Physiological Basis for the Salicylic Acid-Mediated Tolerance of Mungbean (*Vigna radiata*) and Mustard (*Brassica juncea*)

Abstract

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The present thesis comprises of six chapters.

In Chapter 1, the importance of the problem and justifications for the present work undertaken were emphasized.

Chapter 2 is the review of literature. It deals with the relevant literature on the aspects of salinity stress and salicylic acid. The chapter has been divided in sections and sub-sections for better understanding of the work of other researchers in this field of study.

Chapter 3 describes the details of the materials used in the study and methodology adopted to determine various characteristics recorded in the four experiments. Relevant information on the experimental design, location of the study and the environmental conditions during the data sampling times has been mentioned.

Chapter 4 includes the results on crop responses to treatments in the four experiments. The results were statistical analysed and the significance at $P<0.05$ was determined. The treatment means were separated by the Duncan’s Multiple Range Test (DMRT).

In Chapter 5, results have been discussed in the light of observations recorded and supported with the earlier findings, if available on the subject.
undertaken, results obtained, conclusion and proposed future research are given.

Importance of the study undertaken

Salinity is a major limiting environmental factor for plant production. Around the world, 100 million ha or 5% of the arable land is adversely affected by high salt concentrations (Ghassemi et al., 1995). The major causes of the soil salinity are inappropriate irrigation and the use of saline irrigation water. In dry areas, salt concentration increases in the upper soil layer due to high evaporatory water loss that exceeds precipitation (Ebert et al., 2002). Attempts have been made to develop salinity stress tolerant plants. The use of salicylic acid (SA) to induce resistance of plants to abiotic stress has received considerable attention.

SA is a common plant-produced phenolic compound that can function as a plant growth regulator (Arberg, 1981). Exogenous application of SA has been reported to influence several developmental and physiological processes, i.e., seed germination (Cutt and Klessing, 1992), transpiration rate (Larque-Saavedra, 1979), stomatal closure (Rai et al., 1986), membrane permeability (Barkosky and Einhellig, 1993), growth and photosynthesis (El-Tayeb, 2005). SA has also received much attention due to its role in plant responses to abiotic stresses such as ozone (Koch et al., 2000), UV-B (Surplus et al., 1998), heat stress (Clark et al., 2004), drought (Nemeth et al., 2003), oxidative stress (Shim et al., 2003), salt and osmotic stress (Borsani et al., 2001; El-Tayeb, 2005).

In view of the importance of SA in abiotic stress management, it was assumed that tolerant and non-tolerant cultivars of mungbean (Vigna radiata) and mustard (Brassica juncea) would respond differently to SA application and
the capacity of SA to modify tolerance in these types would be different. Therefore, the reported research was undertaken to determine whether or not applying a low concentration of SA to mungbean and mustard plants will reduce any damaging effects caused by salt stress. The response of tolerant and non-tolerant cultivars of mungbean and mustard to SA application under salt stress was determined for their growth behaviour, photosynthetic capacity, biochemical characteristics, activities of antioxidative enzymes and yield attributes.

Mungbean and mustard are important crops and grown in summer and winter season, respectively in India. Mungbean is one of the most important pulse crop and offer an excellent source of high quality of protein. Vitamin C is synthesized in sprouted seeds of mungbean with increment in riboflavin and thiamine. It is a native of India and central Asia. It is grown in these areas since pre-historic period. It is also grown in the parts of Africa and USA and has recently been introduced in Australia. In India, mungbean is grown in an area of 3 million ha with the production of about 1 million tones (www.ikisan.com). Similarly, the other test crop, the oleiferous Brassica is the third most important source of vegetable oil in the world after palm and soybean oil (Zhang et al., 2003). The major mustard oil-producing countries include Canada, China, France, Germany, India and UK. According to a report of USDA, the world oilseed production is 397Mt in 2006-07. This total production is an increase of 9Mt, or 2% on the last season. About 90% of the total land under oilseed cultivation in India is occupied by Brassica juncea (Khan et al., 2007).

