Journal of Bioresource Management

Volume 3 | Issue 1 Article 7

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

Recommended Citation

Shaheen, B., Shaheen, T., Rehman, S. u., Rahman, M. u., Nawaz, M., & Qamar, M. T. (2016). *In silico* Mechanistic Study of Abscisic Acid (ABA) Mediated Drought Tolerance in Crops, *Journal of Bioresource Management, 3* (1).
DOI: https://doi.org/10.35691/JBM.6102.0045
ISSN: 2309-3854 online

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

Authors

Bushra Shaheen, Tayyaba Shaheen, Shahid ur Rehman, Mahmood ur Rahman, Muhammad Nawaz, and Muhammad Tahir ul Qamar

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

Bushra Shaheen[1], Tayyaba Shaheen[1*], Shahid ur Rehman[4], Mahmood ur Rahman[1], Muhammad Nawaz[2], Muhammad Tahir ul Qamar[1,3]

[1]Department of Bioinformatics and Biotechnology, Government College University, Faisalabad 38000, Pakistan

[2]Department of Botany, Government College University, Faisalabad 38000, Pakistan

[3]Center of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture (UAF), Faisalabad 38040, Pakistan

[4]Department 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^{+1}) 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^{+1} ion level will activate the K^{+1} 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^{+1} 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 form 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). ABCG25is 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 parametersForce 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 Glu440showed 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. Thehomology model of AtAAO3, might indicate some restrictions in docking of this structure.

3.2.2. De-conjugation Enzyme AT-BG1

Conjugated ABAcan 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 INSENSTIVE 1), ABAI2 (ABA INSENSTIVE 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 ABAcan 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 (Gutterson 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

1. Alvarado MC, Zsigmond LM, Kovacs I, Cseploe A, Koncz C, Szabados LM (2004). Gene trapping with firefly luciferase in Arabidopsis. Tagging of stress-responsive genes. Plant Physiol. 134, 18-27.
2. Ascenzi R, Gantt JS (1999). Molecular genetic analysis of the drought-inducible linker histone variant in *Arabidopsisthaliana*. Plant Mol Biol. 41, 159–169.
3. Bahrun A, Jensen CR, Asch F, Mogensen VO (2002). Drought induced changes in xylem pH, Ionic composition, and ABA concentration act as early signals in field-grown maize (*Zea mays* L.). J Exp Bot. 53, 251–263.
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.
5. Boursiac Y, Leran S, Corratge-Faillie C, Gojon A, Krouk G, Lacombe B (2013). ABA transport and transporters. Trends Plant Sci. 18, 325-333.

6. Bruce WB, Edmeades GO, Barker TC (2002) Molecular and physiological approaches to maize improvement for drought tolerance. J Exp Bot. 53, 13–25
7. Chaves M, Davies B (2010). Drought effects and water use efficiency: improving crop production in dry environments. Func Plant Biol. 37, 1-3.
8. Crouzet J, Trombik T, Fraysse AS, Boutry M (2006). Organization and function of the plant pleiotropic drug resistance ABC transporter family. Plant Physiol. 580, 1123-1130.
9. Dai A (2011). Drought under global warming: A review. WIREs Climatic Change. 2, 45-65.
10. David TS, Henriques MO, Kurz-Besson C, Nunes J, Valente F, Vaz M, Pereira JS, Siegwolf R, Chaves MM, Gazarini LC, David JS (2007). Water use strategies in two co-occurring Mediterranean evergreen oaks: surviving the summer drought. Tree Physiol. 27, 793-803.
11. Davies WJ, Wilkinson S, Loveys R (2002). Stomatal control by chemical signalling and the exploitation of this mechanism to increase water use efficiency in agriculture. New Phytol. 153, 449–460.
12. Dodd AN, Kudla J, Sanders D (2010). The language of calcium signalling. Annu Rev Plant Biol. 61, 593–620.
13. Freundl E, Steudle E, Hartung W (2000). Apoplastic transport of abscisic acid through roots of maize: effect of the exodermis. Planta. 210, 222–231.
14. Gutterson N, Reuber TL (2004). Regulation of disease resistance pathways by AP2/ERF transcription factors. Curr Opin Plant Biol. 7, 465-471.
15. Iuchi S, Kobayashi M, Taji T, Naramoto M, Seki M, Kato T, Tabata S, Kakubari Y, Yamaguchi-Shinozaki K, Shinozaki K (2007). Regulation of drought tolerance by

gene manipulation of 9-cis-epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in Arabidopsis. Plant J. 27, 325-333.

