Role of Abscisic Acid in Plant Growth and Regulation: An Insilico Study

Ashwini S[1], Ghazala Masood[1], Harshitha B S[1], Sinchana H[1]
Vishnumurthy K A[2*], Swarna M Patra[2], Vidya Niranjan[3]

[1]Dept. of Biotechnology, G M Institute of Technology, Davangere -577006
[2]Dept. of Chemistry, RV College of Engineering, Bengaluru-560059
[3]Dept. of Biotechnology, RV College of Engineering, Bengaluru-560059.

Abstract

Plant hormones are known for releasing chemical signals that induce plant growth and regulation. Among the phytohormones, Abscisic acid (ABA) responds to stress such as salinity, pathogen activity and drought. The present study was designed to understand the growth and regulation, across the root and shoot by using different in-silico tools. In the present study among the 50 enzymes and genes, some were selected for the study based on the role in plant growth and regulation and were verified through homology modelling tools such as Swiss model and for docking studies, Autodock tools were used and helped to understand the behavior of ABA in plant i.e., Arabidopsis Thaliana during stress conditions. Computational models of AtABCB4, AtABCB14, AtABCB19, AtABCG25, AtABCG30, AtABCG31 and AtABCG40 and NCED were used for docking by finding all their role, helps to understand the growth and regulation in Arabidopsis Thaliana.

Keywords*: Arabidopsis Thaliana, ABA, Autodock, Stress hormone personal*

1.0. Introduction

Abscisic acid (ABA) is a fifteen-carbon compound molecule which is also known as sesquiterpene. Where three methyl groups, unsaturated chain and a terminal carboxyl group are attached to a aliphatic ring. ABA is known as a near-universal abiotic stress hormone, involved in diverse abiotic stress response cascades including those related to low temperature, salinity, drought and flooding [1]. ABA shows its involvement in plant development and stress responses such as dormancy and growth regulation, leaf senescence and desiccation tolerance. It is also involved in the regulation of vegetative development, maturation and dormancy onset as the response for various environmental stresses such as

[*] *Mail address: Vishnumurthy K A, Assistant Professor, Department of Chemistry, RV College of Engineering, Bengaluru – 59.*
Email: vishnumurthyka@rvce.edu.in, Ph:9741211557

drought and high-salinity conditions. As a phytohormone ABA antagonistically mediates several plant developments processes [2]. It includes flowering time control, primary root growth, seed germination, dormancy and seed maturation. It exhibits vivipary i.e., precocious germination if seeds are still attached to plant. Vivipary condition can be seen in ABA deficit seeds.

Plant responses to environmental stresses for survival in terrestrial conditions. Plants have developed an elegant system for ABA sensing and early signal transduction mechanisms [3]. So in such conditions to prevent transpirational water loss stomatal closure is completed by its complex signalling cascade. The endogenous level of ABA decreases which reverse the process is caused when water conditions return to normal for growth. Thus understanding the regulation of endogenous ABA levels is crucial in developing plant-improvement strategies for drought tolerance [4].

ABA is a weak acid of pKa=4.7 which is partially present in their protonated form in physiological pH condition. Through apoplastic spaces under normal conditions, ABA can diffuse into the plasma membrane. Hence, it can be transported through adjacent cells. Apoplastic pH increases during drought stress [5] and ABA which is in its freely permeable form decreases. Therefore, transportation of ABA across the plasma membrane from the vascular tissue (site of synthesis) to the guard cells (action site), is necessary and biosynthesis of ABA is induced [6]. ABA is important for plant responses for regulation and germination and development processes. When plants are exposed to water deficiency synthesis of ABA increases greatly. Abscisic acid glucose ester (ABA-GE) is the inactive form of ABA stored in the vacuole and Endoplasmic reticulum. It appears at leaf apoplast during drought conditions and the concentration of the other five conjugates increases significantly. ABA reaches leaf apoplast via ABA transporters i.e., AtABCG25 and AtABCG40. ABA-GE raises the pH level of leaf apoplast to 7.2 and makes it alkaline. It leads to activation as esterase in the leaf cells. Conversion of conjugated ABA to free ABA is done by these activated esterase [7]. Then ABA is released to cytosol and nucleus of guard cell where it binds to ABA receptors Pyrabactin Resistance (PYR)/ Regulatory component of ABA receptor (RCAR) [8].

