SCREENING OF RICE VARIETIES FOR SALT TOLERANCE: INFLUENCE OF WHOLE AND SUBSOIL SALINITY ON CROP PERFORMANCES AND AGRONOMIC MITIGATION MEASURES

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ABSTRACT

Salinity is one of the major abiotic stresses which limit the rice production in coastal areas and in inland where irrigation agriculture is practiced. Inland salinity in rice fields is increasing, and if corrective measures are not taken, rice production could greatly be influenced. Therefore, identification of saline tolerant varieties, development of new salt tolerance varieties and introduction of measures to mitigate filed salinity are the most viable solutions. Therefore, this study was carried out as three separate experiments to (1) screen salinity tolerant rice varieties at seed germination stage, to study seed characteristics associated with seed germination under high saline conditions, and to evaluate seedling establishment under different soil salinity conditions, (2) evaluate effects of whole and sub-soil salinity on rice cultivars and to identify the physiological traits associated with salt tolerance in rice and (3) identify ways to mitigate the field salinity by managing irrigation and fertilization.

Seeds of several traditional, old-improved, new-improved and hybrid rice varieties were soaked in four different saline solutions (0, 3, 25 and 45 dS/m) for nine days and germination was recorded six days after when seeds were transferred from the respective solutions. Absorption of water, sodium and chlorine by seeds was analyzed after soaking the seeds in saline water. Seed-husk thickness and husk density were also measured. Germinated seeds were planted in soils adjusted to different salinity levels of 0, 3, 6 and 10 dS/m using NaCl . The experiment was arranged as three factor factorial design with three replicates. Seedling survival was recorded at 7, 14 and 21 days after sowing (DAS) ,and shoot and root weight was taken at 21 DAS, and leaf and root Na^+ contents were measured.

In the second experiment, Pokkali, Nona Bokra, At354 and Bg300 were grown under salinity levels of 0, 4, 6 and 8 dS/m created separately by adding NaCl and Na_2SO_4 at whole and sub soil salinity levels. Growth, physiological and yield parameters were assessed at regular intervals during the experimental period.

In the third experiment, four rice varieties (Bg 300, Bg 352, At 354 and Pokkali) were tested under three irrigation levels (saturated, field capacity and alternative wetting and drying) with the application of organic matter to mitigate filed salinity. Growth and yield parameters were measured during this experiment.

Results of the first experiment revealed that tested varieties could be categorized into four distinct groups according to its germination ability and seedling survival rate. Pokkali, At 354, Nona Bokra and At 401 could be categorized as tolerant varieties where Pokkali recorded the highest germination under the highest salinity level. A clear relationship was observed between seed sodium absorption percentage and seed germination of varieties. Seed-husk thickness had affected the sodium absorption to the seeds and it determined the germination ability of a variety. Seedling survival ability of rice cultivars decrease with increased salinity level and it is negatively correlated to soil electrical conductivity. Seed germination ability of a rice cultivar after pre-soaking the seeds in high salt solutions has positive relationship to seedling survival under soils with high salt. Thus, seed germinations and early seedling survival can be used to screen rice varieties for salinity tolerance. Shoot and root growth of saline sensitive varieties were drastically reduced under high saline soils where these were not much reduced in saline tolerant varieties. Salt tolerant varieties contained more Na^+ ions in their roots compared to Na^+ ions in their leaves where salt sensitive varieties were not recorded such higher difference. Chloride ion content in leaves of tested varieties varied significantly where saline tolerant cultivars had lower leaf Cl- content compared to saline sensitive varieties.

Growth and yield of rice varieties were significantly affected by the salinity conditions which they were grown. Varietal differences could be observed at early seedling growth and plants were able to survive up to harvesting in all the treatments except NaCl ($8dSm^{-1}$, whole soil) in the second experiment. With the increase of soil salinity , growth and yield reduction could be observed in all tested varieties. Suppression of growth and yield were varied with the variety and the type of salt used to create soil salinity. When increase the salinity level, Pokkali reduced its shoot: root ratio, implying higher root growth compared to shoot growth while Nona Bokra, At354 and Bg300 increased their shoot growth compared to root growth. Greater reduction in plant growth and yield was observed under NaCl compared to Na_2SO_4. Leaf and root sodium content varied among tested varieties. Leaves of saline sensitive variety of Bg300 contained more Na^+ ions than the other varieties where saline tolerant variety Pokkali contained less Na^+ ions in its leaves.

Yield of tested rice varieties varied with different soil amended with organic matter and with different water management practices in the field experiment. Inland salinity is mainly occurred due to the limitation of water in the dry zone and this is evident with the increase of yield under saturated water condition and standing water conditions. Soil amendment treatment with cow dung and charcoaled paddy husk recorded higher yield over other treatments. This implies that inland salinity problem could be reduced by adding organic matter and charcoaled paddy husk a soil amendments and providing adequate water supply.

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CHAPTER I

INTRODUCTION

Salinity is an enormous worldwide problem for agriculture; especially for crops that are grown under irrigation. This is because salt is inhibitory to the growth of a large number of different plants (Cuartero and Fernandez-Munoz, 1999). The extent of land affected due to salinity worldwide is estimated to be more than 900 million hectares which is approximately 6% of the total global land mass, or about 20% of the world's cultivated area (Flowers, 2004). Importantly, around half of the land devoted to the growth of irrigated crops is adversely affected by salt (Gamalero et al, 2009).

The inland salinity affected areas has been on the increase. Irrigation systems are particularly prone to salinization. When irrigated agricultural systems are practiced, solutes from the irrigation water can accumulate and eventually reach levels that have an adverse affect on plant growth. Salinization transforms fertile and productive land to barren land, and often leads to loss of crop production, loss of habitat and reduction of biodiversity. The effects of salinity on agriculture can be dramatic.

In Sri Lanka, although irrigation projects have substantially improved agricultural production, most large-scale projects face salinity problems. Although only a small percentage of the country's land has high and severe salinity problems, there are a significant number of areas affected by medium salinity. These are in danger of becoming highly saline due to inadequate management. Despite the scale of the problem, information on salinity in Sri Lanka is scarce. Studies indicated that crops and land in some areas in Mahaweli System exhibited significant salinity damage and large numbers of farmers in the affected areas cannot cultivate their land fully, while many suffer low productivity (http://web.idrc.ca/en/ev-8266-201-1-DO_TOPIC.html). In addition they found that salinity was having an effect on drinking water quality, human health, and an natural vegetation as well.

Salinity limits vegetative and reproductive growth of plants by inducing physiological dysfunctions and causing widespread direct and indirect harmful effects, such as inhibition of enzyme activity, photosynthesis, absorption of minerals, protein and nucleic acid mainly in non-halophytes even at low salt concentrations. Salt stress inhibited the repair of the photo-damaged PSII (inhibit synthesis of D1 protein). The combination of light and salt stress appeared to inactivate PSII very rapidly as a consequence of their synergistic effects.

Photosynthesis is a primary process in plant productivity and a positive relationship between photosynthesis and yield under saline conditions has been found in different crops (Ashraf and Praveen, 2002). Varying relationships between photosynthesis and growth or yield of salt tolerant and salt sensitive lines of some species have been reported. Studies has also shown that the photosynthetic rate, stomatal conductance and intrinsic water use efficiency of the flag leaf were greater of salt tolerant wheat species compared to salt sensitive (Ashraf and Praveen, 2002). Research projects at Liverpool have investigated details of the physiology and biochemistry of salt tolerance and also looked at methods to screen overall plant performance that could be used in breeding programs. Many other studies have also shown that there were genotypic differences in salt tolerance (Singh and Senadhira, 1986; Singh et al., 1994; Abeysiriwardena, 2004). Tolerance is mostly associating with physiological, anatomical and biochemical variations, which could be exploited to develop new tolerant varieties to successfully overcome salinity problems. Several studies also suggested that salt tolerance in rice is correlated with antioxidant enzyme activities and modulation of the antioxidant activity might be important in the resistance of rice to salt stress. However, these remain to be clarified (Jain et al., 2003).

Some traditional and improved Sri Lankan rice varieties are capable of tolerating salinity conditions better (Singh and Senadhira, 1986; Abeysiriwardena, 2004). Though some preliminary screening studies were carried out and some tolerant varieties were identified; proper guideline for screening, traits associated with tolerance and/or salinity tolerance mechanisms were not investigated adequately. This information is very much essential for future breeding and genetic engineering programs for the development of new salt tolerant rice varieties.

Salinization can also be managed to some extent by changing gnomic management practices such has water and fertilizer management. Therefore, the identification of specific water and fertilizer management practices together with the development of tolerant varieties definitely provide long-term solution to salinity problems.

Therefore, overall objective of the project was to develop precise varietal screening methodology, characterization of varieties to salinity responses, and to find mitigatory measures to overcome salinity problems by using tolerant varieties and other possible agronomic practices.

Specific objectives were to;

a) Study the reaction to salinity of selected traditional, old and new improved, and hybrid rice varieties grown after seeds were soaked in high salt concentrations based on seed germination and seedling growth after establishment.

b) Determine growth, yield, and physiological traits associated with the salt tolerance of selected salt tolerant rice varieties under whole and subsoil salinity conditions.

c) Evaluate productivity of selected salt tolerant and susceptible rice varieties under farmer field conditions with different irrigation and fertilizer management practices to develop potentially salinity mitigatory agronomic practices.

CHAPTER II

REVIEW OF LITERATURE

2.1 Rice Plant and its general characteristics

2.1.1 Seed Germination

Seeds are defined as “the ovule developed after fertilization”. Germination starts with the growth of embryos. There are two definitions of germination. It is defined as the time when the embryo splits the outer membrane of seeds and part of the tissue appears (Tkahashi, 1962., Bewly and Black, 1985). Another view is that germination is the time when the plumule and radicle indicates geotropism; this is the time when the normal growth of next generation can be established (Danjo, 1966).

When rice seeds germinate in air, the radicle grows predominantly. However, when they germinate in water, coleoptiles growth takes place preferentially and radicle growth is prevented. When the coleoptiles tips reach the water surface and encounter oxygen, their elongation ceases and root growth occurs. Even in water, if germination takes place under the presence of light, chlorophyll is formed in the coleoptiles and primary leaves and the photosynthesis proceeds; thus radicle growth is also becomes possible. The growth of the coleoptiles and radicle in water also varies with the rice ecotype (Takahashi, 1978).

2.1.2 Differentiation and development of tiller buds

Tiller buds of rice plants are axillary buds which differentiate at the leaf axils. A tiller bud differentiates at each leaf axils in rice plants. Environment conditions and cultural practices seem to account very little for the differentiation under actual field cultivation. The development of tiller buds after differentiation is greatly affected by environment conditions, as well as varietal characters in regard to the response to environments (Takane *et al*, 1995).

During its growing period, the rice generates and lengthens roots from nodes; these roots often number of several hundreds. In addition, each of the roots has secondary, tertiary and further lateral roots; these roots form a root system. Since a root system consists of old and new roots in different growth stages including the respective lateral roots (Takane *et al.*, 1995).

Rice plant leaves consist of six parts; leaf blade, leaf sheath, ligule, auricule, lamina joint and leaf sheath pulvinus. The leaf blade is the organ of photosynthesis and transpiration, and at the same time is a main source of internal nitrogen for growing parts (Fugiwara *et al.*, 1951; Yoneyama and Sano, 1978). The leaf sheath protects and supports the shoot (Chang, 1964) and serves as a temporal storage of photosynthesis (Tagari *et al.*, 1954). Its photosynthetic capacity is only about one tenth that of the leaf blade (Matsushima *et al*, 1958; Tsuno *et al*, 1975).

2.2 Diversity of salt tolerance among species

Salt tolerance is usually assessed as the percent biomass production in saline versus control conditions over a prolonged period of time. Another criterion of salt tolerance of crops is their yield in saline versus non saline conditions. A survey of salt tolerance of crops, vegetables and fruit trees has been published by Mass and Hoffman (1977), and updated by Francois and Mass (1994). They showed there is threshold salinity for each species below which there is no reduction in yield, and then a regression for the reduction in yield with increasing salinity wide range of tolerance across species.

Differences in salt tolerance between closely related species are difficult to quantify, as the growth reduction depends so much on the period of time over which the plants have grown in saline conditions. Salinity lowers the water potential of the roots, and this quickly causes reduction in growth rate, along with a suit of metabolic changes identical to those caused by water stress (Munns, 2002). Later, there may be salt specific effects that impact on growth and senescence.

The crops show a two-phase growth response to salinity (Munns, 1993). The first phase of growth reduction is quickly apparent, and is due to the salt outside the roots. It can be called as water stress or osmotic phase, for which there is surprisingly little genotypic difference.

Then, there is a second phase of growth reduction, which takes time to develop, and is associated with advanced senescence of older leaves. This presumably results from internal injury due to salt accumulating in these transpiring leaves to excessive levels. If excessive amounts of salt enter a plant, salt will eventually rise to toxic levels in the older transpiring leaves, causing premature senescence and reducing the photosynthetic capacity of the plant to a level that cannot sustain further growth (Munns, 1993).

The cause of injury is probably due to the salt load exceeding the ability of the cells to compartmentalize salts in the vacuole. Salts then rapidly build up in the cytoplasm and inhibit enzyme activity. Alternatively; they might build up in the cell walls and dehydrate the cell. Evidence for ions accumulating to high concentrations in the apoplast of leaves has been found in rice (Flowers *et al.*, 1991). A two-phase growth response has also been shown clearly for maize and wheat cultivars (Crameret *et al.*, 1994).

For rice, a clear distinction has been made between the initial effects of salinity, from which recovery is possible, and the long-term effects that result from accumulation of salt within expanded leaves (Yeo *et al.*, 1991).

The length of time required before growth differences between genotypes can be seen depends on the salinity and the degree of salt tolerance of the species. The second phase will start earlier in plants that are poor excluders of Na^+, such as lupins or beans, and when salinities are higher. It will also start earlier when temperatures are not higher. For the rice plants that are given at high temperatures 10-15 days in salinity is sufficient to generate differences in biomass between genotypes that correlate well with differences in yield (Aslam *et al.*, 1993).

Different plant species and cultivars have differing ability to grow in saline conditions. The following table shows some plant species showing different responses under saline condition (FAO, 2005).

Table 1: Response of plants to salinity under broad sense.

Type of plant	High Tolerance	Medium Tolerance
Field Crops	Barley, Cotton	Rye,wheat,Sunflower,Lupin,Soybean Oats, Millets,Sorghum, Rice, Peanut
Fruits	Date palm	Pomegranate, Fig, Olive,Grape Rockmelon, Mulberry
Vegetables	Beet-root, kale Asparagus,Spinach	Tomato, Broccoli,Cabbage,Cauliflower Sweet corn,Broad bean, Squash Pumpkins, cucumber
Pasture plants	Rhodes grass, Couch,Panic Kikuyu,Almum,Pangola wimmera ryegrass,Lucerne Siratro,Buffel,Guinea	Berseem clover,Snail medic,Phalaris Barrel medic, ,Paspalum Strawberry clover, Sudan grass Perennial ryegrass, Reed canary
Ornamentals	Carnation, Clematis	Bouganvillea, Chrysanthemum Oleander, Hibiscus

2.3 Mechanisms of salt tolerance

Mechanisms of salt tolerance takes place at three levels of organization. They are whole plant, cellular and molecular levels.

2.3.1 Control of salt at the whole plant level

Salt tolerance depends on the ability of the plant to control the transport of salt at five sites as below (Munns *et al.*, 2002).

1. Selectivity of uptake by root cells. It is still unclear which cell types control the selectivity of ions from the soil solution. The initial uptake of Sodium and Chlorine could occur at epidermis, at the endodermis, or if the soil solution flow apopplastically across the root cortex, it would occur at the endodermis.

2. Loading of the xylem. There is evidence for the preferential loading of K+ rather than Na+ by the cells at the stele.

3. Removal of salt from the xylem in the upper parts of the roots, the stem, petiole or leaf sheaths. In many species, Na^+ is retained in the upper part of the root system and in the lower part of the shoot, identically an exchange of K^+ for Na^+ by the cells in the stele of the roots or in the vascular bundles and petioles.

4. Loading of the phloem. There is little translocation of Na^+ or Cl^- in the phloem, particularly in the more tolerant species. This ensures that salt is not exported to growing tissues of the shoot.

5. Excretion through salt glands or bladders. Only halophytes have these specialized cell types.

All halophytes have well-developed mechanisms to control uptake, transport and excretion of salt. Glycophytes rely on the first three mechanisms to various degrees. In perennials, the leaves of which may live for a year or more. Hence, there is greater need to regulate the incoming salt load over a much longer period of time. In annual species whose leaves may live for only one month. They show high shoot: root ratios and high intrinsic growth rates to reduce salt accumulation rates in leaves (Pitman, 1984) and absence of an apoplastic pathway in roots (Garcia *et al.*, 1997).

2.3.2 Control of salt in the Organelle level (Ion compartmentation)

There is no evidence of adaptations in enzymes to the presence of salt (Munns *et al.*, 1983), so mechanisms for salt tolerance at the cellular level involves keeping the salt out of the cytoplasm, and sequestering in the vacuole of the cell. In some leaves it exceeds concentration of 200mM in leaves without functional differences but these concentrations will completely inhibits enzyme activity in vitro (Munns *et al.*, 1983).Generally, Na^+ starts to inhibit most enzymes at a concentration above 100mM. The concentration at which Cl^- become toxic is even less well defined, but is probably in the same range as that of Na^+. If Na^+ and Cl^- are sequestered in the vacuole of the cell, K^+ and organic solutes accumulate in the cytoplasm and organelles to balance the osmotic pressure of the ions in the vacuole. The commonly accumulated organic solutes are proline and glycine betane (Hasegawa *et al.*, 2000).

2.3.3 Control of salt at the molecular level (Ion transporters)

The ion channels and transporters are involved to regulate the net movement of salt across the cell membrane. There is no specific Na^+ transporter, Na^+ entry being gained by competition with other cations; in particular K^+.These non selective channels are affected by Ca^{2+}. These cation channels could allow entry of large amounts of Na^+ from highly saline soil if not adequately regulated (Amtmann and Sunders., 1999). Sodium ions(Na^+) can be effluxed from the cytoplasm through Na^+/H^+ antiporter, driven by the pH gradient across plasmalemna (Blunwald, 2000).

2.4 Effect of salinity on rice

Rice varieties exhibit variability in their sensitivity to salt stress, vegetative growth of some cultivars showing surprisingly high resistance to soil salinity (Munns *et al.*, 2002, Yeo and Flowers., 1983). In most plants, radial transport of sodium to the root-xylem occurs via a symplastic pathway involving loading transporters (Munns *et at.*2002, Tiaz and Zeiger, 1998), but in rice it is proposed to occur preferentially by an apoplastic transpirational bypass pathway (Garcia *et al.*, 1997, Yeo *et al.*, 1987). There are, however, a few reports that implicate K^+ transporters such as HKT1 in Na^+ uptake in rice roots (Garciadeblas *et al.*, 2004, Golldack *et al.*, 2002). Little is understood about mechanisms regulating the net shoot Na^+ uptake in rice, but the negative correlation reported between initial Na^+ uptake into shoots and varietal survival (Yeo and Flowers, 1983) suggests that these mechanisms constitute a significant aspect of tolerance.

Differences in salt tolerance among rice cultivars can also be caused by differential compartmentalization of the Na^+ in the shoot (Yeo and Flowers, 1982). Older leaves have been shown to act as ion sinks that restrict ion entry into meristematic and actively growing; photosynthesizing cells (Yeo and Flowers, 1982). High Na^+ levels in shoot apoplast would lead to osmotic stress and eventually cyto-toxicity (Oertli, 1968). The critical role proposed for this compartment supported by toxic levels of apoplastic Na^+ detected in rice and pea (Flowers *et al.*, 1991, Speer and Kaiser, 1991). Finally, vacuolar sequestration could play a significant role as suggested by salt tolerance conferred on over-expression of the vacuolar Na^+/H^+ antiporter in rice (Fukuda *et al.*, 2004, Ohta *et al.*, 2002).

Calcium (Ca^{2+}) is found to be crucial in altering the ion selectivity of uptake by plants and in enhancing salt tolerance in many species (Epstein, 1961; Rains and Epstein, 1967, Zhu, 2002). The application of Ca^{2+} (as gypsum) is a common management practice for soils with high exchangeable Na^+, and its beneficial effects in saline paddy fields as a reclamation technique.

2.4.1 Effect of salinity on seed germination and early seedling growth

Germination is a crucial stage in the life of many plants and salt tolerance during this phase is critical for the establishment of plants growing in saline soils (Maranon *et al.*, 1989).

For several halophytic and glycophytic species, germination was inhibited by salinity (Marcar, 1987; Maranon *et al.*, 1989; Miyamoto, 1989; Keiffer and Ungar, 1997). Laboratory investigations of seed germination indicated that seeds of most halophytes and glycophytes attain their maximum germination in distilled water and are very sensitive to elevated salinity at the germination and early seedling phase of development (Norlyn and Epstein, 1984; Morgan and Myers, 1989; Khan, 1991). Ghoulam *et al.* (2001) showed that the germination was reduced with increasing salinity levels in *Beta vulgaris* L. and sodium content in the seeds increased with increasing salinity condition. They also observed a reduction of final germination percentage with an increase in the osmotic potential of solutions. At the same osmotic potential, germination was different in NaCl compared to mannitol. This implies that NaCl acts on germination not only by an osmotic effect but also by another effect that could be specific ion toxicity (Ghoulam *et al.*, 2001).

Sirisena *et al.* (2005) showed that germination reduction of rice seeds after seed soaking in high saline solutions and they observed varietal differences at 40 -50 dS/m saline solutions and this method could be used as rapid screening procedure for salinity tolerance for rice. Also they observed that saline tolerant varieties recorded higher germination percentage than saline sensitive varieties at same salinity level. A same pattern in rice was also observed by Dissanayake *et al.* in 2006.

2.4.2 Effect of Salinity on Photosynthesis

Salinity appears to affect two plant processes: water relations and ionic relations. During initial exposure to salinity, plants experience water stress, which in term reduces leaf expansion. During long-term exposure to salinity, plants experience ionic stress, which can lead to premature senescence of adult leaves, and thus a reduction in the photosynthetic area available to continued growth (Cramer and Nowak, 1992). Reduced photosynthesis with increasing salinity is attributed to either stomatal closure, leading to a reduction in intercellular CO_2 partial pressure, or non-stomatal factors (Bethke and Drew, 1992). There is increasing evidence that salinity changes photosynthetic parameters, including osmotic and leaf water potential, transpiration rate, leaf temperature, and relative water content. Salt also affects photosynthetic components such as enzymes, chlorophylls, and carotenoids. Changes in these parameters depend on the severity and duration of stress (Lakshmi *et al.*, 1996; Misra *et al.*, 1997) and on plant species (Dubey, 1994).

Grain growth depends on current assimilation, remobilization of stored pre-anthesis assimilates, and re-translocation of assimilates to grain after anthesis. During grain filling, leaf water potential plays an important role in assimilate production and partitioning (Bradford, 1994).

Results of greenhouse experiments showed that wheat(Mass and Poss, 1989a) and cowpea(Mass and Poss, 1989b) were most sensitive during the vegetative and early reproductive stages, less sensitive during flowering , and least sensitive during grain- filling. Many reports of rice showed effect of salinity at early stage. Lutts *et al* (1995) reported that rice is more sensitive to salinity at the reproductive stage.

Sultana *et al* (1999) showed that rice is more resistant to salinity at the initial stage of imposed salinity but more sensitive with increasing duration of salinity. They studied the response of *Oryza stiva* L. cv.Koshihikari (moderately salt resistant variety) to salinity that NaCl drastically reduced stomatal conductance; this might be attributed to the lower leaf water potential and a reduction in relative leaf water content, which resulted in loss of turgor, which leads to reduced photosynthetic rate. The extent to which stomatal closure affects photosynthetic capacity is indicated by the magnitude of reduction in stomatal conduction (Farquhar and Sharky, 1982). The decline in photosynthesis under salinity attributed partly to reduced stomatal conductance (Nagi and Galiba, 1995; Lakshmi *et al.*, 1996), partly due to a reduction in protein concentration (Sibole *et al.*, 1998) and partly to a decline in photosynthetic pigment concentrations (Kolchevskii *et al.*, 1995) or ionic concentrations (Khan *et al.*, 1997). Yeo *et al.* (1985) also reported that the inhibition of net photosynthesis in rice by salinity is mediated by water deficit in the leaf cells due to accumulation of salt in the apoplast. Thus the decline in photosynthesis was not due to limited CO_2. It was also reported that low photosynthesis under stress was associated with a low demand for photosynthates in the sink, viz, reproductive development, including grains (Karim *et al.*, 1993). Photosynthetic pigments, sugars and protein concentrations of leaves were reduced by salinity and that the effect was aggravated by the long duration by salinity (Sultana *et al.*, 1999).

A decrease in chlorophyll concentration in salinized plants could be attributed to increased activity of the chlorophyll degrading enzyme chlorophyllase (Reddy and Vora, 1986). Ion accumulation in leaves also adversely affected chlorophyll concentration (Yeo and Flowers, 1983). The decrease in carotenoids under salt stress leads to degradation of β-carotene and formation of zeaxanthins, which are apparently involved in protection against photo inhibition (Sharma and Hall, 1991). As salinity adversely influenced the photosynthetic process, photosynthetic production (e.g. sugar) was inhibited. Kerepesi *et al.* (1998) also found that sugar contents of leaves decreased in tolerant genotypes of wheat under NaCl stress but drought stress increased the water soluble carbohydrate which had linked to stress tolerance. Munns and Weir (1981) also reported that the initial changes in osmotic potential were largely due to reducing sugars. Sultana *et al.* (1999) showed that lower photosynthesis is due not solely to lower stomatal conductance or CO_2 fixation but also to the cumulative effects of other non-stomatal and biochemical components.

