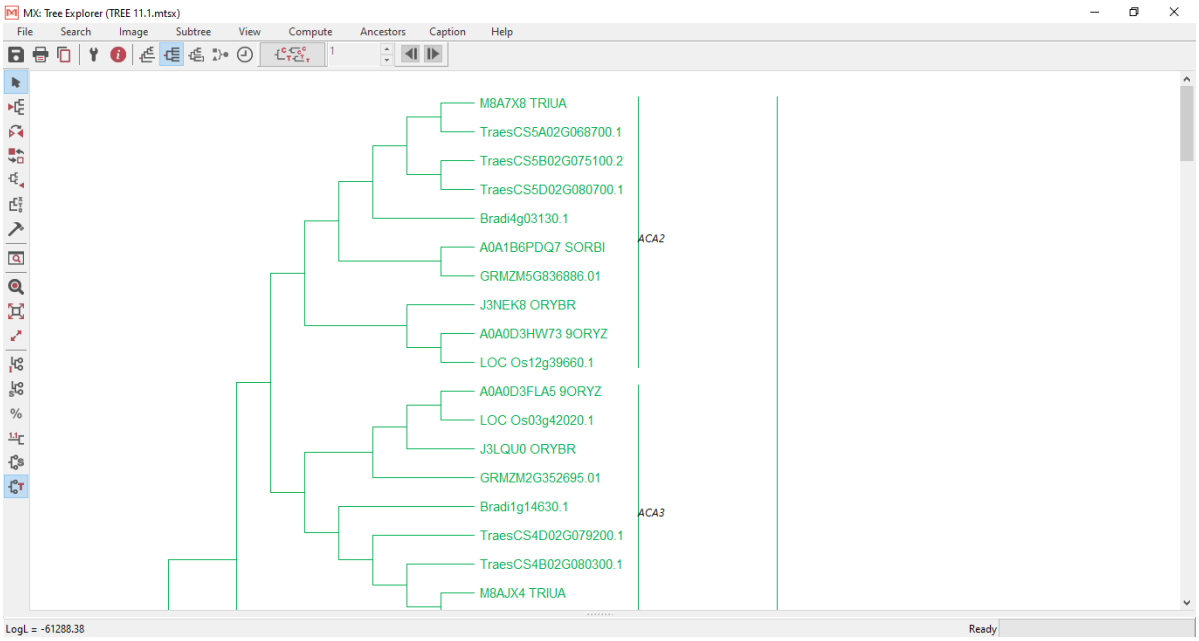


Figure. 3.4. Tree output file

1.2. Identification of conserved motifs

Identification of conserved motifs was performed using online available tool Clustal Omega(Sievers et al., 2018) and the aligned sequences were represented in the GeneDoc(Nicholas, 1997). The conserved motifs were then searched by pressing ‘F9’ key and entering the motifs specific for P-type ATPases in the literature.

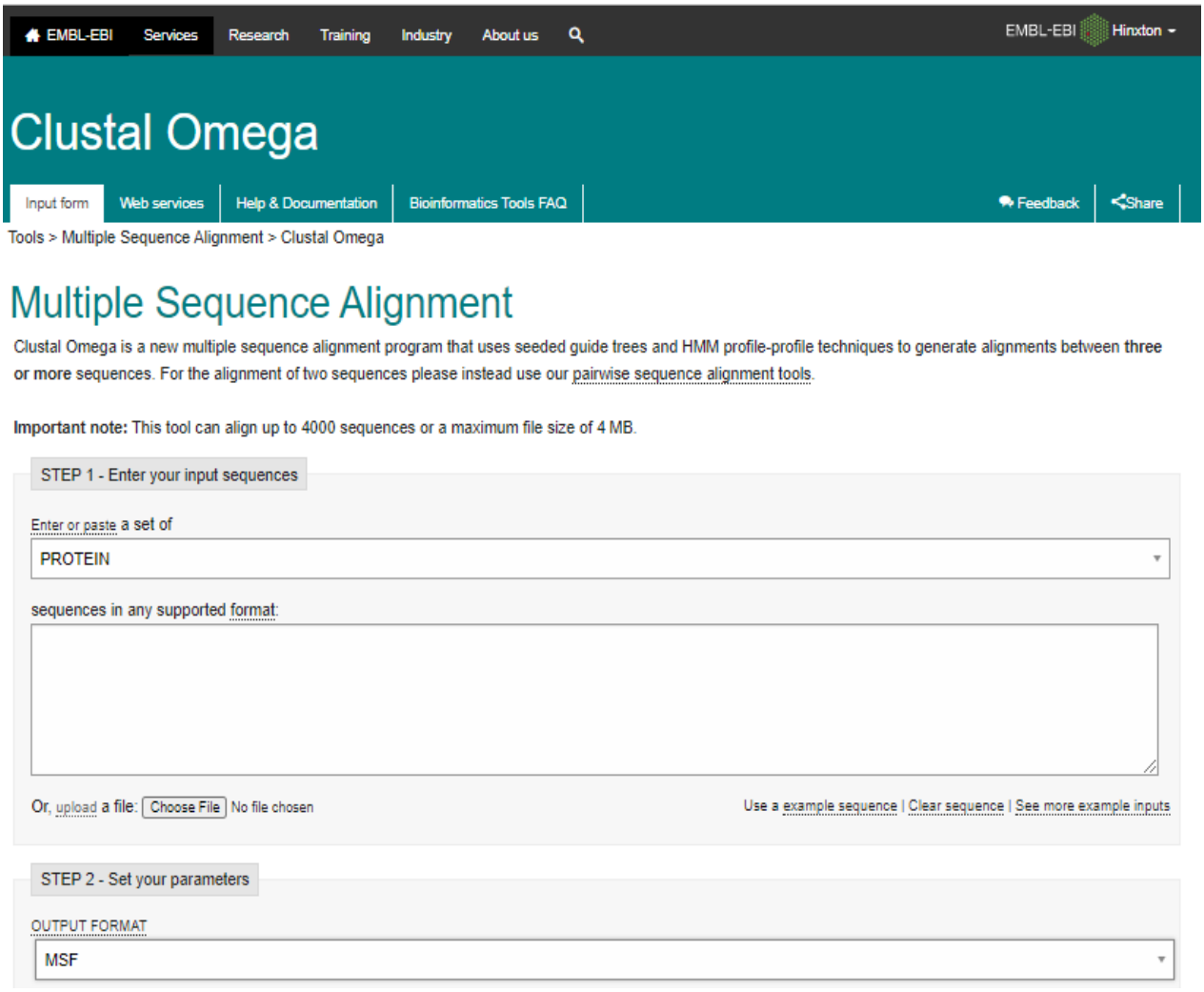


Figure. 3.5. Clustal Omega online Alignment tool

1.3. Identification of subcellular localization of P-type ATPases

The P-type ATPase subcellular localization for *Triticum aestivum* were determined by Plant-mPLoc which predicts sub-cellular localization of plant proteins in multiple locations(Chou *et al.*, 2010) and is represented in the appendix of protein localization. Each amino acid sequence was entered separately to get the result. Another localization tool was used that identifies localization signals of both plant and effector proteins in the cell compartments which is known as 'LOCALIZER' (Sperschneider *et al.*, 2017). Amino acid files of all *T. aestivum* were uploaded separately for results.

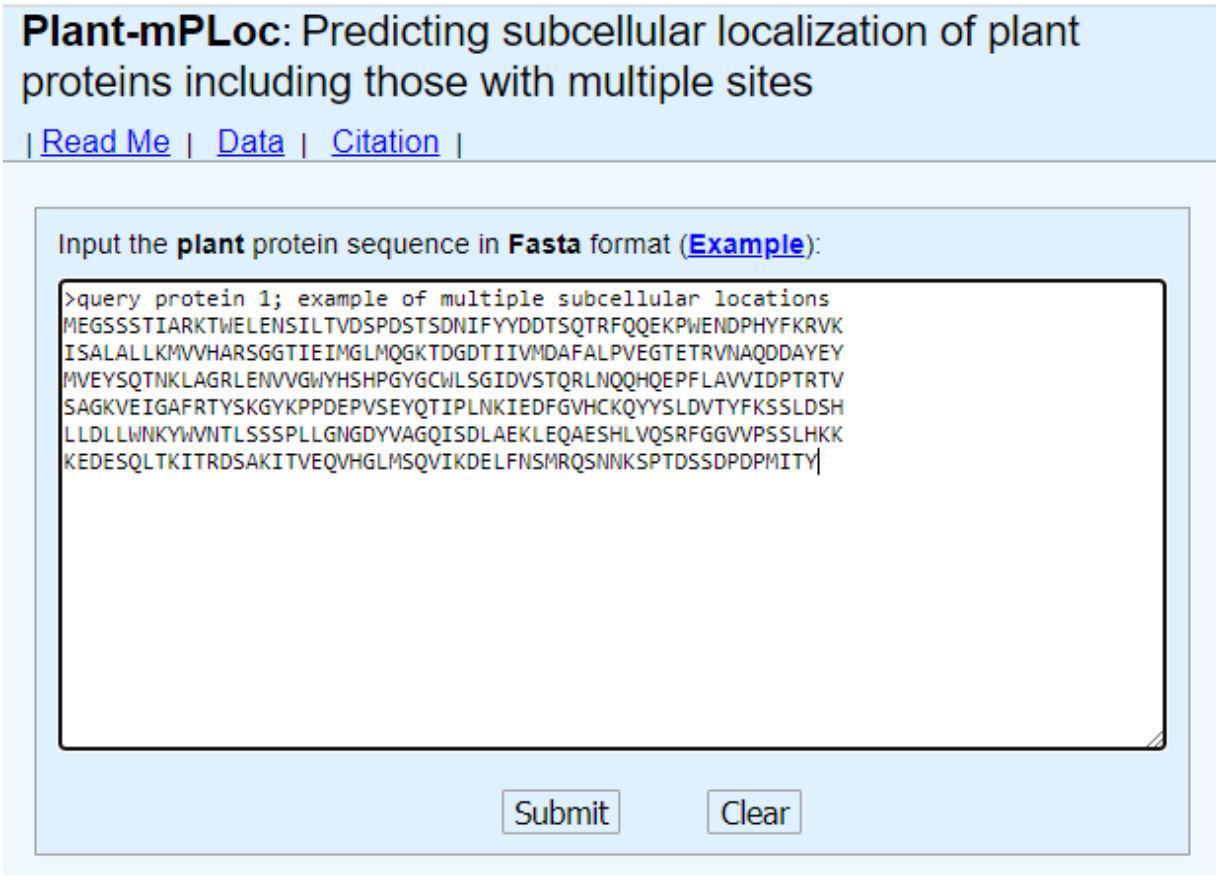


Figure. 3.6. Subcellular localization tool overview

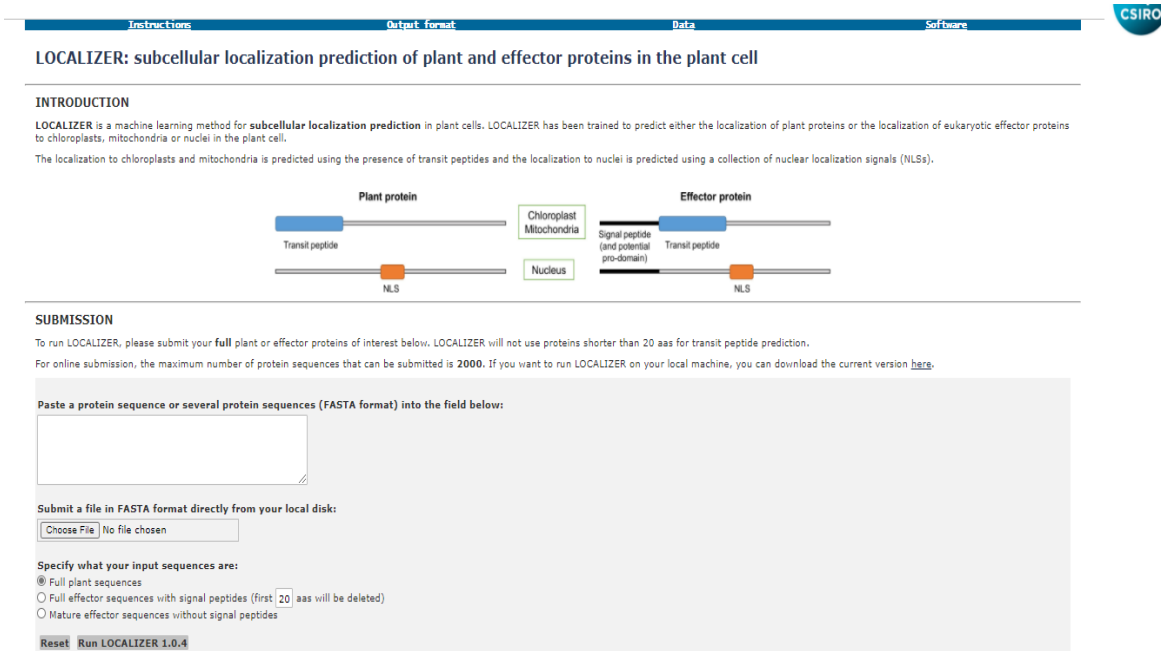


Figure. 3.7. LOCALIZER overview

1.4. Prediction of gene structure of P-type ATPases

Gene structure display server (GSDS 2.0) was used to predict gene structures that encode P-type ATPases for *T. aestivum* to determine the position of introns and exons(Hu *et al.*, 2015). The CDS region and introns were shaded in different colors to be distinguished and shades can be manually changed. the genomic sequence filas well as CDS sequence file must be uploaded simultaneously in designated input boxes, then output format was set to get PNG or SVG format.

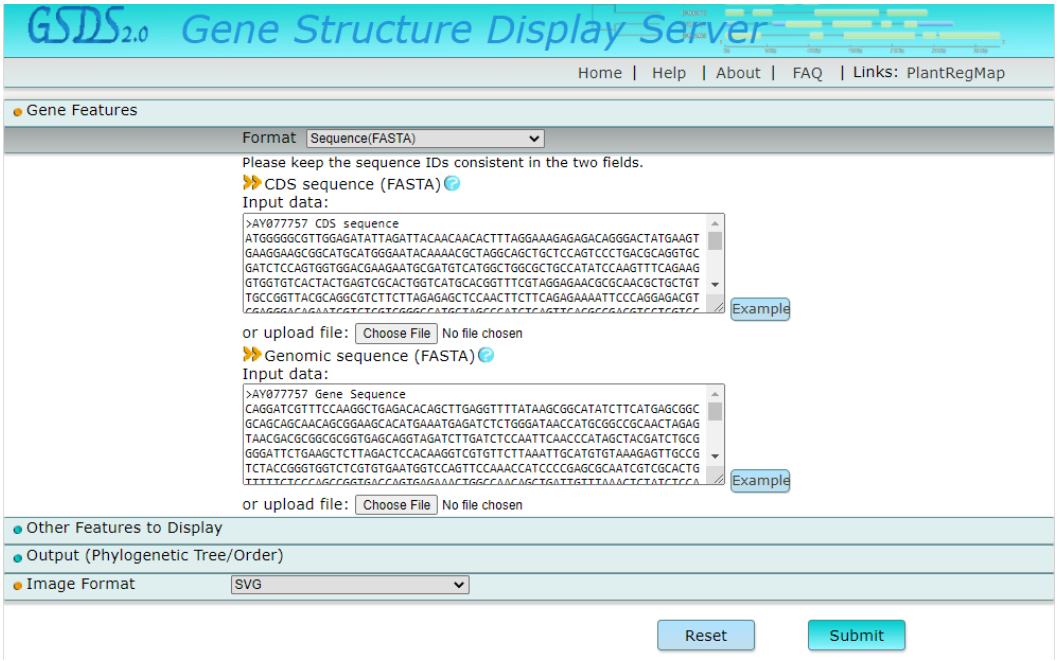


Figure. 3.8. GSDS 2.0 tool overview

Chapter 4

2. Results

2.1. Phylogenetic Analysis

2.1.1. P1B-type ATPase Tree

A phylogenetic tree was constructed using seventy-eight protein sequences (Appendix I) of heavy metal ATPases (*HMAs*) from *Triticum aestivum, Triticum urartu, Oryza sativa, Brachypodium distachyon, Oryza brachyantha, Oryza barthii, Zea mays,* and *Sorghum bicolor* to determine their evolutionary history.

Full-length sequences were retrieved from different sequence databases through BLAST of the query sequence. Three major threads were extending towards nine heavy metal ATPase (*HMAs*) clades from selected species of the grass family. *HMA1* ATPases (indigo color) formed a monophyletic taxon/clade that diverged from the most common recent ancestor containing amino acid sequences from all species along with three *T. aestivum* homoeologs (AA, BB, and DD genome).

HMA3 clade (red color) was short of *Sorghum bicolor* and *Triticum urartu* sequences although it is a fact that *T. urartu,* being a wild progenitor, adds "A genome" to hexaploid bread wheat but the presence of *T. aestivum* in this monophyletic group and absence of *T. urartu* indicated that possibly *HMA3* gene of the former was not translated to form a protein transcript that is why its accession number was not annotated in the sequence database. *HMA4* (arctic blue color) and *HMA5* (brown color) ATPases diverged into paraphyletic taxon/sub-clade which means that they have descended from a common ancestor but unlike monophyletic group not including all the descendants. The presence of these two ATPase protein sequences in paraphyletic taxon reveals that these are structurally and functionally similar.

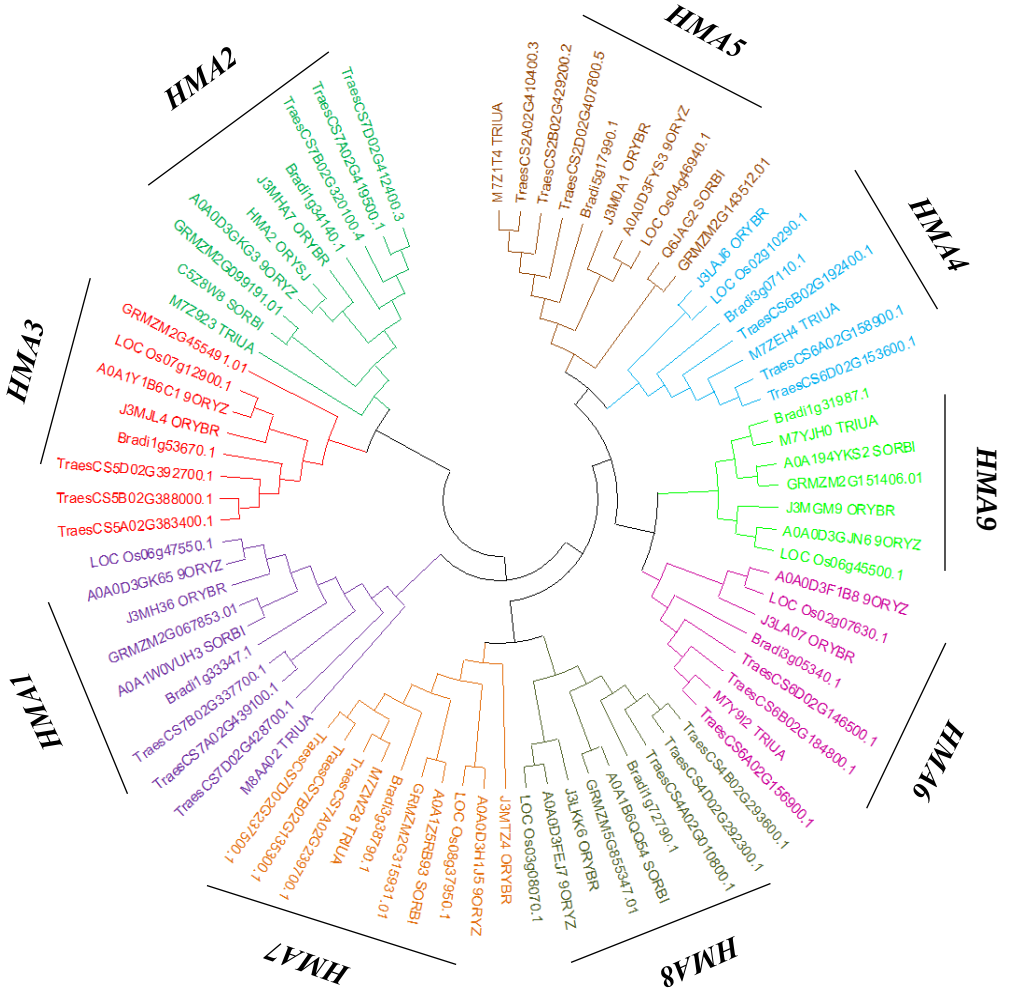


Figure. 4.1.1. P1B-type ATPase phylogenetic tree. The evolutionary history was inferred by using the Maximum Likelihood method and JTT matrix-based model. The tree with the highest log likelihood (-44874.59) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the JTT model, and then selecting the topology with superior log likelihood value. This analysis involved 79 amino acid sequences. There were a total of 1773 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

HMA6 clade (purple color) shows the existence of all heavy metal ATPases except *Zea mays* and *Sorghum bicolor*. It was observed that *Zea mays* is closely related to *O. sativa* and shares a polyphyletic relation but in this case, its absence portrays uncharacterized *HMA6* ATPase in the maize plant and it needs to be reported in the sequence database with full characterization. *HMA9* clade (chartreuse green color) shows evolutionary relatedness with *HMA6* ATPase clade and it is the only heavy metal ATPase group without any homoeolog of *Triticum aestivum.* A large portion of the *T. aestivum* genome does not contain designated gene names and it is also possible that after speciation its *HMA9* ATPases were masked and failed to translate into proper functioning proteins. *HMA7* clade (orange color) showed a close ancestral relationship with *HMA8* clade (hunter green color) which depicts their structural and functional resemblance. *HMA8* ATPase was observed to be missing in *T. urartu* although it was present in its cultivated descendent *T. aestivum.*

2.1.2. P2-type ATPase Tree (*ECAs* and *ACAs*)

The P2-type ATPases tree was created having two major types named as '*ECAs* (Endoplasmic reticulum ATPases)' or 'P-type IIA ATPase' shaded in red color and '*ACAs* (Autoinhibited Calcium ATPases)' or 'P-type IIB ATPase' highlighted in green colored clades (Appendix II). The monocot species present in each clade represented the same ancestry of the P-type ATPase family. *ECAs* consist of *ECA1*, *ECA2*, and *ECA3* ATPases and in the phylogenetic tree all of these subtypes were present in all species except *T. urartu* protein ATPase sequence which was not present in the *ECA3* clade which can either be due to gene loss or presence of gene but the absence of its protein transcript but 'A genome' of *T. aestivum* is present in this clade. P-type IIB ATPases/*ACAs* formed two major roots diverging to 11 sub-clades each representing its particular protein type from *ACA1* to *ACA1*2 except ACA5 ATPases. The following Figure. 4.1.2. illustrates P2-type ATPases of 8 selected species from the Poaceae family.