Based on amino acid sequence identity The PYR/PYL proteins have been classified into subfamilies I, II, and III. Currently full structural elucidation of ABA receptors belonging to subclass I (PYR1, PYL1, and PYL2), even though PYR/PYL members from each subfamily are purified and crystallized [9-11]. After ABA binding this complex initiates a series of

signal transduction step involving the early signalling components Protein Phosphate 2C (PP2Cs) and Sucrose non-fermenting-1-related protein kinase2 (SNRK2s). Protein degradation done by Signal transduction is an irreversible mechanism which requires de novo protein synthesis to accomplish feedback desensitization to the hormone signal. In a highly reversible manner, kinases/phosphatases are the main effector proteins which set the off cellular response to the hormone as in case of ABA [12]. PYR/PYL binds with individual PP2C family members which are the second level of signal regulation after ABA binding to PYR/PYL [13]. In which, this complex will inhibit the activity of PP2C which in turn activates SNRK2 and phosphorylate. SNRK2 is activated by ABA binding to it which controls the ion channels and also regulates the ABA-dependent gene expression in the guard cells and triggers stomatal closure [14].

Under drought stress, the drought signal is first precisely identified by roots. Hence, for a long time, it was believed that ABA synthesis occurs in roots under drought stress to close stomata [15] But now, according to the recent report synthesis of drought-induced ABA occurs in short vasculature [16, 17]. 9-cis-epoxycarotenoid deoxygenase 3 [NCED3] which is a rate-limiting enzyme, induced in cells of vasculature in response to drought signal. ATP-binding cassette (ABC) transporters ABCG25/WBC25 and ABCG40/PDR12 play a major role in inducing stomatal closure under drought stress [8, 18]. ABCG25 transport ABA from the plasma membrane of vascular cells then ABCG40 transport it to the xylem. ABCG25 is mainly expressed in phloem compassion cells. AtABCB14 is responsible for opening and closing of stomata. By transporting malate from the apoplast into guard cells, it maintains stomatal movement, which in turn increasing their osmotic pressure.

To maintain seed dormancy, transportation of ABA from the endosperm of seed coats to the embryo via ABA transporters ABCG25, ABCG40, ABCG30 and ABCG31 [19]. ABCG25 and ABCG31 are the main transporters in the transportation of ABA from the endosperm, while ABCG40 and ABCG30 play an important role in ABA uptake into the embryo.

From the foregoing literature it is evident that ABA plays an important role in plant growth. But complete ABA mediated plant growth is still not studied in detail. Hence present study aiming at the in-silico verification of ABA-mediated plant growth and regulation. In the study, a plant Arabidopsis Thaliana was taken. Amino acids were taken from databases and genes which are responsible for the plant growth are employed to study the synthesis of ABA and its role in the plant growth.

2.0 Materials and Methods

2.1 Tools used for Study

The current study deals with the behavior of ABA regulatory genes, ABA biosynthesis enzymes and complex activated in ABA-mediated growth and regulation. We used bioinformatics Biological-databases and tools such as Pubmed for literature survey, Uniprot KB for retrieval of protein sequence, NCBI Blast for template search (BLAST search), ClustalW for multiple sequence alignment, SAVES5 for Ramachandran plot analysis, ProSa for refinement of model, Swiss model for building homology model, SPDB Viewer for visualization of homology model, YASARA for energy minimization and to remove water molecules, HETATM, RCSB for 3D structure of protein, Autodock, MGL tools for molecular docking and PyMOL, DSV to visualise docked structures and interactions between ligand and protein

2.2 Methodology

2.2.1 Sequence retrieval of ABA family transporters

The plant selected for the ABA study is *Arabidopsis Thaliana.* Retrieval of an amino acid sequence is done from Uniprot databases. Out of 50 regulatory genes and enzymes; some enzymes were selected for the study based on their role in growth and regulation in plants. ABA promoter AtABCB4 regulates the root elongation and the initiation of lateral roots and the development of root hairs [20]. AtABCB19, an auxin transporter helps in auxin transport, root and shoot development [21]. At NCED gene improves the tolerance of plants to dehydration stress and salinity stress and increases the level of photosynthesis. ABCG31 with ABCG25 help to export ABA from endosperm to embryo through ABCG30 and ABCG40 which suppress radical extension and subsequent embryonic growth. ABCG40 imports ABA through plasma membrane mainly in guard cell involved in intracellular ABA signalling pathway which leads to stomatal closure.ABCG30 imports ABA to embryo from endosperm through ABCG25 and ABCG31 mediated ABA export to suppress subsequent embryonic growth and radical extension.

