Chapter 5

Identification and Analysis of SOS2 and SOS3 gene in *Brassica juncea* var CS52.

5.1 Background

Plants have to endure the adverse climatic conditions because of their sessile nature. Soil salinity is a major adverse environmental factor limiting the agricultural productivity by reducing the plant growth and development (Epstein et al. 1980). High concentration of salt in soil results in perturbation of ionic steady state not only for Na\(^+\) and Cl\(^-\) but also for K\(^+\) and Ca\(^{2+}\) (Niu et al. 1995). Uptake of K\(^+\) is also affected by the increased Na\(^+\) content by competing for its enzyme binding sites (Quan et al. 2007). To attain the salt tolerance, plants maintain ionic homeostasis by keeping high level of K\(^+\) and low level of Na\(^+\) in the cytoplasm.

The molecular and genetic characterization of several salt overly sensitive (*sos*) mutants in *Arabidopsis thaliana*, that were defective in K\(^+\)/Na\(^+\) homeostasis, showed the importance of this ionic homeostasis pathway activated in salt stress condition (Liu & Zhu 1980; Liu et al. 2000; Shi et al. 2000). It was observed that *sos1*, *sos2* and *sos3* mutants were specifically hypersensitive to Na\(^+\) and Li\(^+\) ions. The SOS pathway is known to be defined by three protein components SOS1, SOS2 and SOS3. SOS1 is primarily expressed at the root tip epidermis and in xylem parenchyma at the xylem-symplast boundary.
SOS1 also control long-distance Na\(^+\) transport between roots and leaves by mediating the loading and unloading of Na\(^+\) in the xylem and phloem (Zhu et al. 2007). In Arabidopsis, Na\(^+\)/H\(^+\) antiporter present in plasma membrane encoded by SOS1 gene (Shi et al. 2000; Shi et al. 2002; Qiu et al. 2002; Quintero et al. 2002). Mutations in SOS1 rendered Arabidopsis extremely sensitive to growth in high level of NaCl (Zhu et al. 1998; Wu et al. 1996). Activation via phosphorylation of the Na\(^+\)/H\(^+\) antiport activity of SOS1 by salt stress is controlled by SOS3 and SOS2 proteins (Qiu et al. 2002; Quintero et al. 2002).

SOS2 is a serine/threonine protein kinase with a unique regulatory domain at the carboxy terminus and an amino-terminal catalytic domain (Liu et al. 2000). The carboxy-terminal regulatory domain of SOS2 interacts with SOS3 through the FISL motif (Guo et al. 2001). In the presence of calcium, SOS3 activates the substrate phosphorylation activity of SOS2 (Halfter et al. 2000). The presence of SOS3-like Ca\(^{2+}\) sensor/binding proteins (SCaBPs)/calcineurin B-like (CBL) proteins, which was found very similar to the SOS3 proteins (Gong et al. 2004; Kolukisaoglu et al. 2004). SOS3 does not have any enzymatic activity of its own but Ca\(^{2+}\) binding and myristoylation are required for SOS3 function in salt tolerance (Ishitani et al. 2000). SOS3 has been shown to interact physically with SOS2 in yeast two-hybrid assay as well as in vitro. sos3/sos2 double-mutant analysis also indicates that SOS3 and SOS2 function in the same pathway (Halfter et al. 2000).

Earlier SOS3-like Ca\(^{2+}\) sensor/binding proteins were identified in Arabidopsis. SOS3 is the founding member of a unique family of ten EF-hand type calcium binding proteins (Guo et al. 2001) in Arabidopsis. This family of proteins share significant similarities to the B-subunit of calcineurin and animal neuronal calcium sensors (Guo et al. 2001; Ishitani et al. 2000).

