CHAPTER 5

CONCLUSION

Salinity is a major problem in current agriculture and global threat in crop productivity. Salinity directly reduces the growth and yield of crops. Rice is a salt-sensitive crop and severely damaged by excess salt in the soil. Our study emphasized the impact of saline stress on physiological, biochemical, cellular and molecular mechanisms in wild type, salt susceptible and salt tolerant cultivars. Salt stress reduced the growth of plants at higher concentration (100 mM) in rice seedlings. The physiological and biochemical characteristics of plants significantly inhibited at 100 mM concentration of NaCl. Salinity induced ROS and caused the cell death of plants and degraded the nuclei as well as DNA.

We demonstrated the role of natural organic biostimulant PG to alleviate the saline stress and assessed its impact at physiological, biochemical and molecular level. Salinity negatively affected rice growth in both susceptible and tolerant cultivars although in tolerant cultivars exposure to salinity and its effect may be different in different cultivars. Salinity negatively affected the biochemical attributes including pigments and protein in salt susceptible when compared to tolerant cultivars. The enhanced antioxidant enzyme activity in salt susceptible cultivars is a key feature of salinity induced oxidative stress and its effect is higher in susceptible cultivars than in the tolerant cultivars.

In India, cow is considered as a sacred animal in Hindu religion. From the ancient period, cow products have been utilized in agriculture for cultivation. The cow urine is also used to cure various diseases. Earlier workers have reported that PG can possibly enhance the plant development and yield. However, it is important to identify the active ingredients to exploit the molecular mechanism of action of PG. We are the pioneer in using FTIR, HPLC techniques to characterize the PG and to identify the molecular mechanisms for underlying the action of PG. The biochemical and cell
biological analysis depict its role in the enhancement of growth, metabolites, and scavenging activity. The study also revealed the important role of PG in saline soil. It is a potential source to alleviate saline stress in crops and to improve the fertility of soil affected by salt. It is a low cost and effective source of organic manure for the farmers as well as important plant growth regulator enriched with different nutrients and phytohormones. Furthermore, it has eminent scavenging activity which detoxifies the effect of reactive oxygen species and prevents nuclear fragmentation, reduces the senescence-induced cell death and ultimately can improve the growth, development, and yield in plants. Hence, it can be utilized as a potential plant controller to improve the yield in plants growing under saline condition.

However, compounds are extracted, isolated, and characterized from methanolic extract of PG and four different compounds (1-methyl palmitate, 2, 4-Di-tert-butylphenol, 2-methyl propyl pentyl sulfide or Sulfurous acid and 2-4-hexadecanoyloxy-3-hydroxy-5-oxooxolan-2-yl-2-hydroxyethylhexadecanoate) were isolated. The major finding of this study is the isolation of a novel compound (2-4-hexadecanoyloxy-3-hydroxy-5-oxooxolan-2-yl-2-hydroxyethyl hexa decanoate) from PG. We are the pioneer to isolate this compound from the biostimulant PG.

The efficacy of PG1 (1-Methyl Palmitate) was studied in O. sativa seedlings grown under saline stress. The 1-Methyl Palmitate treatment significantly enhanced the photosynthetic pigments, protein content, antioxidant enzyme activities, reduces the nuclear fragmentation, cell death in seedlings grown under saline stress and acted as a potent plant growth promoting compound. The significant finding from this study is cost effective biostimulant can be formulated and supplied to the farmers for sustainable agriculture.

SA and JA are well established signaling molecule and play important role in modulating plant immune responses and play major role in defense during various abiotic and biotic stresses. SA and JA pathway is not an isolated pathway although it is an interconnected network of various well-
regulated pathways. In the present study, we have analyzed the role of SA and JA during salt stress at biochemical, cellular and molecular level.

The investigation reasoned that all physiological, biochemical and development parameters were fundamentally decreased under saline stress. SA and JA application not just lightened the impact of salt stress but also associate with transcription factors including WRKY53, MAPK1, PCD genes, BL-1 and ATG genes.

Autophagy occurs in all eukaryotic cells and protects animals and plants from various abiotic stresses and pathogen infections. Various compounds have been utilized to monitor or inhibit autophagy and CQ is widely used. The current study demonstrated that combination of CQ with NaCl increased the oxidative stress in rice seedlings and acted as an elicitor or activator of oxidative damage. The saline stress reduced the physiological and biochemical parameters of rice and reduced the growth and development. The analysis of gene encoding autophagy was inhibited by the application of CQ in rice seedlings. This may be due to CQ inhibit the fusion of autophagosome with vacuole and impaired the process. To our knowledge, this is the first time CQ has been utilized as a foliar spray to study autophagy in rice during saline stress.

The "self-eating" process of autophagy regulates protein degradation and accelerated under various environmental stresses and contributes nutrient starvation, senescence, and oxidative stress. In the current study, we analyzed saline stress at physiological, biochemical, cellular and molecular aspects in rice seedlings. Our current study revealed the differential expression pattern of CAT, SOD, GPX, BL-1, MAPK1 WRKY53 and various ATG genes. The ATG and PCD genes co-expressed during saline stress. The down-regulation of ATG and PCD genes in different treatments including PG, PG1, SA, and JA suggested that these compounds are efficient in alleviating saline stress. Taken together the results of antioxidant, BL-1, MAPK1 and WRKY53 genes suggested that these genes regulate salt tolerance by interacting with multiple genes and factors.
Activation or expression of ATG genes suggested that autophagy imparts in defense and induced tolerance mechanism during saline stress in plants. To reduce the effect of environmental stresses on crop yield these ATG genes can be utilized as the potential targets for engineering plants to create resistance against saline stress. Finally, the identification of pure compounds from biological sources provides an economical and easy method to identify the potential sources that can replace the use of chemical fertilizers and will be useful for sustainable agriculture. This is the first time we have isolated the biopriming compound PG1 from PG and demonstrated its efficacy in reducing the effects caused by saline stress. The enhancement of biochemical constituents, antioxidant and reduction of autophagy by PG1 also was demonstrated for the first time. The mechanism of action of PG1 was first identified in this work. The study imparts our understanding on salinity, PCD, and autophagy that are co-regulated mechanisms and these mechanisms are critical for plants to tolerate saline stress.