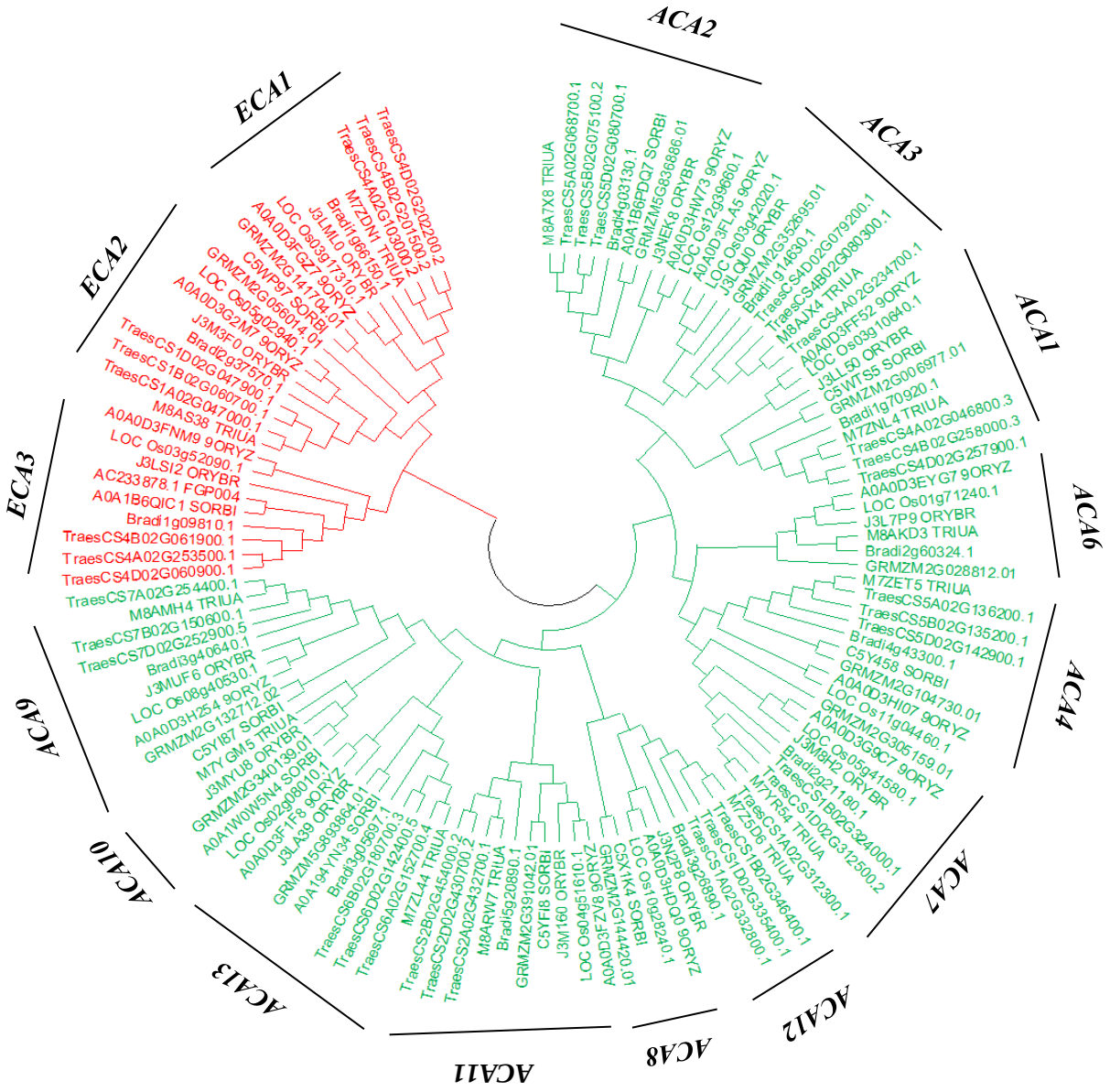


Figure. 4.1.2. P2A/P2B-type ATPase phylogenetic tree. The evolutionary history was inferred by using the Maximum Likelihood method and the JTT matrix-based model. The tree with the highest log likelihood (-61288.38) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the JTT model, and then selecting the topology with superior log likelihood value. This analysis involved 125 amino acid sequences. There were a total of 2736 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

Three main groups were noted in the *ACAs* portion of the evolutionary tree. First, the monophyletic clade of *ACA1, ACA2,* and *ACA3* was sharing a common ancestor and contained all plants' isolates except for *Sorghum bicolor* absence in the *ACA3* clade. Second, *ACA4, ACA6,* and *ACA7* showed a connection while residing the same monophyletic taxon with a few irregularities; *Oryza brachyantha* did not appear in *ACA4* clade, *T. aestivum* and *Sorghum bicolor* was not present in *ACA6* clade and *ACA7* clade was also deficient of *S. bicolor*. It emphasizes the fact that the *Sorghum bicolor* genome is left with most of the uncharacterized calcium ATPases that need to be identified and the same goes to the *T. aestivum* genome.

Third, all the remaining *ACAs* lie under the same thread of monophyletic taxon showing paraphyly (having several lines of descent) to one another. *ACA8* and *ACA1*2 amino acid sequences are aligned to form a single clade although chances were there to form a clade with one type of ATPase. As it has been discussed before in methodology, *Arabidopsis thaliana* and *Oryza sativa* ATPases were reported with designated names in the literature so *ACA8* clade was named based on this plant ATPases but *ACA1*2 is not reported in the *Oryza sativa* genome that's why *ACA1*2 was named with a high sequence identity of corresponding amino acid sequences of Arabidopsis. *Triticum urartu* and *Triticum aestivum* genome were not found to have *ACA8* amino acid sequence.

The rest of the *ACAs* including *ACA9, ACA10, ACA11,* and *ACA13* were present in all the selected species except *ACA10* that lacks most of the plants; *Triticum aestivum, Oryza sativa, Oryza barthii,* and *Brachypodium distachyon.* Interestingly, *Oryza brachyantha* and *Triticum urartu* were present in this clade which are the distant relatives of cultivated rice and wheat.

2.1.3. P3A-type ATPase Tree

Autoinhibited H^+ ATPases (*AHAs*) are also known as P3A-type ATPases which are the plasma membrane proton transporting ATPases and 10 subtypes are shown in Figure.4.1.3.

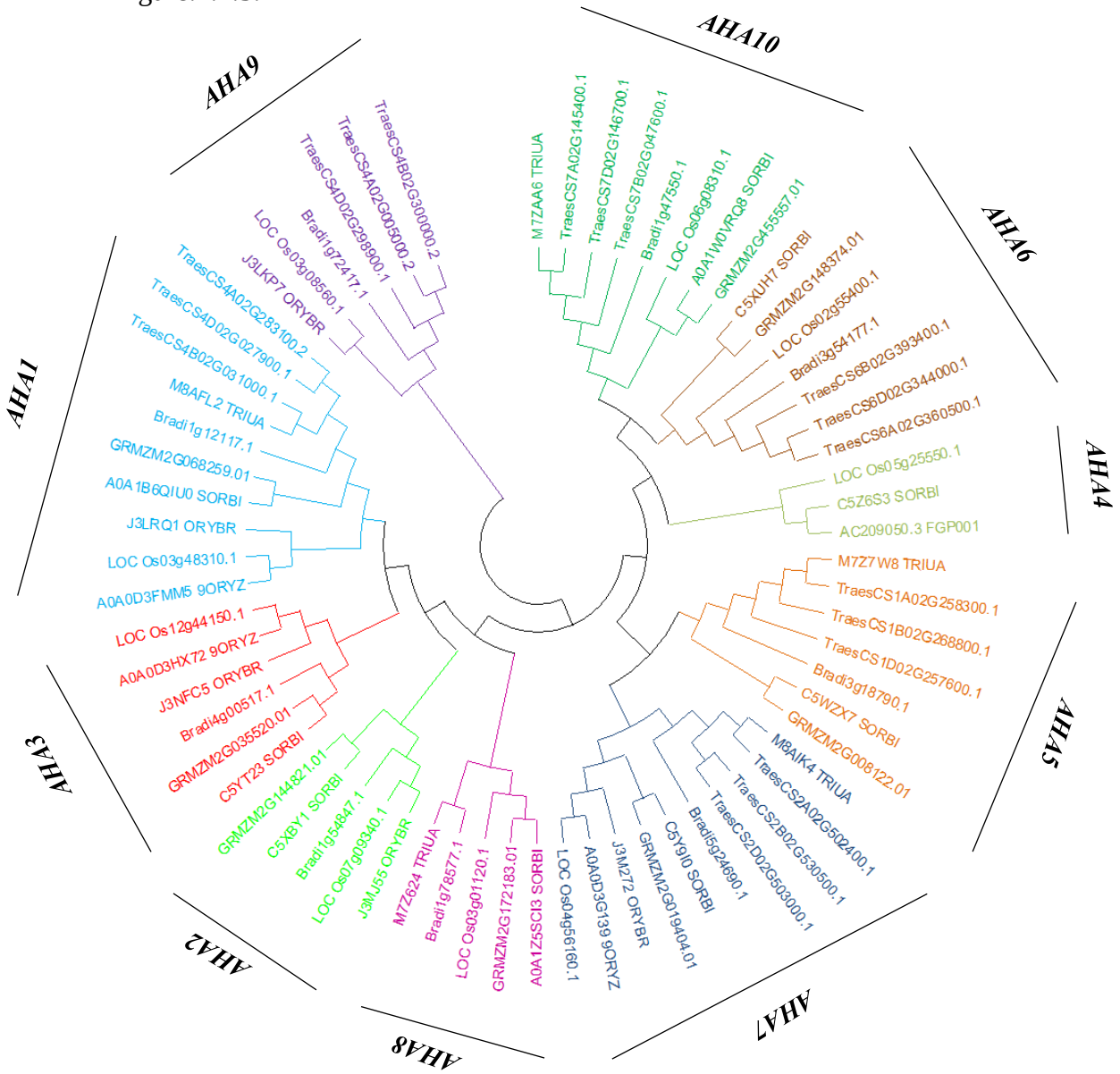


Figure. 4.1.3. P3A-type ATPase tree. The evolutionary history was inferred by using the Maximum Likelihood method and the JTT matrix-based model. The tree with the highest log likelihood (-24672.52) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the JTT model, and then selecting the topology with superior log likelihood value. This analysis involved 67 amino acid sequences. There were a total of 1755 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

This phylogenetic tee was constructed using sixty-seven amino acid sequences (Appendix III) and isolates of *AHA2*, *AHA3*, *AHA4*, and *AHA8* were not found in the *Triticum aestivum* genome though *AHA8* was present in *T. urartu* which is the earlier progenitor of today's wheat plant.

2.1.4. P4-type ATPase Tree

These are also known as phospholipid flippases divided into catalytic(blue) and regulatory(orange) complexes (Figure. 4.1.4) and their information is represented in Appendix IV. There were 8 *ALAs* (Catalytic component of Phospholipid Flippase Complex) in the final evolutionary tree from *ALA1* to *ALA10* without *ALA3* and *ALA7* ATPases. It was noted that *ALA1*, *ALA6*, and *ALA10* clades did not have *Triticum aestivum* sequences. Three main threads in *ALAs* were observed outspreading to form corresponding clades. *ALA1*, *ALA2*, and *ALA8* lie in one major clade showing structural and functional similarity with the most common recent ancestor. *ALA9* and *ALA10* are under one main group. In the same way, *ALA4*, *ALA5*, and *ALA6* diverged from a common ancestor. Only 1 partial sequence of *Brachypodium distachyons* is observed in P4-type ATPases (*ALA1*) that needs more research in its genome as further sequence analysis can give a hint about its conserved domains in other parts of the maize genome. *Oryza sativa* and *Brachypodium distachyon* were present together in all clades of *ALA* showing a polyphyletic relationship.

There were present 5 *ALA* interacting subunits (*ALIS*)/putative regulatory component of phospholipid flippase complex *viz*., *ALIS1*, *ALIS2*, *ALIS3*, *ALIS4*, and *ALIS5* out of which *T. aestivum* was not present in *ALIS1* monophyletic taxon. As *T. urartu* mostly lies together with *T. aestivum* but in this case, only the former is present which indicates gene loss during mutation or recombination or it there is also a likelihood that *ALIS1* in *T. aestivum* is present but not characterized and reported in the wheat database.

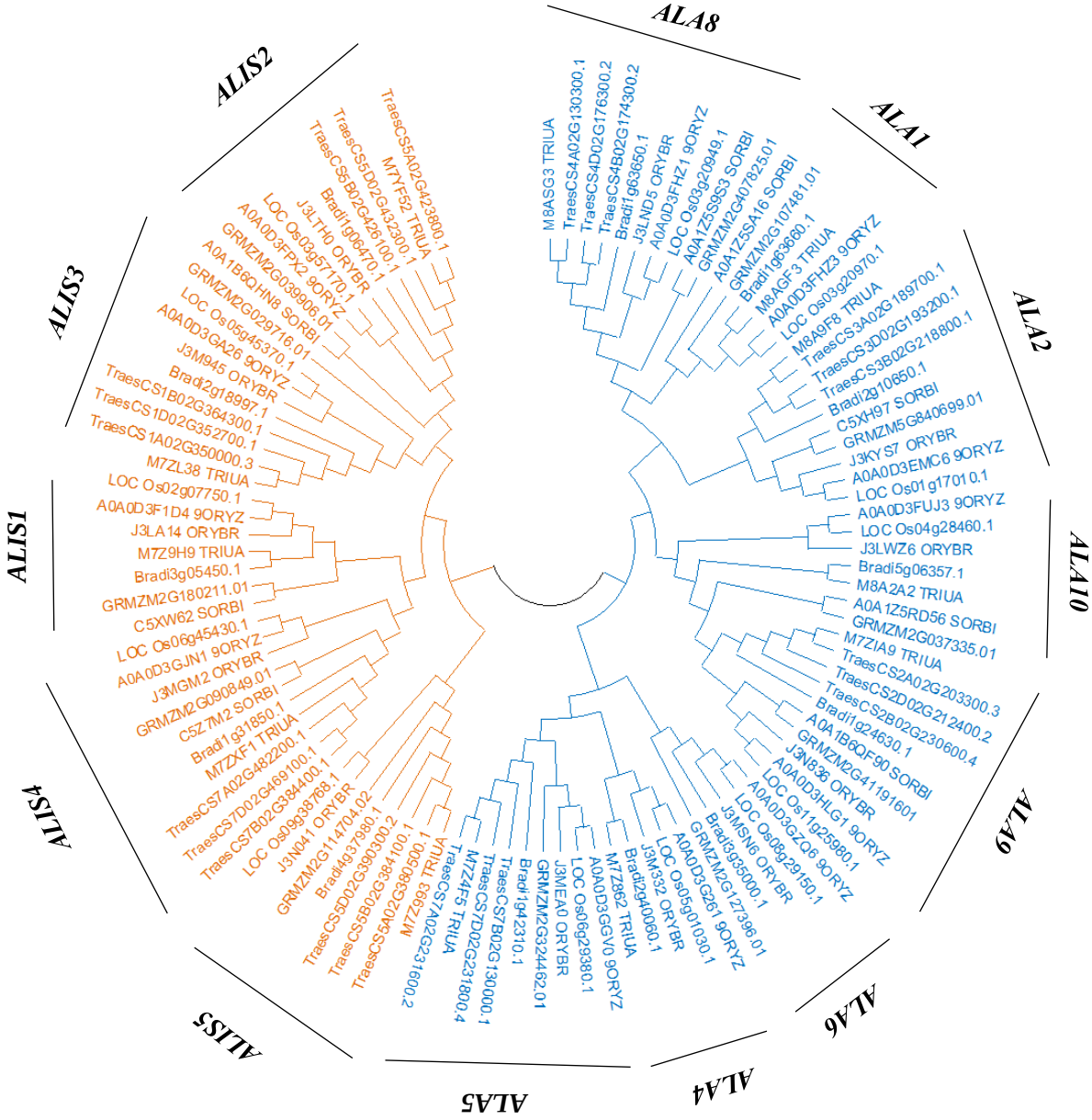


Figure. 4.1.4. P4-type ATPase tree. The evolutionary history was inferred by using the Maximum Likelihood method and the JTT matrix-based model. The tree with the highest log likelihood (-47913.85) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the JTT model, and then selecting the topology with superior log likelihood value. This analysis involved 106 amino acid sequences. There were a total of 1897 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

2.1.5. P5A-type ATPase Tree

There was only 1 putative P5A-type cation transporting ATPase in *Arabidopsis* (ATP5) which was employed to search for corresponding ATPase in Poaceae members (Appendix V). The transporting ligand of P5A-type ATPase is unknown so far but was analyzed to play an essential role in plant fertilization and pollen development.

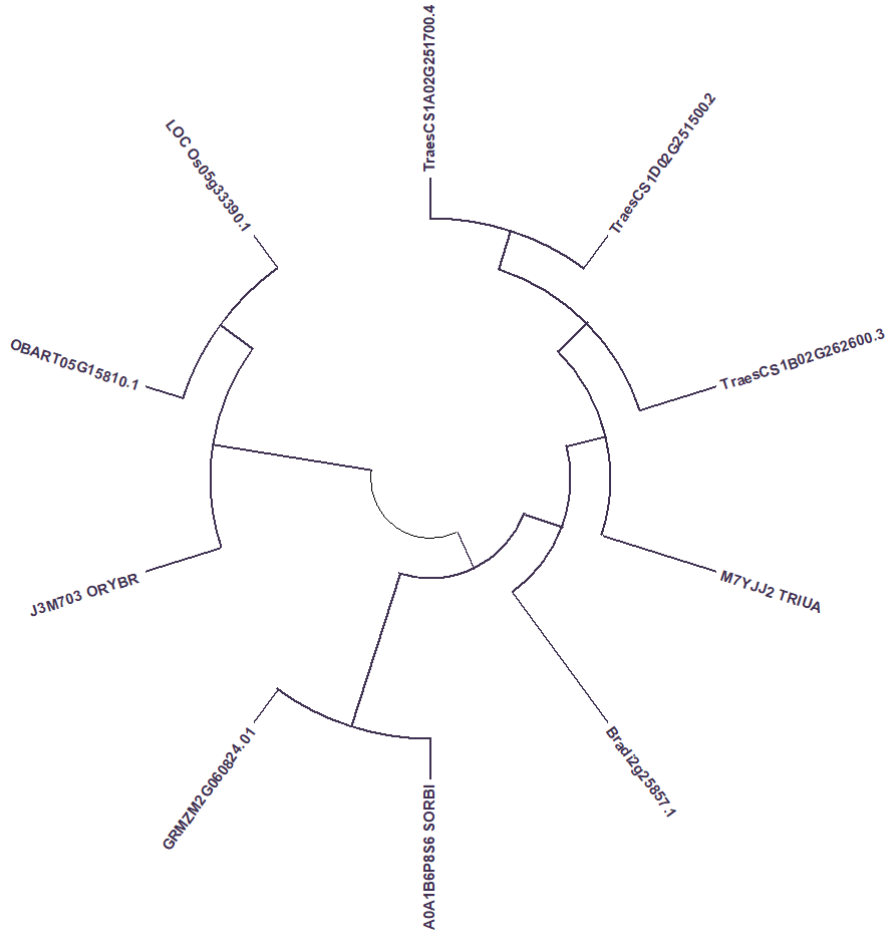


Figure. 4.1.5. P5A-type ATPase tree. The evolutionary history was inferred by using the Maximum Likelihood method and the JTT matrix-based model. The tree with the highest log likelihood (-6136.65) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the JTT model, and then selecting the topology with superior log likelihood value. This analysis involved 10 amino acid sequences. There were a total of 1406 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

It has been observed to have a similar role to lipid flippases (P4-type ATPases). The following phylogenetic tree (Figure. 4.1.5) was constructed by the alignment of ten amino acid sequences in ClustlW and then uploading the alignment file in MEGA X software to reconstruct the tree by the maximum likelihood method. There was a probability of P5A-type ATPase sequences to have the same sequences with other P-type ATPases, so it was confirmed by matching the P5A sequences with all other retrieved sequences, and the results inclined towards a distinct and separate identity of P5A-type ATPase family. In this P5A tree, three homoeologs of *T. aestivum* were in the vicinity of *T. urartu* amino acid sequences. *Oryza sativa* stayed along with *Oryza brachyantha* and *Oryza barthii* as these two are the wild type species.

4.2. Identification of Conserved Motifs for *Triticum aestivum* P-Type ATPases

P-type ATPases contain specific sequences that are the characteristic of this class of ATPases. P-type ATPase pumps of *Triticum aestivum* were aligned by online alignment tool ‘Clustal Omega’ and the results are shown below (Figure. 4.2.1- 4.2.5) for each subfamily of ATPases. Amino acid sequences were used for alignment and conservbed motifs were identified after literature review.

a)

b)

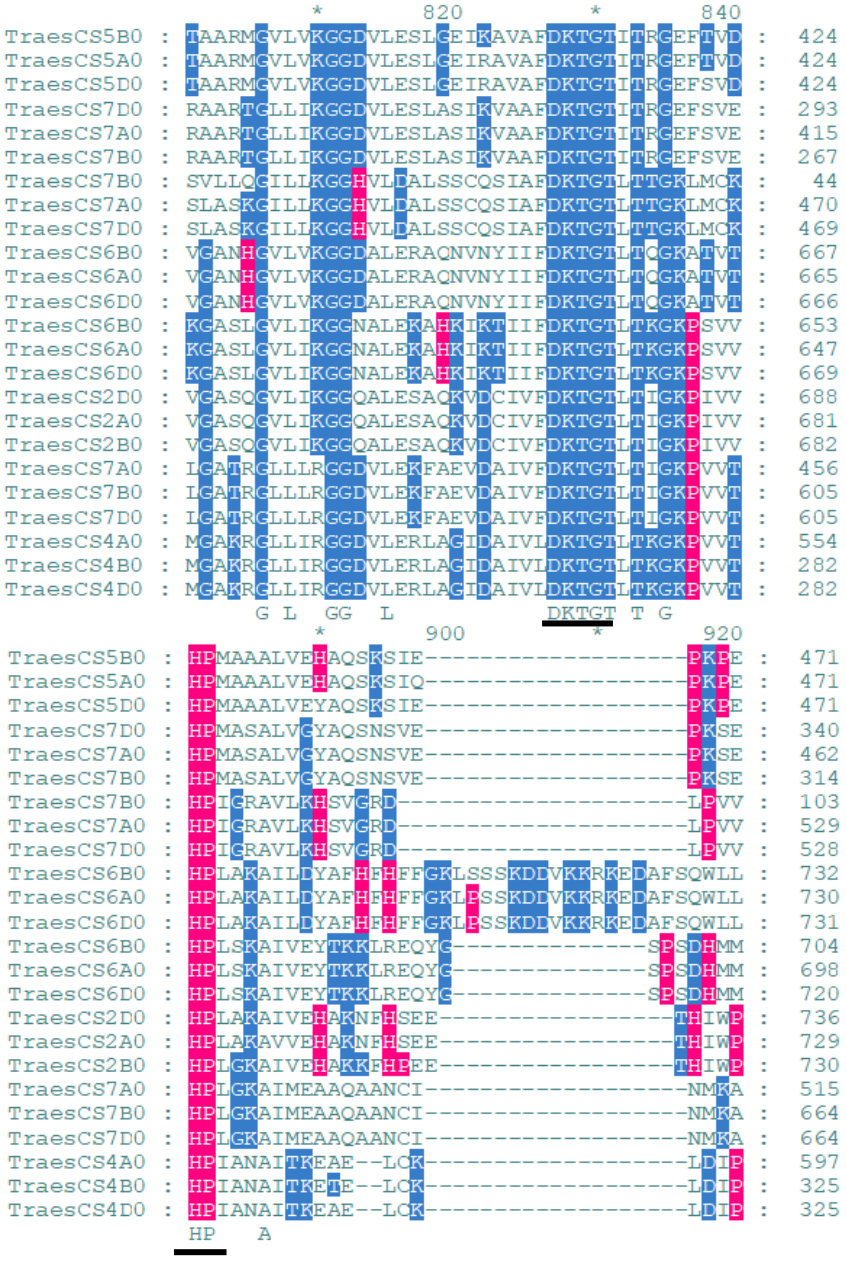
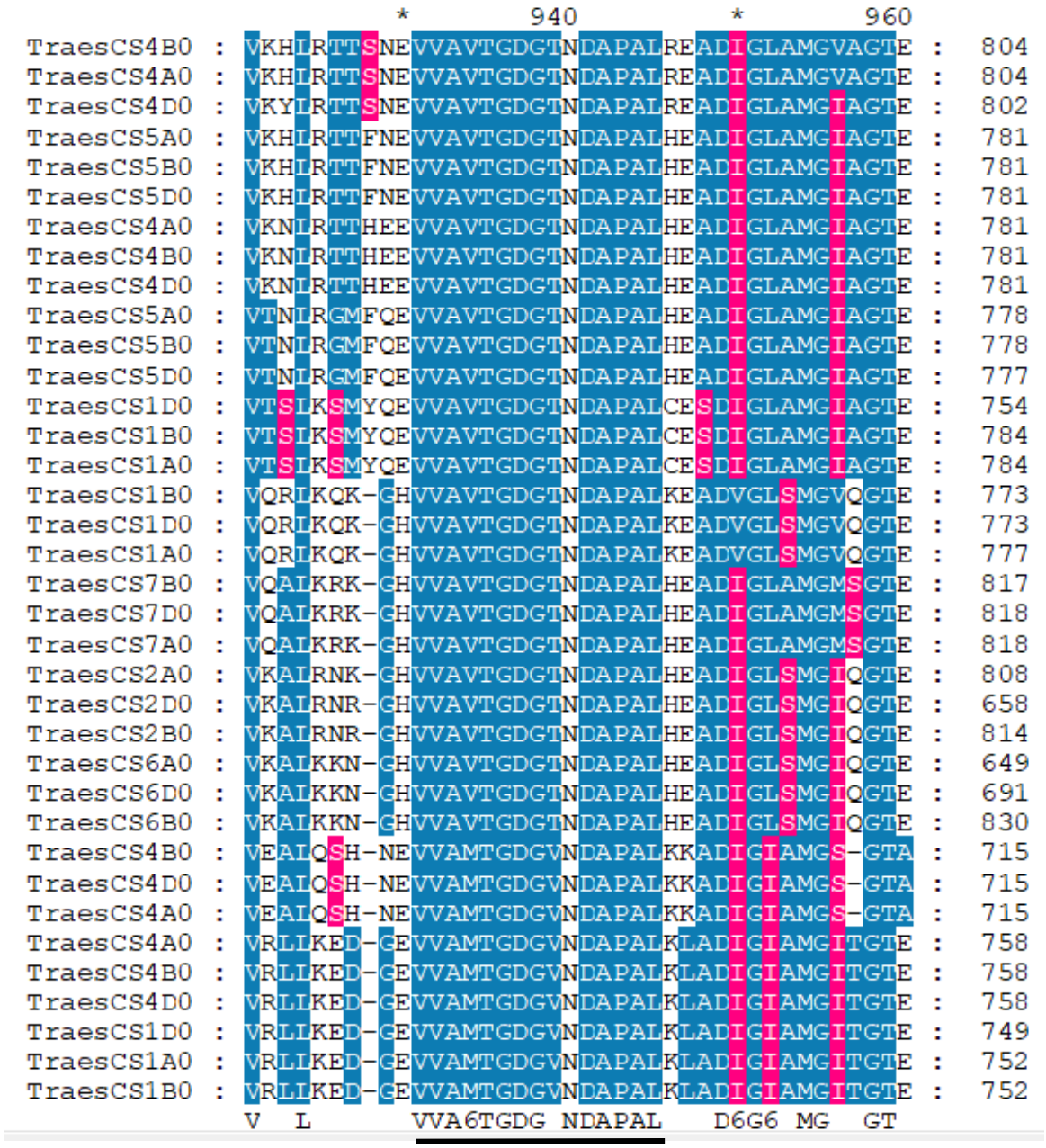
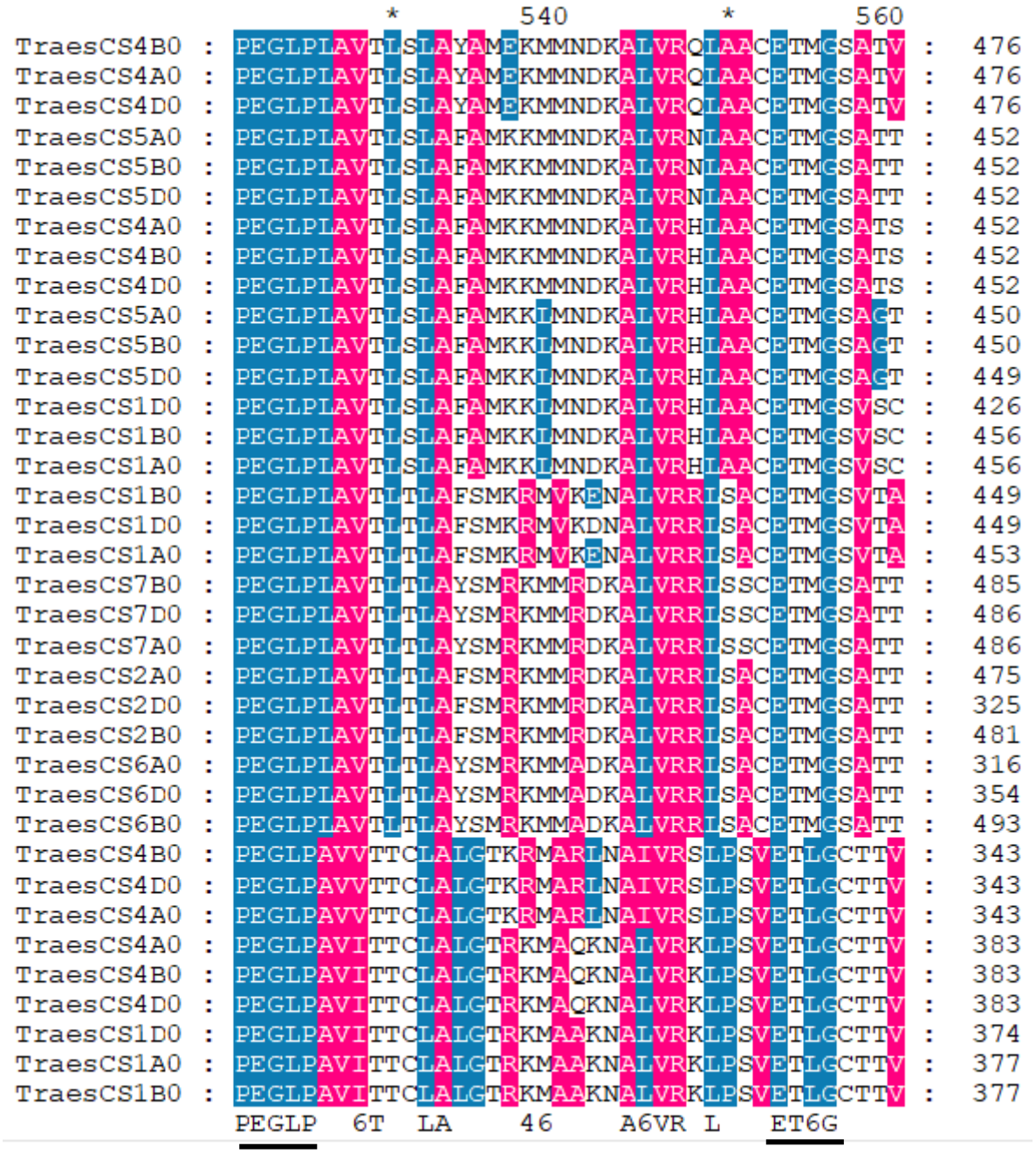


