

SCREENING OF CITRUS ROOTSTOCKS FOR SALT TOLERANCE AT SEEDLING AND CALLUS LEVEL

By

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of the requirements for the degree of:**

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in

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2000

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This is to certify that this dissertation entitled, **“Screening of citrus rootstocks for salt tolerance at seedling and callus level”**, submitted for the degree of Ph.D. in the subject of Plant Breeding of the Chaudhary Charan Singh Haryana Agricultural University, is a bonafide research work carried out by **Mr. Awtar Singh** 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.

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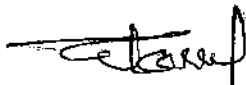
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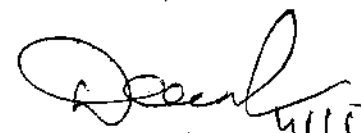
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This is to certify that this dissertation entitled, "Screening of citrus rootstocks for salt tolerance at seedling and callus level", submitted by **Mr. Awtar Singh** to the Chaudhary Charan Singh Haryana Agricultural University, in partial fulfilment of the requirements for the degree of Ph.D., in the subject of Plant Breeding, has been approved by the Student's Advisory Committee after an oral examination on the same, in collaboration with an External Examiner.


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CHAPTER - 1

INTRODUCTION

About one third of the world's irrigated lands and half the lands in arid, semi-arid and coastal regions are affected by salinity and 10 m ha of irrigated lands are abandoned annually owing to excessive salinity (Abrol *et al.*, 1988). In India, 10.1 m ha of land are affected by salinity and/alkalinity problems (Sehgal and Abrol, 1994). Salinity poses several problems for plant growth and development by inducing physiological dysfunctions (Shannon *et al.*, 1994). This warrants for appropriate management options or genetic tailoring of crops to cope up with problem of soil salinity to ensure sustainable food production. Plant species and varieties are, however, known to vary widely in their ability to tolerate salts (Patil *et al.*, 1996).

The knowledge on the genetic basis of salt tolerance is very limited and is usually described as a complex attribute. Markers that are associated with salt tolerance and are easily identified can be used for screening tolerant plants in large breeding populations. Many of the basic mechanisms involved in salt tolerance operate at the cellular level. Therefore, the application of cell culture for the identification and quantification of central physiological attributes to salinity, including

compartmentation, osmoregulation and most important the cost of energy for the operation of the resistance mechanism is desirable.

Tolerance to salinity of crop plants varies greatly, among these the perennials and the fruit trees are most sensitive and vulnerable. The mechanism of salt tolerance is not clearly understood in the plants. Recent investigations on biochemical and physiological parameters, both in whole plants and cell cultures have yielded little information which suggests the presence of several salt tolerance mechanisms in plants. Plants tolerate salinity stress mainly by osmotic adjustments through accumulation of organic and inorganic ions which act as osmoprotectants. Information on genetic basis of salt tolerance is still scarce as breeding for salt tolerance has been done on a rather limited scale.

Citrus is the third most important fruit crop in India, after banana and mango, with an estimated production with 3.79 m tonnes of fruits and area coverage of 0.45 m ha during 1996 (Ghosh,1999). Commercially mandarins, sweet orange and acid limes are most important, sharing 41, 23 and 23 per cent, respectively of all citrus fruits produced in the country. The productivity of citrus orchards in India is 7.8 t/ha as compared to 20-25 t/ha in major citrus producing countries of the world like Brazil, U.S.A., Japan, South Africa, Israel etc. This low productivity is due to many problems related to fungal, viral, bacterial and nematode diseases, insect-pests, poor nutrition and soil related problems including salinity.

Citrus species rank among the most sensitive to soil salinity (Cooper *et al.*, 1951; Bhambota and Kanwar, 1968, 69; Maas and Hoffman,1977;

Maas, 1992), but nevertheless they do exhibit wide variation in the ability to tolerate salinity. The poor plant growth and reduction in fruit yield is common due to salinity. Excess of salts leads to yellowing of leaves, leaf drop and in severe cases, twig die-back and ultimately death of the plant. Salt tolerance is a heritable quantitative character for citrus and is of great importance in identifying and screening salt tolerant germplasm. Large variations in salt tolerance among different *Citrus* species and its relatives have been widely observed (Bhambota and Kanwar, 1969; Rokba *et al.*, 1979; Hassan and Galal, 1989; Singh *et al.*, 1997).

The citrus systems allowed to test parallelism of results *in vivo* and *in vitro* in a wide range of citrus genotypes at different levels of organization. Cell culture techniques and especially *in vitro* selection can provide alternative method for screening and selecting plants which exhibit resistance, or relative tolerance to stresses. By this technique, a large number of cells can be screened rapidly in a relatively small area under controlled environment and nutrient conditions.

The problem of salinity can possibly be overcome either by management of environment, development or improvement of salt tolerant varieties or both. The identification and isolation of salt tolerant germplasm in related species of plants is a basic requirement for a breeding programme. The major drawback in such breeding programmes is the time required for effective selection and difficulty in maintaining background genotype for maximum gene expression.

There is growing realization that a more effective, less costly, non polluting and long lasting adaptive measure lies with the genetic modification of the plant itself that can lead to adaptation of plants to salt environment.

Commercially grown citrus plants are mostly composed of two components, viz. scion and rootstock. Rootstock determines the success or failure of the scion variety grafted on it. It affects the plant growth, fruit quality and adaptation to different soil and climatic conditions. So, the test of performance of rootstock cultivars is essential for successful cultivation of citrus. In earlier works done, the salt tolerance was generally studied at seedling level (*in vivo*) or at cell level (*in vitro*), but not simultaneously at both the levels. So as to offer a valid comparison and to know the effect of organogenesis on salt sensitivity/ tolerance, it is necessary to screen citrus rootstocks at seedling as well as cell level. Therefore, this investigation was planned with the following objectives :

1. To screen the citrus rootstocks for salt tolerance under *in vivo* and *in vitro* conditions
2. To study changes in physiological and morphological characters in the citrus rootstocks as affected by different levels of soil salinity
3. To find out association between *in vivo* and *in vitro* screening for tolerance to salinity using various morphological and physiological parameters

CHAPTER - 2

REVIEW OF LITERATURE

Soil salinity has been a colossal problem for the agricultural production. Vast areas of land in the world and our country have become unfit for cultivation owing to excessive salt concentrations (conc.) in soils, mostly chlorides and sulphates of sodium, calcium and magnesium. Major inhibitory effects of salinity on plants include (i) osmotic inhibition of water availability, (ii) toxic effects of salt ions responsible for causing salinity and nutrient imbalances. Although these are common effects of salinity, but they may depend upon weather conditions, water availability, plant species, type and conc. of ions and physiological stage of plant growth.

Salt tolerance of a plant has been described as a polygenic trait, but its gene products are not well documented. For breeding programmes to develop plant salt tolerance, an understanding of tolerance mechanism for salinity is must. Several efforts have been made to study and improve salt tolerance in plants.

Citrus is grown in many semi-arid climates where rainfall is scanty and irrigation is required for the plant growth. Soils in these areas are often saline to affect the growth and yield of citrus crops. *Citrus* species

are most sensitive to soil salinity (Maas, 1992), however, the capacity of citrus trees to tolerate salinity varies among different species and even among strains within a species (Cooper *et al.*, 1956; Furr *et al.*, 1963; Furr and Ream, 1968; Newcomb, 1978; Singh *et al.*, 1997).

Harmful nature of saline and alkali soils to plants was first recognized by Buffum in 1896. As early as in the year 1900, Loughridge observed that certain orange orchards in California, USA had suffered injury due to irrigation with saline water. Further, Hilgard (1900) concluded that NaCl was the constituent of the irrigation water, which was responsible for injury to those orange trees. After that a great deal of research work had been conducted in different countries on the salinity tolerance and related aspects of citrus to various saline conditions. A review of the work done so far on the topic in present investigation is presented below:

2.1 EFFECT OF SALINITY ON CITRUS

2.1.1 Effect of salinity on seed germination

The effect of salinity on germination of citrus seeds has been studied by many workers and the prominent of these are as under: Caro *et al.* (1973) studied the effect of NaCl solution in four *Citrus* species and reported that the germination was reduced with an increase in salinity. These findings were further supported by Mobayen and Milthorpe (1978), who stated that the seed germination of populations with slower germination was reduced much more by salt stress than those populations in which all seeds germinated rapidly.

Zekri (1993c) reported that increasing salt conc. in the solution delayed and depressed seedling emergence, increased the time to 50 per cent emergence and reduced seedling growth in comparison to non-saline control, but it did not affect number of days between the emergence of the first and last seedlings. Further, according to him, seedling growth was more affected by salinity than the emergence and especially at higher salinity levels. He concluded that shoot Cl^- was more useful than total Cl^- for evaluating the extent of injurious nature of salt stress and was a better parameter in ranking salt tolerance of the rootstocks. His findings further showed that salt tolerance was not a constant character in rootstocks, but varied with the stage of seedling development.

According to Zekri (1993a) effect of salt on seed imbibition and time to first seedling emergence was primarily osmotic, whereas, the effect on final percentage emergence and growth of seedlings was mainly a toxic one. Zekri (1993b, 1994) reported that when seeds of eight citrus rootstocks were sown in a commercial growing medium, salinity generally delayed and reduced seedling emergence, reduced shoot and root biomass and altered the mineral composition of young seedlings, but there was no uniform trend among cultivars between salt tolerance during emergence and that during seedling growth. He concluded that salt tolerance at seedling emergence may not be a useful indicator for rapid screening of citrus cultivars.

El-Desouky and Atawia (1998) reported that germination percentage of seeds decreased and the number of days required for germination were

increased as the level of salinity increased, but these reductions and delays were rootstock specific.

2.1.2 Effect of salinity on plant growth and yield

The major effects of salinity on plants are the growth suppression, leaf injury symptoms and reduction in yield. Vegetative growth and fruit yield decrease in direct proportion to increasing salt conc.

Chapman *et al.* (1969) reported that tree growth was significantly reduced by 30 and 60 meq/l salinities. Further, he reported that fruit borne on trees receiving high salt water were smaller in size. According to Goell (1969) in Shamouti orange trees, the rise in salinity was accompanied by a reduction in flushing frequency of the axillary buds and a tendency towards inhibition of flowering terminals which would eventually reduce yields.

Peynado and Young (1969) stated that trees grew less and exhibited more salt injury under saline conditions. According to El-Kholi *et al.* (1979a) growth of the trees was progressively reduced with increasing Cl^- conc. Dry weight of leaves, roots and stems were significantly reduced compared with the controls. Further, Badawi *et al.* (1979) stated that salinity treatments resulted in lower leaf and dry root weight than the untreated control, whereas, NaCl alone resulted in less increase in dry weight than NaCl and microelements. El-Kholi (1979b) reported that salinity severely reduced plant dry weight and pigment content.

Salinity reduced rind thickness and delayed maturation of fruits, but did not influence other fruit quality characters (Francois and Clark,

1980). Growth of Blood Red and Campbell Valencia budded on different rootstocks was decreased by increasing salinity (Joolka *et al.*, 1980). Mobayen and Milthorpe (1980) observed greater differences between cultivars for leaf senescence and expansion rate and suggested that these criteria could be used for screening seedlings for salinity tolerance.

According to Cherif *et al.* (1981), growth of all parts of sour orange was severely limited by salinity, but symptoms of injury were expressed poorly. However, on Troyer citrange, necrosis was seen, but growth was less adversely affected by lower salinity levels and severely affected by highest salinity level. Reduction in branch diameter under salinity was recorded by Dasberg *et al.* (1985), whereas, Duke *et al.* (1986) reported that shoot and root dry weight decreased with increase in salinity.

Cerda *et al.* (1986) recorded that growth retardation and salt injury symptoms are rootstock dependent, in some scions, their appearance was more while in others less. The results of Attalla (1987) indicated that dry weight of rootstocks decreased with increase in salt conc. The tolerant rootstocks had the lowest leaf : root ratio. Zid and Grignon (1986) proved that mean relative growth rate of young seedlings of *C. aurantium* did not depend on NaCl conc., but its highest conc. induced leaf necrosis and leaf fall. Highest salinity treatment decreased the growth of main branches and root development (Bielorai *et al.*, 1988). Dasberg *et al.* (1988) reported that tree growth was retarded in the plots that received irrigation water with high level of salinity. Further Dasberg *et al.* (1988) reported that main branch growth in cross section was retarded by highest salinity treatment.

Zekri and Parsons (1989) recorded a decrease in shoot : root ratio and increase in root weight per unit length with NaCl conc. A relationship was established between NaCl treatment and relative growth rate and it was concluded that reduction in relative growth rate caused by NaCl treatment was more scion dependent, whereas, defoliation was more rootstock dependent (Banuls *et al.*, 1990). Zekri and Parsons (1990a) concluded that when sour orange seedlings, treated with nutrient solution containing various levels of NaCl alone or in combination with CaSO_4 , CaCl_2 or KCl, had reduced root and dry shoot weight.

Zekri and Parsons (1990b), further reported that seedlings with NaCl stress produced fewer roots and shallower root system. Fibrous root weight/unit length increased. Growth was depressed and stomatal conductance was reduced. Plants had smaller root and shoot dry weights, fewer leaves, shorter height and fewer roots, but an increase in root density (Alva and Syvertsen, 1991). Zekri (1991) concluded that at a relatively low salinity level, shoot and root dry weight, stem cross-sectional area, total leaf area and fibrous root length were reduced by 44-55 per cent.

Garcia-Legaz *et al.* (1993) showed that shoot length of plants on sour orange and *C. volkameriana* had greater reduction with increasing salinity than those on *C. macrophylla*. Boman (1994) reported a reduction in canopy volume of about 7 per cent for each 1.0 dS/m increase in irrigation water salinity over the base level of 0.7 dS/m.

El-Hammady *et al.* (1995) reported that salinity treatments greatly affected plant height, stem diameter and total plant fresh and dry weight

and the effects were increased with increase in salinity, but Rangpur lime seedlings exhibited better growth. Aljuburi (1996) also concluded that salinity influenced relative growth rates, stem diameter, stem and leaf dry weight, but, the extent of effect was rootstock dependent, some were tolerant and others were susceptible.

The Cl^- salts markedly reduced plant growth in scion rootstock combinations whereas, NaNO_3 had very little effect (Banuls *et al.*, 1997). Further, Ruiz *et al.* (1997) reported that salt treatment significantly reduced both relative growth rate and net assimilation rate, whereas, leaf weight ratio showed no definite trend. Fernandez-Ballester *et al.* (1998) concluded that growth reduction was severe or less under salinity depending upon rootstock used.

According to Garcia-Lidon (1998) salinity reduced growth and the least affected rootstock was *C. macrophylla* and best combination for saline conditions was suggested as 'Fino' grafted on *C. macrophylla*. Nastou *et al.* (1999) quantified the effect of NaCl on shoot growth and reported that shoot growth decreased substantially at NaCl conc. above 15 mM. Further, Ruiz *et al.* (1999) concluded that growth response to different salt treatments was primarily affected by osmotic effect, although in *C. macrophylla*, the ionic effect also seemed to be present.

Howie and Lloyd (1989) reported that trees irrigated with high salinity water have reduced flowering intensities and fruit set, slower fruit growth and late maturity of fruits. Kirkpatrick and Bitters (1969) suggested the use of the survival and threshold limit for new top growth and fibrous

roots as a useful first approximation when selecting potentially salt tolerant rootstocks.

Khanna and Kumar (1990) reported that leaf area was reduced in trees after sixty days of irrigation with NaCl or NaCl + Na₂SO₄, but not with Na₂SO₄ alone. After 90 days, shoot growth and leaf number/shoot reduced in treatments with NaCl or NaCl + Na₂SO₄, whereas, Na₂SO₄ treated trees showed no significant changes. None of the treatments had significant effect on stem diameter or tree height. Lloyd and Howie (1989) recorded a reduction in leaf area and extensive abscission of leaves of Washington Navel on orange rootstock.

Shalhevet *et al.* (1974) reported a reduction in yield of the magnitude of 2.3 t/ha (2.3%) with per meq/l increase in Cl⁻ for Shamouti orange on sweet lime and 3.25 t/ha (5.5%) for Valencia orange on sour orange. Francois and Clark (1980) suggested that the yield reduction with increasing salinity was attributed to a reduction in fruit number and not in the fruit size. The reduction in yield was correlated with increased Cl⁻ accumulation by Levy *et al.* (1992). Cole and McCloud (1985) reported that salinity was negatively correlated with yield, but its effect was location specific and salinity during the irrigation, prior to harvest was associated with decreased yield.

Reduction in yield with increase in salinity was also observed by Dasberg *et al.* (1985), Bielorai *et al.* (1988) and Nieves *et al.* (1992). Bielorai *et al.* (1988) reported that fruit quality was not impaired by salinity and the reduction in yield was correlated with number of fruits,

but not with the size of the fruit. A reduction of 9.0 per cent in yield was reported by Bielorai *et al.* (1983), when a treatment with high Cl^- water was given. They also reported that total water uptake by the plant was reduced and that could be the reason for yield reduction.

Lloyd and Howie (1989) reported that Washington Navel on sweet orange rootstock irrigated with 20 mM NaCl/m^3 had greater number of vegetative flushes at the expense of flowering, number of reproductive branches and mixed flushes were reduced by salinity. Maas (1992) concluded that soil salinity significantly limited the citrus production worldwide with grapefruit, lemons and oranges being the most susceptible. Fruit yields were decreased by 13 per cent for each 1.0 dS/m increase in electrical conductivity of saturated soil extract once the salinity exceeds a threshold limit of 1.4 dS/m.

2.1.3 Effect of salinity on chemical composition of plant

Chapman *et al.* (1969) reported that tentative injury at threshold levels in 4 months old spring cycle leaves were 0.3 per cent Cl^- (dry matter) in leaves from non-fruiting terminals and 0.50 per cent in leaves from fruiting terminals. Cl^- accumulated in leaves progressively with age. According to Goell (1969), the effect of Cl^- ions in the citrus leaves was associated with a reduction in leaf longevity due to increased chlorosis, earlier senescence and abscission. Trees on rough lemon had highest leaf Cl^- content and leaf drop rate and a high degree of chlorosis, but the crown volume was greater on this stock. He described such type of tolerance as specific rootstock effect.

Salerno (1975) reported that salt affected leaves contained abnormally high levels of Cl^- . According to Guillen *et al.* (1978) increase in salinity depressed leaf K^+ and Mn^{++} contents and increased N and Mg^{++} , but P, Ca^{++} and Fe^{++} contents were unaffected and further, the effect on ion content was rootstock specific. However, Abdel-Messih *et al.* (1979) recorded that salinity increased Na^+ , Ca^{++} and K^+ levels in leaves of Cleopatra mandarin, sour orange, F₁ hybrids, poor man orange and rough lemon, whereas, leaf Mg^{++} , N and Fe^{++} fell and P was unaffected. They further noticed the rootstock specificity in accumulation of individual ions.

Badawi *et al.* (1979) recorded an increase in plant Cl^- and Na^+ contents in NaCl treatments, but the Fe^{++} , Mn^{++} and B contents remained constant. Further, Mobayen and Milthorpe (1980) stated that Na^+ conc. in leaves was linearly related to conc. in the external solution and increased with time. Na^+ , P, Ca^{++} and Mg^{++} conc. was higher in Bakraie mandarin than in Cleopatra mandarin. Grieve (1983) reported that the most salt tolerant rootstocks were Rangpur lime, Cleopatra mandarin, Carrizo and Troyer citranges and least tolerant was *Poncirus trifoliata*.

Grieve and Walker (1983) reported that plants differed in their leaf Cl^- and Na^+ conc., but no or little difference between root Cl^- and Na^+ conc. The different patterns of accumulation of Cl^- and Na^+ in different species were consistent with the existence of apparently separate mechanisms. Cleopatra mandarin, Rangpur lime, *C. macrophylla* and Appleby Smooth Seville accumulated significantly less Cl^- than *P. trifoliata* and rough lemon.

Walker and Douglas (1983) reported differences between rootstocks in Cl^- conc. in leaves and to a lesser extent in shoots emphasizing marked differences in root to shoot transport of Cl^- . The ability in Cl^- exclusion increased in order Etrog citron, Kharna khatta and Rangpur lime. Further, they observed that root, stem and leaf Na^+ conc. increased with salt treatment but reduction in K^+ occurred in stems and roots only. Walker *et al.* (1983) concluded that Rangpur lime and Cleopatra mandarin restricted uptake and transport of Cl^- to shoots and leaf K^+ conc. remained similar to controls. According to Behboudian *et al.* (1986), scions on Cleopatra mandarin accumulated less Cl^- in their leaves and stems than Valencia on rough lemon and rough lemon seedlings, demonstrating that Cl^- accumulation in shoots was rootstock dependent, but Na^+ accumulation in shoots was more scion dependent. Further, they observed that salinity caused a decrease in K^+ conc. in all roots and shoots.

Walker (1986) reported that trifoliate orange at lower salinity possessed the ability to restrict Na^+ accumulation in leaves, but not at higher salinity. Salt tolerant Cleopatra mandarin plants tended to show a marginal increase in leaf $\text{Na}:\text{K}$ ratio. There was a marked reduction in root to leaf Na^+ transport

It was reported by Zid and Grignon (1986) that NaCl had no effect on K^+ and N . It led to large Na^+ and Cl^- accumulation in leaves increasing with its conc. in the medium. According to Syvertsen *et al.* (1988), increase in conc. of Cl^- in trees on trifoliate orange rootstock was significantly higher than those on sweet orange rootstock. Na^+ and Cl^- in foliage

increased and K^+ decreased with age of the leaves, especially under irrigation with saline water.

Banuls *et al.* (1990) studied salinity effects on some rootstock scion combinations and reported that scions on Cleopatra mandarin accumulated less Cl^- in leaves than on Troyer citrange. The distribution of Cl^- in the whole plant showed that Clementines had a reduced ability to transport Cl^- from roots to leaves. However, Na^+ content was lower in Troyer citrange than in Cleopatra mandarin. It indicated that Troyer citrange restricted the Na^+ transport to scion. Further, they noticed that salinity decreased K^+ , Ca^{++} and Mg^{++} contents in leaves and roots of all the rootstock combinations. It was further confirmed by Alva and Syvertsen (1991) that leaf nutrient values and responses to salinity were rootstock dependent, as trees on sour orange rootstock had higher K^+ and Ca^{++} , but lower Mg^{++} and Cl^- than trees on Carrizo citrange. Leaf K^+ of trees on Carrizo citrange and leaf Mg^{++} of trees on sour orange were reduced by salinity.

Susceptibility of sour orange rootstock to salt stress was observed due to its failure to exclude Cl^- even at low salt levels (Zekri, 1991), but Nieves *et al.* (1992) were of the view that Cl^- accumulation was more dependent on scion. This view was also supported by Garcia-Legaz *et al.* (1993) and according to them salt accumulation in leaves was scion dependent as 'Eureka' accumulated higher Na^+ and Cl^- than 'Fino' and 'Verna', when all were budded onto a common rootstock.

According to Walker *et al.* (1993), both Cl^- and Na^+ could reduce assimilation rates in salt stressed lemon leaves. Increase in leaf Na^+ and

Cl^- accounted for 54-96 per cent of the reduction in osmotic potential in leaves on trees treated with NaCl , whereas, increase in Na^+ and SO_4^{2-} accounted for 33-71 per cent of the reduction in osmotic potential in leaves on trees irrigated with Na_2SO_4 .

Boman (1994) concluded that trees showed excessive foliar Na^+ or Cl^- accumulation at the highest salinity. Trees on Cleopatra mandarin were able to exclude Cl^- better than on other rootstocks. Trees on Carrizo citrange accumulated high levels of Cl^- , but they were most effective in excluding Na^+ . Further, Garcia-Legaz *et al.* (1994) reported that cultivars grafted on *C. macrophylla* had lower Na^+ and Cl^- conc. in leaf, but higher root conc. than other rootstocks. Highest conc. of Na^+ and Cl^- in cultivars was found in 'Eureka' lemon.

According to Banuls and Primo-Millo (1995) scions on Cleopatra mandarin accumulated less Cl^- in their leaves than did scions on Troyer citrange. Also leaf Cl^- conc. in Clementine scions was lower than in Navel orange, when both were grafted on the same rootstock. However, Na^+ conc. was lower in scions on Cleopatra mandarin than on Troyer citrange.

El-Hammady *et al.* (1995) reported that irrigation with saline water increased leaf Mg^{2+} , Na^+ and Cl^- regardless of rootstock, while irrigation with tap water increased Ca^{2+} but the conc. varied between rootstocks. Volkamer lemon seedlings contained higher leaf Mg^{2+} and lowest values of Na^+ and Cl^- indicating such rootstocks could tolerate salinity. Sour orange accumulated higher leaf Na^+ and Cl^- and therefore was considered as susceptible to salinity.

According to Banuls *et al.* (1997) relatively more Cl^- accumulated in leaves and less in roots of *P. trifoliata* than in Cleopatra mandarin, but conc. of Na^+ was higher in Cleopatra mandarin than in *P. trifoliata*. They concluded that salinity effects on growth and gas exchange were induced by external Cl^- supply. Ruiz *et al.* (1997) further supplemented that in addition to osmotic effects and the inhibitory effects of high Cl^- and Na^+ conc., salinity caused an imbalance of essential nutrients. In a significant report, Garcia-Lidon (1998) concluded that none of the rootstocks was able to exclude Cl^- and Na^+ from scion leaves and macro and micro-nutrient conc. was influenced by salinity and rootstock/scion combinations. But Nastou *et al.* (1999) reported that Cl^- accumulation increased with increasing NaCl conc. in nutrient solution while Na^+ accumulation increased markedly at higher conc. of NaCl.

Storey and Walker (1999) reported that salt damage in citrus was associated with accumulation of Na^+ and/or Cl^- in leaf cells. Greater salt resistance of rootstocks, such as Rangpur lime and Cleopatra mandarin was associated with their capacity to limit the accumulation of Cl^- in leaves. Rootstocks like trifoliate orange have a capacity to restrict Na^+ transport to the shoots at low salinity. They further reported that the traits which restrict the root to shoot transport of Cl^- and Na^+ in citrus are heritable. The regulation of leaf Cl^- conc. in citrus appeared to be primarily associated with transport process in roots, e.g. root uptake and unloading of Cl^- at the root symplast/xylem interface. The capacity of rootstocks, such as trifoliate orange to limit Na^+ accumulation in leaves was associated with

reabsorption of Na⁺ from xylem stream in root and basal stem. The extent of recycling of Na⁺ back to root is unknown in citrus.

2.2 RELATIVE SALT TOLERANCE OF CITRUS SPECIES

Salt tolerance of woody plants, particularly of fruit trees was found to be relatively high during early years when vegetative growth was dominant and it declined with the advent of fruiting (Devjatov, 1961). According to Bahodyrov (1956), salinity retarded seed germination and early growth, after which trees became resistant, whereas, Beeftink (1955) observed that tree age had no clear effect on sensitivity to salinity.

Relative tolerance of citrus cultivars and species have been studied by various workers (Cooper *et al.*, 1951; Cooper, 1961, 1962; Bhambota and Kanwar, 1968, 1969; Abbadi, 1969; Singh *et al.*, 1997). Among the citrus and related species, Cleopatra mandarin, *Severinia buxifolia* and Rangpur lime were the most tolerant rootstocks (Cooper, 1961, 1962; Singh *et al.*, 1997) and sour orange, rough lemon, sweet lemon and Cambodian sweet lime were moderately tolerant (Cooper *et al.*, 1951). Holevas and Demetrisdes (1963) found that sweet oranges were least affected, citron and lemons, slightly sensitive, whereas, sour orange and mandarin were observed to be intermediate. Bhambota and Kanwar (1968, 1969) reported that Kharna khatta, galgal (*C. limon*), trifoliate orange and rough lemon were progressively less tolerant to salinity when used as rootstocks for Blood Red sweet orange. When budded on rough lemon, Hamlin was the most tolerant, followed by Valencia Late, Pineapple and Blood Red sweet oranges. A comprehensive list on relative salt tolerance of citrus rootstocks is presented in Table 1.

