Hindawi
Advances in Agriculture
Volume 2023, Article ID 2749859, 9 pages
https://doi.org/10.1155/2023/2749859

Research Article

Effects of D128N Mutation on OsSERK2 in Xa21-Mediated Immune Complex: An *In Silico* Study

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Received 13 February 2023; Revised 18 May 2023; Accepted 12 October 2023; Published 28 November 2023

Academic Editor: Nasim Ahmad Yasin

Receptor-like kinases (RLKs) are plant proteins that form signaling circuits to transduce information through the plant cell membrane to the nucleus and activate processes that direct growth, development, stress response, and disease resistance. Upon sensing various environmental stress stimuli, RLKs interact with specific targets and recruit several other proteins to initiate the defense mechanism. Among many RLK subfamilies, leucine-rich repeat RLKs (LRR-RLKs) are the largest. Xa21, a member of LRR-RLK, is a vital receptor protein in rice plants that binds with bacterial RaxX21-sY, whereas OsSERK2 is a somatic embryogenic receptor kinase (SERK) that acts as a coreceptor in this process. This study focuses on the effect of a substitution mutation of aspartate128 with asparagine128 (D128N) in OsSERK2 on the interdependent binding pattern of the Xa21, RaxX21-sY, and OsSERK2 D128N proteins. The *in silico* results showed that the D128N mutation in OsSERK2 can significantly change the interaction pattern of the critical residues of the OsSERK2 and affects its receptor-ligand (Xa21-RaxX21-sY) interaction in the complex. These findings are expected to significantly contribute to the study of the structural basis of Xa21-mediated immunity and the first layer of plant defense mechanisms, thereby aiding further research on these structures and their phenotypic implications.

1. Introduction

As multicellular sessile organisms that must respond to dynamic environments, plants need to effectuate and react toward numerous internal signals [1] to achieve their growth and metamorphosis [2, 3], as well as to distinguish abundant input signals from their surroundings [4]. Most of these signaling cues are detected on the cell periphery [5, 6], and plants have developed an idiosyncratic group of receptor-like kinases (RLKs) that can transmit extracellular signals throughout the membranes [7, 8].

These receptors are defined as a combination of a signal peptide, an extracellular domain, a transmembrane domain, and a cytoplasmic kinase domain together with the serine/threonine consensus sequence [9]. In *Arabidopsis thaliana*, the number of leucine-rich repeat RLKs (LRR-RLKs) is numerous, originating from 13 subfamilies [10–14]. These LRR-RLKs bind to somatic embryogenesis receptor kinases (SERKs) and form dimers [15, 16]. The binding of SERKs with their respective LRR-RLKs is either ligand-dependent or independent. The best characterized SERK protein is BRI1-associated kinase 1 (BAK1), which binds with LRR-RLK FLS2, EFR, and BRI1. It forms a heterodimer, where ligand binding induces this heterodimerization [16–18]. BAK1 may also bind with RLK7 [19, 20], CORE [21, 22], and other similar receptors and thus become a common SERK coreceptor protein for many other LRR-RLKs and plays a key role as a regulator in plants' innate immunity [23].

Among the LRR-RLKs, Xa21 is an important receptor protein of rice plants that binds with SERK OsSERK2 and forms a heterodimer. The Xa21 ectodomain contains 23 LRRs, a single transmembrane domain with a kinase domain followed by one juxtamembrane (JM) domain [24, 25]. RaxX21, a bacterial peptide secreted by *Xanthomonas oryzae* (*Xoo*), acts as a ligand for Xa21, the ectodomain of which binds with this pathogen-associated molecular pattern (PAMP)

molecule. *Xoo* is the causative agent for bacterial leaf blight disease in the rice plant [26, 27]. The gene *XA21* confers resistance to the multiple isolates of *Xoo* and shows genetic and phenotypic diversity with the other rice plants [28–31].

When RaxX21 is secreted, sulfation occurs in its tyrosine region (RaxX21-sY) [32], which, as a result, gives more stability to the bacterial peptide [33]. After RaxX21-sY binds with the ectodomain of Xa21, it recruits coreceptor SERK protein OsSERK2 and activates the defense signal by forming a heterodimer structure [34, 35]. Interestingly, the Xa21 also can interact with its coreceptor OsSERK2 without the presence of RaxX21-sY [35]. Besides associating with Xa21, OsSERK2 also binds with other rice proteins such as OsBRI1, which is important for brassinosteroid-regulated development, as well as OsFLS2; and Xa3, which are important for initiating the defense mechanism in rice plants [35, 36].

