10. Summary and Conclusion

Environmental stresses are a serious menace to agriculture productivity, especially salt stress at divergent growth stages of crop plants pounced severe damage to growth, development, product quality and yield. It is essential to study plants with resistances to increased salinity in order to produce crops that can survive on salt stress conditions. Many plant species possess exceptional physiological uniqueness which critically influences environmental adaptation. The adaptive mechanisms can be developmental, morphological, physiological or biochemical. However, the adaptive mechanisms under salty conditions of halophytes have not yet been clearly distinguished. In this backdrop, the present study was undertaken to determine the effects of exogenous application of abscisic acid on salinity tolerance in *Suaeda maritima*. For this study seed samples were collected from adjacent bank of Vellar estuary (lat. 11°29′N and long. 79°45′E) in Parangipettai (Tamilnadu). The present study is detailed in five chapters, as under:

- First chapter deals with the molecular identification of plant species through 18S rRNA sequencing and phylogenetic analysis. Through morphological identification, the study species collected from the adjacent bank of Vellar estuary was identified as *Suaeda maritima*. For molecular identification genomic DNA was extracted from the leaf sample and subjected to 18S rRNA sequencing analysis. About ~724bp nucleotide sequences was obtained and submitted to NCBI. BLAST. Maximum identity of 99%-97% with a maximum score of 1291 was derived. Even though the study species was morphologically identified, molecular level identification is yet to be confirmed with the uses of other markers. There are no much previous reports that discusses about the use of 18S rRNA sequencing of *S.maritima* and the present study might be the first to make use of 18S sequencing This study also highlights the importance and need of sequencing with distinct markers to identify these isolates in future studies.

- Multiple sequence alignments were performed and the read length of about 724bp long, although in some instances some base calls were uncertain no insertions, deletions or stop codons were observed in the sequences. The evolutionary history was inferred using the
Neighborhood Joining method. Phylogenetic tree infers that *Suaeda maritima* is closely related to *Spinacia oleracea*, *Oenothera laciniata*, *Beta vulgaris* (Sea beet or Sub sp.*maritima*), *Atriplex canescens* (fourwing saltbush), *Atriplex torreyi Phaulothamnus spinescens*, and *Celosia argentea*. Phylogram showed distinct clade with *Mollugo verticillata* and *Halophytum ameghinoi* (Succulent annual plant) with strong bootstrap support value of 100%. The results provide evidence that *S.maritima* belong to the angiosperm variety of salt tolerant halophytic plants.

- Second chapter deals with the prediction of threshold concentration of salinity and abscisic acid on germination and growth rate. Seed germination was affected by NaCl concentrations. The highest germination percentage was found in control (96%) and there was a gradual decrease observed in increasing concentration of NaCl. However, 300mM NaCl was considered as the threshold concentration. Application of different concentrations of salinity conditions induced a considerable variation in the protein patterns, some protein bands disappeared (~20 to 30kDa) and few proteins selectively increased (55kDa) in higher concentrations. However, 300mM of salinity was considered as the threshold level. The 300mM salinity effects on protein profile of different time period revealed that there were no significant qualitative and quantitative variations in the protein expression pattern.

- All ABA concentrations (10µM, 50µM, 100µM and 150µM) had the potential to increase the effects on all growth parameters. The evaluation of changes in the root-leaf water content, pigment content, protein content and osmolyte accumulation made evident that the ABA application caused a considerable increase. However, 50µM ABA application alone caused stabilized increase in growth, pigment and osmolyte accumulation. Thus, the present investigation confirms that the positive impact of exogenous 50µM ABA was associated with increase in the leaf and root total ABA concentration. The present study suggests that the optimal application of 50µM ABA can benefit plant growth on stress tolerance.
Third chapter deals with the abscisic acid pretreatment effects on growth and osmolyte accumulation under salinity stress. Both leaf and root fresh weight and dry weight decreased in NaCl treatment and there was a gradual increase observed in ABA+NaCl treated samples. The RWC content of the leaf and root of *S.maritima* upon exposure with NaCl showed significant reduction, which gradually increased as 81.21% and 103.13% respectively after treating with ABA.

The determination of leaf cation content in *S. maritima* revealed that, Ca\(^+\) (73.99mg/l) and Na\(^+\) (173.6mg/l) content was significantly high in NaCl treatment than the control and ABA+NaCl treated samples. Highest Mg\(^+\) (107.9mg/l) and K\(^+\) (131.5mg/l) content was recorded in ABA+NaCl treated samples. Root cation content in *S. maritima* revealed that, NaCl treated samples showed very less concentration of Ca\(^+\)(5.89mg/l), K\(^+\) (25.86mg/l) and Mg\(^+\) (7.16mg/l) than the control and ABA+NaCl treated samples. The Na\(^+\) concentration ranged significantly high in NaCl (57.98mg/l) and interestingly in reduced concentration of Na\(^+\) observed in ABA+NaCl (44.99mg/l) treatment.

The chlorophyll “a” content was pointedly showed increase in ABA+NaCl (0.605±0.04mg/FW) treatment and considerably low concentration was found in NaCl (0.526 ± 0.015mg) treated samples. Very Low concentration of chlorophyll “b” was observed in NaCl (3.6±0.26mg/FW) than the ABA+NaCl (4.26±0.20mg/FW) treatment. Highest concentration of total chlorophyll was recorded in ABA+NaCl treatment (0.56±0.04mg/FW) and lowest concentration was observed in NaCl (0.48±0.04mg/FW) treated samples.