After seed initiation, leaf mesophyll tissues are the closest source of sugars and provide current photo-assimilate for the growth and development of seeds. Reductions in photosynthetic pigments, hill reaction, carboxylase enzymes, and chlorophyllase induction under saline conditions led to poor photosynthates formation (Soussi *et al.*, 1998).

The significant increase of accumulation of proline in both salinized leaves and grains is implicated in osmotic adjustment to salinity. Proline is also considered to act as a compatible osmoticum and therefore to be involved in salt resistance (Delauney and verma, 1993).

In general, three major processes operate simultaneously during grain filling: photosynthesis, translocation of photosynthates to the grain, and grain growth. In analyzing the reduction in grain dry matter due to stress, it is necessary to identify which of these processes is the limiting factor. The reduction in dry matter at dough stage might be through inhibition of current photo-assimilation, because salinity reduces the contents of photosynthetic pigments and soluble proteins in the ovaries; this change might cause the decline of ovary photosynthesis leading to the poor sugar production in the ovaries. A reduced number of fertile florets (Khatum and Flowers, 1995) and a lower rate of assimilate translocation from shoot to panicles are responsible for the lower dry weight of panicles and grain. This might be at attributable to the rapid reduction in leaf photosynthesis, which is related to the decrease in photosynthetic pigments. Therefore, translocation of assimilates from stem to grain is the main source as well as limiting factor for growth and development of grain.

Salinity decreased the reserves in leaves, the grain growth rate, and the current dry matter production (Sultana *et al.*, 1999). Similar results were found with maize (Jurgens *et al.*, 1978). Toxic ions in the leaves such as Na^+ may interfere with phloem loading and thus translocation of assimilate reserves was inhibited in salinized plants, independently leaf water potential and relative leaf water content. Carbon assimilation and hence dry matter production were more affected than was remobilization of assimilates during post-anthesis water deficit (Kobata *et al.*, 1992) and they concluded that under salinity, both photosynthesis and translocation of photosynthates to the grain are important for grain growth.

2.5 NaCl-induced Senescence and Chlorophyll fluorescence

Salinity applied at the seedling stage frequently induces premature senescence of leaves (Sahu and Mishra, 1987; Yeo *et al*, 1991). Leaf senescence is most often quantified by decreases in protein or chlorophyll concentration (Chen and Kao, 1991; Chen, Li and Kao, 1991) and by increases in membrane permeability (Dhindsa *et al.*, 1981).Chlorophyll fluorescence kinetics, and more especially the rates of the maximal variable fluorescence (Fv/Fm) which is directly related to PS II photochemical efficiency, may also be modified during ageing (Bjorkman, 1987).

In rice, NaCl salinity lowered the Fv/Fm chlorophyll fluorescence ratio after 47 days of stress and also at the flowering stage (Bohra et al., 1995; Bohra and Doffling, 1993). NaCl decreased chlorophyll concentration in both young and older leaves. Decrease of Fv/Fm ratios coincided with a decrease in chlorophyll-b concentration, although only Chlorophyll-a is involved in fluorescence. Since chlorophyll-b is mainly associated with PS II antenna, a decrease in its concentration could reflect a structural modification of antenna which could explain the NaCl-induced increase in F_0 occurring before any decrease in Fm(Lutts *et al.*,1996)

Most of rice cultivars differing in salt resistance differed in Fm values. This is considered to reflect a reduction in the amount of the primary electron acceptor, Q_A. A decrease in Fm values in stressed plants could result either from photochemical quenching, from non-radiative energy dissipation in the pigment bed or from transfer to PS-I (Bjorkman, 1987; Krause and Weis, 1991).

2.6 Role of Zinc, Calcium and Silicon on salinity damage in rice

Zinc is required for maintenance of integrity of bio-membranes (Marschner, 1995). Under zinc deficient conditions there is a typical increase in plasma membrane permeability of root cell (Welch *et al.,* 1982). Increased membrane permeability in Zn-deficient plants might lead to enhanced chlorine, sodium and boron uptake particularly in salt-affected soils. Thus, under these conditions plant growth and yield decrease (Marschner, 1995). Khoshgoftar et al. (2004) showed that application of Zn increased salt-tolerance of wheat.

2.7 Genetic Variability and sources of salt tolerance

Traditional varieties are reported as the most tolerant to abiotic stresses. varieties Pokkali, Cheriveruppu, Nona Bokra, Damodar and Getu are tolerant of salinity but possess poor agronomic characters; they are tall and some are photosensitive, have poor grain quality and low yielding (Senadhira and Akbar, 1991). Salt tolerant indica rice cultivars seem to have originated or been selected in coastal areas of India (states of kerala and West Bengal); examples are Pokkali, Nona Bokra and Cheriviruppu. An evaluation at IRRI of 250 traditional cultivars collected from Orissa and Tamil Nadu in India has revealed some salt-tolerant types from southeastern coastal areas of India. Among the tolerant cultivars identified, NV, Pat, Solla, and DH were found to have tolerance similar to popular pokkali. Similar tests with coastal rice cultivars of Indonesia, Thailand, and Veitnam identified three highly tolerant types: Ketumbar(Indonesia), Khao Seetha(Thailand) and Soc Nau (Vietnam). Again tolerant mechanisms appear similar to Pokkali. But cultivars like Pat, Ketumbar and Soc Nau have better grain quality(white fine grains, intermediate amylase) than Pokkali(red bold grain, high amylase)(Gregorio *et al.*, 2002).

Attempts have also been made to identify other sources of salt tolerance. Wild species of *Oryza* and the African *Oryza glaberrima* were screened at 2-3 leaf stage at electrical conductivity of 12 dS/m for 14 days. All were highly susceptible with seedling survival range 0-16% while the tolerant control, Nona Bokra showed complete survival (Akbar et al., 1987). However, some wild rice (*Oryza rufipogon*) from Sri Lanka showed salinity tolerance at seedling stage comparable to Pokkali. A distant relative of Oryza (*Porteresia coarctata*); halophyte that needs high salinity (EC of 12 – 18 dS/m) to produce grain, may not be a good source of tolerance in breeding programs because the difficulty in producing fertile F_1, and their requirement for high salinity (Gregario et al., 2002).

2.7.1 Genetics of Salt Tolerance

Genetic studies at IRRI indicated that both additive and dominance effects are important in the inheritance of almost all characters associated with salt tolerance (Mishra et al., 1990; Gregario and Senadhira, 1993; Lee, 1995). At the seedling stage under saline condition, characters associated with salinity tolerance such as shoot length, low Na^+ and high K^+ content in the shoots, and large dry weights of shoots and roots showed highly significant additive effects as the of heritability in these characters is low. Characters at the maturing stage such as plant height and yield per plant showed highly significant additive effects. This suggests the greater importance of additive gene action in the inheritance of these characters (Moeljopawiro and Ikehashi, 1981; Akbar *et al.*, 1986, Mishra *et al.*, 1990).

2.8 Salt Stress-Induced Anti-oxidant activity

Aerobic oxidative metabolism represented a major evolutionary step for living organisms, allowing a more efficient utilization of the energy stored in chemical bonds. On the other hand, the use of molecular oxygen as the final electron acceptor posed a risk of oxidative damage due to production of partially reduced intermediates, known as reactive oxygen species(ROS)(Scandalios, 1993). ROS have the potential to interact non-specifically with many cellular components, triggering peroxidive reactions and causing significant damage to proteins, lipids, and nucleic acids, therefore, their levels must be carefully monitored and controlled in cells(Scandalios, 1993, Halliwell *et al.*,1999). To cope with ROS and to maintain redox homeostasis, living organisms evolved antioxidant defense systems, comprised of enzymatic and non-enzymatic components, which normally maintain ROS balance within the cell. Thus, oxidative stress is established when this balance is disturbed, either through enhanced production and accumulation of ROS or antioxidant defenses depletion (Scandalios, 1993, Dat *et al.*, 2000). In spite of their potential hazards, ROS also exert many important physiological roles such as cell signaling, gene regulation, senescence, programmed cell death, pathogen defense, and others (Dat *et al.*, 2000).

The interest in plant antioxidant defense has increased over the past years. The evidence accumulated thus far indicates oxidative stress as an important consequence of different environmental stresses, including salinity (Dat *et al.*, 2000). Several reports have demonstrated a direct connection between salt stress and altered redox states and antioxidant defenses. Menizes *et al* (2004) revealed that salt stress in rice triggered a defense mechanism against oxidative stress. Antioxidant response patterns operating in different plant species are well conserved, with major antioxidant transcripts being up-regulated in response to salinity. Hence, antioxidant defenses are concertedly regulated to ensure proper protection against ROS generated after salt exposure (Menizes *et al.*, 2004).

2.9 Chlorine and its characteristics on plant growth

Chlorine (Cl) is an essential micronutrient for higher plants, and a minimal requirement for crop growth of 1 g kg^{-1} dry weight has been suggested (Marschner, 1995). This quantity can generally be supplied by rainfall, and chlorine-deficient plants are rarely observed in agriculture or nature. However, high tissue chlorine concentrations can be toxic to crop plants, and may restrict the agriculture of saline regions (Xu *et al.*, 2000).

Chlorine inputs to soils occur mainly as a result of depositions of chloride (Cl^-) from rain water, fertilizer applications (KCl), irrigation water, sea spray, dust and air pollution (Hewitt and Smith, 1974; Xu et al., 2000).

Chlorine is present mainly as Cl^-, although plants do contain compounds with covalently bound chlorine (Engvild, 1986). Chloride is a major osmotically active solute in the vacuole and is involved in both turgor and osmoregulation. In the cytoplasm it regulates the activities of enzymes. Chloride also acts as a counter anion, and Cl^- fluxes are implicated in the stabilization of membrane potential, the regulation of pH gradients and electrical excitability.

The growth of many plants is reduced substantially in chloride-free media (Broyer *et al.*, 1954; Xu *et al.*, 2000), and environmental factors that enhance growth rate increase susceptibility to chlorine deficiency(Ozanne *et al.*, 1957).deficiency causes reduced leaf growth and wilting, followed by chlorosis, bronzing and, finally, necrosis. Roots become stunted and the development of laterals is suppressed. Fruits are decreased in number and size (Xu *et al.*, 2000).

2.9.1 Chloride toxicity

The growth responses of plants to high chloride concentrations in the external medium can be divided into four categories (Greenway and Munns, 1980). Species can be grouped into, **(1)** Halophytes (defined as the native flora of saline soils), which can be sub divided into ; species whose growth is stimulated (e.g. Sueda maritime, Atriplex nummularia) or species whose growth is little affected by 200mM of chloride(e.e. Atriplex hastate, Spartina spp., Sugar beet); **(2)** non- halophytes(glycophytes)whose growth is reduced substantially by 100mM chloride, which can be sub divided into tolerant(e.g. *Festuca rubris*, cotton, barley), intermediate(e.g. tomato) and sensitive(e.g. soybeans, beans) and **(3)** very salt-sensitive non-halophytes(e.g. citrus). Many important cereal, vegetable and fruit crops are susceptible to Cl^- toxicity during cultivation. This is a major constraint to horticultural production on irrigated or saline soils (Mass and Hoffman, 1977; Xu *et al.*, 2000). The critical tissue Cl^- concentration for toxicity is about 4-7 and 15-50 mg g-1 dry weight for chloride-sensitive and chloride-tolerant plant species respectively.

Differences between cultivars to withstand chloride toxicity are frequently related to the ability to restrict chloride transport to the shoot. This has been observed in soybean (Glycine max; Abel, 1969), wheat (Triticum aestivum; Bernad et al., 1974), barley (Greenway and Munns, 1980), stone fruit trees (Bernatein et al., 1956) grape vine (Vitis spp; Antcliff *et al.*, 1983) and citrus (Storey and Walker, 1999). The trait is heritable (Abel, 1969; Sykes, 1992) and, in soybean, it is determined by a single gene (Abel, 1969). Thus there is the opportunity for simple breeding and genetically modified (GM) approaches to limit chloride accumulation and to generate plants that withstand chloride toxicity (Garcia and Primo, 1995).

2.9.2 Chloride Distribution within the plant

There is considerable variation in the ability of plants to accumulate chloride (Cram, 1976; Greenway and Munns, 1980). Halophytes generate turgor by accumulating high chloride concentrations in plant tissues (340 to 475mM), and fluctuations in osmotic pressure during their growth, or in response to environmental challenges, are usually affected by changes in NaCl concentration. By contrast, glycophytes growing in natural habitats have much lower chlorite in plant tissues (7 to 70mM), and chloride is generally only a minor component of their cell-sap osmotic pressure.

There are also large differences in tissue chloride concentrations between different parts of the same plant. In general, the older leaves of non-halophytes grown at high salinity have higher tissue-chloride concentration than lower leaves (Greenway and Munns, 1980). This is probably the result of a combination of rapid growth and low transpiration in young expanding leaves, coupled with the continued uptake and minimal recycling of Cl^- in young leaves is thought to be important for salinity tolerance of glycophytes. By contrast, in halophytes, such as sugar beet, *Atriplex hastate* or the salt-tolerant garss *Leptocloa fusca*, all leaves have similar $[Cl^-]_{tissue}$ (Greenway and Munns, 1980). In both glycophytes and halophytes, floral tissues generally have lower $[Cl^-]_{tissue}$ than other shoot parts. Tissues that are fed predominantly through the phloem tend to have the lowest $[Cl^-]_{tissue}$. Hence, the $[Cl^-]_{tissue}$ of fruits and seeds is generally low (Xu et al., 2000). However, the exact value may be depending upon previous Cl^- fertilization of the crop and on the plant species or cultivar (Hewitt and Smith, 1974).

Plants acquire most of their chlorine from the soil solution as the Cl^- anion and delivered to the shoot. Within the root, the mature root has a higher $[Cl^-]_{tissue}$ than regions close to the tip (Storey and Walker, 1987).

2.10 Strategies for increasing salt tolerance of crops

i) Searching amongst natural diversity within the species, or closely related and inter fertile species.

ii) Genetic engineering

With both methods, back crossing into cultivars or advanced breeding lines will be required. In the field, the major drawback is the heterogeneous nature of salinity within paddocks and between sites.

Traits for salt tolerance that have been used to screen germplasm collections have included rates of $Na^+$and Cl^- accumulation in leaves, degree of heat injury, seedling root length and germination percentage. sodium accumulation in leaves has been shown to regulate relate to salt tolerance in genotypes of rice(Yeo and Flowers,1986) and diploid wheat(Schachtman *et al.*,1991).Some success has been reported for molecular markers for salt tolerance in rice(IRRI,1997).

CHAPTER III

Materials and Method

Three separate experiments were carried out to screen the rice varieties for salinity tolerance at germination stage, to evaluate selected rice varieties for whole and subsoil salinity conditions and to manage saline effected soils using different management practices at farmer fields.

3.1 Experiment one:

- Identification of salt tolerant rice varieties at the seed germination stage and its relationship to seed-husk thickness and ion absorption.
- Effect of salinity on early seedling establishment of rice.

3.1.1 Experiment Location:

The experiment was carried out under laboratory conditions at Rice Research and Development Institute, Batalagoda, Ibbagamuwa, Sri Lanka and chemical analyses were done at the Department of Crop Science, faculty of Agriculture, University of Peradeniya, Sri Lanka from February to July in 2007.

3.1.2 Description of the Experiment

Rice varieties were screened for salinity tolerance at the germination stage using sixteen improved (Bg300, Bg304, Bg350, Bg380, Bg94-1, Bg407-H, Bw302, Bw78, Bg750, Bg450, At353, At354, At303, At401, H-10 and H-4) and ten traditional rice varieties(Pokkali, Nona Bokra, Rathel, Seeraga Samba, Bandara Heththewa, Murungakayan, Hondarawalu, Pachchaperumal, Periyakarappan and Dikwee). Pokkali and At354 were used as resistant check varieties. Seeds of selected varieties were obtained from the Rice Research and Development Institute, Batalagoda, Ibbagamuwa and Plant Genetic Resource Centre, Gannoruwa, Sri Lanka.

3.1.3 Experimental Design

A two factor factorial experiment, factors being the cultivars (26 varieties listed above) and salinity levels [seed soaked for nine days in 25 and 45 dS/m NaCl respectively, prior to germination and control (no soaking in NaCl before germination)] was laid out in a complete randomized design (CRD) with three replicates.

3.1.4 Germination Test

3.1.4.1 Preparation of saline solutions

Sodium chloride solutions with different electrical conductivity (EC) levels, 3, 25 and 45dS/m were prepared by dissolving AR grade sodium chloride in distilled water to represent solutions with different levels of salinity. Equal volumes of these solutions were poured into petri-dishes containing 100 seeds of each variety, as three replicates per variety. Seeds were kept in these solutions for 9 days. Seeds soaked with distilled water without NaCl was used as the control.

3.1.4.2 Germination count

Nine days after soaking, seeds were taken out and washed thoroughly to remove the film of salt solution around the seeds and placed in Petri-dishes with a pieces of wet blotting paper lined at the bottom. Number of germinated seeds, abnormally germinated seeds and non-germinated seeds were counted after six days. If seeds had both well developed radicle and coleoptile on the sixth day, germination was considered as normal. If there was no formation of radicle and coleoptile, such seeds were considered as non-germinated.

3.2 Sodium and Chlorine analysis

A separate seed sample from selected varieties based on the germination ability under saline water (representative varieties including salt tolerant, moderately tolerant, susceptible and highly susceptible) were soaked with desired level of NaCl to give the correct EC level to measure sodium and chloride content. Then seeds were air dried and followed by oven drying at 60^0C for 24 hours. Absorption of sodium and chloride by seeds was analyzed after careful de-husking of seeds and using rice grains (kernel).

3.2.1 Sodium analysis

Finely ground dried kernels of rice (1g) was taken into a crucible. Then, it was put in a furnace at 200^0C for one hour. After that, temperature was increased up to 450^0C for one hour and sample was allowed to cool. Then, sample was taken out from the furnace and 5ml of 6M HNO_3 acid added into the crucible and boiled on a hot plate. While boiling the mixture, 5ml of 3M HNO_3 was added to the crucible. Then, the sample was allowed to cool and mixture was filtered into a 50ml volumetric flask while washing the residues with 1% HNO_3 and volume up to 50ml using distilled water. Finally, Na^+ content of the extraction was analyzed using flame photometer (Vanrast et al., 1999).

3.2.2 Chlorine Analysis

Dried rice grains (kernels) were finely ground using a grinder. A sample of 1 to 2 grams was placed in a porcelain evaporating basin and mixed it with about one quarter of its weight with calcium oxide (CaO) and sufficient distilled water to make a thin paste. Then, samples were placed in a muffle furnace and gradually raised the temperature up to 550^0C and kept for 90 minutes. Sample was removed from the muffle furnace and allowed to cool. Then, hot distilled water (15ml) was added while warming the evaporating basin on a hot plate. Mixture was filtered in to 250ml Erlenmeyer flask while rinsing the residues of the sample with five 10ml portions of hot distilled water.

Then, acetic acid drops were added to the filtrate until the solution is about pH 6 to 7. Five drops of potassium chromate solution were added and titrated with standardized 0.05N silver nitrate until the first permanent reddish brown color appeared (Yoshida et al., 1971).

3.3 Water Absorption Analysis

Four grams of rice seeds of different varieties were weighed and soaked in 50ml saline water (45 dS/m) with three replicates for each variety. Nine days after soaking, seeds were removed from the solution and surface water was removed using a blotting paper and weighed. Then, water absorption was calculated for each variety.

3.4 Measurements of seed-husk thickness

Thickness of the seed-husk was measured using the vernier-caliper. Husk of the flat side of the seed of different varieties were carefully measured preventing ridges being in contact with surface of the vernier.

3.5 Data Analysis

Germination data were analyzed using the logistic procedure where other parameters were analyzed using ANOVA procedure in SAS.

3.6 Experiment – II (Trial – 1): Determination of growth, yield and physiological traits associated with salt tolerance of rice varieties under whole and sub-soil salinity conditions.

This experiment was carried out as two trials during two seasons.

3.6.1 Specific objectives:

This experiment was done to determine the influence of whole and sub-soil salinity levels on growth, development, physiological parameters and yield of rice to identify specific traits associated with salt tolerance.

3.6.2 Experimental Location

This experiment was carried out inside a plant cage at rice Research and Development Institute, Batalagoda, Ibbagamuwa, from August, 2007 to July 2008. The experimental site belongs to agro ecological region of intermediate zone in low country (IL_{1a}).

3.6.3 Experimental Design

The experiment was arranged as a three factor factorial using a completely randomized design with three replicates. The factor one was soil salinity induced using NaCl and Na_2SO_4. Rice varieties were tested under four levels of salinity. These were the control without adding any salt, add NaCl to raise soil electrical conductivity (EC) up to 4 and 8dS/m, respectively and adding Na_2SO_4 to raise soil EC up to 8 dS/m (Table 1).

Second factor was depth of soil treated with salts. Pots were either filled with saline soil to the whole or filled 45 cm (sub-soil; only up to 45 cm of 60 cm soil column, leaving 15 cm at the top) with saline soil and top half with normal soil (Table-2) (this is to simulate salinity development in the subsoil layer of most of irrigated agricultural lands).

The third factor was varieties. Four varieties were selected based on the stress response results of the experiment one representing salt susceptible (Bg300), salt tolerant (Pokkali and Nona Bokra) and one salt tolerant new improved variety (At354).

3.6.4. Preparation of the growing pots

60 cm long with 12 cm diameter Z-lone pipes (PVC) were used as pots. Bottom end was sealed using an end-cap. Soils were taken from RRDI upland fields and similar amount of soil was added to each pot. Germinated seeds (four seeds) of respective varieties were planted and all management practices were carried out as recommended by the Department of Agriculture.

Table 2: Type of salt used, EC, soil depth (treated with salt) and the amount of salt added for different treatment combinations.

Treatment	Salt	Electrical Conductivity(dS/m)	Soil Depth	Added Salt Amount(grams)
T1	(control)	-	-	no salt added
T2	NaCl	4	whole-soil	4.9
T3	NaCl	4	sub-soil	3.6
T4	Na_2SO_4	8	sub-soil	19.31
T5	NaCl	8	sub-soil	9.16
T6	Na_2SO_4	8	whole-soil	25.81
T7	NaCl	8	whole-soil	12.24

3.6.5 Determination of soil salinity level

Soils were taken from the Batalagoda (upland area) and air dried and sieved using a 2mm wire mesh. A preliminary soil electrical conductivity (EC) adjustment was done to raise the soil EC using NaCl and Na_2SO_4. Then, required amounts of salt were added to raise the EC in the soil column. To fill the whole soil column, 6.35kg of soil was needed. In whole-soil treated pots, whole soil amount was treated with relevant salt and in sub-soil treated samples only sub-soil (4.75kg) was treated with relevant salt and the rest was filled with non-salted soil. Then, pots were kept for incubation for two weeks.

3.6.6. Crop Management

Four varieties tested in this experiment named, Pokkali, Nona Bokra, At354 and Bg300.Ten pre-germinated seeds were planted in a pot and thinned the seedlings at two weeks after planting leaving two plants per pot. Fertilizer application was done according to the recommendations of the department of agriculture, Sri Lanka (Table-3).

Table 3: Fertilizer Recommendation to achieve 6 tons / ha by Department of Agriculture, Sri Lanka (Dry and Intermediate Zone).

		kg/ha		
Time of Application	**Nitrogen**	**K_2O**	**P_2O_5**	**Zn**
Basal	5	40	20	1
2 weeks after sowing	35	*	*	*
5 weeks after sowing	55	*	*	*
7 weeks after sowing	25	*	20	1

Watering was done to keep the soil of the pot at saturated level according to the demand by plants. An Insecticide was applied (Marshal 20SC - Carbosulfan 200g/l SC) to control the paddy bug attack. Diseases were not observed during the growing period.

3.6.7. Measurements

3.6.7.1 Growth parameters

Number of tillers per pot was recorded at heading stage. Leaf area of two plants per each treatment was taken at heading stage using leaf area meter (LI 3100c) following destructive sampling procedure. Time taken to heading and maturity were recorded at relevant times.

3.6.7.2 Physiological parameters

Leaf photosynthesis of the youngest fully matured leaves were recorded at maximum tillering stage of the plants using portable photosynthetic system (Licor6400).Chlorophyll fluorescence was recorded at maximum tillering stage using the fluoro-meter of the youngest fully matured leaves(leaves were covered with black paper in the days before the reading was taken). Leaf chlorophyll content was estimated using SPAD meter at two weeks' intervals.

3.6.7.3 Yield parameters

Total biomass, root weight and shoot weight were taken at heading and at physiologically maturity stage. Grain yield was recorded at physiological maturity.

3.6.7.4 Chemical analysis

Destructive samples that were taken at the heading stage (two plants per each treatment) were used for Sodium and Chlorine analysis of plant samples and soil samples for pH and electrical conductivity.

3.6.7.5 Procedures for Chemical analysis

Sodium and chlorine analysis was done following the same method in experiment-1.

3.6.7.6 Data Analysis

Data analysis was done using the ANOVA procedure in SAS.

3.7 Experiment – II (Trial-2)

The first pot experiment was repeated once from February, 2008 to July, 2008 season with some alterations to the previous treatments. In the first pot trial, all plants were dead in treatment 4(NaCl, 8dSm^{-1}, whole-soil level). Therefore, this treatment was removed from second pot trial and treatments were arranged as in table-3. Additional two treatments were tested in second pot trial with NaCl at 6dSm^{-1} level.

Preparation of growing pots and soil, adjusting the soil EC, watering, fertilizing, measurements of growth, physiological and yield parameters and data analysis were done as same as the trial-1. Deferent treatments which were investigated listed in table-4.

Table 4: Type of salt used, EC, soil depth and the amount of salt added for different treatment combinations.