In rice plants, OsSERK2 acts as a functional homolog of BAK1, transphosphorylating the kinase domains of these rice receptor proteins [35, 37]. Additionally, the structure of OsSERK2 is highly similar to the BAK1 coreceptor. The short-curved solenoid ectodomain of OsSERK2, composed of an N-terminal LRRNT and five LRRs, has a 67% similarity with the BAK1 [37, 38]. Moreover, the binding pattern of OsSERK2 and BAK1 reveals that both of the SERK coreceptors bind with their respective pattern recognition receptor proteins (PRRs) at the concave side of their ectodomains [38–41].

The mutation in BAK1 of Asp122 to asparagine alters its interaction with its respective PRRs [42]. A recently produced crystallographic structure of FLS2-BAK1 and BRI1-BAK1 has revealed that, although mutation in Asp122 alters the overall interaction, there is no direct contact of this particular residue with FLS2 or BRI1 [38, 40]. Moreover, this residue was not predicted to be glycosylated in the D122N mutant of BAK1 [37, 43], which suggests that mutation in this particular residue indirectly influences binding by altering the position of residues near it. Asp128 residue of OsSERK2 forms hydrogen bonds with Ser126 and a salt bridge with Arg152. Mutation in Asp128 alters the binding of this residue with Arg152; in this case, Arg152 interacts with the residue Glu174 [37]. This aspartate is conserved among all the rice SERK proteins and *A. thaliana*. Asp128 in rice OsSERK2 is the corresponding residue of BAK1 and is located in the LRR3 region of OsSERK2 [37]. However, the impact of this mutation in the Xa21 LRR-RaxX21-sY-OsSERK2 LRR complex is yet to be elucidated. We hypothesize that the substitution mutation of aspartate128 with asparagine128 (D128N) in OsSERK2 affects the interdependent binding pattern of the Xa21, RaxX21-sY, and OsSERK2 proteins in rice plants.

Molecular dynamics (MD) simulation is a useful method for studying the dynamic characteristics of proteins at the atomic level [44]. With the help of modern computers, simulations can now be performed for several nanoseconds, allowing for the identification of the major factors contributing to atomic fluctuations [45]. This makes MD simulations an appealing method for locating flexible areas in proteins that could be targeted for stability improvement [45, 46]. Additionally, the flexibility of protein structures plays a key role in determining their interactions with other proteins. By using atomic-level MD simulations, it is possible to analyze both the structure and dynamics of protein–protein complexes [47]. Numerous studies have been performed to date to see the effect of mutation on protein–protein interaction using the MD simulation [48–51].

In this study, we employed a detailed *in silico* approach, including MD simulation, to analyze the impact of the Asp128 mutation in OsSERK2 in the Xa21-mediated immune complex. The objectives of this study were to investigate the effect of this mutation in OsSERK2 on its interaction with Xa21 and RaxX21-sY, determine the impact of the mutation on the receptor–ligand interaction between Xa21 and RaxX21-sY in the complex, and provide insight into the molecular mechanisms underlying the receptor–ligand interactions in plant defense response pathways.

2. Materials and Methods

2.1. Mutating Coreceptor OsSERK2. In our previous study, OsSERK2 LRR was docked with the PRR Xa21 LRR and PAMP RaxX21-sY [24]. Multiple *in silico* modeling approaches were used to predict the 3D model of a full plant PRR protein, Xa21, and only the LRR part of Xa21 was examined for the interaction [24]. OsSERK2 LRR (PDB ID: 4q3g) was obtained from Protein Data Bank [37, 52]. In the current study, the OsSERK2 LRR of that complex was point mutated using rotamer tools of UCSF Chimera [53], where the Dunbrack library was used [54]. The point mutation was performed to mutate 128th positioned aspartate into asparagine. The most probable form of asparagine (probability = 0.473) was selected for this position, and then a new PDB file was created for MD simulation. The mutated coreceptor was named OsSERK2 D128N, and these three protein-containing complex was named Xa21 LRR-RaxX21-sY-OsSERK2 D128N LRR.