Table 1. Relative salinity tolerance of citrus and related species

<i>Citrus</i> species	Tolerant	Moderately tolerant	Susceptible	Reference
Cleopatra mandarin	Yes	-	-	Furr and Ream, 1968; Joolka and Singh, 1979; Grieve, 1983; Grieve and Walker, 1983; Walker <i>et al.</i> , 1983; Behboudian <i>et al.</i> , 1986; Cerda <i>et al.</i> , 1986; Zekri, 1991; Banuls and Primo-Millo, 1995; Banuls <i>et al.</i> , 1997; Singh <i>et al.</i> , 1997; Storey and Walker, 1999.
	-	Yes	-	Mobayen and Milthorpe, 1980; El-Hammady <i>et al.</i> , 1995.
	-	-	Yes	Joolka <i>et al.</i> , 1980
Rangpur lime	Yes	-	-	Furr and Ream, 1968; Joolka and Singh, 1979; Grieve, 1983; Grieve and Walker, 1983; Walker <i>et al.</i> , 1983; Walker and Douglas, 1983; El-Hammady <i>et al.</i> , 1995; Storey and Walker, 1999.
	-	-	Yes	Joolka <i>et al.</i> , 1980
Rough lemon	Yes	-	-	Joolka <i>et al.</i> , 1980
	-	-	Yes	Joolka and Singh, 1979; Grieve and Walker, 1983; Behboudian <i>et al.</i> , 1986; Singh <i>et al.</i> , 1997.
Trifoliate orange	-	-	Yes	Mobayen and Milthorpe, 1980; Grieve, 1983; Grieve and Walker, 1983; Walker, 1986; Syvertsen <i>et al.</i> , 1988; Banuls <i>et al.</i> , 1997; Singh <i>et al.</i> , 1997; Storey and Walker, 1999.
<i>C. macrophylla</i>	Yes	-	-	Grieve and Walker, 1983; Garcia-Legaz <i>et al.</i> , 1993; 1994; Garcia-Lidon, 1998.
	-	-	Yes	Cerda <i>et al.</i> , 1986; Fernandez-Ballester <i>et al.</i> , 1998; Ruiz <i>et al.</i> , 1999.
Sour orange	-	-	Yes	Cherif <i>et al.</i> , 1981; Cerda <i>et al.</i> , 1986; Zekri and Parsons, 1990b; Zekri, 1991; Garcia-Legaz <i>et al.</i> , 1993; 1994; El-Hammady <i>et al.</i> , 1995; Fernandez-Ballester <i>et al.</i> , 1998.
Troyer citrange	Yes	-	-	Cherif <i>et al.</i> , 1981; Grieve, 1983.
	-	-	Yes	Joolka <i>et al.</i> , 1980; Banuls <i>et al.</i> , 1990; Banuls and Primo-Millo, 1995

<i>Citrus</i> species	Tolerant	Moderately tolerant	Susceptible	Reference
<i>C. volkameriana</i>	Yes	-	-	El-Hammady <i>et al.</i> , 1995
	-	Yes	-	Garcia-Legaz <i>et al.</i> , 1993,94; El-Hammady <i>et al.</i> , 1995.
Kharna Khatta	-	-	Yes	Walker and Douglas, 1983
Sunki mandarin	Yes	-	-	Furr and Ream, 1968
Shekwasha mandarin	Yes	-	-	Furr and Ream, 1968
Carrizo citrange	Yes	-	-	Grieve, 1983
Barkaie	Yes	-	-	Mobayen and Milthorpe, 1980

2.3 SALT TOLERANCE LIMIT

A salt content of 0.05 per cent was considered to be upper limit for orange groves irrigated with saline water (de Boxio, 1963). Salt conc. more than 0.11 per cent was highly detrimental to orange plant growth (Bhambota and Kanwar, 1970). Cooper and Peynado (1953) found that in salt treatment, there was a decrease in growth of both young and old citrus trees.

2.4 BREEDING AND SCREENING OF CITRUS ROOTSTOCKS FOR SALT TOLERANCE

Different techniques have been used by different workers. Some used *in vivo* techniques and the others used *in vitro* techniques. Amongst the *in vivo* techniques, seed germination in saline soil or the seeds irrigated with saline water under artificial conditions, seedling screening in salinized plots/pots were common. Some workers had used cuttings for screening purposes. Both types of conditions i.e. soil salinity and irrigation with saline water had been used in majority of screening programmes. NaCl had been used as a principal salt. Experiment duration varied from 6 weeks of salt stress to several years in the field evaluation.

Under *in vitro* screening, as such these techniques had not been used in citrus for screening against salt tolerance, but they were used for selection of salt tolerant cell lines. In citrus, the techniques of callus induction and regeneration of plants were well documented but there may be differences for different species and sources of explants.

2.4.1 *In vivo* screening for salt tolerance

Considerable genetic variability in salt uptake and tolerance had been noted in citrus, especially between rootstocks. For screening of rootstocks for salt tolerance, seed germination is the first technique.

A. Seed germination under saline conditions

Seeds of Troyer citrange, bitter orange, *C. macrophylla* and Kinnow mandarin were immersed for 12 hrs in NaCl solution at four conc. between zero and 2.4 g/l by Caro *et al.* (1973) and they recorded that all rootstocks showed a similar trend in germination as the salinity levels increased. Mobayen and Milthorpe (1980) studied the germination behaviour of *P. trifoliata* and Cleopatra and Bakraie mandarins seeds in solutions of NaCl or Na₂SO₄ of zero, -250, -500 or -750 J/kg water potential and reported that seed germination of populations with slow germination was reduced much more by salt stress than that of populations in which all seeds germinated quickly.

Zekri (1993c) sowed the seeds of three rootstocks in a green house and irrigated with different salinity treatments (5, 10, 20, 40 and 80 mmol/l) by adding NaCl and CaSO₄ in a ratio of 4:1 to Hoagland's solution. He concluded that salt tolerance was not a constant character in rootstocks, but varied with the stage of seedling development. In another experiment of Zekri (1993a), dry or water imbibed seeds of Cleopatra mandarin, sour orange and *P. trifoliata* were sown in trays containing seed compost covered by vermiculite and watered by control, or 50 mM NaCl or mannitol at 17 g/l or PEG at 130 g/l. From these results, he concluded that the effect of

salts on seed imbibition and time to emergence of first seedlings was primarily osmotic, whereas, the effect on the final percentage emergence and seedling growth was mainly a toxic one.

Zekri (1993b) used cellular trays containing a commercial growing medium covered with vermiculite, for seed germination of sour orange, *C. volkameriana*, Ridge Pineapple, Cleopatra mandarin, Carrizo and Troyer citranges, Swingle citrumelo and rough lemon. The seeds were irrigated with 10 per cent Hoagland's solution containing 50 or 100 mmol NaCl/m³ or 50 mmol NaCl + 50 mmol CaSO₄/m³ and concluded that no uniform trend was found among cultivars between salt tolerance during emergence and that during seedling growth.

In a green house study, seeds of eight rootstocks were sown in trays by Zekri (1994) and irrigated with 4 salinity levels viz., control (100 ppm NaCl), 3000 ppm NaCl, 6000 ppm NaCl and 3000 ppm NaCl + 3500 ppm CaSO₄. The addition of CaSO₄ enhanced the emergence of the first seedling, final percentage emergence and improved seedling growth. No uniform trend was found in the relationship between salt tolerance during emergence and that during seedling growth. He suggested that salt tolerance at emergence may not be a useful indicator for rapid screening of citrus cultivars.

B. Breeding/selection for salt tolerance at whole plant level

Furr and Ream (1968) reported that when a highly salt-tolerant variety, such as Rangpur lime, was crossed with one of low tolerance, such as Rubidoux trifoliate orange, the progeny exhibited varying degrees

of salt tolerance, indicating heritable nature of salt tolerance in citrus. Further, they used Cl⁻ tolerant selections such as Cleopatra mandarin, Rangpur lime, Sunki mandarin and Shekwasha mandarin for breeding salt tolerant rootstocks. They concluded that inheritance of salt tolerance was a quantitative trait and produced some F₁ seedlings which were as tolerant to salinity as Cleopatra mandarin.

Bhambota and Kanwar (1969) conducted experiments in sandy loam soil with a total salt concentration of about 1900 ppm and reported that Kharna khatta, galgal, trifoliolate orange and rough lemon were progressively less tolerant to salinity when used as rootstocks for Blood Red sweet orange. A progeny (Swingle citrumelo) from a cross between Duncan grapefruit and *Poncirus trifoliata* was reported as moderately tolerant to salinity by Hutchinson (1974).

Ream and Furr (1976) screened hybrids having one parent as Cleopatra mandarin or Rangpur lime alongwith sour orange and reported that 20 hybrids were as tolerant to salinity as the tolerant parents. Patil and Bhambota (1978) studied the behaviour of six citrus rootstocks grown at salinity levels of 2-8 mmhos/cm in pots and reported Cleopatra mandarin, Jatti Khatti and Jullundhari Khatti as the most tolerant rootstocks based on per cent mortality, stem and root growth and leaf number.

Rokba *et al.* (1979) used saline irrigation water (NaCl + CaCl₂) and screened the citrus rootstocks and hybrids. Cleopatra mandarin proved to be the most salt tolerant roostock. Mobayen and Milthorpe (1980) grew seedlings of three rootstocks in sand and subjected them to NaCl conc.

from 2.6-152.6 mol/m³ in basic nutrient solution for 4 or 6 weeks. Bakraie mandarin, followed by Cleopatra mandarin were reported as salt tolerant. Further, they suggested that leaf abscission and expansion rates may provide useful indices for screening seedlings for salinity tolerance and leaf analysis being less satisfactory.

When seedlings of *Citrus reticulata* and *C. grandis* were grown at NaCl conc. of 0, 200, 300, 400, 500, 1200 or 2000 ppm, seedlings of *C. grandis* were more tolerant to high NaCl conc. (Harahap, 1984). Syvertsen *et al.* (1988) used mature Valencia orange trees grafted on *P. trifoliata* or sweet orange and irrigated them with river water or river water + NaCl. They reported the specific effect of rootstocks/scions on ion accumulation and growth of the trees under salinity.

Gallasch and Dalton (1989) conducted the experiment in a shade house, using highly saline irrigation water (8700 μ S/cm conductivity) and screened 28 imported rootstocks along with three rootstocks widely used in Australia. Fifteen of the imported rootstocks showed no significant reductions after 84 days of salt treatment and were, therefore considered as highly salt tolerant. The response of five citrus rootstocks to soil salinity was investigated in green house studies by Hassan and Galal (1989). When seedlings were 6 months old, salt treatment was applied. The salt solution consisted of Ca, Mg, Na, K, CO₃, HCO₃⁻, Cl and SO₄⁻. Rangpur lime was recorded as the most tolerant rootstock to salinity.

When seedlings of Cleopatra mandarin, sour orange, rough lemon and Troyer citrange, growing in pots in green house were supplied with

nutrient solutions containing NaCl, KCl or CaCl₂ at 20 or 40 meq/l, seedling growth was progressively affected by all salt levels. NaCl had the most adverse effect followed by KCl and CaCl₂. Cleopatra mandarin was least affected and rough lemon and Troyer citrange were the most affected, whereas sour orange was intermediate (Salem and El-Khorieby, 1989).

According to Chen *et al.* (1990), the salt injury to citrus plants was due to high conc. of Cl⁻, Na⁺ or HCO₃⁻ in soil and it was possible to solve the problem by using salt tolerant rootstocks as the absorption and accumulation of Cl⁻ and Na⁺ are controlled by rootstock. Further, they stated that salt tolerance is a heritable quantitative character in citrus, so, it is of great importance to identify and screen the salt tolerant germplasm, which can be used directly as rootstocks or breeding material.

Nieves *et al.* (1991) grew one year old Verna and Fino lemon cultivars budded onto sour orange or Alemow in nutrient solutions containing 2 (control), 40 or 80 mmol NaCl/m³ for 75 days. Growth of all combinations was affected by increased salinity, but the effect was more severe on Alemow. They concluded that differences in the parameters determined in response to salinity were attributable to the different capacity of each specific rootstock/scion combination to import Cl⁻ and Na⁺ to leaves.

Sykes (1992) reported selection of new salt excluding citrus hybrids which performed better than their parents when grafted with a common scion and grown in artificially salinized field plots. Lea-Cox and Syvertsen (1993) grew five months old Cleopatra mandarin and *C. volkameriana* seedlings in pots of fine sand and irrigated with either non-saline (ECe =

0.23 dS/m) or saline ($E_{ce} = 6.13$ dS/m) by using 3:1 NaCl: CaCl₂ solution over a four weeks period. *Citrus volkameriana* was reported as more tolerant to salinity than Cleopatra mandarin.

Singh *et al.* (1997) screened six months old seedlings of 20 rootstocks in a pot experiment against four levels of Cl⁻ in irrigation water viz., control, 300, 600 and 900 ppm Cl⁻ through NaCl applied upto two months and reported the existence of strainal variability within a rootstock. Khanna and Kumar (1990) grew two year old budlings of Nagpur mandarin budded on rough lemon in pots containing salinized soil from control (0.3 mmhos/cm), NaCl at 3, 6 and 9 mmhos/cm, Na₂SO₄ at 3, 6 and 9 mmhos/cm and NaCl + Na₂SO₄ at 3, 6 and 9 mmhos/cm. They reported that NaCl was more harmful than the Na₂SO₄.

In a pot experiment with seedlings of four rootstocks irrigated with control, 300, 600, 900 or 1200 ppm Cl⁻ through NaCl for 75 days, it was reported that trifoliate orange was the most tolerant rootstock to salinity (Srivastava *et al.*, 1998). Levy *et al.* (1999) studied the effect of irrigation with saline water on six citrus genotypes in a short term field experiment against salinity levels ranging from 2.0 to 6.4 dS/m. Cleopatra mandarin was observed as the best Cl⁻ excluder.

2.4.2 *In vitro* selection and breeding for salt tolerance

Kochba and Spiegel-Roy (1980) selected ovular callus lines of Shamouti orange which had increased tolerance to NaCl after treating ovular callus with gamma-rays (8-16 kr). The selected lines had more rapid growth rate and were stable after removal of selection pressure.

Embryoids were developed from the selected callus lines. Further, Ben-Hayyim and Kochba (1982) reported that the selected lines were able to grow in presence of 0.2 M NaCl. They observed that the growth curve of the selected salt tolerant lines in presence of NaCl showed a longer lag period, slightly slower rate of increase in fresh weight and about 50 per cent yield as compared with the same line in absence of the salt.

According to Kochba *et al.* (1982) the increased salt tolerance of callus lines of Shamouti and sour orange was retained even after three consecutive transfers in medium without salt. The NaCl selected lines also grew well on medium containing Na_2SO_4 or K_2SO_4 but poorly on a medium containing KCl. They further reported that embryoids regenerated from salt tolerant callus survived and grew better in saline medium than embryoids regenerated from the original salt sensitive callus. Further, Ben-Hayyim and Kochba (1983) described that the NaCl tolerant ovular callus line of Shamouti orange proved to be a true cell line variant.

Matsumoto and Yamaguchi (1984) reported the development of seven salt tolerant lines from EMS treated material and the lines with highest salt tolerance were derived from the material which had undergone the longest exposure to EMS.

Ben-Hayyim *et al.* (1985) compared the selected NaCl-tolerant cell lines with non-selected lines in their growth and internal ion content. All the selected lines took up similar or even larger amounts of Na^+ and Cl^- than NaCl-sensitive cell lines, but selected lines maintained higher growth rate and K^+ content than the non-selected lines when grown under similar

NaCl conc. Selection for tolerance to NaCl in *Citrus sinensis* and *C. aurantium* was also attempted by Spiegel-Roy and Ben-Hayyim (1985). They selected tolerant cells in media containing upto 0.17 M NaCl. Embryoids from a selected line of *C. sinensis* showed greater tolerance for NaCl in medium than comparable embryoids of unselected line. Further, they compared plantlets from one tolerant line with plantlets from unselected controls and reported that the selected lines had greater adaptation to salt (0.085 M to 0.12 M of NaCl) and a lesser degree of leaf burn symptoms.

Plant regeneration from a NaCl-selected salt-tolerant callus culture of Shamouti orange was attempted by Ben-Hayyim and Goffer (1989) and they concluded that NaCl interfered with the regeneration process, with embryogenesis and/or embryo development into plantlets. Its presence during callus growth probably changed the balance of phytohormones which was later manifested in plant regeneration. They further reported that citrus salt tolerant callus yielded salt-tolerant embryos and salt tolerant calli derived from regenerated plantlets, which indicated acquisition of salt tolerance on the whole plant level.

Deng *et al.* (1989) developed ovular callus in the medium and protoplasts were isolated and irradiated with various mutagens and again calli were induced and NaCl tolerant cell lines were selected after 6-7 passages of irradiated callus in medium containing 0.8 per cent NaCl and plantlets were regenerated. They further observed that selected mutants exhibited higher proline accumulation, lower peroxidase activity and altered Na⁺ and Cl⁻ absorption as compared to original callus lines.

Two models were obtained by computer aided fitting of the base-survival curves of cultured cells after exposure to gamma rays and EMS by Deng *et al.* (1990). They reported the selection of NaCl tolerant lines of Jincheng and Taoyecheng by subjecting their nucellar calli to gamma-rays and EMS followed by 10 passages of selection *in vitro*. The selected lines were reported stable in salt tolerance, accumulated more proline, maintained higher level of K⁺ and absorbed less Cl⁻ and Na⁺ than the original calli. Protoplasts were isolated from the selected calli and they were developed into embryoids which were later germinated into plantlets.

Citrus limonum embryogenic calli were selected on medium of several levels of NaCl by Piqueras and Hellin (1992). They reported that unselected and selected tolerant callus lines showed marked differences in their growth curves and salt tolerance. The maximum level of tolerance achieved completely inhibited the unselected line.

Garcia-Agustin and Primo-Millo (1992) reported the regeneration of plants from ovular callus of Troyer citrange which was subjected to EMS. Nucellar tissue cultured to induce embryoids and plantlets were regenerated and regenerated plants were exposed to 45 mM of NaCl. Three possible mutants were selected and these were further propagated by cuttings and were exposed to increasing salinity levels. The selected plants showed better growth, less leaf damage and lower Cl⁻ and Na⁺ but higher K⁺ levels in leaves than wild type. They further reported the recovery of a NaCl tolerant plant of Troyer citrange, which grew faster, accumulated more Na⁺ in shoots and accompanied by a significant reduction in K⁺.

conc. and concluded that this plant restricted accumulation of Na⁺ in leaves by concentrating it in shoots and roots (Garcia-Agustin and Primo-Millo, 1995). Similarly, Spiegel Roy and Saad (1997) reported that in green house experiments irrigated with saline water (upto 2000-2500 mg Cl⁻/litre), plants from tolerant cell line (R₁) showed increased plant height and higher plant survival compared to wild line.

Piqueras *et al.* (1996) reported the changes in antioxidant enzymes and organic solutes associated with adaptation of citrus cells to salt stress. The selected callus accumulated more Na⁺ and Cl⁻ than non-selected one. Proline and total sugars, mainly sucrose were higher in tolerant selected lines and they also had higher choline and glycine betaine.

Wanas *et al.* (1999) reported screening of seedlings from five rootstocks *in vitro*. The seedlings were subcultured at 4-weekly intervals to solid or liquid MS medium containing progressively increasing conc. (0-5000 ppm) of a mixture of NaCl, CaCl₂ and MgCl₂. Seedlings demonstrated a progressive degree of adaptation to salinity as they were subcultured. Plantlets, which survived exposure to 5000 ppm salinity *in vitro* also survived exposure to 4000 ppm salinity in sand culture.

Tusa *et al.* (1988) tested the behaviour of 14 sour orange accessions towards *Phytophthora* gummosis under *in vivo* and *in vitro* conditions and reported a correlation between two series of results. Similarly, Geraci *et al.* (1988) used culture filtrates of *Phoma tracheiphilla* to test lemon resistance to mal secco disease in order to develop an *in vitro* technique for rapid screening. The resistance of 14 lemon clones was tested by

treating seeds with culture filtrates of the fungus. The significant correlation between the results of *in vitro* screening tests and those of previous field inoculations proved the reliability of the technique.

CHAPTER - 3

MATERIALS AND METHODS

The present investigation was carried out during 1998-99 and 1999-2000 in the Department of Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar.

3.1 MATERIAL

3.1.1 Seed Material

The seeds of the three citrus rootstocks viz., Rangpur lime (*Citrus limonia* Osbeck), rough lemon (*C. jambhiri* Lush.) and trifoliate orange (*Poncirus trifoliata* Raf.) collected from National Research Centre for Citrus, Nagpur, Maharashtra. The seeds were collected from a single tree for each rootstock. These rootstocks were selected for the present investigation because, rough lemon is the most commonly used rootstock for citrus in India, Rangpur lime is known for producing quality fruits from the scions grafted on it and is presently replacing rough lemon for mandarins and sweet orange and trifoliate orange is a hardy rootstock, resistant to *Phytophthora* foot and root rot, citrus tristeza virus and citrus nematode and is being used as a leading rootstock in countries like Japan.

The seeds were extracted from fruits, washed with water and air dried under shade and were treated with Bavistin @ 2g/100g seeds. The seeds

were stored in refrigerator at 4°C till they were used for various experiments.

3.1.2 Chemicals and Glassware

The chemicals used in the present study were of high purity and quality and were purchased from Hi-media, BDH, Glaxo, E' Merck and s.d. Fine Chemicals Ltd., companies.

All the glassware used during the investigation was of borosilicate quality and obtained either from Borosil India Limited or Corning Glass Company.

3.1.3 Collection of soil

The soil was collected from the sand dunes from village Ludas near Hisar. The collected soil was passed through 2 mm sieve and subjected to analysis in order to determine its mechanical and chemical composition. The details of mechanical and chemical analysis of soil are given below :

(a) Mechanical analysis		(b) Chemical analysis	
(i) Sand	87%	(i) Organic carbon	0.05%
(ii) Silt	8.3%	(ii) pH	7.0
(iii) Clay	4.7%	(iii) Electrical conductivity of saturated extract	0.65 dS/m
(iv) Texture	Sandy		

3.2 METHODS

The present investigation was carried out in three sets of experiments:

3.2.1 *In vivo* and *in vitro* seed germination of different rootstocks under different salinity regimes

3.2.1A *In vivo* seed germination

(a) Preparation of saline soil

Five kg of soil was taken in a pot lined with polyethylene bag. The salinity was developed in the soil as per the procedure given below and the salts used for the development of salinity are described in Table 2.

The salts were taken in a 500 ml beaker and dissolved in water and final volume was made to 15 l. From this solution 1.5 l/pot was added to pots containing 5 kg of soil. After drying, this soil was thoroughly mixed.

(b) Filling of pots with soil

After development of desired salinity levels, the soil was dried and mixed thoroughly. The earthen pots of 2 kg capacity were lined with polyethylene bags and 1.5 kg of soil was filled in it.

(c) Sowing of seeds

The seeds of all the rootstocks were sown after treatment with Bavistin @ 2g/100g seeds. The seeds were sown at a depth of one cm. After sowing, the seeds were covered with the soil. The seeds were sown in three replications with 15 seeds/replication in factorial completely randomized design (CRD).

Table 2. Development of different salinity regimes in pot soil

Salt	Quantity used (g)									
	Chloride dominated salinity					Sulphate dominated salinity				
	Control	2 dS/m	4 dS/m	6 dS/m	8 dS/m	Control	2 dS/m	4 dS/m	6 dS/m	8 dS/m
NaCl	-	10.959	21.918	31.563	46.029	-	-	-	-	-
Na ₂ SO ₄	-	-	-	-	-	-	15.979	31.959	47.938	66.581
CaCl ₂	-	3.440	6.891	9.923	14.477	-	4.134	8.269	12.404	17.234
MgCl ₂	-	2.867	5.718	8.235	12.002	-	8.006	16.013	24.019	33.352
MgSO ₄	-	13.865	27.731	39.933	58.235	-	11.092	22.185	33.277	46.218

(d) Irrigation in pots

The irrigation in pots was provided whenever required with deionized water.

(e) Data recording

The following observations were recorded for this experiment :

(i) Initiation of seed germination : It was recorded when the first seedling emerged.

(ii) Per cent seed germination : It was recorded at four days interval and finally when maximum seed germination occurred i.e. after 90 days of sowing.

(iii) Seedling height (cm) : Recorded from soil level in the pot to the tip of upper most leaf of the seedling.

(iv) Stem diameter (cm) : It was recorded with the help of Vernier calliper at 0.5 cm above the soil level.

(v) Number of leaves/plant : Recorded at the time of termination of the experiment.

(vi) Fresh aerial and root biomass (g)/plant : Recorded at the time of termination of the experiment.

(vii) Dry aerial and root biomass(g)/plant : The aerial parts and roots were dried in oven at 60°C till the weight became constant and then weighed.

3.2.1B *In vitro* seed germination**(a) Sterilization of water, glassware and instruments**

Glassware was thoroughly washed with running tap water, rinsed with distilled water and dried in oven at 180°C for 30 min. The glassware viz. pipettes, beakers, petridishes, flasks etc. and scalpels and forceps were

heat sterilized in an oven at 180°C for 2 hrs. The distilled water was sterilized in an autoclave at 1.5 kg cm⁻² at 121°C temperature for 15 min. In order to maintain aseptic conditions, all the culture manipulations were carried out in a laminar clean air work station.

(b) Preparation and storage of culture media and stock solutions

During the course of investigation, Murashige and Skoog (1962) medium (MS) was used. Basal MS media with or without growth hormones were used for seed germination (Table 3). For creation of salt stress, the media were supplemented with different conc. of NaCl. All the constituents of the medium were mixed and final volume was made after testing pH. The pH of medium was adjusted to 5.75 with the help of 1N hydrochloric acid and 1N sodium hydroxide. For solidification of the medium, agar was used. After dissolving agar by heating, the medium was poured either in bottles or in test tubes. The bottles were plugged with lids and test tubes were plugged with cotton plugs, and autoclaved at 1.5 kg cm⁻² at 121°C temperature for 15 min. After autoclaving, the medium was stored in dark at 20-25°C and used within 10 days of preparation.

Stock solutions (1x, 5x, 10x, 100x) of growth hormones, vitamins, amino acids, chelating agents, major and minor salts were prepared separately. These stock solutions were stored in refrigerator at 1-4°C. All the stocks were utilized within a month of their preparation.

(c) Culturing of seeds

The seeds were washed thoroughly with running tap water. After removing both the seed coats (outer and inner), the seeds were taken in a

Table 3. Composition of Basal MS (Murashige and Skoog, 1962) medium

Constituents		MS (mg l ⁻¹)
Major salts	KNO ₃	1900
	NH ₄ NO ₃	1650
	KH ₂ PO ₄	170
	MgSO ₄ ·7H ₂ O	370
	CaCl ₂ ·2H ₂ O	440
Minor salts	MnSO ₄ ·4H ₂ O	16.90
	KI	0.83
	ZnSO ₄ ·7H ₂ O	8.6
	H ₃ BO ₃	6.2
	NaMoO ₄ ·2H ₂ O	0.25
	CoCl ₂ ·6H ₂ O	0.025
	CuSO ₄ ·5H ₂ O	0.025
Vitamins	Myo-inositol	100.00
	Thiamine-HCl	2.0
	Pyridoxine-HCl	0.5
	Glycine	0.5
	Nicotinic acid	0.5
Chelating agents	Na ₂ EDTA	37.3
	FeSO ₄ ·7H ₂ O	27.8
Other's	Sucrose	30,000
	Agar	8,000
	pH	5.75

piece of muslin cloth and washed thoroughly with distilled water. Then the seeds were disinfected by soaking in 0.1 per cent HgCl_2 (w/v) solution for 8 min. After that the HgCl_2 solution was decanted and seeds were washed 5-6 times with sterilized single glass distilled water to remove the traces of HgCl_2 . Sterilized seeds were put in test tubes containing basal MS media with varying conc. of NaCl. Two seeds/test tube were placed in media. Four replications were used and each replication was having 10 tubes. The seeds were grown at a temperature of $25 \pm 0.2^\circ\text{C}$ with a 16/8 (light/dark cycle) hr photo period. The transferring of seeds into MS medium was carried out in a laminar air flow cabinet.

