In silico Mechanistic Study of Abscisic Acid (ABA) Mediated Drought Tolerance in Crops

Bushra Shaheen  
Government College University, Faisalabad, Pakistan

Tayyaba Shaheen  
Government College University, Faisalabad, Pakistan, tayaba_pgmb@yahoo.com

Shahid ur Rehman  
University of Agriculture, Faisalabad, Pakistan

Mahmood ur Rahman  
Government College University, Faisalabad, Pakistan

Muhammad Nawaz  
Government College University, Faisalabad, Pakistan, muhammadnawaz@gcuf.edu.pk

See next page for additional authors

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In silico mechanistic study of abscisic acid (ABA) mediated drought tolerance in crops

Bushra Shaheen¹, Tayyaba Shaheen¹*, Shahid ur Rehman⁴, Mahmood ur Rahman¹, Muhammad Nawaz², Muhammad Tahir ul Qamar¹,³

¹Department of Bioinformatics and Biotechnology, Government College University, Faisalabad 38000, Pakistan
²Department of Botany, Government College University, Faisalabad 38000, Pakistan
³Center of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture (UAF), Faisalabad 38040, Pakistan
⁴Department of Poultry Sciences, University of Agriculture (UAF), Faisalabad 38040, Pakistan

* Corresponding authors:

Dr. Tayyaba Shaheen; Email: tayaba_pgmb@yahoo.com

Dr. Muhammad Nawaz, ; Email : muhammadnawaz@gcuf.edu.pk
Abstract:
Drought is one of the major environmental stresses that produces adverse effects on plants. Vast areas of agricultural land is susceptible to drought. Drought induced yield loss of crops has negative effects on the economy of a country. Among phytohormones, Abscisic Acid (ABA) induces abiotic stress tolerance in plants, and directs a complex regulatory network involving multiple transporters, up-regulation of ABA biosynthesis genes and various signalling pathways that enable plants to withstand low water availability. The current study was designed to understand ABA synthesis, its transport across the plant during stress and its mechanism to induce stomatal closure by using different in silico tools, because the complete ABA mediated drought tolerance has not yet been reported. In the current study, seven transporters, four ABA biosynthesis enzymes, deconjugation enzyme and a core complex of ABA signalling was verified through Modeller 9.10 and Molecular Operating Environment (MOE). The Intel [R] Xenon [R] CPU-E5420 @ 2.50 GHz system with 4 GB of RAM and the 11.4 (X 86_64) operating system was used for molecular docking. Protein-ligand interactions were analysed by the LigPolt feature of MOE. Docking studies helped to understand the behaviour of ABA biosynthesis enzymes, ABA transporters and ABA core complex, which in turn helps to comprehend the whole mechanism of ABA synthesis in plants during drought stress. Computational models of AtABCG11, AtBG1, AtABCG12, AtABCB14, AtABCG22, AtABCG25, AtABCG32, AtABCG40, NCED, ABA2 and AAO were used for docking. Docking analysis has shown promising results for all the models, except AtABCG11 and AtABCG12. Residues of AtABCG11 and AtABCG12 did not show binding with the ABA, as these transporters are involved in cuticle formation. Findings of this study will strengthen the work on ABA drought tolerance in plants and help to produce drought resistant crops globally.
Keywords: Drought, ABA, Arabidopsis, drought resistant crops
1. Introduction

Among the several biotic and abiotic stresses, drought is a major reason of decline in photosynthetic rate and causes disturbances in physiological processes which may lead to poor growth and low yield in crops (Chaves and Davies, 2010; Yang et al., 2009; Ma et al., 2009). Worldwide drought affected area is 36%, while areas under temporary drought conditions is more than 64% (Xoconostle-Cazares et al., 2010; Dai, 2011). Most physiological pathways suggests that Abscisic Acid (ABA) plays a vital role in regulating plant development, growth, functioning and signalling, being a major chemical root-shoot stress signal under drought conditions (Bruce et al., 2002). During drought, ABA biosynthesis and stimulation of ABA via transporters causes an increase in root to shoot stress signal from the roots to leaf (Bahrun et al., 2002). Previous studies on ABA described the presence of five conjugates of ABA in the xylem sap of well-watered sunflower plants (Gutterson and Reuber, 2004; Xiong et al., 2015). While in plants under drought, the sixth conjugate, namely ABA-GE, appears and the concentration of the other conjugates rises significantly. From roots conjugated, ABA reaches leaf apoplast via ABA transporters, i.e. AtABCG25, AtABCG40 (Boursiac et al., 2013).

