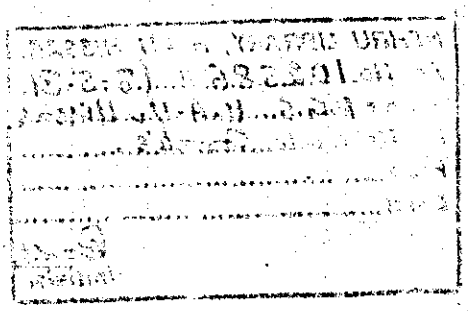
STUDIES ON SALT TOLERANCE IN GUAVA



DISSERTATION

SUMITTED TO THE HARYANA AGRICULTURAL UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE
OF

DOCTOR OF PHILOSOPHY

IN

HORTICULTURE

By

M. K. KAUL

DEPARTMENT OF HORTICULTURE
COLLEGE OF AGRICULTURE
HISSAR

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THIS DISSERTATION IS

DEDICATED TO

PROFESSOR B.P. STROGONOV

IN APPRECIATION OF

HIS ABUNDANT CONTRIBUTIONS TO

SALINITY KNOWLEDGE

Dr.K.S. Chauhan,
Professor & Head,
Department of Horticulture,
Haryana Agricultural University,
Hissar.

CERTIFICATE- I

This is to certify that dissertation entitled " Studies on salt tolerance in guava " submitted for the degree of Doctor of Philosophy in the subject of Horticulture of the Haryana Agricultural University, is a bonafide research work carried out by Shri M.K.Kaul under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

K.S.Chauhan
(K.S.Chauhan) 6.3.87
Major Advisor

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I feel a great pleasure in expressing my reverenced gratitude to Dr.K.S.Chauhan, Professor & Head,Department of Horticulture, Haryana Agricultural University, for his untiring guidance, continuous encouragement and full help throughout the study. His endless patience in discussing all sort of problems, constructive criticism in preparation of this manuscript and inculcating in me the habit of independent thinking is also greatly acknowledged.

I am highly indebted to the members of my Advisory Committee, namely Dr.O.P.Dhankar, Dr.J.P.S.Dhindsa, Dr.H.N.Krishnamurthy, Dr.H.C.Sharma,Dr.I.S.Singh and Dr.H.S.Nainawati for their help, valuable suggestions during the course of study and for going through the manuscript critically.

I highly appreciate and greatly acknowledge Dr.A.P.Khera, Dr.T.S.Kathpal, Dr.Maharaj Singh and Dr.H.R.Manchanda for their encouragement,whole-hearted cooperation and able suggestions during the tenure of this study.

I owe my special debt of gratitude to all those my friends who helped me in various capacities during the tenure of this study.

Last,but not the least, I heartly acknowledge and cordially appreciate the subdued spirit of cooperation,timely encouragement and sacrifices made by my parents, brothers and sisters during the course of present study.

Merit fellowship awarded by Haryana Agricultural University is duly acknowledged.

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CHAPTER - I

INTRODUCTION

Salinity is one of the acute problems faced by agriculturists throughout the world. In India, this problem has taken serious dimensions with the introduction of canal irrigation, especially where the water table is high and underground water is of poor quality. In fact about 7 million hectares of land is influenced with salinity in different parts of the country, of which 5,25,000 hectares exist in Haryana State alone (Abrol and Bhumbla,1970).

The ability of the plants to withstand excess soluble salts in the rhizosphere is of great interest to agriculturists in the semi-arid and arid regions of world. It affects almost all the physiological and metabolic processes in the plants in an adverse manner. However, the exact mechanism of its action is not yet fully understood. Moreover, very little work of this kind has been done on fruit plants.

Deleterious effects of saline conditions on plant growth in general are attributed to (i) increase in osmotic pressure of the rooting medium (ii) specific ion effect and (iii) decrease absorption of some essential nutrients. The extent of injury done to plant by salinization of medium varies with the type of predominent ion, their concentration, the physiological stage of plant growth at which it is exposed to salinity and the plant species.

There are very few tropical and subtropical fruits like guava which have good salt tolerance.This fruit can be grown on marginal land with less care and can withstand

drought conditions well. It is also a fruit of high nutritive value particularly in relation to high vitamin C content. It is also a good source of other vitamins, minerals and pectin. Although, an important crop, very little work has been done on the salt tolerance of guava, especially with reference to its physiological basis. Moreover, reclaimation of saline soil is a long term process and the immediate solution is to grow salt tolerant crops in such soils by evaluating the rapid method of screening the salt tolerant crops under such conditions.

It was, therefore, thought worthwhile to study the effect of various levels of salinity on guava plant with the following objectives:

1. To determine the salt tolerance limit of guava to chloride and sulphate types of salinity.
2. To study the specific ion injury due to sodium, chloride and sulphate ions.
3. To study the nutritional imbalance associated with salt damage.
4. To study the metabolic disturbances caused by salts and their relationship with growth and development of guava.

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CHAPTER - II

REVIEW OF LITERATURE

The literature on the effects of salt stress on plant growth and development, physiology and metabolism has been reviewed laying main emphasis on the parameters which are closely related to the subject of present study. Loughridge (1901) pointed out that the different plant species were able to tolerate different salt concentrations. He further reported that fruit trees are relatively salt sensitive as compared to cereals and forage crops. Since then a great deal of research work has been conducted and extensively reviewed by many workers (Magistad, 1945; Hayward and Wadleigh, 1949; Arnold, 1955; Bernstein and Hayward, 1958; Asana, 1961; Bernstein, 1962; Slatyer, 1967; Levitt, 1972 and Mass and Hoffman, 1977). However, no one has paid any attention to salinity aspect of guava except Gupta and Bhambota (1968). The work done on various crops, with special emphasis on fruit crops, is reviewed under the following heads.

Relative salt tolerance of fruit crops

The response of various plant species and even varieties of some species to saline conditions, varies. The varietal differences in some of the cases have been found to be quite significant (Ayers, 1952; Bishnoi and Pancholy, 1980; Malik et al., 1977; Puntamakar et al., 1970 and Udovenko, 1975). Magistad and Christiansen (1944) found that fig, grapes and olive were moderately salt tolerant, whereas, citrus, apple pear and drupaceous fruits had low salt tolerance. Fruit crops have been classified into three groups viz. high salt

tolerant, medium salt tolerant and highly salt sensitive crops (U.S. salinity laboratory staff,1953). Date-palm has the highest salt tolerance pomegranate, fig, olive and grape are medium salt tolerant and apple, pear, orange, grapefruit, stone fruits, strawberry, lemon and avocado are highly salt sensitive crops. Great variation in the salt tolerance of fruit crops have alsobeen reported by Avon, 1953; Rocha and Flores, 1958; Ehlig and Bernstein,1959 and Vander Berg,1950. Oganesjan (1953) reported that Z. jujuba and Punica granatum as most tolerant species, whereas Diaspyros kaki is reported to be least tolerant. Raheja (1962) gave the order of tolerance for sensitive citrus crops i.e. Lemon > Sweet lime > Nagpur oranges. Ivanov (1969) observed that on saline soils, apple grew better than pears and of the stone fruits, apricot, myrobalan and sour cherry grew well, plum worse and sweet cherry and peach poorly. Oganesjan (1953) found that the concentrations of total soluble salts which proved fatal for apricot and apples were 2 and 1.2 per cent respectively. Almond, plum, pears resembled apricots, whereas, peaches resembled apples in their salt tolerance limits. Bernstein (1965) has also given a list of limits of chloride tolerance and critical salinity levels for deciduous and sub-tropical fruit trees. Desai (1975) reported that guava plants can survive well under sulphate type of salinity upto 0.525 per cent salt and under chloride and carbonate type of salinity upto 0.350 per cent salt. Makhija et al. (1980) reported that

six months old seedlings of guava could survive well under salinization upto 7.5 mmhos/cm whereas at 10.5 mmhos/cm only 75 per cent of the seedling survived.

Relative toxicity of different constituents of saline soils

The excess soluble salts most frequently found in saline soils, consists of cations-sodium, calcium, magnesium and anions - chloride and sulphate. The toxicity of salt is directly correlated to their permeability into the cell. So, every salt has a distinct effect on the plant. Sargeev (1953) arranged salts according to their adverse effects on seed germination in the following order, Na_2CO_3 > balanced solution > NaCl > Na_2SO_4. Strogonov (1962) reported that carbonates are much more toxic than chloride and sulphate. However, soil salinity is usually caused by chloride and sulphate. Therefore, the work done on its specific effects on fruit crops, is reviewed separately in the following pages.

Chloride

Chloride is blamed more frequently than any other ion for the severe injurious effects and plays major role in inhibiting growth of plants on saline soils. The chloride limits differ in different fruit crops. Brown et al. (1963) observed that chloride salt was more toxic to peach and other stone fruit trees. Sideris and Young (1954) observed terminal leaf necrosis in pineapple due to chloride. Leaf burn of avocado due to chloride accumulation has been reported by Fenn et al. (1970). Salerno (1975) reported that chloride caused tip drying

in citrus trees. Pandey et al. (1971) reported the leaf scortch of mango due to excess of chloride. The incipient chlorosis, accompanied by burning and drying of leaf margin in mango is produced by chloride injury (Bhambota et al.,1963). The sensitivity of Senna (Cassia sp.) was correlated with the high rate of chloride accumulation in the tissue, resulting in specific chloride injury (Ayoub,1977). Makhija et al. (1980) reported that increasing concentration of chloride were injurious to guava plant.

Sulphate

Sulphate ions are usually less toxic than chloride to plant (Thorne and Peterson,1954). The application of SO_2 gas to vines caused leaf drop (Ruiz,1969). Gupta and Nauriyal (1973) observed toxicity symptoms of sulphate on grapevine under higher sulphate treatment. Older leaves were affected first and abscission of foliage occurred as soon as nearly half of the leaf margin developed necrosis. Ehlig (1960) reported magnesium deficiency in grapes with high sulphate concentration. The injurious symptoms of sulphate in mango have been reported. It caused slight darkening and browning at the tips and margins of the leaves, often encircling the entire leaf (Bhambota et al., 1963). Divate (1974) observed loss of dark green colour in the grape leaves subjected to high sulphate concentration.

Sodium

Large amounts of sodium in the soil is toxic to plants and adversely affects the plant growth. Sodium exerts secondary effect on plant growth through adverse structural

modification of soil. Appreciable amount of Na on the soil exchangeable complex makes it dispersed and puddled, thereby causing poor aeration and low water availability (U.S.salinity lab.1953). Martin et al. (1959) reported that 13 per cent exchangeable sodium slightly reduced the growth and injured peach seedlings. Smith (1963) observed the decline of orange trees with the accumulation of sodium upto 2500 ppm in 4-5 month old seedlings. Hayward and Wadleigh (1949) reported that the accumulation of sodium in the plant may be associated with a depression in the accumulation of other cations to the extent that their content may be below adequate levels and an unfavourable cation balance may be built up. Symptoms of Na^+ injury in fruit plants include appearance of chlorotic areas in leaves (Lunt, 1966). Ayers et al. (1951) have described sodium scorch of avocado leaves. Black (1967) noted leaf spot damage in avocado due to excess of sodium. Bernstein (1965) reported that in citrus, sodium toxicity caused well defined leaf necrosis. Harding et al. (1956) correlated leaf burn and defoliation symptoms to sodium toxicity in citrus. In apricot, Halsely et al. (1958) observed that sodium scorch is associated with curling and burning of leaf margin. Petrosjan et al. (1967) reported that pomegranate and quince showed relatively resistance to Na salt.

Physiological basis of growth inhibition due to salinity

Substrate salinity affects plant growth in two ways,

(i) by increasing the osmotic pressure of the media(Bernstein,

1961, 1963; Sands and Clarke,1977) and (ii) by disturbing the normal mineral nutrition of the plant (Strogonov et al., 1970). However, different views have been put forth to explain the detrimental effects of salts on plant growth. Reduced growth of plants under saline conditions is caused by osmotic inhibition of water absorption, toxic effects of ion, nutritional imbalance, production of toxic substances within the plant and many other ill effects on plant metabolism. According to water relation theory due to increased osmotic pressure of soil solution, availability of water to plant is decreased. This theory has been supported by Eaton (1941), Hayward and Spurr (1944), Wadleigh and Ayers (1945) and Danielson and Russell (1957). But,these results do not reconcile with the results of Philips (1958), according to whose theory", physiological drought" develops when solution cannot penetrate into the cell. Salts penetrate into the cell, cause an increase in the osmotic pressure of cell and hence increase in water uptake, Inhibition of plant growth under such condition is explained by him as being due to toxic effects of salts.

Both nutrients and non-nutrients are absorbed by the plant under salinization so as to keep nutritional balances but the non-nutrients are an unnecessary ballast. The penetration of these salts into plant is mainly limited by the roots. However, the permeability of root tissues to determine the salt resistance of plant is maintained upto a certain

level, beyond which a sort of salt burst occurs, thereby causing poisoning and ultimately death to plant (Strogonov, 1962). Strogonov and Ostapenko (1946) demonstrated that salt poisoning is a result of accumulation of toxic intermediates i.e. accumulation of nitrogenous substances like ammonia and H_2O_2. Putrescine accumulation in the plant organs has been reported by Strogonov and Shevyakova (1961). A unified theory of salinity has been put forth by O'Leary (1971), according to which, the resistance of roots to water flow increases in plants grown on salt solution decreases hormone delivery thereby, hormonal balance is disturbed in leaves. It is reflected by increased rigidity of cell walls. During this time plant accumulates solutes and osmotic pressure of cell sap is increased apparently (osmotic adjustment) which maintains the osmotic gradient necessary for water absorption.

Effect of salinity on germination, growth and development

a), Germination: Fruit plants are more sensitive during germination and emergence than at later stages of growth. However, salinity delays germination at lower levels, whereas at higher levels, it reduces the final germination percentage as well (Ayers and Hayward,1948; Bhumbla and Singh,1965; Bhatti,1972; Malik et al.,1977; Hasson-Porath et al.,1972; Odegbaro and Smith,1969; Panigarh et al.,1978; Prisco et al., 1978; Thomas and Iyengar,1971; Ungar,1974, 1978; Ram,1979; Bishnoi and Pancholy,1980). These effects have been considered

to be mainly osmotic at lower levels but appear to be primarily ionic at higher levels of salinity (Tulaikov, 1922; Khudairi, 1958; Macke and Ungar,1971; Prisco and O'Leary,1970; Tur et al., 1980). While working on Deglet Noor date seeds, Hewitt (1963) observed that germination of date seed was reduced only at 30,000 ppm and above. Bahodyrov (1956) reported that germination of mulberry seeds in saline soils was retarded. Bangash (1977) while doing pot experiment with NaCl salinization from 0.05- 0.80 per cent, said that Zizyphus jujuba was most tolerant species. Dhankar et al. (1978) reported that Z. rotundifolia seed germination was reduced to 50 per cent at 6 mmhos/cm with almost complete inhibition at 12 mmhos/cm ECe.

Puntamakar et al. (1970) found that the relative toxicity of different salts at 5-10 mmhos/cm conductivity on germination of several varieties of wheat was in ascending order $CaCl_2$, Na_2SO_4, NaCl, $NaHCO_3$ and Na_2CO_3.

b) Plant growth and development: Salinity in general results in stunted growth, restricted lateral shoot development, reduction in size of leaves and fruit, decrease in fresh and dry weights of different plant parts, decreases in number and size of seeds and finally yield (El-Karouri,1979; Tal, 1971). However, salinity adversely effects plant growth and the reduction in plant growth is more or less proportional to the salt concentration present in the saline soil. Cooper and Peynado (1959) reported that salt treatment decreased

growth in both young and old citrus trees. More mature plants of grapevine with well developed root system grow better on top soil salinity as compared to young plants (Gildiev,1950). Obbink and Alexander (1973) correlated growth and chloride accumulation of six grape cultivars and reported that as chloride in media increased, shoot length was decreased. Furr et al. (1966) reported that growth rate of young date palm irrigated with water containing 6000 ppm salt was about 50 per cent to that of untreated control. Tursunov (1970) observed reduced growth and leaf area of grapes with increased salinity . Heikal et al. (1980) reported the decrease in dry weight of castor bean and sunflower plants by 40 to 80 meq/l NaCl salinity. Sanchez-Conde and Azuara (1980) showed decrease in fresh and dry weight in Zea mays by 5 atm. $MgSO_4$. Nieman and Poulsen (1971) reported suppression of growth in pea and other vegetables due to sodium chloride. Bhambota and Kanwar (1970) observed that growth of Blood Red budded on rough lemon was reduced with the increase in salt concentration. Furr and Ream (1968) reported growth depression of date plant with the increase in salinity. Joolka (1976) found reduced growth, number of leaves, leaf area and dry weight in citrus with increasing levels of salt salinity. Jindal et al. (1976) reported mango tree growth was adversely affected with high levels of salinity. Leaf injury increased with salt concentration and at 10 mmhos/cm ECe almost all the leaves were injured. Reduced stem girth of orange plant

due to sodium chloride was observed by Chapman et al. (1968). Downton (1977) reported in grapes that salinity led to reducedgrowth whereas foliar symptoms of salt toxicity were absent. Nasr et al. (1977) while evaluating the effect of salinity on growth of plum and peach found that trunk growth, shoot length, fresh weight of plant organs were reduced with the increased salinity. Grape fruit showed inverse relationship between growth and electrical conductivity of soil saturation extract (Pearson et al., 1957). Wigdor et al. (1958) reported that dry weight of branches, leaves, roots and trunk diameter of pummeols reduced under sodium chloride salinity. He further attributed it to the reduced uptake of water by plant and thus affecting cell enlargement. Nauriyal and Gupta (1967) and Gupta and Nauriyal (1973) indicated that dry weight of roots and shoots in grapevine is reduced due to NaCl, Na_2SO_4 and Na_2CO_3. Tur et al. (1980) reported that growth of rice seedlings was depressed more by Na_2SO_4 than NaCl at iso-osmotic concentrations.

All the above mentioned effects of salinity on plant growth appear to operate via the inhibitory effect of salinity on the rates of cell division alongwith the decrease in cell enlargement (Gaidmakina, 1973; Nieman, 1965).

