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In- silico Structural & Molecular characterization of Pectin methylesterase from *Arabidopsis thaliana*

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Abstract
Pectin methylesterase (PME) is a ubiquitous cellwall- associated enzyme present in plants & certain micro-organisms. PME is a hydrolytic enzyme that catalyzes demethylesterification of the pectin homogalacturonan which present in the plant cell wall producing pectate and methanol. It has been proved that PME enzyme plays important role in seed germination of *Arabidopsis thaliana* allowing water uptake. The present *in-silico* study includes retrieval of sequence of PME of *A. thaliana* & its analysis followed by prediction of its domain, secondary structure & 3D structure which is further validated by PROCHECK analysis using various *Insilco* tools & servers. The results showed that the PME is alkaline, cationic enzyme having two domains PMEI & pectin esterase. In the secondary structure, it contains more % of random coils than helices & sheets & the most favorable region of predicted 3D structure is 86.1%. These findings will put insight into its functional role in seed germination which will be helpful to find the natural inhibitor & also to design the novel inhibitor to stop the seed germination process.

Keywords: *In-silico*, pectin methylesterase, de-methylesterification, domain, Homology modeling, Ramchandran plot etc.

Introduction
Arabidopsis thaliana (mouse-ear cress or Arabidopsis) is a model plant used for studying plant sciences, including genetics, evolution, population genetics, and plant development [1-3]. The plant's small size and rapid lifecycle are also advantageous for research. The small size of the plant is convenient for cultivation in a small space, and it produces many seeds. Also, as an individual plant can produce several thousand seeds; that take about six weeks from germination to mature seed. Each of the above criteria leads to *A. thaliana* being valued as a model organism. It has been proved that PME enzyme plays important role in seed germination of the model plant *Arabidopsis thaliana* by demethylesterification of homogalactur-onans pectin present in the cell wall [4]. The little study is known about the role of PMEs in the germination of angiosperm seeds, although one study has characterized their role in the dormancy breakage and germination of conifer seeds [5]. Seed germination is a mechanism, in which morphological and physiological alterations result in activation of the embryo in plant growth & development. Before germination, seed absorbs water, resulting in the expansion and elongation of seed embryo [6]. The plant cell wall consists mainly of a hydrated gel matrix of hemicellulosic and pectic polysaccharides, as well as cellulose, along with proteins and aromatic substances [7-9]. Cell wall pectins are found either as homogalacturonans or as substituted molecules, the rhamnogalacturonans I and II as well as xylogalacturonan. Composed of a linear chain of 1, 4-linked a-D-galacturonic acid (GalUA) residues, the homogalacturonans can be methylesterified at the C-6 carboxylic acid groups of the GalUA residues [7, 9]. Pectinmethylesterase (PME) (EC 3.1.1.11) is a ubiquitous cellwall- associated enzyme catalyzes reactions according to the double-displacement mechanisms, de-esterification through transferring the C6 carboxyl groups in the pectin-PME complexes to water molecules altering the degree and pattern of methyl esterification and trans acylation through transferring the C6 carboxyl groups to the hydroxyl groups of another pectin molecules and resulting in the formation of high molecular weight pectins with new non-methoxy ester linkages which facilitate plant cell wall modification and subsequent breakdown allowing water uptake [10]. In this reaction methanol is produced as a byproduct in addition with pectic substances. This enzyme is widely used in juice and fruit beverage industries to improve the quality of the process [11]. Pectinase preparations (such as Olivex) are also used in olive oil industry to increase the oil extraction output and to improve certain olive oil quality indicators [12, 13]. Another application of combinational use of PME, other pectinases and cellulases is the peeling of fruits. Even though *A. thaliana* is a model plant, the physicochemical & structural details of its PME are not available. In the absence of this experimental structure it gives the physicochemical & structural details of PME of *A. thaliana* by *in-silico* approach. The present study focuses on the primary structure of PME, analysis of its physicochemical properties using PROTPARAM tool & identification of its domains in Pfam database. Further the secondary structure of PME was predicted using SOPMA tool in which the content of helices, sheets & random coils were enlisted. The 3D structure is predicted in SWISS MODEL server & finally the structure is validated to prove best model by using PROCHECK server.