(d) Data recording

The method of data recording and observations recorded were similar to that of *in vivo* seed germination.

3.2.2 Evaluation of salinity tolerance of citrus rootstocks

(a) Raising of seedlings of citrus rootstocks

The seeds of citrus rootstocks were sown in wooden trays containing a mixture of sand and soil (1:1). After three months of sowing, nucellar seedlings were separated from zygotic ones on the basis of visual observations and were transplanted individually in polyethylene bags containing a mixture of sand and soil (1:1). The seedlings were irrigated whenever required. To maintain the vigorous growth of the seedlings, they were fertilized regularly and sprays of insecticides were given to keep the seedlings free from insect-pests.

(b) Preparation of saline soil

Two types of salinities were created in the soil viz Cl^- dominated salinity and SO_4^{--} dominated salinity by addition of a group of salts (Table 2) as per the procedure described earlier. The earthen pots were lined with polyethylene bags to avoid leakage of water. Five kg of dried soil was put in each pot. Saline solutions were prepared by dissolving the salts in 15 l of deionized water and 1.5 l of this solution was added to each pot. In control pots, only 1.5 l of deionized water was added. On one side of the pot, one plastic pipe, 1.2' long and of 1" diameter was inserted, which was having perforations at the bottom end.

(c) Transplanting of seedlings in pots

As the condition of the soil reached, eight months old seedlings were planted bare-rooted in pots. In each pot one seedling was planted and in each salinity level, three replications/rootstock were used in factorial CRD.

(d) Irrigation in seedlings

The seedlings were irrigated with deionized water whenever necessary. The irrigation was provided once from the surface of the soil and once through the plastic pipe to maintain the balance of the salts in the soil. To avoid the entry of rain water in pots, the roof of the screen house was covered with polyethylene sheet.

(e) Maintenance of seedlings

To maintain the temperature and humidity in the screen house, the sides were covered with gunny bags, which were sprayed with water thrice a day during summer months. Weeding in pots was done regularly.

Insecticide sprays (with monocrotophos @ 0.5 ml/l and endosulphon @ 1.0 ml/l) were given whenever incidence of leaf miner and lemon butterfly larvae was observed. The seedlings were uniformly fertilized three times with a mixture of macro and micro-nutrients through irrigation water (50 g urea+200 g single super phosphate+ 50 g muriate of potash+ 20 g each of Zn, Fe and Mn dissolved in 10 l of water and 250 ml of this solution was added to each seedling). This experiment was terminated after eight months of seedling transplantation in saline media.

(f) Data recording

The following observations were recorded in this experiment :

(f1) Observations recorded at the time of initiation of the experiment

(i) Plant height (cm) : Measured from soil level in the pot to the tip of upper most leaf.

(ii) Stem diameter (cm) : Measured with the help of Vernier calliper, one cm above the soil level in pot.

(iii) Number of leaves/plant : Recorded at the time of initiation of the experiment.

(iv) Number of branches/plant : Number of branches/plant was recorded at the time of initiation of the experiment.

(v) Leaf area (cm²) : Leaf area was recorded with the help of leaf area meter.

(f2) Appearance of salt toxicity symptoms : Appearance of salt toxicity symptoms was recorded at seven days interval.

(f3) Extent of leaf scorching : Leaf scorching was recorded on a visual scale of 1, 5 and 10 per cent leaf burning.

(f4) Mortality of plants : The plant mortality was recorded at weekly interval.

(f5) Final observations : The following observations were recorded at the time of termination or after the termination of the experiment.

(f5a) Growth parameters

- (i) Plant height (cm)
- (ii) Number of branches/plant
- (iii) Total number of leaves/plant
- (iv) Number of affected leaves/plant
- (v) Number of dead leaves/plant
- (vi) Number of healthy leaves/plant
- (vii) Leaf area (cm²) : Leaf area was measured with the help of leaf area meter. Five leaves were used/replication.
- (viii) Stem diameter (cm) : Stem diameter was recorded with the help of Vernier calliper
- (ix) Fresh leaf, stem and root weight (g) : They were measured after uprooting the plants.
- (x) Dry leaf, stem and root weight (g) : The leaves, stem and roots were dried in an oven at 60°C till a constant weight reached and then weighed.

(f5b) Estimation of chemical constituents

Dried samples of aerial parts (leaves + stem) and roots were analysed for Na⁺, K⁺, Ca⁺⁺, Mg⁺⁺, Cl⁻ and SO₄⁻ ions content.

Digestion of samples for estimation of Na⁺, K⁺, Ca⁺⁺, Mg⁺⁺ and SO₄⁻

The dried samples were ground to a fine powder with the help of a grinder. 500 mg of ground powder for leaf+stem and root of each seedling

was taken in a 100 ml flask. Twenty ml of diacid (4 parts nitric acid + 1 part perchloric acid) were added to each sample. The flasks were kept for overnight. Next day the samples were digested in fumehood at a temperature of around 200°C till a clear solution was obtained. After cooling, the volume was made to 50 ml and filtered through Whatman Filter No. 1. The filtered samples were kept in air tight plastic bottles till they were analysed for Na^+ , K^+ , Ca^{++} , Mg^{++} and SO_4^- content.

Digestion of samples for estimation of Cl^- content

For digestion of samples and for estimation of Cl^- content, the method of Chhabra (1973) was followed. Fifty mg of finely ground dry sample was taken in 100 ml glass beaker alongwith 10 ml of supporting electrolyte (0.5 M KNO_3 and 0.5M HNO_3). It was continuously stirred for 15 min. using a magnetic stirrer. The coloured extract so obtained was decolorised by addition of Darco G60 (charcoal) and filtered. The filtrate so obtained was used for the estimation of Cl^- .

Estimation of sodium and potassium

Sodium and potassium content was determined from the samples already digested by using Flamephotometer.

Estimation of calcium and magnesium

Calcium and magnesium content was determined by Versenate method of Chang and Bray (1951), by using the already digested samples.

Estimation of chloride

Chloride content was determined by using the method of Chhabra (1973) from the pre-digested samples.

Estimation of sulphate

Total sulphate was determined by Turbidity method using Spectronic-20 (Chesnin and Yien, 1950).

3.2.3 *In vitro* screening of citrus rootstocks at callus level

(a) Raising of seedlings for source of explants for initiation of callus

The seedlings were either raised in the pots in a screen house or the seeds were germinated *in vitro* for taking explants for callus induction.

(b) Callus initiation and development

The callus was initiated from hypocotyl and internodal stem segments of *in vivo* or *in vitro* grown seedlings of the citrus rootstocks. The internodal stem segments and root segments were taken from *in vitro* grown seedlings when they were 14-18 days old. Internodal stem segments from *in vivo* grown seedlings were taken from the freshly growing flushes/shoots (measuring 10 cm in length). The leaves and thorns were removed and the shoots were thoroughly washed with single glass distilled water containing few drops of teepol. The shoots were then sterilized with 0.1% HgCl_2 (w/v) for 8-10 min. After that the shoots were thoroughly washed with sterilized single glass distilled water to remove the traces of HgCl_2 . The sterilized shoots were placed in sterilized Petri dishes and internodal stem segments, 4-5 mm long were cut aseptically and placed on modified MS media containing varying conc. of growth hormones, sucrose or other chemicals. All the operations were carried out in a laminar air flow cabinet.

Internodal stem segments and root segments (4-5 mm) from 14-18 days old *in vitro* grown seedlings were taken aseptically and placed on

callus induction media. The culture bottles and test tubes containing explants for callus induction were cultured in dark at $25 \pm 0.2^\circ\text{C}$. Callus induction was observed regularly. The induced calli were subcultured after three weeks in fresh media.

(c) Salt stress to calli of citrus rootstocks

MS modified medium supplemented with varying conc. of NaCl viz. control (no NaCl), 0.05M, 0.10M, 0.15M and 0.20M was used for salt stress experiment. A weighed amount of callus was placed in salt stress medium aseptically. In each test tube, only one weighed callus was put. Four replications were used for each treatment and each replication was consisted of ten tubes. The experimental design followed was factorial CRD.

(d) Data recording

The following observations were recorded as described below :

(d1) Number of explants cultured

Total number of explants/rootstock cultured on different media were recorded.

(d2) Per cent callus development

It was calculated as :

$$\frac{\text{Number of explants that induced callus}}{\text{Total number of explants cultured}} \times 100$$

(d3) Callus weight (g)

Initial callus weight (g) : Fresh callus weight (g) before putting it in stress medium.

Final callus weight (g) : Fresh callus weight (g) after completion of the salt stress.

Dry callus weight (g) : The calli (after termination of experiment) were dried in an oven at 60°C till constant weight was obtained and then weighed.

(d4) Callusing index : It was calculated as :

$$\frac{\text{Final callus weight (g)}}{\text{Initial callus weight (g)}}$$

(d5) Estimation of chemical constituents

The dried calli of each rootstock at every salt conc. was ground to fine powder and was analysed for Na^+ , K^+ , Ca^{++} , Mg^{++} , SO_4^{--} and Cl^- contents as described earlier.

3.2.4 Regeneration of plantlets from calli of different rootstocks

The calli from different citrus rootstocks were placed on regeneration media. The calli were subcultured after every fifteen days. The per cent calli under went regeneration and number of shoots/calli was recorded.

3.2.5 Statistical methods

The experiments were laid out in factorial CRD (2 factors and 3 factors) and the statistical analysis was done with the help of a computer.

Statistical parameters

Mean, standard error of difference between two means and critical differences (C.D.) at 5 per cent level of significance were calculated with the help of a computer by using OP Stat programme.

CHAPTER - 4

EXPERIMENTAL RESULTS

The present investigation was carried out to screen different citrus rootstocks against salinity using *in vivo* and *in vitro* techniques. The salient results obtained are described below :

4.1 *In vivo* seed germination of citrus rootstocks at different salinity regimes

During the present investigation, seeds of three citrus rootstocks viz; rough lemon, Rangpur lime and trifoliate orange were sown at different salinity levels viz; control, 2, 4, 6 and 8 dS/m of Cl⁻ dominated salinity under screen house conditions to test their tolerance to salinity.

The reaction of citrus rootstocks to salinity under screen house conditions is given in Table 4. Seed germination was maximum (77.40%) in Rangpur lime, followed by rough lemon (63.93%), whereas, it was minimum (17.27%) in trifoliate orange. In general, with an increase in salinity, there was a reduction in per cent germination (Plate 1 to 3), as maximum germination was recorded in control and minimum in 8 dS/m. In other levels, moderate germination was recorded. The reduction in germination was rootstock specific and this was minimum in Rangpur lime (100 to 58.00%), followed by rough lemon (95.33 to 31%), whereas, in trifoliate orange, germination was not recorded beyond 4 dS/m.

Table 4. Effect of different levels of salinity on per cent seed germination after sixty days of sowing in citrus rootstocks

Rootstock	Salinity level (dS/m)	Per cent germination
Rough lemon	Control	95.33 (77.54)
	2	95.33 (77.97)
	4	60.00 (50.78)
	6	38.00 (38.05)
	8	31.00 (33.81)
	Mean	63.93 (55.63)
Rangpur lime	Control	100.00 (90.00)
	2	89.00 (70.80)
	4	71.00 (57.43)
	6	69.00 (56.37)
	8	58.00 (49.61)
	Mean	77.40 (64.84)
Trifoliate orange	Control	53.33 (46.92)
	2	22.00 (26.96)
	4	11.00 (19.32)
	6	0.00 (0.00)
	8	0.00 (0.00)
	Mean	17.27 (18.64)
CD at 5% for	Rootstocks	2.03
	Salinity levels	2.63
	Rootstocks x Salinity levels	4.55

Values in parentheses are the angular transformed values.



Plate 1. *In vivo* germination in rough lemon seeds



Plate 2. *In vivo* germination in Rangpur lime seeds



Plate 3. *In vivo* germination in trifoliate orange seeds

The data on the effect of salinity on spread of germination in citrus rootstocks is given in Table 5 and Fig.1 to 3. The perusal of data in Table 5, indicated that the increase in salinity reduced and delayed the germination. For initiation of germination, rough lemon required minimum days (14, 14, 14, 22 and 26), followed by Rangpur lime (22, 22, 26, 26 and 30) at control, 2, 4, 6 and 8 dS/m salinity levels, respectively. Trifoliate orange took maximum days (30) for initiation of germination at all the levels of salinity. The days required for maximum germination were not directly related with rootstock, however, for maximum germination, minimum days were taken at control in all the rootstocks, but with increase in salinity, there was a reduction in per cent germination in all the rootstocks, minimum in Rangpur lime (100 to 53%), moderate in rough lemon (95 to 33%) and maximum in trifoliate orange (53 to 0%).

Table 6 depicts the effect of salinity on plant characters and the effects were expressed as per cent increase/decrease over the control. In plant height, minimum decrease on an average basis was recorded in Rangpur lime (-10.05%), followed by rough lemon (-18.46%) and trifoliate orange (-44.11%). With an increase in salinity, there was a decrease in plant height, but its magnitude was different in different rootstocks. Reduction in plant height in different salt treatments occurred maximum in trifoliate orange (zero to -100.00%), followed by rough lemon (zero to -42.72%) and minimum in Rangpur lime (zero to -23.58%), when salinity increased from control to 8 dS/m salinity level. At 2 dS/m salinity level, there was an increase in plant height over control, but it was significant only in Rangpur lime.

Table 5. Effect of different levels of salinity on number of days taken for initiation of germination and maximum germination in citrus rootstocks

Rootstock	Salinity level (dS/m)	Initiation of germination		Maximum germination	
		Time in days	%germination	Time in days	% germination
Rough lemon	Control	14	5	38	95
	2	14	2	54	96
	4	14	2	50	60
	6	22	2	54	38
	8	26	4	70	33
Rangpur lime	Control	22	29	38	100
	2	22	5	66	89
	4	26	7	90	75
	6	26	2	62	58
	8	30	2	50	53
Trifoliate orange	Control	30	7	50	53
	2	30	7	78	24
	4	30	5	58	11
	6		No germination		
	8		No germination		

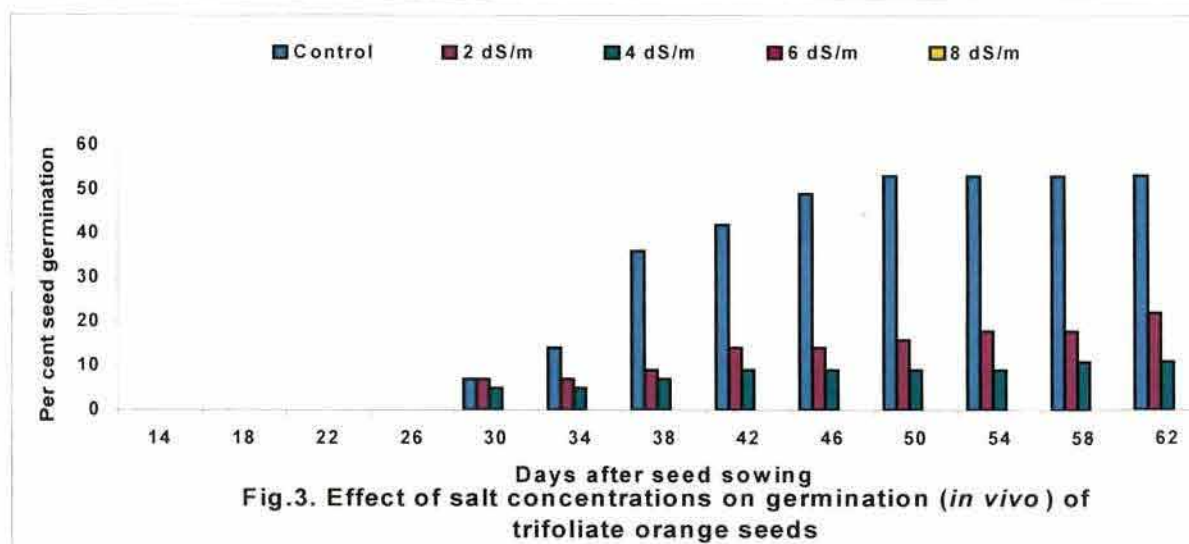
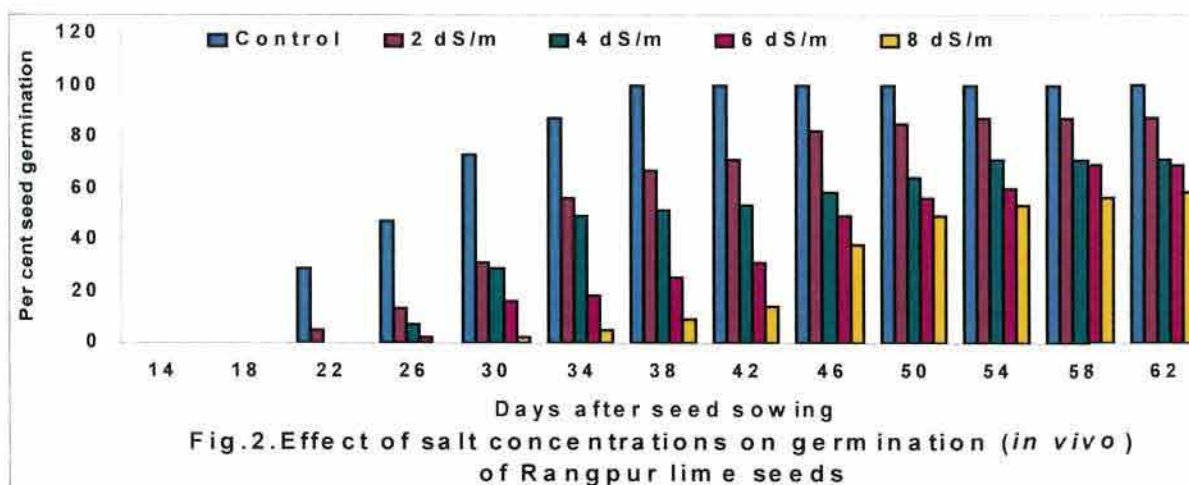
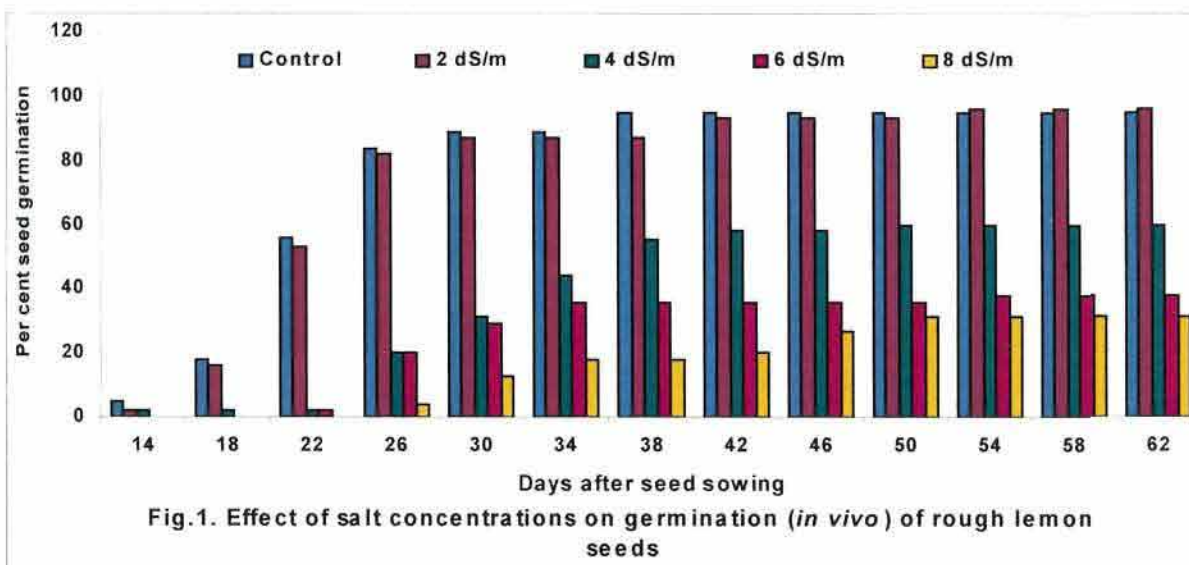


Table 6. Effect of different levels of salinity on plant growth in citrus rootstocks

Rootstock (A)	Salinity level(B) (dS/m)	Per cent increase/decrease in					Total fresh biomass
		Plant height	Stem diameter	No. of leaves	Fresh aerial biomass	Fresh root biomass	
Rough lemon	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	2	0.58 (2.42)	-21.88 (-27.87)	0.73 (3.85)	-13.08 (-20.90)	-12.93 (-13.90)	-13.28 (-20.38)
	4	-15.04 (-22.58)	-47.20 (-43.27)	4.00 (9.81)	-29.82 (-33.01)	-46.64 (-43.04)	-35.09 (-36.23)
	6	-35.10 (-36.19)	-70.44 (-57.06)	-12.85 (-13.07)	-67.96 (-55.77)	-70.50 (-57.26)	-66.25 (-54.51)
	8	-42.72 (-40.81)	-76.20 (-60.86)	-20.49 (-26.83)	-72.92 (-58.75)	-81.50 (-64.58)	-75.50 (-60.40)
	Mean	-18.46 (-19.43)	-43.14 (-37.81)	-5.72 (-5.25)	-36.76 (-33.69)	-42.31 (-35.76)	-38.02 (-34.31)
Rangpur lime	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	2	6.05 (9.32)	0.49 (2.86)	-10.18 (-18.21)	12.60 (13.91)	6.59 (8.91)	8.49 (9.36)
	4	-11.35 (-18.94)	-11.16 (-23.40)	-5.00 (-10.45)	-12.85 (-13.83)	-18.80 (-25.66)	-15.28 (-21.93)
	6	-21.35 (-26.26)	-16.22 (-16.67)	-11.67 (-16.45)	-29.00 (-32.10)	-28.98 (-30.68)	-29.38 (-32.09)
	8	-23.58 (-28.70)	-15.33 (-15.77)	6.85 (-13.42)	-25.83 (-28.79)	-38.03 (-36.81)	-13.25 (-20.04)
	Mean	-10.05 (-12.92)	-8.44 (-10.60)	-4.00 (-11.71)	-11.02 (-12.16)	-15.84 (-16.85)	-9.88 (-12.94)
Trifoliate orange	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	2	-7.89 (-14.89)	-2.14 (-6.53)	-6.72 (-14.90)	3.36 (6.42)	-20.40 (-26.53)	-6.93 (-15.16)
	4	-12.65 (-20.81)	-45.69 (-42.55)	-2.83 (-3.05)	-42.26 (-40.55)	-61.67 (-53.08)	-51.76 (-46.02)
	6	-100.00 (-90.00)	-100.00 (-90.00)	-100.00 (-90.00)	-100.00 (-90.00)	-100.00 (-90.00)	-100.00 (-90.00)
	8	-100.00 (-90.00)	-100.00 (-90.00)	-100.00 (-90.00)	-100.00 (-90.00)	-100.00 (-90.00)	-100.00 (-90.00)
	Mean	-44.11 (-43.14)	-49.57 (-45.82)	-41.19 (-39.59)	-47.78 (-42.83)	-56.41 (-51.92)	-51.74 (-48.24)
CD at 5% for	A	5.40	8.06	10.11	8.25	10.31	6.84
	B	6.97	10.40	13.05	10.65	13.31	8.83
	A x B	12.07	18.01	22.60	18.45	N.S	15.29

Values in parentheses are the angular transformed values.

contd..

Table 6. contd.

Rootstock (A)	Salinity level (B) (dS/m)	Per cent increas/decrease in		
		Dry aerial biomass	Dry root biomass	Total dry biomass
Rough lemon	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	2	-21.01 (-27.06)	0.62 (-1.70)	-12.55 (-20.17)
	4	-44.31 (-41.71)	-51.70 (-45.89)	-47.47 (-43.67)
	6	-65.58 (-54.27)	-57.03 (-49.13)	-59.64 (-50.67)
	8	-67.33 (-55.23)	-72.84 (-58.80)	-68.31 (55.87)
	Mean	-39.65 (-35.66)	-36.19 (-31.10)	-37.59 (-34.06)
Rangpur lime	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	2	1.24 (0.95)	6.82 (9.05)	2.64 (5.78)
	4	-27.48 (-31.42)	-25.60 (-29.85)	-27.39 (-31.32)
	6	-39.92 (-38.96)	-16.27 (-23.00)	-33.69 (-34.38)
	8	-36.69 (-36.88)	-40.91 (-38.54)	-39.12 (-38.19)
	Mean	-20.57 (-21.26)	-15.19 (-16.47)	-19.51 (-19.62)
Trifoliate orange	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	2	-6.39 (-13.95)	-36.68 (-36.69)	-21.02 (-27.12)
	4	-57.58 (-49.39)	-69.20 (-56.32)	-63.26 (-52.72)
	6	-100.00 (-90.00)	-100.00 (-90.00)	-100.00 (-90.00)
	8	-100.00 (-90.00)	-100.00 (-90.00)	-100.00 (-90.00)
	Mean	-52.79 (-48.67)	-61.18 (-54.60)	-56.86 (-51.97)
CD at 5% for				
	A	6.43	9.98	7.93
	B	8.31	12.89	10.24
	A x B	14.38	22.33	17.73

Values in parentheses are the angular transformed values.

Stem diameter was significantly affected by salinity levels. Minimum mean effect of salinity on stem diameter was observed in Rangpur lime (-8.44%), followed by rough lemon (-43.14%) and trifoliate orange (-49.57%). In general, with increase in salinity, there was an increase in reduction over control in stem diameter. Minimum decrease of stem diameter was recorded in Rangpur lime (zero to -15.33%), followed by rough lemon (zero to -76.00%) and trifoliate orange (zero to -100%), as the salinity increased from control to 8 dS/m. There was decrease in stem diameter of rough lemon with increase in salinity, but it was non significant in 2 dS/m and 4 dS/m, 4 dS/m and 6 dS/m and 6 dS/m and 8 dS/m salinity levels. The reduction from 4 dS/m to 8 dS/m salinity level in Rangpur lime was not significant. At 2 dS/m a non-significant increase in stem diameter over control was observed in Rangpur lime. In trifoliate orange, the decrease at 2 dS/m was non significant, but at upper levels of salinity the decrease was- significant.

Mean per cent decrease over control in number of leaves/plant was significantly higher in trifoliate orange (-41.91%), and minimum in Rangpur lime (-5.72%), but decrease in Rangpur lime and rough lemon was non-significant. In rough lemon, there was an increase in leaf number upto 4 dS/m, but it was not different to that of control. However, decrease was non-significant in rough lemon at 6 dS/m and 8 dS/m. Minimum decrease over control in number of leaves/plant at highest salinity level was observed in Rangpur lime, followed by rough lemon and trifoliate orange, but this decrease was not consistent at different salinity levels within a rootstock.

There was minimum mean reduction in fresh aerial bio-mass over control in Rangpur lime (-11.02%), followed by rough lemon (-36.76%) and trifoliate orange (-47.78%). As the salinity increased, fresh aerial bio-mass decreased, but this decrease was non-significant between 6 and 8 dS/m in all the rootstocks. At 8 dS/m, the reduction was maximum in trifoliate (-100%), followed by rough lemon (-72.92%) and Rangpur lime (-25.83%). In rough lemon, non-significant decrease was recorded between 2 and 4 dS/m and 6 and 8 dS/m. In Rangpur lime, there was an increase at 2 dS/m, but it was non significant from control. Again, in Rangpur lime, there was a reduction in fresh aerial bio-mass from 4 dS/m to 8 dS/m, but it was non-significant. In trifoliate orange, there was increase at 2 dS/m, and significant decrease was observed at higher salinity levels.

