

**MITIGATION OF SALT STRESS IN TOMATO BY
EXOGENOUS APPLICATION OF CALCIUM**

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**MITIGATION OF SALT STRESS IN TOMATO BY
EXOGENOUS APPLICATION OF CALCIUM**

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CERTIFICATE

This is to certify that the thesis entitled “**MITIGATION OF SALT STRESS IN TOMATO BY EXOGENOUS APPLICATION OF CALCIUM**” submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE** in **AGRICULTURAL BOTANY**, embodies the results of a piece of *bona fide* research work carried out by **KHURSHEDA PARVIN**, Registration. No. **07-02437** under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information as has been availed of during the course of this investigation has duly been acknowledged.

Dated:
Dhaka, Bangladesh

(Prof. Dr. Kamal Uddin Ahamed)
Supervisor

DEDICATED
TO
MY
BELOVED PARENTS

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MITIGATION OF SALT STRESS IN TOMATO BY EXOGENOUS APPLICATION OF CALCIUM

ABSTRACT

A pot experiment was conducted in the Horticulture Farm of Sher-e-Bangla Agricultural University, Dhaka, during the period from October 2013 to March 2014 to study the salt stress mitigation in tomato by exogenous application of calcium (Ca^{2+}). The two factors experiment was laid out in Randomized Complete Block Design (RCBD) with four replications. Factor A is different levels of salinity induced by sodium (Na^+) viz. 0, 2, 4, 6 and 8 dSm^{-1} and factor B is different concentration of Ca^{2+} viz. 0, 5, 10 mM. The total treatment combinations were 15 (5×3). The experimental results showed that salt stress significantly affects morphology, physiology, yield contributing characters and yield of tomato. Plant height, leaf number and branch number per plant were reduced with increased levels of salinity mostly at 6 and 8 dSm^{-1} . Salinity also adversely affected the leaf and stem dry weight (gm), leaf area (cm^2), leaf chlorophyll content, number of flower plant^{-1} , number of dropped flower plant^{-1} , number of fruit plant^{-1} and also fruit weight plant^{-1} mostly at 8 dSm^{-1} . Salt treatment greatly increased the uptake of Na^+ and decreased both potassium K^+ and Ca^{2+} uptake in the leaves of tomato. Exogenous application of Ca^{2+} significantly mitigates the adverse effects of salinity on plant biomass production or morphology, physiology and yield. The plant height, leaf number plant^{-1} , branch number plant^{-1} , leaf area (cm^2) plant^{-1} , dry weight of shoot plant^{-1} (gm), leaf chlorophyll content as measured in SPAD value, the highest number of flower and fruit plant^{-1} , fruit weight plant^{-1} were increased with the application of calcium than the control or without calcium. In addition, the uptake of Na^+ decreased and uptake of Ca^{2+} and K^+ increased in tomato shoot while plants were treated with Ca^{2+} under salt stress. The yield of tomato is gradually decreasing with the increasing levels of salinity. Interestingly, the rate of reduction of yield of tomato was decreased with Ca^{2+} in response to different saline conditions and the lowest yield was recorded at the highest salinity (8 dSm^{-1}) along with without Ca^{2+} . The present study also showed that the highest fruit yield recorded with without salt and 5 mM Ca^{2+} treatment combination which was statistically similar with control treatment combination. These results are consistent with the findings of regulation of ion uptake in presence or absence of Ca^{2+} at different levels of Na^+ stress. Therefore, this experiment suggests that Ca^{2+} can effectively mitigate the deleterious effect of Na^+ stress in tomato cultivation.

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LIST OF ACRONYMS

ABA	Abscisic acid
AEZ	Agro- Ecological Zone
Anon.	Anonymous
AOS	Active Oxygen Species
ASC	Ascorbic acid
BARI	Bangladesh Agricultural Research Institute
BAU	Bangladesh Agricultural University
BBS	Bangladesh Bureau of Statistics
BINA	Bangladesh Institute of Nuclear Agriculture
Ca	Calcium
CaCl ₂	Calcium Chloride
Cl	Chlorine
cm	Centi-meter
cm ²	Centimeter square
CO ₂	Carbon di oxide
CuSO ₄ .2H ₂ O	Copper Sulphate dehydrate
CV	Coefficient of Variance
cv.	Cultivar (s)
DAS	Days after sowing
DAT	Days After Transplanting
⁰ C	Degree Centigrade
df	Degree of freedom
dSm ⁻¹	Dessisimen per meter
DW	Dry Weight
EC	Electrical conductivity
<i>et al.</i>	And others
FAO	Food and Agricultural Organization
FAOSTAT	Food and Agricultural Organization Statistics
Fe	Iron
GA	Gibberellins
GK	Glutamyl kinase

LIST OF ACRONYMS (Contd.)

gm	Gram (s)
hr	Hour(s)
IAA	Indole Acidic Acid
JA	Jasmonic acid
K	Potassium
K ₂ O	Potassium Oxide
Kg	Kilogram (s)
KMP	Potassium Mono Phosphate
LRWC	Leaf Relative Water Content
LSD	Least Significant Difference
m	Meter
m ²	Meter squares
Mg	Magnesium
mg	Milligram
ml	Milliliter
mm	Millimeter
mM	Millimolar
N	Nitrogen
Na	Sodium
NaCl	Sodium Chloride
No.	Number
NS	Non significant
OM	Organic matter
%	Percentage
P	Phosphorus
pH	Negative Logarithm of hydrogen ion concentration
ppm	Parts per million
RCBD	Randomized complete block design
ROS	Reactive Oxygen Species
S	Sulphur
SA	Salicylic acid

LIST OF ACRONYMS

SAU	Sher-e- Bangla Agricultural University
Si	Silicon
t ha ⁻¹	Ton per hectare
TDM	Total Dry mass
var.	Variety
Wt.	Weight
WUC	Water Uptake Capacity

CHAPTER I

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is one of the most important vegetable crops grown throughout the world including Bangladesh under both field and greenhouse conditions. Tomato is a nutritious vegetable crop generally grown in the winter season (December -April) in Bangladesh. It is the most popular vegetable and consumed as a raw salad, cooked or as processed food item such as Sauce, Ketchup, Jam, Jelly, Pickles Soup etc. In terms of human health, tomato is a major component in the daily diet and constitutes an important source of minerals, vitamins and antioxidants, like lycopene, which acts as an anti-carcinogen. In addition, it contains vitamins A, B and C, potassium (K), iron and calcium along with low fat contents and zero cholesterol levels. The world dedicated 4.8 million hectares in 2012 for tomato cultivation and the total production was about 161.8 million tons. The leading top ten tomato producer country in the world are China, India, United States, Turkey, Egypt, Iran, Italy, Spain, Brazil and Mexico (FAO, 2012). The productivity of crops is not increasing in parallel with the food demand due to changing environmental factors both biotic and abiotic. A vast number of insect pests including various fungal, bacterial and virus diseases such as early blight, late blight Fusarium wilt, bacterial wilt, bacterial spot, tobacco mosaic virus, leaf curl, spotted wilt, etc. are serious problem which are biotic stress for crop production.

Various abiotic environmental stresses such as drought, high or low temperature, salinity, flooding, metal toxicity, etc., which pose serious threat to world agriculture. It has been reported that abiotic stresses reduced the crop growth and yield more than 50% among which salinity is one of the most brutal environmental factors which is increasing day by day due to anthropogenic activities and hamper the agricultural productivity including tomato (Tanji, 2002). Salinity is a threat to agriculture all over the world (Flowers and Colmer, 2008). It is observed that over 800Mha of soil is salt-affected in the world (FAO, 2005). Salinity is one of the major abiotic stress factors that limit the plant growth as well as fruit yield. It induces osmotic and toxic effects leading to physiological, morphological and biochemical modifications; it causes growth inhibition, crop yield reduction, lower rate of photosynthesis and respiration, nutritional deficiencies and inhibition of protein synthesis (Ashraf and Foolad, 2007). These phenomena have been observed in agricultural and horticultural crops,

including tomato (Juan *et al.*, 2005).

Salinity disturbs the physiology of plants by changing the metabolism of plants (Garg *et al.*, 2002). Salinity also injures cells in transpiring leaves, thus reducing growth of wheat plant (Munns, 2005). Salinity badly reduces leaf area, accumulation of dry matter content and also reduces net rate of CO₂ assimilation (Barnardo *et al.*, 2000). Many reports showed that salinity impairs the metabolism in plant tissue that altered growth performance and physiological process to provide tolerance against salinity (Sairam and Tyagi, 2004; Mahajan and Tuteja, 2005). Separately, plants have developed a well-organized defense mechanism of biochemical and physiological processes to protect themselves from the salinity-induced damages including antioxidant responses, ionic homeostasis, and/or osmoregulation (Hasegawa *et al.*, 2000; Parida and Das, 2005). It is well known that the basal or foliar application of Ca²⁺, Mg²⁺, K⁺, proline, glycine-betaine, salicylic acid can mitigate the adverse effects of salinity.

Calcium (Ca) is a signaling molecule and second messenger which is increased in the cytosol by activating influx channel both in the plasma membrane and tonoplast and plays a significant role in mediating mechanisms involved in recognition and response to abiotic stresses in plants (Kader and Lindberg, 2010). In addition, Hussain *et al.* (2010) and Lazof and Bernstein, 1999) reported that Ca²⁺ restrict the entry of Na⁺ into the plant cells under sodium stress. The Ca²⁺ has a pivotal participation in salt stress signaling that controls ion homeostasis pathways (Yokoi *et al.*, 2002). It was confirmed by Ca²⁺-dependent activation of phosphatase leading to transcription of the ENA1 gene, which encodes the P-type ATPase (Mendoza *et al.*, 1994). Components of signal recognition and transduction pathways initiate the action of a calcium sensor on a protein kinase that affects the activity of Na⁺/H⁺ antiporter (Shi *et al.*, 2000). These findings suggest that calcium can mitigate the sodium toxicity of plant. Many authors stated that exogenous calcium alleviates stress in greengram, soybean, linseed (Manivannan *et al.* 2007; Arshi *et al.*, 2010, Khan *et al.*, 2010). In addition, the gypsum (CaSO₄. 2H₂O) has been exogenously applied to the saline soil for improving the soil chemical, leading to enhance the crop productivity (Cha-um *et al.*, 2011).

However, the studies investigating the role of calcium in regulating the physiological and biochemical process in response to salinity induced stress in tomato are largely lacking. With this background, a study has been envisaged to understand the role of

Ca²⁺ in mitigating salt stress-induced response in tomato. Improvement in growth performance with addition of calcium to salt-stressed cowpea (Murillo-Amador *et al.*, 2006), Jerusalem artichoke (Xue *et al.*, 2008), linseed (Khan *et al.*, 2010), bean, loquat and anger rootstocks (García-Legaz *et al.*, 2008) were observed earlier. So in the present context, the role of Ca²⁺ to minimize the effect of salt toxicity in the reduction of yield and quality of tomato fruits is essential to investigate.

Bangladesh is an over populated country and to fullfil the current need of tomato for added population, the yield of tomato needs to be increased and saline prone area must be undertaken in tomato cultivation through proper use of mitigating substance to mitigate the toxicity of salt. However, to my knowledge little is known about the studies investigating the role of exogenous Ca²⁺ in improving the morphophysiology, yield and quality of tomato under different levels of Na stress in Bangladesh.

OBJECTIVES

Therefore this study has been taken under consideration to achieve the following objectives:

1. To investigate the morphophysiological and yield contributing characters and yield of tomato under salt stress.
2. To examine the role of Ca²⁺ on mitigation of salt stress in tomato in view of improvement of morphophysiological, yield contributing characters and yield of tomato.

CHAPTER II

REVIEW OF LITERATURE

Salinity is a great problem in the coastal region of Bangladesh, where a vast area remains fallow for long time. Tomato is an important crop plant which supply Vitamin C as well as used as a vegetables by the people of Bangladesh. It is a great source of Vitamin C for poor people of the coastal area. The scientists of Bangladesh are conducting different experiments to adopt different crops in the saline area; tomato is one of theme. Very limited research works have been conducted to adapt tomato crop in the saline area of Bangladesh. An attempt has been made to find out the performance of tomato at different levels of salinity as well as to find out the possible mitigation ways by using calcium in the saline stressed tomato plants. To facilitate the research works different literatures have been reviewed in this chapter under the following headings.

Effect of salinity on morphological characters of plant

Mohammad *et al.* (1998) conducted a pot experiment of Tomato where Tomato seedlings (cv. *riogrande*) were grown in 500 ml glass jars containing Hoagland's solutions which were salinized by four levels of NaCl salt (0, 50, 100 and 150 mM NaCl) and/or enriched with three P levels (0.5, 1 and 2 mM P) making nine combination The results indicate that increasing salinity stress was accompanied by significant reductions in shoot weight, plant height, number of leaves per plant.

Alaa El-Din Sayed Ewase (2013) said that, a pot experiment was carried out to study the effect of salinity stress on plants growth of Coriander (*Coriandrum sativum* L.) by the selection method. For this purpose, four treatments of different concentrations of NaCl were used, namely, 0, 1000, 2000, 3000 and 4000 ppm of NaCl. The following parameters of: plant length, number of leaves, roots number and length were recorded. The Obtained results showed that all growth parameters were reduced by increasing the NaCl concentration. Coriander plants were found to resist salinity up to the concentration of 3000 ppm NaCl only.

Nawaz *et al.* (2010) reported that applications of salt in the growth medium caused reduction in shoot length of sorghum cultivars. Under saline conditions 50 mM proline was more effective to reduce the effect of NaCl than 100 mM proline in both cultivars. Proline level 50 mM showed 26.58% and 11.78% increased shoot length as

compared to NaCl stresses plants. However, high concentration of proline (100 mM) was not so much effective as compared to low concentration i.e. 50 mM.

Jafari (2009) studied the interactive effects of salinity, calcium and potassium on physio-morphological traits of sorghum (*Sorghum bicolor* L.) in a green-house experiment. Treatments included 4 levels of NaCl (0, 80, 160, and 240 mM NaCl), 2 levels of CaCl₂ (0 and 20 mM), and 2 levels of KCl (0 and 20 mM). Salinity substantially reduced the plant growth as reflected by a decrease in the plant height, shoot and root weight.

Memon *et al.* (2007) conducted a pot experiment on silty clay loam soil at Sindh Agriculture University, in Tando Jam, Pakistan. Sarokartuho variety of Sorghum (*Sorghum bicolor* L.) was continuously irrigated with fresh (control) and marginally to slightly saline EC 2, 3, 4 and 5 (dSm⁻¹) waters. Increasing water salinity progressively decreased plant height and fodder yield (fresh and dry weight) per plant. Javaid *et al.* (2002) investigated the salinity effect (0, 20, 50 and 75 mM NaCl) on plant height in four rice variety and reported that salinity affects the morphological characters of the studied plants and plant height decreased with increased salinity levels. Similar results were also reported by Thakral *et al.* (2001) in twenty nine Ethiopian mustard (*Brassica carinata*) and by Uddin *et al.* (2005) in *B. campestris*.

Babu and Thirumurugan (2001) conducted a pot experiment to study the effect of salt priming on growth and development of sesame under induced salinity condition. Salinity was induced by addition of 35, 70 and 140 mM NaCl solution to create three levels of salinity and observed that plant height decreased with the increased salinity level. Similar results were also observed by many researchers in sesame by Ragiba (2000).

El-Midaoui *et al.* (1999) conducted a greenhouse experiment with three sunflower cultivars (cv. Oro 9, Flamme pinto and Ludo) under four salinity levels of 0, 50, 75 and 100 mM NaCl. They reported that plant growth was adversely affected by increasing salinity. Similar results were also reported by Steduto *et al.* (2000) in sunflower.

Ponnamperuma and Bandyopathy (1980) reported that symptoms of salinity injury in rice were found as stunted growth. Plant height was progressively decreased with increase in salinity levels as reported by Zeng (2004). Similar result was reported by most of the workers (Khan *et al.*, 1997; Powar and Mehta, 1997; Hossain, 2002; Sen,

2002; Choi *et al.*, 2003; Islam, 2004; Natarajan *et al.*, 2005; Hossain, 2006; Rana, 2007). On the other hand, Natarajan *et al.* (2005) reported that plant height decrement was lesser in salt tolerant varieties than salt susceptible varieties.

Milne (2012) studied on the effects of 30 and 60 mM NaCl on Lettuce (*Lactuca sativa* L.), grown in soilless culture, with additions of 0, 1, 2 and 4 mM Si was evaluated. Height, leaf number, weight, chlorophyll content and elemental analysis of plants were examined.

Uddin *et al.* (2005) studied salt tolerance on nine *Brassica juncea* varieties along with one *Brassica carinata* variety. They also found that number of branch decreased with increased salinity in all the studied Brassica species.

BINA (2008) studied the screening of wheat varieties for growth and yield attributes contributing to salinity tolerance and reported that wheat varieties of high yielding and tolerant group recorded a higher value of number of effective tillers plant⁻¹.

Mortazainezhad *et al.* (2006) had observed that tiller number decreased with increasing salinity levels imposed at all growth stages in rice. Soil salinity affects the growth of rice plant. But the degree of deleterious effect may vary on the growth stages of plant. During germination rice is tolerant, but it becomes very sensitive during the early seedling stage. Similar result was also reported by many workers in rice (LingHe *et al.*, 2000; Burman *et al.*, 2002; WeonYoung *et al.*, 2003; Islam, 2004; Rashid, 2005; Karim, 2007). LingHe *et al.* (2000) further reported that decreased tiller number was the major causes of yield loss.

Saberi (2009) studied the response of two forage sorghum varieties, Speedfeed and KFS4 to salinity and irrigation frequency. Two varieties were grown under salinity levels of 0, 5, 10 and 15 dSm⁻¹. Salinity, irrigation frequency and variety significantly affected the number of ratoon tillers. The number of tillers declined with increase in salinity and with less frequent irrigation. Speed feed variety produced a higher number of tillers than KFS4. Parti *et al.* (2002) conducted an experiment where salinity levels of 4, 8 and 12 dSm⁻¹ were obtained from adding chloride and sulphate salts of sodium, calcium and magnesium. All salinity treatments affected plant growth considerably

Saberi *et al.* (2011) conducted an experiment where two forage sorghum (*Sorghum bicolor* L. Moench) varieties (Speedfeed and KFS4) were grown under salinity levels of 0, 5, 10 and 15 dSm⁻¹. Maximum number of leaves was produced in non-saline soil

(13.5 leaves plant⁻¹) with normal irrigation (12.4 leaves plant⁻¹). Low soil water and high salinity reduced the number of leaves as well as the number of tillers produced.

Saberi *et al.* (2011) conducted a pot experiment where two forage sorghum varieties (Speedfeed and KFS4) were grown under salinity levels of 0, 5, 10 and 15 dSm⁻¹. Leaf area of plants were also reduced in response to salinity and decreasing soil water availability, while the suppressive effect was magnified under the combined effect of the two factors. Salinity and water stress significantly affected the total leaf area of ratoon crop. The maximum total leaf area was obtained in the control treatment but with increasing salinity and infrequent irrigation, this parameter was found to decrease. Maximum leaf area of 1167 mm² plant⁻¹ was attained in plants with normal irrigation, without water stress. Under effects of salinity 5, 10 and 15 dSm⁻¹ the leaf area was reduced by 7, 12 and 17%, respectively.

Islam (2004) conducted a pot experiment to study the effect of salinity (3, 6, 9, 12 and 15 dSm⁻¹) on growth and development of rice under induced salinity condition and observed that number of leaves decreased with the increased salinity level. Similar result was also observed by Rashid (2005) in rice.

Netondo *et al.* (2004) conducted an experiment where sorghum plants were grown in sand culture under controlled greenhouse conditions. The NaCl concentrations in complete nutrient solution were 0 (control), 50, 100, 150, 200, and 250 mM. Salinity significantly reduced leaf area by about 86% for both varieties of sorghum and these decreases were similar for the two sorghum varieties.

Angrish *et al.* (2001) conducted a pot experiment and observed that increasing levels of chloride (0-12 dSm⁻¹) and sulfate salinity decreased leaf number of wheat plants. Similarly, Khan *et al.* (1997) reported that leaf number and leaf area were seriously decreased by salinity in rice.

Chakraborti and Basu (2001) conducted a pot experiment to study the effect of salinity (0, 6 and 9 dSm⁻¹) on growth and development of sesame under induced salinity condition and observed that number of leaves decreased with the increased salinity level. Moreover, Javaid *et al.* (2002) investigated the salinity effect (0, 20, 50 and 75 mM NaCl) on plant height, stem diameter, TDM, leaf number and leaf area in four *Brassica* species and reported that salinity affected the morphological characters of the studied plants and leaf number as well as leaf area decreased with increased salinity levels.

El-Midaoui *et al.* (1999) conducted a greenhouse experiment with three sunflower cultivars (cv. Oro 9, Flamme pinto and Ludo) under four salinity levels of 0, 50, 75 and 100 mM NaCl. They reported that plant growth was adversely affected by increasing salinity. Among the studied parameters, leaf number and leaf area were mostly affected (72%) followed by plant height (67%).

Salinity stress may cause the death of the plant as well as hinder growth depending on tolerance, may cause chlorosis and necrotic stains and also decrease yield and quality (Hasegawa *et al.* 1986; Mer *et al.* 2000).

Sixto *et al.* (2005) stated that depending on increasing salinity levels, decrease in vegetative growth parameters has been observed in plants. Decrease in root, stem and shoot developments, fresh & dry stem and root weights; leaf area and number and yield have been observed in plants subject to salinity stress.

Munns (2005); Munns and Tester (2008). reported that salt-induced osmotic stress is the major reason of growth reduction at initial stage of salt stress, while at later stages accumulation of Na⁺ occurs in the leaves and reduces plant growth.

Çiçek and Çakırlar (2002) observed salt stress caused a significant decrease in shoot length, fresh and dry weights of shoot and leaf area of both cultivars with the increase of stress treatments.

Parida and Das (2005) observed salt stress affects some major processes such as root/shoot dry weight and Na⁺/K⁺ ratio in root and shoots.

Jampeetong and Brix (2009) and Gorai *et al.* (2010) reported that, various plant growths and development processes viz. seed germination, seedling growth, flowering and fruiting are adversely affected by salinity, resulting in reduced yield and quality.

Munns & Tester (2008) observed that osmotic effect, which develops due to increasing salt concentration in the root medium, is a primary contributor in growth reduction in the initial stages of plant growth. This stage can be characterized by reduction in generation of new leaves, leaf expansion, development of lateral buds leading to fewer braches or lateral shoots formation in plants.

Hasegawa *et al.* (1986), Okubo and Saturatarini (2000) reported that defoliation is frequently observed under increasing salinity stress conditions.

Munns (2002) observed that when salt concentration increases inside the plant, the salt starts to accumulate inside the older leaves and eventually they die. If these older leaves die at a rate greater than that at which new leaves generate, it reduces the

capacity of plants to supply the carbohydrate requirements of younger leaves leading to reduction in their growth rate (Munns *et al.* 2006). This phase may be recognized by the appearance of some specific symptoms of plant damage in the leaves such as color change, tip burn, marginal necrosis and succulence (Munns & Tester, 2008).

Hernandez *et al.* (2003) reported that salt stress inhibited cell division and cell expansion, and consequently leaf expansion. Growth of leaf area is inhibited by salinity (Brungnoli and Lauteri, 1991; Alberico and Cramer, 1993).

Liu *et al.* (2008) reported significant reduction in the dry biomass of halophyte *Suaeda salsa* when exposed to different concentration of NaCl under different water regimes.

Ali (2004) conducted a research on Salt tolerance in eighteen advanced rice genotypes was studied under an artificially salinized ($EC = 8.5 \text{ dSm}^{-1}$) soil conditions after 90 days of transplanting. The results showed that the yield per plant, and number of productive tillers, panicle length and number of primary braches per panicle of all the genotypes were reduced by salinity.

Pasternak *et al.* (1979) reported that flowering of tomato plants is delayed by salinity.

Shannon and Grieve (1999) reported that salinity changes the roots structure by reducing their length and mass, therefore roots may become thinner or thicker.

Maas (1986) and Bolarin *et al.* (1993) reported that, all stages of plant development including seed germination, vegetative growth and reproduction show sensitivity to salt stress and economic yield is reduced under salt stress.

Effect of salinity on Physiological Attributes of plant

Salinity arrests the cell cycle transiently by reducing the expression and activity of cyclins and cyclin-dependent kinases that results in fewer cells in the meristem, thus limiting growth (West *et al.* 2004).

Memon *et al.* (2007) experimented on sarokartuho variety of sorghum that was continuously irrigated with fresh (control) and marginally to slightly saline EC 2, 3, 4 and 5 (dSm^{-1}) waters in a pot experiment. Saline water treated plants contained more Na^+ , less K^+ and showed lower leaf K^+/Na^+ ratio.

Netondo *et al.* (2004) studied two Kenyan sorghum varieties, Serena and Seredo, were grown in a greenhouse in quartz sand supplied with a complete nutrient solution to which 0 (control), 50, 100, 150, 200, and 250 mM NaCl was added. Results showed that roots and stems accumulated substantial amounts of sodium, saturating at

150 mM external NaCl. Accumulation of K^+ and Ca^{2+} in the roots, stems, and leaves was strongly inhibited by salinity. Leaves continuously accumulated sodium, which was preferentially deposited in the sheaths. Mature leaves contained more Ca^{2+} and Mg^{2+} than young ones. The two sorghum varieties appear to sequester Na^+ predominantly in roots, stems, leaf sheaths, and older leaf blades.

Cicek and Cakirlar (2002) observed the effect of salinity on physiological attributes of maize cultivars. They found that salinity caused a marked decrease in shoot length, fresh and dry weight, leaf area of maize plants.

Salinity adversely affects reproductive development by inhibiting microsporogenesis and stamen filament elongation, enhancing programmed cell death in some tissue types, ovule abortion and senescence of fertilized embryos. In *Arabidopsis*, 200 mM NaCl stress causes as high as 90% ovule abortion (Sun *et al.*, 2004).

Hamayun (2010) reported that, the adverse effects of NaCl induced salt stress on growth attributes and endogenous levels of gibberellins (GA), abscisic acid (ABA), jasmonic acid (JA) and salicylic acid (SA) soybean cv. Hwangkeumkong was showed. Chlorophyll content was significantly decreased in response 70 mM and 140 mM concentrations of NaCl.

Ali *et al.* (2004) conducted a research on Salt tolerance in eighteen advanced rice genotypes was studied under an artificially salinized ($EC=8.5 \text{ dSm}^{-1}$) soil conditions after 90 days of transplanting. The results showed that the chlorophyll concentration was reduced by salinity.

Netondo *et al.* (2004) said that, salinity affects photosynthesis mainly through a reduction in leaf area, chlorophyll content and stomatal conductance, and to a lesser extent through a decrease in photosystem II efficiency. Low availability of soil water leads to a transient loss of turgor in plants. It reduces cell elongation, which causes a reduction in leaf expansion, as well as other plant parts, ultimately leading to stunting of the plants (Hernandez *et al.* 1999).

Netondo *et al.* (2004) conducted an experiment where sorghum plants were grown in sand culture under controlled greenhouse conditions. The NaCl concentrations in complete nutrient solution were 0 (control), 50, 100, 150, 200, and 250 mM. Chlorophyll a and b, net assimilation, stomatal conductance, and transpiration rate decreased significantly with the increase in salinity, and these decreases were similar for the two sorghum varieties.

Netondo *et al.* (2004) conducted an experiment where sorghum plants were grown in sand culture under controlled greenhouse conditions. Leaf growth, gas exchange, and chlorophyll fluorescence of two sorghum (*Sorghum bicolor* L. Moench) varieties, Serena and Seredo, were measured in response to increasing NaCl concentration. The NaCl concentrations in complete nutrient solution were 0 (control), 50, 100, 150, 200, and 250 mM. . The results indicate that salinity affected photosynthesis per unit leaf area indirectly through stomatal closure, and to a smaller extent through direct interference with the photosynthetic apparatus. In addition, salinity decreases whole plant photosynthesis by restricting leaf area expansion. This effect starts from low levels of salinity, in contrast to that of net photosynthesis per unit leaf area, which occurs at higher levels of NaCl concentration. It has been stated that depending on increasing salinity levels decrease in chlorophyll amount have been observed in plants subject to salinity stress.

Iyengar and Reddy (1996) explained several factors contributing to decline in photosynthetic rate. A physiological water deficit and a reduction in water potential lower the photosynthetic efficiency of plants under salinity stress, thus lowering the net carbon assimilation rate.

Many reports have indicated reduced photosynthesis under increasing concentrations (0 mM - 100 mM) of NaCl (Romeroaranda *et al.* 2001; Soussi *et al.* 1998).

A significant decline in the net photosynthesis is an immediate effect of stomatal closure coupled with photorespiration in plants exposed to high salinity stress. This short term response to salinity exposure lasts for 24 - 48 hr and completely ceases photosynthesis (Parida *et al.* 2005).

Reddy *et al.* (1992) reported a significant reduction in stomatal conductance and CO₂ assimilation rate in salt stressed *Salicornia brachiata* Roxb. Plants which prevented optimal activities of several enzymes in Calvin cycle. Similarly, salt stress aggravated photo-inhibition and delayed recovery of photosynthetic apparatus in wheat cultivars (Mishra *et al.* 1991). As reviewed by Paul and Foyer (2001), for conservation of the resources and energy, feedback signaling may regulate the rate of photosynthesis due to growth inhibition, and the balance between sources and sinks.