The total free amino acid (96±4µg/ml FW) and MDA content (46.46±1.005 nmol/ml) were found to be more in NaCl treated samples and less amount of MDA content was noted in ABA+NaCl (28.33±0.97nmol/ml) treated leaf samples. Highest proline content was observed in NaCl treated sample (180.89 ± 13.48 µg/g FW) and highest Glycine betaine (GB) content was recored in ABA+NaCl treated sample (204.17±5.59µg/ml FW) The abscisic acid pretreatment enhanced the growth, water relations, ion content and
positively stimulated the organic and inorganic osmolyte accumulation of *Suaeda maritima* under salinity stress.

- Fourth chapter deals with the impact of exogenous abscisic acid on the antioxidant activity under salinity stress. Higheast SOD (2.67 X10^4 U mg\(^{-1}\) protein), APX (1.13 U mg\(^{-1}\) protein) and GST (4750µmoles/min/mg protein) activity were observed in ABA+NaCl treated samples. Salt stress (NaCl) significantly affected the enzyme activities. Highest CAT (0.3X10^4 µmols/mg of protein) and POX (21.86 U mg\(^{-1}\) protein) activity were recorded in NaCl treated samples and there was no significant effects observed in ABA pre treatment (ABA+NaCl). The present study confirms that there is a drastic increased antioxidant activity in ABA pretreated samples. It is also clear that ABA pre treatment decreased the salinity induced oxidative stress by stimulating the activity of antioxidant enzymes.

- Fifth chapter deals with the Proteomic approaches on identification of salt tolerance proteins regulated by abscisic acid pretreatment under salinity stress. The SDS PAGE analysis of soluble proteins revealed that the protein peptides of 108kDa, 22kDa and 6.8kDa were absent and the 15kDa and 22kDa showed less intensity in control and in ABA treatment. But these proteins were accumulated with more intensity in NaCl and ABA pretreated leaves. In the present investigation no much variation was observed between NaCl (salt stress) and ABA+NaCl (ABA pretreatment under salt stress) treatment.

- Comparative proteomic approach between control and ABA treated group revealed that, 100 spots were differentially expressed and 17 spots matched with each other, of which 15 spots showed increased accumulation and single spot showed decreased accumulation in the ABA-treated leaves than the control. Between control and NaCl treated groups, 200 spots were differentially expressed and 27 spots matched with each other, of which six spots showed increased and eight protein spots showed decreased accumulation in the stress-treated leaves than the control. Between control and ABA+NaCl treated group, 93 spots were differentially expressed and 23 spots matched with each other in which nine
spots showed increased expression and single spot showed decreased accumulation in the ABA+NaCl treated leaves than the control. Comparative analysis between NaCl and ABA+NaCl treated leaves showed 91 unique protein spots and 21 protein spots matched with each other, of which 8 spots were up-regulated and 3 protein spots (spots, 14, 1 and 3) were down regulated in ABA+NaCl treated leaves.

- For identification the protein spot of 5.25 PI/48MW excised and digested with trypsin and successfully identified using MALDI-TOF/TOF MS analysis. That spot matched with a significant score of 94% in the closely related SKP1-like protein 1A (Sesamum indicum).

- Sixth chapter deals with the In-silico analysis of the salt responsive protein of SKP1-like protein 1A. Motif search, integrated protein structure and conserved domain search revealed that protein sequence belongs to SKP1 super family.

- Protein sequence showed 166 amino acids length and its molecular weight was 18347.6 and theoretical pl was 4.53. The maximum number of amino acids present in the sequence were Alanine (12.7%) Glutamine (10.8%) and Aspargene (9%). The total numbers of negatively charged residues (Aspartic acid + Glutamic acid) were thirty three while the total numbers of positively charged residues (Arginine + Lysine) were 19. Atomic composition was computed as C (802), H (1281), N (209), O (267) and S (7). The instability index was computed to be 46.64. The grand average of hydropathicity (GRAVY) was calculated as -0.336.

- The secondary structural analysis of the protein showed that alpha helix (Hh) was found to be most frequent (63.86%), followed by random coil (30.72%). Extended strand (Ee) was found to be least frequent (5.42%). The dominance of the coiled regions indicates the high level of conservation and stability of the protein structure.

- Highest Sequence similarity, resolution value and the stereochemical quality was observed in model 3 (PDB ID: 3ogl (Arabidopsis thaliana)) and was selected as a best template model for the target sequence of SKP1-like protein1A and finally tertiary
structure of the protein was visualized. The sequence analysis revealed that SKP1-like protein1A protein might be involved in ubiquitine dependent protein degradation in stress related signaling and response mechanism. Thus this results aid to the experimental data and helps to built up a complete view of SKP1-like protein1A protein role in plant stress response.

As a conclusion, exogenous abscisic acid pretreatment can reduce the damaging effects of salt stress via improved antioxidant enzymes, enhancement of the biosynthesis of photosynthetic pigments and thereby increasing the general growth rate; as well as enhancing the accumulation of nontoxic metabolites (protein, free amino acids, proline and glycine betaine). The present study thus reports for the first time that abscisic acid induced the differential expression of proteins under salt stress in a natural halophyte. This study also highlights the direct link between ubiquitination and the response of plant to salt stress. Therefore, exogenous application of ABA in crop plants may play a vital role and can act as an alternative way to improve the productivity when cultivated under salt stress. In the scientific aspect, this study may pave new ways for the researchers across the globe in targeting the right gene(s) for genetic engineering of crop plants with improved salinity tolerance.