Experimental Results

The results of the experiments are summarized below:

Experiments 1 and 2

Experiment 1 was conducted on mungbean and Experiment 2 on mustard to assess the effects of salinity stress (0, 50 and 100mM NaCl) on four
cultivars. The purpose of the study was to select tolerant and non-tolerant cultivars on the basis of their growth, photosynthetic and yield characteristics at 20 and 40DAS in mungbean and at 30, 60 and 90DAS in mustard. Yield characteristics were determined at 60DAS in mungbean and 120DAS in mustard. The design of the experiments was randomized block design. Growth characteristics determined were: root length, root fresh mass, root dry mass, leaf fresh mass, leaf dry mass, leaf area and plant dry mass. Photosynthetic characteristics were: carbonic anhydrase activity and net photosynthetic rate. At harvest, yield characteristics determined were: pod length, pod number per plant, seed number per pod and seed yield.

Experiment 1 (2003): Maximum reductions in the growth and photosynthetic characteristics were noted with 100mM NaCl at 20 and 40DAS in all the cultivars of mungbean. Upto maturity stage, treatment of 100mM NaCl proved deleterious and plants did not survive in this treatment. Plants treated with 50mM NaCl, thus exhibited a significant and maximum decrease over control on yield characteristics. Therefore, 50mM NaCl concentration was considered suitable to assess tolerance of the cultivars, and this concentration was used in further experiments. The cultivar Tram exhibited maximum decrease followed by T44 whereas Pusa Vishal registered lowest decrease followed by PBM54. The order of the tolerance of the cultivars to salinity stress was Pusa Vishal > PBM54 > T44 > Tram.

Experiment 2 (2003-2004): The effect of 100mM NaCl decreased the growth and photosynthetic characteristics maximally and was more conspicuous on all the cultivars of mustard at 30, 60 and 90DAS sampling times. However, the effect of 100mM NaCl on yield characteristics was detrimental and the plants could not survive. The cultivars, Alankar and Pusa Bold had significantly more growth, photosynthetic and yield characteristics than Sakha and PBM16 under 50mM NaCl concentration. The order of the
suitability of the cultivars to salinity stress in terms of growth, photosynthetic and yield characteristics was Alankar > Pusa Bold > Sakha > PBM16.

**Experiments 3 and 4**

Experiments 3 and 4 were conducted based on the findings of Experiments 1 and 2. The aim of the experiments was to study the effects of exogenous application of salicylic acid in alleviating salinity stress and the physiological processes associated changes with the salicylic acid treatment on tolerant and non-tolerant cultivars of mungbean (Experiment 3) and mustard (Experiment 4). From the results of Experiment 1, Pusa Vishal and Tram cultivars of mungbean were categorized as tolerant and non-tolerant cultivars, respectively. Similarly, from Experiment 2 it was clear that Alankar and PBM16 cultivars of mustard were tolerant and non-tolerant, respectively. This has also been detailed out in earlier pages that the treatment of 100mM NaCl was deleterious for both the crops. Therefore, the plants were burned at maturity stages of the crops. Both the experiments (3 and 4) were confined with the use of 0 or 50mM NaCl for growing plants and the application of 0.0, 0.1, 0.5 and 1.0mM SA on foliage at 15DAS on tolerant and non-tolerant cultivars was studied on ameliorating salt stress effects. In these two experiments growth, photosynthetic, biochemical and yield characteristics were studied. The time of sampling for these characteristics for mungbean was 20, 40 and 60DAS, and 30, 60, 90 and 120DAS for mustard. The activities of antioxidative enzymes were also studied in both the crops at first sampling time. Growth characteristics were similar as in earlier experiments. Photosynthetic characteristics were : carbonic anhydrase activity, net photosynthetic rate, stomatal conductance, intercellular CO₂ concentration and the contents of chlorophyll and carotenoid. Biochemical characteristics were : concentration of sodium, chloride, nitrogen, phosphorus, potassium and calcium. The activities of antioxidative enzymes assayed were : catalase, superoxide dismutase, glutathione reductase and ascorbate peroxidase. Yield
characteristics were similar as in Experiments 1 and 2. The design of the experiments was randomized block design.