Effect of salinity on different physiological aspects

a) Water relations

(i) Relative water content/ internal water deficit: The problem of water supply and water exchange of plants growing

on saline soils has till now been inadequately studied. Meiri et al. (1971) reported that chloride type of salinity induces succulance character due to increased hydration of tissue, whereas, sulphate salinization causes an apparent dehydration of tissues and organs. Moreover, the temperature of the leaves from chloride-sulphate varient was lower than that of sulphate-chloride one. Comparison of leaf temperature at different levels of water supply shows that the temperature increases as the water supply becomes worse. The increase in water content of cell under chloride salinity occurs both in vacuole and protoplasm. Water supply under sulphate is ensured by an intensive development of root system and water conducting system in root and stem. Plants from chloride salinity have a higher suction force in their leaves and a higher osmotic pressure in their cell sap than plants from sulphate type of salinity (Strogonov, 1962). Prisco and O' Leary (1973) reported that the relative water content of salt treated plant was lower than that of control, in bean plants. Nieman (1962) observed increase in water content of plants grown on chloride salinity. Ram (1979) also reported that plants under chloride type of salinity had more relative water content as compared to plants under sulphate type of salinity. Repp (1961) considered that salinity throws out the balance between intake and water consumption out of adjustment, the water balance, therefore, increases and assimilation of metabolites ceases.

(ii) Leaf-diffusive resistance: Salt and water stress conditions are known to decrease the rate of transpiration in various plant species (Gale et al., 1967; Turner,1974; Weatherley and Slatyer,1957; McCree,1974). Salinity responses to transpiration in different plant species are quite variable because of the differences in stomatal behaviour under varying environmental conditions. Boyer (1965) reported that if internal osmotic adjustment occurs following stress imposition, stomata behave in a fashion very similar to control plants due to turger recovery. Types of salt exert different effects on the rate of respiration. Aceves -N et al. (1975) reported 10 fold increase in leaf diffusive resistance to water vapour at -12 bar salt in wheat. Both NaCl and $CaCl_2$ behaved similarly although the effect of $CaCl_2$ was somewhat larger at intermediate salinity levels. Kaplan and Gale (1972) showed that salinization produced little or no increase in stomatal resistance of the upper leaf surface but a large increase of the lower surface of Atriplex halimus. Several workers have shown that the adaxial and abaxial surfaces of the plant respond differently not only to their environment but also to water stress (Jordan et al.,1975; Brady et al.,1975). In most cases, the adaxial tended to close earlier and at a lower water stress than the abaxial one. On the other hand, Sanchez-Diaz and Kramar (1971) and Duniway (1975) found no such differences between adaxial and abaxial leaf surfaces.

Endogenous ABA content of leaf increased several folds with increasing stress (Wright,1969; Wright and Hiron,1969) and a concomitant increase in diffusive resistance of stomata. Walton et al. (1977) suggested that rate of ABA content, determined the stomatal aperture and the stomata apparently do not function normally untill the ABA content of leaves returns to its original value.

(iii) Respiration : Various workers have given different results regarding rate of respiration under salinity. Lapina and Popov (1970) observed a reduction in dark respiration of tomato leaves under NaCl salinization. Tur et al. (1980) reported that NaCl decreases respiration in rice seedlings. Similar results in rate of respiration was reported by Flowers (1972), Goris (1969), Porath and Poljakoff-Mayber (1968) whereas Lapina and Bikmukhametova (1972) found an increase in respiration rate of corn grown under NaCl or Na_2SO_4 salinization. According to Slatyer (1967), increase in the rate of respiration directly provides mechanism for growth suppression, affecting net assimilation rate. Increase in respiration can be expected as a result of the energy requirement for selective ion absorption in the presence of high external substrate concentration. Livme and Levin (1966), Ziv (1968), Morozovskiz and Kabanov (1970) observed increased rate of respiration. Porath and Poljakoff -Mayber (1964) reported that mitochondria from NaCl treated pea plants showed higher rate of oxygen uptake. The route of respiration was changed from glycolytic to pentose-phosphate shunt in seedling of barley, cotton and

pea (Goris, 1969; Porath and Poljakoff-Mayber, 1968). Divate (1974) observed increased rate of respiration in grape leaves due to salinity. Nieman (1962) found that in 12 crops species studied, the respiration rate was more sensitive to NaCl and tended to increase in both tolerant and sensitive species as the concentration of salt in saline media was increased. Pokrovskaia (1958) reported that respiration rate was increased at higher salinity levels.

(iv) Chlorophyll content: The adverse effect of salt on the changes in pigment concentration in plant depend upon the specific nature of ion, plant species and stage of plant growth and development. Salinization with NaCl and Na_2SO_4 has been reported to reduce leaf chlorophyll content in number of plants (Singh and Mangal, 1971; Lapina and Popov, 1970; Ponomareva et al. 1971 and Reddy and Das, 1978). This is due to decrease in the rate of metabolism caused by high concentration of chloride (Genkel, 1954; Greenway, 1965; Strogonov, 1962). Furthermore, Sivtsev et al. (1973) while working on tomato proposed that the increase in the hydrolytic activity of chlorophyllase may be regarded as one of the cause of this decrease. Bhambota and Kanwar (1970) observed that the chlorophyll content in the leaves of Blood Red grafted on rough lemon fell as salinity increased. They further added that this decrease in chlorophyll content was accompanied by higher 'Na' and lower 'Mg' and 'Fe' uptake. Passera and Albuzia (1978) reported an increase in the chlorophyll content of wheat leaves under salt stress. Similar increase in chlorophyll

content of Pea leaves under salt stress were reported by Nieman (1962) and Sheoran (1975) in mung and chickpea. However, Kim (1958) reported that chlorophyll content of raddish, cabbage and lettuce seedlings was progressively reduced by increasing concentration either of NaCl or Na_2SO_4. Weimberg (1975) reported that the rate of chlorophyll formation in etiolated pea seedlings exposed to light in salinized media was slower than the unsalinized control plant. In grapes, the loss of dark green colour of leaves near the margins and between the veins followed by yellowing of leaves due to CO_3 while SO_4 caused loss of green colour at the tip and between veins (Gupta and Nauriyal, 1973). Ponomareva et al. (1971) suggested that the stability of the bond between chlorophyll and protein lipid complex is reduced under NaCl salinization. Soil salinization with NaCl and Na_2SO_4 (4.4 atm.) decreased the chlorophyll content on per plant basis whereas Na_2SO_4 increased but NaCl decreased on mg/g basis in tomato (Lapina and Popov, 1971). Lapina and Popov (1970) noticed concurrent changes in the structure of chloroplast i.e. increase in the transparency of protein stroma and disorientation of the Lamella system. Inhibition to the normal development of lamellar system in the chloroplast was noticed by Ziv (1968). Prisco and O'Leary (1972) observed the decay of chlorophyll and protein in bean leaves subjected to NaCl salinity.

Effect of salinity on metabolites

Very little work has been done on the effect of salinity

of sulphur containing amino acids was inhibited under both NaCl and Na_2SO_4 salinization. According to Kessler and Smir (1968) in sweet lime seedlings, the solution of salt strongly inhibited RNA synthesis and protein turnover. In sweet lime and cleopatera mandarin, chloride had a depressing effect on protein level. Decreased synthesis of DNA, RNA and protein under saline conditions results in reduced rate of cell division (Nieman and Poulson, 1964; Prisco and O'Leary, 1973).

According to Saakjan and Petrosjan (1964) increased salinity and alkalinity by Na_2SO_4 caused reduction in RNA and protein N in vine leaves. Severely stressing pea plants (Pisum sativum L.) with NaCl caused very little change in the amounts of DNA, RNA/g of organic matter in leaves and did not prevent the labelled adenine into RNA (Kabanov et al., 1973). Smillie and Krotkov (1961) reported a loss of nucleic acid and protein during the maturation of pea leaves.

The increased level of ammonia and free amino acids in horsebean plants, growing under saline conditions, decreases back but remain little higher than the control plant even after seven days of transfer to normal nutrient solution. Similarly, the decreased protein level increases and sucrose content decreases, but these metabolic changes are not fully repaired after shift to salt free condition in hoursebean (Strogonov et al., 1970).

Organic acid content

Organic acid content of the plant is influenced by the rate of ion absorption as well as the type of ion being

absorbed (Jacobsen, 1955). By using $^{14}CO_2$ incorporation into organic acid and varying salt concentration and type of ion absorbed, Torii and Laties (1966) suggested that the delivery of ion into the vacuole effectively removed organic acid from cytoplasm by forming the salt of acid in vacuole and this results in more organic acid being synthesized. Williams (1960) observed higher levels of organic acids in halophytes and other plants associated with dry climate and high salt. He further added, a strong correlation between organic acid level, type of ion and salt concentration in the nutrient medium, NaCl was principal salt absorbed and level of organic acid was present correspondingly high. Ruiz (1969) reported that application of SO_2 gas to Tempainlo vines caused slightly higher melic acid content and lower tartaric acid content compared to untreated and manually defoliated control.

Enzymes

Activity of number of enzymes and their isoenzymes have been studied under salinity but the results were controversial. Peroxidase activity was not affected at moderate NaCl concentrations but decreased at higher concentration in wheat seedlings (El-Fouly and Jung, 1972). High salt concentrations of 0.1 - 0.5 M NaCl and Na_2SO_4 decreased the peroxidase activity in wheat, corn and pea (Vasile, 1963). Maliwal and Paliwal (1972) also reported decrease in peroxidase activity in okra and sponge gourd under saline conditions. Similar

results were reported by Gaidamakina (1973) in sunflower. On the contrary increase in activity of peroxidase was reported under NaCl or Na_2SO_4 type of salinity in pea roots and seedlings (Pokrovskaia, 1958; Strogonov, 1962; Rokova et al., 1969 and Weimberg, 1970).

Increasedactivity of amylase has been reported by Strogonov (1962) and El-Fouly and Jung (1972) under NaCl and Na_2SO_4 salinization.

Effect of salinity on mineral composition of plant

The mineral metabolism of the plant gets affected by salinity i.e. the excess of some ion in soil would prevent the uptake or availability of other present in relatively small amount and secondly by their own excessive uptake because of their relative abundance in the media. In order to study the effect of salinity on mineral metabolism single salt solution have been quite popular as we can find out clearly the effect of cations and aninos responsible for salinization, on the uptake of ions and their own uptake.

Under NaCl salinization, the uptake of calcium, magnesium, potassium and phosphorus is reduced and an accumulation of Na and Cl occur (EL-Kadi et al., 1971; Hasson-Porath et al., 1972). However, Shah and Nadeem (1976) reported that increasing salinity increased P, Ca, Mg, Na, Cl and SO_4 content and decreased K content of grain and straw of lentil plant. Similar results were reported in different crop plants (Cevda et al., 1979; Storey and Jones, 1977). Ehlig (1960) observed

deficiency of magnesium in grapes due to high concentration of sulphate in the saline media. Shimose (1968) while studying the physiology of salt injury found that leaf content of K, Ca, Mg, fell when concentrations of NaCl and Na_2SO_4 were increased in saline media. The decrease in Ca was more under Na_2SO_4. Rains (1972) put forth the view that if sulphate replaces chloride as accompanying ion, sodium absorption is depressed. However, Bhambota et al. (1963) in their study on mango, observed higher uptake of Na due to NaCl followed by Na_2CO_3 and Na_2SO_4. Similar results were obtained by Gupta and Bhambota (1968) in guava.

The phosphorus uptake has also been reported to be reduced by salinity (Zhukovskaya, 1973). Buckman and Brady (1969) suggested that if pH of soil increases above 7, phosphate uptake is reduced due to formation of calcium phosphate. Mattson (1969) reported that salinity created with $CaCl_2$ in four soils, increased phosphorus in pea while reduced it in barley, reduced the ratio of di to monovalent cations in pea and increased them in barley. Sands and Clarke (1977) reported that $CaCl_2$ and NaCl damage to radiate-pine was associated with chloride excess and an induced phosphorus deficiency. Throne and Peterson (1954) mentioned about low availability of P in saline and alkaline soils. Reduced P content of leaves due to saline water was observed by Fakhr (1961). The entry of ^{32}P into the organs of barley and tomato was reduced by chloride and sulphate type of salinity (Matukhin and Zhukovskii, 1961).

Increasing chloride concentration decreased the K content in peacan seedlings (Faruque,1968); cherry and peach(Dilley et al.,1958) and grapefruit (Peynado and Young,1963) and decreased calcium and increased Mn in cherry and peach (Dilley et al. , 1958). Ortuno et al. (1971) found out that under the effect of high chloride concentration in the soil, the Na, K, Ca and Mg levels were higher in young veena orange leaves than in mature senescening leaves.

Sulphate, beside its accumulation in toxic amounts in different parts of plant, also affects the other constituents of the plant. With the increase in sulphate concentration there was decrease in Ca content (Billinges,1962 and Zusman,1956), Mg content (Ehlig,1960 and Peyanodo and Young,1963) and K content to deficiency levels (Peyando and Young, 1963). They further observed that boron content of grapefruit was low, while contrary results have been reported by Dilley et al. (1958) in apple, cherry and peach. Ortuno et al. (1971) observed that cation sum decreased until leaf maturity and rose slightly subsequently.

The growth effects of sodium was additive in trifoliate seedlings (Martin et al.,1969). Cooper and Peynado (1953) found no clear and consistent relationship between chlorosis and Cl, Na or Ca content. Bower and Wadleigh (1949) observed decreased accumulation of Ca, Mg, K in plants growing on high levels of sodium in the soil. Petrosjan et al. (1967) found the salinity reduced the K and P content of necrotic tissues of vine, mulberry and quince leaves.

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CHAPTER- III

MATERIALS AND METHODS

The present study was carried out at the experimental orchard of department of Horticulture, Haryana Agricultural University, Hissar, during the year 1979-80.

The seeds of cv. Lucknow-49 (Sardar) guava were sown in February, 1979, in earthen pots filled with dry garden soil and FYM in 3:1 ratio. After germination only one plant was retained in each pot. To prevent leaching pot holes were blocked. Then, in the month of February, 1980, these plants were transplanted alongwith earth ball to the cement pots (30 x 30 x 45 cm). These pots were filled with 60 kg of sandy loam soil. To prevent the leaching of the salts, pots were pasted inside with coal tar and then lined with 400 gauze imprevious polythene sheet.

On 20th of February, 1980, sixty-five pots with plants of uniform vigour were selected and arranged in five replications. Salts on mmhos basis were given through water on the saturation percentage of the soil. Mixture of sodium chloride, sodium sulphate and calcium chloride were in the proportion of 3:1:4. Analysis of soil used prior to addition of salt has been given in Appendix-I.

Experimental details

Salts

1. Control (no salt)
2. Sodium chloride (NaCl)
3. Sodium sulphate (Na_2SO_4)
4. Calcium chloride ($CaCl_2$)

5. Mixture ($NaCl + Na_2SO_4 + CaCl_2$)

Conductivity = 6, 9,12 *mmhos/cm* ECe

Replications = Five

Treatments = 13 treatments inclusive of control

(Four salts x 3 conductivities) + control

Total number of pots = 13 x 5 = 65

Design = CRD factorial

Experiment-I: Effect of different salinity treatments on the germination and growth of guava seeds

Seed sowing

Seeds of freshly harvested fruits of cv.Lucknow-49 guava were taken out and then sown in the artificially prepared saline soils in the aluminium trays (15 x 10 x 2 inches). Two hundred seeds in each tray in four rows were sown making 50 seeds in each row.

Germination

The data on the number of seeds germinated was recorded at weekly interval after the first seed germinated. The per cent seeds germinated and overall survival percentage were calculated after 79 days i.e. two weeks later when germination under control was almost complete.

Radical length

The length of root was measured in cm at the end of experiment.

Hypocotyl length

At the end of experiment hypocotyl length was measured and calculated in terms of hypocotyl length in cm.

Number of secondary root

Number of secondary roots were counted for each treatment at the termination of experiment.

Experiment-II: Effect of different salinity treatments on growth, physiological and biochemical changes in plant

Sampling technique

Fifth and sixth leaves from the apex were taken for estimation of chlorophyll, respiration, protein, leaf area, enzyme, organic acid, proline and free amino acid determinations. Fourth leaf was taken for leaf-water potential, relative water content, and leaf-diffusive resistance. For nucleic acid determinations second leaf was taken. Duplicate samples were taken for all the determinations.

Determinations of proline, respiration, relative water content, enzymes and nucleic acid content were done two times i.e. in the last week of May,1980 and again in the last week of September,1980.

Growth observations

(a) Cumulative plant growth

(i) The height of all the branches of plants including main stem under different salts treatments was taken at monthly interval and summed up. Total height was represented as cumulative plant growth at monthly interval.

(ii) Main stem height: Height of main stem was taken from the marked base upto last leaf opened. The initial

height of the plant taken before the addition of salt was substracted from over all height.

(b) Stem diameter

The diameter of plant was measured in centimetres with the help of vernier-callipers at the marked base of plant at monthly intervals.

(c) Leaf area

Area of leaves taken out for various biochemical determinations was worked out. At the end of experiment leaves of the fifth and sixth position from the apex were collected and the area was measured with the help of leaf area meter. Finally, average area of leaf was calculated.

(d) Fresh weight

At the end of the experiment the plants were cut at the ground level and separated into stem and leaves. Each part was weighed and weight was recorded as g/plant.

The root system was taken out carefully by taking out polythene sheet alongwith soil and roots. Then, roots were removed carefully after washing with water and dried on the absorbant paper. Weight was recorded as g/plant.

(e) Dry weight:

The stem, leaves and roots were dried at 80°C for 48 hours. The dry weight of sample was recorded when there was no further loss of weight during dried.

(f) Root length

Length of primary and secondary roots were taken separately at the end of experiment.

Nature and extent of injury

The growth of plant under different treatments were frequently watched and the symptoms of injury, whenever, noticed were recorded separately for each treatment. The extent of injury was determined by percentage of mortality of plants under various treatments.

Plant analysis

Preparation of samples

The leaves were dried at 80°C for 48 hours and finely ground. Different nutrients from these samples were determined according to the procedure adopted for respective estimation.

Except for chloride and sulphate, the digestion/extraction procedure was common for rest of the elements viz. phosphorus, sodium, potassium, calcium and magnesium. For nitrogen, H_2SO_4 was used in place of HNO_3 with $HClO_4$ in the ratio 9:1. Rest of the procedure was same.

Digestion

Two hundred mg of dried and well ground material was taken in a 50 ml conical flask to which 10 ml of 4:1 HNO_3 and $HClO_4$ (60%) mixture were added. The flasks were heated gently over a hot plate for about half an hour till whole of the material gets dissolved and solution became colourless. The digest, thus, obtained was cooled and final volume made to 50 ml with distilled water.