Arabidopsis and Brassica have diverged 14.5 - 20.4 million years ago from a common ancestor (Bowers et al. 2003). In the family Brassicaceae, co-linear chromosome segments were observed using comparative genetic mapping analysis (Kowalski et al. 1994; Lagercrantz et al. 1996; Schmidt et al. 2001). Similar approaches have revealed linkage arrangements between Arabidopsis and Brassica oleracea (Lukens et al. 2003). It has been observed that the genomes of Brassica
species have duplicated, perhaps triplicated counterparts of the corresponding homeologous segments of *Arabidopsis* (O’Neill & Bancroft 2000; Rana *et al*. 2004). *Brassica* is one of the core genera in the family Brassicaceae. Since the size of *Brassica* genome (529-696 Mb for the diploids and 1068-1284 Mb for polyploids) (Johnston *et al*. 2005) is much larger than that of *Arabidopsis* (125 Mb), therefore it is speculated that in *Brassica* some novel gene interactions might have evolved through the process of sub-functionalization and/or neo-functionalization of paralogs (Lynch & Force 2000; He & Zhang 2005; Roth *et al*. 2007). Recently initiated Mulational Brassica genome project (MBGP)(www.brassicagencode.org) and *Brassica rapa* Genome sequencing project (BrGSP) (www.brassicarapa.org) are aiming to completely sequence the genome of *Brassica*. Comparative analysis between *Arabidopsis* and *Brassica* coupled with the functional analysis could greatly contribute towards a better understanding of the genetic architecture for the conserved as well as the evolved traits in Brassicaceae.

In an earlier attempt to understand salinity stress tolerance, it was reported that SOS pathway-related genes shows strong correlation in *Brassica* species (Kumar *et al*. 2008). In order to develop complete understanding of the SOS3 protein in *Brassica juncea*, we have performed sequence and structural analyses. The phylogenetic study showed the homology of the SOS3 protein with those of SOS3 members in *Arabidopsis*, *Thellungiella halophila*. The complete study of SOS3 protein in *Brassica* has established the existence of SOS pathway in *Brassica*.

5.2 Method

5.2.1 Sequence analysis of BjSOS3 protein

The sequence of BjSOS3 was extracted from NCBI (Accession No. ABB52103). The SOS3 proteins were searched from *Arabidopsis*, *Brassica juncea* and *Thellungiella halophila* genome using PSI-BLAST (Altschul *et al*. 1990) program. All the SOS3 protein sequences were then aligned using MUSCLE-multiple sequence alignment software (Edgar 2004). The sequences from *Brassica* were named as
accession id obtained from the Brassica rapa genome gateway prefixed with Brassica. TIGR ids’ were used for the naming sequences obtained from the Arabidopsis genome. ‘Bj’ or ‘Th’ abbreviation were used for the SOS3 sequences obtained from Brassica juncea and Thallungiela halophila respectively. Using the multiple sequence alignment, bootstrap analyses were performed with 2000 replicas. The parsimonious tree was calculated using protpars program from Phylip package (version 3.6) (Felsenstein 1989). A consensus tree was obtained, and unrooted tree was plotted using drawtree program from the phylip package. The final alignments were prepared using Jalview (Waterhouse et al. 2009).

5.2.2 Molecular Modelling of BjSOS2 and BjSOS3

The secondary structures of BjSOS2 and BjSOS3 protein were predicted using JNET secondary structure prediction server (http://barton.ebi.ac.uk/jpred2) (Cuff & Barton 1999; Cole et al. 2008), PREDATOR (Protein secondary structure prediction from sequences) (Kabsch & Sander 1983; Frishman & Argos 1995; Frishman & Argos 1996; Frishman & Argos 1997), STRIDE (STRuctural IDEntification) (Frishman & Argos 1995), PSIPRED (Protein Structure Prediction server) (http://bioinf.cs.ucl.ac.uk) (Bryson et al. 2005).

The fold analysis of BjSOS2 and BjSOS3 protein were performed using FUGUE (Sequence structure homology recognition) (Shi et al. 2001), GenTHREADER (Jones 1999; McGuffin & Jones 2003), 3DPSSM (Kelley et al. 2000) and phyre server (http://www.sbg.bio.ic.ac.uk). The architectural motifs and the topology of proteins with known three-dimensional structure were analysed according to SCOP (Structure Classification Of Protein) (Murzin et al. 1995) and CATH (Orengo et al. 1997; Cuff et al. 2009) classifications.

