DOCTORAL THESIS

Nutrio-physiological studies on saline and alkaline toxicities and tolerance in Foxtail millet (*Setaria italica* L.) and Proso millet (*Panicum miliaceum* L.)

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GRADUATE SCHOOL OF BIOSPHERE SCIENCE

HIROSHIMA UNIVERSITY

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HIROSHIMA UNIVERSITY

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DECLARATION

This is to certify that the thesis titled **''Nutrio-physiological studies on saline and alkaline toxicities and tolerance in Foxtail millet (*Setaria italica* L.) and Proso millet (*Panicum miliaceum* L.)**'' is the original record of the research work done by the candidate, Mr. Md. Sohidul Islam. The thesis was accepted in partial fulfillment of the requirement for Doctor of Agriculture.

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DEDICATION

.....who would be the most proud person today to know that his dearest grandson has been fulfilled his dream which he dreamt before my birth, my late grandfather-a man of inspiration, my real mentor, ***M. Abdul Jabber Pramanik,*** *a small dedication to his name.....*

ABBREVIATIONS

AS	Alkaline stress
CA	Citric acid
Chl	Chlorophyll
DMRT	Duncan multiple range test
DW	Dry weight
ELR	Electrolyte leakage rate
FW	Fresh weight
gs	Stomatal conductance
LAR	Leaf area ratio
LSD	Least significant difference
NAR	Net assimilation rate
OA	Organic acid
Pn	Photosynthetic rate
Pro	Proline
RGR	Relative growth rate
RWC	Relative water content
SE	Standard error
SS	Saline stress
TSS	Total soluble sugar
Tr	Transpiration rate
Ψ_{LW}	Leaf water potential

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Soil salinity and alkalinity

Salinity is generally defined as the presence of excessive amounts of soluble salts that inhibit or affect the normal functions needed for plant growth. Sodium chloride (NaCl), sodium sulphate (Na_2SO_4), sodium nitrate ($NaNO_3$), magnesium sulphate ($MgSO_4$), magnesium chloride ($MgCl_2$), potassium sulphate (K_2SO_4), calcium carbonate ($CaCO_3$) etc. are present in saline soils, although NaCl and Na_2SO_4 cause most of the salt problems for higher plants in nature (Kawanabe and Zhu, 1991). In addition, saline soils are those with electrical conductivity (ECe) more than 4 dS m^{-1} (equivalent to 40 mM NaCl), exchangeable sodium percentage (ESP) of less than 15% and pH below 8.5 (USDA, 1954; Szabolcs, 1994). On the other hand, alkaline (sodic) soils are those which have ECe of less than 4 dS m^{-1}, ESP greater than15% and pH greater than 8.5 (USDA, 1954; Szabolcs, 1994). The most predominant salts in alkaline soils are $NaHCO_3$ and Na_2CO_3 which induce much stronger destructive effects on plants than neutral salts (NaCl and Na_2SO_4) (Yang et al., 2008a). Alkaline soils are prone to water logging because of their low water infiltration capacity, exposure to soil erosion and the spread of alkalinity and soluble salts into adjoining areas and poor in hydraulic conductivity (Rengasamy, 2002, 2006). Na^+, K^+, Ca^{2+}, and Mg^{2+} are the main cations of dissoluble mineral salts, and Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-}, and NO_3^- are the corresponding main anions in saline and alkaline soils, which come from neutral salts or alkaline salts (Läuchli and Lüttge, 2002).

1.2 Causes of soil salinity and alkalinity

There are two major types of salinity and alkalinity based on groundwater processes found all over the world namely, primary (natural) and secondary (artificial) (Ghassemi et al., 1995). The source of primary salinization is mostly seawater, since it contains around 500 mM NaCl (Taiz and Zeiger, 2002). Salt accumulation is high when water table is less than 1.5 m below the soil surface (Rengasamy, 2006). However, this threshold depth may vary

depending on soil hydraulic properties and climatic conditions. Therefore, the area in close proximity to the sea is vulnerable to salinity especially, those with tidal water flowing over the areas. The problem becomes acute when tidal water goes away and soil becomes dry. It may also happen in areas that come in contact with seawater through rivers, canals and creeks. Moreover, cyclones, like those which occurred in Bangladesh in 1991, 2007 (*Sidr*), and 2009 (*Aila*) or exceptionally high tides, for example, the recent *Tsunami* occurred in Indonesia (2004) and in Japan (2011) pushed the saline water front further inland and into the groundwater. In secondary salinity (irrigation associated salinity), salts introduced by poor quality irrigation water are stored within the root zone due to insufficient leaching, low hydraulic conductivity of soil layers as found in heavy clay soils and sodic soils. High evaporative conditions also accelerate secondary salinization. Use of highly saline effluent water and improper drainage and soil management increases the risk of salinity in irrigated soils. Rengasamy (2006) described another type of salinity named non-groundwater-associated salinity (NAS) which occurs when salts are introduced by rain, weathering, and aeolian deposits are stored within the soil solum. In drier climatic zones, where water table is deep (solum layers) and drainage is poor, salt stores are usually found there. However, poor hydraulic properties of shallow solum layers can lead to the accumulation of salts in the topsoil and subsoil layers affecting agricultural productivity. In regions where sodic soils are predominant, this type of salinity is a common feature.

1.3 Saline and alkaline affected area

The United Nations Environment Program (UNEP) estimated that approximately 20% of the world's agricultural land and nearly 50% of all irrigated land are adversely affected by soil salinity (Flowers and Yeo, 1995). It is a worldwide problem, but most acute in North and Central Asia, Australia and South America (Pessarakli, 1999). Some of the most serious

problems occur in semi-arid regions associated with the great river systems of South-East Asia. In Bangladesh, over 30% of the net cultivable areas lie in the coastal zone of Bay of Bengal, of which approximately 53% are affected by varying degrees of salinity (Haque, 2006). The salt affected area in the coastal zone of the country was about 0.83 million ha in 1966-76, which expanded to 3.1 million ha over the last three decades (Haque, 2006). In addition, more area in that zone is expected to become saline in the future due to increase in sea water level as a consequence of the greenhouse effect. The other concern is that the area under irrigation is increasing worldwide day-by-day leaving more areas vulnerable to salinity stress. As estimated by FAO, about 20-30 million ha of irrigated lands worldwide were seriously damaged in 2002 due to the build-up of salts (Martínez-Beltrán and Manzur, 2005). Moreover, in the same investigation it was also reported that every year 0.25-0.50 million ha of irrigated lands worldwide are lost from production due to build-up of salts and alkali.

1.4 Plant categories under saline and alkaline environment

Plants are classified as glycophytes or halophytes according to their capacity to grow on high saline-alkaline medium. Halophytes are native to saline soils (around 500 mM NaCl) and able to complete their life cycle in that environment (Colmer et al., 2006). Glycophytes or non-halophytes, on the other hand, cannot survive at a high salt concentration. Most of the agricultural crops are glycophytes and cannot tolerate salt-stress, although some of them like sugar beet, barley, wheat etc. can tolerate salt to some extent. To achieve salt-tolerance, the foremost task is either to prevent or alleviate the damage, or to re-establish the homeostatic conditions in the new stressful environment.

1.5 Impacts on agricultural land and production

The salinization and alkalization of soil are widespread environmental problems. In some areas, alkalization of the soil as a result of $NaHCO_3$ and Na_2CO_3 accumulation may be

a more severe problem than soil salinization caused by neutral salts such as NaCl and Na_2SO_4. For example, in northeast China, more than 70% of the land areas are alkaline grassland (Kawanabe and Zhu, 1991), and only a few alkaline-tolerant halophytes can survive there (Zheng and Li, 1999). The distribution of saline-sodic and sodic soils on more than half a billion ha worldwide, warrants attention for their efficient, economical and environmentally acceptable management practices to be taken. Most of the salt affected lands lie in the arid and semiarid environment (Khan et al., 2010). Saline and sodic soils exist in over 100 countries, and cover about 10 % of total arable lands (Läuchli and Lüttge, 2002). The area in the close vicinity of the seashore is prone to salt stress and thus, agricultural production in those areas is reduced. Salt problem in agricultural crops, however, commonly develops in the irrigated areas when salts from the irrigation water build up in the root zone. Out of the total world's cropland, nearly 17% are under irrigation, but irrigated agriculture contributes to more than 30% of the total agricultural production (Hillel, 2000). Since the cropland under irrigation has substantially been increasing as discussed earlier, salt stress in irrigated agriculture is a major concern for world food security. The crop production through irrigated agriculture is increasingly being emphasized across the globe in response to escalating food demands in the face of the adverse consequence of global climate change. Many workers stipulate that the success or failure of any irrigated agriculture is determined by the extent to which salt and sodium problems are controlled (Muya et al., 2009).

1.6 Effects on plant growth and productivity

Soil salinity and alkalinity influence plant growth by inducing adverse effects on different physiological and metabolic processes, ultimately diminishing growth and yield (Yang et al., 2008a, 2009a). Saline and alkaline stresses induce specific changes in morphology and anatomy of the cells, tissues and organs (Li et al., 2009). The mechanisms

responsible for reduction in plant growth under salt stress are: 1) osmotic stress, 2) specific ion toxicity, and 3) nutritional imbalance.

1.6.1 Osmotic stress

Saline and alkaline stresses present in plant growth media exert high osmotic pressure and reduce soil water potential making water unavailable to plants (Munns et al., 2006). This reduces cell turgor, photosynthetic rate (Pn) and ultimately reduces activity of cell division and elongation and overall plant growth (Saqib et al., 2004).

1.6.2 Specific ion toxicity

The primary cause of growth reduction due to excessive amount of certain ions (Na^+ and Cl^-) under salt stress is termed as specific ion toxicity (Guo et al., 2009; Li et al., 2010).

1.6.3 Nutritional imbalance

Salinity and alkalinity reduced plant growth and development through nutritional imbalance (Yang et al., 2007): N accumulation is reduced due to interaction between Na^+ and NH_4^+ and / or between Cl^- and NO_3^- that ultimately reduces growth and yield of the crops (James et al., 2006); low solubility of Ca-P minerals (Qadir and Schubert, 2002); interference in the acquisition of K^+ by the roots (Suhayda et al., 1990); the low concentration of Ca^{2+} (Cakmak, 2005) and Mg^{2+} (Hu and Schmidhalter, 1997). In addition, micronutrient deficiencies are also very common under salt-alkali stress owing to high pH (Zhu et al., 2004).

1.7 Urgent need to address the saline and alkaline problems

The world population is increasing rapidly and may reach 7 to 9.3 billion by the year 2050 (http://www.unfpa.org/swp/200/), whereas the crop production is decreasing rapidly because of the negative impact of various environmental stresses; therefore, it is now very important to develop stress tolerant varieties to cope with this upcoming problem of food security. Among stresses, abiotic stress is the principal cause of decreasing average yield of

major crops by more than 50%, which causes losses worth hundreds of million dollars in each year (Mahajan and Tuteja, 2005). Soil degradation caused by salinization and sodification is of universal concern. According to the FAO Land and Nutrition Management Service, over 6% of the world's land is affected by either salinity or sodicity which accounts for more than 800 million ha of land (FAO, 2008). This problem manifests itself especially in arid and semiarid regions with poorly drained soils because of continual addition of salts with irrigation practices (Ayars and Tanji, 1999). Flowers and Yeo (1995) showed that the UNEP estimated 20 % of the agricultural land and 50% of the cropland in the world are salt-stressed. El-Kharbotly et al. (2003) mentioned that salinity imposes serious environmental problems that affect grassland cover and the availability of animal feed in arid and semi-arid regions. Therefore, the amelioration of saline sodic-soils is of great importance to restore these degraded soils and make them suitable for agriculture.

1.8 Approaches to improve stress tolerance

Plants being generally characterized by a high degree of homeostatic plasticity in response to salinity and alkalinity stresses have evolved a number of adaptive strategies to overcome such abiotic stresses (Bartels and Sunkar, 2005). The most common type of osmotic adjustment in plant cells involves accumulation of compatible solutes like proline (Vinocur and Altman, 2005) and exudation of organic acids in cytoplasm (Rhodes and Hanson, 1993). The compatible solutes and organic acids which are commonly employed as osmoprotectants, can lower the osmotic potential in cells without interfering with the metabolic processes or protein structuring and functioning, and consequently, maintain water content of cells under stresses (Yancey et al., 1982).

Proline

Proline is a well-known compatible solute that plays a pivotal role in osmotic adjustment in plants by helping maintain sufficient cell turgor for growth (Nanjo et al., 2003), and exogenous proline is known to mitigate the detrimental effects of Na and improve growth and survival under various stresses (Okuma et al., 2004; Sun and Hong, 2010a). It is synthesized from glutamate by the actions of the two enzymes, pyrroline-5-carboxylate synthetase (P5CS) and pyrroline-5-carboxylate reductase (P5CR) in higher plants (Delauney and Verma, 1993) (Fig. 1.1). It is reported that proline acts as free radical scavengers and / or enzyme protectant (Hoque et al., 2007). It is also reported that proline protects higher plants against salt/osmotic stresses by adjusting osmotic pressure (Chinnusamy et al., 2005; Vinocur and Altman, 2005). In contrast, Moftah and Michel (1987) reported that proline content could not be use as a sensitive indicator of salt stress. Similarly, a negative relationship between proline accumulation and salt tolerance was observed by Ashraf (1989) in *Vigna mungo*, and in tomato by Aziz et al. (1998). Salt resistant rice cultivars accumulated lesser amount of proline than the salt sensitive ones (Lutts, et al., 1999), while salt sensitive species of tomato accumulated more than in tolerant wild relatives (Tal et al., 1979). However, in view of these contrasting reports on the role of proline in salt tolerance, its use as selection criterion for salt tolerance has been questioned and which should be further investigated.

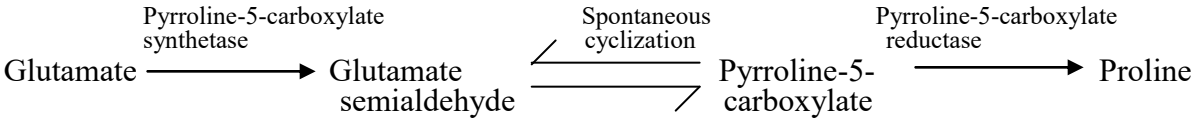

Fig.1.1 Biosynthetic pathway of proline in higher plants.

Citric acid

Citric acid (CA) is a symmetric tricarboxylic acid involved in the tricarboxylyc caid (TCA) cycle. It is the product of the citrate synthase, an enzyme of the mitochondrial oxidative TCA cycle and does not accumulate under normal growth conditions (Goldberg et

al., 2006). Early research on stress tolerance indicates that organic acid metabolism correlates closely with the mechanism of alkali tolerance (Shi and Sheng, 2005). It is reported that citric acid accumulation increased under alkali stress in *Puccinellia tenuiflora* (Guo et al., 2010), in rice (Wang et el., 2011), in sea buckthorn (Chen et al., 2009), in alfalfa roots under salt stress (Fougére et al., 1991), and in cotton plants under drought stress (Timpa et al., 1986). It has a strong relationship with stress tolerance of heavy metal (Zeng et al., 2008; Mailloux et al., 2008). It has also been reported that plants often combat or overcome aluminum toxicity by accumulating organic acids in their cells or by secreting them from their root tips (Larsen et al., 1998). The metabolism of organic acids is also reported to play a crucial part in the plant's response to iron deficiency (López-Millán et al., 2000) and phosphorus deficiency (Watt and Evans, 1999), as well as to promote uranium uptake (Ebbs et al., 1998). However, no study has yet examined the relationship of citric acid and stress tolerance of Foxtail millet and Proso millet under saline and alkaline conditions.

1.8.1 Exogenous proline to alleviate saline and alkaline stresses

Exogenous application of proline can play an important role in enhancing plant stress tolerance. This role can be in the form of either osmoprotection (Handa et al., 1986) or cryoprotection (Santarius, 1992). For example, in various plant species growing under saline conditions, exogenously-supplied proline facilitated osmoprotection and growth (Yancey, 1994), protected cell membranes from salt-induced oxidative stress by enhancing activities of various antioxidants in soybean (Yan et al., 2000), acted as a protectant of enzymes and membranes in tobacco (Okuma et al., 2000), increased activities of superoxide dismutase and peroxidase, which contributed to increase its salt tolerance (Hua and Guo, 2002), decreased Na^+ and Cl^- accumulations and an increase in growth in barley (Lone et al., 1987), promoted Ca uptake in *Phaseolus* seedlings (Rana and Rai, 1996), relieved salt toxicity in barley

plantlets by changing salt transport from root to shoot (Lone et al., 1987), increased K content and alleviated salt stress effects in *Vigan radiata* (Kumar et al., 1990) and (Tipirdamaz and Karakullucku, 1993), increased K uptake in *Raphanus* seedlings by 15% (Khanna,1998), and stabilized the plasma membrane (Mansour, 1998). In general, accumulation of proline in the cytoplasm is associated with a reduction in the concentration of toxic ions and an increase in the cytosolic water volume (Cayley et al., 1992). In contrast to the above findings on beneficial effects of exogenous application of proline, there are a few reports cautioning its use. For example, exogenous application of proline did not influence Na^+ and Cl^- accumulation in rice leaves (Lutts et al., 1996) and in wheat (Colmer et al., 1995). It caused damages to ultra-structures of chloroplast and mitochondria in *Arabidopsis* plants (Hare et al., 2002) and exacerbated the deleterious effects of salt on rice (Garcia et al., 1997). The role of proline in salt tolerance needs to be further elucidated before considering it as a salt tolerance indicator. However, in spite of its positive and negative roles on salt tolerance and crop production, very little attention has been paid to the responses of Foxtail millet and Proso millet under exogenous application of proline.

1.8.2 Exogenous citric acid to alleviate saline and alkaline stress

It has been reported that organic acids (OA) like citric acid has a potential role as metabolically-active solutes in osmotic adjustment, balance of cation exchange, and pH homeostasis under saline and alkaline conditions (Guo et al., 2010; Wang et el., 2011). In recent years, reports have shown that some alkali-tolerant halophytes accumulate high concentrations of OA under alkali stress (Yang et al., 2008b), but not in alkali sensitive maize (Qu and Zhao, 2004), or the alkali tolerant halophyte *Suaeda salsa* (Qu and Zhao, 2003). However, no evidence exists regarding the effects of exogenous application of citric acid to stress tolerance of glycophytes under SS and AS conditions except the report of Sun

and Hong (2010a) in halophytes (*Leymus chinensis* Trin.) who reported that exogenous citric acid can mitigate the saline and alkaline stress as like proline. Although there is evidence that exogenous application of citric acid to the hydroponic solution alleviated the inhibitory effect of toxic Al on root extension in cotton (Hue et al., 1986) and shoot growth in corn (Bartlett and Riego, 1972). These reports demonstrated that exogenous application of citric acid might have a positive role on stress responses of crop plants.

1.9 Aim of the study

The aim of the present study was to investigate the mechanisms of salt tolerance of Foxtail millet (*Setaria italica* L.) and Proso millet (*Panicum miliaceum* L.) which are particularly important food grains and fodder crops grown in arid and semi-arid regions. The water requirements of these millets are very low as compared to the other major cereals which allowed them to grow successfully in the drought prone areas, such as the northern parts of Bangladesh. Considering their significant roles of the food security and the expanding salt problem in the vast areas of the country, the Bangladesh Government recently approved research approaches towards developing high-yielding crops that can be grown in the salt affected areas. To enable for growing such crops, it is necessary to know how tolerant plants are able to adapt in saline and alkaline conditions. During recent decades, research on the stress responses of halophytic plants has aided our understanding of the mechanisms of stress adaption and stress tolerance in plants. But very little attention has been paid to the responses to saline stress and alkaline stress in the glycophytes. In spite of their versatile uses and adaptation in drought prone areas, these crops have not yet been properly addressed or studied under saline and alkaline conditions as like other halophytes and glycophytes. Based on the results of Sun and Hong (2010a), it can be hypothesized that citric acid is a component of the stress response and that exogenous citric acid can improve salt tolerance by stimulating

plant growth and metabolic activities. The present study was, therefore, conducted to identify the saline and alkaline tolerance of Foxtail millet and Proso millet by comparing to their growth and metabolic responses under saline and alkaline conditions and to explore the potentiality of the sensitive one cultivated with or without the application of exogenous citric acid and proline and to compare the effects of citric acids and proline by judging the growth and metabolic responses. In order to achieve these aims, the objectives of this work were as follows:

1) To investigate the nature of tolerance of Foxtail millet and Proso millet under saline and alkaline environments,

2) To assess whether exogenous application of citric acids and proline could alleviate the adverse effects of saline stress (SS) and alkaline stress (AS), and

3) To find out the strategies how citric acid and proline ameliorate saline and alkaline stresses.

CHAPTER 2

COMPARATIVE STUDIES ON GROWTH AND PHYSIOLOGICAL RESPONSES TO SALINE AND ALKALINE STRESSES OF FOXTAIL MILLET (*Setaria italica* L.) AND PROSO MILLET (*Panicum miliaceum* L.)

2.1 INTRODUCTION

Environmental stresses adversely affect growth and productivity of plants, particularly those which are sensitive to salinity and alkalinity. These stresses cause severe changes in growth, physiology and metabolism of plants, thus threatening the cultivation of plants around the globe (Lunde et al., 2007). According to an estimate, the world's land surface occupies about $13.2x10^9$ ha, no more than $7.0x10^9$ ha are potentially arable, and only $1.5x10^9$ ha are currently cultivated. Of the cultivated area, about $0.34x10^9$ ha (23%) are saline and another $0.56x10^9$ ha (37%) are sodic (Tanji, 1990). The loss of potentially cultivable land is likely to increase over the next 20 years and threats the world food supply. For example, in the northeast of China, area of alkalinized grassland has reached more than 70% (Kawanabe and Zhu, 1991); because soil salinization and alkalinization frequently co-occur, the conditions in naturally salinized and alkalinized soils are very complex; the total salt contents, their composition and the proportion of neutral to alkaline salts may vary in different soils. Grain productivity through green revolution has reached a ceiling, whereas the world population continues to grow (Akhtar and Saqib, 2008). Therefore, improving crop yields in normal and less productive soils, including saline and alkaline soils by combating those stresses is highly desirable to feed the ever-increasing population.

Plants under saline conditions encounter three inevitable factors (Islam, 2001). First, salt decreases osmotic potential of soil solution effectively generating water stress for plants. It can result in specific ion toxicity due to excess accumulation of Na^+ or Cl^- in plant cells, which is the second effect on plants. Lastly, the interaction of salts with mineral nutrients may result in nutrient imbalances and deficiencies (Munns and Tester, 2008). Halophytes cope with this situation by actively taking up Na^+, and compartmentalizing Na^+ into vacuoles, which acts as an osmoticum to maintain the water potential gradients necessary for

continuous water uptake (Ehret and Plant, 1999). These plants also generate a higher level of osmotically active compounds (proline, glycine betaine, etc.) in the cells in order to sustain adequate osmotic gradients for water uptake (Hasegawa et al., 2000). To induce tolerance against toxic Na^+ sensed by plants, the regulation of K^+ uptake and / or prevention of Na^+ entry, efflux of Na^+ from the cell, and utilization of Na^+ for osmotic adjustment are the strategies commonly used by plants to maintain desirable Na^+/ K^+ ratios in the cytosol (Glenn and Brown, 1999). The Na^+, K^+, Ca^{2+}, and Mg^{2+} are the main cations of dissoluble mineral salts, and Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-}, and NO_3^- are the corresponding main anions in saline and alkaline soils, which come from neutral salts or alkaline salts (Läuchli and Lüttge, 2002). Alkaline salts ($NaHCO_3$ and Na_2CO_3) induce much stronger destructive effects on plants than neutral salts (NaCl and Na_2SO_4) (Shi and Yin, 1993). When salinized soil contains HCO_3^- and / or CO_3^{2-}, which raise the soil pH, plants suffer damaging effects of both saline and alkaline stresses (Yang et al., 2008a).

The contributory role of proline to osmotic adjustment has been reported by many researchers (Ashraf and Foolad, 2007). Proline has also been considered as a carbon and nitrogen source for growth, a stabilizer for the membrane and some macromolecules and also a free radical scavenger under stress conditions (Okuma et al., 2000). However, to date, researches into salt stress have emphasized NaCl as the main contributing factor to proline accumulation, but there is very little published information available regarding this issue under alkaline stress condition.

Proso millet (*Panicum miliaceum* L.) is an important forage species of the largest genus *Panicum*, which includes more than 400 species (Roshevits, 1980). This plant naturally grows in hot and dry areas where a high salt content is the characteristic of most soils and it has been cultivated for both its high food and feed value. Foxtail millet (*Setaria italica* L.) is

also widely cultivated in arid and semi-arid regions as a food and fodder crop. The morpho-physiological, cellular and molecular responses of many crop species to salinity/alkalinity stresses have been extensively investigated but, unfortunately, millets like Foxtail and Proso millets have not been explored in this way to date. Therefore, the present study was aimed to assess inter-species variation in saline and alkaline tolerance of Foxtail millet and Proso millet in their vegetative stage.

2.2 MATERIALS AND METHODS

2.2.1 Plant material and culture conditions

Seeds of Foxtail millet (*Setaria italica* L. cv: *BARI kaun-3*) and Proso millet (*Panicum miliaceum* L., cv: *BARI china-1*) were collected from Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh. Seeds of both species were surface-sterilized with 5% thiophanate-methyl for 5 min and air-dried. Seeds were sown into 5 L plastic pots containing a soil mixture of granite regosol soil and perlite (2:1 v/v). After germination, 20 uniform seedlings were kept at an identical distance in each pot. Pots were maintained under greenhouse conditions. Plants were irrigated with nutrient solution at each watering using an irrigation system. The basal nutrient solution contained 8.3 mM NO_3-N, 0.8 mM NH_4-N, 0.5 mM P_2O_5, 2.2 mM K_2O, 0.7 mM MgO, 2.1 mM CaO, 11 μM MnO, 5 μM B_2O_3 and 13 μM Fe. To simulate saline stress (SS) and alkaline stress (AS) conditions in nature (Chen et al., 2009; Liu et al., 2010), two stress treatments were applied: neutral salts of NaCl and Na_2SO_4 (9:1 molar ratio) and alkaline salts of $NaHCO_3$ and Na_2CO_3 (9:1 molar ratio). At four weeks after sowing, plants were subjected to stress treatments every day until water was drained-out from the bottom of the pot. Before applying 100 mM SS and AS treatments for 7 days, plants were subjected to SS and AS of 25, 50 and 75 mM concentrations every 3 days alternatively for the hardening of plants. The pH/EC (S m^{-1}) of

saline and alkaline solutions was 6.9/1.217 and 9.2/0.930, respectively. Each treatment was applied to three replicates located randomly in the greenhouse in order to avoid positional effects.