In barley, short term exposure to high salinity leads to an immediate and significant drop in stomatal conductance, due to osmotic stress and local synthesis of ABA (Fricke *et al.* 2004 and 2006).

Mostafa (2004) observed that at low and moderate salinity levels, sugars and consequently the total carbohydrates are decreased. Soluble protein is generally decreased in response to salinity (Parida *et al.* 2002 and Abed-Latef, 2005).

It has also been reported that high salt concentration either causes an increase in the N-contents and high protein content in some glycophytic plants (Abed El- Baki, 1996; Jones and Mac Millan, 1987) or increase in soluble proteins (Shaddad *et al.* 2005).

The Number of N-containing compounds accumulating in plants subjected to environmental stress (Robe, 1990 and Kuznetsov *et al.* 2007). The most frequently accumulating compounds include amides (glutamine and asparagines), amino acids (arginine, proline, citrulline and ornithine) and the amine putrescine. Amino acids such as proline, asparagine and amino butyric can play important roles in osmotic adjustment of plant under saline conditions (Gilbert *et al.* 1998).

Proline accumulation might be used as an indicator in selection for withstanding saline stress through the involvement in osmoregulation (Haroun, 2002 and Ueda *et al.* 2007).

Additionally, proline accumulation under stress conditions may be caused by induction of proline biosynthesis enzymes, reduction the rate of proline oxidation conversion to glutamate, decrease utilization of proline in proteins synthesis and enhancing proteins turnover (Claussen, 2005).

The relative ability of the plant or plant organ to stimulate the accumulation of cytosolutes in its tissues (osmotic adjustment) will partially determine its tolerance to stress conditions (Karimi *et al.* 2005 and Kukreja *et al.* 2005). The marked increase in soluble saccharides as well as soluble protein and tissue water contents in shoots might indicate the superiority of shoots and even spikes over roots to alleviate the imposed salt stress, either via osmotic adjustment or by conferring desiccation resistance to plant cells (Handa *et al.* 1983 and Erdei and Taleisnik, 1993).

Salinity stress is known to result in an excess production of reactive oxygen species (ROS), oxidative damage and a change in concentrations of antioxidants (Bor *et al.* 2003; Sekmen *et al.* 2007; Gao *et al.* 2008). Consequently, ROS are good cellular indicators of stress (Mittler, 2002).

Plant stresses, including salinity stress, are known to disturb cellular homeostasis, enhancing the production of ROS (Dat *et al.* 2000). Additionally, osmotic stress, one of the foremost stresses associated with high salinity levels, has shown to cause the

production of ROS (Xiong and Zhu, 2002).

Although ROS have roles as signaling molecules, active generation of which can be initiated by abiotic stresses (Desikan *et al.* 2005).

Increases in GSH concentrations are known to be associated with salinity stress (Ruiz & Blumwald, 2002; Leyva *et al.* 2011).

This is interesting when considering that, contrary to conventional thought, active plant growth cessation, as opposed to stress limiting growth, has been linked to surviving adverse environmental conditions (Harberd *et al.* 2009), including salinity stress (Magome *et al.* 2008).

GSH are well known antioxidants, and higher concentrations would infer superior antioxidative defence (Tausz *et al.* 2004), and would therefore logically result in a decrease in ROS concentrations brought about by salinity stress (Foyer *et al.* 2005).

Soil salinity affects plant physiology by way of injurious effects of toxic ions, osmotic stress, reduced water use efficiency and the resulting nutrient imbalance (Sairam and Tyagi, 2004; Grewal, 2010). Moreover, saline-induced stress in plants can imbalance reactive oxygen species, such as superoxide, hydrogen peroxide, and the hydroxyl radical, producing oxidative damage to lipids, proteins, and nucleic acids (Schwanz *et al.* 1996; Halliwell and Gutteridge, 1999).

Haghighi *et al.* (2012) conducted a study to evaluate the effectiveness of salinity on seed germination and growth characteristics of tomato seeds. A laboratory experiment was performed on completely randomized design with two levels of salinity (25 and 50 mM NaCl). A laboratory experiment was performed on completely randomized design with two levels of salinity (25 and 50 mM NaCl) and 2 concentration of Si (1 and 2 mM) with 4 replications. Germination percentage, germination rate, seedling shoot and root length, fresh and dry weight of seedling and mean germination time was measured. Seed germination of *Lycopersicon esculentum* L. was significantly affected by salinity levels, Si and their interaction. Germination characteristics of tomato seeds decreased drastically increasing by NaCl concentrations. However, 1 mM Si had positive effects on seed germination characteristics and improved germination percentage, germination rate and mean germination time. Si alleviated the harmful effect of salinity stress on tomato seed germination at almost all germination characteristics.

Zuccarini (2008) studied the effect of Si on *Phaseolus vulgaris* L. under two level of

salinity (30 and 60 mM). His results showed that salinity decreased stomatal conductance and net photosynthetic rate.

Osmotic stress (drought problem), ion imbalances, particularly with Ca and K, and the direct toxic effects of ions on the metabolic process are the most important and widely studied physiological impairments caused by salt stress (Zhu, 2001; Munns, 2002; Munns *et al.* 2006). Salt stress, like many abiotic stress factors, also induces oxidative damage to plant cells catalyzed by reactive oxygen species (Mittler, 2002; Demidchik *et al.* 2003; Azevedo-Neto *et al.* 2006).

In barley, short term exposure to high salinity leads to an immediate and significant drop in stomatal conductance, due to osmotic stress and local synthesis of ABA (Fricke *et al.* 2004 & 2006).

A significant decrease in the stomatal density of tomato plants was recorded when treated with 70 mM of NaCl in a sand culture experiment (Romero-Aranda *et al.* 2001).

Tomato and alfalfa leaves showed a significant reduction in total chlorophyll content, when exposed to salinity levels of 100 mM of NaCl (Khavarinejad and Mostofi, 1998).

Lacerda (2003) studied seedlings of two sorghum cultivars, one salt tolerant (CSF 20) and the other salt sensitive (CSF 18) that were grown in nutrient solution containing 0, 50, and 100 mmol litre⁻¹NaCl for seven days. The higher decrease in the P seen in the salt-sensitive cultivar was mostly due to higher accumulation of Na⁺ and Cl⁻ that probably exceeded the amount needed for the osmotic adjustment.

Lacerda (2001) studied the effects of high NaCl concentration on plant growth, on inorganic solute transfer to shoot and on the accumulation and distribution of inorganic solutes (Cl⁻, Na⁺, K⁺, Ca²⁺ and Mg²⁺) were evaluated to better understand the mechanism of salt tolerance in two sorghum cultivars (salt tolerant; ST87-11-ST88-03-ST89-02-ST90-01-ST91-03-ST91-B-Ca92B and salt sensitive: 51-Ca84-B1-Ca87-B2-BCa89). NaCl at 0 and 100 mM were added in 25 mM increments. At 0, 4 and 8 days after the beginning of NaCl treatment, samples were collected to evaluate the root dry matter yield and to determine ion contents in the shoot. The Na⁺ and Cl⁻ transfer rates to the shoot during the experimental period of 0 to 4 days increased an average of about five times with increasing saline treatment in both cultivars. The sensitive cultivar showed higher Na⁺ plus Cl⁻ transfer rates to the shoot, especially in

the beginning of the stress application and greater accumulation of these ions in the leaves.

Bavei (2011) studied the tolerance of sorghum varieties in terms of fresh weight, ion accumulations, proline content and peroxidase activity was analyzed in this study. Three sorghum varieties, Payam, Kimia, and Jambo, differing in salt tolerance, were grown in a greenhouse-hydroponic culture with a complete nutrition solution to which 0, 50, 100, 150 and 200 mM NaCl was added. Plant roots and leaves were harvested at 15 and 30 days after treatment and subjected to analysis. Clear decline in K^+ and Ca^{2+} concentrations and increase in Na^+ and proline contents were observed in the root and leaf tissues at each NaCl concentration in all varieties during the NaCl treatment.

Azooz (2004) studied the salt tolerance of 3 sorghum (*Sorghum bicolor*) cultivars (Dorado, Hagen Shandawil and Giza 113) and their responses to shoot spraying with 25 ppm IAA. The differences in the tolerance of the sorghum cultivars were associated with large differences in K^+ rather than in Na^+ , which was found to be similar in the whole plant. The youngest leaf was able to maintain a higher K^+ content than the oldest leaf. Consequently, the K^+/Na^+ ratios were higher in the most salt tolerant cultivar Dorado than in the other sorghum cultivars, and in the youngest than in the oldest leaf. In conformity with this mechanism, the stimulatory effect of the exogenous application of IAA was mostly associated with a higher K^+/Na^+ ratio.

Potassium (K) uptake by plant roots is often suppressed by sodium (Na) in the growth medium, whose damage may be moderated by calcium (Ca^{2+}). There is a debate if K influx could be used as an index to salinity tolerance and the reliability of its determination by the ion depletion method. Silberbush (2001) studied two sorghum (*Sorghum bicolor*) varieties (Hegari and NB-9040), that differ in their salt tolerance, grew for 28 days in nutrient solutions with 0, 25, 50 and 75 mM NaCl. Depletion of K^+ concentration in the solutions was then determined over time, and K net influx was calculated from K^+ concentration depletion between two time steps. The procedure was repeated four times. K influx data of the four reps were fitted by the least-squares procedure to the equation is the calculated coefficients. For each variety, the fitted equation indicated a decrease in K influx affinity to C with the increase in the NaCl concentration in the growth solution. This effect was obtained in the salinity sensitive NB9-040 in lower NaCl concentration than in the tolerant Hegari.

Thimmaiah (2002) carried out an experiment where sorghum (*Sorghum bicolor*) was grown under different levels of salinity (1, 2, 4, 6, 8 and 12 dSm⁻¹) in irrigation water and investigated for yield and yield components and biochemical composition. K⁺ and Ca²⁺ content, protein content and total amylolytic enzyme activity differed significantly due to salinity. However, these parameters were, more or less, at par with each other in the range of 2 to 8 dSm⁻¹. Among the chemical constituents, increased salinity levels increased Ca²⁺ content and decreased K⁺ content.

Silberbush (2001) studied that Potassium (K⁺) uptake by plant roots is often suppressed by sodium (Na) in the growth medium, whose damage may be moderated by calcium (Ca²⁺). There is a debate if K⁺ influx could be used as an index to salinity tolerance and the reliability of its determination by the ion depletion method. Two sorghum (*Sorghum bicolor*) varieties (Hegari and NB- 9040), that differ in their salt tolerance grew for 28 days in nutrient solutions with 0, 25, 50 and 75 mM NaCl. In addition, the effect of Ca was determined in the presence of 2 and 5 mM Ca²⁺ with plants grown in 50 mM NaCl.

Netondo (2004) studied two Kenyan sorghum varieties, Serena and Seredo, were grown in a greenhouse in quartz sand supplied with a complete nutrient solution to which 0 (control), 50, 100, 150, 200, and 250 mM NaCl was added. Magnesium concentration of the roots was minimally impaired but that of the stems and leaves was strongly affected. Mature leaves contained more Ca²⁺ and Mg²⁺ than young ones.

Thimmaiah (2002) conducted an experiment where sorghum was grown under different levels of salinity (1, 2, 4, 6, 8 and 12 dSm⁻¹) in irrigation water and investigated for yield and yield components and biochemical composition. Mg²⁺ content and invertase enzyme activity were unaffected by salinity. Magnesium concentration of the roots was minimally impaired but that of the stems and leaves was strongly affected.

High Na⁺ in soil solution causes intracellular K⁺ deficiency due to competition and leads to K⁺/Na⁺ disequilibrium (Kronzucker and Britto, 2008; Pardo and Rubio, 2011). High concentration of NaCl caused reductions in Ca²⁺ and Mg²⁺ levels in a number of plants (Khan *et al.* 1999 & 2000).

Werner and Finkelstein (1995) reported that plants tend to take up more Na⁺ and exclude K⁺ with increasing NaCl concentration in the in vitro conditions. Parida *et al.* (2004) have reported a significant increase in Na⁺ and Cl⁻ contents in leaf, stem and

root of mangrove *Bruguiera parviflora* without any significant endogenous alteration of K^+ and Fe^{2+} . Decrease in Ca^{2+} and Mg^{2+} content of leaves has also been reported by salt accumulation in this species.

The K^+/Na^+ ratio has been used as an index for sodium toxicity in plant tissues, because it is assumed that activity of K^+ requires some enzymes (Cramer *et al.* 1994), and higher K^+/Na^+ ratio indicates less Na^+ toxicity. Some authors reported that K^+/Na^+ ratio is decreased under salt stress (Gadallah, 1999; Haroun, 2002).

A significant accumulation of Na^+ in organs when plants were grown in saline solutions, while the concentration of K^+ , Ca^{2+} , and Mg^{2+} decreased with also length of internodes and leaf area significantly decreased with increased salinity, and net photosynthesis declined dramatically as well (Wang *et al.*, 1997).

Ionic imbalance occurs in the cells due to excessive accumulation of Na^+ and Cl^- and reduces the uptake of other mineral nutrients, such as K^+ , Ca^{2+} and Mn^{2+} (Karimi *et al.*, 2005).

A higher level, salinity limits the concentration of K^+ and Ca^{2+} in the leaves and roots of *Brassica napus* (canola) cultivars (Ulfat *et al.*, 2007; Ashraf and Ali, 2008).

High concentration of Na^+ and Cl^- ions in soil solution reduced the uptake of K^+ ions which ultimately caused K^+ deficiency in plants. K^+ deficiency result in chlorosis and then necrosis in plant leaves (Gopal and Dube, 2003).

Moreover, numerous studies have revealed that salt stress can reduce K^+ , Ca^{2+} and N accumulation in different crop plants, e.g. wheat (Raja *et al.* 2006) sunflower (Akram *et al.*, 2007), radish, cabbage (Jamil *et al.*, 2007) and canola (Ulfat *et al.*, 2007). Salinity reduces nutrient availability as well as transport to the growing regions of the plant, thereby affecting the quality of both vegetative and reproductive organs. For example, higher concentrations of Na^+ in soil decreased the Ca^{2+} activity in the external medium leads to limit its availability in *Celosia argentea* (Carter *et al.*, 2005).

In view of these reports, it is quite clear that salt stress limits the accumulation of essential nutrients such as K^+ , Mg^{2+} and Ca^{2+} while increases the concentration of Na^+ in most of crop species thereby resulting in reduced growth and yield. This argument is further supported by a number of studies in which it was found that exogenous application of salt-induced deficient nutrient such as Ca, K or N can mitigate the adverse effects of salinity on growth of many crops e.g., wheat, sunflower and beans

etc. (Shabala *et al.* 2003; Akram *et al.* 2007; Mahmood, 2011).

Cicek and Cakirlar (2002) observed the effect of salinity on physiological attributes of maize cultivars. They found that salinity caused a marked decrease in relative water contents of maize plants. They further concluded that amount of proline, Na^+ and Na^+/K^+ ratio increased under salt stress condition.

Other effects of osmotic stress include inhibition of root growth, decrease in stomatal conductance leading to reduction in the rate of photosynthesis (Munns, 1993; Munns *et al.* 2002).

The ion toxicity occurs when certain ionic species from irrigation water make their way into the plant, altering K^+/Na^+ ratios, and increasing Na^+ and Cl^- ion concentrations to those that are detrimental to plants because of their negative effects on important processes in plants, including enzymatic activity, protein metabolism, and balance of plant growth regulators (Munns *et al.* 2002; Tester & Davenport, 2003).

Eisa (2012) conducted an experiment where *Chenopodium quinoa* plants were grown in a hydroponic quick check system with 0, 100, 200, 300, 400, and 500 mM NaCl (equivalent to 0, 20, 40, 60, 80 and 100% seawater salinity). Higher salinities considerably reduced plant growth, with maximum reduction of 82% observed at 500 mM NaCl. The plants were able to reduce the leaf water potential below the soil water potential. This was associated with substantial decrease in osmotic potential mainly by Na^+ and Cl^- . The net photosynthesis rates were greatly decreased by high salinity, being 28% of initial control values at 500 mM NaCl. Salt-induced photosynthesis inhibition was accompanied with a decrease in transpiration rates but also with improved water use efficiency. Salt-induced growth reduction is presumably due to low photosynthate supply as a consequence of impaired photosynthetic capacity.

Hu *et al.* (1997) showed that salinity significantly increased sodium and chloride concentration in leaves and stems of wheat, while the concentration of K^+ , Ca^{2+} , Mg^{2+} and NO_3^- decreased. Both K^+ and Ca^{2+} are required in the external growth medium to maintain the selectivity and integrity of the cell membrane.

Soil salinity affects plant growth and development by way of osmotic stress, injurious effects of toxic Na^+ and Cl^- ions and to some extent Cl^- and SO_4^{2-} of Mg^{2+} and nutrient imbalance caused by excess Na^+ and Cl^- ions (Sairam *et al.* 2004).

High Na^+ in soil solution causes intracellular K^+ deficiency due to competition and

leads to K^+/Na^+ disequilibrium (Kronzucker and Britto, 2008; Pardo and Rubio, 2011).

High concentration of NaCl caused reductions in Ca^{2+} and Mg^{2+} levels in a number of plants (Khan *et al.* 1999 & 2000).

Patel *et al.* (2010) reported that, Salinity induced a significant increase in Na^+ , Cl^- and proline concentrations, while reduced the accumulation of K^+ and Ca^{2+} in leaves of all the cultivars of cowpea.

Essa (2002) reported that the main response of the plant to salt stress is a change in Ca^{2+} homeostasis and attributed that the salt tolerance of plants is their ability to avoid Na^+ toxicity and to maintain Ca^{2+} and K^+ concentrations.

Sodium is not considered an essential element for plants and plants accumulate Na^+ at the expense of Ca^{2+} and K^+ in saline conditions (Kuiper, 1984).

According to Greenway and Munns (1986), the reduction in K^+ concentration could inhibit growth by reducing the capacity for osmotic adjustment and turgor maintenance or by adversely affecting metabolic functions.

Effect of salinity on yield and yield contributing characters of plant:

Admans and Ho (1989) and Vanleperen (1996) conducted three different experiments at different time to find out the effect of salinity on tomato and they reported separately that, the number of cluster plant⁻¹ was reduced both with high salinity and long salinization periods in case of tomato. Whereas Mubarak (2011) also observed the fruit size reduced with salinity over control.

Hajer *et al.* (2006) and Cuartero and Munoz (1999) conducted two different experiment separately on tomato under saline condition and reported that fruit yield decreased with increased salinity separately.

Cuartero and Munoz (1990) observed salinity adversely affected the fruits number plant⁻¹ of tomato under different levels of salinity. The number of fruits plant⁻¹ was gradually reduced with increased levels of salt.

Grunberg *et al.* (1995) reported that fruit set could be decreased because of low number of pollen grains flower⁻¹ in plant under salt stress, extra flower production would be inhibited (Saito and Ito, 1974).

Jonson *et al.* (1992) reported that individual fruit weight decreased with increased salinity due to the reason of high salinity lowering the water potential in the plant which will reduce the water flow into the fruit and therefore the rate of fruit expansion.

Belda and Ho (1993) conducted an experiment on tomato and reported that salinity reduced the xylem development in tomato fruit but since the tomato fruit has a very low transpiration rate, only a small proportion of the water input come via the xylem (Ho *et al.* 1987) thus reduced the individual fruit size as well as weight.

Gonzalez- Fernandez and Cuartero (1993) reported that a 10% reduction in fruit weight is caused following irrigation with 5-6 dSm⁻¹ water, a 30% reduction with 8dSm⁻¹ and about 40% at higher ECE.

Hajer *et al.* (2006); Cruz and Cuartero (1990); Cuartero and Munoz (1999) reported that the effect of NaCl salinity stress on the growth of tomato plants was reflected in lower fresh and as well as dry weights.

Khavarinejad and Mostofi (1998) reported that chlorophyll content was reduced with higher salinity levels at all growth stage where the reduction rate was greater at vegetative growth stage than maturity stage in tomato.

Rafat and Rafiq (2009) reported that, total chlorophyll content in tomato plant proportionally decreased with the increase in salinity levels up to 0.4% sea salt solution (EC 5.4 dSm⁻¹).

Amini and Ehsanpour (2006) reported decrease in chlorophyll content in tomato cultivar due to salt stress.

Brungnoli and Lauteri(1991), Alberioco and Cramer (1993) reported that growth of leaf area is inhibited by salinity.

Hamayun *et al.* (2010) reported that, the adverse effects of NaCl induced salt stress on growth attributes and endogenous levels of gibberellins (GA), abscisic acid (ABA), jasmonic acid (JA) and salicylic acid (SA) soybean cv. Hwangkeumkong was showed. 1000 seed weight and yield significantly decreased in response 70 mM and 140 mM concentrations of NaCl.

Abdullah *et al.* (2001) conducted an experiment for finding out the effect salinity stress on seed set of IR-28 rice under different salinity levels and found that panicle length was significantly decreased due to salinity stress.

Karim (2007) conducted an experiment to investigate the effect of different salinity levels (0, 6, 9 and 12 dSm⁻¹) and reported that all parameters including panicle length decreased with increased salinity levels. Panicle length was adversely affected by soil salinity levels as reported by most of the researchers (Islam *et al.* 1998; Hossain, 2002; Islam, 2004; Natarajan *et al.* 2005; Rana, 2007).

The responses of forage sorghum (*Sorghum bicolor* L. Moench) varieties to salinity and irrigation frequency were studied from December 2007 to December 2009 by Saberi *et al.* (2011). Increased salinity significantly reduced forage dry yield from 44.09 gm plant⁻¹ in the control to 32.76 g plant⁻¹ at salinity with 15 dSm⁻¹. For every one unit increase in salinity, the forage yield decreased by 5.2 units and for every one unit increase in water stress (irrigation frequency), the forage yield decreased by 3.6 units. The variety Speedfeed had higher total dry mass than KFS4 under well-watered conditions but KFS4 performed better than Speedfeed under water stress. For both varieties, infrequent watering reduced dry matter and biomass accumulation, but increased water use efficiency (WUE) (6.88).

Gain *et al.* (2004) conducted pot experiment to study the effect of different levels of salinity (0, 7.81, 15.62, 23.43 and 31.25 dSm⁻¹) on growth and yield attributes of rice and reported that total dry mass decreased gradually with increased levels of salinity. Campos *et al.* (1999) grew rice varieties in nutrient solution with NaCl and reported that total dry mass (TDM) was decreased with increasing salinity. Similar result was also reported by many workers in rice (Gill and Singh, 1995; Islam *et al.* 1998; Islam *et al.* 2004; Cristo *et a.* 2001; Hossain, 2002; Islam, 2004; Hossain, 2006; KyuSeeong *et al.* 2007).

Netondo *et al.* (2004) conducted an experiment to determine how salinity affects growth, water relations, and accumulation of cations of nutritional importance in various organs of grain sorghum. Two Kenyan sorghum varieties, Serena and Seredo, were grown in a greenhouse in quartz sand supplied with a complete nutrient solution to which 0 (control), 50, 100, 150, 200, and 250 mM NaCl was added. The 250 mM NaCl treatment significantly reduced the relative shoot growth rates, measured 25 days after the start of salt application, by 75 and 73%, respectively, for Serena and Seredo, and stem dry weight by 75 and 53%.

A field experiment was conducted by Leena (2003) in Vadodara, Gujarat, India to test the effect of salt stress on *Sorghum bicolor*. Though there was a reduction in the chlorophyll content of the plants subjected to salt stress, the fresh and dry weights of the plants were reduced only at the earlier stages.

Parti *et al.* (2002) conducted an experiment where salinity levels of 4, 8 and 12 dSm⁻¹ were obtained from adding chloride and sulphate salts of sodium, calcium and magnesium. All salinity treatments affected plant growth considerably. The dry matter weight was maximum at 4 dSm⁻¹ and beyond this level, a constant decreased with increased salinity in TDM, plant height and siliqua plant⁻¹ was observed.

Muralidharudu *et al.* (1997) found that majority of field crops were sensitive in dry matter production to high salinity levels at different growth stages.

Uddin *et al.* (2005) conducted an experiment to study salt tolerance of *B. napus* and *B. campestris* varieties under saline conditions (1.2-11.5 dSm⁻¹) and observed that siliqua number and seeds siliqua⁻¹ decreased with increased salinity.

Sultana *et al.* (1999) evaluated the effect of salinity on rice at reproductive stage and observed that low concentration of assimilate in the leaves and poor translocation of assimilates from source to sink resulted less number of filled grain set while reverse trend was observed in unfilled grain number panicle⁻¹.

Gain *et al.* (2004) studied the effect of salinity (0, 7.81, 15.62, 23.43 and 31.25 dSm⁻¹) on yield attributes and yield in rice and reported that number of spikelet panicle⁻¹ decreased with increasing salinity levels but the decrement was less in salt tolerant varieties than salt susceptible varieties. This statement was supported many workers (Ahmed *et al.* 1988; Islam *et al.* 1998; Islam *et al.* 2004; Islam, 2004; Hossain, 2006).

Chakraborti and Basu (2001) studied salt tolerance ability in 9 sesame varieties under saline condition and reported that capsule per plant, seeds per capsule and seed yield decreased under saline condition in all studied varieties of sesame.

Ali *et al.* (2005) conducted a pot experiment with three salinity levels (0, 6 and 9 dSm⁻¹) and observed that 1000-seed weight decreased with increased salinity level in sesame. Again, Thakral *et al.* (1996) studied six *B. carinatus* species under 0-125 meq L⁻¹ chloride solution and observed that siliqua plant⁻¹, 1000-seed weight and seed yield decreased under salinity.

Gain *et al.* (2004) studied the effect of salinity (0, 7.81, 15.62, 23.43 and 31.25 dSm⁻¹) on yield attributes and yield in rice and reported that 1000-grain decreased with increasing salinity levels but the decrement was less in salt tolerant varieties than salt susceptible varieties. Sen (2002) conducted a pot experiment with three salinity levels (3, 6 and 9 dSm⁻¹) and observed that 1000-grain weight decreased with increased salinity level in rice. Similar result was also reported by Abudullah *et al.* (2001) in rice who observed that 1000-grain weight decreased with increased salinity levels. Again, Ali and Awan (2004) opined that generally, salinity caused a marked reduction in yield and yield components in rice except a few highly tolerant varieties.

Thimmaiah (2002) grew sorghum (*Sorghum bicolor*) under different levels of salinity (1, 2, 4, 6, 8 and 12 dSm⁻¹) in irrigation water and investigated for yield and yield components and biochemical composition. Seed and straw yield, seed weight per ear, N, P, K and Ca content, protein content and total amylolytic enzyme activity differed significantly due to salinity. However, these parameters were, more or less, at par with each other in the range of 2 to 8 dSm⁻¹. The 1000-seed weight, Mg²⁺ content and invertase [beta-fructofuranosidase] enzyme 14 activity were unaffected by salinity. Except 1000-seed weight, yield and yield components decreased significantly at 12 dSm⁻¹ salinity.

Rana (2007) carried out a pot experiment with 5 levels of salinity (0, 3, 6, 9 and 12 dS/m) of three rice varieties viz., BRRIdhan42, STM-1 and STM-2 and reported that plant height, number of tillers hill⁻¹, TDM hill⁻¹, leaf area hill⁻¹, root dry weight hill⁻¹ and yield contributing characters and yield decreased significantly with increase in salinity levels. Among the advanced rice lines BRRIdhan-42 showed more tolerance for all studied parameters compared to STM-1 and STM- 2.

Karim (2007) conducted an experiment to investigate the effect of different salinity levels (0, 6, 9 and 12 dSm⁻¹) and reported that all parameters including grain yield decreased with increased salinity levels. The yield was decreased due to production of decreased number of effective tillers hill⁻¹, decreased number of grains panicle⁻¹ and 1000-seed weight. Similar result was also reported by many researchers (Islam *et al.* 1998., Hossain, 2002; Sen, 2002; Islam *et al.* 2004; Rashid, 2005; Hossain, 2006).

Gain *et al.* (2004) reported that elevated salinity levels significantly decreased the total and filled grains panicle⁻¹ and 1000-grain weight which resulted decrease grain yield. Similarly, Soliman *et al.* (1994) conducted a greenhouse experiment and found

that grain yield reduced under saline condition. Similar result was also reported by Rashid (2005) in rice. Again, Hossain (2002) carried out an experiment with rice to know the effect of salinity (0, 3, 6 and 9 dSm⁻¹) on growth, yield attributes and yields, and reported that grain yield decreased with increasing salinity levels.

Ali *et al.* (2003) conducted an experiment to know the effect of four levels of salinity (0, 3.0, 4.5 and 6.5 dSm⁻¹) on plant biomass production, leaf area and yield attributes of two soybean varieties viz. Ertou and S-95-1. They found that salinity induced a marked reduction in yield attributes like siliqua plant⁻¹ and 1000-seed weight and seed yield.