16. Johnson JM, Reichelt M, Vadassery J, Gershenzon J, Oelmüller R (2014). An Arabidopsis mutant impaired in intracellular calcium elevation is sensitive to biotic and abiotic stress. BMC Plant Biol. 14, 162.

17. Kang J, Hwang JU, Lee M, Kim YY, Assmann SM, Martinoia E, Lee Y (2010). PDR-type ABC transporter mediates cellular uptake of the phytohormoneabscisic acid. Proc Natl Acad Sci. 107, 2355–2360.

18. Kuromori T, Miyaji T, Yabuuchi H, Shimizu H, Sugimoto E, Kamiya A, MoriyamaY, Shinozaki K (2010). ABC Transporter AtabcG25 Is Involved in Abscisic Acid Transport and Responses. Proc Natl Acad Sci. 107, 2361–2366.

19. Lee S, Lan W, Buchanan B, Luan S (2009). A Protein Kinase-Phosphatase Pair Interacts With An Ion Channel to Regulate ABA Signaling In Plant Guard Cells. Proc Natl Acad Sci. 106, 21419–21424.

20. Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, Christmann A, Grill E (2009). Regulators of PP2C phosphatase activity function as abscisic acid sensors. Science. 324, 1064–1068.

21. MacRobbie EAC (2000). ABA activates multiple Ca^{2+} uxes in stomatal guard cells, triggering Vacuolar K+ (Rb^{+}) Release. Proc Natl Acad Sci USA. 97, 12361-12368.

22. Nagasaki A, Uyeda TQP (2008). Screening of genes involved in cell migration in Dictyostelium. Exp Cell Res. 314, 1136-1146.

23. Park SY, Fung P, Nishimura, N, Jensen D, Fujii H, Zhao Y (2009). Abscisic acid inhibits type 2C Protein phosphatases via the PYR/PYL family of START proteins. Science. 324, 1068–1071.

24. Reiland S, Messerli G, Baerenfaller K, Gerrits B, Endler A, Grossmann J, Gruissem W, Baginsky S (2009). Large-scale Arabidopsis phosphoproteome profiling reveals novel chloroplast kinase substrates and phosphorylation networks. Plant Physiol. 150, 889-903

25. Schroeder JI, Allen GJ, Hugouvieux V, Kwak JM, Waner D (2001). Guard cell signal transduction. Annu. Rev. Plant Physiol. Plant Mol Biol. 52, 627–58.

26. Seo M, Koshiba T (2002). Complex regulation of ABA biosynthesis in plants. Trends Plant Sci. 7, 41-48.

27. Sirichandra C, Wasilewska A, Vlad F, Valon C, Leung J. (2009). The Guard Cell as a Single-Cell Model towards Understanding Drought Tolerance and Abscisic Acid Action. J Exp Bot. 605, 1439-1463.

28. Sutton F, Paul SS, Wang XQ, Assmann SM (2000). Distinct abscisic acid signaling pathways for modulation of guard cell versus mesophyll cell potassium channels revealed by expression studies in xenopuslaevis oocytes. Plant Physiol. 124, 223-230.

29. Tan BC, Schwartz SH, Zeevaart JAD, Mc-Carty DR (1997). Genetic control of abscisic acid biosynthesis in maize. Proc Natl Acad Sci USA. 94, 12235–12240.

30. Ukitsu H, Kuromori T, Toyooka K, Goto Y, Matsuoka K, Shinozaki K (2007). Cytological and biochemical analysis of COF1, an Arabidopsis mutant of an ABC transporter gene. Plant Cell Physiol. 48, 1524-1533.

31. Umezawa T, Sugiyama N, Mizoguchi M, Hayashi S, Moyuga F, Shinozaki Y. (2009). Type 2C Protein Phosphatases Directly Regulate Abscisic Acid-Activated Protein Kinases In Arabidopsis. Proc Natl Acad Sci. 106, 17588–17593.