Methods
i) Retrieval of PME (Pectin methylesterase sequence) from Protein database
The pectin methylesterase protein sequence (PME) from *Arabidopsis thaliana* was retrieved from Uniprot database [14]. UniProtKB is public protein database which contains the

amino acid sequences of proteins. The sequence was retrieved & saved in FASTA file format with its Accession ID.

ii) Physicochemical analysis of PME by Protparam tool

Physicochemical properties of PME were performed by using ProtParam analysis tool which on ExPASy server [15]. It allows the computation of various physical and chemical parameters for a given protein The computed parameters includes amino acid composition, molecular weight, theoretical pI, Instability index, Grand average of hydropathicity.

iii) Identification of functional domain in PME from Pfam database

Domain is the most important factor governing the protein folding into the structure. The domain of the PME protein was predicted from the Pfam domain database which contains the information about protein families & domains [16].

iv) Secondary structure prediction and analysis of PME

The secondary structure of PME was predicted by SOPMA secondary structure prediction method [17]. SOPMA stands for self-optimized prediction method with alignment for the prediction of helix, strands and coils of the protein sequence.

v) Prediction, Validation & Visualization of 3D structure of PME

The 3D structure of PME was predicted by using Swiss-model server [18-21]. The selection of template was accomplished by protein BLAST using PDB database having identity more than 30%. The evaluation and validation of generated model was performed with PROCHECK server on PDBSum database [22] and predicted model was visualized by Rasmol visualization tool [23].

Results & Discussions

i) Retrieval of amino acid sequence of PME of *A. thaliana* from protein database

Pectin methylesterase [Uniprot ID: Q5MFV8] sequence from *Arabidopsis thaliana was* retrieved from UniProtKB database with its 595 amino acids and saved in FASTA format which shown as below,

>sp|Q5MFV8|PME5_ARATH Pectinesterase 5
OS=Arabidopsis thaliana GN=PME5 PE=1 SV=2
MIGKVVVSVASILLIVGVAIGVVAYINKNGDANLSPQM
KAVRGICEATSDKASCVKTLEPVKSDDPNKLIKAFMLA
TRDAITQSSNFTGKTEGNLGSGISPNNKAVLDYCKKVF
MYALEDLSTIVEEMGEDLNQIGSKIDQLKQWLTGVYN
YQTDCLDDIEEDDLRKTIGEGIASSKILTSNAIDIFHTVV
SAMAKLNLKVEDFKNMTGGIFAPSDKGAAPVNKGTPP
VADDSPVADPDGPARRLLEDIDETGIPTWVSGADRKLM
TKAGRGSNDGGARIRATFVVAKDGSGQFKTVQQAVNA
CPEKNPGRCIIHIKAGIYREQVIIPKKKNNIFMFGDGARK
TVISYNRSVKLSPGTTTSLSGTVQVESEGFMAKWIGFK
NTAGPMGHQAVAIRVNGDRAVIFNCRFDGYQDTLYVN
NGRQFYRNIVVSGTVDFIFGKSATVIQNSLIVVRKGNKG
QFNTVTADGNEKGLAMKIGIVLQNCRIVPDKKLAAERL
IVESYLGRPWKKFSTTVIINSEIGDVIRPEGWKIWDGESF
HKSCRYVEYNNRGPGAITNRRVNWVKIARSAAEVNDF
TVANWLGPINWIQEANVPVTLGL

ii) Physicochemical analysis of PME by PROTPARAM tool

Physicochemical composition of PME was analyzed by using ProtParam analysis tool on ExPASy server. The physiochemical parameters were tabulated in Table no. 1. As per table instability index is 26.12 classifies the PME is instable, on the basis theoretical pI the PME is basic in nature, as there are only presence of positively charged amino acids and the PME is cationic in nature.

Table 1: Physicochemical parameters of PME.

Sr.no.	Parameters	Values
1.	Number of amino acids	595
2.	Molecular weight	64728.14
3.	Theoretical pI	9.07
4.	Instability index	26.12
5.	Grand average of hydopathicity	-0.184
6.	No. of positively charged amino acids	74
7.	No. of negatively charged amino acids	62

iii) Identification of functional domain in PME from Pfam database

The functional domain of PME was predicted by Pfam database which shows two domains PMEI and pectinesterase which is shown in Fig.no.1 & the regions of two domains are shown in Table no. 2. The PMEI region may act as an autoinhibitory domain and prevent untimely PME activity during transport. It has been implicated in the regulation of fruit development, carbohydrate metabolism and cell wall extension. It may also be involved in inhibiting microbial pathogen PMEs. It has been observed that it is often expressed as a large inactive pre-protein. The next domain pectinesterase catalyses the de-esterification of pectin into pectate and methanol. Pectinesterase plays an important role in cell wall metabolism during fruit ripening. Plant pectinesterases are regulated by pectinesterase inhibitors, which are ineffective against microbial enzymes.

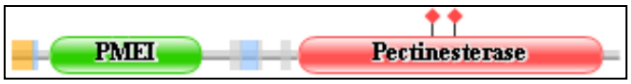

Fig 1: Domains of PME predicted in Pfam database.