Rivelli *et al.* (2002) conducted an experiment during 1997 and 1998 in Metaponto (MT), Italy, to evaluate the growth and yield response of paper sorghum (*Sorghum bicolor* x *S. dochna*) to irrigation with saline water (EC 0.9, 5, 5 plus leaching requirement, and 10 dSm⁻¹). A significant increase in soil salt concentration (EC) was measured at the end of the growing cycle. Winter rain and the application of leaching requirement were not sufficient for soil reclamation. A significant decrease in yield was observed due to saline stress. In 1997, the total epigeous dry matter decreased from 3900 g/m² in the control to 2450 g/m² of the most saline treatment. In 1998, sorghum sown in the same spot as the previous year produced lower yield than in 1997 due to higher salt accumulation in the soil. The soil threshold limit for salinity was 3.5 dS/m; where for each unitary decrease of soil EC a relative yield decrease of 9.4% was measured. Paper sorghum can be classified between moderately tolerant and moderately sensitive crops.

Chakraborti and Basu (2001) studied salt tolerance ability in 9 sesame varieties under saline condition and reported that capsules plant⁻¹, seeds capsule⁻¹ and seed yield decreased under saline condition in all studied varieties of sesame.

Nasiruddin and Rahman (1980) found that the reduction of yield in aman rice by 13% was due to use of salt concentration of 4 to 8 m mhos/cm. They also reported that the yield reduction was 62%, caused by the application of salt concentration of >15 m mhos cm⁻¹ in Khulna area of Bangladesh.

Debnath (2003) and Rahman (2003) worked with mustard to know the effect of different levels of salinity (0, 5, 7, 10 and 15 dSm⁻¹) on yield attributes and dry matter partitioning and reported that harvest index decreased with increased salinity levels.

Again, Hossain (2002) conducted a pot experiment with three salinity levels (0, 6 and

9 dSm⁻¹) and observed that harvest index decreased with increase of salinity level in rice. Similarly, Islam (2004) reported that harvest index decreased with the increase of salinity level in rice. Again, Hossain (2006) worked with rice to know the effect of different levels of salinity (0, 6, 9, and 15 dSm⁻¹) on yield attributes and dry matter partitioning and reported that harvest index decreased with increased salinity levels. Similar result was also reported by Rana (2007) in rice.

From the above review of literature, it may be concluded that salinity has marked effect on plant growth and development as well as yield of crops.

Role of Ca²⁺ to mitigate the saline toxicity:

It has been mentioned in many reports that the proline was mostly accumulated when plant growth was ceased (Lutts *et al.* 1996 and Joly *et al.* 2000). The function of this osmoprotectant is presumed to be protective, with a role in scavenging free radicals (Mansour, 2000). Minimization of reactive oxygen (ROS) as a result of inhibition of photosynthesis and maximization of their removal (scavenging) is likely to be an important response to high salinity, among other stresses (Zhu, 2001). When plants are subjected to stress, the amount of ROS in the cells increases which bring oxidative stress to crops (Xiong and Zhu, 2002)

Calcium nutrition plays an important role in the maintenance of a high growth rate under saline conditions (Marschner, 1995). Several reports show a significant role of Ca in improving the salt tolerance of plants. In studies on the soybean and cucumber, an additional supply of Ca to salt-stressed plants improved the salt tolerance of plants by reducing Na uptake and transport (Dabuxilatu and Ikeda, 2005). According to Husain *et al.* (2004), the major role of Ca in increasing the salt tolerance of plants is related to its inhibitory effect on the xylem loading of Na and thus decreases in shoot Na concentration.

Song *et al.* (2006) reported that high levels of external Ca are essential for the maintenance of high root uptake and shoot accumulation of Ca and K on saline soils and thus for avoiding salinity damage in plants as shown in rice plants.

The growth and yield of tomato is significantly reduced by high salinity (Feigin *et al.* 1987; Shalhevet and Hsiao, 1986; Smith, *et al.* 1992). The response of tomato to salinity is variable according to lines and cultivars (Shannon *et al.* 1987). Evlagon, *et al.* (1992) found that the root length was reduced by 54% after 4 days exposure to 0.1 strength Hoagland's solution salinized with 100 mM NaCl, while surface area was

reduced by 20% when 100 mM Ca was added to the salinized solution. Tomato shoot and fruit physiological responses to salt stress conditions have been extensively investigated (Cruz *et al.* 1990; Mitchell *et al.* 1991; Niedziela *et al.* 1993).

Tal and Shannon (1987) reported that salinity stress reduces elongation rate of the main stem in tomato. Cruz *et al.* (1990) reported that shoot length is one of the most reliable response indicators for a wide range of tomato genotypes under salinity stress. Significant reductions in flesh and dry weight of tomato shoots were reported in response to salinity stress (Bolarin *et al.* 1991 and 1993).

Cruz *et al.* (1990) reported that the effect of salinity on plants was expressed as reduced shoot dry weight because vegetative growth is the most widely used index in studies on salt tolerance in tomato. In addition, slower growth due to slower leaf expansion rates of sugar beet and cotton was reported by Shalhevet and Hsiao (1986). Leaf osmotic potential was reported to decrease (more negative) with the increased salinity stress in 'Micro-Tom' tomato (Smith *et al.* 1992). Peroxidases have been shown to play a big role in plant responses to stress conditions (Sherf and Kolattuudy, 1993). Adapted tomato cells were reported to grow in the presence of 15 g L⁻¹ NaCl and increased peroxidase activity was reported in response to salt stress (Sancho *et al.* 1996).

Tzortzakis (2010) reported that, Salinity either of soil or of irrigation water causes disturbance in plant growth and nutrient balance and reduces crop yields. The effects of NaCl salinity and/or calcium or potassium level on the plant growth and severity of gray mold (*Botrytis cinerea* [De Bary] Whetzel) were investigated in endive (*Cichorium endivia* L., cv. Green Curled) grown with the nutrient film technique under greenhouse conditions during early spring. Plants were supplied with nutrient solutions containing 40 mmol L⁻¹ of sodium chloride (NaCl) and/or 10 mmol L⁻¹ potassium sulphate (K₂SO₄). Additionally, plants treated with foliar spray of 15 mmol L⁻¹ calcium nitrate [(CaNO₃)₂] or distilled water. Salinity or K and Ca enrichment mainly affected the upper part of endive plants and reduced leaf area. However, when salinity combined with either K or Ca enrichment, the negative impact of salinity on plant growth was reversed. Salinized and/or K and Ca enriched, plants did not differ in plant biomass, leaf/root ratio, leaf fresh weight, leaf number, and root length. Salinity did not have any impacts on photosynthetic rate, stomatal conductance, and intercellular CO₂ concentration. Indeed, photosynthetic rate and stomatal conductance

increased with Ca foliar application and decreased with K while the opposite effects were observed for the intercellular CO₂ concentration. Total nutrient uptake was reduced 2-fold in salt-treated plants compared to controls. No symptoms of tip-burn or blackheart were recorded throughout the experimental study. Endive grown in the nutrient film technique had tolerance to NaCl salinity, and this method could be used to exploit saline water in soilless culture. These findings also suggest that a proper management of the salt concentration of the nutrient solution plus external elemental enrichment may provide an efficient tool to improve the quality of leafy vegetables with little effect on yield.

Gobinathan (2009) *Pennisetum* plants were grown with NaCl and CaCl₂ in order to study the effect of CaCl₂ on NaCl induced oxidative stress in terms of osmolyte concentration, proline (PRO)-metabolizing enzymes. The plants were treated with solutions of 100 mM NaCl, 100 mM NaCl with 5 mM CaCl₂ and 5 mM CaCl₂ alone. Groundwater was used for irrigation of control plants. Plants were uprooted randomly on 40 days after sowing (DAS). NaCl-stressed plants showed increased glycine betaine (GB) and PRO contents, decreased proline oxidase (PROX) activity and increased glutamyl kinase (GK) activity when compared to control. Addition of CaCl₂ to NaCl-stressed plants lowered the PRO concentration by increasing the level of PROX and decreasing the gamma-GK activities. Calcium ions increased the GB contents. CaCl₂ appears to confer greater osmoprotection by the additive role with NaCl in GB accumulation.

Manivannan *et al.* (2007) worked on the ameliorating effect of calcium chloride on sodium chloride-stressed plants of *Vigna radiata* L. Wilczek. Plants were treated with solutions of 100 mM NaCl, 100 mM NaCl with 5 mM CaCl₂, or 5 mM CaCl₂. Groundwater was used for irrigation as the control. Plants were harvested randomly 30 and 50 days after sowing. NaCl and CaCl₂-stressed plants showed reduced growth as indicated by decreased root length, stem length, total leaf area and dry weight. Proline and glycinebetaine content and the activity of the antioxidant enzymes superoxide dismutase, ascorbate peroxidase and catalase were increased under treatment with NaCl alone and CaCl₂ alone. When CaCl₂ was combined with NaCl, CaCl₂ altered the overall plant metabolism to ameliorate the deleterious effects of NaCl stress and increased the vegetative growth of the plants.

Hameda and Ahmed (2013) a greenhouse experiment was carried out to study the

response of presoaked tomato seeds (*Lycopersicon esculentum* Mill. var. *Cerasiforme*) in freshly prepared ascorbic acid (50 ppm ASC) or distilled water (control) for 12 h at natural environmental conditions, to reduce the effect of salinity stress. Generally, the tomato seeds germination occurred after 3 days, while, the germination rate (%) were more faster after soaking the seeds in ascorbic acid (ASC) compared with control (soaked in distilled water). NaCl salt-stress treatments caused a reduction in all growth parameters (fresh and dry weights of plant, leaf area and number per plant) compared control, particularly at high NaCl level (8000 ppm) more reduced. In the meantime, ascorbic acid had reduced the effect NaCl salinity stress on all growth parameters. Photosynthetic pigments (chlorophyll a & b and carotenoids) and chloroplast efficiency were increasing with salinity stress, but the response was more pronounced at 8000 ppm NaCl whether alone or combined with ascorbic acid. Also, salinity stress treatments tended to increase all of the total available carbohydrates (Monosaccharide, Disaccharides & polysaccharides), nitrogenous components (protein, amino acids & proline), antioxidase, (catalase, peroxidase & superoxide dismutases) enzymes activities and inorganic mineral elements (Na^+ , K^+ , N^{3+} , P^{3+} , Ca^{2+} , Mg^{2+} & Cl^-) but after soaked the seeds in ascorbic acid (+ASC), these components tended to increased more. Application of NaCl salinity-stress on tomato plant induced the synthesis of nitrogenous components (protein, amino acids, proline), whereas, the tomato seeds soaked before planting in ascorbic acid (ASC) which leads to remarkably increasing more for all nitrogenous components, antioxidase, carbohydrates and inorganic mineral elements content.

Lolaei *et al.* (2012) stated the effects of salinity and supplied calcium chloride on growth and leaf ions concentration of tomato (*Lycopersicon esculentum* L.) were investigated in Gorgan, Iran. A factorial experiment was conducted based on RCBD with four NaCl levels (0, 50, 100, and 150 mM) and four CaCl_2 levels (0, 100, 200 and 300 mg L^{-1}). Data of growth, yield and leaf's Ca, K, and Na content were subjected to analyze of variance. The results showed that fruit yield decreased under salinity stress. Increasing Ca^{2+} concentration in the nutrient solution increased the fruit yield. Leaf Ca^{2+} , K^+ , and N content decreased under salinity stress. Tomato in its response to nutrient solution, salinized with sodium chloride and calcium chloride. The results obtained from this experiment show that salinity stress caused a significant reduction in plant growth, leaf number and fruit weight.

Al-Moshileh (2004) reported that the application of chelated calcium (10% calcium) at the rates of 0, 250 and 500 mg Ca²⁺ kg⁻¹ soil on potato plants irrigated with saline water (1000, 3000, 6000 and 9000 ppm NaCl) was studied in a pot experiment. Also, an application of calcium chelate (10% calcium) at the rates of 0, 500 and 1000 mg Ca²⁺ kg⁻¹ soil on potato plants irrigated with saline ground water (538, 945, 1652 and 2044 ppm), was studied in some farms within Al-Qassim area. The results indicated that the vegetative growth characters such as plant height, leaf area and number of leaves per plant, and tuber yield were significantly decreased as the salinity level of irrigated water increased. Plants given 500 mg Ca²⁺ kg⁻¹ soil had a higher yield than plants without applying calcium or with 250 mg Ca²⁺ kg⁻¹ soil in the pot experiment, whereas plants given 500 mg Ca²⁺ plant⁻¹ under water salinity level of 945 ppm in the farms experiment had a higher yield than plants without calcium application. Therefore, calcium application might improve potato yield and mitigate the effects of salt stress during plant growth and development. The potato yield increased about 33% and 10% by increasing calcium application from 500 to 1000 mg/plant at the highest salinity levels 1652 and 2044 ppm, respectively.

Hussein (2012) reported that, irrigation with high salinity water influences plant growth, production of photosynthetic pigments and total phenols, leading to reduction in crop yield and quality. The objective of this study was to investigate the effects of potassium (K) foliar application in mitigating the negative effects of salt stress on pepper plants. A greenhouse experiment was conducted to investigate the effects of foliar application of potassium (K) on pepper plants grown with different salinity water irrigation (3000 and 6000 ppm as compared to tap water with salinity level of 300 ppm). Irrigation using high salinity water decreased plant height, biomass production, and fruit yield as compared to those of the plants irrigated by tap water. Photosynthetic pigments and total phenols increased in the former as compared to those of the latter plants. The most serious affect was for the plants under highest salinity irrigation (6000 ppm) as compared to that of the plants under moderate salinity irrigation (3000 ppm). Foliar application of potassium mono phosphate (KMP) at 200 ppm concentration increased the plant growth, biomass production, and fruit yield. Chlorophyll a content and total phenols increased significantly with foliar application of 100 ppm KMP. Further increase in foliar KMP concentration to 200 ppm had no significant benefits on photosynthetic pigments and total phenols content.

This study demonstrated that foliar application of KMP, to some extent, mitigated the negative effects of high salinity water irrigation on pepper plant growth and fruit yield.

Anbu and Sivasankaramoorthy (2014) worked on a pot culture was carried out with *Oryza sativa* L. vari-Co-39, to investigate the effects of supplementary calcium chloride on plants grown at NaCl (50mM) concentration. Treatments were: (1) Control: nutrient solution alone (C); (2) nutrient solution plus 50mM sodium chloride (NaCl); (3) nutrient solution plus 10mM calcium chloride (CaCl₂); (4) nutrient solution plus 15mM calcium chloride (CaCl₂); (5) nutrient solution and 50 mM NaCl plus supplementary 10 mM CaCl₂ (NaCl + CaCl₂); and (6) 50 mM NaCl plus additional mixture of 15 mM CaCl₂ in nutrient solution (NaCl + CaCl₂). The plants grown under salt stress produced low dry weight and relative water content than those grown in standard nutrient solution and in CaCl₂ alone. Supplemental calcium chloride added to nutrient solution containing salt significantly improved growth and relative water content. Membrane permeability increased with high NaCl application and these increases in root membrane permeability were decreased with supplementary Ca. The concentration of chloride (Cl) increases highly for all treatments. Sodium (Na) concentration in plant tissues increased in both shoots and roots at high NaCl treatment. Application of supplementary Ca lowered Na concentration. Concentrations of Ca, K and N were at deficient ranges in the plants grown at high NaCl levels and these deficiencies were corrected by supplementary Ca. The ameliorating effect of Ca on growth and physiological variables could reduce the negative effect of salinity of *Oryza sativa* L. plants.

Sivasankaramoorthy (2013) observed a great increase of K⁺/Na⁺ and Ca²⁺/Na⁺ ratios was observed when CaCl₂ was applied alone. The K⁺ and Ca²⁺ contents decreased under NaCl stress; but NaCl + CaCl₂ treatment reduced the extent of decrease caused by NaCl (Arshi *et al.*, 2010). High Na⁺ concentration in root zone inhibits uptake and transport of Ca²⁺ and thus subsequently, salt stressed plants have lower Ca²⁺ /Na⁺ ratios (Ashraf and Akhtar, 2004). Calcium has been shown to ameliorate the adverse effects of salinity on plants (White and Broadley, 2003). Calcium is well known to have regulatory roles in metabolism (Epstein, 1998) and sodium ions may compete with calcium ions for membrane binding sites. Therefore, it has been suggested that high calcium levels can protect the cell membrane from the adverse effects of salinity

(Rengel, 1992). Addition of Ca^{2+} increased K^+ / Na^+ ratios in tomato (Levent *et al.*, 2007).

Calcium is an essential plant nutrient and has a role in metabolic activities, like stabilization of membranes, signal transduction through second messenger, and control of enzyme activity in *Cassia angustifolia* (Arshi *et al.* 2006). Ca^{2+} can help to remediate the adverse effect of salinity on plants. It helps in maintaining membrane integrity and ion-transport regulation and is essential for K^+/Na^+ and $\text{Ca}^{2+}/\text{Na}^+$ selectivity (Maathuis and Amtmann, 1999); in *Cornus stolonifera* (Renault, 2005). Elevated Ca^{2+} concentration in nutrient solution mitigates the adverse effects of NaCl by inhibiting Na^+ uptake (Kaya *et al.*, 2002) and reducing membrane leakage (Tuna *et al.* 2007). K^+ concentrations reduced by salinity, can be restored to adequate levels by an additional supply of calcium, as it protects cell membranes from adverse effect of Na^+ and minimizes the leakage of cytosolic potassium. Calcium plays a vital role in the regulation of ionic relations in plants and in improving the soil physical conditions (Qadir *et al.* 2001).

A high cytosolic K^+ / Na^+ ratio is an essential requirement for plant growth in high salt concentrations (Zhu, 2003).

Chaum *et al.* (2012) reported that Calcium (Ca) is a signaling molecule that plays an active role in regulating various mechanisms involved in recognition and response to abiotic stresses in plants. However, not much has been done to evaluate its role in regulating physiological and biochemical process in response to salt-induced stress. Two rice genotypes viz. Pokkali, salt tolerant and IR29 salt susceptible, grown on liquid Murashige and Skoog medium (MS) supplied by 1.98 mM CaCl_2 (control) were compared to 2 (3.96 mM), 4 (7.92 mM) and 8 (15.84 mM) folds exogenous CaCl_2 pretreatment subsequently exposed to 200 mM NaCl salt stress. Thus, the present investigation evaluated the potential of exogenous calcium chloride (CaCl_2) supply in improving the growth performance and photosynthetic ability in salt stressed rice. In IR29 salt susceptible rice, leaf area of salt-stressed seedling was significantly recovered by exogenous application of 7.92 mM CaCl_2 , which was greater by 1.38-folds over that in 1.98 mM CaCl_2 application. Exogenous CaCl_2 (7.92 mM) enhanced proline accumulation in both Pokkali (3.26 $\mu\text{mol g}^{-1}$ FW) and IR29 (4.37 $\mu\text{mol g}^{-1}$ FW) genotypes, and reduced relative electrolyte leakage thereby indicating its positive role in membrane stability. Treatment of 7.92 mM CaCl_2 significantly

enhanced the photosynthetic abilities, including maximum quantum yield of PSII (Fv/Fm), photon yield of PSII, photochemical quenching (qP) and net photosynthetic rate (Pn), in two genotypes of salt-stressed rice seedlings, especially in salt susceptible IR29 genotypes. The study concludes that an exogenous application of 7.92mM CaCl₂ significantly enhanced the photosynthetic abilities and overall growth performances in the photoautotrophic growth of salt-stressed rice seedlings. Exogenous calcium in the culture media may absorb by root tissues, transfer to whole plant and function as salt defense mechanisms including calcium signaling in the abscisic acid (ABA) regulation system and calcium sensing in stomatal closure when plant subjected to salt stress.

Howladar and Rady (2012) studied the effect of coating the seeds with calcium paste before sowing, on plant growth, yield, the contents of some antioxidants and the activities of carbonic anhydrase and nitrate reductase in the *Pisum sativum* L. leaves under the influence of NaCl stress. NaCl stress reduced plant growth, photosynthetic pigment levels, ascorbic acid and calcium contents, and the activities of carbonic anhydrase and nitrate reductase. In contrast, proline and sodium contents were increased. These results are negatively reflected in the yield components. However, seed coating with calcium paste reduced the toxic effects of NaCl on plant growth and yield by increasing leaf pigments, ascorbic acid, proline contents and enzymatic activities. This study clearly highlights the effects of calcium paste as a seed coat in mitigating the phytotoxicity of NaCl stress in pea plants.

Soualem *et al.* (2014) studied the effect of calcium sulfate (CaSO₄) supply under salt stress was studied in two populations of *Atriplex halimus* from two locations (coastal western Algeria (Oran) and continental semi-arid zone (Djelfa)) contrasted for salinity gradients. The plants were grown in pots and subjected to salt stress (0, 300 or 500 mM NaCl) with a supply of (5 or 10mM) of CaSO₄. Growth, mineral, proline and soluble sugars contents were measured. The results showed a reduction in growth with increasing NaCl concentration. The impact of salinity was more pronounced on the inland population than the coastal one. The leaves Na⁺ content increased with increasing salt stress and led to reduced plant growth. In response to the intensity of salt stress and CaSO₄ supply, plants accumulated more soluble sugars, proline and K⁺.

This accumulation was more pronounced at high concentrations of NaCl and CaSO₄ in both populations. Our results emphasized that supply of CaSO₄ reduced the inhibitory effects of NaCl.

Jaleel *et al.* (2008) reported that, in a pot culture experiment, the effect of calcium chloride (CaCl₂) as an ameliorating agent on sodium chloride (NaCl) stress was studied in *Dioscorea rotundata* plants. Plants were raised in pots and exposed to salinity stress (80 mM NaCl) with or without 5 mM CaCl₂. NaCl-stressed plants showed decreased protein and total sugars, and increased free amino acid and proline content. When NaCl treatment was combined with CaCl₂, overall plant metabolism was altered, with increased antioxidant enzyme activity, paving the way for partial amelioration of oxidative stress caused by salinity.

Barakat (2011) reported that, *Vicia faba* L. plant was grown in a pot experiment to study the positive role of CaCl₂ on NaCl induced stress in terms of growth parameters, metabolic, cation contents and cell wall degrading enzymes in different plant organs. The salinity treatments were having an osmotic potential of (0.0; -0.23; -0.46; -0.92 and -1.15 MPa), respectively. A hundred mL of 10 mM CaCl₂ were added to the previous concentrations and harvested after 11 weeks old. The data revealed that, NaCl treatments reduced the growth parameters; which most sensitive in root than shoot. Organic cytosolutes were much higher in root than shoot organ except for protein accumulation. The amount of inorganic cytosolutes (Na⁺ and Ca²⁺) in general increased markedly in shoot than root and vice versa for K⁺ and Mg²⁺. CaCl₂ treatment alone induces these parameters than control one. Mixed salts of NaCl and CaCl₂ positively improve the aforementioned parameters with varying degrees depending on the organs. While root seems to be the more sensitive organ for growth parameters measured, it also seems most accumulator organ than shoot for many metabolites. For the ionic contents, shoot and root varies between the mono and divalent cations. Cell wall degrading enzymes significantly and progressively increased as salinity level of treated plants increased. However, CaCl₂ treatments induced a significant reduction in the activity of these enzymes when compared with their respective NaCl treatments. The ameliorative percentage due to calcium application of stressed faba bean on growth parameters ranges from 17.53 to 79.55% for metabolites from 8.69 to 194.91 for ionic status from 9.94 to 56.67% and for cell

wall degrading enzymes from 16.76 to 39.15%. These data leads to strongly recommend adding CaCl_2 to saline environment to decrease the deleterious effects of salinity.

Externally supplied Ca^{2+} reduces the toxic effects of NaCl, presumably by facilitating a high K^+/Na^+ selectivity (Liu and Zhu 1998 and Abdel Latef, 2011).

Sunukumar *et al.* (2011). Worked on Active Oxygen Species (AOS) synthesis and its scavenging were investigated in the control, different concentrations of NaCl and NaCl + CaCl_2 stressed *Amaranthus tricolor* L. and *Phaseolus vulgaris* L. AOS such as superoxide anion and H_2O_2 content showed a steady increase in the plants of all NaCl treated media compared to control. When the salinized media were supplemented with CaCl_2 the AOS level drastically decreased compared to the corresponding plants grown on salt alone. Similarly, the activity of antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, catalase and glutathione reductase under salt stress were higher in NaCl + CaCl_2 supplemented media than the plants on the salinized media alone. This suggested that the alleviation effect of calcium under saline condition was through modulation of the enzyme complexes that accelerate the rate of antioxidant enzymes biosynthesis under salt stress. Similarly, the level of lipid peroxidation was found to be lower in plants of all NaCl + CaCl_2 media than control.

Jafari (2009) studied the interactive effects of salinity, calcium and potassium on physio-morphological traits of sorghum (*Sorghum bicolor* L.) in a greenhouse experiment. Treatments included 4 levels of NaCl (0, 80, 160, and 240 mM NaCl), 2 levels of CaCl_2 (0 and 20 mM), and 2 levels of KCl (0 and 20 mM). Number of leaves was significantly affected by NaCl, while elevated calcium promoted total number of leaves, particularly at high levels of NaCl. The interaction effect of Ca^{2+} and K^+ improve leaf generation only at 160 mM NaCl. Salt stress, increased mortality of leaves exposed to salinity. Application of Ca^{2+} and K^+ didn't make up the adverse effects of salinity, in comparison to control.

Khan (2013) reported that salinity reduced the growth of wheat plants. When K and N were applied as foliar spray on the wheat plant, it reduced the effect of salinity and increased the plant growth such as plant height, leaf number plant fresh and dry weight and physiological attributes such as chlorophyll content of wheat plants. Similarly, grains yield is also decreased by salinity but foliar application of K and N mitigated the salinity effect on grains yield.

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted during the period from October 2013 to March 2014. The materials and methods those were used and followed for conducting the experiment have been presented under the following headings.

3.1 Experimental site

This study was conducted in the Horticulture Farm of Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. The location of the experimental site is 23°74'N latitude and 90°35'E longitude at an altitude of 8.6 meter above the sea level (Anon., 2004), which have been shown in the Appendix III.

3.2 Characteristics of soil

The soil of the experimental area belongs to the Modhupur Tract (Anon., 1988) under AEZ No. 28. The characteristics of the soil under the experiment were analyzed in the Laboratory of Soil science Department, SAU, Dhaka and details of soil characteristics have been presented in Appendix I.

3.3 Climatic condition of the experimental site

The experimental site is situated in the subtropical monsoon climatic zone, which is characterized by heavy rainfall during the months from April to September (Kharif season) and scanty of rainfall during rest of the year (Rabi season). Plenty of sunshine and moderately low temperature prevail during October to March (Rabi season), which are suitable for growing of tomato in Bangladesh. The weather information regarding temperature, rainfall, relative humidity and sunshine hours prevailed at the experimental site during the cropping season October 2013 to April 2014 have been presented in Appendix II.

3.4 Planting materials

Seedlings of 30 days of BARI Tomato-5 were used. The seedlings of tomato were grown at the nursery of Horticulture Farm in Sher-e-Bangla Agricultural University. BARI Tomato-5, a high yielding variety of Tomato was developed by the Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, Bangladesh. It was released in 1996. Its total duration is about 95-100 days after transplanting.

3.5 Treatments of the experiment

The experiment consisted of two factors:

Factor A: Different doses of sodium (Na^+) with irrigation water

Factor (B): Different doses of Ca^{2+}

- | | |
|--|-----------------------------------|
| i. 0 dSm^{-1} marked as S_0 (control) | i. 0 mM marked as C_0 (control) |
| ii. 2 dSm^{-1} marked as S_1 | ii. 5mM marked as C_1 |
| iii. 4 dSm^{-1} marked as S_2 | iii. 10mM marked as C_2 |
| iv. 6 dSm^{-1} marked as S_3 | |
| v. 8 dSm^{-1} marked as S_4 | |

Total 15 treatment combinations were as follows:

S_0C_0 : Without Salt+ Without Calcium
 S_0C_1 : Without Salt +5 mM Calcium
 S_0C_2 : Without Salt +10 mM Calcium
 S_1C_0 : 2 dSm^{-1} Salt+ Without Calcium
 S_1C_1 : 2 dSm^{-1} Salt+ 5 mM Calcium
 S_1C_2 : 2 dSm^{-1} Salt+ 10 mM Calcium
 S_2C_0 : 4 dSm^{-1} Salt+ Without Calcium
 S_2C_1 : 4 dSm^{-1} Salt+ 5 mM Calcium
 S_2C_2 : 4 dSm^{-1} Salt+ 10mM Calcium
 S_3C_0 : 6 dSm^{-1} Salt+ Without Calcium
 S_3C_1 : 6 dSm^{-1} Salt+ 5 mM Calcium
 S_3C_2 : 6 dSm^{-1} Salt+ 10 mM Calcium
 S_4C_0 : 8 ds/m Salt+ Without Calcium
 S_4C_1 : 8 dSm^{-1} Salt+ 5 mM Calcium
 S_4C_2 : 8 dSm^{-1} Salt+ 10 mM Calcium

3.6 Design and layout of the experiment

The two factors experiment was laid out in Randomized Complete Block Design (RCBD) with five levels of salinity and three levels of calcium. Four replications were maintained in this experiment. The total number of unit pots was 60 (15×4). Each pot was 35 cm (14 inches) in diameter and 30 cm (12 inches) in height. The experiment was placed under vinyl house which was made by bamboo with polythene roof and pots were kept on the bamboo made frame of 70 cm height.

