



Comprehensive *In-Silico* Study Of PLAT Domain Containing Protein 1 Upregulated From Salinity Stress In *Oryza Sativa*

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	Abstract Salinity stress is one of the most detrimental causes of productivity loss and death among plants including crops. Worldwide, rice is the most common staple food and also, most commonly under stressed crop. Plant protein with single PLAT (Polycystin-1, Lipoxygenase, Alpha-toxin and Triacylglycerol lipase) domain and PLAT plant stress protein family are found in most angiosperms. The exact mechanism of PLAT plant protein isn't assuredly understood yet available reports suggest, overexpressed PLAT1 protein enhances abiotic stress, especially temperature and salt tolerance along with heavy metal stress tolerance in plants. In <i>Arabidopsis</i> and tobacco plant, PLAT1 appeared to be a positive mediator for growth under non-stressed condition, ABA signalling and ER stress tolerance. PLAT1 is also present in <i>Oryza sativa</i> both in <i>indica</i> and <i>japonica</i> group. Most of the proteins were predicted, hence lacking proper PDB structure. So, this study intended to find the possible structural resemblance between PLAT1 both in <i>Oryza</i> and <i>Arabidopsis</i> . Possibly to understand the nature of mechanism by building homology models by using SWISSMODEL and comparing sequences by MSA. Thus, encouraging further scientific use and proteomic study in order to improve salinity stress tolerance and crop yielding. Keywords: <i>Arabidopsis</i> , <i>Homology modelling</i> , <i>Multiple Sequence alignment</i> , <i>Plant stress protein</i> , <i>PLAT1</i>
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1. Introduction

Among all the cereals, rice is considered the most salt sensitive, therefore called as glycophytes [1, 2]. The early seedling stage and later reproductive stages are majorly susceptible to salinity bringing about the loss of yield, even premature mortality [1, 3-5]. Osmotic and ionic stress due to salinity stress in plants, invoke different types of adaptogenic responses to the very extend of molecular, cellular, metabolic and physiological core as withstanding mechanism [6-7]. Antioxidant metabolism activation, protein modifications, increase in energy and biomolecule metabolism are among distinct countereffects of the salinity stress [4,6,8-9]. Among these responses, effects of proteins are the most enigmatic so far considered. Proteins with particular domain are the interesting players in this field. PLAT is considered among one of them. The PLAT (Polycystin-1, Lipoxygenase, Alpha-toxin and Triacylglycerol lipase) is an intercellular domain usually forms a β -sandwich having four strands and each composed of two sheets. PLAT resides not only in various types of membrane or lipid associated multi domain proteins, but in proteins bearing either a single PLAT domain or repeat [8,10-13].

Because of its similarity to the C2 domain which is involved actively in Ca^{2+} signalling and having several stress response elements towards biotic and abiotic stress, the PLAT domain was suggested to function in protein-protein interactions in addition to protein-membrane interactions [8,10-11,13]. PLAT domain proteins have some experimental data but are available only in the animal field, as these proteins from plants are under-researched. Although, according to the available reports, genes encoding PLAT domain proteins have been isolated from several plant species [9,13-17]. Intriguingly, one of these studies advocates that proteins, belong to the PLAT-plant-stress protein family (Conserved Domain cd1754) are ubiquitously present in angiosperms, bearing a single PLAT domain. This indicates that PLAT-plant stress proteins in general could promote tolerance towards stress responses, despite no available data from functional studies in homologous systems. PLAT domain further differentiated into PLAT-1, PLAT- 2 and PLAT-3. It is reported that in *Arabidopsis*, PLAT -1 is expressed under abiotic stress condition such in salt and cold; whereas PLAT-2 is also expressed in moderated salinity stress but usually expressed in younger seedlings. Studies suggest no evidence of PLAT-3 is associated with abiotic stress induction.

Recent, studies also suggest that PLAT plant stress and a plant dehydrin family (DHN) protein, both are involved in stress tolerance and showed hiked amount under salinity treatment as well as during heavy metal stress [23].

2. Methodology

2.1 Sequence extraction and alignment

In-silico assessment of PLAT plant proteins related to abiotic stress, especially related to salinity and temperature stress, were obtained from various literature study [10]. The available data in FASTA format retrieved from Uniprot -KB/SWISSPROT (<https://www.uniprot.org/>) has been used for sequence study. Further a BLAST database was created considering the homology study of the following protein sequence. Multiple sequence alignment (MSA) was done using CLUSTER-OMEGA version 1.2.4 (<https://www.ebi.ac.uk/Tools/msa/clustalo>) has been used for the concerned sequences.

2.2 Homology modelling

The 3D structure of PLAT-1protein wasn't available in PDB format as usually they were predicted, so homology models of this protein for both *Arabidopsis* and *Oryza sativa* has been built using SWISS MODEL (<https://swissmodel.expasy.org/>) tool. The superimposition between the proteins, ie; the template PDB structure and the newly built protein structures, was checked by using SUPERPOSE(<http://superpose.wishartlab.com>) [19] server.