Table 2: Starting & ending regions of PME domains.

Sr. no.	Domains	Start	End
1	PMEI	39	186
2	Pectinesterase	283	580

vi) Secondary structure prediction and analysis of PME

The secondary structure of PME was predicted by SOPMA Secondary Structure Prediction method. Secondary structural elements Alpha helices, strands & coils were enlisted in following Table no.3. The table shows the PME has more number of coils that is 33.78% followed by alpha helices 29.87% and strands 24.92%.

Table 3: Secondary structure of PME.

Sr.no	Secondary structure	No. of residues	percentage
1.	Alpha helices	178	29.92
2.	Extended strands	148	24.87
3.	Random coils	210	33.78

v) Prediction, Validation & Visualization of 3Dimensional structure of PME

The homology modeling of PME from *A. thaliana* was obtained through SWISS MODEL server using resolution 1.8 A˚ structure of PME from *Daucus carota* (PDB Id:1gq8.1 Chain A) as a template. The evaluation and validation of generated model were executed with PROCHECK server on PDBSum database which is shown in Fig.no.2. Validation of the predicted PME from *A. thaliana* by PROCHECK analysis showed that 86.1% of the residues of PL model were present in the most favoured region followed by 13.9% in the allowed region, 0% in generously allowed region and disallowed region respectively of Ramachandran plot which are shown in Table no.4. Further the predicted structure was visualized by Rasmol viewer which is shown in Fig.no.3.

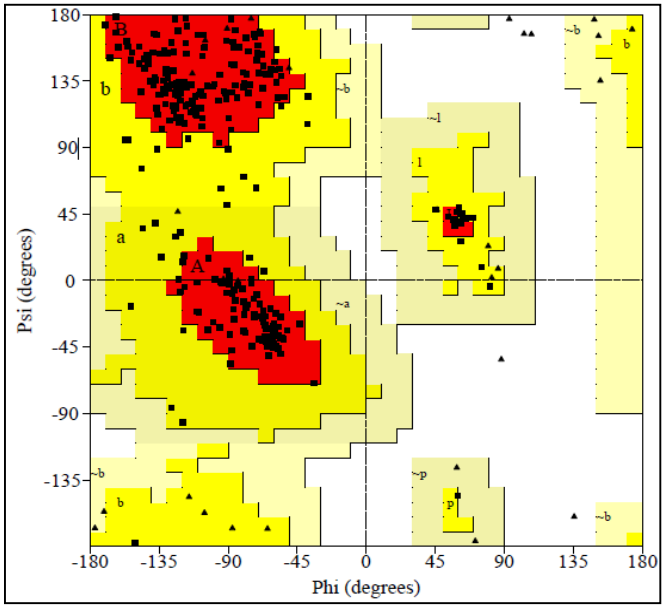

Fig 2: Ramchandran plot of Predicted 3d structure of PME.

Table 4: Residue no. & its % in different regions of Ramchandran plot of PME.

Sr. no.	Regions	Residue no.	%
1.	most favoured regions [A,B,L]	236	86.1%
2.	additional allowed regions [a,b,l,p]	38	13.9%
3.	generously allowed regions [~a,~b,~l,~p]	0	0
4.	disallowed regions	0	0

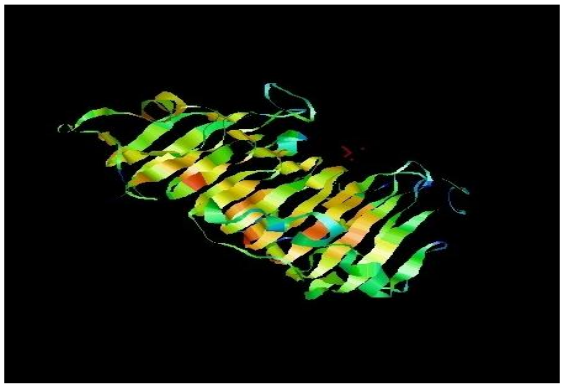

Fig 3: Predicted 3D structure of PME viewed in RASMOL.

Conclusions

The present preliminary investigation mainly leads to understand the basic primary, secondary structure and tertiary structure of PME using various *in-silico* tools and techniques. The primary structure illustrates that the PME is a enzyme with its 595 amino acids. The physicochemical properties depict that the PME is alkaline, stable, cationic protein. The secondary structure reveals that PME consist of a helix, a sheet and random coil structure within its short stretch of residues. The 3D structure predicted by SWISS-MODEL was validated using PROCHECK, the percentage of most favorable region was 86.1%. This present study put molecular insight into the further studies to find the structural and functional properties of this PME enzyme to find or design the novel inhibitors of seed germination process of unwanted & poisonous plants.

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