Figure. 4.2.1. P1B-type ATPase conserved motifs

a)


940
960
TraesCS4B0 : VKHLRTTSNEVVAVTGDGTNDAPALREADIGLAMGVAGTE : 804
TraesCS4A0 : VKHLRTTSNEVVAVTGDGTNDAPALREADIGLAMGVAGTE : 804
TraesCS4D0 : VKYLRTTSNEVVAVTGDGTNDAPALREADIGLAMGIAGTE : 802
TraesCS5A0 : VKHLRTTFNEVVAVTGDGTNDAPALHEADIGLAMGIAGTE : 781
TraesCS5B0 : VKHLRTTFNEVVAVTGDGTNDAPALHEADIGLAMGIAGTE : 781
TraesCS5D0 : VKHLRTTFNEVVAVTGDGTNDAPALHEADIGLAMGIAGTE : 781
TraesCS4A0 : VKNLRTTHEEVVAVTGDGTNDAPALHEADIGLAMGIAGTE : 781
TraesCS4B0 : VKNLRTTHEEVVAVTGDGTNDAPALHEADIGLAMGIAGTE : 781
TraesCS4D0 : VKNLRTTHEEVVAVTGDGTNDAPALHEADIGLAMGIAGTE : 781
TraesCS5A0 : VTNLRGMFQEVVAVTGDGTNDAPALHEADIGLAMGIAGTE : 778
TraesCS5B0 : VTNLRGMFQEVVAVTGDGTNDAPALHEADIGLAMGIAGTE : 778
TraesCS5D0 : VTNLRGMFQEVVAVTGDGTNDAPALHEADIGLAMGIAGTE : 777
TraesCS1D0 : VTSLKSMYQEVVAVTGDGTNDAPALCESDIGLAMGIAGTE : 754
TraesCS1B0 : VTSLKSMYQEVVAVTGDGTNDAPALCESDIGLAMGIAGTE : 784
TraesCS1A0 : VTSLKSMYQEVVAVTGDGTNDAPALCESDIGLAMGIAGTE : 784
TraesCS1B0 : VQRLKQK-GHVVAVTGDGTNDAPALKEADVGLSMGVQGTE : 773
TraesCS1D0 : VQRLKQK-GHVVAVTGDGTNDAPALKEADVGLSMGVQGTE : 773
TraesCS1A0 : VQRLKQK-GHVVAVTGDGTNDAPALKEADVGLSMGVQGTE : 777
TraesCS7B0 : VQALKRK-GHVVAVTGDGTNDAPALHEADIGLAMGMSGTE : 817
TraesCS7D0 : VQALKRK-GHVVAVTGDGTNDAPALHEADIGLAMGMSGTE : 818
TraesCS7A0 : VQALKRK-GHVVAVTGDGTNDAPALHEADIGLAMGMSGTE : 818
TraesCS2A0 : VKALRNK-GHVVAVTGDGTNDAPALHEADIGLSMGIQGTE : 808
TraesCS2D0 : VKALRNR-GHVVAVTGDGTNDAPALHEADIGLSMGIQGTE : 658
TraesCS2B0 : VKALRNR-GHVVAVTGDGTNDAPALHEADIGLSMGIQGTE : 814
TraesCS6A0 : VKALKKN-GHVVAVTGDGTNDAPALHEADIGLSMGIQGTE : 649
TraesCS6D0 : VKALKKN-GHVVAVTGDGTNDAPALHEADIGLSMGIQGTE : 691
TraesCS6B0 : VKALKKN-GHVVAVTGDGTNDAPALHEADIGLSMGIQGTE : 830
TraesCS4B0 : VEALQSH-NEVVAMTGDGVNDAPALKKADIGIAMGS-GTA : 715
TraesCS4D0 : VEALQSH-NEVVAMTGDGVNDAPALKKADIGIAMGS-GTA : 715
TraesCS4A0 : VEALQSH-NEVVAMTGDGVNDAPALKKADIGIAMGS-GTA : 715
TraesCS4A0 : VRLLKED-GEVVAMTGDGVNDAPALKLADIGIAMGITGTE : 758
TraesCS4B0 : VRLLKED-GEVVAMTGDGVNDAPALKLADIGIAMGITGTE : 758
TraesCS4D0 : VRLLKED-GEVVAMTGDGVNDAPALKLADIGIAMGITGTE : 758
TraesCS1D0 : VRLLKED-GEVVAMTGDGVNDAPALKLADIGIAMGITGTE : 749
TraesCS1A0 : VRLLKED-GEVVAMTGDGVNDAPALKLADIGIAMGITGTE : 752
TraesCS1B0 : VRLLKED-GEVVAMTGDGVNDAPALKLADIGIAMGITGTE : 752
V L VVA6TGDG NDAPAL D6G6 MG GT

b)


* 540 * 560
TraesCS4B0 : PEGLPLAVTLSLAYAMEKMMNDKALVRQLAACETMGSATV : 476
TraesCS4A0 : PEGLPLAVTLSLAYAMEKMMNDKALVRQLAACETMGSATV : 476
TraesCS4D0 : PEGLPLAVTLSLAYAMEKMMNDKALVRQLAACETMGSATV : 476
TraesCS5A0 : PEGLPLAVTLSLAFAMKKMMNDKALVRNLAACETMGSATT : 452
TraesCS5B0 : PEGLPLAVTLSLAFAMKKMMNDKALVRNLAACETMGSATT : 452
TraesCS5D0 : PEGLPLAVTLSLAFAMKKMMNDKALVRNLAACETMGSATT : 452
TraesCS4A0 : PEGLPLAVTLSLAFAMKKMMNDKALVRHLAACETMGSATS : 452
TraesCS4B0 : PEGLPLAVTLSLAFAMKKMMNDKALVRHLAACETMGSATS : 452
TraesCS4D0 : PEGLPLAVTLSLAFAMKKMMNDKALVRHLAACETMGSATS : 452
TraesCS5A0 : PEGLPLAVTLSLAFAMKKLMNDKALVRHLAACETMGSAGT : 450
TraesCS5B0 : PEGLPLAVTLSLAFAMKKLMNDKALVRHLAACETMGSAGT : 450
TraesCS5D0 : PEGLPLAVTLSLAFAMKKLMNDKALVRHLAACETMGSAGT : 449
TraesCS1D0 : PEGLPLAVTLSLAFAMKKLMNDKALVRHLAACETMGSVSC : 426
TraesCS1B0 : PEGLPLAVTLSLAFAMKKLMNDKALVRHLAACETMGSVSC : 456
TraesCS1A0 : PEGLPLAVTLSLAFAMKKLMNDKALVRHLAACETMGSVSC : 456
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TraesCS1D0 : PEGLPLAVTLTLAFSMKRMVKDNALVRRLSACETMGSVTA : 449
TraesCS1A0 : PEGLPLAVTLTLAFSMKRMVKENALVRRLSACETMGSVTA : 453
TraesCS7B0 : PEGLPLAVTLTLAYSMRKMMRDKALVRRLSSCETMGSATT : 485
TraesCS7D0 : PEGLPLAVTLTLAYSMRKMMRDKALVRRLSSCETMGSATT : 486
TraesCS7A0 : PEGLPLAVTLTLAYSMRKMMRDKALVRRLSSCETMGSATT : 486
TraesCS2A0 : PEGLPLAVTLTLAFSMRKMMRDKALVRRLSACETMGSATT : 475
TraesCS2D0 : PEGLPLAVTLTLAFSMRKMMRDKALVRRLSACETMGSATT : 325
TraesCS2B0 : PEGLPLAVTLTLAFSMRKMMRDKALVRRLSACETMGSATT : 481
TraesCS6A0 : PEGLPLAVTLTLAYSMRKMMADKALVRRLSACETMGSATT : 316
TraesCS6D0 : PEGLPLAVTLTLAYSMRKMMADKALVRRLSACETMGSATT : 354
TraesCS6B0 : PEGLPLAVTLTLAYSMRKMMADKALVRRLSACETMGSATT : 493
TraesCS4B0 : PEGLPAVVTTCLALGTKRMARLNAIVRSLPSVETLGCTTV : 343
TraesCS4D0 : PEGLPAVVTTCLALGTKRMARLNAIVRSLPSVETLGCTTV : 343
TraesCS4A0 : PEGLPAVVTTCLALGTKRMARLNAIVRSLPSVETLGCTTV : 343
TraesCS4A0 : PEGLPAVITTCLALGTRKMAQKNALVRKLPSVETLGCTTV : 383
TraesCS4B0 : PEGLPAVITTCLALGTRKMAQKNALVRKLPSVETLGCTTV : 383
TraesCS4D0 : PEGLPAVITTCLALGTRKMAQKNALVRKLPSVETLGCTTV : 383
TraesCS1D0 : PEGLPAVITTCLALGTRKMAAKNALVRKLPSVETLGCTTV : 374
TraesCS1A0 : PEGLPAVITTCLALGTRKMAAKNALVRKLPSVETLGCTTV : 377
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c)

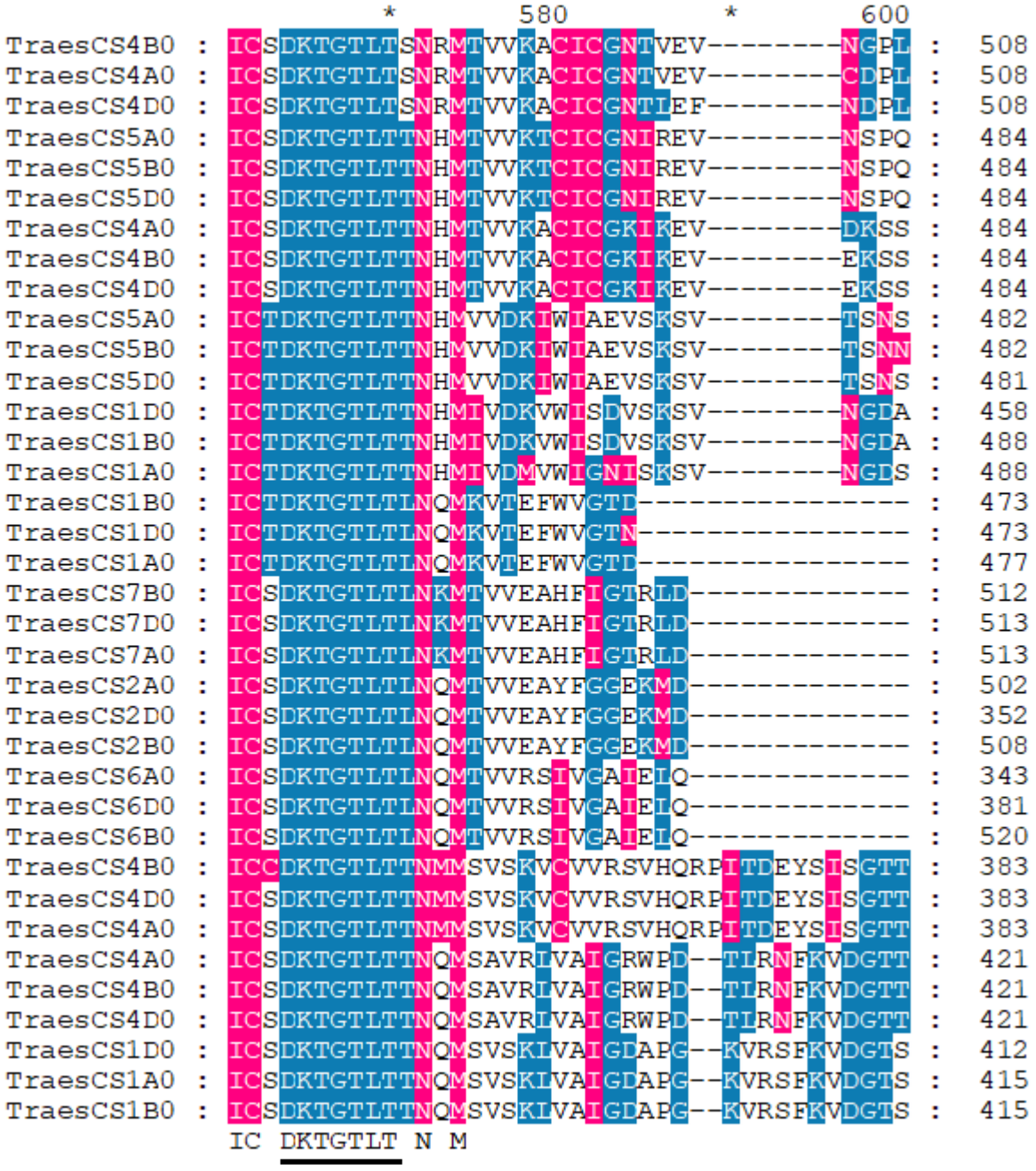
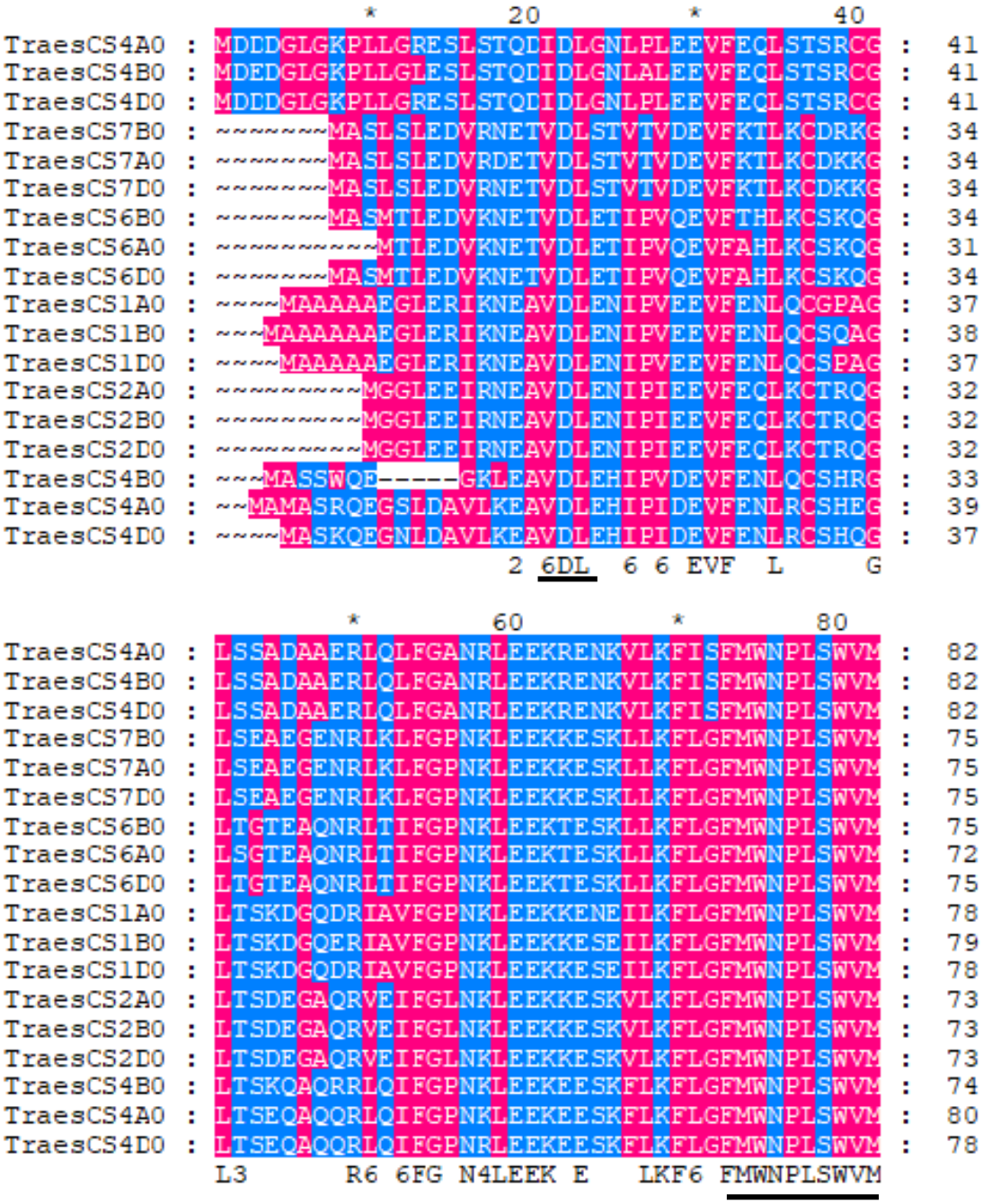