2.2. MD Simulation of Xa21 LRR, RaxX21-sY, and OsSERK2 D128N LRR Complex. The GROMACS software suite (version 5.1) [55] was used to carry out the MD simulation process of the complex Xa21 LRR-RaxX21-sY-OsSERK2 D128N LRR. GROMOS 54a7 [56] united force field was applied for the simulation process. A cubic box with a distance of 1 Å between the surfaces and edges of the complex was set for the solvation. The complex was then solvated using the SPC water model [57] using the *gmx solvate* tool, and *gmx genion* was used to neutralize the system, and then energy minimization was performed using the *gmx grompp* tool. Then, the system was equilibrated for 2 ns NVT and 1 ns NPT, respectively, setting the temperature at 300 K and pressure at 1 atm. Finally, a 100 ns MD simulation was run for the system using the *gmx grompp* tool. To analyze the stability of the complex over the simulation period, the GROMACS *gmx rms* tool was used, and the GROMACS *gmx rmsf* tool was used to analyze individual residual fluctuations. Moreover, to analyze the radius of gyration (Rg) and hydrogen bonds, the GROMACS *gmx gyrate*, and *gmx hbond* tool were used, respectively.

2.3. Investigation of the Interaction of Xa21 LRR and RaxX21-sY with OsSERK2 D128N LRR. The binding pattern of OsSERK2 D128N LRR with its PRR Xa21 LRR and respective PAMP RaxX21-sY was examined using UCSF Chimera. To analyze different types of interactions alongside salt-bridge, the protein interaction calculator (PIC) [58] and evaluating the salt bridges in proteins (ESBRI) [59] web tools were used. The PIC website uses the coordinates of a protein's or assembly's 3D structure to calculate numerous interactions, including disulfide bonds, contacts between hydrophobic residues, ionic interactions, hydrogen bonds, aromatic–aromatic interactions, aromatic–sulfur interactions, and cation-interactions inside protein or between proteins in a complex. Interactions are computed using standard and publicly available criteria. The convenience of accessing inter-residue interaction calculations in a single location is a benefit of employing a PIC server. It also calculates the available surface area and the distance between a residue and the protein's surface. In contrast, ESBRI is a web-based software tool used for calculating salt bridge interactions of protein complexes [59].

2.4. Analysis of the Mutated Residue of OsSERK2 D128N LRR. Using the UCSF Chimera *clash and contact* tool, the interactions of Asp128 of OsSERK2 and Asn128 of OsSERK2 D128N with their neighboring residues were observed, where atoms of the selected residues were designated. Also, by using the UCSF Chimera *distance measurement* tool, the distances between interactive atoms were visualized.

3. Results and Discussion

3.1. Changes in the Behavior of the Mutated Residue. We first checked our designed OsSERK2-mutated structure with the crystal OsSERK2 D128N (4q3i) structure and found both structures similar (Figure S1). We then proceeded with our designed structure to do the MD simulation. The previously solved crystal structure of OsSERK2 showed that due to this mutation, the salt bridge interaction of Arg152 with Asp128 was demolished [37]. Our study also found the same phenomena (Figures 1(a) and 1(b)). We observed that Arg152 of OsSERK2 D128N shifted its salt bridge interaction with Glu174 (Figure 1(c)) (Table S1), which also supports the previous structural study [37]. Again, we noticed Ser126 had a hydrogen bond with Asp128 in OsSERK2 (Figure 1(b)), but in the case of Os-SERK2 D128N, that interaction was discontinued (Figure 1(c)), which also justifies the previous study [37]. These changes in intraprotein interaction might lead to several changes in the interaction of the prominent residues of the complex of OsSERK2 with its PRR Xa21 and respective PAMP RaxX21. Previous studies have shown that a similar mutation in BAK1 (*BAK1 elg*, which results in aspartate 122 substitutions with asparagine) maintains its interaction with its receptor BRI1 even if their concentration is very low [42]. However, in a recent structural and biochemical study, it was observed that this mutation can disrupt BAK1's ability to interact with the ectodomain of BRI1 receptor pseudo kinases [60] and can lead to the stabilization of certain SERK3 residues [38, 60]. Moreover, this mutation could also lead to the impairment of its (*BAK1 elg*) ligand-induced association with FLS2 [42].

3.2. Changes in Interactions of Prominent Residues of the Proteins. Our previous study showed that Arg185 and Arg230 from Xa21, Val2, and Lys15 from RaxX21-sY, and Lys164 from OsSERK2 act as prominent residues for the wild Xa21 LRR-RaxX21-sY-OsSERK2 LRR complex (having wild OsSERK2 LRR) [24]. For this wild complex, Arg185 of Xa21 makes a hydrogen bond with Leu52 of OsSERK2, and Arg230 makes a hydrogen bond with Asp6 of RaxX21-sY within 3.5 Å (Figure 2(a)). Moreover, Val2 and Lys15 of RaxX21-sY form hydrogen bonds with Asn331, and Cys382 of Xa21, respectively (Figure 2(b)). Lys164 of OsSERK2 interacts with Asp565 of Xa21 by forming an ionic bond within 4 Å (Figure 2(c)) [24].