Similar trend for fresh root bio-mass was obtained in all the rootstocks. Least reduction in mean fresh root bio-mass over control was recorded in Rangpur lime (-15.84%), followed by rough lemon (-42.31%) and trifoliate orange (-56.41%). In general, with increase in salinity, there was reduction in fresh root bio-mass, but this decrease was statistically non-significant.

Minimum reduction over control in mean total fresh bio-mass occurred in Rangpur lime (-9.88%), followed by rough lemon (-38.02%) and trifoliate orange (-51.74%). With the increase in salinity, there was a decrease in total fresh bio-mass and this decrease was maximum in trifoliate orange (zero to -100%), followed by rough lemon (zero to -75.50%) and Rangpur lime (zero to -13.25%). In Rangpur lime, there

was non-significant increase in total fresh bio-mass over control. Maximum reduction for total fresh bio-mass in Rangpur lime (-29.38%) was observed at 6 dS/m, but it was not significantly different from both 4 dS/m and 8 dS/m.

Per cent decrease in mean dry aerial bio-mass over control was maximum in trifoliate orange (-52.79%), followed by rough lemon (-39.65%) and Rangpur lime (-20.57%). In Rangpur lime, it was from no reduction at control to -39.92 per cent at 6 dS/m. In rough lemon, it was from zero at control to -67.33 per cent at 8 dS/m, whereas, in trifoliate orange, the reduction was from zero at control to 100.00 per cent at 6 and 8 dS/m. In Rangpur lime, an increase at 2 dS/m was recorded, but it was not significantly different from control. The reduction in dry aerial bio-mass from 4 dS/m onward was non-significant among themselves in rough lemon and Rangpur lime.

The decrease in mean dry root bio-mass was minimum in Rangpur lime (-15.19%), followed by rough lemon (-36.19%) and maximum in trifoliate orange (-61.18%). As the salinity increased, there was a decrease in dry root bio-mass except at 2 dS/m in rough lemon and Rangpur lime. In rough lemon, the reduction was from zero at control to -72.84 per cent at 8 dS/m, but the reduction between control and 2 dS/m and 4 to 8 dS/m was non-significant. There was an increase in dry root weight at 2 dS/m in Rangpur lime, but it was non-significant. While there was no reduction ~~was~~ in control, it was -40.91 per cent at 8 dS/m in Rangpur lime, but the reduction at 4 to 8 dS/m was non-significant. Similarly, the reduction at 4

to 8 dS/m in rough lemon was non-significant. In trifoliate orange, the decrease was from zero (control) to -100 per cent (8 dS/m).

Minimum per cent decrease over control in mean total dry bio-mass was recorded in Rangpur lime (-19.51%), followed by rough lemon (-37.59%) and trifoliate orange (-56.86%). In all the three rootstocks increase in per cent reduction over control in total dry bio mass was observed with increase in salinity. The reduction in rough lemon was from zero (control) to -68.31 per cent (8 dS/m), but the reduction from 4 to 8 dS/m was non-significant. There was a non-significant increase in total dry bio-mass at 2 dS/m in Rangpur lime over control. The reduction of total dry bio-mass from 4 to 8 dS/m was also non-significant. In trifoliate orange, all the salinity levels significantly reduced the total dry bio-mass.

4.2 *In vitro* seed germination of citrus rootstocks at different salt concentrations

In this study, seeds of three citrus rootstocks were sown under *in vitro* conditions. The seeds of trifoliate orange gave very poor germination in MS medium under *in vitro* conditions. To improve the germination, different conc. of GA_3 were incorporated into the germination medium (Table 7). In MS_2 medium, there was maximum germination (84.31%) and maximum percentage (51.11%) of grown up seedlings. The addition of GA_3 at low conc. (1 mg/l) was found adequate for improving the germination of trifoliate orange seeds. Higher conc. were similar to control or even gave poor response than the control.

Table 7. Effect of different GA_3 concentration in germination medium on germination of trifoliolate orange seeds (two months after seed sowing)

Media code *	No. of seeds sown	No. of seeds germinated	Per cent germination	Grown up seedlings	Per cent grown up seedlings
MS_0	49	23	46.94	6	26.09
MS_2	51	43	84.31	22	51.11
MS_x	22	9	40.91	-	-
MS_y	16	1	6.25	-	-
MS_z	15	8	53.33	2	25.00

* MS_0 = MS basal medium
 MS_2 = MS + 1 mg/l GA_3
 MS_x = MS+2.5 mg/l GA_3
 MS_y = MS+ 5 mg/l GA_3
 MA_z = MS + 10 mg/l GA_3

The seeds of three rootstocks were grown *in vitro* at different salt conc. viz; control, 0.05, 0.10, 0.15 and 0.20 Molar of NaCl in the growth medium to test their tolerance to salinity.

The perusal of data in Table 8, indicated that the rootstocks behaved differently in different salt conc. for seed germination. Maximum mean germination was observed in Rangpur lime (71.60%) (Plate 5) followed by rough lemon (57.13%) (Plate 4), but germination was very poor in trifoliate orange (13.00%) (Plate 6). As the salt conc. increased, there was a decrease in per cent seed germination and this decrease was quite evident in trifoliate orange, where seed germination did not occur beyond control. The reduction in germination was significant in rough lemon and Rangpur lime with increase in salt conc., but the decrease was comparatively low in Rangpur lime at all the salt conc. The germination in Rangpur lime was from 100 per cent at control to 22.00 per cent at 0.20 M, but in rough lemon, it was from 94.00 per cent at control to zero per cent at 0.20M conc.

The effect of increasing salt conc. on number of days taken for initiation of germination and maximum germination in different citrus rootstocks is given in Table 9. The perusal of data in Table 9 revealed that at initiation of germination, there was very less difference in different citrus rootstocks, but with increase in salt conc., there was reduction in initiation of germination. In rough lemon, it was 31 per cent at control and 5 per cent at 0.15 M on 14th day of seed sowing (Fig. 4). In Rangpur lime, it was 64 per cent on 14th day in control and only 9 per cent in 0.15

Table 8. Effect of different levels of salt concentration on per cent seed germination after sixty days of sowing in citrus rootstocks

Rootstock	Salt conc. (Molar)	Per cent germination
Rough lemon	Control	94.00 (75.95)
	0.05	89.00 (70.74)
	0.10	56.67 (48.83)
	0.15	46.00 (42.70)
	0.20	0.00 (0.00)
	Mean	57.13 (47.65)
Rangpur lime	Control	100.00 (90.00)
	0.05	100.00 (90.00)
	0.10	81.00 (64.22)
	0.15	55.00 (47.88)
	0.20	22.00 (27.96)
	Mean	71.60 (64.01)
Trifoliate orange	Control	65.00(53.73)
	0.05	0.00 (0.00)
	0.10	0.00 (0.00)
	0.15	0.00 (0.00)
	0.20	0.00 (0.00)
	Mean	13.00 (10.75)
CD at 5% for	Rootstocks	1.11
	Salt conc.	1.45
	Rootstocks x Salt conc.	2.49

Values in parentheses are the angular transformed values.



Plate 4. *In vitro* germination in rough lemon seeds

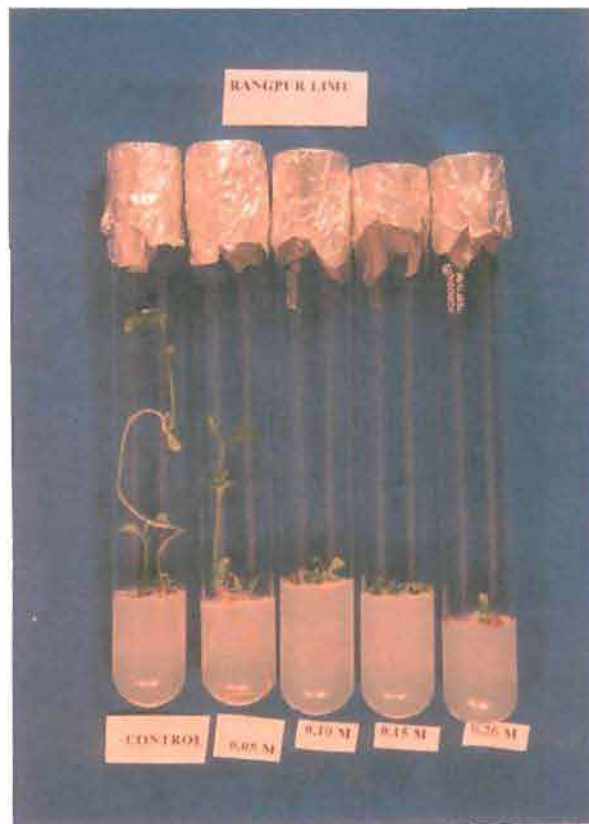


Plate 5. *In vitro* germination in Rangpur lime seeds

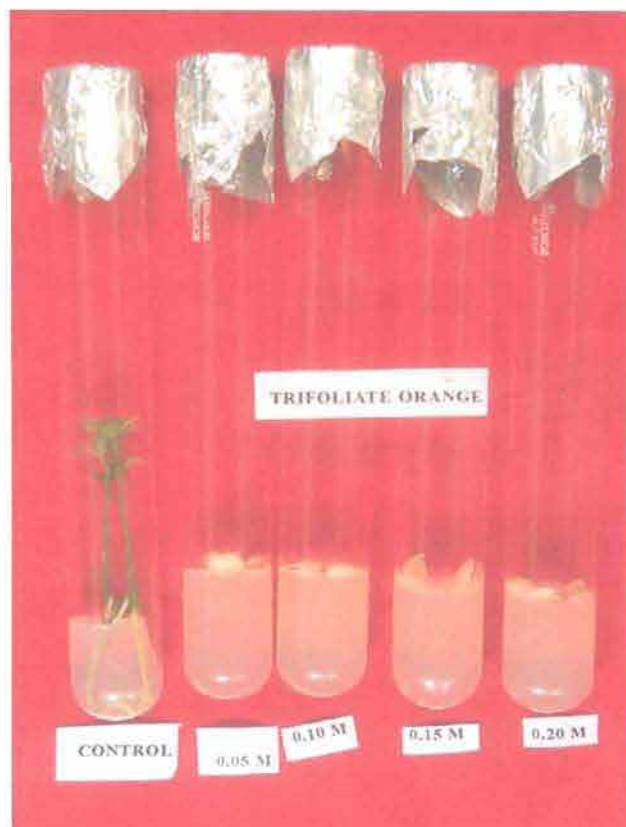


Plate 6. *In vitro* germination in trifoliate orange seeds

Table 9. Effect of different levels of salt concentration on number of days taken for initiation of germination and maximum germination in citrus rootstocks

Rootstock	Salt conc. (Molar)	Initiation of germination		Maximum germination	
		Time in days	%germination	Time in days	% germination
Rough lemon	Control	14	31	38	94
	0.05	14	29	46	89
	0.10	14	15	38	57
	0.15	14	5	58	46
	0.20			No germination	
Rangpur lime	Control	14	64	38	100
	0.05	14	56	34	100
	0.10	14	19	50	81
	0.15	14	9	66	52
	0.20	22	9	45	22
Trifoliate orange	Control	22	17	46	65
	0.05			No germination	
	0.10			No germination	
	0.15			No germination	
	0.20			No germination	

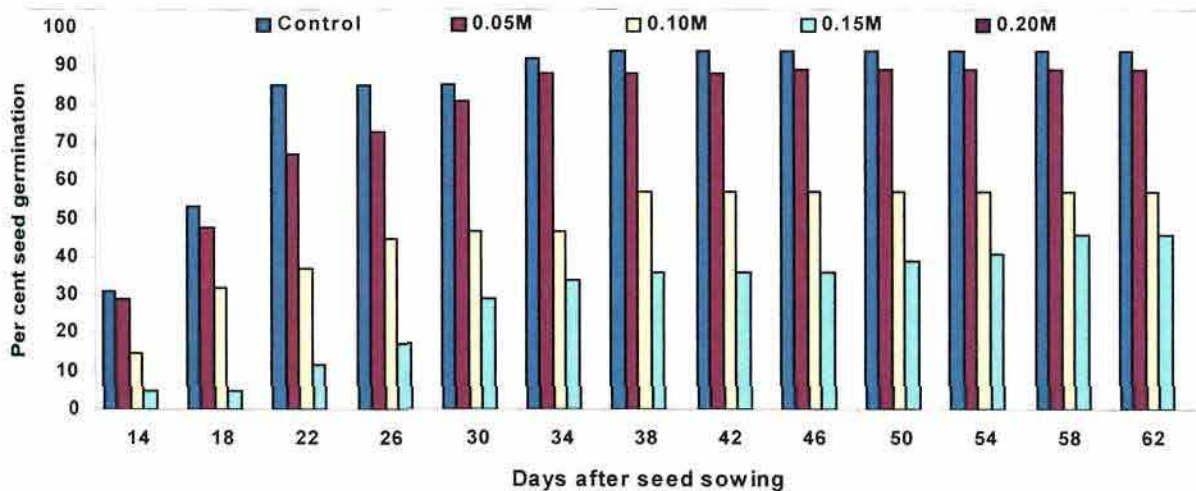


Fig.4. Effect of salt concentrations on germination (*in vitro*) of rough lemon seeds

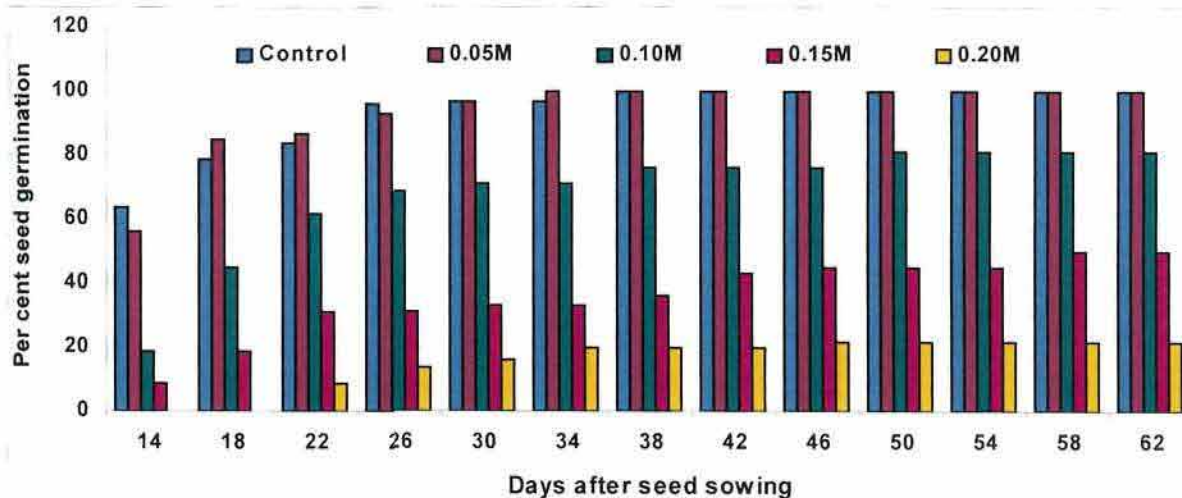


Fig.5. Effect of salt concentrations on germination (*in vitro*) of Rangpur lime seeds

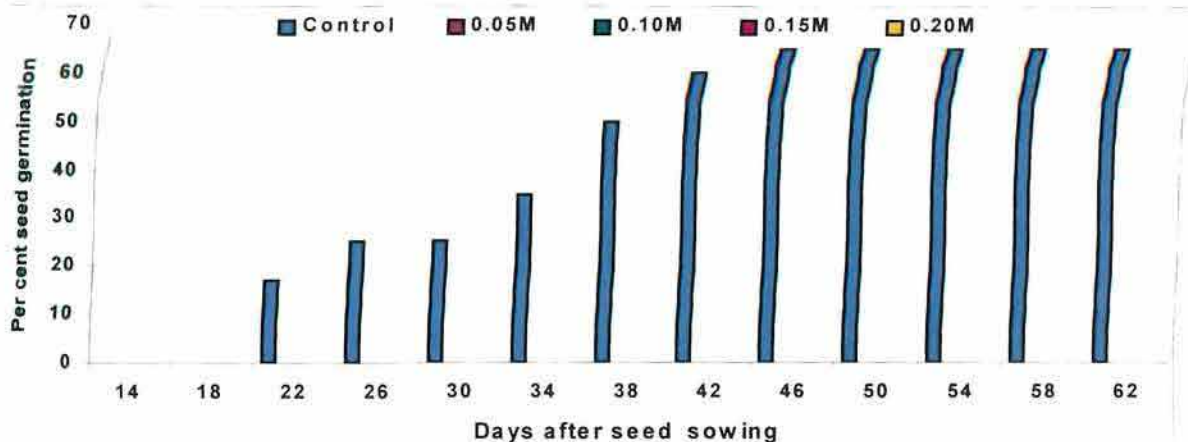


Fig.6. Effect of salt concentrations on germination (*in vitro*) of trifoliate orange seeds

and 0.20 M on 14th and 22nd days respectively (Fig.5). For maximum germination, it took 38 days for 94 per cent germination in control and 58 days for 46 per cent germination in 0.15 M in rough lemon and in Rangpur lime, 38 days for 100 per cent germination in control and 45 days for only 22 per cent germination in 0.20 M salt conc. In trifoliate orange, initiation of germination was recorded on 22nd day and maximum germination on 46th day and beyond control, none of the seeds germinated in high salt levels (Fig.6). So, it is evident that increase in salt conc., reduced and delayed the seed germination.

The results presented in Table 10 depict the effect of salt conc. on plant characters. With an increase in salt conc., a reduction in all the plant characters was observed, except for number of leaves/plant in case of rough lemon.

Maximum plant height (2.814 cm) was recorded in rough lemon, followed by Rangpur lime (1.874 cm) and minimum in trifoliate orange (0.931 cm). As the salt conc. increased, there was more decrease in plant height was more in rough lemon, where it reduced from 7.739 cm in control to 0.344 cm in 0.15 M, but the decrease was less in Rangpur lime, where it was from 4.675 cm in control to 0.433 cm in 0.15 M conc.

Maximum number of leaves/plant was recorded in Rangpur lime (2.239), followed by rough lemon (1.821) and trifoliate orange (0.911). In rough lemon, there was a marginal increase in number of leaves/plant at 0.05 M, but it was non-significant as compared to control. The reduction in number of leaves was more in rough lemon as the leaves/plant decreased

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Table 10. Effect of different levels of salt concentration on plant characters in citrus rootstocks

Rootstock		Salt conc.		Plant characters								
(A)	(B)	(Molar)	(B)	Plant height (cm)	Stem diameter (cm)	No. of leaves/plant	Fresh aerial biomass (g)/plant	Fresh root biomass (g)/plant	Total fresh biomass (g)/plant	Dry aerial biomass (g)/plant	Dry root biomass (g)/plant	Total dry biomass (g)/plant
Rough lemon	Control			7.739	0.133	2.553	0.123	0.051	0.173	0.019	0.008	0.027
	0.05			5.133	0.129	2.833	0.083	0.040	0.123	0.012	0.006	0.018
	0.10			0.853	0.127	2.387	0.016	0.018	0.035	0.003	0.004	0.007
	0.15			0.344	0.133	1.333	0.007	0.017	0.024	0.003	0.003	0.006
	0.20			0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Rangpur lime	Mean			2.814	0.105	1.821	0.046	0.025	0.070	0.007	0.004	0.011
	Control			4.675	0.125	3.277	0.064	0.058	0.122	0.009	0.009	0.018
	0.05			3.211	0.112	2.943	0.043	0.030	0.074	0.008	0.007	0.015
	0.10			1.050	0.100	2.777	0.010	0.010	0.020	0.002	0.002	0.004
	0.15			0.433	0.068	2.200	0.005	0.007	0.012	0.001	0.001	0.002
Trifoliate orange	0.20			0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Mean			1.874	0.081	2.239	0.024	0.021	0.046	0.004	0.004	0.008
	Control			4.654	0.132	4.557	0.078	0.123	0.200	0.014	0.024	0.038
	0.05			0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	0.10			0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
CD at 5% for	0.15			0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	0.20			0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Mean			0.931	0.026	0.911	0.016	0.025	0.040	0.003	0.005	0.008
	A			0.255	0.007	0.329	0.008	N.S	0.012	0.002	N.S	0.002
	B			0.329	0.009	0.424	0.010	0.006	0.015	0.002	0.001	0.003
AxB				0.571	0.016	0.735	0.018	0.010	0.026	0.004	0.002	0.005

from 2.553 in control to 1.333 in 0.15 M, however, the decrease was less in Rangpur lime, where the leaves/plant reduced from 3.27 in control to 2.200 in 0.15 M.

Highest fresh aerial bio-mass (g/plant) was recorded in rough lemon (0.046), followed by Rangpur lime (0.024) and trifoliate orange (0.016). The reduction in fresh aerial bio-mass was more in rough lemon as compared to Rangpur lime with the increase in salt conc. In rough lemon, it decreased from 0.123 g/plant in control to 0.007 g/plant in 0.15 M conc., whereas, in Rangpur lime, it was from 0.064 g/plant in control to 0.005 g/plant in 0.15 M salt conc.

In all the rootstocks, mean fresh root bio-mass (g)/plant was not significantly different from each other. With the increase in salt conc., there was decrease in fresh root bio-mass, but this decrease was less in rough lemon (from 0.051 in control to 0.017 g/plant in 0.15 M) and more in Rangpur lime (from 0.058 in control to 0.007 g/plant in 0.15 M salt conc.).

The mean total fresh bio-mass (g)/plant was recorded maximum in rough lemon (0.079 g/plant), followed by Rangpur lime (0.046 g/plant), which was similar to trifoliate orange. The increase in salt conc., resulted in a decrease in total fresh bio-mass/plant, but this decrease was genotype specific. Rangpur lime exhibited more decrease in total fresh bio-mass, where it decreased from 0.122 g/plant at control to 0.012 g/plant at 0.15 M and rough lemon showed less decrease (from 0.173 in control to 0.024 g/plant in 0.15M). In both these rootstocks, the drastic reduction was recorded at 0.10M salt conc.

Maximum mean dry aerial bio-mass (g)/plant was exhibited by rough lemon (0.007 g/plant) followed by Rangpur lime (0.004 g/plant) and trifoliate orange (0.003 g/plant). The dry aerial bio-mass in Rangpur lime and trifoliate orange was more or less the same. Maximum reduction in dry aerial bio-mass (g)/plant was recorded in Rangpur lime with increase in salt conc., where the decrease was from 0.009 g/plant in control to 0.001 g/plant in 0.15 M, but, the reduction in rough lemon was less as it decreased from 0.019 g/plant in control to 0.003 g/plant in 0.15 M.

Similarly, the decrease in dry root bio-mass was recorded for Rangpur lime and rough lemon. This decrease was more in Rangpur lime (0.009 in control to 0.001 g/plant in 0.15 M), and less in rough lemon (0.008 in control to 0.003 g/plant in 0.15 M).

Mean total dry bio-mass (g)/plant was maximum in rough lemon (0.011 g/plant), followed by Rangpur lime and trifoliate orange (0.008 g/plant). With an increase in salt conc., there was a decrease in total dry bio-mass, but this decrease was less in rough lemon (0.027 in control to 0.006 g/plant in 0.15 M) and more in Rangpur lime (0.018 in control to 0.002 g/plant in 0.15 M). The decrease was maximum at 0.10 M in both the rootstocks.

4.3 Screening of citrus rootstock seedlings for salt tolerance

Seedlings of all the three citrus rootstocks were planted in salinized soil at different salinity levels viz; control, 2, 4, 6 and 8 dS/m under the screen house. Two types of salinities, namely chloride (Cl^-) dominated and sulphate (SO_4^-) dominated, were used and data were recorded.

4.3.1 Effect of salinity on plant characters

The data on per cent mortality of seedlings in different salinity types and levels are presented in Table 11. The perusal of data in Table 11 revealed that mortality of rootstock seedlings was more in SO_4^- dominated salinity, but Cl^- dominated salinity proved to be more deleterious, as seedlings in the later salinity type died at an early age. In trifoliate orange, none of the seedlings survived beyond control (Plate 7 to 16) in both salinity types. In rough lemon, mortality started at 4 dS/m in both salinity types (Plate 7 to 16), whereas, in Rangpur lime, seedling mortality was observed at 6 and 8 dS/m only in SO_4^- dominated salinity and at 8 dS/m in Cl^- dominated salinity (Plate 7 to 16).

The data pertaining to effect of salinity on plant height (cm) and stem diameter (cm) are presented in Table 12. These characters were expressed as per cent of control. The mean plant height was significantly higher in SO_4^- dominated (64.04%) as compared to Cl^- dominated (54.99%) salinity. Overall height was maximum in Rangpur lime (89.41%), followed by rough lemon (58.31%) and trifoliate orange (45.19%). The interaction between salinity types and rootstocks was significant, indicating differential performance of rootstocks in different salinity types. With the increase in salinity a decrease in plant height was recorded, but it was lower in Rangpur lime (from 100 at control to 72.30% at 8 dS/m), followed by trifoliate orange (100 to 22.84%) and rough lemon (100 to 18.96%).

Mean stem diameter (cm) was higher in Cl^- dominated (66.30%) than SO_4^- dominated (61.98%) salinity. Highest mean per cent of stem diameter

Table 11. Effect of increasing salinity on per cent mortality of plants in citrus rootstocks

Rootstock	Salinity level (dS/m)	Per cent mortality of plants					
		Cl ⁻ dominated salinity			SO ₄ ²⁻ dominated salinity		
		Mortality (%)	Days		Mortality (%)	Days	
Rough lemon	Control	0	-		0	-	
	2	0	-		0	-	
	4	33.33	181		33.33	216	
	6	66.67	131		100.00	156	
	8	100.00	114		100.00	153	
Rangpur lime	Control	0	-		0	-	
	2	0	-		0	-	
	4	0	-		0	-	
	6	0	-		66.67	210	
	8	66.67	170		66.67	187	
Trifoliate orange	Control	0	-		0	-	
	2	100.00	71		100.00	83	
	4	100.00	50		100.00	71	
	6	100.00	27		100.00	59	
	8	100.00	27		100.00	39	



Plate 7. Growth of citrus rootstock seedlings (Control)



Plate 8. Growth of citrus rootstock seedlings (2 dS/m Cl⁻)



Plate 9. Growth of citrus rootstock seedlings (2 dS/m SO₄⁻²)



Plate 10. Growth of citrus rootstock seedlings (4 dS/m Cl⁻)



Plate 11. Growth of citrus rootstock seedlings (4 dS/m SO₄⁻²)



Plate 12. Growth of citrus rootstock seedlings (Control)



Plate 13. Growth of citrus rootstock seedlings (6 dS/m Cl⁻)



Plate 14. Growth of citrus rootstock seedlings (6 dS/m SO₄⁻²)



Plate 15. Growth of citrus rootstock seedlings (8 dS/m Cl⁻)



Plate 16. Growth of citrus rootstock seedlings (8 dS/m SO₄⁻²)

Table 12. Effect of salinity on plant height (cm) and stem diameter (cm) expressed as per cent of control in citrus rootstocks

Root-stock (B)	Plant height (cm)					Stem diameter (cm)								
	Salinity type (A)*		Mean (B x C)			Salinity type (A)*		Mean (B x C)						
	A ₁ A ₂		Control (dS/m)	2 (dS/m)	4 (dS/m)	6 (dS/m)	8 (dS/m)	A ₁ A ₂		Control (dS/m)	2 (dS/m)	4 (dS/m)	6 (dS/m)	8 (dS/m)
	Mean (A ₁ x B)	Mean (A ₂ x B)						Mean (A ₁ x B)	Mean (A ₂ x B)					
Pooled mean		Pooled mean		Pooled mean		Pooled mean								
Rough lemon	100.00 (52.83)	87.42 (56.65)	44.50 (77.54)	40.68 (39.55)	18.96 (24.89)	58.31 (54.74)	100.00 (65.28)	86.16 (69.14)	80.61 (63.97)	70.00 (56.86)	48.76 (44.47)	77.11 (64.89)		
Rangpur lime	100.00 (75.61)	86.41 (81.68)	97.60 (73.54)	90.76 (79.51)	72.30 (62.34)	89.41 (78.65)	100.00 (79.97)	93.44 (81.01)	84.67 (70.84)	80.39 (67.16)	70.02 (57.42)	85.70 (73.29)		
Trifoliolate orange	100.00 (36.54)	46.36 (53.78)	31.93 (47.73)	24.84 (28.38)	22.84 (26.30)	45.19 (45.16)	100.00 (53.65)	82.51 (65.30)	55.45 (48.47)	36.33 (37.00)	25.88 (30.48)	60.03 (54.25)		
Pooled mean (A)	-	(64.04)	-	-	-	-	(66.30)	-	-	-	-	-		
Pooled mean (C)	100.00 (90.00)	73.40 (66.27)	58.01 (54.31)	52.09 (49.15)	38.03 (37.84)			100.00 (90.00)	87.37 (71.82)	73.58 (61.09)	62.24 (53.67)	48.22 (44.12)		

C.D. at 5%

A = 8.03, B = 9.84, C = N.S., A x B = 13.91,
A x C = N.S.; B x C = 22.00 , A x B x C = N.S.