Once ABA-GE arrives at the leaf apoplast, it raises the pH level of leaf apoplast to 7.2 and makes it alkaline. Alkaline pH of leaf apoplast serves as a signal to initiate the apoplastic mechanism of ABA-GE transport and also leads to the activation of esterase in the leaf cells. These activated esterase convert conjugated ABA to free ABA, thus increasing the level of ABA in leaf cells (Freundl et al., 2000; Ascenzi and Gantt, 1999). Now free ABA will bind to the core, signalling a complex Pyrabactin Resistance (PYR)/Regulatory Component of ABA Receptor (RCAR) in the cytosol and nucleus of guard cells (Kuromori et al., 2010). After ABA binding, this complex will inhibit the activity of Protein Phosphatase 2C (PP2Cs),
which in turn activates SNF1-Related Protein Kinase 2s (SnRKs) and phosphorylate, the main targets of the ABA signalling mechanism (Park et al., 2009). Recent progress in understanding early ABA signal transduction has led to the construction of a PYR/RCAR–PP2C–SnRK2 signal transduction model. In the absence of ABA, PP2Cs inhibit protein kinase (SnRK2) activity through the removal of activating phosphates (Lee et al., 2009). SnRK2 (activated via ABA binding) controls the ion channels, regulates the ABA dependent gene expression in the guard cells and triggers stomata closure (Umezawa et al., 2009). ABA in the guard cell will bind with receptors of the right guard cell and prompt elevation of cytosolic Ca$^{2+}$ ion concentration through the extracellular influx of Ca$^{2+}$ ions influx and its release from intracellular stores. The influx of calcium and the higher level of cytoplasmic Ca$^{2+}$ ion concentration inside the cell are important for guard cell ABA signal transduction (Johnson et al., 2014).

Movement of Ca$^{2+}$ ions towards the guard cell activates the S-type and R-type ion channels and depolarizes the guard cell membrane. Activation of slow and rapid ion channels and depolarization of the cell membrane will open the potassium (K$^{+}$) ion conduits (Sutton et al., 2000). Elevation of Ca$^{2+}$ ions cytoplasm will activate vacuolar potassium ion channels and generate potassium ions efflux from the guard cell (MacRobbie, 2000). During stomata closure, more than 90% of the ions released from the guard cell must be released from vacuoles into the cytosol. The higher K$^{+}$ ion level will activate the K$^{+}$ ions efflux towards the cytoplasm through vacuolar ion channels. The higher level of Ca$^{2+}$ in the cell will inhibit the hydrogen ion (H$^{+}$) efflux by inactivating proton-extruding H$^{+}$ ATPases of plasma membrane and inward movement of K$^{+}$ ion in the nucleus of the guard cell (Schroeder et al., 2001). Initiation of ion efflux and inhibition of stomata opening processes provide the mechanistic basis for ABA-induced stomata closure. After all above mentioned steps, the cell’s pH will increase (alkaline), and water will move outwards from the guard cell, which
will lower the turgidity of the guard cell and lead to stomal closure (Sirichandra et al., 2009; Dodd et al., 2010).

Taking into account the whole ABA mediated stress tolerance, the current study was designed to structurally analyse the up regulated genes of drought, their respective enzymes, the ABA transporters and the ABA core complex. The purpose was to study the involvement of ABA in triggering the guard cell responses during drought stress. It would be helpful to find the role of ABA in guard cell opening and closing. In the current study, seven transporters, four ABA biosynthesis enzymes, deconjugation enzyme and the core complex of ABA signalling was verified through the Modeller 9.10 and Molecular Operating Environment (MOE). Docking studies will help to understand the behaviour of ABA biosynthesis enzymes, ABA transporters and the ABA core complex, which in turn helps to comprehend the whole mechanism of ABA synthesis in plants during drought stress. Understanding of ABA mediated drought tolerance via in silico analysis may pave the way to understand the drought tolerance mechanism.