3.7 Seedling raising

A common procedure was followed in raising of seedlings in the seedbed. Tomato Seedlings were raised in one seedbed on a relatively high land at Horticulture Farm of Sher-e-Bangla Agricultural University, Dhaka. The size of the seedbed was 3m× 1 m. The soil was well prepared with spade and made into loose friable and dried mass to obtain fine tilth. All weeds and stubbles were removed and 5 kg well rotten cowdung was applied during seedbed preparation. The seeds were sown in the seedbed at 16 November, 2013 to get 30 days old seedlings. Germination was visible 3 days after sowing of seeds. After sowing, seeds were covered with light soil to a depth of about 0.6 cm. Heptachlor 40 WP was applied @ 4 kg ha⁻¹ around each seedbed as precautionary measure against ants and worm. The emergence of the seedlings took place within 5 to 6 days after sowing. Necessary shading by banana leaves was provided over the seedbed to protect the young seedlings from scorching sun or heavy rain. Weeding, mulching and irrigation were done from time to time as and when required and no chemical fertilizer was used in this seedbed.

3.8 Pot preparation

A ratio of 1:3 well rotten cow dung and soil were mixed and pots were filled 15 days before transplanting. Silt Loam soils were used for pot preparation. All 60 pots were filled on October 2013. Weeds and stubbles were completely removed from the soil.

3.9 Uprooting and Transplanting of Seedlings

Healthy and uniform 30 days old seedlings were uprooted separately from the seedbed and were transplanted in the experimental pots in the afternoon of 16 December, 2013 maintaining two seedlings in each pot. The seedbed was watered before uprooting the

seedlings from the seedbed so as to minimize damage to roots with ensuring maximum retention of roots. The seedlings were watered after transplanting. Shading was provided using banana leaf sheath for three days to protect the seedlings from the hot sun and removed after seedlings were established.

3.10 Application of the treatments

Tomato plants were treated with 0, 2, 4, 6 and 8 dsm^{-1} salinity levels which were maintained by adding 0, 12, 27, 48 and 58 g of sodium chloride (NaCl) respectively per pot containing 10 kg soil. These total amounts of salts were applied through irrigation water in three splits at 30, 50 and 70 DAT. As a Na^+ stress mitigation agent, Ca^{2+} was used in the form of $\text{CaSO}_4 \cdot 0.5\text{H}_2\text{O}$ at 0, 5 and 10 mM concentration with irrigation water at 30, 50 and 70 DAT.

3.11 Intercultural operations

3.11.1 Irrigation

Light watering was provided with water cane immediately after transplanting the seedlings and this technique of irrigation was used as every day at early morning and sometimes also in evening throughout the growing period. But the frequency of irrigation became less in harvesting stage. Irrigation in those days when treatment was applied was done at evening as salt was applied with irrigation water. The amount of irrigation water was limited up to that quantity which does not leached out through the bottom. As such the salinity status was maintained in the desired level.

3.11.2 Staking

When the plants were well established, staking was given to each plant by bamboo sticks for support to keep them erect.

3.11.3 Weeding

Weeding was done whenever it was necessary, mostly in vegetative stage.

3.11.4 Plant Protection Measures

Melathion 57 EC was applied @2 ml L^{-1} of water against the insect pests like cutworm, leaf hopper, fruit borer and others. The insecticide application was made fortnightly after transplanting and was stopped before second week of first harvest.

Furadan 10G was also applied during pot preparation as soil insecticide. During foggy weather precautionary measure against disease attack of tomato was taken by spraying Diathane M-45 fortnightly @2 gm L⁻¹ of water at the early vegetative stage. Ridomil gold was also applied @ 2 gmL⁻¹ of water against blight disease of tomato.

3.12 Harvesting

Fruits were harvested at 3 days interval during early ripe stage when they developed slightly red color. Harvesting was started from 11 March, 2014 and was continued up to 1st week of April 2014.

3.13 Recording of Data

Experimental data were recorded from 40 days after transplanting and continued until harvest. The following data were recorded during the experimental period.

A. Morphological characters

1. Plant height (at different days after transplanting)
2. Leaf Number plant⁻¹ (at different days after transplanting)
3. No. of branches plant⁻¹ (at different days after transplanting)
4. Leaf Area plant⁻¹ (cm²)

B. Physiological characters

5. Leaves dry Wt. (gm) plant⁻¹
6. Stem dry Wt. (gm) plant⁻¹
7. Chlorophyll content -SPAD reading
8. Stomatal conductance
9. Na⁺ (% of DW basis) content in leaves
10. K⁺ (% of DW basis) content in leaves
11. Ca²⁺ (% of DW basis) content in leaves
12. K⁺/Na⁺ ratio
13. Ca²⁺/Na⁺ ratio

C. Yield contributing and yield characters

14. No. of flower clusters plant⁻¹
15. No. of flowers cluster⁻¹
16. No. of flowers plant⁻¹
17. No. of fruits per plant⁻¹
18. No. of fruits per cluster⁻¹
19. No. of fruits per plant⁻¹
20. Length of fruit
21. Diameter of fruit

22. Wt. of individual fruit
23. Fruit Wt. plant⁻¹ (kg)
24. Yield (t ha⁻¹)

3.14 Detailed Procedures of Recording Data

A brief outline of the data recording procedure followed during the study is given below:

A. Morphological characters

1. Plant height (cm)

Plant height was measured at 40, 50, 60, and 75 DAT. The height of the plant was determined in centimeter by measuring the distance from the soil surface to the tip of the highest leaf.

2. Leaf No. plant⁻¹

Leaf number was counted at 40, 50, 60 and 75 DAT. The number of leaves plant⁻¹ was counted from each plant.

3. No. of branches plant⁻¹

The total number of branches plant⁻¹ was counted from each plant at 45 DAT, 60 DAT and 75 DAT. There is no option to make average value from collected value due to only one plant was maintained per pot.

4. Leaf Area plant⁻¹ (cm²)

Leaf area was measured by non-destructive method using CL-202 Leaf Area Meter, (USA). Mature leaves were measured all the time and were expressed in cm².

5. Leaves dry wt. (gm) plant⁻¹

After harvesting, all the leaves of plant were collected and sliced into very thin pieces and were put into envelop and placed in oven maintaining at 70 °C for 72 hours. The samples were then transferred into desiccators and allowed to cool down at room temperature. The final weight of the sample was taken in gram.

6. Stem dry wt. (gm) plant⁻¹

After harvesting, stems of plant were collected and sliced into very thin pieces and were put into envelop and placed in oven maintaining at 70 °C for 72 hours. The sample was then transferred into desiccators and allowed to cool down at room temperature. The final weight of the sample was taken in gram.

B. Physiological characters

7. Chlorophyll content-SPAD reading

Leaf chlorophyll content was measured using a hand-held chlorophyll content SPAD meter (CCM-200, Opti-Science, USA). At each evaluation the content was measure 5 times from five leaves at different positions plant⁻¹ and the average was used for analysis.

8. Stomatal conductance

Stomatal conductance was measured using a portable photosynthesis system ADC LC pro+4 (UK) which is a non-destructive method. Mature leaf were measured all time and expressed in molm⁻²s⁻¹.

9. Na⁺ (% of DW basis) content in leaves

Sodium content was measured from digest sample of leaves of tomato by using the flame photometer. The concentrations were measured by using standard curves and expressed as percentage.

10. K⁺ (% of DW basis) content in leaves

Potassium content was measured from digest sample of leaves of tomato by using the flame photometer. The concentrations were measured by using standard curves and expressed as percentage.

11. Ca²⁺ (% of DW basis) content in leaves

Potassium content was measured from digest sample of leaves of tomato by using the flame photometer. The concentrations were measured by using standard curves and expressed as percentage

12. K⁺/ Na⁺ ratio

K⁺/ Na⁺ ratio in leaves of tomato was measured from the Na⁺ and K⁺ content in leaves.

13. Ca²⁺ / Na⁺ ratio

Ca²⁺/ Na⁺ ratio in leaves of tomato was measured from the Ca²⁺ and Na⁺ content in leaves.

C. Yield contributing and yield characters

14. No. of flower clusters plant⁻¹

The number of flower clusters produced plant⁻¹ was counted and recorded.

15. No. of flowers cluster⁻¹

The number of flower produced cluster⁻¹ was counted on the basis of flower cluster plant⁻¹.

16. No. of flowers plant⁻¹

The number of flower plant⁻¹ was counted and recorded.

17. No. of fruits per cluster⁻¹

The number of fruits cluster⁻¹ was counted from the plant.

18. No. of fruits per plant⁻¹

The number of fruits plant⁻¹ was counted and recorded.

19. Number of dropped flower plant⁻¹

The number of dropped flower plant⁻¹ was calculated by subtracting the total number of fruits plant⁻¹ from the total number of flowers plant⁻¹

20. Length of fruit (cm)

The length of fruit was measured with a slide calipers from the neck of the fruit to the bottom of 10 fruits from each plant and their average was taken and expressed in cm.

21. Diameter of fruit (cm)

Diameter of fruit was measured at middle portion of 10 fruits from each plant with a slide calipers. Their average was taken and expressed in cm.

22. Wt. of individual fruit

Among the total number of fruits during the period from first to final harvest, fruit was considered for determining the individual fruit weight by the following formula:

$$\text{Weight of individual fruit (gm)} = \frac{\text{Total weight of fruits}}{\text{Total number of fruits}}$$

23. Fruit wt. plant⁻¹ (kg)

Fruit weight of tomato plant⁻¹ was calculated from the whole fruit plant⁻¹ and was expressed in kilogram (kg)

24. Yield (t ha⁻¹)

Yield hectare⁻¹ of tomato fruits was calculated by converting the weight of plant yield into hectare on the basis of total plant population of tomato hectare⁻¹ and expressed in ton.

3.15 Chemical Analysis of leaf and Soil samples

1. Soil sample

Soil samples were analyzed for both physical and chemical properties in the laboratory of the Soil Science department, SAU, Dhaka, Bangladesh. The properties studied included pH, organic matter, available Na, K and Ca. The soil was analyzed following standard methods. Particle-size analysis of soil was done by Hydrometer method (Bouyoucos, 1951) and soil pH was measured with the help of a glass electrode pH meter using soil water suspension of 1:2.5 as described by Jackson (1962). Available Na, K and Ca content were measured by flame photometer.

2. Plant sample

A sub sample weighing 0.5 g was transferred into a dry, clean 100 ml digestion vessel. Ten ml of di-acid (HNO₃: HClO₄ in the ratio 2:1) mixture was added to the flask. After leaving for a while, the flasks were heated at a temperature slowly raised to 200⁰C. Heating were stopped when the dense white fumes of HClO₄ occurred. The content of the flask were boiled until they were became clean and colorless. After cooling, the content was taken into a 50 ml volumetric flask and the volume was made up to the mark with de-ionized water. Na, K and Ca were determined from this digest by using different standard methods.

3.16 Statistical Analysis

All the data collected on different parameters were statistically analyzed following the analysis of variance (ANOVA) technique using MSTAT-C computer package program and the mean differences were adjudged by least significant difference (LSD) test at 5% level of significance (Gomez and Gomez, 1984).

CHAPTER IV

RESULTS AND DISCUSSION

The results of the study on the effect of salinity levels and calcium on morphological, yield and yield contributing characters with physiological and biochemical properties of tomato have been presented and possible interpretations have been made in this chapter.

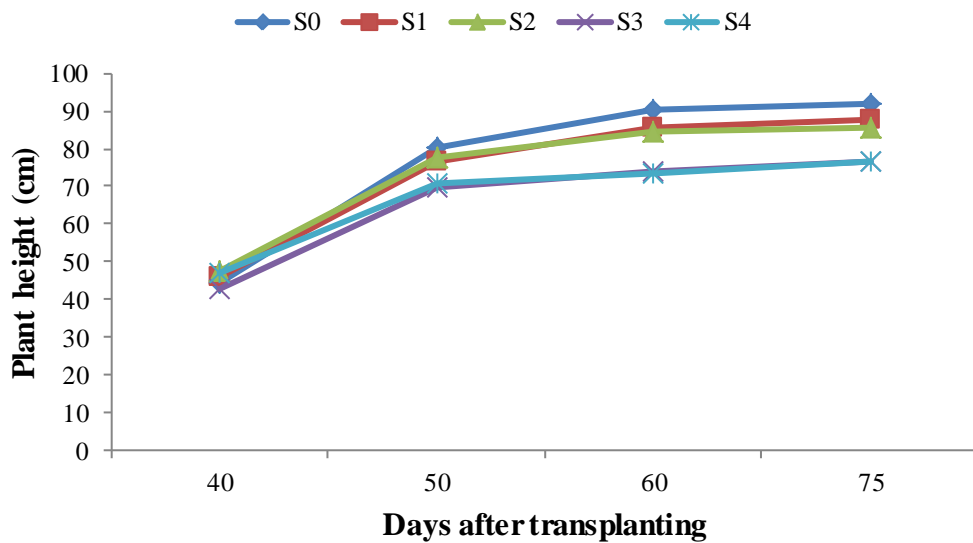
4.1 Effect of salinity and calcium on plant morphological characters

4.1.1 Plant height

The effect of NaCl stress on the growth of tomato plant is reflected firstly in plant height. From previous research and findings, now it is well established that the salinity strongly regulated the plant growth and development by decreasing plant height, leaves number, leaf area, branches number etc. (Nawaz *et al.* 2010; Islam 2004; Mohammad *et al.* 1998). As natural plant height increased with increasing age but decreased with increasing salinity in tomato. The plant height varied significantly ($p \leq 0.01$) due to the effect of salinity stresses observed at 40, 50, 60 and 75 DAT with statistically significant variation (Figure 1 and Appendix IV). The results of this study showed that salinity significantly reduced the plant height of tomato at different days after transplanting (DAT) and the reduction was quite incremental with the increase of NaCl concentrations. At 40 DAT, the highest plant height (47.67 cm) was found from S₂ and the lowest value (42.75 cm) was observed with S₃ treatment. At 50 DAT, the highest plant height (80.50 cm) was recorded in case of S₀ and the lowest value (69.83 cm) was found from S₃. At 60 DAT, the highest plant height (90.58 cm) was recorded from S₀ and the lowest value (73.67 cm) was observed in case of S₄ with statistically identical result (73.92 cm) to S₃. At 75 DAT, the highest plant height (91.75 cm) was found from S₀ where the lowest value (76.58 cm) was found from S₄ which was statistically similar (76.83 cm) to S₃. The natural plant height increased with increasing age but decreased with increasing salinity in tomato. Similar results were also recorded by many other authors like Mohammad *et al.* (1998) in tomato, Jafari (2009) and Nawaz (2010) in sorghum, Milne (2012) in lettuce, Alaa El-din Sayed Ewase (2013) in coriander etc. The reduction of plant height may be due to inhibitory behavior of salt stress on cell division and cell expansion (Hernandez *et al.*, 2003).

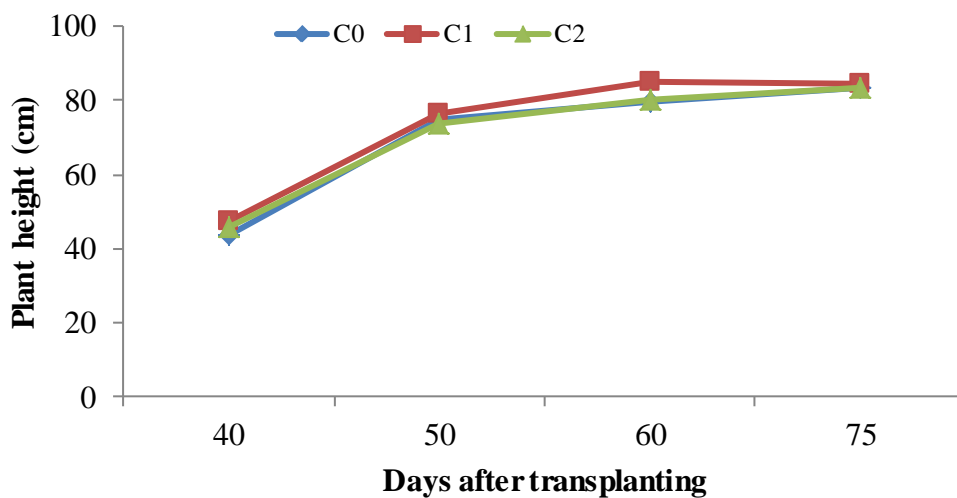
In this study, different dose of calcium such as 0(C₀), 5 mM (C₁) and 10 mM (C₂) were used to investigate the effect of calcium to reduce the saline toxicity in plant height. As shown in Figure 2 and appendix IV, calcium significantly increased plant height at 40, 50, 60 and 75 DAT. At 40 DAT the highest plant height (47.30 cm) was found from C₁ and the lowest value (43.50 cm) was recorded from C₀. At 50 DAT, the plant height (76.65 cm) was found from C₁ where the lowest value (73.90 cm) was recorded from C₂. At 60 DAT, the highest plant height (84.90cm) was recorded from C₁ and the lowest value (79.50 cm) was found from C₀. At 75 DAT, the highest plant height (84.65 cm) was recorded from C₁ and the lowest value (83.30 cm) was found from C₂ which was statistically similar (83.40 cm) to C₀. From this experiment it was observed that calcium increased the plant height as compared with control where the best result was found from 5mM concentration of calcium. Manivannan *et al.* (2007) also reported that calcium increased the plant height. Al-Moshileh (2004) also reported the same result in case of potato.

The results of the present study showed that, the interaction effect between salinity stress and calcium as mitigation agent on plant height was significant at 40, 50, 60 and 75 DAT (Table 1 and appendix IV). At 40 DAT the highest plant height (49.50 cm) was found from S₀C₁ and the lowest value (38.50 cm) was recorded in case of S₀C₀. At 50 DAT, the plant height (83.00 cm) was found from S₀C₁ where the lowest value (67.50 cm) was recorded from S₄C₀ which was statistically similar (68.25 cm) to S₃C₂. At 60 DAT, the highest plant height (97.00 cm) was recorded from S₀C₁ and the lowest value (66.75 cm) was found from S₄C₀. At 75DAT, the highest plant height (95.25 cm) was found from S₀C₁ where the lowest value (74.25 cm) was recorded from S₃C₀ which was statistically similar (75.25 cm) to S₄C₀.



S_0 = without salt, $S_1 = 2 \text{ dSm}^{-1}$, $S_2 = 4 \text{ dSm}^{-1}$, $S_3 = 6 \text{ dSm}^{-1}$, $S_4 = 8 \text{ dSm}^{-1}$

Figure 1: Effect of salinity levels on plant height (at different days after transplanting, $LSD_{0.05} = 0.4505, 0.5714, 0.645$ and 1.421 for 40 DAT, 50 DAT, 60 DAT and 75 DAT respectively) of tomato



C_0 = No calcium, $C_1 = 5 \text{ mM}$, $C_2 = 10 \text{ mM}$

Figure 2: Effect of calcium levels on plant height (at different days after transplanting, $LSD_{0.05} = 0.349, 0.4426, 0.4997$ and 1.101 for 40 DAT, 50 DAT, 60 DAT and 75 DAT respectively) of tomato

Table 1: Interaction effect of salinity and calcium levels on plant height (at different days after transplanting) of tomato

Treatment combinations	Plant height at different days after transplanting (cm)			
	40	50	60	75
S ₀ C ₀	38.50 i	77.50 d	85.50 d	88.50 cd
S ₀ C ₁	49.50 a	83.00 a	97.00 a	95.25 a
S ₀ C ₂	45.25 f	81.00 b	89.25 b	91.50 b
S ₁ C ₀	44.75 f	79.25 c	85.50 d	89.00 c
S ₁ C ₁	48.75 abc	79.50 c	85.75 d	86.25 de
S ₁ C ₂	43.50 g	71.25 h	85.00 d	87.75 cd
S ₂ C ₀	47.50 d	81.25 b	88.00 c	90.00 bc
S ₂ C ₁	46.50 e	77.25 d	83.00 e	82.00 f
S ₂ C ₂	49.00 ab	75.00 e	82.00 ef	85.25 e
S ₃ C ₀	42.25 h	68.50 i	71.75 i	74.25 h
S ₃ C ₁	43.25 g	72.75 g	81.00 f	82.25 f
S ₃ C ₂	42.75 gh	68.25 ij	69.00 j	77.00 g
S ₄ C ₀	44.50 f	67.50 j	66.75 k	75.25 gh
S ₄ C ₁	48.50 bc	70.75 h	77.75 g	77.50 g
S ₄ C ₂	48.00 cd	74.00 f	76.50 h	77.00 g
LSD (0.05)	0.7803	0.9897	1.117	2.462
F-test value	**	**	**	**
CV (%)	1.2	0.92	0.96	2.06

S₀ = without salt, S₁ = 2 dSm⁻¹, S₂ = 4 dSm⁻¹, S₃ = 6 dSm⁻¹, S₄ = 8 dSm⁻¹
C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

4.1.2 Number of leaves plant⁻¹

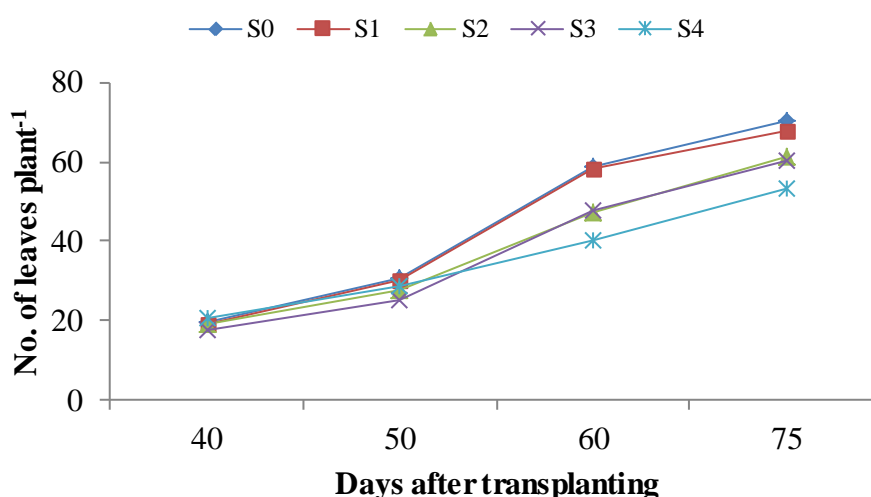
The leaf number is the very important character for plant growth and development as leaf is the main photosynthetic organ. Salinity adversely affected the production of leaf number per plant in tomato.

The results of this experiment showed that different concentration of salt have significant effect on number of leaves per plant of tomato at different DAT such as 40, 50, 60 and 75 DAT (Figure 3 and Appendix V). Number of leaves in each plant was decreased due to increasing level of salt. At 40 DAT maximum numbers of leaves plant⁻¹ (20.58) was found in case of S₄ and the lowest value (17.75) from S₃. At 50 DAT, the highest number of leaves (30.92) was recorded from S₀ and the lowest value (25.17) was found from S₃. At 60 DAT, the highest number of leaves (58.92) was observed in case of S₀ whereas the lowest value (40.33) was found from S₄. At 75 DAT, the highest number of leaves plant⁻¹ (70.50) was recorded from S₀ which was statistically similar (67.75) to S₁, and the lowest value (53.50) was found in case of S₄. Similar observation was also observed by Alaa El-Din Sayed Ewase (2013) who reported that number of leaves plant⁻¹ decreased with the increase of NaCl concentration in coriander. Mohammad *et al.* (1998) also reported increasing salinity stress accompanied by significant reduction in number of leaves plant⁻¹. Jafari (2009), Saberi *et al.* (2011b), Islam (2004), and Angrish *et al.* (2001) also obtained reduced leaves number plant⁻¹ under salinity stress.

A significant effect of calcium on the number of leaves plant⁻¹ of tomato at 40, 50, 60 and 75 DAT was found (Figure 4 and Appendix V). At 40 DAT, the highest number of leaves plant⁻¹ (20.30) was found from C₂ and the lowest value (18.15) from C₀. At 50 DAT, the highest number of leaves plant⁻¹ (29.40) was recorded from C₀ and the lowest one (28.00) from C₁ which was statistically similar (28.35) to C₂. At 60 DAT, the highest number of leaves (51.00) was observed in case of C₁ where the lowest value (49.90) was recorded from C₀. At 75 DAT, The highest number of leaves plant⁻¹ (64.15) was recorded from C₁ whereas the lowest value (61.90) was found from C₂ which was statistically similar (61.95) to C₀. From this experiment it was found that, the number of leaves was gradually increased with the increase in age with the supplementation of calcium along with salt. Thus these results suggested that the calcium application increased the number of leaves by reducing the effect of salt. This fact was supported by other authors like Tzortzakis (2010) in leafy vegetables, Lolaei

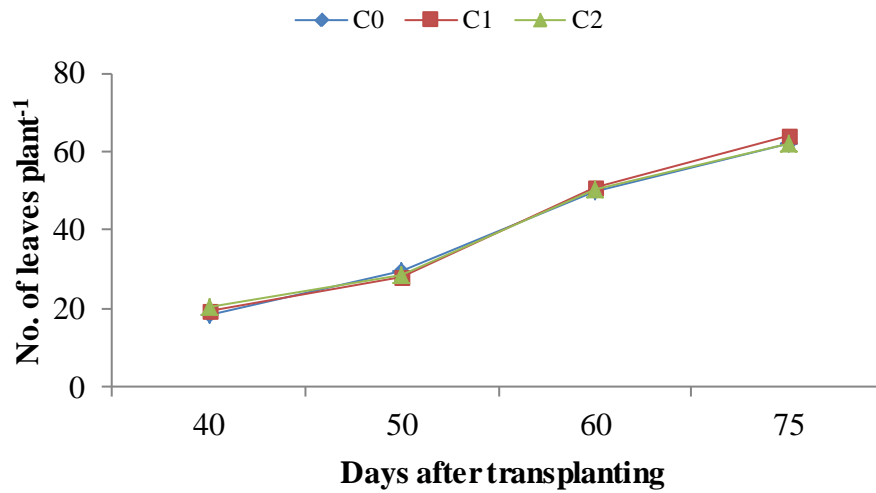
et al. (2012) in tomato and Al- Mohshileh (2004) in potato.

The combined effect of salinity and calcium on the number of leaves plant⁻¹ was significantly reflected at 40, 50 and 60 DAT and was non-significant at 75 DAT (Table 2 and Appendix V). At 40 DAT, the highest number of leaves plant⁻¹ (23.25) was found from S₄C₂ and the lowest value (16.00) was found from S₃C₀. At 50 DAT, the highest number of leaves plant⁻¹ (35.00) was found from S₀C₀ and the lowest value (24.25) was found from both S₃C₀ and S₃C₁. At 60 DAT, the highest number of leaves plant⁻¹(63.75) was found from S₁C₁ and the lowest value (38.50) was found from S₄C₁. At 75 DAT, the highest number of leaves plant⁻¹ (71.00) was found from S₁C₁ which was statistically similar to S₀C₀ (69.00), S₀C₁ (65.00), S₀C₂ (69.25), S₁C₀ (70.50), S₁C₂ (70.00), S₂C₀ (64.00), S₂C₁ (60.25), S₃C₀ (64.25) and S₃C₁ (61.75). The lowest value (52.25) was found from S₄C₁ which was statistically identical to S₂C₁ (60.25), S₂C₂ (59.50), S₃C₁ (61.75), S₃C₂ (55.00), S₄C₀ (52.50) and S₄C₂ (55.75).