32. Verrier PJ, Bird D, Burla B, Dassa E, Forestier C, Geisler M, Klein M, Kolukisaoglu HU (2008). Plant ABC proteins - a unified nomenclature and updated inventory. Trends Plant Sci. 13, 151-159
33. Wojtkowiak A, Witek K, Hennig J, Jaskolski M (2012). Two high-resolution structures of potato endo-1,3-?-glucanase reveal subdomain flexibility with implications for substrate binding. Acta Crystallogr. 68, 713-723.
34. Xiong JL, Kong HY, Akram NA, Bai X, Ashraf M, Tan RY, Turner NC (2015). 24-epibrassinolide increases growth, grain yield and β-ODAP production in seeds of well-watered and moderately water-stressed grass pea. Plant Growth Regul. 78 (2), 217-231.
35. Xoconostle-Cazares B, Ramirez-Ortega FA, Flores-Elenes L, Ruiz-Medrano R (2010) Drought tolerance in crop plants. Am J Plant Physiol. 5, 1-16.
36. Yang S, Vanderbeld B, Wan J, Huang Y (2010). Narrowing Down the Targets: Towards Successful Genetic Engineering of Drought-Tolerant Crops. Mol Plant. 3, 469-490.

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

Transporter Name	**Database Accession Number and Number of Amino acid**	**Model similarity and Template Structure**	**Ramachandran plot favoured region residues**
ABC transporter G family member 11 (*At* ABCG11)	Q8RXN0 703 AA	Model 42% Template 4i99A	78%
ABC transporter G family member 12 (*At* ABCG12)	**Q9C8K2 687 AA**	Model 37% Template4aytA	78%
ABC transporter B family member 14 (*At* ABCB14)	Q9C7F2 1247 AA	Model 49% Template 3nh6A	86%
ABC transporter G family member 22 (*At* ABCG22)	Q55DA0 615 AA	Model 32% Template 1vciA	77%
ABC transporter G family member 25 (*At* ABCG25)	**Q84TH5 662 AA**	Model 42% Template 4i99A	77%
ABC transporter G family member 32	**O81016** 1425 AA	Model 42% Template 2cvfB	

(*At* ABCG32)			
ABC transporter G family member 40 (*At* ABCG40)	Q9M9E1 1423 AA	Model 39% Template 2d62A	77%
NCED 9-cis-epoxycarotenoid-dioxygenase (NCED3)	**Q9LRR7** 599 AA	Model 90% Template 3npeA	85%
Alcohol dehydrogenase/reductase SDR (At ABA2)	**Q9C826** 285 AA.	Model 49% Template 2bgkA	91%
ABA aldehyde oxidase (AAO) (AtAAO3)	**Q7G9P4**	Template 1fo4A Template 1w4gA	84%
(BETA-1, 3-GLUCANASE 1) *At* BG1	**Q70C53** **338 AA**	1GHZ	90%

Table 2: List of core signaling complex and response to ABA binding and guard cell response

Structure Name	**Ligands**		**PDB & UniProt KB Ids**	
Ligand-free PYL2 (PYL2 RCAR14 At2g26040).	ABA	C_{15} H_{20} O_4	3KDH	080992
	PYV	C_{16} H_{13} Br N_2 O_2 S		
Crystal structure of abscisic acid receptor PYL1	ABA	C_{15} H_{20} O_4	3KAY	Q8VZS8
	PYV	C_{16} H_{13} Br N_2 O_2 S		
The Abscisic acid receptor PYR1 in complex with Abscisic Acid	ABA	C_{15} H_{20} O_4	3KN0	O49686
	PYV	C_{16} H_{13} Br N_2 O_2 S		