2. Materials and Methods

2.1. Sequence Retrieval of ABA-G and B Family Transporters

Amino acid sequences of ABA transporters were collected from UniProt databases. Sequence collection was done because of their role in drought tolerance in plants. Out of seven ATP binding cassettes transporters, AtABCG11 and AtABCG32 have potential roles in the formation of the cuticle layer during drought (Crouzet et al., 2006). AtABCG12 is also helpful in the regulation of drought stress in the presence of AtABCG11 (Ukitsu et al., 2007; Reiland et al., 2009). A member of the ABCG family, AtABCG22 and AtABCB14 transporter were present in shoot and leaf respectively, and controlled the stomata opening and closing (Nagasaki et al., 2008). ABCG25 is expressed mainly in vacuolar tissue, where ABA is
synthesized (Verrier et al., 2008). Uniprot Accession numbers of all these transporters are given in Table 1.

2.2. Sequence Information of Up Regulated Genes, Enzymes Involved In ABA Biosynthesis and De-conjugation

Amino acid sequences of enzymes, i.e. NCED and AtBG1 (BETA-1, 3-GLUCANASE 1), were retrieved from the UniProt database given in Table 1. The above mentioned enzymes were reported to be involved in ABA biosynthesis and its de-conjugation. Drought stress has shown the up-regulation expression of NCED3, AtABA2 and AtBG1 genes in Arabidopsis thaliana (Iuchi et al., 2001; Alvarado et al., 2004; Seo and Koshiba, 2002). Oxidative cleavage of major epoxycarotenoid 9-cis neoxanthin by the 9-cis-epoxycarotenoid dioxygenase (NCED) yields a C15 intermediate-xanthoxin. The AtABA2 (ABA deficient 2) gene encodes short-chain alcohol dehydrogenase/reductase (SDR). ABA de-conjugation plays a significant role in providing an ABA pool for plants that allows them to adjust to changing physiological and environmental conditions. Up-regulation of AtBG1 (Beta-1, 3-Glucanase 1) protein leads to more production of ABA as it is responsible for the release of ABA from ABA-GE (glucose ester) (Wojtkowiak et al., 2012).

2.3. Sequence and Structure information of ABA receptors ABA Core Complex signalling Pathway

Amino acid sequence and structure information of ABA receptors involved in the core complex signalling pathway was collected from the UniProt and Protein databank respectively. The UniProt database is used for amino acid sequences retrieval, while structural data was taken from the Protein Databank. Details related to the particular structure along with its coding gene and ligand is given in Table 2.
2.4. Homology Modelling

The MODELLER 9.10 tool was used for homology modelling of three dimensional structures of protein. Structures of ABA transporters and enzymes related to the biosynthesis of ABA were not available. Homology modelling of all the ABA transporters, up regulated genes, and ABA biosynthesis enzymes was performed through MODELLER 9.10. The model with lowest DOPE value was selected and visualized with Chimera 1. Model evaluation was performed in a PDB sum database. The Z-score and Ramachandran plot of our desired model was calculated through the PDB sum Generate.

2.5. Molecular Docking

Two ligand databases were designed using the Molecular Operating Environment Software (MOE, 2009) for molecular docking. One database was of conj-ABA and ABA which was used for docking of ABA transporters. The other database was of PYV and ABA designed for the docking of core signalling complex of ABA receptor. Both databases were saved in .mdb format. Three-dimensional (3D) structures of respective proteins were opened in MOE one by one and removal of water molecules, 3D protonation and energy minimization was done by using MOE with the parameters Force Field: MMFF94X+Solvation, Gradient: 0.05, and Chiral Constraint: Current Geometry. These energy minimized structures were further used as receptors for docking analysis. After ligand database preparation and proteins structure refinement, respective receptors were docked against the respective ligand database. The selected parameters used to calculate the score and interaction of ligand molecules were the Rescoring function: London dG, Placement: Triangle matcher, Retain: 10, Refinement: Force field, and Rescoring 2: London dG. Most appropriate interactions of ligand molecules with targets were chosen for further analysis on the basis of their S score and Root-Mean-Square Deviation (RMSD) values.
3. Results

The current study deals with the behaviour of ABA transporters, ABA biosynthesis enzymes and core signalling complex activated in ABA mediated drought tolerance. Computational methods like modelling and docking were employed to understand the whole mechanism of drought tolerance. An Intel [R] xenon [R] CPU-E5420 @ 2.50 GHz system with 4 GB of RAM and the 11.4 (X 86_64) operating system was used for molecular docking. Homology modelling was performed by using Modeller 9.10 and Protein ligand docking was performed with MOE (Molecular Operating Environment) software. Protein-ligand interactions were analysed by using the LigPolt feature of MOE.