The three-dimensional structure of BjSOS3 protein was modeled in a stepwise procedure, starting with the identification of template structures with PDB id: 1VIF.pdb, 2EHB.pdb, 2ZFD.pdb and 1UHN.pdb from Arabidopsis while BjSOS2 protein was modelled using 2EUE.pdb, 2FH9.pdb and 2H6D.pdb as templates. The identified templates were obtained from protein structure database (PDB) (http://www.rcsb.org) and were aligned using structure alignment software STAMP (Russell & Barton 1992). The aligned structures were used as a profile
for aligning the target sequence using ClustalX (Larkin et al. 2007). An automated comparative modelling program MODELLER9v7 (Sali & Blundell 1993; Eswar et al. 2000; Fisher et al. 2000; Marti-Renom et al. 2000) was then used to generate a 100 all-atom model by alignment of the target sequence with the selected template sequence in an alignment file. The best model was chosen on the basis of the stereochemistry quality report generated using PROCHECK (Morris et al. 1992; Laskowski et al. 1993) and side chains were optimized using SCWRL 3.0 (Canutescu et al. 2003). The bond distances and dihedral angle restraints on the target sequences were derived from its alignment with the template three-dimensional structures. The spatial restraints and the energy minimization steps were performed within Modeller using the CHARMM22 force field for proper stereochemistry of proteins. Further evaluation of the modeled BjSOS2 and BjSOS3 structure was done using the PROSA-Web (Sippl 1993; Weiderstaein & Sippl 2007). Molecular visualization and analysis of the final model were carried out with VMD (Humphrey et al. 1996).

5.3 Result and Discussion

5.3.1 Identification of three dimensional folds in BjSOS2

To create model of BjSOS2, we first performed BLAST searches against Protein Data Bank (PDB) for proteins with similar sequence and known 3D structure using 448 residue long sequence of SOS2 from Brassica juncea (ABM66448). The identified templates were then used to model the SOS2 protein using threading approach (See Methods). The conserved domain in both the sequences were identified using Pfam database. The analysis of the results obtained from Pfam showed the presence of two conserved domain Pkinase (PF00069) and NAF/FISL domain (PF03822) in the BjSOS2 protein sequence. The presence of conserved domains identified in Pfam searches, were also confirmed in searches against CDD (Conserved domain database). The results obtained showed that the major secondary structure and fold region of SOS2 protein sequence were found to be well conserved.
5.3.2 Structural analysis of SOS2 protein

The comparison of BjSOS2 and AtSOS2 protein sequence with that of SOS2 proteins sequences in other plant species revealed the presence of conserved residues. BjSOS2 protein was observed to possess 10 $\beta$-strands and 16 $\alpha$-helices (Figure 5.1). SOS2 protein consist of two distinct structural domains namely kinase domain and NAF domain. Analysis of kinase domain of SOS2 revealed that kinase domain of SOS2 resembles that of Snf1 domain of members of the Snf1/AMP-activated kinase (AMPK) family. The Snf1 domain/ AMP-activated kinase (AMPK) family was found conserved in all eukaryotes and play fundamental role in cellular responses to metabolic stress (Hardie et al. 1998; Carling 2004). The kinase domain showed the presence of ATP binding conserved residues Lys88 and Glu117. The high degree of sequence conservation with respect to the other kinase proteins shows that the kinase domain of the SOS2 protein has highly homologous structure. Earlier analysis has revealed that the phosphorylation of activation loop of SOS2 protein leads to its activation (Hanks & Hunter 1995; Johnson et al. 1996).

The other distinct domain observed in BjSOS2 was NAF/FISL domain. Earlier analysis of AtSOS2 protein has shown that C-terminal region consist of FISL (also known as NAF) and PPI motif (Sanchez-Barrena et al. 2007). Analysis show that SOS2 protein is constitutively active when the FISL motif of the protein is removed (Guo et al. 2001; Qiu et al. 2002). Various binding analysis of SOS3 with SOS2 protein shown that NAF/FISL domain play major role in their interaction. The NAF domain was observed to fit into the cleft formed by SOS3 (Sanchez-Barrena et al. 2007). In BjSOS2 protein, Asn312, Ala313, Phe319, Ile292 and Leu319 was found conserved as observed in other CIPK (NAF/FISL domain) family. Analysis of secondary structure revealed that Asn312 and Phe319 are involved in formation of the loop connecting the N-terminus of the FISL/NAF motif. Structural analysis suggest that these residues get buried on interaction between SOS2 and SOS3 proteins (Sanchez-Barrena et al. 2007). The conservation of PPI structural motif of BjSOS2 protein with that of other SOS2 member proteins suggests that BjSOS2 protein have similar folds that assists in phosphate binding.
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Figure 5.1: Sequence alignment of SOS2 proteins in *Arabidopsis* (AtSOS2), *Brassica juncea* (BjSOS2), shows presence of conserved residues.