S₀ = without salt, S₁ = 2 dSm⁻¹, S₂ = 4 dSm⁻¹, S₃ = 6 dSm⁻¹, S₄ = 8 dSm⁻¹

Figure 3: Effect of salinity levels on leaves number plant⁻¹(at different days after transplanting, LSD_{0.05} = 0.5636, 0.5178, 0.5779 and 6.412 for 40DAT, 50DAT, 60 DAT and 75 DAT respectively) of tomato



C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

Figure 4: Effect of calcium levels on leaves number plant⁻¹ (at different days after transplanting, LSD_{0.05} = 0.4366, 0.4011, 0.4476 and 12.42 for 40DAT, 50DAT, 60 DAT and 75 DAT respectively) of tomato

**Table 2: Interaction effect of salinity and calcium levels on leaves number plant⁻¹
(at different days after transplanting) of tomato**

Treatment combination	Number of leaves plant ⁻¹ at different days after transplanting							
	40		50		60		75	
S ₀ C ₀	18.75	ef	35.00	a	57.00	d	69.00	ab
S ₀ C ₁	19.25	de	27.75	fg	58.00	cd	65.00	abc
S ₀ C ₂	21.25	b	30.00	d	61.75	b	69.25	ab
S ₁ C ₀	19.25	de	33.25	b	59.00	c	70.50	ab
S ₁ C ₁	20.25	c	28.75	e	63.75	a	71.00	a
S ₁ C ₂	18.25	f	28.25	ef	52.25	ef	70.00	ab
S ₂ C ₀	18.50	ef	28.25	ef	51.50	f	64.00	abc
S ₂ C ₁	19.25	de	30.25	d	42.00	h	60.25	abcd
S ₂ C ₂	20.00	cd	25.25	i	47.50	g	59.50	bcd
S ₃ C ₀	16.00	g	24.25	j	41.75	h	64.25	abc
S ₃ C ₁	18.50	ef	24.25	j	52.75	e	61.75	abcd
S ₃ C ₂	18.75	ef	27.00	gh	48.25	g	55.00	cd
S ₄ C ₀	18.25	f	26.25	h	40.25	i	52.50	d
S ₄ C ₁	20.25	c	29.00	e	38.50	j	52.25	d
S ₄ C ₂	23.25	a	31.25	c	42.25	h	55.75	cd
LSD _(0.05)	0.9762		0.8969		1.001		11.11	
F-test value	**		**		**		NS	
CV (%)	3.54		2.20		1.39		12.42	

S₀ = without salt, S₁ = 2 dSm⁻¹, S₂ = 4 dSm⁻¹, S₃ = 6 dSm⁻¹, S₄ = 8 dSm⁻¹

C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

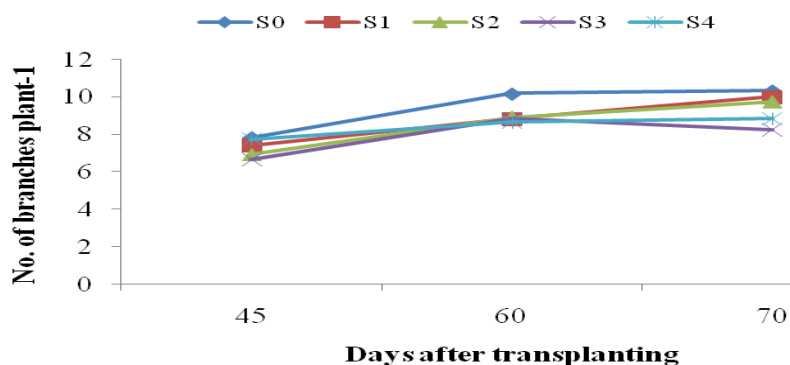
4.1.3 Number of branches plant⁻¹

Number of branches plant⁻¹ of tomato was significantly affected by the different levels of salinity at 45, 60 and 75 DAT (Figure 5 and Appendix VI). The highest number of branches plant⁻¹ (7.833) was found from S₀ which was statistically similar (7.75 and 7.417) to both S₄ and S₁. The lowest value (6.667) was recorded from S₃ which was statistically similar (6.917) to S₂. At 60 DAT, the highest number of branches plant⁻¹ (10.17) was observed from S₀ and the lowest value (8.667) was found from S₄ which was statistically similar (8.833, 8.917 and 8.833) to S₁, S₂, and S₃. At 75 DAT, where the highest Number of branches plant⁻¹ (10.33) was found from S₀ which was statistically similar (10.00 and 9.75) to both S₁ and S₂. The lowest value (8.25) was recorded from S₃ which was statistically similar (8.833) to S₄. Uddin *et al.*, 2005 also found that number of branch decreased with the increased salinity in *Brassica* species. Similar observation was also found in rice where tiller number decreased in response to salinity which was reported by Mortazainezhad *et al.*, 2006. Many other authors like LingHe *et al.*, 2000; Burman *et al.*, 2002; WeonYoung *et al.*, 2003; Islam, 2004; Rashid, 2005; Karim, 2007 also reported the similar results in rice.

A significant effect of calcium was found on the number of branches plant⁻¹ of tomato at 45, 60 and 75 DAT (Figure 6 and Appendix VI). At 45 DAT, the highest number of branches plant⁻¹ (7.70) was found from C₀ which was statistically similar (7.45) to C₁ and the lowest value (6.80) was recorded from C₂. At 60 DAT, where the highest number of branches plant⁻¹ (9.35) was observed from C₁ which was statistically similar (9.30) to C₀. The lowest value (8.60) was found from C₂. And at 75 DAT, the highest number of branches plant⁻¹ (10.05) was found from C₀ which was statistically identical (9.30) to C₁ and the lowest value (8.95) was found from C₂ which was also statistically similar (9.30) to C₁.

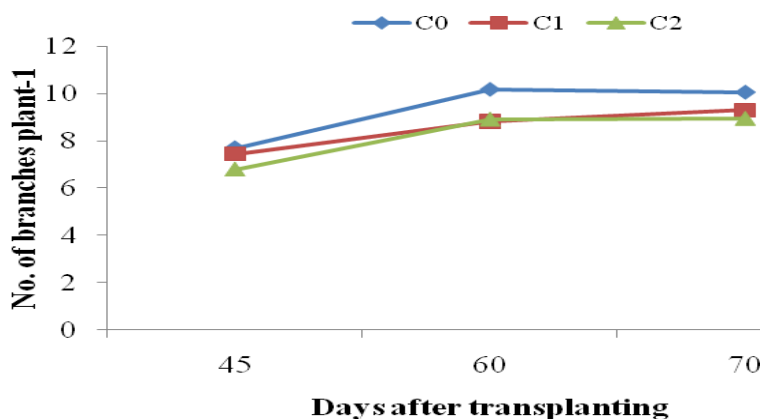
The combined effect of salinity and calcium on number of branches plant⁻¹ of tomato exhibited a significant effect at 45 DAT and not at both 60 and 75 DAT (Table 3 and Appendix VI). At 45 DAT, the highest number of branches plant⁻¹ (8.75) was found from S₀C₀ which was statistically similar (8.25 and 8.25) to both S₁C₀ and S₁C₁. The lowest value (6.75) was found from S₂C₀, S₂C₁, S₂C₂, S₃C₀, S₃C₁ and S₃C₂ which was also statistically similar (7.00) to S₀C₂. At 60 DAT, the highest number of branches plant⁻¹ (10.50) was found from S₀C₁ which was statistically similar (10.25 and 9.75) to both S₀C₀ and S₀C₂. The lowest value (8.000) was found from S₂C₂ which was statistically similar (8.25, 8.50 and 8.75) to S₁C₂, S₃C₂, S₄C₁, S₄C₂ and S₃C₀. At 75

DAT, the highest number of branches plant⁻¹(11.25) was found from S₁C₀ which was statistically similar (11.00, 10.75, 9.75, 10.00, 9.75 and 9.50) to S₀C₀, S₀C₁, S₁C₂, S₂C₀, S₂C₁ and S₂C₂. The lowest value (7.75) was found from S₃C₂ which was statistically similar to S₀C₂ (9.25), S₁C₁ (9.00), S₂C₂ (9.50), S₃C₀ (8.75), S₃C₁ (8.25), S₄C₀ (9.25), S₄C₁ (8.75) and S₄C₂ (8.50).



S₀ = without salt, S₁ = 2 dSm⁻¹, S₂ = 4 dSm⁻¹, S₃ = 6 dSm⁻¹, S₄ = 8 dSm⁻¹

Figure 5: Effect of salinity levels on branches number plant⁻¹(at different days after transplanting, LSD_{0.05} = 0.436, 0.4769 and 1.138 for 45DAT, 60 DAT and 75 DAT respectively) of tomato



C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

Figure 6: Effect of calcium levels on branches number plant⁻¹(at different days after transplanting, LSD_{0.05} = 0.3377, 0.3694 and 0.8813 for 45DAT, 60 DAT and 75 DAT respectively) of tomato

Table 3: Interaction effect of salinity and calcium levels on branches number plant⁻¹ (at different days after transplanting) of tomato

Treatment combination	Number of branches plant ⁻¹ at different days after transplanting		
	45	60	75
S ₀ C ₀	8.750 a	10.25 ab	11.00 ab
S ₀ C ₁	7.750 bc	10.50 a	10.75 abc
S ₀ C ₂	7.000 cd	9.750 abc	9.250 bcde
S ₁ C ₀	8.250 ab	9.000 cdef	11.25 a
S ₁ C ₁	8.250 ab	9.250 cde	9.000 cde
S ₁ C ₂	5.750 e	8.250 fg	9.750 abcd
S ₂ C ₀	7.250 cd	9.500 bcd	10.00 abcd
S ₂ C ₁	6.750 d	9.250 cde	9.750 abcd
S ₂ C ₂	6.750 d	8.000 g	9.500 abcde
S ₃ C ₀	6.500 de	8.750 defg	8.750 de
S ₃ C ₁	6.750 d	9.250 cde	8.250 de
S ₃ C ₂	6.750 d	8.500 efg	7.750 e
S ₄ C ₀	7.750 bc	9.000 cdef	9.250 bcde
S ₄ C ₁	7.750 bc	8.500 efg	8.750 de
S ₄ C ₂	7.750 bc	8.500 efg	8.500 de
LSD _(0.05)	0.7551	0.8259	1.971
F-test value	**	NS	NS
CV (%)	7.23%	6.37%	14.64%

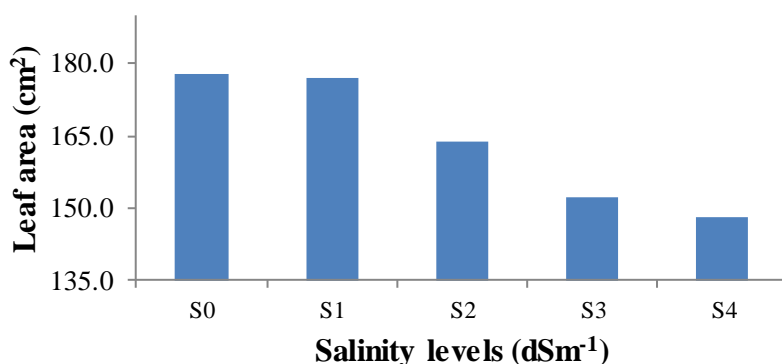
S₀ = without salt, S₁ = 2 dSm⁻¹, S₂ = 4 dSm⁻¹, S₃ = 6 dSm⁻¹, S₄ = 8 dSm⁻¹
C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

4.1.4 Leaf area plant⁻¹

Leaf area plant⁻¹ was significantly affected by different salinity levels. Leaf area (cm²) decreased with increasing concentration of salinity in tomato (Figure 7 and Appendix VII). The maximum leaf area (178.1 cm²) was recorded from control, S₀ (without salt) treated plant which was statistically similar (176.9 cm²) to S₁ where the minimum leaf area (148.2 cm²) was found from S₄ (8 dSm⁻¹). Similar result was also reported by Saberi *et al.* (2011b), Munns and Tester (2008), Sixto *et al.*, (2005), Netondo *et al.* (2004), Brungnoli and Lauteri(1991), Alberioco and Cramer (1993). According to Hernandez *et al.* (2003) salt stress inhibited the cell division and cell expansion, consequently leaf expansion and as a result leaf area is reduced.

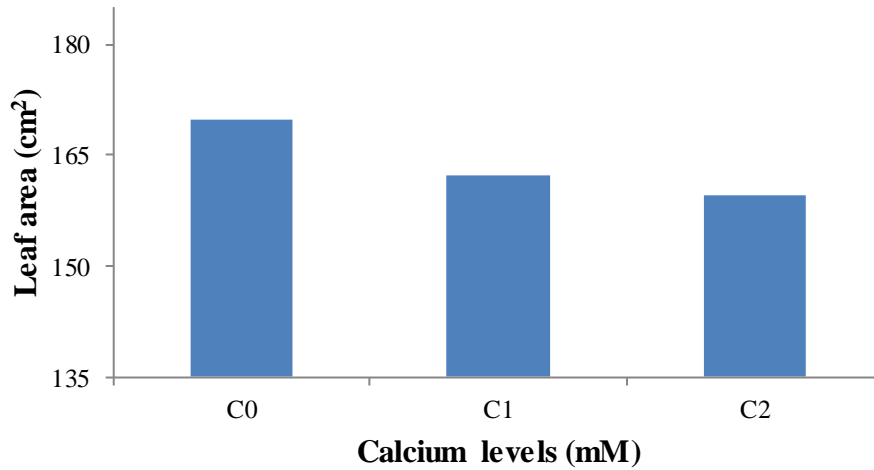
Different levels of calcium affected significantly on leaf area plant⁻¹ (Figure 8 and Appendix VII). The highest leaf area plant⁻¹(169.9 cm²) was found from C₀ where the lower leaf area (159.6 cm²) plant⁻¹ was recorded from C₂.

The combined effect of salt and calcium was a significant effect on the leaf area plant⁻¹ (Figure 9 and Appendix VII). The maximum leaf area (189.7 cm²) was recorded from S₀C₂ which was statistically similar (187.2) to S₀C₀. The leaf area was found higher with 10 mM concentration of calcium in case of 0, 2 and 4dSm⁻¹ treated plant and with increasing salinity level such as 6 and 8 dSm⁻¹ treated plants showed higher leaf area in 5mM concentration of calcium. From the above results it can be concluded that the calcium has important role in mitigating salt stress.



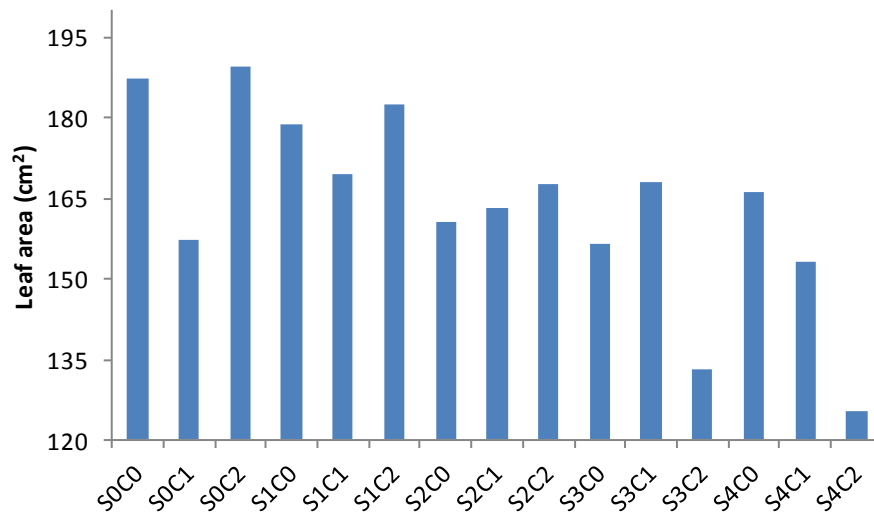
S₀ = without salt, S₁ = 2 dSm⁻¹, S₂ = 4 dSm⁻¹, S₃ = 6 dSm⁻¹, S₄ = 8 dSm⁻¹

Figure 7: Effect of salinity levels on leaf area (cm²) of tomato (LSD_{0.05} = 2.924)



C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

Figure 8: Effect of calcium levels on leaf area (cm²) of tomato (LSD_{0.05} = 2.265)



S₀ = without salt, S₁ = 2 dSm⁻¹, S₂ = 4 dSm⁻¹, S₃ = 6 dSm⁻¹, S₄ = 8 dSm⁻¹

C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

Figure 9: Effect of salinity and calcium levels on leaf area (cm²) of tomato (LSD_{0.05} = 5.065)

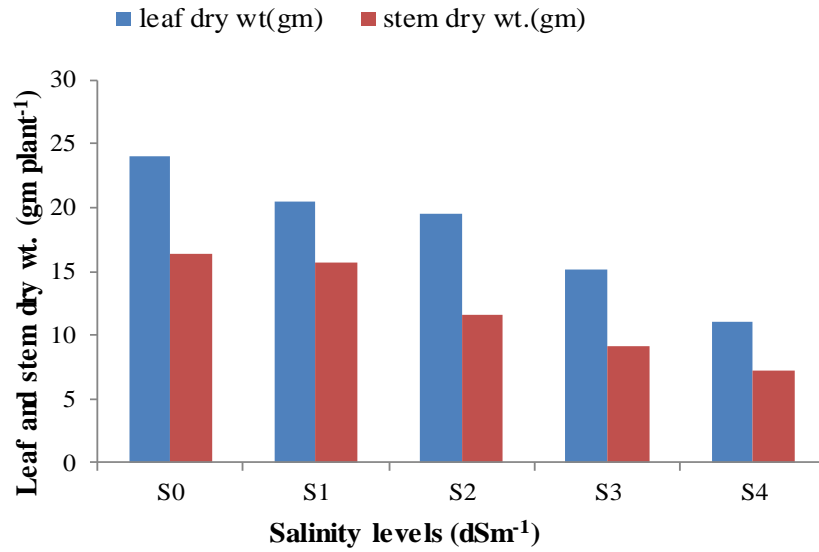
4.2 Effect of salinity and calcium on plant physiological characters

4.2.1 Dry weight of leaves and stems plant⁻¹

NaCl induced changes in dry matter production in different parts of tomato plant. Accumulation of dry matter in leaves and stems of tomato plant at harvesting time due to the influence of different saline levels have been studied in this experiment. It was found that, leaves and stems dry weight decreased with the increased salinity. There were significant effects on leaves and stems dry weight with the different levels of salinity (Figure 10 and Appendix VII). The highest leaves dry weight (24.09 gm) and stems dry weight (16.40 gm) were found in S₀ (control) treatment whereas S₄ (8 dSm⁻¹) treatment of salinity showed the lowest leaves (10.99gm) and stem dry weight (7.217gm). Similar results were also reported by several authors such as Hajor *et al.*, (2009); Memon *et al.* (2007), Cruz and Cuartero, (1990); Cuartero and Munoz (1990) in tomato, Aziz (2003) for mungbean, Sixto *et al.* (2005), Liu *et al.* (2008). They reported that, the effect of NaCl salinity stress on the growth of tomato plant was resulted in lower dry weight. Decreased in leaves dry weight does not seem to be due to a reduction in leaves number (Cruz and Cuartero, 1989) but due to a reduction in leaf area which can be reduced proportionately more than the stems dry weight (Vanleperen, 1996).

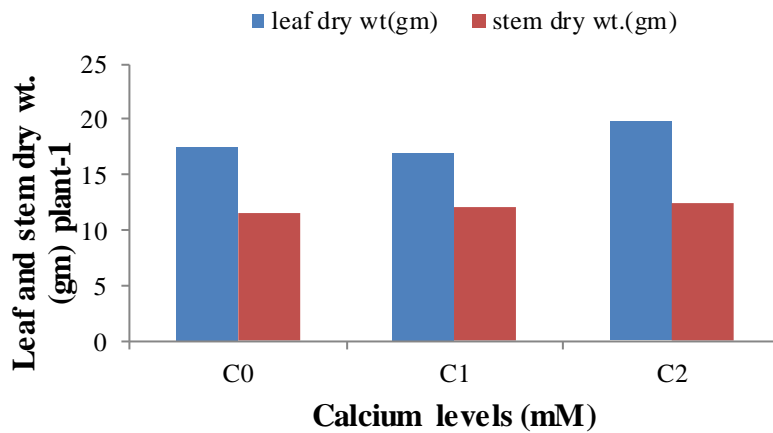
A significant effect of calcium on dry matter production of leaves and stems of tomato has been shown in Figure 11 and Appendix VII. The minimum leaves (16.91gm) and stems (11.51 gm) dry weights were recorded in C₁ and C₀ treatments respectively. But maximum leaves (19.79 gm) and stems (12.39gm) dry weight was found in case of C₂. It is suggested that calcium influenced in the increase dry weight of leaves and stems of tomato plant. Manivannan *et al.* (2007) reported that, calcium had the ameliorative effect on salt stress and increased the total dry weight of plant through increasing the vegetative growth of plant.

In interaction effect of salinity and calcium on leaves and stems dry weight exhibited a significant effect (Table 4 and Appendix VII). The highest leaves dry weight (25.27gm) and stems dry weight (17.02gm) was found in S₀C₂ and S₀C₁ respectively where both the lowest leaves dry weight (8.575gm) and stems dry weight (6.375gm) was found in S₄C₀.



S₀ = without salt, S₁ = 2 ds/m, S₂ = 4 ds/m, S₃ = 6 ds/m, S₄ = 8 ds/m

Figure 10: Effect of salinity levels on leaf and stem dry weight (gm plant⁻¹) of tomato (LSD_{0.05} = 2.079 and 0.2644 for leaf and stem dry weight respectively)



C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

Figure 11: Effect of calcium levels on on leaf and stem dry weight (gm plant⁻¹) of tomato (LSD_{0.05} = 1.61 and 0.2048 for leaf and stem dry weight respectively)

Table 4: Interaction effect of salinity and calcium levels on leaves and stems dry weight plant⁻¹ tomato

Treatment combinations	Leaves dry wt. (gm)	Stem dry wt. (gm)
S ₀ C ₀	24.77 ab	16.48 b
S ₀ C ₁	22.23 abc	17.02 a
S ₀ C ₂	25.27 a	15.70 c
S ₁ C ₀	21.92 abc	15.50 cd
S ₁ C ₁	20.33 cd	16.38 b
S ₁ C ₂	19.10 cde	15.23 d
S ₂ C ₀	20.40 cd	12.38 f
S ₂ C ₁	16.98 de	9.450 h
S ₂ C ₂	21.35 bc	13.02 e
S ₃ C ₀	11.70 f	6.825 j
S ₃ C ₁	16.38 e	10.88 g
S ₃ C ₂	17.48 de	9.550 h
S ₄ C ₀	8.575 f	6.375 j
S ₄ C ₁	8.650 f	6.825 j
S ₄ C ₂	15.75 e	8.450 i
LSD _(0.05)	3.601	0.458
F-test value	**	**
CV (%)	13.97%	2.67%

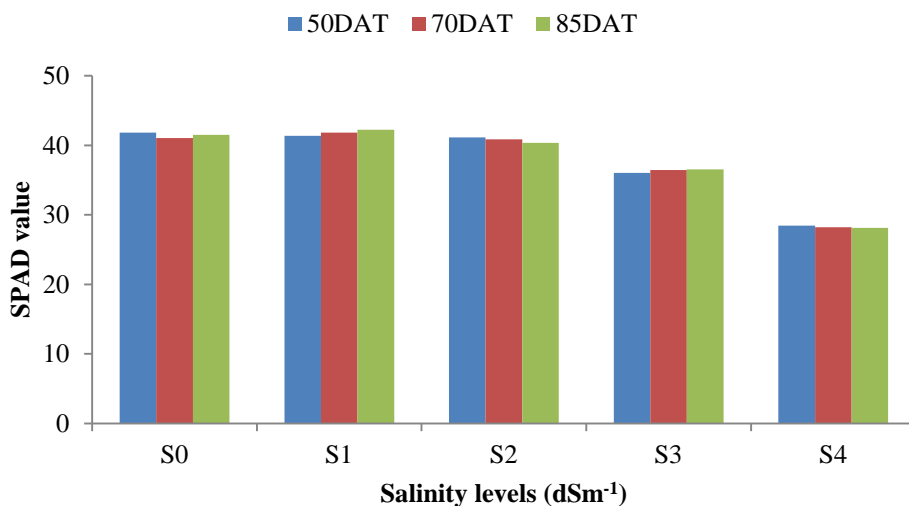
S₀ = without salt, S₁ = 2 dSm⁻¹, S₂ = 4 dSm⁻¹, S₃ = 6 dSm⁻¹, S₄ = 8 dSm⁻¹
C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

4.2.2 Leaf chlorophyll content

There was a clear effect of salinity on the leaf chlorophyll content of tomato plant at 50, 70 and 85 DAT (Figure 12 and Appendix XI). The chlorophyll content (SPAD reading) in leaves of tomato decreased with increasing salinity levels. At 50 DAT, the highest chlorophyll content (41.83 SPAD units) was recorded from S_0 which was statistically similar (41.36 and 41.11 SPAD units) to both S_1 and S_2 respectively and the lowest value (28.46 SPAD units) was found from S_4 . At 70 DAT, the highest chlorophyll content (41.84 SPAD units) was found from S_1 which was statistically similar (41.06 and 40.84 SPAD units) to both S_0 and S_2 and the lowest value (28.23 SPAD units) was observed in case of S_4 . And at 85 DAT, the highest chlorophyll content (42.24 SPAD units) was recorded from S_1 which was statistically similar (41.52 and 40.35 SPAD units) to both S_0 and S_2 whereas the lowest value (28.14 SPAD units) was found from S_4 . From these results, it was found that the high levels of salinity (8 dSm^{-1}) induced a significant decrease in the total chlorophyll content as compared to control plants. The total chlorophyll content of the leaves of tomato plant exhibits a little increase when grown at 2 and 4 dSm^{-1} . Chlorophyll content was significantly reduced at 6 dSm^{-1} , these results were also supported by Nahar (2014). Salinity reduced the total chlorophyll content in leaves which was also supported by Shahid et al. (2012), Netondo *et al.* (2004), Ali (2004), Amini and Ehsanpour (2006) etc.

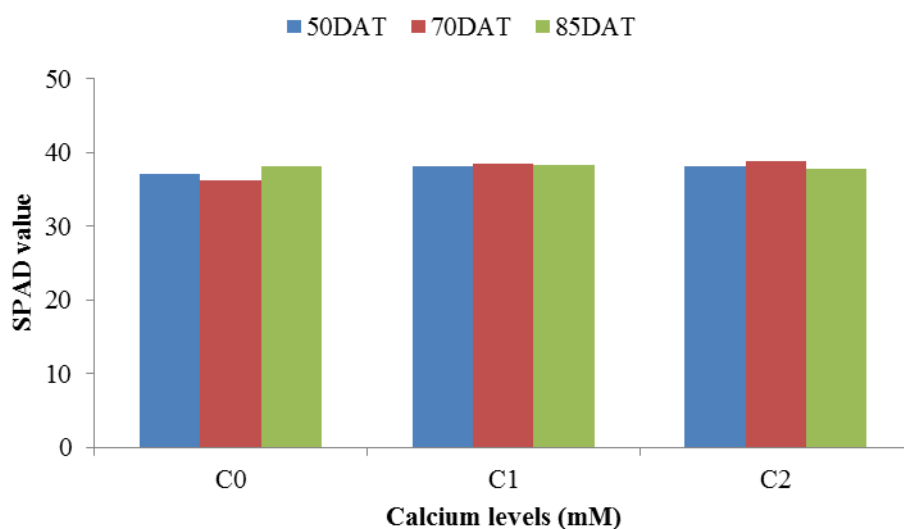
Significant effect of calcium on leaf chlorophyll content of tomato plant was found at 70 and 85 DAT and non-significant at 50 DAT (Figure 13 and Appendix XI). The highest value (38.16 SPAD units) was found from C_2 and lowest value (37.04 SPAD units) was recorded from C_0 , but both of these were statistically similar (38.08 SPAD units) to C_1 . At 70 DAT, the highest chlorophyll content (38.53 SPAD units) was found from C_1 which was statistically similar (38.31 SPAD units) to C_2 . The lowest value (36.22 SPAD units) was recorded from C_0 . Then at 85 DAT, where the highest chlorophyll content (38.79 SPAD units) was found from C_1 which was similar (37.80 SPAD units) to C_2 and the lowest value (36.69 SPAD units) was recorded from C_0 which was identical (37.80) to C_2 . Thus, calcium reduced the toxic effect on leaf chlorophyll content which was supported by Howladar and Rady (2012). This study suggests that, exogenous Ca^{2+} supply improves the total chlorophyll content in plant which was strongly related to the fruits weight plant⁻¹ as well as to yield of tomato.

The interaction effect between salinity and calcium levels on leaf chlorophyll content of tomato plant was statistically significant at 50, 70 and 85 DAT (Table 5 and Appendix XI). At 50 DAT, the highest leaf chlorophyll content (43.05 SPAD units) was found from S_0C_0 which was statistically similar (42.63, 39.83, 40.80, 41.58, 41.70, 41.78, 41.30, 40.25, 39.85 and 39.45 SPAD units) to S_0C_1 , S_0C_2 , S_1C_0 , S_1C_1 , S_1C_2 , S_2C_0 , S_2C_1 , S_2C_2 , S_3C_1 and S_3C_2 . The lowest value (25.02 SPAD units) was found from S_4C_0 which was statistically identical (28.83 SPAD units) to S_3C_0 . At 70 DAT, the highest value (43.47 SPAD units) was observed in S_1C_1 which was statistically similar (41.17, 41.75, 40.25, 40.47, 41.58, 41.70, 41.05, 40.22 and 41.03 SPAD units) to S_0C_0 , S_0C_1 , S_0C_2 , S_1C_0 , S_1C_2 , S_2C_0 , S_2C_1 , S_3C_1 and S_3C_2 . The lowest value (26.15 SPAD units) was found from S_4C_1 which was statistically identical (28.10, 29.65 and 28.90 SPAD units) to S_3C_0 , S_4C_0 and S_4C_2 . And at 85 DAT, where the highest value (44.17 SPAD units) was found from S_1C_1 which was statistically similar (41.57, 43.17, 41.22, 41.33, 40.67, 40.58, 40.63, and 40.10 SPAD units) to S_0C_0 , S_0C_1 , S_1C_0 , S_1C_2 , S_2C_0 , S_2C_1 , S_3C_1 and S_3C_2 . The lowest value (25.40 SPAD units) was recorded from S_4C_1 which was statistically similar (27.95 and 28.92) to both S_3C_0 and S_4C_2 .