3. Results and Discussion:

3.1 Sequence Analysis

The sequence assay of the concerned PLAT domain containing protein -1 present both in *Arabidopsis thaliana* (accession no. AT4G39730) and *Oryza sativa* (NCBI accession no. XP_015634852.1) showed better similarities, The obtained MSA result showed possible similar homology for PLAT-1 of both *Arabidopsis thaliana* and *Oryza sativa* (Fig 1).

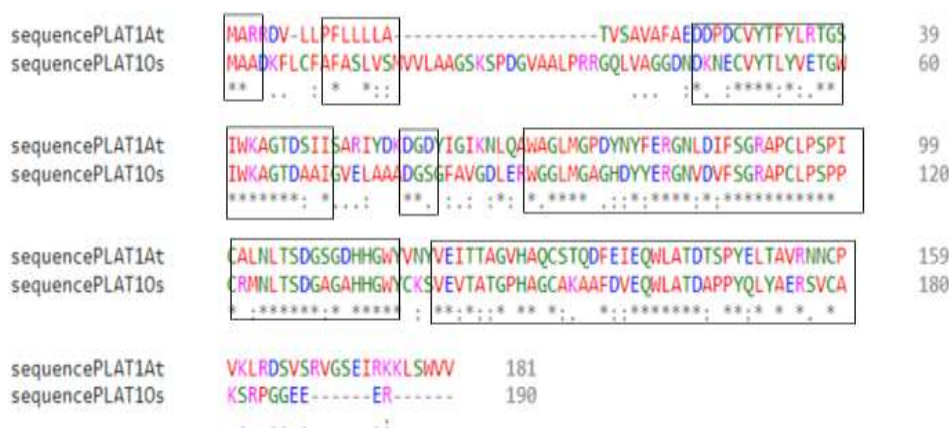


Fig 1. MSA of PLAT 1 of *Arabidopsis thaliana* (seq PLAT1At) and *Oryza sativa* (seq PLAT1Os), the conserved domain sequences are shown within the boxes

Another multiple sequence alignment (Fig 2) was obtained using the PLAT-1 protein of *Oryza sativa* and other PLAT -1 domain containing proteins found in *japonica* group, although there are similar kind of proteins can be found in *indica* group but most of them are hypothetical in nature.

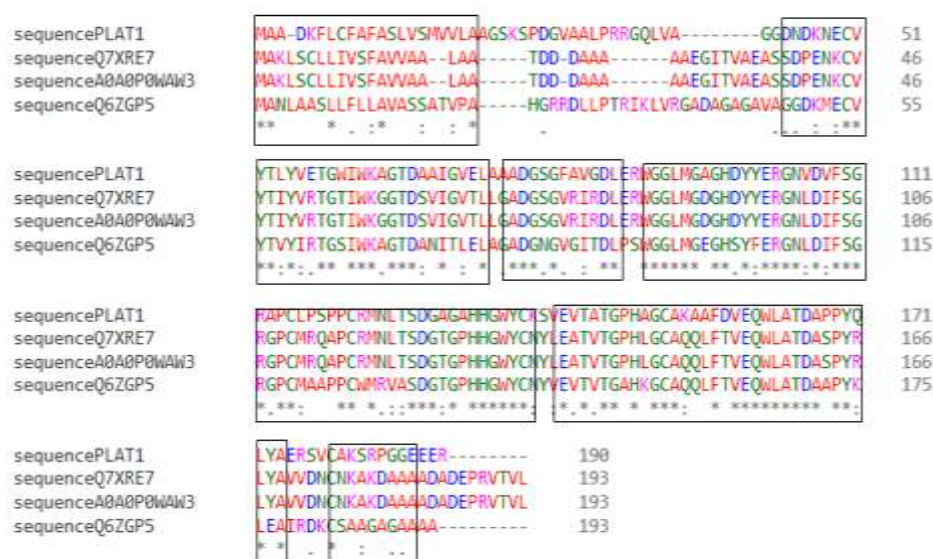


Fig 2. MSA of PLAT 1 of *Oryza sativa* (seqPLAT1) and PLAT domain containing proteins of *japonica* (seqQ7XRE7, A0A0P0WAW3, Q6ZGP5); the conserved domain sequences are shown within the boxes

3.2 Protein three-dimensional Structure

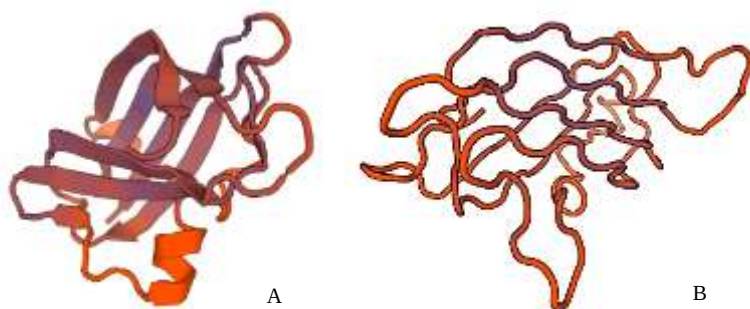


Fig 3: Three-dimensional structure of PLAT 1 of *Arabidopsis* (A) & *Oryza* (B)