3.1. Homology Modelling

Homology modelling was performed in Modeller 9.10 and computational models of AtABCG11, AtBG1, AtABCG12, AtABCB14, AtABCG22, AtABCG25, AtABCG32, AtABCG40, NCED, ABA2 and AAO were created. On the basis of an analysis having a structural resolution of at least 2.0 Angstroms and R factor no greater than 20.0, good quality models were designed by Modeller 9.10 shown in Figure 1 and detailed results are shown in Table 1. The 3D structures of stomal closure protein of the drought resistant gene provides correct information on their biological function/mechanism and evolutionary relationships. For the docking study, generated models of ABA up-regulated genes, transporters and receptors were selected and further docked with their appropriate ligand databases.

3.2. Molecular Docking

3.2.1. Results of up regulated Genes and Enzymes Involved in ABA Biosynthesis and De-conjugation
In ABA biosynthesis, NCED3 acts on a precursor of ABA, namely Epoxycartenoid 9-cis-neoxanthin, and converts it into xanthonin. Therefore, epoxycartenoid 9-cis-neoxanthin was used as the ligand in docking studies to check the interaction of active site residues involved in the catalytic activity of NCED. Out of the nineteen residues of active site, only His169, Glu114, His218, Thr226, Phe430, Asn439 and Glu440 showed great exposure to the ligand. They are supposed to be involved in the activity of NCED Figure 2 (1A, 1B). Increases in the level of conjugated ABA occurs due to the up-regulation expression of At ABA2, which encodes Alcohol dehydrogenase (ABA2). The 3D model of Alcohol dehydrogenase (ABA2) is used in docking studies in order to check the binding of Xanthoxin. Xanthoxin is the substrate of the Alcohol dehydrogenase (ABA2) which is converted into conjugate ABA (ABA-aldehyde). Docking results show the structure of Alcohol dehydrogenase (ABA2) binds with Xanthoxin via Lys209, Asn278, Ser280, Val168, Tyr204, Phe274, Phe281 and Phe284 residues Figure 2 (2A, 2B). In the presence of the AAO enzyme, abscisic aldehyde is converted into ABA by the up-regulation of the AtAAO3 gene in drought stress. Residues of AtAAO3 gene did not show binding with the ABA aldehyde. The homology model of AtAAO3, might indicate some restrictions in docking of this structure.

3.2.2. De-conjugation Enzyme AT-BG1

Conjugated ABA can act as an additional root-to-shoot stress signal. ABA-GE is transported to an even greater extent under stress conditions; the conjugate can be considered a long-distance signal (Hansen and Dorffling, 1999). Fourteen residues are present in the active site. Three residues are directly linked with conjugated ABA, i.e. Asn4, Ser35, and Glu222 as shown in Figure 3 (A, B). Translocation of endogenous ABA-GE must occur within the root Symplastic during drought. Once it has arrived at the xylem parenchyma cells, the conjugated ABA is released into the xylem elements. Moreover, the symplastic mechanism helps the
transport of mostly conjugated Abscisic acid to the leaf cells where it may serve as an emergency supply in case of stress, specifically drought stress, while apoplast is a barrier for conjugated ABA.