On the contrary, due to the D128N mutation in OsSERK2, these prominent residues changed their interaction patterns. The previous interaction of Arg185 and Arg230 of Xa21 got discontinued; instead, Arg230 of Xa21 established a new hydrogen bond with Asp56 of OsSERK2 D128N (Figure 2(d)) (Table S2). Val2 from RaxX21-sY formed a hydrophobic interaction with Phe354 of Xa21, and Lys15 bonded with Asn383 of Xa21 by forming a hydrogen bond (Figure 2(e)) (Tables S2 and S3). No bond formation of Lys164 from OsSERK2 D128N is found within 4 Å in this mutated complex (Figure 2(f)). These changes in interactions of the prominent residues point to the overall interaction pattern in the complex due to the mutation. Our findings support the previous finding of the mutation effect on the similar protein SERK3, where it was observed that mutation in this protein hampers the interaction between $SERK3^{D122N}$ and BRI1 receptor pseudo kinases. In the finding, it was shown that $SERK3^{D122}$ plays an important role in the interaction between $SERK3^{R146}$ and $BRI1^{E749}$. This is due to the fact that $SERK3^{D122}$ stabilizes the conformation of $SERK3^{R146}$, which then makes polar contact with $BRI1^{E749}$. If the corresponding Asp128 is mutated to asparagine in rice SERK2, it alters these interactions. $SERK3^{D122}$ also positions $SERK3^{E98}$ for interaction with $BRI1^{T750}$, which is replaced by isoleucine in bri1-102 loss-of-function mutants. These findings suggest that $SERK3^{D122}$ is in contact with several critical residues involved in the formation of the brassinosteroid signaling complex [60]. Also, the corresponding D122N mutation in BAK1 might lead to the impairment of its (*BAK1 elg*) ligand-induced association with FLS2 [42].

3.3. Root Mean Square Deviation (RMSD), RMS Fluctuation (RMSF), Rg, and Hydrogen Bonds of the Xa21 LRR-RaxX21-sY-OsSERK2 D128N LRR Complex. The stability of the complex was measured in terms of deviations by analyzing the RMSD after performing a 100 ns MD simulation. The backbone of the complex showed the least variable RMSD in the simulated system. It deviated from 0.0004 to 0.75 nm during the entire simulation period (Figure 3(a)). The complex's standard deviation was 0.05 nm. The average deviation of the complex is 0.49 nm, which indicates that the Xa21 LRR-RaxX21-sY-OsSERK2 D128N LRR complex is stable and favorable. Moreover, we utilized Rosetta [61] to relax and measure the energy of the after-simulated complexes, and it also aligns with the results we got. It showed a lower total