2.2.2 Plant sampling and measurements

Plants in each pot were sampled and separated into the leaves, stems (culms) and roots before the application of treatments and at 16 d after treatment initiation. The separated segments were wiped with tissue towel paper to remove moisture and their fresh weights were measured. The fresh samples were kept frozen in liquid nitrogen, then freeze-dried and we measured the dry weight. Dry samples were ground into fine powder using a vibrating sample mill (Model TI-100, Heiko Seisakusho Ltd., Tokyo, Japan) for chemical analysis. Leaf samples were taken in triplicate from a composite pool of physiologically mature leaves of each genotype. The leaf area was measured using a leaf area meter (AMM-5 type leaf area meter, Hayashi-Denko, Tokyo, Japan) and the leaves were oven-dried at 80°C for 72 h and the dry weight was determined. The leaf area ratio was calculated as the total leaf area per unit leaf dry mass. The RGR was calculated using the method of Kingsbury et al. (1984). The RWC of the leaf was estimated according to the method of Saneoka et al. (1995). The Na and K concentrations were determined after digestion by nitric acid–hydrogen peroxide, using a flame photometer (ANA 135, Eiko Instruments Inc., Tokyo, Japan). The Ca and Mg concentrations were determined using an atomic absorption spectrophotometer (U-3310 Hitachi Co. Ltd., Tokyo, Japan). Proline was determined spectrophotometrically following the ninhydrin method described by Bates et al. (1973), using L-proline as a standard. The total N content was determined using a Kjeldahl nitrogen digester and distillator (Kjeldatherm Type TT100 & Vapodset Type 20, Gerhardt, Germany).

2.2.3 Statistical analysis.

Data were examined using one-way ANOVA and presented as the mean ± S.E. for each treatment and species (n=3). Multiple comparisons of means of data among the treatments within the plants were performed using Duncan's test at the 0.05 significance level (all tests were performed with SPSS Version 16.0 for Windows).

2.3 RESULTS

2.3.1 Plant growth

The plant dry matter yield of both Foxtail millet and Proso millet declined with SS and AS and the decline was mostly caused by a reduction in leaf and stem biomass. However, Proso millet produced a significantly greater amount of dry matter than Foxtail millet (Fig. 2.1). A marked relative reduction (37 and 62% under SS and AS, respectively) in the shoot dry mass was observed in Foxtail millet, as compared to Proso millet (22 and 45%, respectively). Moreover, decreases of 40 and 17% more than the control were also recorded for root dry mass under the AS condition in Foxtail millet and Proso millet, respectively. The values for the root/shoot ratio increased with the stress treatments and reached a maximum in Foxtail millet under the AS condition (data not shown). The RGR and NAR of both species decreased significantly under AS condition. The reduction percentages of RGR and NAR of alkaline treated Foxtail millet were 44 and 33%, whereby 31 and 27% in the case of Proso millet, respectively (Table 2.1). It is noteworthy that a noticeable reduction of the LAR was observed only in Foxtail millet under AS but no statistical differences were observed among the treatments in Proso millet.

2.3.2 Relative water content

Stress treatments caused a significant decrease in the RWC and rate of reduction was greater in AS than in SS in both tested species (Fig. 2.2). The relative reduction was more

marked in Foxtail millet than in Proso millet. The RWC was almost the same between the two species under control treatment (87 and 88% in Foxtail millet and Proso millet, respectively), however, under stress conditions, it tended to be lower in Foxtail millet (70 and 61% under SS and AS, respectively) than in Proso millet (74 and 68% under SS and AS, respectively).

2.3.3 Ionic status

Sodium

The Na concentrations in leaves, stems and roots increased under both stresses, and the increases under the AS condition were significantly greater than those under SS in all of the plant parts of both species with the exception of the roots of Foxtail millet, which accumulated a significantly higher concentration of Na under the SS condition (Table 2.2). Compared to Proso millet, the leaves of Foxtail millet accumulated 1.55 times more Na under the SS condition and 1.61 times more Na under the AS condition (Table 2.3). Interestingly, the roots of Proso millet accumulated a higher amount (40%) of Na (2.67 times higher) than the roots of Foxtail millet (15%) under AS (Table 2.3).

Potassium

The AS caused a significant decrease in the K concentration of the studied plant segments in both species except for the leaves of Proso millet. The leaves of both species achieved the highest concentration of K under the SS condition compared to the other treatments (Table 2.4). Significantly lower concentrations of K were observed in all of the plant parts in Foxtail millet under AS compared to under SS; however, this tendency was found only in the roots but not in the leaves and stems of Proso millet.

Na / K ratio

The ratio of Na / K increased under both stresses and it was higher under AS compared to SS in all of the plant parts (Table 2.5).

Calcium

The calcium concentration was noticeably reduced by SS and AS in the leaves and by AS in the stems of Foxtail millet. The leaves accumulated a higher concentration than the stems and roots of both species (Table 2.6). Saline stress caused a significant decrease in root Ca concentration of both species, whereas AS increased the roots Ca concentration more markedly in Foxtail millet. The relative reduction due to stresses was greater in Foxtail millet than in Proso millet.

Magnesium

The Mg concentration was decreased significantly by the stresses in the leaves and roots of both species (Table 2.7). The relative inhibition was greater (39 and 52% under SS and AS) in the roots of Foxtail millet than Proso millet (23 and 40% under SS and AS, respectively) but the rates of reduction were more in the leaves and stems of Proso millet than Foxtail millet. The significant inhibition was mainly observed in the stems of AS treated plants of both species.

2.3.4 Nitrogen and proline

The total N content decreased in all plant parts under both stresses and the reductions were more severe in AS than in SS (Fig. 2.3). Significant reductions were observed in Foxtail millet under both SS and AS, showing values (relative reduction plant^{-1}) of 25 and 63%, respectively. However, a significant reduction (54%) was observed only under AS but not under SS (14%) in Proso millet. The proline concentration increased under SS and AS conditions and the increase was greater under SS than under AS for both species (Fig. 2.4). Furthermore, these results demonstrated that Foxtail millet produced 14.7 and 12.6 times more than the control under SS and AS conditions, respectively; while those values in Proso millet were only 5.2 and 2.3.

2.4 DISCUSSION

2.4.1 Plant growth

The decreased biomass weights of plants under saline and alkaline conditions are correlated with the reduced leaf area, which results in decreases of photosynthetic area and Pn (Yang et al., 2008a). It is thought that a decreased Pn under stress could have reduced the shoot growth and development, thus finally leading to lower biomass production compared to the control (Campbell and Nishio, 2000). In the present study, the lower stress-induced reduction of growth in Proso millet compared with Foxtail millet (Fig. 2.1) might be attributed to the lower reduction of the RGR (SS:21/13% and AS:44/31% for Foxtail millet/Proso millet, respectively) and also NAR in the salt-stressed plants (Table 2.1). These results indicate that Proso millet is a comparatively saline and alkaline tolerant species with the inhibitory effect of alkalinity being stronger than that of salinity. It is suppose that a high pH appearing in the rhizosphere might be a primary factor for a more pronounced inhibition of plant growth by disturbing some mineral nutrition and other physiological functions. This finding is also in agreement with the previous studies (Sharma et al., 2001; Nuttall et al., 2003). The reduction of plant growth at a higher saline concentration was mainly due to the reduction of the photosynthetic area as reported by Marcelis and Van-Hooijdonk (1999) and James et al. (2002). The other factors mainly depend on the cumulative effects of leaf water and osmotic potential, biochemical constituents (Dixit and Chen, 2010), contents of photosynthetic pigments (Koyro, 2006) and ion toxicities in the cytosol (James et al., 2006). The RGR value reflects the life-sustaining activities of plants, and is considered an optimum index for degrees of stress and plant responses to stresses. Severe salt stress generally leads to growth arrest and even to death of plants (Parida and Das, 2005). In the present study, the decreases of RGR under AS (44 and 31% in Foxtail millet and Proso millet, respectively) were greater than that under SS (21 and 13% in Foxtail millet and Proso millet, respectively) (Table 2.1).

This more injurious effect by AS compared with SS is consistent with the previous study reported by Yang et al. (2007). The RGR is the product of NAR and LAR, where NAR is largely the net result of carbon gain (Pn) and carbon losses (respiration) expressed per unit leaf area. The alkaline stress exerts the same stress factors as SS but under AS plants have to deal with the stress of an elevated pH. The AS induced severe reductions in water content in plants (Fig. 2.2). These results indicate that high pH due to AS in the soil surrounding the roots might cause damage to root structures and functions such as reduced water uptake (Fig. 2.2), and inability to prevent accumulation of Na (Table 2.3) and to uptake the essential elements like K, Ca, Mg showing reduced concentrations (Tables 2.4, 2.6 and 2.7) following reduced LAR and NAR (Table 2.1). These may be the main reasons explaining the lower RGR value under AS than under SS of Foxtail millet and Proso millet. The injurious effects of salinity are commonly thought to be a result of low water potentials and ion toxicities (Munns, 2002).

2.4.2 Relative water content

Under saline conditions, plants suffer from osmotic shock due to lower osmotic potential and synthesize different metabolites to maintain turgor (Orcutt and Nilsen, 2000). However, in this study, the RWC decreased under SS and AS, and a more marked reduction was also observed under AS in Foxtail millet compared to Proso millet (Fig. 2.2), which may represent the cumulative effects of a greater reduction in the leaf area and LAR, as well as severe damage to root structures by a higher concentration of Na. Nonetheless, Foxtail millet plants have to face a more pronounced water deficit under AS, imposed by a low external water potential due to a higher concentration of Na accumulation in extracellular regions reaching a toxic threshold, causing severe damage to plant tissues. Our results suggest that

the better water relation in plant under stress conditions obviously contributed to the maintaining of higher plant growth in Proso millet than in Foxtail millet.

2.4.3 Ionic status

Under saline conditions, halophytes usually accumulate inorganic ions in vacuoles to decrease the cell water potential because energy consumption to absorb inorganic ions is far less than that needed to synthesize organic compounds (Moghaieb et al., 2004; Shi and Sheng, 2005), and they generally compartmentalize Na^+ in vacuoles to avoid Na^+ toxicity in the cytosol (Serrano and Rodriguez-Navarro, 2001; Zhu, 2003). Additionally, halophytes usually absorb Na^+ and inhibit K^+ uptake under saline and alkaline stresses (Tammam et al., 2008). In this study, Na concentration was induced under both stresses in all the plant segments (Table 2.2) and K concentration was reduced in the stems and roots of both species (Table 2.4), indicating that there is a competitive inhibition between the absorption of Na and K. However, in leaves, the concentration of Na and K increased under SS, which implies that there was no competitive inhibition for absorption Na and K in leaves. No competitive inhibition between Na^+ and K^+ uptake was observed by Saneoka et al. (1995, 1999) in maize and wheat. The acquisition of K was inhibited more by AS than by SS of both species, possibly due to the high pH under AS which increased the interference with the selective absorption of K to Na in roots and elevated intracellular Na concentration to a toxic level. A more markedly decreased acquisition of K in *Chloris virgata* under AS than under SS was noticed by Yang et al. (2008a). Recently some investigations also reported that both Na and K concentrations increased with elevating salinity in the shoots of *Suaeda glauca* and *Kochia sieversiana* (Yang et al., 2007, 2008c), in the leaf blade of bread wheat (Hidhab) (Benderradji et al., 2011). Thus, the pattern of Na and K accumulation to SS and AS in halophytes may be varied by their genotypic nature. Those antagonistic-synergistic effects

for uptaking Na and K may need to investigate further. The Na / K ratios have been shown to increase with rising salinity in many halophytes (Yang et al., 2007; 2008b) and a high Na / K ratio implies metabolic disorders (Brady et al., 1984). In the present study, AS sharply increased the Na / K ratio and Foxtail millet showed higher ratios than Proso millet (Table 2.5). It is thought that the severe depressive effect of alkalinity over salinity on plant growth could be related to a greater increase of Na and decline of K concentration in aerial plant parts. Proso millet restricted the transportation of Na from roots to shoots resulted in a higher ratio of Na / K in Proso millet roots. Yang et al. (2008a) reported the similar results whereby a high pH caused by alkaline stress may enhance interference with the selective absorption of Na / K in roots and may increase intracellular Na to a toxic level. The Ca^{2+} and Mg^{2+} accumulation is inhibited by salt stress in many plants (Yousif et al., 2010). In this observation, the Ca concetration was inhibited significantly in the Foxtail millet leaves under SS and AS, and stems under AS. In case of Proso millet, the inhibition was insignificant in the leaves under SS and stems under both stresses (Table 2.6), indicating that Proso millet is more tolerant than Foxtail millet. The Mg concentration also decreased in the leaves and roots of both species under SS and AS and the extent of decreases under AS were higher than under SS (Table 2.7). It may be due to the high pH under AS reducing the availability of Ca and Mg in the root zones by precipitating them into $CaCO_3$ and $MgCO_3$.

2.4.3 Nitrogen and proline

Decreased nitrogen uptake under SS and AS conditions may be due to the interaction between Na^+ and NH_4^+ and / or between Cl^- and NO_3^- that ultimately reduces the growth of crops. Moreover, the lower accumulation of Na^+ in Proso millet as compared to Foxtail millet is thought to be the result of a higher N uptake due to the reduced antagonistic effects of Na^+-NH_4^+ in roots and the lower influence of Na^+ on NH_4^+ loading into the xylem. Na^+-NH_4^+ / Cl^-

-NO_3^- interactions under stresses from a biochemical perspective indicate a decreased N accumulation that ultimately reduces growth and yield of crops as described by Bar et al. (1997). N deprivation adversely affects plant growth and development by reducing the photosynthetic area (James et al., 2002), having cumulative effects on the leaf water and osmotic potential (Munnns, 2002), and increasing ion toxicities in the cytosol (James et al., 2006). In this case, It is to predict that a more markedly decreased leaf area, RWC and increased Na^+ accumulation under AS in Foxtail millet induced higher-level inhibition of the NAR, ultimately mediated by a reduced nitrogen content (Fig. 2.3). The roles of proline have been widely reported as cell osmotic adjustment, membrane stabilization and the detoxification of injurious ions and correlation with stress tolerance in plants exposed to salt stress (Ashraf and Foolad, 2007; Tammam et al., 2008). It is evident from our study that the proline concentration of both species increased under SS and AS (Fig. 2.4). These results suggest that the induction of proline is related to the changes in not only salinity, but also alkalinity. It is common for proline to be correlated with stress tolerance (Kavi Kishor et al., 2005; Younis et al., 2009) but the significance of proline accumulation in osmotic adjustment is still being debated and varies according to the species (Rodriguez et al., 1997). These results indicate that the increment of proline concentration is not only being osmolyte and protectant, but it may also have other roles related to alkaline stress, which should be further investigated.

Proso millet showed a more favorable leaf area, LAR, NAR, RGR and Na-K levels under saline and alkaline conditions by reducing stress-induced changes in all physiological and biochemical functions. Meanwhile, the deleterious effects of alkaline stress on all plant traits were always higher than that of saline stress, and thus Proso millet may have evolved specific mechanisms to tolerate saline and alkaline stresses and these should be investigated further.

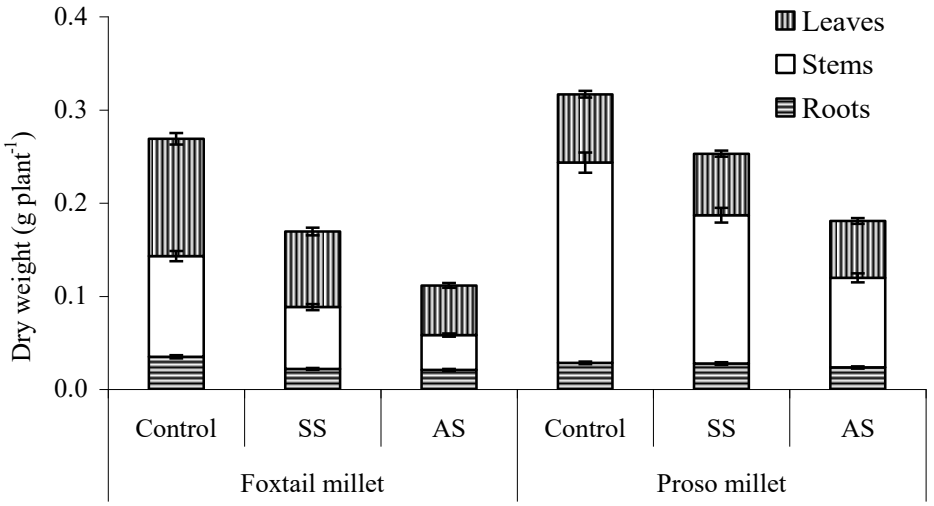

Fig 2.1 Effects of SS and AS on the dry weight of leaves, stems and roots of Foxtail millet and Proso millet. The values are the means (± S.E) of three replicates.

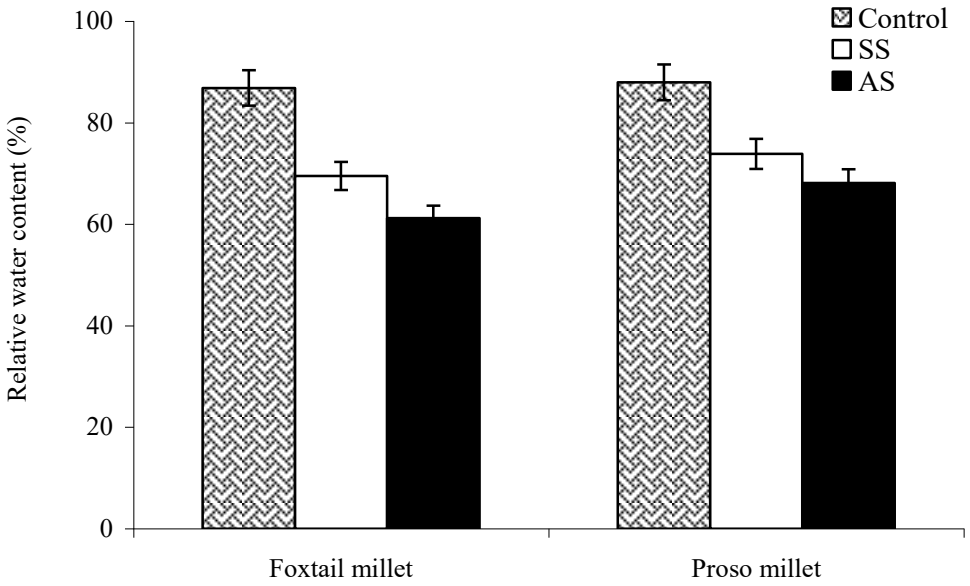

Fig. 2.2 Effects of SS and AS on the RWC in the leaves of Foxtail millet and Proso millet. The values are the means (± S.E) of three replicates.

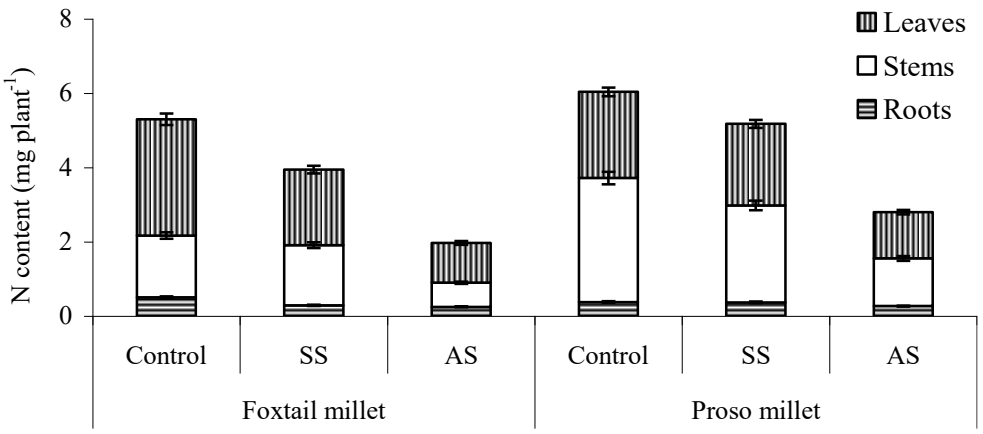

Fig. 2.3 Effects of SS and AS on total N content in the leaves, stems and roots of Foxtail millet and Proso millet. The values are the means (± S.E) of three replicates.

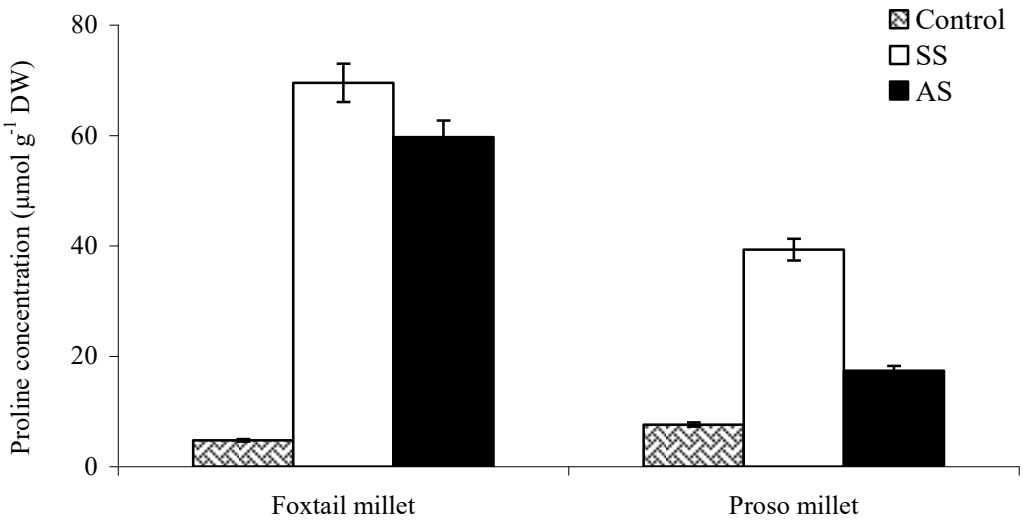

Fig. 2.4 Effects of SS and AS on the proline concentration in the leaves of Foxtail millet and Proso millet. The values are the means (± S.E) of three replicates.

Table 2.1 Effects of SS and AS on the RGR, NAR and LAR of Foxtail millet and Proso millet. The values are the means (± S.E) of three replicates.

Growth parameters	Foxtail millet			Proso millet		
	Control	SS	AS	Control	SS	AS
RGR (mg g^{-1} day^{-1})	59.3±4.04^{a}	46.7±5.67^{ab}	33.2±4.58^{b}	54.2±0.58^{a}	46.9±1.53^{ab}	37.5±4.63^{b}
NAR (mg cm^{-2} day^{-1})	0.110±0.01^{a}	0.091±0.01^{ab}	0.074±0.00^{b}	0.140±0.01^{a}	0.122±0.00^{ab}	0.102±0.02^{b}
LAR (cm^{2} g^{-1})	537.6±11.5^{a}	513.3±6.57^{ab}	448.2±12.72^{b}	389.4±12.46^{a}	385.4±7.60^{a}	367.9±6.94^{a}

Table 2.2 Effects of SS and AS on Na concentration (mg g^{-1} DW) in the leaves, stems and roots of Foxtail millet and Proso millet. The values are the means (± S.E) of three replicates.

Genotypes	Treatments	Na		
		Leaves	Stems	Roots
Foxtail millet	Control	0.86±0.04^{c}	1.72±0.09^{c}	1.85±0.29^{c}
	SS	26.30±1.81^{b}	28.60±1.53^{b}	20.44±1.79^{a}
	AS	41.02±3.93^{a}	37.67±4.15^{a}	13.60±0.93^{b}
Proso millet	Control	0.84±0.02^{c}	1.56±0.05c	2.45±0.11^{c}
	SS	8.53±0.38^{b}	10.92±0.79^{b}	21.38±1.01^{b}
	AS	19.28±1.76^{a}	22.14±1.00^{a}	28.73±2.43^{a}

Table 2.3 Effects of SS and AS on Na accumulation (mg plant^{-1}) in the leaves, stems and roots of Foxtail millet and Proso millet. The values are the means of three replicates.

Genotypes	Treatments	Na			
		Leaves	stems	Roots	Total
Foxtail millet	Control	0.11	0.22	0.23	0.56
		(19)	(39)	(42)	(100)
	SS	2.11	2.36	1.70	6.16
		(34)	(38)	(28)	(100)
	AS	2.17	1.99	0.71	4.88
		(45)	(40)	(15)	(100)
Proso millet	Control	0.06	0.11	0.18	0.36
		(17)	(32)	(51)	(100)
	SS	0.55	0.70	1.29	2.54
		(22)	(27)	(51)	(100)
	AS	1.17	1.35	1.70	4.22
		(28)	(32)	(40)	(100)

( ): Na partitioning as percentage in the leaves, stems and roots

Table 2.4 Effects of SS and AS on K concentration (mg g^{-1} DW) in the leaves, stems and roots of Foxtail millet and Proso millet. The values are the means (± S.E) of three replicates.

Genotypes	Treatments	K		
		Leaves	Stems	Roots
Foxtail millet	Control	38.84±1.55^{a}	42.88±1.80^{a}	3.31±0.41^{a}
	SS	41.15±2.01^{a}	36.46±2.82^{a}	2.84±0.427^{a}
	AS	32.95±0.34^{b}	23.50±2.48^{b}	0.88±0.07^{b}
Proso millet	Control	16.27±0.62^{b}	27.61±0.65^{a}	5.89±0.09^{a}
	SS	20.20±0.87^{a}	20.10±1.11^{b}	3.50±0.31^{b}
	AS	18.19±0.37^{ab}	19.67±0.28^{b}	1.87±0.12^{c}

Table 2.5 Effects of SS and AS on Na / K ratio in the leaves, stems and roots of Foxtail millet and Proso millet. The values are the means of three replicates.

Genotypes	Treatments	N / K		
		Leaves	Stems	Roots
Foxtail millet	Control	0.03	0.04	0.56
	SS	0.76	0.79	7.20
	AS	1.49	1.60	15.45
Proso millet	Control	0.02	0.06	0.42
	SS	0.72	0.54	6.04
	AS	1.15	1.13	15.36

Table 2.6 Effects of SS and AS on Ca concentration (mg g^{-1} DW) in the leaves, stems and roots of Foxtail millet and Proso millet. The values are the means (± S.E) of three replicates.

Genotypes	Treatments	Ca		
		Leaves	Stems	Roots
Foxtail millet	Control	2.84 ± 0.00^{a}	1.94 ± 0.06^{a}	0.84 ± 0.08^{b}
	SS	2.44 ± 0.09^{b}	1.64 ± 0.13^{ab}	0.59 ± 0.01^{c}
	AS	2.12 ± 0.07^{c}	1.48 ± 0.10^{b}	1.12 ± 0.05^{a}
Proso millet	Control	2.19 ± 0.02^{a}	1.47 ± 0.15^{a}	1.12 ± 0.03^{a}
	SS	2.16 ± 0.04^{ab}	1.35 ± 0.10^{a}	0.86 ± 0.03^{b}
	AS	2.07 ± 0.03^{b}	1.18 ± 0.04^{a}	1.17 ± 0.09^{a}

Table 2.7 Effects of SS and AS on Mg concentration (mg g^{-1} DW) in the leaves, stems and roots of Foxtail millet and Proso millet. The values are the means (± S.E) of three replicates.