Figure. 4.2.2. P2-type ATPase conserved motifs

a)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TraesCS4B0 : MDEDGLGKPLLGLESLSTQDIDLGNLALEEVFEQLSTSRCG : 41
TraesCS4D0 : MDDDGLGKPLLGRESLSTQDIDLGNLPLEEVFEQLSTSRCG : 41
TraesCS7B0 : ~~~~~~~~MASLSLEDVRNETVDLSTVTVDEVFKTLKCDRKG : 34
TraesCS7A0 : ~~~~~~~~MASLSLEDVRDETVDLSTVTVDEVFKTLKCDKKG : 34
TraesCS7D0 : ~~~~~~~~MASLSLEDVRNETVDLSTVTVDEVFKTLKCDKKG : 34
TraesCS6B0 : ~~~~~~~~MASMTLEDVKNETVDLETIPVQEVFTHLKCSKQG : 34
TraesCS6A0 : ~~~~~~~~~~~MTLEDVKNETVDLETIPVQEVFAHLKCSKQG : 31
TraesCS6D0 : ~~~~~~~~MASMTLEDVKNETVDLETIPVQEVFAHLKCSKQG : 34
TraesCS1A0 : ~~~~MAAAAAEGLERIKNEAVDLENIPVEEVFENLQCGPAG : 37
TraesCS1B0 : ~~~MAAAAAAEGLERIKNEAVDLENIPVEEVFENLQCSQAG : 38
TraesCS1D0 : ~~~~MAAAAAEGLERIKNEAVDLENIPVEEVFENLQCSPAG : 37
TraesCS2A0 : ~~~~~~~~~~MGGLEEIRNEAVDLENIPIEEVFEQLKCTRQG : 32
TraesCS2B0 : ~~~~~~~~~~MGGLEEIRNEAVDLENIPIEEVFEQLKCTRQG : 32
TraesCS2D0 : ~~~~~~~~~~MGGLEEIRNEAVDLENIPIEEVFEQLKCTRQG : 32
TraesCS4B0 : ~~~MASSWQE-----GKLEAVDLEHIPVDEVFENLQCSHRG : 33
TraesCS4A0 : ~~MAMASRQEGSLDAVLKEAVDLEHIPIDEVFENLRCSHEG : 39
TraesCS4D0 : ~~~~MASKQEGNLDAVLKEAVDLEHIPIDEVFENLRCSHQG : 37
2 6DL 6 6 EVF L G
TraesCS4A0 : LSSADAAERLQLFGANRLEEKRENKVLKFISFMWNPLSWVM : 82
TraesCS4B0 : LSSADAAERLQLFGANRLEEKRENKVLKFISFMWNPLSWVM : 82
TraesCS4D0 : LSSADAAERLQLFGANRLEEKRENKVLKFISFMWNPLSWVM : 82
TraesCS7B0 : LSEAEGENRLKLFGPNKLEEKKESKLLKFLGFMWNPLSWVM : 75
TraesCS7A0 : LSEAEGENRLKLFGPNKLEEKKESKLLKFLGFMWNPLSWVM : 75
TraesCS7D0 : LSEAEGENRLKLFGPNKLEEKKESKLLKFLGFMWNPLSWVM : 75
TraesCS6B0 : LTGTEAQNRLTIFGPNKLEEKTESKLLKFLGFMWNPLSWVM : 75
TraesCS6A0 : LSGTEAQNRLTIFGPNKLEEKTESKLLKFLGFMWNPLSWVM : 72
TraesCS6D0 : LTGTEAQNRLTIFGPNKLEEKTESKLLKFLGFMWNPLSWVM : 75
TraesCS1A0 : LTSKDGQDRIAVFGPNKLEEKKENEILKFLGFMWNPLSWVM : 78
TraesCS1B0 : LTSKDGQERIAVFGPNKLEEKKESEILKFLGFMWNPLSWVM : 79
TraesCS1D0 : LTSKDGQDRIAVFGPNKLEEKKESEILKFLGFMWNPLSWVM : 78
TraesCS2A0 : LTSDEGAQRVEIFGLNKLEEKKESKVLKFLGFMWNPLSWVM : 73
TraesCS2B0 : LTSDEGAQRVEIFGLNKLEEKKESKVLKFLGFMWNPLSWVM : 73
TraesCS2D0 : LTSDEGAQRVEIFGLNKLEEKKESKVLKFLGFMWNPLSWVM : 73
TraesCS4B0 : LTSKQAQRRLQIFGPNKLEEKEESKFLKFLGFMWNPLSWVM : 74
TraesCS4A0 : LTSEQAQQRLQIFGPNKLEEKEESKFLKFLGFMWNPLSWVM : 80
TraesCS4D0 : LTSEQAQQRLQIFGPNRLEEKEESKFLKFLGFMWNPLSWVM : 78
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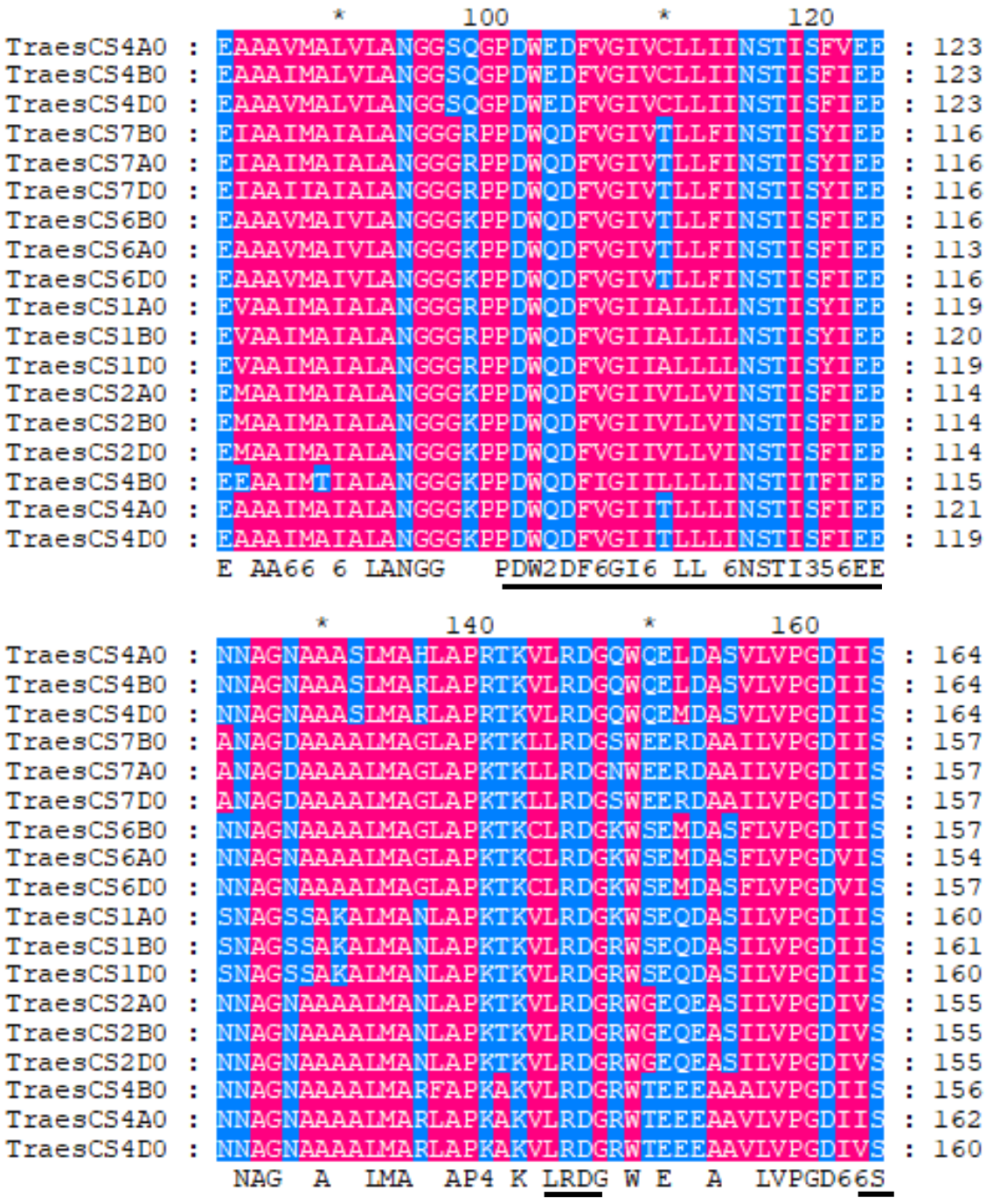
b)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TraesCS4D0 : EAAAVMALVLANGGSQGPDWEDFVGIVCLLIINSTISFIEE : 123
TraesCS7B0 : EIAAIMAIALANGGGRPPDWQDFVGIVTLLFINSTISYIEE : 116
TraesCS7A0 : EIAAIMAIALANGGGRPPDWQDFVGIVTLLFINSTISYIEE : 116
TraesCS7D0 : EIAAIIAIALANGGGRPPDWQDFVGIVTLLFINSTISYIEE : 116
TraesCS6B0 : EAAAVMAIVLANGGGKPPDWQDFVGIVTLLFINSTISFIEE : 116
TraesCS6A0 : EAAAVMAIVLANGGGKPPDWQDFVGIVTLLFINSTISFIEE : 113
TraesCS6D0 : EAAAVMAIVLANGGGKPPDWQDFVGIVTLLFINSTISFIEE : 116
TraesCS1A0 : EVAAIMAIALANGGGRPPDWQDFVGIIALLLLNSTISYIEE : 119
TraesCS1B0 : EVAAIMAIALANGGGRPPDWQDFVGIIALLLLNSTISYIEE : 120
TraesCS1D0 : EVAAIMAIALANGGGRPPDWQDFVGIIALLLLNSTISYIEE : 119
TraesCS2A0 : EMAAIMAIALANGGGKPPDWQDFVGIIVLLVINSTISFIEE : 114
TraesCS2B0 : EMAAIMAIALANGGGKPPDWQDFVGIIVLLVINSTISFIEE : 114
TraesCS2D0 : EMAAIMAIALANGGGKPPDWQDFVGIIVLLVINSTISFIEE : 114
TraesCS4B0 : EEAAIMTIALANGGGKPPDWQDFIGIILLLLINSTITFIEE : 115
TraesCS4A0 : EAAAIMAIALANGGGKPPDWQDFVGIITLLLINSTISFIEE : 121
TraesCS4D0 : EAAAIMAIALANGGGKPPDWQDFVGIITLLLINSTISFIEE : 119
E AA66 6 LANGG PDW2DF6GI6 LL 6NSTI356EE
* 140 * 160
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TraesCS4B0 : NNAGNAAASLMARLAPRTKVLRDGQWQELDASVLVPGDIIS : 164
TraesCS4D0 : NNAGNAAASLMARLAPRTKVLRDGQWQEMDASVLVPGDIIS : 164
TraesCS7B0 : ANAGDAAAAALMAGLAPKTKLLRDGSWEERDAAILVPGDIIS : 157
TraesCS7A0 : ANAGDAAAAALMAGLAPKTKLLRDGNWEERDAAILVPGDIIS : 157
TraesCS7D0 : ANAGDAAAAALMAGLAPKTKLLRDGSWEERDAAILVPGDIIS : 157
TraesCS6B0 : NNAGNAAAAALMAGLAPKTKCLRDGKWSEMDASFLVPGDIIS : 157
TraesCS6A0 : NNAGNAAAAALMAGLAPKTKCLRDGKWSEMDASFLVPGDVIS : 154
TraesCS6D0 : NNAGNAAAAALMAGLAPKTKCLRDGKWSEMDASFLVPGDVIS : 157
TraesCS1A0 : SNAGSSAKALMANLAPKTKVLRDGKWSEQDASILVPGDIIS : 160
TraesCS1B0 : SNAGSSAKALMANLAPKTKVLRDGRWSEQDASILVPGDIIS : 161
TraesCS1D0 : SNAGSSAKALMANLAPKTKVLRDGRWSEQDASILVPGDIIS : 160
TraesCS2A0 : NNAGNAAAAALMANLAPKTKVLRDGRWGEQEASILVPGDIVS : 155
TraesCS2B0 : NNAGNAAAAALMANLAPKTKVLRDGRWGEQEASILVPGDIVS : 155
TraesCS2D0 : NNAGNAAAAALMANLAPKTKVLRDGRWGEQEASILVPGDIVS : 155
TraesCS4B0 : NNAGNAAAAALMARFAPKAKVLRDGRWTEEEAAALVPGDIIS : 156
TraesCS4A0 : NNAGNAAAAALMARLAPKAKVLRDGRWTEEEAAVLVPGDIIS : 162
TraesCS4D0 : NNAGNAAAAALMARLAPKAKVLRDGRWTEEEAAVLVPGDIVS : 160
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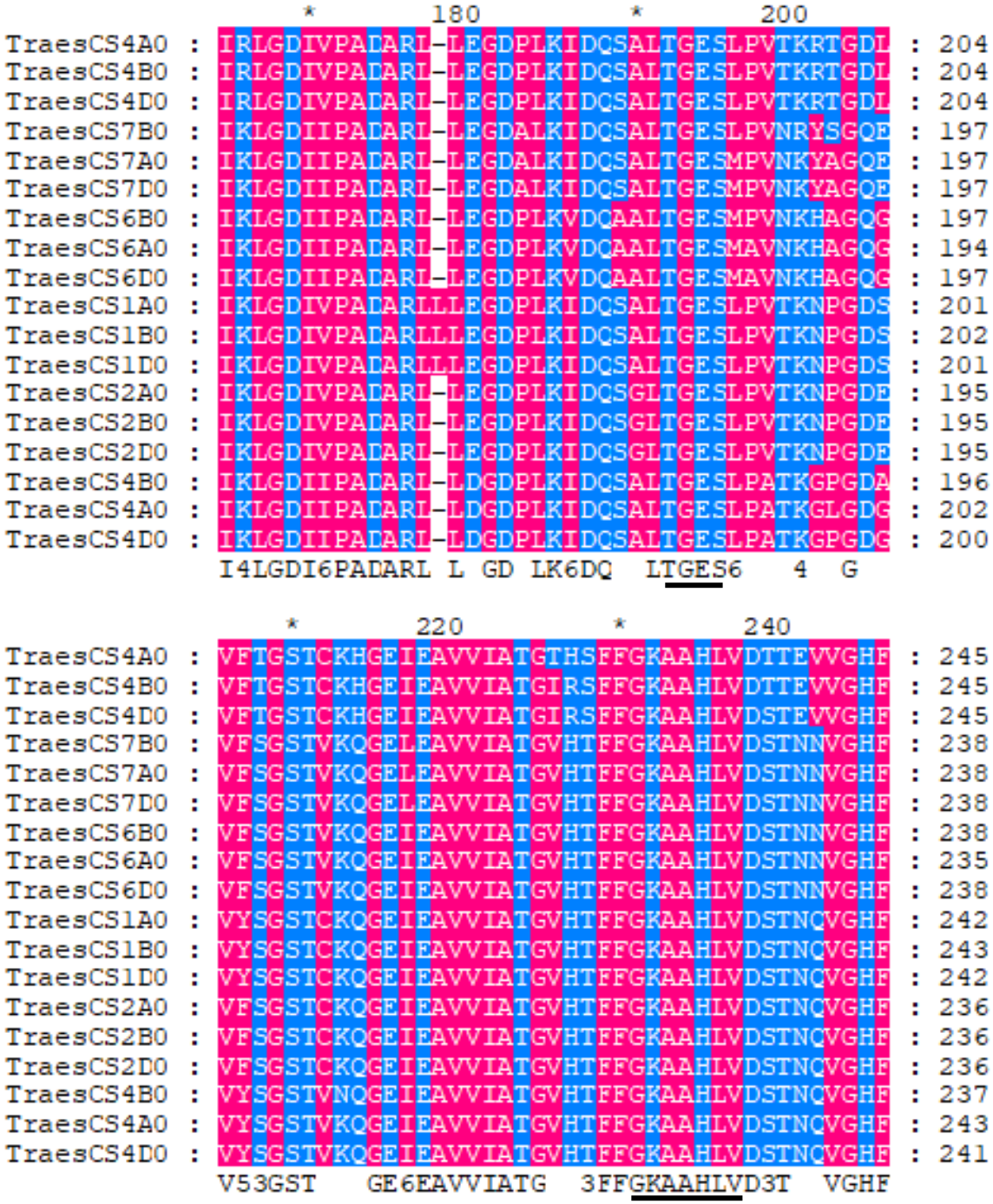
c)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TraesCS4B0 : IRLGDIVPADARL-LEGDPLKIDQSALTGESLPVTKRTGDL : 204
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TraesCS7B0 : IKLGDIIPADARL-LEGDALKIDQSALTGESLPVNRYSGQE : 197
TraesCS7A0 : IKLGDIIPADARL-LEGDALKIDQSALTGESMPVNKYAGQE : 197
TraesCS7D0 : IKLGDIIPADARL-LEGDALKIDQSALTGESMPVNKYAGQE : 197
TraesCS6B0 : IKLGDIIPADARL-LEGDPLKVDQAALTGESMPVNKHAGQG : 197
TraesCS6A0 : IKLGDIIPADARL-LEGDPLKVDQAALTGESMAVNKHAGQG : 194
TraesCS6D0 : IKLGDIIPADARL-LEGDPLKVDQAALTGESMAVNKHAGQG : 197
TraesCS1A0 : IKLGDIVPADARLLLEGDPLKIDQSALTGESLPVTKNPGDS : 201
TraesCS1B0 : IKLGDIVPADARLLLEGDPLKIDQSALTGESLPVTKNPGDS : 202
TraesCS1D0 : IKLGDIVPADARLLLEGDPLKIDQSALTGESLPVTKNPGDS : 201
TraesCS2A0 : IKLGDIVPADARL-LEGDPLKIDQSGLTGESLPVTKNPGDE : 195
TraesCS2B0 : IKLGDIVPADARL-LEGDPLKIDQSGLTGESLPVTKNPGDE : 195
TraesCS2D0 : IKLGDIVPADARL-LEGDPLKIDQSGLTGESLPVTKNPGDE : 195
TraesCS4B0 : IKLGDIIPADARL-LDGDPLKIDQSALTGESLPATKGPGDA : 196
TraesCS4A0 : IKLGDIIPADARL-LDGDPLKIDQSALTGESLPATKGLGDG : 202
TraesCS4D0 : IKLGDIIPADARL-LDGDPLKIDQSALTGESLPATKGPGDG : 200
I4LGDI6PADARL L GD LK6DQ LTGES6 4 G
* 220 * 240
TraesCS4A0 : VFTGSTCKHGEIEAVVIATGTHSFFGKAAHLVDTTEVVGHF : 245
TraesCS4B0 : VFTGSTCKHGEIEAVVIATGIRSFFGKAAHLVDTTEVVGHF : 245
TraesCS4D0 : VFTGSTCKHGEIEAVVIATGIRSFFGKAAHLVDSTEVVGHF : 245
TraesCS7B0 : VFSGSTVKQGELEAVVIATGVHTFFGKAAHLVDSTNNVGHF : 238
TraesCS7A0 : VFSGSTVKQGELEAVVIATGVHTFFGKAAHLVDSTNNVGHF : 238
TraesCS7D0 : VFSGSTVKQGELEAVVIATGVHTFFGKAAHLVDSTNNVGHF : 238
TraesCS6B0 : VFSGSTVKQGEIEAVVIATGVHTFFGKAAHLVDSTNNVGHF : 238
TraesCS6A0 : VFSGSTVKQGEIEAVVIATGVHTFFGKAAHLVDSTNNVGHF : 235
TraesCS6D0 : VFSGSTVKQGEIEAVVIATGVHTFFGKAAHLVDSTNNVGHF : 238
TraesCS1A0 : VYSGSTCKQGEIEAVVIATGVHTFFGKAAHLVDSTNQVGHF : 242
TraesCS1B0 : VYSGSTCKQGEIEAVVIATGVHTFFGKAAHLVDSTNQVGHF : 243
TraesCS1D0 : VYSGSTCKQGEIEAVVIATGVHTFFGKAAHLVDSTNQVGHF : 242
TraesCS2A0 : VFSGSTCKQGEIEAVVIATGVHTFFGKAAHLVDSTNQVGHF : 236
TraesCS2B0 : VFSGSTCKQGEIEAVVIATGVHTFFGKAAHLVDSTNQVGHF : 236
TraesCS2D0 : VFSGSTCKQGEIEAVVIATGVHTFFGKAAHLVDSTNQVGHF : 236
TraesCS4B0 : VYSGSTVNQGEIEAVVIATGVHTFFGKAAHLVDSTNQVGHF : 237
TraesCS4A0 : VYSGSTVKQGEIEAVVIATGVHTFFGKAAHLVDSTNQVGHF : 243
TraesCS4D0 : VYSGSTVKQGEIEAVVIATGVHTFFGKAAHLVDSTNQVGHF : 241
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d)

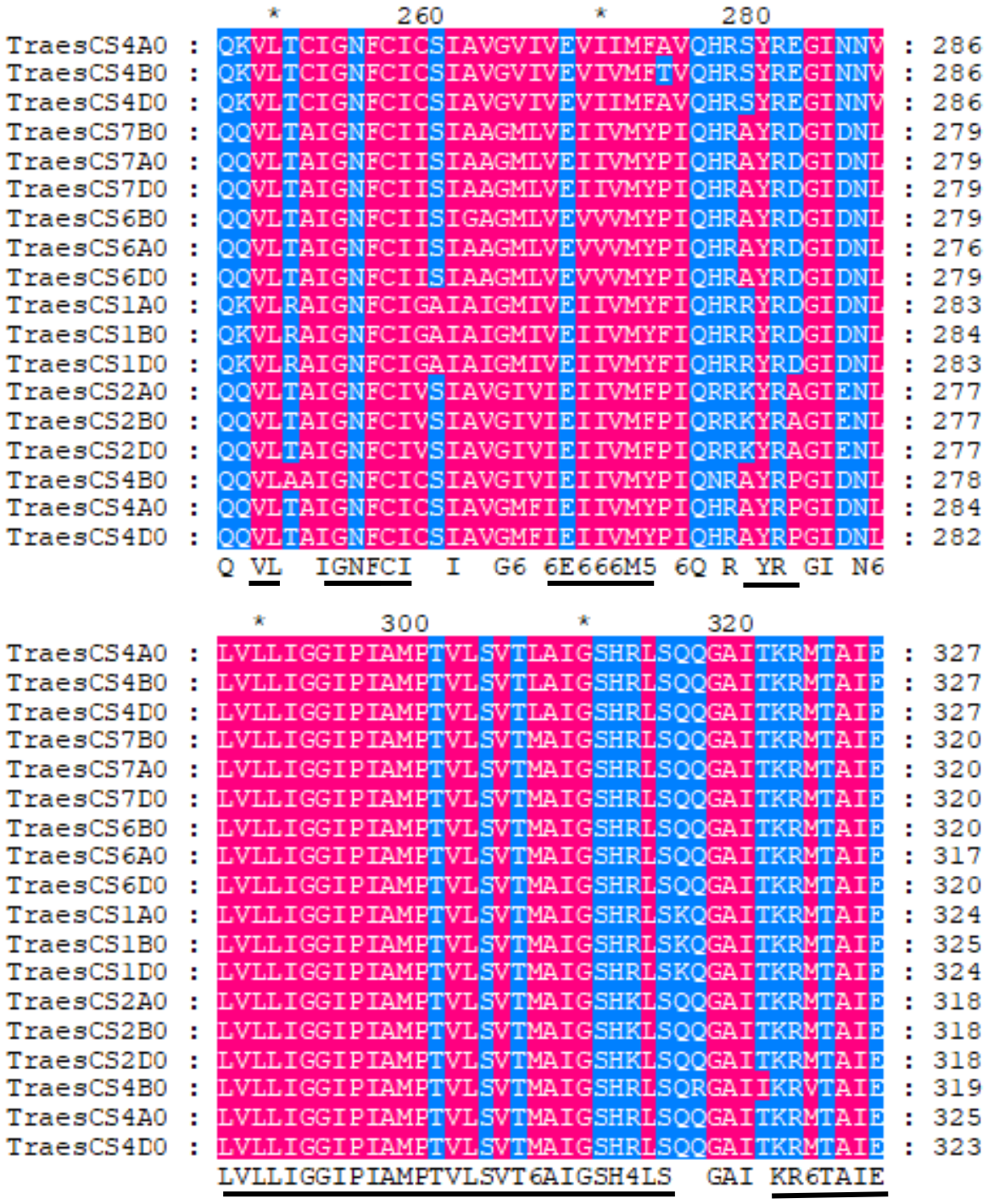
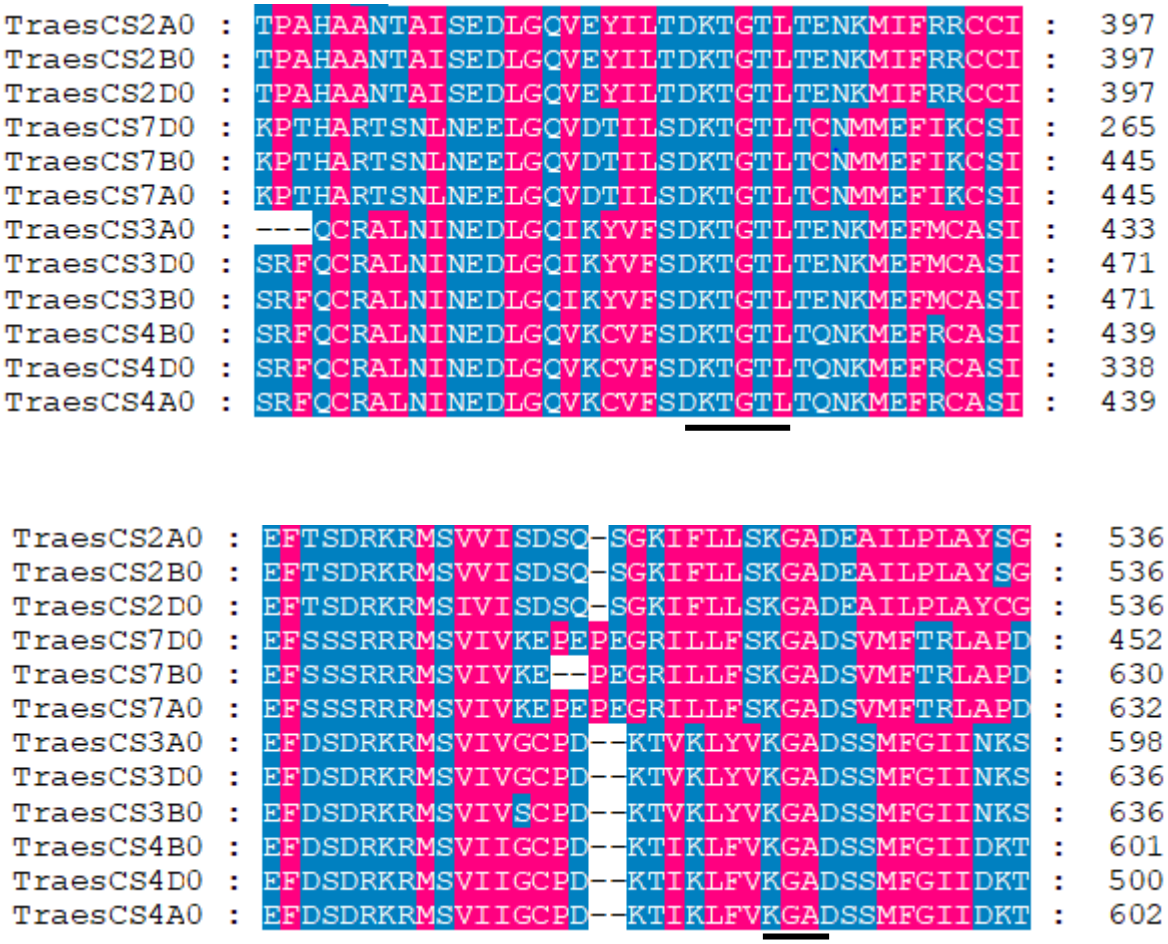