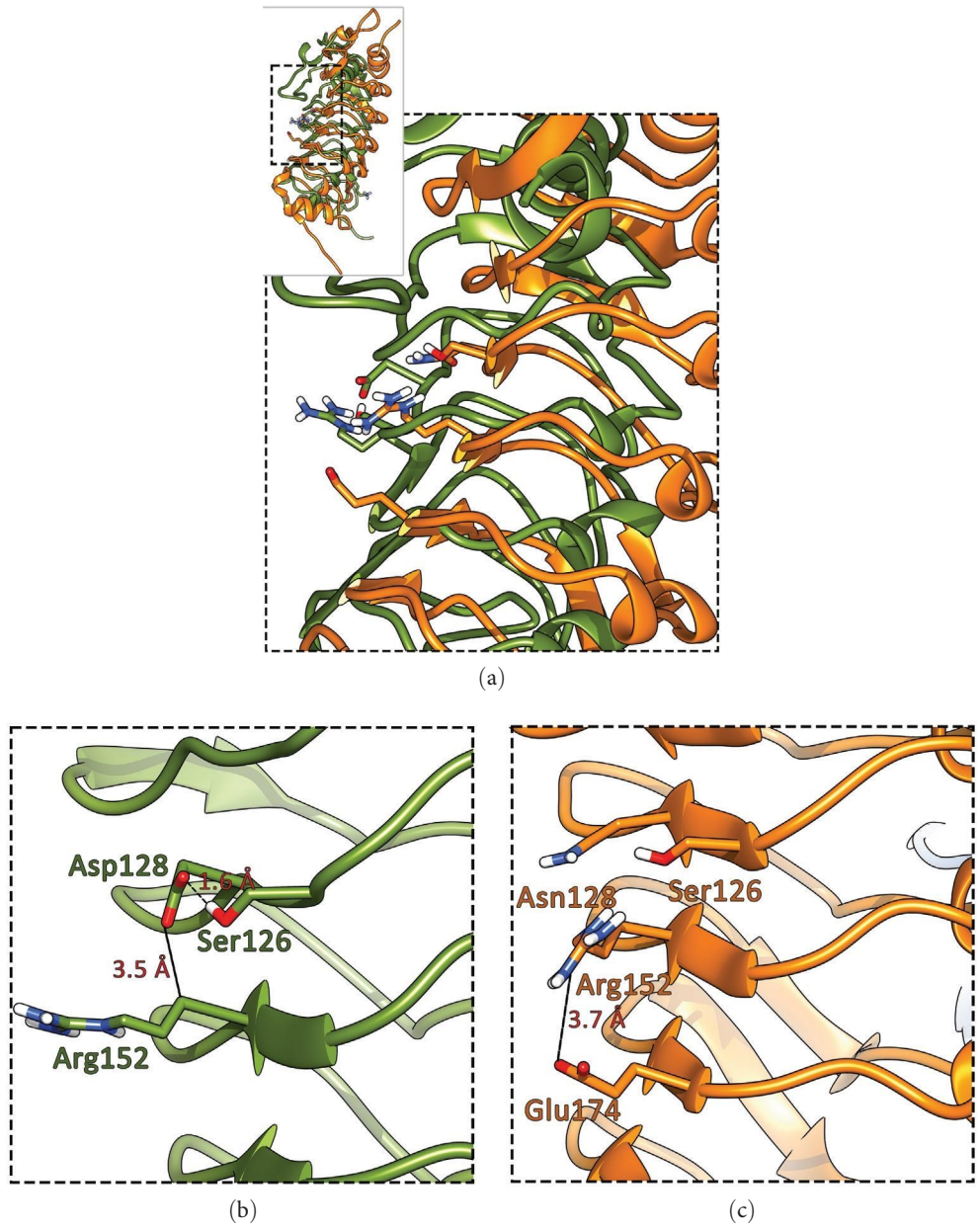

Figure 1: Changes in the interaction of the mutated residue with the neighboring residues. (a) The superimposed cartoon structure of OsSERK2 and OsSERK2 D128N focused on the mutated area. (b) Cartoon structure of OsSERK2, where Asp128 formed an s hydrogen bond with Ser126 and a salt bridge interaction with Arg152 of itself. (c) Cartoon structure of OsSERK2 D128N, where mutated residue Asn128 has no interaction with Ser126 and Arg152. Rather, Arg152 formed a salt bridge interaction with Glu174 (Table S1). Cartoon: OsSERK2 LRR (green), OsSERK2 D128N LRR (orange); stick: Asp128/Asn128, Arg152, Ser126, Glu174; both structures were evaluated after 100 ns MD simulation.

score (1,685.627 kJ/mol) for the mutated complex where fa_atr (Lennard–Jones attractive between atoms in different residues) contributed the most (−4,728.319 kJ/mol) to minimize the energy (Table S4).

From the 100 ns MD trajectories, the RMSFs of the residues of Xa21 LRR, RaxX21-sY, and OsSERK2 D128N LRR were calculated. Analyses of the overall results revealed that most residues fluctuated by less than 0.21 nm for Xa21 LRR and 0.13 nm for OsSERK2 D128N LRR (Figures 3(b) and 3(c)). Moreover, the residues stated as prominent with low RMSFs, in our previous study [19], showed slightly higher in the mutated complex. For Xa21, Arg185, and Arg230, which showed a different binding pattern, exhibited high RMSF than the average RMSF of Xa21 LRR (Figure 3(b)). Also, for RaxX21-sY, Val2, and Pro14, which were considered important residues for binding with Xa21, showed similar phenomena. Furthermore, Lys164 of OsSERK2 D128N had a low RMSF with a value of 0.14 nm (Figure 3(c)). On the other hand, the mutated residue Asn128 of OsSERK2 D128N showed a very low RMSF value (0.08 nm) alongside Arg152

