Influence of Sodium Chloride (NaCl) Stress Growth and insects identification of Rice (*Oryza Sativa L.*)

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Abstract

Abiotic stress is a major issue all around the world, producing tremendous crop losses. To address this issue, a study was conducted to investigate the influence of sodium chloride (NaCl) stress on seed germination, plantlet development (root, shoot length, and fresh, dry weight), cell damage (cell membrane stability), insects population and SDS-PAGE analysis of CO 47 rice cultivars. Salt stress was found to have a negative impact on seed germination and germination rate. The variety rice demonstrated an ongoing decline in root and shoot length from control to 100 mM Sodium chloride stress, but a significant drop in shoot and root length was seen at 150 mM saline stress. With increasing salt stress, root and shoot dry weight decreased gradually. Cell damage increased gradually from control to 150 mM salt concentration. SDS-PAGE study revealed no differences in the protein banding pattern of rice leaf proteins under salt stress and control.

Keywords: Salinity, Cell membrane stability, Rice, SDS-PAGE, protein, Cell damage, insects.

Introduction

Salinity is the most severe concern to crop production worldwide (Sahi, 2006). Natural and salty resources of water are the chief damaging factors in waterless and semi-arid area of the global (Binzel, 1994). Salinity is one of the important ecological issues that damage yield development and cultivated efficiency. Salinity covered 953 million ha of total area that is about 8 percent of cultivated region (Szabolcs, 1979; Singh, 2009). Among all the other crop species, rice is temperately sensitive to salt in the field. Rice is one of the most commonly cultivated crops in seaside regions normally under water with salty seawater (Akbar et al., 1972; Mori and Kinoshita, 1987).The salt concentration increased the germination rate and reduced germination percentage (Ashraf et al., 2002). The fresh and dry weight of roots and shoots decrease with increase of salt stress (Dkhil and Denden, 2010). In high salt concentration germination, root and shoot length and fresh and dry weight of rice reduced upto 50 percent (Anuradha and Rao, 2002).

In India sustainable crop production is endangered by several factors including soil degradation, moderate salinities and use of salty ground water. In Pakistan 6.8 million ha area are effect from salinity and about 40,000 ha area are affected from salinity each year (Khan 1998). Rice (Oryza sativa L.) is the most important staple food globally, and Asia contributes more than 90% of global rice production and consumption. In Asian countries, India produced different kinds of fragrant rice varieties (Rabbani et al., 2008). Rice grows as a major crop for 11,500 years and it presently sustains almost one half of the world population (Wu et al., 2004). It has also been employed as a foreign exchange earning product (Anonymous, 2009). Production of rice is badly affected due to deterioration of soil and water reservoirs around the world; there is an urgent demand to combat these environmental factors. Therefore, the current study was designed to investigate the effect of salt stress on seed germination and seedling growth of rice CO 47.

Materials and Methods

Plant Materials

Rice CO 47 variety seeds were obtained from the Coimbatore campus of the Tamil Nadu Agricultural University (TNAU).

Sterilization

The seeds were surface sterilized in 3.5 percent Sodium hypochlorite for 5 minutes after being extracted from vigorous and healthy plants.

Seed Germination and Seedling Growth

To study the effect of salt stress on germination and seedling growth, healthy and mature seeds were germinated in Petri plates contained double filter paper soaked with 20 mL distilled water or 50, 100 and 150mM NaCl solution. During that period seeds were kept in dark condition under room temperature (30 °C) for 4 days so that all seeds can germinate. Seeds germination reading was taken after every 12 hours up to 4 days (96 hours). After 4 days seeds were transferred to white fluorescent light (16 hours light period/ day) at room temperature (32 °C) and allowed to grow for further 3 days. Root, shoot length and root, shoot fresh and dry weight of 10 days old seedling were then measured and recorded.

Hydroponics culture Experiment

The seeds were primarily germinated in distilled water for conducting hydroponics culture. The seeds were kept in distilled water until they were fully germinated. After ten days the young seedling with two leaves were then transferred to plastic trays filled with Hoagland's solution (Hoagland and Arnon, 1950). The growing seeds with shoots and roots were allowed to be fully grown in Hoagland's solution for three weeks. During these weeks the Hoagland solution was renewed after every week and with continuous maintenance of pH at 5.6 to 5.9.After three weeks, stress of sodium chloride of different concentration i.e. control, 50, 100, 150 and 200mM were given to the sterilized fully growing plants for one week. Whole experiment was conducted under green house condition. After one week of stress treatment, the plant were harvested and chopped in liquid nitrogen to get fine powder for further analysis.