Treatment	Salt	Electrical Conductivity(dS/m)	Soil Depth
T1(control)	-	-	whole-soil
T2	NaCl	4	whole-soil
T3	NaCl	4	sub-soil
T4	NaCl	8	sub-soil
T5	Na_2SO_4	8	whole-soil
T6	Na_2SO_4	8	sub-soil
T7	NaCl	6	whole-soil
T8	NaCl	6	sub-soil
T9	Na_2SO_4	4	whole-soil
T10	Na_2SO_4	4	sub-soil

3.8 Experiment – III: Mitigation of soil salinity in farmer fields by adopting irrigation and fertilizing techniques.

3.8.1 Specific objective:

This experiment was carried out to evaluate rice varieties selected in the experiment one and two for their resistance ability, growth, development and productivity under salt affected farmer field with possible management of irrigation and fertilization.

3.8.2 Experimental Locations

This experiment was carried out at salt affected paddy field in Polonnaruwa District (Adaptive Research Institute, Kaduruwela) where water was supplied by major irrigation system which is belonged agro ecological region of low country dry zone (DL1c) from late May, 2008 to October, 2008(Yala season, 2008). This experiment was repeated as same treatments at Maho area (IL1b) in Kurunegala Distric.

3.8.3 Experimental Design

Experiment was arranged as a split plot design with three replicates. The factors were arranged as follows.

Main plot factor: Irrigation Management

Three irrigation levels were used during the growing season.

1. Irrigated to maintain standing water all season(at least to keep one inch of water level)
2. Keeping the soil at saturated level during all growing season.

3. Conventional way of managing the irrigation in the region by imposing several dry spells during the growing season.

Split plot factors: Fertilizer and different varieties in a factorial arrangement.

Fertilizer:

1. Cow dung (2 tons/Ac) with Nitrogen, Phosphorous and Potassium as recommended by Department of Agriculture, Sri Lanka (T1).
2. Cow dung (2 tons/Ac) and Charcoaled paddy-husk(250 kg/Ac) with Nitrogen, Phosphorous and Potassium as recommended by Department of Agriculture, Sri Lanka (T2).
3. Nitrogen, Phosphorous and Potassium as recommended by the Department of Agriculture, Sri Lanka (T3).

Varieties

1. Bg 300 as susceptible to salt.
2. Bg 352 as popular variety in Polonnaruwa District.
3. At 354 as New Improved Variety which is tolerant to salt.
4. Pokkali as known salt tolerant traditional variety.

Plot size: 18 m^2 (6m x 3m) for each treatment.

3.8.4: Crop Management

Field was arranged as a split plot design. Row seeding was done where each variety was laid in two adjacent rows in one plot and all four varieties per each plot. Spacing was done as 20cm x 20cm. Cow-dung and charcoaled paddy husk were added to the plots after second ploughing and field was kept for one week before seeding.

Thinning of seedlings was done two weeks after seeding leaving one plant per hill. Water management was done to keep different treatments at standing, saturate and alternate wetting and drying. Paddy Bug attack was reported and it was controlled by applying an insecticide (Marshal).

3.8.5 Measurements

3.8.6 Growth parameters

Number of tillers per hill was recorded at heading stage. Leaf area was taken at heading stage using leaf area meter (LI 3100c) from four plants per each treatment combination following a destructive sampling procedure. Time taken to heading and maturity were recorded at relevant times.

3.8.7 Yield parameters

Total biomass, root weight and shoot weight were taken at heading and at physiologically maturity stage. Grain yield was recorded at physiological maturity.

3.8.8 Initial soil properties

Soil pH and EC of Polennaruwa field was 6.8 and 3.2 dS/m repectively. Soil pH of Maho field was 6.7 and EC was 3.4dS/m.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 EXPERIMENT –I

4.1.1 Germination at different salinity levels

Germination was reduced across all varieties when the seeds were soaked in saline solutions. The germination % was not significantly different at 0 dSm^{-1} and 3 dSm^{-1} saline conditions (Fig-1). However there was a reduction in germination in seeds soaked in 25 dSm^{-1} and 45 dSm^{-1} saline solutions and the germination percentages were significantly different when the seeds were soaked in 45 dSm^{-1} saline solutions ($P=0.001$). Varieties could be categorized in to four distinct groups based on the percentage seed germination at 45 dSm^{-1} as >50% (tolerant), 50-20% (moderately tolerant), 20-5% (susceptible) and <5% (highly susceptible). Interestingly, most salt tolerant variety, Pokkali recorded the highest germination percentage among the tested varieties and it was as high as 86 percent on average, whereas At-354 and At-401 and Nona Bokra had germination percentages of 29, 21 and 20 %, respectively (Table-5).

Based on the proposed varietal grouping, the variety Pokkali is categorized as tolerant to salinity. Moderately tolerant group includes At 354, At 401 and Nona Bokra and the susceptible group includes H-10, Rathel, H-4, Bg750, At353, Bg450, Seeraga Samba, Bandara Heththewa, Bg 407 H, Bw 302, Bw78 and At303.Varieties recorded the least germination were grouped as highly susceptible to salinity which includes Bg300, Pachchaperumal, Bg304, Bg94-1, Periyakarappan, Dikwee, Bg 380, Bg 350, Hondarawalu and Murungakayan. Varietal ranking for salinity tolerance observed in this experiment is in agreement with the finding of Abeysiriwardena (2004) who had ranked Pokkali as highly tolerant and At 354, Nona Bokra and At 401 as tolerant and Bg 450 as moderately susceptible. The susceptible group of Abeysiriwardena (2004) included Bg 94-1, Bg 300, Bg 403 and Bg 350. Response of germination to salinity varies among rice varieties and decreased as the duration of pre-soaking in saline water increased (Abeysiriwardena, 2004).

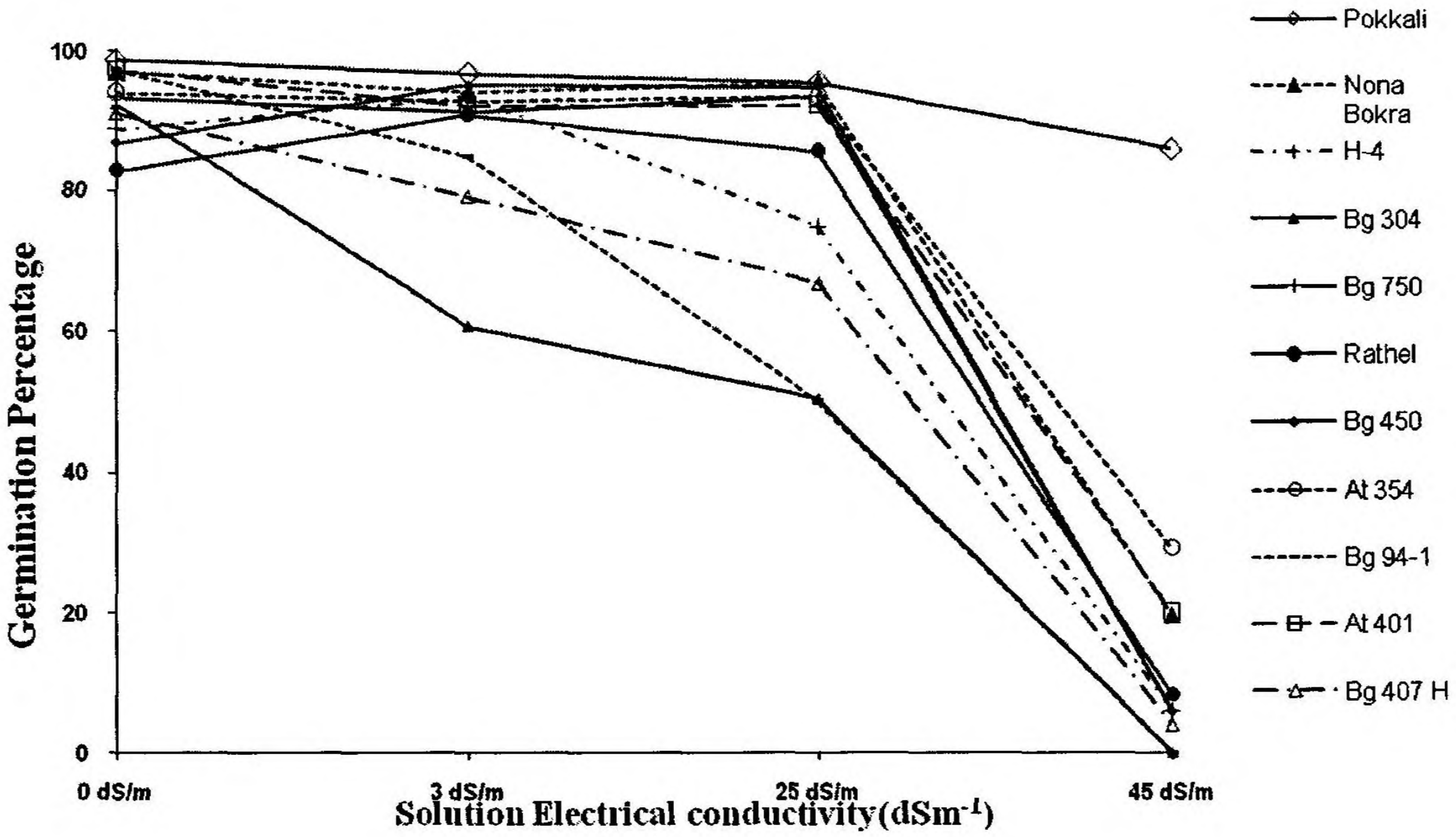


Figure 1: Variation in seed germination of rice varieties under different saline conditions (only few representative varieties from susceptible and highly susceptible groups are shown in this figure).

A similar pattern was also observed by Sirisena and Abeysiriwardena (2005) but they have ranked Bg 94-1 as moderately tolerant though it had very low germination after pre-soaking at 45 dSm^{-1} EC. This might be due to differences in pre-soaking of seeds in their experiment which was nine days pre-soaking in saline water followed by two day soaking in de-ionized water whereas in this experiment, seeds were pre-soaked for nine days only in saline water. Results of germination were also consistent with Dissanayaka and Wijeratna (2006) who used 21dSm^{-1} saline solution to screen the salinity tolerant rice varieties.

Regulation of seed germination by environmental factors is important in both ecological and agricultural contexts. The interaction between environment stresses and endogenous dormancy mechanisms determine whether a particular seed will germinate under given conditions (Bradford *et al.*, 1992). Fully imbibed, non dormant seeds can be expected to initiate radicle growth after a lag period related to the temperature. If the water potential of the imbibitions-medium is reduced, germination will be delayed or prevented (Hegarty, 1978). Sodium Chloride (NaCl) may readily cross the cell membrane into the cytoplasm of the cell. Entering ions decrease seed osmotic potential which facilitates the hydration of the seed (Katembe *et al.*, 1998). Rates of water uptake by rice seed is reduced with increasing salinity. Rice seeds attained full imbibitions in 48 hours at 150 mM salinity and at 250 mM salinity; seeds reached the critical moisture content by 72 hours (Alam *et al.*, 2003). This suggests that increasing salinity delays the time to attain critical moisture content of rice seeds and thereby the onset of germination. The critical moisture content for germination of rice seeds is around 25 – 30% and it may differ among varieties and with different seed sizes and husk characteristics (Alam *et al.*, 2003).

4.1.2 Variation in water absorption and seed germination at high salinity level

Seeds of all the varieties tested had absorbed higher amount of water when seeds were soaked in distilled water compared to saline water (45 dS/m). In distilled water, all the varieties had absorbed 30 – 40 % water. The variety Pokkali absorbed the highest water

Table 5: Seed germination percentages of different rice varieties at EC of 0, 3, 25 and 45 $dS^{-1}m$ and variety reaction to salinity. Grouping of varieties were based on the sensitivity to salinity.

Variety	Electrical conductivity of the salt solution				Reduction of seed germination(%) at 45 dSm^{-1}	Reaction to salinity
	0 dSm^{-1}	3 dSm^{-1}	25 dSm^{-1}	45 dSm^{-1}		
	Seed germination (%)					
Pokkali	99 ± 1.1	97	95	86 ± 3.2	13	Tolerant
At 354	94 ± 6.0	93	91	29 ± 2.1	65	Moderately Tolerant
Nona Bokra	94 ± 1.0	94	83	20 ± 2.1	74	Moderately Tolerant
At 401	97 ± 2.3	92	92	21 ± 1.0	76	Moderately Tolerant
H-10	97 ± 2.3	60	31	10 ± 1.0	87	Susceptible
Rathel	91 ± 2.0	91	85	8 ± 1.5	83	Susceptible
Seeraga Samba Bandara	95 ± 2.1	68	60	7 ± 2.0	86	Susceptible
Heththawa	94 ± 1.5	99	96	7 ± 2.0	87	Susceptible
Bg 407 H	95 ± 1.5	87	85	8 ± 1.5	87	Susceptible
Bw 302	95 ± 3.0	88	79	10 ± 2.0	85	Susceptible
Bw 78	94 ± 2.1	89	82	11 ± 3.1	83	Susceptible
H-4	93 ± 4.5	53	25	6 ± 3.5	87	Susceptible
Bg 750	93 ± 2.1	93	91	6 ± 2.0	87	Susceptible
Bg 450	93 ± 2.6	95	91	6 ± 4.0	87	Susceptible
At 353	97 ± 1.0	96	96	6 ± 2.5	89	Susceptible
At 303	96 ± 2.0	93	93	6 ± 2.0	88	Susceptible
Murungakayan	99 ± 1.0	92	91	3 ± 1.1	96	Highly susceptible
Hondarawalu	96 ± 3.1	89	87	4 ± 1.5	92	Highly susceptible
Bg 380	93 ± 2.3	81	80	3 ± 1.0	90	Highly susceptible
Bg 350	99 ± 1.0	100	95	1 ± 1.0	98	Highly susceptible
Pachchaperumal	94 ± 1.5	91	86	2 ± 1.5	92	Highly susceptible
Bg 300	95 ± 2.3	97	88	2 ± 2.0	93	Highly susceptible
Bg 304	92 ± 1.5	50	38	0	92	Highly susceptible
Periyakarappan	95 ± 3.1	90	81	0	95	Highly susceptible
Bg 94-1	97 ± 4.6	85	50	0	97	Highly susceptible
Dikwee	98 ± 2.5	97	92	0	98	Highly susceptible

content (30.3%) when soaked in the high saline solution while variety Dikwee(26.2) recorded the lowest absorption (Table 6). Though, seeds of all varieties absorbed water more than their critical level (25–30%) for germination, clear differences in germination were observed among varieties which may be due to toxicity effects of absorbed ions.

There is no clear relationship among varieties between water absorption by individual seeds after 9 days of soaking in saline water and their germination percentage ($R^2=0.21$). Lack of such relationship could be partly due to absorption of water above critical level by seeds of all varieties. Some varieties like Pokkali recorded 30.3% water absorption and 86 % germination whereas At 354 and At 353 recorded 29 % and 6 % germination respectively while having 29.8 % water absorption (Table 6).

Table 6: Water absorption and reduction in water absorption at 45dSm^{-1} over pure water by individual seeds of different varieties after seed soaking in pure (0 dSm^{-1}) and saline water (45dSm^{-1}) for 9 days and seed germination at saline water (45dSm^{-1}) of different rice varieties.

	Water absorption (%) by seeds on initial dry weight basis		Reduction in water absorption (%)by seeds	Seed germination(%) at
Variety	0 dS/m*	45 dS/m*	at 45 dS/m	45 dS/m
Dikwee	31.7^{g}	26.2^{e}	5.5	0
Bg 94-1	36.4^{e}	29.8^{b}	6.6	0
Bg 300	37.8^{bcd}	28.6^{d}	9.2	0
Bg 380	37.9^{cbd}	28.7^{d}	9.2	3
Bg 450	34.8^{f}	29.4^{c}	5.4	6
At 353	38.2^{b}	29.8^{b}	8.4	6
H-4	38.3^{ced}	28.7^{d}	9.6	6
Nona Bokra	38.0^{cb}	28.6^{d}	9.4	20
At 401	36.8^{ed}	29.8^{b}	7.0	21
At 354	37.8^{cbd}	29.8^{b}	8.0	29
Pokkali	39.5^{a}	30.3^{a}	9.2	86

*Means followed by the same letter within a column are not significantly different at the 5% probability level.

4.1.3 Accumulation of Na and Cl ions in seeds

All the varieties absorbed more sodium ions when seeds were soaked in saline water. Sodium absorption between varieties was significantly different in 45 dSm^{-1} EC solutions ($P < 0.05$). Some varieties such as Bg 450 recoded 300 ppm more salt compared to Pokkali and Nona Bokra (Table 7). It was evident that seed germination was significantly reduced when more sodium was accumulated in seeds. Correlation analysis revealed that there was a stronger relationship among varieties between percentage increases in sodium absorption (% of Na at 45 dS/m compared to Na at 0 dS/m) and germination percentage at 45 dSm^{-1} ($P<0.05$, $R^2=0.56$) (Fig. 2).

4.1.4 Seed properties and ion absorption and germination

Seed-husk thickness varied among varieties and sodium absorption was reduced with increasing husk thickness ($R^2=0.82$) (Fig. 3). Higher salt accumulation inside the seeds affected germination of rice varieties (Fig.2).

Chloride accumulation was also increased when the seeds were soaked in high salinity levels and showed a significant variation among varieties (Table 7). However, there was no clear relationship between the amount of chloride absorption in to seeds and the germination percentage ($P>0.05$, $R^2=0.069$).

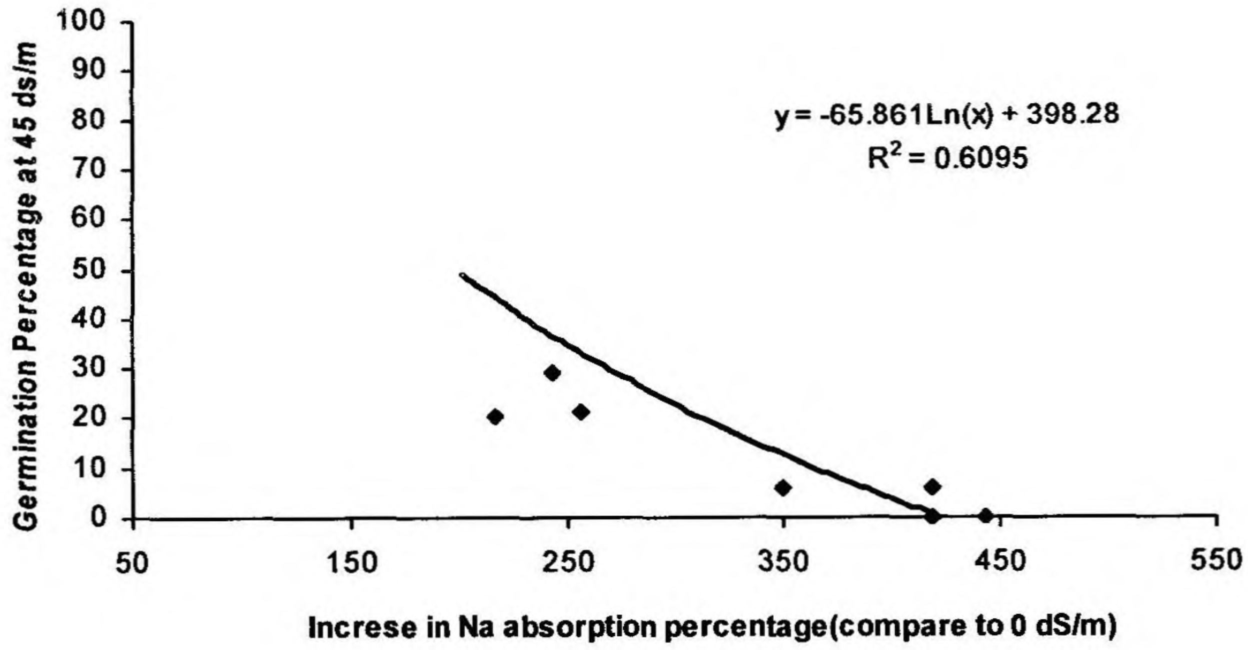


Figure 2: Relationship between seed germination at 45 dSm^{-1} and percentage increase in Na absorption at 45dSm^{-1} over 0 dSm^{-1} in rice.

Table 7: Seed Sodium and Chloride concentrations at 0 and 45$dsm^{-1}$and their percentage increases at 45dSm^{-1} over pure water of de-husked rice grains after seeds were soaked in pure (0 dSm^{-1}) and saline water (45 dSm^{-1}) for nine days.

	Na absorption(ppm)		Increase of Na	Cl Absorption (%)		Increase of Cl
Variety	0 dS/m*	45 dS/m*	at 45 dS/m(%)	0 dS/m*	45 dS/m*	at 45 dS/m(%)
Pokkali	233.0^{a}	703.0^{d}	201.7	0.35^{b}	0.41^{c}	16.3
Nona						
Bokra	200.0^{a}	633.3^{d}	216.7	0.39^{b}	0.42^{c}	9.1
At 354	233.3^{a}	800.0^{c}	242.9	0.37^{ab}	0.42^{c}	14.3
At 401	177.8^{b}	633.3^{d}	256.2	0.32^{c}	0.44^{b}	38.3
Bg 380	177.8^{b}	800.0^{d}	349.9	0.50^{a}	0.64^{a}	28.6
Bg 450	233.3^{a}	1050.0^{a}	350.1	0.28^{ef}	0.32^{g}	12.5
At 353	122.2^{b}	633.3^{d}	418.2	0.27^{f}	0.35^{e}	33.3
Dikwee	122.2^{b}	633.3^{d}	418.2	0.30^{d}	0.34^{f}	11.8
H-4	122.2^{b}	633.3^{d}	418.2	$0.28d^{e}$	0.34^{f}	18.8
Bg 300	177.8^{b}	966.0^{b}	443.3	0.23^{g}	0.37^{d}	61.5
Bg 94-1	177.8^{b}	966.0^{b}	443.3	0.35^{b}	0.50^{h}	40.0

*Means followed by the same letter within a column letter are not significantly different at the 5% probability level

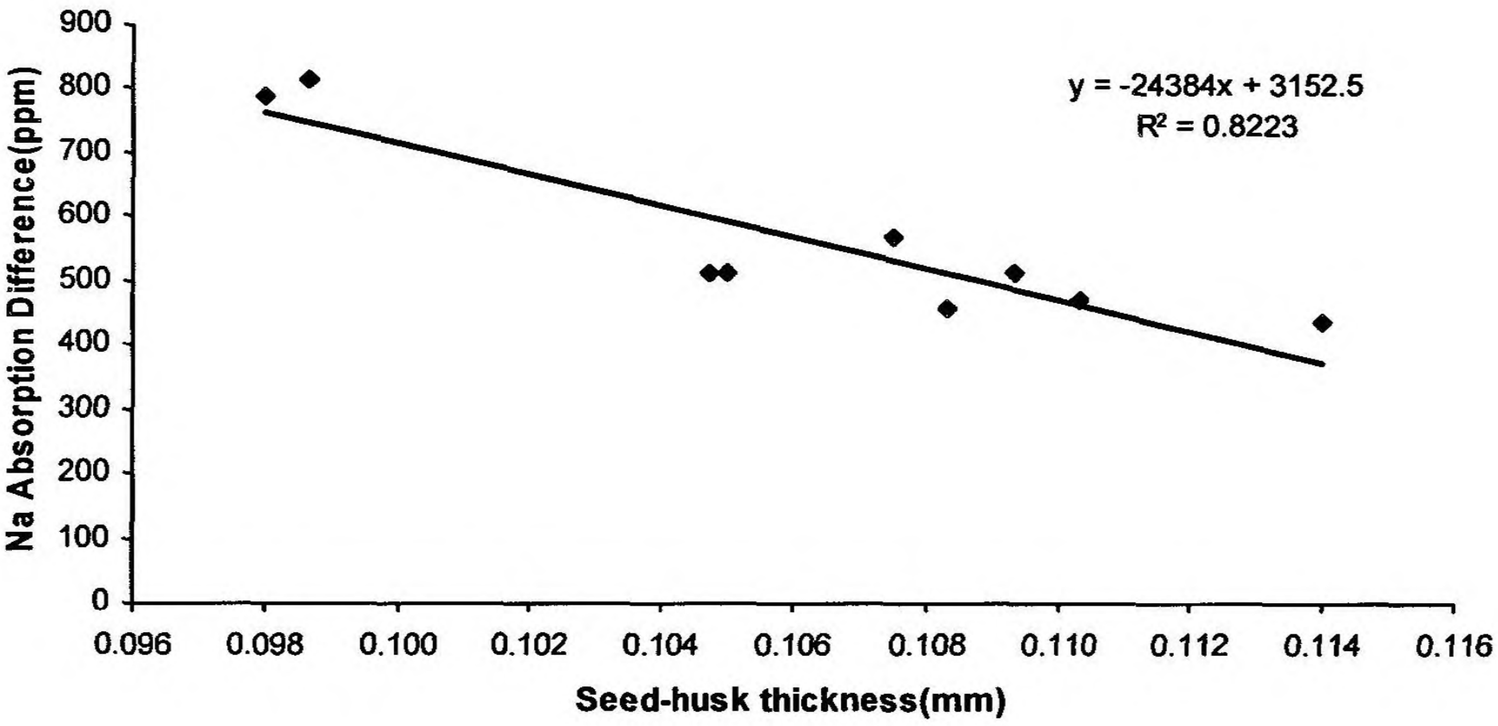


Figure 3: Relationship between increased Sodium absorption (ppm) at 45dSm^{-1} over 0 dSm^{-1} and seed-husk thickness in rice.

Individual seed size as measured by seed weight was significantly different among the varieties tested. However seed size has no relation with the germination ability of a particular variety under saline conditions (Table 8).

Age class of a particular variety has no effect on its germination ability under saline conditions (Table 8).

Table 8: Effect of seed size as measured by the seed weight ratio to the smallest seed, Rathel and age class on reaction to salinity of rice.