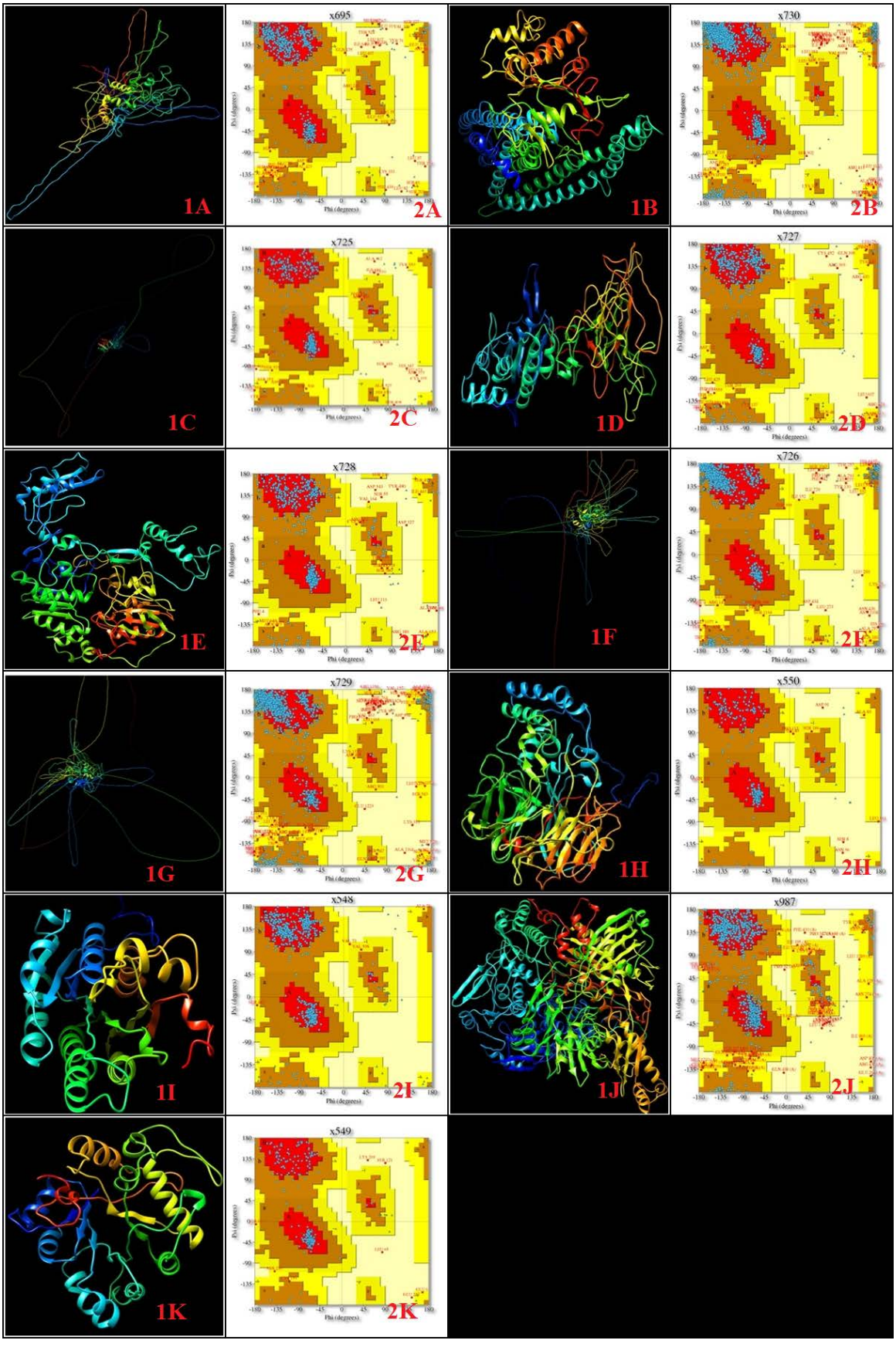
1A
x695
2A
1B
x730
2B
1C
x725
2C
1D
x727
2D
1E
x728
2E
1F
x726
2F
1G
x729
2G
1H
x550
2H
1I
x348
2I
1J
x987
2J
1K
x549
2K

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).