Nitrogen

The nitrogen content was determined by the method of Lindner (1944).

Phosphorus

P Content was estimated by Venedo-molybdophosphoric yellow colour method, described by Jackson (1973).

Sodium and potassium

The Na and K content were determined by the Flame photometer.

Calcium and magnesium

These were determined by versenate method of Cheng and Bray (1951).

Chloride

The chloride content was determined by the method of Ramsay et al. (1955).

Extraction

200 mg of finely ground dry material was boiled in water for one hour and the volume of the extract was made to 50 ml. This was filtered and filterate was used for chloride estimation.

Sulphur

Two gm of plant material was digested according to the procedure described by AOAC (1950) and final volume was made to 100 ml. To 50 ml of aliqot two drops of methyl red and 2 ml of concentrated HCl were added; volume was made to 100 ml which was boiled. Excess of barrium chloride was added drop by drop. It was concentrated to 50 ml on a water bath, cooled, filtered and the ppt of $BaSO_4$ was dried and weighed. Amount of sulphate was expressed as percentage on dry weight basis.

Determination of physiological and biochemical parameters

The procedure adopted for determination of various physiological aspects i.e. leaf-water potential, leaf-diffusive resistance, respiration, relative water content, chlorophyll and metabolism studies like proline, amino acids, protein, enzymes, nucleic acid and organic acid are given below:

a) Leaf water potential

Leaf water potential was measured with a pressure chamber (Scholander et al., 1964) as modified to take leaf alongwith petiole (Turner et al., 1971) available from RMS Instrumentation Corporation, Oregon, USA. Leaf alongwith petiole was taken and put in pressure chamber. The cut end was inserted through hole made in rubber compression gland. The cut end was then observed through a magnifying glass as the gas pressure in pressure bomb was raised by admitting nitrogen under pressure. The pressure at which xylem sap appears at the cut end of petiole was regarded as equal to the water-potential of leaf cells and readings were recorded as leaf-water potential in bars. Leaf water potential was taken 100 days after transplanting at 11.00 A.M. at 25 days interval.

b) Leaf diffusive resistance

Leaf resistance was measured with diffusion porometer. Adaxial and abaxial leaf resistance were measured separately. It was expressed in seconds per centimeter. Leaf diffusive

resistance was taken after 100 days of transplanting at 25 days interval in the morning at 11.00 A.M.

Preparation of sample for chlorophyll and respiration

Leaf samples from 5th and 6th position from the apex were collected between 6.30-8.30 A.M. They were placed in polythene bags and kept in ice box immediately.The samples were cleaned and rinsed with distilled water when used for determination. The excess of adhering water was soaked with the help of tissue paper.

Determination of chlorophyll

Five hundred mg of leaf sample was crushed and ground thoroughly in a glass pistal and mortar using 80 per cent acetone (Arnon,1949). A little amount of potassium carbonate was added to neutralize acids present in the cell, since in the presence of acidity in the cell, chlorophyll changes to pheophytin. After the extraction was over, it was centrifuged and volume of supernatant made to 100 ml in the volumetric flask, with the addition of acetone. Absorbance values at 663 mμ and 645 mμ wave length were recorded by using Bausch and Lamb Spectronic-20 colorimeter. Total chlorophyll, chlorophyll 'a' and chlorophyll 'b' were determined and expressed as mg/g fresh weight by using the absorption coefficient in the formula given by Arnon (1949).

Total chlorophyll = 20.2D 645 + 8.02D 663

Chlorophyll'a' = 12.7D 663 - 2.669D 645

Chlorophyll'b' = 22.9D 645 - 4.68D 663

c) Respiration

Standard Warburg technique (Umbrett et al., 1971) was used for measurement of respiration rate in the leaves. Two discs each of 0.44 cm^2 area were cut from middle portion of leaf in between the veins and were floated on 1 ml of 0.1 M phosphate buffer (pH 7.0) in the Warburg flask. 0.2 ml of 20% KoH was added in central cavity alongwith the filter paper strip. It was run for 15 minutes as blank and then the stopper were closed and uptake of oxygen was measured twice at 20 minute interval at 27°C. The results obtained have been expressed as µl of oxygen taken /cm^2/hour.

Relative water content

Three leaves per replicate at random from 5th and 6th position were taken and immediately their fresh weight was taken. Then, it was soaked in water for 24 hours. After 24 hours, turgid weight was taken and then put in the oven at 80°C for 48 hours and then dry weight was recorded when there was no further loss of weight. The relative water content was calculated by the following formula:

$$\frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

Biochemical estimations

a) Proline content : Proline content of plant leaf was determined by colourimeteric method (Bates et al.,1973).

b) Free amino acids: Free amino acid content was estimated spectrophotometerically according to method of Yemm and Cocking (1955).

c) Protein: Protein content was estimated by method given by Lowry et al. (1951) using Folin cio-calteau reagent.

d) Estimation of organic acid: Two g of fresh leaves were taken and ground in 20 ml of distilled water and to the extract 2 ml of concentrated HCl was added. Afterwards, organic acids were extracted in petroleum ether with the help of separating funnel. Petroleum ether fraction was evaporated on a water bath. Then, organic acids were dissolved in distilled water and titrated with 0.1 N NaOH. The amount of organic acid was expressed as milliequivalents of NaOH.

e) Amylase : Amylase was assayed according to methods of Shuster and Gifford (1962) with slight modification.

Reagent I: Soluble starch: 67 mg of soluble starch, 0.06 M NaH_2PO_4 and 200 µM $CaCl_2$ were added to distilled water to make a total volume of 100 ml, boiled for one minute and filtered . The supernatant was used as substrate.

Reagent II: Iodine reagent: A stock solution was prepared by adding 6 g of KI and 600 mg of I_2 in 100 ml of H_2O. One ml of the stock solution was diluted to 100 ml with 0.1 N HCl.

Procedure

One ml of the appropriatelydiluted enzyme was incubated with 1 ml of soluble starch solution. Reaction was terminated after five minutes by adding one ml of iodine reagent. After making the volume to 7 ml with distilled water O.D. was read

at 620 nm against reagent blank. Initial O.D. was recorded using the assay mixture in which enzyme was replaced by distilled water.

One enzyme unit was defined as the amount of enzyme required to decrease. O.D. by 0.1 under the given conditions.

Peroxidase

Peroxidase activity was measured by the method of Seevers et al. (1971) following spectrophotometrically the change in absorbance at 470 nm due to oxidation of O-dianisidine in the presence of H_2O_2 and enzyme. A 0.1 ml aliquot of properly diluted enzyme was incubated with 1.8 ml of 0.05 M sodium acetate buffer, pH 4.5, 0.1 ml of O-dianisidine (0.25% in methanol) and 0.2 ml of 0.2 M H_2O_2. An equivalent volume of buffer was substituted for H_2O_2 in the reference. Readings were recorded after 3 minutes. Unit was defined as the amount of enzyme which causes a change of 0.1 in O.D. under the given condition.

Nucleic acid

Nucleic acid extraction was carried out according to the methods of Cherry (1962) and Nieman and Poulson (1963). The preliminary extraction for removing various types of phosphates was done following the procedure suggested by Cherry (1962) except at the first two steps when extraction was done in enthanol (Nieman and Poulsen, 1963) instead of methanol.

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CHAPTER - IV

EXPERIMENTAL RESULTS

The results of the studies conducted are presented in the ensuing chapter.

1. Germination studies

1.1. Germination

Increasing salt stress in the media progressively delayed and even inhibited the germination of guava seeds (Table 1a). Germination was first noticed 21 days after sowing under 6 mmhos/cm Na_2SO_4 salinity. Na_2SO_4 (6 mmhos showed maximum germination (18.5%) followed by control (12.5%) at 28 days after sowing. However, there was maximum germination (86.5%) in control at 42 days. Rest of the treatments recorded 25 per cent germination at 42 days. At 12 mmhos/cm conductivity there was no germination in NaCl, $CaCl_2$ and mixture of salt whereas it was 20% with sodium sulphate. Even at 6 mmhos/cm conductivity the germination was reduced to 47%, 47.5% and 53% with NaCl, mixture of salt and $CaCl_2$, respectively whereas it was as high as 74.5% in Na_2SO_4 salinity at the same conductivity.

After 70 days of sowing, maximum germination was obssrved under control followed by Na_2SO_4, $CaCl_2$, Na Cl at 6 mmhos/cm and Na_2SO_4 at 9 mmhos/cm treatments respectively (Table 1b). Seedling survival was maximum (94.0%) after 79 days under control whereas under different salinization treatments only Na_2SO_4 (6 mmhos/cm) had more than 43 per cent survival.This was followed by $CaCl_2$ and NaCl,6 mmhos/cm each with 31 and 25.5 per cent seedling survival, respectively. Lowest survival percentage (16.0) was observed under mixture of salts.

Table 1a: Effect of different salts and their concentrations on the germination of guava seeds

Salt	Conductivity (mmhos/cm)	Seed germination (%)							
		Days after sowing							
		21	28	35	42	49	56	63	70
Control	0.08	0.0	12.5	49.0	86.5	99.0	99.0	99.0	99.0
NaCl	6	0.0	0.0	5.0	22.5	44.5	46.5	47.0	47.0
	9	0.0	0.0	2.0	11.5	19.0	20.5	20.5	20.5
	12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Na_2SO_4	6	6.5	18.5	38.0	53.0	71.5	74.5	74.5	74.5
	9	0.0	4.0	13.5	19.0	34.5	43.5	45.5	45.5
	12	0.0	0.0	8.5	10.5	17.0	19.5	19.5	20.0
$CaCl_2$	6	0.0	0.0	12.0	24.0	43.5	49.0	52.0	53.0
	9	0.0	0.0	9.5	21.5	26.5	28.0	28.0	28.0
	12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mixture	6	0.0	0.0	9.0	16.0	40.5	43.5	47.0	47.5
	9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 1b: Effect of different salts and their concentrations on final germination and survival of seedlings

Salt	Conductivity (mmhos/cm)	Germination after 70 days of sowing (%)	Survival after 79 days of sowing (%)
Control	0.08	99.0	94.0
NaCl	6	47.0	25.5
	9	20.5	6.0
	12	0.0	0.0
Na_2SO_4	6	74.5	43.0
	9	45.5	19.5
	12	20.0	3.5
$CaCl_2$	6	53.0	31.0
	9	28.0	7.5
	12	0.0	0.0
Mixture	6	47.5	16.0
	9	0.0	0.0
	12	0.0	0.0

1.2. Hypocotyl length, root length and number of secondary roots

The data presented in Table 2 reveals that different salts and their concentrations reduced the hypocotyl length and root length differently. Although the maximum hypocotyl length (3.4 cm) was observed under control but at 6 mmhos/cm conductivity it was more in Na_2SO_4 (2.4 cm) followed by mixture (1.9 cm), NaCl(1.3 cm) and $CaCl_2$ (1.0 cm). At 9 mmhos/cm it was again high in Na_2SO_4 followed by $CaCl_2$ and NaCl (0.5 cm, each).

Same is true in case of root length. The maximum root length (6.9 cm) was recorded in control. Among the different salts, Na_2SO_4 at 6 mmhos/cm had maximum root length (5.5 cm) whereas $CaCl_2$ and NaCl at this conductivity had only 3.7 cm and 4.4 cm root length. At 9 mmhos/cm conductivity, Na_2SO_4 had 4.4 cm root length followed by $CaCl_2$ (3.8 cm) and NaCl (1.9 cm). Critical view of the data showed that root length under all treatments was more as compared to hypocotyl length.

The number of secondary roots was maximum under control whereas, under salinity treatments, Na_2SO_4 was followed by $CaCl_2$, NaCl and mixture of salts at 6 mmhos/cm with 5.0, 3.6, 2.8 and 2.9 roots, respectively. Higher conductivity of all the salts suppressed the growth as well as number of secondary roots.

2. Survival percentage of transplanted seedlings

Under all the salinity levels except 12 mmhos/cm, the survival percentage was more than 50 per cent (Table 3). In

Table 2: Effect of different salts and their concentrations on hypocotyl length, radical length and number of secondary roots

Salt	Conductivity (mmhos/cm)	Hypocotyl length (cm)	Radical length (cm)	Number of secondary roots per seedling
Control	0.08	3.4	6.9	6.0
NaCl	6	1.3	3.7	2.8
	9	0.5	1.9	0.0
	12	0.0	0.0	0.0
Na_2SO_4	6	2.4	5.5	5.0
	9	1.0	4.4	1.8
	12	0.4	2.1	0.0
$CaCl_2$	6	1.0	4.4	3.6
	9	0.5	3.8	0.9
	12	0.0	0.0	0.0
Mixture	6	1.9	3.5	2.0
	9	0.0	0.0	0.0
	12	0.0	0.0	0.0

Table 3: Mean survival of transplanted guava seedlings under different salinity levels

Salt	Conductivity (mmhos/cm)	Survival (%)
Control	0.08	100
NaCl	6	100
	9	70
	12	-
$NaSO_4$	6	100
	9	100
	12	-
$CaCl_2$	6	100
	9	80
	12	-
Mixture	6	100
	9	60
	12	-

addition to control, NaCl, $CaCl_2$, mixture of salts and Na_2SO_4, all at 6 mmhos/cm and later both at 6 and 9 mmhos/cm had cent per cent survival. However, under NaCl, $CaCl_2$ and mixture of salts (all at 9 mmhos/cm) 80, 70 and 60 per cent survival was observed. At 12 mmhos/cm conductivity the survival was nil, irrespective of salt.

3. Symptoms of injury

Plants under different salinity levels were kept under watch and symptoms of injury were recorded separately for each treatment.

After 20 days of treatment, slight leaf scorch was noticed under 12 mmhos/cm $CaCl_2$. After 26 days, leaf scorch occurred in 12 mmhos/cm NaCl and mixture of salts. After 42 days, slight loss of dark green colour was noticed under 12 mmhos/cm Na_2SO_4. Therefore, leaf-scorch was observed in higher conductivities of Na_2SO_4, NaCl, CaCl and mixture of salts. Leaf scorch gradually extended towards the base of theleaf. Defoliation was invariably noticed where more than 75 per cent of leaf was scorched. Leaf defoliation was more quick under Na_2SO_4 than chloride type of salinity. Under chloride type of salinity in most of the cases the leaves remained attached even after whole leaf was scorched and this was especially true with $CaCl_2$ treatment. Burning at the tip and margins was observed at higher conductivity. The nature of symptoms differed in every salt treatment. In most of the cases, complex nature of injurious symptoms were observed.The clear cut symptoms noticed under different salinization are given below:

Symptoms of high chloride injury

Leaf scorch was first noticed at the margins of older leaves which extended to the base of leaf. Defoliation occurred when more than 3/4th leaf was scorched. Inword curling of leaf was observed. Margins beneath the tip started curling first. Partial death of plant was observed at higher conductivity and afterwards whole plant died. Plants under NaCl and $CaCl_2$ (both at 9 mmhos/cm) shed all their leaves by the end of June and by the end of July new buds sprouted thereby, meaning that plant has entered into quiescence because of shock at higher conductivity. Incipient chlorosis followed by burning of leaf margin was also noticed. Death of apical portion was seen prior to death of plant.

Sulphate injury

The symptoms were noticed at 9 mmhos/cm and 12 mmhos/cm conductivity. Loss of dark green colour was observed which was uniform on the leaf. Occasional yellow spots were also seen. Slight burning of tips and margin was noticed. Leaf burning spots were not uniform but in patches. Detachment of leaf was quick as compared to leaf under chloride salinization.

4. Growth parameters

4.1. Effect on main stem height and cumulative plant growth

All the salts, in general, reduced the main stem height and cumulative plant growth over control (Table 4).

The minimum stem height was observed under $CaCl_2$ and NaCl whereas maximum stem height was observed under control.

Table 4: Effect of different salts and their concentrations on main stem height and cumulative plant growth

Salt	Conductivity (mmhos/cm ECe)							
	Main stem height (cm)				Cumulative plant growth (cm)			
	6	9	12*	Mean	6	9	12*	Mean
NaCl	51.3	35.1	18.9	43.2	278.4	176.9	62.8	227.6
Na_2SO_4	60.2	41.2	23.4	50.7	321.4	215.6	66.9	268.5
$CaCl_2$	47.1	35.9	18.6	41.5	287.1	181.6	61.1	234.3
Mixture	55.5	39.4	19.9	47.5	296.6	196.2	62.3	246.8
Mean	53.5	37.9	20.2		295.9	192.8	63.3	
Control		79.1				442.5		

	C.D.at		C.D. at	
	1%	5%	1%	5%
Control Vs Treatment	3.7	2.8	191.4	141.2
Salt x Conductivity	N.S.	3.7	N.S.	N.S.
Conductivity	2.4	1.9	N.S.	94.1
Salt	3.5	2.6	N.S.	N.S.

*Values at 12 mmhos are not statistically calculated since all plants died after June,80.