Cell Membrane Stability

From each treatment fresh leaves were taken and sliced in twenty pieces of 1cm. These fine strips were placed in test tube with twenty ml distilled water. The test tubes were incubated at 10 ºC for 24h, followed by warming at 25 ºC. The electro-conductivity (C1) was measured and then autoclaved for 40 min to determined the electro-conductivity (C2) again. Cell membrane stability was calculated by using formula, where C refers to electro-conductivity one and two (Jamil et al., 2011). EC = C1/C2×100

Analysis of Proteins by SDS-PAGE

Rice plant proteins were analyzed by SDS-PAGE (Sodium dodecyle sulfate Polyacrylamide Gel Electrophoresis) using the standard protocol of Laemmli (1970) with slight modification. Electrophoresis was carried out in discontinuous system using 10% separating gel and 4% stacking gel.

Results

Effect of Salt stress on seed germination

The results demonstrated that untreated and salt-treated seedlings' germination patterns had very minor variations. In CO 47, the maximum germination percentage for untreated seeds was 100%, whereas under salt stress of 200 mM, it fell to 60%. (Fig.1). In the control group, seeds began to germinate in thirty-six hours, but they took 48 hours for the rest of the salt treatment group to begin. As the salt content rose in CO 47, salt stress also decreased seed germination. At 200 mM salinity, the maximum germination percentage dropped to 60% from a maximum of 100% in the control. Seed germination began in the control and 50mM salt stressed conditions after 36 and 48 hours, respectively, but it took 72 hours to witness seed germination in the 100, 150, and 200mM salt stressed conditions.

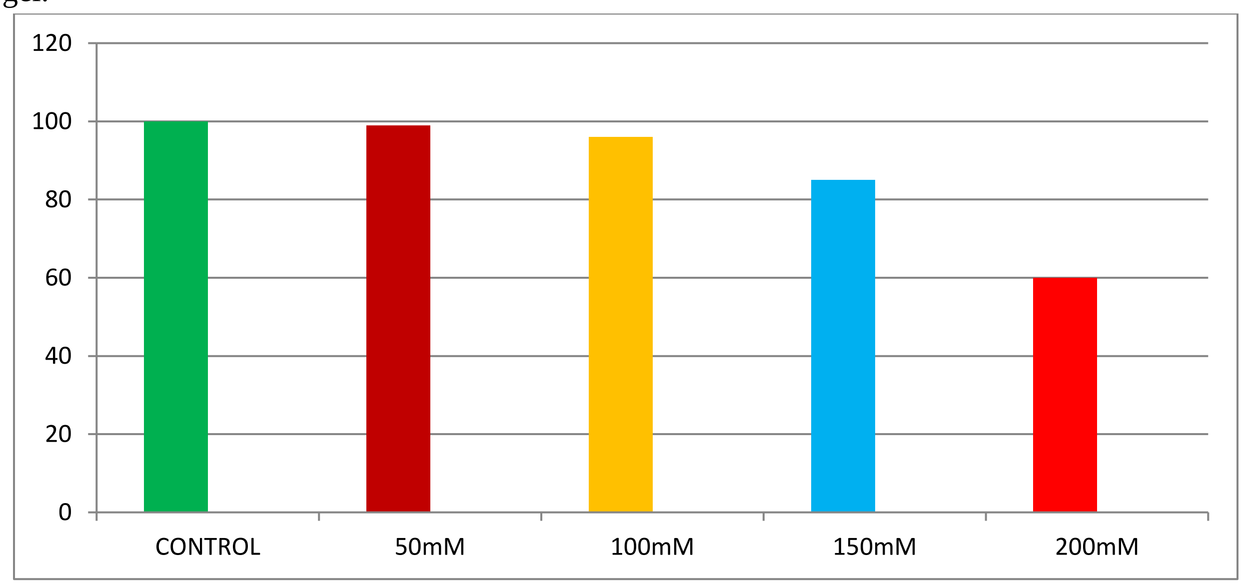


Fig -1 Effect of Salt stress on seed germination

Effect of Salt Stress on Root and Shoot Length

Seedlings that were ten days old were cultivated for three weeks at various salt concentrations. After harvest, the lengths of the roots and shoots were measured. The lengths of the plantlets' roots and branches were found to be shorter when there was more salt present. From control to 100mM NaCl stress, CO 47's root length continued to diminish, but from 150 to 200mM NaCl, a regular decline in root length was seen (Fig.2&3). The effects of NaCl on CO 47's shoot length were comparable to those of NaCl on its roots. From control to 100mM, there was a steady reduction in the length of the shoots, but at 200mM, there was a considerable reduction.