Variety	Seed size	Sensitivity to salinity	Age Class (months)
Rathel	1^{i}	Susceptible	4 - 4 1/2
Bg 450	1.033^{i}	Susceptible	4 - 4 1/2
Dikwee	1.501^{h}	Highly Susceptible	4 - 4 1/2
Bg 304	1.754^{g}	Highly Susceptible	3
Pokkali	1.933^{f}	Tolerant	3 1/2
Bg 300	1.965^{f}	Highly Susceptible	3
H-10	2.040^{e}	Susceptible	3
At 353	2.062^{ed}	Susceptible	3 1/2
Bg 94-1	2.093^{d}	Highly Susceptible	3 1/2
At 354	2.142^{c}	Moderately Tolerant	3 1/2
At 401	2.187^{c}	Moderately Tolerant	4 - 4 1/2
H-4	2.206^{b}	Susceptible	4 - 4 1/2
Nona Bokra	2.240^{b}	Moderately Tolerant	4 - 4 1/2
Bg 750	2.314^{a}	Susceptible	2 - 2 1/2
Pachchaperumal	2.338^{a}	Susceptible	3 1/2

*significant at 5% probability level weight

Seed Size – Seed weight ratio to the smallest seed – Rathel, Any means followed by the same letter are not significantly different at the 5% probability level

4.2 Seedling Survival under saline condition and its relationship with germination of rice seeds under high saline solutions:

Varieties showed significant differences in seedling survival rates at different soil electrical conductivities and seedling survival was decreased with increased soil salinity and time after planting (Table 9). The interaction between varieties and soil-electrical conductivity levels on seedling survival were significantly different at 7, 14 and 21 days after seeding ($p<0.05$) (Figure 4).

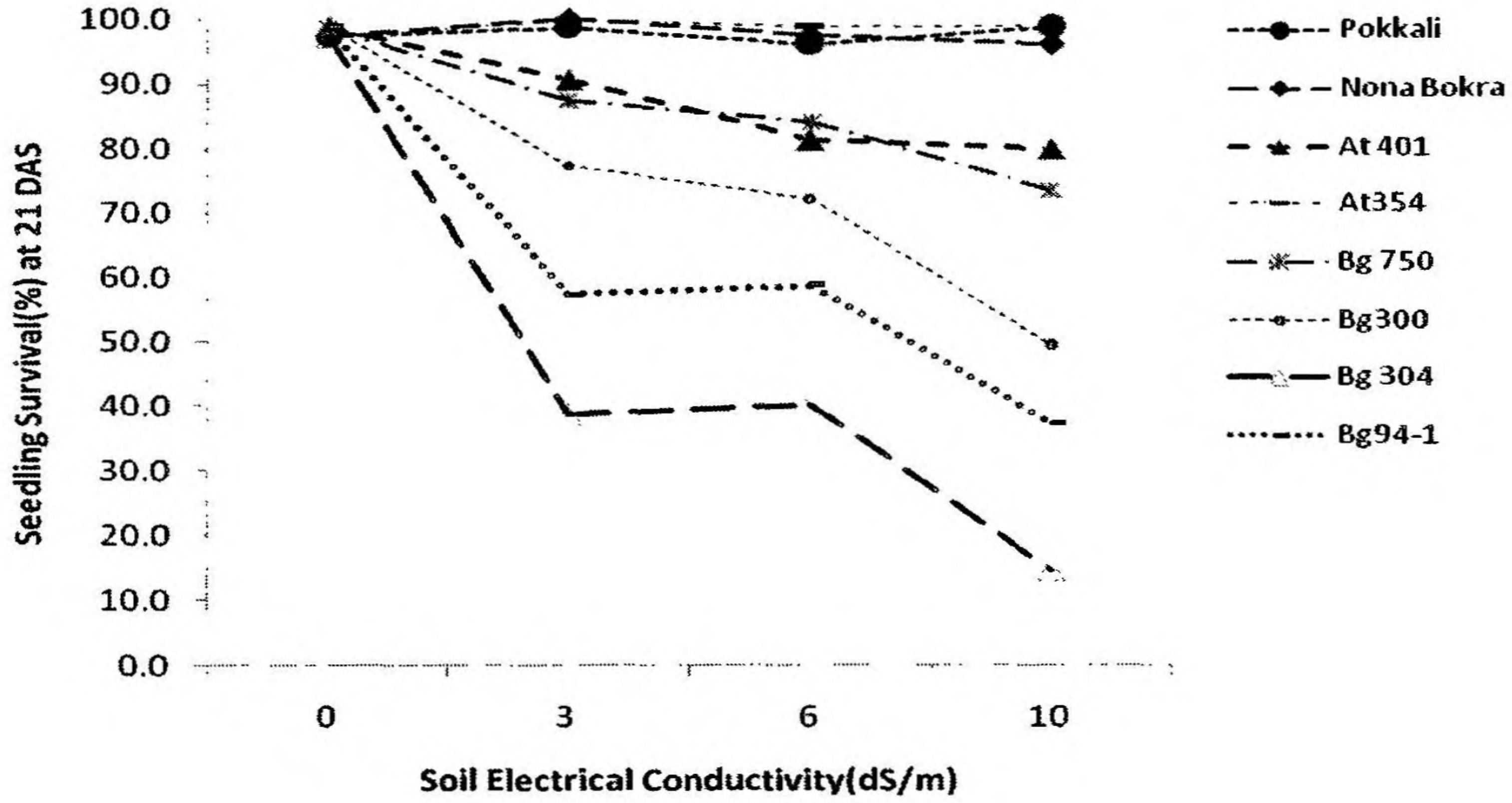


Figure 4: Variation in seedling survival under different soil salinity levels at 21 DAS.

Almost all the varieties decreased their seedling survival rate with the increase of soil salinity level (Figure 4). In the control (0dSm^{-1}), there was no significant difference in seedling death up to 21 DAS and a normal growth was observed. Suggesting that under no stress there was no difference in seeds used for the experiment. However, with increasing soil salinity up to 3dSm^{-1}, varieties showed significant differences in their seedling survival and seedling growth. Seedling death was greater at 21DAS (Figure4).

Average seedling survival rate at 6dSm^{-1} salinity level was lower than that of 3dSm^{-1} level in all the tested varieties and the difference between varieties was greater than 3dSm^{-1} soil salinity level. Some known salinity resistance varieties recorded higher seedling survival than known saline susceptible varieties at this level.

Average seedling survival rate was lowest at 10dSm^{-1} salinity level than other levels and varietal differences were highest at this level. Saline resistance varieties (Pokkali, Nona

Bokra and At354) showed 100% seedling survival at 7DAS where Bg300, Bg304 and Bg94-1 showed seedling survival of 73.3%, 38.7% and 60% respectively. Seedling death was higher at 14DAP than 7DAP at 10 dSm^{-1}. Most significant differences were observed at 21DAP in all the varieties tested. Seedling survival of Pokkali, Nona Bokra, At353, At401 and At354 were 90.6%, 88%, 77%, 76% and73.3% respectively where seedling survival of Bg300, Bg304 and Bg94-1 were 6.6%, 2.6% and 2.6% respectively (Figure 4).

Reduction in survived seedlings was lowest in traditional varieties; Pokkali and Nona Bokra followed by new improved varieties (At353, At401 and At354). Susceptible varieties showed greater reduction in seedling death under saline conditions (Table 9).

These findings are in accordance with the results of Sirisena and Abeysiriwardena (2005). Rice varieties exhibit variability in their reaction to salt stress, vegetative growth of some cultivars show surprisingly high resistance to soil salinity (Munns *et al.,* 2002). Salinity applied at seedling stage frequently induces premature senescence of leaves (Yeo *et al.*, 1991). Higher rates of salt accumulation in more sensitive varieties lead to leaf senescence. This further inhibits new growth in susceptible varieties compared to resistant varieties (Neumann P., 1997). With increase in duration under salt stress, plants accumulate more salt and this might be the cause of higher leaf senescence and seedling death.

Table 9: Reduction in seedling survival percentage under saline conditions compared to 0 dSm^{-1} at 21 DAS.

Variety	Reduction in seedling survival(%) compared to the control(0dSm^{-1})		
	3 dSm^{-1}	6 dSm^{-1}	10dSm^{-1}
Pokkali	1.3	1.3	6.7
Nona Bokra	0.0	1.3	9.3
At353	5.3	22.6	21.3
At 401	8.0	28.0	22.6
At354	0.0	4.0	24.0
Bandara Heththewa	17.3	37.3	46.6
At303	12.0	40.0	58.6
H-4	80.0	61.3	61.3
Rathel	32.0	86.7	62.7
Bg350	17.3	62.6	72.0
Bg 750	33.3	64.0	78.6
Bg 450	46.6	73.3	84.0
Dikwee	29.3	26.7	85.3
Bg380	33.3	53.3	88.0
Bg300	29.3	54.6	92.0
Bg 304	74.7	73.4	93.4
Bg94-1	60.0	60.0	96.0

Seedling survival was greatly affected at 10dSm^{-1} soil salinity level. Higher seedling survival recorded by saline tolerant varieties such as Pokkali ((91%), Nona Bokra(88%), At 353(77%), and At 401(76%). Seedling survival of other varieties was less than 50 percent (Table 9). Results of seedling survival were also consistent with Sirisena and Abeysiriwardena(2005) who used 8dSm^{-1} soil electrical conductivity to check the resistance.

Table 10: Germination percentage of rice varieties (from experiment-I) at 0 dSm^{-1} and 45 dSm^{-1}EC solutions and seedling survival percentage at 0 dSm^{-1} and 10 dSm^{-1} soil EC levels.

Variety	Germination(%) under saline solutions		Seedling Survival (%) after 21 DAS	
	0 dS/m	45 dS/m	0 dS/m	10 dS/m
Pokkali	99 ± 1.0	86 ± 3.2	97 ± 2.31^{a}	91 ± 9.2^{a}
Nona Bokra	94 ± 1.0	20± 2.1	97 ± 2.31^{a}	88 ± 8.0^{a}
At 354	94 ± 6.0	29 ± 2.1	97 ± 2.31^{a}	73 ± 8.3^{abc}
At 401	94 ± 2.3	21 ± 1.0	99 ± 2.31^{a}	76 ± 4.0^{ab}
At 303	96 ± 2.0	6 ± 2.0	99 ± 2.31^{a}	40 ± 4.0^{bcde}
At 353	97 ± 1.0	6 ± 2.5	99 ± 2.31^{a}	77 ± 2.3^{ab}
Bg 750	93 ± 2.1	6 ± 2.0	99 ± 2.31^{a}	20 ± 8.5^{efg}
Bandara Heththewa	94 ± 1.5	7 ± 2.0	99 ± 2.31^{a}	52 ± 4.0^{abcd}
Bg 350	99 ± 1.0	1 ± 1.0	99 ± 2.31^{a}	27 ± 6.1^{def}
Dikwee	98± 2.5	0	97 ± 2.31^{a}	12 ± 6.7^{8ghi}
Rathel	91 ± 2.0	8 ± 1.5	97 ± 2.31^{a}	35 ± 8.1^{def}
Bg 300	95 ± 2.3	2 ± 2.0	99 ± 2.31^{a}	7 ± 4.5^{hi}
Bg 380	93 ± 2.3	3 ± 1.0	97 ± 2.31^{a}	9 ± 3.3^{ghi}
Bg 450	93 ± 2.0	6 ± 4.0	99 ± 2.31^{a}	15 ± 6.5^{fgh}
Bg 94-1	97 ± 3.0	0	99 ± 2.31^{a}	3 ± 4.62^{i}
Bg 304	92 ± 1.5	0	96 ± 0^{a}	3 ± 4.62^{i}

- Significant at p= 0.05, any means followed by same letter are not significantly different at the p=0.05.

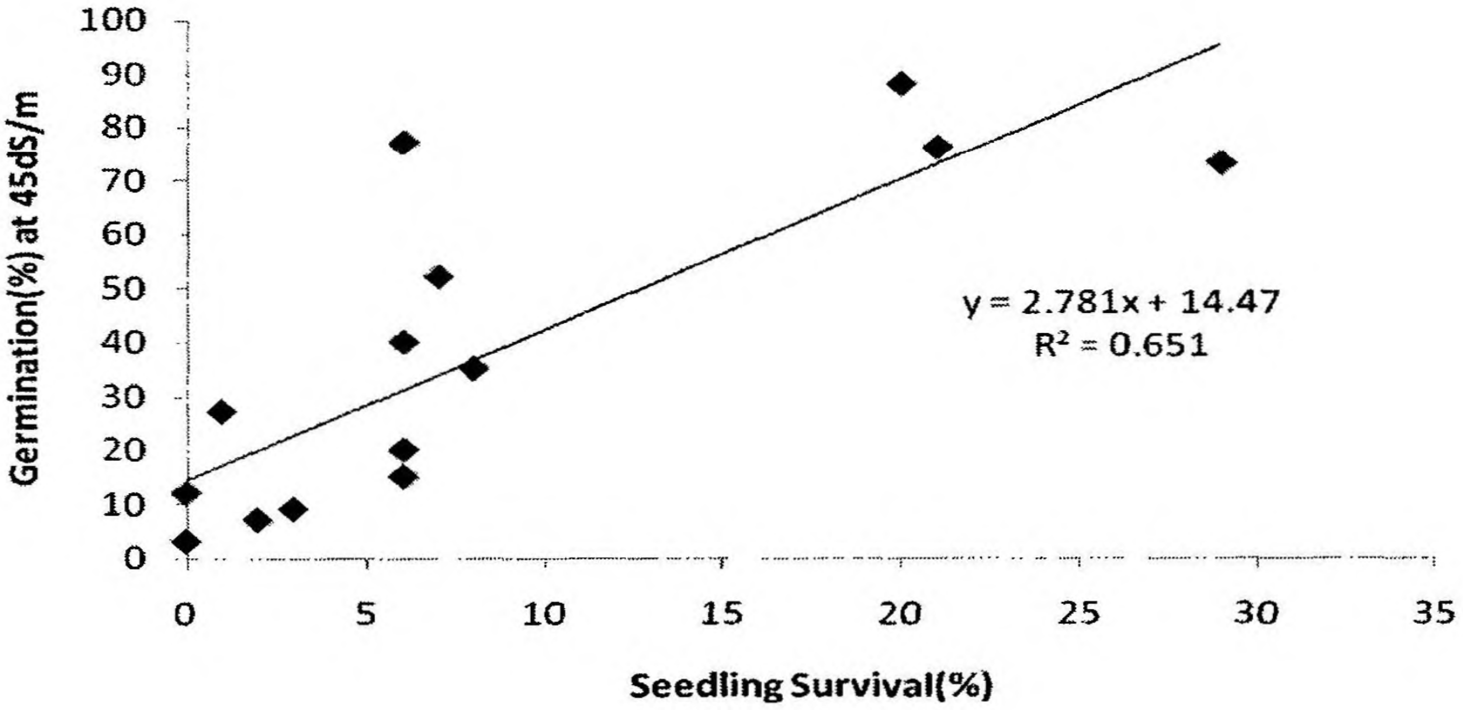


Figure 5: Relationship between seed germination percentage of rice varieties after seed soaking in saline solution(45dSm^{-1}) and their seedling survival rate at 10 dSm^{-1} soil salinity level.

Seed germination at 45dSm^{-1}and seedling survival rate at high soil salinity (10dSm^{-1}) was closely related (R^2=0.651) except At303 which showed low seed germination (6%) but recorded higher seedling survival (77%). Pokkali recorded the highest germination and highest seedling survival among tested varieties (Figure 5).

4.2.1 Seedling Growth

Number of survivals did not vary among tested varieties up to 21 days after planting in the control (no salt added) and normal plant growth was observed during this period. Almost all the seedlings survived up to 21 days after establishment, but differences in growth responses could be observed among varieties implying their inherent growth differences under normal growing conditions.

The highest shoot weight recorded by Bg304 in the control at 21 DAS where other varieties categorized into another group according to shoot weight (figure 6). Shoot weight of most saline susceptible varieties was reduced with increased soil salinity where Bg304 and Bg94-1 recorded no plant survival, Rathel and Bg300 recorded very low shoot weight where Nona Bokra recorded the highest shoot weight followed by Pokkali and At353.

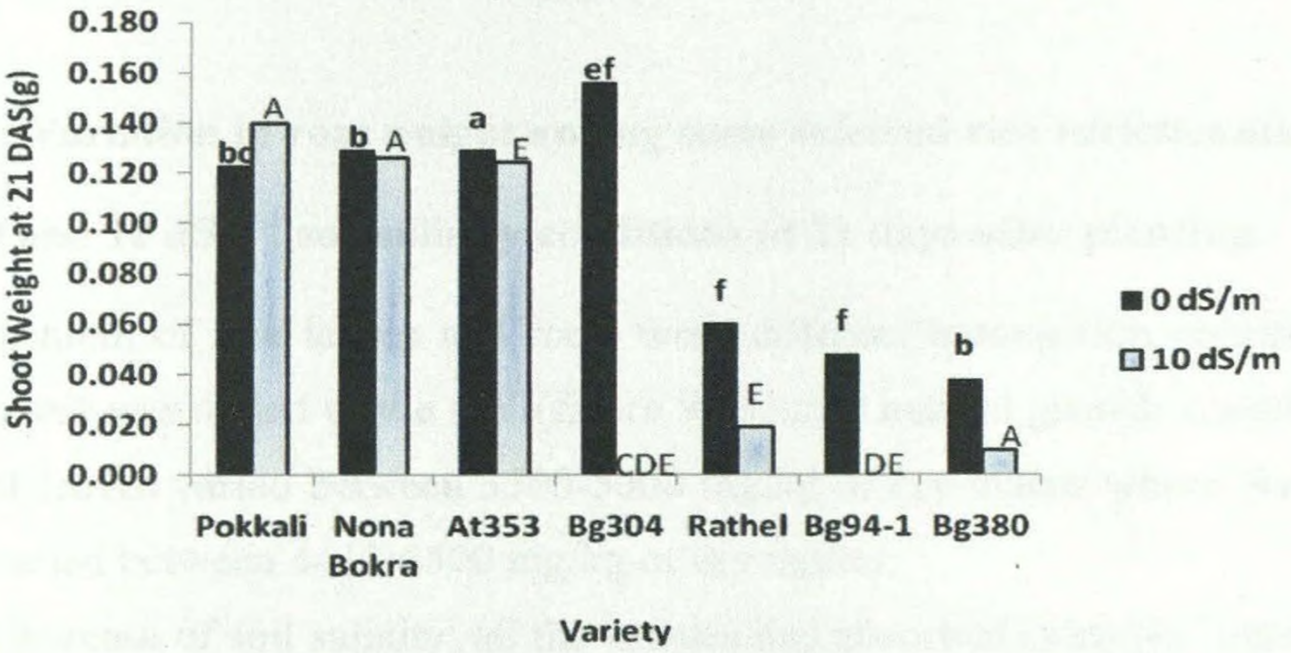


Figure 6: Variation in shoot weight among some selected rice varieties under control ($0 dSm^{-1}$) and $10 dSm^{-1}$ soil salinity conditions at 21 days after planting.

Root weight showed significant differences among tested varieties in the control implying their inherent variability in rooting ability. Pokkali recorded highest root weight followed by Nona Bokra and At353. When increase the soil salinity level, root weight of Bg304, Bg94-1, Rathel and Bg300 was reduced drastically where root weight of Nona Bokra, pokkali and At353 remain unchanged (Fig 7).

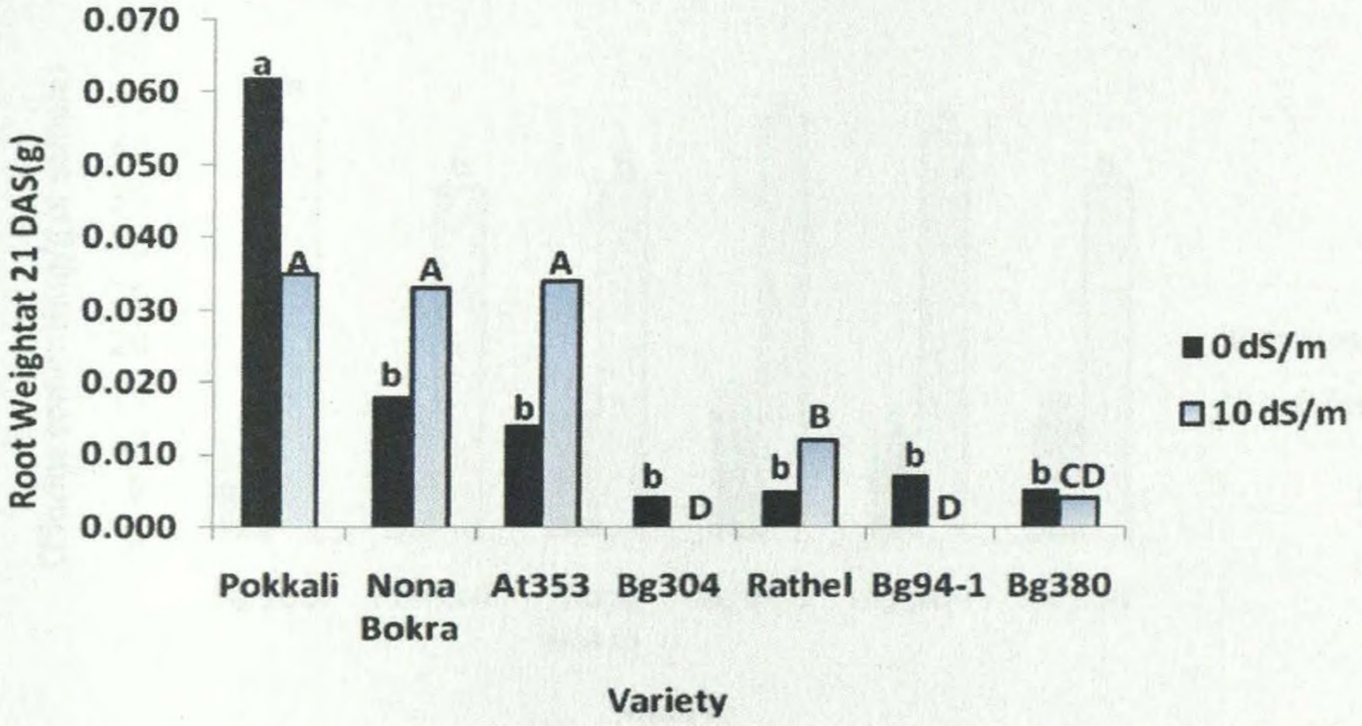


Figure 7: Variation in root weight among some selected rice varieties under control ($0dSm^{-1}$) and 10 dSm^{-1} soil salinity conditions at 21 days after planting.

Sodium content of rice leaves and roots were different among rice varieties in the control where no salt was added to the soil (figure 9). Under normal growth conditions Na^+ (mean) content of leaves varied between 3500-5000 mg/kg of dry matter where Na^+ (mean) content of roots varied between 4417-6500 mg/kg of dry matter.

With the increase of soil salinity, all the varities had absorbed more Na^+ ions compared to the control. Varieites having greater resistance to salinity recorded lesser sodium content in their leaves compared to salt sensitive varieties(figure-9). Most tolerant varieties, Pokkali and Nona Bokra recorded lower Na^+ content(6500 & 5666.7 mg Na^+/kg dry matter respectively) in their leaves compared to susceptible varieties, Bg300, Bg304 and Bg94-1. The most salt tolerant varieties such as Pokkali, Nona Bora and At 354 recorded higher Na^+ content in their roots compared to their Na^+ content in leaves where salt sensitive varieties recorded lower root Na^+ content compared to their Na^+ content in leaves.

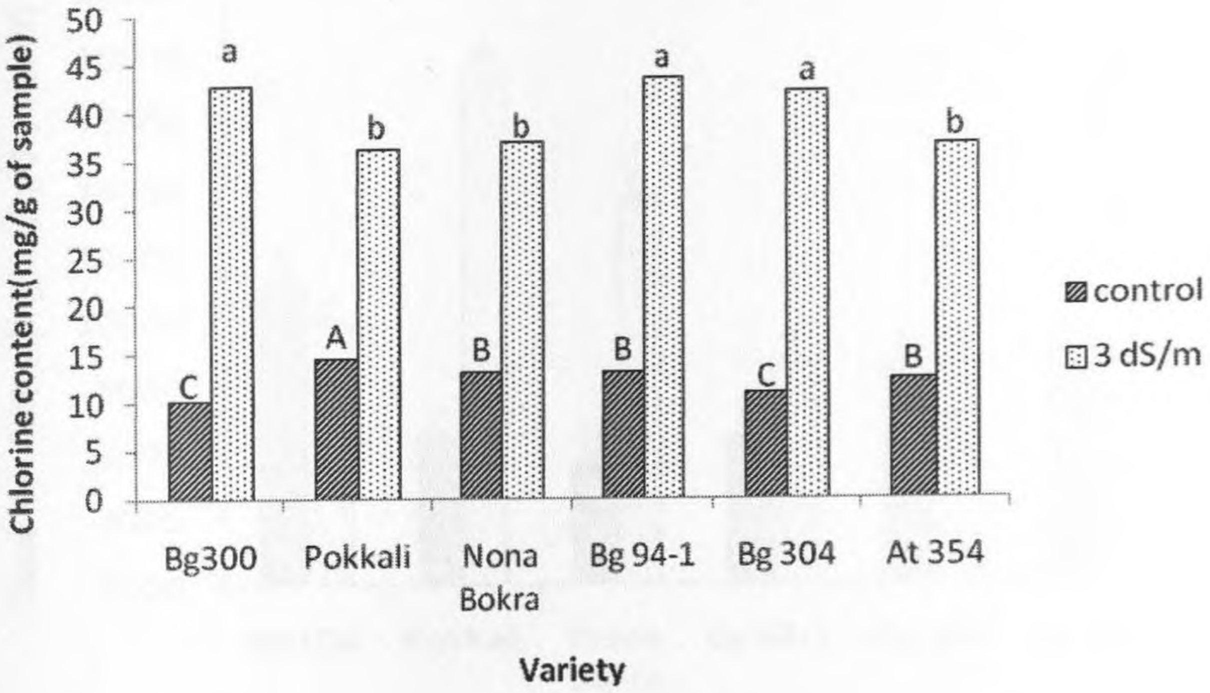


Figure 8: : Chloride ion concentraion of the leaves of some selected rice varieties under 0 dSm^{-1} and 3 dSm^{-1} soil salinity levels at 30days after planting.