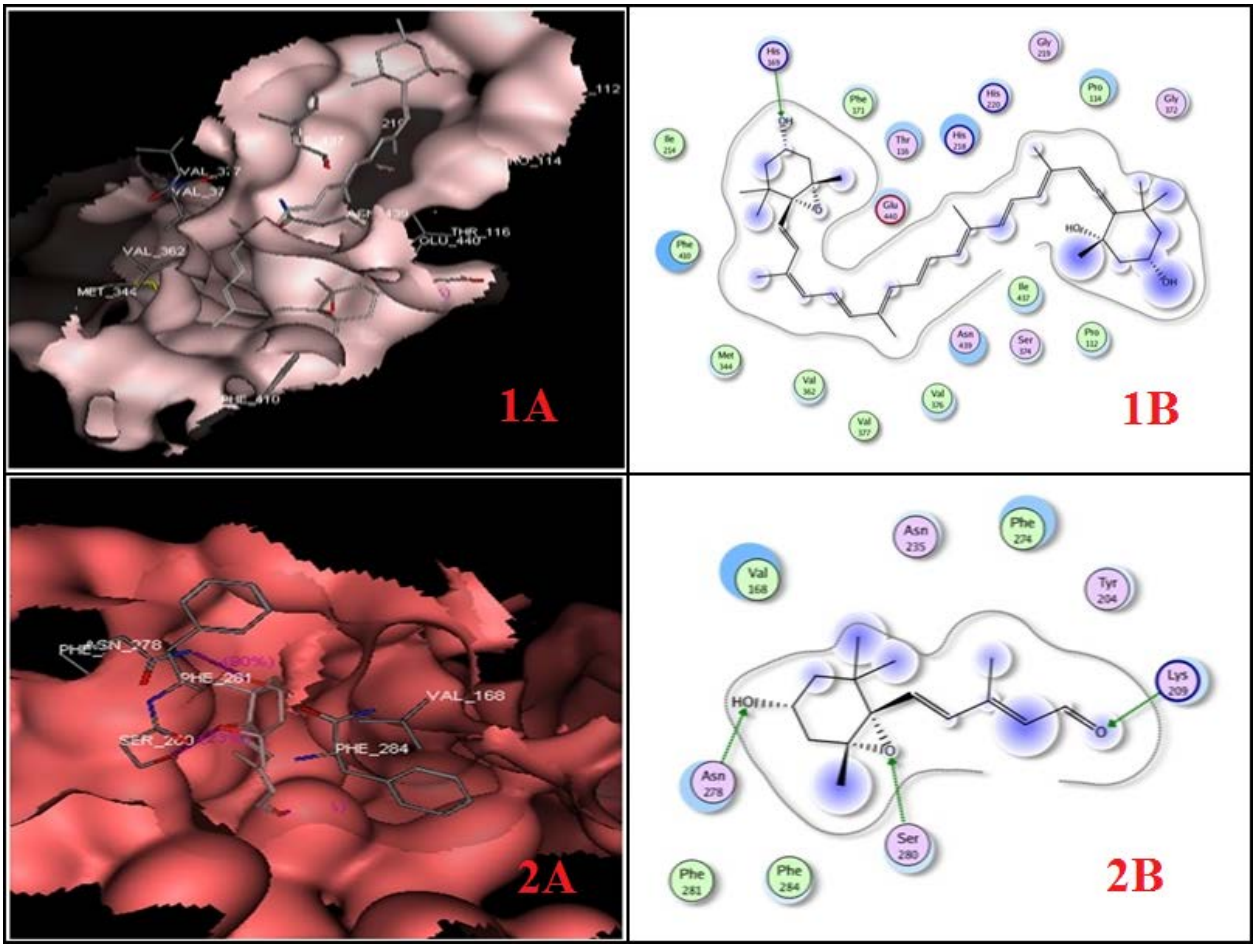

Figure 2: (1A, 1B) Docked structure and Protein Ligand interaction diagram of NCED3 and epoxycartenoid 9-cis-neoxanthin, (2A, 2B) Docked structure and Ligand interaction diagram of Alcohol dehydrogenase and xanthonin