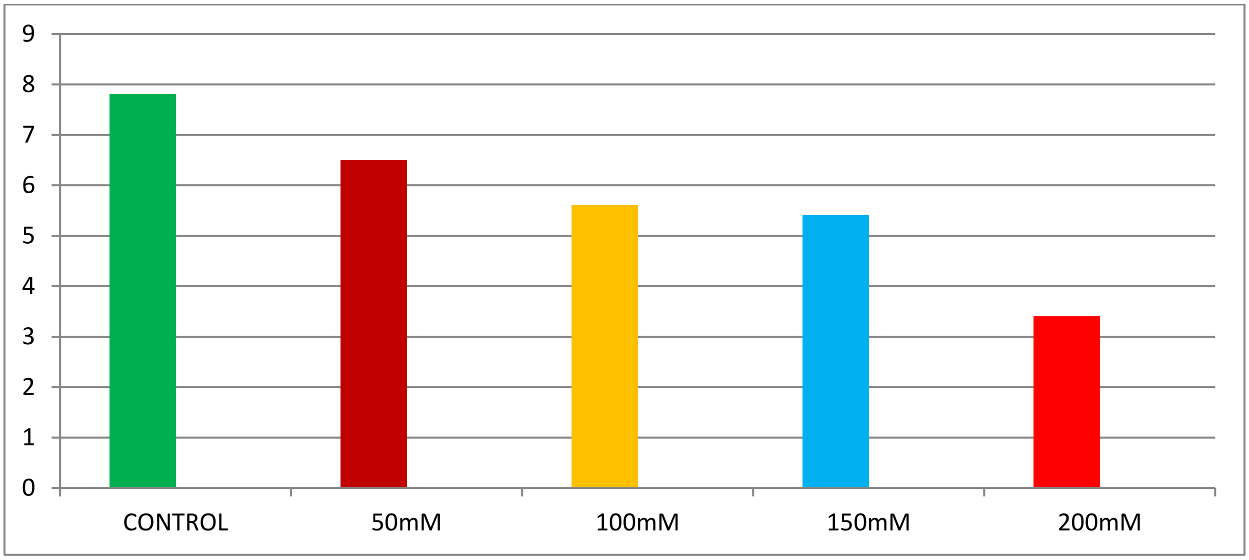


Fig -2 Effect of Salt Stress on Shoot Length

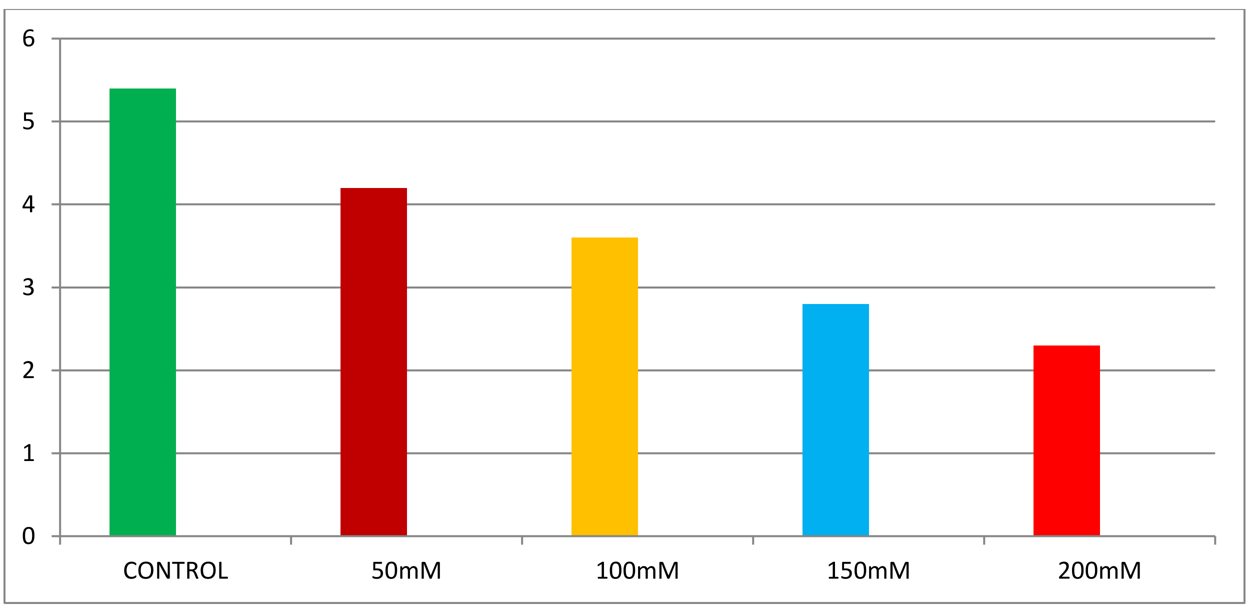


Fig -3 Effect of Salt Stress on Root Length

Effect of Salt Stress on Root/Shoot Fresh and Dry Weight

Following the measurement of lengths, the average weights of the roots and shoots were determined using a digital balance. Findings showed that salinity significantly reduced the fresh weight of both kinds' roots and shoots when compared to the control (Figs.4&5). Dry weights of the root and shoot similarly demonstrated a gradual decrease in CO 47 as the salt concentration increased.