Genotypes	Treatments	Mg		
		Leaves	Stems	Roots
Foxtail millet	Control	1.92±0.10[a]	1.50±0.09[a]	0.33±0.02[a]
	SS	1.33±0.16[b]	1.12±0.09[b]	0.20±0.02[b]
	AS	1.26±0.05[b]	0.71±0.09[c]	0.16±0.01[b]
Proso millet	Control	2.82±0.14[a]	0.99±0.03[a]	0.80±0.03[a]
	SS	1.79±0.17[b]	0.91±0.14[a]	0.62±0.01[b]
	AS	1.34±0.07[b]	0.37±0.03[b]	0.48±0.01[c]

CHAPTER 3

GROWTH AND METABOLIC RESPONSES OF FOXTAIL MILLET (*Setaria italica* L.) AND PROSO MILLET (*Panicum miliaceum* L.) TO SALINE AND ALKALINE STRESSES

3.1 INTRODUCTION

Soil salinity and alkalinity seriously affect about 932 million ha of land globally, reducing productivity in about 100 million ha in Asia alone (Rao et al., 2008). In many agricultural areas of Asia, alkalinity (high pH) is an important factor limiting crop productivity (Wang et al., 2011). More than 70% of the land area in northeast China is alkaline grassland, where the soil becomes alkaline as a result of hydrolysis of two carbonates ($NaHCO_3$ and Na_2CO_3) (Yang et al., 2007). Salt stress in soil generally involves osmotic stress and ion-induced injury (Munns, 2002), and there is an additional high pH effect with alkali stress. A high-pH environment surrounding the roots can cause metal ions and phosphorus to be precipitated, with loss of the normal absorptive functions of the roots and the destruction of the root cell structure (Li et al., 2009). Alkali stress can inhibit the absorption of inorganic anions such as Cl^-, NO_3^- and $H_2PO_4^-$, greatly affects the selective absorption of K^+-Na^+, and disrupts the ionic balance (Yang et al., 2007, 2008b, 2009a). Thus, plants in alkaline soil must cope with physiological drought and ion toxicity, and should also maintain intracellular ion balance and regulate pH outside the roots.

To date, research of salt stress still emphasizes NaCl as the main subject, but it is rapidly developing towards various aspects such as Na^+ metabolism (Serrano et al., 1999), molecular biology of salt resistance genes (Quesada et al., 2002), and salt stress signal transduction (DeWald et al., 2001), and so on. However, there are only a few reports regarding alkaline stress on crop plants and it has been reported that alkali stress more severely affects on the plant growth and metabolism than salt stress (Ma et al., 2007; Yang et al., 2008a; Liu et al., 2008).

Reclamative and preventive measures for rendering saline-alkaline affected soils fit for crop production are usually expensive and generally considered temporary solutions.

Crops differ considerably in their ability to tolerate salinity-sodicity and these intergenic differences can be exploited for selecting the crops that produce satisfactory yield under given levels of root zone salinity and sodicity (Koyama *et al*., 2001). Therefore, selection and breeding of species / cultivars tolerant to salinity is a feasible and economical approach for utilizing salt affected soils (Munns *et al*., 2006). Substitution of salt-tolerant crop species for sensitive species is still practiced in all saline growing areas of the world. However, the success of this approach depends on the presence of genetic variation in the gene pool of inter-intra species.

It has been reported in the previous chapter that Foxtail millet is more sensitive than Proso millet under 100 mM saline and alkaline conditions, especially in more deleterious alkaline conditions. In fact, Foxtail millet could not survive longer under that AS condition making it unavailable for measurement of physiological attributes. Therefore, the present study was undertaken using lower levels of SS and AS (50 and 75 mM) for closely investigating the growth, membrane stability, water status, photosynthetic pigments and gas exchange characters, mineral composition and organic metabolites of Foxtail millet and Proso millet.

3.2 MATERIALS AND METHODS

3.2.1 Plant material and culture conditions

The collection of seeds of Foxtail millet (*Setaria italica* L.) and Proso millet (*Panicum miliaceum* L.) was described in Chapter 2. The seeds were surface-sterilized with 5% thiophanate-methyl for 5 min and air-dried and sown into 1 L plastic pots containing a soil mixture of granite regosol soil and perlite (2:1 v/v). Six uniform seedlings were kept after germination at an identical distance in each pot. Plants were irrigated with basal nutrient

solution containing 8.3 mM NO_3-N, 0.8 mM NH_4-N, 0.5 mM P_2O_5, 2.2 mM K_2O, 0.7 mM MgO, 2.1 mM CaO, 11 μM MnO, 5 μM B_2O_3 and 13 μM Fe. Two neutral salts of NaCl and Na_2SO_4 (9:1 molar ratio) and two alkaline salts of $NaHCO_3$ and Na_2CO_3 (9:1 molar ratio were used to simulate saline stress (SS) and alkaline stress (AS) conditions in nature). At six weeks after sowing, plants were subjected to stresses twice a day until water was drained-out from the bottom of the pot. Plants were subjected to 25 mM SS and AS for 3 d and 50 mM SS for next 3 d for the hardening of plants before applying original treatments. The pH and EC (S m^{-1}) of SS solutions was 6.5 and 1.217, respectively while in AS solution the values were 9.0 and 0.930, respectively. Each treatment was applied to four replicates located randomly in the greenhouse in order to avoid positional effects.

3.2.2 Plant sampling and measurements

Before the application of treatments and at 14 d after treatment initiation, plants in each pot were sampled and separated into the leaves, stems and roots. The separated plant parts were wiped with tissue towel paper to remove moisture and their fresh weights were measured. The fresh samples were kept frozen in liquid nitrogen, freeze-dried and measured the dry weight. Dry samples were ground into fine powder using a vibrating sample mill (Model TI-100, Heiko Seisakusho Ltd., Tokyo, Japan) for chemical analysis. Leaf samples were taken in a composite pool of physiologically mature leaves of each genotype. The leaf area was measured using a leaf area meter (AMM-5 type leaf area meter, Hayashi-Denko, Tokyo, Japan) and the leaves were oven-dried at 80°C for 72 h and the dry weight was determined. The leaf area ratio was calculated as the total leaf area per unit leaf dry mass. The relative water content (RWC) of the leaf was estimated according to the method of Saneoka et al. (1995). The Na and K concentrations were determined after digestion by nitric acid–hydrogen peroxide, using a flame photometer (ANA 135, Eiko Instruments Inc., Tokyo,

Japan). Ca and Mg concentrations were determined using an atomic absorption spectrophotometer (U-3310 Hitachi Co. Ltd., Tokyo, Japan). Fresh plant materials (0.5 g) were randomly sampled to determine Chl concentrations in acetone (80%) extracts spectrophotometrically as described by Zhu (1993). Proline was determined spectrophotometrically following the ninhydrin method described by Bates et al. (1973), using L-proline as a standard. The total N content was determined using a Kjeldahl nitrogen digester and distillator (Kjeldatherm Type TT100 & Vapodset Type 20, Gerhardt, Germany).

3.2.3 Measurement of leaf water potential and photosynthetic rate

The leaf water potential (Ψ_{LW}) was measured according to the method described by Saneoka et al. (1995), using the uppermost fully expanded leaf employing a pressure chamber (Daiki-Rika Instruments, Tokyo, Japan) at 14 d after the initiation of the salt treatments. Fourteen days after the treatments, the photosynthetic rate (Pn), stomatal conductance and transpiration (Tr) of the third uppermost fully expanded leaves from the top of the plants were determined by using a portable open gas exchange system (LI-6400P model of Li-Cor, Inc., Lincoln, NE, USA). The photosynthetic photon flux density was maintained at 1,000 μmol m^{-2} s^{-1}. The temperature of the leaf was 25°C and the ambient CO_2 concentration of the measurement chamber was 380 μL L^{-1} while measurements were taken.

3.2.4 Membrane permeability

Membrane permeability can be reflected by electrolyte leakage rate (ELR) which was measured with the method described by Lutts *et al*. (1996). Fresh leaves (1 g) were cut into pieces of 5 mm length and equally placed into test vials containing 30 ml deionized water. The vials were incubated at 25°C on a rotary shaker for 2 h, and then the initial electrical conductivity (EC_1) was measured using a DDS-11C conductivity meter (Hongyi Company, Shanghai, China). Then the vials were autoclaved at 120°C for 20 min to release all

electrolytes and finally cooled to 25^{o}C for the measurement of the electrical conductivity (EC_2). ELR can be defined as follows:

$$ELR\ (\%) = EC_1 / EC_2 \times 100$$

3.2.5 Determination of sugar and starch content

The soluble sugars were extracted by boiling 50 mg of dry powdered plant material with 10 ml of 80% ethanol at 80^{o}C for 20 mins. A clear extract was obtained by centrifugation at 3000 rpm for five min and collected into 50 ml beaker. This step was repeated for the second and third time and the collected supernatant was heated at 80^{o}C to remove ethanol. Then the residues were kept into a 50 ml volumetric flask and made up with distilled water and the aliquot was taken for the estimation of the content of soluble sugar with anthrone reagent by spectrophotometer (U-2001, Hitachi, Japan) using D-glucose solution as a standard, according to the method of Yemm and Willis (1954). The residues after ethanolic extraction were dissolved in perchloric acid (9.2 and 4.6 N) and the collected supernatant transferred into 100 ml volumetric flask and made up with distilled water. The aliquot was taken for the estimation of the content of starch with anthrone reagent by spectrophotometer (U-2001, Hitachi, Japan) using glucose solution as a standard.

3.2.6 Statistical analysis

Data were examined using one-way ANOVA and presented as the mean ± S.E. for each treatment and species (n=4). Multiple comparisons of means of data among different saline and alkaline treatments within the plants were performed using Duncan's test at the 0.05 significance level (all tests were performed with SPSS Version 16.0 for Windows).

3.3 RESULTS

3.3.1 Plant growth

Plant height and leaf area decreased with the increasing salinity and alkalinity in both species, however 50 mM SS did not reduce the plant height (Fig. 3.1) and leaf area (Fig. 3.2) significantly in Proso millet but reduced the leaf area significantly in Foxtail millet plants. Plant dry weight decreased by 30/41% at 50 mM SS/AS and 42/53% at 75 mM SS/AS treatments in Foxtail millet. On the other hand, in Proso millet, it decreased by 22/36% under 50 mM SS/AS and 30/43% relative to the control under 75 mM SS/AS treatments (Fig. 3.3).

3.3.2 Electrolyte leakage rate

Electrolyte leakage rate, which is attributed to the damaged leaf membranes resulting from the SS and AS, increased gradually with increasing salinity and alkalinity in both species. In the case of Foxtail millet, the injury increased (over the unstressed control) 51 and 93% at 50 and 75 mM of SS, and 118 and 161% under AS at 50 and 75 mM of AS, respectively. On the other hand, injury in Proso millet leaves intensified under the same stresses, showing an increase of 11 and 40% at 50 and 75 mM of SS, and 54 and 73% at 50 and 75 mM of AS, respectively (Fig. 3.4).

3.3.3 Water status

The RWC decreased remarkably with increasing SS and AS in both crops and the rate of reduction was higher in Foxtail millet than in Proso millet (Fig. 3.5). AS reduced RWC more severely than SS did in all levels of stresses, and the higher level of AS (75 mM) reduced the RWC in Foxtail and Proso millet to 80 and 86% of the control, respectively. The effect of 75 mM SS was almost similar to that of 50 mM AS on this trait.

The leaf water potential (Ψ_{LW}) declined significantly with the intensification of SS and AS in both species, and the reduction was greater under AS than under SS. Foxtail millet reduced 1.4/2.6-fold at 50 mM SS/AS and 1.7/3.4-fold at 75 mM SS/AS treatments. On the

other hand, Proso millet reduced similarly at 1.3/1.7 and 1.5/2.6-fold at 50 and 75 mM SS/AS, respectively (Fig. 3.6).

3.3.4 Chlorophylls and gas exchange characters

Chlorophylls concentration significantly decreased with increasing stresses and the decrease was greater in Foxtail millet under AS conditions (Fig. 3.7). The photosynthesis (Pn), stomatal conductance (gs) and transpiration rate (Tr) of Foxtail millet decreased significantly under SS and AS conditions and the rate of reduction was greater in AS than in SS at all levels (Table 3.1). On the contrary, no significant variations of those parameters were observed under 50 mM SS in Proso millet, however but the reduction was remarkable under all other treatments (Table 3.1). Foxtail millet showed more reduced values of Pn, gs and Tr in all treatment conditions compared with those of Proso millet.

3.3.5 Ionic status

Sodium

Under SS and AS conditions, Foxtail and Proso millet plants acquired significantly higher concentration of Na in all plant segments as compared to unstressed plants. On the other hand, Proso millet acquired very less concentration of Na as compared to Foxtail millet under SS and AS conditions (Table 3.2). The roots of Foxtail millet transported a greater amount of accumulated Na to the leaves under SS and AS conditions (Table 3.3). In contrast, Proso millet transported very less amount of Na from roots to leaves in both conditions.

Potassium

The K concentration in the roots of both species gradually decreased with increasing salinity and alkalinity, and a significant reduction was observed in Foxtail millet under both stresses while in Proso millet only under alkaline stress (Table 3.4). The concentration in the leaves and stems of Foxtail millet under SS conditions remain unchanged from control plants

but significantly reduced under AS conditions. The leaves of Foxtail millet accumulated higher amount of K than the leaves of Proso millet. On the other hand, the roots of Foxtail millet accumulated lower amount of K than the roots Proso millet (Table 3.5).

Na / K ratio

The ratio of Na / K increased with increasing the levels of SS and AS in all the plant segments of both species. The ratio was higher under AS compared to SS and Foxtail millet showed greater values than did Proso millet (Table 3.6).

Calcium

The leaves of Foxtail millet contained higher concentration of Ca than the leaves of Proso millet; on the contrary, it was higher in the roots of Proso millet than in the roots of Foxtail millet (Table 3.7). Under AS conditions, the leaves of Foxtail millet and Proso millet accumulated 66-81% and 39-42% Ca, respectively (Table 3.8). The relative reduction due to stresses was greater under AS than under SS and as well as greater in Foxtail millet than in Proso millet.

Magnesium

The stems of Foxtail millet contained higher concentration of Mg than the Proso millet stems; on the other hand, the leaves and roots of Proso millet acciquisited higher concentration than the leaves and roots of Foxtail millet (Table 3.9). The relative reduction due to stresses was greater under AS than in SS. Foxtial millet leaves accumulated higher amount of Mg in AS than in SS conditins; on the other hand, Proso millet accumulated higher amont in SS than in AS (Table 3.10).

3.3.6 Total nitrogen

The total N concentration in leaves and roots decreased gradually with increasing stresses and the reductions were more severe in AS than in SS, as well as more in the leaves than in the roots (Figs. 3. 8 and 3.9). However, Foxtail millet was severely affected under

both SS and AS, whereas in Proso millet this was true only under AS conditions. The leaves of both species accumulated greater amount of N over control under stress conditions (Table 3.11).

3.3.7 Proline

The proline content increased under SS and AS conditions and the increase was greater under AS than under SS in both species (Fig. 3.10). Furthermore, Foxtail millet produced higher concentration of proline relative to its control than the Proso millet.

3.3.8 Total soluble sugar and starch

Total soluble sugar (TSS) in the leaves of Foxtail millet increased significantly with increasing salinity and alkalinity. On the other hand, the TSS of Proso millet increased significantly only under alkaline stress (Fig 3.11). Starch content in the leaves decreased significantly with increasing intensity of SS and AS in both species except in Proso millet under 50 mM SS condition (Fig. 3.12). The relative reduction was higher in Foxtail millet than in Proso millet.

3.4 DISCUSSION

3.4.1 Plant growth

Plant height, leaf area and dry matter accumulation are ideal indicators of plant growth. In this study, all three indicators were inhibited under both SS and AS conditions and the effects of alkalinity were more severe than those of salinity (Figs. 3.1, 3.2 and 3.3). The relative reductions of those growth parameters were greater in Foxtail millet than those of Proso millet. The effects of saline stress on the membrane permeability (Fig. 3.4) and RWC (Fig. 3.5) were slight, while, alkaline stress induced severe reductions in RWC and sharply increased ELR. The results can be explained as SS generally involves osmotic stress and ion-

induced injury, whereas alkalinity exerts the same stress factors, even in less concentration of AS, with the added influence of high pH in the root zone that is involved in inhibiting plant growth intensely. These results were also in agreement with the previous studies reported by Shi and Sheng (2005); Yang et al., (2007). Many of the published data have shown that high pH is a key factor in limiting plant growth and development under alkaline conditions (Yang et al., 2008a, 2009a,b).

3.4.2 Electrolyte leakage rate

The AS induced injurious effect was greater than that of SS at the same levels of stresses, and is consistent with previous reports (Shi and Yin, 1993; Yang et al., 2007). Proso millet showed higher membrane stability under the same stress conditions, especially more deleterious AS conditions, indicating its higher tolerance in comparison with Foxtail millet. The injurious effects of salinity are commonly thought to be a result of low water potentials and ion toxicities (Munns, 2002). The high pH under AS may have triggered the damaging effects on root cell structure and functions such as the absorption of more Na ion (Tables 3.2 and 3.3) and a sharp increase in ELR (Fig. 3.4).

3.4.3 Water status

It is reported that plants usually can reduce RWC as a quick and economical approach to osmotic adjustment in response to osmotic stress (Lissner et al., 1999). The RWC decreased significantly with increasing salinity and alkalinity, with the extent of reductions under AS greater than that under SS (Fig. 3.5). However, the RWC of Proso millet was greater compared to Foxtail millet in both SS and AS conditions, indicating that Proso millet faced less stress induced by SS and AS through the increasing Ψ_{LW} and less accumulation of toxic Na.

The more reduced Ψ_{LW} in Foxtail millet under AS as compared to SS conditions (Fig. 3.6) indicated the desiccation of cell that resulted in limited water availability (Fig. 3.5) for cell expansion processes. Higher accumulation of Na in Foxtail millet under AS conditions might also be another reason for reducing the Ψ_{LW}. The severe reduction of gs and Tr are closely correlated with changes in Ψ_{LW} under salt stress (Koyro, 2006) and salt-alkali stress (Liu et al., 2010).

3.4.4 Gas exchange characters

Pn, gs and Tr of a plant usually decrease with increasing salinity or alkalinity (Yang et al., 2009a,), and it has been reported that alkaline stress, even at low alkalinity (15 mM), limited the photosynthesis of barley (Yang et al., 2009b) and wheat (Yang et al., 2008c). However, it is observed that the Pn and Tr of Proso millet did not decrease under 50 mM SS (Table 3.1). Zhang and Mu (2009) found similar results and concluded that the Pn, gs and Tr of *Lathyrus quinquenervius* did not decrease under moderate (30 mM) saline stress or alkaline stress. The more reduced Pn under higher salinity probably results from a reduction in intracellular CO_2 partial pressure caused by stomatal closure or of non-stomatal factors (Bethke and Drew, 1992). The non-stomatal factors mainly depend on the cumulative effects of leaf water potential and osmotic potential, reduced photosynthetic area (Marcelis and Van-Hooijdonk, 1999), contents of photosynthetic pigments (Fig. 3.7) and ion toxicities in the cytosol (Zhang and Mu, 2009). The results of the present study showed that the inhibitory effects of AS on gas exchange characters were greater than those of SS at the same levels of stress and Proso millet performed well under AS conditions, indicating its higher tolerances compared to Foxtail millet.

3.4.5 Ionic status

Under saline stress, plants usually accumulate high concentrations of Na^+ in vacuoles to reduce cell water potential (Munns and Tester, 2008), simultaneously inhibiting K^+ absorption (Shi and Sheng 2005). The lower concentration of Na and the higher conentration of K and as well as the lower Na / K ratio in plants have been considered good physiological trait indicators of salt tolerance in plants (Morsy *et al*., 2007; Kaya *et al*., 2007). However, the Na concentrations in the leaves and stems of Proso millet were very low under all treatments as compared to Foxtail millet whereas K concentrations in the stems and roots of Proso millet were almost the same as the Foxtail millet. Therefore, the lower Na / K in Proso millet indicated its high tolerance than Foxtail millet. The selectivity of low Na / K ratio in plants is an important control mechanism and is also a selection criterion for salt tolerance (Wenxue et al., 2003). The ability of plant to limit Na^+ transport into the shoot is critically important for the maintenance of high elongation cells from the toxic effects of Na^+ (Razmjoo et al., 2008). This could be attributed to the ability of root to exclude Na^+ from the xylem sap flowing to the shoot, which would result to the better growth of shoot than root (Kaya et al., 2007). In the present study, the roots of Proso millet accumulated greater amount of Na than the roots of Foxtail millet under stresses but Proso millet transported less amount of Na to the leaves than Foxtail millet (Table 3.3), proving that Proso millet is more tolerant than Foxtail millet.

The Mg (the key component of chlorophyll) and Ca (maintains membrane stability) accumulation in many plants are inhibited by salt stress (Khan, 2001). However, in this study, Foxtail millet accumulated more Ca in the leaves and less in the roots. On the other hand, Proso millet accumulated almost equal amounts in the leaves and stems and less in roots but greater amount than Foxtail millet roots (Table 3.8). The Mg concentration also decreased in both species under SS and AS and the extent of decreases under AS was higher than that

under SS. It may be due to the high pH under AS which reduced the availability of Ca and Mg in the root zones by precipitating them into $CaCO_3$ and $MgCO_3$.

3.4.6 Total nitrogen

Nitrogen is one of the most essential elements and plays an important role in the maintenance of intracellular ionic balance and osmotic adjustment when plants are subjected saline and alkaline stress (Yang et al., 2007, 2008b, 2009a). Salt stress reduces N uptake in many plants due to the antagonistic effect between Na^+ and NH_4^+ and / or NO_3^- and Cl^- (Parida and Das, 2004). In this study, the N concentration in the leaves and roots gradually decreased with increasing salinity and alkalinity in both species, and the reduction was greater under AS than under SS (Figs. 3.8 and 3.9). The reduction of N concentration was insignificant in Proso millet at 50 mM SS, whereas it was significant under all levels of AS in both species. Alkaline stress might interfere the uptake or metabolism of NO_3^-. It has been proposed that NO_3^- uptake is mediated by a H^+ / NO_3^- symport mechanism, which relies on the transmembrane proton gradient (Crawford and Glass, 1998). The reduction in NO_3^- in the root under alkali stress might be related to the lack of external protons due to the high pH.

3.4.7 Proline

It has been widely reported that plants under stresses accumulate compatible solutes such as proline for osmotic adjustment and detoxification of injurious ions (Kavi Kishor et al., 2005; Tammam et al., 2008). In this study, the proline concentration of both species increased with increasing SS and AS (Fig 3.10). It is common for proline to be correlated with stress tolerance (Ashraf and Foolad, 2007; Younis et al., 2009) but the significance of proline accumulation in osmotic adjustment is still being debated and varies according to the species (Rodriguez et al., 1997). Therefore, the role of proline on alkaline stress tolerance should be further investigated.

3.4.8 Total soluble sugar and starch

Plants under stress conditions accumulated compatible solutes like total soluble sugar to adjust the osmotic stress (Jiménez-Bremont et al., 2006; Yang et al., 2007; Khadri et al., 2007; Palma et al., 2009) and decreased starch content (Murakeozy et al., 2003). In this study, soluble sugar concentration increased significantly with increasing SS and AS. The increase in TSS concentration was greater under AS than under SS suggesting that AS might induce more severe stress and plants accumulated more TSS to adjust to osmotic shock (Fig. 3.11) On the other hand, decreased starch concentration might be related to the lower RWC, Ψ_{LW} and ultimately the reducing photosynthetic activities which yielded lower starch (Fig. 3.12).

In conclusion, Proso millet showed more capability to survive under SS and AS conditions as compared to Foxtail millet based on almost all plant traits examined. The more tolerant ability of Proso millet under SS and AS conditions, especially more destructive alkaline conditions, might be related to its genetic ability and it should be emphasized to investigate this crop further on genetic aspects.

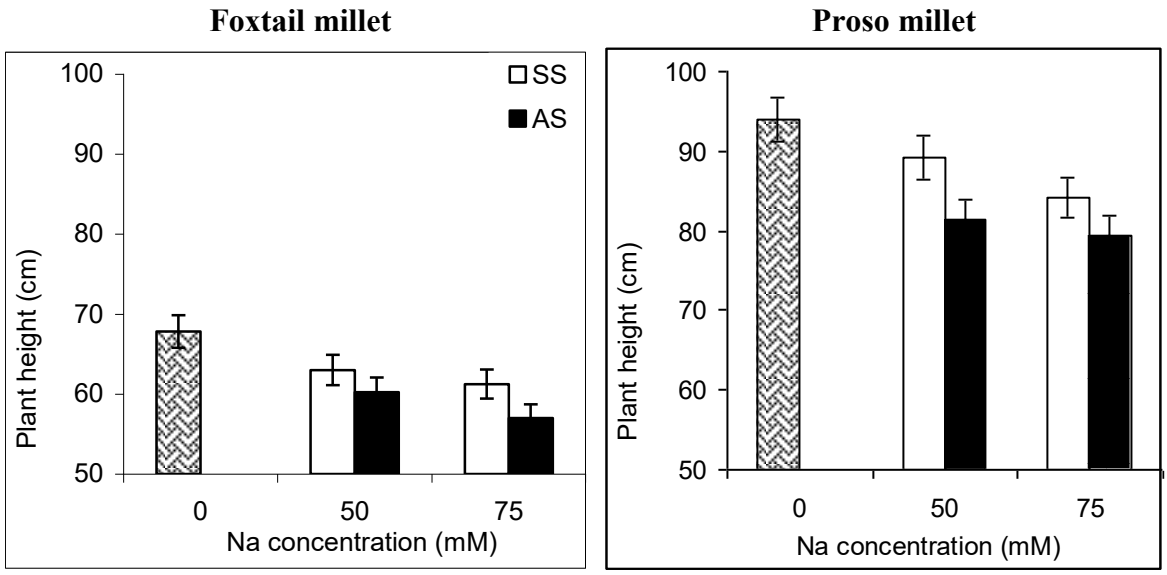

Fig. 3.1 Effect of SS and AS on the plant height of Foxtail millet and Proso millet. The values are the means (± S.E) of four replicates.