S₀ = without salt, S₁ = 2 dSm⁻¹, S₂ = 4 dSm⁻¹, S₃ = 6 dSm⁻¹, S₄ = 8 dSm⁻¹

Figure 12: Effect of salinity levels on Leaf chlorophyll content (SPAD units) of tomato (LSD_{0.05} = 2.637, 2.131 and 2.371 for 50DAT, 70 DAT and 85 DAT respectively)



C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

Figure 13: Effect of calcium levels on Leaf chlorophyll content (SPAD units) of tomato (LSD_{0.05} = 2.043, 1.651 and 1.836 for 50DAT, 70 DAT and 85 DAT respectively)

Table 5. Interaction effect of salinity and calcium levels on chlorophyll content (SPAD reading) at different days after transplanting of tomato

Treatment combination	Leaf chlorophyll content (SPAD reading) at different days after transplanting		
	50	70	85
S ₀ C ₀	43.05 a	41.17 ab	41.57 ab
S ₀ C ₁	42.63 a	41.75 ab	43.17 ab
S ₀ C ₂	39.83 a	40.25 ab	39.80 b
S ₁ C ₀	40.80 a	40.47 ab	41.22 ab
S ₁ C ₁	41.58 a	43.47 a	44.17 a
S ₁ C ₂	41.70 a	41.58 ab	41.33 ab
S ₂ C ₀	41.78 a	41.70 ab	40.67 ab
S ₂ C ₁	41.30 a	41.05 ab	40.58 ab
S ₂ C ₂	40.25 a	39.78 b	39.80 b
S ₃ C ₀	28.83 bc	28.10 c	28.92 cd
S ₃ C ₁	39.85 a	40.22 ab	40.63 ab
S ₃ C ₂	39.45 a	41.03 ab	40.10 ab
S ₄ C ₀	30.77 b	29.65 c	31.08 c
S ₄ C ₁	25.02 c	26.15 c	25.40 d
S ₄ C ₂	29.58 bc	28.90 c	27.95 cd
LSD _(0.05)	4.567	3.691	4.106
F-test value	**	**	**
CV (%)	8.48%	6.86%	7.62%

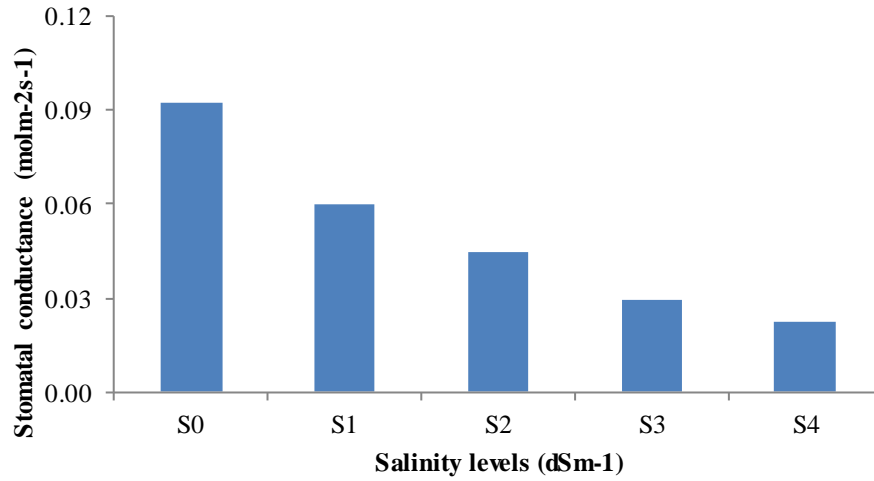
S₀ = without salt, S₁ = 2 dSm⁻¹, S₂ = 4 dSm⁻¹, S₃ = 6 dSm⁻¹, S₄ = 8 dSm⁻¹
C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

4.2.3 Stomatal conductance ($\text{molm}^{-2}\text{s}^{-1}$):

Stomatal conductance was significantly affected by different levels of salinity (Figure 14 and Appendix XII). The highest stomatal conductivity ($0.09 \text{ molm}^{-2}\text{s}^{-1}$) was recorded from S_0 and the lowest value ($0.02 \text{ molm}^{-2}\text{s}^{-1}$) was observed in case of S_4 which was statistically similar to S_1 ($0.06 \text{ molm}^{-2}\text{s}^{-1}$), S_2 ($0.06 \text{ molm}^{-2}\text{s}^{-1}$) and S_3 ($0.03 \text{ molm}^{-2}\text{s}^{-1}$) respectively. From this experiment it was observed that the stomatal conductance decreased with the increase of salinity levels. Zuccarini (2008) showed that salinity decreased growth, stomatal conductance and net photosynthetic rate. It was reported that short term exposure to high salinity leads to an immediate and significant drop in stomatal conductance, due to osmotic stress and local synthesis of ABA in barley (Fricke *et al.*, 2004; 2006). These findings were also supported by many others author like Reddy *et al.* (1992) and Netondo *et al.* (2004a).

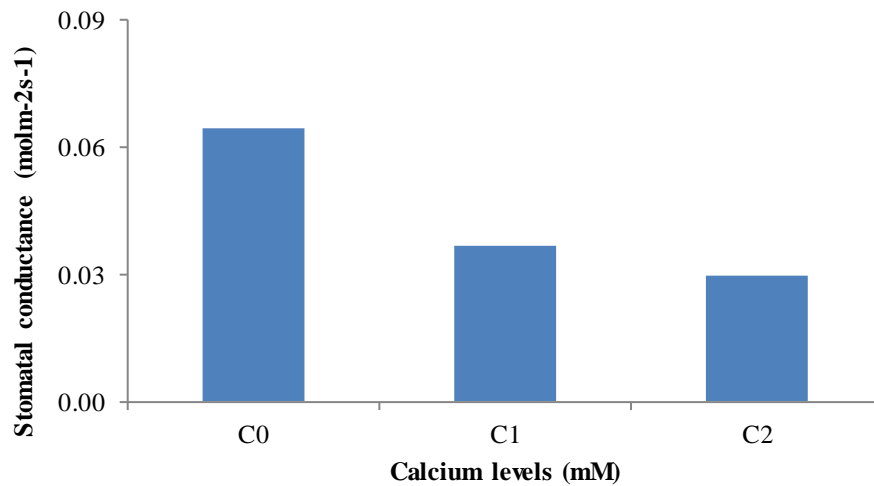
Different levels of calcium significantly affected the stomatal conductance (Figure 15 and Appendix XII). The highest stomatal conductance ($0.06 \text{ molm}^{-2}\text{s}^{-1}$) was found from C_0 and the lowest value ($0.03 \text{ molm}^{-2}\text{s}^{-1}$) was recorded from C_2 which was statistically similar ($0.04 \text{ molm}^{-2}\text{s}^{-1}$) to C_1 . It was observed that the stomatal conductance was not increased by the application of calcium which was dissimilar to the findings of Tzortzakis (2010).

Interaction effect of different salinity and calcium levels showed significant difference on stomatal conductance of tomato (Figure 16 and Appendix XII). The highest stomatal conductance ($0.17 \text{ molm}^{-2}\text{s}^{-1}$) was recorded from S_0C_0 . The lowest value ($0.01 \text{ molm}^{-2}\text{s}^{-1}$) was observed in S_4C_2 which was statistically similar to S_0C_2 , S_1C_0 , S_1C_1 , S_1C_2 , S_2C_0 , S_2C_1 , S_3C_0 , S_3C_1 , S_3C_2 , S_4C_0 and S_4C_1 . These results showed that higher salinity levels reduced stomatal conductance which were not influenced by application of Ca^{2+} .



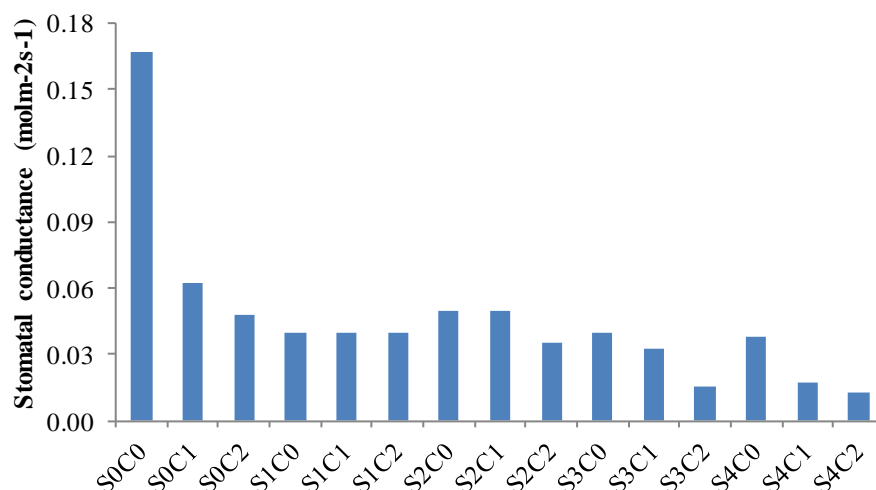
S₀ = without salt, S₁ = 2 dSm⁻¹, S₂ = 4 dSm⁻¹, S₃ = 6 dSm⁻¹, S₄ = 8 dSm⁻¹

Figure 14: Effect of salinity levels on stomatal conductance (molm⁻²s⁻¹) of tomato (LSD_{0.05} = 0.026).



C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

Figure 15: Effect of calcium levels on stomatal conductance (molm⁻²s⁻¹) of tomato (LSD_{0.05} = 0.020)



S₀ = without salt, S₁ = 2 dSm⁻¹, S₂ = 4 dSm⁻¹, S₃ = 6 dSm⁻¹, S₄ = 8 dSm⁻¹
 C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

Figure 16: Effect of salinity and calcium levels on stomatal conductance (molm⁻²s⁻¹) of tomato (LSD_{0.05} = 0.045).

4.2.4 Na⁺ (%) content in leaves

A significant ($p \leq 0.01$) difference in Na⁺ was also observed in studied tomato leaves under different levels of salinity (Figure 17 and Appendix XII). The highest Na⁺ (0.6076%) content in leaves was recorded from S₄ whereas the lowest value (0.2408%) was found in control (S₀) treatment. So, the results showed that, salinity increased the concentration of Na⁺ in leaves with increasing levels of salinity. As higher salinity levels provided more available Na⁺ in soil, the Na⁺ content in leaves tended to increase. Memon *et al.* (2007) observed that saline water treated plants contained more Na⁺ than plants grown in non-saline water. This result was supported by Lolaei *et al.* (2012), Bavei (2011), Netondo *et al.* (2004), Lacerda *et al.* (2003) results in sorghum.

Different levels of calcium significantly decreased the Na⁺ content in leaves over the control plant (Figure 18 and Appendix XII). The highest Na⁺ content (0.4030%) in leaves was recorded from C₀ which was statistically similar (0.3965%) to C₁. The lowest Na⁺ content (0.3770%) was found from C₂ which was also statistically similar (0.3965%) to C₁. Calcium application gradually decreased the Na⁺ accumulation rate in leaves under saline condition is supported by Anbu and Sivasankaramoorthy (2014), Kaya *et al.* (2002), Qadir *et al.* (2001), Lolaei *et al.* (2012), Dabuxilatu and Ikeda (2005) etc.

The interaction effect between salinity and calcium on Na⁺ content in leaves was statistically significant ($p \leq 0.01$) (Figure 19 and Appendix XII). The highest Na⁺ content (0.6125%) in leaves was observed in S₄C₁ which was statistically similar (0.6100%) and (0.5975%) to S₄C₂ and S₄C₀ respectively. The lowest value (0.2300%) was recorded from S₀C₂ which was statistically similar (0.2450%) and (0.2475%) to S₄C₀ and S₄C₁ respectively. The plants accumulated more Na⁺ in leaves with the increasing levels of salinity. But in case of C₂ (10mM) Na⁺ was 0.2300% at control condition but it increased up to 0.6100% at 8 dSm⁻¹ salinity level which is a mitigation mechanisms of calcium to salinity.

4.2.5 K⁺ content (%) in leaves

The effect of salinity was statistically significant on K⁺ content in leaves ($p \leq 0.01$) (Figure 17 and Appendix XII). The highest K⁺ content (1.038%) was recorded in S₀ (control). The lowest K⁺ content (0.7467%) was found from S₄ (8dSm⁻¹). K⁺ content decrease was accompanied by increase in Na⁺ content, indicating an apparent antagonism between Na⁺ and K⁺. These results strongly followed by the Na⁺ (%) contents in leaves and it was clear that increased Na⁺ (%) decreased the K⁺ (%). Netondo *et al.* (2004) also reported that accumulation of K⁺ in leaves was strongly inhibited by salinity and this similar observation was supported by Memon *et al.* (2007), Bavei *et al.* (2011), Thimmaiah (2004) etc.

K⁺ content in leaves of tomato under salinity was significantly affected by calcium treatments (Figure 18 and Appendix XII). The highest K⁺ content (0.9330%) was observed in C₁ which was statistically similar (0.9070%) to C₀. The lowest K⁺ content (0.8455%) was recorded in C₂ which was also statistically similar (0.9070%) to C₀. It was observed that K⁺ content in leaves was increased with calcium application upto 5 mM which decreased at 10 mM calcium. So exogenous application of Ca²⁺ increased the K⁺ content in leaf which is strongly supported by Soualem *et al.* (2014), Anbu and Sivasankaramoorthy (2014), Arshi *et al.* (2010), Lolaei *et al.* (2012), Levent *et al.* (2007). Elevated Ca²⁺ concentration in nutrient solution mitigates the adverse effects of NaCl by inhibiting Na⁺ uptake (Kaya *et al.*, 2002) and reducing membrane leakage (Tuna *et al.*, 2007). It was reported that K⁺ concentrations reduced by salinity, can be restored to adequate levels by an additional supply of calcium, as it protects cell membranes from adverse effect of Na⁺ and minimizes the leakage of cytosolic potassium (Qadir *et al.*, 2001).

The interaction between salinity levels and calcium levels had significant effect on K^+ content in leaves of tomato (Figure 19 and Appendix XII). The highest K^+ content (1.178%) was recorded in S_0C_0 . The lowest K^+ content (0.6625%) was recorded in S_4C_2 which was statistically similar (0.7650%, 0.7750% and 0.8025%) to S_3C_1 , S_4C_0 and S_4C_1 respectively. C_1 increased K^+ accumulation in leaves at 2, 4 and 8 dSm^{-1} salinity levels except 6 dSm^{-1} where increased K^+ accumulation was found by C_2 . So, these results strongly suggest that calcium excludes toxic Na^+ and includes more non-toxic K^+ in leaves of plant which is one of the important mitigation behaviors of calcium.

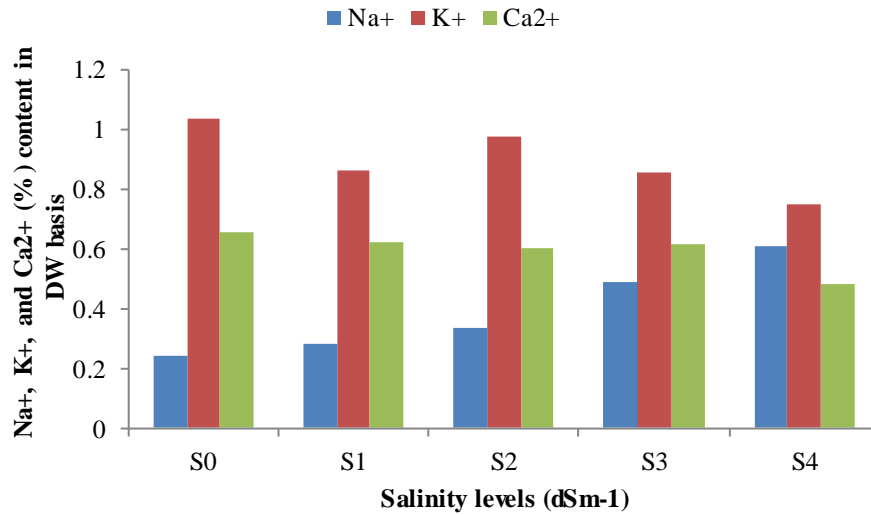
4.2.6 Ca^{2+} content (%) in leaves

The effect of salinity was statistically significant on Ca^{2+} content of leaves of tomato (Figure 17 and Appendix XII). The highest Ca^{2+} content (0.6542%) was found from S_0 which was statistically similar (0.6208%, 0.6042% and 0.6167%) to S_1 , S_2 and S_3 respectively. The lowest Ca^{2+} content (0.4833%) was recorded from S_4 . Tomato leaves accumulated less amount of Ca^{2+} at higher salinity level. It was found that Ca^{2+} (%) content in leaves as DW basis was gradually decreased with the decrease of K^+ (%) along with increased levels of salinity. This result is consistent with the findings of Rabbi (2004) in mungbean and also with Netondo *et al.* (2004), Bavei *et al.* (2011), Karimi *et al.* (2005) etc.

Different levels of calcium significantly increased the Ca^{2+} content in leaves of tomato over the control plant (Figure 18 and Appendix XII). The highest Ca^{2+} content (0.6575%) was recorded at C_1 treatment which was statistically similar (0.6325%) to C_2 and the lowest Ca^{2+} content (0.4975%) was recorded at C_0 . Ca^{2+} ameliorates detrimental effects of toxic Na^+ and the Ca^{2+} content will be improved by applying calcium and this results was also supported by Anbu and Sivasankaramoorthy (2014), Arshi *et al.* (2010), Lolaei *et al.* (2012).

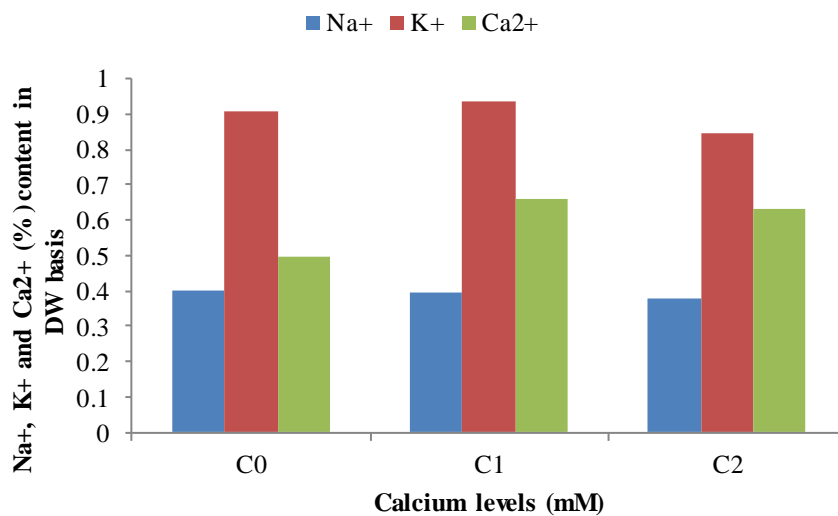
The interaction between salinity and calcium levels had significant effect on Ca^{2+} in leaves of tomato (Figure 19 and Appendix XII). The highest Ca^{2+} content (0.7125%) was found in case of S_0C_1 which was statistically similar (0.7125%, 0.7000% and 0.7000%) to S_1C_2 , S_2C_1 and S_3C_2 respectively. The lowest Ca^{2+} content (0.4000%) was observed in S_4C_0 which was also statistically similar (0.4750%, 0.4875%, 0.5000% and 0.5000%) to S_3C_0 , S_2C_0 , S_1C_0 and S_4C_2 respectively. Thus calcium increased the Ca^{2+} content in leaves in different saline conditions which improved the

K^+ content resulting somewhat salinity tolerance.



S₀ = without salt, S₁ = 2 dSm⁻¹, S₂ = 4 dSm⁻¹, S₃ = 6 dSm⁻¹, S₄ = 8 dSm⁻¹

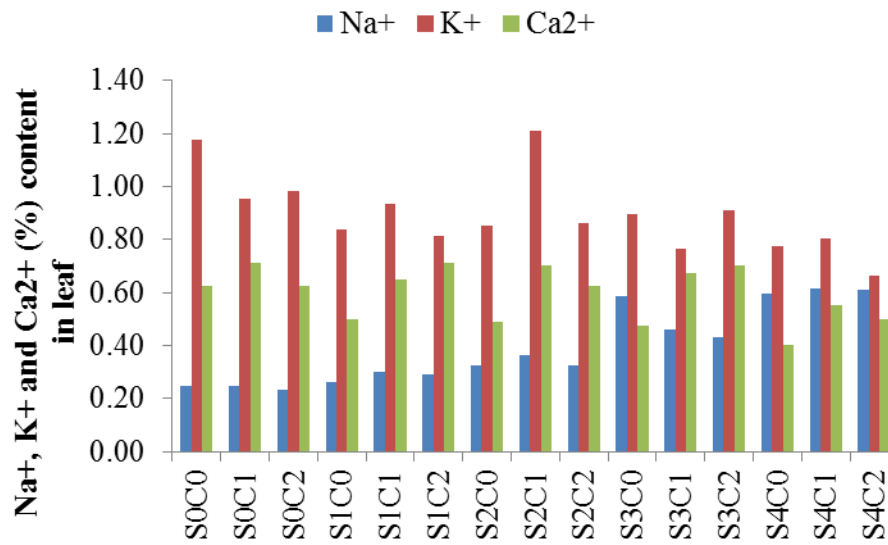
Figure 17: Effect of salinity levels on Na⁺, K⁺ and Ca²⁺ content (%) in leaves of tomato (LSD_{0.05} = 0.026, 0.086 and 0.074 for Na⁺, K⁺ and Ca²⁺ content respectively).



C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

Figure 18: Effect of calcium levels on Na⁺, K⁺ and Ca²⁺ content (%) in leaves of

tomato ($LSD_{0.05} = 0.020, 0.067$ and 0.057 for Na^+ , K^+ and Ca^{2+} content respectively)



S_0 = without salt, $S_1 = 2 \text{ dSm}^{-1}$, $S_2 = 4 \text{ dSm}^{-1}$, $S_3 = 6 \text{ dSm}^{-1}$, $S_4 = 8 \text{ dSm}^{-1}$
 C_0 = No calcium, $C_1 = 5 \text{ mM}$, $C_2 = 10 \text{ mM}$

Figure 19: Effect of salinity and calcium levels on Na^+ , K^+ and Ca^{2+} content (%) in leaves of tomato ($LSD_{0.05} = 0.045, 0.15$ and 0.13 for Na^+ , K^+ and Ca^{2+} content respectively)

4.2.7 K⁺/Na⁺ ratio in leaves

A significant ($p \leq 0.01$) difference in K⁺/Na⁺ was observed in studied tomato leaves under different levels of salinity (Figure 20 and Appendix XII). The highest K⁺/Na⁺ (4.317) was observed in S₀ and the lowest value was found from S₄ (8 dSm⁻¹). The results showed that the K⁺/Na⁺ content in leaves of tomato gradually decreased with the increase of salinity levels. Under saline condition, plants uptake more toxic Na⁺ with less Non-toxic K⁺. High Na⁺ in soil solution causes intracellular K⁺ deficiency due to competition and leads to K⁺/Na⁺ disequilibrium (Kronzucker and Britto, 2008; Pardo and Rubio, 2011). Similar results were also mentioned by Gadallah (1999), Haroun (2002), Cicek and Cakirlar (2002), Munns *et al.* (2002), Tester and Davenport (2003).

K⁺/Na⁺ content in leaves of tomato under salinity was significantly affected by calcium treatments (Figure 21 and Appendix XII). The highest K⁺/Na⁺ ratio (2.795) was observed in case of C₀ and the lowest value (2.535) was found from C₂. It was observed that more Na⁺ ions were found from non- calcium treated plant and calcium reduced the Na⁺ translocation to the shoot portion. It was reported that application of Ca²⁺ mitigates the toxic effect of NaCl and a greater increase in K⁺/Na⁺ ratios was observed (Sivasankaramoorthy, 2013; Lolaei *et al.* 2012; Liu and Zhu, 1998 and Abdel Latef, 2011, Levent *et al.* 2007). Earlier research workers commented that calcium is thought to improve the K⁺/Na⁺ selectivity of membrane (Marschnar, 1995) and prevent the cell from invasion of toxic ions (Cramer *et al.* 1987).

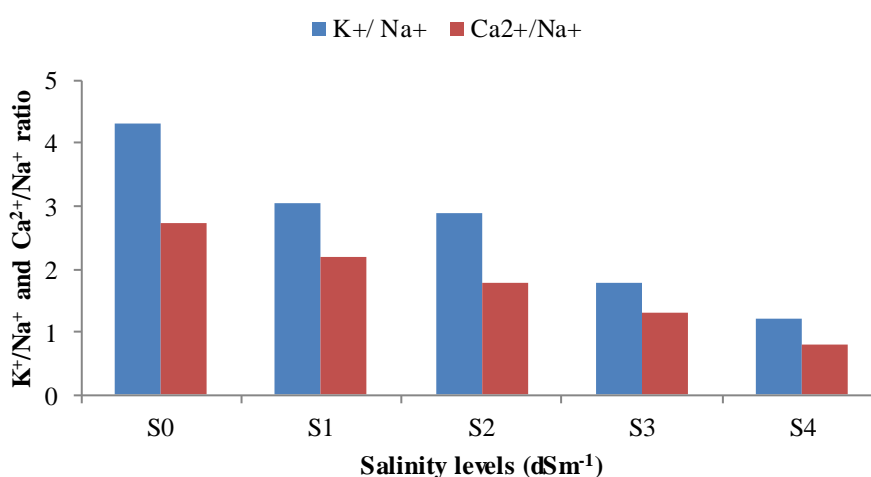
The interaction between salinity and calcium levels had significant effect on K⁺/Na⁺ ratio in leaves of tomato (Figure 22 and Appendix XII). The highest K⁺/Na⁺ ratio (4.900) was found from S₀C₀. The lowest value (1.025) was recorded from S₄C₂ which was statistically similar (1.350 and 1.303) to both S₄C₀ and S₄C₁ respectively.

4.2.8 Ca²⁺/Na⁺ ratio in leaves

The effect of salinity was statistically significant on Ca²⁺/Na⁺ ratio in leaves of tomato (Figure 20 and Appendix XII). The highest Ca²⁺/Na⁺ ratio (2.724) was found in case of S₀ which was gradually decreased with the increase of salinity levels. The lowest value (0.7958) was recorded from S₄, possibly the higher levels of salinity decreased the Ca²⁺ uptake, whereas the Na⁺ uptake was increased. Ca²⁺/Na⁺ ratio was also decreased with increased salinity concentration which was reported by Patel *et al.* (2010), Shabala *et al.* (2003), Hu *et al.* (1997) etc.

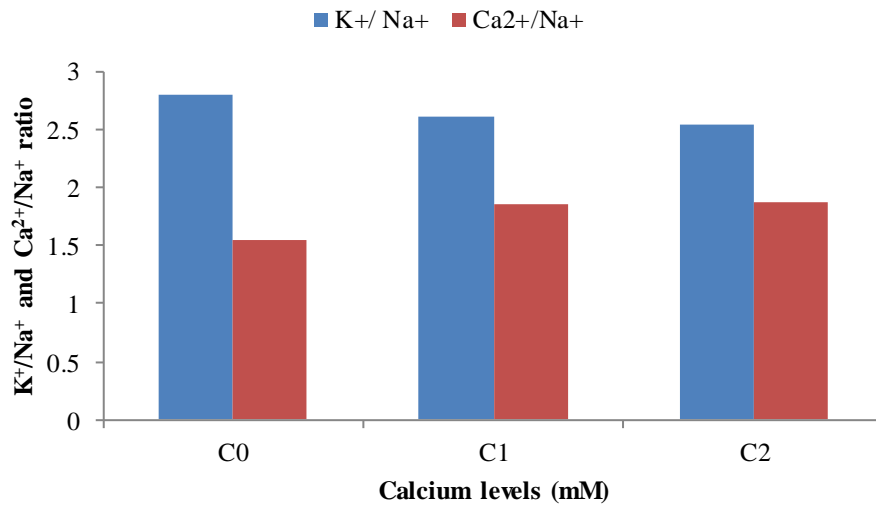
Different levels of calcium significantly increased the $\text{Ca}^{2+}/\text{Na}^+$ ratio in leaves of tomato over the control plant (Figure 21 and Appendix XII). The highest $\text{Ca}^{2+}/\text{Na}^+$ ratio (1.880) was recorded from C_2 which was statistically similar (1.849) to C_1 . Whereas the lowest $\text{Ca}^{2+}/\text{Na}^+$ ratio (1.549) was found from C_0 . A great increase of $\text{Ca}^{2+}/\text{Na}^+$ ratios was observed by Sivasankaramoorthy (2013) and Lolaei *et al.* (2012) when Ca^{2+} was applied. The results for $\text{Ca}^{2+}/\text{Na}^+$ ratios suggest that Ca^{2+} might have played an important role in maintaining the proper functioning of biological membranes integrity with ion-transport regulation as reported by Maathuis and Amtmann (1999) and their permeability was influenced similarly as reported by Kent and Lauchli (1985), thereby resulting in relatively normal growth.

Salinity and calcium levels did not significant effect on $\text{Ca}^{2+}/\text{Na}^+$ ratio in leaves of tomato in case of their interaction effect (figure 22 and Appendix XII). The highest $\text{Ca}^{2+}/\text{Na}^+$ ratio (2.855) was found from S_0C_1 which was statistically similar (2.715 and 2.602) to both S_0C_2 and S_0C_0 respectively. From the figure 22, it was clear that calcium increased the $\text{Ca}^{2+}/\text{Na}^+$ ratio in leaves of tomato under saline condition, normally which was decreased with increased levels of salinity.