2.2.2 Sequence information of the regulated genes and enzymes

Sequence retrieval of AtABCB4, AtABCB14, AtABCB19, ABCG25, ABCG30, ABCG31, ABCG40 and AtNCED from UniProt, given in the Table 1.In ABA biosynthesis pathway the enzyme NCED is involved. The epoxycarotenoid 9-cis neoxanthin was cleaved by the 9-cis-epoxycarotenoid dioxygenase (NCED) which gives a C15 intermediate-

xanthoxin. To get adapted to changing environmental condition and physiological stress, De-conjugation of ABA plays an important role in providing an ABA puddle for plants. In negative regulation, PP2C inhibits the K+ potassium channel and involved in the regulation of seed dormancy. In ABA signaling pathway SnRK2 protein kinases are involved in the gene-regulation as it leads to activation of ABA regulated promotes template identification was done by comparative searching, selection of a homolog with the best score as a template by NCBI BLAST. Downloading of template protein in FASTA format and multiple sequence alignment is done through Clustal W**,** Selection of most matched sequences from multiple sequence alignment result.

2.2.3 Homology Modeling and Refinement

A homology model of the ABA regulatory genes was constructed by using the Software SWISS-MODEL. The model was built according to the target sequence, an alignment file and 3D structure of the template protein data bank (PDB) and was viewed by SPDB viewer. Refinement of the model was done by performing energy minimization using Yasara software. Model was visualized through Discovery studio visualizer. The Z-score and Ramachandran plot of model was calculated through SAVES 5 and ProSa.

2.2.4 Molecular Docking

For docking study, 3D structure of protein is retrieved from PDB and ligand structure is retrieved form PubChem. The selected compounds were retrieved in 3-Dimensional format and saved as SD file format. Docking study was performed using AutoDock tools by removal of the water molecule, Energy minimization and 3D protonation. This energy minimized structures were further used as the receptor for docking studies, the ligand preparation and structure refinement of protein. Docking was performed against the respective receptors and the ligand database. The ligand molecule with high affinity, with the most appropriate interaction was chosen for further analysis of Root mean square difference (RMSD). The receptor and ligand are aligned to compare their displacement and conformational changes. Then, results are reported in root mean square deviation (RMSD). The confirmation with highest binding affinity is compared to itself, so the RMSD value obtained is zero Angstrom.

3.0 Results and discussion

3.1 Homology Modeling

Homology modeling was performed in Swiss-Model and computational models of AtABCB4, AtABCB14, AtABCB19, AtABCG25, AtABCG30, AtABCG31, AtABCG40, and NCED were created. Structural resolutions were approximately nearly 2.0 Armstrong, good quality models were designed by the Swiss model which is shown in Table 1 and the further detailed results are shown in Fig. 1 to 3. The 3-Dimensional structures of seed dormancy of the drought resistance, stomatal closure protein and regulatory gene provide proper information on their functions, mechanism and the relationship between them. For docking studies, models of ABA receptors and transporters were selected and docked with ligand databases.