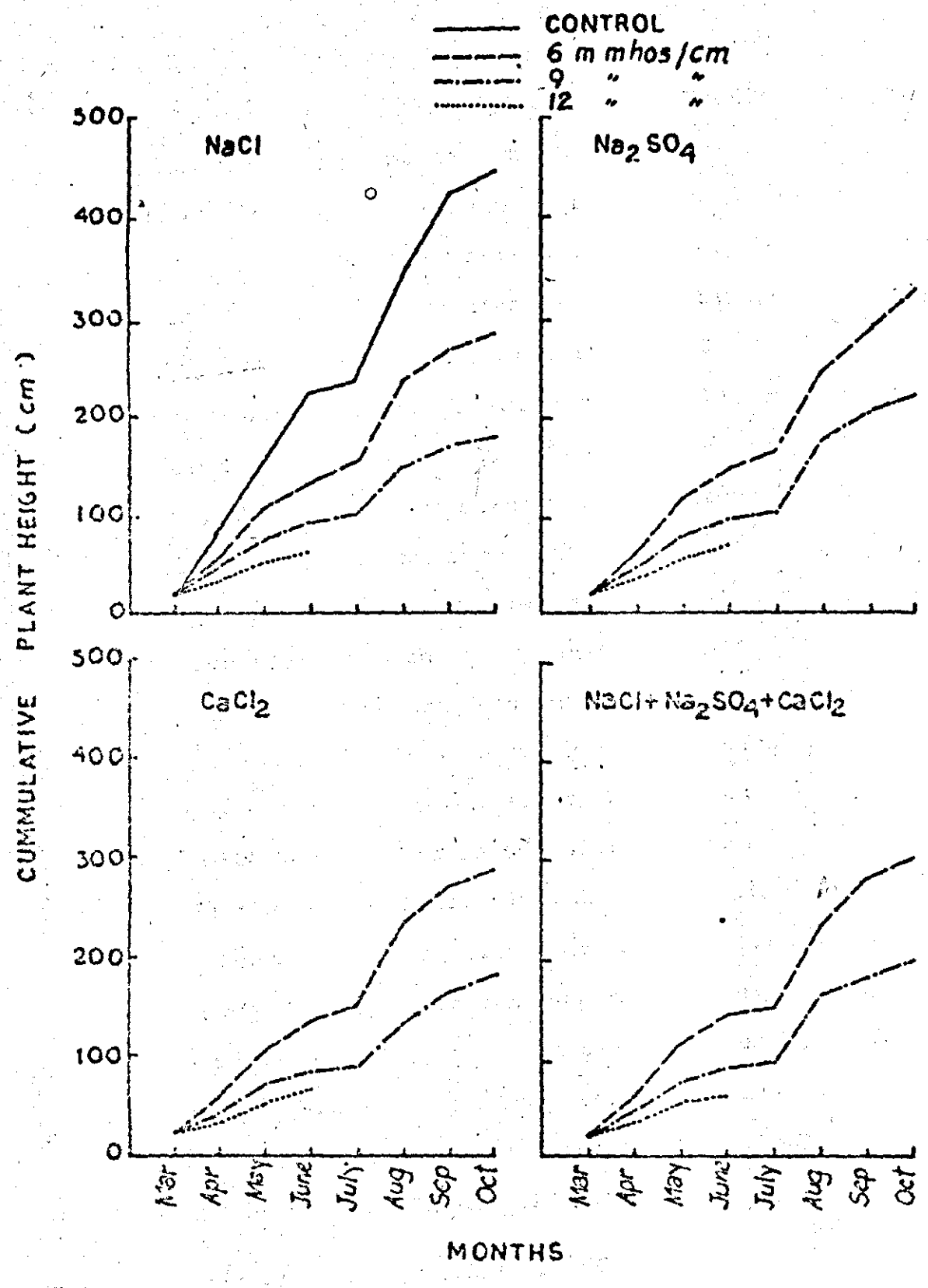

FIG. 1 EFFECT OF DIFFERENT SALINITY LEVELS ON CUMMULATIVE PLANT HEIGHT

Among the different salts, irrespective of conductivity, $CaCl_2$ had minimum plant height closely followed by NaCl. Both the salts had significantly lower stem height over the Na_2SO_4 and mixture of salts. Increase in conductivity significantly reduced the stem height. Interaction between salt and conductivity was significant. NaCl and $CaCl_2$ at 9 mmhos/cm were more suppressive than Na_2SO_4 at the same conductivity, with 35.1 , 35.9 and 41.2 cm, respectively. Moreover, $CaCl_2$, at 6 mmhos/cm was significantly more inhibitive than NaCl at same conductivity.

A perusal of data in Table 4 and Fig.1 reveal that cumulative plant growth was maximum (442.5 cm) in control while minimum was recorded under NaCl (227.6 cm). Among the different salts, there was no significant difference in the cumulative plant growth, however, Na_2SO_4 showed maximum plant height followed by mixture of salts, $CaCl_2$ and NaCl. Increase in conductivity significantly reduced the cumulative plant growth. However, interaction between conductivity and salts was not significant, yet maximum cumulative plant growth was observed under Na_2SO_4 at 6 mmhos/cm followed by mixture of salts, $CaCl_2$ and NaCl at the same conductivity. The minimum cumulative plant growth was recorded under NaCl at 9 mmhos/cm followed by $CaCl_2$, mixture of salts and Na_2SO_4.

4.2. Effect on stem diameter

Irrespective of conductivity, different salts reduced the stem diameter significantly (Table 5,Fig.2). Maximum

Table 5: Effect of different salts and their concentrations on stem diameter (cm)

Salt	Conductivity (mmhos/cm ECe)			
	6	9	12	Mean
NaCl	1.06	0.75	0.33	0.91
Na_2SO_4	1.20	1.08	0.36	1.14
$CaCl_2$	1.10	0.91	0.34	1.01
Mixture	1.15	1.03	0.36	1.09
Mean	1.13	0.94	0.35	
Control	1.50			

C.D. at 1%

Control VS Treatment	=	0.03
Salt x Conductivity	=	0.04
Salt	=	0.03
Conductivity	=	0.02

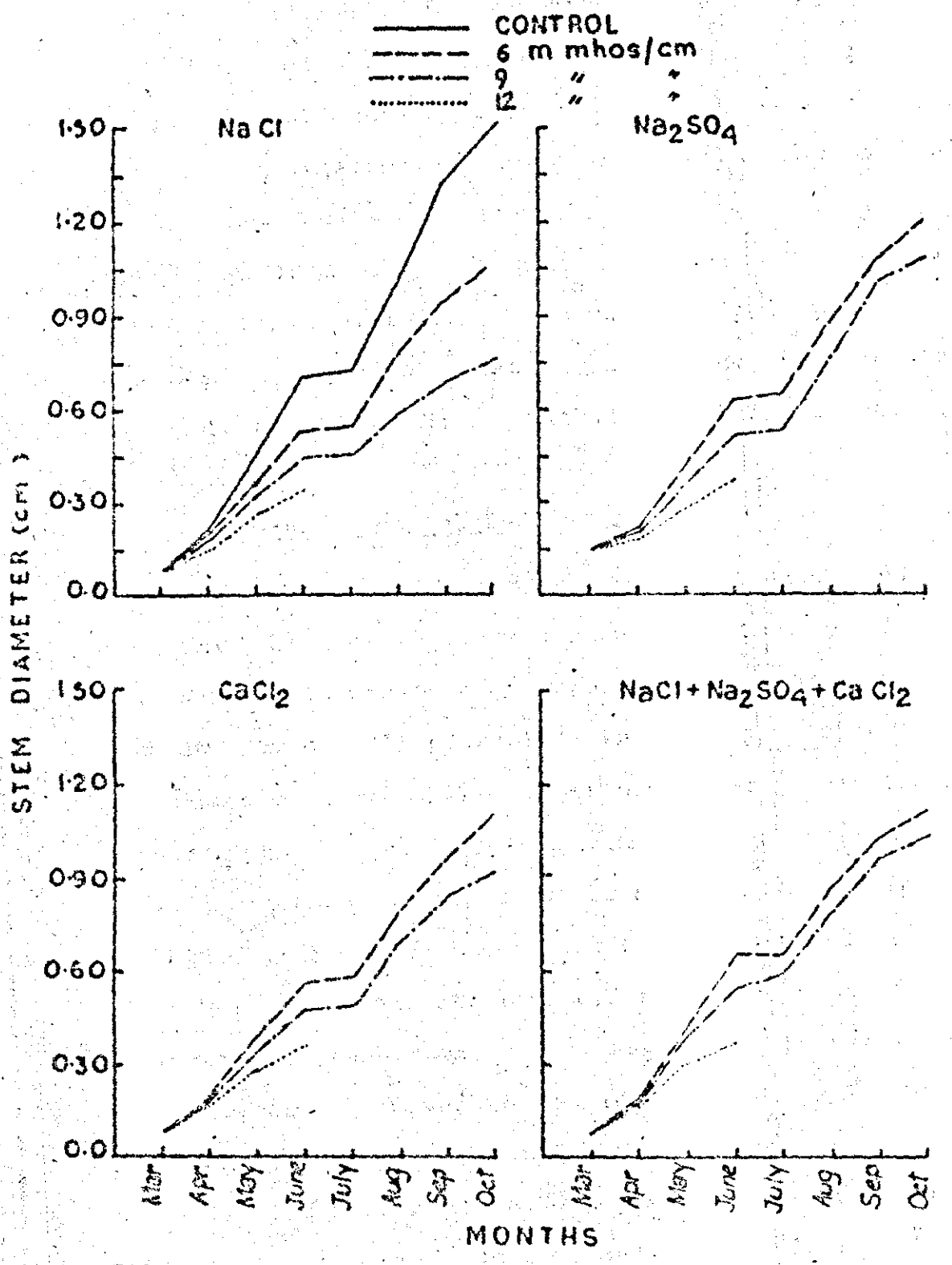

FIG. 2. EFFECT OF DIFFERENT SALINITY LEVELS ON STEM DIAMETER

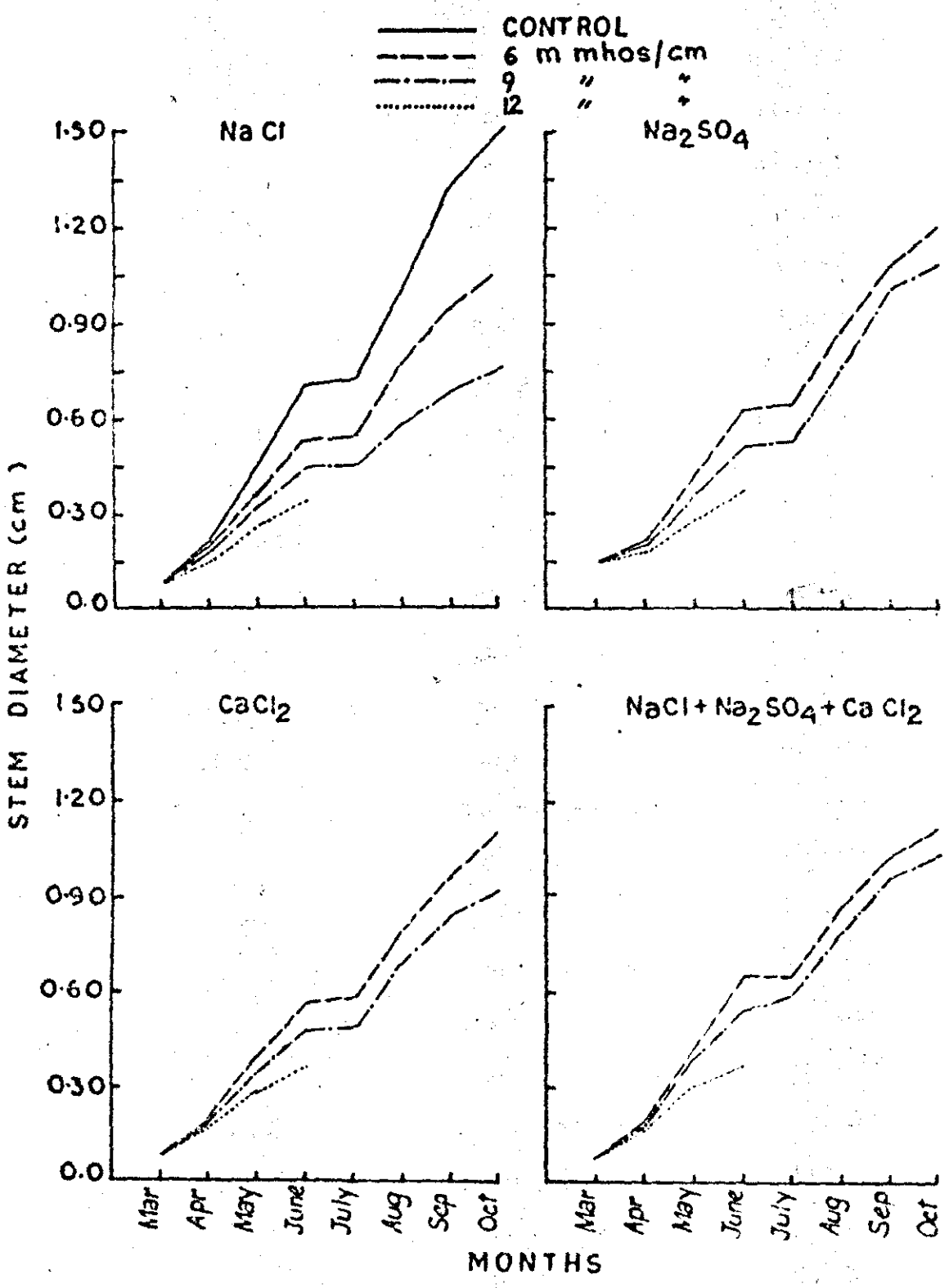

FIG. 2. EFFECT OF DIFFERENT SALINITY LEVELS ON STEM DIAMETER

stem diameter (1.50 cm) was recorded under control, whereas minimum stem diameter (0.75 cm) was recorded under NaCl. Increase in conductivity reduced the stem diameter, irrespective of salt. The interaction between salt and conductivity was significant. Sodium chloride at 6 mmhos/cm was more suppressive than NaCl at 9 mmhos. Maximum increase in stem diameter, under different salts was recorded under Na_2SO_4 (6 mmhos/cm) followed by same conductivity of mixture of salts, $CaCl_2$ and 9 mmhos/cm Na_2SO_4 with 1.20, 1.15, 1.10 and 1.08 cm, respectively.

4.3 Total number of leaves

The number of leaves present on each plant under different salinity levels was counted at the end of experiment and the data is presented in Table 6. The nature and conductivity of salt present in the soil significantly affected the number of leaves. The number of leaves ranged from 490 (control) to 174(NaCl, 6 mmhos/cm). Conductivity levels above 6 mmhos/cm, irrespective of salts used was significantly effective in reducing the number of leaves per cent. Moreover, there was a significant interaction between salt and conductivity as regards to number of leaves. Na_2SO_4 (6 mmhos/cm) had maximum (383) number of leaves as compared to other salts at the same conductivity. This was followed by mixture of salt, $CaCl_2$, NaCl(all at 6 mmhos/cm) Na_2SO_4, mixture of salt, $CaCl_2$ and NaCl(at 9 mmhos/cm) with 350, 307, 286, 260, 217, 182 and 174 leaves, respectively.

Table 6: Effect of different salts and their concentrations on total number of leaves per plant

Salt	Conductivity (mmhos/cm ECe)		
	6	9	Mean
NaCl	286 (16.90)*	174 (13.17)	230.0 (15.04)
Na_2SO_4	383 (19.58)	260 (16.12)	321.5 (17.85)
$CaCl_2$	307 (17.52)	182 (13.48)	244.5 (15.50)
Mixture	350 (18.70)	217 (14.73)	283.5 (16.72)
Mean	331.5 (18.18)	208.3 (14.38)	
Control		490 (23.13)	

C.D. at 1%

Control VS Treatment = 0.15
Salt x Conductivity = 0.20
Salt = 0.14
Conductivity = 0.10

*Values in parenthesis are square root transformation values.

4.4. Effect on leaf area

The different salinity levels significantly affected leaf area (Table 7). The leaf area was maximum (71.7 sq.cm) under control whereas in different salts it varied from 54.6 to 66.3 sq.cm/leaf. The leaf area was significantly reduced at the conductivity level of 9 mmhos/cm. The interaction between salt and conductivity was significant. The reduction in leaf area was lesser under Na_2SO_4 (9 mmhos/cm) as compared to NaCl (6 mmhos/cm). Among various salts, leaf area was minimum under mixture of salts (9 mmhos/cm) and maximum under Na_2SO_4 (6 mmhos/cm).

4.5. Fresh and dry weight of leaf

The data pertaining to fresh and dry weight of leaf are presented in Table 8.

The fresh weight of leaf significantly decreased under all salinity levels as compared to control. Among various salts, $CaCl_2$ had maximum (0.616 g) fresh weight, followed by NaCl, Na_2SO_4 and mixture of salts. Increase in conductivity had more deliterious effect on fresh weight. Interaction between salt and conductivity was significant. Chloride salinization at 6 mmhos/cm had more fresh weight as compared to sulphate salinization(6 mmhos/cm) but reverse was true with increase in conductivity.

Contrary to fresh weight, dry weight was significantly higher under Na_2SO_4 treatment as compared to mixture of salt and chloride salinization. However, all the salts significantly

Table 7: Effect of different salts and their concentrations on leaf area (sq.cm./leaf)

SALT	Conductivity (mmhos/cm ECe)		
	6	9	Mean
NaCl	61.6	57.6	59.6
Na_2SO_4	66.3	63.2	64.7
$CaCl_2$	64.8	59.4	62.1
Mixture	62.6	54.6	58.6
Mean	63.8	58.7	
Control	71.7		

C.D.at 1%

Control VS Tretment = 0.8

Salt x Conductivity = 1.0

Salt = 0.7

Conductivity = 0.5

Table 8: Effect of different salts and their concentrations on fresh weight, dry weight and fresh weight/dry weight of leaf

Salt	Conductivity (mmhos/cm ECe)								
	Fresh weight of leaf (g)			Dry weight of leaf (g)			Fresh wt./dry wt. ratio		
	6	9	Mean	6	9	Mean	6	9	Mean
NaCl	0.614	0.576	0.595	0.152	0.135	0.144	4.04	4.27	4.16
Na_2SO_4	0.592	0.586	0.589	0.166	0.152	0.159	3.57	3.85	3.71
$CaCl_2$	0.645	0.586	0.616	0.166	0.135	0.151	3.89	4.35	4.12
Mixture	0.580	0.557	0.569	0.144	0.126	0.135	4.03	4.43	4.23
Mean	0.608	0.576		0.157	0.137		3.88	4.23	
Control	0.662			0.197			3.36		

	C.D. at		C.D. at 1%	C.D.at	
	1%	5%		1%	5%
Control VS Treatment	0.04	0.03	0.004	0.12	0.09
Salt x Conductivity	N.S.	0.04	0.006	0.16	0.12
Conductivity	0.03	0.02	0.003	0.08	0.06
Salt	N.S.	0.03	0.004	0.12	0.09

reduced the dry weight of leaf as compared to control. The dry weight varied from 0.197 to 0.126 g. Increase in conductivity also significantly reduced the dry weight content of leaf. Na_2SO_4 and $CaCl_2$ at 6 mmhos/cm showed same amount of dry weight, whereas, $CaCl_2$ and $NaCl_2$ at 9 mmhos/cm had equal dry matter accumulation on per leaf basis.

On the basis of fresh weight/dry weight ratio, Na_2SO_4 was noticed to be less deliterious in reducing dry weight as compared to mixture of salts and chloride salinization.

4.6. Fresh and dry weight of stem

The perusal of the data given in Table 9 would indicate that fresh weight as well as dry weight of stem was significantly more under control in comparison to different salinity levels. However, Na_2SO_4 had more fresh weight as compared to other salts.