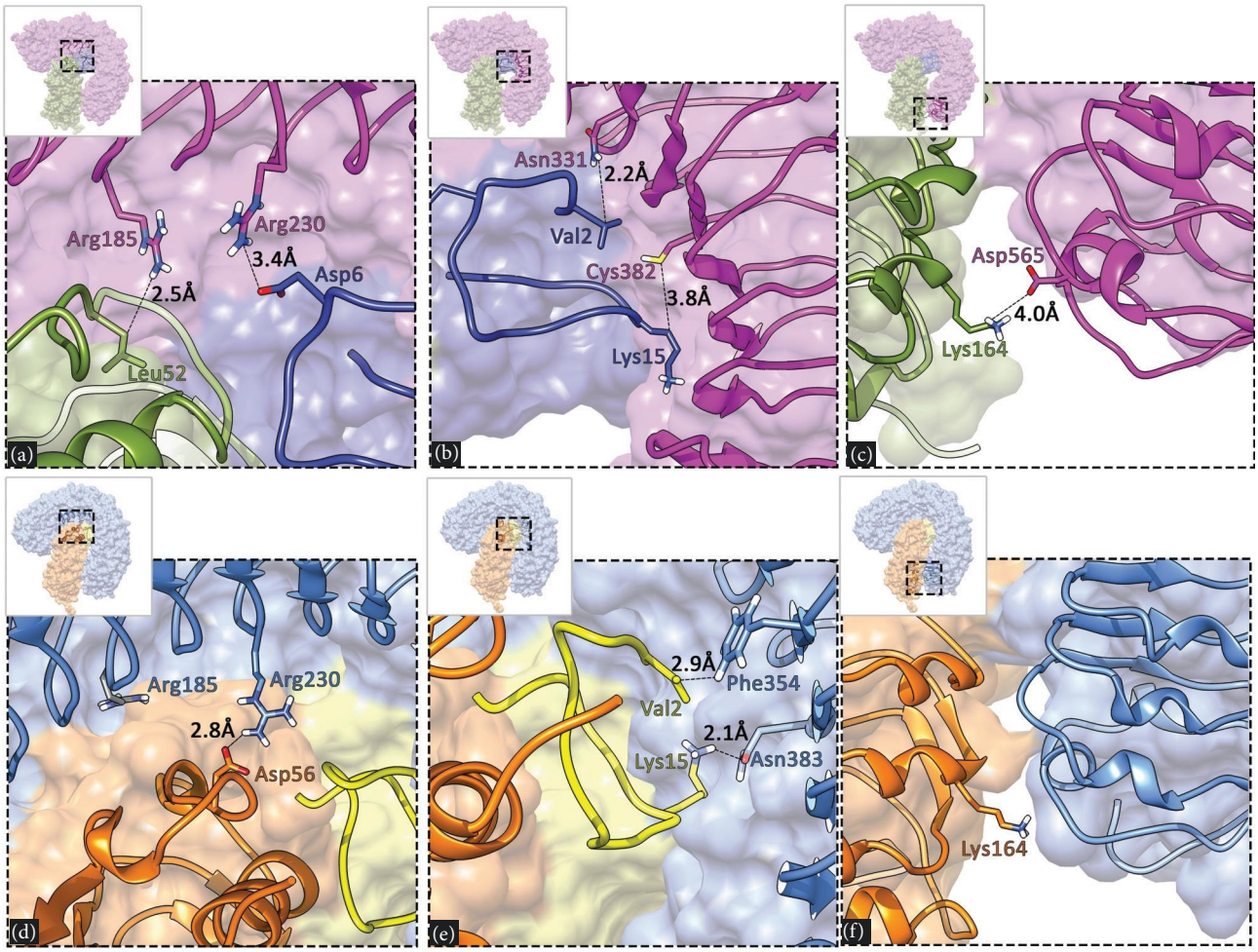

FIGURE 2: Changes in interactions of prominent residues of the complex. (a) Arg185 and Arg 230 of Xa21 form hydrogen bonds with Leu52 of OsSERK2 and Asp6 of RaxX21-sY in the wild complex. (b) Val2 and Lys15 of RaxX21-sY form hydrogen bonds with Asn331 and Cys382 of Xa21 in the wild complex. (c) Lys164 of OsSERK2 forms an ionic bond with Asp565 of Xa21 in the wild complex. (d) Arg185 of Xa21 discontinues the previous interaction, and Arg230 of Xa21 forms a new hydrogen bond with Asp56 of OsSERK2 D128N in the mutated complex (Table S2). (e) Val2 and Lys15 of RaxX21-sY form a new hydrophobic interaction with Phe354 and hydrogen bond Asn383 of Xa21 in the mutated complex (Tables S2 and S3). (f) Lys164 of OsSERK2 D128N shows no interaction within 4.0 Å in the mutated complex. Cartoon: Xa21-RaxX21-sY-OsSERK2 complex where Xa21 (magenta), RaxX21-sY (blue), and OsSERK2 (green), and Xa21-RaxX21-sY-OsSERK2 D128N complex where Xa21 (light blue), RaxX21-sY (yellow), and OsSERK2 D128N (orange); stick: prominent and interacting residues of both complex; all the complexes were investigated after 100 ns MD simulation.

and Ser126. The overall RMSD and RMS fluctuation of Xa21 LRR, RaxX21-sY, and OsSERK2 D128N LRR seem less fluctuating and more stable than the wild complex.