### 5.3.3 Analysis of SOS3 sequences

To gain insight into the SOS3 protein in Brassicaceae, the SOS3 sequences from *Brassica rapa*, *Arabidopsis*, *Thellungiella halophila* and *Brassica juncea* were aligned (see Method). The alignment of the sequence showed the presence of four conserved EF domain (Figure 5.2). The sequences from *Brassica rapa* obtained from *Brassica* genome gateway showed the presence of large gap regions in the SOS3 protein sequences. In otherwise conserved EF1 hand of SOS3 in *Brassica rapa* showed deletions as some of the amino acid residues were found missing as compared to the SOS3 sequences from *Arabidopsis*, *Brassica juncea*
5.3 Result and Discussion

and *Thellungiella halophila*.

![Sequence alignment of SOS3 proteins](image)

Figure 5.2: Sequence alignment of SOS3 proteins in *Brassica rapa*, *Arabidopsis*, *Brassica juncea* and *Thellungiella halophila*, shows presence of conserved EF-hand motif.

It was observed from the sequence analysis that in EF1 motif of SOS3 protein in *Brassica rapa* shows the presence of Glycine in place of Serine followed by missing residues, which was otherwise observed to be present in SOS3 sequence of *Arabidopsis*, *Brassica juncea* and *Thellungiella*. This shows that in *Brassica rapa* SOS3 protein might have evolved distinctly. Other conserved domain EF2, EF3 and EF4 domain were found to be conserved. In *Arabidopsis*, SOS3 protein sequences were found to have variations but EF hand domains were found to be conserved in all the sequences. The EF2 domain shows well conserved residues, but found to have some variations in *Brassica rapa* SOS3 with respect to the SOS3 sequences in *Arabidopsis*, *Brassica juncea* and *Thellungiella*. The EF3 and EF4 domain also showed similar pattern of conservation as that of EF2 domain. The EF domain in *Brassica juncea* shows conserved DxSLKxDGxEKREE sequence where x can be any amino acid. The consensus sequences for N-myristoylation (i.e., MGxxxS, where the letter x can be any amino acid) (Towler *et al.* 1988) can be found in all the SOS3 sequences except for the *Brassica rapa* SOS3 sequences. It has been earlier shown that the SOS3 is myristoylated at the N-terminus and
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![Diagram of unrooted parsimony tree of SOS3 sequences from Arabidopsis, Brassica rapa, Brassica juncea and Thellungiella halophila.]

that the myristoylation is necessary for SOS3 function in plant salt tolerance (Ishitani et al. 2000).

The unrooted parsimonious tree analysis of the SOS3 protein shows that in *Brassica rapa* SOS3 protein sequence have evolved distinctly, while *Arabidopsis* SOS3 sequences shows variations as also observed in the sequence analysis (Figure 5.3). Unrooted tree plotted from all SOS3 aligned sequences shows the presence of four clades. As expected with the kind of variations in the *Arabidopsis* SOS3 proteins sequences, they does not follow the same clade. *Arabidopsis* SOS3 sequences were found to have significant identity (52% to 96%) among themselves. The SOS3 protein in *Brassica rapa* were found to be in one clade as they share high level of sequence identity (85%) among themselves as seen in the multiple sequence alignment as well. *Brassica juncea* SOS3 protein sequence have been observed to have 50% - 66% identity with the SOS3 protein sequences in *Brassica rapa*, *Arabidopsis* and *Thellungiella halophila*.

5.3.4 Identification of three-dimensional folds in BjSOS3

To create a model for BjSOS3, we first performed a BLAST search against the Protein Data Bank (PDB) for proteins with similar sequences and known 3D structures using the 188-residue-long BjSOS3 sequence obtained from NCBI.
The search result identified structures of SOS3 in *Arabidopsis* that were taken as possible templates for modeling BjSOS3 using the threading approach. The threading approach helps to assess the compatibility of the target sequence with the available protein folds based not only on sequence similarities but also on structural considerations (Bujnicki 2003; Godzik 2003). The conserved domain search against the conserved domain database, CDD (Marchler-Bauer *et al.* 2009) showed the presence of calcium binding EF-hand motif of EFh superfamily. For further identification of all the folds and domains, fold-recognition servers such as 3DPSSM were used. Results from the servers show that the models of *Arabidopsis* can be good templates with significant scores. The structures of SOS3 from *Arabidopsis* were used as a template for modeling. These results show that the major secondary structure and fold region in BjSOS3 was found to be well conserved.