The fresh weight of stem varied from 512.9 g (control) to 198.8 g (NaCl, 9 mmhos/cm). Increase in conductivity significantly reduced the fresh weight of stem. Na_2SO_4 at 6 mmhos/cm showed significant increase in stem weight (378.4 g) as compared to other salinity treatments.

Similarly, in case of stem dry weight, control significantly had more dry matter as compared to other salinity treatments. NaCl at 9 mmhos/cm significantly reduced the dry matter accumulation in stem as compared to other salts. Contrary to fresh weight, $CaCl_2$ at 6 mmhos/cm had more dry weight than mixture of salts, however, the difference was not significant.

Table 9: Effect of different salts and their concentrations on fresh weight, dry weight and fresh weight / dry weight ratio of stem

Salt	Conductivity (mmhos/cm ECe)								
	Fresh weight of stem (g)			Dry weight of stem (g)			Fresh weight/dry weight ratio		
	6	9	Mean	6	9	Mean	6	9	Mean
NaCl	280.8	198.8	239.8	64.5	48.4	56.4	4.36	4.11	4.24
Na_2SO_4	378.4	254.4	316.4	104.6	78.3	91.4	3.62	3.25	3.44
$CaCl_2$	313.9	222.3	268.1	84.8	58.0	71.4	3.70	3.84	3.77
Mixture	341.8	233.9	287.9	82.5	66.0	74.2	4.14	3.55	3.85
Mean	328.7	227.4		84.1	62.6		3.96	3.69	
Control	512.9			123.5			4.15		

	C.D.at 1%	C.D.at 1%	C.D.at 1% & 5%
Control VS Treatment	9.4	4.6	N.S.
Salt x Conductivity	12.5	6.1	N.S.
Conductivity	6.3	3.0	N.S.
Salt	8.9	4.3	N.S.

Among the different salinity treatments, Na_2SO_4 had the highest dry matter content at both the conductivity levels whereas other salts had comparatively lower dry matter. However, at 6 mmhos/cm $CaCl_2$ and mixture of salt behaved similarly but at 9 mmhos/cm there was variation in dry matter content between these two treatments. Statistically there was no significant difference in dry matter content of stem under Na_2SO_4 at 9 mmhos/cm and mixture of salt at 6 mmhos/cm conductivity.

Contrary to fresh weight and dry weight, the increase in conductivity did not reduce the fresh weight to dry weight ratio. Higher fresh weight/dry weight ratio (4.36) under NaCl at 6 mmhos/cm was closely followed by control, $CaCl_2$ mixture of salts (both at 6 mmhos/cm) and NaCl at 9 mmhos/cm.

4.7. Fresh weight and dry weight of roots

The data presented in Table 10 show that both, fresh and dry weight were significantly decreased under different salinity levels.

Control with 186.42 g fresh weight was followed by 6 mmhos/cm Na_2SO_4 and $CaCl_2$ with 138.25 g and 123.90 g fresh weight of root, respectively. NaCl was significantly more injurious as compared to other salts. Higher conductivity of Na_2SO_4 was less deterious as compared to NaCl and mixture of salt.

The dry weight, among the different salts, was significantly higher under Na_2SO_4 salinization while reverse was true under NaCl. Further more, higher conductivity had significant effect on dry weight reduction. The interaction between salt and

Table 10: Effectof different salts and their concentrations on fresh weight, dry weight and fresh weight/dry weight ratio of root

Salt	Conductivity (mmhos/cm ECe)								
	Fresh weight of root (g)			Dry weight of root (g)			Fresh weight/dry weight ratio		
	6	9	Mean	6	9	Mean	6	9	Mean
NaCl	107.19	88.35	99.77	24.42	17.77	21.10	4.39	4.98	4.69
Na_2SO_4	138.25	117.97	128.11	34.56	29.49	32.03	4.00	4.00	4.00
$CaCl_2$	123.90	97.58	110.74	28.61	22.08	25.34	4.33	4.44	4.39
Mixture	117.67	94.36	106.01	29.94	23.16	26.55	3.94	4.09	4.01
Mean	121.75	99.56		29.38	23.13		4.17	4.38	
Control	186.42			45.00			4.15		

	C.D.at 1%	C.D.at 1%	C.D.at 1%
Control VS Treatment	3.09	1.63	0.17
Salt x Conductivity	4.11	2.17	0.23
Conductivity	2.06	1.09	0.12
Salt	2.91	1.54	0.16

conductivity was significant, but Na_2SO_4 (9 mmhos/cm), mixture of salts and CaCl(6 mmhos/cm) were not significantly different, when compared with each other.

Contrary to fresh and dry weight, there was no significant difference in fresh weight/dry weight ratio under Na_2SO_4 and $CaCl_2$ at two conductivity levels, however, the differences were significant when compared with each other.

4.8. Length of primary and secondary root

The length of primary and secondary roots under different salinity levels was measured and the data is presented in Table 11.

The nature and conductivity of salt greatly affected the primary root growth. The maximum root length (36.9 cm) was observed under control. However, $CaCl_2$ recorded significantly more primary root length (25.6 cm) among different salinity levels. NaCl showed significant reduction in primary root length (19.3 cm). Increase in conductivity significantly suppressed the root length. Except Na_2SO_4 and mixture of salt at 9 mmhos/cm, the interaction between salt and conductivity was significant. The length of secondary root among various salts varied from 38.7 cm($CaCl_2$, 6 mmhos/cm) to 21.2 cm (NaCl, 9 mmhos/cm). However, among salts, Na_2SO_4 and $CaCl_2$ were almost equally effective in reducing secondary root length. The highest (54.3 cm) secondary root length under control was followed by $CaCl_2$, mixture of salts and Na_2SO_4 (all at 6 mmhos/cm) with 38.7 cm, 36.6 cm and 35.2 cm, respectively. The interaction between salt and conductivity was significant

Table 11: Effect of different salts and their concentrations on the length of primary and secondary root

Salt	Conductivity (mmhos/cm ECe)							
	Length of primary root (cm)				Length of secondary root (cm)			
	6	9	12	Mean	6	9	12	Mean
NaCl	24.5	21.2	11.4	19.3	29.3	21.2	9.3	19.9
Na_2SO_4	30.0	24.0	13.8	22.6	35.2	28.2	13.4	25.6
$CaCl_2$	34.6	26.3	15.8	25.6	38.7	29.2	12.5	26.8
Mixture	31.4	24.0	15.7	23.7	36.6	31.8	14.4	27.6
Mean	30.4	28.4	14.2		34.9	27.6	12.4	
Control	36.9				54.3			

	C.D.at 1%	C.D.at 1%
Control VS Treatment	1.6	2.2
Salt x Conductivity	2.2	3.0
Conductivity	1.1	1.5
Salt	1.3	1.8

Table 12: Effect of different salts and their concentrations on rate of respiration (O_2 uptake in µl/sq. cm./hour)

Salt	Conductivity (mmhos/cm ECe)						
	At 90 days after transplanting				At 210 days after transplanting		
	6	9	12	Mean	6	9	Mean
NaCl	12.93	13.25	13.85	13.34	11.76	12.56	12.16
Na_2SO_4	9.86	10.47	12.78	11.03	8.47	10.55	9.51
$CaCl_2$	12.59	13.46	13.80	13.28	11.28	12.56	11.92
Mixture	10.27	11.74	13.29	11.76	9.50	10.92	10.21
Mean	11.41	12.23	13.43		10.25	11.65	
Control	9.15				8.34		

	C.D.at 1%	C.D.at 1%
Control VS Treatment	0.10	0.08
Salt x Conductivity	0.13	0.11
Conductivity	0.07	0.05
Salt	0.08	0.08

Table 13: Effect of different salts and their concentrations on relative water content (%) of leaf

Salt	Conductivity (mmhos/cm ECe)						
	At 90 days after transplanting				At 210 days after transplanting		
	6	9	12	Mean	6	9	Mean
NaCl	85.2	86.0	84.9	85.4	91.8	92.7	92.3
Na_2SO_4	82.9	82.2	80.2	81.8	89.9	88.6	89.2
$CaCl_2$	84.6	85.8	84.8	85.1	91.7	92.1	91.9
Mixture	84.0	83.1	81.2	82.7	90.2	89.7	90.0
Mean	84.17	84.3	82.9		90.9	90.8	
Control	83.6				90.3		

	C.D.at 1%	C.D.at 1%
Control VS Treatment	0.09	0.09
Salt x Conductivity	0.14	0.12
Conductivity	0.08	0.06
Salt	0.08	0.08

salts. The increase in conductivity significantly increased the RWC under chloride salinization, but at 12 mmhos RWC was significantly lowered. Contrary to this, Na_2SO_4 and mixture of salts reduced the RWC with the increase in conductivity. Under different salinity levels, highest RWC (86.0%) was observed under NaCl (6 mmhos/cm) and the lowest RWC(80.2%) under Na_2SO_4 (12 mmhos/cm). Similar trend was observed at 210 days after transplanting. However, RWC after 210 days of transplanting was more than that at 90 days after transplanting under all treatments.

5.3 Leaf water potential

Water potential of the leaves declined with increasing conductivity levels of all the salts at different sampling dates (Fig.3) Appendix III). The reduction in leaf water potential with varying levels of NaCl, Na_2SO_4, $CaCl_2$ and mixture of salts was comparable. There was a reduction of -5 to -10 bars in leaf water potential under all treatments at 12 mmhos/cm conductivity at 110 days after transplanting. However, differences were reduced to -1 to -2 bars at the final date of sampling. Na_2SO_4 at 6 and 9 mmhos/cm conductivity showed lower leaf water potential as compared to NaCl at same conductivities. On last sampling day, control and NaCl at 6 mmhos/cm leaves had almost equal (-12.34 and -12.67, respectively) water potential whereas the sodium sulphate at the same conductivity had -14.83 bars. The maximum ρ - 15.67 bars water potential was observed under Na_2SO_4 at 9 mmhos/cm at 190 days after transplanting.

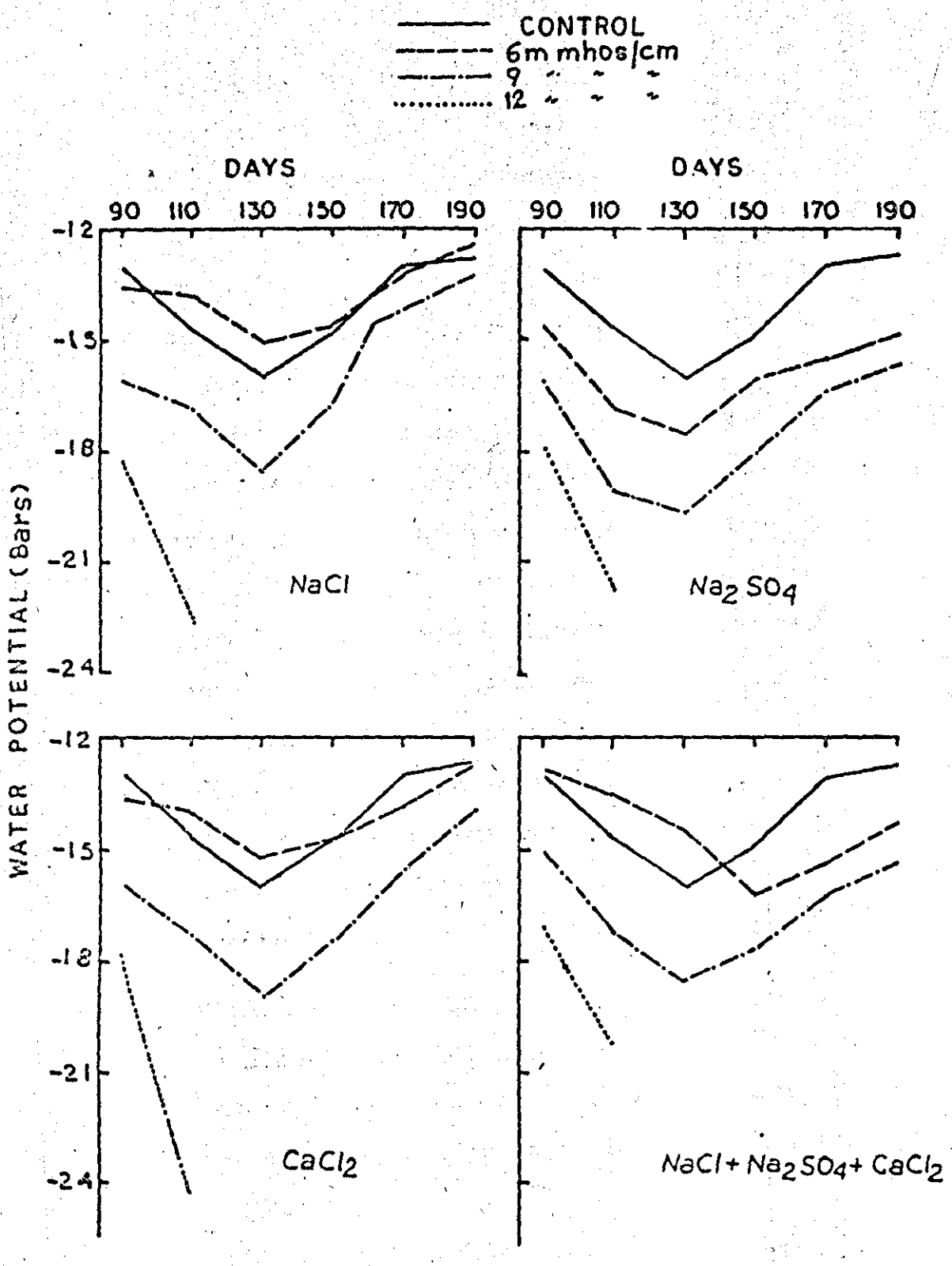

FIG. 3 EFFECT OF DIFFERENT SALINITY LEVELS ON LEAF WATER POTENTIAL

5.4. Diffusive resistance of leaf

The data regarding diffusive resistance of adaxial and abaxial leaf surfaces are presented in Fig. 4a and 4b and Appendix IV. A perusal of data reveals that there was an increase in lower and upper leaf surfaces with the increase in salinity levels, irrespective of type of salt. However, diffusive resistance of upper leaf surface showed a consistent decrease with advanced sampling dates. On last sampling date $CaCl_2$ at 9mmhos/cm conductivity showed maximum (3.58 sec/cm) diffusive resistance which was closely followed by NaCl (3.51 sec/cm) at the same conductivity.

Diffusive resistance of lower leaf surface, unlike the upper surface, showed an increase with the time in control plants as well as in all the salts. At the final sampling date, greater resistance with chloride salinity at higher conductivity levels was recorded as compared to sulphate salinity. Diffusive resistance of lower surface was higher than that of upper surface, both in control as well as in different salts.

6. Biochemical estimates

6.1. Chlorophyll contents

Chlorophyll content of fully expanded leaf decreased significantly under different salinity levels, as compared to control (Table 14).

Significantly high contents of chlorophyll 'a', chlorophyll 'b' and total chlorophyll were recorded under NaCl salinization as compared to other salinity treatments. However, highest