Due to lack of proper PDB structure of PLAT 1 in plants specially of *Arabidopsis* and *Oryza*, three dimensional structures were built using Swiss model server. The newly built homology model for PLAT 1 of *Arabidopsis* (Fig 3A) showed the query coverage about 87%, with 28.16% sequence identity between the target and template. The best template was found from allene oxide synthase-lipoxygenase protein (pdb id 4qwt.1. A). Validation of the model was done by using SAVES (<https://saves.mbi.ucla.edu/>) [20, 21]. The validation result showed 100% of the residues owning average 3D-1D score was ≥ 0.2 , a sign of good quality model. Ramachandran plot and Errat value 89.57% indicating a good model.

The newly built homology model for PLAT 1 of *Oryza* (Fig 3B) showed the query coverage of 87%, with 26.42% sequence identity between the target and template. The best template was found from allene oxide synthase-lipoxygenase protein (pdb id 2fgq.1.A).

Likewise the PLAT 1 of *Arabidopsis* model, this model of PLAT 1 of *Oryza* showed a validation result of 100% of residues having average 3D-1D score was ≥ 0.2 , indicating a good quality model. The Ramachandran plot and Errat value 64.588, also supported the goodness of the model.

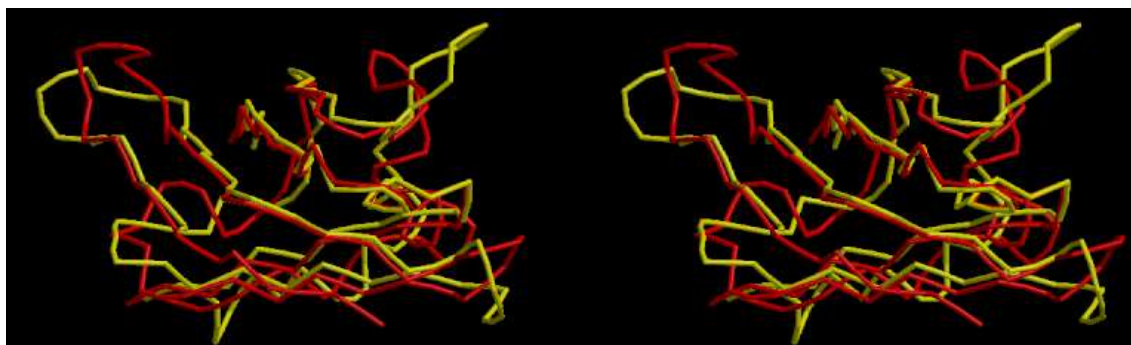


Fig 4: Superimposed three-dimensional structure of PLAT 1 of *Arabidopsis* (red) & *Oryza* (yellow)

The structural comparison of PLAT-1 between *Arabidopsis* and *Oryza* in superimposed condition revealed slightly higher RMSD value *i.e*; 3.78Å displaying overall good imposed similarity (Fig 4).

The superimposition confirmed the structural similarities of PLAT-1 between the two genera, might be indicating similar working mechanism against the salinity stress. The BLAST result of PLAT-1 of *Oryza* have given several related PLAT domains containing proteins in *Oryza*, while in the *indica* group all were either predicted without evidences or hypothetical. In *japonica* group four consecutive proteins were selected having PLAT-1 domain. The accession no. and details (Table 1.) are given below:

Table 1. List of the proteins along with their accession number

Accession No.	Protein Name	Organism	Amino acid Seq. Length
A0A0P0WAW3	Os04g0456300 protein	<i>Oryza sativa</i> subsp. <i>japonica</i>	97
Q6ZGP5	Os02g0753300 protein	<i>Oryza sativa</i> subsp. <i>japonica</i>	193
Q7XRE7	Os04g0456700 protein	<i>Oryza sativa</i> subsp. <i>japonica</i>	193



Fig 5: Three-dimensional structure of PLAT domain containing proteins of *Oryza japonica* group: Q7XRE7 (5A); A0A0P0WAW3 (5B) & Q6ZGP5 (5C)

Three dimensional structures of all these four proteins are built using Swiss model server and validated with SAVES server. The three obtained model validation turned out ideal with 84-97% of residues having 3D-1D score ≥ 0.2 .

4. Conclusion

This study showed the sequence similarities in PLAT -1of *Arabidopsis* and *Oryza* indicating possible similar work mechanism towards salinity stress. The newly built models might open the future prospects regarding these proteins. During the homology model building, PLAT-1 both in *Arabidopsis* and *Oryza* showed more or less good shared similarities with the allene oxide synthase-lipoxygenase (AOS) protein. Reported studies show, AOS acts actively towards JA biosynthesis and biotic and abiotic stresses usually by forming LOX2-AOS-AOC2 complex [22]. This can also lead to a question whether AOS and PLAT can work as a team in plant stress defense.

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6. References

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