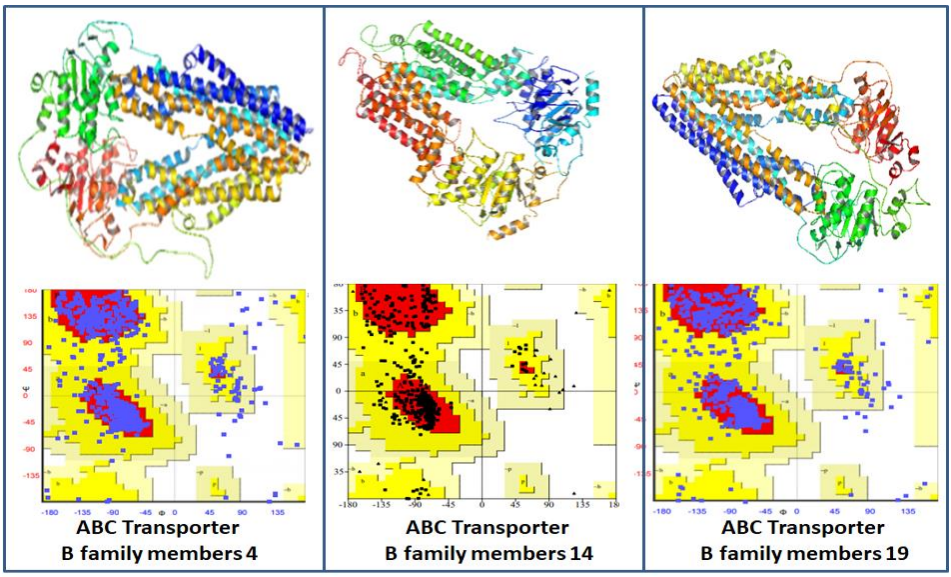

Fig. 1. Homology model and Ramachandran plots of ABC Transporter B family members through Swiss-model

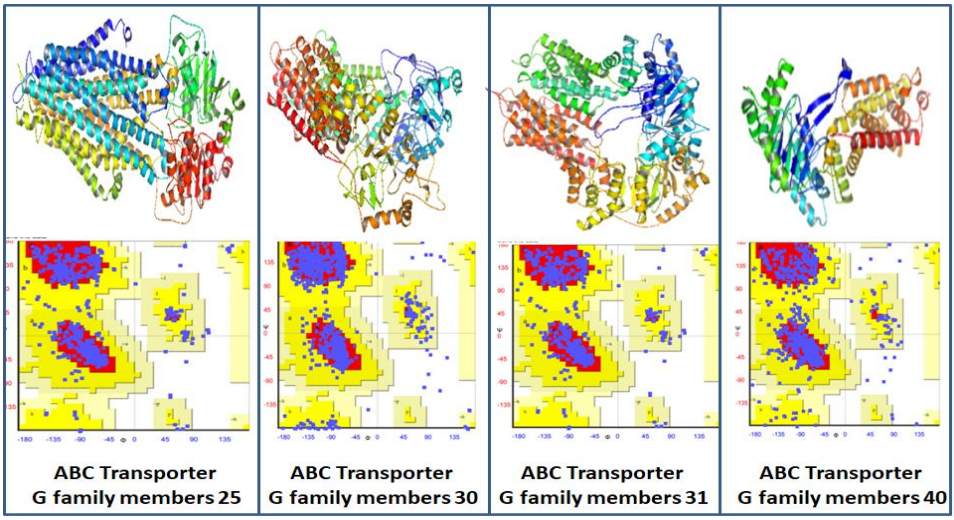

Fig. 2. Homology model and Ramachandran plots of ABC Transporter G family members through Swiss-model

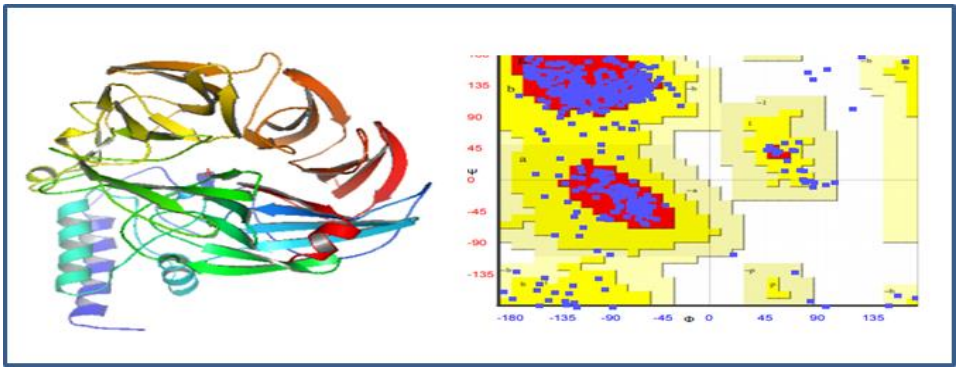

Fig. 3. Homology model and Ramachandran plots NCED9-cis-epoxycarotenoid-dioxygenase through Swiss-model

Table 1. List of transporters, genes and enzymes for homology modelling through Swiss-model.