Figure. 4.2.3. P3A-type ATPases conserved motifs

a)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TraesCS2B0 : TPAHAANTAISEDLGQVEYILTDKTGTLTENKMIFRRCCI : 397
TraesCS2D0 : TPAHAANTAISEDLGQVEYILTDKTGTLTENKMIFRRCCI : 397
TraesCS7D0 : KPTHARTSNLNEELGQVDTILSDKTGTLTCNMMEFIKCSI : 265
TraesCS7B0 : KPTHARTSNLNEELGQVDTILSDKTGTLTCNMMEFIKCSI : 445
TraesCS7A0 : KPTHARTSNLNEELGQVDTILSDKTGTLTCNMMEFIKCSI : 445
TraesCS3A0 : ---QCRALNINEDLGQIKYVFSDKTGTLTENKMEFMCASI : 433
TraesCS3D0 : SRFQCRALNINEDLGQIKYVFSDKTGTLTENKMEFMCASI : 471
TraesCS3B0 : SRFQCRALNINEDLGQIKYVFSDKTGTLTENKMEFMCASI : 471
TraesCS4B0 : SRFQCRALNINEDLGQVKCVFSDKTGTLTQNKMEFRCASI : 439
TraesCS4D0 : SRFQCRALNINEDLGQVKCVFSDKTGTLTQNKMEFRCASI : 338
TraesCS4A0 : SRFQCRALNINEDLGQVKCVFSDKTGTLTQNKMEFRCASI : 439
TraesCS2A0 : EFTSDRKRMSVVISDSQ-SGKIFLLSKGADEAILPLAYSG : 536
TraesCS2B0 : EFTSDRKRMSVVISDSQ-SGKIFLLSKGADEAILPLAYSG : 536
TraesCS2D0 : EFTSDRKRMSIVISDSQ-SGKIFLLSKGADEAILPLAYCG : 536
TraesCS7D0 : EFSSSRRRMSVIVKEPEPEGRILLFSKGADSVMFTRLAPD : 452
TraesCS7B0 : EFSSSRRRMSVIVKE--PEGRILLFSKGADSVMFTRLAPD : 630
TraesCS7A0 : EFSSSRRRMSVIVKEPEPEGRILLFSKGADSVMFTRLAPD : 632
TraesCS3A0 : EFDSDRKRMSVIVGCPD--KTVKLYVKGADSSMFGIINKS : 598
TraesCS3D0 : EFDSDRKRMSVIVGCPD--KTVKLYVKGADSSMFGIINKS : 636
TraesCS3B0 : EFDSDRKRMSVIVSCPD--KTVKLYVKGADSSMFGIINKS : 636
TraesCS4B0 : EFDSDRKRMSVIIGCPD--KTIKLFVKGADSSMFGIIDKT : 601
TraesCS4D0 : EFDSDRKRMSVIIGCPD--KTIKLFVKGADSSMFGIIDKT : 500
TraesCS4A0 : EFDSDRKRMSVIIGCPD--KTIKLFVKGADSSMFGIIDKT : 602

b)

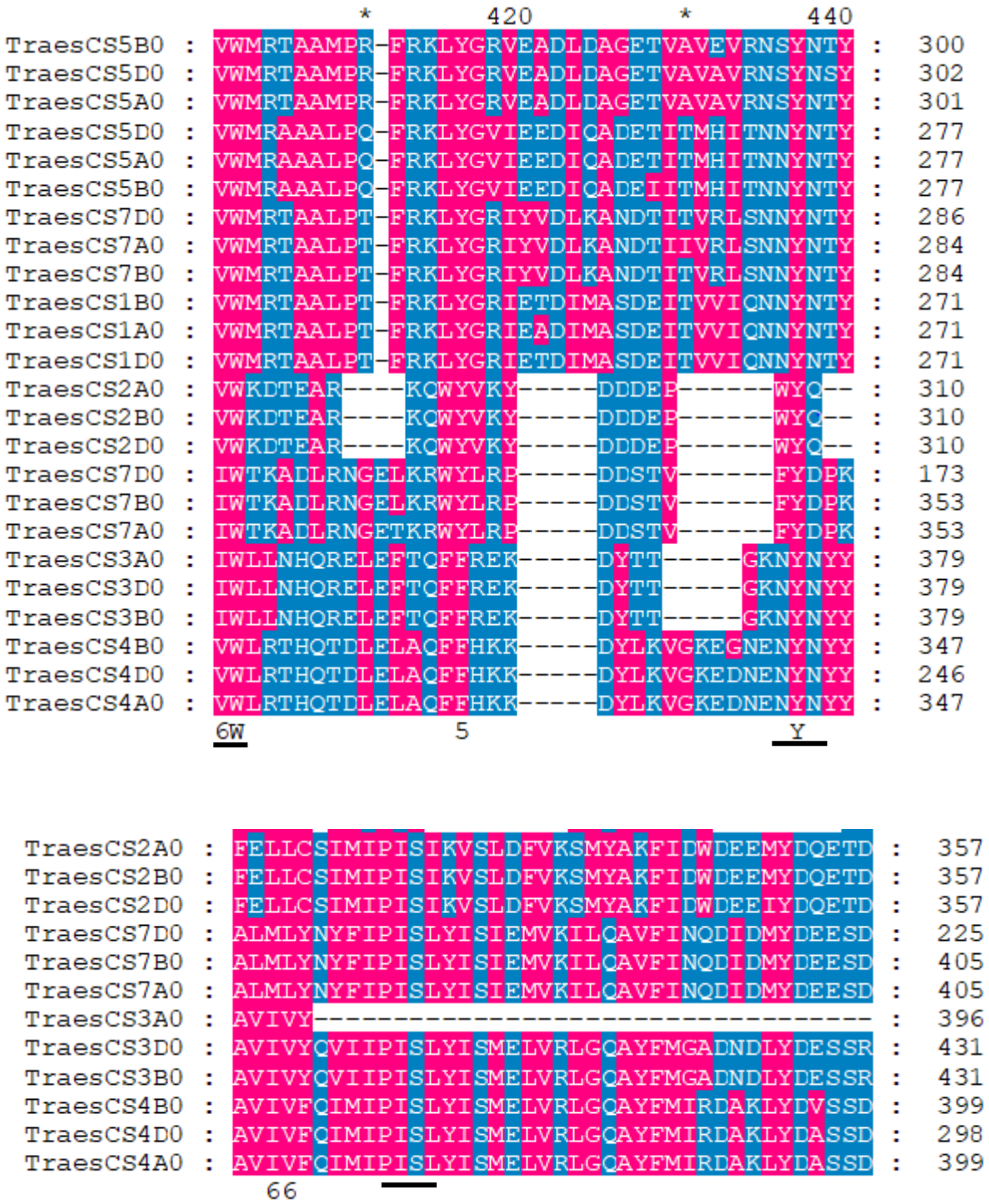


Figure. 4.2.4. P4-type ATPases conservd motifs

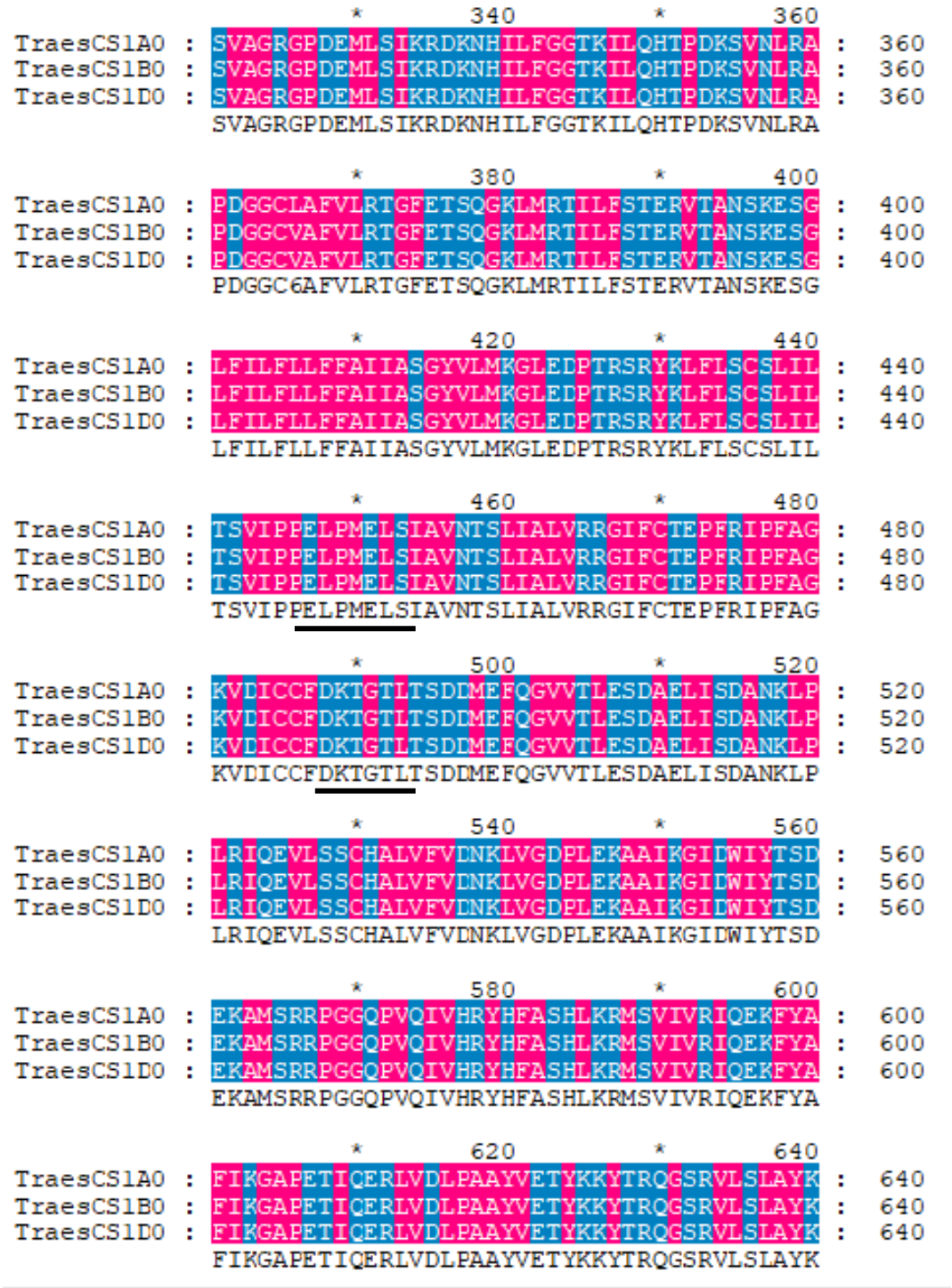


Figure. 4.2.5. P5A-type ATPases conserved motifs

4.3. Prediction of Sub-cellular Localization of *T. aestivum* P-type ATPases

P-type ATPase proteins are localized in different subcellular compartments according to the specific function they perform. 'Plant mPLOc' was used to predict the location of *T. aestivum* P-type ATPases (Appendix VI). Localization of plant protein signals were determined by an online available tool lnown as 'LOCALIZER' which used a number of Nuclear localization signals (NLS) in nucleus and chloroplast/ mitochondria localization was predicted through the presence of transit peptides (Figure. 4.3.1- 4.3.5). The red bars depict the absence of transit peptides/ NLS signals.

```
# LOCALIZER 1.0 Predictions
# -----------------
Identifier                  Chloroplast          Mitochondria         Nucleus
TraesCS6A02G158900.1[HMA4]  -                    -                    -
TraesCS6B02G192400.1[HMA4]  -                    -                    -
TraesCS6D02G153600.1[HMA4]  -                    -                    -
TraesCS5A02G383400.1[HMA3]  -                    -                    Y (RRRR)
TraesCS5B02G388000.1[HMA3]  -                    -                    -
TraesCS5D02G392700.1[HMA3]  -                    -                    -
TraesCS2A02G410400.3[HMA5]  -                    -                    Y (PVAKRKG)
TraesCS2B02G429200.2[HMA5]  -                    Y (0.888 | 1-34)     Y (AAAED,PVAKRKG)
TraesCS2D02G407800.5[HMA5]  Y (0.993 | 1-39)     -                    Y (PVAKRKG)
TraesCS7A02G439100.1[HMA1]  Y (1.0 | 1-41)       -                    Y (RRRR)
TraesCS7B02G337700.1[HMA1]  -                    -                    -
TraesCS7D02G428700.1[HMA1]  Y (1.0 | 1-41)       -                    Y (PRRRR)
TraesCS6A02G156900.1[HMA6]  -                    -                    Y (KKRK,LGEV)
TraesCS6B02G184800.1[HMA6]  -                    -                    Y (KKRK,LGEV)
TraesCS6D02G146500.1[HMA6]  -                    -                    Y (KKRK,LGEV)
TraesCS7A02G419500.1[HMA2]  -                    -                    Y (RRIPKAIKLARRTHR)
TraesCS7B02G320100.4[HMA2]  -                    -                    Y (RRIPKAIKLARRTHR)
TraesCS7D02G412400.3[HMA2]  Y (0.856 | 1-38)     Y (0.943 | 1-23)     Y (RRIPKAIKLARRTHR,RRRPAADRAQERRRPPPA,RRGGCRRRPAADRAQERRR)
TraesCS7A02G239700.1[HMA7]  -                    -                    -
TraesCS7B02G135300.1[HMA7]  Y (1.0 | 1-41)       -                    Y (KRPR)
TraesCS7D02G237500.1[HMA7]  Y (1.0 | 1-41)       -                    Y (KRPR)
TraesCS4A02G010800.1[HMA8]  Y (0.983 | 1-31)     Y (0.995 | 1-21)     -
TraesCS4B02G293600.1[HMA8]  -                    -                    -
TraesCS4D02G292300.1[HMA8]  -                    -                    -
```

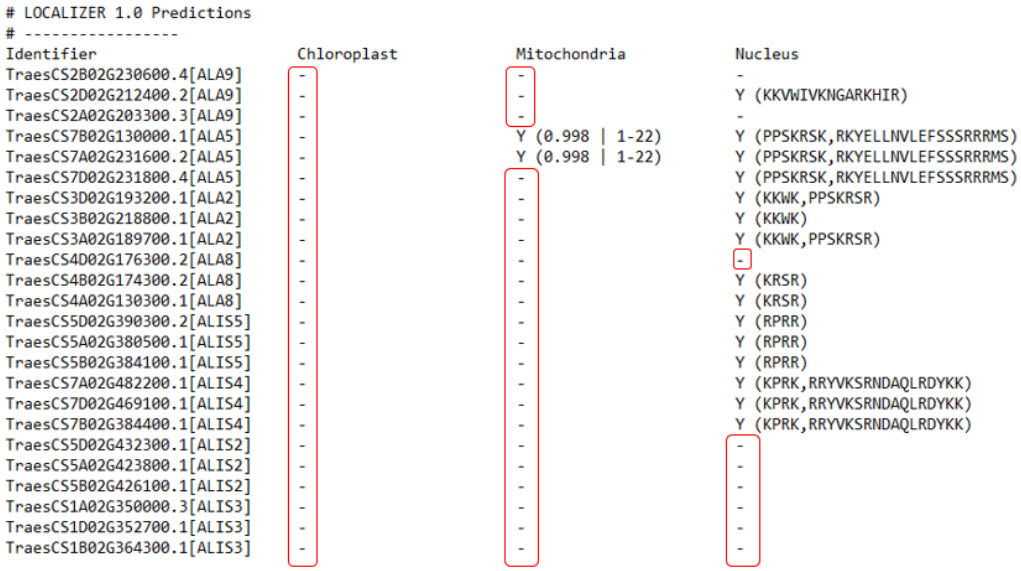
Figure. 4.3.1. Subcellular localization of P1B-type ATPases

Identifier	Chloroplast	Mitochondria	Nucleus
TraesCS1B02G346400.1[ACA12]	Y (0.971 | 1-57)	-	Y (RRRRESFGGNTYPKPRPK,KRSCKVLHVEAFNSDKKRS)
TraesCS1A02G332800.1[ACA12]	Y (0.944 | 1-42)	-	Y (RRRRESFGGNTYPKPKPK,KRSCKVLHVEAFNSDKKRS)
TraesCS7D02G252900.5[ACA9]	-	-	Y (RGGKRFR,RRDVFGANTYPRKKRKSI)
TraesCS7B02G150600.1[ACA9]	-	-	Y (RGGKRFR,RRDVFGANTYPRKKRKNI)
TraesCS7A02G254400.1[ACA9]	-	-	Y (RGGKRFR,RRDVFGANTYPRKKRKNI)
TraesCS6A02G152700.4[ACA13]	-	-	Y (KKKK)
TraesCS6D02G142400.5[ACA13]	-	-	Y (KKKK)
TraesCS6B02G180700.3[ACA13]	-	-	Y (KKKK,KKEEEKEQIRRKIRA,RRWRQAALVLNASRRFRY)
TraesCS2D02G430700.2[ACA11]	-	-	Y (RKNAFGANTYPRKKGRS)
TraesCS2A02G432700.1[ACA11]	-	-	Y (KKEAQKEEVIRKIRA,RKNAFGSNTYPRKKGRS,KKWRQAALVLNASRRFRY)
TraesCS2B02G454000.2[ACA11]	-	-	Y (KKEAQKEEVIRKIRA,RKNAFGSNTYPRKKGRS,KKWRQAALVLNASRRFRY)
TraesCS1B02G324000.1[ACA7]	-	-	Y (RRVRVEPFNSVKKKMS,RRAVGLVVRNRRRRFRE,RRFREFSALGAIDDAQRRR)
TraesCS1D02G312500.2[ACA7]	-	-	-
TraesCS1A02G312300.1[ACA7]	-	-	Y (RRVRVEPFNSVKKKMS,RRAVGLVVRNRRRRFRE,RRFREFSALGAIDDAQRRR)
TraesCS5A02G136200.1[ACA4]	-	-	Y (KRRS,RRWRSAVGSLVVKNRRRRF)
TraesCS5D02G142900.1[ACA4]	-	-	Y (KRRS,RRWRSAVGSLVVKNRRRRF)
TraesCS4A02G046800.3[ACA1]	-	-	Y (RKVVGVVKNPKRRFRF,RKATTLLKVEPFNSAKKRM)
TraesCS4B02G258000.3[ACA1]	-	-	Y (RKVVGVVKNPKRRFRF,RKATTLIKVEPFNSAKKRM)
TraesCS4D02G257900.1[ACA1]	-	-	Y (RKVVGVVKNPKRRFRF,RKATTLIKVEPFNSAKKRM)
TraesCS4B02G080300.1[ACA3]	Y (0.999 | 1-51)	-	Y (KRKR,KRQETKIVKVEPFNSVKKR)
TraesCS4A02G234700.1[ACA3]	Y (0.999 | 1-51)	-	Y (RRNPCGAAKTERPDRRGFR,KRQETKIVKVEPFNSVKKR)
TraesCS5A02G068700.1[ACA2]	-	-	Y (RRWRKLCSVVKNPKRRFRF,KRAETKIAKVEPFNSTKKR)
TraesCS5B02G075100.2[ACA2]	-	-	Y (RRWRKLCSVVKNPKRRFRF,KRAETKIAKVEPFNSTKKR)
TraesCS5D02G080700.1[ACA2]	-	-	Y (RRWRKLCSVVKNPKRRFRF,KRAETKIAKVEPFNSTKKR)
TraesCS5B02G135200.1[ACA4]	-	-	Y (KRRS,RRWRSAVGSLVVKNRRRRF)
TraesCS1D02G335400.1[ACA12]	Y (0.988 | 1-58)	-	Y (RRESFGANTYPKPRPK,KRSCKVLHVEAFNSDKKRS)
TraesCS4D02G079200.1[ACA3]	Y (0.993 | 1-52)	-	Y (RRNPCGAAKTERPDRRGFR,KRQESKIVKVEPFNSVKKR)
TraesCS1A02G047000.1[ECA2]	-	-	Y (PKRRLKKQKGE)
TraesCS1B02G060700.1[ECA2]	-	-	Y (PKRRLKKQKGE)
TraesCS1D02G047900.1[ECA2]	-	-	Y (PKRRLKKQKGE)
TraesCS4D02G060900.1[ECA3]	-	-	Y (KPRK,RRPRAWSFPLRLWRR)
TraesCS4B02G061900.1[ECA3]	-	-	Y (KPRK,RRPQAWSFPLRLWRR)
TraesCS4A02G253500.1[ECA3]	-	-	Y (KPRK,RRPRAWSFPLRLWRR)
TraesCS4B02G201500.2[ECA1]	-	-	-
TraesCS4D02G202200.2[ECA1]	-	-	-
TraesCS4A02G103000.2[ECA1]	-	-	-

Figure. 4.3.2. Subcellular localization of P2B-type ATPases

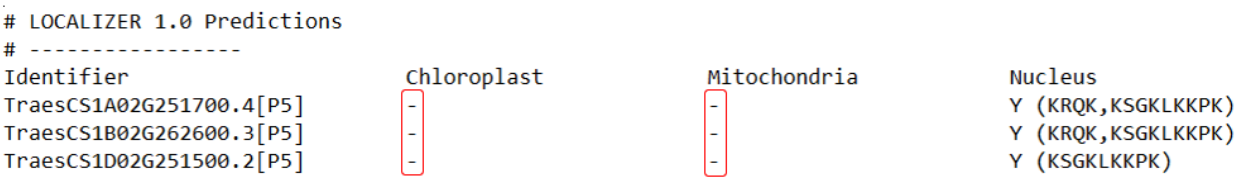
Identifier	Chloroplast	Mitochondria	Nucleus
TraesCS4A02G283100.2[AHA1]	-	-	-
TraesCS4B02G031000.1[AHA1]	-	-	-
TraesCS4D02G027900.1[AHA1]	-	-	-
TraesCS1A02G258300.1[AHA5]	-	-	-
TraesCS1B02G268800.1[AHA5]	-	-	-
TraesCS1D02G257600.1[AHA5]	-	-	-
TraesCS6A02G360500.1[AHA6]	-	-	-
TraesCS6B02G393400.1[AHA6]	-	-	-
TraesCS6D02G344000.1[AHA6]	-	-	-
TraesCS2A02G502400.1[AHA7]	-	-	-
TraesCS2B02G530500.1[AHA7]	-	-	-
TraesCS2D02G503000.1[AHA7]	-	-	-
TraesCS4A02G005000.2[AHA9]	-	-	-
TraesCS4B02G300000.2[AHA9]	-	-	-
TraesCS4D02G298900.1[AHA9]	-	-	Y (KRAR,RRRR)
TraesCS7A02G145400.1[AHA10]	-	-	-
TraesCS7B02G047600.1[AHA10]	-	-	-
TraesCS7D02G146700.1[AHA10]	-	-	-

Figure. 4.3.3. Subcellular localization of P3A-type ATPases



```
# LOCALIZER 1.0 Predictions
# -----------------
Identifier                  Chloroplast     Mitochondria            Nucleus
TraesCS2B02G230600.4[ALA9]  -               -                       -
TraesCS2D02G212400.2[ALA9]  -               -                       Y (KKVWIVKNGARKHIR)
TraesCS2A02G203300.3[ALA9]  -               -                       -
TraesCS7B02G130000.1[ALA5]  -               Y (0.998 | 1-22)        Y (PPSKRSK,RKYELLNVLEFSSSRRRMS)
TraesCS7A02G231600.2[ALA5]  -               Y (0.998 | 1-22)        Y (PPSKRSK,RKYELLNVLEFSSSRRRMS)
TraesCS7D02G231800.4[ALA5]  -               -                       Y (PPSKRSK,RKYELLNVLEFSSSRRRMS)
TraesCS3D02G193200.1[ALA2]  -               -                       Y (KKWK,PPSKRSR)
TraesCS3B02G218800.1[ALA2]  -               -                       Y (KKWK)
TraesCS3A02G189700.1[ALA2]  -               -                       Y (KKWK,PPSKRSR)
TraesCS4D02G176300.2[ALA8]  -               -                       -
TraesCS4B02G174300.2[ALA8]  -               -                       Y (KRSR)
TraesCS4A02G130300.1[ALA8]  -               -                       Y (KRSR)
TraesCS5D02G390300.2[ALIS5] -               -                       Y (RPRR)
TraesCS5A02G380500.1[ALIS5] -               -                       Y (RPRR)
TraesCS5B02G384100.1[ALIS5] -               -                       Y (RPRR)
TraesCS7A02G482200.1[ALIS4] -               -                       Y (KPRK,RRYVKSRNDAQLRDYKK)
TraesCS7D02G469100.1[ALIS4] -               -                       Y (KPRK,RRYVKSRNDAQLRDYKK)
TraesCS7B02G384400.1[ALIS4] -               -                       Y (KPRK,RRYVKSRNDAQLRDYKK)
TraesCS5D02G432300.1[ALIS2] -               -                       -
TraesCS5A02G423800.1[ALIS2] -               -                       -
TraesCS5B02G426100.1[ALIS2] -               -                       -
TraesCS1A02G350000.3[ALIS3] -               -                       -
TraesCS1D02G352700.1[ALIS3] -               -                       -
TraesCS1B02G364300.1[ALIS3] -               -                       -
```

Figure. 4.3.4. Subcellular localization of P4-type ATPases



```
# LOCALIZER 1.0 Predictions
# -----------------
Identifier                  Chloroplast     Mitochondria            Nucleus
TraesCS1A02G251700.4[P5]    -               -                       Y (KRQK,KSGKLKKPK)
TraesCS1B02G262600.3[P5]    -               -                       Y (KRQK,KSGKLKKPK)
TraesCS1D02G251500.2[P5]    -               -                       Y (KSGKLKKPK)
```

Figure. 4.3.5. Subcellular localization of P5A-type ATPases

4.4. Gene Structure Prediction for *T. aestivum* P-type ATPases

Gene structure prediction performed by Gene Structure Display Server (GSDS) gave insight about the genetic features of *T. aestivum* P-type ATPase genes, showing their exonic and intronic positions. The genomic and CDS sequence were entered in the designated input boxes in fasta format and output results are shown below (Figure. 4.4.1- 4.4.5) and number of exons are represented in appendix VI along with protein length and size.