3.2.3. Results of Drought Related Transporters

AtABCB14 responsible for stomata opening and closing were docked first with ABA. Fifteen residues are present in the active site. Among fifteen residues, Arg427, Leu300, Glu430, and Glu251 are showing interaction with ABA in Figure 4(1A, 1B). After this, AtABCB14 was docked first with conjugated ABA. Twenty residues are present in the active site. Five residues are directly interacting with conjugated ABA, i.e. Arg302, Arg427, Ser260, Glu251, Asn259, and Glu430, shown in Figure 4(2A, 2B). Docking results of AtABCG22 with ABA shows an interaction of four residues with ABA, i.e. Gln395, Ser415, Arg430 and Arg583, given in Figure 4(3A, 3B). Docking results of AtABCG22 with ABA-GE shows an interaction of Gln395 and Arg430 out of 9 active site residues. Both residues are acting as side chain acceptors as shown in Figure 4(4A, 4B). Active site residues Lys334 and Ser388 of AtABCG25 bind with ABA as shown in Figure 4(5A, 5B), while Gly610, Lys611 and Ser608 bind ABA-GE given in Figure 4(6A, 6B). AtABCG32 docked with ABA and conjugated ABA one by one. Two residues are directly linked, i.e. Arg903 and Tyr907, in results of AtABCG32 docked with ABA as shown in Figure 4(7A, 7B), and conjugated ABA binds three residues, i.e. Arg903, Asn491 and Asp466, as shown in Figure 4(8A, 8B). AtABCG40 docked with ABA and out of five residues of the active site, only one residue is directly linked with ABA, i.e. Arg411, as shown in Figure 4(9A, 9B). The model of AtABCG40 did not show any interaction with conjugated ABA as shown in Figure 4(10A, 10B). Therefore, it is proven from the docking result that AtABCG40 transporter is not involved in the transportation of conjugated ABA, while other results support the AtABCB14,
AtABCG22, AtABCG25 and AtABCG32 involvement in ABA and conjugated ABA transport pathways. Kang et al (2010) reported the role of all these ABA transporters as exporters as well as importers.

### 3.2.4. Core Signalling Complex of ABA

In leaf free, ABA binds to the core signalling complex pyrabactin resistant (PYR)/regulatory component of ABA receptor (RCAR) of cytosol and the nucleus of guard cells. This complex will inhibit the activity of Protein Phosphatase 2C (PP2Cs), the negative regulator of ABA signalling, i.e. ABI1 (ABA INSENSITIVE 1), ABAI2 (ABA INSENSITIVE 2). On inhibition of protein Phosphatase 2C (PP2Cs), SNF1-RELATED PROTEIN KINASE 2s (SnRKs) will be activated and phosphorylate the main targets of the ABA signalling mechanism. SnRK2 will control the ion channels, regulate the ABA dependent gene expression in the guard cells and trigger stomata closure (Sirichandra et al., 2009). Regulation of guard cells opening and closing is the evolutionary process to maintain water at equilibrium. In drying soil, the stress hormone Abscisic acid concentration increased due to the stimulus of low leaf/root water potential (Davies et al., 2005; David et al., 2007). The docking results of the core complex with ABA show a potential interaction with each other. Therefore, results support the wet lab studies reported by Sirichandra et al., (2009) and Tan et al., (1997). Interaction analysis results of PYL, PVP, ABA, PYR and PYV are shown in Figure 5.

### 4. Discussions

Drought is an environmental stress of sufficient duration that produces a plant water deficit, which in turn causes a disturbance of physiological processes. Drought tolerant plants can survive in stress by lowering their metabolic functions. In drought tolerant plants, Abscisic acid (ABA), a plant stress hormone, induces the closure of the leaf stomata, thereby reducing water loss through transpiration, and decreasing the rate of photosynthesis. The mechanism of
ABA is selected due to its significant role in plants, especially in drought tolerance (Bruce et al., 2002).

Synthesis of ABA starts with the breakdown of precursor of the C40 carotenoid. After this cleavage, intermediate xanthoxin is converted to ABA through ABA-aldehyde. Conversion of intermediate xanthoxin into ABA through ABA-aldehyde is basically a two-step mechanism which involves several enzymes, i.e. NCED3, SDR and AAO (Bahrun et al., 2002). In the current study, all those transporters were selected whose involvement in the drought tolerance is reported in the review studies of Kang et al., (2010). The purpose of selecting all transporters was to define their involvement in drought tolerance via docking studies. Docking studies helped us to elucidate those transporters that are directly linked to the ABA-mediation of drought. First, homology modelling was performed and computational models of AtABCG11, AtBG1, AtABCG12, AtABCB14, AtABCG22, AtABCG25, AtABCG32, AtABCG40, NCED, ABA2 and AAO were created. AtABCG11 and AtABCG12 were docked with ABA one by one. The poorly docked structure of AtABCG11 and AtABCG12 with ABA was obtained by MOE docking. Residues of AtABCG11 and AtABCG12 did not show binding with the ABA, as this transporter is involved in cuticle formation (Crouzet et al., 2006). Docking results of AtABCG40, AtABCB14, AtABCG22, AtABCG25 and AtABCG32 proved that the AtABCG40 transporter is not involved in the transportation of conjugated ABA, while other results support the AtABCB14, AtABCG22, AtABCG25 and AtABCG32 involvement in ABA and conjugated ABA transport pathways. Blum et al (2009) reported the role of all these ABA transporters as exporters as well as importers. Conjugated ABA can act as an additional root-to-shoot stress signal. ABA-GE is transported to an even greater extent under stress conditions; the conjugate can be considered a long-distance signal (Guttersson and Reuber, 2004). Docking results also proved that the de-conjugation Enzyme AT-BG1 and ABA have interactions. The docking results of the core complex pyrabactin
resistant (PYR)/regulatory component of ABA receptor (RCAR) with ABA show potential interactions with each other. Therefore, results supported the wet lab studies reported by Sirichandra et al., (2009) and Tan et al., (1997).