### 5.3.5 Comparative modeling of BjSOS3

X-ray resolved structures of SOS3 protein from *Arabidopsis* were obtained from Protein Data Bank (PDB). Hence, SOS3 protein from *Brassica juncea* was modeled based on SOS3 from *Arabidopsis* using a comparative modelling strategy (Tramontano 1998). Since the protein structures used for the alignment and modeling have well conserved structural motifs and regions, and functional information is also available, multiple sequence alignment obtained from the known sequences provided a reasonable approach to comparative structure modelling. In order to verify the quality of the sequence alignment and optimise the position of gaps, corresponding positions from secondary structures were used. Experimentally determined structures were aligned and used as a profile for aligning the target sequence and to obtain an alignment, which was then used for modeling BjSOS3. To generate a 3D model of BjSOS3, a set of 100 structural models were generated using MODELLER9v7. Ramachandran plots were generated for BjSOS3 structures to determine deviations from normal bond lengths, dihedrals and nonbonded atom-atom distances, and with a view of comparing the BjSOS3 model against the SOS3 structure solved by X-ray crystallography. The plot shows that the modeled BjSOS3 (Figure 5.4) has 92% residues in most favourable
regions with the remaining 8% of residues occurring in allowed regions. None of the residue was found to lie in the disallowed region. This is expected for crystallographic models with at least 2.2Å resolutions. X-ray resolved structures give comparable stereochemical data: that of the SOS3 protein in Arabidopsis was found to be 91% favourable, with 9% in the additional allowed region. The PROCHECK result summary showed 6 out of 186 residues labeled, while the torsion angles of the side chain designated by χ1-χ2 plots showed only 2 labeled residues out of 124. All main-chain and side-chain parameters were found to be in the ‘better region’. G-factor is essentially a log odds score based on the observed distribution of stereochemical parameters such as main chain bond angles, bond length and phi-psi torsion angles. The score for G-factors should be above -0.50 for a reliable model. The observed G-factor scores of the present model were 0.05 for dihedral bonds, -0.11 for covalent bonds and -0.01 overall. The distribution of the main chain bond lengths and bond angles were 99% and 94% within limits. Conclusively, the modeled structure of BjSOS3 was found to be comparable to the structurally resolved SOS3 as structural motifs are conserved. The PROSA-Web analysis plots shows z-score of -6.35 for the modeled BjSOS3 protein. The PROSA energy plots and z-score further confirm the quality of modeled structure (Figure 5.5).
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5.3.6 Structural Analysis of BjSOS3

The X-ray structure of the SOS3 protein in *Arabidopsis* has already been solved by Sancha ez-Barrena et al., 2005, which shows that the overall fold of an SOS3 protein is almost identical with that found for SCaBP1/AtCBL2 (Nagae et al. 2003) and the homologous structures of calcineurin B (CnB) (Cyert & Thorner 1992) and neuronal calcium sensor (NCS1) (Schaad et al. 1996). It consists of two domains joined by a short linker. Each domain is formed by a pair of EF-hand motifs (Sachez-Barrena et al. 2005). SOS3 protein in *Brassica juncea* shows all α-helices in its modeled structure while in *Arabidopsis* SOS3, two β-sheets were observed to be present along with the α-helices (Figure 5.6(a), 5.6(b)). These β-sheets play a major role in cooperative binding of Ca\(^{2+}\) between the EF hands which was also observed in the other proteins in the EF hand superfamily (Zhang et al. 1995; Kuboniwa et al. 1995; Finn et al. 1995; Verdino et al. 2002). This structural distinctiveness is due to the difference of amino acid residue 116 and 171.