The compactness of the complex, which can be determined by analyzing the radius of gyration, did not change noticeably over time, suggesting that the protein was not going through significant conformational changes. The mean of Rg of the complex was 3.22 nm, where 3.20–3.25 nm compactness was observed after 25 ns of simulation (Figure 3(d)).

For Xa21 LRR and RaxX21-sY, the total number of hydrogen bonds remained largely constant during the simulation. However, the number of hydrogen bonds between Xa21 LRR and OsSERK2 D128N LRR is getting higher, indicating more stronger interaction. For Xa21-LRR and RaxX21-sY, around five hydrogen bonds, were observed after 25 ns period till the end of the MD simulation (Figure 3(e)). On the other hand, between Xa21 LRR and OsSERK2 D128N LRR, the number of hydrogen bonds got increased after 25 ns, and more than 15 hydrogen bonds were observed at the end of the simulation (Figure 3(f)). These findings indicate that due to mutation in OsSERK2, Xa21 LRR interacts more prominently with OsSERK2. These results also support the previous work, where it was observed that mutation in the coreceptor could lead to the stabilization of certain SERK3 residues. However, it also was observed that mutation in this protein hampers the interaction between $SERK3^{D122N}$ and BRI1 receptor pseudo kinases [38, 60]. The corresponding D122N mutation in BAK1 also led to the impairment of its (*BAK1 elg*) ligand-induced association with FLS2 [42].