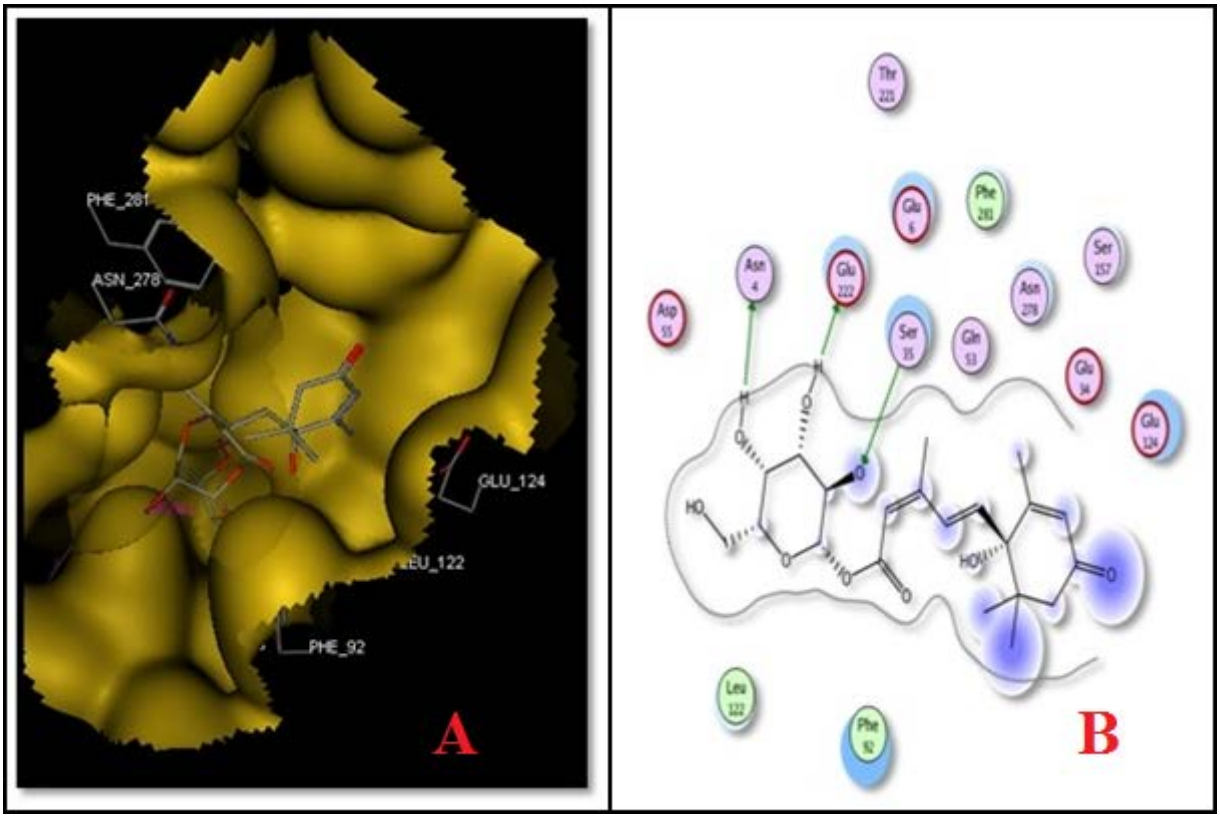

Figure 3: (A) Docked structure ofDe-conjugation Enzyme AT-BG1 and ABA**, (B)** Ligand interaction diagram of De-conjugation Enzyme AT-BG1 and ABA.

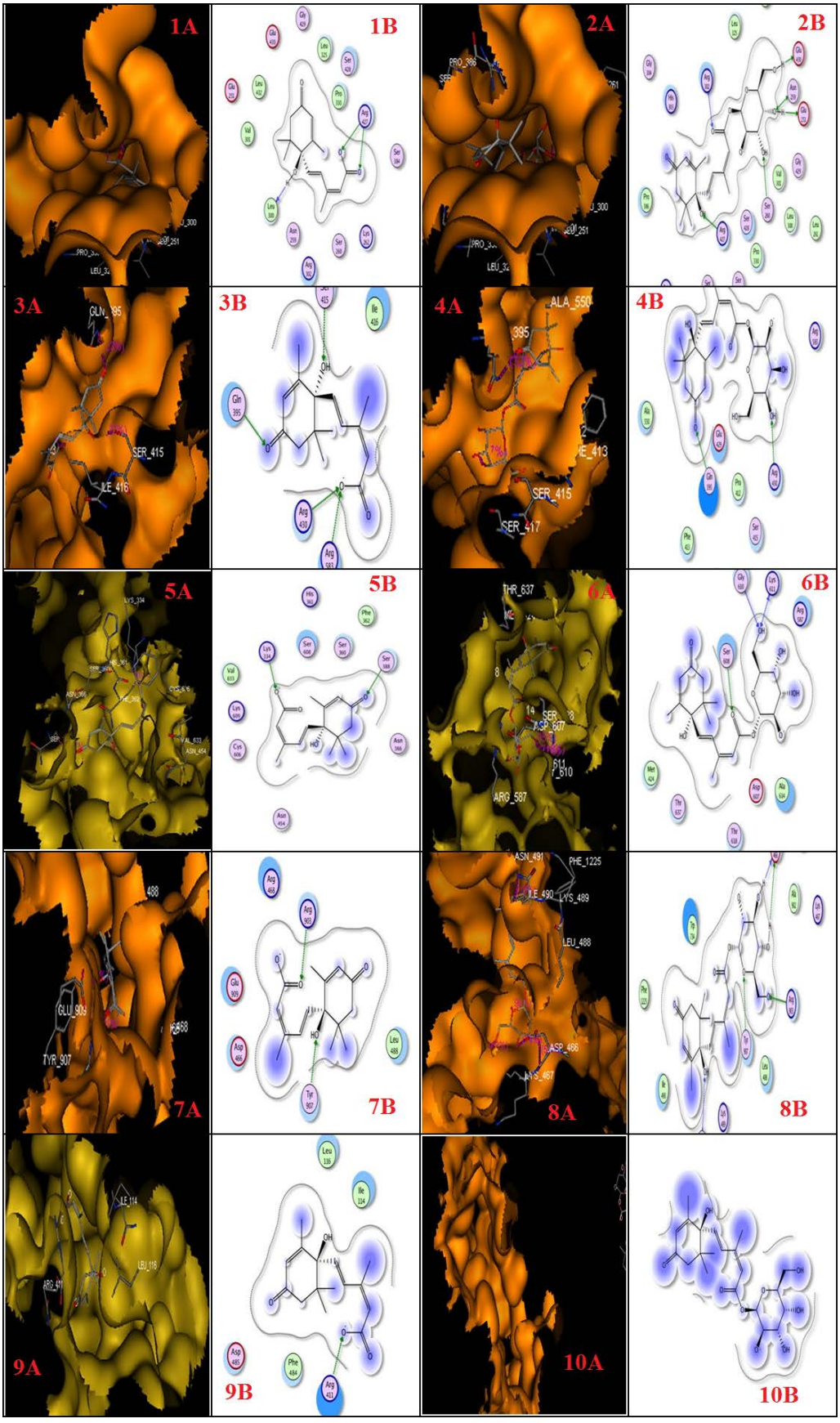
1A
1B
2A
2B
3A
GLN_395
SER_415
ILE_416
3B
4A
ALA_550
SER_415
SER_417
4B
5A
5B
6A
THR_637
ARG_587
6B
7A
GLU_309
TYR_307
7B
8A
ASN_491
PHE_1225
LYS_489
LEU_488
ASP_486
8B
9A
LEU_118
9B
10A
10B