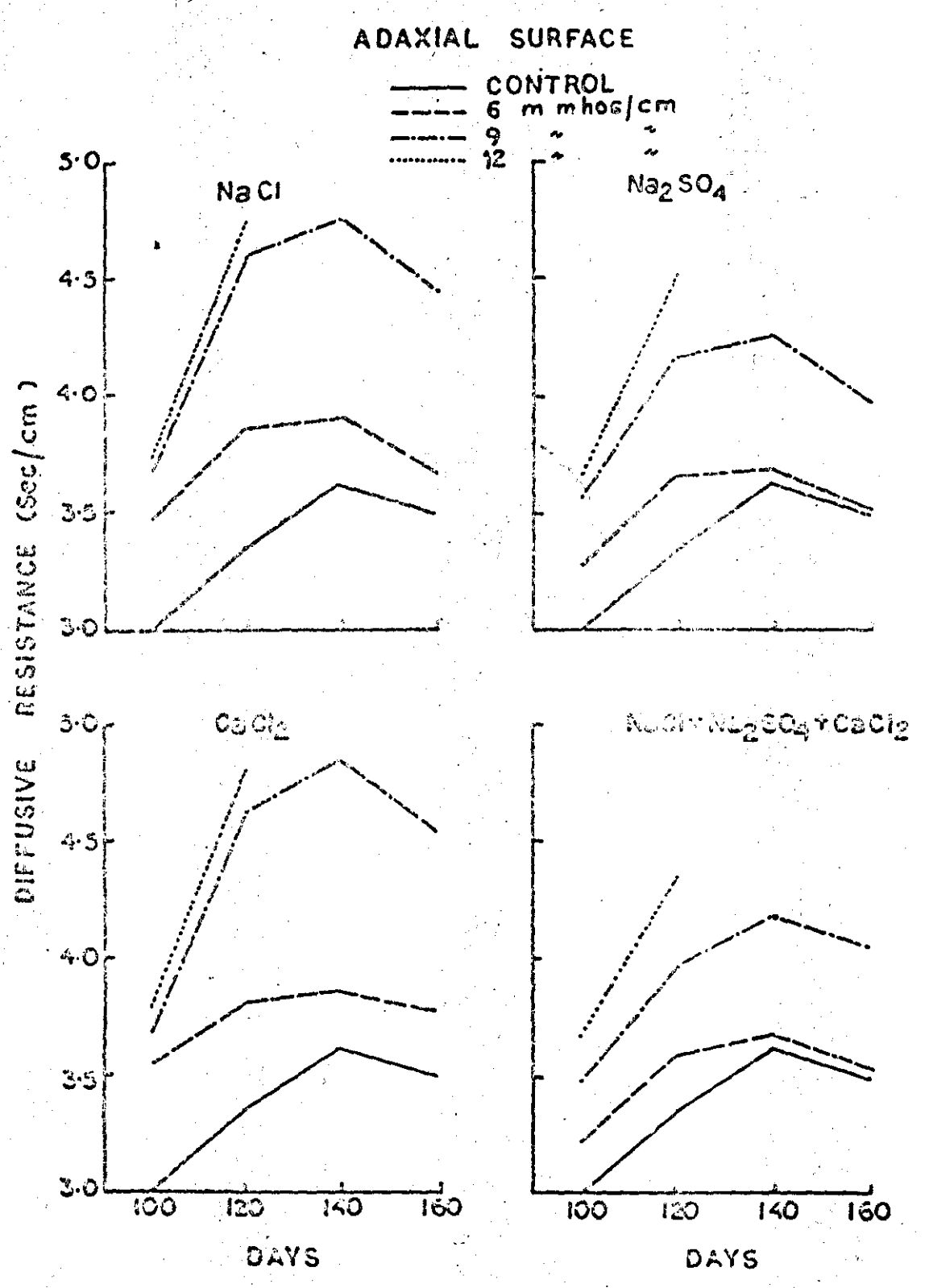

FIG. 4D. EFFECT OF DIFFERENT SALINITY LEVELS ON LEAF DIFFUSIVE RESISTANCE

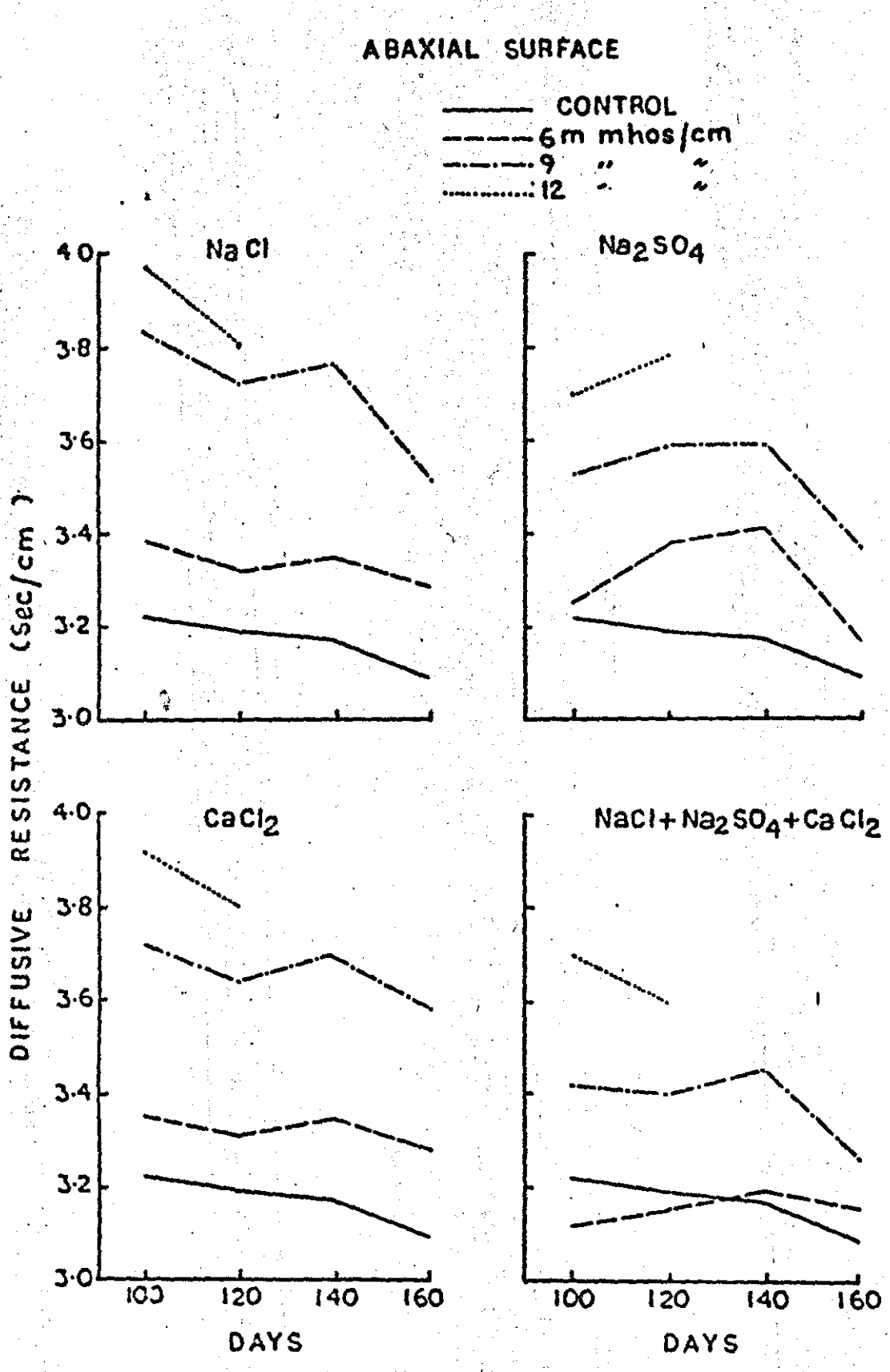

FIG.4b. EFFECT OF DIFFERENT SALINITY LEVELS ON LEAF DIFFUSIVE RESISTANCE

Table 14: Effect of different salts and their concentrations on chlorophyll content (mg/g fresh weight)

Salt	Conductivity (mmhos/cm ECe)								
	Chlorophyll 'a'			Chlorophyll 'b'			Total chlorophyll		
	6	9	Mean	6	9	Mean	6	9	Mean
NaCl	1.693	1.550	1.622	0.735	0.668	0.702	2.43	2.22	2.32
Na_2SO_4	1.583	1.548	1.566	0.673	0.654	0.664	2.25	2.20	2.23
$CaCl_2$	1.590	1.468	1.529	0.704	0.624	0.664	2.30	2.09	2.19
Mixture	1.561	1.547	1.554	0.676	0.669	0.673	2.24	2.22	2.23
Mean	1.607	1.528		0.697	0.654		2.30	2.18	
Control	1.971			0.933			2.91		

	C.D.at 1%	C.D. at 1%	C.D.at 1%
Control VS Treatment	0.007	0.009	0.03
Salt x Conductivity	0.010	0.012	0.04
Conductivity	0.005	0.006	0.02
Salt	0.007	0008	0.03

content of chlorophyll was obtained under control. Chlorophyll 'b' under Na_2SO_4 and $CaCl_2$ types of salinity were same (i.e. 0.664 mg/g). Chlorophyll content was inversely proportional to conductivity. Chlorophyll 'a', chlorophyll 'b' and total chlorophyll content were significantly high under $CaCl_2$ (6 mmhos/cm) as compared to Na_2SO_4 at same conductivity. $CaCl_2$ (9 mmhos/cm) showed minimum (2.09 mg/g) and NaCl (6 mmhos/cm) had maximum (2.43 mg/g) total chlorophyll contents.

6.2. Free proline content

Proline content significantly increased under different salinity levels at 90 days as well as 210 days after transplanting, as compared to control (Table 15).

At 90days after transplanting, the maximum proline content (187.1 µg/g)was under NaCl (12 mmhos/cm) while minimum (56.9 µg/g) was recorded under Na_2SO_4 (6 mmhos/cm).There was a significant increase in proline under different salinity levels, except under Na_2SO_4 at 6 and 9 mmhos/cm, as compared to control. Irrespective of salt; proline content increased with the increase in conductivity. The interaction between salt and conductivity was significant. NaCl at 6 mmhos/cm showed more proline content (90.2 µg/g) than Na_2SO_4 at 12 mmhos/cm (87.8 µg/g). However, the differences between the values were not statistically significant. Mixture of salts (12 mmhos/cm) showed significant decrease in proline content as compared to individual salts of chloride salinization at 9 mmhos/cm.

Similar trend was observed even at 210 days after transplanting. There was a general increase in proline content

Table 15: Effect of different salts and their concentrations on free proline content (ug/g fresh weight)

Salt	Conductivity (mmhos/cm ECe)						
	At 90 days after transplanting				At 210 days after transplanting		
	6	9	12	Mean	6	9	Mean
NaCl	90.2	172.3	187.1	149.9	135.2	155.7	145.4
Na_2SO_4	56.9	68.1	87.8	70.9	76.2	87.2	81.7
$CaCl_2$	84.1	141.0	159.6	128.2	129.2	159.2	144.2
Mixture	78.6	110.7	129.7	106.3	112.5	131.6	122.0
Mean	77.4	123.0	141.1		113.3	133.4	
Control	74.0				80.8		

	C.D. at 1%	C.D.at 1%
Control VS Treatment	4.9	7.6
Salt x Conductivity	5.8	10.2
Conductivity	2.9	5.1
Salt	3.1	7.2

under different salinity levels except under NaCl salinization. Moreover, at 210 days the proline content was more under Na_2SO_4 (9 mmhos/cm) salinity as compared to control.

6.3 Free amino acids

The perusal of data presented in Table 16 clearly indicates that all salinity levels significantly increased free amino acids (mg/g fresh weight) as compared to control.

Out of different salts, mixture of salts showed significant increased in free amino acid content followed by $CaCl_2$, NaCl and Na_2SO_4. Increase in free amino acid content was linear to conductivity. However, NaCl (9 mmhos/cm) showed maximum (1.51 mg/g) free amino acid content as compared to $CaCl_2$ and other salts at same conductivity. Interestingly, NaCl at 6 mmhos showed minimum increase (1.19 mg/g) in free amino acids as compared to other salinity levels. Reverse was true with Na_2SO_4 i.e. significant increase in free amino acids was observed under Na_2SO_4 at 6 mmhos/cm than its 9 mmhos/cm conductivity.

6.4. Total protein: Significant decrease in protein content (mg/g fresh weight) was recorded under different salinity as compared to control (Table 17).

The minimum protein content (20.5 mg/g) was recorded under NaCl (9 mmhos/cm). While comparatively higher (30.1 mg/g) content was observed under Na_2SO_4 (6 mmhos/cm) out of different salinity treatments. NaCl significantly reduced the protein followed by $CaCl_2$, mixture of salts and Na_2SO_4. Increase in

Table 16: Effect of different salts and their concentrations on free amino-acids (mg/g fresh wt.)

Salt	Conductivity (mmhos/cm ECe)		
	6	9	Mean
NaCl	1.19	1.51	1.35
Na_2SO_4	1.33	1.26	1.30
$CaCl_2$	1.31	1.45	1.38
Mixture	1.38	1.43	1.41
Mean	1.30	1.41	
Control	0.96		

C.D.at 1%

Control VS Treatment	=	0.04
Salt x Conductivity	=	0.05
Conductivity	=	0.02
Salt	=	0.03

Table 17: Effect of different salts and their concentrations on total insoluble proteins (mg/g fresh wt.)

Salt	Conductivity (mmhos/cm ECe)		
	6	9	Mean
NaCl	25.2	20.5	22.8
Na_2SO_4	30.1	24.8	27.4
$CaCl_2$	25.7	22.5	24.1
Mixture	26.9	21.6	24.3
Mean	27.0	22.4	
Control	31.9		

C.D. at 1%

Control VS Treatment	=	1.3
Salt x Conductivity	=	1.7
Conductivity	=	0.9
Salt	=	1.2

conductivity significantly reduced the protein content, irrespective of salt used. Although, the interaction between salt and conductivity was significant yet, NaCl and $CaCl_2$, each at 6 mmhos/cm had practically thesame protein content. The difference in the protein content under Na_2SO_4 (9 mmhos/cm) and NaCl (6 mmhos/cm) was not significant.

6.5. Organic acid content

The data pertaining to organic acid content (meq of NaOH/ 100 g) is presented in Table 18.

A significant increase over control was recorded under different salinity levels. The maximum increase in organic acid content (4.99 meq NaOH/100 g) was observed under NaCl (9 mmhos/cm) and lower content (4.59 meq NaOH/ 100 g) under Na_2SO_4 (6 mmhos/cm). NaCl significantly increased the organic acid content which was followed by $CaCl_2$, mixture of salts and Na_2SO_4, respectively. The increase in organic acid content was observed with the increase in conductivity. The interaction between the salt and conductivity significantly affected the organic acid content. Na_2SO_4 at 9 mmhos/cm had the same organic acid content as that found under mixture at 6mmhos/cm. However, NaCl at 6 mmhos/cm significantly increased organic acid content as compared to Na_2SO_4 at 9 mmhos/cm.

6.6. Enzymatic studies

6.6.1. Peroxidase activity: The data with respect to effect of different salinity levels on the peroxidase activity (enzyme unit/g fresh weight) at 85 days and 205 days after transplanting were recorded and is presented in Table 19.

Table 18: Effect of different salts and their concentrations on organic acids content (meq of NaOH/ 100 g fresh weight)

Salt	Conductivity (mmhos/cm ECe)		
	6	9	Mean
NaCl	4.83	4.99	4.91
Na_2SO_4	4.59	4.66	4.63
$CaCl_2$	4.75	4.91	4.83
Mixture	4.66	4.72	4.69
Mean	4.71	4.82	
Control	4.44		

	C.D. at 1%
Control VS Treatment =	0.04
Salt x Conductivity =	0.05
Conductivity =	0.02
Salt =	0.03

Table 19: Effect of different salts and their concentrations on peroxidase activity (Enzyme units/g fresh wt.)

Salt	Conductivity (mmhos/cm ECe)						
	At 85 days after transplanting				At 205 days after transplanting		
	6	9	12	Mean	6	9	Mean
NaCl	942.7	1161.7	1228.7	1111.0	674.7	729.3	702.0
Na_2SO_4	811.3	1081.3	1313.3	1068.7	586.0	636.0	611.0
$CaCl_2$	890.7	1174.3	1382.0	1149.0	707.0	752.7	729.8
Mixture	838.3	1072.7	1280.3	1063.8	584.0	691.0	637.5
Mean	870.8	1122.5	1301.1		637.9	702.3	
Control	586.7				547.7		

	C.D.at 1%	C.D.at 1%
Control VS Treatment	8.8	7.4
Salt x Conductivity	12.0	9.8
Conductivity	6.0	4.9
Salt	6.9	6.9

An overall significant increase under all salinity levels at 85 days as well as at 205 days after transplanting, was recorded as compared to control. Among the various salinity levels, highest peroxidase activity (1382 E.U/g) was recorded under $CaCl_2$ (12 mmhos) while lower activity (811.3 enzyme unit/g) was observed under Na_2SO_4 (6 mmhos/cm). The control recorded lowest (586.7 enzyme unit/g) peroxidase activity as compared to all salinity levels. Among various salts, plants under $CaCl_2$ treatment showed maximum peroxidase activity followed by NaCl, Na_2SO_4 and mixture of salts. Furthermore, increase in conductivity increased the peroxidase activity.

Similar results were recorded at 205 days after transplanting. However, the overall activity of peroxidase was reduced under all salinity treatments as compared to activity observed at 85 days after transplanting.

6.6.2. Amylase activity: The amylase activity was significantly increased under all salinity levels at 85 days and 210 days after transplanting as is obvious from Table 20.

All the treatments significantly increased amylase activity over control. The maximum amylase activity (577 enzyme unit/g) was recorded under NaCl at 9 mmhos/cm while the minimum (305.7 enzyme unit/g) was recorded under control. Increase in conductivity upto 9 mmhos significantly increased enzyme activity. At 12 mmhos/cm conductivity of NaCl the enzyme activity was reduced significantly as compared to 9 mmhos/cm NaCl treatment. However, it was still more than under NaCl at

Table 20: Effect of different salts and their concentrations on α-Amylase activity (Enzyme units/g fresh wt.)

Salt	Conductivity (mmhos/cm ECe)						
	At 85 days after transplanting				At 205 days after transplanting		
	6	9	12	Mean	6	9	Mean
NaCl	480.3	577.0	549.7	535.7	355.3	414.7	385.0
Na_2SO_4	335.3	319.3	293.3	316.0	263.0	307.0	285.0
$CaCl_2$	457.3	531.7	538.3	509.1	333.7	382.7	358.2
Mixture	376.0	425.0	444.0	415.0	293.3	320.0	306.7
Mean	412.3	463.3	456.3		311.3	356.1	
Control	305.7				289.3		

	C.D.at 1%	C.D. at 1%
Control VS Treatment	7.5	7.8
Salt x Conductivity	10.2	10.4
Conductivity	5.1	5.2
Salt	5.9	7.3

6 mmhos/cm. The chloride type of salinization showed higher activity of amylase under NaCl at 6 mmhos/cm than under Na_2SO_4 at 6,9 and 12 mmhos/cm.

Similar trend was observed at 205 days after transplanting. However, the activity, in general, was reduced.

6.6.3 RNA and DNA content at 95 days after transplanting: The data presented in Table 21 clearly reveal that both RNA and DNA contents wers significantly reduced under all treatments as compared to control.

Among the different salts, NaCl significantly affected RNA content followed by mixture , $CaCl_2$ and Na_2SO_4. Content of RNA significantly decreased with the increase in conductivity. Lowest RNA content (0.88 mg/g) was recorded under NaCl (12 mmhos/cm) while highest RNA content was found under Na_2SO_4 (6 mmhos/cm) However, there was no significant difference in RNA content under Na_2SO_4 and $CaCl_2$, each at 6 mmhos/cm, with 1.76 mg/g and 1.71 mg/g respectively.

Contrary to RNA, DNA content was more reduced under Na_2SO_4 salinization followed by NaCl, mixture of salt and $CaCl_2$. Higher conductivity levels had significantly lower DNA content. Among different salinity levels NaCl (6 mmhos/cm) observed maximum (0.300 mg/g) DNA content while minimum DNA content (0.207 mg/g) was also obtained under same salt at 12 mmhos/cm.

The data regarding RNA, DNA ratio revealed that this ratio was significantly reduced under NaCl followed by $CaCl_2$, mixture of salt and Na_2SO_4. Chloride type of salts were more deliterous than sulphate salinization.

Table 21: Effect of different salts and their concentrations on RNA, DNA and RNA/DNA ratio after 95 days of transplanting

Salt	Conductivity (mmhos/cm ECe)											
	RNA content (mg/g fresh wt.)				DNA content (mg/g fresh wt.)				RNA/ DNA ratio			
	6	9	12	Mean	6	9	12	Mean	6	9	12	Mean
NaCl	1.57	1.28	0.88	1.24	0.300	0.278	0.207	0.262	5.24	4.60	4.26	4.70
Na_2SO_4	1.76	1.42	1.03	1.40	0.285	0.254	0.225	0.255	6.20	5.59	4.56	5.45
$CaCl_2$	1.71	1.37	0.98	1.35	0.294	0.276	0.234	0.268	5.82	4.97	4.20	5.00
Mixture	1.67	1.38	0.96	1.34	0.289	0.266	0.234	0.263	5.79	5.18	5.09	5.35
Mean	1.68	1.36	0.96		0.292	0.269	0.225		5.76	5.08	4.53	
Control	1.98				0.306				6.48			

	C.D.at		C.D.at		C.D.at	
	1%	5%	1%	5%	1%	5%
Control VS Treatment	0.09	0.07	0.015	0.011	0.41	0.3
Salt x Conductivity	N.S.	0.09	0.020	0.015	0.28	0.21
Conductivity	0.06	0.05	0.010	0.008	0.32	0.24
Salt	0.07	0.05	0.012	0.009	N.S.	0.41

6.6.4. RNA and DNA content at 215 days after transplanting

Under all salinity levels of RNA content (mg/g fresh weight) decreased significantly whereas there was no significant difference in RNA content (Table 22).