At 6dSm^{-1} soil salinity level, varieties absorbed Na$^+$ ions in high quantities which caused greater leaf Na$^+$ content resulting higher death of the plants (figure 10). Differences in salt tolerance among rice cultivars can also be caused by differential compartmentalization of the Na$^+$ in the shoot (Yeo and Flowers, 1983). High Na$^+$ levels in shoot apoplast would lead to osmotic stress and eventually cyto-toxicity. Salt concentration in individual leaves of non-halophytes usually increases greatly with time (Walker *et al.*, 1982). Non halophytes show no signs of regulation of the salt concentrations in their leaves (R. Munns and Termaat A., 1986).

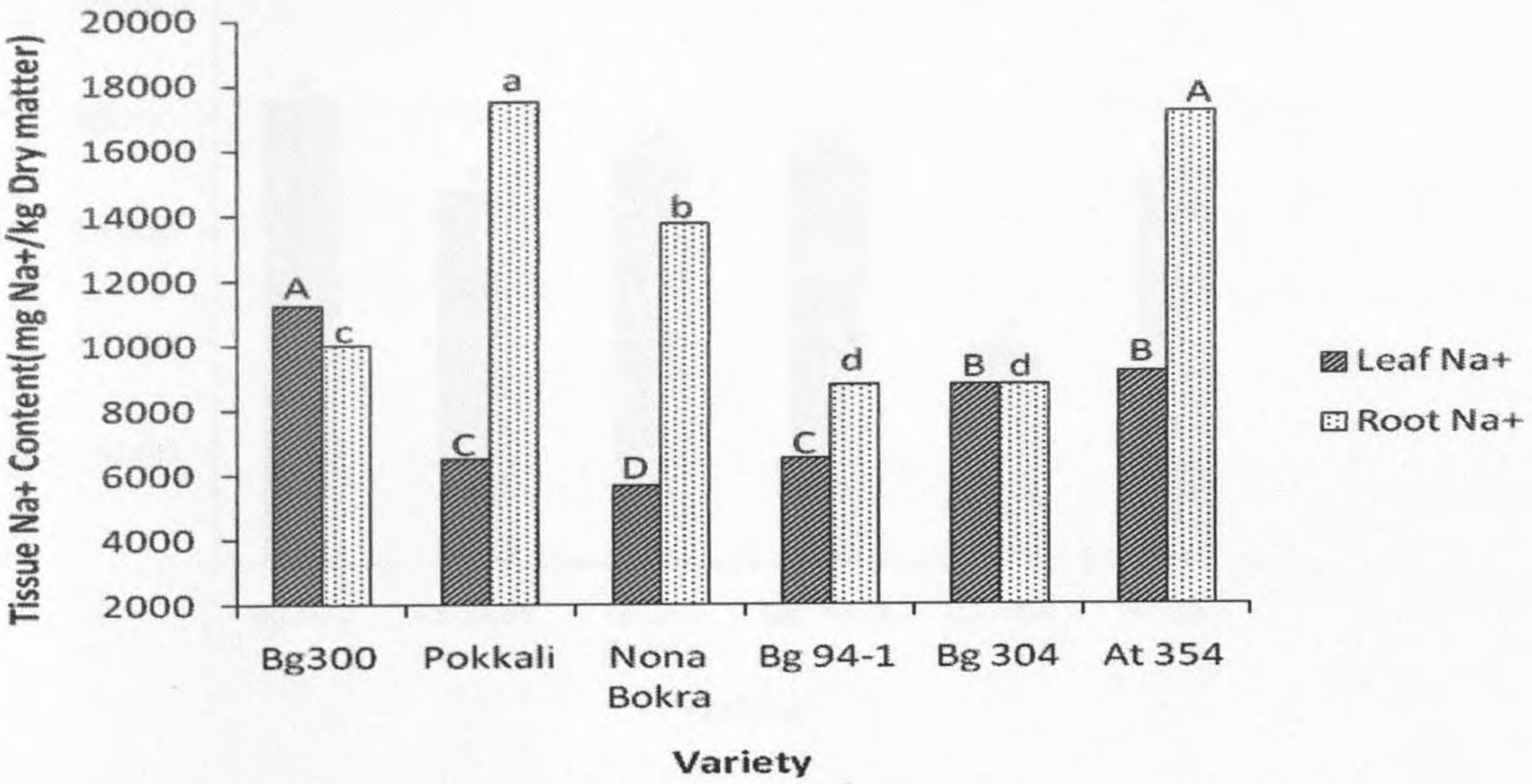


Figure 9: Sodium ion concentraion of the leaves and roots of some selected rice varieties under 3dSm^{-1} soil salinity level at 30days after planting.

Chlorine content of rice leaves increased drastically at 3 dSm^{-1} soil salinity level compared to that of the control (fig. 8). Highly saline susceptible varieties such as Bg300, Bg304 and Bg 94-1 contained more chloride ions compared to saline tolerant varieties such as Pokkali and Nona Bokra. In the control, Cl$^-$ content of leaves of tested varieties was within the range of 10-15 mg per gram of leaf weight, and this was significantly increased (35-45 mg/g of leaf) at 3 dSm^{-1} soil salinity level. There is considerable variation in the ability of plants to accumulate chloride (Cram, 1976; Greenway and Munns, 1980). Differences between varieties to withstand chloride toxicity are frequently related to the ability to restrict chloride transport to the shoot. This has been observed in soybean (Abel, 1969), wheat (Bernad *et al.*, 1974), barley (Greenway and Munns, 1980), stone fruit trees (Bernatein *et al.*, 1956) grape vine (Vitis spp; Ancliff *et al.*, 1983) and citrus (Storey and Walker, 1999).

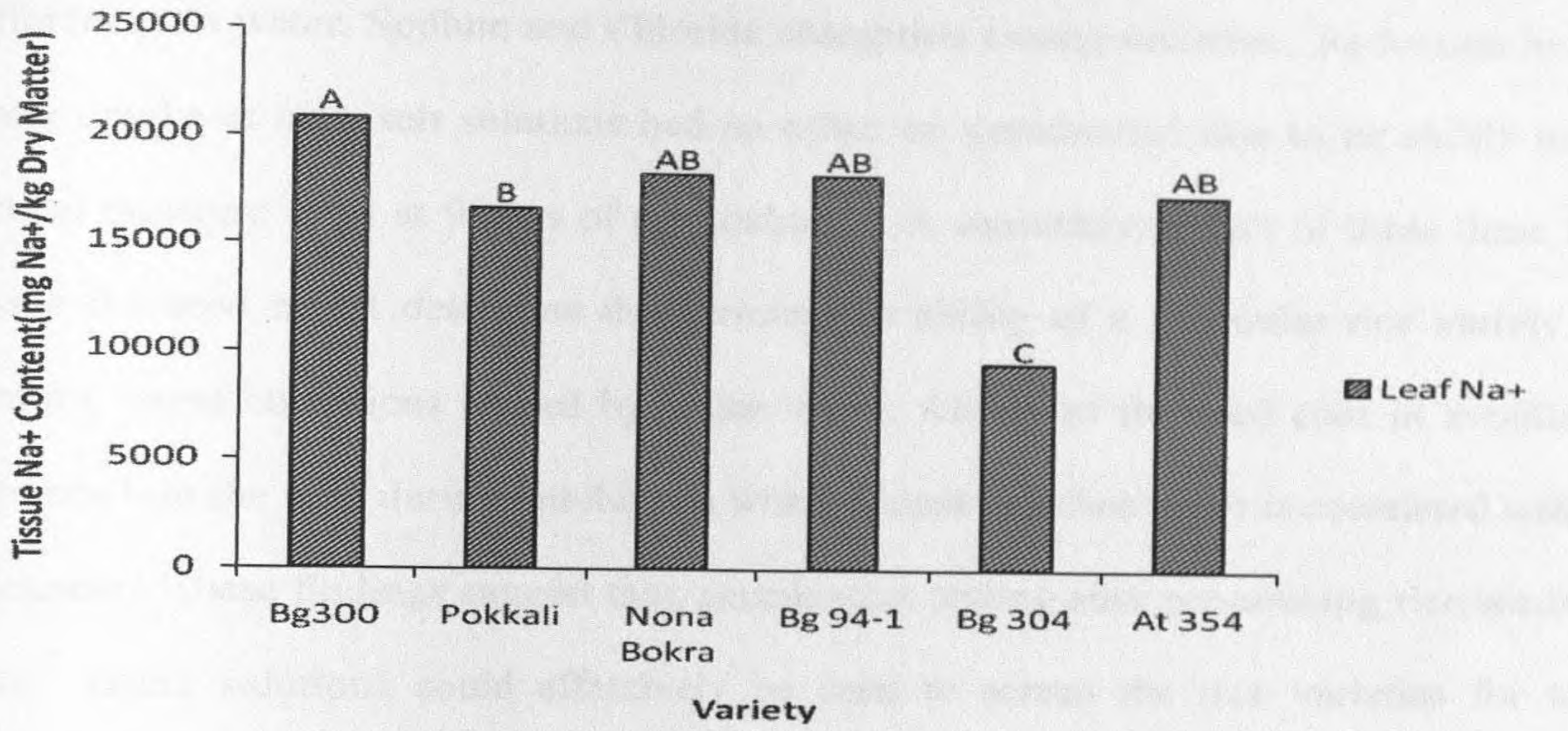


Figure 10: Sodium ion concentraion of the leaves of some selected rice varieties under 6 dSm^{-1} soil salinity level at 30days after planting.

Differences between cultivars to withstand chloride toxicity are frequently related to the ability to restrict chloride transport to the shoot. This has been observed in soybean (Abel, 1969), wheat (Bernad *et al.*, 1974), barley (Greenway and Munns, 1980), stone fruit trees (Bernatein *et al.*, 1956) grape vine (Vitis spp; Antcliff *et al.*, 1983) and citrus (Storey and Walker, 1999).

4.2.2 Conclusion

Pre-soaking of rice seeds using 45 dSm^{-1} NaCl solution can be used to screen rice varieties for salinity tolerance at the seed germination level. Nine days pre-soaking of rice seeds in saline water will reduce the water absorption and increase the Sodium and Chloride content in seeds. Sodium is absorbed to the seeds along with imbibitions of water. There are differences in water, Sodium and Chloride absorption among varieties. Reduction in rate of water uptake at high salt solutions had no effect on germination due to its ability to attain critical moisture level at 9 days of pre soaking. A cumulative effect of these three factors inside the seed might determine the germination ability of a particular rice variety under osmotic stress conditions caused by saline water. Ability of the seed coat in avoiding salt entrance into the seed during imbibitions when soaked in saline water is correlated with husk thickness. These findings suggest that, germination testing after pre-soaking rice seeds in 45 dSm^{-1} saline solutions could effectively be used to screen the rice varieties for salinity tolerance.

Seedling survival ability of rice varieties decrease with increased soil salinity level and it is negatively correlated to soil electrical conductivity. Seed germination ability of a rice variety after pre-soaking the seeds in high salt solutions has positive relationship to seedling survival under soils with high salt. Thus, seed germination and early seedling survival can be used to screen rice varieties for salinity resistance. Shoot and root growth of saline susceptible varieties were drastically reduced under high saline soils where these were not much reduced in saline tolerant varieties. Salt tolerant varieties contained more Na^+ ions in their roots compared to Na^+ ions in their leaves where salt sensitive varieties were not recorded such higher difference. Chloride ion content in leaves of tested varieties varied significantly where saline tolerant cultivars had lower leaf Cl^- content compared to saline susceptible varieties.

4.3 Experiment – II: Determination of growth, yield and physiological traits associated with salt tolerance of rice varieties under whole and sub-soil salinity conditions.

4.3.1: Trial - 1

Growth of all the plants was retarded under saline conditions compared to non salt-treated condition (control) at the age of one month and clear visual symptoms of salt damage were observed on plants (Table-11). In the control (T1), salinity symptoms could not be observed where highest damage was observed in all the tested varieties in the T7 leading no survival of plants in all the tested varieties. Severity of salt damage was varied with the salt type used to create saline condition. NaCl treated plants recorded greater damage compared to N_2SO_4 treated plants at same soil electrical conductivity and similar soil depth. This indicates that the damage of salinity to rice plants created by NaCl is greater than that of Na_2SO_4 (Table 11). When the salt was added to whole soil column, salinity damage was increased compared to the soil which treated up to subsoil level in the same variety.

Table 11: Reaction to salinity during seedling stage of rice as measured by the rank (4 weeks after planting).

Treatment	Variety and Rank *			
	Bg300	Pokkali	Nona Bokra	At354
T1(control)	1	1	1	1
T2(NaCl, 4dS/m,whole soil)	4	2	3	3
T3(NaCl,4dS/m,sub soil)	2	1	1	2
T4(NaCl,8dS/m,sub soil)	2	2	2	2
T5(Na_2SO_4,8dS/m,whole soil)	3	2	2	2
T6(Na_2SO_4,8dS/m,sub soil)	2	1	2	1
T7(NaCl,8dS/m,whole soil)	6	6	6	5

*Higher the rank higher the plant damage.

Leaf area (mean of tested four varieties) at maximum tillering was significantly different under different salinity treatments (Fig.12). Treatments were categorized into three groups as

A, B & C according to the leaf area. Lowest leaf area was observed in T4 (NaCl, $8dSm^{-1}$, sub soil). NaCl had greater influence on leaf area compared to Na_2SO_4 at similar soil electrical conductivity. Leaf area of T4 (NaCl, $8dSm^{-1}$, sub soil) was lower than that of T6 (Na_2SO_4, $8dSm^{-1}$, sub soil). Leaf area of tested varieties was varied under different treatments, implying different varietal response to salt stress ($p<0.05$). Highest leaf area was recorded in the control of all the varieties and it was decreased with increased soil salinity level. At354 recorded the highest leaf area compared to other varieties along all treatments. In tested varieties, leaf area of whole-soil treated treatments had severe effects compared to that in subsoil salt treated treatments (figure 12 & 13).

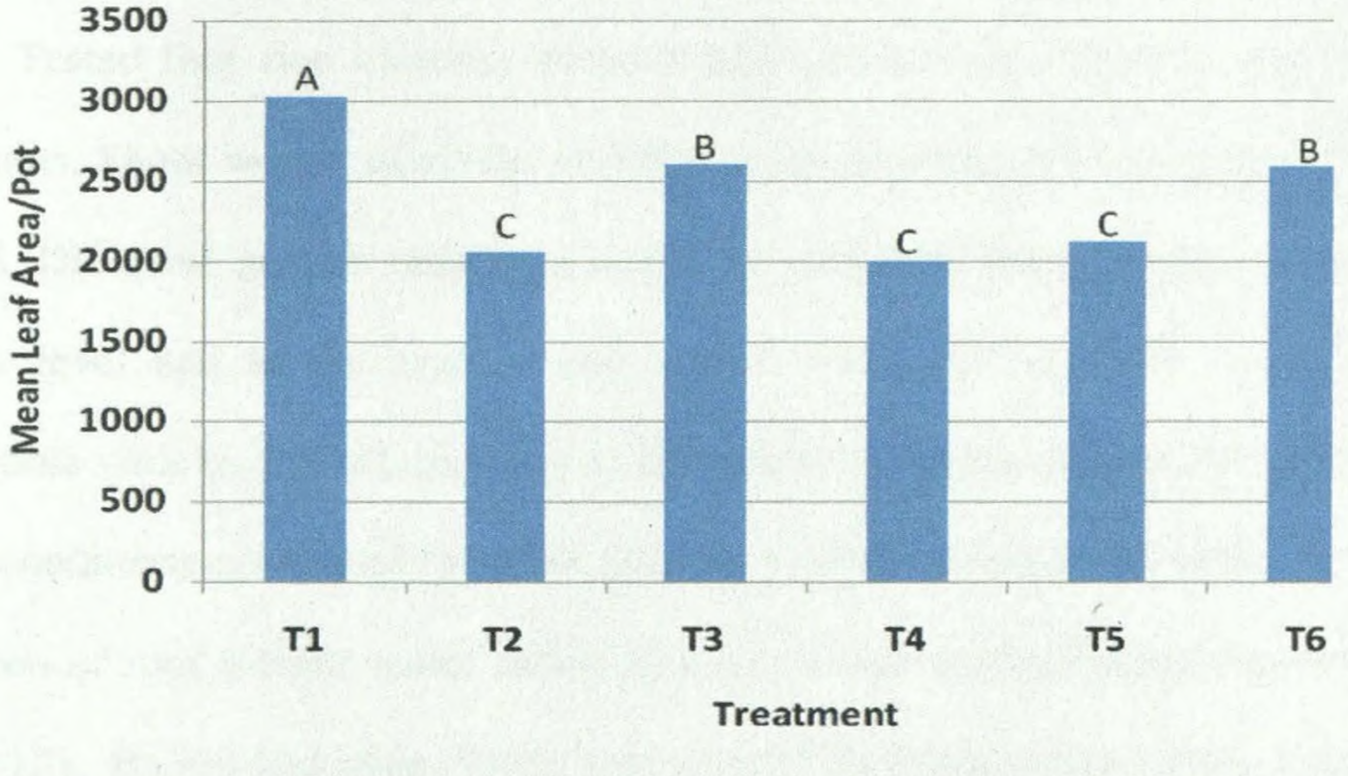
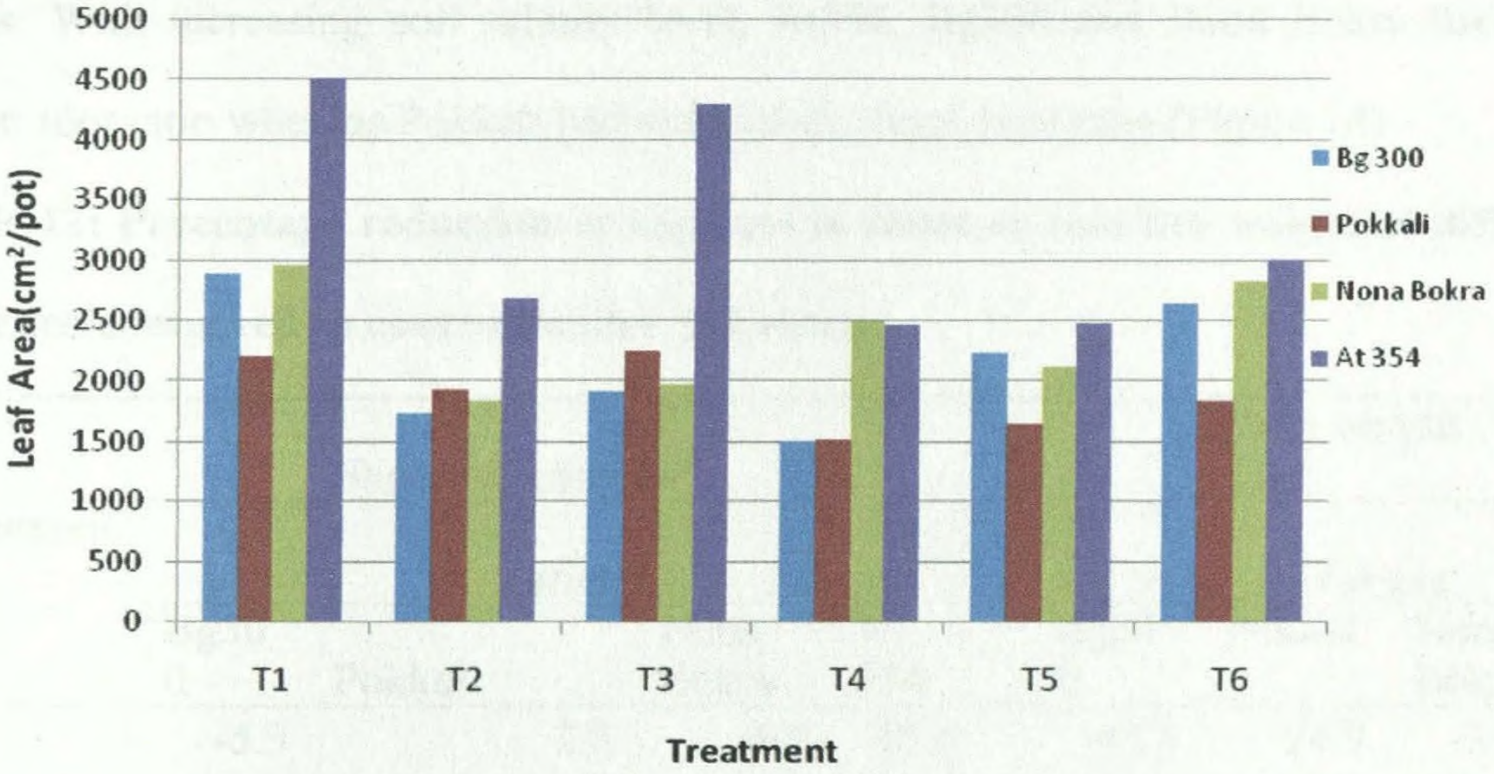


Figure 12: Leaf area(mean) at maximum tillering under different salinity treatments.



. **Figure 13: Leaf area of rice varieties at heading under different salinity treatments.**

Tested four rice varieties showed different growth responses under different saline conditions. Shoot weight of all the varieties reduced under saline conditions compared to the control. Different growth responses could be observed among tested varieties against soil salinity level and to the type of salt which was used to create salinity. Known saline susceptible variety, Bg300, reduced its both shoot biomass and root biomass significantly in saline conditions compared to saline tolerant variety (Pokkali and Nona Bokra). Percentage reduction of root weight under saline conditions was more predominant compared to shoot (Table 12). Bg300 and Nona Bokra had reduced its shoot weight where Pokkali showed low variability in shoot weight under salt stress. However, shoot weight of At354 was increased along all treatments. Percentage reduction in root weight was highest in Bg300 and followed by Nona Bokra and At354 where as root weight of Pokkali was increased. Relative reduction in shoot and root weight of a particular variety had determined its shoot: root ratio under salt stress. With increasing soil salinity level, At354, Bg300 and Nona Bokra had increased shoot: root ratio whereas Pokkali had reduced its shoot: root ratio (Figure 14).

Table 12: Percentage reduction or increase in shoot or root dry weight of different rice varieties(compared to control) under salt stress.

Treatment	Shoot weight (%)*				Root weight (%)*			
	Variety				Variety			
	Bg300	Pokkali	Nona Bokra	At 354	Bg300	Pokkali	Nona Bokra	At 354
T2	-5.3	2.7	-5.8	15.6	-47.6	24.9	-32.2	-6.3
T3	-19.5	-1.4	-17.2	16.1	-32.9	4.2	-21.3	-2.1
T4	-13.3	-0.1	-3.7	17.6	-32.9	25.7	-41.5	-3.4
T5	-16.3	0.0	-13.0	2.3	-51.0	1.4	-21.4	-8.8
T6	-17.0	3.8	-10.8	0.4	-44.4	13.7	-34.8	-0.4

- Percentage=[(Shoot weight or Root weight in treatment - Shoot weight or Root weight in control)/ Shoot weight or Root weight in control]*100

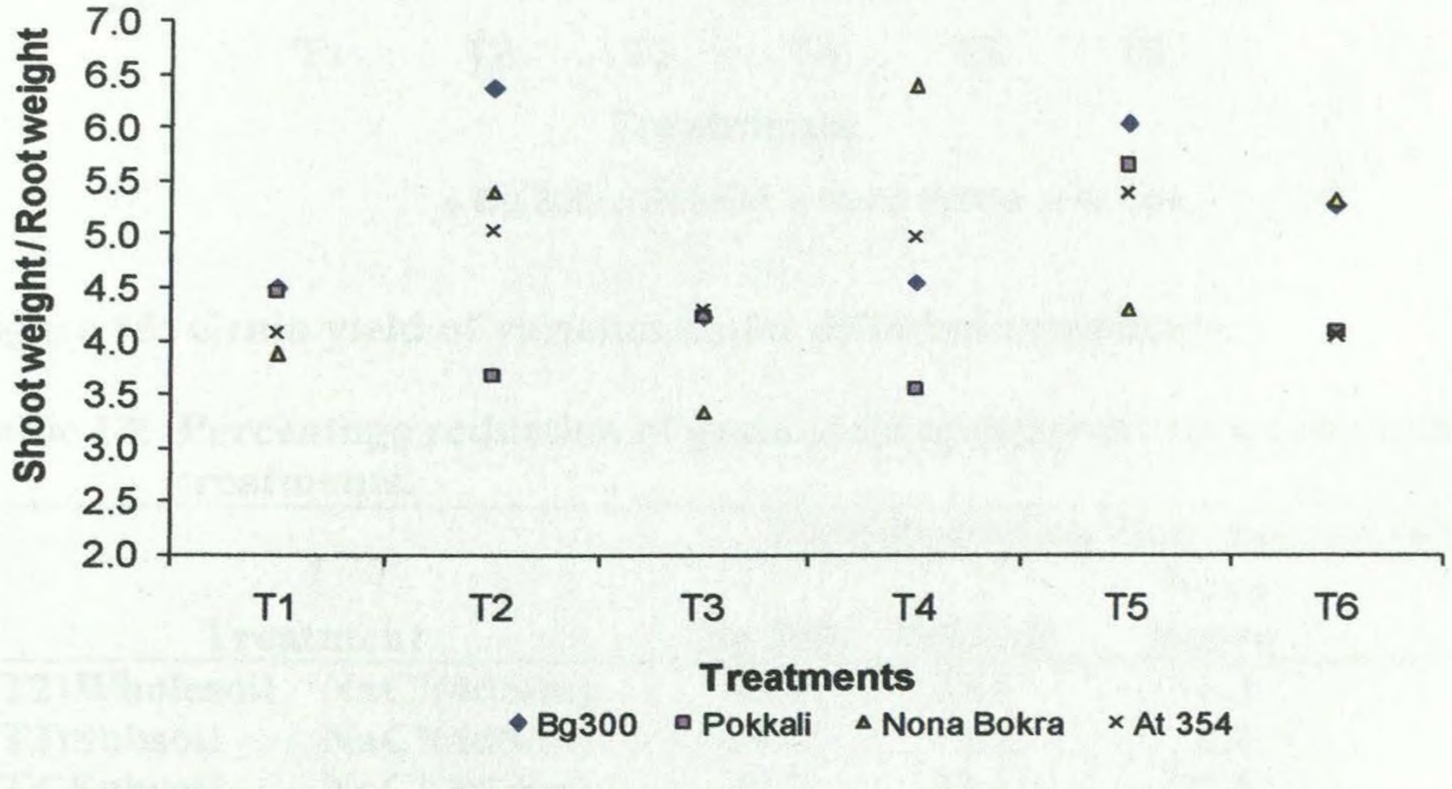


Figure 14: Shoot: root weight ratio of varieties at heading under different treatments.