Drought is an environmental stress of sufficient duration to produce a plant water deficit, which in turn causes a disturbance of physiological processes. The current study focuses on in silico verification of ABA mediated drought tolerance in plants. Much work is available on drought tolerance, but the complete ABA mediated drought tolerance was not reported before. Molecular modelling and docking analysis will improve the understanding of ABA synthesis and its transport across plants during drought stress. Findings of this study would intensify the work on ABA drought tolerance in plants and would help to produce drought resistant crops.

References


4. Blum A (2009). Effective use of water (EUW) and not water use efficiency (WUE) is the target of crop yield improvement under drought stress. Field Crop Res. 112, 119-123.


Table 1: List of transporters, up regulated genes and de-conjugation enzyme for homology modelling through Modeller

<table>
<thead>
<tr>
<th>Transporter Name</th>
<th>Database Accession</th>
<th>Model similarity and Template Structure</th>
<th>Ramachandran plot favoured region residues</th>
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<td>ABC transporter G family member 11</td>
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<td>78%</td>
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Table 2: List of core signaling complex and response to ABA binding and guard cell response

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<th>PDB &amp; UniProt KB Ids</th>
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<td>The Abscisic acid receptor PYR1 in complex with Abscisic Acid</td>
<td>ABA</td>
<td>C_{15} \text{H}<em>{20} \text{O}</em>{4}</td>
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Figure 1: Models and Ramachandran plots of At ABCG11 (1A,2A), At BG1 (1B,2B), At ABCG12 (1C,2C), At ABCB14 (1D,2D), At ABCG22 (1E,2E), At ABCG25 (1F,2F), At ABCG32 (1G,2G), At ABCG40 (1H,2H), NCED (1I,2I), ABA2 (1J,2J) and AAO (1K,2K).

Figure 2: (1A, 1B) Docked structure and Protein Ligand interaction diagram of NCED3 and epoxycartenoid 9-cis-neoxanthin, (2A, 2B) Docked structure and Ligand interaction diagram of Alcohol dehydrogenase and xanthonin.
Figure 3: (A) Docked structure of De-conjugation Enzyme AT-BG1 and ABA, (B) Ligand interaction diagram of De-conjugation Enzyme AT-BG1 and ABA.
**Figure 4:** (1A, 1B; 2A, 2B; 3A, 3B; 4A, 4B; 5A, 5B; 6A, 6B; 7A, 7B; 8A, 8B; 9A, 9B; 10A, 10B) Docked Models and Protein Ligand interaction diagrams of $^1$AtABCB14 and ABA; $^2$AtABCB14 and ABA-GE; $^3$AtABCG22 and ABA; $^4$AtABCG22 and ABA-GE; $^5$AtABCG25 and ABA; $^6$AtABCG25 and ABA-GE; $^7$AtABCG32 and ABA; $^8$AtABCG32 and ABA-GE; $^9$AtABCG40 and ABA; $^{10}$AtABCG40 and ABA-GE respectively.

**Figure 5:** Docking complex and Lig X interaction diagram of PYL1 and ABA (A1, A2), PYL1 and PYV (B1, B2), PYL2 and ABA (C1, C2), PYL2 and PYV (D1, D2), PYR and ABA (E1, E2), PYR and PYV (F1, F2)