In *Arabidopsis*, SOS3 protein sequence was found to have Arginine at position 130 which corresponds to the lysine at position 116 which might have resulted in
Figure 5.6: Cartoon representation (a) and electron density map (b) of BjSOS3 protein showing conserved EF domain with Ca$^{2+}$ ion. The N-terminal domain was observed to have $\alpha$-helix (H1) in BjSOS3 protein.

the absence of $\alpha$-helix at that position. Similarly at position 171, lysine is present in Arabidopsis SOS3 while in BjSOS3, arginine is present in the corresponding position which might have prevented the formation of the secondary structure. The RMSD between the template and the modeled structure is 0.3 showing that the two structures are quite similar and comparative. The structure is basically composed of H1 domain at the N-terminal followed of EF domains. The classical EF-hand motif is characterized by a sequence of 12 residues involved in Ca$^{2+}$ binding (Figure 5.7). It has been earlier shown that the residues in position X, Y, Z and -Z ligate Ca$^{2+}$ via side-chain oxygen donors (Sachez-Barrena et al. 2005). The
5.4 Analysis of SOS3-SOS2 complex

The complex of calcium binding SOS3 protein with C-terminal regulatory domain of SOS2 kinase protein can help in understanding the mechanism of Ca$^{2+}$ sensing of SOS3 protein and its interaction with SOS2 kinase protein. Docking analysis of C-terminal regulatory moiety of BjSOS2 kinase protein (FISL/NAF domain) with BjSOS3 protein revealed that the interaction is stabilized majorly due to hydrophobic interactions (Figure 5.9(a)). Earlier analysis of the AtSOS2 and AtSOS3 interaction in has also shown the role of hydrophobic interaction in
5.4 Analysis of SOS3-SOS2 complex

Figure 5.8: A comparison of the sequence of the EF Ca$^{2+}$-binding sites of SOS3 and the classical EF-hand superfamily of BjSOS3 and SOS3 protein in *Arabidopsis*. Residues involved in Ca$^{2+}$ binding are highlighted by X, Y, Z, -X, -Y, -Z according to a classical EF hand. Red, Green, magenta and purple colors stands for EF1, EF2, EF3 and EF4 motif respectively.

stabilizing the Ca-SOS3:SOS2 complex (*Sachez-Barrena et al. 2005*). The exact role of Ca$^{2+}$ the interaction is still not known but previous analysis has also shown that the calcium binding in SOS3 leads to the enhancements of its hydrophobic character (*Zhang et al. 1995*). Similarly, Ca$^{2+}$ was not observed to be directly involved in interaction between SOS2-SOS3 protein in *B. juncea*. The large loop segment between EF1, EF2 and EF3, EF4 forms an open conformation that helps in binding of FISL/NAF domain of SOS2 with SOS3 (Figure 5.9(b)). Further analysis also revealed hydrogen bond interaction between Lys57 of SOS3 protein with Arg332 of SOS2 FISL/NAF domain (Figure 5.9(a)).
Figure 5.9: (a) Representation of SOS2-SOS3 complex in *Brassica juncea* (b) Schematic representation of conserved residues playing major role in the SOS2-SOS3 complex in *Brassica juncea*

## 5.5 Conclusion

Genetic and biochemical studies in *Arabidopsis* identified salt overly sensitive (SOS) pathway for Na\(^+\) homeostasis and salt tolerance (Hasegawa *et al.* 2000; Zhu 2003). The transcript abundance of SOS pathway related genes under salinity stress in *Brassica* species were investigated (Kumar *et al.* 2008). The understanding of the structure of SOS3 protein can give an insight to the Ca\(^{2+}\) sensing mechanism and its mediation to the signaling in salt stress condition.

With the structure available for SOS3 protein in *Arabidopsis* and SOS mechanism recently established in *Brassica juncea*, the understanding of sequence and structural variation in Brassicaceae can assist in elaborating the characteristic structural features of SOS3 protein.

Sequence analysis and phylogenetic analysis of the SOS3 protein in *Brassica rapa, Brassica juncea, Arabidopsis* and *Thellungiella halophila* revealed the presence of conserved domain essential for Ca\(^{2+}\) binding and also the myristoylation sites. Comparison of AtSOS3 protein structure with that of BjSOS3 revealed the presence of two \(\beta\)-sheets in AtSOS3 which was found absent in the modeled
BjSOS3 protein. This structural distinctiveness was attributed to the variation of residues at the sequence level in AtSOS3 and BjSOS3. Structural analysis also confirmed the presence of EF-hand type calcium binding motifs which plays an important role in the sensing $\text{Ca}^{2+}$ as signal that promotes the dimerization of the macromolecule which further transmit the $\text{Ca}^{2+}$ as signal to SOS2, thus initiating the salt stress response cascade. Analysis of complex between C-terminal regulatory moiety of kinase SOS2 and SOS3 protein revealed that the hydrophobic interaction in stabilizing the complex.