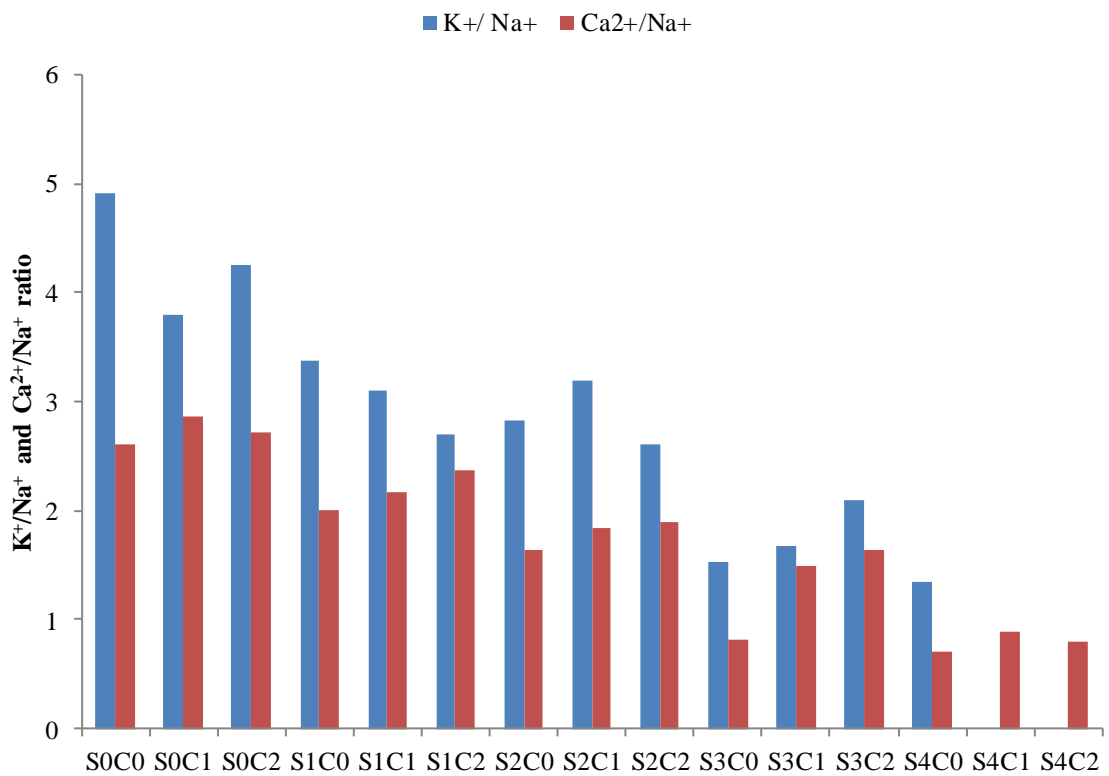
S_0 = without salt, $\text{S}_1 = 2 \text{ dSm}^{-1}$, $\text{S}_2 = 4 \text{ dSm}^{-1}$, $\text{S}_3 = 6 \text{ dSm}^{-1}$, $\text{S}_4 = 8 \text{ dSm}^{-1}$

Figure 20: Effect of salinity levels on K^+/Na^+ and $\text{Ca}^{2+}/\text{Na}^+$ ratio in leaves of tomato (LSD_{0.05} = 0.208 and 0.247 for K^+/Na^+ and $\text{Ca}^{2+}/\text{Na}^+$ ratio respectively)



C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

Figure 21: Effect of calcium levels on K⁺/Na⁺ and Ca²⁺/Na⁺ ratio in leaves of tomato ((LSD_{0.05} = 0.161 and 0.191 for K⁺/Na⁺ and Ca²⁺/Na⁺ ratio respectively)



S₀ = without salt, S₁ = 2 dSm⁻¹, S₂ = 4 dSm⁻¹, S₃ = 6 dSm⁻¹, S₄ = 8 dSm⁻¹

C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

Figure 22: Effect of salinity and calcium levels on K^+/Na^+ and Ca^{2+}/Na^+ ratio in leaves of tomato ((LSD_{0.05} = 0.361 and 0.428 for K^+/Na^+ and Ca^{2+}/Na^+ ratio respectively)

4.3 Effect of salinity and calcium on yield contributing and yield characters

4.3.1 Number of flower clusters plant⁻¹

There was a significant difference in number of flower clusters plant⁻¹ at different levels of salinity (Table 6 and Appendix VIII). The highest number of flower clusters plant⁻¹ (14.58) of tomato was found in control plants (S₀) and the lowest number of cluster was recorded from 2dSm⁻¹ (S₁) of salinity. This report was similar to other studies (Admans and Ho, 1989 and Vanleperen, 1996) in case of tomato.

Significant variation was observed for number of flower clusters plant⁻¹ of tomato for different levels of calcium (Table 6 and Appendix VIII). The highest flower clusters plant⁻¹ (15.10) was found in C₂ treated plants and control treated plants showed the lowest flower clusters plant⁻¹ (12.05).

Number of flower clusters plant⁻¹ varied significantly for the interaction of salinity and calcium levels (Table 7 and Appendix VIII). The highest number of flower clusters plant⁻¹ (17.75) was found from S₄C₂, while the lowest number (10.25) was obtained from S₄C₀.

4.3.2 Number of flowers cluster⁻¹

Number of flowers cluster⁻¹ of tomato showed significant variation for different salinity levels (Table 6 and Appendix VIII). The highest number of flowers cluster⁻¹ (5.392) was observed from S₀ treatment and the lowest number (4.670) was found from S₄ which was statistically similar to S₁ (4.949), S₂ (4.911) and S₃ (4.753) respectively. Salinity reduced the flowers cluster⁻¹.

Different levels of calcium varied significantly for number of flowers cluster⁻¹ of tomato (Table 6 and Appendix VIII). The highest number of flowers cluster⁻¹ (5.192) was found from C₀ (control) treatment which was statistically similar (4.988) to C₁ and the lowest number was (4.687) recorded from C₃ which was statistically similar (4.988) to C₁.

Interaction effect of salinity and calcium showed significant differences for number of flowers cluster⁻¹ (Table 7 and Appendix VIII). The highest number of flowers cluster⁻¹ (5.905) was recorded from S₀C₁ which was statistically similar to S₀C₀ (5.472), S₂C₂

(5.177), S₃C₁ (5.188) and S₄C₀ (5.238) respectively. On the other hand, the lowest number (4.273) was obtained from S₃C₂ which was statistically identical to S₀C₂ (4.798), S₁C₁ (4.972), S₁C₂ (4.757), S₂C₀ (5.023), S₂C₁ (4.532), S₃C₀ (4.797), S₄C₁ (4.342) and S₄C₂ (4.430) respectively.

4.3.3 Number of fruits cluster⁻¹

Significant variation was recorded for the number of fruits cluster⁻¹ of tomato due to different levels of salinity (Table 6 and Appendix VIII). The highest number of fruits cluster⁻¹ (3.704) was obtained from S₃ which was statistically similar (3.668, 3.665 and 3.495) to S₀, S₁ and S₂ respectively, while the lowest number (3.123) was obtained from S₄.

Application of different levels of calcium on tomato for number of fruits cluster⁻¹ has no significant effect (Table 6 and Appendix VIII). The highest number of fruits cluster⁻¹ (3.572) was recorded from C₁ which was statistically similar (3.563 and 3.458) to C₀ and C₂ respectively. The lowest value (3.458) was found from C₂.

Interaction effect of salinity and calcium differed significantly for number of fruits cluster⁻¹ (Table 7 and Appendix VIII). The highest number of fruits cluster⁻¹ (4.068) was found from S₀C₀ which was statistically similar to S₁C₁ (3.820), S₁C₂ (3.777), S₃C₀ (3.950) and S₃C₁ (3.650) and lowest value (2.957) was recorded from S₄C₀ which was statistically similar (3.275 and 3.138) to S₄C₁ and S₄C₁.

Table 6. Effect of salinity and calcium levels on number of flower clusters plant⁻¹, number of flowers cluster⁻¹ and number of fruits cluster⁻¹ of tomato

Treatment Combination	No. of Flower Clusters plant ⁻¹	No. of Flowers Cluster ⁻¹	No. of Fruits Cluster ⁻¹
S ₀	14.58 a	5.392 a	3.668 a
S ₁	13.08 c	4.949 b	3.665 a
S ₂	13.33 bc	4.911 b	3.495 a
S ₃	13.67 b	4.753 b	3.704 a
S ₄	13.75 b	4.670 b	3.123 b
LSD (0.05)	0.4853	0.436	0.2458
F-test value	**	**	**
CV (%)	4.30%	10.73%	8.46%
C ₀	12.05 c	5.129 a	3.563 a

C ₁	13.90 b	4.988 ab	3.572 a
C ₂	15.10 a	4.687 b	3.458 a
LSD _(0.05)	0.3759	0.3377	0.1904
F-test value	**	*	NS
CV (%)	4.30%	10.73%	8.46%

S₀ = without salt, S₁ = 2 dSm⁻¹, S₂ = 4 dSm⁻¹, S₃ = 6 dSm⁻¹, S₄ = 8 dSm⁻¹
C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

Table 7. Interaction effect of salinity and calcium levels on number of flower clusters plant⁻¹, number of flowers cluster⁻¹ and number of fruits cluster⁻¹ of tomato

Treatment Combination	No. of Flower Clusters plant⁻¹	No. of Flowers Cluster⁻¹	No. of Fruits Cluster⁻¹
S ₀ C ₀	13.25 def	5.472 ab	4.068 a
S ₀ C ₁	15.75 b	5.905 a	3.513 cde
S ₀ C ₂	14.75 c	4.798 bcde	3.425 cde
S ₁ C ₀	12.50 f	5.117 bcd	3.398 cde
S ₁ C ₁	13.25 def	4.972 bcde	3.820 abc
S ₁ C ₂	13.50 de	4.757 bcde	3.777 abc
S ₂ C ₀	13.75 de	5.023 bcde	3.445 cde
S ₂ C ₁	13.25 def	4.532 cde	3.600 bcd
S ₂ C ₂	13.00 ef	5.177 abcd	3.440 cde
S ₃ C ₀	10.50 g	4.797 bcde	3.950 ab
S ₃ C ₁	14.00 cd	5.188 abc	3.650 abcd
S ₃ C ₂	16.50 b	4.273 e	3.513 cde
S ₄ C ₀	10.25 g	5.238 abc	2.957 f
S ₄ C ₁	13.25 def	4.342 e	3.275 def
S ₄ C ₂	17.75 a	4.430 de	3.138 ef
LSD _(0.05)	0.8406	0.7551	0.4257
F-test value	**	*	*
CV (%)	4.30%	10.73%	8.46%

S₀ = without salt, S₁ = 2 dSm⁻¹, S₂ = 4 dSm⁻¹, S₃ = 6 dSm⁻¹, S₄ = 8 dSm⁻¹
C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

4.3.4 Number of flowers plant⁻¹

Number of flowers plant⁻¹ of tomato showed significant differences with different levels of salinity (Figure 23 and appendix X). The highest number of flowers plant⁻¹ (74.25) was observed from S₀, where the lowest number (61.50) was recorded from S₄. Number of flowers plant⁻¹ gradually reduced with the increased levels of salinity through dropping of flowers and also consistent with flowers cluster⁻¹.

Significant variation was recorded for the number of flowers plant⁻¹ of tomato for different doses of calcium (Figure 24 and Appendix X). The maximum number of flowers plant⁻¹(70.60) was found from C₂. Again the lowest number (59.85) was obtained from C₀. Application of calcium increased the number of flowers plant⁻¹.

Interaction effect of salinity level with calcium showed significant variation in terms of number of flowers plant⁻¹(Figure 25 and Appendix X).The highest number of flowers plant⁻¹ (80.50) was observed in S₄C₂ and the lowest number (46.00) was recorded from S₄C₀.

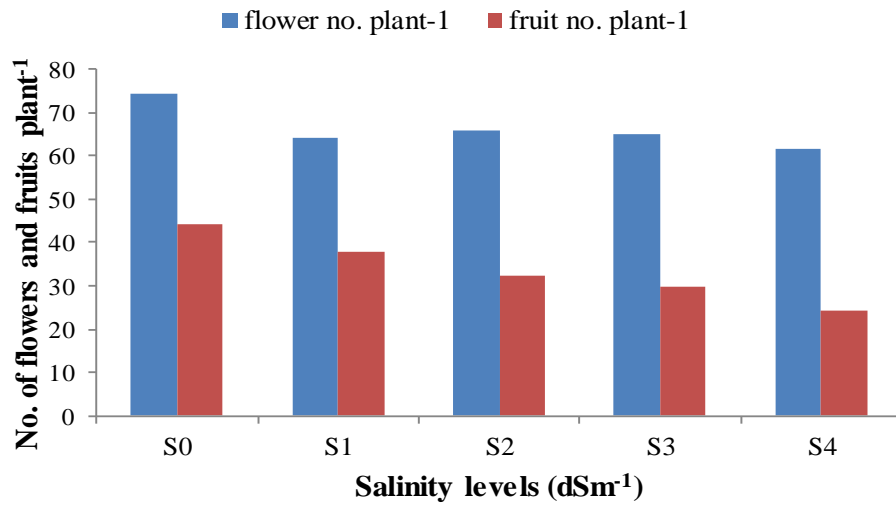
4.3.5 Number of fruits plant⁻¹

Number of fruits plant⁻¹ of tomato showed significant differences in response to different levels of salinity (Figure 23 and Appendix X). The highest number of fruits plant⁻¹ (44.17) was recorded from S₀ (control) and the lowest number (24.42) was observed from S₄ (8dSm⁻¹). Salinity reduced the number of fruits plant⁻¹ which was also consistent with the number of flowers plant⁻¹ and ultimately reduced the fruit yield which is also supported by Hamayun (2010). According to Sun and Hauster (2004), salinity adversely affects reproductive development by inhibiting microsporogenesis and stamen filament elongation, enhancing programmed cell death, ovule abortion and senescence of fertilized embryo.

Statistically significant variation was recorded for number of fruits plant⁻¹ of tomato after the application of different levels of calcium (Figure 24 and Appendix X). The highest number of fruits plant⁻¹ (34.65) was observed from C₂ and the lowest value (33.15) from C₁ and which was statistically similar to C₀. Number of fruits plant⁻¹ increased with the increased levels of calcium. This trend was also related with the number of flowers plant⁻¹.

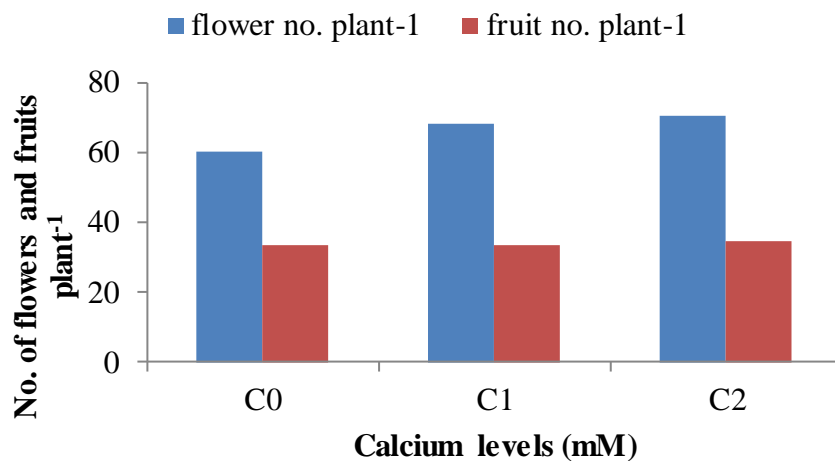
Number of fruits plant⁻¹ varied significantly for the interaction effect of different salinity and calcium levels (Figure 25 and Appendix X). The highest number of fruits plant⁻¹ (52.25) was recorded from S₀C₀ and the lowest (22.00) was obtained from

S₄C₀.



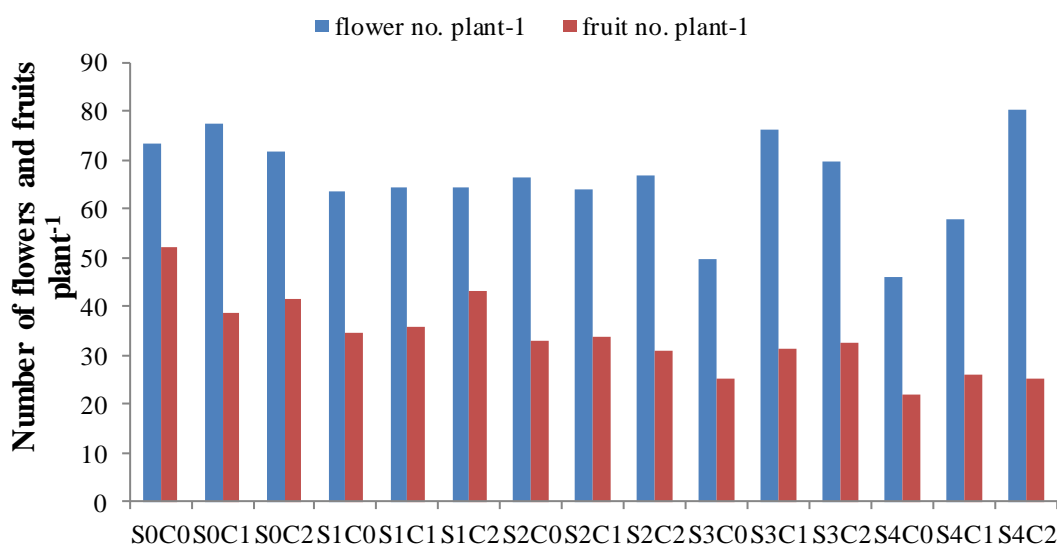
S₀ = without salt, S₁ = 2 dSm⁻¹, S₂ = 4 dSm⁻¹, S₃ = 6 dSm⁻¹, S₄ = 8 dSm⁻¹

Figure 23: Effect of salinity levels on flower and fruit number plant⁻¹ of tomato (LSD_{0.05} = 0.915 and 0.849 for flower no. and fruit no. plant⁻¹ respectively).



C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

Figure 24: Effect of calcium levels on flower and fruit number plant⁻¹ of tomato (LSD_{0.05} = 0.709 and 0.657 for flower no. and fruit no. plant⁻¹ respectively).



S₀ = without salt, S₁ = 2 dSm⁻¹, S₂ = 4 dSm⁻¹, S₃ = 6 dSm⁻¹, S₄ = 8 dSm⁻¹
 C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

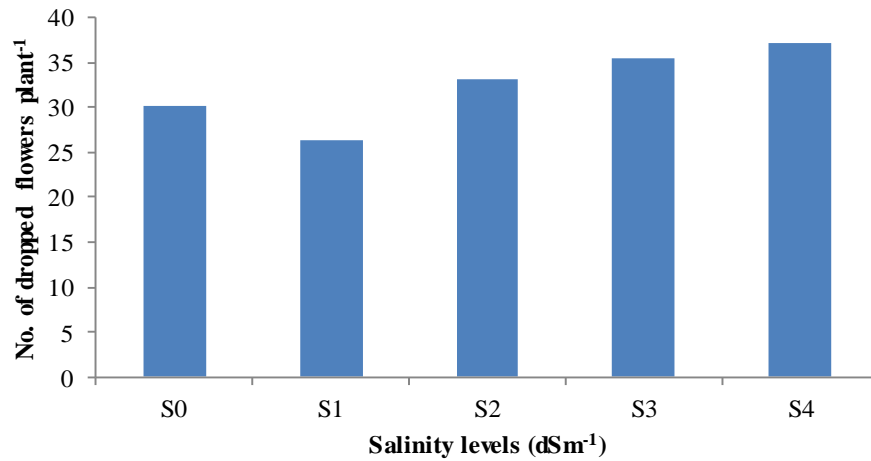
Figure 25: Combined effect of salinity and calcium levels on flower and fruit number plant⁻¹ of tomato ((LSD_{0.05} = 1.586 and 1.47 for flower no. and fruit no. plant⁻¹ respectively)

4.3.6 Number of dropped flowers plant⁻¹

Influence of different levels of salinity on number of dropped flowers plant⁻¹ of tomato varied significantly (Figure 26 and Appendix X). The highest number of dropped flowers plant⁻¹ (37.08) was recorded from S₄ and the lowest number (26.33) was found from S₁. Salinity increased the number of dropped flowers with increased levels of salinity resulting the lower number of fruits plant⁻¹ as well as yield.

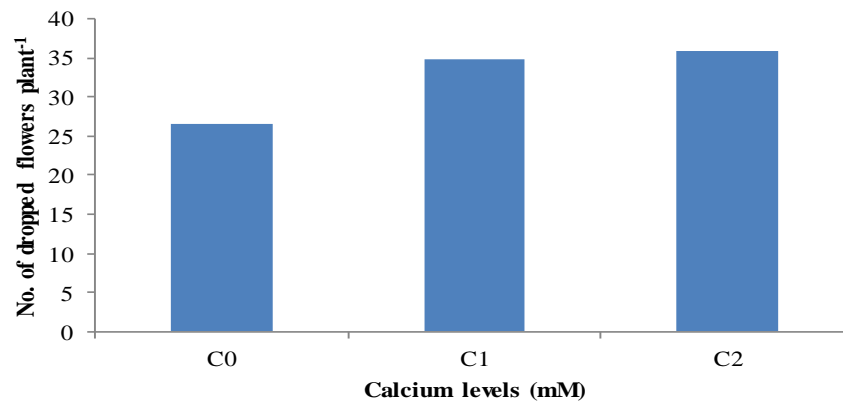
Statistically significant variation was found for number of dropped flowers plant⁻¹ of tomato due to application of different levels of calcium (Figure 27 and Appendix X). The highest number of dropped flowers plant⁻¹ (35.95) was found from C₂. The lowest value (26.55) was observed from C₀. The results obtained from this experiment showed that the highest flower dropping was happened with 10 mM Ca²⁺ application where this concentration of calcium is also responsible for highest number of flower plant⁻¹ and thus produced highest fruit yield plant⁻¹.

Interaction effect of salinity and calcium showed significant differences in case of number of dropped flowers plant⁻¹ (Figure 28 and Appendix X). The highest number of dropped flowers plant⁻¹(55.25) was recorded from S₄C₂ and the lowest value (21.25) was observed from S₀C₀ which was statistically similar to S₁C₂ (21.50).



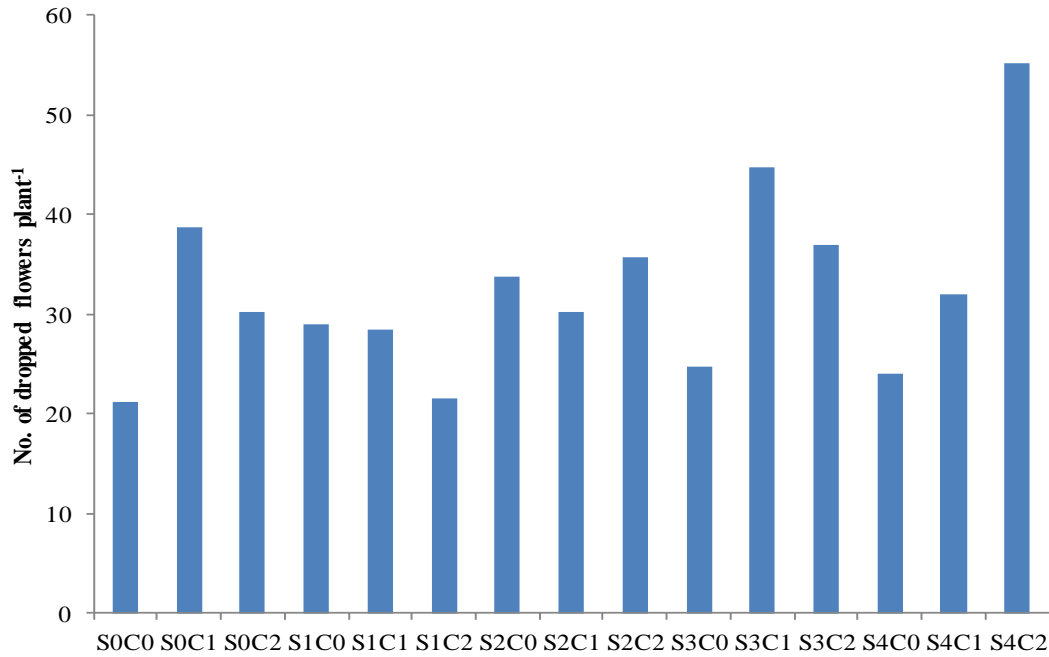
S₀ = without salt, S₁ = 2 dSm⁻¹, S₂ = 4 dSm⁻¹, S₃ = 6 dSm⁻¹, S₄ = 8 dSm⁻¹

Figure 26: Effect of salinity levels on number of dropped flowers plant⁻¹ of tomato (LSD_{0.05} = 1.03)



C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

Figure 27: Effect of calcium levels on number of dropped flowers plant⁻¹ of tomato ((LSD_{0.05} = 0.785)



S₀ = without salt, S₁ = 2 dSm⁻¹, S₂ = 4 dSm⁻¹, S₃ = 6 dSm⁻¹, S₄ = 8 dSm⁻¹
 C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

Figure 28: Effect of salinity and calcium levels on number of dropped flowers plant⁻¹ of tomato ((LSD_{0.05} = 1.755)

4.3.7 Fruit length (cm)

Different salinity levels exhibited significant variation in fruit length (cm) of individual fruit (Table 8 and Appendix IX). From Table 8, it was evident that the maximum fruit length (3.317 cm) was found from control (S₀) whereas the minimum (2.925 cm) was obtained from S₄ (8dSm⁻¹). It was observed in almost all cases that relative large size fruits were obtained from control plants and gradual small size fruits were obtained from increased salinity levels due to its inhibitory effect on cell expansion. Calcium had significant effect on the fruit length (Table 8 and Appendix IX). The longest fruit (3.225 cm) was produced from C₁ and the shortest fruit (3.095 cm) was produced from C₀ which was statistically identical (3.115) to C₂.

There were significant interaction effects between different salinity levels and calcium levels in case of fruit length of tomato (Table 9 and Appendix IX). The maximum fruit length (3.575 cm) was found from the treatment combination of S₀C₁. The minimum fruit length (2.900 cm) was obtained from S₄C₁ which was statistically similar to S₄C₀ (2.950 cm) and S₄C₂ (2.925 cm)

4.3.8 Fruit diameter (cm)

Diameter of individual tomato fruit varied significantly for the different levels of salinity (Table 8 and Appendix IX). The highest fruit diameter (3.092 cm) was recorded from control (S_0) whereas the lowest fruit diameter (2.700 cm) was found from S_4 (8 dSm⁻¹). Salinity decreased the fruit size.

Statistically significant difference was observed for fruit diameter of tomato due to the application of different levels of calcium (Table 8 and Appendix IX). The highest fruit diameter (2.960 cm) was recorded from C_1 which was statistically identical to C_0 (2.925 cm). Again the lowest value (2.880 cm) was observed from C_2 which was also similar to C_0 (2.925 cm).

Salinity and calcium levels significantly affected fruit diameter of tomato (Table 9 and Appendix IX). The highest fruit diameter of fruit (3.300 cm) was recorded from S_0C_1 whereas the lowest fruit diameter (2.600 cm) was found from S_4C_1 which was statistically identical (2.700cm) to S_4C_2 .

4.3.9 Individual fruit weight (gm)

Individual fruit weight of tomato varied significantly due to influence of the different levels of salinity (Table 8 and Appendix IX). The highest individual fruit weight (18.87 gm) was found from S_0 (control). The lowest weight (14.51 gm) was obtained from S_4 . The results obtained from this experiment showed that salinity stress caused a significant reduction in fruit weight which was also reported by Lolaei *et al.* (2012). This behavior also responsible for reduction of fruit weights plant⁻¹. Salinity reduced the individual fruit weight by inhibiting the cell division and rate of fruit expansion due to the lower water potential in the plant which will reduce the water flow into the fruit as reported by Jonson *et al.* (1992). It was reported that the low water content of fruit appeared to be the result of an osmotic effect rather than a toxic effect of NaCl. It was studied that salinity reduced xylem development in tomato fruit (belda and Ho, 1993) but since the tomato fruit has a very low transpiration rate, only a small proportion of the water input came via the xylem (Ho *et al.*, 1987).

Application of different levels of calcium showed statistically significant differences for the weight of individual fruit of tomato (Table 8 and Appendix IX). The highest individual fruit weight (18.19 gm) was recorded from C_1 and the lowest value (15.11 gm) was obtained from C_2 which was lower (15.62 gm) than C_0 .

The fact that increasing Ca^{2+} concentration increased the individual fruit weight was also supported by Lolaei *et al.* (2012), where 5 mM concentration gave the better result than 10 mM of Calcium.

Significant variation was recorded for the interaction effect of salinity and calcium for the weight of individual fruit of tomato (Table 9 and Appendix IX). The highest weight (23.10 gm) was recorded from S_0C_1 whereas the lowest value (12.42 gm) was found from the S_4C_2 .

4.3.10 Fruits weight (kg) plant⁻¹

Fruits weight plant⁻¹ varied significantly due to different levels of salinity (Table 8 and Appendix IX). The highest fruits weight (0.8275 kg) was observed from S_1 and the lowest value (0.3542 kg) was recorded from S_4 . Number of fruits plant⁻¹ and individual fruit weight were decreased with increased levels of salinity and that's why fruit weight plant⁻¹ also decreased under high salinity. Similar observation was also reported by Lolaei *et al.* (2012) in tomato, Ali (2004) in rice.

Statistically significant variation was recorded for different levels of calcium in terms of fruits weight plant⁻¹ (Table 8 and Appendix IX). The highest yield plant⁻¹ (0.6175 kg) was found from C_1 and the lowest value (0.5210 kg) was obtained from C_0 which was statistically similar (0.5265 kg) and closely related with C_2 . Fruits weight was also increased with the supply of calcium and highest result was recorded from 5 mM of Ca^{2+} . This result suggests that calcium reduced the toxic effect of salinity and increased the fruit weight in tomato which agrees with the result of Lolaei *et al.* (2012).

Interaction effect of salinity and calcium levels showed significant differences for fruits weight plant⁻¹ of tomato (Table 9 and Appendix IX). The maximum fruit weight plant⁻¹ (0.9000 kg) was recorded from S_0C_1 which was statistically similar (0.8575 kg) to S_0C_0 whereas the lowest value (0.3150 kg) was observed from both S_4C_0 and S_4C_2 .