Yield of all the varieties treated with salt were reduced compared to the control. Yield reduction was higher in whole-soil treatments compared to subsoil treatments at same electrical conductivity (EC). At the same EC level, yield was reduced in NaCl treated samples compared to Na_2SO_4 treatments. In the control, Bg300 recorded greatest yield followed by Nona Bokra, At354 and Pokkali. The greatest yield reduction observed in Bg300 (42.2%), followed by At354 (39.2%), Nona Bokra (27.8%) and Pokkali (22.1) at subsoil salinity of 8dS/m (Figure 15 and Table 13).

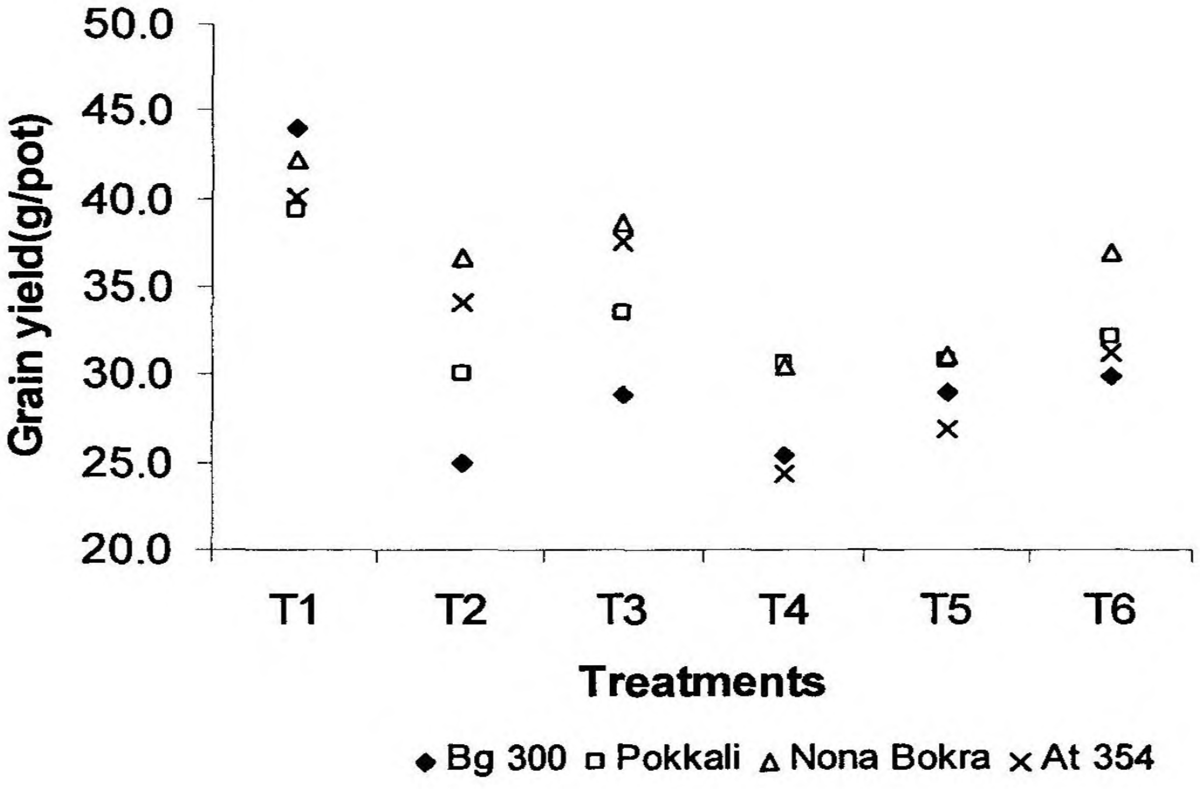


Figure 15: Grain yield of varieties under different treatments.

Table 13: Percentage reduction of grain yield of different varieties under different treatments.

Treatment		Yield Reduction %(compared to control)			
		Bg 300	Pokkali	Nona Bokra	At 354
(T2)Wholesoil	NaCl(4dS/m)	43.4	23.6	13.3	15.0
(T3)Subsoil	NaCl(4dS/m)	34.4	15.2	8.8	6.4
(T4)Subsoil	NaCl(8dS/m)	42.2	22.1	27.8	39.2
(T5)Wholesoil	Na_2SO_4(8dS/m)	34.3	22.0	26.5	32.8
(T6)Subsoil	Na_2SO_4(8dS/m)	32.1	18.5	12.6	22.1

4.3.2: Trial - 2

Growth of all the plants was retarded with saline conditions compared to non salt-treated plants (control) at the early vegetative stage. At the age of one month, clear visual symptoms were observed due to salinity damage. Severe symptoms could be observed in NaCl treated plants at 6 dSm^{-1} whole-soil (T5) and 8 dSm^{-1} NaCl subsoil (T6) (table 14). Damage caused by NaCl was greater than that of Na_2SO_4 at same electrical conductivity. But the plants of all the treatments were able to survive under above saline conditions up to maturity with different growth and yield responses.

Table 14: Ranking of varieties by visual symptoms for salinity damage at one month after seeding.

Treatment			Variety and Average scoring*			
			Bg300	Pokkali	Nona Bokra	At 354
T1	wholesoil	Control	1	1	1	1
T2	wholesoil	NaCl(4 dS/m)	3	2	3	3
T3	subsoil	Na_2SO_4(4dS/m)	2	1	1	1
T4	subsoil	NaCl(6 dS/m)	3	2	3	3
T5	wholesoil	NaCl(6 dS/m)	4	3	3	3
T6	subsoil	NaCl(8 dS/m)	3	2	3	3
T7	wholesoil	Na_2SO_4(4dS/m)	2	1	1	1
T8	subsoil	NaCl(4 dS/m)	2	1	1	2
T9	wholesoil	Na_2SO_4(8dS/m)	3	2	2	2
T10	subsoil	Na_2SO_4(8dS/m)	2	1	2	1

*Damage increases when scoring is increased

Tested varieties were shown different growth responses with different salinity levels and the type of salt. Plants which were treated with NaCl showed a greater damage compared to Na_2SO_4 treatments at same salinity level. Known saline susceptible variety, Bg300, reduced its both root biomass and shoot biomass significantly compared to saline tolerant varieties (pokkali). When increasing the salinity level, Pokkali was reduced its shoot: root ratio, implying higher root growth compared to shoot growth while Nona Bokra, Bg300 and At354 were increased their shoot growth compared to root growth (figure 16). These results are in accordance with the first pot experiment.

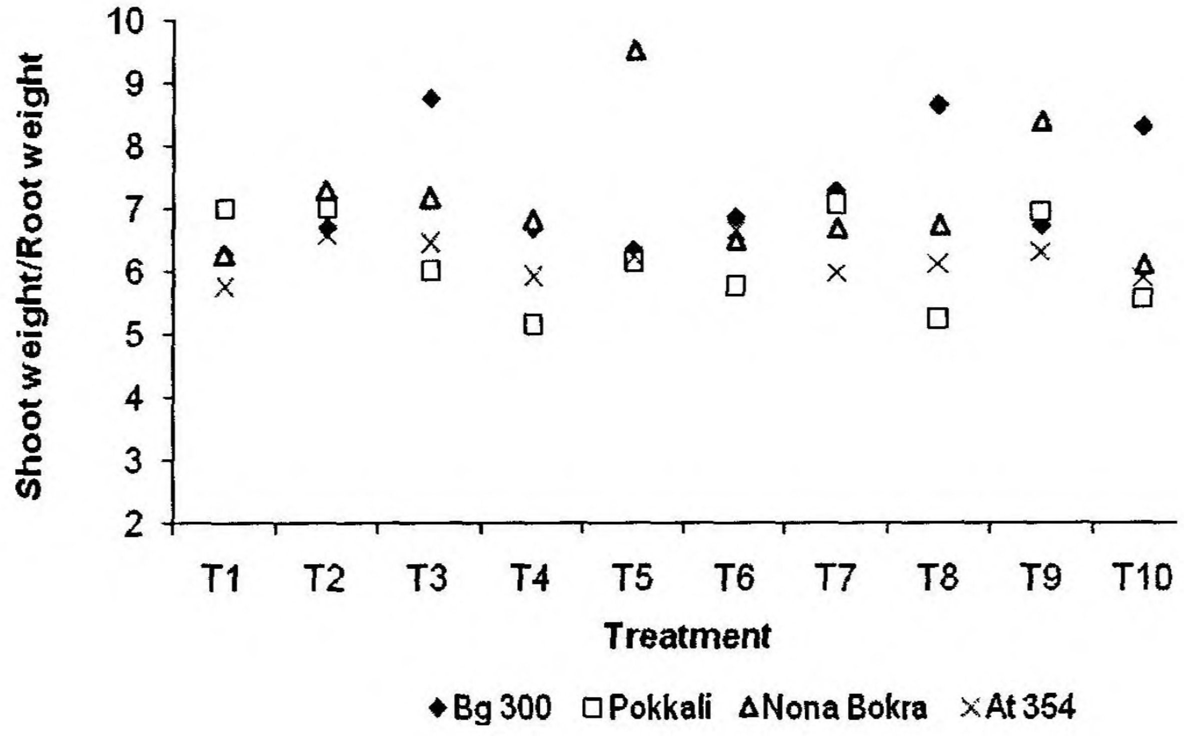


Figure 16: Shoot: Root ratio of varieties at heading under different treatments.

Yield of all the tested varieties were reduced under saline conditions compared to the control. Yield reduction was greater at whole-soil treatments compared to subsoil conditions at the same electrical conductivity (EC). At the same EC level, yield of NaCl treated plants were decreased than Na_2SO_4 treated plants (Table 15).The highest yield recorded by Pokkali (48grams/pot) followed by Bg300(45.2grams/pot), Nona Bokra(36.4grams/pot) and At 354(33.8grams/pot) in the control.

The yield reduction was highest in Bg300 in all the treatments where T5 recorded the highest yield reduction for Bg300 (65.5%) compared to control (Table 16).yield reduction percentage (compared to control) was lowest in Pokkali under salt stress and followed by Nona Bokra and At354.

Table 15: Average yield of the tested varieties under different treatments.

Treatment Number	Variety & Average Yield(grams/pot)			
	Bg 300	Pokkali	Nona Bokra	At 354
T1	45.26	48.00	36.40	33.87
T2	20.73	30.06	24.16	21.56
T3	30.30	40.30	25.66	30.40
T4	24.10	35.30	32.60	15.46
T5	15.60	20.23	16.03	17.80
T6	18.15	29.50	20.36	17.65
T7	29.96	39.80	25.00	25.56
T8	28.05	38.13	24.83	28.60
T9	16.33	25.70	22.73	12.43
T10	20.53	29.40	23.80	21.50

Table 16: Yield reduction of varieties under different treatments compared to control.

Treatment Number	Variety & Yield reduction(%) compared to control			
	Bg 300	Pokkali	Nona Bokra	At 354
T1	*	*	*	*
T2	54.2	37.4	18.5	36.3
T3	33.1	16.0	13.5	10.2
T4	46.8	26.5	10.4	54.4
T5	65.5	57.9	45.9	47.4
T6	59.9	38.5	31.3	47.9
T7	33.8	17.1	15.7	24.5
T8	38.0	20.6	16.3	15.6
T9	63.9	46.5	23.3	63.3
T10	54.6	38.8	19.7	36.5

Leaf sodium content and root sodium content were significantly different ($p<0.005$) at maximum tillering among varieties tested. Sodium Sulfate treated plants were absorbed more sodium than Sodium Chloride treated plants at same electrical conductivity (Figure 17 & Figure 18).

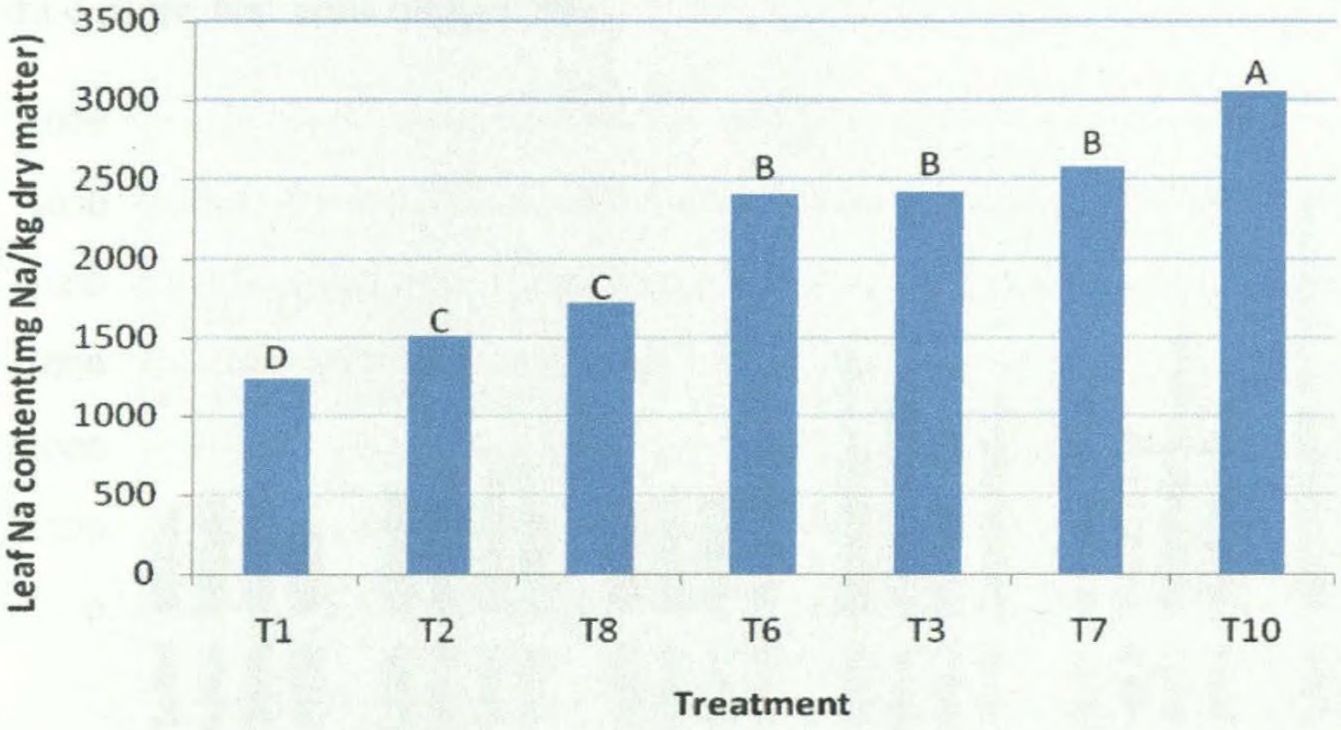


Figure 17: Mean Leaf Sodium content under different treatment.

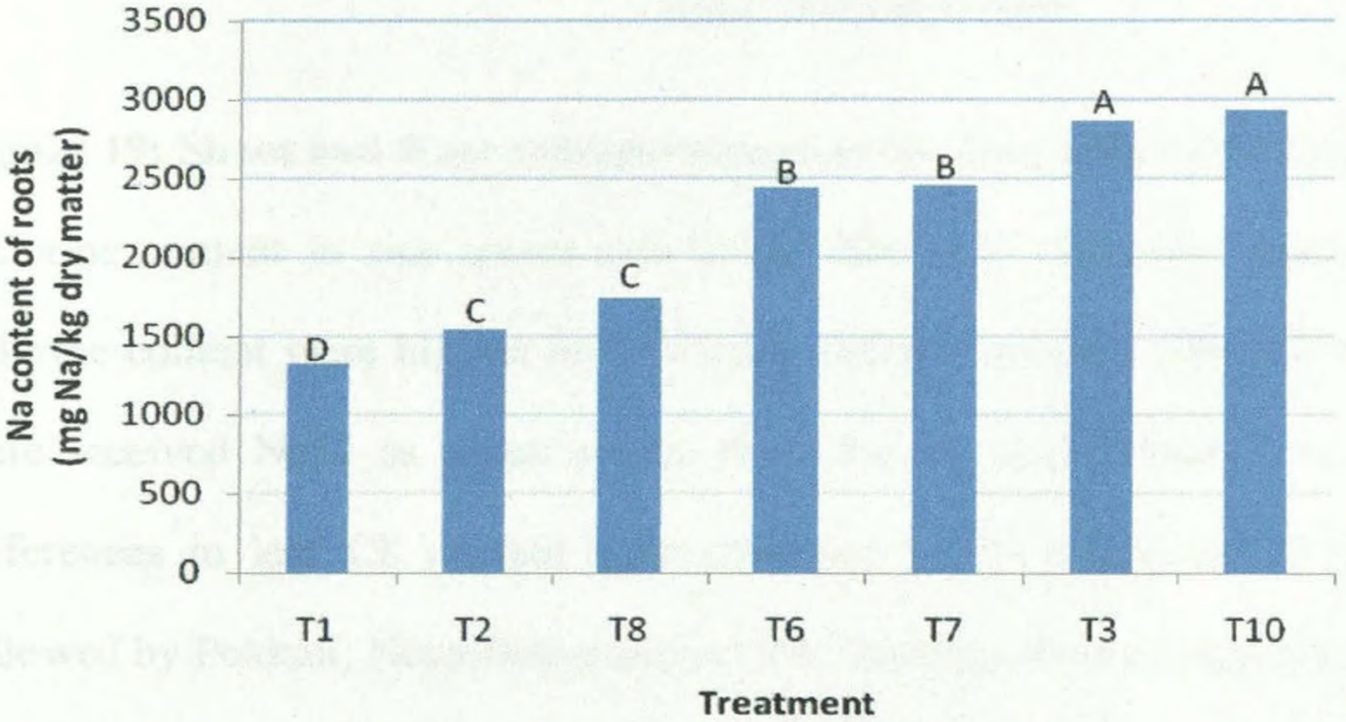


Figure 18: Mean Root Sodium content under different treatments.

Sodium content of leaves and roots were significantly different at 5% probability level. Most susceptible variety (Bg300) recorded more Na^+ in leaves compared to other varieties but its roots contained lesser Na$^+$ ions compared to other varieties. Saline tolerant varieties contained lesser amount of Na$^+$ ions in the leaves compared to Bg300 while their roots contained more Na$^+$ ions (figure 19).

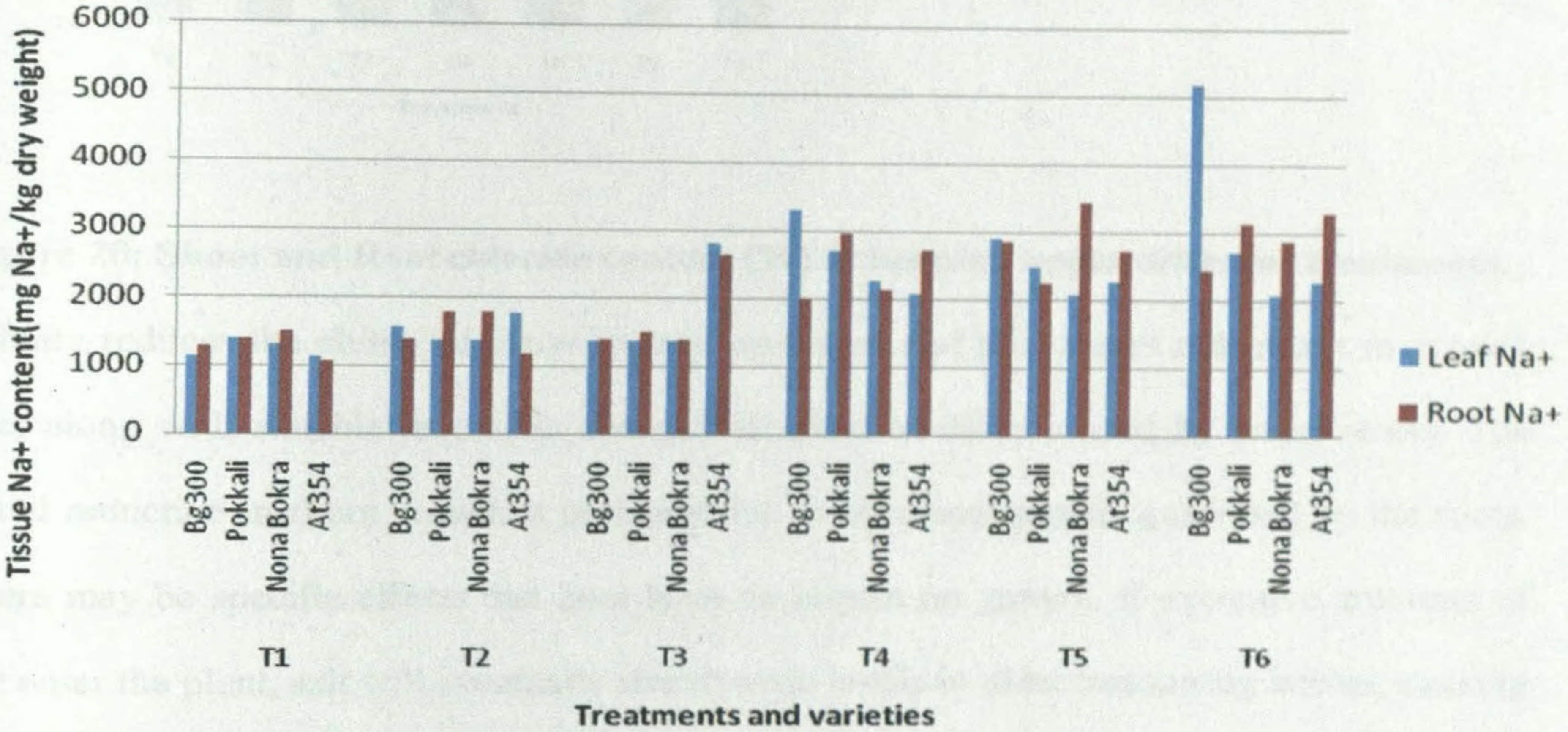


Figure 19: Shoot and Root sodium content at heading under different treatments.

Chlorine content in rice leaves was lower than that of roots (Figure 20). Leaf and root chlorine content were highest in T4 (NaCl, 8dSm^{-1}, subsoil) followed by T2 and T3 which were received NaCl as saline source than that of Na_2SO_4treatments. Significant varietal differences in leaf Cl$^-$ content were observed in T4 where Bg300 recorded highest Cl$^-$ followed by Pokkali, Nona Bokra and At354. Varieties showed significant differences in root chlorine content in T4, T2 and T3 where significant differences were not observed in T1, T6, T9 and T10. Highest root chlorine content was observed in Pokkali and Nona Bokra followed by At354 and Bg300.

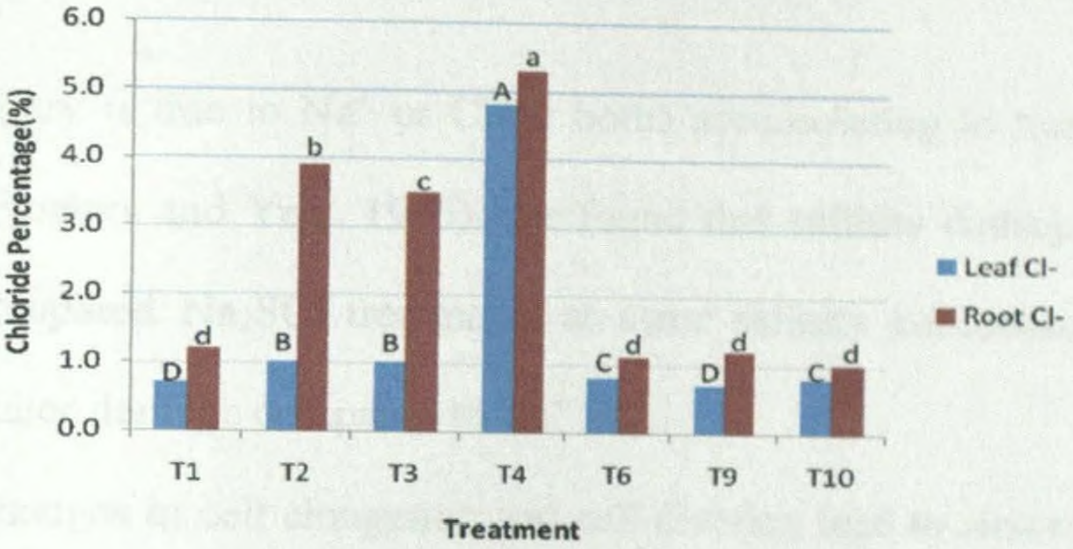


Figure 20: Shoot and Root chloride content (%) at heading under different treatments.

Salinity reduces the ability of plants to take up water, and this causes reductions in growth rate, along with suitable metabolic changes identical to those caused by water stress. The initial reduction in shoot growth is probably due to hormonal signals generated by the roots. There may be specific effects that later have an impact on growth, if excessive amounts of salt enter the plant, salt will eventually rise to toxic levels in older transpiring leaves, causing premature senescence, and reduce the photosynthetic area(Munns, R.,2002). So our results in accordance with above phenomena reducing the rice plant growth at higher saline conditions compared to low saline conditions.