Among various salts, mixture of salt significantly reduced the RNA content followed by NaCl, $CaCl_2$ and Na_2SO_4. However, the differences in NaCl, $CaCl_2$ and Na_2SO_4 salinization were not significant. The lowest RNA content (1.69 mg/g) was observed under mixture at 9 mmhos/cm. While highest (2.16 mg/g) RNA content was recorded under Na_2SO_4 and $CaCl_2$, each at 6 mmhos/cm.

There was no significant difference in the DNA content under different salinity levels as compared to control. However, 0.357 mg/g DNA was recorded under control followed by $CaCl_2$, NaCl, Na_2SO_4 and mixture of salt.

Among different salts, the Na_2SO_4 showed significantly higher RNA/DNA ratio followed by $CaCl_2$, NaCl and mixture of salt. Increase in conductivity significantly decreased the RNA/DNA ratio. There was no significant difference in RNA/DNA ratio at 9 mmhos/cm and 6 mmhos/cm of Na_2SO_4 and NaCl salinization, respectively.

7. Mineral composition

7.1 Nitrogen

The data concerning per cent nitrogen in leaves, presented in Table 23, clearly reveals that salinity significantly increased nitrogen content over control. Different salts behaved differently regarding nitrogen content of leaves. Increase in

Table 22: Effect of different salts and their concentrations on RNA, DNA and RNA/DNA ratio after 215 days of transplanting

Salt	Conductivity (mmhos/cm ECe)								
	RNA content mg/g fresh wt.			DNA content mg/g fresh wt.			RNA/DNA ratio		
	6	9	Mean	6	9	Mean	6	9	Mean
NaCl	2.09	1.89	1.99	0.337	0.317	0.327	6.21	5.96	6.09
Na_2SO_4	2.16	1.95	2.06	0.324	0.320	0.322	6.67	6.09	6.38
$CaCl_2$	2.16	1.92	2.04	0.340	0.327	0.334	6.36	5.87	6.12
Mixture	1.85	1.69	1.77	0.307	0.287	0.297	6.03	5.89	5.96
Mean	2.09	1.86		0.327	0.313		6.32	5.95	
Control	2.42			0.357			6.78		

	C.D.at		C.D.at 1% & 5%	C.D. at	
	1%	5%		1%	5%
Control VS Treatment	0.10	0.07	N.S.	0.31	0.22
Salt x Conductivity	N.S.	0.09	N.S.	N.S.	0.30
Conductivity	0.06	0.05	N.S.	0.20	0.15
Salt	0.09	0.07	N.S.	0.29	0.21

Table 23: Effect of different salts and their concentrations on nitrogen content(%) of guava leaves

Salt	Conductivity (mmhos/cm ECe)		
	6	9	Mean
NaCl	2.41	2.36	2.39
Na_2SO_4	2.23	2.31	2.27
$CaCl_2$	2.43	2.44	2.44
Mixture	2.28	2.34	2.31
Mean	2.34	2.36	
Control	2.20		

	C.D. at	
	1%	5%
Control VS Treatment	0.03	0.02
Salt x Conductivity	0.04	0.03
Conductivity	0.02	0.02
Salt	0.03	0.02

conductivity resulted in increased nitrogen content under Na_2SO_4 and mixture of salt whereas NaCl salinity caused significant reduction with increase in conductivity. Calcium chloride recorded significantly more (2.44%) nitrogen per cent followed by NaCl (2.39%) , mixture of salt (2.31%) and Na_2SO_4 (2.27%). The interaction between salt and conductivity was significant. But, increasing of $CaCl_2$ salinity had no significant effect on nitrogen content in leaves. The maximum nitrogen content (2.44%) was recorded under $CaCl_2$ (9 mmhos/cm), followed by its lower conductivity. However, the differences were not significant. Plants under NaCl treatment (6 mmhos/cm) recorded higher nitrogen content (2.41%) than at 9 mmhos/cm. The N content was increased comparatively more under chloride salinization than under sulphate salinization.

7.2. Phosphorus

The perusal of data in Table 24 reveals that P content of leaves decreased with increasing levels of salinity in the soil. P content was significantly higher with Na_2SO_4 salinity as compared to chloride type of salinity. The interaction between salt and conductivity was significant, however, the differences within salts of $CaCl_2$ and Na_2SO_4 salinity at 6 mmhos/cm and 9 mmhos/cm were not significant.

7.3. Potassium

Control leaves significantly showed more (1.22%) K as compared to all salinity levels (Table 24). Increase in conductivity significantly decreased the K content. Among

Table 24: *Effect of different salts and their concentrations on phosphorus and potassium content of leaves*

Salt	Conductivity (mmhos/cm ECe)					
	Phosphorus (%)			Potassium (%)		
	6	9	Mean	6	9	Mean
NaCl	0.17	0.16	0.16	0.89	0.70	0.79
Na_2SO_4	0.19	0.19	0.19	0.93	0.82	0.87
$CaCl_2$	0.17	0.17	0.17	0.90	0.74	0.82
Mixture	0.17	0.16	0.17	0.92	0.79	0.85
Mean	0.18	0.17		0.91	0.76	
Control	0.21			1.22		

	C.D.at 1%	C.D.at 1%
Control VS Treatment	0.01	0.02
Salt x conductivity	0.01	0.02
Conductivity	0.004	0.01
Salt	0.01	0.014

the different salts, NaCl leaves had lowest K followed by $CaCl_2$, mixture of salts and Na_2SO_4. The content of K at different salinity levels varied from 0.70 per cent (NaCl at 9 mmhos/cm) to 0.93 per cent (Na_2SO_4 at 6 mmhos/cm). The interaction between salt and conductivity was found to be significant.

7.4. Calcium and magnesium

The data pertaining to calcium and magnesium percentage in guava leaves under different salinity levels are presented in Table 25.

The nature and conductivity of salt present in soil significantly affected the calcium content in leaves as compared to control. Among the various salts, $CaCl_2$ leaves accumulated highest content of calcium followed by mixture of salts, Na_2SO_4 and NaCl, respectively. The interaction between salt and conductivity was significant. At 9 and 6 mmhos/cm, $CaCl_2$ showed maximum Ca content (2.94% and 2.42% respectively) in leaves followed by mixture of salts at 9 and 6 mmhos/cm, whereas with NaCl and Na_2SO_4 salinity calcium content of leaves decreased significantly with increase in salinity.

Contrary to calcium, magnesium content in leaf was found to be significantly lower under sulphate salinization followed by $CaCl_2$, mixture of salts and NaCl. The increase in conductivity decreased 'mg' content of leaf. The interaction between salt and conductivity was significant. Among different salinity levels, the maximum absorption of Mg content (0.913%) was observed under NaCl (6 mmhos/cm) while it was minimum (0.697%) under Na_2SO_4 at 9 mmhos/cm.

Table 25: Effect of different salts and their concentrations on calcium and magnesium content of leaves

Salt	Conductivity (mmhos/cm ECe)					
	Calcium (%)			Magnesium (%)		
	6	9	Mean	6	9	Mean
NaCl	1.46	1.30	1.38	0.913	0.823	0.868
Na_2SO_4	1.59	1.38	1.49	0.784	0.697	0.741
$CaCl_2$	2.42	2.94	2.68	0.815	0.768	0.792
Mixture	2.07	2.27	2.17	0.847	0.792	0.820
Mean	1.89	1.97		0.840	0.770	
Control	1.84			1.004		

	C.D.at 1%	C.D.at 1%
Control VS Treatment	0.03	0.004
Salt x Conductivity	0.04	0.006
Conductivity	0.02	0.003
Salt	0.03	0.004

Table 26: Effect of different salts and their concentrations on chloride, sodium and sulphate content of leaves

Salt	Conductivity (mmhos/cm ECe)								
	Chloride (%)			Sodium (%)			Sulphate (%)		
	6	9	Mean	6	9	Mean	6	9	Mean
NaCl	0.44	0.67	0.56	0.30	0.45	0.38	0.19	0.17	0.18
Na_2SO_4	0.18	0.18	0.18	0.26	0.37	0.32	0.30	0.36	0.33
$CaCl_2$	0.56	0.71	0.64	0.11	0.11	0.11	0.18	0.17	0.18
Mixture	0.37	0.42	0.40	0.22	0.26	0.24	0.21	0.23	0.22
Mean	0.39	0.50		0.22	0.30		0.22	0.23	
Control	0.18			0.11			0.20		

	C.D. at 1%	C.D.at 1%	C.D.at 1%
Control VS Treatment	0.01	0.01	0.01
Salt x Conductivity	0.01	0.01	0.01
Conductivity	0.003	0.003	0.003
Salt	0.004	0.004	0.004

7.5. Chloride, sodium and sulphate

The data regarding chloride, sodium and sulphate content in leaves grown under different salinity levels was determined and are presented in Table 26.

The perusal of data clearly indicates that increase in salinity significantly increased chloride content in leaves. The maximum increase was observed under $CaCl_2$ salinity followed by NaCl and mixture of salt, irrespective of conductivity. No difference in chloride content was observed between control and Na_2SO_4 salinity at both the conductivity levels.

The sodium content of leaf was also found increased with increase in salt concentration in soil in general and with sodium dominant salinity in particular (Table 26). Pronounced increase in Na content of leaves was observed with NaCl indicating an increase of four times as the conductivity increased from 0.08 mmhos/cm (control) to 9 mmhos/cm. An increased Na content was also observed with Na_2SO_4 and mixture of salt.

The data in Table 26, regarding sulphate content indicates that among the four salts applied, the sulphate content in the leaf was found to be highest (0.36%) under Na_2SO_4 type of salinity (9 mmhos/cm) followed by Na_2SO_4, mixture of salts and NaCl (each at 6 mmhos/cm) with 0.3 per cent, 0.21 per cent and 0.19 per cent, respectively. The content of sulphate found under Na_2SO_4 at 9 mmhos/cm was just double of that found under $CaCl_2$ at 6 mmhos/cm. Regarding the effect of individual salt, an increasing trend was apparent in Na_2SO_4 type of salinity whereas reverse was true under NaCl and $CaCl_2$ salinity.

CHAPTER - V

DISCUSSION

The present work was undertaken with the objective to study the effect of different salts and their concentrations on germination , growth, mineral composition, water relations and metabolic changes in guava.

Effect of salinity on germination and seedling growth

The results of present study indicated that nature and concentration of salts not only delayed but also inhibited the seed germination. Na_2SO_4 salinity was not as injurious to germination as NaCl, $CaCl_2$ and mixture of salts. At 12 mmhos/cm there was no germination under mixture of salt, NaCl and $CaCl_2$ whereas, it was 20 per cent with Na_2SO_4. Mixture of salts proved more deleterious even at 6 mmhos/cm. Ayers and Hayward (1948) also found that higher salinity levels aggravated the delay in emergence and also decreased the final germination percentage. Similar results have been reported in mulberry (Bahodyrov, 1956), dates (Hewitt, 1963) and Zizyphus rotundifolia (Dhankar et al., 1978).

In the present study, the reduced and differential germination percentage of guava seeds under different salts at lower levels can be attributed to osmotic effect of salts. A general inhibition of germination at higher concentration of salts may be due to slowed hydration of seeds under different ions. The slower and reduced rate of hydration may have reduced the rate of hydrolysis of reserve food material which in turn affected germination. However, a higher germination percentage with sodium sulphate as compared to

sodium chloride, calcium chloride and mixture of salts suggests thatthe effect of salinity on germination is not merely an osmotic one but ions responsible for salinization also have their own effect. Decrease in rate of seed germination in response to salinity has been well documented (Hasson-Porath et al.,1972; Ungar,1974,1978;Panigarh et al. , 1978) and each species appears to have thresh-hold suction value for its germination (Hunter and Erickson,1952).Prisco and O'Leary (1970) and Tur et al. (1980) suggested that the effect of salts is mainly osmotic at lower levels but appears to be primarily ionic at higher levels. Sheoran (1975) observed that increase in salinity (upto 10 mmhos/cm) slowed down the hydration of cotyledons of moong and gram which in turn reduced the germination percentage.

Hypocotyl length, root length and number of secondary roots were significantly depressed by the type of salt and increasing salt concentration.Growth of embryo axis and roots were greatly reduced with chloride type of salinity as compared to sulphate type. Secondary root emergence is more sensitive to salt stress. A complete inhibition of secondary roots at higher conductivity levels of salts was observed. It seems likely that at lower salt concentration primarily the cell elongation is inhibited while at higher concentrations in addition cell division is also affected. Hasson-Porath et al. (1972) also reported that sodium chloride was more inhibitory to pea seedling growth than sodium sulphate.This was attributed to complete osmotic adaptation of plants to sodium sulphate whereas it was incomplete to sodium

chloride. Guardiola and Sutcliffe (1972) suggested that due to initial delay in commencement of hydrolytic processes in the cotyledons under salt stress, the rate of hydrolysis may not become limiting to growth of embryo axis at later stage, but the reduced metabolic activity of the embryonic axis itself could be the main inhibiting factors. In addition to these factors, the accumulation of ions in the leaves may have also affected the normal metabolism of seedling thereby reducing the growth.

Effect of salinity on growth and development of transplanted guava plants

Higher concentrations of different salts in soil adversely affected the plant growth. Salinity significantly decreased the height, stem diameter, leaf number, leaf area, fresh and dry weight of various plant parts and the length of primary and secondary roots of guava (Tables 4 to 11).Higher the concentration of salt, greater the suppression of growth, however, the degree of suppression was dependent on the nature of salt. In the present study, chloride type of salinity proved more injurious than sulphate type of salinity. Similar results have been reported in various fruit trees viz.guava (Gupta and Bhambota,1968), orange (Furr and Ream,1968); grapes (Gupta and Nauriyal,1973), plum and peach (Nasr et al., 1977), dates (Furr and Ream,1968) and mango (Bhambota et al.,1963). However, fresh weight per leaf basis was maximum under chloride type of salinity than that of sulphate type whereas reverse is true for dry weight. The increase in fresh weight under

chloride type of salinity is mainly due to more accumulation of water. The higher relative water content as well as leaf water potential under chloride type of salinity as compared to sulphate type of salinity observed in the present investigation supports this view. Therefore,mere " Physiological drought" under the influence of salinity as it is frequently accounted for does not appear to hold good.

Toxic effects of salts cause metabolic disturbances within the plant, is very clear from the present investigations. Nitrogen metabolism including nucleic acid content is greatly affected. In addition, nutritional imbalance and hunger there in created cannot be isolated from its effect on plant growth. The higher accumulation of sodium, chloride and sulphate and decrease in uptake of phosphorus, potassium, calcium and magnesium under sodium chloride and sodium sulphate type of salinity are direct reflection on reduced growth of guava plant. Sulphate type of salinity is far less injurious as compared to that of chloride type of salinity. This may be because of the fact that plants grown under sulphate type of salinity, generally, are characterized by a well developed conductive system in their roots along their axis while in chloride type of salinity, the water conducting tissue is less differentiated and vessels are of small diameter (Strogonov(1962). He further suggested that under sulphate type of salinity in cotton, the water expenditure on transpiration depends on the degree of stunting of plants. He further found that the more stunted the plant is the less water it would expend on transpiration.In

the present study it has been observed that plants under chloride type of salinity were more stunted as compared to plants under sulphate type of salinity.This is because the evaporation surface in greatly stunted guava plants is much smaller than the normal plants. Furthermore, relative water content of leaf is more under chloride salinity than under sulphate salinity. The high amount of relative water content is due to increased diffusive resistance of leaf under chloride type of salinity as compared to sulphate type of salinity. The higher diffusive resistance causes lesser transpiration and lower uptake of water which in turn may increase the temperature within the plant (Strogonov,1962). Thus, increased temperature may be additive to the toxic effects of salts and thereby growth is affected. Furthermore, possibly the hormonal balance is disturbed and ultimately affects the normal plant growth.

Many workers have ascribed the reduced plant growth on saline media to the osmotic inhibition of water absorption, toxic effect of ions, nutritional imbalance, production of toxic substances in plants and ill effects on plant metabolism. Wadleigh and Gauch(1944) and Wadleigh and Ayers (1945) suggested that increase in osmotic pressure of soil solution, by the presence of different salts in the media, decreased the water uptake of plants, thus, creating a "physiological drought".This in turn results in suppressed plant growth. Contrary to these ~~plants~~ results, Philips (1958) reported that 'physiological drought' develops only when solution cannot pass across the cell. Si

salts penetrate into the cells, cause an increase in osmotic pressure of cell, henceforth the water uptake increases. O'Leary (1971) putforth an unified theory on this controversial issue stating that resistance of roots to water uptake increases under salinity, thereby, hormonal transportation from roots to leaves is reduced. This in turn disturbs hormonal balance within the plant. At this time plant accumulates solutes and osmotic pressure of cell sap increases which maintains the osmotic gradient necessary for water absorption. Thus, it can be postulated that lower water uptake under salinity is not the main factor that suppresses the growth. Van Den Berg (1952) pointed out that salt tolerance of some crops mainly depends upon their adaptability to toxic effects. Toxic accumulation of salinity ions and nutrient imbalance in plant due to excess of these ions in soil and plant has been reported by various workers (Hayward and Bernstein, 1958; Allison, 1964).

Effect of salinity on water relations of guava plant

The results regarding water relations as affected by salinity show that chloride type of salinity induced more relative water content of leaves while sulphate type of salinity decreased it (Table 13, Fig. 3, 4a and Fig. 4b). These results are in agreement with those of Desai (1975), Nieman (1962), Divate (1974), Meiri et al. (1971) and Prisco and O'Leary (1973). Leaf water potential decreased under both chloride and sulphate type of salinity. Although, maximum decrease was observed in plants from sulphate type of salinity. Similarly, leaf diffusive resistance

is increased in different salts but higher resistance was observed from chloride type of salinity. Nature of salt produced little increase in resistance of abaxial surface but a large increase in diffusive resistance of adaxial surface. Kaplan and Gale (1972) also showed that salinization produced little or no effect in stomatal resistance of upper surface but a large increase in lower surface. Aceves-N et al.(1975) reported 10 fold increase in leaf diffusive resistance to water vapour at -12 bar salt in wheat. Both NaCl and $CaCl_2$ behave similarly although the effect of $CaCl_2$ was somewhat larger at intermediate salinity levels. In the present study too, $CaCl_2$ is somewhat more effective in increasing the resistance.