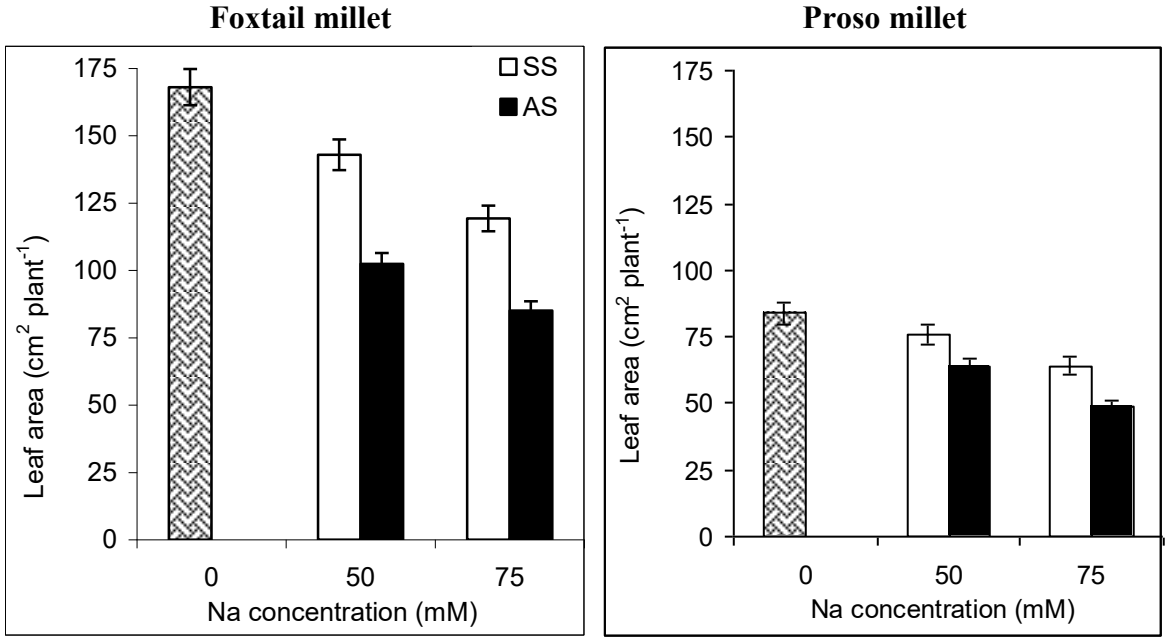

Fig. 3.2 Effect of SS and AS on the leaf area of Foxtail millet and Proso millet. The values are the means (± S.E) of four replicates.

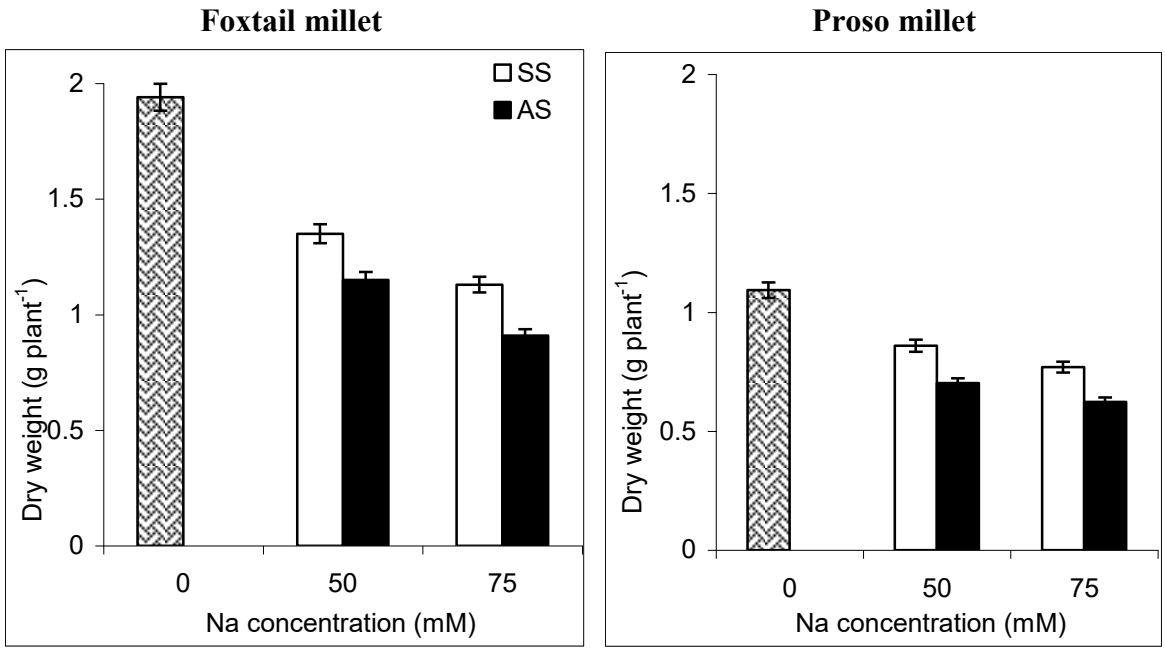

Fig. 3.3 Effects of SS and AS on the plant dry weight of Foxtail millet and Proso millet. The values are the means (± S.E) of four replicates.

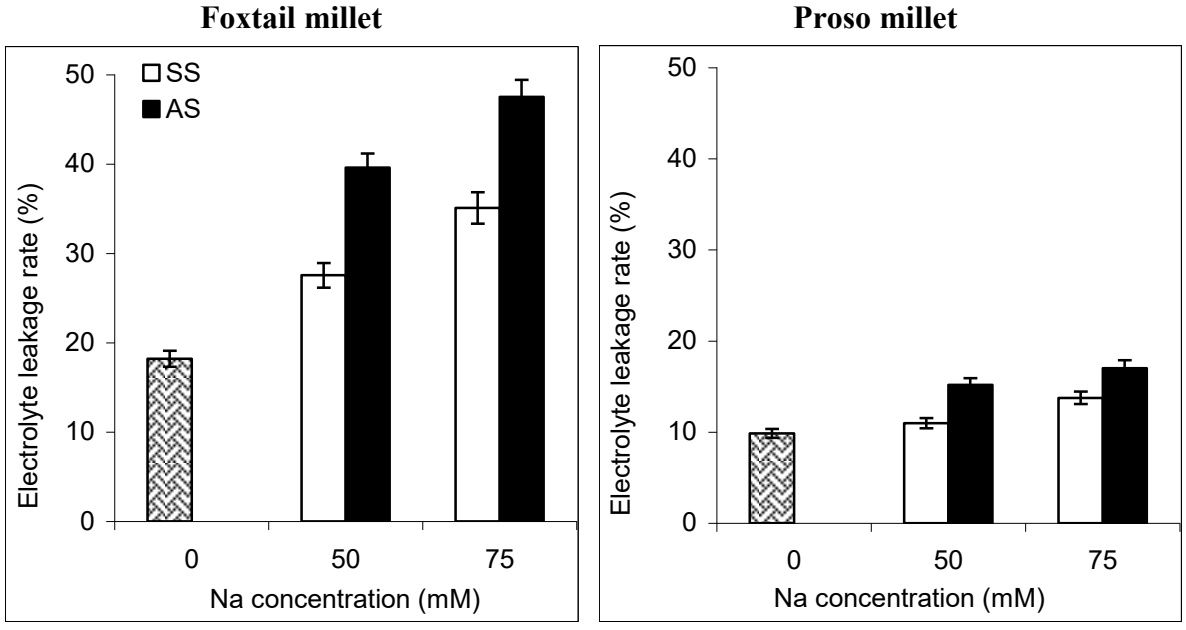

Fig. 3.4 Effects of SS and AS on the electrolyte leakage rate in the leaves of Foxtail millet and Proso millet. The values are the means (± S.E) of four replicates.

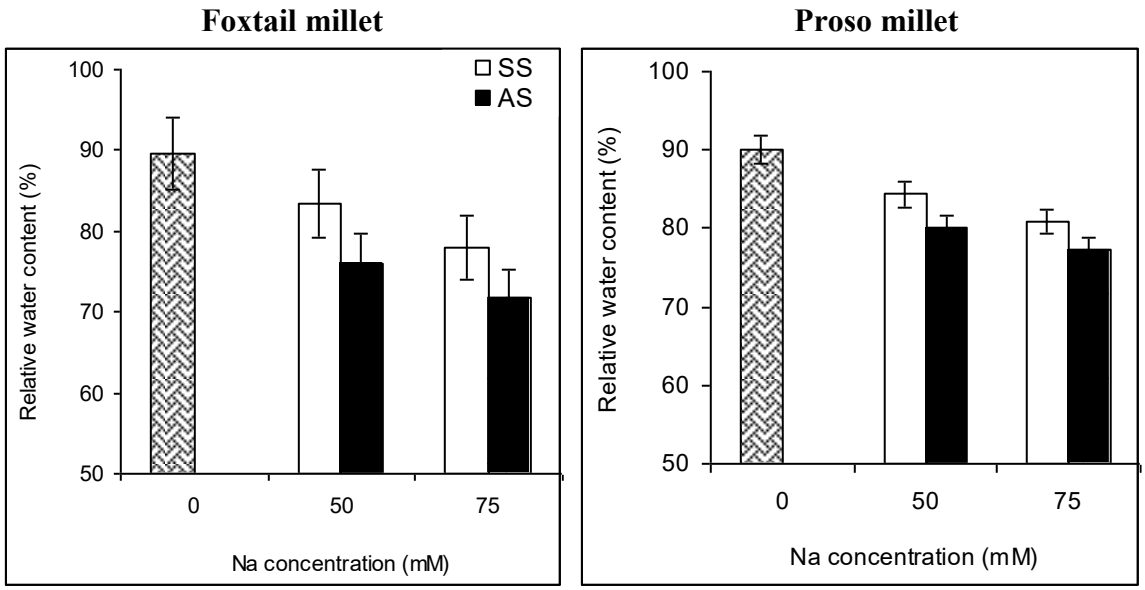

Fig. 3.5 Effects of SS and AS on the relative water content in the leaves of Foxtail millet and Proso millet. The values are the means (± S.E) of four replicates.

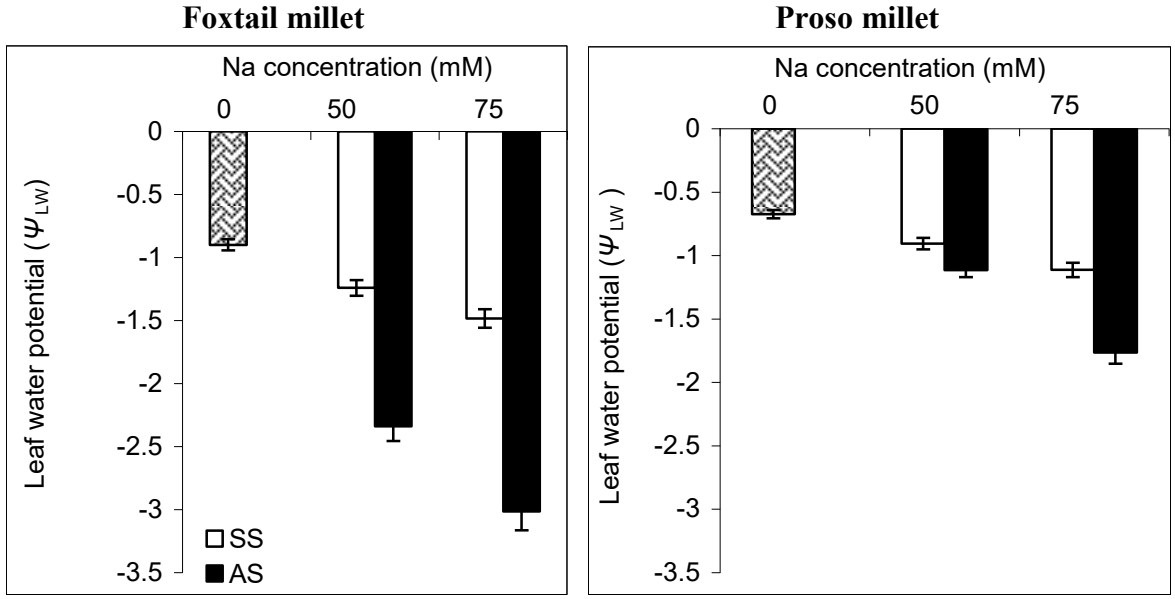

Fig. 3.6 Effects of SS and AS on the leaf water potential of Foxtail millet and Proso millet. The values are the means (± S.E) of four replicates.

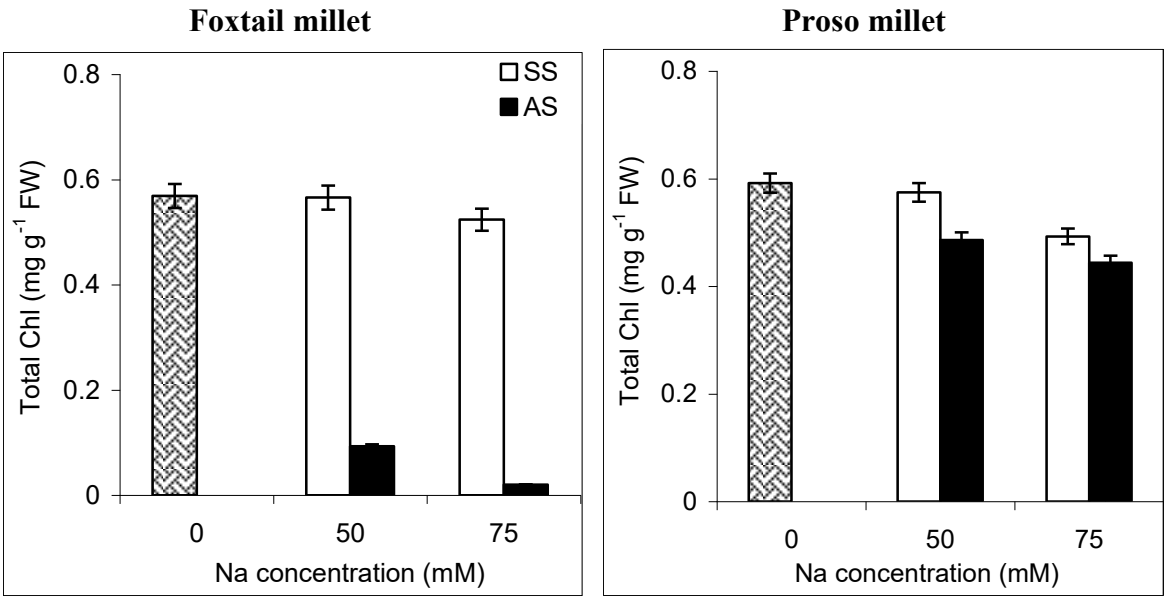

Fig. 3.7 Effects of SS and AS on the total Chl (a+b) of Foxtail millet and Proso millet. The values are the means (± S.E) of four replicates.

Table 3.1 Effects of SS and AS on the photosynthesis (Pn), stomatal conductance (gs), and transpiration rate (Tr) of Foxtail millet and Proso millet. The values are means (± S.E) of three replicates.

Genotypes	Treatment groups	Treatments (mM)	Pn (μmol CO_2 m^{-2} s^{-1})	gs (mol m^{-2} s^{-1})	Tr (mmol m^{-2} s^{-1})
Foxtail millet	Control	0	23.15±0.70^{a}	0.138±0.01^{a}	3.28±0.21^{a}
	SS	50	15.52±0.21^{b}	0.075±0.01^{b}	2.00±0.04^{b}
		75	7.06±1.30^{c}	0.038±0.00^{c}	0.97±0.08^{c}
	AS	50	1.21±0.10^{d}	0.007±0.00^{d}	0.19±0.08^{d}
		75	0.11±0.01^{d}	0.007±0.00^{d}	0.17±0.01^{d}
Proso millet	Control	0	20.23±2.98^{a}	0.165±0.04^{a}	2.87±0.45^{a}
	SS	50	16.05±0.07^{a}	0.118±0.00^{ab}	2.36±0.17^{ab}
		75	9.35±0.10^{b}	0.069±0.00^{bc}	1.71±0.02^{bc}
	AS	50	5.91±0.33^{bc}	0.057±0.00^{c}	1.28±0.13^{cd}
		75	3.24±0.11^{c}	0.028±0.00^{c}	0.68±0.12^{c}

In a column, values with the same letter are not significantly different, and that with different letters are significantly different at $P<0.05$ using Duncan test, values are means ±S.E (n=3).

Table 3.2 Effects of SS and AS on Na concentration (mg g^{-1} DW) in the leaves, stems and roots of Foxtail millet and Proso millet. The values are the means (± S.E) of four replicates.

Genotypes	Treatment groups	Treatments (mM)	Na		
			Leaves	Stems	Roots
Foxtail millet	Control	0	0.083±0.02^{d}	1.17±0.13^{e}	4.43±0.41^{d}
	SS	50	5.14±0.47^{c}	13.34±0.12^{d}	15.16±0.31^{b}
		75	14.95±1.82^{b}	19.00±1.04^{c}	17.76±0.45^{a}
	AS	50	21.17±0.53^{a}	22.34±0.52^{b}	9.05±0.24^{c}
		75	23.55±0.80^{a}	25.96±0.68^{a}	10.36±0.25^{c}
Proso millet	Control	0	0.42±0.06^{c}	0.89±0.14^{d}	5.83±0.70^{d}
	SS	50	2.05±0.03^{b}	5.21±0.28^{c}	12.21±0.37^{c}
		75	4.57±0.39^{a}	10.54±0.87^{a}	16.03±0.14^{a}
	AS	50	3.06±0.19^{b}	7.74±0.47^{b}	14.19±0.44^{bc}
		75	5.15±0.19^{a}	10.01±0.67^{a}	15.27±0.28^{ab}

In a column, values with the same letter are not significantly different, and that with different letters are significantly different at $P<0.05$ using Duncan test, values are means ±S.E (n=4).

Table 3.3 Effects of SS and AS on Na accumulation (mg $plant^{-1}$) in the leaves, stems and roots of Foxtail millet and Proso millet. The values are the means of four replicates.

Genotypes	Treatment groups	Treatments (mM)	Na			
			Leaves	Stems	Roots	Total
Foxtail millet	Control	0	0.05 (2)	1.05 (37)	1.74 (61)	2.85 (100)
	SS	50	2.30 (16)	8.97 (61)	3.42 (23)	14.69 (100)
		75	6.12 (31)	9.98 (51)	3.41 (18)	19.51 (100)
	AS	50	9.19 (43)	10.88 (51)	1.21 (6)	21.28 (100)
		75	7.88 (38)	11.39 (55)	1.38 (7)	20.65 (100)
Proso millet	Control	0	0.08 (8)	0.48 (47)	0.46 (45)	1.02 (100)
	SS	50	0.39 (12)	2.17 (69)	0.60 (19)	3.16 (100)
		75	0.75 (14)	4.07 (73)	0.75 (13)	5.57 (100)
	AS	50	0.42 (11)	2.81 (74)	0.56 (15)	3.79 (100)
		75	0.51 (12)	3.20 (74)	0.60 (14)	4.30 (100)

( ): Na partitioning as percentage in leaves, stems and roots.

Table 3.4 Effects of SS and AS on K concentration (mg g^{-1} DW) in the leaves, stems and roots of Foxtail millet and Proso millet. The values are the means (± S.E) of four replicates.

Genotypes	Treatment groups	Treatments (mM)	K		
			Leaves	Stems	Roots
Foxtail millet	Control	0	29.51±0.24^{b}	32.17±0.15^{a}	4.73±0.31^{a}
	SS	50	33.88±0.23^{a}	31.66±0.76^{a}	3.42±o.13^{b}
		75	33.98±0.34^{a}	31.38±1.19^{a}	3.37±0.19^{b}
	AS	50	26.36±0.90^{c}	25.17±0.79^{c}	1.36±0.06^{c}
		75	27.84±1.03^{bc}	25.17±1.34^{b}	1.29±0.01^{c}
Proso millet	Control	0	14.08±0.24^{b}	21.39±0.72^{b}	7.59±0.78^{a}
	SS	50	18.87±0.27^{a}	24.24±0.11^{a}	7.32±0.35^{a}
		75	19.63±0.32^{a}	25.17±0.91^{a}	7.07±0.22^{a}
	AS	50	19.26±0.30^{a}	20.43±0.56^{bc}	4.14±0.17^{b}
		75	19.20±0.67^{a}	19.29±0.33^{c}	3.53±0.14^{b}

In a column, values with the same letter are not significantly different, and that with different letters are significantly different at $P<0.05$ using Duncan test, values are means ±S.E (n=4).

Table 3.5 Effects of SS and AS on K accumulation (mg $plant^{-1}$) in the leaves, stems and roots of Foxtail millet and Proso millet. The values are the means of four replicates.

Genotypes	Treatment groups	Treatments (mM)	K			
			Leaves	Stems	Roots	Total
Foxtail millet	Control	0	18.91 (38)	29.01 (58)	1.86 (4)	49.77 (100)
	SS	50	15.16 (41)	21.29 (57)	0.77 (2)	37.22 (100)
		75	13.91 (45)	16.49 (53)	0.65 (2)	31.05 (100)
	AS	50	11.44 (48)	12.26 (51)	0.18 (1)	23.88 (100)
		75	9.32 (45)	11.04 (54)	0.17 (1)	20.53 (100)
Proso millet	Control	0	2.80 (19)	11.55 (77)	0.60 (4)	14.95 (100)
	SS	50	3.58 (25)	10.10 (72)	0.36 (3)	14.04 (100)
		75	3.24 (249	9.71 (73)	0.33 (2)	13.28 (100)
	AS	50	2.65 (26)	7.42 (72)	0.16 (2)	10.23 (100)
		75	1.91 (23)	6.16 (75)	0.14 (2)	8.21 (100)

( ): K partitioning as percentage in leaves, stems and roots.

Table 3.6 Effects of SS and AS on Na / K in the leaves, stems and roots of Foxtail millet and Proso millet. The values are means of four replicates.

Genotypes	Treatment groups	Treatments (mM)	Na / K		
			Leaves	Stems	Roots
Foxtail millet	Control	0	0.003	0.04	0.94
	SS	50	0.15	0.42	4.44
		75	0.44	0.61	5.27
	AS	50	0.83	0.89	6.65
		75	0.95	1.12	8.03
Proso millet	Control	0	0.03	0.04	0.82
	SS	50	0.11	0.22	1.67
		75	0.23	0.42	2.09
	AS	50	0.16	0.38	3.43
		75	0.34	0.52	4.33

Table 3.7 Effects of SS and AS on Ca concentration (mg g^{-1} DW) in the leaves, stems and roots of Foxtail millet and Proso millet. The values are means (± S.E) of four replicates.

Genotypes	Treatment groups	Treatments (mM)	Ca		
			Leaves	Stems	Roots
Foxtail millet	Control	0	1.75 ± 0.03^{a}	0.39 ± 0.03^{a}	0.48 ± 0.02^{b}
	SS	50	1.55 ± 0.05^{b}	0.33 ± 0.02^{b}	0.61 ± 0.03^{a}
		75	1.35 ± 0.09^{bc}	0.41 ± 0.02^{a}	0.46 ± 0.03^{b}
	AS	50	1.36 ± 0.09^{bc}	0.15 ± 0.04^{b}	0.46 ± 0.03^{b}
		75	1.29 ± 0.06^{c}	0.41 ± 0.02^{a}	0.29 ± 0.05^{c}
Proso millet	Control	0	1.00 ± 0.01^{a}	0.38 ± 0.01^{a}	0.92 ± 0.04^{a}
	SS	50	0.98 ± 0.06^{a}	0.36 ± 0.01^{ab}	0.78 ± 0.02^{b}
		75	0.89 ± 0.03^{a}	0.32 ± 0.03^{b}	0.69 ± 0.03^{c}
	AS	50	0.70 ± 0.05^{b}	0.33 ± 0.01^{ab}	0.81 ± 0.01^{b}
		75	0.68 ± 0.03^{d}	0.22 ± 0.01^{c}	0.60 ± 0.01^{d}

In a column, values with the same letter are not significantly different, and that with different letters are significantly different at $P<0.05$ using Duncan test, values are means ±S.E (n=4).

Table 3.8 Effects of SS and AS on Ca accumulation (mg plant^{-1}) in the leaves, stems and roots of Foxtail millet and Proso millet. The values are means of four replicates.

Genotypes	Treatment groups	Treatments (mM)	Ca			
			Leaves	Stems	Roots	Total
Foxtail millet	Control	0	1.12	0.35	0.19	1.66
		(%)	(68)	(21)	(11)	(100)
	SS	50	0.69	0.22	0.14	1.05
			(66)	(21)	(13)	(100)
		75	0.55	0.22	0.09	0.86
			(65)	(25)	(10)	(100)
	AS	50	0.59	0.07	0.06	0.72
			(81)	(10)	(9)	(100)
		75	0.43	0.18	0.04	0.65
			(66)	(28)	(6)	(100)
Proso millet	Control	0	0.20	0.21	0.07	0.48
			(42)	(43)	(15)	(100)
	SS	50	0.19	0.15	0.04	0.37
			(50)	(40)	(10)	(100)
		75	0.15	0.12	0.03	0.30
			(48)	(41)	(11)	(100)
	AS	50	0.10	0.12	0.03	0.25
			(39)	(48)	(13)	(100)
		75	0.07	0.07	0.02	0.16
			(42)	(44)	(14)	(100)

( ): Ca partitioning as percentage in leaves, stems and roots.

Table 3.9 Effects of SS and AS on Mg concentration (mg g^{-1} DW) in the leaves, stems and roots of Foxtail millet and Proso millet. The values are means (± S.E) of four replicates.

Genotypes	Treatment groups	Treatments (mM)	Mg		
			Leaves	Stems	Roots
Foxtail millet	Control	0	2.32±0.03^{a}	1.33±0.02^{b}	0.50±0.01^{a}
	SS	50	2.09±0.02^{ab}	1.49±0.01^{a}	0.55±0.02^{a}
		75	2.07±0.02^{ab}	1.46±0.04^{a}	0.51±0.03^{a}
	AS	50	1.97±0.13^{b}	1.14±0.03^{c}	0.35±0.01^{b}
		75	1.95±0.07^{b}	1.06±0.01^{c}	0.29±0.00^{b}
Proso millet	Control	0	3.71±0.15^{a}	0.98±0.01^{a}	2.18±0.07^{a}
	SS	50	3.57±0.03^{ab}	0.96±0.05^{a}	2.03±0.01^{b}
		75	3.30±0.12^{b}	0.90±0.03^{ab}	1.89±0.02^{b}
	AS	50	2.71±0.12^{c}	0.63±0.02^{c}	1.62±0.05^{c}
		75	2.66±0.09^{d}	0.83±0.05^{b}	1.50±0.05^{c}

In a column, values with the same letter are not significantly different, and that with different letters are significantly different at $P<0.05$ using Duncan test, values are means ±S.E (n=4).

Table 3.10 Effects of SS and AS on Mg accumulation (mg plant^{-1}) in the leaves, stems and roots of Foxtail millet and Proso millet. The values are means of four replicates.

Genotypes	Treatment groups	Treatments (mM)	Mg			
			Leaves	Stems	Roots	Total
Foxtail millet	Control	0	1.49	1.20	0.20	2.88
		(%)	(52)	(41)	(7)	(100)
	SS	50	0.94	1.00	0.12	2.06
			(45)	(49)	(6)	(100)
		75	0.85	0.77	0.10	1.71
			(49)	(45)	(6)	(100)
	AS	50	0.85	0.56	0.05	1.46
			(59)	(38)	(3)	(100)
		75	0.65	0.46	0.04	1.16
			(57)	(40)	(3)	(100)
Proso millet	Control	0	0.74	0.53	0.17	1.44
			(51)	(37)	(12)	(100)
	SS	50	0.68	0.40	0.10	1.18
			(58)	(34)	(8)	(100)
		75	0.54	0.35	0.09	0.98
			(56)	(35)	(9)	(100)
	AS	50	0.37	0.23	0.06	0.67
			(56)	(34)	(10)	(100)
		75	0.26	0.27	0.06	0.59
			(45)	(45)	(10)	(100)

( ): Mg partitioning as percentage in leaves, stems and roots.