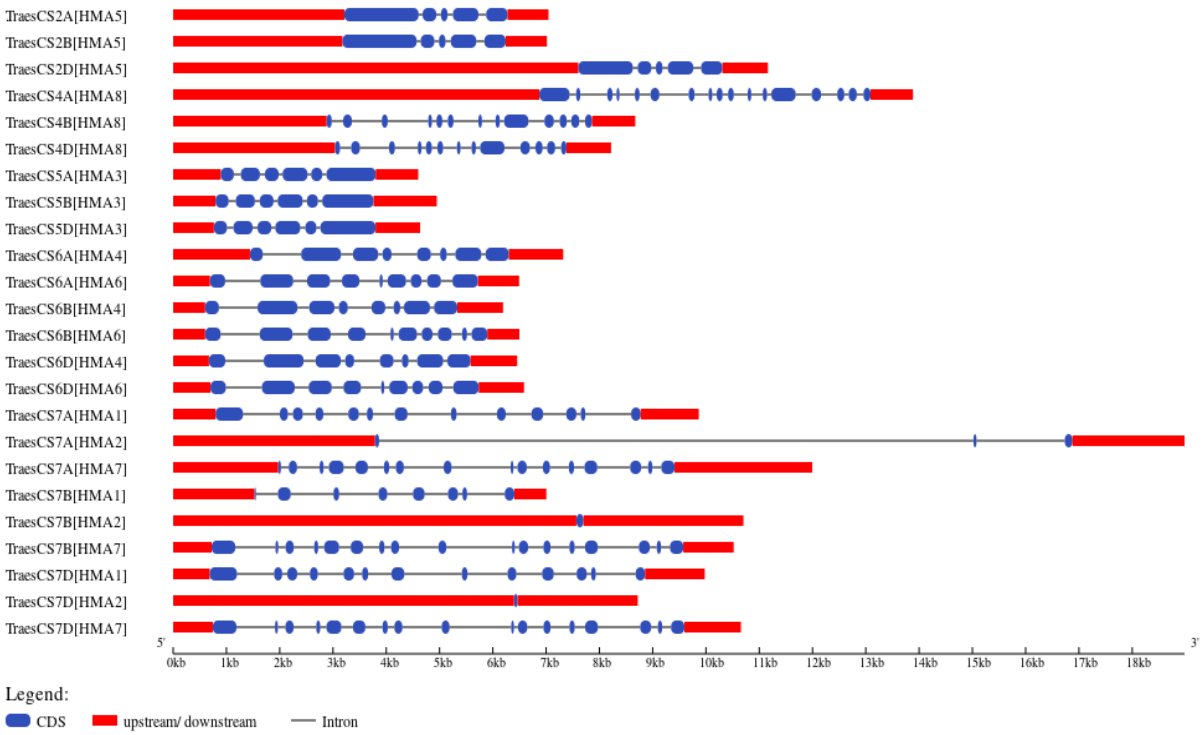


Figure. 4.4.1. Gene structure prediction of P1B-type ATPases

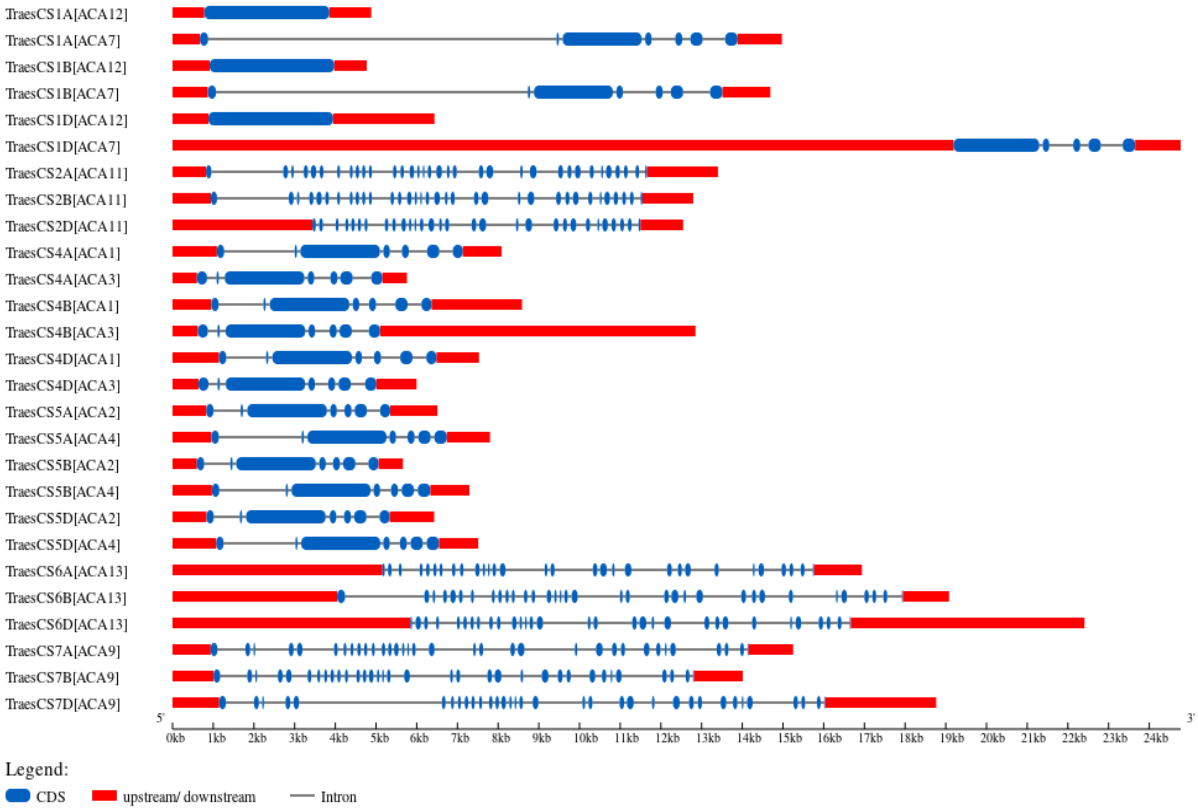


Figure. 4.4.2. a) Gene structure prediction of *ACAs*

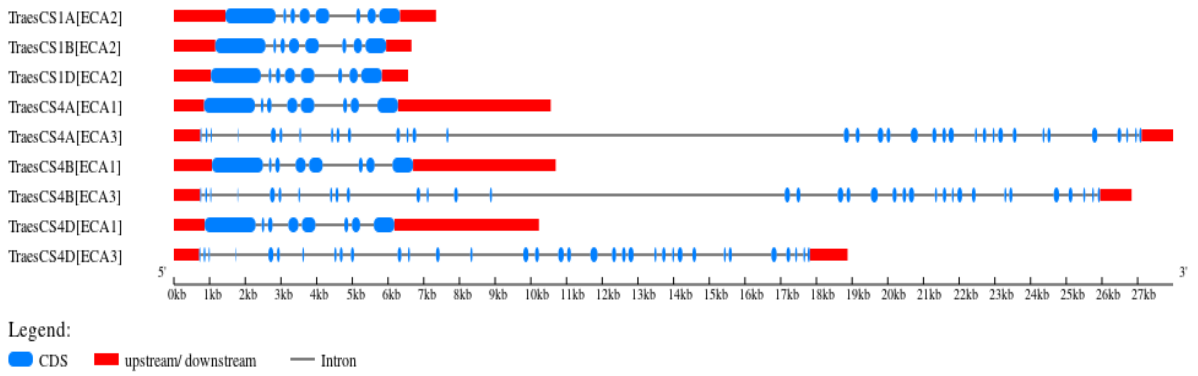


Figure. 4.4.2. b) Gene structure prediction of *ECAs*

Figure. 4.4.3. Gene structure prediction of P3A-type ATPases

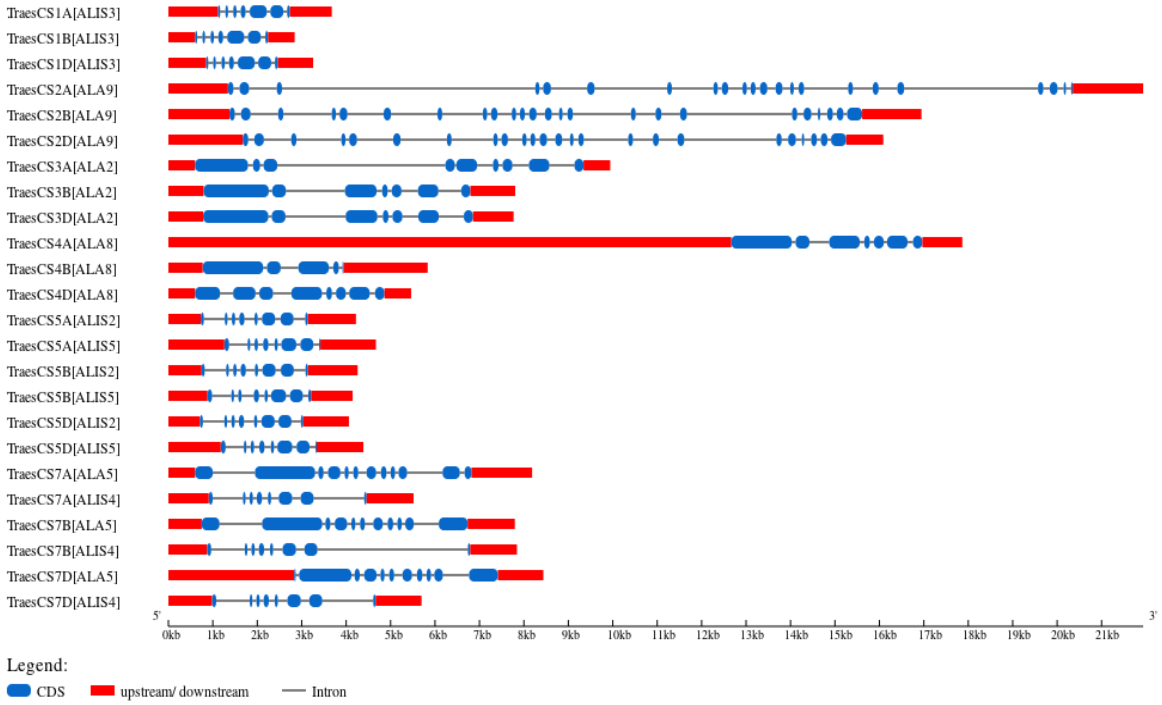


Figure. 4.4.4. Gene structure prediction of P4-type ATPases

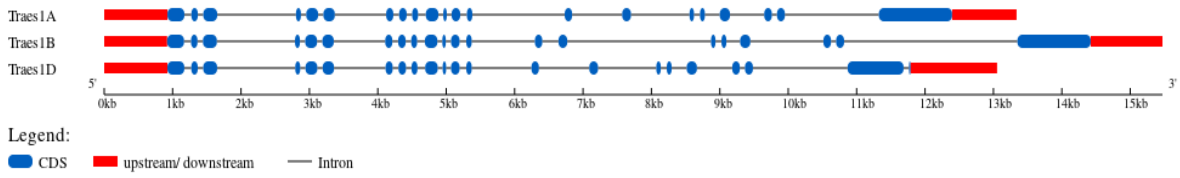


Figure. 4.4.5. Gene structure prediction of P5A-type ATPases

Chapter 5

Discussion

Plant genome sequencing has provided an opportunity to researchers for investigating structural and functional traits of crop plants that are the vital source of food for the ever-increasing world population and improvement in the cultivars can be a beneficial source of high yield and disease-resistant plants(Edwards *et al.*, 2010). Sequencing technologies such as next-generation sequencing (NGS), have made it easier to see through the genome of plants, exploring their developmental traits, adaptation to the changing environment, and many more(Bolger *et al.*, 2014). *Arabidopsis thaliana* was the first plant to be sequenced due to its smallest genome size and less repetitive sequences(Kaul *et al.*, 2000). After two years of *A. thaliana* genome sequencing, the first draft of the rice genome was published by whole genome sequencing technique as it is considered a model grass plant with a genome of 420Mb(Goff *et al.*, 2002a). *Brachypodium distachyon* is also contemplated as a model grass due to its small genome size (355Mb) and quick multiplication time and comparative analysis of *Brachypodium* genome with that of *Oryza sativa* and *Sorghum bicolor* presented a definite evolutionary history in the diversified grass genome(Initiative, 2010).

Bread wheat is an economically important Pooideae grass occupying 220 million hectares of cultivated area worldwide with the production rate of 750 metric megatons per year, it is also a chief food crop in Pakistan due to which its *In silico* analysis must be the topmost priority to check for phylogenetic history for crop improvement but allohexaploid genome (17Gb) of wheat has repetitive sequences and intergenic regions which are largely non-uniform leaving the un-assembled genome which remained the major cause of a large portion of the uncharacterized genome after the first draft was published(Alaux *et al.*, 2018; Balfourier *et al.*, 2019; Consortium, 2014). Homoeology is the term that is widely used for triplicated genomes (AA, BB, and DD) in hexaploid wheat because wheat homoeologs are the pair of genes/ homologous genes that came into being through speciation and then allopolyploidy derived them from three distinct species to bring them simultaneously in the same

genome and genetic pieces of evidence are also present to prove that hybridization of tetraploid *Triticum turgidum* (AABB) with diploid wheat *Aegilops tauschii* resulted in the formation of hexaploid *Triticum aestivum* (AABBDD) where AA genome came from wild einkorn wheat *Triticum urartu*(Glover *et al.*, 2016; McFadden *et al.*, 1946).

The genome of *T. urartu* can be used for the functional and evolutionary studies of hexaploid wheat and BAC-by-BAC (Bacterial Artificial Chromosome) sequencing technique was utilized to generate genome sequence of einkorn wheat with scaffold sequences of 4.86 Gb, a number very close to the estimated 4.94 Gb genome showing a synteny with *O. sativa, B. distachyon* and *S. bicolor* moreover 41,507 protein coding genes were reported(Ling *et al.*, 2018).

Plant breeders take advantage of the crop wild relatives (CWR) that provide an enormous gene pool for introducing genetic variations in domesticated crop plants that have lost most of the genomic regions due to bottleneck during its domestication that's why conservation of genomic isolates from wild species is of prime importance(Brozynska *et al.*, 2016).

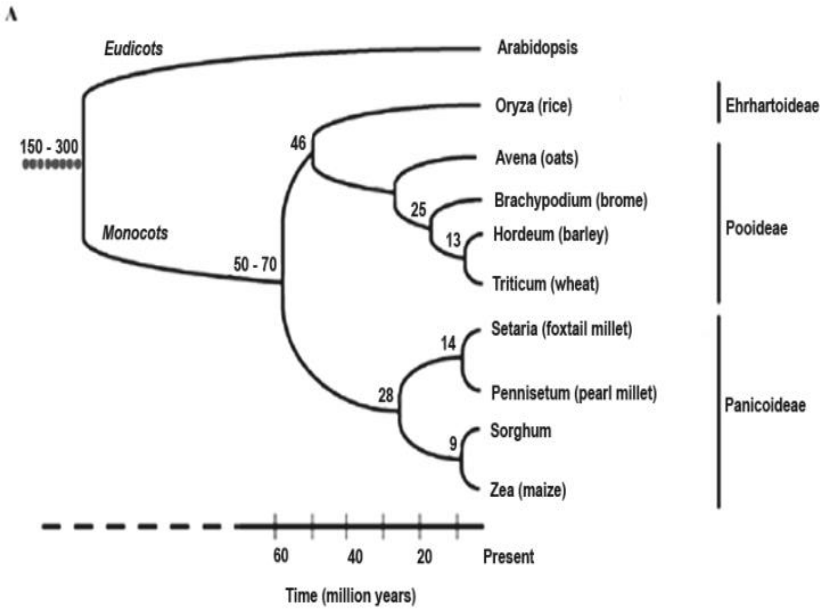


Figure. 5.1. Evolutionary relatedness of *Zea mays* to cereal crops

Oryza brachyantha is a unique plant of the genus with FF genome and rapid life cycle being the closest relative, diverged from *Oryza sativa* 15 million years ago(Jacquemin *et al.,* 2013a; Ricachenevsky *et al.,* 2018). *Oryza barthii* is the wild progenitor of African cultivated rice (*Oryza glaberrima*) has beneficial traits of biotic and abiotic stress tolerance accompanied with weed competitiveness that is useful to form high yielding hybrid varieties with Asian rice named as NERICA (New Rice for Africa) with disease resistance capacity(Z.-M. Li *et al.*, 2011; Sarla *et al.,* 2005). *Zea mays* is agronomically important crop worldwide, also a model organism for grass family is a key player for genetic and cytogenetic research and several mutant stocks of maize plant are developed to study the Poaceae plant species having an evolutionary relationship (Figure. 5.1.) where divergence of rice and maize occurred ~50 million years ago and that of maize and *Sorghum* ~9 million years ago(Bolot *et al.,* 2009; Strable *et al.,* 2009).

Monophyly, paraphyly and polyphyly are three widely used terms in cladistics. Monophyletic taxon is a group of species in one clade originating from a single ancestor whereas paraphyletic group does not contain all progenies from recent predecessor. Polyphyletic taxon does not contain the common ancestor from which all members of the clade are diverged. There was a number of P-type ATPases absent in the selected Poaceae members that demands an increased pace of research in systems biology of these plants. *HMA2* sequence was not present in *Oryza sativa* amino acid sequence genome, which was retrieved from Aramemnon database, but reported in MSU database (locus ID: LOC_Os02g10290.1) and UniProtKb (name: *HMA2*_ORYSJ) with sequence length 1067 and an article was also published on the functional analysis of Os*HMA2*(Satoh-Nagasawa *et al.*, 2012). *HMA3* ATPase was not present in *Sorghum bicolor* and *Triticum urartu* which means both of them have syntenic regions with gene loss or they are not annotated so far(Talini *et al.*, 2020). *HMA4* and *HMA5* share a paraphyletic relationship while *HMA6* clade does not contain *Zea mays* and *Sorghum bicolor* which may be due the reason of genetic predisposition or gene loss(Albalat *et al.*, 2016). *HMA9* is the only heavy metal ATPase that does not comprise any genome of *T. aestivum* because its sequences is not fully annotated in any database. *HMA8* amino acid sequence is not seems to be catalogued in *T. urartu*.

Calcium/magnesium transporting ATPases are also of prime importance for plant growth and development. *T. urartu* genome did not show *ECA3* amino acid sequence probably due to evolutionary events or malfunctioning in the gene transcript do not form the protein transcript. *ACA3, ACA4* and *ACA12* clades were deficient of *S. bicolor, O. brachyantha* and *O. sativa,* respectively. The plant species of *T. urartu* and *T. aestivum* were not found to be present in *ACA8* clade. *ACA10* clade was short of half the selected species; *T. aestivum, O. barthii, O. sativa* and *B. distachyon* but *O. brachyantha* and *T. urartu* were present leaving the question mark for *Triticum* and *Oryza* species do not present there as these two are the wild progenitors. Hitherto, most of the genome needs to be annotated with regard to P-type ATPases. Plasma membrane proton ATPases are important for the maintaining potential difference and transport across plasma membrane of plant cell. Hexaploid bread wheat was not present in four AHA clades and rice was missing in *AHA5* clade. It is an intriguing fact to know that *AHA9* monophyletic taxon is the closest to most recent ancestral node. The catalytic component of three lipid flippases; *ALA1, ALA6* and *ALA10* clades did not comprise of *T. aestivum* and one regulatory component i.e., *ALIS1* not found for bread wheat though einkorn wheat was resent in that clade. Amazingly, *ALA2* amino acid sequence from *B. distachyon* was the only partial length sequence in the pool of full length P-type ATPases that arose a prospect to investigators to indulge into the re-sequencing of *Brachypodium* genome as done for rice plant to check for candidate genes(Huang *et al.*, 2009)

There is only one P5A-type ATPase reported for the model plant *Arabidopsis* so far. All the selected species were retrieved for the designated amino acid sequence and each retrieved P5A-ATPase was distinct in its accession and sequence so the probability of similarity with other P-type ATPase sequences was ruled out. It has functional similarity with other p-type ATPases as it contains low affinity calcium binding site present in cytosol and this domain is specific to P-type ATPases but its transporting ligand is unknown(Sørensen et al., 2012).

Protein transportation from cytosol to particular organelles require N-terminal transit peptides in case of chloroplast/mitochondria and nuclear signals in case of nucleus that recognize and help carry the specific protein cargo(Jarvis, 2008).

Effector proteins are secreted by pathogens while attacking on the cell thereby mimic the function of transit peptides to control over the cellular machinery because transit peptides don't show conserved sequences that's why various *in silico* approaches are present to determine the localization of plant proteins or effector proteins within the organelles and 'LOCALIZER' is one of them showing good estimate with precision. Localization of *Triticum aestivum* P-type ATPase proteins is also determined by this programme. Localizer executes a simple searching engine for eukaryotic nuclear localization signals (NLS) while WoLF PSORT and YLoc programmes perform homology search which is not much specific approach, hence LOCALIZER shows higher accuracy as compared to these programmes. The 'D genome' of wheat *HMA5* has probability 0.993 of containing the chloroplast transit peptide at position 1-39 in this sequence with no mitochondrial localization signal but NLS (PVAKRKG) is present. The same interpretation of results could be done for other subfamilies of P-type ATPases. It is an important fact to know that all the calcium ATPases (*ACAs*/*ECAs*) had NLS except *ACA7* from D-genome and also no mitrochondrial transit peptite was predicted. It is also noticeable that only one P3A-type ATPase i.e., *AHA9* from D-genome had NLS while no other *AHAs* showed presence of either transit peptides or NLS. P4-type ATPases (*ALAs*/ALIS) did not contain any chloroplast transit peptide sequence while only two mitochondrial transit peptide signals were predicted in 7B and 7D genome of *ALA5* sequence. P5A proteins only showed NLS in all the three genomes (A, B and D) while chloroplast and mitochondrial transit peptide signals were lacking in the sequences.

Plant protyein subcellular localization was also performed by 'Plant mPLoc' which is user friendly predictor of multiplex proteins that can exist simultaneously at multiple subcelllar locations. It predicts proteins location at 12 sites; cell wall, cell membrane, extracellular, cytoplasm, ER, Golgi apparatus, peroxisomes, chloroplast, mitochondria, vacuole, plastid and nucleus. All the *TaHMAs* predicted to localize in cell membrane except *HMA2* that also resides in nucleus along with cell membrane and *HMA1*, localized in the chloroplast only. It is interesting to note that Ta*ECA3* was predicted to reside in the cell membrane though this is contradictory to its natural existence in the ER which raises question about its being an ECA of *T. aestivum* but it was also the same sequence that higher percentage identity (97.4%, 97.5% and 97.6%

for A, B and D genome, respectively) and homology with the *Brachypodium ECA3* potein sequences. Further experimental analysis can be conduceted to verify this paradigm. Ta*ACA1*,2 and 4 showed multiple location sites i.e., chloroplast, ER and vacuole while Ta*ACA9*, 11, 12 and 13 showed single location in the cell membrane only. All the Ta*AHAs* were predicted to localize in the cell membrane only. All the P4-type ATPase catalytic and regulatory (*ALAs*/ALIS) sites were in cell membrane with the exception of Ta*ALIS2* and Ta*ALIS4* that showed multiplex sites in cell membrane, cell wall and nucleus. The presence of regulatory component (ALIS) in the nucleus verifies its regulatory role. The TaP5 ATPase was predicted to be present in the cell membrane depicting their role as a transmembrane pump but its specific transpoting ligand is still a question mark.

The prediction of gene structure helps researchers to annotate and predict their functions evolutionary history and position and introns and exons. The Gene structure display server (GSDS version 2.0) was used to predict gene structures of *T. aestivum* ATPases and number of exons vary in each P-type ATPase. *TaHMA7* and *TaHMA8* had higher number of exons. Ta*ECA3*, Ta*ACA9*, 11 and 13 possess higher no. of exons among P2-type ATPases. Ta*AHA1*, 7 and 9 and Ta*ALA9* contained highest exonic regions. P5A sequence showed highly conserved region 21 exons in 1A and 1B genome each while 22 exonic positions in 1D genomic sequence.