Experiment 3 (2004): Salt stress led to a significant reduction in growth and photosynthetic characteristics, nitrogen, phosphorus, potassium and calcium concentrations and yield characteristics of both the cultivars. The cultivar Tram exhibited a higher reduction than Pusa Vishal. The treatment of 50mM NaCl increased sodium and chloride concentrations in Pusa Vishal and Tram at 20 and 40DAS, and the accumulation was higher in Tram than Pusa Vishal. Exposure of plants to 50mM NaCl increased the activities of antioxidative enzymes in both the cultivars but to a higher degree in Pusa Vishal than Tram. The treatment of 0.5mM SA was found most effective in alleviating salinity stress on growth, photosynthetic, biochemical and yield characteristics. The application of 0.5mM SA increased growth and photosynthetic characteristics, nitrogen, phosphorus, potassium and calcium concentrations and yield characteristics of mungbean. In both the cultivars i.e. Pusa Vishal (tolerant) and Tram (non-tolerant) the increases were greater under non-saline (control) conditions than under saline conditions (50mM NaCl) at 20 and 40DAS. The positive effect of 0.5mM SA application was also found as it decreased sodium and chloride concentrations under both saline and non-saline conditions. The activities of antioxidative enzymes of both the cultivars further increased significantly with 0.5mM SA under both saline and non-saline conditions. In Pusa Vishal, at initial growth stage i.e. 20DAS, the application of 0.5mM SA increased potassium and calcium concentrations of plants grown under 50mM NaCl which was higher than control. However, in Tram, the increase was noted only for potassium concentration. At later growth stage, photosynthetic characteristics, nitrogen, potassium and calcium concentrations were found higher than control with the application of 0.5mM SA on Pusa Vishal plants treated with 50mM NaCl. In Tram, only potassium
concentration was found increased with 0.5mM SA of 50mM NaCl treated plants.

Experiment 4 (2004-2005): Application of 0.5mM SA increased growth and photosynthetic characteristics, nitrogen, phosphorus, potassium and calcium concentrations, activities of antioxidative enzymes and yield characteristics of Alankar (tolerant) and PBM16 (non-tolerant) cultivars grown under non-saline (control) conditions. Non-salinized plants treated with 0.5mM SA maintained a higher growth and photosynthetic characteristics, nitrogen, phosphorus, potassium and calcium concentrations and yield characteristics than salinized plants at all the stages, indicating adverse effect of the NaCl salinity in tolerant (Alankar) as well as non-tolerant (PBM16) cultivars. Application of 0.5mM SA decreased the concentrations of sodium and chloride in both tolerant (Alankar) and non-tolerant (PBM16) cultivars, under normal and saline conditions at all the sampling times. Growth and photosynthetic characteristics, nitrogen, phosphorus, potassium and calcium concentrations and yield characteristics decreased significantly with 50mM NaCl in both the cultivars but more adverse effects of salinity were found on PBM16. However, the concentrations of sodium and chloride and the activities of antioxidative enzymes increased with 50mM NaCl in both the cultivars. Application of 0.5mM SA helped to reduce the adverse effects of salinity. SA alleviated the salt stress effects when applied on plants treated with 50mM NaCl. In both the cultivars, application of 0.5mM SA restored the decrease in characteristics caused by salinity stress and even increased over control at 30DAS. Nitrogen and potassium concentrations and activities of antioxidative enzymes were increased in comparison to the respective control. The application of 0.5mM SA under saline conditions also increased the calcium concentration in Alankar. At 60DAS, the treatment of 0.5mM SA on Alankar enhanced the photosynthetic characteristics, nitrogen, potassium and calcium concentrations of plants grown under 50mM NaCl. In PBM16, only two characteristics
nitrogen and potassium concentrations increased at 60DAS. At 90DAS, in Alankar, the growth and photosynthetic characteristics, nitrogen, potassium and calcium concentrations increased over control with the application of 0.5mM SA on plants treated with 50mM NaCl. In PBM16, the increase was found only for nitrogen and potassium concentrations. In Alankar, the yield characteristics were found higher than control with 0.5mM SA application on plants treated with 50mM NaCl.

The present chapter is followed by an up-to-date bibliography of the literature cited in the text.