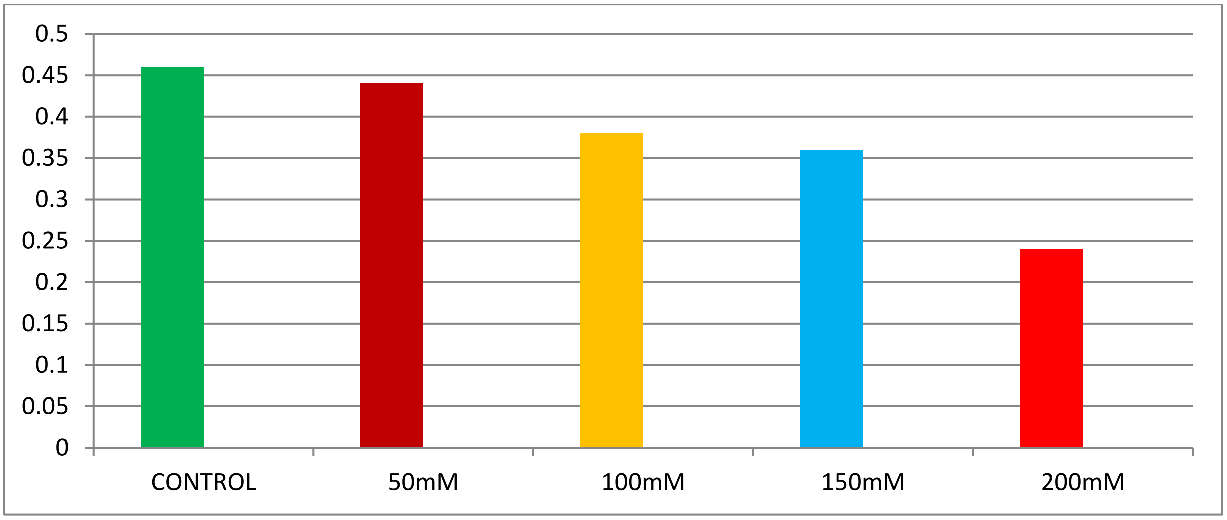


Fig -4 Effect of Salt Stress on Shoot Fresh and Dry Weight

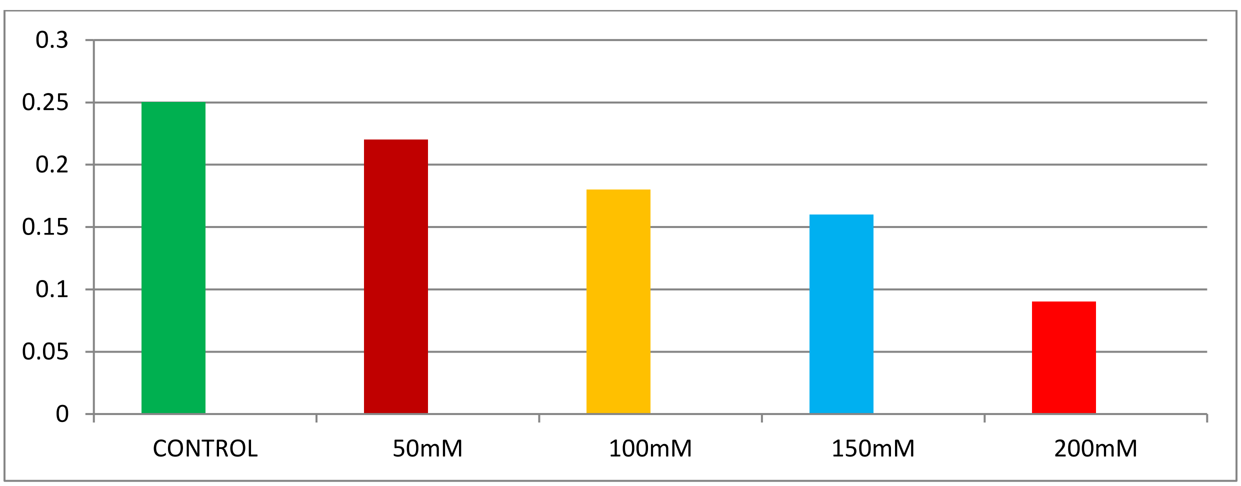


Fig -5 Effect of Salt Stress on Root Fresh and Dry Weight

Effect of Salt Stress on Cell membrane stability

Salt stress showed considerable effect on cell membrane stability of rice leaves of both varieties. The cell membrane was ruptured due to high salt concentration. High electric conductivity was noticed with the increasing concentrations showing increase in cell injury. The cell injury was high at

150 mM NaCl solution as compared to other salt solution in both varieties (Fig. 6).