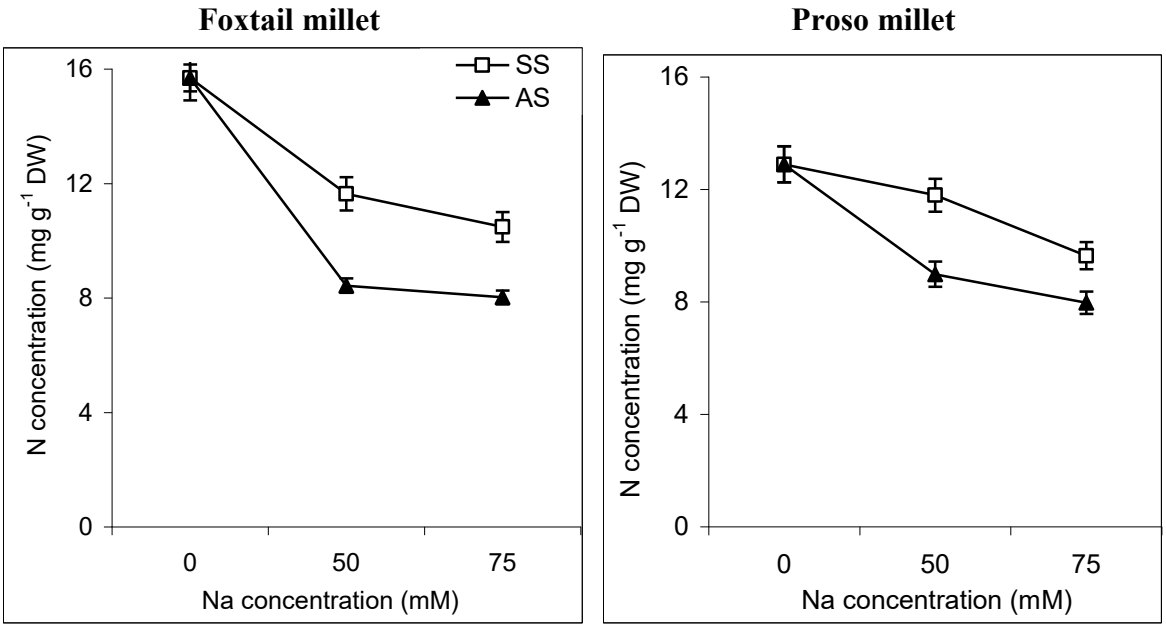

Fig. 3.8 Effects of SS and AS on the total nitrogen concentration in the leaves of Foxtail millet and Proso millet. The values are the means (± S.E) of four replicates.

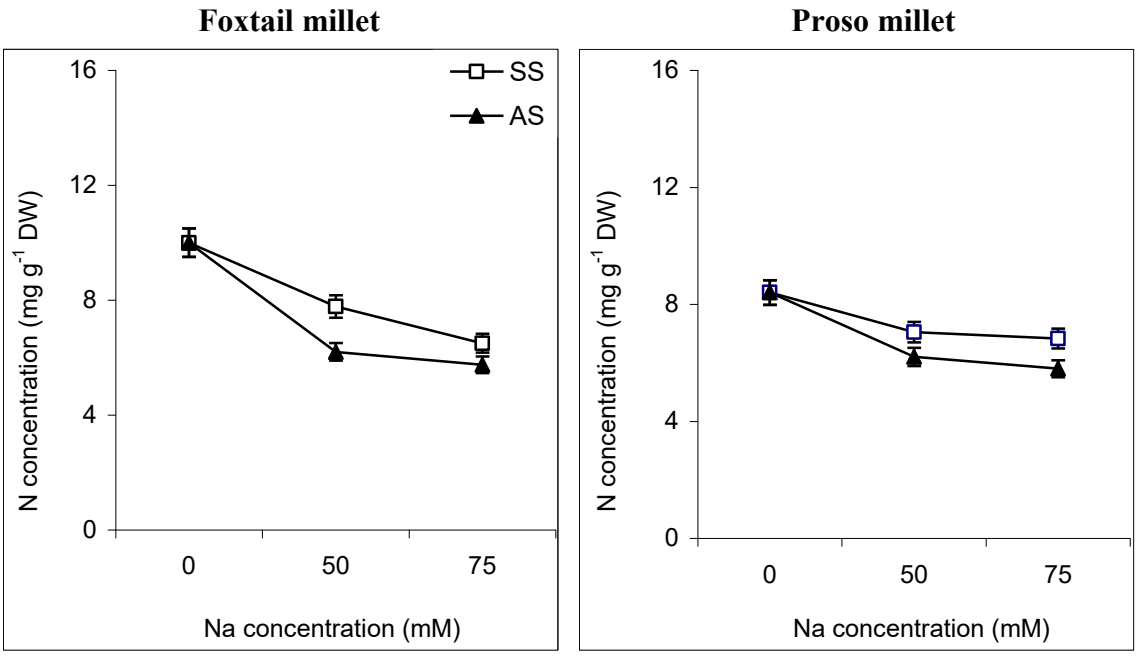

Fig. 3.9 Effects of SS and AS on the total nitrogen concentration in the roots of Foxtail millet and Proso millet. The values are the means (± S.E) of four replicates.

Table 3.11 Effects of SS and AS on N accumulation (mg plant^{-1}) in the leaves and roots of Foxtail millet and Proso millet. The values are means of four replicates.

Genotypes	Treatment groups	Treatments (mM)	N		
			Leaves	Roots	Total
Foxtail millet	Control	0	10.05 (72)	3.93 (28)	13.99 (100)
	SS	50	5.21 (75)	1.75 (25)	6.96 (100)
		75	4.29 (77)	1.25 (23)	5.54 (100)
	AS	50	3.66 (82)	0.83 (18)	4.49 (100)
		75	2.68 (78)	0.76 (22)	3.45 (100)
Proso millet	Control	0	2.57 (80)	0.63 (20)	3.20 (100)
	SS	50	2.24 (87)	0.35 (13)	2.59 (100)
		75	1.59 (83)	0.32 (17)	1.91 (100)
	AS	50	1.24 (84)	0.24 (16)	1.48 (100)
		75	0.74 (77)	0.23 (23)	0.97 (100)

( ): N partitioning as percentage in leaves and roots.

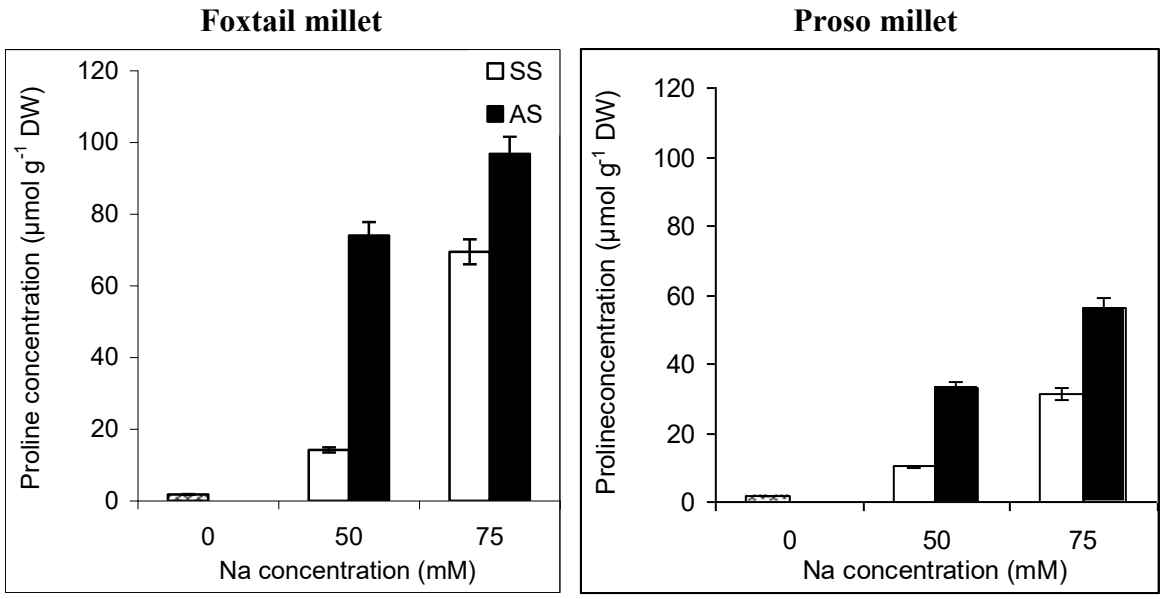

Fig. 3.10 Effects of SS and AS on the proline concentration in the leaves of Foxtail millet and Proso millet. The values are the means (± S.E) of four replicates.

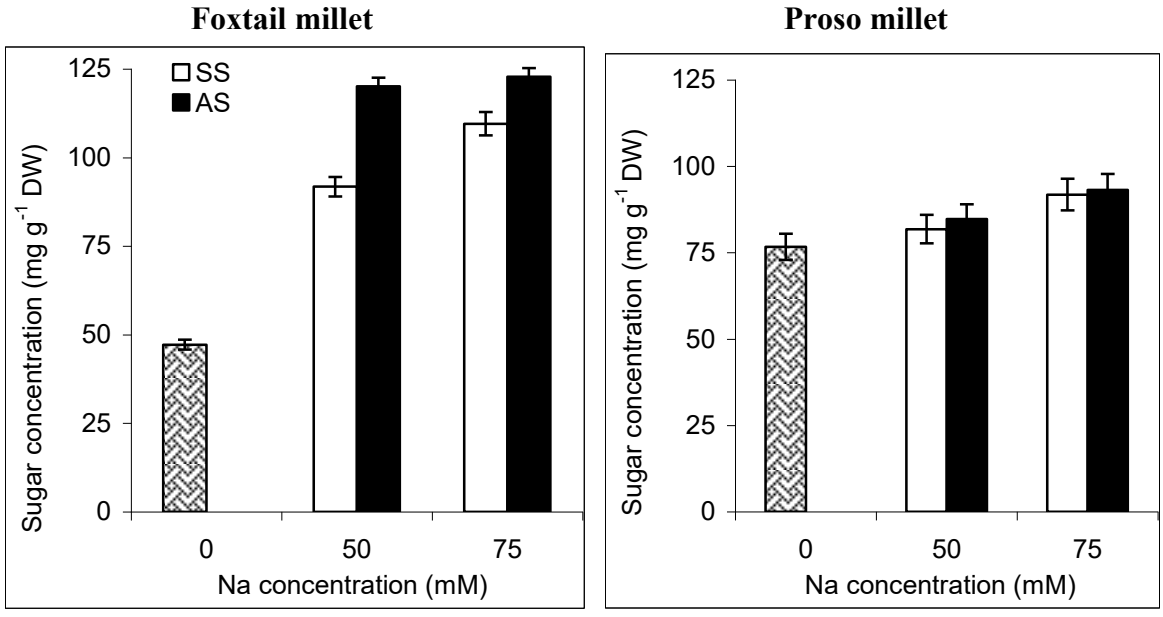

Fig. 3.11 Effects of SS and AS on the sugar concentration in the leaves of Foxtail millet and Proso millet. The values are the means (± S.E) of four replicates.

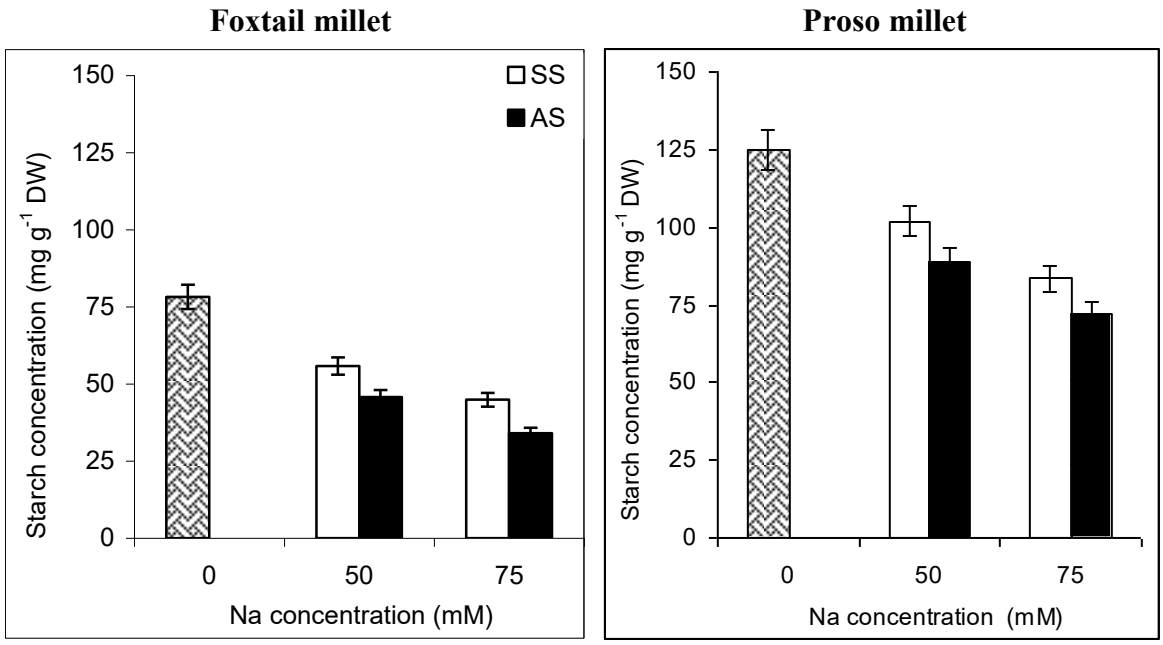

Fig. 3.12 Effects of SS and AS on the starch concentration in the leaves of Foxtail millet and Proso millet. The values are the means (± S.E) of four replicates.

CHAPTER 4

EFFECTS OF EXOGENOUS APPLICATION OF CITRIC ACID AND PROLINE TO FOXTAIL MILLET AND ANALYSIS OF STRESS TOLERANCE FACTORS UNDER SALINE AND ALKALINE CONDITIONS

4.1 INTRODUCTION

Arable lands are subjected to salinization and alkalinization day by day in a alarming rate. Increasing food demand from the fast growing human population reminds us to ameliorate the harmful effects of salinity and alkalinity on agricultural lands by using various strategies. Most of the crop species are glycophytes which generally show limited growth and development under saline and alkaline conditions. High salinity and alkalinity cause reduction of plant growth, enhancement of soluble sugars, organic acids and proline in many glycophytic crops like wheat (Guo et al., 2009), *Lathyrus quinquenervius* (Zhang and Mu, 2009), barley (Yang et al., 2009a) and sunflower (Liu et al., 2010). Glycophytic species employ different strategies to withstand saline and alkaline stress. The increase in salt resistance may involve protection of cell and organelle membranes (Mansour and Salama, 2004) and the accumulation of some protector components (Mansour, 2000).

Plants are generally characterized by a high degree of homeostatic plasticity in response to environmental stresses, thereby optimizing growth and development in a way that maximizes their opportunities for survival and reproduction. The higher plants have evolved a number of adaptive strategies to overcome such abiotic stresses (Tester and Davenport, 2003; Bartels and Sunkar, 2005). The most common type of osmotic adjustment in plant cells involves the accumulation of compatible solutes and exudation of organic acids in cytoplasm (Rhodes and Hanson, 1993). Compatible solutes and organic acids which are commonly employed as osmoprotectants can lower the osmotic potential for cells without interfering with the metabolic processes or protein structuring and functioning and, consequently, maintain the water content of the cells under stresses (Yancey et al., 1982). Proline and citric acid (CA) may act as modulators by suppressing or enhancing the stress responses of plants (Sun and Hong, 2010a,b).

Proline is a well-known compatible solute which plays a pivotal role in osmotic adjustment in plants by helping to maintain sufficient cell turgor for growth (Nanjo et al., 1999). Exogenous proline is known to mitigate the detrimental effects of Na and improve growth and survival under various stresses (Okuma et al., 2004; Sun and Hong, 2010b). It is reported that proline acts as free radical scavengers and / or enzyme protectant (Okuma et al., 2002; Hoque et al., 2007). It is also reported that proline protects higher plants against salt / osmotic stresses, not only by adjusting osmotic pressure (Chinnusamy et al., 2005; Vinocur and Altman, 2005), but also by stabilizing many functional units such as complex II electron transport (Hamilton and Heckathorn, 2001), membranes and proteins (McNeil et al., 1999; Yan et al., 2000) and enzymes (Makela et al., 2000). Until now, little information has been reported about proline-related stress defense mechanisms that help to maintain plant growth and antioxidant enzyme activities under alkaline conditions.

Citric acid is an important organic acid for plant growth and has apparent relationships with stress tolerance. Metabolism and accumulation of CA increased under salt stress in alfalfa (Fougére et al., 1991); under alkali stress in *Puccinellia tenuiflora* (Guo et al., 2010), in rice (Wang et el., 2011), in sea buckthorn (Chen et al., 2009), and under drought stress in cotton plants (Timpa et al., 1986). Phosphorus fixation and its precipitation as insoluble compounds are considered to be one of the major constraints to crop production in alkaline soils. Organic acid can manipulate the availability of P either indirectly through promoting the growth of microorganisms and the subsequent mineralization of organic P (Richardson, 1994), or directly by inducing shifts in rhizosphere pH, shifting chemical equilibrium in soil solution and inducing the dissolution of sparingly soluble P minerals (Hocking, 2001). The effectiveness of organic acids to mobilize soil P depends on the number of its carboxyl groups they possess and tends to follow the series tricarboxylic >

dicarboxylic > monocarboxylic acid (Jones, 1998). CA contains three carboxylic groups and varying negative charges which favors for uptake of nutrients and detoxification of metals (Al, Fe etc.) (Jones, 1998). However, no study has yet examined the relationship of CA and stress tolerance in glycophytic crops under saline and alkaline conditions. On the other hand, there are some lines of evidence that exogenous application of CA alleviated the inhibitory effect of toxic Al on root extension in cotton (Hue et al., 1986) and shoot growth in corn (Bartlett and Riego, 1972). Recently Sun and Hong (2010a) reported that exogenous CA can mitigate the saline and alkaline stress in halophytes (*Leymus chinensis* Trin.) like proline. However, to the best of my knowledge no evidence exists regarding the exogenous application of CA to the stress tolerance of glycophytic crops under SS and AS conditions.

It has been reported in the previous chapters that Foxtail millet is comparatively more sensitive than Proso millet under saline and alkaline conditions, especially in alkaline condition. However, no study has yet been examined the ameliorating effects of exogenous proline and CA on plant growth and metabolism under saline and alkaline conditions. Therefore, the present study was conducted to investigate the effects of exogenous application of CA and proline on the growth, membrane stability, water status, photosynthetic apparatus, minerals composition, organic metabolites of Foxtail millet plant under saline and alkaline conditions.

4.2 MATERIALS AND METHODS

4.2.1 Plant material and culture conditions

Seeds of Foxtail millet (*Setaria italica* L. cv: *BARI kaun-3*) were surface-sterilized with 5% thiophanate-methyl for 5 min and air-dried. Seeds were sown into 1 L plastic pots containing a soil mixture of granite regosol soil and perlite (2:1 v/v). After germination, 6

uniform seedlings were kept at equal distances in each pot. Pots were maintained under greenhouse conditions. Plants were irrigated with nutrient solution at each watering using an irrigation system. The basal nutrient solution contained 8.3 mM NO_3-N, 0.8 mM NH_4-N, 0.5 mM P_2O_5, 2.2 mM K_2O, 0.7 mM MgO, 2.1 mM CaO, 11 μM MnO, 5 μM B_2O_3 and 13 μM Fe. To simulate saline stress (SS) and alkaline stress (AS) conditions in nature, two stress treatments were applied: neutral salts of NaCl and Na_2SO_4 (9:1 molar ratio) and alkaline salts of $NaHCO_3$ and Na_2CO_3 (9:1 molar ratio). At six weeks after sowing, plants were subjected to stresses every day until water was drained-out from the bottom of the pot. Before applying 75 mM SS and 50 mM AS stresses, plants were subjected to SS and AS of 25, and 50 mM concentrations every 3 d alternatively for the hardening of plants.

4.2.2 Application of exogenous CA and proline treatments

Two levels of citric acids (Citric Acid Monohydrates), CA1 and CA2, and proline (L-Proline) at the rate of 0.25 (CA1), 0.5 (CA2) and 0.5 mM, respectively, were applied with the SS and AS solutions on alternate days. The pH and EC (S m^{-1}) of saline solutions were 6.4~6.5 and 1.0~1.2 and those of alkaline solution were 9.0~9.1 and 0.850~0.900, respectively. Each treatment was applied to four replicates located randomly in the greenhouse in order to avoid positional effects.

4.2.3 Plant sampling and measurements

Plants in each pot were sampled and separated into the leaves, stems (culms) and roots before the application of treatments and at 14 d after treatment initiation. The separated segments were wiped with tissue towel paper to remove moisture and their fresh weights were measured. The fresh samples were kept frozen in liquid nitrogen, then freeze-dried and their dry weight measured. Dry samples were ground into fine powder using a vibrating sample mill (Model TI-100, Heiko Seisakusho Ltd., Tokyo, Japan) for chemical analysis.

Leaf samples were taken in triplicate from a composite pool of physiologically matured leaves. The leaf area was measured using a leaf area meter (AMM-5 type leaf area meter, Hayashi-Denko, Tokyo, Japan) and the leaves were oven-dried at 80°C for 72 h and the dry weight was determined. The leaf area ratio was calculated as the total leaf area per unit leaf dry mass. The RWC of the leaf was estimated according to the method of Saneoka et al. (1995). The Na and K concentrations were determined after digestion by nitric acid–hydrogen peroxide using a flame photometer (ANA 135, Eiko Instruments Inc., Tokyo, Japan). The Ca, Mg and Fe concentrations were determined using an atomic absorption spectrophotometer (U-3310 Hitachi Co. Ltd., Tokyo, Japan). Aliquots of the fresh plant materials (0.5 g) were randomly sampled to determine Chl concentrations in acetone (80%) extracts spectrophotometrically as described by Zhu (1993). Proline was determined spectrophotometrically following the ninhydrin method described by Bates et al. (1973) using L-proline as a standard. CA was measured with the enzymatic bioanalysis method using spectrophotometer followed by Mollering and Gruber (1966). The total N concentration was determined using a Kjeldahl nitrogen digester and distillator (Kjeldatherm Type TT100 & Vapodset Type 20, Gerhardt, Germany). Phosphorus was determined spectrophotometrically following the molybdenum reaction solution described by Chen et al. (1956).

4.2.4 Measurement of leaf water potential and photosynthetic rate

The leaf Ψ_{LW} was measured according to the method described by Saneoka et al. (1995) using the uppermost fully expanded leaf employing a pressure chamber (Daiki-Rika Instruments, Tokyo, Japan) at 14 d after the initiation of the salt treatment. The Pn, gs and Tr of the third uppermost fully expanded leaves from the top of the plants were determined by using a portable open gas exchange system (LI-6400P model, Li-Cor, Inc., Lincoln, NE, USA). The photosynthetic photon flux density was maintained at 1,000 μmol m^{-2} s^{-1}. The

temperature of the leaf was 25°C and the ambient CO_2 concentration of the measurement chamber was 380 μL L^{-1} when measurements were taken.

4.2.5 Membrane permeability

Membrane permeability can be expressed by electrolyte leakage rate (ELR) which was measured with the method described by Lutts *et al*. (1996). Fresh leaves (1 g) were cut into pieces of 5-mm length and equally placed into test vials containing 30 ml deionized water. The vials were incubated at 25°C on a rotary shaker for 12 h, and then the initial electrical conductivity (EC_1) was measured using a DDS-11C conductivity meter (Hongyi Company, Shanghai, China). Then the vials were autoclaved at 120°C for 20 min to release all electrolytes and finally cooled to 25°C for the measurement of the electrical conductivity (EC_2). ELR can be defined as follows:

$$ELR\ (\%) = EC_1 / EC_2 \times 100$$

4.2.6 Determination of sugar and starch content

The soluble sugars were extracted by boiling 50 mg of dry powdered plant material with 10 ml of 80% ethanol at 80°C for 20 mins. A clear extract was obtained by centrifugation at 3000 rpm for 5 min and collected into a 50 ml beaker. This step was repeated twice and the collected supernatant was heated at 80°C to remove ethanol. Then the residues were kept in a 50 ml volumetric flask and made up with distilled water and the aliquot was taken for the estimation of the content of soluble sugar with the anthrone reagent by spectrophotometer (U-2001, Hitachi, Japan) using D-glucose solution as a standard according to the method of Yemm and Willis (1954). The residues after ethanolic extraction were dissolved in perchloric acid (9.2 and 4.6 N) and the supernatant collected into a 100 ml volumetric flask and made up with distilled water. The aliquot was taken for the estimation

of the content of starch with the anthrone reagent by spectrophotometer (U-2001, Hitachi, Japan) using D-glucose solution as a standard.

4.2.7 Statistical analysis.

Data were examined using one-way ANOVA and presented as the mean ± S.E. for each treatment and species (n=4). Multiple comparisons of means of data among different saline and alkaline treatments within the plants were performed using Duncan's test at the 0.05 significance level (all tests were performed with SPSS Version 16.0 for Windows).

4.3 RESULTS

4.3.1 Plant growth

Plant growth was evaluated using the height and leaf area and a significant difference in plant height was observed among the SS and AS treated plants and untreated plants (Fig. 4.1). The plant height increased remarkably and consistent with the control plants with the exogenous application of CA1, CA2 and proline increased under SS conditions and CA1 and CA2 under AS conditions. SS and AS treatments significantly reduced the leaf area and dry weight as compared to unstressed plants with the reduction more in SS than in AS plants. The use of exogenous CA and proline a little bit reversed the reduced leaf area in all cases (Fig. 4.2). Plant dry weight under SS and AS decreased 42 and 47% (relative to the control), respectively (Fig. 4.3). However, due to the addition of exogenous CA1, CA2 and proline the reduction in SS plants were 28, 23 and 30%, and in AS plants 34, 33 and 40%, respectively. The role of CAs in affecting plant growth is a little bit more significant than proline.

4.3.2 Electrolyte leakage rate (ELR)

The ELR is a good strain index as it reflects the degree of plant injury as a result of environmental stresses. It substantially increases in the leaves of SS and AS treated plants as

compared to unstressed plants and the injury can be more severe in AS plants than in SS plants (Fig. 4.4). Application of CAs and proline significantly reduced the injury in the stressed plants and the rate of reduction was greater in AS condition, and CA is better than proline in maintaining membrane permeability under both conditions.