A = 3.14, B = 3.84, C = 4.96, A x B = 5.43
A x C = N.S., B x C = 8.59, A x B x C = N.S.

*A₁ = Chloride dominated salinity; A₂ = Sulphate dominated salinity; C = Salinity level.
Values in parentheses are the angular transformed values.

over control was recorded in Rangpur lime (85.70%), followed by rough lemon (77.11%) and trifoliolate orange (60.03%). The significance of interaction between salinity types and rootstocks indicated that the rootstocks behaved differently in different salinity types. As the salinity increased, the stem diameter decreased significantly. Maximum decrease from control to 8 dS/m was recorded in trifoliolate orange (100 to 25.88%), followed by rough lemon (100 to 48.76%) and Rangpur lime (100 to 70.02%).

The effects of increase in salinity on total number of leaves/plant and healthy leaves/plant expressed as per cent of control are presented in Table 13. Perusal of results in Table 13 indicated that rough lemon and Rangpur lime did not differ significantly from each other for total number of leaves/plant, but had significantly higher number of leaves than trifoliolate orange (63.63%). The interaction between salinity types and rootstocks was significant, indicating that rootstocks responded differently in different salinity types. The interaction between rootstocks and salinity levels was non-significant.

The number of healthy leaves/plant expressed as per cent of control was highest in Rangpur lime (59.45%) followed by rough lemon (38.60%) and trifoliolate orange (20.00%). The significant interaction between salinity types and rootstocks revealed that different rootstocks behaved variably under different saline conditions. As the salinity increased the number of healthy leaves/plant decreased, whereas, there was not much difference for number of healthy leaves/plant in Cl^- dominated and SO_4^{2-} dominated salinity.

Table 13. Effect of salinity on total number leaves and healthy leaves/plant expressed as per cent of control in citrus rootstocks

Root-stock (B)	Healthy leaves/plant									
	Total number of leaves/plant					Mean (B x C)				
	Salinity type (A)*		Pooled mean			Salinity type (A)*		Mean (B x C)		
	A ₁ Mean (A ₁ x B)		A ₂ Mean (A ₂ x B)			A ₁ Mean (A ₁ x B)		A ₂ Mean (A ₂ x B)		
	Control	2	4	6	8	Control	2	4	6	8
	(dS/m)	(dS/m)	(dS/m)	(dS/m)	(dS/m)	(dS/m)	(dS/m)	(dS/m)	(dS/m)	(dS/m)
Rough lemon	100.00 (80.46)	100.23 (90.00)	99.97 (93.74)	86.79 (72.81)	70.52 (57.80)	91.50 (80.89)	100.00 (90.00)	68.90 (56.39)	22.25 (20.81)	1.85 (0.00)
Rangpur lime	100.00 (89.04)	97.12 (87.97)	98.50 (89.27)	105.41 (93.43)	89.38 (82.88)	98.08	100.00 (90.00)	74.90 (65.04)	58.82 (50.25)	53.87 (46.10)
Trifoliolate orange	100.00 (56.62)	58.84 (50.36)	57.70 (50.08)	54.44 (48.24)	47.18 (43.38)	63.63 (56.41)	100.00 (90.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Pooled mean (A)	(75.37)	-	-	-	-	-	(35.24)	(34.45)	-	-
Pooled mean (C)	100.00 (90.00)	85.40 (76.14)	85.39 (77.70)	82.21 (71.49)	69.03 (61.35)	-	100.00 (90.00)	47.93 (40.47)	27.02 (23.69)	18.57 (16.45)
C.D. at 5%	A = N.S., B = 12.80, C = N.S., A x B = 18.09, A x C = N.S.; B x C = N.S., A x B x C = N.S.									
	A = N.S., B = 6.51, C = N.S., A x B = 9.21 A x C = N.S., B x C = 14.57, A x B x C = N.S.									

*A₁ = Chloride dominated salinity; A₂ = Sulphate dominated salinity; C = Salinity level.
 Values in parentheses are the angular transformed values.

The data in Table 14 revealed that the number of affected leaves and dead leaves/plant varied with the increase in salinity. Salt affected leaves/plant were highest in Rangpur lime (5.60), moderate in rough lemon (3.37) and minimum in trifoliolate orange (1.00). In rough lemon and Rangpur lime, initially, there was an increase in number of affected leaves/plant up to 4 dS/m, but after that the number decreased.

Dead leaves/plant (mean) were highest in rough lemon (18.30) and trifoliolate orange (16.83) followed by Rangpur lime (12.67). The rootstocks behaved significantly different with salinity types as is evident from significant interaction between salinity types and rootstocks. The significance of higher order interaction of salinity types x rootstocks x salinity levels indicated the differential response of rootstocks in salinity types and at different salinity levels. With the increase in salinity, there was an increase in dead leaves/plant, though the extent of decrease was different in different rootstocks.

The perusal of the data in Table 15 revealed that Rangpur lime had maximum mean number of branches/plant (6.47), followed by rough lemon (4.23) and trifoliolate orange (3.10). The rootstocks behaved differently with salinity types. As salinity increased, in general, there was a reduction in number of branches/plant. The reduction for number of branches/plant was from 5.33 to 4.00 in rough lemon, from 8.00 to 4.83 in Rangpur lime and from 4.33 to 2.50 in trifoliolate orange, as salinity increased from control to 8 dS/m.

Table 14. Effect of salinity on number of affected and dead leaves/plant in citrus rootstocks

Root-stock (B)	Affected leaves/plant						Dead leaves/plant															
	Salinity type (A)*		Mean (B x C)				Salinity type (A)*		Mean (B x C)													
			Control	2	4	6			8	Control	2	4	6	8								
	Mean		(dS/m)				Mean		(dS/m)													
	(A ₁ x B)		(A ₁ x B)				(A ₂ x B)		(A ₂ x B)													
	A ₁	A ₂					A ₁	A ₂					A ₁	A ₂								
	Mean		Mean				Mean		Mean				Mean		Mean				Mean		Mean	
	(A ₁ x B)		(A ₁ x B)				(A ₂ x B)		(A ₂ x B)				(A ₁ x B)		(A ₁ x B)				(A ₂ x B)			
	Control		Control				Control		Control				Control		Control				Control			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00		0.00				0.00			
	0.00		0.00				0.00		0.00				0.00									

For leaf area (cm^2)/leaf, significant difference between Rangpur lime and rough lemon was not observed (Table 15), but, both of them differed from trifoliate orange (3.15 cm^2). Interaction between salinity levels and rootstocks was significant. As the salinity increased, leaf area decreased, but the decrease was genotype specific. Maximum decrease occurred in rough lemon (27.33 in control to 8.22 cm^2 in 8 dS/m), followed by Rangpur lime (19.73 to 9.78 cm^2), but decrease was not significant in trifoliate orange (3.47 to 3.00 cm^2).

The data pertaining to fresh leaf weight (g)/plant and dry leaf weight (g)/plant, expressed as per cent of control are presented in Table 16. For fresh leaf weight (g)/plant, Rangpur lime and rough lemon differed significantly from trifoliate orange. The rootstocks behaved differently under different saline conditions as is evident from significant interaction between salinity types and rootstocks. With increase in salinity, a decrease in fresh leaf weight (g)/plant was observed. The decrease was maximum in trifoliate orange (100 to 7.58%), moderate in rough lemon (100 to 27.33%) and minimum in Rangpur lime (100 to 44.59%) at control to 8 dS/m salinity levels, respectively.

Similarly, the dry leaf weight (g)/plant expressed as per cent of control was not significantly different in Rangpur lime and rough lemon, but, both differed from trifoliate orange. Salinity levels and rootstocks interaction was evident for this character. A clear cut decrease was observed in dry leaf weight (g) with the increase in salinity levels, but, this decrease was genotype specific. Minimum decrease was recorded in

Table 16. Effect of salinity on fresh and dry leaf weight (g)/plant expressed as per cent of control in citrus rootstocks

Root-stock (B)	Fresh leaf weight(g)/plant					Dry leaf weight (g)/plant				
	Salinity type (A)*		Mean (B x C)			Salinity type (A)*		Mean (B x C)		
	A ₁ Mean (A ₁ x B)	A ₂ Mean (A ₂ x B)	Control	2 (dS/m)	4 (dS/m)	6 (dS/m)	8 (dS/m)	Pooled mean (B)	A ₁ Mean (A ₁ x B)	A ₂ Mean (A ₂ x B)
Rough lemon	(65.05)	(60.09)	100.00 (90.00)	96.08 (81.74)	77.40 (62.73)	53.35 (47.05)	27.23 (31.33)	70.81 (62.57)	(60.76)	(58.21)
Rangpur lime	(66.70)	(62.07)	100.00 (90.00)	89.61 (79.02)	70.68 (57.69)	63.60 (53.42)	44.59 (41.81)	73.70 (64.39)	(67.65)	(59.01)
Trifo- liate orange	(32.96)	(34.53)	100.00 (90.00)	17.80 (24.94)	13.18 (21.18)	8.43 (16.74)	7.58 (15.87)	29.40 (33.75)	(40.44)	(47.81)
Pooled mean (A)	(54.90)	(52.23)							(56.28)	(55.01)
Pooled mean (C)			100.00 (90.00)	67.83 (61.90)	53.75 (47.20)	41.79 (39.07)	26.47 (29.67)			
C.D. at 5%										

A = N.S.; B = 4.79, C = N.S.; A x B = 6.78;
A x C = N.S.; B x C = 10.72, A x B x C = N.S.

A = N.S.; B = 4.94; C = 6.38.; A x B = 6.99
A x C = N.S.; B x C = 11.05; A x B x C = N.S.

*A₁ = Chloride dominated salinity; A₂ = Sulphate dominated salinity; C = Salinity level.
Values in parentheses are the angular transformed values.

Rangpur lime (100 in control to 50.25% in 8 dS/m), moderate in rough lemon (100 in control to 28.83% in 8 dS/m) and severe in trifoliate orange (100 in control to 20.89% in 8 dS/m).

The data presented in Table 17 reveal the effect of salinity on fresh and dry stem bio-mass (g)/plant expressed as per cent of control in citrus rootstocks. Maximum fresh stem bio mass(g)/plant was recorded in Rangpur lime (82.03%) which was significantly higher than rough lemon (58.67%) and trifoliate orange (51.67%). The rootstocks behaved differently in different salinity types. Fresh stem bio-mass (g)/plant expressed as per cent of control decreased with increase in salinity, but the decrease was genotype specific. Minimum reduction was recorded in Rangpur lime (100 in control to 50.28% in 8 dS/m), followed by trifoliate orange (100 in control to 28.83% in 8 dS/m) and rough lemon (100 in control to 28.60% in 8 dS/m). There was an increase at 2 dS/m (116.41%) in fresh stem bio-mass of Rangpur lime.

Maximum dry stem bio-mass (g)/plant expressed as per cent of control was recorded in Rangpur lime (86.02%) but the dry bio-mass in trifoliate orange (65.97%) and rough lemon (61.81%) was not significantly different from each other. In this case also, the rootstock behaviour differed with salinity types. Increase in salinity levels led to a reduction in dry stem bio-mass. Maximum reduction was recorded in rough lemon (100 to 35.71%) and trifoliate orange (100 to 41.44%), but minimum in Rangpur lime (100 to 51.64%) from control to 8 dS/m, respectively. For this character also, an increase at 2 dS/m was recorded in Rangpur lime.

The observations on fresh and dry root weight (g)/plant expressed as per cent of control are presented in Table 18. Data revealed that fresh root weight was maximum in Rangpur lime (76.12%), followed by rough lemon (59.22%) and trifoliate orange (49.80%) and this was significantly higher in SO_4^- -dominated than in Cl^- dominated salinity. The rootstocks behaved differently in different salinity types. A decrease in fresh root weight in correspondence to an increase in salinity was recorded. The decrease was highest in rough lemon (100 to 26.92%), followed by trifoliate orange (100 to 30.73%) and Rangpur lime (100 to 40.96%), as the salinity increased from control to 8 dS/m. The Rangpur lime showed an increase in fresh root weight at 2 dS/m.

Mean dry root weight (g)/plant was more in SO_4^- -dominated salinity than the Cl^- dominated salinity and different rootstocks showed varying performance with the different salinity types. Significant differences were not observed for dry root weight between Rangpur lime (75.40%) and trifoliate orange (77.04%), but both were having higher dry root weight than rough lemon (62.27%), when expressed as per cent of control. With an increase in salinity, dry root weight was reduced. Minimum reduction was recorded in trifoliate orange (100 in control to 59.70% in 8 dS/m), but maximum reduction was recorded in rough lemon (100 in control to 31.77% in 8 dS/m). For this character also, an increase at 2 dS/m was observed in comparison to control in Rangpur lime.

4.3.2 Effect of salinity on ion content in plant parts

The data pertaining to ions viz; Na^+ , K^+ , Ca^{++} , Mg^{++} , Cl^- and SO_4^- content in aerial parts as well as in roots of citrus rootstocks are presented

Table 18. Effect of salinity on fresh and dry root weight (g)/plant expressed as per cent of control in citrus rootstocks

Root-stock (B)	Fresh root weight(g)/plant					Dry root weight (g)/plant								
	Salinity type (A)*		Mean (B x C)			Pooled mean (B)	Salinity type (A)*		Mean (B x C)			Pooled mean (B)		
	A ₁ Mean (A ₁ x B)	A ₂ Mean (A ₂ x B)	Control	2 (dS/m)	4 (dS/m)		8 (dS/m)	A ₁ Mean (A ₁ x B)	A ₂ Mean (A ₂ x B)	Control	2 dS/m		4 dS/m	8 dS/m
Rough lemon	100.00 (52.61)	83.26 (55.15)	53.89 (66.48)	32.01 (34.45)	26.92 (31.07)	59.22 (53.88)	100.00 (90.00)	81.94 (65.68)	49.86 (44.76)	31.77 (33.92)	62.27 (55.64)			
Rangpur lime	100.00 (64.80)	106.01 (69.63)	75.24 (95.40)	58.40 (50.02)	40.96 (39.70)	76.12 (67.21)	100.00 (90.00)	107.66 (96.65)	67.62 (55.67)	54.13 (47.56)	47.59 (43.63)	75.40 (66.70)		
Trifo- liate orange	100.00 (46.07)	49.13 (50.60)	39.09 (44.49)	30.05 (35.04)	30.73 (33.55)	49.80 (48.34)	100.00 (90.00)	89.49 (72.54)	71.79 (58.21)	64.22 (53.28)	59.70 (50.66)	77.04 (64.94)		
Pooled mean (A)	(58.46)					(60.17)	(64.68)							
Pooled mean (C)	100.00 (90.00)	79.47 (68.79)	56.07 (48.98)	40.15 (39.84)	32.87 (34.77)		100.00 (90.00)	93.03 (78.29)	63.09 (52.88)	55.38 (48.22)	46.35 (42.73)			
C.D. at 5%														

A = 3.68 B = 4.51, C = N.S.; A x B = 6.38;
A x C = N.S.; B x C = 10.09, A x B x C = N.S.

A = 4.18.; B = 5.13; C = N.S.; A x B = 7.25
A x C = N.S.; B x C = 11.46; A x B x C = N.S.

A = 4.18.; B = 5.13; C = N.S.; A x B = 7.25
A x C = N.S.; B x C = 11.46; A x B x C = N.S.

A = 3.68 B = 4.51, C = N.S.; A x B = 6.38;
A x C = N.S.; B x C = 10.09, A x B x C = N.S.

*A₁ = Chloride dominated salinity; A₂ = Sulphate dominated salinity; C = Salinity level.
Values in parentheses are the angular transformed values.

in Tables 19 to 25. Table 19 depicts the Na^+ content in aerial parts and roots. Na^+ content was higher in SO_4^- type (0.855%) than Cl^- type (0.654%) of salinity. Minimum Na^+ content was recorded in Rangpur lime (0.460%), followed by rough lemon (0.802%) and trifoliate orange (1.002%). Rootstocks behaved differently in various salinity types. With increase in salinity, Na^+ content also increased and this increase was highest at 8 dS/m (1.231%). As the salinity progressed upwards, Na^+ content increased, but this increase was genotype specific. At highest salinity level (8 dS/m), Rangpur lime maintained minimum Na^+ in aerial parts (1.057%) in comparison to trifoliate orange (1.302%) and rough lemon (1.336%). Rangpur lime tended to maintain low Na^+ , as there was very less increase from control to 6 dS/m (0.146 to 0.381%). The increase in Na^+ was gradual with increase in salinity in rough lemon and trifoliate orange.

As for as Na^+ content in roots is concerned, Rangpur lime had lowest Na^+ (0.149%), followed by trifoliate orange (0.192%) and rough lemon (0.294%). Na^+ absorption was higher in SO_4^- type (0.236%) than the Cl^- type (0.202%) salinity. Rootstocks behaved differently in different salinity types and at different levels of salinity, which is evident from the interaction of salinity types x rootstocks x salinity levels. With increase in salinity, Na^+ content increased in roots, but this increase was not of the same magnitude in all the rootstocks. This increase was from 0.063 in control to 0.214 per cent in 8 dS/m in Rangpur lime, from 0.171 to 0.253 per cent in trifoliate orange and from 0.094 to 0.624 per cent in rough lemon.

The data related to the effects of salinity on per cent K^+ content in aerial parts and roots of the rootstocks are given in Table 20. It is evident from the data that K^+ content in aerial parts was higher in SO_4^{2-} dominated salinity (0.953%) than Cl^- dominated salinity (0.935%). Rangpur lime (0.950%) and trifoliate orange (0.953%) had higher K^+ than rough lemon (0.931%) and differential reaction of rootstocks was observed in different salinity types as well as at different salinity levels. As the salinity increased from control to 8 dS/m, K^+ content decreased from 1.178 to 0.741 per cent. This decrease in K^+ content was rootstock specific. In rough lemon it decreased from 0.972 to 0.799 per cent, in Rangpur lime from 1.312 to 0.729 per cent and in trifoliate orange from 1.250 to 0.694 per cent, as the salinity increased from control to 8 dS/m.

In roots, maximum K^+ was recorded in trifoliate orange (0.471%), followed by rough lemon (0.222%) and Rangpur lime (0.209%). The interactions between salinity types x rootstocks, rootstocks x salinity levels and salinity types x rootstocks x salinity levels were significant, indicating differential performance of rootstocks in different salinity types and at different salinity levels. As the salinity level increased, K^+ decreased in rough lemon (0.244 in control to 0.184% in 8 dS/m), and trifoliate orange (1.042 in control to 0.319% in 8 dS/m), but increased in Rangpur lime roots (0.176 in control to 0.237% in 8 dS/m), indicating that Rangpur lime tried to maintain K^+ content in roots with increase in salinity.

The data presented in Table 21 are related to Na : K ratio in aerial parts as well as roots of citrus rootstocks. The ratio in aerial parts was

Table 20. Effect of salinity on per cent potassium content in aerial parts and roots of citrus rootstocks

Root-stock (B)	Aerial parts					Roots				
	Salinity type (A)*		Mean (B x C)			Salinity type (A)*		Mean (B x C)		
	A_1	A_2	Control	2	4	A_1	A_2	Control	2	4
	Mean ($A_1 \times B$)	Mean ($A_2 \times B$)	(ds/m)	(ds/m)	(ds/m)	Mean ($A_1 \times B$)	Mean ($A_2 \times B$)	ds/m	ds/m	ds/m
Pooled mean	Pooled mean (B)					Pooled mean (B)				
Rough lemon	0.958	0.903	0.972	1.007	0.973	0.903	0.799	0.931	0.211	0.234
Rangpur lime	0.971	0.929	1.312	0.972	0.938	0.799	0.729	0.950	0.216	0.202
Trifoliate orange	0.877	1.028	1.250	1.117	0.902	0.798	0.694	0.953	0.470	0.472
Pooled mean (A)	0.935	0.953						0.299	0.302	
Pooled mean (C)			1.178	1.032	0.938	0.833	0.741		0.487	0.251
C.D. at 5%									0.227	0.291

A = 0.0106; B = 0.0129, C = 0.0167; A x B = 0.0183;
A x C = 0.0236; B x C = 0.0289, A x B x C = 0.0409

* A_1 = Chloride dominated salinity; A_2 = Sulphate dominated salinity; C = Salinity level.

Table 21. Effect of salinity on sodium : potassium ratio in aerial parts and roots of citrus rootstocks

Aerial parts										Roots						
Root-stock (B)	Salinity type (A)*		Mean (B x C)				Pooled mean (B)	Salinity type (A)*		Mean (B x C)				Pooled mean (B)		
	A ₁ Mean (A ₁ x B)	A ₂ Mean (A ₂ x B)	Control	2 (dS/m)	4 (dS/m)	6 (dS/m)		8 (dS/m)	A ₁ Mean (A ₁ x B)	A ₂ Mean (A ₂ x B)	Control	2 dS/m	4 dS/m		6 dS/m	8 dS/m
Rough lemon	0.738	1.057	0.255	0.519	0.808	1.247	1.659	0.898	1.101	1.726	0.386	1.044	0.982	1.229	3.426	1.413
Rangpur lime	0.504	0.619	0.111	0.318	0.439	0.479	1.460	0.561	0.594	0.829	0.359	0.854	0.516	0.908	0.921	0.712
Trifo- liate orange	1.092	1.225	0.534	0.743	1.122	1.549	1.844	1.158	0.581	0.540	0.164	0.480	0.908	0.500	0.749	0.560
Pooled mean (A)	0.778	0.967							0.759	1.031						
Pooled mean (C)			0.300	0.527	0.790	1.091	1.634				0.303	0.793	0.802	0.879	1.699	
C.D. at 5%																
A = 0.0218; B = 0.0267, C = 0.0344; A x B = 0.0377; A x C = 0.0487; B x C = 0.0596, A x B x C = 0.0843 A = 0.0428; B = 0.0524; C = 0.0676.; A x B = 0.0741, A x C = 0.0957; B x C = 0.1172; A x B x C = 0.1657																

C.D. at 5%

A = 0.0218; B = 0.0267, C = 0.0344; A x B = 0.0377;
 A x C = 0.0487; B x C = 0.0596, A x B x C = 0.0843

A = 0.0428; B = 0.0524; C = 0.0676; A x B = 0.0741,
 A x C = 0.0957; B x C = 0.1172; A x B x C = 0.1657

*A₁ = Chloride dominated salinity; A₂ = Sulphate dominated salinity; C = Salinity level.

higher in SO_4^- salinity (0.967) than Cl^- salinity (0.778). Rangpur lime had the lowest ratio (0.561), followed by rough lemon (0.898) and trifoliate orange (1.158). As the salinity increased, this ratio in aerial parts also increased gradually. The significance of interaction between salinity types x rootstocks and salinity types x rootstocks x salinity levels revealed the differential performance of rootstocks in different salinity types environment and at different salinity levels. The increase in the ratio was minimum in Rangpur lime (0.111 in control to 1.460 in 8 dS/m), followed by rough lemon (0.255 in control to 1.659 in 8 dS/m) and maximum increase was recorded in trifoliate orange (0.534 in control to 1.844 in 8 dS/m).

In roots of rootstocks, minimum Na : K ratio was observed in trifoliate orange (0.560), followed by Rangpur lime (0.712) and rough lemon (1.413). Na:K ratio was higher in SO_4^- dominated salinity (1.031) than Cl^- dominated salinity (0.759). The interactions of salinity types x rootstocks, rootstocks x salinity levels and salinity types x rootstocks x salinity levels were significant. In roots, with the increase in salinity, the increase in ratio was maximum in rough lemon (0.386 to 3.426), followed by Rangpur lime (0.359 to 0.921) and trifoliate orange (0.164 to 0.749). In trifoliate orange, the ratio was significantly lower than Rangpur lime at all the levels except at 4 dS/m.

Per cent Ca^{++} content in aerial parts and roots of citrus rootstocks is presented in Table 22. In aerial parts higher Ca^{++} content was recorded in Cl^- dominated salinity (3.023%) than SO_4^- dominated salinity (2.519%).

Table 22. Effect of salinity on per cent calcium content in aerial parts and roots of citrus rootstocks

Root-stock (B)	Aerial parts					Roots				
	Salinity type (A)*		Mean (B x C)			Salinity type (A)*		Mean (B x C)		
	A ₁	A ₂	Control	2	4	A ₁	A ₂	Control	2	4
	Mean (A ₁ x B) (A ₂ x B)	Mean (A ₂ x B)	(dS/m)	(dS/m)	(dS/m)	Mean (A ₁ x B) (A ₂ x B)	Mean (A ₂ x B)	(dS/m)	(dS/m)	(dS/m)
Pooled mean (B)			8	6	4	8	6	4	6	8
			(dS/m)	(dS/m)	(dS/m)	(dS/m)	(dS/m)	(dS/m)	(dS/m)	(dS/m)
Rough lemon	3.220	2.650	3.700	3.450	3.000	2.625	1.900	2.935	1.870	2.400
Rangpur lime	3.083	2.560	3.800	3.058	2.950	2.350	1.950	2.822	1.860	2.400
Trifoliate orange	2.767	2.347	2.933	2.500	2.300	2.650	2.400	2.557	1.660	1.300
Pooled mean (A)	3.023	2.519							1.953	1.797
Pooled mean (C)			3.478	3.003	2.750	2.542	2.083	2.033	2.100	1.792
C.D. at 5%									1.733	1.717

A = 0.0802; B = 0.0982 C = N.S.; A x B = 0.1389;
A x C = 0.1794; B x C = 0.2197; A x B x C = 0.3107

A = 0.0589; B = 0.0721; C = 0.0931; A x B = 0.1020,
A x C = 0.1317; B x C = 0.1613; A x B x C = 0.2281

*A₁ = Chloride dominated salinity; A₂ = Sulphate dominated salinity; C = Salinity level.

Maximum mean Ca^{++} content was recorded in rough lemon (2.935%), followed by Rangpur lime (2.822%) and trifoliate orange (2.557%). Rootstocks behaved differently in different saline conditions and at different salinity levels. With the increase in salinity, there was decrease in Ca^{++} content, but this decrease was rootstock specific. Minimum decrease was recorded in trifoliate orange (2.933 to 2.400%), followed by Rangpur lime (3.800 to 1.950%) and rough lemon (3.700 to 1.900%) as the salinity increased from control to 8 dS/m.

In roots significantly higher Ca^{++} was also recorded in Cl^- dominated salinity (1.953%) than SO_4^- dominated (1.797%) salinity. Rough lemon contained higher Ca^{++} (2.045%) than Rangpur lime (1.89%) and trifoliate orange (1.69%). There was a decrease in Ca^{++} content with increase in salinity, but this decrease was not significant beyond 4 dS/m. In Rangpur lime, the decrease in Ca^{++} content was gradual with increase in salinity (2.400 to 1.250%), but in rough lemon, no definite trend in the decrease was observed. In contrast, an increase over control was recorded in trifoliate orange with increase in salinity, but at different extent between salinity levels. Here the Ca^{++} increased from 1.300 per cent in control to 2.100 per cent in 8 dS/m.