The results of the experiments show that the water exchange of plant on soils of the same conductivity, is determined by the type of salinity. The plants raised on sulphate type of salinity showed lower water content and higher rate of transpiration than the plants raised on chloride type of salinity. Possibly the plants from sulphate type of salinity absorb water more intensively from soil and expend it more intensively than the plants from chloride type of salinity. This change in rate of water exchange can be considered as a specific response of the plant to the effect of chloride and sulphate ions. It is worthwhile to mention here that adequate water supply under sulphate type of salinity is ensured by an intensive development of root system with much larger lateral roots and rootlets bearing root hair. However, root development under calcium chloride salinization too was

better but rootlets bearing root hair were practically missing/ absent, indicating that the root hair are important and more sensitive parameter under salt stress. Also the higher absorption and higher rate of transpiration consequently under sulphate salinization resulted in better water circulation within plant. Thus, leading to a self cooling of leaves which in turn caused lower toxic effect of salt. Contrarily, diffusive resistance is much more increased in $CaCl_2$ salinity because of high chloride content resulting in lowering the rate of transpiration and reducing the water circulation and hence no self-cooling of leaves and ultimately toxic effect of salt is increased. Isakova and Chkoniya (1936) showed that the soil was salinized with NaCl, the rate of transpiration of Bochmeria nivea decreased but reverse was true with sodium sulphate. Strogonov (1962) interpreted that the amount of mobile and easily exchangeable water increased under sulphate salinized soils while under chloride, water was exchanged with difficulty and hence more relative water content was observed. He further reported that high water uptake and high rate of transpiration under sulphate salinized soils lowered the temperature of leaf by self cooling and this in turn lowered the toxic effect of salts.

The increase in relative water content and leaf water potential in later stages is probably due to leaching effect of salts through rain water since the experiments were conducted in the open. Higher concentrations of all the salts drastically

reduced the leaf water potential and increased the diffusive resistance, ultimately causing death of the plant. Probably, the leaf-water potential is reduced to a point where both osmotic potential and turgor pressure became limiting for growth, causing death of plant.

Effect of salinity on metabolism of guava plant

Disturbance in the normal metabolic processes of guava plant under different salts was observed in the present study i.e. various salts caused an increase in free amino acid content particularly proline, total organic acids, the activities of amylase and peroxidase and decrease in total insoluble protein, chlorophyll content and nucleic acid content of the leaves. Metabolic disturbances have been reported to be an indirect effect of salinity (Bernstein, 1962; Passingham and Kriedemann, 1970; Nieman and Poulson, 1971 and Desai, 1975).

Marked increase in proline content in the leaves is observed under chloride type of salinity as compared to sulphate salinity, indicating that the proline accumulation is primarily an ionic rather than an osmotic effect. Moreover, it is interesting to note that the highest proline accumulation is in the leaves taken at 90 days after transplanting which consequently coincides with the period of fall in water potential of leaf. Thus, an increase in proline content could also result due to internal water content. Chu et al. (1976) also reported that proline accumulation in barley is initiated and maintained by fall in internal water potential but the rate of proline synthesis is

influenced by the internal concentration of certain ions i.e. Mg^{2+} and possibly Ca^{2+} promoting and cl^{-} being neutral, Na^{+}, K^{+} and SO_4^{-} being inhibitory. So, the less proline content recorded in the present study under sulphate salinity appear to be due to greater inhibition of its synthesis as a result of higher concentration of SO_4^{2-} and reduced Mg^{2+} ions. Stewart and Bogess (1978) reported that oxidation of proline was inhibited by NaCl whereas only a slight inhibition was observed with Na_2SO_4. This might also explain the lower proline content under sulphate salinity. Three main factors may cause proline to accumulate under salt stress. First, there may be a stimulation of proline synthesis from glutamic acid; second, proline oxidation may be inhibited; third, incorporation of free proline into protein may be impaired. Singh et al. (1973) and Stewart and Bogess (1978) reported that there is a stimulation of proline synthesis from glutamic acid, perhaps due to loss of feed back inhibition of synthesis of intermediate Δ^{1} -Pyrroline-5-carboxylate (P-5-C) and proline oxidation is inhibited. When stress is relieved the proline levels decline rapidly in viable tissues due to oxidation via P-5-C and also the incorporation of glutatmate into protein. Hence, the decrease in proline content, at the second sampling (at 210 days after transplanting) in the leaves may possibly be because the plants had adapted themselves to the salinity conditions.

An increase in the free amino acids and decrease in the protein content indicates that salt stress may have either

accelerated the degradation of protein or impaired the incorporation of amino acids into protein or both. The reduction in protein content is more under chloride salinity, suggesting the high hydrolytic activity vis-a-vis low synthetic activity of proteins under chloride salinization as compared to sulphate salinity. The inhibition of protein synthesis in the guava plant leaves under salt stress may be attributed to excessive increase in ionic concentration in cells. Reduced protein synthesis and enhanced hydrolysis under saline conditions is well documented (Nieman and Poulson, 1964; Nieman, 1965 and Udovenko et al., 1973). Kahane and Poljakoff-Mayber (1968) showed that both uptake and incorporation of labelled amino acids into protein of pea roots were reduced by presence of salts in the incubation medium. Similar results were reported by Benzioni et al. (1967) in tobacco leaves. Sheoran (1975) reported an increase in protease activity under saline conditions whereas the protein content was decreased and free amino acids increased. Nieman and Poulson (1971) suggested that salinity suppresses chloroplast development which most likely accounts for reduced rate of protein synthesis in salt affected leaves.

A decrease in the nucleic acid content is observed with the increase in salinity. More pronounced effect was on RNA content. The decrease in nucleic acid content of plant under salinity may be due to their reduced synthesis and/or increased rate of degradation as reported by Nieman and Poulson (1964) and Prisco and O'Leary (1972). Kesseler et al.

(1964) reported that salinity treatment strongly suppressed RNA and DNA content. A decrease in RNA content was attributed to the increased activity of cytoplasmic RNase, whereas decrease in DNA content could be due to its impaired synthesis. Rouser and Hansen (1966) observed that salinity stress caused leakage of divalent cations (Mg^{2+}) which normally act as stabilizer of ribosome against endogenous nuclease. As a result of such leakage, lower content of nucleic acid resulted in the plants under saline conditions. In the present study too a decrease in Ca^{2+} and Mg^{2+} was observed under all salts except $CaCl_2$ where Ca was high.

Reduction in chlorophyll content of leaves under salt stress could be because of the nutritional deficiencies, water stress and suppression of chloroplast. The reduction in chlorophyll content was more under sulphate than chloride salinization. This is probably because of the more reduction in Mg^{2+} content observed under sulphate type of salinity, since Mg^{2+} is the mineral constituent of chlorophyll molecule. Deleterious effects due to salinity in the photosynthetic apparatus like suppression of chloroplast development and change in lamellar structure has been reported by Nieman and Poulson (1971) and Lapina and Popov (1970). In view of the above facts, it is obvious to have reduced chlorophyll content in leaves from plants under salt stress, observed in present course of study.

An increase in the rate of respiration observed is an indication of growth suppression and increased hydrolytic activity in plants under saline stress. The increased rate of respiration may be because of the increased energy requirement of the plant for the selective absorption of ions and uptake of water under salinity conditions. Slatyer (1967) and Desai (1975) reported the increased respiration rate under saline media. Energy requirement of plants increased for water uptake of selective ion absorption under salt stress conditions.

An increase in the activity of amylase and peroxidase observed in the present study suggested increased hydrolytic activity. Salinity is known to affect the peroxidase activity. Although, contradictory reports have been reported regarding peroxidase activity under salinity (El-Fouly and Jung,1972; Maliwal and Paliwal,1972; Weimberg ,1970). Strogonov (1964) showed that peroxidase must play a role in oxidation and accumulation of substances, leading to melanin formation from tyrosine in necrotic areas of cotton leaves under sodium chloride salinization. The increase in amylase activity observed may be due to increased starch content. Desai (1975) reported increase in starch and carbohydrate content in the leaves of guava plant under various salinity treatments. Similar reports have been published by Gauch and Eaton (1942), King (1954) and Strogonov (1962) in different crops under salinity. They also reported that the increased carbohydrate level may be

due to the fact that these might have accumulated more rapidly than their utilization for the formation of new cells and tissues.

The increase in organic acid content as affected by salinity shows that organic acid content in the plant is influenced by the rate of ions absorbed as well as by the type of ions being absorbed. Torii and Laties (1966) suggested that delivery of ions into the vacuole effectively removed organic acid from cytoplasm by forming salt of acid in vacuole and this results in increased synthesis of organic acids being synthesized. It is also possible that excess of organic acids are utilized as substrate for the increased respiration in plant under saline medium.

Effect of salinity on mineral composition of leaves

An obvious disturbance in mineral composition of plants occur under different salts. In the present study, the imbalance was found in the level of mineral elements. Chloride type of salinity affected more as compared to the sulphate type. These results are in agreement with those of Makhija et al. (1980) and Desai (1975). There was an increase in the nitrogen content under all salts. Chloride uptake was highest under calcium chloride type of salinity followed by NaCl and mixture of salts. Sodium content was highest in NaCl followed by Na_2SO_4. Calcium content was highest only in plants raised under treatments of calcium salt. Similarly, sulphate was maximum in Na_2SO_4 salinity plants. The contents of P, K, Ca, Mg decreased under all

salts. This increase or decrease in leaf mineral content is generally proportional to concentrations of salts present in the substrate. Moreover, the mineral composition of leaves indicate that the extent to which ion accumulation and ion imbalance is involved in suppressing the plant growth.

Increase in nitrogen content under different salts may be attributed to the marked increase in the free amino acid content, particularly proline. Strogonov (1962) put forth that nitrogen metabolism of plants grown under saline conditions differs from normal plants because of the higher breakdown of protein, resulting in the formation and accumulation of intermediary nitrogenous substances. Reduction in P,K, Ca and Mg observed under NaCl and Na_2SO_4 salinities in the present study have also been reported by Ehlig,(1960), Shimose (1968) and Desai (1975). The reduced uptake of these elements is probably due to the high sodium content in soil. Sodium has been reported to cause deficiency of these elements. Firstly, because sodium is comparatively loosely held in the exchangeable form, so mostly Na^+ ions are released to the soil in the fractional exchange because of its high percentage in the soil. Secondly, at high pH values, usually associated with excess sodium, the soil solution contains high carbonate and biocarbonate (however, this is not the reason in the present study). Thirdly, on competitive basis Na excludes these ions from absorption and sodium is absorbed as an alternative cation due to mass action (Blac 1967). The higher content of Ca under $CaCl_2$ and mixture

Conclusion:

The germination of guava seed is inhibited under saline media, especially under mixture of salts and chloride type of salinity. The inhibition of seed germination was probably to reduced rate of hydration and mobilization of reservefood material from the cotyledons to embryo axis. The inhibition of seedling growth under salt stress is the resultant of reduced cell division, enlargement, toxicity due to ions and disturbed metabolism.

Suppression of growth of guava plants under saline conditions was due to number of factors viz. reduction in cell division and elongation, adverse effect on root development particularly on root hair, low dry matter production, reduced water status, suppressed synthesis of protein, nucleic acids and increased respiration and hydrolytic activity.

Proline accumulation seems to be an index of salt injury rather than tolerance; because of the fact that marked accumulation of proline took place only under chloride type of salinity and latter was more injurious than sulphate type of salinity.

Nature and concentration of salts are more important than salinity in general. The harmful effects of different salts may be primarily attributed to accumulation of Na^+, Cl^- and SO_4^- ions.

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CHAPTER- VI

SUMMARY

The effects of four salts ($NaCl$, Na_2SO_4, $CaCl_2$ and their mixture) at different concentrations (6,9,12 mmhos/cm) on seed germination, growth of transplanted seedlings, water relations, biochemical changes and mineral composition of guava (Psidium guajava L.) seedlings were studied. The results are summarised as given below:

Increasing salt stress in the media progressively delayed and even inhibited the germination of guava seeds. At higher conductivity levels especially under mixture of salts the germination was totally inhibited. Seedling growth was also inhibited at higher concentrations especially under chloride type of salts.

The growth of transplanted guava seedlings in terms of plant height, stem diameter, number of leaves, leaf area and dry matter accumulation was greatly affected under NaCl followed by $CaCl_2$, mixture of salts and Na_2SO_4.

All the salt treatments caused injury to the leaves and the extent of injury increased with the increasing salt concentration. Under 12 mmhos/cm ECe plants did not survive.

The primary and secondary root growth was maximum inhibited by NaCl, followed by mixture of salts, $CaCl_2$ and Na_2SO_4.

Respiration rate was significantly increased with increase in conductivity at 90 days after transplanting. At

210 days, respiration rate under different salinity levels decreased, however, it was significantly higher than control.

Irrespective of conductivity, the relative water content was significantly higher under NaCl, followed by $CaCl_2$ and mixture of salts. Water potential of the leaves declined with increasing the conductivity levels of all the salts at different sampling dates. An increase in diffusive resistance was observed under both lower and upper leaf surfaces. However, upper surface showed a consistent decrease with advanced sampling date. Diffusive resistance of lower surface was more than that of upper surface.

Proline content significantly increased under different salinity levels at 90 days as well as 210 days after tranplanting. Highest proline content was observed under NaCl treatment.

Chlorophyll, protein and nucleic acid contents decrased with increasing concentration of salts especially chloride were more deleterious than sulphate type.

All the concentrations of salts increased the nitrogen content. The P, K, Ca and Mg were reduced significantly under all salt treatments. However, Ca was higher in the leaves under $CaCl_2$ treatment.

The leaves of guava seedlings absorbed constituent ions of salts (Na_2SO_4, $NaCl_2$, $CaCl_2$) in toxic amounts. The accumulation of these ions increased with increasing concentration of the respective salt in the soil.

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* Original not seen.

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APPENDIX- I

Physico-chemical characteristics of the soil used

Texture	Sandy soil
Saturation per cent	30
pH	8.5
Conductivity	0.08 mmhos/cm ECe
Organic carbon	0.03 per cent
Available phosphorus	21 lbs/ acre
Available potash	296 lbs/acre

APPENDIX - II

Amount of salts added for preparing soil of different conductivity levels

Desired salinity (mmhos/cm)		Total salt concentration meq/litre	Observed concentration (mmhos/cm)
NaCl	6	60	6.0
	9	90	8.6
	12	120	11.7
Na_2SO_4	6	60	6.1
	9	90	8.8
	12	120	12.2
$CaCl_2$	6	60	5.9
	9	90	8.7
	12	120	11.9
Mixture of salts	6	60	6.1
	9	90	8.3
	12	120	12.3

APPENDIX- IV

Effect of different salts and their concentrations on the leaf diffusive resistance (sec/cm)

Salt	Days after transplanting							
	Abaxial surface				Adaxial surface			
	100	120	140	160	100	120	140	160
Control	3.22	3.19	3.17	3.09	3.00	3.25	3.62	3.50
NaCl								
6 ECe	3.38	3.32	3.35	3.28	3.47	3.87	3.90	3.66
9 ECe	3.83	3.72	3.76	3.51	3.68	4.61	4.75	4.44
12 ECe	3.97	3.81	-	-	3.73	4.80	-	-
Na_2SO_4								
6 ECe	3.25	3.38	3.41	3.17	3.28	3.66	3.68	3.51
9 ECe	3.53	3.59	3.59	3.37	3.56	4.17	4.25	3.97
12 ECe	3.70	3.78	-	-	3.65	4.53	-	-
$CaCl_2$								
6 ECe	3.35	3.31	3.34	3.28	3.55	3.81	3.87	3.78
9 ECe	3.72	3.64	3.69	3.58	3.69	4.64	4.84	4.54
12 ECe	3.92	3.80	-	-	3.78	4.84	-	-
$NaCl + Na_2SO_4 + CaCl_2$								
6 ECe	3.12	3.15	3.19	3.15	3.22	3.60	3.69	3.55
9 ECe	3.42	3.40	3.45	3.26	3.48	3.98	4.18	4.06
12 ECe	3.70	3.60	-	-	3.67	4.38	-	-

STUDIES ON SALT TOLERANCE IN GUAVA

By

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(An abstract of the dissertation presented in partial fulfilment of the requirements for the degree of Ph.D.., Haryana Agricultural University, Hissar).

Studies were conducted on guava (Psidium guajava L.) cv. Lucknow-49 (Sardar), grown under four different salts viz. NaCl, Na_2SO_4, $CaCl_2$ and mixture of these three salts, each at three conductivity levels i.e. 6, 9 and 12 mmhos/cm. Increasing salinity, with all the salts, progressively delayed as well as reduced germination. Chloride salinity was more injurious than sulphate salinity. Salinity depressed the radical length, hypocotyl length and number of secondary roots.

Survival of transplanted plants decreased with increasing salinity levels. The plants did not survive at 12 mmhos/cm conductivity, irrespective of salt. Salinity suppressed the shoot and root growth. Chloride salinity was more suppressive than sulphate salinity. Plant height, stem diameter, number of leaves, leaf area, fresh and dry weight of leaves, stem and root were significantly reduced. Plant water status decreased whereas leaf diffusive resistance increased under salt stress. The decrease in leaf water potential was more under sulphate salinity than under chloride salinity while reverse was true for leaf diffusive resistance. Rate of respiration was more under salt stress, maximum being under chloride salinity. Chlorophyll content decreased under salt stress. A major shift in metabolism under salt stress was involved. Proline content was high under chloride salinity whereas protein content reduced under all the salts. Hydrolytic activity increased under salinity. Activity of amylase and peroxidase increased under all salts. Nucleic acid content also decreased particularly RNA content. The extent of reduction was more under chloride salinity.

Nitrogen content was more under salt stress. The contents of P, K, Ca and Mg significantly decreased under salt stress, except Ca content which increased under $CaCl_2$ salinity. The specific injury due to accumulation of Na, Cl, SO_4 in toxic amounts, disturbed metabolism and nutrient imbalance seems to be the main effects of salinity on guava plant.