4.3.3 Water status

Relative water content (RWC)

The RWC in the leaves exposed to SS and AS conditions decreased significantly and the rate of reductions under AS conditions was greater than those under SS (Fig. 4.5). However, the uses of CA and proline have significantly increased the RWC in the leaves of SS and AS plants and the rate of increment were greater in SS than in AS plants. Nonetheless, no statistical differences were observed among the treatments but the effectiveness of containing the water in the leaf cells was higher in CA treated plants, while proline was less effective under AS condition.

Leaf water potential

The stress treatments caused a significant reduction of the Ψ_{LW} in the plants and the extent of the reductions under AS was greater (2.6-fold) than that under SS (1.6-fold) (Fig. 4.6). However, CA and proline addition led to significant improvement that were almost equal between SS (24~26%) and AS (17~26%) conditions. There was no significant difference among the treatments of CA1, CA2 and proline under both SS and AS conditions. Proline was a less responsive treatment under AS condition where it did not induce a significant increase as it did to SS plants.

4.3.4 Chlorophylls and gas exchange characters

Under stressful conditions, the plants undergo a rapid and significant effect on the chlorophyll concentration and gas exchange characteristics (Pn, gs and Tr) as compared to

the unstressed plants while the damaging effects were more severe in AS than in SS plants (Tables 4.1 and 4.2). The addition of CA and proline significantly relieved the effects of saline stress based on these parameters. The role of proline was less effective under AS condition. No remarkable differences were observed between CA1 and CA2 in all traits examined.

4.3.5 Ionic status

Sodium

Na concentrations in the leaves, stems and roots increased significantly with the intensification of stresses, and the increases under the AS condition were significantly greater than those under SS in all plant segments with the exception of the roots, which contained a significantly higher concentration of Na (two times more) under the SS condition (Table 4.3). SS plants transported a lesser amount of accumulated Na (32%) from roots to leaves, contrary to AS plants which were unable to retain the Na that it transported greater amount (43%) from roots to leaves (Table 4.4). However, exogenous application of CA and proline effectively reduced the Na concentrations in all plant segments and proline was less effective in the leaves and stems under SS and in the roots under AS conditions. CA and proline reduced the Na accumulation in the leaves (Table 4.4).

Potassium

AS caused a significant decrease in the K concentration in all plant segments and a similar trend was observed only in the roots of SS plants. The shoots (leaves and stems) achieved greater concentration of K than roots under both stress conditions and significantly lower concentrations of K were observed in all plant parts under AS compared to those under SS (Table 4.5). However, the use of CA and proline slightly increased K concentration in the leaves and roots under AS conditions and significant increase was observed in the roots of SS

plants. K uptake by the roots was also increased with the exogenous application of CA and proline (Table 4.6).

Na / K ratio

The ratio of Na / K increased under both stresses and it was greater under AS compared to SS in all plant parts (Table 4.7). Compared with the untreated stressed plants, the use of CAs and proline decreased the ratio in all cases except for the stem of SS plants treated with proline.

Calcium

Calcium concentration was reduced significantly in the leaves and stems by AS, and in the stems by SS (Table 4.8). The leaves contained a higher concentration than the stems and roots under both stresses. However, application of CA and proline led to increase Ca concentration in all plant parts and a remarkable increase was observed in the leaves and roots (except proline) of SS treated plants and in the stems and roots (except CA1) of AS treated plants (Table 4.8). Ca accumulation in the roots and stems was also increased by the application of CA and proline under AS conditions (Table 4.9).

Magnesium

SS and AS reduced the Mg concentration in all plant segments but a remarkable reduction was observed only in the stems of alkaline stressed plants (Table 4.10). The application of CA significantly enhanced Mg concentration in all plant parts under SS condition and CA1 treated stems under AS condition.

Iron

SS and AS remarkably decreased Fe concentration in all plant parts, and the decrease was greater under AS than that of SS condition (Table 4.12). However, exogenous CA and proline slightly increased Fe concentration in all plant parts and significant increase was observed in roots and leaves under SS and in roots and stems under AS conditions. Fe uptake increased a little bit in the leaves under SS and roots under AS conditions (Table 4.13).

4.3.6 Phosphorus

Phosphorus (P) concentration in the leaves and roots of SS and AS plants decreased and the reduction was greater under AS than that of SS conditions (Table 4.14). However, the exogenous CA significantly increased P concentration in the leaves and roots under SS and in the roots under AS conditions. P accumulation in the stems and roots also increased in CA and proline treated plants under AS conditions (Table 4.15), and the relative influence of CA on P acquisition was higher than that of proline.

4.3.7 Total nitrogen

Total N concentrations were dropped as a result of exposure to the stresses relative to the controls and the relative reduction was higher in AS plants than in SS plants (Figs. 4.7). With exogenous application of CA and proline in stressed plants, the situation improved significantly, increased N concentration compared to those of stressful plants except in the leaves of SS plants (Fig. 4.7) where CA and proline induced a little but non-significant increase. N accumulation in the roots also increased due to application of CA and proline (Table 4.16).

4.3.8 Citric acid

Compared to unstressed leaves, internal citric acid concentration increased significantly under SS and AS conditions and the increase was remarkably higher in AS than in SS conditions (Fig. 4.8). However, the exogenous application of CA and proline in the AS plants significantly reduced internal CA concentration as compared to stressed (AS) plants. There was no remarkable decrease of internal CA concentration in salt stressed plants with the exogenous CA and proline application.

4.3.9 Proline

Proline concentration in the leaves significantly increased under saline and alkaline conditions than in unstressed plants (Fig. 4.9). Exogenous application of CA1 and CA2 and proline significantly reduced the internal proline concentration in the leaves of both SS and AS treated plants. Exogenous proline significantly reduced internal proline concentration only in SS treated plants. There were no statistical differences among the different levels of CAs and proline under SS conditions but proline treated plants produced more internal proline (49.61 μmol g^{-1} DW) than CA1 and CA2 treated plants did (48.05 and 45.46 μmol g^{-1} DW). Similar pattern of responses on the internal proline was also observed under AS conditions with the greater difference in the concentration between proline treated and CAs treated plants. No statistical difference was observed between stressed (control) and proline-treated stressed plants.

4.3.10 Total soluble sugar and starch

The sugar and starch contents in the leaves were significantly influenced by SS and AS treatments (Fig. 4.10). The highest amount of sugar content was recorded in both stressful situations, and the application of CAs and proline was found to be effective in reducing the sugar content. No significant variation was observed among the treatments under SS condition but CA1 reduced the sugar content effectively under AS condition. SS and AS markedly reduced the starch content in the leaves and the application of exogenous CAs and proline effectively counteracted the stresses by producing a greater amount of starch; proline was markedly less effective than CAs in increasing starch concentration under AS condition (Fig. 4.11).

4.4 DISCUSSION

4.4.1 Plant growth

Plant height, leaf area and dry matter accumulation are ideal indicators of plant growth. In this study, dry matter accumulation and leaf area were inhibited under both SS and AS conditions, and the effects of alkalinity were more severe (Figs. 4.1, 4.2 and 4.3). This can be explained as SS generally involves osmotic stress and ion-induced injury, whereas alkalinity exerts the same stress mechanisms even in low concentration of AS. The added influence of high pH in the root zone further inhibits plant growth intensely. These results were in agreement with the previous studies reported by Munns (2002), James et al. (2002), Shi and Sheng (2005). Many of the published data show high pH as a key factor in limiting plant growth and development under alkaline conditions (Yang et al., 2007, 2008a, c, 2009a). However, with the exogenous application of CA or proline, the height of the plants increased slightly under SS condition but increased significantly under AS condition, particularly in the case of CA2 treatment. The leaf area as well as dry matter of plants significantly increased with the addition of exogenous CA under both stresses. Sun and Hong (2010a) also reported the similar results in *Leymus chinensis* (Trin.) Tzvel., the Chinese lyme grass.

4.4.2 Electrolyte leakage rate (ELR)

Electrolyte leakage rate is an important plant traits to asses the tissue injury in plant under stress. Generally, intensifying stress causes increasing injury of plasma membranes thus leading to increasing ELR. The use of ELR to document the degree of stress injury to plants has been reported by many authors (Surjus and Durand, 1996; Li et al., 2010). The membrane permeability decreased with the intensification of SS and AS, and the severity of damage higher in AS plants (Fig. 4.4). AS also induced severe reductions in water content (Fig. 4.5), leaf water potential and a sharp increase in ELR indicating that high-pH from AS might have damaged root structure and functions as seen in reduced absorption of water (Fig. 4.5) and leaf water potential (Fig. 4.6), which may have caused decreasing membrane

permeability. However, the addition of exogenous CA and proline to plants under SS and AS conditions significantly reduced the leakage rate (Fig. 4.4). Stress impaired membrane permeability (increasing electrolyte leakage rate) was alleviated by the application of proline and glycinebetaine as reported by Mansour (1998) and Gadallah (1999) and by the application of salicylic acid as reported by Stevens *et al*. (2006) and Tuna et al. (2007). Those solutes might provide protection against the destabilization of proteins and membranes.

4.4.3 Water status

Relative water content

The reduced water content in the leaves of plants subjected to SS and AS improved with the application CAs and proline (Fig. 4.5) possibly due to the inhibition of water efflux through the effects of those solutes on the destabilization of membrane (Fig. 4.4) and reduced transpiration rate (Table 4.2). Nonetheless, those solutes may be involved in osmoregulation. Many authors reported that the reduced water content as a result of SS and AS can be alleviated with the addition of exogenous proline (Sun and Hong, 2010a, b), proline and glycinebetaine (Gadallah, 1999; Ahmed et al., 2010) and CA (Sun and Hong, 2010a).

Leaf water potential

The Ψ_{LW} sharply decreased under AS treatment as compared to SS treatment (Fig. 4.6). The restoration of better plant water status and inhibition of Na accumulation under CAs and proline treatments revealed the capacity for osmotic adjustment, which allows the growth to continue under stress conditions. This result is in agreement with the previous studies of Huber (2003) and Ahmed et al. (2010). The gs and Tr are closely correlated with changes in water potential under salt stress (Koyro et al., 2006) and salt-alkali stress (Liu et al., 2010).

4.4.4 Chlorphylls and gas exchange characters

SS and AS significantly reduced Pn compared to the control plants with AS causing a marked reduction of Pn as compared to SS conditions (Table 4.2). This phenomenon was also observed by many authors in both halophytes and glycophytes (Zhang and Mu, 2009; Li et al., 2010; Liu et al., 2010). The reduced plant Pn under stresses is generally considered a result of either reduced intracellular CO_2 partial pressure caused by stomatal closure, (Bethke and Drew, 1992), reduced photosynthetic pigments (Koyro et al., 2006) or ion toxicities in the cytosol (James et al., 2006). This finding pointed that changes of gs and Tr under SS and AS (more effective) might be a response to decreased Ψ_{LW} (Fig. 4.6). However, this may also be related to physiological drought that was caused by the reduction of water uptake by plants (Fig. 4.5). High pH caused by alkaline conditions may seriously affect stomatal opening and closing, and gas exchange. Leaf area directly affects photosynthetic production (Yang et al., 2008b) thereby affecting growth and metabolism (Sheng et al., 1999). Improved photosynthesis in stressed plants by exogenous proline and CA application could be associated with an increase in gs along with Tr and chlorophyll concentrations (Tables 4.1 and 4.2). No evidence was found in the case of CA effects on Pn as well as on gs and Tr but the capability to significantly improve Pn under stress conditions has been reported for other solutes such as proline (Lopéz-Climent et al., 2008; Ahmed et al., 2010), glycinebetaine (Yang and Lu, 2005; Dubey, 2005; Raza et al., 2006; Nawaz and Ashraf, 2010) and ascorbic acid (Khodary, 2004). The increased Pn in CA and proline treated stressed plants might also be related to increased photosynthetic pigments (Table 4.1). This is in agreement with the results of Athar and Ashraf (2005) and Lawlor and Cornic (2002).

4.4.5 Ionic status

The primary physiological response of plants to osmotic stress is to undergo osmotic adjustments by the accumulation of ions in the vacuole. The metabolism of Na^+ and K^+ is an

important component of salt stress (Cheeseman, 1988) and it is essential to maintain lower Na^+ and higher K^+ in the cytoplasm for enzymatic processes (Munns and Tester, 2008). Usually, Na^+ increases and K^+ decreases in salt stressed plants (de Lacerda et al., 2003). This result showed that Na concentration increased and K concentration decreased in all plant parts under SS (except in the leaves) and AS conditions (Tables 4.3 and 4.5), a phenomenon perhaps related to plasma membrane being destroyed more severely by alkaline stress, which was demonstrated here in the form of increased ELR (Fig. 4.4). However, stressed plants receiving CA and proline substantially reduced Na concentration in all plant parts compared to untreated stressed plants. This might be aided through decreased transpiration rate, causing fewer ions to be carried through the transpiration stream. Exogenous application of proline resulted in a decrease in Na^+ and Cl^- accumulations and an increase in growth in barley (Lone et al., 1987). On the other hand, K concentration was increased in the leaves and roots by the application of exogenous CA and proline probably due to the reduced competitive inhibition between Na and K. This is in agreement with Gadallah (1999) using vaba bean, Kumar and Sharma (1989) using *Vigna radiata* and Khanna (1998) using *Raphanus* seedlings.

The lower Na / K ratio in plants has been considered a physiological trait indicator of salt tolerance in plants (Morsy et al., 2007). The results in this study conformed to those obtained by Chartzoulakis et al. (2002) and Kaya et al. (2007). The selectivity of low Na / K ratio in plants is an important control mechanism and a selection criterion for salt tolerance (Wenxue et al., 2003; Cuin et al., 2003). The reduced Ca, Mg and Fe concentrations due to SS and AS were revised by the exogenous CA and proline (Tables 4.8, 4.10 & 4.12). Rana and Rai (1996) showed that the exogenous proline promoted Ca uptake in *Phaseolus* seedlings. The CA efficiently mobilizes the di and tri-valent cations like Ca, Mg and Fe into soil solutions (Jones and Darrah, 1994).

4.4.6 Phosphorus

The reduced P concentration under SS and AS conditions was alleviated and increased by the addition of exogenous CA and proline and the relative influence of CA was higher than that of proline (Table 4.14). The CA has been hypothesized to be involved in the mobilization and solubilization of poorly soluble nutrients like P, reduced rhizosphere pH and increased activities of soil microbes which influenced the availability of P. This resul is in agreement with the previous studies of Micales (1997) and Jones et al. (2003).

4.4.7 Total nitrogen

Nitrogen is one of the most essential elements in all biological materials, playing an important role in the maintenance of intracellular ionic balance and osmotic adjustment when plants are under salt or alkali stress (Yang et al., 2007, 2008b, 2009a,b). Total N concentration is reduced more in AS than in SS plants. An explanation for this may be due to more concentration of Na under AS that competes with N in the form of NH_4^+ (Fig. 4.7) resulting higher accumulation of N in the roots (Table 4.16). Proline activated the acquisition of N more than CA in the roots of SS plants. This is in agreement with Kumar et al. (1990) who reported that exogenous proline mitigated the antagonistic effects of Na^+-NH_4^+ / Cl^--NO_3^-, and relaxed stresses and improved plant growth.

4.4.8 Citric acid

Plants induce intrinsic stress defense molecules such as organic materials to counteract environmental stresses (Shlizerman et al., 2007). In particular, the exudation of CA has been reported to be closely-related with alkaline stress (Timpa et al., 1986), high salinity (Fougére et al., 1991), aluminum poisoning (Ma and Furukawa, 2003), iron stress (Shlizerman et al., 2007). In this study, alkaline stressed plants increased CA concentration

prominently in the leaves. However, exogenous CA reduced the internal CA concentration by deducting stress damage effects (Fig. 4.9).

4.4.9 Proline

Biosynthesis of compatible solutes in the cytosol for osmotic adjustment is one of the primary physiological responses of plants under stress conditions. Accumulated proline under SS and AS is usually considered as an organic-compatible osmolyte, a protecting agent for the activity of intracellular macromolecules (Tang, 1989) and a nitrogen source for plant growth (Okuma et al., 2000). In this study, proline content increased under both stresses and it is more pronounced under lower level of AS (50 mM) than higher levels of SS (75 mM) (Fig. 4.10). This suggests that the induction of proline synthesis is related to the severity of the stress, which is induced by high pH. However, the use of exogenous CA and proline in SS and AS plants remarkably reduced the proline concentration. Exogenously-supplied proline provided osmoprotection (Csonka and Hanson, 1991; Yancey, 1994), protected cell membranes from salt-induced oxidative stress (Yan et al., 2000), increased activities of superoxide dismutase and peroxidase, (Hua and Guo, 2002), decreased Na^+ and Cl^- accumulations, and facilitated growth (Lone et al., 1987).

4.4.10 Total soluble sugar and starch

Total soluble sugar (TSS) concentration in the leaves significantly increased, on the other hand, starch concentration decreased under stressful situations. The increments and decreases of those solutes were greater under AS as compared to SS (Fig. 4.11). It is well documented that plants under osmotic stress conditions accumulate compatible solutes for osmotic adjustment. Plants grown under AS conditions faced more severe stress and produced more TSS than those under SS conditions. This result is in agreement with the studies of Jiménez-Bremont et al. (2006), Khadri et al. (2007) and Palma et al. (2009).

Stressed plants treated with CAs and proline revealed a significant reduction of elevated TSS by protecting enzymes and membranes thereby reducing stress (Okuma et al., 2000; El-Tayeb, 2005). On the other hand, the decrease of starch concentration the stressed plants (Fig. 4.11) might be related to the reduced photosynthetic activities. Similar results were also reported by Rathert (1985) and Murakeozy et al. (2003).

Under SS and AS conditions plant growth arrested and decreased due to damaging root structure and functions (increased ELR), maintained lower water status (reduced RWC and Ψ_{LW}), inhibited metabolism and uptake of nutrients (reduced N, P, K, Ca, Mg and Fe), reduced Pn (by damaging chlorophylls and lower water) and unable to restrict toxic Na accumulation. However, the exogenous application of CA and proline remarkably improved all the aforementioned plant traits and maintained good plant growth. CA and proline contain more carboxylic groups and varying negative charges which favor the complexation of metal cations in solution and the displacement of anions from the soil matrix by the mobilisation and uptake of nutrients and the detoxification of metals, microbial proliferation in the rhizosphere, and the dissolution of soil minerals. CA also involves in energy production as intermediates in the tricarboxylic (TCA) cycle, balances cation charges or for maintaining osmotic potential, governs primarily C fixation. The role of CA in alleviating AS should be further investigated in other crops.

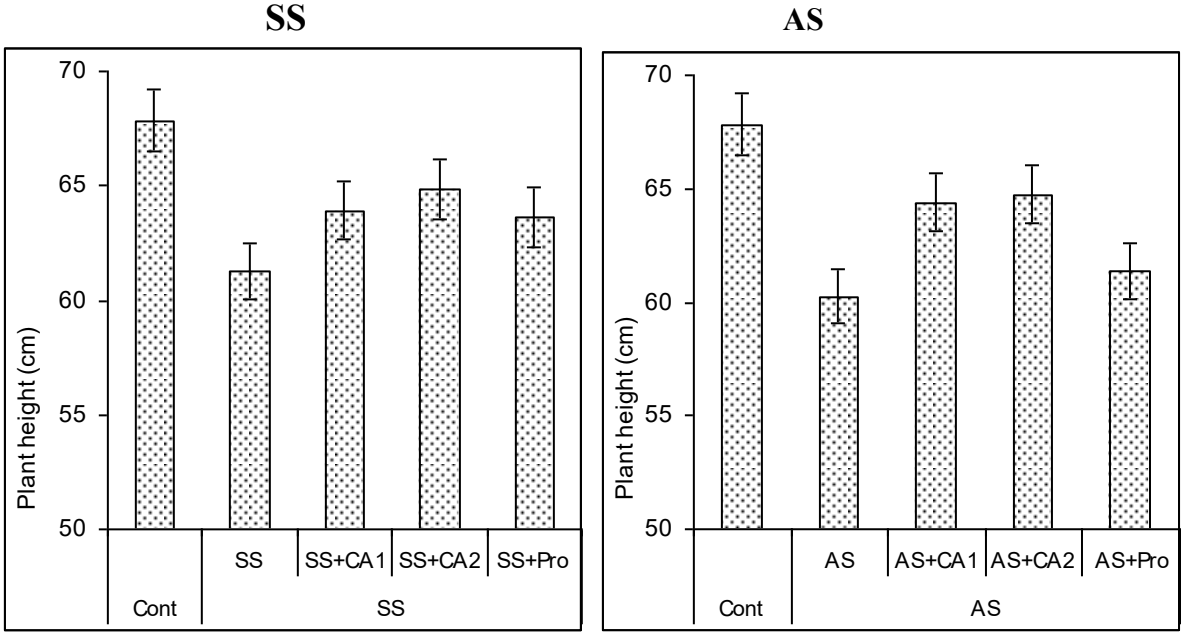

Fig. 4.1 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on plant height of Foxtail millet under SS and AS conditions. The values are the means (± S.E) of four replicates.

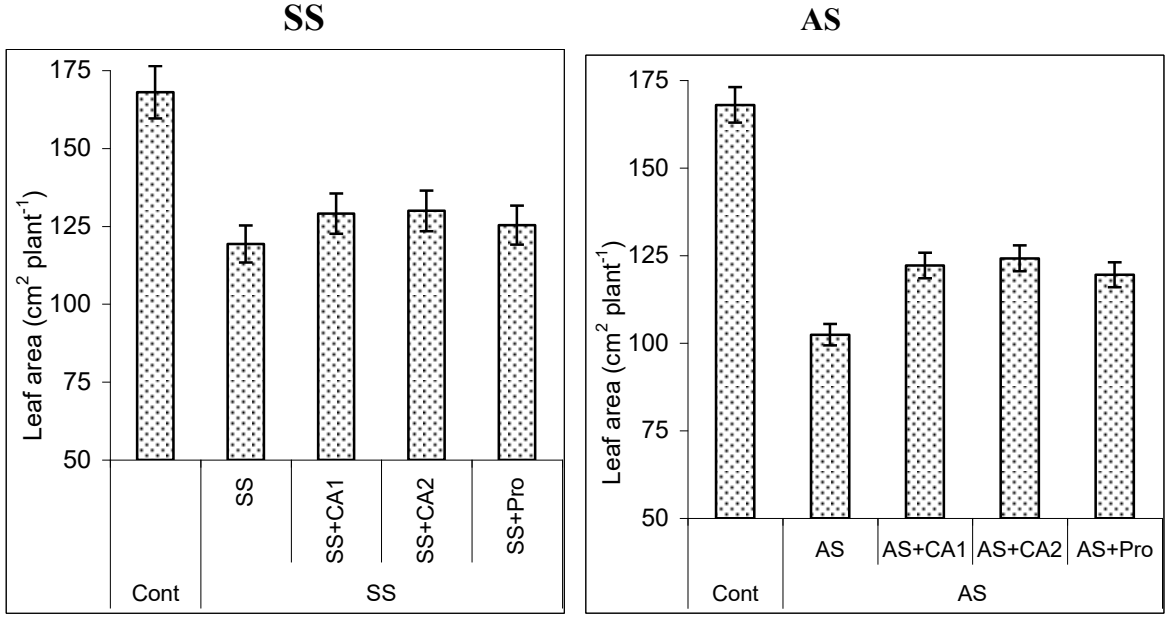

Fig. 4.2 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on the leaf area of Foxtail millet under SS and AS conditions. The values are the means (± S.E) of four replicates.

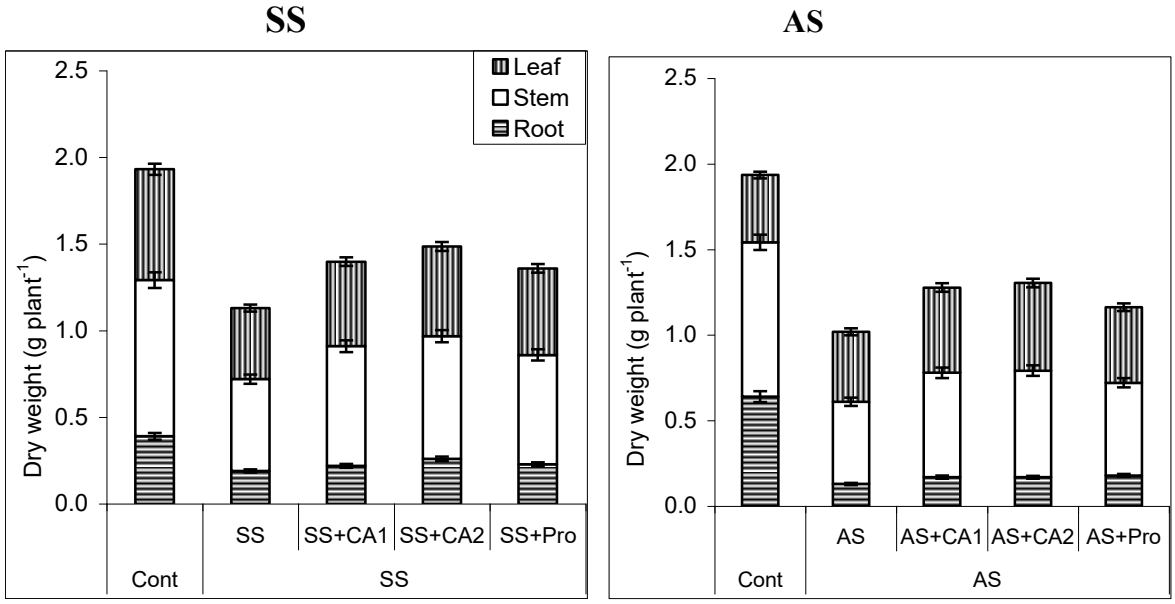

Fig. 4.3 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on the plant dry weight of Foxtail millet under SS and AS conditions. The values are the means (± S.E) of four replicates.

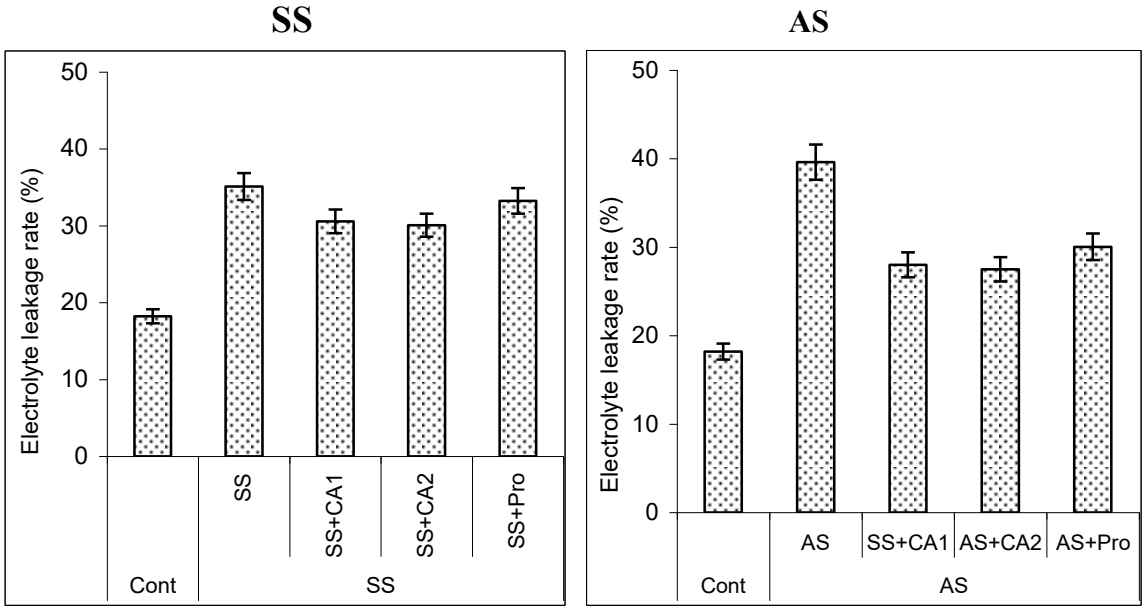

Fig. 4.4 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on the electrolyte leakage rate in the leaves of Foxtail millet under SS and AS conditions. The values are the means (± S.E) of four replicates.