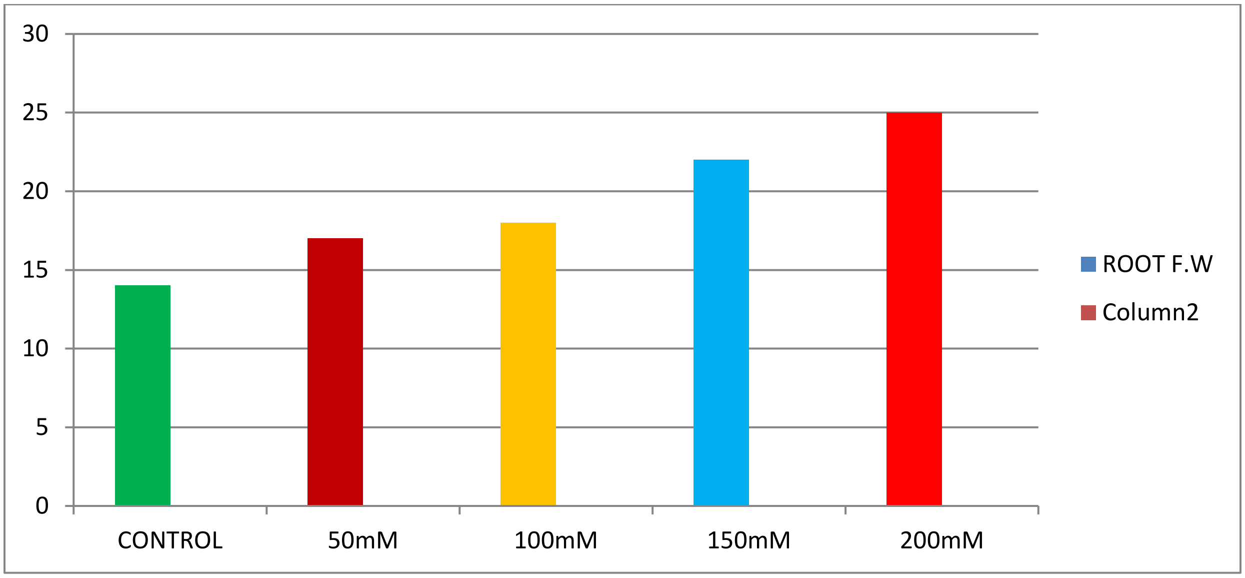


Fig -6 Effect of Salt Stress on Cell membrane stability

Effect of Salt Stress on Protein analysis by SDS-PAGE

Protein profile analysis of both varieties (CO 47) were between 20 kDa and 60 kDa. There were total four protein bands observed in all treatments (Control, 50, 100 150 and 200mM) in the electrophoretic gel. In four protein bands, three major bands and one minor band were observed. The protein banding was compared with a standard molecular weight marker. First two major bands lied in 60 kDa and 