Salt tolerant plants differ from salt sensitive ones having a low rate of Na^+ and Cl^- transport to leaves, and the ability to compartmentalize these ions in vacuoles to prevent their build up in cytoplasm or cell walls and thus avoid toxicity (Munns, R., 2002). Varieties which are generally considered as salt tolerant (Pokkali and Nona Bokra) recorded higher root Na^+ ion content compared to leaf Na^+ content while saline susceptible variety, Bg300 recorded higher leaf Na^+ content compared to its root Na^+ content. This might suggest that Pokkali and Nona Bokra had reduced transport of Na^+ ions to their leaves to survive in saline conditions. Salt injury is due to Na^+ or Cl^-(or both) accumulating in transpiring leaves to excessive levels (Flowers and Yeo., 1986). We found that salinity damage is greater under NaCl treatments compared Na_2SO_4 treatments at same salinity condition. That implies the Cl^- ions cause major damage compared to Na^+ ions.

Changes in cell elongation and cell division lead to slower leaf appearance and smaller final size, and leaf growth is more affected than root growth (Munns R., 2000).Rapid and transient reductions in leaf expansion rates after a sudden increase in salinity have been recorded in rice (Yeo et al., 1991), maize (Neuman., 1993) and wheat and barley (Passioura and Munns., 2000). Leaf growth is often more reduced than root growth by salinity, phenomenon in common with dry soil (Hsiao and Xu 2000). During vegetative growth of rice, plant height, straw weight, number of tillers per plant, dry weight of roots, and root length are all adversely affected by salinity, but all the growth parameters are not affected equally(Akbar et al., 1972).

4.3.3 Leaf Photosynthesis under salt stress:

Leaf net photosynthetic rate was significantly different with different treatments ($p<0.05$). Highest photosynthetic rate was observed in the control where no salt was added (26-29 $\mu mol\ CO_2 m^{-2} S^{-1}$). With the increase of soil salinity level, leaf photosynthesis was reduced significantly among the tested varieties. Photosynthetic rate was varied with different varieties and the salt type which were used to induce soil electrical conductivity.

Difference of leaf photosynthetic rate could not be observed among tested varieties in the control at 5% probability level and it remained at the maximum value along all the treatments investigated (figure-1).

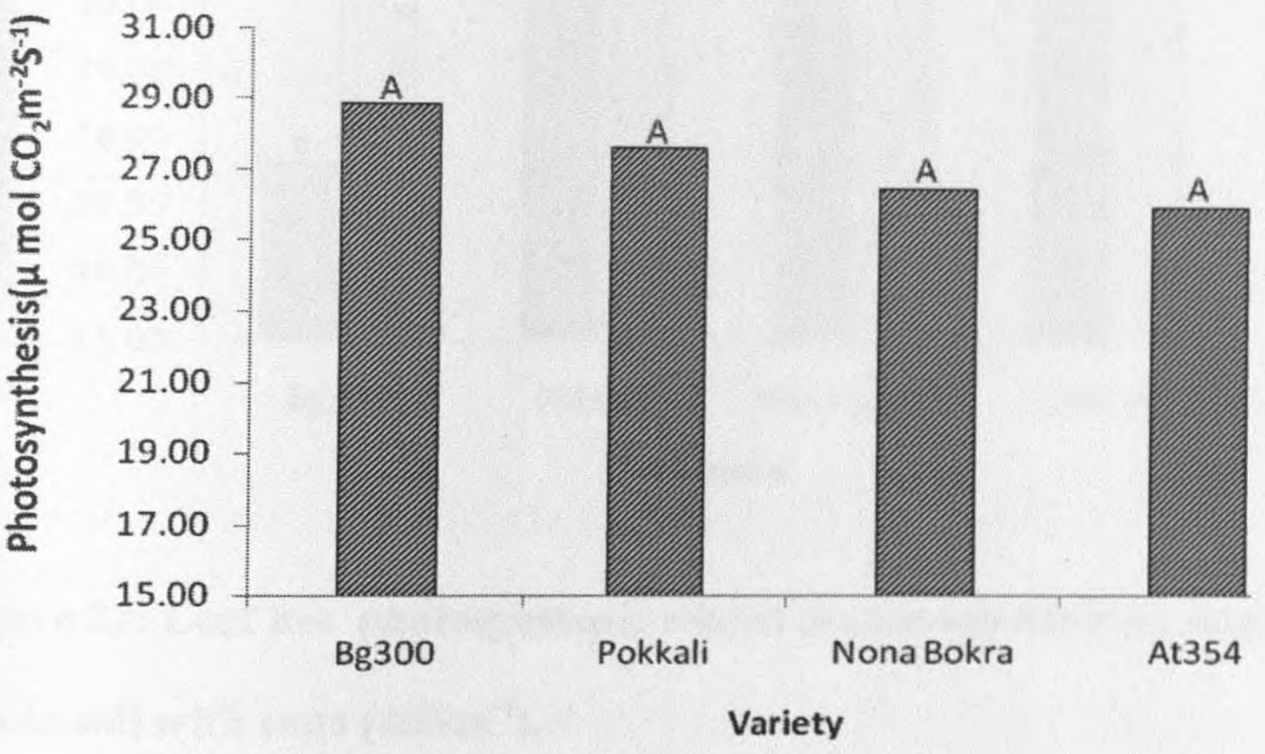


Figure 21: Leaf photosynthesis rate of different varieties at maximum tillering stage (Control).

Net photosynthetic rate (Pn) of rice leaves was higher in Na_2SO_4 treatments compared to NaCl treatments. Varieties could be categorized into two groups at NaCl treatment where At354, Pokkali and Nona Bokra recorded higher Pn than Bg300. In the Na_2SO_4 treated plants, Pn was highest in Pokkali and followed by At354, Nona Bokra and Bg300 (Figure 21).

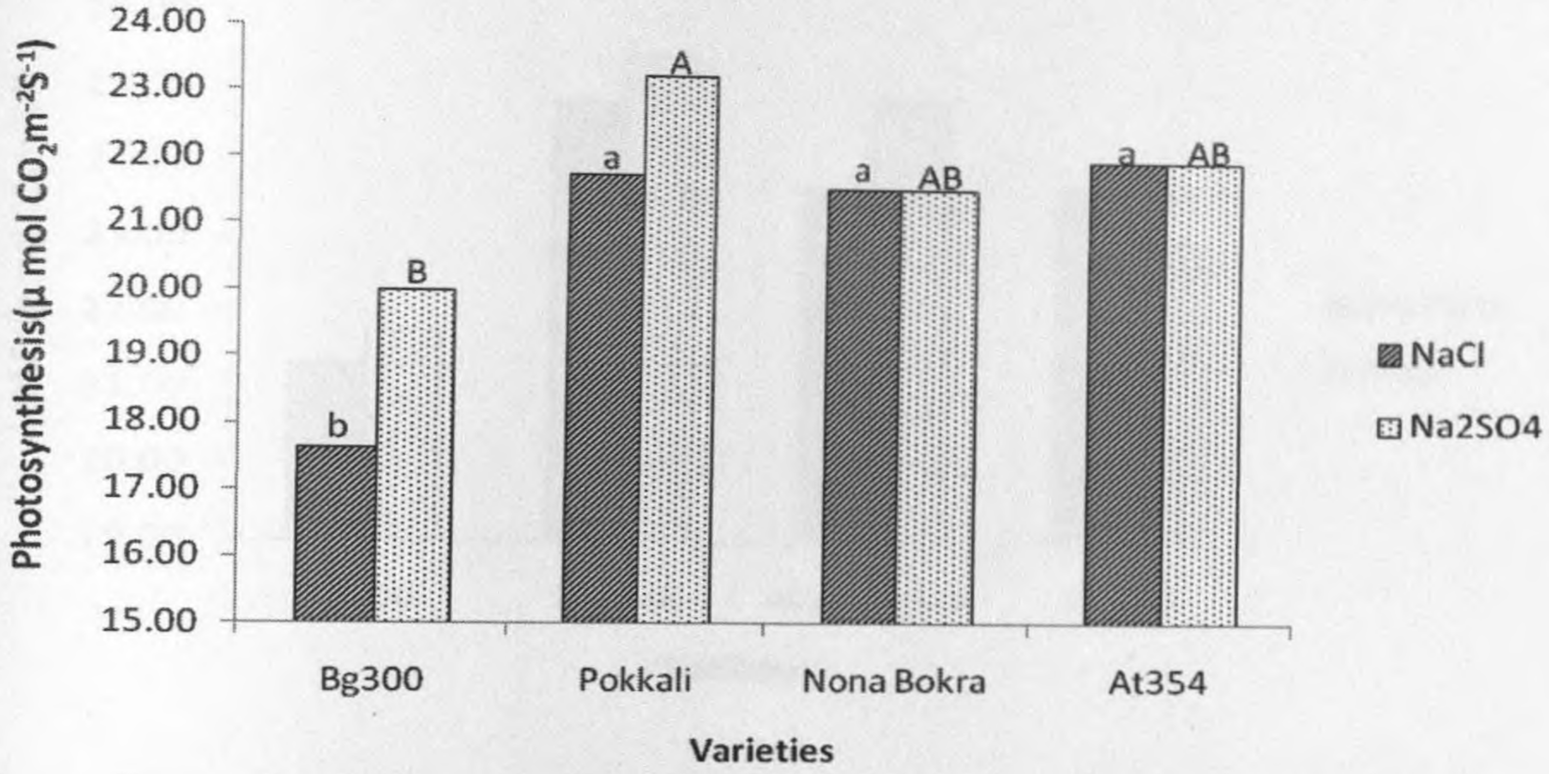


Figure 22: Leaf net photosynthesic rate at maximum tillering stage which was treated whole-soil with salts (4dSm^{-1}).

Net photosynthetic rate of tested varieties was higher in sub soil conditions than that of whole soil conditions (figure 22 & 23). In the subsoil treatments, Pokkali recorded the highest Pn and followed by Nona Bokra and At 354 and least Pn recorded by the saline susceptible variety, Bg300. NaCl treated plants showed greater reduction of Pn compared to Na_2SO_4 treated plants at 4dSm^{-1} sub-soil salinity level.

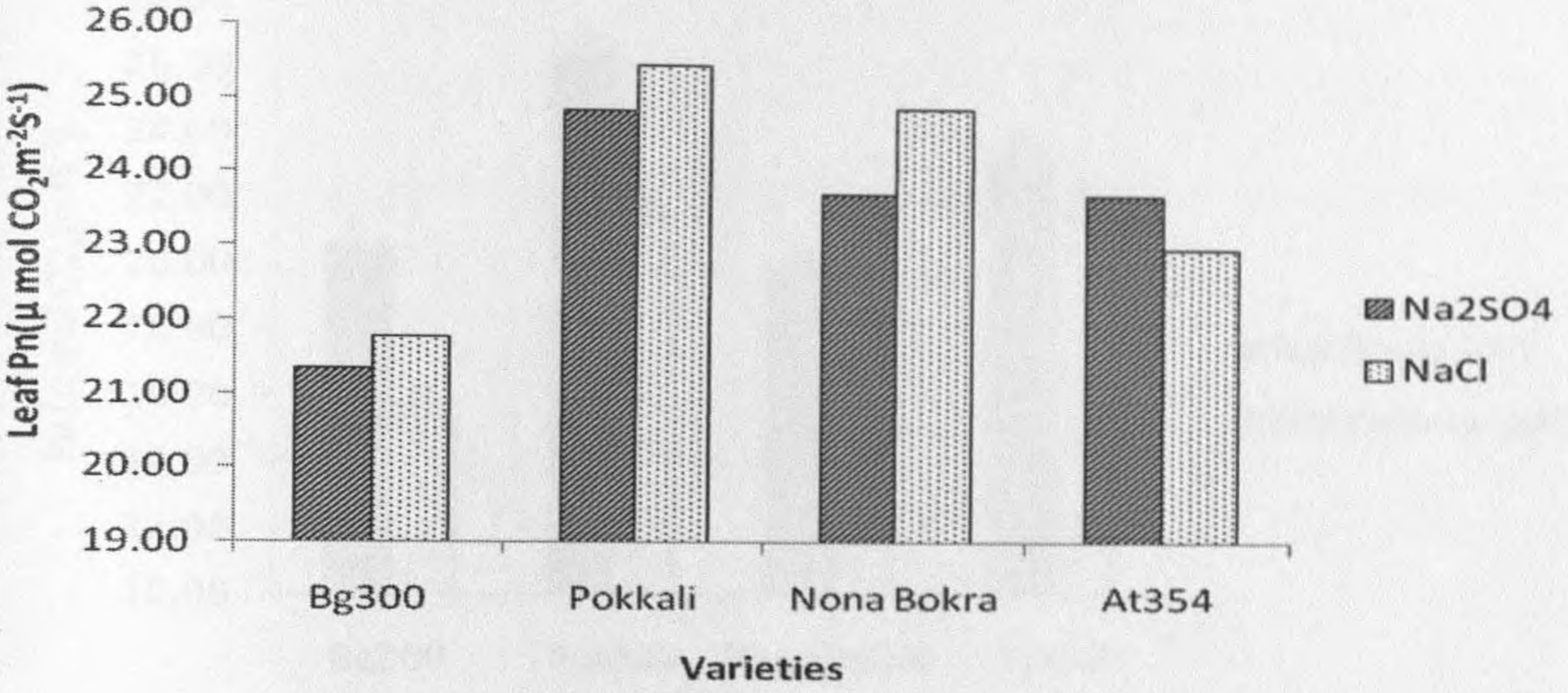


Figure 23:Leaf net photosynthetic rate at maximum tillering stage which was treated sub-soil (4dSm^{-1}).

When increase the soil electrical conductivity from 4dSm^{-1} to 6dSm^{-1}, reduction in Pn was significant. Highest reduction in Pn was recorded by NaCl at 6 dS m^{-1} compared to all other treatments. Here also, whole-soil treated plants were reduced their Pn compared to sub-soil treatments(Figure 24).

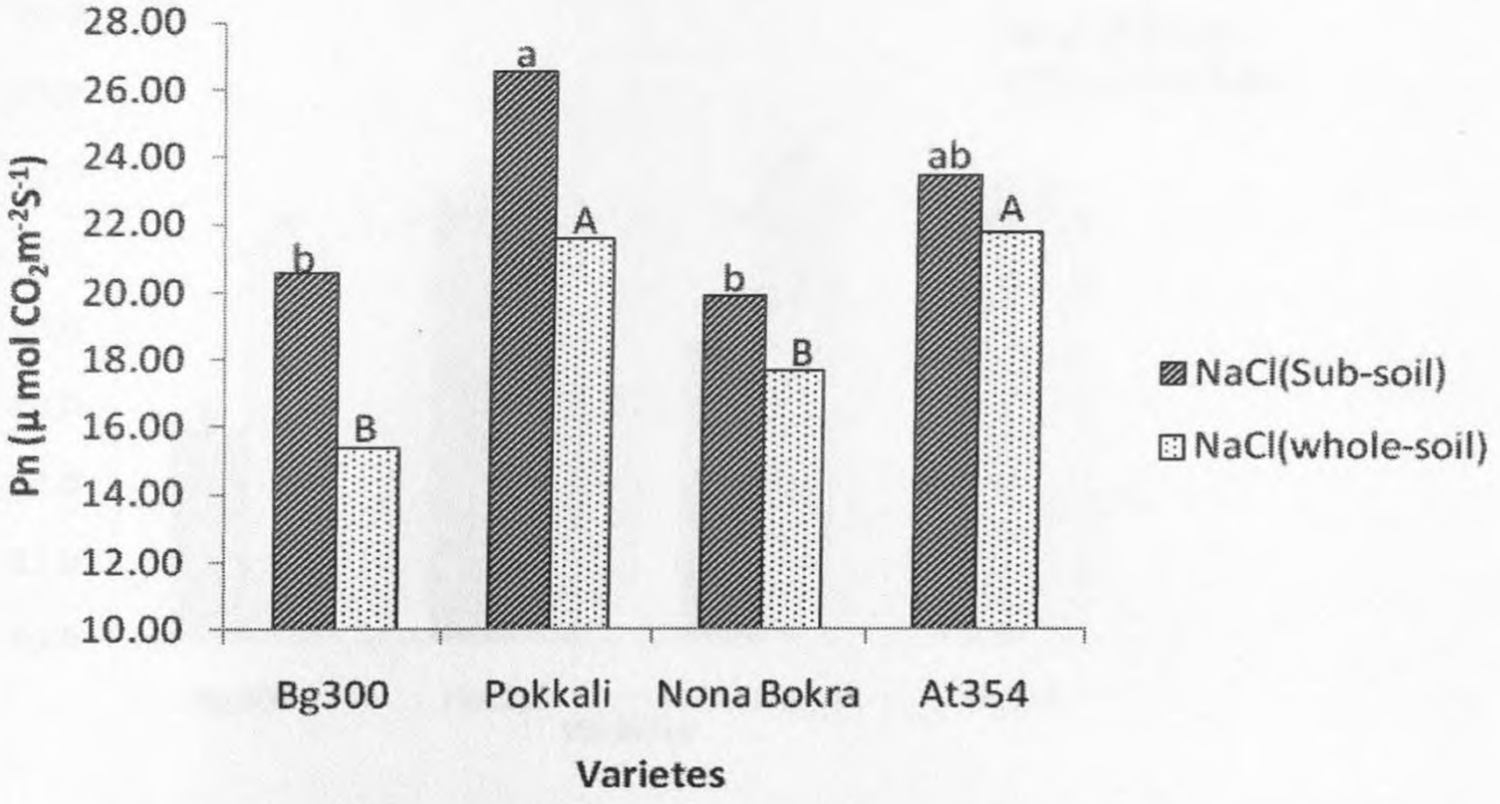


Figure 24: Leaf net photosynthesic rate at maximum tillering stage which was treated with NaCl (6dSm^{-1}).

Rice plants were unable to survive under 8 dSm^{-1} whole-soil treated with NaCl but they were able to survive under sub-siol treatment at same salinity level. Reduction of Pn was higher at NaCl sub-soil treatment compared Na_2SO_4 sub-soil treatment(Figure 25). In the Na_2SO_4 8dSm^{-1} whole-soil treatment, plants recorded lower yield compared to sub-soil at same salt and salinity level(figure 26).

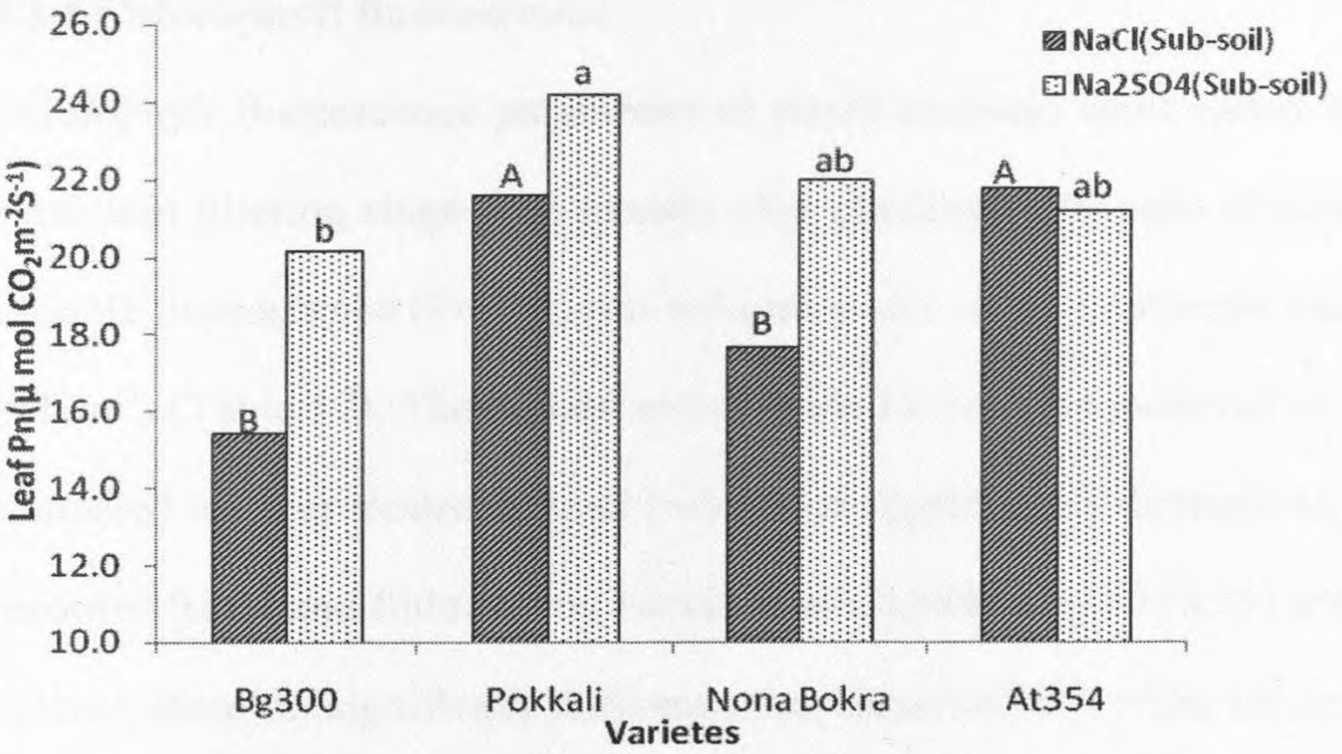


Figure 25: Leaf net photosynthetic rate at maximum tillering stage under 8 dSm^{-1}(sub soil).

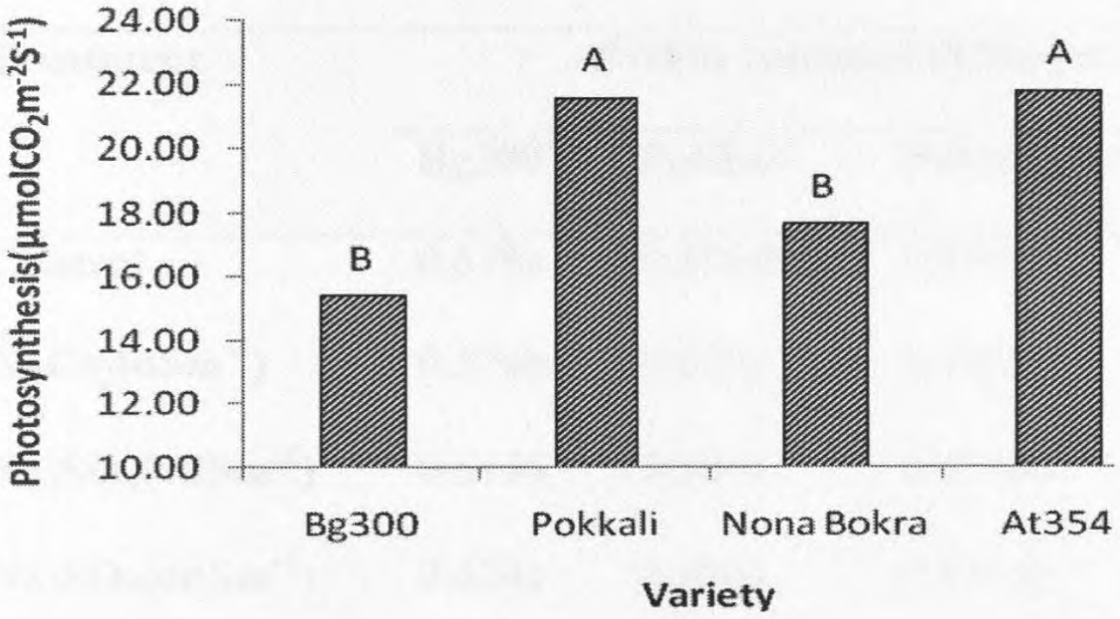


Figure 26: Leaf net photosynthesis at maximum tillering (Na_2SO_4, 8dSm^{-1}, whole-soil).

4.3.4 Chlorophyll fluorescence

Chlorophyll fluorescence parameters of tested varieties were varied with treatments at their maximum tillering stage (two months after planting). The ratio of maximum fluorescence to variable fluorescence (Fv/Fm) was reduced under saline treatments except Pokkali at Na_2SO_4 ($4dSm^{-1}$) (Table 17). The highest reduction in Fv/Fm was observed in NaCl $4dsm^{-1}$ treatment compared to other treatments and Fv/Fm was significantly different at $p=0.05$, where Pokkali recorded 0.607 and followed by Nona Bokra (0.584), Bg300 (0.57) and At354 (0.541). In the control, there no significant difference was observed in Fv/Fm values. With the increase of soil salinity, greater reduction of Fv/Fm values could be observed.

Table 17: Fv/Fm values under different treatments at maximum tillering stage.

Treatment	Fv/Fm values of different varieties			
	Bg300	Pokkali	Nona Bokra	At354
Control	0.676a	0.696ab	0.685b	0.694a
NaCl($4dSm^{-1}$)	0.57ab	0.607a	0.584a	0.541b
Na_2SO_4($4dSm^{-1}$)	0.613b	0.704a	0.654ab	0.674a
Na_2SO_4($4dSm^{-1}$)	0.624c	0.684a	0.676ab	0.663b

The basal non-variable chlorophyll fluorescence (F_O) varied with different varieties. F_O was increased in Bg300 under saline treatments where other varieties did not show a pattern on increase or decrease compared to the control. The basal non-variable chlorophyll fluorescence of Pokkali and Nona Bokra recorded as lowest values compared to Bg300 and

At354. This implies the greater damage to photosynthetic apparatus in Bg300 and At354 compared to Pokkali and Nona Bokra (Table 18).

Table 18: Basal non-variable fluorescence (F_0) of rice varieties under salt stress.

Treatment	F_0 values of different varieties			
	Bg300	Pokkali	Nona Bokra	At354
Control	77.56a	74.67a	72.57a	81.0a
NaCl(4dS/m)	88.55a	67.0b	67.0b	83.77a
Na_2SO_4(4dS/m)	79.5a	67.9a	65.1a	75.55a
Na_2SO_4(4dS/m)	78.55a	67.4a	71.0a	75.05a

The maximum chlorophyll fluorescence (Fm) was not showed any pattern with soil salinity but it was changed under different soil salinity levels (Table 19).