Data pertaining to Mg^{++} content in aerial parts and roots of citrus rootstocks, are presented in Table 23. It was revealed that maximum Mg^{++} in aerial parts was recorded in Rangpur lime (0.774%), followed by rough lemon (0.723%) and trifoliate orange (0.684%). The interactions between salinity types and rootstocks and salinity types x rootstocks x salinity

Table 23. Effect of salinity on per cent magnesium content in aerial parts and roots of citrus rootstocks

Root-stock (B)	Aerial parts								Roots																							
	Salinity type (A)*				Mean (B x C)				Pooled mean				Salinity type (A)*				Mean (B x C)				Pooled mean											
	A ₁		A ₂		Control		2		4		6		8		A ₁		A ₂		Control		2		4		6		8					
	Mean		Mean		(dS/m)		(dS/m)		(dS/m)		(dS/m)		(dS/m)		Mean		Mean		dS/m		dS/m		dS/m		dS/m		dS/m					
	(A ₁ x B)		(A ₂ x B)												(A ₁ x B)		(A ₂ x B)															
Rough lemon	0.670	0.776	1.120	0.900	0.720	0.605	0.270	0.723	1.630	1.838	1.780	2.315	1.860	1.785	0.930	1.734																
Rangpur lime	0.732	0.816	1.080	1.020	0.780	0.570	0.420	0.774	2.064	2.352	3.240	1.650	1.740	1.020	3.390	2.208																
Trifoliate orange	0.672	0.696	1.200	0.540	0.630	0.510	0.540	0.684	2.808	1.524	1.140	1.500	2.220	2.040	3.930	2.166																
Pooled mean (A)	0.691	0.763							2.167	1.905																						
Pooled mean (C)			1.133	0.820	0.710	0.562	0.410				2.053	1.822	1.940	1.615	2.750																	
C.D. at 5%																																
																	A = 0.0115; B = 0.0141; C = 0.0182; A x B = 0.0199; A x C = 0.0257; B x C = 0.0315; A x B x C = 0.0445															
																	A = 0.0450; B = 0.0551; C = 0.0712; A x B = 0.0780, A x C = 0.1007; B x C = 0.1233; A x B x C = 0.1744															
																	* A ₁ = Chloride dominated salinity; A ₂ = Sulphate dominated salinity; C = Salinity level.															

A = 0.0115; B = 0.0141; C = 0.0182; A x B = 0.0199;
A x C = 0.0257; B x C = 0.0315; A x B x C = 0.0445

A = 0.0450; B = 0.0551; C = 0.0712; A x B = 0.0780,
A x C = 0.1007; B x C = 0.1233; A x B x C = 0.1744

*A₁ = Chloride dominated salinity; A₂ = Sulphate dominated salinity; C = Salinity level.

levels were significant. With an increase in salinity, Mg^{++} content in aerial parts reduced (1.133 in control to 0.410% in 8 dS/m). Rangpur lime and rough lemon maintained Mg^{++} in aerial parts as the decrease was less with the increase in salinity levels, but in trifoliate orange, the decrease was sharp i.e. from 1.200 in control to 0.540 per cent in 2 dS/m.

In roots of rootstocks significantly higher Mg^{++} was recorded in Cl^- dominated (2.167%) than the SO_4^{--} dominated (1.905%) salinity. Rangpur lime (2.208%) and trifoliate orange (2.166%) were having higher mean Mg^{++} than rough lemon (1.734%). Definite trend of increase or decrease in Mg^{++} content was not observed at increasing salinity levels. In rough lemon, it increased at 2 dS/m and after that it decreased and reached a minimum value at 8 dS/m (0.93%). In Rangpur lime, no definite trend of decrease or increase was observed, but in trifoliate orange, Mg^{++} content increased with increase in salinity.

The results on effects of salinity on per cent Cl^- content in aerial parts and roots of citrus rootstocks are depicted in Table 24. Perusal of the data revealed that Cl^- content in aerial parts was higher in SO_4^{--} dominated salinity (0.676%) than Cl^- dominated (0.632%) salinity. Rangpur lime had minimum (0.588%) Cl^- in aerial parts, followed by trifoliate orange (0.613%) and rough lemon (0.761%). The interactions of salinity types x rootstocks and salinity types x rootstocks x salinity levels were significant, indicating differential absorption of Cl^- in different salinity types and its levels. As the salinity increased, Cl^- content also increased in aerial parts. The Cl^- conc. increased from 0.500 per cent in control to

Table 24. Effect of salinity on per cent chloride content in aerial parts and roots of citrus rootstocks

Root-stock (B)	Aerial parts										Roots										
	Salinity type (A)*					Mean (B x C)					Salinity type (A)*				Mean (B x C)						
	Pooled mean					Pooled mean					Pooled mean				Pooled mean						
	A ₁	A ₂	Control	2	4	6	8	A ₁	A ₂	Control	2	4	6	8	A ₁	A ₂	Control	2	4	6	8
	Mean (A ₁ xB)	Mean (A ₂ xB)	(dS/m)	(dS/m)	(dS/m)	(dS/m)	(dS/m)	Mean (A ₁ xB)	Mean (A ₂ xB)	(dS/m)	(dS/m)	(dS/m)	(dS/m)	(dS/m)	Mean (A ₁ xB)	Mean (A ₂ xB)	(dS/m)	(dS/m)	(dS/m)	(dS/m)	(dS/m)
Rough lemon	0.843	0.680	0.500	0.670	0.717	0.830	1.090	0.761	0.360	0.464	0.280	0.390	0.700	0.440	0.250	0.412					
Rangpur lime	0.508	0.668	0.380	0.500	0.580	0.640	0.840	0.588	0.775	0.636	0.840	0.560	0.713	0.663	0.750	0.705					
Trifoliate orange	0.546	0.680	0.280	0.500	0.640	0.665	0.980	0.613	0.309	0.404	0.340	0.313	0.560	0.260	0.310	0.357					
Pooled mean (A)	0.632	0.676							0.481	0.501											
Pooled mean (C)			0.387	0.557	0.646	0.712	0.970				0.487	0.421	0.658	0.454	0.437						
C.D. at 5%																					

A = 0.0342; B = 0.0419; C =0.0540; ; A x B =0.0592;
A x C =N.S.; B x C = 0.0860; A x B x C = 0.1324;

A = N.S.; B = 0.0420; C = 0.0542; A x B =0.0594;
A x C = 0.0766; B x C = 0.0938; A x B x C= 0.1327.

A = N.S.; B = 0.0420; C = 0.0542; A x B = 0.0594;
A x C = 0.0766; B x C = 0.0938; A x B x C = 0.1327.

*A₁ = Chloride dominated salinity; A₂ = Sulphate dominated salinity; C = Salinity level.

1.090 per cent at 8 dS/m in rough lemon, from 0.280 per cent in control to 0.980 per cent at 8 dS/m in trifoliate orange and from 0.380 per cent to 0.840 per cent in Rangpur lime. In all rootstocks, the increase in Cl^- content in aerial parts was high at higher salinity level (8 dS/m).

Mean Cl^- content in roots was low in trifoliate orange (0.357%) and rough lemon (0.412%), but high in Rangpur lime (0.705%). Though the conc. of Cl^- increased or decreased in roots of all the rootstocks, definite trend was not observed with the increase in salinity levels. A direct relationship between Cl^- content and seedling mortality was observed in different rootstocks (Fig. 8).

The results on SO_4^{--} content in aerial parts and roots of citrus rootstocks are given in Table 25. Results showed that SO_4^{--} content in aerial parts was significantly higher in SO_4^{--} dominated salinity (0.031%) than Cl^- dominated salinity (0.025%). Rough lemon had lowest SO_4^{--} (0.021%), followed by Rangpur lime (0.028%) and trifoliate orange (0.035%). Rootstocks behaved differently in different salinity types. The interaction between salinity types x rootstocks x salinity levels was significant. As the salinity increased, initially there was an increase in SO_4^{--} content (control to 4 dS/m), but it decreased at 6 dS/m and finally increased at 8 dS/m. In rough lemon, SO_4^{--} content increased from 0.018 per cent in control to 0.024 per cent in 8 dS/m, in Rangpur lime, the conc. of SO_4^{--} decreased from 0.036 per cent in control to 0.022 per cent in 2 dS/m, but then increased at higher salinity levels. In trifoliate orange, the increase in SO_4^{--} content was recorded up to 4 dS/m, then decreased at 6

Table 25. Effect of salinity on per cent sulphate content in aerial parts and roots of citrus rootstocks

Aerial parts										Roots							
Root-stock (B)	Salinity type (A)*		Mean (B x C)				Pooled mean		Salinity type (A)*		Mean (B x C)				Pooled mean		
	A ₁	A ₂	Control	2	4	6	8	A ₁	A ₂	Control	2	4	6	8	A ₁	A ₂	
	Mean		(dS/m)				(dS/m)	(dS/m)	Mean		(dS/m)				(dS/m)	Mean	
	(A ₁ xB)	(A ₂ xB)						(A ₁ xB)	(A ₂ xB)						(A ₁ xB)		
Rough lemon	0.020	0.023	0.018	0.018	0.022	0.025	0.024	0.021	0.015	0.010	0.014	0.013	0.014	0.011	0.012	0.013	
Rangpur lime	0.027	0.028	0.036	0.022	0.024	0.029	0.027	0.028	0.014	0.019	0.015	0.013	0.014	0.018	0.021	0.016	
Trifoliate orange	0.028	0.041	0.018	0.039	0.046	0.030	0.039	0.035	0.016	0.027	0.013	0.030	0.022	0.015	0.027	0.021	
Pooled mean (A)	0.025	0.031							0.015	0.018							
Pooled mean (C)			0.024	0.026	0.031	0.028	0.030				0.014	0.018	0.017	0.015	0.020		
C.D. at 5%																	
A = 0.0015; B = 0.0019; C = 0.0027; ; A x B = 0.0027; A x C = 0.0034; B x C = 0.0042; A x B x C = 0.0059.																	
A = 0.0011; B = 0.0014; C = 0.0018; A x B = 0.0020; A x C = 0.0026; B x C = 0.0031; A x B x C = 0.0044.																	

C.D. at 5%

A = 0.0015; B = 0.0019; C = 0.0027; A x B = 0.0027;
 A x C = 0.0034; B x C = 0.0042; A x B x C = 0.0059.

*A₁ = Chloride dominated salinity; A₂ = Sulphate dominated salinity; C = Salinity level.

A = 0.0011; B = 0.0014; C = 0.0018; A x B = 0.0020;
 A x C = 0.0026; B x C = 0.0031; A x B x C = 0.0044.

dS/m, but again increased at 8 dS/m level. It is obvious that although definite trend of increase was not observed, but rootstocks generally accumulated higher SO_4^- at higher salinities in aerial parts of rootstocks.

In roots also, almost similar trend was followed in different rootstocks, but there was no change in SO_4^- concentration in rough lemon, whereas, in Rangpur lime and trifoliate orange, there was an increase in the SO_4^- content with increase in salinity.

4.4 Screening of citrus rootstocks for salt tolerance at callus level

4.4.1 Induction of callus

Effect of different growth media and source of explant on per cent callus induction in different citrus rootstocks is given in Table 26. The callus was induced from three types of explants viz; internodal stem segments from *in vivo* grown seedlings and internodal stem segments and root segments from *in vitro* grown seedlings. Per cent callus induction was highest from the explant internodal stem segments taken from *in vitro* grown seedlings in all the rootstocks. Three callus induction media were tried and callus was induced on all of them. But maximum callus induction was recorded on MSA medium from all sources of explants and in all rootstocks, however, the response to callus induction on this medium was genotype specific, the maximum being in rough lemon (88.46%), followed by trifoliate orange (73.33%) and Rangpur lime (71.77%), when the explants were derived from internodal stem segments of *in vitro* grown seedlings. So, MSA medium [MS + sucrose (30 g/l), NAA (2 mg/l), 2,4-D (2 mg/l) and kinetin (0.5 mg/l) and internodal stem segments from *in vitro* grown seedlings were used for further studies.

Table 26. Effect of growth media and source of explant on per cent callus induction in citrus rootstocks

Rootstock	Media* code	Per cent callus induction			
		<i>In vivo</i> grown seedlings		<i>In vitro</i> grown seedlings	
		Stem seg.	Root seg.	Stem seg.	Root seg.
Rough lemon	MS _A	50.86	-	88.46	37.50
	MS _B	30.12	-	60.00	23.16
	MS _C	36.52	-	67.06	24.12
Rangpur lime	MS _A	28.12	-	71.77	32.96
	MS _B	3.15	-	45.00	14.55
	MS _C	9.28	-	18.08	17.42
Trifoliate orange	MS _A	29.20	-	73.33	8.00
	MS _B	13.68	-	17.53	-
	MS _C	25.71	-	30.36	3.18

*MS_A = MS+Sucrose (30 g/l), NAA (2 mg/l), 2,4-D (2 mg/l) and kinetin (0.5 mg/l)

*MS_B = MS+Sucrose (50 g/l), NAA (2 mg/l), 2,4-D (2 mg/l) and kinetin (0.5 mg/l)

*MS_C = MS+Sucrose (30 g/l), NAA (2 mg/l), 2,4-D (2 mg/l), kinetin (0.5 mg/l) and ME (0.5 g/l)

4.4.2 Imposition of salt stress in culture media

Different conc. of NaCl, viz; control (no NaCl), 0.05, 0.10, 0.15 and 0.20 M were added to the growth medium. A known weight of callus was placed in stress medium and a salt stress for six weeks was imposed. The effects of salt stress on callus characteristics are depicted in Table 27 and Plate 17 to 19.

With the passage of time, there was increase in final callus weight (g) in all the rootstocks at all the salt levels, except in trifoliate orange, where final callus weight was less than the initial callus weight after 0.15 M. The mean final callus weight (g) was recorded significantly higher in rough lemon (0.150 g) followed by trifoliate orange (0.130 g) and Rangpur lime (0.107 g). With the increase in salt conc., there was a decrease in final callus weight. This decrease was maximum in trifoliate orange (0.241 g in control to 0.073 g in 0.20 M), followed by rough lemon (0.229 g in control to 0.150 g in 0.20 M), and least in Rangpur lime (0.135 g in control to 0.109 g in 0.20 M).

The mean dry callus weight (0.025 g) was maximum in rough lemon, followed by trifoliate orange (0.021 g) and Rangpur lime (0.018 g). With the increase in salt conc., the dry callus weight was reduced, but it was salt level and genotype specific. Maximum reduction was observed in trifoliate orange (0.030 g in control to 0.015 g in 0.20 M), followed by rough lemon (0.034 g in control to 0.019 g in 0.20 M). Minimum reduction was recorded in Rangpur lime (0.024 g in control to 0.018 g in 0.20 M).

Table 27. Effect of different levels of salt concentration on callus characteristics in citrus rootstocks

Rootstock (A)	Salt		Callus characteristics				
	conc. (Molar) (B)	Initial callus weight (g)	Final callus weight(g)	Dry callus weight(g)	Callusing index	Per cent increase in weight	Per cent decrease in weight
Rough lemon	Control	0.146	0.229	0.034	1.577	57.430	0.00 (0.000)
	0.05	0.142	0.168	0.030	1.183	18.310	68.11 (55.630)
	0.10	0.113	0.128	0.024	1.133	13.273	76.89 (61.273)
	0.15	0.101	0.111	0.016	1.00	9.893	62.78 (65.537)
	0.20	0.112	0.115	0.019	1.027	2.380	95.86 (77.393)
Rangpur lime	Mean	0.123	0.150	0.025	1.204	20.257	60.73 (51.967)
	Control	0.115	0.135	0.024	1.170	17.097	0.00 (0.000)
	0.05	0.087	0.097	0.018	1.117	11.483	32.85 (34.937)
	0.10	0.089	0.097	0.018	1.090	8.990	47.42 (43.527)
	0.15	0.095	0.099	0.014	1.040	4.153	75.71 (60.467)
Trifoliolate orange	0.20	0.106	0.109	0.018	1.027	2.510	85.35 (67.553)
	Mean	0.098	0.107	0.018	1.089	8.847	48.27 (41.297)
	Control	0.124	0.241	0.030	1.943	94.353	0.00 (0.000)
	0.05	0.106	0.158	0.026	1.493	49.063	47.99 (43.857)
	0.10	0.090	0.100	0.020	1.110	11.037	88.24 (71.813)
C.D. at 5% for	0.15	0.091	0.076	0.014	0.837	-16.483	115.72 (111.493)
	0.20	0.111	0.073	0.015	0.657	-34.237	136.29 (125.240)
	Mean	0.104	0.130	0.021	1.208	20.747	77.65 (70.481)
	A		0.002	0.001	0.020	1.941	2.334
	B		0.003	0.001	0.026	2.505	3.014
	AxB		0.005	0.002	0.045	4.339	5.220

Values in parentheses are the angular transformed values.



Plate 17. Callus growth in rough lemon



Plate 18. Callus growth in Rangpur lime

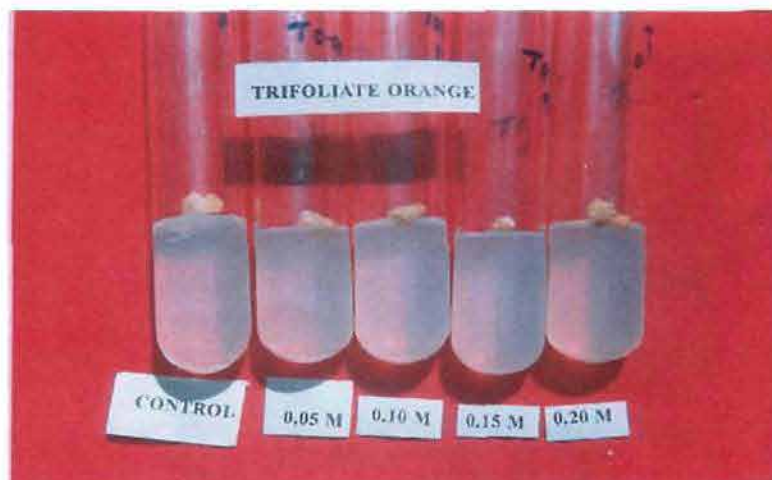


Plate 19. Callus growth in trifoliate orange

To overcome the effect of difference in initial callus weight, the callusing index was calculated. The mean callusing index was maximum in trifoliate orange (1.208) but it was non-significantly different from rough lemon (1.204), and least callusing index was observed in Rangpur lime (1.089). With the increase in salt conc., the callusing index reduced which was maximum in trifoliate orange, where it reduced from 1.943 in control to 0.657 in 0.20 M, followed by rough lemon (1.577 in control to 1.027 in 0.20 M), but least reduction was recorded in Rangpur lime (1.170 in control to 1.027 in 0.20 M).

When per cent increase in final callus weight over the initial callus weight was calculated, maximum increase was recorded in trifoliate orange (20.747%) and rough lemon (20.257%) and minimum in Rangpur lime (8.847%). This increase was reduced with increase in salt conc. Maximum reduction was recorded in trifoliate orange (94.353 in control to -34.237% in 0.20 M), followed by rough lemon (57.430 in control to 2.380% in 0.20 M), but least per cent reduction was recorded in Rangpur lime (17.09 in control to 2.510% in 0.20 M conc.) (Fig.7).

For better elucidation, per cent decrease in final callus weight over the control was determined. Here minimum reduction was recorded in Rangpur lime (48.27%), followed by rough lemon (60.73%), whereas, trifoliate orange exhibited the highest decrease (77.65%). This decrease increased successively with the increase in salt conc. Minimum reduction over control at highest salt conc. (0.20 M) was recorded in Rangpur lime (85.35%), followed by rough lemon (95.86%) and trifoliate orange (136.29%).

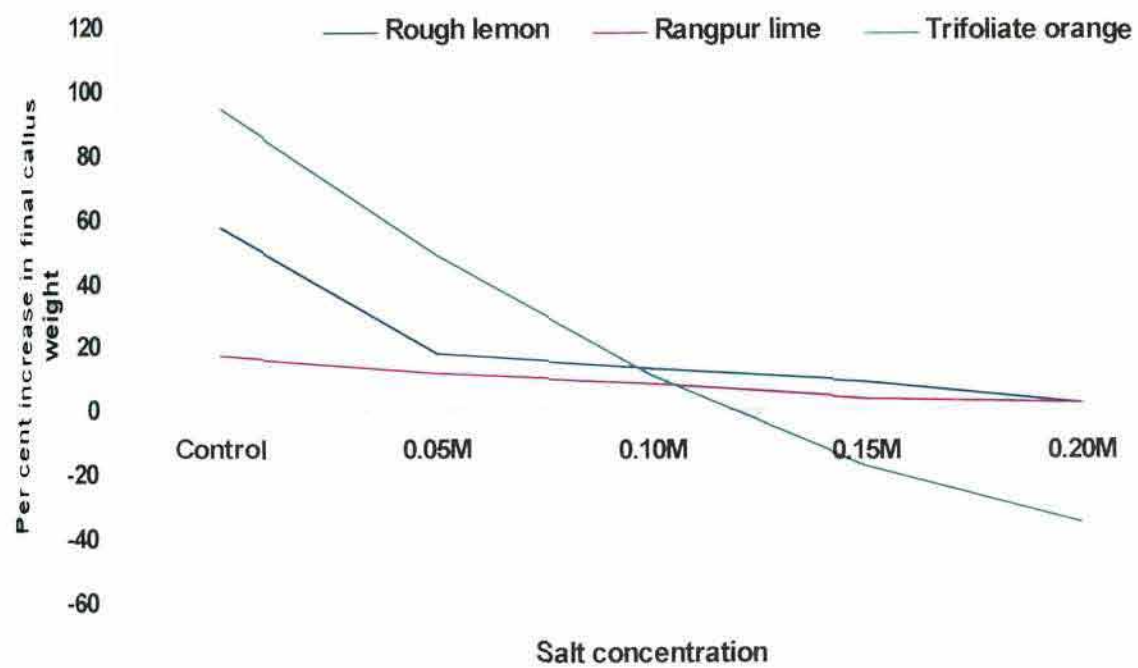


Fig.7. Effect of salt concentrations on per cent increase in final callus weight in citrus rootstocks

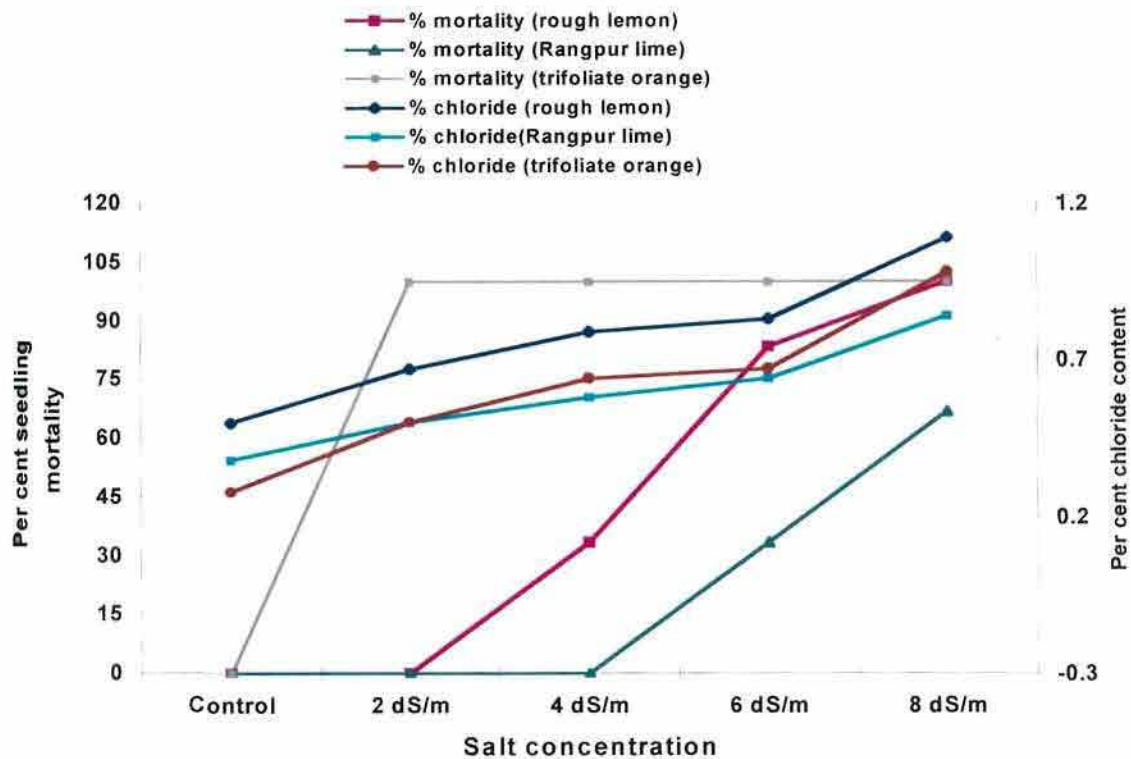


Fig.8. Relationship between seedling mortality and chloride content in citrus rootstocks

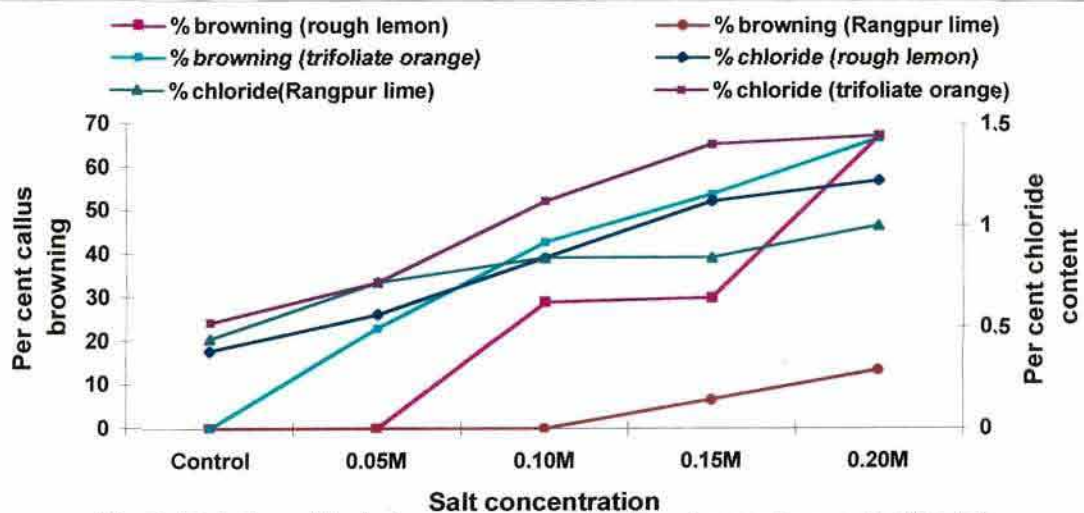


Fig.9. Relationship between per cent callus browning and chloride content in citrus rootstocks

4.4.3 Effect of salt concentration on ion content in callus of citrus rootstocks

The data related to ion content in callus of citrus rootstocks are presented in Table 28. It is apparent from the data that Na^+ content was significantly higher in callus of rough lemon (1.558%), followed by Rangpur lime (1.322%) and trifoliate orange (1.261%). As the conc. of salt increased, Na^+ content also increased. Highest Na^+ conc. was recorded at highest salt level in all the rootstocks. The increase was from 0.190 at control to 2.870 per cent in 0.20 M in Rangpur lime and from 0.140 in control to 2.307 per cent in 0.20 M in trifoliate orange.

The mean value of K^+ content was also significantly higher in rough lemon (1.999%), followed by Rangpur lime (1.474%) and trifoliate orange (1.147%). With an increase in salt conc., a decrease in K^+ content was observed in all the rootstocks and this decrease was genotype specific. Maximum reduction in K^+ content was recorded in rough lemon, where it reduced from 2.730 in control to 1.460 per cent in 0.15 and 0.20 M. At highest level of salt, the decrease from control was similar in trifoliate orange and Rangpur lime, however, at low conc. of salt, Rangpur lime maintained higher K^+ content.