Conclusion

It may be concluded that NaCl treatment decreased growth, photosynthetic and yield characteristics of tolerant and non-tolerant cultivars of mungbean and mustard. The salt treatment caused an accumulation of sodium and chloride to a higher extent and the essential nutrients, nitrogen, phosphorus, potassium to a lesser extent. The tolerant cultivars exhibited lesser decrease than the non-tolerant cultivars of both the crops when treated with NaCl. The application of 0.5mM SA increased growth and photosynthetic characteristics, nitrogen, phosphorus, potassium and calcium concentrations and yield characteristics of mungbean and mustard. In tolerant and non-tolerant cultivars, the increases were greater under non-saline (control) conditions than under saline conditions. The positive effect of 0.5mM SA application was found as it decreased sodium and chloride concentrations in both tolerant and non-tolerant cultivars under saline and non-saline conditions. The activities of antioxidative enzymes of both the cultivars increased significantly with SA application under both saline and non-saline conditions. Application of 0.5mM SA helped to reduce the adverse effects of salinity in both the crops. SA alleviated the salt stress effects when applied on plants treated with 50mM NaCl.
In tolerant and non-tolerant cultivars, application of 0.5mM SA restored the decrease in characteristics caused by salinity stress and even increased over control for few characteristics. Salt-induced reduction in growth and finally yield characteristics in mungbean and mustard was improved by the foliar application of 0.5mM SA in both tolerant and non-tolerant cultivars. This improvement in the above characteristics due to SA was associated with improved photosynthetic capacity. The changes in net photosynthetic rate due to SA application were due to metabolic factors, other than photosynthetic pigments and leaf carotenoids. The tolerant cultivars exhibited higher growth and photosynthetic traits than non-tolerant cultivars under saline conditions, which could explain the ability of salt tolerant cultivars to show better yield characteristics under salt stress than non-tolerant cultivars. SA also maintained higher activities of antioxidative enzymes under salt stress and better synergy among the enzymes helped to reduce the active oxygen species level and damage caused by it. It may be suggested that 0.5mM SA could be used as a potential growth regulator to improve plant growth, photosynthetic and yield characteristics under salt stress.

**Future Research**

Salinity is a limiting environmental factor for plant production, and is becoming more prevalent in agricultural soil due to several reasons. The study reported in the thesis shows that maximum reduction in the growth and photosynthetic characteristics were noted with 100mM NaCl in all the cultivars of mungbean and mustard. The treatment of 100mM NaCl was so intense that it proved detrimental on yield characteristics and the plants could not survive. SA plays an important role in abiotic stress tolerance, and considerable interests have focused on SA due to its ability to induce a protective effect on plants under stress. Plants respond to stress by the synthesis of signaling molecules. These activate a range of signal transduction pathways. Several such signaling molecules have been identified in plants. The study of interaction of these...
molecules with SA may provide fruitful information on the influence of SA on plants under normal conditions and its potential in alleviating salt stress effects. The signaling molecules may be ABA, calcium, jasmonic acid and ethylene. High salt concentration triggers an increase in levels of plant hormones such as ABA. ABA is responsible for the alteration of salt-stress genes. ABA has been found to alleviate the inhibitory effect of NaCl on photosynthesis, growth and translocation of assimilates. ABA promotes stomatal closure by rapidly altering ion fluxes in guard cells under stress conditions. Other ABA actions involve modifications of gene expression, and the analysis of ABA-responsive promoters has revealed diversity of potential cis-acting regulatory elements. The nature of the ABA receptors remains unknown. The combined biophysical, genetic and molecular approaches have led to considerable progress in the characterization of more downstream signaling elements. In particular, substantial evidence points to the importance of reversible protein phosphorylation and modification of cytosolic calcium levels and pH as intermediates in ABA signal transduction. Increase of Ca\(^{2+}\) uptake is associated with the rise of ABA under salt stress and thus contributes to membrane integrity maintenance, which enables plants to regulate uptake and transport under high levels of external salinity in the longer term. Jasmonates also have important roles in salt tolerance. Jasmonates are generally considered to mediate signaling, such as defense responses, flowering, and senescence. However, factors involved in the jasmonate signal-transduction pathway remain unclear. Ethylene is now considered as a plant hormone regulating growth and photosynthetic responses in plants. Evidences indicate that it plays a prominent role in managing abiotic stress (Druege, 2006). Further study on the interaction of SA, ABA and ethylene may provide in-depth insight into the role of SA in alleviating salt stress.