Chapter 6

Conclusion and Future Prospects

This research has provided for the first time, *in silico* analysis of all the five sub-families (P1B, P2, P3A, P4 and P5A) of P-type ATPase pumps from eight members of the Poaceae family with a special consideration to *Triticum aestivum* which is a staple crop of Pakistan and also used worldwide. Phylogenetic analysis gave insight about the different species of the *Poacea* having homology and evolutionary relationship. Yeast-two-hybrid approach can be performed in future to analyze their functional characteristics. *HMAs* can be used for heavy metal resistant in plants by conducting wet lab experiments and Calcium ATPases are helpful in studying cell signaling. *AHAs* can be used for plamsa membrane proton studies for salt tolerant plants while lippid flippases play their role in vesicle budding that can be further studied in context of hexaploid wheat. Genetic engineering of these genes in wheat and other plants can pave the way for biotic and abiotic resistant and highquality crops. Wheat genome database should be re-investigated for more annotated genes as their computational analysis helps in characterization of their functional and structural features. There is a lot to discover about P5 ATPases as their transporting ligand is still unknown despite of the experiments carried out in yeast *Spf1* which is one of the putative P5 ATPase in yeast.

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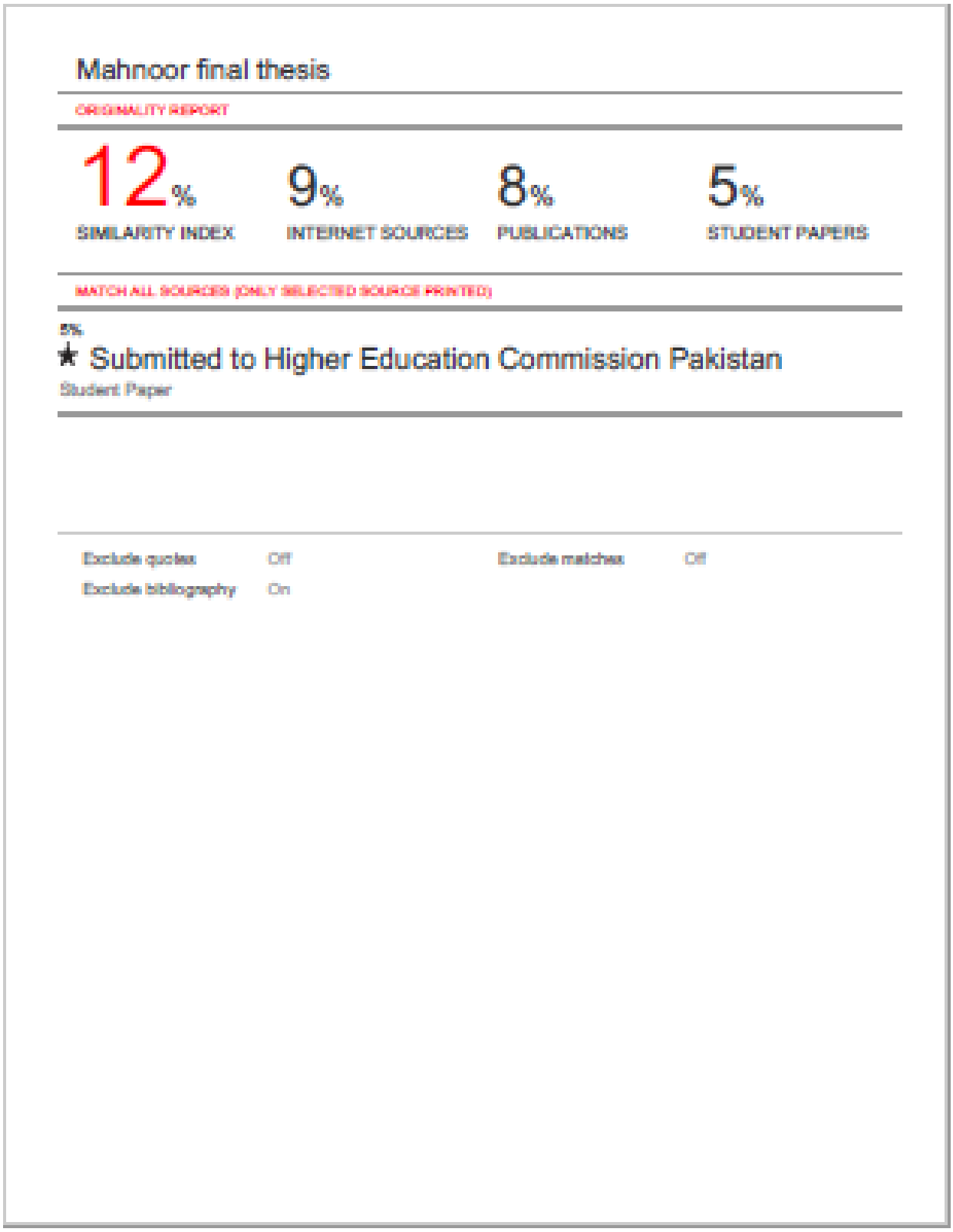

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Appendix

Appendix I. P1B-type ATPases from Selected Monocots

Sr.no.	Taxon	Accession Number	Sequence length	Database
		HMA1		
1	*Triticum aestivum*	TraesCS7A02G439100	828	Ensembl Plant
2	*Triticum aestivum*	TraesCS7B02G337700	402	Ensembl Plant
3	*Triticum aestivum*	TraesCS7D02G428700	826	Ensembl Plant
4	*Oryza sativa*	LOC_Os06g47550 .1	822	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi1g33347 .1	819	ARAMEMNON/MIPS
6	*Triticum urartu*	M8AA02_TRIUA	718	UniProtKB
7	*Oryza brachyantha*	J3MH36_ORYBR	831	UniProtKB
8	*Oryza barthii*	A0A0D3GK65_9ORYZ	822	UniProtKB
9	*Zea mays*	GRMZM2G067853 .01	823	ARAMEMNON/Gramene
10	*Sorghum bicolor*	A0A1W0VUH3_SORBI	502	UniProtKB
		HMA2		
1	*Triticum aestivum*	TraesCS7A02G419500	985	Ensembl Plant
2	*Triticum aestivum*	TraesCS7B02G320100	876	Ensembl Plant
3	*Triticum aestivum*	TraesCS7D02G412400	882	Ensembl Plant
4	*Brachypodium distachyon*	Bradi1g34140 .1	1039	ARAMEMNON/MIPS
5	*Triticum urartu*	M7Z923_TRIUA	968	UniProtKB
6	*Oryza brachyantha*	J3MHA7_ORYBR	1044	UniProtKB
7	*Oryza barthii*	A0A0D3GKG3_9ORYZ	1069	UniProtKB
8	*Sorghum bicolor*	C5Z8W8_SORBI	1069	UniProtKB
9	*Zea mays*	GRMZM2G099191 .01	1099	ARAMEMNON/Gramene
10	*Oryza sativa*	*HMA2*_ORYSJ	1067	UniProtKB
		HMA3		
1	*Triticum aestivum*	TraesCS5A02G383400	816	Ensembl Plant
2	*Triticum aestivum*	TraesCS5B02G388000	829	Ensembl Plant
3	*Triticum aestivum*	TraesCS5D02G392700	853	Ensembl Plant
4	*Oryza sativa*	LOC_Os07g12900 .1	1004	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi1g53670 .1	819	ARAMEMNON/MIPS
6	*Oryza brachyantha*	J3MJL4_ORYBR	902	UniProtKB
7	*Oryza barthii*	A0A1Y1B6C1_9ORYZ	1004	UniProtKB
8	*Zea mays*	GRMZM2G455491 .01	927	ARAMEMNON/Gramene

		HMA4		
1	*Triticum aestivum*	TraesCS6A02G158900	974	Ensembl Plant
2	*Triticum aestivum*	TraesCS6B02G192400	980	Ensembl Plant
3	*Triticum aestivum*	TraesCS6D02G153600	996	Ensembl Plant
4	*Oryza sativa*	LOC_Os02g10290 .1	978	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi3g07110 .1	981	ARAMEMNON/MIPS
6	*Triticum urartu*	M7ZEH4_TRIUA	980	UniProtKB
7	*Oryza brachyantha*	J3LAJ6_ORYBR	976	UniProtKB
		HMA5		
1	*Triticum aestivum*	TraesCS2A02G410400	1011	Ensembl Plant
2	*Triticum aestivum*	TraesCS2B02G429200	994	Ensembl Plant
3	*Triticum aestivum*	TraesCS2D02G407800	1000	Ensembl Plant
4	*Oryza sativa*	LOC_Os04g46940 .1	1002	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi5g17990 .1	999	ARAMEMNON/MIPS
6	*Triticum urartu*	M7Z1T4_TRIUA	901	UniProtKB
7	*Oryza brachyantha*	J3M0A1_ORYBR	999	UniProtKB
8	*Oryza barthii*	A0A0D3FYS3_9ORYZ	1042	UniProtKB
9	*Zea mays*	GRMZM2G143512 .01	999	ARAMEMNON/Gramene
10	*Sorghum bicolor*	Q6JAG2_SORBI	1002	UniProtKB
		HMA6		
1	*Triticum aestivum*	TraesCS6A02G156900	997	Ensembl Plant
2	*Triticum aestivum*	TraesCS6B02G184800	972	Ensembl Plant
3	*Triticum aestivum*	TraesCS6D02G146500	998	Ensembl Plant
4	*Oryza sativa*	LOC_Os02g07630 .1	1012	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi3g05340 .1	996	ARAMEMNON/MIPS
6	*Triticum urartu*	M7Y9I2_TRIUA	950	UniProtKB
7	*Oryza brachyantha*	J3LA07_ORYBR	904	UniProtKB
8	*Oryza barthii*	A0A0D3F1B8_9ORYZ	993	UniProtKB
		HMA7		
1	*Triticum aestivum*	TraesCS7A02G239700	803	Ensembl Plant
2	*Triticum aestivum*	TraesCS7B02G135300	952	Ensembl Plant
3	*Triticum aestivum*	TraesCS7D02G237500	952	Ensembl Plant
4	*Oryza sativa*	LOC_Os08g37950 .1	959	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi3g38790 .1	954	ARAMEMNON/MIPS

6	*Triticum urartu*	M7ZW28_TRIUA	973	UniProtKB
7	*Oryza brachyantha*	J3MTZ4_ORYBR	807	UniProtKB
8	*Oryza barthii*	A0A0D3H1J5_9ORYZ	862	UniProtKB
9	*Zea mays*	GRMZM2G315931 .01	928	ARAMEMNON/Gramene
10	*Sorghum bicolor*	A0A1Z5RB93_SORBI	787	UniProtKB
HMA8				
1	*Triticum aestivum*	TraesCS4A02G010800	890	Ensembl Plant
2	*Triticum aestivum*	TraesCS4B02G293600	618	Ensembl Plant
3	*Triticum aestivum*	TraesCS4D02G292300	607	Ensembl Plant
4	*Oryza sativa*	LOC_Os03g08070 .1	885	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi1g72790 .1	891	ARAMEMNON/MIPS
6	*Oryza brachyantha*	J3LKK6_ORYBR	728	UniProtKB
7	*Oryza barthii*	A0A0D3FEJ7_9ORYZ	910	UniProtKB
8	*Zea mays*	GRMZM5G855347 .01	442	ARAMEMNON/Gramene
9	*Sorghum bicolor*	A0A1B6QQ54_SORBI	900	UniProtKB
HMA9				
1	*Oryza sativa*	LOC_Os06g45500 .1	1003	ARAMEMNON/MSU
2	*Brachypodium distachyon*	Bradi1g31987 .1	1012	ARAMEMNON/MIPS
3	*Triticum urartu*	M7YJH0_TRIUA	945	UniProtKB
4	*Oryza brachyantha*	J3MGM9_ORYBR	1006	UniProtKB
5	*Oryza barthii*	A0A0D3GJN6_9ORYZ	902	UniProtKB
6	*Zea mays*	GRMZM2G151406 .01	400	ARAMEMNON/Gramene
7	*Sorghum bicolor*	A0A194YKS2_SORBI	1007	UniProtKB

Appendix II. P2-type ATPases

Sr.no.	Taxon	Accession Number	Sequence length	Database
		ACAs		
		ACA1		
1	*Triticum aestivum*	TraesCS4A02G046800.3	1020	Ensembl Plant
2	*Triticum aestivum*	TraesCS4B02G258000.3	1020	Ensembl Plant
3	*Triticum aestivum*	TraesCS4D02G257900.1	1020	Ensembl Plant
4	*Oryza sativa*	LOC_Os03g10640.1	1019	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi1g70920.1	1020	ARAMEMNON/MIPS
6	*Triticum urartu*	M7ZNL4_TRIUA	1020	UniProtKB
7	*Oryza brachyantha*	J3LL50_ORYBR	1031	UniProtKB
8	*Oryza barthii*	A0A0D3FF52_9ORYZ	1019	UniProtKB
9	*Zea mays*	GRMZM2G006977.01	1020	ARAMEMNON/Gramene
10	*Sorghum bicolor*	C5WTS5_SORBI		UniProtKB
		ACA2		
1	*Triticum aestivum*	TraesCS5A02G068700.1	1020	Ensembl Plant
2	*Triticum aestivum*	TraesCS5B02G075100.2	1020	Ensembl Plant
3	*Triticum aestivum*	TraesCS5D02G080700.1	1020	Ensembl Plant
4	*Oryza sativa*	LOC_Os12g39660.1	1020	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi4g03130.1	1019	ARAMEMNON/MIPS
6	*Triticum urartu*	M8A7X8_TRIUA	946	UniProtKB
7	*Oryza brachyantha*	J3NEK8_ORYBR	1020	UniProtKB
8	*Oryza barthii*	A0A0D3HW73_9ORYZ	1020	UniProtKB
9	*Sorghum bicolor*	A0A1B6PDQ7_SORBI	1020	UniProtKB
10	*Zea mays*	GRMZM5G836886.01	539	ARAMEMNON/Gramene
		ACA3		
1	*Triticum aestivum*	TraesCS4A02G234700.1	1052	Ensembl Plant
2	*Triticum aestivum*	TraesCS4B02G080300.1	1052	Ensembl Plant
3	*Triticum aestivum*	TraesCS4D02G079200.1	1050	Ensembl Plant
4	*Oryza sativa*	LOC_Os03g42020.1	1033	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi1g14630.1	1020	ARAMEMNON/MIPS
6	*Triticum urartu*	M8AJX4_TRIUA	1536	UniProtKB

7	*Oryza brachyantha*	J3LQU0_ORYBR	986	UniProtKB
8	*Oryza barthii*	A0A0D3FLA5_9ORYZ	1033	UniProtKB
9	*Zea mays*	GRMZM2G352695.01	1034	ARAMEMNON/Gramene
10	*Zea mays*	GRMZM5G836886.01	539	ARAMEMNON/Gramene
ACA4				
1	*Triticum aestivum*	TraesCS5A02G136200.1	1036	Ensembl Plant
2	*Triticum aestivum*	TraesCS5B02G135200.1	1036	Ensembl Plant
3	*Triticum aestivum*	TraesCS5D02G142900.1	1036	Ensembl Plant
4	*Oryza sativa*	LOC_Os11g04460.1	1017	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi4g43300.1	1035	ARAMEMNON/MIPS
6	*Triticum urartu*	M7ZET5_TRIUA	998	UniProtKB
7	*Oryza barthii*	A0A0D3HI07_9ORYZ	985	UniProtKB
8	*Zea mays*	GRMZM2G104730.01	1379	ARAMEMNON/Gramene
9	*Sorghum bicolor*	C5Y458_SORBI		UniProtKB
ACA6				
1	*Oryza sativa*	LOC_Os01g71240.1	1043	ARAMEMNON/MSU
2	*Brachypodium distachyon*	Bradi2g60324.1	1051	ARAMEMNON/MIPS
3	*Triticum urartu*	M8AKD3_TRIUA	985	UniProtKB
4	*Oryza brachyantha*	J3L7P9 _ORYBR	1042	UniProtKB
5	*Oryza barthii*	A0A0D3EYG7_9ORYZ	1043	UniProtKB
6	*Zea mays*	GRMZM2G028812.01	1065	ARAMEMNON/Gramene
ACA7				
1	*Triticum aestivum*	TraesCS1A02G312300.1	1042	Ensembl Plant
2	*Triticum aestivum*	TraesCS1B02G324000.1	1042	Ensembl Plant
3	*Triticum aestivum*	TraesCS1D02G312500.2	1012	Ensembl Plant
4	*Oryza sativa*	LOC_Os05g41580.1	1073	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi2g21180.1	1041	ARAMEMNON/MIPS
6	*Triticum urartu*	M7YR54_TRIUA	992	UniProtKB
7	*Oryza brachyantha*	J3M8H2_ORYBR	1038	UniProtKB
8	*Oryza barthii*	A0A0D3G9C7_9ORYZ	1073	UniProtKB
9	*Zea mays*	GRMZM2G305159.01	1041	ARAMEMNON/Gramene
ACA8				
1	*Oryza sativa*	LOC_Os10g28240.1	1035	ARAMEMNON/MSU
2	*Oryza brachyantha*	J3N2P8_ORYBR	1049	UniProtKB

3	*Oryza barthii*	A0A0D3HDQ0_9ORYZ	1032	UniProtKB
4	*Zea mays*	GRMZM2G144420.01	1026	ARAMEMNON/Gramene
5	*Sorghum bicolor*	C5X1K4_SORBI	1012	UniProtKB
		ACA9		
1	*Triticum aestivum*	TraesCS7A02G254400.1	1083	Ensembl Plant
2	*Triticum aestivum*	TraesCS7B02G150600.1	1082	Ensembl Plant
3	*Triticum aestivum*	TraesCS7D02G252900.5	1083	Ensembl Plant
4	*Oryza sativa*	LOC_Os08g40530.1	605	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi3g40640.1	1094	ARAMEMNON/MIPS
6	*Triticum urartu*	M8AMH4_TRIUA	1525	UniProtKB
7	*Oryza brachyantha*	J3MUF6_ORYBR	1086	UniProtKB
8	*Oryza barthii*	A0A0D3H254_9ORYZ	1016	UniProtKB
9	*Zea mays*	GRMZM2G132712.02	657	ARAMEMNON/Gramene
10	*Sorghum bicolor*	C5YI87_SORBI	1087	UniProtKB
		ACA10		
1	*Triticum urartu*	M7YGM5_TRIUA	1050	UniProtKB
2	*Oryza brachyantha*	J3MYU8_ORYBR	1053	UniProtKB
3	*Zea mays*	GRMZM2G340139.01	1051	ARAMEMNON/Gramene
4	*Sorghum bicolor*	A0A1W0W5N4_SORBI	1004	UniProtKB
		ACA11		
1	*Triticum aestivum*	TraesCS2A02G432700.1	1081	Ensembl Plant
2	*Triticum aestivum*	TraesCS2B02G454000.2	1087	Ensembl Plant
3	*Triticum aestivum*	TraesCS2D02G430700.2	931	Ensembl Plant
4	*Oryza sativa*	LOC_Os04g51610.1	1088	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi5g20890.1	1082	ARAMEMNON/MIPS
6	*Triticum urartu*	M8ARW7_TRIUA	999	UniProtKB
7	*Oryza brachyantha*	J3M160_ORYBR	1084	UniProtKB
8	*Oryza barthii*	A0A0D3FZV8_9ORYZ	1013	UniProtKB

9	*Zea mays*	GRMZM2G391042.01	1090	ARAMEMNON/Gramene
10	*Sorghum bicolor*	C5YFI8_SORBI	1092	UniProtKB
ACA12				
1	*Triticum aestivum*	TraesCS1A02G332800.1	1024	Ensembl Plant
2	*Triticum aestivum*	TraesCS1B02G346400.1	1020	Ensembl Plant
3	*Triticum aestivum*	TraesCS1D02G335400.1	1020	Ensembl Plant
4	*Triticum urartu*	M7Z5D6_TRIUA	671	UniProtKB
5	*Brachypodium distachyon*	Bradi3g26890.1	1025	ARAMEMNON/MIPS
ACA13				
1	*Triticum aestivum*	TraesCS6A02G152700.4	917	Ensembl Plant
2	*Triticum aestivum*	TraesCS6B02G180700.3	1097	Ensembl Plant
3	*Triticum aestivum*	TraesCS6D02G142400.5	958	Ensembl Plant
4	*Oryza sativa*	LOC_Os02g08010.1	591	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi3g05697.1	1027	ARAMEMNON/MIPS
6	*Triticum urartu*	M7ZL44_TRIUA	1130	UniProtKB
7	*Oryza brachyantha*	J3LA39_ORYBR	1088	UniProtKB
8	*Oryza barthii*	A0A0D3F1F8_9ORYZ	1084	UniProtKB
9	*Zea mays*	GRMZM5G893864.01	400	ARAMEMNON/Gramene
10	*Sorghum bicolor*	A0A194YN34_SORBI	1091	UniProtKB
ECAs				
ECA1				
1	*Triticum aestivum*	TraesCS4A02G103000.2	1062	Ensembl Plant
2	*Triticum aestivum*	TraesCS4B02G201500.2	1062	Ensembl Plant
3	*Triticum aestivum*	TraesCS4D02G202200.2	1062	Ensembl Plant
4	*Oryza sativa*	LOC_Os03g17310.1	1062	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi1g66150.1	1062	ARAMEMNON/MIPS
6	*Triticum urartu*	M7ZDN1_TRIUA	382	UniProtKB
7	*Oryza brachyantha*	J3LML0_ORYBR	1010	UniProtKB
8	*Oryza barthii*	A0A0D3FGZ7_9ORYZ	1058	UniProtKB
9	*Zea mays*	GRMZM2G141704.01	372	ARAMEMNON/Gramene
10	*Sorghum bicolor*	C5WP97_SORBI	1061	UniProtKB
ECA2				
1	*Triticum aestivum*	TraesCS1A02G047000.1	1057	Ensembl Plant
2	*Triticum aestivum*	TraesCS1B02G060700.1	1057	Ensembl Plant
3	*Triticum aestivum*	TraesCS1D02G047900.1	1054	Ensembl Plant

4	*Oryza sativa*	LOC_Os05g02940.1	373	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi2g37570.1	1038	ARAMEMNON/MIPS
6	*Triticum urartu*	M8AS38_TRIUA	848	UniProtKB
7	*Oryza brachyantha*	J3M3F0_ORYBR	1057	UniProtKB
8	*Oryza barthii*	A0A0D3G2M7_9ORYZ	1055	UniProtKB
9	*Zea mays*	GRMZM2G056014.01	1052	ARAMEMNON/Gramene
10	*Sorghum bicolor*	Sb09g001850.1	1058	UniProtKB
		ECA3		
1	*Triticum aestivum*	TraesCS4A02G253500.1	1000	Ensembl Plant
2	*Triticum aestivum*	TraesCS4B02G061900.1	1000	Ensembl Plant
3	*Triticum aestivum*	TraesCS4D02G060900.1	1000	Ensembl Plant
4	*Oryza sativa*	LOC_Os03g52090.1	1217	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi1g09810.1	1002	ARAMEMNON/MIPS
6	*Oryza brachyantha*	J3LSI2_ORYBR	1000	UniProtKB
7	*Oryza barthii*	A0A0D3FNM9_9ORYZ	1078	UniProtKB
8	*Zea mays*	AC233878.1_FGP004	884	ARAMEMNON/Gramene
9	*Sorghum bicolor*	A0A1B6QIC1_SORBI	1000	UniProtKB