Table 19: Maximum chlorophyll fluorescence (Fm) under different treatments at maximum tillering.

Treatment	Fm values of different varieties			
	Bg300	Pokkali	Nona Bokra	At354
Control	247.0a	217.17a	178.43ab	239.4ab
NaCl(4dS/m)	200.65a	215.3a	163.2a	192.1a
Na_2SO_4(4dS/m)	268.4a	175.95b	189.05b	232.45ab
Na_2SO_4(4dS/m)	251.4a	179.5b	219.5a	222.95a

4.3.5 Conclusion:

Growth and yield of rice varieties were significantly affected by the salinity conditions which they were grown. Varietal differences could be observed at early seedling growth but the plants could be able to survive up to harvesting in all the treatments in second pot trial. With the increase of soil electrical conductivity, growth and yield reduction could be observed in all tested varieties. Suppression of growth and yield were varied with variety and the type of salt used to create soil salinity. When increasing the salinity level, Pokkali reduced its shoot: root ratio, implying higher root growth compared to shoot growth while Nona Bokra, At354 and Bg300 increased their shoot growth compared to root growth. Greater reduction in plant growth and yield was observed under NaCl compared Na_2SO_4. Leaf and root sodium content varied among tested varieties, a saline sensitive variety; leaves of Bg300 contained more Na^+ ions than the other varieties where saline tolerant variety (Pokkali) contained less Na^+ ions in its leaves.

4.4 EXPERIMENT- 3

4.4.1 FIELD TRIAL – I

The main effects investigated (soil water condition and variety) were significantly affected the leaf chlorophyll content (measured as SPAD reading) at heading ($p < 0.05$). There was no difference of leaf chlorophyll content between standing water condition and saturated water condition but it was lower in alternative wetting and drying condition. However, treatments investigated had no effect on leaf chlorophyll content. The interaction between soil water conditions and treatments was not significantly different among tested varieties ($p=0.13$). Chlorophyll content of varieties tested was significantly different at 5% probability level, implying that there an inherent variability in chlorophylls of tested varieties. The two way interactions (variety*treatment) and three way interactions (variety*treatment* soil water condition) were also not significantly different. The average SPAD values(chlorophyll content) along soil water conditions and treatments of the varieties Bg300, At354, Pokkali and Nona Bokra were 41.1, 38.1, 37.5 and 34.1 respectively.

Main effects of variety, soil water condition and organic matter treatments (addition of organic matter) had a significant impact on the rice grain yield under field conditions. The soil water condition had affected the grain yield significantly at 5% probability level ($p<0.05$). Different organic matter treatments investigated had affected the grain yield of rice ($p=0.04$). The interaction effect between soil water condition and treatments also significantly affected on yield ($p<0.05$) and three way interactions among the treatments, variety and soil water condition were also significant ($p<0.05$).

The yield of rice varieties under different water conditions and organic matter treatments can be illustrated as in figure 27

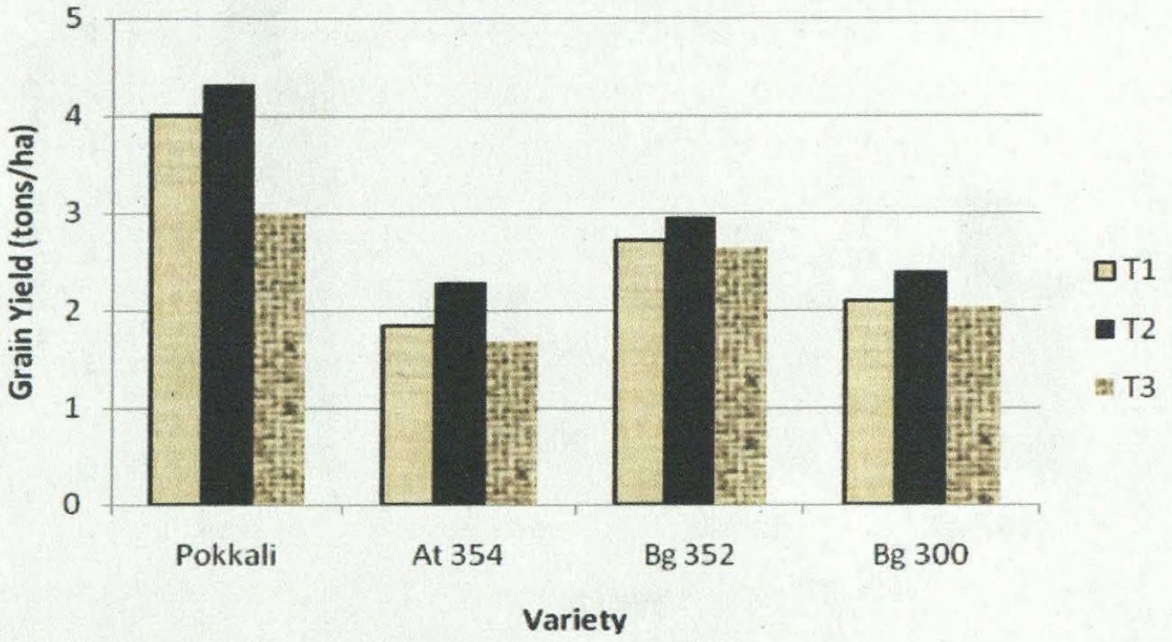


Figure 27: The yield of different varieties under different treatmenmts at standing water condition(T1-Cowdung+N,P,K; T2- Cowdung+N,P,K+Charcoaled paddy husk;T3- N,P,K only).

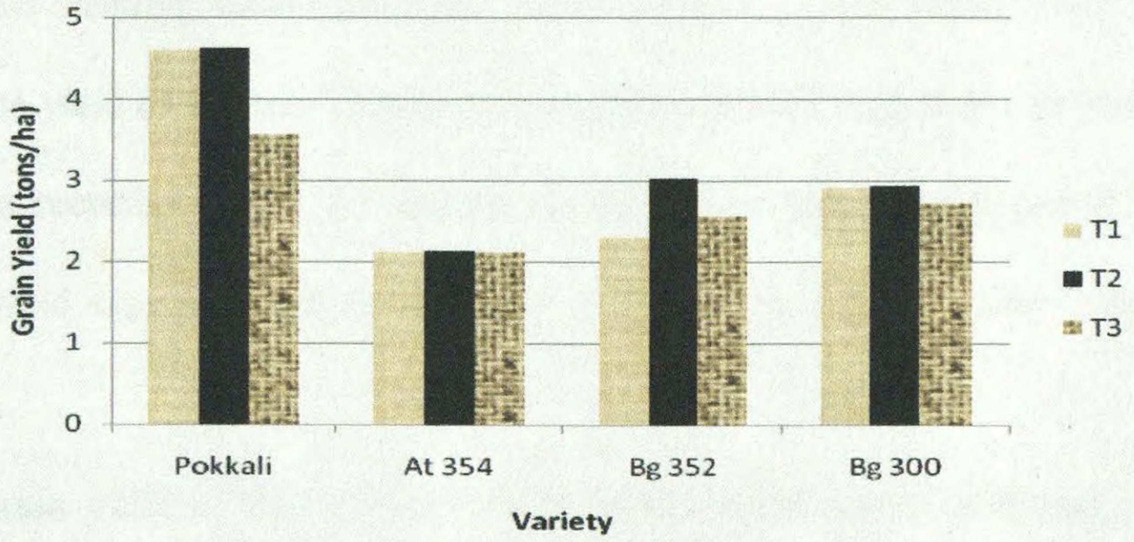


Figure 28: The yield of different varieties under different treatmenmts at saturated water condition.

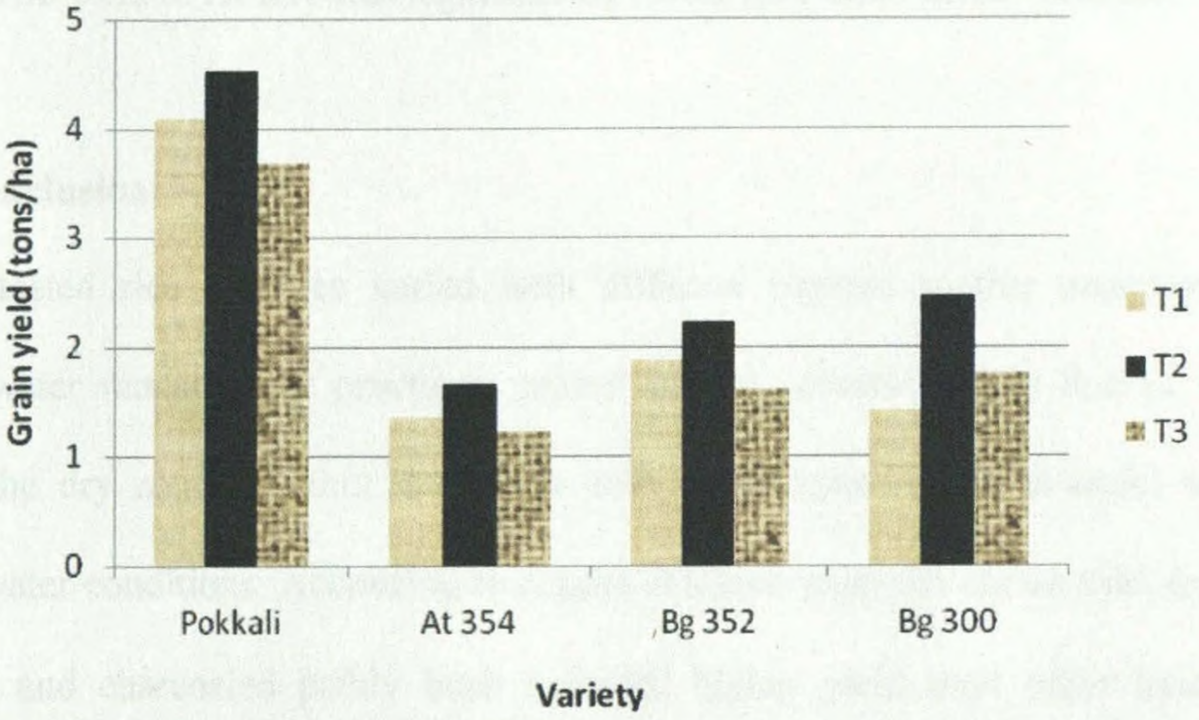


Figure 29: The yield of different varieties under different treatmenmts at alternative wetting and drying condition.

All the tested varieties recorded the highest yield under the treatment two (T2 – Cow dung + charcoaled paddy-husk + N, P, K)) compared to T1 (Cow dung + N, P, K) and T3 (N, P, K) under standing water condition. Grain yield of T2 was significantly higher than T1 and T3 and the yield of T1 and T3 was not significantly different at 5% probability level. Lowest yield was recorded under T3 across all the tested varieties compared to T1 and T3.The highest yield was recorded by Pokkali compared to other varieties under standing water condition.

Grain yield of the tested varieties were significantly different under different soil water conditions with different treatments and could be categorized into three groups according to the yield ($p<0.05$). Pokkali recorded the highest yield in all the treatments under all water conditions. Bg 352 recorded a greater yield than Bg300 but grouped into the same category. The yield of At 354 was significantly lower than other three varieties.

4.4.1.1 Conclusion:

Yield of tested rice varieties varied with different organic matter treatments and with different water management practices. Inland salinity occurs mainly due to limitation of water in the dry zone and this is evident with the increase of yield under saturated and standing water conditions. According to results obtained from the above trial, treatment with cow dung and charcoaled paddy husk recorded higher yield over other treatments. This implies that inland salinity problem in this location could be rectified by adding organic matter and charcoaled paddy husk.

4.4.2 FIELD TRIAL - II

Yield of tested varieties were significantly different among treatments at 5% probability level. The effect of major plot factor (soil water condition) on grain yield was significant at 5% probability level. Yield response of varieties was varied with treatments and soil water conditions tested ($p<0.05$).

The varietal response under different water conditions and treatments can be illustrated as in following graphs.

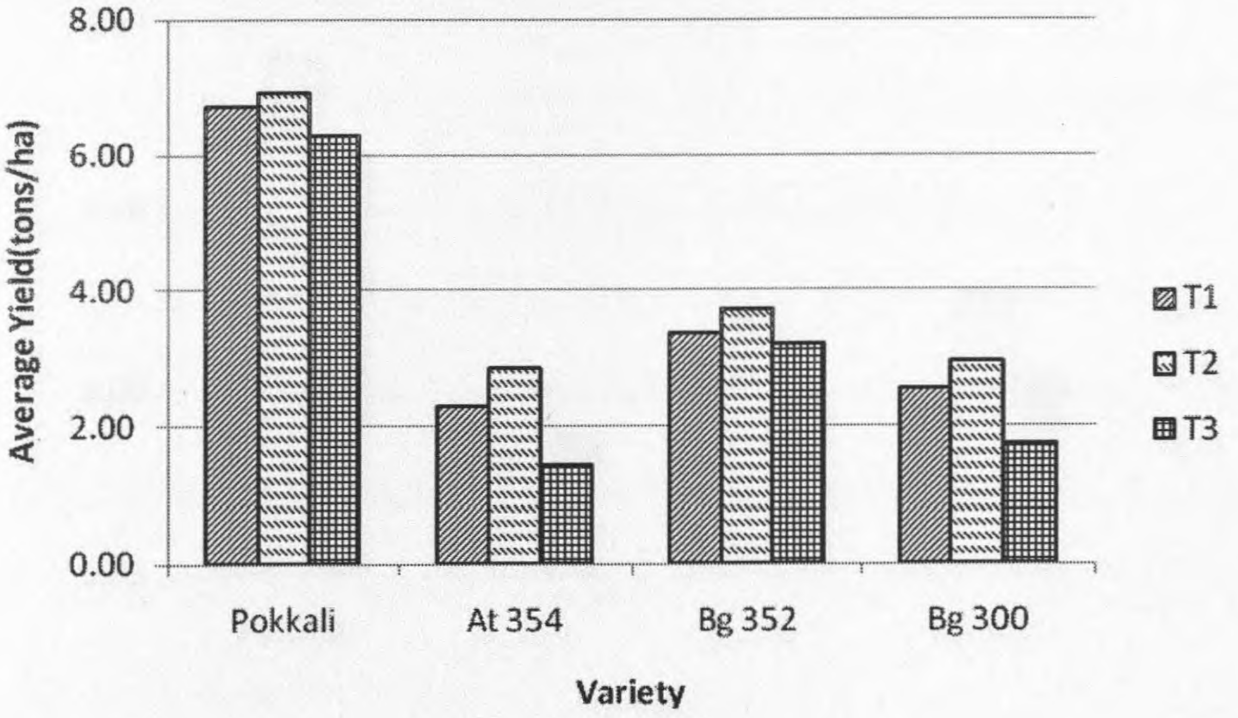


Figure 30: The yield of different varieties under different treatmenmts at Standing water condition.

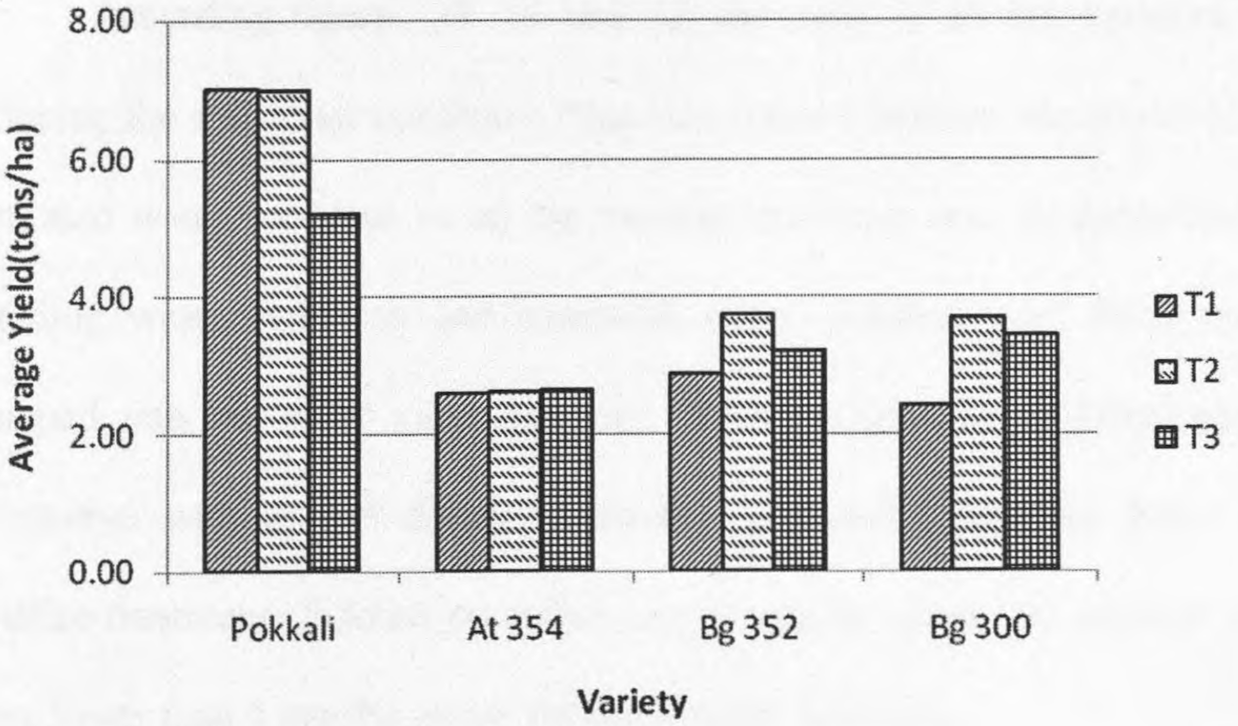


Figure 31: The yield of different varieties under different treatmenmts at saturated water condition.

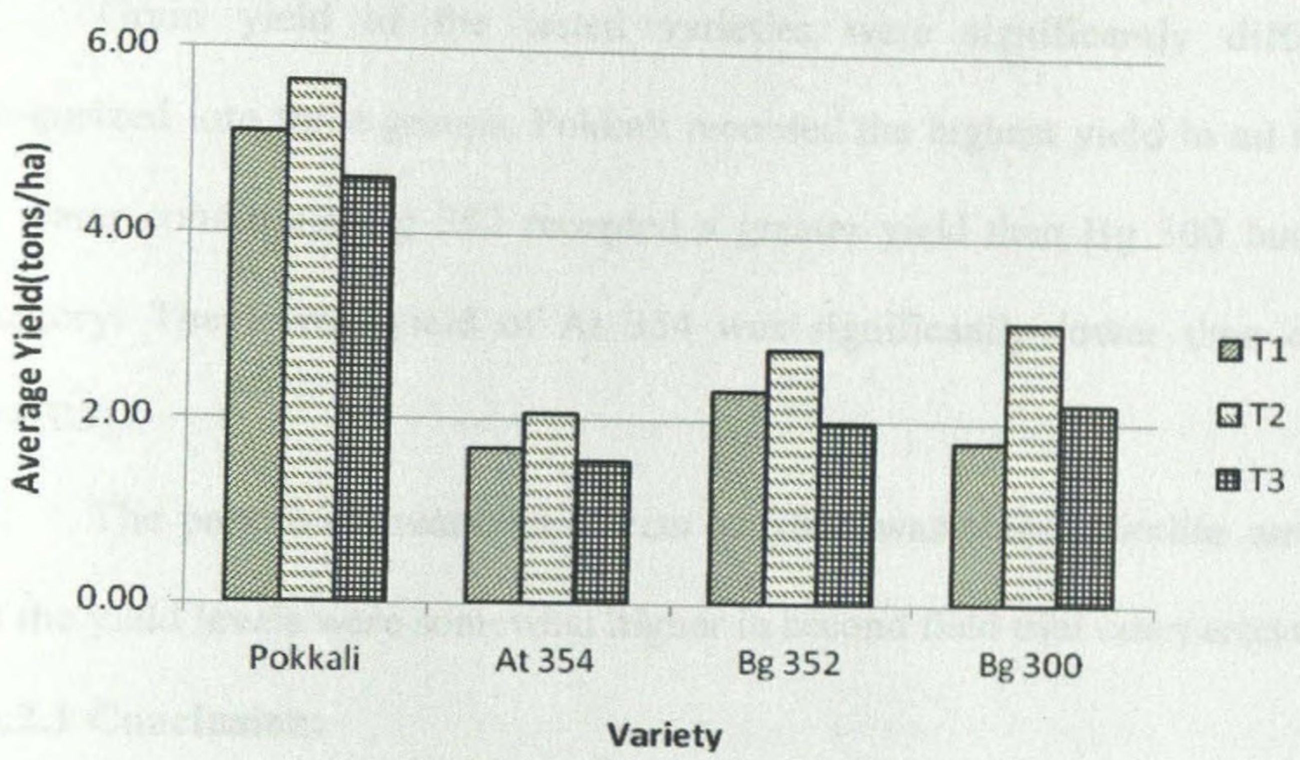


Figure 32: The yield of different varieties under different treatmenmts at alternative wetting and drying condition.

According figures 30, 31 and 32, the yield of all the varieties were reduced with reducing the soil water condition. Standing water condition has recorded a higher yield than saturated water condition in all the varieties but there was no significant difference among standing water condition and saturated water condition and these two conditions were grouped into the same yield category. Varieties recorded a lower yield ($p<0.05$) in the alternative wetting and drying treatment compared other two water treatments. Across fertilize treatments Pokkali recorded over 6 tons/ha where the yield of other three varieties were lower than 4 tons/ha under standing water condition.

Treatment two (T2) recorded the highest yield compared to treatment-1 and treatment-3 in all the varieties across all three water conditions. Three treatments were separated into three groups and yield was significantly different at 5% probability level.

Grain yield of the tested varieties were significantly different ($p<o.o5$) and categorized into three groups. Pokkali recorded the highest yield in all the treatments under all water conditions. Bg 352 recorded a greater yield than Bg 300 but grouped into same category. The lowest yield of At 354 was significantly lower than other three varieties ($p<0.05$).

The pattern of treatment effects on yield was almost similar among varieties tested but the yield levels were somewhat higher in second field trial compared to first filed trial.

4.4.2.1 Conclusion:

Yield of tested rice varieties was varied with different treatments and with different water management practices. Inland salinity is mainly occurred due to limitation of water in the dry zone and intermediate zone and this can be described by the increase of yield under saturated water condition and standing water condition. According to the results obtained from the above trial, treatment with cow dung and charcoaled paddy husk recorded higher yield over other treatments. This implies that inland salinity problem in this location could be reduced by adding organic matter and charcoaled paddy husk.

4.5 General Discussion and Conclusions

Nine days pre-soaking of rice seeds in saline water will reduce the water absorption and increase the Sodium and Chloride content in seeds. Sodium is absorbed to the seeds along with imbibitions of water. There are varietal differences in absorption of water, sodium and chloride. Reduction in rate of water uptake at high salt solutions had no effect on germination due to its ability to attain critical moisture level at 9 days of pre soaking. A cumulative effect of these three factors inside the seed might determine the germination ability of a particular rice variety under osmotic stress conditions caused by NaCl solution. Ability of the seed coat in avoiding salt entrance into the seed during imbibitions when it's soaked in saline water is correlated with husk thickness.

Seedling survival ability of rice varieties decrease with increased soil salinity level and it is negatively correlated to soil electrical conductivity. Seed germination ability of a rice variety after pre- soaking rice seeds in 45 dSm^{-1} saline solutions has positive relationship to seedling survival under soils with high salt. Thus, seed germination and early seedling survival can be used to screen rice varieties for salinity resistance.

Growth and yield of rice varieties were significantly affected by the salinity conditions which they were grown. With the increase of soil electrical conductivity, growth and yield reduction could be observed in all tested varieties. Suppression of growth and yield were varied with variety and the type of salt used to create soil salinity. When increasing the salinity level, Pokkali reduced its shoot: root ratio, implying higher root growth compared to shoot growth while Nona Bokra, At354 and Bg300 were increased their shoot growth compared to root growth. Yield of tested rice varieties also varied greatly based on the nature of salinity causing agents and how they are distributed in the soil column. Whole-soil column salinity greatly reduce growth and yield of all varieties compared to sub-soil salinity, but the reduction at both cases were lower for salinity tolerant varieties of Pokkali, Noan-bokra and At 354 compared to other varieties. Salinity developed using NaCl and Na_2SO_4 sources revealed that Cl^- ions caused more toxic effects than that of sulphate ions.

Salt tolerant variety contained more Na^+ ions in their roots(Pokkali) compared to Na^+ ions in their leaves where as salt sensitive variety(Bg300) was not recorded such higher difference. Chloride ion content in leaves of tested varieties varied significantly where saline tolerant cultivars had lower leaf Cl^- content compared to saline susceptible varieties.

Net photosynthetic rate (Pn) of rice leaves was higher in Na_2SO_4 treatments compared to NaCl treatments at same soil EC. Varieties could be categorized into two groups at NaCl treatment where At354, Pokkali and Nona Bokra recorded higher Pn than Bg300. In the Na_2SO_4 treated plants, Pn was highest in Pokkali and followed by At354, Nona Bokra and Bg300. These suggest that Chloride ions cause more damage to leaf photosynthesis than that of sodium ions. This might cause the ultimate yield reduction in rice plants under saline conditions.

Yield of tested rice varieties was varied with different soil amendment treatments and with different water management practices. Inland salinity is mainly occurred due to limitation of water in the dry zone and intermediate zone and this can be described by the increase of yield under saturated water condition and standing water condition. According to the results obtained from the above field trials, treatment with cow dung and charcoaled paddy husk recorded higher yield over other treatments. This implies that inland salinity problem could be reduced by adding organic matter and charcoaled paddy husk successfully. Charcoaled paddy husk treated plants showed no lodging of varieties, Pokkali and Nona Bokra under field conditions whether these are normally lodged under field conditions. This would be partly responsible for the higher yield in Pokkali compared to other varieties under field conditions.

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