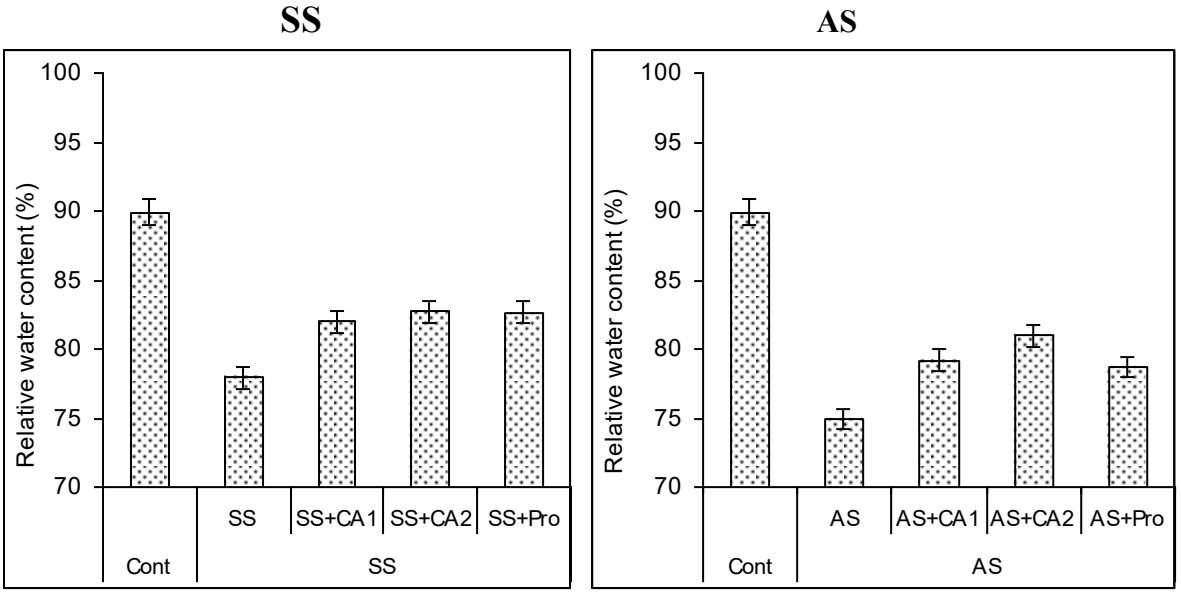

Fig. 4.5 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on the relative water content in the leaves of Foxtail millet under SS and AS conditions. The values are the means (± S.E) of four replicates.

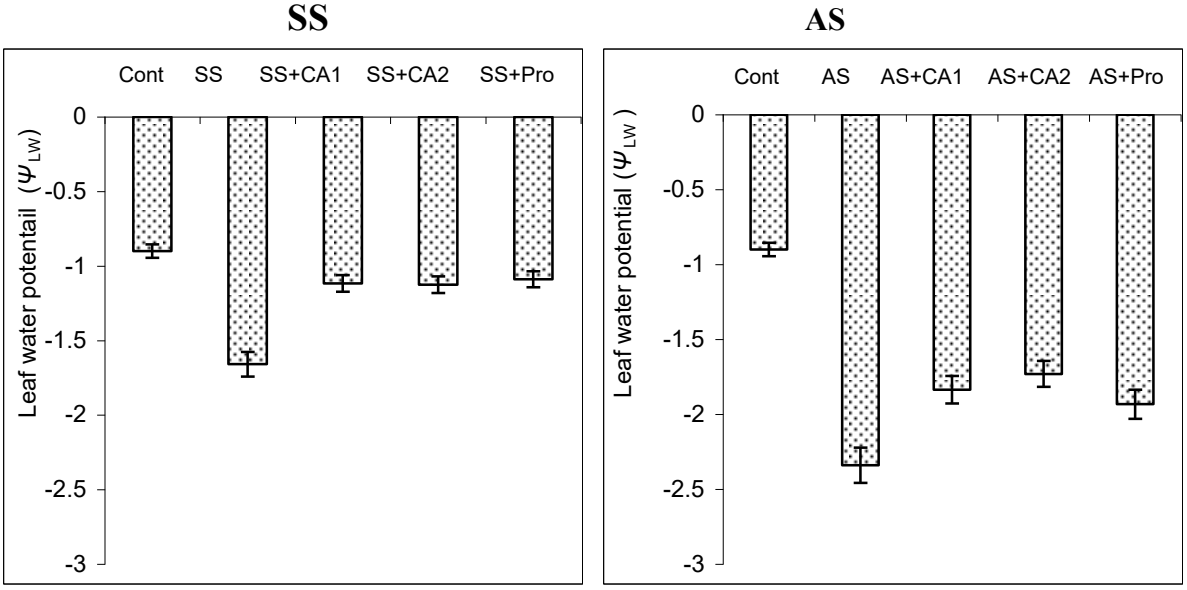

Fig. 4.6 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on leaf water potential of Foxtail millet under SS and AS conditions. The values are the means (± S.E) of four replicates.

Table 4.1 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on Chl a, Chl b, total Chl and Chl a/b ratio of Foxtail millet under SS and AS conditions. The values are the means (± S.E) of three replicates.

Treatment groups	Treatments	Chlorophylls			
		Chl a (mg g^{-1})	Chl b (mg g^{-1})	Total Chl	Chl a/b
Control		0.210±0.13^{abc}	0.359±0.36^{a}	0.569±0.45^{a}	0.59±0.13^{d}
SS	SS	0.210±0.08^{abc}	0.314±0.09^{b}	0.524±0.72^{b}	0.68±0.39^{cd}
	SS+CA1	0.216±0.10^{ab}	0.375±0.52^{a}	0.591±0.53^{a}	0.58±0.02^{d}
	SS+CA2	0.223±1.53^{a}	0.384±0.44^{a}	0.607±0.38^{a}	0.58±0.17^{d}
	SS+Proline	0.209±4.15^{abc}	0.372±0.27^{a}	0.581±0.26^{a}	0.56±0.01^{d}
AS	AS	0.068±0.24^{e}	0.025±0.07^{e}	0.093±0.30^{e}	2.69±0.11^{a}
	AS+CA1	0.182±0.86^{c}	0.117±0.98^{c}	0.299±1.74^{c}	1.63±0.15^{c}
	AS+CA2	0.189±1.19^{bc}	0.108±0.74^{c}	0.298±1.93^{c}	1.76±0.05^{c}
	AS+Proline	0.152±0.63^{d}	0.077±0.35^{d}	0.229±0.98^{d}	2.00±0.07^{b}

In a column, values with the same letter are not significantly different, and those with different letters are significantly different at P<0.05 using Duncan test, values are means ±S.E (n=3).

Table 4.2 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on photosynthesis (Pn), stomatal conductance (gs) and transpiration rate (Tr) of Foxtail millet under SS and AS conditions. The values are the means (± S.E) of three replicates.

Treatment groups	Treatments	Pn	gs	Tr
		(μmol CO_2 m^{-2} s^{-1})	(mol m^{-2} s^{-1})	(mmol m^{-2} s^{-1})
Control		23.15±0.70^{a}	0.138±0.01^{a}	3.28±0.21^{a}
SS	SS	7.06±1.30^{c}	0.038±0.00^{cd}	0.97±0.08^{cd}
	SS+CA1	12.78±1.71^{b}	0.059±0.01^{b}	1.45±0.24^{b}
	SS+CA2	12.18±0.70^{b}	0.061±0.00^{b}	1.37±0.09^{bc}
	SS+Pro	11.63±0.20^{b}	0.051±0.00^{b}	1.31±0.26^{bc}
AS	AS	1.21±0.01^{e}	0.007±0.00^{e}	0.19±0.04^{e}
	AS+CA1	3.68±0.40^{d}	0.026±0.01^{d}	0.46±0.06^{d}
	AS+CA2	3.59±0.31^{d}	0.025±0.00^{d}	0.42±0.02^{d}
	AS+Pro	2.89±0.38^{de}	0.010±0.00^{e}	0.26±0.04^{de}

In a column, values with the same letter are not significantly different, and those with different letters are significantly different at P<0.05 using Duncan test, values are means ±S.E (n=3).

Table 4.3 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on Na concentration (mg g^{-1} DW) in the leaves, stems and roots of Foxtail millet under SS and AS conditions.

Treatment groups	Treatments	Na		
		Leaves	Stems	Roots
Control		0.09±0.02^{g}	1.17±0.13^{e}	4.43±0.41^{e}
SS	SS	14.95±1.83^{cd}	19.00±1.03^{bc}	17.62±0.43^{a}
	SS+CA1	9.37±0.49^{f}	15.75±0.89^{d}	16.01±0.50^{b}
	SS+CA2	10.16±1.09^{ef}	15.79±1.16^{d}	16.98±0.49^{ab}
	SS+Pro	12.56±0.72^{de}	17.38±0.72^{cd}	15.78±0.61^{b}
AS	AS	21.04±0.61^{a}	22.59±0.38^{a}	9.05±0.24^{c}
	AS+CA1	17.63±0.97^{bc}	19.72±0.64^{b}	7.42±0.17^{d}
	AS+CA2	18.26±0.54^{ab}	19.92±0.33^{b}	7.36±0.29^{d}
	AS+Pro	17.42±1.34^{bc}	20.12±0.60^{b}	8.95±0.50^{c}

In a column, values with the same letter are not significantly different, and those with different letters are significantly different at $P<0.05$ using Duncan test, values are means ±S.E (n=4).

Table 4.4 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on Na accumulation (mg $plant^{-1}$) in the leaves, stems and roots of Foxtail millet under SS and AS conditions.

Treatment groups	Treatments	Na			
		Leaves	Stems	Roots	Total
Control		0.06 (2)	1.05 (37)	1.74 (61)	2.85 (100)
SS	SS	6.12 (31)	9.98 (51)	3.38 (17)	19.48 (100)
	SS+CA1	4.58 (24)	10.90 (57)	3.52 (19)	19.00 (100)
	SS+CA2	5.26 (25)	11.17 (54)	4.42 (21)	20.85 (100)
	SS+Proline	5.94 (29)	10.99 (54)	3.60 (18)	20.54 (1009
AS	AS	9.18 (43)	10.95 (51)	1.28 (6)	21.42 (100)
	AS+CA1	8.87 (40)	12.07 (54)	1.29 (6)	22.22 (100)
	AS+CA2	9.05 (41)	11.94 (54)	1.25 (6)	22.24 (100)
	AS+Proline	7.75 (39)	10.93 (54)	1.45 (7)	20.12 (100)

( ): Na partitioning as percentage in leaves, stems and roots.

Table 4.5 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on K concentration (mg g^{-1} DW) in the leaves, stems and roots of Foxtail millet under SS and AS conditions.

Treatment groups	Treatments	K		
		Leaves	Stems	Roots
Control		29.51±0.46^{ab}	32.17±0.15^{a}	4.73±0.31^{a}
SS	SS	33.98±0.34^{a}	31.38±1.19^{a}	3.37±0.19^{c}
	SS+CA1	33.08±1.02^{a}	30.86±0.86^{ab}	4.14±0.32^{b}
	SS+CA2	32.09±0.90^{a}	28.51±1.20^{b}	3.50±0.11^{c}
	SS+Pro	31.97±0.58^{a}	28.08±0.54^{b}	3.38±0.10^{c}
AS	AS	25.35±0.51^{c}	25.24±0.79^{c}	1.36±0.06^{d}
	AS+CA1	26.85±0.90^{bc}	24.12±0.25^{c}	1.76±0.03^{d}
	AS+CA2	26.74±1.03^{bc}	24.15±1.18^{c}	1.58±0.02^{d}
	AS+Pro	26.36±0.34^{bc}	24.57±1.38^{c}	1.47±0.13^{d}

In a column, values with the same letter are not significantly different, and those with different letters are significantly different at $P<0.05$ using Duncan test, values are means ±S.E (n=4).

Table 4.6 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on K accumulation (mg $plant^{-1}$) in the leaves, stems and roots of Foxtail millet under SS and AS conditions.

Treatment groups	Treatments	K			
		Leaves	Stems	Roots	Total
Control		18.91 (38)	29.00 (58)	1.86 (4)	49.78 (100)
SS	SS	13.91 (45)	16.49 (53)	0.65 (2)	31.05 (100)
	SS+CA1	16.15 (42)	21.55 (56)	0.91 (2)	38.62 (100)
	SS+CA2	16.63 (44)	20.16 (53)	0.91 (2)	37.71 (100)
	SS+Proline	15.95 (46)	17.76 (51)	0.77 (2)	34.48 (100)
AS	AS	11.44 (48)	12.23 (51)	0.18 (1)	23.85 (100)
	AS+CA1	12.75 (46)	14.78 (53)	0.27 (1)	27.80 (100)
	AS+CA2	13.43 (48)	14.45 (51)	0.25 (1)	28.14 (100)
	AS+Proline	11.95 (47)	13.35 (52)	0.31 (1)	25.60 (100)

( ): K partitioning as percentage in leaves, stems and roots.

Table 4.7 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on Na / K ratio in the leaves, stems and roots of Foxtail millet under SS and AS conditions.

Treatment groups	Treatments	Na / K		
		Leaves	Stems	Roots
Control		0.003	0.04	0.94
SS	SS	0.44	0.61	5.23
	SS+CA1	0.28	0.51	3.88
	SS+CA2	0.32	0.55	4.85
	SS+Pro	0.39	0.62	4.67
AS	AS	0.83	0.90	6.65
	AS+CA1	0.66	0.82	4.70
	AS+CA2	0.68	0.82	5.01
	AS+Pro	0.65	0.82	5.09

Table 4.8 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on Ca concentration (mg g^{-1} DW) in the leaves, stems and roots of Foxtail millet under SS and AS conditions.

Treatment groups	Treatments	Ca		
		Leaves	Stems	Roots
Control		1.75 ± 0.03^{ab}	0.41 ± 0.02^{a}	0.48 ± 0.02^{cd}
SS	SS	1.35 ± 0.09^{c}	0.38 ± 0.03^{ab}	0.45 ± 0.02^{d}
	SS+CA1	1.94 ± 0.09^{a}	0.45 ± 0.00^{a}	0.59 ± 0.02^{ab}
	SS+CA2	1.85 ± 0.11^{a}	0.44 ± 0.03^{a}	0.57 ± 0.03^{abc}
	SS+Pro	1.79 ± 0.03^{a}	0.42 ± 0.03^{a}	0.46 ± 0.07^{cd}
AS	AS	1.36 ± 0.09^{c}	0.15 ± 0.04^{d}	0.46 ± 0.03^{cd}
	AS+CA1	1.45 ± 0.03^{c}	0.30 ± 0.01^{c}	0.56 ± 0.01^{abc}
	AS+CA2	1.54 ± 0.07^{bc}	0.33 ± 0.02^{bc}	0.64 ± 0.03^{ab}
	AS+Pro	1.41 ± 0.09^{c}	0.28 ± 0.02^{c}	0.68 ± 0.04^{a}

In a column, values with the same letter are not significantly different, and those with different letters are significantly different at $P<0.05$ using Duncan test, values are means ±S.E (n=4).

Table 4.9 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on Ca accumulation (mg plant^{-1}) in the leaves, stems and roots of Foxtail millet under SS and AS conditions.

Treatment groups	Treatments	Ca			
		Leaves	Stems	Roots	Total
Control		1.75	0.19	0.35	2.29
		(77)	(8)	(15)	(100)
SS	SS	1.35	0.09	0.21	1.66
		(82)	(5)	(13)	(100)
	SS+CA1	1.94	0.13	0.31	2.38
		(81)	(5)	(13)	(100)
	SS+CA2	1.85	0.15	0.31	2.31
		(80)	(6)	(13)	(100)
	SS+Proline	1.79	0.11	0.26	2.16
		(83)	(5)	(12)	(100)
AS	AS	1.36	0.06	0.07	1.49
		(91)	(4)	(5)	(100)
	AS+CA1	1.44	0.10	0.18	1.72
		(84)	(6)	(11)	(100)
	AS+CA2	1.54	0.11	0.19	1.85
		(83)	(6)	(11)	(100)
	AS+Proline	1.41	0.12	0.15	1.68
		(84)	(7)	(9)	(100)

( ): Ca partitioning as percentage in leaves, stems and roots.

Table 4.10 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on Mg concentration (mg g^{-1} DW) in the leaves, stems and roots of Foxtail millet under SS and AS conditions.

Treatment groups	Treatments	Mg		
		Leaves	Stems	Roots
Control		2.32±0.09^{abc}	1.33±0.02^{b}	0.50±0.01^{bc}
SS	SS	2.07±1.76^{bc}	1.36±0.04^{b}	0.49±0.02^{c}
	SS+CA1	2.50±0.04^{a}	1.54±0.04^{a}	0.58±0.01^{a}
	SS+CA2	2.48±1.81^{a}	1.48±o.06^{a}	0.56±0.02^{ab}
	SS+Pro	2.40±3.93^{ab}	1.47±0.03^{a}	0.51±0.03^{bc}
AS	AS	1.97±1.76^{c}	1.14±0.03^{d}	0.34±0.01^{cd}
	AS+CA1	2.26±0.02^{abc}	1.28±0.06^{bc}	0.37±0.02^{c}
	AS+CA2	2.30±0.38^{abc}	1.19±0.03^{cd}	0.35±0.01^{c}
	AS+Pro	2.18±1.76^{abc}	1.17±0.04^{cd}	0.35±0.03^{c}

In a column, values with the same letter are not significantly different, and those with different letters are significantly different at P<0.05 using Duncan test, values are means ±S.E (n=4).

Table 4.11 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on Mg accumulation (mg plant^{-1}) in the leaves, stems and roots of Foxtail millet under SS and AS conditions.

Treatment groups	Treatments	Mg			
		Leaves	Stems	Roots	Total
Control		1.49 (52)	1.20 (42)	0.20 (7)	2.89 (100)
SS	SS	0.85 (50)	0.77 (45)	0.09 (5)	1.71 (100)
	SS+CA1	1.22 (51)	1.06 (44)	0.13 (5)	2.41 (100)
	SS+CA2	1.29 (52)	1.05 (42)	0.15 (6)	2.48 (100)
	SS+Proline	1.20 (53)	0.93 (42)	0.12 (5)	2.25 (100)
AS	AS	0.86 (59)	0.55 (38)	0.05 (3)	1.46 (100)
	AS+CA1	1.14 (59)	0.72 (38)	0.06 (3)	1.92 (100)
	AS+CA2	1.16 (60)	0.72 (37)	0.06 (3)	1.93 (100)
	AS+Proline	0.97 (56)	0.69 (40)	0.07 (4)	1.73 (100)

( ): Mg partitioning as percentage in leaves, stems and roots.

Table 4.12 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on Fe concentration (µg g^{-1} DW) in the leaves, stems and roots of Foxtail millet under SS and AS conditions.

Treatment groups	Treatments	Fe		
		Leaves	Stems	Roots
Control		306.5±22.9^{a}	206.9±13.1^{a}	2824.0±0.1^{a}
SS	SS	228.5±10.0^{cd}	166.2±4.4^{bc}	1948.4±0.1^{d}
	SS+CA1	266.4±11.5^{bc}	177.3±6.3^{bc}	2197.3±22.8^{bc}
	SS+CA2	267.0±11.5^{bc}	177.6±11.4^{bc}	2020.7±32.2^{cd}
	SS+Pro	270.4±15.3^{ab}	160.1±2.1^{bc}	2005.2±1.4^{cd}
AS	AS	214.1±7.8^{d}	158.1±2.7^{cd}	1827.5±24.9^{d}
	AS+CA1	221.9±5.4^{d}	173.6±10.2^{bc}	1981.8±27.2^{cd}
	AS+CA2	221.2±9.4^{d}	187.2±4.7^{b}	1954. 5±25.7^{d}
	AS+Pro	236.3±10.8^{bcd}	160.0±2.5^{bc}	2248.2±18.8^{b}

In a column, values with the same letter are not significantly different, and that with different letters are significantly different at $P<0.05$ using Duncan test, values are means ±S.E (n=4).

Table 4.13 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on Fe accumulation (μg plant^{-1}) in the leaves, stems and roots of Foxtail millet under SS and AS conditions.

Treatment groups	Treatments	Fe			
		Leaves	Stems	Roots	Total
Control		196.4 (13)	186.6 (12)	1110.8 (74)	1493.7 (100)
SS	SS	93.5 (17)	87.3 (16)	373.7 (67)	554.6 (100)
	SS+CA1	130.1 (18)	122.7 (17)	483.7 (66)	736.5 (100)
	SS+CA2	138.4 (18)	125.6 (16)	526.4 (67)	790.4 (100)
	SS+Proline	134.9 (19)	101.2 (15)	457.8 (66)	693.9 (100)
AS	AS	92.9 (22)	76.6 (19)	244.8 (59)	414.3 (100)
	AS+CA1	111.6 (20)	106.2 (19)	343.3 (61)	561.0 (100)
	AS+CA2	111.1 (20)	108.6 (20)	331.6 (60)	551.3 (100)
	AS+Proline	105.1 (18)	86.9 (15)	395.3 (67)	587.3 (100)

( ): Fe partitioning as percentage in leaves, stems and roots.

Table 4.14 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on P concentration (mg g^{-1} DW) in the leaves, stems and roots of Foxtail millet under SS and AS conditions.

Treatment groups	Treatments	P		
		Leaves	Stems	Roots
Control		1.75±0.04^{a}	2.01±0.04^{a}	0.98±0.04^{a}
SS	SS	1.46±0.01^{c}	1.84±0.05^{ab}	0.87±0.03^{b}
	SS+CA1	1.60±0.04^{b}	2.06±0.12^{a}	0.94±0.03^{ab}
	SS+CA2	1.53±0.01^{bc}	1.94±0.07^{a}	0.96±0.01^{a}
	SS+Pro	1.52±0.05^{bc}	1.89±0.04^{a}	0.91±0.01^{ab}
AS	AS	1.12±0.06^{d}	1.44±0.02^{c}	0.57±0.03^{d}
	AS+CA1	1.17±0.04^{d}	1.62±0.06^{bc}	0.65±0.01^{c}
	AS+CA2	1.24±0.03^{d}	1.62±0.05^{bc}	0.66±0.02^{c}
	AS+Pro	1.15±0.05^{d}	1.55±0.11^{c}	0.70±0.02^{c}

In a column, values with the same letter are not significantly different, and that with different letters are significantly different at P<0.05 using Duncan test, values are means ±S.E (n=4).

Table 4.15 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on P accumulation (mg plant^{-1}) in the leaves, stems and roots of Foxtail millet under SS and AS conditions.

Treatment groups	Treatments	P			
		Leaves	Stems	Roots	Total
Control		1.12 (34)	1.76 (54)	0.39 (12)	3.27 (100)
SS	SS	0.60 (34)	1.01 (57)	0.16 (9)	1.76 (100)
	SS+CA1	0.78 (32)	1.43 (59)	0.20 (8)	2.41 (100)
	SS+CA2	0.79 (33)	1.38 (58)	0.21 (9)	2.39 (100)
	SS+Proline	0.76 (35)	1.17 (54)	0.23 (11)	2.16 (100)
AS	AS	0.49 (38)	0.70 (56)	0.08 (6)	1.26 (100)
	AS+CA1	0.59 (35)	1.00 (59)	0.11 (7)	1.71 (100)
	AS+CA2	0.63 (37)	0.96 (57)	0.11 (7)	1.70 (100)
	AS+Proline	0.51 (33)	0.90 (59)	0.12 (8)	1.54 (100)

( ): P partitioning as percentage in leaves, stems and roots.

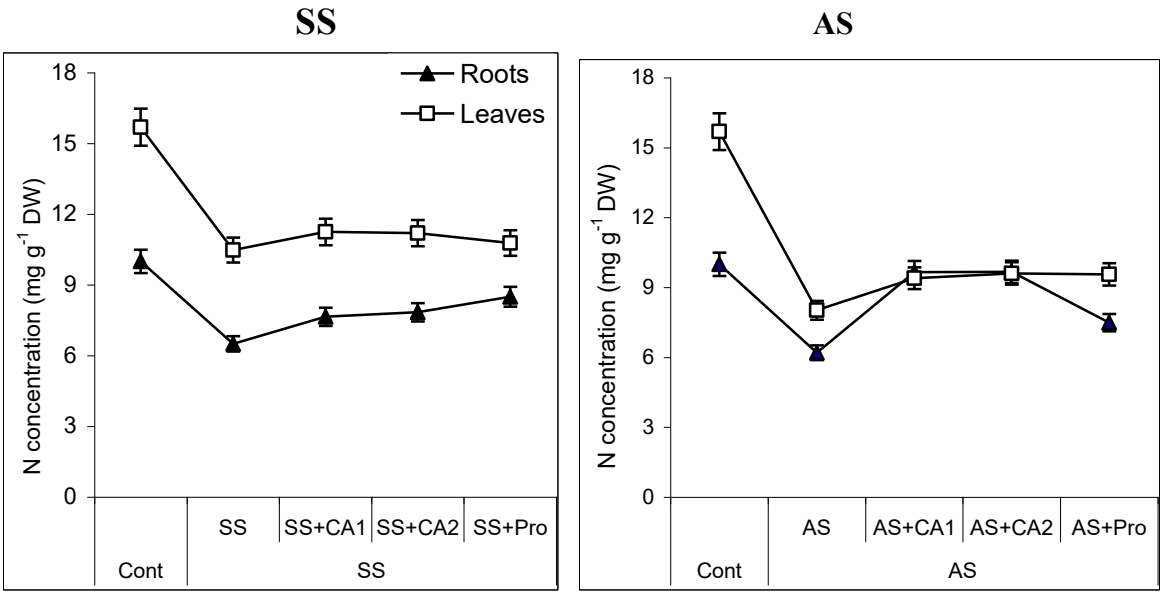

Fig. 4.7 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on the total N concentration in the leaves and roots of Foxtail millet under SS and AS conditions. The values are the means (± S.E) of four replicates.