Mean $\text{Na} : \text{K}$ ratio was lowest in rough lemon (0.956), followed by Rangpur lime (1.038) and trifoliate orange (1.314). With the increase in salt conc., this ratio also increased, but differently in different rootstocks. The ratio in rough lemon increased from 0.070 in control to 1.970 in 0.20 M, in Rangpur lime from 0.120 at control to 2.410 at 0.20 M and in trifoliate orange from 0.090 at control to 2.660 at 0.20 M.

Table 28. Effect of different salt concentration on ion content in callus of citrus rootstocks

Rootstock (A)	Salt conc. Molar) (B)	Per cent ion content in callus						
		Na ⁺	K ⁺	Na:K ratio	Ca ⁺⁺	Mg ⁺⁺	Cl ⁻	SO ₄ ⁻
Rough lemon	Control	0.190	2.730	0.070	0.600	0.540	0.380	0.070
	0.05	0.920	2.240	0.420	0.800	0.417	0.560	0.050
	0.10	1.380	2.107	0.660	0.600	0.300	0.840	0.050
	0.15	2.430	1.460	1.660	0.800	0.240	1.113	0.060
	0.20	2.870	1.460	1.970	0.600	0.360	1.220	0.040
	Mean	1.558	1.999	0.956	0.680	0.371	0.823	0.054
Rangpur lime	Control	0.190	1.670	0.120	1.000	0.360	0.440	0.053
	0.05	0.870	1.740	0.500	0.833	0.240	0.720	0.060
	0.10	1.330	1.530	0.870	0.493	0.540	0.840	0.050
	0.15	1.880	1.460	1.290	0.693	0.120	0.840	0.050
	0.20	2.340	0.970	2.410	0.700	0.180	1.000	0.050
	Mean	1.322	1.474	1.038	0.744	0.288	0.760	0.053
Trifoliate orange	Control	0.140	1.567	0.090	1.200	0.600	0.520	0.080
	0.05	0.750	1.320	0.570	0.700	0.240	0.720	0.060
	0.10	1.230	1.130	1.090	0.740	0.530	1.120	0.050
	0.15	1.880	0.853	2.160	1.000	0.600	1.400	0.060
	0.20	2.307	0.867	2.660	1.240	0.440	1.440	0.080
	Mean	1.261	1.47	1.314	0.976	0.482	1.040	0.066
C.D. at 5% for	A	0.023	0.047	0.050	0.039	0.031	0.069	N.S
	B	0.030	0.061	0.065	0.050	0.040	0.089	N.S
	AxB	0.052	0.106	0.113	0.087	0.070	0.155	N.S

Ca^{++} content was recorded significantly higher in trifoliate orange (0.976%), followed by Rangpur lime (0.744%) and rough lemon (0.680%). No definite trend in the increase or decrease of Ca^{++} in rough lemon was observed with increase in salinity. In Rangpur lime Ca^{++} content decreased up to 0.10 M and then it started increasing from 0.15 M to 0.20 M. Similarly, for trifoliate orange, Ca^{++} content decreased up to 0.05 M and then increased in 0.10 M to 0.20 M.

Mg^{++} content was highest in trifoliate orange (0.482%) followed by rough lemon (0.371%) and Rangpur lime (0.288%). With the increase in salt conc., Mg^{++} content decreased from 0.540 at control to 0.360 per cent at 0.20 M in rough lemon, from 0.360 at control to 0.180 per cent at 0.20 M in Rangpur lime and from 0.600 at control to 0.440 per cent at 0.20 M in trifoliate orange. The decreasing trend in Mg^{++} was generally observed in rough lemon and Rangpur lime, but not clearly in trifoliate orange.

Similarly, higher Cl^- content was recorded in trifoliate orange, followed by rough lemon and Rangpur lime, but the difference between rough lemon and Rangpur lime was not significant. With the increase in salt conc. in growth medium, there was an increase in Cl^- content of callus. Minimum increase in Cl^- content was recorded in Rangpur lime (0.440 in control to 1.000% in 0.20 M), followed by rough lemon (0.380 in control to 1.122% in 0.20 M) and trifoliate orange (0.520 at control to 1.440% at 0.20 M). At higher salt conc., Rangpur lime tended to maintain the Cl^- content.

Significant differences for SO_4^{2-} content were not observed either between rootstocks or among different salt conc. within a rootstock.

4.4.4 Appearance of browning in callus cultures under salt stress

The data related to appearance of browning in callus of citrus rootstocks with increase in salt conc. in growth medium are presented in Table 29. In trifoliate orange, browning of callus started even at 0.05 M on 35th day of stress. In rough lemon, it appeared at 0.10 M on 35th day and in Rangpur lime, the browning started on 28th day and that to after 0.15 M. As the salt conc. increased, the browning of callus also increased, but its extent was different in different rootstocks. The browning of callus was observed at all the salt conc. (0.05 to 0.20 M) in trifoliate orange, at three (0.10, 0.15 and 0.20 M) in rough lemon and at two (0.15 and 0.20 M) in Rangpur lime. Maximum browning was recorded at highest (0.20 M) salt conc. (67.00% in rough lemon, 66.66% in trifoliate orange and 13.33% in Rangpur lime). A direct relationship was observed between Cl^- content and browning of callus in different citrus rootstock (Fig. 9).

4.4.5 Plant regeneration from callus of citrus rootstocks

Plant regeneration from callus was tried in all the rootstocks and the results are presented in Table 30 and Plates 20 and 21. Five media were tried for plant regeneration. From the perusal of data, it is evident that regeneration was there only in Rangpur lime and rough lemon, but not in trifoliate orange. In rough lemon, maximum plantlet regeneration from callus was recorded in MS_G medium (27.27%), and in this medium, the number of shoots/callus was 2.00. In Rangpur lime, maximum regeneration was obtained on MS_r medium with 2.33 shoots/callus.

Table 29. Effect of different salt concentration on appearance of browning (in days) in callus of citrus rootstocks

Rootstock	Salt conc. (Molar)	Per cent browning after days of initiation of salt stress						
		7	14	21	28	35	42	
Rough lemon	Control	0	0	0	0	0	0	
	0.05	0	0	0	0	0	0	
	0.10	0	0	0	0	27.00	29.00	
	0.15	0	0	0	10.00	23.33	30.00	
	0.20	0	0	0	32.67	55.00	67.00	
Rangpur lime	Control	0	0	0	0	0	0	
	0.05	0	0	0	0	0	0	
	0.10	0	0	0	0	0	0	
	0.15	0	0	0	6.64	6.64	6.64	
	0.20	0	0	13.33	13.33	13.33	13.33	
Trifoliate orange	Control	0	0	0	0	0	0	
	0.05	0	0	0	0	15.38	23.07	
	0.10	0	14.29	21.42	21.42	42.86	42.86	
	0.15	15.38	30.79	30.79	38.45	46.16	53.85	
	0.20	33.33	41.67	58.33	58.33	66.66	66.66	

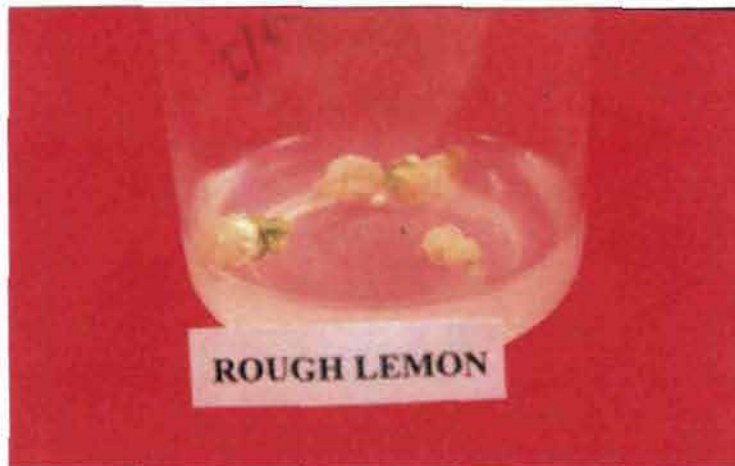


Plate 20. Plantlet regeneration from callus
in rough lemon

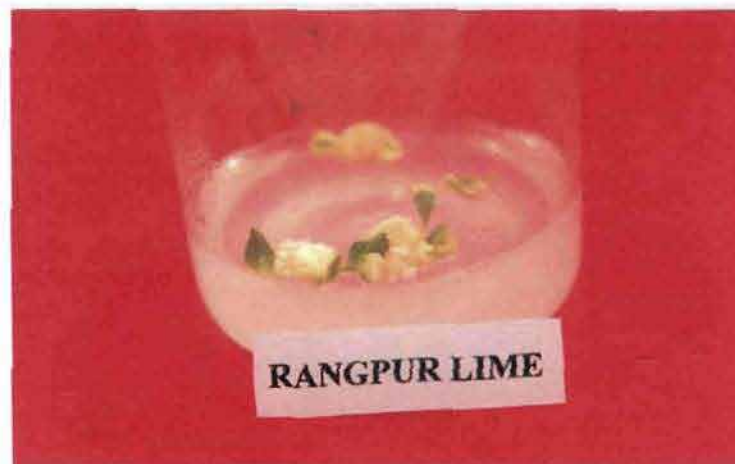


Plate 21. Plantlet regeneration from callus
in Rangpur lime

Table 30. Effect of media on plant regeneration from callus of citrus rootstocks

Rootstock	Media code*	Number of calli		Per cent regeneration	Number of shoots/ callus
		Cultured	Regenerated		
Rough lemon	MS _A	21	4	19.05	2
	MS _D	21	0	0	0
	MS _E	19	0	0	0
	MS _F	22	4	18.18	2
	MS _G	22	6	27.27	2
Rangpur lime	MS _A	17	3	17.65	1
	MS _D	22	6	27.27	2.67
	MS _E	21	4	19.05	1.33
	MS _F	16	8	50.00	2.33
	MS _G	22	6	27.27	2
Trifoliata orange	MS _A	17	0	0	0
	MS _D	15	0	0	0
	MS _E	18	0	0	0
	MS _F	17	0	0	0
	MS _G	16	0	0	0

*MS_A = MS+NAA (2 mg/l), 2,4-D (2 mg/l), Kinetin (0.5 mg/l)MS_D = MS+NAA (10 mg/l), Kinetin (0.5 mg/l)MS_E = MS+2,4-D (2 mg/l), Kinetin (0.5 mg/l)MS_F = MS+Kinetin (1 mg/l), BAP (0.5 mg/l), ME (0.5 g/l)MS_G = MS+NAA (0.5 mg/l), BAP (3 mg/l), ME (0.5 g/l)

CHAPTER - 5

DISCUSSION

Citrus fruits are grown in 49 countries throughout the tropical and sub-tropical regions of the world, covering an area of about 2.9 m ha. Among fruit plants, citrus fruits rank third in area and production in India. Mandarins (*Citrus reticulata* Blanco), sweet orange (*Citrus sinensis* (L.) Osbeck), acid lime (*Citrus aurantifolia* Swingle) and lemon (*Citrus limon* Tanaka) are commercially important citrus fruits. Despite the wide genetic diversity, the productivity of citrus in India remained low. Therefore, there is need to improve citrus on priority.

Citrus fruits are commercially grown as grafted plants onto a rootstock, which imparts resistance/tolerance to most of the biotic and abiotic stresses and also determines the success/failure of the scion variety grafted onto it. Rough lemon (*Citrus jambhiri* Lush.) is the most commonly used rootstock for citrus in India, but, this rootstock is susceptible to soil salinity and produces poor quality fruits. Owing to the salinity sensitivity of citrus as a whole, citrus rootstocks are required to be developed which can be grown atleast on some saline soils. Conventional breeding methods are difficult to apply for the improvement of citrus rootstocks for salinity

tolerance, due to polygenic nature of the salinity tolerance and also the problems of heterozygosity, nucellar embryony and long juvenility. Therefore, searching the sources of resistance/tolerance for salinity among the existing diversity is an important and comparatively easy method of breeding, but it requires a lot of time, space and labour. On the other hand, under *in vitro* conditions, large populations can be screened in a limited space and time.. In citrus, protocols for *in vitro* culture/multiplication are available (Gill et al., 1990,1994 1995; Raman et al. 1987, 1992). To explore the possibility whether these techniques can be used in a similar manner as to that of *in vivo* screening techniques, the present study was conducted. The results obtained herein are discussed below:

In the present investigation attempts were made to screen different citrus rootstocks for salinity tolerance under *in vivo* and *in vitro* conditions. In this context, three different experiments were conducted.

- *In vivo* and *in vitro* seed germination of different citrus rootstocks under different salinity regimes.
- Evaluation of salinity tolerance of citrus rootstocks.
- *In vitro* screening of citrus rootstocks at cellular level.

5.1 *In vivo* and *in vitro* seed germination of different citrus rootstocks under different salinity regimes

Although a number of accessions in citrus and its related genera have been screened for salinity tolerance, but progress in screening has been less satisfactory, as the screening was done at different levels of

organization i.e. either at seed, seedling, whole plant or callus level, but not simultaneously at all the levels.

In first experiment, three rootstocks were screened for salt tolerance using *in vivo* and *in vitro* seed germination, by creating different salinity levels. Under *in vitro* conditions, the seeds of trifoliate orange germinated poorly. The addition of GA_3 in the medium improved the germination of trifoliate orange seeds when added in lower conc. in comparison to higher conc. Increase in seed germination by addition of GA_3 in the media at lower conc. in trifoliate orange seeds was also reported by Abdalla *et al.* (1978), in rough lemon and Kharna Khatta seeds by Singh *et al.* (1979), in Swingle citrumelo by Ono *et al.* (1995) and in sour orange by Yousif *et al.* (1989), whereas, higher conc. of GA_3 reduced seed germination (Ono *et al.*, 1995).

In the present study, it was observed that seed germination was affected in all the rootstocks by salinity, but minimum affected rootstock was Rangpur lime. Increase in salt conc. reduced the germination in all the rootstocks, but the reduction was minimum in Rangpur lime followed by rough lemon and trifoliate orange. In trifoliate orange, none of the seeds germinated beyond 4 dS/m under *in vivo* conditions and except control under *in vitro* conditions. Similar results related to reduction in seed germination in different rootstocks were reported by various workers (Caro *et al.*, 1973; Mobayen and Milthorpe, 1978; Zekri, 1993b,c; Zekri, 1994; El-Desouky and Atawia, 1998).

With an increase in salinity, there was delay in initiation of germination and maximum germination, but this delay was rootstock specific under *in vivo* conditions. Similar trend of germination was also observed under *in vitro* conditions, where, the increase in salt conc. reduced and delayed the germination. Germination of seed populations with slow germination was reduced more by salinity stress than those populations in which all the seeds germinated quickly (Mobayen and Milthorpe, 1978). Similar observations were recorded in trifoliate orange, where germination was late and final germination percentage was also very poor. Trifoliate orange (*Poncirus trifoliata*) was reported sensitive to salts by various workers (Mobayen and Milthorpe, 1980; Grieve and Walker, 1983; Syvertsen *et al.*, 1988; Storey and Walker, 1999). The effect of salts on seed imbibition and time of emergence of first seedling was primarily osmotic, whereas, the effect on final percentage seed emergence and growth of seedlings was primarily a toxic (Zekri, 1993a). Therefore, the reduction and delay in seed germination might be due to osmotic effects of salts on seed germination, which can affect the imbibition of seeds.

On the basis of germination studies, it was clear that Rangpur lime was tolerant to salinity, rough lemon was moderately tolerant and trifoliate orange was susceptible. Further, with change in growth environment, a change in pattern of germination was observed, but the general trend remained the same in both the environments i.e. under *in vivo* and *in vitro* conditions.

For vegetative growth characters of plants, reduction in all the plant characters viz., plant height (cm), stem diameter (cm), number of leaves, fresh aerial, root and total biomass (g) and dry aerial, root and total biomass(g) was recorded in all the rootstocks with an increase in salt conc., but, the rate of reduction varied among the rootstocks. Reduction in all the plant characters with increase in salinity was also reported by Zekri (1993b,c); Zekri (1994) and El-Desouky and Atawia (1998).

Minimum reduction in plant characters was observed in Rangpur lime, followed by rough lemon and trifoliate orange. There was some sort of reverse trend between Rangpur lime and rough lemon for some characters, when both the environments were considered together, which might be attributed to the environmental effects. Rangpur lime was also reported as tolerant by El-Desouky and Atawia (1998). Trifoliate orange proved to be very susceptible to salinity under both the environments. The reason for reduction in all the plant characters might be due to dehydration and visible leaf injuries. Addition of salts increased the conc. of Na^+ and Cl^- in all the parts at higher salt conc. which might be responsible for poor plant growth of the seedlings, especially at higher salinity, because these elements are toxic to plants. There is alteration of biological activities of the phytohormones (cytokinins, gibberellins and auxins) due to salinity, which might be responsible for poor growth of the seedlings (El-Desouky and Atawia, 1998).

An increase for most of the plant characters was recorded at lower salinity level i.e. upto 2 dS/m in Rangpur lime under *in vivo* conditions.

This type of increase suggested a certain threshold limit for the character expression and beyond this limit, there was reduction in the plant growth.

Under *in vitro* conditions, a reduction in all the plant characters was recorded, but to a different extent in different rootstocks as compared to *in vivo* conditions. This may be attributed to differences in prevailing environmental conditions.

From the pattern of reduction in plant growth characters, Rangpur lime was considered as tolerant, rough lemon as moderately tolerant and trifoliate orange as susceptible to salinity.

5.2 Evaluation of salinity tolerance of citrus rootstocks

Comparative evaluation of seedling mortality in SO_4^{--} and Cl^- dominated salinity regimes revealed that the overall mortality rate, although was higher in the former, but at a slower pace, as it took 39 to 153 days for 100 per cent mortality in trifoliate orange and rough lemon, respectively. On the other hand, Cl^- dominated salinity caused seedling mortality at much faster pace, as it took 27 to 114 days for 100 per cent mortality in trifoliate orange and rough lemon. In Cl^- dominated salinity, seedling mortality was considerably less in Rangpur lime as compared to SO_4^{--} dominated salinity. Walker *et al.* (1993) reported that increase in leaf Na^+ and Cl^- accounted for 54-96 per cent of the reduction in osmotic potential in leaves on trees treated with NaCl , whereas, increase in Na^+ and SO_4^{--} accounted for 33-71 per cent of the reduction in osmotic potential in leaves on trees irrigated with Na_2SO_4 . Hence, Na_2SO_4 can be considered less injurious to plants than NaCl which are the two different salts used

in two different types of salinities in the present study. Na_2SO_4 was reported less toxic in comparison to NaCl (Mobayen and Milthorpe, 1980).

Mortality of seedlings was minimum in Rangpur lime, followed by rough lemon and trifoliolate orange. Rangpur lime as salt tolerant rootstock was also reported by various workers (Furr and Ream, 1968; Hassan and Galal, 1989; Storey, 1995; Singh *et al.*, 1997 ; Storey and Walker, 1999). Mortality of seedlings increased with increase in salinity, but in trifoliolate orange, none of the seedlings survived beyond the control in both types of salinity. In rough lemon, mortality of seedlings started at 4 dS/m in both the salinity types, whereas, in Rangpur lime, seedling mortality occurred only at 6 and 8dS/m in SO_4^- dominated salinity and at 8 dS/m only in Cl^- dominated salinity. Patil and Bhambota (1978) reported mortality of all the rootstock seedlings at 8 dS/m. They also reported less mortality in Rangpur lime as compared to rough lemon and considered Rangpur lime and Cleopatra mandarin as tolerant rootstocks to salinity. Hence, Rangpur lime was considered as tolerant, rough lemon as moderately tolerant and trifoliolate orange as susceptible rootstock to salinity. These results are in confirmity with the findings of Patil and Bhambota (1978).

The Cl^- and Na^+ conc. in aerial parts might have increased, which became toxic to plants, especially at higher salinity levels. Under high salinity, the water availability to plants is reduced due to osmotic effect of salts (Bielorai *et al.*, 1983; Ruiz *et al.*, 1999) which may lead to mortality of seedlings due to water stress. The salinity reduces both relative growth

rate and net assimilation rate (Ruiz *et al.*, 1997), which might have resulted in mortality of seedlings. However, the tolerance limit for different rootstocks varied, but trifoliate orange was very susceptible as none of the seedlings could survive beyond the control treatment.

Reduction in all the plant characters viz., plant height (cm), stem diameter (cm), number of leaves, leaf area (cm²), fresh leaf, stem and root biomass(g) and leaf, stem and root biomass (g) was observed in all the rootstocks at all the levels of salinity and in both the salinity types. Plant height (cm), fresh and dry root weight (g) were higher in SO₄⁻ dominated salinity and stem diameter (cm) was higher in Cl⁻ dominated salinity. For other characters, differences were not observed between the salinity types. In most of the characters, the interaction between salinity types and rootstocks was significant indicating that the rootstocks performed differently in different salinity types.

Increase in salinity level resulted in a decrease in all the plant characters viz., plant height (cm), stem diameter (cm), total number of leaves/plant, healthy leaves/plant, number of branches/plant, leaf area (cm²)/leaf, fresh and dry leaf, stem and root weight (g)/plant, but this reduction was rootstock specific. However, number of affected leaves and dead leaves/plant, increased with increase in salinity. These results were in accordance of the results reported by Walker and Douglas (1983), Banuls *et al.* (1990) and Aljuburi (1996).

The reduction in plant characters with an increase in salinity was minimum in Rangpur lime, followed by rough lemon and trifoliate orange.

Similar results were also reported by other workers (Furr and Ream, 1968; Bhambota and Kanwar, 1969; Patil and Bhambota, 1978; Hassan and Galal, 1989; Storey, 1995; Singh *et al.*, 1997). Fresh and dry stem and root biomass (g)/plant increased at low salinity in Rangpur lime, indicating some sort of threshold limit of salinity for these characters, beyond which, there is reduction. These results also revealed that Rangpur lime was tolerant, rough lemon was moderately tolerant and trifoliate orange was susceptible to salinity.

Leaf abscission and expansion can provide better indices for screening salt tolerance of rootstocks (Mobayen and Milthorpe, 1980). The differences in differential reduction of the plant characters may be due to different tolerance limits of these rootstocks for salinity. Hence, Rangpur lime can better tolerate salinity and therefore, considered as salinity tolerant rootstock.

Growth reductions in plant characters due to salinity can in some instances be attributed to salinity induced adverse changes in leaf water relations reducing the photosynthesis (Walker *et al.*, 1983). This suggested that osmotic adjustment, and therefore, turgor maintenance might be one factor which determines the photosynthetic performance and explains the observed influence on the reduction of growth under water stress.

Sodium content in aerial parts and roots was higher in SO_4^{--} dominated salinity as compared to Cl^- dominated salinity. As the salinity increased, Na^+ content also increased in both the parts, but significantly only in aerial parts. Increase in Na^+ content with increase in salinity was

also reported by various workers (Zid and Grignon, 1986; Walker and Douglas, 1983; Badawi *et al.*, 1979; Syvertsen *et al.*, 1988; El-Hammady *et al.*, 1995; Abdel-Messih *et al.*, 1979; Mobayen and Milthorpe, 1980).

In aerial parts as well as in roots, Na^+ content was minimum in Rangpur lime. In aerial parts, rough lemon had less Na^+ than trifoliate orange, but in roots, trifoliate orange had more Na^+ than rough lemon. At low salinity levels, Rangpur lime contained less Na^+ than other rootstocks, but at higher salinity (8 dS/m), it was unable to restrict Na^+ accumulation in aerial parts. Citrus tolerance to salinity was reported to be associated with rootstock's ability to restrict accumulation of Cl^- and Na^+ (Cerdeña *et al.*, 1976; Mobayen and Milthorpe, 1980; Walker *et al.*, 1983). In roots, trifoliate orange had more Na^+ , so it restricted Na^+ transport to shoots and leaves, but not at higher salinities. The present observation was in contrast to earlier reports, where trifoliate orange was referred to as a Na^+ excluder (Syvertsen and Yelenosky, 1988). This may be due to higher levels of salinity used in the present study.

Potassium content was higher in aerial parts in SO_4^{2-} dominated salinity as compared to Cl^- dominated salinity. As the salinity increased, K^+ content decreased in aerial parts, but this trend was not observed in roots at different levels of salinity. Decrease in K^+ content in aerial parts was also recorded by Guillen *et al.* (1978), Behboudian *et al.* (1986), Walker (1986), Syvertsen *et al.* (1988) and Banuls *et al.* (1990). K^+ content in aerial parts was comparable in Rangpur lime and trifoliate orange and both had higher K^+ than rough lemon, but in roots, maximum K^+ was

recorded in trifoliate orange followed by rough lemon and Rangpur lime. It was concluded that Rangpur lime transported more K^+ to aerial parts and very less K^+ was retained in the roots.

Less reduction of K^+ in aerial parts of Rangpur lime with increasing salinity might be due to its tolerance, as very less K^+ was replaced by Na^+ . K^+ is not an injurious ion in leaves and it helped to maintain the turgor, which under salinity is a common phenomenon. So, the conc. of K^+ in aerial parts seemed to be an important factor in determining the salt tolerance of a rootstock.

Na: K ratio was higher in SO_4^{2-} dominated salinity than Cl^- dominated salinity. In aerial parts, this ratio was minimum in Rangpur lime, followed by rough lemon and trifoliate orange, but in roots, minimum ratio was observed in trifoliate orange, followed by Rangpur lime and rough lemon. As the salinity increased, this ratio also increased in both aerial parts and roots, but this increase was less in Rangpur lime as compared to rough lemon and trifoliate orange. Increase in Na:K ratio with increase in salinity was also reported by Walker (1986) and Syvertsen *et al.* (1988).

In aerial parts Rangpur lime maintained this ratio to a lower limit at lower salinity levels (upto 6 dS/m). Hence, this ratio in aerial parts seemed to be directly related with mortality of seedlings, because, particularly in Rangpur lime, when this ratio was high (at 8 dS/m), seedling mortality was recorded. This ratio represented the ability of a rootstock to absorb more K^+ with increase in salinity, which mitigated the effect of increase in Na^+ in aerial parts. At lower salinities, Rangpur lime had minimum

ratio, therefore, indicating its role in determining tolerance to salinity. The ability of trifoliate orange to restrict Na^+ accumulation at lower salinity levels was not evident in this study as the rootstock had very high increase in this ratio with increase in salinity.

Calcium content was higher in Cl^- dominated salinity than SO_4^{2-} dominated salinity and it was higher in rough lemon, followed by Rangpur lime and trifoliate orange in aerial parts as well as in roots. As the salinity increased, Ca^{++} content decreased in both aerial parts and roots, but the decrease was significant in roots only. The decrease was less in trifoliate orange, but high in rough lemon and Rangpur lime. In trifoliate orange, there was an increase in Ca^{++} content at higher salinity. These results were in accordance with the results reported by Abdel-Messih *et al.* (1979), Gallasch and Dalton (1989) and Banuls *et al.* (1990). But Guillen *et al.* (1978) reported no effect of salinity on Ca^{++} content, whereas, Mobayen and Milthorpe (1980) reported that Ca^{++} was higher in more tolerant rootstocks.

In roots, the decrease in Ca^{++} content was higher in Rangpur lime, so this rootstock transported more Ca^{++} to aerial parts, whereas, trifoliate orange retained it in roots. It was concluded that with increase in salinity Ca^{++} content was decreased. Ahmed and Al-Shurafa (1984) found that Ca^{++} , K^+ , Na^+ and Cl^- levels were more variable under salinity and reported that these were the elements where changes in relationship occurred when the salinity increased from lower to higher side. The differences in results of the earlier and present study may be attributed to different types of salinity and perhaps due to higher levels of salinity used in the present study.

Magnesium content was higher in SO_4^- dominated salinity in aerial parts and in Cl^- dominated salinity in roots. In aerial parts, maximum Mg^{++} content was recorded in Rangpur lime followed by rough lemon and trifoliate orange, but in roots, there was no difference between Rangpur lime and trifoliate orange, however, both had higher Mg^{++} than rough lemon. With increase in salinity, there was a decrease in Mg^{++} in aerial parts, but similar trend was not observed in roots. These results are in confirmity with the findings reported earlier (Abdel-Messih *et al.*, 1979 ; Banuls *et al.*, 1990).