Appendix III. P3A-type ATPases

Sr.no.	Taxon	Accession Number	Sequence length	Database
		AHA1		
1	*Triticum aestivum*	TraesCS4A02G283100.2	959	Ensembl Plant
2	*Triticum aestivum*	TraesCS4B02G031000.1	922	Ensembl Plant
3	*Triticum aestivum*	TraesCS4D02G027900.1	957	Ensembl Plant
4	*Oryza sativa*	LOC_Os03g48310.1	956	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi1g12117.1	956	ARAMEMNON/MIPS
6	*Triticum urartu*	M8AFL2_TRIUA	1009	UniProtKB
7	*Oryza brachyantha*	J3LRQ1_ORYBR	956	UniProtKB
8	*Oryza barthii*	A0A0D3FMM5_9ORYZ	968	UniProtKB
9	*Zea mays*	GRMZM2G068259.01	1149	ARAMEMNON/Gramene
10	*Sorghum bicolor*	A0A1B6QIU0_SORBI	954	UniProtKB
		AHA2		
1	*Oryza sativa*	LOC_Os07g09340.1	957	ARAMEMNON/MSU
2	*Brachypodium distachyon*	Bradi1g54847.1	956	ARAMEMNON/MIPS
3	*Oryza brachyantha*	J3MJ55_ORYBR	957	UniProtKB
4	*Sorghum bicolor*	C5XBY1_SORBI	956	UniProtKB
5	*Zea mays*	GRMZM2G144821.01	949	ARAMEMNON/Gramene
		AHA3		
1	*Oryza sativa*	LOC_Os12g44150.1	956	ARAMEMNON/MSU
2	*Brachypodium distachyon*	Bradi4g00517.1	959	ARAMEMNON/MIPS
3	*Oryza brachyantha*	J3NFC5_ORYBR	1321	UniProtKB
4	*Oryza barthii*	A0A0D3HX72_9ORYZ	1020	UniProtKB
5	*Sorghum bicolor*	C5YT23_SORBI	956	UniProtKB
6	*Zea mays*	GRMZM2G035520.01	956	ARAMEMNON/Gramene
		AHA4		
1	*Oryza sativa*	LOC_Os05g25550.1	972	ARAMEMNON/MSU
2	*Zea mays*	AC209050.3_FGP001	924	ARAMEMNON/Gramene
3	*Sorghum bicolor*	C5Z6S3_SORBI	953	UniProtKB
		AHA5		
1	*Triticum aestivum*	TraesCS1A02G258300.1	957	Ensembl Plant
2	*Triticum aestivum*	TraesCS1B02G268800.1	958	Ensembl Plant
3	*Triticum aestivum*	TraesCS1D02G257600.1	957	Ensembl Plant

4	*Brachypodium distachyon*	Bradi3g18790.1	953	ARAMEMNON/MIPS
5	*Triticum urartu*	M7Z7W8_TRIUA	1039	UniProtKB
6	*Zea mays*	GRMZM2G008122.01	982	ARAMEMNON/Gramene
7	*Sorghum bicolor*	C5WZX7_SORBI	959	UniProtKB
AHA6				
1	*Triticum aestivum*	TraesCS6A02G360500.1	947	Ensembl Plant
2	*Triticum aestivum*	TraesCS6B02G393400.1	871	Ensembl Plant
3	*Triticum aestivum*	TraesCS6D02G344000.1	950	Ensembl Plant
4	*Oryza sativa*	LOC_Os02g55400.1	884	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi3g54177.1	950	ARAMEMNON/MIPS
6	*Zea mays*	GRMZM2G148374.01	951	ARAMEMNON/Gramene
7	*Sorghum bicolor*	C5XUH7_SORBI	951	UniProtKB
AHA7				
1	*Triticum aestivum*	TraesCS2A02G502400.1	951	Ensembl Plant
2	*Triticum aestivum*	TraesCS2B02G530500.1	951	Ensembl Plant
3	*Triticum aestivum*	TraesCS2D02G503000.1	951	Ensembl Plant
4	*Oryza sativa*	LOC_Os04g56160.1	951	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi5g24690.1	951	ARAMEMNON/MIPS
6	*Triticum urartu*	M8AIK4_TRIUA	953	UniProtKB
7	*Oryza brachyantha*	J3M272_ORYBR	951	UniProtKB
8	*Oryza barthii*	A0A0D3G139_9ORYZ	951	UniProtKB
9	*Zea mays*	GRMZM2G019404.01	951	ARAMEMNON/Gramene
10	*Sorghum bicolor*	C5Y9I0_SORBI	951	UniProtKB
AHA8				
1	*Oryza sativa*	LOC_Os03g01120.1	970	ARAMEMNON/MSU
2	*Brachypodium distachyon*	Bradi1g78577.1	976	ARAMEMNON/MIPS
3	*Triticum urartu*	M7Z624_TRIUA	897	UniProtKB
4	*Zea mays*	GRMZM2G172183.01	543	ARAMEMNON/Gramene
5	*Sorghum bicolor*	A0A1Z5SCI3_SORBI	993	UniProtKB
AHA9				
1	*Triticum aestivum*	TraesCS4A02G005000.2	933	Ensembl Plant
2	*Triticum aestivum*	TraesCS4B02G300000.2	892	Ensembl Plant
3	*Triticum aestivum*	TraesCS4D02G298900.1	945	Ensembl Plant

4	*Oryza sativa*	LOC_Os03g08560.1	956	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi1g72417.1	973	ARAMEMNON/MIPS
6	*Oryza brachyantha*	J3LKP7_ORYBR	955	UniProtKB
AHA10				
1	*Triticum aestivum*	TraesCS7A02G145400.1	946	Ensembl Plant
2	*Triticum aestivum*	TraesCS7B02G047600.1	946	Ensembl Plant
3	*Triticum aestivum*	TraesCS7D02G146700.1	946	Ensembl Plant
4	*Oryza sativa*	LOC_Os06g08310.1	869	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi1g47550.1	946	ARAMEMNON/MIPS
6	*Triticum urartu*	M7ZAA6_TRIUA	538	UniProtKB
7	*Zea mays*	GRMZM2G455557.01	857	ARAMEMNON/Gramene
8	*Sorghum bicolor*	A0A1W0VRQ8_SORBI	857	UniProtKB

Appendix IV. P4-type ATPases

Sr.no.	Taxon	Accession Number	Sequence length	Database
Catalytic Component of Phospholipid Flippase Complex (*ALAs*)				
ALA1				
1	*Oryza sativa*	LOC_Os03g20970.1	715	ARAMEMNON/MSU
2	*Brachypodium distachyon*	Bradi1g63660.1	671*	ARAMEMNON/MIPS
3	*Triticum urartu*	M8AGF3_TRIUA	640	UniProtKB
4	*Oryza barthii*	A0A0D3FHZ3_9ORYZ	688	UniProtKB
5	*Zea mays*	GRMZM2G107481.01	728	ARAMEMNON/Gramene
6	*Sorghum bicolor*	A0A1Z5SA16_SORBI	1228	UniProtKB
ALA2				
1	*Triticum aestivum*	TraesCS3A02G189700.1	1113	Ensembl Plant
2	*Triticum aestivum*	TraesCS3B02G218800.1	1162	Ensembl Plant
3	*Triticum aestivum*	TraesCS3D02G193200.1	1163	Ensembl Plant
4	*Oryza sativa*	LOC_Os01g17010.1	1175	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi2g10650.1	1169	ARAMEMNON/MIPS
6	*Triticum urartu*	M8A9F8_TRIUA	233	UniProtKB
7	*Oryza brachyantha*	J3KYS7_ORYBR	1176	UniProtKB
8	*Oryza barthii*	A0A0D3EMC6_9ORYZ	1141	UniProtKB
9	*Sorghum bicolor*	C5XH97_SORBI	1180	UniProtKB
10	*Zea mays*	GRMZM5G840699.01	1178	ARAMEMNON/Gramene
ALA4				
1	*Oryza sativa*	LOC_Os05g01030.1	1189	ARAMEMNON/MSU
2	*Brachypodium distachyon*	Bradi2g40060.1	1216	ARAMEMNON/MIPS
3	*Triticum urartu*	M7Z862_TRIUA	1102	UniProtKB
4	*Oryza brachyantha*	J3M332_ORYBR	1180	UniProtKB
ALA5				
1	*Triticum aestivum*	TraesCS7A02G231600.2	1205	Ensembl Plant
2	*Triticum aestivum*	TraesCS7B02G130000.1	1203	Ensembl Plant
3	*Triticum aestivum*	TraesCS7D02G231800.4	1025	Ensembl Plant
4	*Oryza sativa*	LOC_Os06g29380.1	1207	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi1g42310.1	1203	ARAMEMNON/MIPS

6	*Triticum urartu*	M7Z4F5 TRIUA	1302	UniProtKB
7	*Oryza brachyantha*	J3MEA0_ORYBR	1209	UniProtKB
8	*Oryza barthii*	A0A0D3GGV0_9ORYZ	1207	UniProtKB
9	*Zea mays*	GRMZM2G324462.01	1201	ARAMEMNON/Gramene
ALA6				
1	*Oryza sativa*	LOC_Os08g29150.1	1171	ARAMEMNON/MSU
2	*Brachypodium distachyon*	Bradi3g35000.1	1150	ARAMEMNON/MIPS
3	*Oryza brachyantha*	J3MSN6_ORYBR	1032	UniProtKB
4	*Oryza barthii*	A0A0D3GZQ6_9ORYZ	1123	UniProtKB
5	*Zea mays*	GRMZM2G127396.01	403	ARAMEMNON/Gramene
ALA8				
1	*Triticum aestivum*	TraesCS4A02G130300.1	1122	Ensembl Plant
2	*Triticum aestivum*	TraesCS4B02G174300.2	833	Ensembl Plant
3	*Triticum aestivum*	TraesCS4D02G176300.2	1020	Ensembl Plant
4	*Oryza sativa*	LOC_Os03g20949.1	1124	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi1g63650.1	1124	ARAMEMNON/MIPS
6	*Triticum urartu*	M8ASG3_TRIUA	1021	UniProtKB
7	*Oryza brachyantha*	J3LND5_ORYBR	1114	UniProtKB
8	*Oryza barthii*	A0A0D3FHZ1_9ORYZ	1124	UniProtKB
9	*Zea mays*	GRMZM2G407825.01	1122	ARAMEMNON/Gramene
10	*Sorghum bicolor*	A0A1Z5S9S3_SORBI	1122	UniProtKB
ALA9				
1	*Triticum aestivum*	TraesCS2A02G203300.3	912	Ensembl Plant
2	*Triticum aestivum*	TraesCS2B02G230600.4	1105	Ensembl Plant
3	*Triticum aestivum*	TraesCS2D02G212400.2	1105	Ensembl Plant
4	*Oryza sativa*	LOC_Os11g25980.1	1107	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi1g24630.1	1103	ARAMEMNON/MIPS
6	*Triticum urartu*	M7ZIA9_TRIUA	1134	UniProtKB
7	*Oryza brachyantha*	J3N836_ORYBR	1105	UniProtKB
8	*Oryza barthii*	A0A0D3HLG1_9ORYZ	1026	UniProtKB
9	*Zea mays*	GRMZM2G411916.01	412	ARAMEMNON/Gramene
10	*Sorghum bicolor*	A0A1B6QF90_SORBI	1103	UniProtKB

ALA10				
1	*Oryza sativa*	LOC_Os04g28460.1	803	ARAMEMNON/MSU
2	*Brachypodium distachyon*	Bradi5g06357.1	1097	ARAMEMNON/MIPS
3	*Triticum urartu*	M8A2A2_TRIUA	362	UniProtKB
4	*Oryza brachyantha*	J3LWZ6_ORYBR	1074	UniProtKB
5	*Oryza barthii*	A0A0D3FUJ3_9ORYZ	897	UniProtKB
6	*Zea mays*	GRMZM2G037335.01	527	ARAMEMNON/Gramene
7	*Sorghum bicolor*	A0A1Z5RD56_SORBI	1080	UniProtKB
Regulatory Component of Phospholipid Flippase Complex (ALIS)				
ALIS1				
1	*Oryza sativa*	LOC_Os02g07750.1	350	ARAMEMNON/MSU
2	*Brachypodium distachyon*	Bradi3g05450.1	349	ARAMEMNON/MIPS
3	*Triticum urartu*	M7Z9H9_TRIUA	449	UniProtKB
4	*Oryza brachyantha*	J3LA14_ORYBR	350	UniProtKB
5	*Oryza barthii*	A0A0D3F1D4_9ORYZ	334	UniProtKB
6	*Zea mays*	GRMZM2G180211.01	349	ARAMEMNON/Gramene
7	*Sorghum bicolor*	C5XW62_SORBI	349	UniProtKB
ALIS2				
1	*Triticum aestivum*	TraesCS5A02G423800.1	344	Ensembl Plant
2	*Triticum aestivum*	TraesCS5B02G426100.1	344	Ensembl Plant
3	*Triticum aestivum*	TraesCS5D02G432300.1	344	Ensembl Plant
4	*Oryza sativa*	LOC_Os03g57170.1	351	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi1g06470.1	346	ARAMEMNON/MIPS
6	*Triticum urartu*	M7YF52_TRIUA	291	UniProtKB
7	*Oryza brachyantha*	J3LTH0_ORYBR	353	UniProtKB
8	*Oryza barthii*	A0A0D3FPX2_9ORYZ	351	UniProtKB
9	*Zea mays*	GRMZM2G039906.01	306	ARAMEMNON/Gramene
10	*Sorghum bicolor*	A0A1B6QHN8_SORBI	345	UniProtKB
ALIS3				
1	*Triticum aestivum*	TraesCS1A02G350000.3	340	Ensembl Plant
2	*Triticum aestivum*	TraesCS1B02G364300.1	340	Ensembl Plant
3	*Triticum aestivum*	TraesCS1D02G352700.1	340	Ensembl Plant

4	*Oryza sativa*	LOC_Os05g45370.1	341	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi2g18997.1	342	ARAMEMNON/MIPS
6	*Triticum urartu*	M7ZL38_TRIUA	466	UniProtKB
7	*Oryza brachyantha*	J3M945_ORYBR	389	UniProtKB
8	*Oryza barthii*	A0A0D3GA26_9ORYZ	341	UniProtKB
9	*Zea mays*	GRMZM2G029716.01	339	ARAMEMNON/Gramene
ALIS4				
1	*Triticum aestivum*	TraesCS7A02G482200.1	351	Ensembl Plant
2	*Triticum aestivum*	TraesCS7B02G384400.1	351	Ensembl Plant
3	*Triticum aestivum*	TraesCS7D02G469100.1	353	Ensembl Plant
4	*Oryza sativa*	LOC_Os06g45430.1	358	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi1g31850.1	353	ARAMEMNON/MIPS
6	*Triticum urartu*	M7ZXF1_TRIUA	341	UniProtKB
7	*Oryza brachyantha*	J3MGM2_ORYBR	371	UniProtKB
8	*Oryza barthii*	A0A0D3GJN1_9ORYZ	358	UniProtKB
9	*Zea mays*	GRMZM2G090849.01	348	ARAMEMNON/Gramene
10	*Sorghum bicolor*	C5Z7M2_SORBI	352	UniProtKB
ALIS5				
1	*Triticum aestivum*	TraesCS5A02G380500.1	359	Ensembl Plant
2	*Triticum aestivum*	TraesCS5B02G384100.1	370	Ensembl Plant
3	*Triticum aestivum*	TraesCS5D02G390300.2	360	Ensembl Plant
4	*Oryza sativa*	LOC_Os09g38768.1	351	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi4g37980.1	379	ARAMEMNON/MIPS
6	*Triticum urartu*	M7Z993_TRIUA	317	UniProtKB
7	*Oryza brachyantha*	J3N041_ORYBR	349	UniProtKB
8	*Zea mays*	GRMZM2G114704.02	385	ARAMEMNON/Gramene

Note: *Partial length sequence

Appendix V. P5A-type ATPases

Sr.no.	Taxon	Accession Number	Sequence length	Database
P5A				
1	*Triticum aestivum*	TraesCS1A02G251700.4	1174	Ensembl Plant
2	*Triticum aestivum*	TraesCS1B02G262600.3	1174	Ensembl Plant
3	*Triticum aestivum*	TraesCS1D02G251500.2	1098	Ensembl Plant
4	*Oryza sativa*	LOC_Os05g33390.1	1203	ARAMEMNON/MSU
5	*Brachypodium distachyon*	Bradi2g25857.1	1174	ARAMEMNON/MIPS
6	*Triticum urartu*	M7YJJ2_TRIUA	1119	UniProtKB
7	*Oryza brachyantha*	J3M703_ORYBR	1174	UniProtKB
8	*Oryza barthii*	OBART05G15810.1	1384	Ensembl Plant
9	*Zea mays*	GRMZM2G060824.01	1171	ARAMEMNON/Gramene
10	*Sorghum bicolor*	A0A1B6P8S6_SORBI	1165	UniProtKB

Appendix VI. *Triticum aestivum* P-type ATPases Subcellular Localization Table

Sr. no.	Accession No.	Length (Amino acids)	No. of Exons	Mass of amino acid sequence (Da)	Cellular Localization (Plant mPLoc)
P1B-type ATPases					
HMA1					
1.	TraesCS7A02G439100.1	828	13	88,131	Chloroplast
	TraesCS7B02G337700.1	402	8	42,548	Chloroplast
	TraesCS7D02G428700.1	826	13	84,435	Chloroplast
HMA2					
2.	TraesCS7A02G419500.1	985	3	106,456	Cell membrane, Nucleus
	TraesCS7B02G320100.4	876	1	94,774	Cell membrane, Nucleus
	TraesCS7D02G412400.3	882	1	96,103	Cell membrane, Nucleus
HMA3					
3.	TraesCS5A02G383400.1	816	6	85,658	Cell membrane
	TraesCS5B02G388000.1	829	6	86,817	Cell membrane
	TraesCS5D02G392700.1	853	6	89,091	Cell membrane
HMA4					
4.	TraesCS6A02G158900.1	974	8	105,106	Cell membrane
	TraesCS6B02G192400.1	980	8	105,627	Cell membrane
	TraesCS6D02G153600.1	996	8	107,410	Cell membrane
	HMA5				
5.	TraesCS2A02G410400.3	1011	5	109,106	Cell membrane
	TraesCS2B02G429200.2	994	5	107,491	Cell membrane
	TraesCS2D02G407800.5	1000	5	108,175	Cell membrane
HMA6					
6.	TraesCS6A02G156900.1	997	9	106,854	Cell membrane
	TraesCS6B02G184800.1	972	10	104,694	Cell membrane
	TraesCS6D02G146500.1	998	9	107,150	Cell membrane
HMA7					
7.	TraesCS7A02G239700.1	803	16	85,188	Cell membrane
	TraesCS7B02G135300.1	952	17	100,020	Cell membrane
	TraesCS7D02G237500.1	952	17	99,938	Cell membrane

HMA8					
8.	TraesCS4A02G010800.1	890	17	93,266	Cell membrane
	TraesCS4B02G293600.1	618	13	64,812	Cell membrane
	TraesCS4D02G292300.1	607	13	63,605	Cell membrane
P2A-type ATPases					
ECA1					
9.	TraesCS4A02G103000.2	1062	8	115,769	Endoplasmic reticulum
	TraesCS4B02G201500.2	1062	8	115,812	Endoplasmic reticulum
	TraesCS4D02G202200.2	1062	8	115,769	Endoplasmic reticulum
ECA2					
10.	TraesCS1A02G047000.1	1057	8	114,338	Endoplasmic reticulum
	TraesCS1B02G060700.1	1057	8	114,402	Endoplasmic reticulum
	TraesCS1D02G047900.1	1054	8	114,146	Endoplasmic reticulum
ECA3					
11.	TraesCS4A02G253500.1	1000	34	109,621	Cell membrane
	TraesCS4B02G061900.1	1000	34	109,562	Cell membrane
	TraesCS4D02G060900.1	1000	34	109,558	Cell membrane
P2B-type ATPases					
ACA1					
12.	TraesCS4A02G046800.3	1020	7	110,254	Chloroplast, Endoplasmic reticulum, Vacuole
	TraesCS4B02G258000.3	1020	7	110,239	Chloroplast, Endoplasmic reticulum, Vacuole
	TraesCS4D02G257900.1	1020	7	110,257	Chloroplast, Endoplasmic reticulum, Vacuole

ACA3					
14.	TraesCS4A02G234700.1	1052	7	113,563	Chloroplast, Endoplasmic reticulum
	TraesCS4B02G080300.1	1052	7	113,413	Chloroplast, Endoplasmic reticulum
	TraesCS4D02G079200.1	1050	7	113,343	Chloroplast, Endoplasmic reticulum
ACA4					
15.	TraesCS5A02G136200.1	1036	7	113,303	Chloroplast, Endoplasmic reticulum, Vacuole
	TraesCS5B02G135200.1	1036	7	113,228	Chloroplast, Endoplasmic reticulum, Vacuole
	TraesCS5D02G142900.1	1036	7	113,170	Chloroplast, Endoplasmic reticulum, Vacuole
ACA7					
16.	TraesCS1A02G312300.1	1042	7	114,411	Chloroplast, Vacuole
	TraesCS1B02G324000.1	1042	7	114,439	Chloroplast, Vacuole
	TraesCS1D02G312500.2	1012	5	114,416	Chloroplast, Vacuole
ACA9					
17.	TraesCS7A02G254400.1	1083	33	119,144	Cell membrane
	TraesCS7B02G150600.1	1082	33	118,988	Cell membrane
	TraesCS7D02G252900.5	1083	33	119,009	Cell membrane
ACA11					
18.	TraesCS2A02G432700.1	1081	34	117,380	Cell membrane
	TraesCS2B02G454000.2	1087	34	117,801	Cell membrane
	TraesCS2D02G430700.2	931	30	101,205	Cell membrane

ACA12					
19.	TraesCS1A02G332800.1	1024	1	110,237	Cell membrane
	TraesCS1B02G346400.1	1020	1	109,937	Cell membrane
	TraesCS1D02G335400.1	1020	1	109,900	Cell membrane
ACA13					
20.	TraesCS6A02G152700.4	917	30	99,754	Cell membrane
	TraesCS6B02G180700.3	1097	34	119,791	Cell membrane
	TraesCS6D02G142400.5	958	31	104,306	Cell membrane
P3A-type ATPases					
AHA1					
21.	TraesCS4A02G283100.2	959	21	105,482	Cell membrane
	TraesCS4B02G031000.1	922	21	101,150	Cell membrane
	TraesCS4D02G027900.1	957	21	105,415	Cell membrane
AHA5					
22.	TraesCS1A02G258300.1	957	9	104,926	Cell membrane
	TraesCS1B02G268800.1	958	9	105,029	Cell membrane
	TraesCS1D02G257600.1	957	9	104,890	Cell membrane
AHA6					
23.	TraesCS6A02G360500.1	947	3	103,932	Cell membrane
	TraesCS6B02G393400.1	871	2	95,412	Cell membrane
	TraesCS6D02G344000.1	950	3	104,192	Cell membrane
AHA7					
24	TraesCS2A02G502400.1	951	15	104,661	Cell membrane
	TraesCS2B02G530500.1	951	15	104,734	Cell membrane
	TraesCS2D02G503000.1	951	15	104,734	Cell membrane
AHA9					
25.	TraesCS4A02G005000.2	933	18	100,825	Cell membrane
	TraesCS4B02G300000.2	892	17	96,055	Cell membrane
	TraesCS4D02G298900.1	945	18	102,444	Cell membrane
AHA10					
26.	TraesCS7A02G145400.1	946	3	103,644	Cell membrane
	TraesCS7B02G047600.1	946	3	103,731	Cell membrane
	TraesCS7D02G146700.1	946	3	103,548	Cell membrane

P4-type ATPases (Catalytic Component)					
ALA2					
27.	TraesCS3A02G189700.1	1113	9	123,777	Cell membrane
	TraesCS3B02G218800.1	1162	7	129,510	Cell membrane
	TraesCS3D02G193200.1	1163	7	129,517	Cell membrane
ALA5					
28.	TraesCS7A02G231600.2	1205	12	136,485	Cell membrane
	TraesCS7B02G130000.1	1203	11	136,354	Cell membrane
	TraesCS7D02G231800.4	1025	11	116,161	Cell membrane
ALA8					
29.	TraesCS4A02G130300.1	1122	7	125,080	Cell membrane
	TraesCS4B02G174300.2	833	5	92,422	Cell membrane
	TraesCS4D02G176300.2	1020	8	113,766	Cell membrane
ALA9					
30.	TraesCS2A02G203300.3	912	22	102,909	Cell membrane
	TraesCS2B02G230600.4	1105	24	124,515	Cell membrane
	TraesCS2D02G212400.2	1105	24	124,567	Cell membrane
P4-type ATPases (Regulatory Component)					
ALIS2					
31.	TraesCS5A02G423800.1	344	8	38,790	Cell membrane
	TraesCS5B02G426100.1	344	8	38,837	Cell membrane, Cell wall, Nucleus
	TraesCS5D02G432300.1	344	8	38,790	Cell membrane, Nucleus
ALIS3					
32.	TraesCS1A02G350000.3	340	7	37,154	Cell membrane
	TraesCS1B02G364300.1	340	7	37,327	Cell membrane
	TraesCS1D02G352700.1	340	7	37,196	Cell membrane
ALIS4					
33.	TraesCS7A02G482200.1	351	8	39,636	Cell membrane, Cell wall
	TraesCS7B02G384400.1	351	8	39,594	Cell membrane, Cell wall

	TraesCS7D02G469100.1	353	8	39,890	Cell membrane, Cell wall, Nucleus
ALIS5					
34.	TraesCS5A02G380500.1	359	8	39,548	Cell membrane
	TraesCS5B02G384100.1	370	8	40,375	Cell membrane
	TraesCS5D02G390300.2	360	8	39,730	Cell membrane
P5A-type ATPase					
35.	TraesCS1A02G251700.4	1174	21	131,454	Cell membrane
	TraesCS1B02G262600.3	1174	21	131,436	Cell membrane
	TraesCS1D02G251500.2	1098	22	122,355	Cell membrane