58 kDa. The third minor band lied in 40 kDa and fourth major band lied in 20 kDa. Salt stress showed no significant variation on protein banding pattern of both varieties (Fig.7).

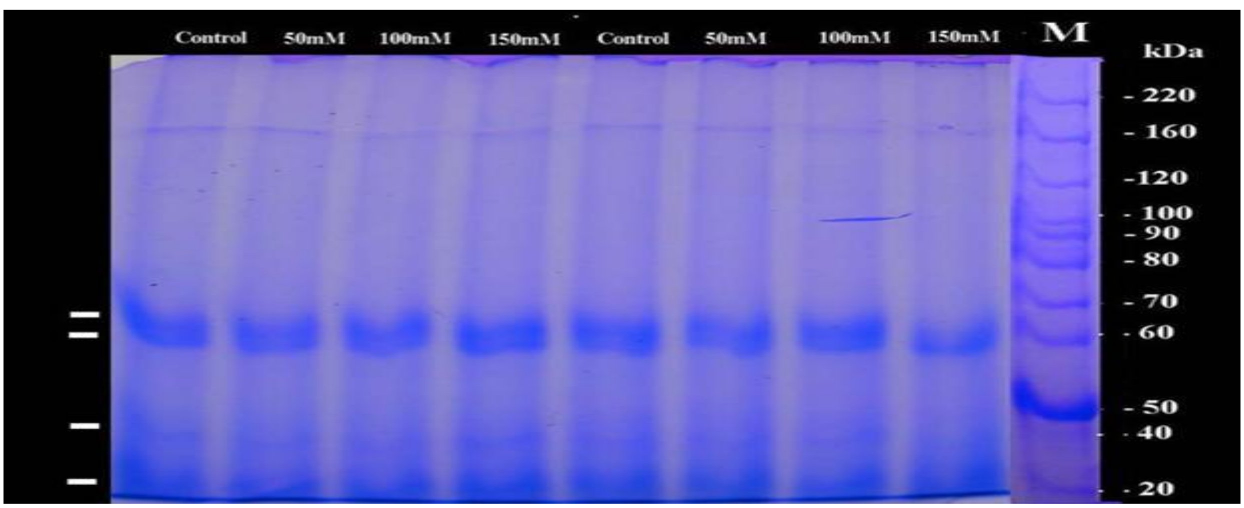
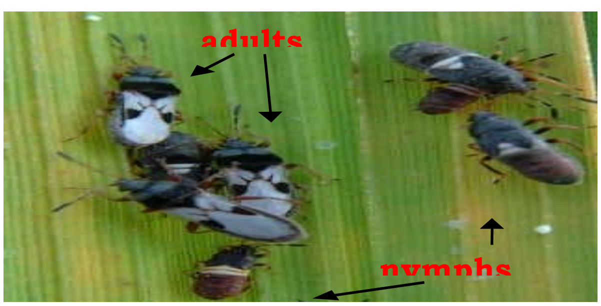


Fig.7 SDS-PAGE page profile of CO-47

Photography	Insect Name
	**Rice Water Weevil** *(Lissorhoptrus oryzophilus)*
	**Rice Stink Bug** (*Oebalus pugnax*)
	**Fall Armyworm** *(Spodoptera frugiperda)*
	**Chinch Bug** **(***Blissus leucopterus leucopterus)*
Photography	**Insect Name**