Transporter Name (Database Accession Number)	Model similarity percentage, Number of Amino Acid)	Ramachandran plot(% of favored region)
ABC Transporter B family member 4 AtABCB4 (080725)	(49%, 1286)	94.34%
ABC transporter B family member 14, AtABCB14(Q9C7F2)	(49%, 1247)	-89.16%
ABCTransporter B family 19 AtABCB19(Q9LJX0)	(39%, 1252)	-94.61%
ABC Transporter G family member 25 AtABCG25(Q84TH5)	(42%, 662)	-95.86%
ABC Transporter G family member 30 AtABCG30(Q8GZ52)	(41%, 1400)	-86.79%
ABC Transporter G family member 31, AtABCG31(Q7PC88)	(9%, 1426)	-89.15%
ABC transporter G family member 40, AtABCG40(Q9M9EI)	(33%, 1423)	-95.48%
NCED9-cis-epoxycarotenoid-dioxygenase NCED3(Q8VY26)	(90%, 570)	-91.39%

3.2 Docking

An inactive form of ABA, ABA-GE is converted to ABA by esterase which is activated by increased pH condition in leaf cells. Hence, here we take ABA-GE as ligand and esterase as a receptor for docking studies. Out of 16 active residues, ASN32, GLN30 shows covalent bond, GLY122, GLY35, GLN30 shows conventional hydrogen bond. These residues show great exposure to ligand whereas other residues show Vander Walls, Pi-

Alkyl, and Pi-Sulphur interaction with ligand shown in figure Table 2 (A1, A2). Free ABA binds to PYR or PYL. In docking study of PYR1 receptor with ABA ligand, there are 21 active residues. In that LYS86 shows conventional hydrogen bond, ASN197 shows unfavorable donor-donor interaction with hydrogen of the ligand. Whereas other residues show Vander Waals, alkyl and Pi-alkyl interaction with ligand in Table 2 (B1, B2). We found 14 active residues in protein-ligand interaction between PYL2 receptor and ABA. In that, HIS119, VAL114 exhibits carbon-hydrogen bond, ASN173, GLU98 exhibits conventional hydrogen bond. So, these are the most interacting active residues with the ligand. Remaining residues exhibit alkyl, Pi-Sigma and Vander Waals interaction Table 2 (C1, C2). ABA binds to PP2C to inhibit its activity. Docking results show the structure of PP2C binds with ABA via 7 active residues i.e., PHE210, VAL320, ASN211, LYS201, LYS206, PRO318 and GLU319 shown in Table 2(D1, D2). SNRK2 is activated by binding of ABA to it and ABA also inhibits the binding of PP2C to SNPRK2. There are 9 residues in the active site. In that ARG119, LYS280, GLU115, ARG246 exhibit conventional hydrogen bond, ASN281 exhibit unfavorable donor-donor interaction and the rest exhibit Vander Waals interaction with ligand Table 2 (E1, E2). This activated SNRK2 controls ion channels and triggers stomatal closing.