Table 8. Effect of salinity and calcium levels on fruit length, fruit diameter, individual fruit weight and fruit weight plant⁻¹ of tomato

Treatment combination	Fruit length (cm)	Fruit diameter (cm)	Individual fruit Wt. (gm)	Fruit /plant (kg)	wt.
S ₀	3.317 a	3.092 a	18.87 a	0.8275 a	
S ₁	3.183 b	3.008 b	16.62 b	0.6033 b	
S ₂	3.150 b	2.867 c	15.57 c	0.5200 c	
S ₃	3.150 b	2.942 b	15.98 c	0.4700 d	
S ₄	2.925 c	2.700 d	14.51 d	0.3542 e	
LSD _(0.05)	0.05211	0.06893	0.5791	0.02605	
F-test value	**	**	**	**	
CV (%)	2.10%	2.96%	4.31%	5.19%	
C ₀		2.925 ab	15.62 b	0.5210 b	
C ₁	3.095 b	2.960 a	18.19 a	0.6175 a	
C ₂	3.225 a	2.880 b	15.11 c	0.5265 b	
LSD _(0.05)	3.115 b	0.05339	0.4485	0.02018	
F-test value	0.04036	**	**	**	
CV (%)	**	2.96%	4.31%	5.19%	

S₀ = without salt, S₁ = 2 dSm⁻¹, S₂ = 4 dSm⁻¹, S₃ = 6 dSm⁻¹, S₄ = 8 dSm⁻¹

C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

Table 9. Interaction effect of salinity and calcium levels on fruit length, fruit diameter, individual fruit weight and fruit weight plant⁻¹ of tomato

Treatment combination	Fruit length(cm)	Fruit diameter(cm)	Individual fruit Wt.(gm)	Fruit plant ⁻¹ (kg)	Wt.
S ₀ C ₀	2.950 cd	2.950 cd	15.94 de	0.8575 a	
S ₀ C ₁	3.300 a	3.300 a	23.10 a	0.9000 a	
S ₀ C ₂	3.025 bc	3.025 bc	17.57 bc	0.7250 b	
S ₁ C ₀	2.900 de	2.900 de	14.73 fgh	0.5025 d	
S ₁ C ₁	3.050 bc	3.050 bc	18.55 b	0.6500 c	
S ₁ C ₂	3.075 b	3.075 b	16.57 cd	0.6575 c	
S ₂ C ₀	2.850 def	2.850 def	14.44 gh	0.4675 de	
S ₂ C ₁	2.900 de	2.900 de	17.21 c	0.6200 c	
S ₂ C ₂	2.850 def	2.850 def	15.07 efg	0.4725 de	
S ₃ C ₀	3.125 b	3.125 b	18.51 b	0.4625 de	
S ₃ C ₁	2.950 cd	2.950 cd	15.50 ef	0.4850 d	
S ₃ C ₂	2.750 fg	2.750 fg	13.93 h	0.4625 de	
S ₄ C ₀	2.800 efg	2.800 efg	14.51 fgh	0.3150 f	
S ₄ C ₁	2.600 h	2.600 h	16.59 cd	0.4325 e	
S ₄ C ₂	2.700 gh	2.700 gh	12.43 i	0.3150 f	
LSD _(0.05)	0.1194	0.1194	1.003	0.04513	
F-test value	**	**	**	**	
CV (%)	2.96%	2.96%	4.31%	5.19%	

S₀ = without salt, S₁ = 2 dSm⁻¹, S₂ = 4 dSm⁻¹, S₃ = 6 dSm⁻¹, S₄ = 8 dSm⁻¹

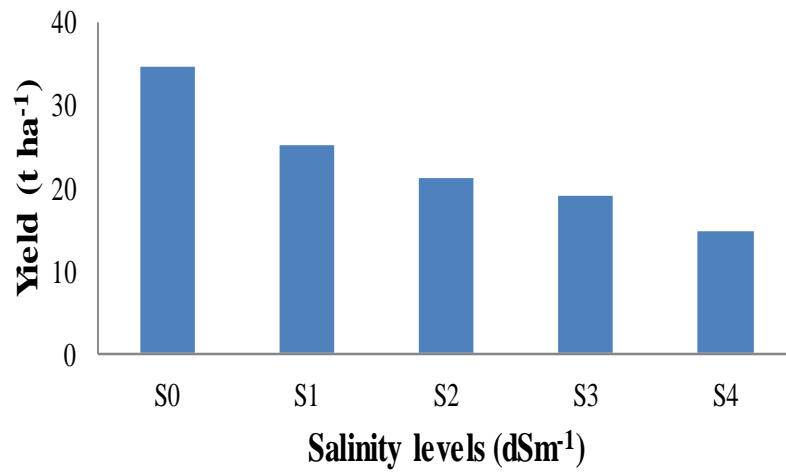
C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

3.3.11 Yield (t ha⁻¹)

Significant variation was recorded for yield (t ha⁻¹) due to the different salinity levels (Figure 29 and Appendix IX). The highest yield (34.44 t ha⁻¹) was obtained from S₀ whereas the lowest (14.85 t ha⁻¹) value from S₄. The result showed the gradual decrease of yield with the increased levels of salinity, which was strongly supported by Hajer *et al.* (2006), Cuartero and Munoz (1999) in tomato and also Lolaei *et al.* (2012), Khan (2013) etc. Tzortzakis (2010) reported that, salinity either of soil or of irrigation water causes disturbance in plant growth and nutrient balance and reduces crop yields. At relatively lower salinity levels, the yield reduction observed is caused mainly by a reduction in the average fruit weight; the declining number of fruits explains the main portion of yield reduction at higher levels of salinity.

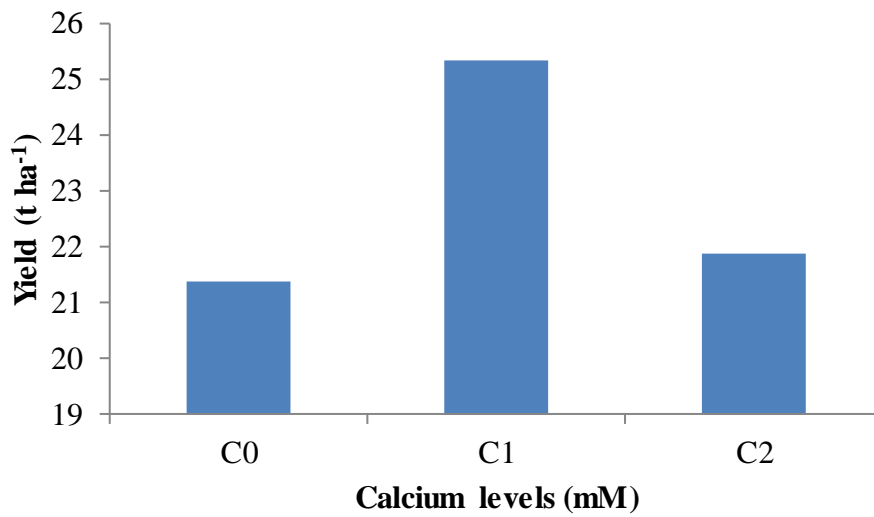
Application of different calcium levels on yield (t ha⁻¹) of tomato plant showed the statistically significant effect (Figure 30 and Appendix IX). The highest yield (25.35 t ha⁻¹) was obtained from C₁ whereas the lowest yield (21.39 t ha⁻¹) was found from C₀ which was statistically similar (21.88 t ha⁻¹) to C₂. These results suggest that the application of calcium increased the tomato fruits yield compared to control but 10mM concentration of calcium gave comparatively lower yield than 5mM. Lolaei *et al.* (2012) reported that, application of Ca²⁺ in the nutrient solution increased the fruit yield of tomato. The present work showed that under both non-saline and saline conditions, the exogenous application of Ca²⁺ alone, through the rooting medium, had an ameliorative effect on yield of tomato.

Combined effect of different salinity levels and calcium levels showed significant differences for yield (t ha⁻¹) of tomato (Figure 31 and Appendix IX). The highest yield (37.50 t ha⁻¹) was obtained from S₀C₁. The lowest yield (13.16 t ha⁻¹) was observed from S₄C₀ which was statistically similar (13.19) to S₄C₂.



S₀ = without salt, S₁ = 2 dSm⁻¹, S₂ = 4 dSm⁻¹, S₃ = 6 dSm⁻¹, S₄ = 8 dSm⁻¹

Figure 29: Effect of salinity levels on yield (t ha⁻¹) of tomato (LSD_{0.05} = 1.005).



C₀ = No calcium, C₁ = 5 mM, C₂ = 10 mM

Figure 30: Effect of Calcium levels on yield (t ha⁻¹) of tomato ((LSD_{0.05} = 0.778)



S_0 = without salt, $S_1 = 2 \text{ dSm}^{-1}$, $S_2 = 4 \text{ dSm}^{-1}$, $S_3 = 6 \text{ dSm}^{-1}$, $S_4 = 8 \text{ dSm}^{-1}$

C_0 = No calcium, $C_1 = 5 \text{ mM}$, $C_2 = 10 \text{ mM}$

Figure 31: Combined effect of salinity and calcium levels on yield (t ha⁻¹) of tomato (LSD_{0.05} = 1.74).

CHAPTER V

SUMMARY AND CONCLUSION

The experiment was conducted in the Horticulture Farm of Sher-e-Bangla Agricultural University, Dhaka, during the period from October 2013 to April 2014 to find out the mitigation of salt stress in tomato by exogenous application of calcium. In this experiment, the treatments consisted of five different salinity levels viz. S_0 = without salt (dSm^{-1}), $S_1= 2 \text{ dSm}^{-1}$, $S_2 = 4 \text{ dSm}^{-1}$, $S_3= 6 \text{ dSm}^{-1}$, $S_4=8 \text{ dSm}^{-1}$, and three different levels of calcium viz. $C_0 = 0\text{mM}$, $C_1= 5\text{mM}$ and $C_2 = 10 \text{ mM}$. The experiment was laid out in two factors Randomized complete Block Design (RCBD) with four replications. Data on different growth parameters, physiological parameters and yield with yield contributing characters of tomato were recorded. The collected data were statistically analyzed for evaluation of the treatment effect. A significant variation among the treatments was found while different salinity levels and calcium levels were applied in different combinations.

There are significant differences among the influence of different levels of salinity in case of almost all the parameters. In this experiment, tomato plants were subjected to salinity by applying saline water at three different days in the life cycle of tomato plant to keep the soil in saline condition. Plant grown on normal soil (control treatment) showed the maximum height more or less over the growth period whereas the lowest height was recorded from 8 dSm^{-1} treated plants. At 50, 60 and 75 DAT, the highest plant height was 80.50, 90.58 and 91.75 cm respectively under a controlled condition whereas the lowest height was 69.83, 73.67 and 76.58 cm at 6dSm^{-1} , 8dSm^{-1} and 8dSm^{-1} respectively. The maximum number of leaves plant^{-1} was 20.58, 30.92, 58.92 and 70.50 at 40, 50, 60 and 75 DAT with S_4 (4 dSm^{-1}), S_1 (2 dSm^{-1}), S_0 (control) and also control respectively whereas the lowest was 17.75, 25.17, 40.33 and 53.50 with 6, 6, 8 and 8dSm^{-1} respectively. Maximum number of branches plant^{-1} was 7.83, 10.17 and 10.33 at 45, 60 and 75 DAT under controlled treatment respectively, whereas the lowest was 6.67, 8.67 and 8.25 with 6, 8 and 6 dSm^{-1} respectively. The highest leaf and stem dry weight (24.09 and 16.40 gm) was recorded from controlled plant whereas the lowest (10.99 and 7.22 gm) was recorded with 8 dSm^{-1} . The maximum leaf area plant^{-1} (178.1 cm^2) was observed from controlled treatment whereas the lowest (148.2 cm^2) was found from 8 dSm^{-1} . The leaf chlorophyll content was degraded with the increase of salinity whereas the maximum

chlorophyll content was recorded from no or low levels of salt with minimum from 8 dSm⁻¹. The highest K⁺ (1.038%), Ca²⁺ (0.6542%), K⁺/Na⁺ (4.317) and Ca²⁺/Na⁺ (2.724) was recorded from controlled treatment whereas maximum Na⁺ (0.6067%) at 8 dSm⁻¹. The lowest K⁺, Ca²⁺, K⁺/Na⁺ and Ca²⁺/Na⁺ was recorded from 8 dSm⁻¹ whereas minimum Na⁺ at controlled treatment. The maximum flower clusters plant⁻¹ (14.58), number of flowers plant⁻¹ (74.25), number of fruits plant⁻¹ (44.17), individual fruit weight (18.87gm), fruit weight plant⁻¹ (0.83kg) was recorded from control which favoured the higher yield and the lowest value of all these parameters were found from 8 dSm⁻¹ thus also produced the lowest yield. 8 dSm⁻¹ salinity level was responsible for maximum number of dropped flowers plant⁻¹ (37.08) whereas the lowest (26.33) was recorded from 2 dSm⁻¹.

Calcium significantly influenced maximum parameters selected for data collection. At 40, 50, 60 and 75 DAT the highest plant height (47.30, 76.65, 84.90 and 84.65 cm) was obtained from C₁ (5mM Ca²⁺) over the control plants. The maximum number of leaves plant⁻¹ was 20.30, 29.40, 51 and 64.15 at 40, 50, 60 and 75 with C₂, C₀, C₁, and C₁ respectively. Then maximum number of branches plant⁻¹ was 7.70, 9.35 and 10.05 at 45, 60 and 75 DAT with C₀, C₁ and C₀ respectively. The maximum leaf area plant⁻¹ (169.9 cm²) was observed from C₀ whereas the lowest (159.6 cm²) was found from C₂. The highest leaf and stem dry weight (19.79 and 12.39 gm) was recorded from C₂ over the control plants. The leaf chlorophyll content was increased with the calcium application where the maximum chlorophyll content (38.53 and 38.79 SPAD unit) was recorded from C₁ at 70 and 85 DAT. The highest K⁺ (0.9330%), Ca²⁺ (0.6575%), K⁺/Na⁺ (2.795) and Ca²⁺/Na⁺ (1.880) was recorded from C₁, C₁, C₀ and C₂ respectively whereas the maximum Na⁺ (0.4030%) was recorded from C₀. The lowest K⁺ (0.8455%), Ca²⁺ (0.4975%), K⁺/Na⁺ (2.535) and Ca²⁺/Na⁺ (1.549) were recorded from C₂, C₀, C₂ and C₀ respectively whereas the minimum Na⁺ (0.3770%) from C₂. The maximum flower clusters plant⁻¹ (15.10), number of flowers plant⁻¹ (70.60), number of fruits plant⁻¹ (34.65) was recorded from C₂ and the highest individual fruit weight (18.19gm), fruit weight plant⁻¹ (0.62kg) were recorded from C₁ which facilitated to higher yield (25.35 t ha⁻¹) in C₁ over the controlled treatment. Maximum number of dropped flowers plant⁻¹ (35.95) was recorded from C₂ over the controlled treatment and the maximum number of flowers plant⁻¹ was also recorded from C₂.

The combinations of salinity and calcium significantly influenced almost all the

parameters. The highest plant height (49.50, 83.97 and 95.25 cm at 40, 50, 60 and 75 DAT respectively) were recorded from S_0C_0 treatment combinations whereas the lowest value (38.50, 67.50, 66.75 and 74 cm) were observed from S_0C_0 , S_4C_0 , S_4C_0 and S_3C_2 treatments respectively. At 40, 50, 60 and 75 DAT, maximum number of leaves plant⁻¹ 23.25, 35, 63.75 and 71 was recorded from S_4C_2 , S_0C_0 , S_1C_1 and S_1C_0 respectively and the lowest value 16, 24.25, 38.50 and 52.25 was recorded from S_3C_0 , S_3C_0 , S_4C_1 and S_4C_1 respectively. The interaction effect of salinity and calcium did not exhibit statistically significant effect on the number of branches plant⁻¹. The maximum leaf area plant⁻¹ (189.7 cm²) was observed in S_0C_2 whereas the lowest (125.2 cm²) was found from S_4C_2 . The highest leaf and stem dry weight (25.27 and 17.02 gm) was recorded from S_0C_2 and S_0C_1 whereas the lowest (8.58 and 6.38 gm) was recorded from S_4C_0 . The maximum leaf chlorophyll content (43.47 and 44.17 SPAD unit) was recorded from S_1C_1 at 70 and 85 DAT. The highest K⁺ (1.178%), Ca²⁺ (0.71%), K⁺/Na⁺ (4.9) and Ca²⁺/Na⁺ (2.86) were recorded from S_0C_0 , S_0C_1 , S_0C_0 and S_0C_1 respectively and the maximum Na⁺ (0.61%) from S_4C_1 . The lowest K⁺ (0.66%), Ca²⁺ (0.40%), K⁺/Na⁺ (1.02) and Ca²⁺/Na⁺ (0.70) were recorded from S_4C_2 , S_4C_0 , S_4C_2 and S_4C_0 respectively whereas the minimum Na⁺ (0.23%) from S_0C_2 . Maximum flower clusters plant⁻¹ (17.75), number of flowers plant⁻¹ (80.50), number of fruits plant⁻¹ (52.25) were recorded from S_4C_2 , S_4C_2 and S_0C_0 respectively and the highest individual fruit weight (23.10 gm), fruit weight plant⁻¹ (0.90 kg) were recorded from S_0C_1 which facilitated to higher yield (37.50 t ha⁻¹) in C₁. Maximum number of dropped flower plant⁻¹ (55.25) was recorded from S_4C_2 with minimum from S_0C_0 , whereas the maximum number of flowers plant⁻¹ was also recorded from S_4C_2 .

Conclusion

Considering the above mentioned results, it may be concluded that, the yield of tomato was gradually decreased by the increase of salinity levels and this reduction rate was decreased by exogenous supply of calcium. Among the calcium levels, almost 5 mM showed the highest result in growth, physiology and yield parameters as compared to 10 mM. Salt treatment increased Na⁺ concentration significantly in leaves of plant whereas K⁺ and Ca²⁺ concentration were decreased as such showed the toxicity of salinity. This toxicity symptom were reduced by calcium through increasing K⁺ and Ca²⁺ concentration by lowering Na⁺ concentration.

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APPENDICES

Appendix I: Physical and chemical composition of soil sample

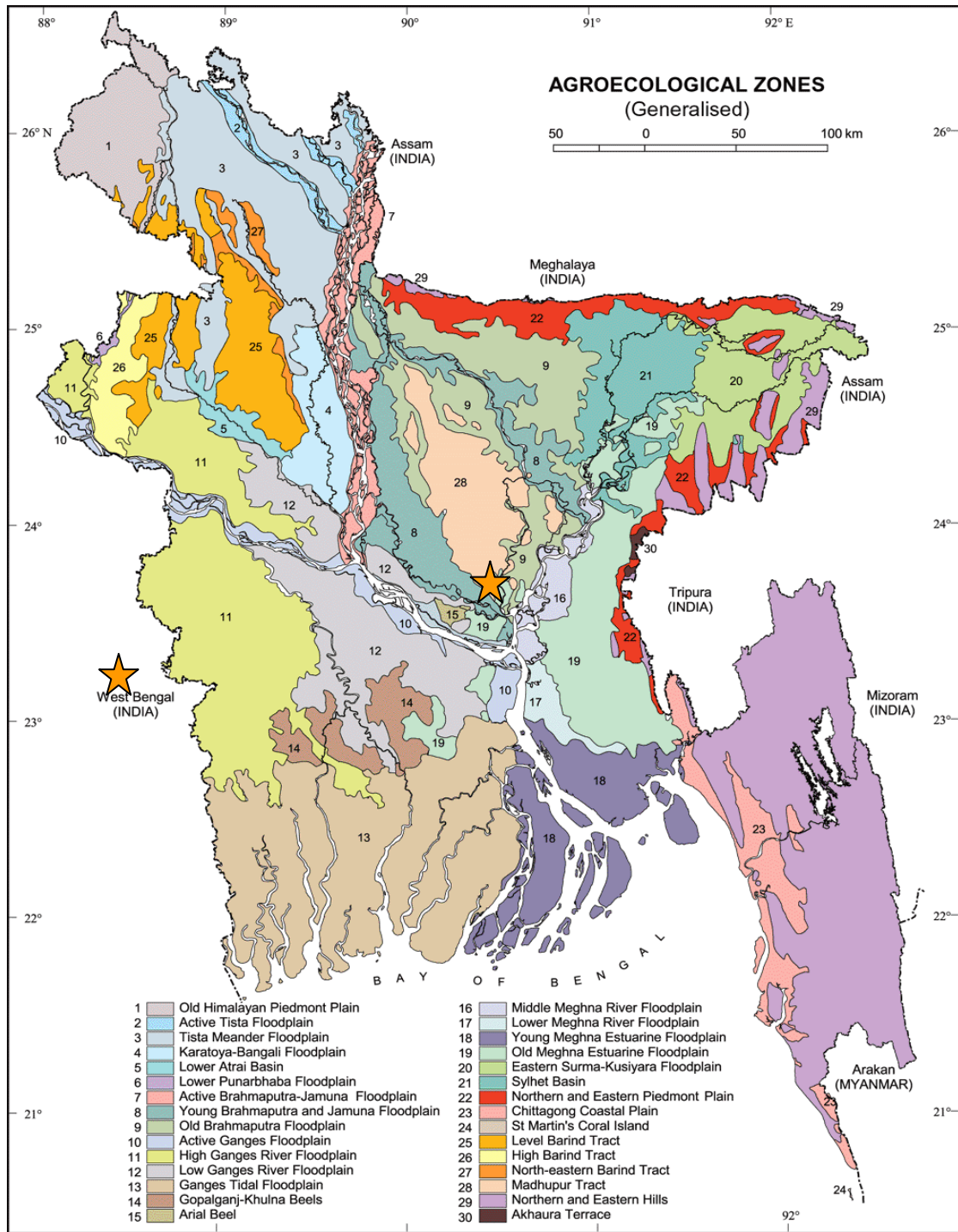
Characteristics	Value
% Sand	20.84
% Silt	57.46
% Clay	21.7
Textural class	Silt loam
pH	6.9
Organic matter (%)	0.86
Available K (ppm)	25
Available Na (ppm)	70

Appendix II: Monthly record of air temperature, rainfall, relative humidity and sunshine hours of the experimental site during the period from October 2013 to April 2014

Year	Month	Average Air temperature (°C)		Total rainfall (mm)	Average RH (%)	Average sunshine hours
		Maximum	Minimum			
2013	October	30.8	14.85	44.64	67.82	7.48
	November	28.1	6.88	15.6	58.18	7.85
	December	25.36	6.12	0.62	54.3	6.85
2014	January	25	12	18	46	9
	February	28	13	31	37	8
	March	33	16	58	38	7
	April	35	23	103	42	6

Source: Weather station, Sher-e-Bangla Agricultural University, Dhaka-1207.

Appendix III: Experimental location on the map of agro-ecological zones of Bangladesh



Appendix IV: Analysis of variance of the data on plant height of tomato under different salinity and calcium levels

Sources of variation	Degrees of freedom	Mean square of plant height (cm) at				
		30DAT	40DAT	50DAT	60DAT	75DAT
Replication	3	0.639NS	1.144**	1.183NS	0.328NS	0.417NS
Factor A	4	19.858**	47.125**	257.233**	674.125**	547.608**
Factor B	2	0.017NS	72.800**	39.317**	168.617**	13.517**
Ax B	8	14.683**	26.675**	46.921**	78.512**	49.558**
Error	42	0.556	0.299	0.481	0.613**	2.976

*significance at 5% level of probability

**significance at 1% level of probability

NS-non significant

Appendix V: Analysis of variance of the data on number of leaves plant⁻¹ of tomato under different salinity and calcium levels

Sources of variation	Degrees of freedom	Mean square of Number of leaves per plant at				
		25DAT	35DAT	45DAT	60DAT	75DAT
Replication	3	0.756NS	0.194NS	0.550NS	0.778NS	87.200NS
Factor A	4	2.275**	12.767**	59.625**	768.892**	536.042**
Factor B	2	0.650NS	23.617**	10.617**	6.067**	33.017NS
Ax B	8	1.150NS	6.054**	33.825**	95.004**	30.204NS
Error	42	0.660	0.468	0.395	0.492	60.569

*significance at 5% level of probability

**significance at 1% level of probability

NS-non significant

Appendix VI: Analysis of variance of the data on number of branches plant⁻¹ of tomato under different salinity and calcium levels

Sources of variation	Degrees of freedom	Mean square of Number of branches per plant at		
		45DAT	60DAT	75DAT
Replication	3	0.328NS	0.061NS	0.467NS
Factor A	4	3.142**	4.500**	8.975**
Factor B	2	4.317**	3.517**	6.317*
Ax B	8	1.879**	0.413NS	1.087NS
Error	42	0.280	0.335	1.907

*significance at 5% level of probability

**significance at 1% level of probability

NS-non significant

Appendix VII: Analysis of variance of the data on leaf area, leaf and stem dry weight plant⁻¹ of tomato under different salinity and calcium levels

Sources of variation	Degrees of freedom	Mean square of		
		leaf area (cm ²)	leaf dry wt. (gm)	stem dry wt. (gm)
Replication	3	78.265**	5.862NS	0.802**
Factor A	4	2246.429**	307.875**	193.752**
Factor B	2	568.463**	46.576**	4.043**
Ax B	8	990.143**	24.720**	8.875**
Error	42	12.598	6.368	0.103

*significance at 5% level of probability

**significance at 1% level of probability

NS-non significant

Appendix VIII: Analysis of variance of the data on number of flower clusters plant⁻¹, No. of flower cluster⁻¹ and No. of fruits cluster⁻¹ of tomato under different salinity and calcium levels

Sources of variation	Degrees of freedom	Mean square of		
		No. of Flower Clusters plant ⁻¹	No. of Flowers Cluster ⁻¹	No. of Fruits Cluster ⁻¹
Replication	3	0.061NS	0.350NS	0.347**
Factor A	4	3.892**	0.939**	0.703**
Factor B	2	47.217**	1.021*	0.080NS
Ax B	8	13.529**	0.657*	0.239**
Error	42	0.347	0.280	0.089

*significance at 5% level of probability

**significance at 1% level of probability

NS-non significant

Appendix IX: Analysis of variance of the data on fruit length, fruit diameter, individual fruit weight, fruit weight plant⁻¹ and yield of tomato under different salinity and calcium levels

Source of variation	Degrees of freedom	Mean square of				
		Fruit length (cm)	Fruit diameter (cm)	Individual fruit wt.(gm.)	Fruit Wt. plant ⁻¹ (Kg.)	Yield (t ha ⁻¹)
Replication	3	0.002NS	0.009NS	0.691NS	0.001NS	0.760NS
Factor A	4	0.238**	0.267**	31.646**	0.376**	664.390**
Factor B	2	0.098**	0.032**	54.387**	0.059**	93.060**
Ax B	8	0.039**	0.081**	15.989**	0.014**	21.154**
Error	42	0.004	0.007**	0.494	0.001	1.487

*significance at 5% level of probability

**significance at 1% level of probability

NS-non significant

Appendix X: Analysis of variance of the data on No. of flowers plant⁻¹, No. of fruits plant⁻¹ and No. of dropped flowers plant⁻¹ of tomato under different salinity and calcium levels

Source of variation	Degrees of freedom	Mean square of		
		No. of flowers plant ⁻¹	No. of fruits plant ⁻¹	No. of dropped flowers plant ⁻¹
Replication	3	2.994NS	1.811NS	2.561NS
Factor A	4	277.892**	689.525**	223.275**
Factor B	2	629.150**	13.650**	528.200**
Ax B	8	350.379**	91.587**	335.075**
Error	42	1.233	1.061	1.513

*significance at 5% level of probability

**significance at 1% level of probability

NS-non significant

Appendix XI: Analysis of variance of the data on leaf chlorophyll content of tomato under different salinity and calcium levels

Source of variation	Degrees of freedom	Mean square of leaf chlorophyll content (SPAD unit) at		
		50DAT	70DAT	85DAT
Replication	3	19.988NS	12.377NS	6.310NS
Factor A	4	390.675**	388.443**	404.647**
Factor B	2	7.705NS	32.446**	21.964NS
Ax B	8	50.292**	51.571**	52.148**
Error	42	10.244	6.691	8.280

*significance at 5% level of probability

**significance at 1% level of probability

NS-non significant

Appendix XII: Analysis of variance of the data on biochemical changes and stomatal conductance of tomato under different salinity and calcium levels

Source of variation	Degrees of freedom	Mean square of					
		Na ⁺ (%)	K ⁺ (%)	Ca ²⁺ (%)	K ⁺ /Na ⁺	Ca ²⁺ /Na ⁺	Stomatal conductance (molm ⁻² s ⁻¹)
Replication	3	0.000	0.005NS	0.006NS	0.009NS	0.040NS	0.001NS
Factor A	4	0.280**	0.154**	0.052**	17.411**	6.715**	0.010**
Factor B	2	0.004**	0.040*	0.148**	0.354**	0.665**	0.007**
Ax B	8	0.007**	0.063**	0.010NS	0.544**	0.102NS	0.003**
Error	42	0.001	0.011	0.008	0.064	0.090	0.001

*significance at 5% level of probability

**significance at 1% level of probability

NS-non significant