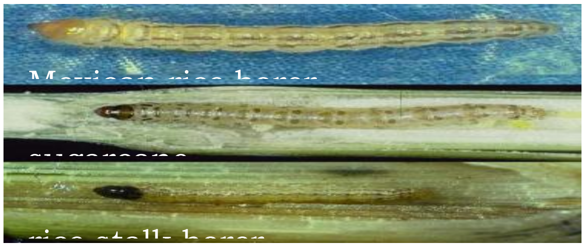
	**Stalk Borers** *(Diatraea saccharalis)*
	**Grasshoppers** (*Orthoptera*)
	**Blister Beetles** *(Epicauta texana)*
	**Leafhoppers** *Cicadellidae (Graminella nigrifrons)*

Fig.8 Insects identification of paddy field

Discussion and conclusion

Seed germination is a complex process and it plays a major role in the life cycle of plant (Saritha et al, 2007). Seed germination decreased as the level of salt increases (Dkdil et al, 2010; Saddiqi et al, 2007). The germination of the seeds was negatively affected by salt concentration, whereas the seeds germinated in salt solution took more time to germinate as compared to control (Figure 1). Inhibition of seed germination occurred with salt concentration which could be due to ion toxicity (Huang and Redmann, 1995). Seed germination delayed as the level of salt increases whereas higher level of salt concentration reduced the final germination percentage (Gloulam and Fares, 2001). Similar kind of result had also been shown by Lima et al. (2003), according to them the viability of seeds under salt stress was decreased as the concentration of salt increased. The germination and final seed germination percentage decreased with the decrease of the water movement into the seeds during imbibitions under salt stress (Hadas, 1977). As the salt concentration increased to 150mM, the germination rate and percentage of seed germination is reduced (Alam et al., 2004). It has been investigated that the germination of seeds, survival of rice seedling and overall plant growth is reduced due to high salt concentration (Zing et al., 2000, Narale et al., 1969). Salt also increases the time for germination (Jamil et al., 2007). A decrease in both percentage and rate of seed germination at high level of salinity could be attributed to process of osmosis, ion toxicity and lack of nutrients in the soil (Redmann, 1994).For salt stress the root and shoots are most important parameters because roots are in direct contact with soil and absorb water from soil while shoot supply it to the rest of the plant (Jamil and Rha, 2007). As the roots are in the direct contact with surrounding, they are first to encounter the saline medium and are potentially the first site of damage or first line of defense under salt stress. Our result showed that there was decreased in root length of both the varieties with the increasing concentration of salt stress (Figure 2B). Mer et al. (2005) reported that by increasing the salinity, plomule length in wheat, barley pea and cabbage seeds decreased. Our results showed that there was decrease in shoot length of both varieties with the increasing concentration of salt stress (Figure 2A). However these results are not similar with those of Cramer et al. (1985) who reported that roots were less sensitive to salt than shoots. The observed reduction in shoot length in salinized conditions was possible due to many reasons. One possibility is that photosynthesis reduced by salinity which in turn limited the supply of carbohydrate needed for growth (Alam et al., 2004). A second possibility is that salinity decreased the root and shoots growth by decreasing turger in expending tissue resulting from lower water potential in root medium (Alam et al,. 2004). A third is that the root show response to salinity was to down regulate shoot growth via long distance signal (Alam et al,. 2004). The fourth possibility is that a disturbance in mineral supply, either an excess or deficiency, induced by changes in concentration of specific ions in the growth medium, might have directly affected growth (Alam et al,. 2004).Cell injury

analysis is another parameter which is affected by salt concentration. According to Jamil et al. (2010) the cell injury increases with increasing salt concentration. Our result showed that salt concentration increases the injury analysis correlated with Jamil et al. (2010). SDS-PAGE is considered to be a particularly reliable method because storage proteins are largely independent of environmental fluctuations (Hanada et al., 2002). In the present study no significant variation was observed in (CO-47) in all treatments (Figure 7).A total of four bands were obtained and all the bands were common in both varieties. The bands were appeared on the gel divided into two categories; category 1st consists of major bands and second encircles minor bands on the glance (sharpness) of the bands. In four protein bands, three major bands and one minor band were observed. Kong-ngern *et al*. (2005) compared salt treated rice plants to control plants. In opposite, the level of sensitive rice remained unaffected with salt treatment. In control and salt therapy, one of the prominent bands in their banding patterns is 21 kDa. One notable band in our results is around 20 kDa in both the control and salt treatments, indicating that our results are similar to those of Kong-ngern et al. It would also be the next frontier for integration-free improvement of crop traits with salinity stress
Tolerance as a target.

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