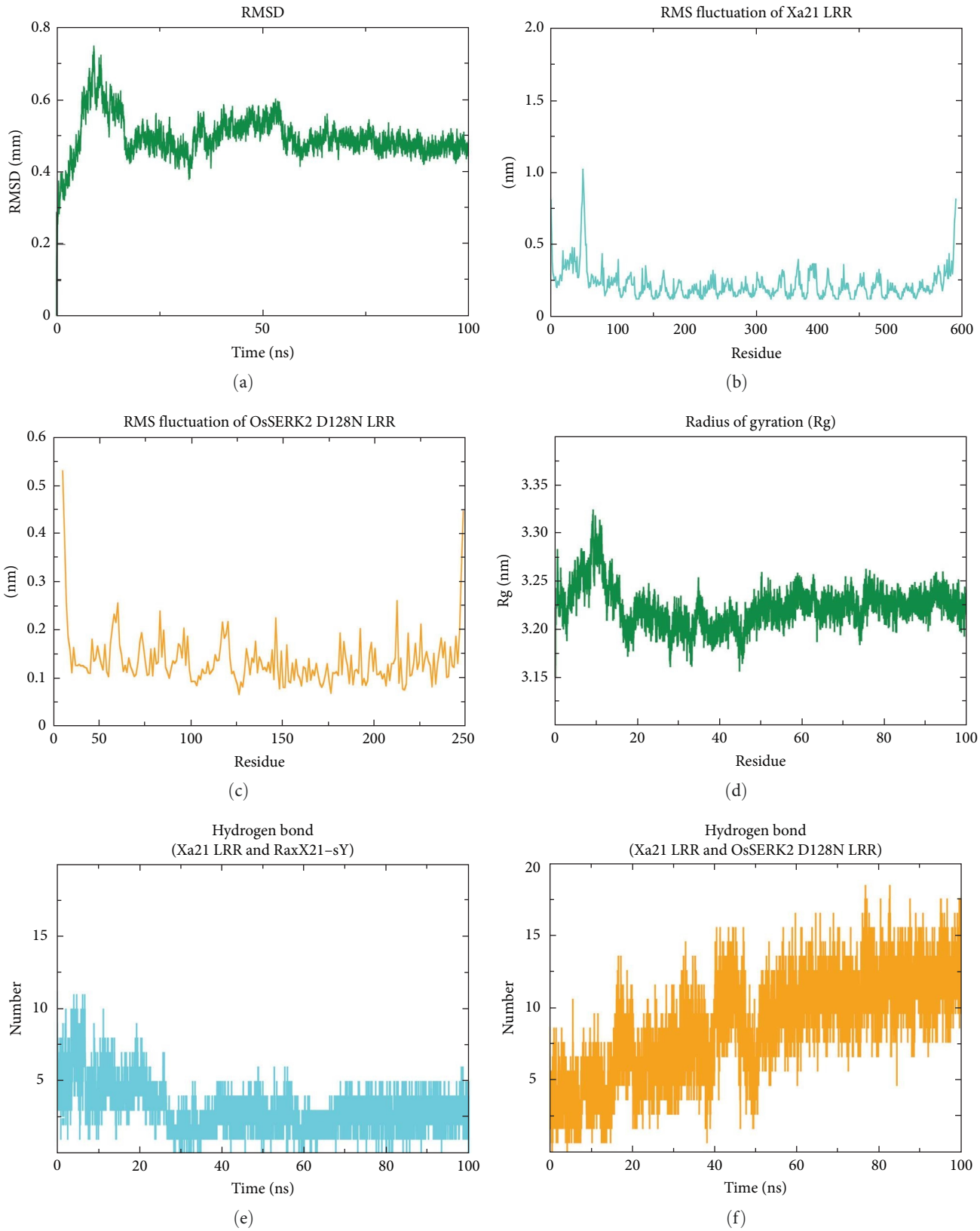

Figure 3: RMS deviation (RMSD), RMS fluctuation (RMSF), radius of gyration (Rg), and hydrogen bonds. (a) RMSD of the complex Xa21 LRR-RaxX21-sY-OsSERK2 D128N LRR from the 100 ns trajectory. (b) RMS fluctuations of the residues of Xa21 LRR from the 100 ns trajectory. (c) RMS fluctuations of the residues of OsSERK2 D128N LRR from the 100 ns trajectory. (d) Radius of gyration (Rg) of the complex Xa21 LRR-RaxX21-sY-OsSERK2 D128N LRR from the 100 ns trajectory. (e) Hydrogen bond between Xa21 LRR and RaxX21-sY from the 100 ns trajectory. (f) Hydrogen bond between Xa21 LRR and OsSERK2 D128N LRR from the 100 ns trajectory.

4. Conclusions

In this study, we have extensively investigated the effects of a D128N mutation in OsSERK2 in the Xa21-mediated immune complex. One of the key findings was the behavioral changes in the amino acid residues of the Xa21, RaxX21-sY, and OsSERK2 proteins due to the mutation. Though with this *in silico* study, we successfully showed the effect of D128N mutation in OsSERK2 itself and in the Xa21-mediated defense complex, a wet-lab structure-based approach (X-ray crystallography or cryogenic electron microscopy) is crucial to verify these data further. Also, an investigation of the changes in the phenotypic expression of the rice plant due to this mutation is pivotal. The exploration of this substitution mutation in OsSERK2 that affects its interaction with Xa21 and RaxX21-sY highlights the complexity of these signaling networks and suggests potential targets for engineering plant resistance to bacterial pathogens. We firmly believe these findings will significantly contribute to those structures and phenotypic study and, therefore, can aid the scientific community in studying further the structural basis of Xa21-mediated immunity and, in general, the first layer of the plant defense mechanism.

Data Availability

The data used to support the fundings of this study are included in the article and within the supplementary information file.

Disclosure

A preprint has previously been published [62].

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

S.H. and M.F.A. conceptualized and supervised the project. R.I.A. and M.M. performed the computational work and analysis. M.M. designed the study. R.I.A. and M.M. co-wrote the manuscript. S.H. and M.F.A. revised the manuscript. All authors have read and agreed to publish the manuscript.

Acknowledgments

The authors thank Marzia Khatun, Tasfia Tawhid Supti, Ramen Chowdhury, and Tanvir [21] for their continuous support throughout the research.

Supplementary Materials

Supplementary material contains protein interaction calculation and scores measured by Rosetta after and before the relaxation of the complexes. Figure S1: superimposed structure of our designed OsSERK2 and the crystal OsSERK2 D128N (4q3i). Table S1: salt bridge interaction of different residues from OsSERK2 D128N LRR. Table S2: side chain–side-chain hydrogen bonds between Xa21 LRR (chain A), RaxX21-sY (chain B), and OsSERK2 D128N LRR (chain C) within 3 Å. Table S3: hydrophobic interactions between Xa21 LRR (chain A), RaxX21-sY (chain B), and OsSERK2 D128N LRR (chain C) within 5 Å. Table S4: measuring the score of the complexes before and after relaxation using Rosetta. Table S5: software and server used in this study. (*Supplementary Materials*)

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