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.

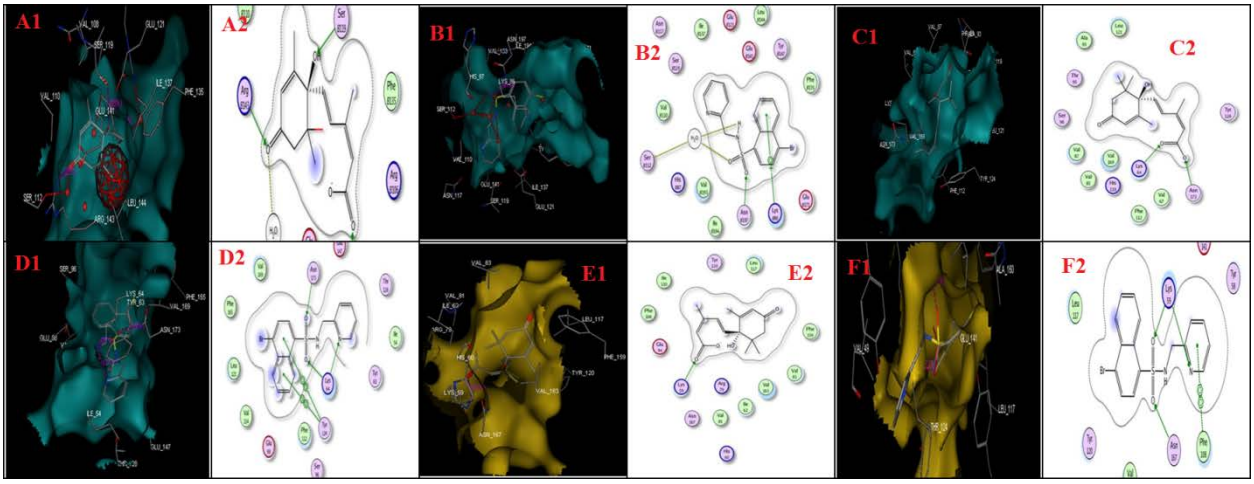

Figure 5: Docking complex and Lig X interaction diagram of PYL1 and ABA (A1, A2), PYL1 and PYV (B1, B2), PYL2 and ABA (C1, C2), PYL2 and PYV (D1, D2),PYR and ABA (E1, E2), PYR and PYV(F1, F2)