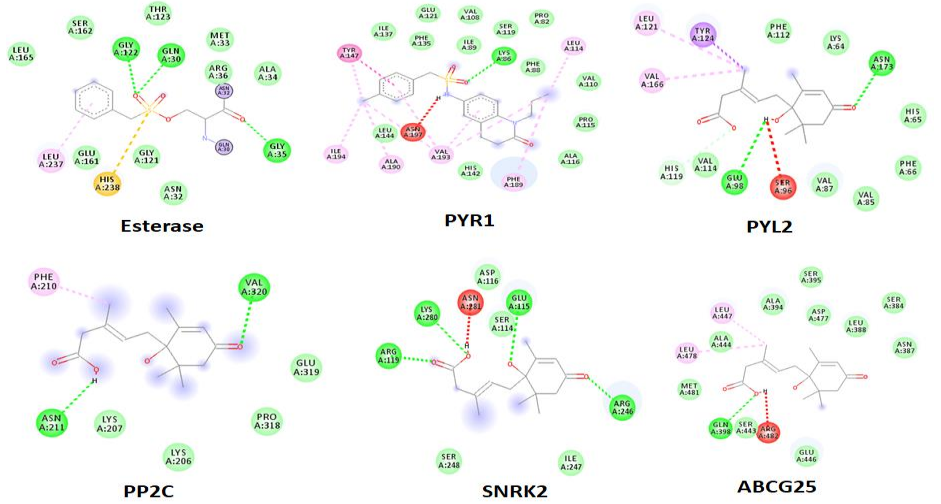

Fig. 4. Protein ligand interactions after subjected to docking with Esterase, PYR1, PYL2, PP2C, SNRK2 and ABCG25

During drought condition, synthesis of drought-induced ABA takes place in shoot vasculature. Transportation of ABA from the site of synthesis to the site of action is perfected by ATP-binding cassette (ABC) transporters.

ABCG25 takes ABA from plasma membrane of vascular cells to xylem. In docking study of ABCG25 and ABA, there are 14 residues in the active site. In which, GLN398 exhibit conventional hydrogen bond, LEU447 and LEU478 exhibit alkyl interaction, ARG482 exhibit unfavorable donor-donor interaction and rest over residues exhibit Vander Waals interaction with ligand Table 2 (F1, F2). ABCG40 takes up ABA from xylem to the site of action. The result of the docking study of ABCG40 with ABA shows an interaction of 13 residues with ABA. In that, HIS924 show carbon-hydrogen bond, TYR1163 shows Pi-alkyl interaction and the remaining residues show Vander Waals interaction with ligand in Table 2. ABA induces AtABCB14 which is responsible for stomatal opening and closing.

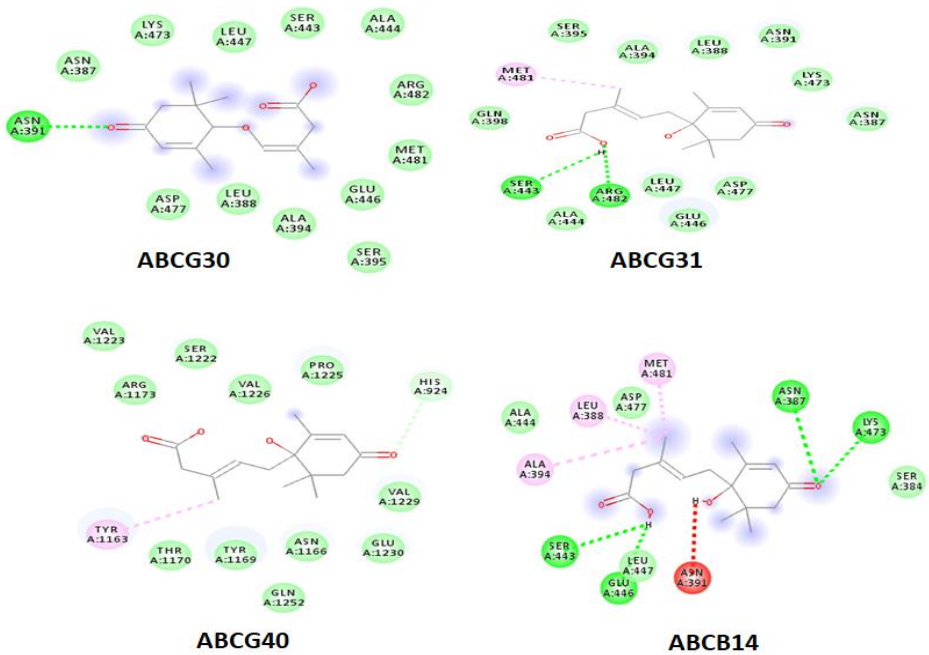

Fig. 5. Protein ligand interactions after subjected to docking with ABCG30, ABCG31, ABCG40 and ABCB14 protein with receptor

Docking result of AtABCB14 and ABA shows 12 residues in the active site. In that, LEU447, GLU446, SER443 exhibit conventional hydrogen bond, ASN391 exhibit unfavorable donor-donor interaction, MET481, LEU388, ALA394 exhibit alkyl interaction and remaining residues exhibit Vander Waals interaction shown in Table 2 (J1, J2). In maintaining seed dormancy, transportation of ABA from the endosperm of seed coats to an embryo via ABCG25 and ABCG31. Docking result of ABCG25 with ABA exhibits 14 residues in the active site. In which, GLN398 exhibit

conventional hydrogen bond, LEU447 and LEU478 exhibit alkyl interaction, ARG482 exhibit unfavorable donor-donor interaction and rest over residues exhibit Vander Waals interaction with ligand shown in Table 2 (F1, F2).