Table 4.16 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on N accumulation (mg plant^{-1}) in the leaves, stems and roots of Foxtail millet under SS and AS conditions.

Treatment groups	Treatments	N		
		Leaves	Roots	Total
Control		10.05 (72)	3.93 (28)	13.98 (100)
SS	SS	4.30 (78)	1.21 (22)	5.50 (100)
	SS+CA1	5.50 (76)	1.71 (24)	7.21 (100)
	SS+CA2	6.00 (74)	2.14 (26)	8.14 (100)
	SS+Proline	5.36 (74)	1.91 (26)	7.27 (100)
AS	AS	3.66 (82)	0.82 (18)	4.47 (100)
	AS+CA1	4.76 (75)	1.58 (25)	6.34 (100)
	AS+CA2	4.84 (75)	1.63 (25)	6.47 (100)
	AS+Proline	4.25 (76)	1.31 (24)	5.56 (100)

( ): N partitioning as percentage in leaves, stems and roots.

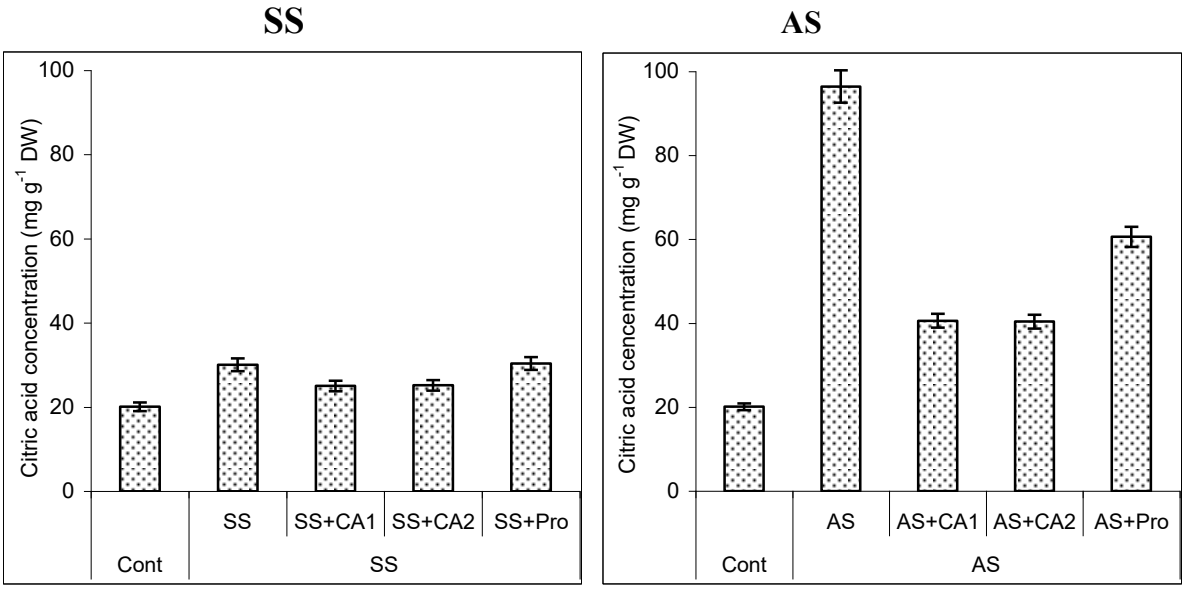

Fig. 4.8 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on the internal citric acid concentration in the leaves of Foxtail millet under SS and AS onditions. The values are the means (± S.E) of four replicates.

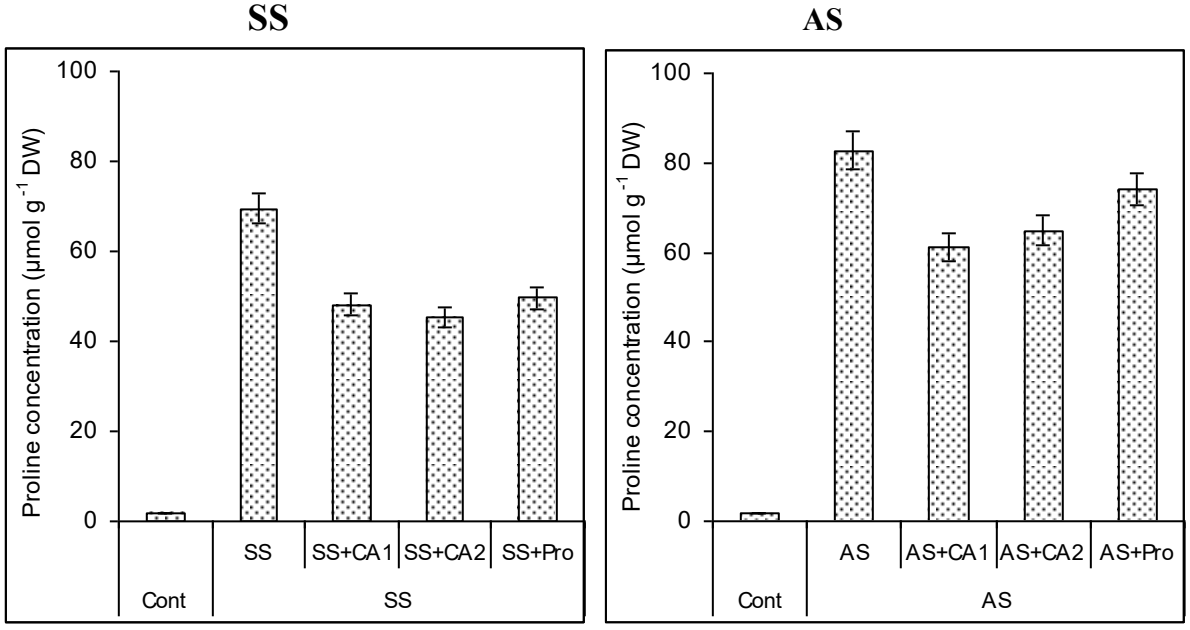

Fig. 4.9 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on the internal proline concentration in the leaves of Foxtail millet under SS and AS conditions. The values are the means (± S.E) of four replicates.

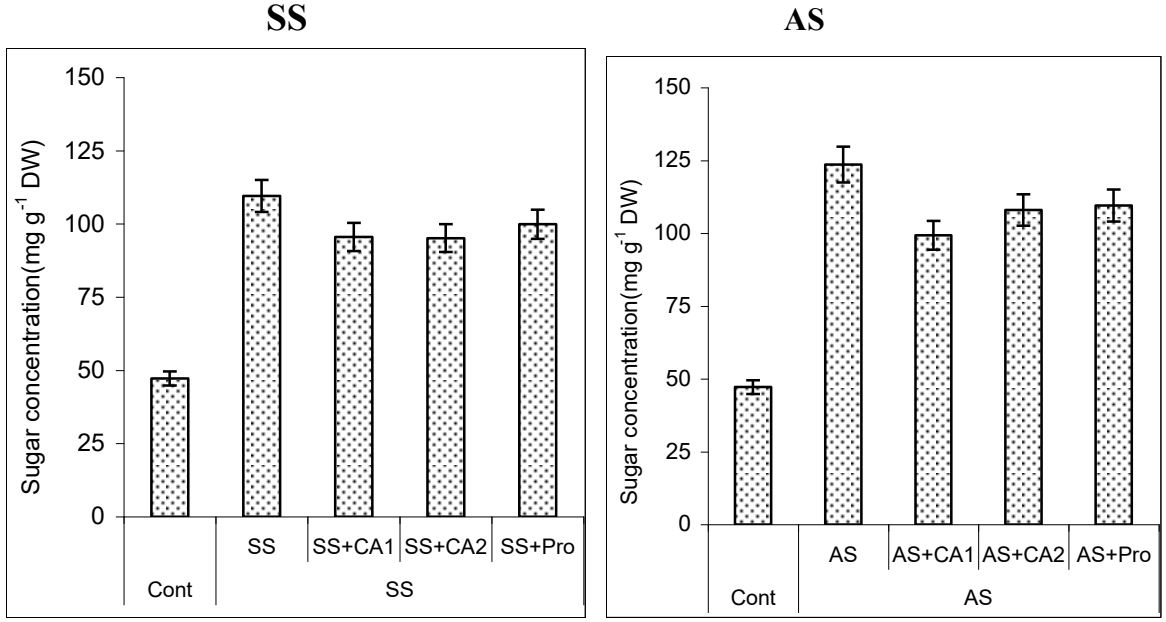

Fig. 4.10 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on the sugar content in the leaves of Foxtail millet under SS and AS conditions. The values are the means (± S.E) of four replicates.

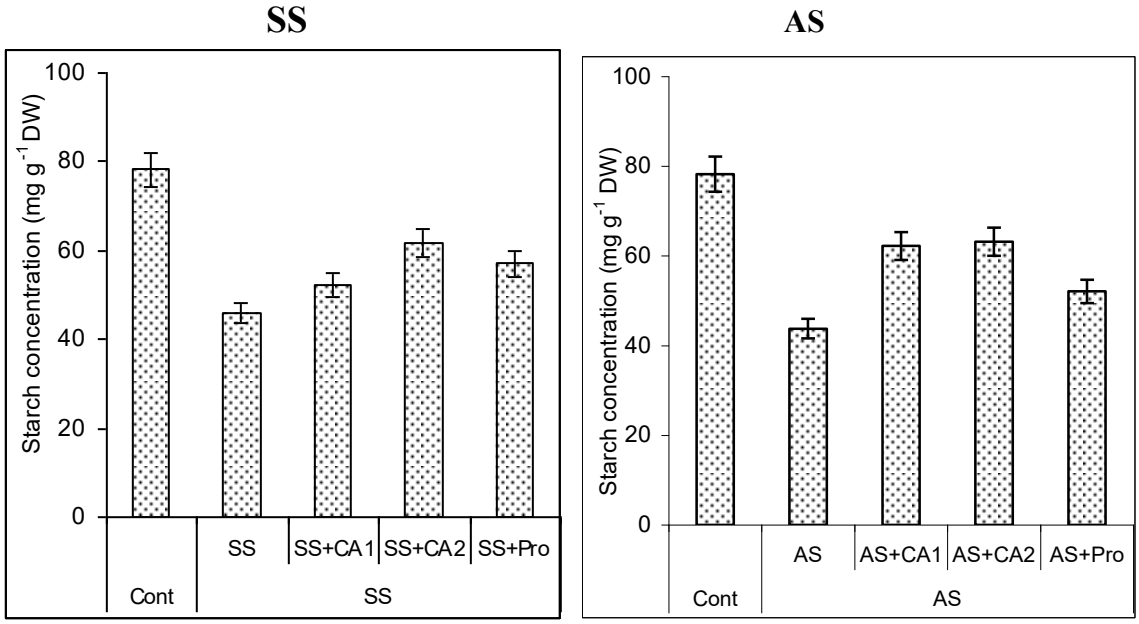

Fig. 4.11 Effect of CA1 (0.25 mM), CA2 (0.5 mM) and proline (0.5 mM) on the starch content in the leaves of Foxtail millet under SS and AS conditions. The values are the means (± S.E) of four replicates.

CHAPTER 5

GENERAL DISCUSSION

5.1 GENERAL DISCUSSION

5.1.1 Comparative studies on saline and alkaline stresses of Foxtail millet (*Setaria italica* L.) and Proso millet (*Panicum miliaceum* L.) and analysis their stress tolerance factors

Proso millet is comparatively more tolerant than Foxtail millet under high levels of SS and AS conditions (100 mM). Due to exposure to higher concentration of AS as well as the early application to the plant (4 weeks seedlings), Foxtail millet damaged early under AS condition, making it unavailable for the measurements of physiological attributes. Therefore, another study was conducted using lower concentration of SS and AS (50 and 75 mM) applied to six weeks plants to investigate in details and combined results are discussed this section.

The biomass production per plant significantly decreased with increasing stresses and the relative reduction was higher in Foxtail millet than in Proso millet under AS conditions. The reduced dry matter is correlated with the reduced leaf area, RWC, Ψ_{LW} (Munns, 2002) and increased ELR (Zhang and Mu, 2009) as a result of stresses. The effects of stress were higher in Foxtail millet as indicated its lower plant growth compared with Proso millet. The high pH in the root zone under AS was involved in inhibiting plant growth. These results were in agreement with the previous studies reported by Yang et al. (2007, 2008a, 2009a).

Plants reduce RWC as a quick and economical approach to osmotic adjustment in response to osmotic stress (Lissner et al., 1999). However, a greater reduction in the leaf area and in Foxtail millet indicates that it is a more sensitive species compared to Proso millet. The higher RWC in Proso millet under AS noticed its higher tolerance compared with Foxtail millet. The redeced Ψ_{LW} under salt-alkali stress is closely correlated with the reduction of gs and Tr (Liu et al., 2010).

It is reported that the Pn, gs and Tr of plants usually decrease with increasing salinity or alkalinity (Yang et al., 2009a,b). In this study lower level of AS (50mM) inhibited Pn, gs and Tr severely than the higher levels of SS (75 mM) in both species. This result is in agreement with Zhang and Mu (2009) who found that the Pn, gs and Tr of *Lathyrus quinquenervius* did not decrease under moderate (30 mM) saline stress as alkaline stress did.

The Na concentration was increased by SS and AS in all plant segments and K concentration was reduced in the stems and roots by the higher concentration SS and AS . This result indicates that there was no competitive inhibition between Na and K uptake in Proso millet species. Similar results were observed by Saneoka et al. (1995, 1999) in maize and wheat and Benderradji et al. (2011) in bread wheat. The high pH under AS might increase the interference with the selective absorption of K to Na in roots and elevated intracellular Na concentration to a toxic level for Foxtail millet. The lower ratio of Na / K in Proso millet indicated its high tolerance compared with Foxtail millet. The ability of plant to limit Na^+ transport into the shoot is critically important for the maintenance of high plant growth from the toxic effects of Na^+ (Razmjoo et al., 2008). Proso millet transported less amount of Na from root to shoot than Foxtail millet did, which might explain higher tolerance of Proso millet than Foxtail millet. The Ca and Mg concentration decreased more in AS plants. The high pH under AS reduced the availability of Ca and Mg in the root zones by precipitating them into $CaCO_3$ and $MgCO_3$. This result is in agreement with the results of Shi and Sheng (2005) and Yousif et al. (2010).

The N concentration decreased under SS and AS conditions and a marked reduction was observed in Foxtail under AS conditions. A lower concentration of N in Foxtail millet is thought to be the result of a higher accumulation of Na in plants which competes more with N as Na^+-NH_4^+ and / or NO_3^- and Cl^- in the uptake site of roots. The higher influence of Na^+

on NH_4^+ loading into the xylem ultimately reduced the N accumulation as reported by Bar et al. (1997), Marcelis and Van-Hooijdonk, (1999) and James et al. (2006).

Plants under stresses accumulate compatible solutes such as proline for osmotic adjustment and detoxification of injurious ions (Tammam et al., 2008), and proline accumulation in plants is correlated with stress tolerance (Younis et al., 2009). The greater concentration of proline in Foxtail millet indicates that Foxtail millet faced more stress than Proso millet. However, under higher stress conditions, SS induced more proline than AS but in lower stress conditions, AS induced more than SS although it was established that AS is more destructive than SS in both experiments. It might be due to the fact that higher concentration of AS severely damaged plant growth and Foxtail millet damaged earlier, stopping their accumulation of proline while the accumulation was still continued in plants under SS.

Plants under stress conditions accumulate TSS to adjust to osmotic stress (Palma et al., 2009). The increase of TSS concentration was greater under AS than under SS. This might be due to AS inducing higher stress forcing plants to accumulate more TSS to adjust to osmotic shock as like proline. The lower concentration of TSS in Proso millet indicates that SS and AS cause less stress effects in this species than in Foxtail millet. On the contrary, starch concentration decreased with increasing stresses and relative reduction was higher in Foxtail millet than in Proso millet under AS. This result is in agreement with previous result of Murakeozy et al. (2003). The reduced starch might be related to the lower RWC, water potential and ultimately the reduction of photosynthetic activities which yielded lower starch. The higher starch in Proso millet under stresses indicates its higher tolerance than Foxtail millet.

5.1.2 Effects of exogenous application of citric acid and proline to Foxtail millet and analysis of stress tolerance factors under saline and alkaline conditions

The effects of exogenous application of CA and proline on the growth, membrane stability, water status, gas exchange characters, mineral composition and organic metabolites of SS and AS sensitive Foxtail millet are presented (Chapter 4) and discussed here.

Plant height, leaf area and dry weight significantly reduced under SS and AS conditions. However, with the exogenous application of CA and proline significantly increased dry matter of plants. This result is consistent with the previous reports that different antioxidants like CA and ascorbic acid mitigated salt and alkaline stresses and enhanced tolerance in *Leymus chinensis* (Trin.) (Sun and Hong, 2010a) and in soybean (Sheteawi, 2007).

The ELR increased sharply under SS and AS which indicates that the high-pH might damage root structure. However, the addition of exogenous CA and proline to plants under SS and AS conditions significantly reduced the ELR, with CA being more effective than proline. It has been reported that stress-impaired membrane permeability was revised by the application of proline and salicylic acid (Stevens *et al*., 2006; Tuna et al., 2007).

The RWC decreased 13% under SS and 17% under AS with the intensification of stresses. However, the application of CA and proline improved the RWC in the leaves and the recovery rate was little bit higher under SS plants. Many authors reported that the reduced water content due to saline and alkaline stresses was alleviated by the addition of exogenous proline (Sun and Hong, 2010b), proline and glycinebetaine (Ahmed et al., 2010), and CA (Sun and Hong, 2010a). Azooz (2009) reported similar results for salicylic acid. Exogenous application of CAs and proline effectively revived the reduced Ψ_{LW} with proline being less effective than CA. These results are in agreement with the previous studies of Huber (2003) and Ahmed et al. (2010).

SS and AS significantly reduced Pn, gs and Tr and AS led to marked reduction of Pn as compared to SS conditions which was supported by many authors both in halophytes and glycophytes (Li et al., 2010; Liu et al., 2010). The high pH caused by AS may seriously affect stomatal opening and closing thereby reducing Pn. However, Pn, gs and Tr were revived in the stressed plants by exogenous proline and CA. This result is in agreement with the results of Sun and Hong (2010a,b) and Farouk (2011). Salicylic acid also effectively stimulated photosynthetic activities in salt stressed maize plant (Khodary, 2004) and in apple leaves (Liu et al., 1999).

The application of CA and proline substantially reduced the Na accumulation in the leaves compared to untreated stressed plants. This might occur through the decreased Tr, causing fewer ions to be carried through the transpiration stream. It has been reported that SS and AS can have either inhibiting effects (Tammam et al., 2008), and inducing effects (Benderradji et al., 2011) on K uptake of plants. This is in agreement with Gadallah (1999) and Khanna (1998). Exogenous CA and proline decreased Na concentration remarkably which influenced the reduction of the Na / K ratio in the presence of SS and AS. The lower Na / K ratio in plants has been considered a physiological trait indicator of salt tolerance in plants (Morsy et al., 2007). The reduced Ca amd Mg uptake due to SS and AS was revised by the exogenous CA and proline. Rana and Rai (1996) showed that the exogenous proline promoted Ca and Fe uptake in *Phaseolus* seedlings.

N and P concentration decreased more in AS than in SS plants and exogenous CA and proline relaxed the stresses and increased their uptake. Proline activated the acquisition of N more effectively than CA in the roots under SS, but very less effective under AS. Internal CA and proline concentration increased more under AS than under SS. This suggests that the induction of CA and proline synthesis is related to the severity of the stress, which is induced by pH. However, the application of exogenous CA and proline remarkably reduced internal

CA and proline concentration, which be might be a stress coping. Exogenously-supplied proline provides osmoprotection (Yancey, 1994), protects cell membranes from salt-induced oxidative stress (Yan et al., 2000), increases activities of superoxide dismutase and peroxidase (Hua and Guo, 2002), decreases Na^+ and Cl^- accumulations, and facilitate growth (Lone et al., 1987).

TSS concentration in the leaves significantly increased under SS and AS. Plants grown under AS induced more TSS than those under SS as reported by Khadri et al. (2007) and Palma et al. (2009). Stressed plants treated with CA and proline revealed a significant reduction of elevated TSS. Similar results were also observed by Okuma et al. (2000) and El-Tayeb (2005). On the other hand, starch concentration decreased in the stressed plants, which might be related to the reduced photosynthetic activities and inhibition of photo assimilates to the growing regions. Similar results were also reported by Rathert (1985), Murakeozy et al. (2003). Nonetheless, the added CA and proline played an important role in starch synthesis and, thus, lessened the pressure on the photosynthetic chain by reducing toxic ions and causing an increase in the cytosolic water volume (Cayley et al., 1992). Khan *et al*. (2003), Khodary (2004) and Yildirim *et al*. (2008) concluded that exogenous salicylic acid application remarkably controlled the increased TSS and decreased starch in opposite directions in salt stressed plants.

Due to containing more carboxylic groups and varying negative charges of CA allows the complexation of metal cations in solution and the displacement of anions from the soil matrix by the mobilisation and uptake of nutrients (N, K, P, Ca, Mg and Fe) and the detoxification of metals (Jones, 1998), microbial proliferation in the rhizosphere, and the dissolution of soil minerals (Marschner, 1995). CA and proline enhance to maintain good water status in the rhizospheres which helps to revived root activities for uptaking required nutrients.

SUMMARY

The salinization and alkalization of soil are widespread environmental problems that lead to loss of agricultural land day by day. Great awareness should be generated in the world for the utilization of that degraded land for crop production to meet the needs of the fast expanding population. Therefore, development of proper technologies to grow crops in degraded soils has become extremely essential. To achieve that goal, the foremost task is to identify the salt-alkali tolerant species and then to prevent or alleviate the stress damage under stressful environments. Foxtail millet (*Setaria italica* L.) and (*Panicum miliaceum* L.) are important food and fodder grain crops grown in arid and semi-arid regions. Growth responses of many crops to salinity stress have been extensively investigated but unfortunately millets like Foxtail millet and Proso millet, which are naturally adapted to drought stress, have not been explored in alkaline stress, to date. The present study was, therefore, conducted to 1) investigate the nature of the tolerance of Foxtail millet and Proso millet under saline and alkaline environments, 2) assess whether exogenous application of citric acids and proline could alleviate the adverse effects of saline stress (SS) and alkaline stress (AS), and 3) find out the strategies how these compounds ameliorate saline and alkaline stresses.

1. Comparative studies on saline and alkaline stresses of Foxtail millet and Proso millet and analysis their stress tolerance factors

To achieve the first objective, Foxtail millet and Proso millet were grown in 100 mM saline and alkaline conditions. For more clarification and confirmation about the tolerances, this experiment was repeated with imposing lower concentration (50 and 75 mM) of SS and AS to observe some physiological attributes.

The data indicated that the reduction of all plant parameters were more pronounced in Foxtail millet and less in Proso millet in both the stressful situations. The biomass production per plant significantly decreased with the increasing salinity and alkalinity in both species and Proso millet produced a significantly greater amount of dry matter than Foxtail millet in both stressful situations. The stress-induced injurious effect on the electrolyte leakage rate (ELR) was greater in Foxtail millet than in Proso millet. The reduction of relative water content (RWC) was more marked in Foxtail millet than in Proso millet. The leaf water potential (Ψ_{LW}) decreased with the intensity of saline stress and alkaline stress and the reductions in Foxtail millet were greater under alkaline stress than saline stress conditions, indicates that Foxtail millet is a sensitive species compared to Proso millet. The inhibitory effect of alkaline stress on the photosynthetic rate (Pn), stomatal conductance (gs) and transpiration rate (Tr) were greater than that of saline stress and the inhibition was higher in Foxtail millet than in Proso millet. Foxtail millet accumulated greater concentration of Na under the saline stress and alkaline stress conditions as compared to Proso millet. The roots of Proso millet attained a higher concentration of Na than the roots of Foxtail millet. The K concentration was reduced in the stems and roots of both species by the higher concentration (100 mM) of saline stress and alkaline stress but did not reduce significantly in the leaves and stems of Proso millet at lower concentration of saline stress and alkaline stress (50 and 75 mM) as Foxtail millet did under alkaline conditions. This result indicates that there was no competitive inhibition between Na^+ and K^+ uptake in Proso millet species. Foxtail millet showed greater values of Na / K ratios than Proso millet except in the alkaline stress treated Proso millet roots. These results suggested that Proso millet is more tolerant to saline stress and alkaline stress than Foxtail millet due to a higher ability of maintain the root function for the uptake and supply of water to shoot under both stress conditions, and a lower accumulation of sodium and its transportation from root to leaves.

2. Effects of exogenous application of citric acid and proline to Foxtail millet and analysis of stress tolerance factors under saline and alkaline conditions

It is important to study the effective management practices that can improve stress tolerances of plants. The effects of exogenous application of citric acid (CA) and proline on the growth, membrane stability, water status, photosynthetic apparatus, mineral composition and organic metabolites in Foxtail millet under saline stress and alkaline stress studied. Plant dry weight significantly reduced under both stress conditions and the percentage of reduction was greater under alkaline stress condition. However, exogenous application of CA and proline significantly increased plant dry matter, and proline was less effective under alkaline stress condition. Saline and alkaline stresses increased ELR, and with the addition of exogenous CA and proline significantly reduced the leakage rate. The application of external CA and proline improved RWC in the leaves and recovery rate was almost similar between saline stressed and alkaline stressed plants, although it was little bit higher in saline stressed plants. Alkaline stress sharply decreased Ψ_{LW} and exogenous application of CA and proline effectively reestablished the Ψ_{LW}.

The Na concentration increased in all plant parts under both stress conditions and the increase was greater under alkaline stress condition. However, CA and proline substantially reduced the Na concentration in all plant parts compared to untreated stressed plants, and CA was more effective than proline in reducing Na accumulation in leaves and also transport from root to leaves. N, P, Ca, Mg and Fe uptake were decreased under both stress conditions, however uptake of these nutrients was increased by the application of exogenous CA and proline under both stress conditions. The total soluble sugar concentration in the leaves significantly increased and the increment was greater under AS compared to saline stress. CA and proline application significantly reduced the total soluble sugar concentration, however

starch concentration decreased in both stress conditions, which indicated the CA and proline played an important role in starch synthesis under stress conditions.

3. Conclusion

Proso millet showed more capability to survive under both stress conditions as compared to Foxtail millet regarding of almost all plant traits examined. Foxtail millet accumulated greater concentration of Na under saline and alkaline stress conditions in the leaves and stems, and showed greater values of Na / K ratios as compared to Proso millet. Proso millet maintained a higher photosynthetic activities and a higher water status under both stress conditions due to supply of required water in shoot. The exogenous application of CA and proline alleviated saline and alkaline stress damages. CA and proline application increased water content, N, P Ca, Mg and Fe accumulation and reduced Na accumulation in leaves under saline and alkaline conditions. These results suggested that CA and proline application enhanced plant growth due to more water and nutrients uptake, and reduced toxic sodium accumulation in leaves resulting increased salt tolerance.

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