In docking study of ABCG31 and ABA, there are 14 active residues. In that, SER443 and ARG482 show conventional hydrogen bond, MET481 shows alkyl interaction and leftover residues show Vander Waals interaction with the ligand shown in Table 2 (H1, H2). Further, ABCG40 and ABCG30 are involved in up taking of ABA into the embryo. We found 13 active residues in protein-ligand interaction between ABCG40 and ABA. In that, HIS924 show carbon-hydrogen bond, TYR1163 show Pi-alkyl interaction and the remaining residues show Vander Waals interaction with ligand Table 2. In docking study of ABCG30 with ABA, there are 13 residues in the active site. In that, ASN391 exhibit conventional hydrogen bond and remaining residues exhibit Vander Waals interaction with the ligand shown in Table 2 (G1, G2).

Table 2. Receptors and ligands, their binding affinity after subjecting to molecular docking analysis

No	Protein/Receptor	Docked ligands with protein	Binding affinity, Kcal/mol
1	Esterase	A1 (Esterase-ABA-GE)	A2 (-7.2)
2	PYR1	B1 (PYR1-ABA)	B2 (-8.0)
3	PYL2	C1 (PYL2-ABA)	C2 (-7.3)
4	PP2C	D1 (PP2C-ABA)	D2 (-5.2)
5	SNRK2	E1 (SNRK2-ABA)	E2 (-5.7)
6	ABCG25	F1 (ABCG25-ABA)	F2 (-7.1)
7	ABCG30	G1 (ABCG30-ABA)	G2 (-6.6)
8	ABCG31	H1 (ABCG31-ABA)	H2 (-7.0)
9	ABCG40	I1 (ABCG40-ABA)	I2 (-6.9)
10	ABCB14	J1 (ABCB14-ABA)	J2 (-6.5)

4.0 Conclusion

The current study deals with the in-silico verification of ABA-mediated plant growth and regulation. Molecular modelling and docking studies

helped in understanding the ABA synthesis and its transportation across plant during growth in certain stress conditions. In growth and regulation of plants, plant hormones affect the plant life from Maturation, flowering, fruit setting and from phototropism to leaf fall. Every cell in the plant has the potential to produce plant hormones, they can be either transported to other parts of the plant body or can act in their origin (cell). ABA was first believed to be the agent in causing abscission, but now it's believed to play a minor role in abscission. ABA plays a major role in stressful conditions; an important function of ABA is to promote development; it is involved in the conversion or growth of apical meristem into a bud (dormant). Low moisture in the soil causes an increase in ABA which causes closure of stomata and the reduction of water loss.

Synthesis of abscisic acid is carried out by the breakdown of 40C compound, after this cleavage, Xanthoxin intermediate is converted into ABA-Aldehyde later to ABA which involves many enzymes; NCED3. The selection of transporters helps to define the growth and regulation via docking. Docking studies help us to understand the direct link to ABA-mediated growth. The homology modelling was carried out and the computational models of the ABCB4, ABCB14, ABCB19, ABCG25, ABCG30, ABCG31, ABCG40 and NCED3 were created. Docking results of ABCG40, ABCG25 involves in the transportation of ABA and showed the involvement ABA transportation pathway. ABCG25 and ABCG31 are the main transporters in the transportation of ABA from the endosperm. While ABCG40 and ABCG30 play an important role in ABA uptake into the embryo. These ABA transporters were reported as importers as well as exporters and also act in the root to shoot signaling. The Docking results of the core complex Pyrabactin resistance (PYR) / regulatory component of ABA receptor (RCAR) / Pyrabactin resistance like (PYL) receptor with ABA shows a dormant interaction with each other.

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