The decrease in Mg^{++} content with increase in salinity was gradual and low in Rangpur lime than other two rootstocks, as far as aerial parts were concerned, but not in roots. Similarly, Mobayen and Milthorpe (1980), reported higher Mg^{++} content in Bakraie mandarin. It seemed that maintenance of Mg^{++} in aerial parts with increase in salinity was also an important factor for governing salt tolerance in Rangpur lime.

As Mg^{++} is an important constituent of chlorophyll, its reduction might had affected the photosynthesis and resulted in less deposition of carbohydrates, followed by less growth and death of the plants. As Rangpur lime was able to maintain high Mg^{++} in comparison to other rootstocks, there was more photosynthesis and growth, showing tolerance to salinity.

Chloride content was higher in SO_4^- dominated salinity compared to Cl^- dominated salinity in aerial parts, but in roots, no difference was observed. Minimum Cl^- from roots to aerial parts was transported in Rangpur lime, followed by trifoliate orange and rough lemon, but in roots,

Rangpur lime contained the highest Cl^- content. There was an increase in Cl^- conc. with the increase in salinity. Similar results were also reported by various workers (Salerno, 1975; Zid and Grignon, 1986; Syvertsen *et al.*, 1988; Storey and Walker, 1999).

Rangpur lime retained more Cl^- in roots and transported less to aerial parts, whereas, in other rootstocks, its transport was more to aerial parts. Upto 6 dS/m, Rangpur lime tended to restrict Cl^- accumulation in aerial parts, whereas, in rough lemon and trifoliate orange, this phenomenon was not observed. So, accumulation of Cl^- in aerial parts seemed directly linked to the tolerance or susceptibility of a rootstock to salinity.

Chloride content in aerial parts of Rangpur lime was lower than other rootstocks and hence it was tolerant to salinity. As the conc. of Cl^- in roots of Rangpur lime was higher, it restricted the transport of Cl^- from roots to aerial parts. Other rootstocks failed to do so and hence, were susceptible to salinity. Tolerance of Rangpur lime to salinity on the basis of its ability to restrict Cl^- accumulation in leaves was also reported by Grieve (1983), Grieve and Walker (1983), Walker and Douglas (1983), Walker *et al.* (1983) and Storey and Walker (1999).

The inability of rough lemon and trifoliate orange to restrict Cl^- accumulation in aerial parts might be the reason for their susceptibility to salts. The effect of Cl^- ion in citrus leaves is associated with a reduction in leaf longevity, earlier senescence and abscission, which led to the reduction in photosynthesis, resulting in less carbohydrate deposition and ultimately poor growth and death of the plants (Goell, 1969).

Higher Cl^- content in SO_4^- dominated salinity might be due to longer survival of plants in this type of salinity. As the mortality of seedlings is a function of osmotic and ionic stress, the plants in Cl^- dominated salinity might have died due to the toxic effects of salts, whereas, in SO_4^- dominated salinity, the plants might have died due to toxic and osmotic effects of salts.

Sulphate content was higher in SO_4^- dominated salinity as compared to Cl^- dominated salinity in both aerial parts and roots. With an increase in salinity, there was increase in SO_4^- content in aerial parts but this trend was not observed in roots. Rough lemon accumulated less SO_4^- , followed by Rangpur lime and trifoliate orange in aerial parts as well as in roots. With the increase in salinity, there was an increase in SO_4^- content in aerial parts in rough lemon and trifoliate orange, but in roots, the increase was recorded in Rangpur lime and trifoliate orange. It can be concluded that SO_4^- conc. played a less important role in determining the tolerance/susceptibility to salinity. Similar observations were made by Khanna and Kumar (1990) where they reported that NaCl was more harmful than Na_2SO_4 . Walker *et al.* (1993) reported that increase in Na^+ and Cl^- accounted for 54-96 per cent of the reduction in osmotic potential in leaves, whereas, increase in Na^+ and SO_4^- accounted for 33-71 per cent of the reduction in osmotic potential in leaves. So, SO_4^- effect becomes cumulative alongwith Cl^- and Na^+ , although its conc. in leaves may not be different in different rootstocks.

5.3 *In vitro* screening of citrus rootstocks for salt tolerance at cellular level

In this experiment, the calli of three rootstocks were screened *in vitro* at different salt conc. in the growth medium. Internodal stem segments and MS_A [MS + sucrose (30 g/l), NAA (2 mg/l), 2,4-D (2 mg/l) and kinetin (2 mg/l)] medium were found adequate for callus induction in all the rootstocks. Internodal stem segments, particularly from *in vitro* grown seedlings were found suitable explants for callus induction, though the frequency of callus induction was different in different rootstocks, indicating the medium specificity for a particular genotype.

Callus had been induced from different explants and on different media by several workers (Bhansali and Arya, 1978; Gmitter, 1986; Raman *et al.*, 1987, 1992; Oh *et al.*, 1991; Song *et al.*, 1991; Koc and Can, 1992; Gill *et al.*, 1990, 1994, 1995; Chakravarty and Goswami, 1999). The media requirement depended on the source of explant used and the cultivar under study, as recorded in the present study. When the internodal stem segments from *in vitro* grown seedlings were taken, they were found superior also by different workers (Raman *et al.*, 1987, 1992; Gill *et al.*, 1990, 1994, 1995).

Mean callus weight after salt stress treatment was highest in rough lemon, followed by trifoliate orange and Rangpur lime. This indicated an ability of a genotype to grow under *in vitro* conditions at different growth rates. With an increase in salt conc., there was a decrease in final callus weight (g), dry callus weight (g), callusing index and per cent increase in final callus weight over the initial callus weight, but this decrease was

rootstock specific. Ben-Hayyim and Goffer (1988) also reported that growth rate as well as gain in fresh callus weight was inhibited by salt.

Maximum reduction in final callus weight (g) was recorded in trifoliolate orange, followed by rough lemon and Rangpur lime. These results are in accordance with Piqueras and Hellin (1992), who reported that unselected and tolerant callus lines showed marked differences in their growth curves and salt tolerance.

Rough lemon had the highest dry callus weight (g), followed by trifoliolate orange and Rangpur lime. The decrease in dry callus weight (g) with increase in salt conc. was less in Rangpur lime, moderate in rough lemon and more in trifoliolate orange. In trifoliolate orange and rough lemon, at higher salt conc. water content was relatively less, since there was less difference between fresh and dry callus weight (g). This might be due to less water absorption or exosmosis at higher salt conc.

Mean callusing index was higher in trifoliolate orange and rough lemon as compared to Rangpur lime, but with the increase in salt conc. the decrease in callusing index was highest in trifoliolate orange, followed by rough lemon and Rangpur lime. So, this factor can be considered as a better criterion for screening of rootstocks for salt tolerance under *in vitro* conditions.

Increase in final callus weight (g) over the initial callus weight (g) was highest in trifoliolate orange and rough lemon and low in Rangpur lime, but with the increase in salt conc., there was a decrease in this increase, which was severe in trifoliolate orange as it exhibited negative growth at

high salt conc. (0.15 and 0.20 M), moderate in rough lemon and low in Rangpur lime. At higher salt conc. the decrease in callus weight (g) over the control was more than 100 per cent in trifoliate orange indicating that the callus of this rootstock lost water due to exosmosis. On the basis of these observations, it was concluded that Rangpur lime was tolerant, rough lemon was moderately tolerant and trifoliate orange was susceptible to salinity.

The decrease in final callus weight (g) might be attributed to both osmotic and toxic effects of the salt. The first loss in callus weight (g) seemed to be osmotic, because under stress, water availability was reduced. The maintenance of higher callus weight (g) seemed to be the ability of Rangpur lime to maintain higher water content at higher salinity level. In trifoliate orange, the loss in final callus weight (g) might be attributed to the exosmosis of water from callus.

As the growth of Rangpur lime callus continued at higher salt conc. at higher rate, this rootstock was considered as tolerant and in rough lemon, the growth was less, so, it was moderately tolerant and in trifoliate orange, there was a negative growth i.e. loss in initial callus weight (g), indicated its susceptibility to salinity. Spiegel-Roy and Ben-Hayyim (1985) reported that selected cell lines for salt stress could be grown in media containing higher conc. of salts, whereas, the non-selected lines could not be grown. Similar results were also reported by Kochba *et al.* (1982) in salt tolerant cell lines of Shamouti orange. Ben-Hayyim and Kochba (1982) reported that growth curves of the selected salt tolerant lines in response to NaCl

stress showed a longer lag period, a slightly slower rate of increase in fresh weight and about 50 per cent yield compared to the same line in absence of the salt, whereas, non-selected lines could not grow in such a high salt conc. In our study also, growth of Rangpur lime callus was less affected by increase in salt conc., hence it was considered tolerant in comparison to other two rootstocks. The negative growth in trifoliate orange might be due to exosmosis, as at intermediate and higher salinities, the differences in dry callus weight (g) were less in comparison to fresh callus weight (g) at the same salt conc.

Minimum Na^+ content in callus was recorded in trifoliate orange, followed by Rangpur lime and rough lemon. With the increase in salt conc., Na^+ content in callus also increased, but to a different extent in different rootstocks. Maximum increase in Na^+ content was recorded in rough lemon. Less accumulation of Na^+ by tolerant cell lines was also reported by Deng *et al.* (1993) and Ben-Hayyim and Kochba (1983), whereas, higher accumulation was reported by Ben-Hayyim *et al.* (1985) and Spiegel-Roy and Saad (1997).

At low salt conc. (0.05 M), trifoliate orange restricted accumulation of Na^+ in cells, but not at higher salt conc., so at lower salt conc. trifoliate orange seemed to be a Na^+ excluder. These results are in agreement with the findings of Syvertsen and Yelenosky (1988) at whole plant level. It was concluded that at low salinity, Na^+ accumulation may not be consequential, but at higher salinity, it might have affected the callus growth.

Maximum K^+ content was recorded in rough lemon followed by Rangpur lime and trifoliate orange. K^+ content in calli decreased with increase in salt conc., but this decrease was severe in trifoliate orange, followed by Rangpur lime and rough lemon. Ben-Hayyim *et al.* (1985) reported that exposure of NaCl-sensitive and tolerant lines to equal conc. of NaCl resulted in a greater loss of K^+ from sensitive lines, indicating that growth and ability to retain high levels of internal K^+ were correlated. It is obvious also, because K^+ is a useful element, and it should not be replaced or less replaced by Na^+ . Spiegel-Roy and Saad (1997) and Deng *et al.* (1993) also reported much higher K^+ in salt tolerant selected lines than the non-selected ones.

Na:K ratio was maximum in rough lemon, followed by Rangpur lime and trifoliate orange, but this ratio increased with increase in salinity, indicating replacement of K^+ by Na^+ . Rangpur lime maintained this ratio at a lower level under moderate salinity in comparison to other rootstocks. Therefore, maintenance of this ratio at a lower level appeared to be an important factor in deciding tolerance to salinity and as such, this ratio can be used as a criterion for screening of citrus rootstocks for salt tolerance.

Mean Ca^{++} content was maximum in trifoliate orange, followed by Rangpur lime and rough lemon. As salinity increased, there was a change in Ca^{++} content in callus, but definite trend was not observed in different rootstocks. Similar trend was also observed at whole plant level in the present study.

Mean Mg^{++} content was maximum in trifoliate orange, followed by rough lemon and Rangpur lime, indicating differential absorption of this ion by different rootstocks. With the increase in salt conc., Mg^{++} content decreased in calli of all the rootstocks, but to a different extent. At moderate salinity, Rangpur lime tended to maintain higher Mg^{++} . Similar observations were recorded at whole plant level in the present study. Hence, Mg^{++} content in cells appeared to be associated with salt tolerance of a rootstock.

Maximum mean Cl^{-} content was recorded in trifoliate orange callus, followed by rough lemon and Rangpur lime. A proportionate increase in Cl^{-} content in callus was recorded with an increase in salt conc. in all the rootstockss, but this increase was maximum in trifoliate orange followed by rough lemon and Rangpur lime. Increase in Cl^{-} content in salt tolerant lines was also reported by Ben-Hayyim *et al.* (1985), Spiegel-Roy and Saad (1997) and Piqueras *et al.* (1996). Ben-Hayyim and Kochba (1983) reported that accumulation of K^{+} and Cl^{-} accompanied the inhibition of growth and the toxic effect was due to the accumulation of Cl^{-} . Hence Cl^{-} was considered to be a more deleterious ion for citrus and considered to be a crucial factor in determining the tolerance or susceptibility of rootstocks to salinity.

Browning of callus in salt stress medium seemed to be directly related to the salinity tolerance of a rootstock as minimum browning was recorded in Rangpur lime followed by rough lemon and trifoliate orange. Hence, per cent browning of callus can be considered as a useful criterion

for screening of citrus rootstocks for salt tolerance under *in vitro* conditions. As in salt sensitive and at higher salt conc., accumulation of Cl^- was there, which is a toxic ion and hence, browning of callus appeared to be directly linked to Cl^- content in cells, which killed the cells and these cells turned brown.

SO_4^- content was not different in calli between the rootstocks and salt levels and hence was considered less important ion for determining the tolerance of rootstock calli to salinity. Similar observations for SO_4^- content were recorded at whole plant level in this study.

All the screening techniques namely, *in vivo* and *in vitro* seed germination at different salinity levels, screening at seedling and callus level were observed equally effective for screening of citrus rootstocks for salt tolerance. Hence, for the screening of large germplasm collections and hybrids, where, either space or seed material is a problem, screening at cell level can be used, because, it requires less space and from a single seed, large number of explants can be taken.

Regeneration of plantlets from calli is important for a screening and selection programme. In the present study, plantlet regeneration was achieved in rough lemon and Rangpur lime, but not in trifoliate orange. Out of the five media used for regeneration, MS_G [MS +NAA (0.5 mg/l), BAP (3mg/l) and malt extract (0.5g/l)] for rough lemon and MS_F [MS + kinetin (1 mg/l), BAP (0.5 mg/l) and malt extract (0.5 g/l) for Rangpur lime were observed satisfactory, as there was maximum plantlet regeneration on these media. This study indicated that media requirement

for regeneration was genotype specific which was in accordance with the earlier reports (Kochba and Spiegel-Roy, 1977; Bhansali and Arya, 1978; Barlass and Skene, 1982; Matsumoto and Yamaguchi, 1984; Raman *et al.*, 1987, 1992; Oh *et al.*, 1991; Song *et al.*, 1991; Koc *et al.*, 1992; Garcia-Agustin and Primo-Millo, 1992; Gill *et al.*, 1990, 1994, 1995).

Implications in Citrus Improvement

It was expected that no material contribution in terms of plant material would emanate from the present studies, however, this can be regarded as systematic attempt to elucidate differences in salt tolerance levels and possibly the mechanisms at callus and plant level of the same genotype. Wherever, the tolerance limits of different rootstocks were in consonance at callus as well as plant level, the screening at callus level would not only be easy, but reliable too, as was the case with Rangpur lime. In other two cases, where the callus/tissue responses and whole plant responses differ, this warrants the impact of organogenesis and/or variations in mechanisms for osmotic adjustment. In such cases, screening at both the levels would embark on identifying true resistance under *in vivo* conditions. This combination of screening would increase the level of precision and minimize confounding effects of environmental variations on deciphering stress tolerance levels. In the present studies, two important elements of salinity problem encountered in real agricultural situation were considered. Results depicted interesting features. Although, on overall basis, SO_4^- dominated salinity caused more seedling mortality over time and space, however, it was slow and would permit management options to

overcome it with some degree of success. On the other hand, Cl^- dominated salinity appeared more lethal and being fast would permit lesser options for management. The relative differences (increase) in Na:K ratio and decrease in Mg^{++} levels over different kinds of salinity and rootstocks revealed that rootstocks were able to manifest different levels of homeostasis, and hence, salt tolerance. Rangpur lime depicted somewhat maintenance of Mg^{++} levels and Na: K ratio over different salt conc. in SO_4^- and Cl^- dominated salinities. This can be a potential candidate as donor for genes regulating salt tolerance limits either through higher deposition of salts in vacuolar system permitting normal metabolic activities of the cellular system and/or possess mechanisms for osmotic adjustment. Thus, the present study can be regarded as a stepping stone to unravel biological facts for salt tolerance in citrus and warrants for supplementary studies on continuous basis.

CHAPTER - 6

SUMMARY

The present study entitled, "Screening of *Citrus* rootstocks for salt tolerance at seedling and callus level" was undertaken during 1998-99 and 1999-2000 at the Department of Plant Breeding, CCS Haryana Agricultural University, Hisar. The major objectives of the study were : to screen the citrus rootstocks for salt tolerance under *in vivo* and *in vitro* conditions; to study the changes in physiological and morphological characters in citrus as affected by different levels of soil salinity and to find out association between *in vivo* and *in vitro* screening for tolerance to salinity using various morphological and physiological parameters.

In first experiment, seeds of three rootstocks viz., rough lemon, Rangpur lime and trifoliate orange were sown under *in vivo* and *in vitro* conditions. The salt levels used for *in vivo* seed germination were control, 2, 4, 6 and 8 dS/m of Cl^- dominated salinity in soil and for *in vitro* seed germination were control, 0.05, 0.10, 0.15 and 0.20 M of NaCl in growth media. Observations on initiation of germination, maximum germination and plant characters were recorded. In second experiment, eight months old seedlings of three rootstocks were planted in pots under screen house in Cl^- dominated and SO_4^- dominated salinity at control, 2, 4, 6 and 8 dS/m.

Appearance of salt toxicity symptoms, mortality of plants and changes in plant characters were recorded and dried aerial parts and root samples were analysed for Na^+ , K^+ , Ca^{++} , Mg^{++} , Cl^- and SO_4^- contents. In the third experiment, the callus was induced from internodal stem segments and stressed for six months at control, 0.05, 0.10, 0.15 and 0.20 M of NaCl in growth media. Changes in callus characteristics were recorded and dried calli were analysed for Na^+ , K^+ , Ca^{++} , Mg^{++} , Cl^- and SO_4^- contents. Plantlet regeneration from calli was attempted using different regeneration media.

Trifoliate orange seeds germinated poorly under *in vitro* conditions, but germination was improved by the addition of low conc. of GA_3 (1 mg/l). Increase in salinity/salt conc. reduced and delayed the seed germination in all the rootstocks, but maximum reduction occurred in trifoliate orange followed by rough lemon and Rangpur lime. Further, increase in salt conc. caused a decrease in all the plant characters viz., plant height (cm), stem diameter (cm), number of leaves/plant, fresh aerial and root bio-mass (g)/plant and dry aerial and root bio-mass (g)/plant. But, in general, the reductions in Rangpur lime were minimum, moderate in rough lemon and severe in trifoliate orange. The rootstocks exhibited environmental specificity for germination and seedling growth under *in vivo* and *in vitro* conditions. Rangpur lime at low salinity levels indicated a threshold level beyond which its growth was reduced.

For the rootstock seedlings, Cl^- dominated salinity proved to be more harmful than SO_4^- dominated salinity, because in Cl^- dominated salinity, seedling mortality occurred at a faster pace. Mortality of seedlings

increased with increase in salinity, but, minimum mortality occurred in Rangpur lime and that to at higher salinity levels (6 and 8 dS/m) and maximum mortality occurred in trifoliolate orange, where none of the seedlings survived beyond the control. Further, increase in salinity resulted in decrease in the plant characters, but the decrease was minimum in Rangpur lime, moderate in rough lemon and severe in trifoliolate orange. At low salinity level (2 dS/m), Rangpur lime exhibited an increase in growth characters. Rangpur lime was considered tolerant, rough lemon moderately tolerant and trifoliolate orange susceptible to salinity.

Minimum Na: K ratio in aerial parts was maintained by Rangpur lime, followed by rough lemon and trifoliolate orange. As the salinity level increased, Na^+ and Cl^- increased, K^+ and Mg^{++} decreased in aerial parts, but the increases and decreases were less in Rangpur lime and maximum in trifoliolate orange. Na: K ratio and Cl^- content in aerial parts determined the salt tolerance of rootstocks. Both these values were maintained at lower level in Rangpur lime.

When callus was induced in rootstocks, inter-nodal stem segments as explants from *in vitro* grown seedlings and MS_A medium were found adequate. As the salt conc. increased in culture media, callus growth decreased, but the decrease was less in Rangpur lime, moderate in rough lemon and higher in trifoliolate orange. Similarly, as the time and conc. of salt increased, browning of callus also increased, but Rangpur lime exhibited minimum browning and trifoliolate orange exhibited maximum browning. Increase in salt conc. in medium caused an increase in Na^+ ,

Na: K ratio and Cl^- content, but a decrease in K^+ and Mg^{++} , however, increase/decrease was less in Rangpur lime and more in rough lemon and trifoliate orange. Callus growth, callus browning percentage, Cl^- content and Na: K ratio were observed as reliable criteria for screening of salt tolerance. Regeneration from callus in different rootstocks exhibited a media specificity.

From all these studies, Rangpur lime was observed as tolerant, rough lemon as moderately tolerant and trifoliate orange as susceptible to salinity. Hence, these techniques can be used for screening for salt tolerance in citrus rootstocks.

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APPENDIX-1

Mean original values (A x B x C) of characters which were subjected to statistical analysis in case of two salinity types

Salinity level (dS/m)	Salinity type					
	Chloride dominated			Sulphate dominated		
	Rough lemon	Rangpur lime	Trifoliate orange	Rough lemon	Rangpur lime	Trifoliate orange
Plant height (% of control)						
Control	100.00	100.00	100.00	100.00	100.00	100.00
2	81.55	78.16	32.39	93.28	94.65	60.33
4	45.70	100.21	16.18	43.30	94.98	47.67
6	35.80	86.49	12.14	45.56	95.03	37.54
8	14.91	68.50	7.48	23.00	76.10	38.20
Stem diameter (% of control)						
Control	100.00	100.00	100.00	100.00	100.00	100.00
2	88.64	101.85	79.98	83.67	85.02	85.04
4	80.72	93.67	49.73	80.49	75.66	61.16
6	67.30	88.60	41.17	72.69	72.17	31.49
8	51.97	70.73	24.51	45.54	69.60	27.24
Total number of leaves/plant (% of control)						
Control	100.00	100.00	100.00	100.00	100.00	100.00
2	93.25	93.83	46.52	107.20	100.40	71.15
4	93.24	88.72	70.73	106.70	108.27	44.67
6	88.10	112.18	54.27	85.48	98.64	56.60
8	82.76	95.12	48.99	58.28	83.63	45.36
Number of healthy leaves/plant (% of control)						
Control	100.00	100.00	100.00	100.00	100.00	100.00
2	76.47	74.11	0.00	61.33	75.69	0.00
4	36.17	60.43	0.00	8.33	57.21	0.00
6	0.00	33.82	0.00	3.70	73.92	0.00
8	0.00	11.76	0.00	0.00	7.58	0.00
Number of affected leaves/plant						
Control	3.33	1.33	5.00	3.33	1.33	5.00
2	5.00	4.67	0.00	7.00	7.67	0.00
4	6.00	6.00	0.00	7.00	11.67	0.00
6	2.00	8.67	0.00	0.00	7.00	0.00
8	0.00	3.00	0.00	0.00	4.67	0.00
Fresh leaf weight (g)/plant (% of control)						
Control	100.00	100.00	100.00	100.00	100.00	100.00
2	98.44	89.16	16.53	93.72	90.06	19.06
4	82.66	77.71	13.03	72.14	63.64	13.33
6	62.26	76.67	6.82	42.43	50.52	10.03
8	21.11	41.20	6.37	33.34	47.98	8.78

Dry leaf weight (g)/plant (% of control)						
Control	100.00	100.00	100.00	100.00	100.00	100.00
2	84.22	87.31	38.34	87.97	75.47	39.30
4	81.92	82.32	31.83	57.98	61.06	38.84
6	60.45	72.21	12.09	47.37	53.24	38.89
8	24.81	49.02	11.17	32.85	51.48	30.61

Fresh stem biomass(g)/plant (% of control)						
Control	100.00	100.00	100.00	100.00	100.00	100.00
2	76.71	103.28	51.59	83.32	129.53	57.00
4	51.25	80.21	40.65	55.85	91.77	42.49
6	31.45	77.13	33.84	30.86	70.11	33.43
8	30.91	47.51	28.02	26.29	50.04	29.64

Dry stem biomass (g)/plant (% of control)						
Control	100.00	100.00	100.00	100.00	100.00	100.00
2	78.46	110.03	73.89	77.99	128.48	76.31
4	52.86	89.21	60.09	53.63	86.88	62.17
6	44.67	72.45	49.84	39.06	69.83	54.57
8	39.62	50.51	39.69	31.80	52.77	43.19

Fresh root weight (g)/plant (% of control)						
Control	100.00	100.00	100.00	100.00	100.00	100.00
2	82.08	103.69	43.84	84.43	108.32	54.42
4	49.65	73.17	32.55	58.13	77.30	45.62
6	31.17	56.71	30.50	32.85	60.09	35.49
8	23.75	35.25	26.06	30.09	46.66	35.39

Dry root weight (g)/plant (% of control)						
Control	100.00	100.00	100.00	100.00	100.00	100.00
2	81.25	101.59	85.05	82.63	113.72	93.93
4	43.52	63.95	70.89	56.20	71.28	72.69
6	36.48	52.55	64.71	59.09	55.71	63.72
8	21.12	48.57	56.06	42.41	46.60	63.33

Leaf area (cm ²)/ leaf						
Control	27.33	19.73	3.47	27.33	19.73	3.47
2	13.55	13.31	3.18	22.74	16.05	3.19
4	11.49	12.34	3.05	11.95	14.86	3.08
6	8.92	16.99	3.02	7.37	12.33	3.04
8	8.92	11.16	3.01	7.51	8.40	3.00

Number of branches/plant						
Control	5.33	8.00	4.33	5.33	8.00	4.33
2	4.67	8.67	2.67	3.33	7.67	3.33
4	4.33	4.33	3.33	4.00	7.00	2.33
6	4.00	6.00	2.33	3.33	5.33	3.33
8	3.33	4.33	2.33	4.67	5.33	2.67

Per cent Na ⁺ content in aerial parts						
Control	0.248	0.146	0.674	0.248	0.146	0.674
2	0.599	0.344	0.818	0.449	0.274	0.818
4	0.599	0.369	0.818	0.954	0.449	1.227
6	0.886	0.356	1.025	1.364	0.406	1.364
8	1.022	0.954	0.954	1.650	1.159	1.650

Per cent Na ⁺ content in roots						
Control	0.094	0.063	0.171	0.094	0.063	0.171
2	0.217	0.155	0.111	0.202	0.184	0.150
4	0.146	0.094	0.150	0.272	0.146	0.295
6	0.312	0.094	0.208	0.356	0.264	0.155
8	0.362	0.267	0.369	0.886	0.160	0.137

Per cent K ⁺ content in aerial parts						
Control	0.972	1.312	1.250	0.972	1.312	1.250
2	1.111	0.972	0.984	0.903	0.972	1.250
4	1.042	0.972	0.972	0.903	0.903	0.972
6	0.903	0.833	0.694	0.903	0.764	0.903
8	0.764	0.764	0.625	0.833	0.694	0.764

Per cent K ⁺ content in roots						
Control	0.244	0.176	1.042	0.244	0.176	1.042
2	0.203	0.191	0.162	0.197	0.205	0.547
4	0.162	0.191	0.206	0.257	0.271	0.272
6	0.257	0.209	0.547	0.289	0.194	0.250
8	0.188	0.312	0.391	0.181	0.163	0.247

Na: K ratio in aerial parts						
Control	0.255	0.111	0.534	0.255	0.111	0.534
2	0.541	0.354	0.831	0.497	0.282	0.955
4	0.575	0.380	0.982	1.041	0.498	1.263
6	0.983	0.427	1.587	1.510	0.531	1.511
8	1.338	1.249	1.526	1.981	1.671	2.161

Na: K ratio in roots						
Control	0.386	0.359	0.164	0.386	0.359	0.164
2	1.071	0.812	0.686	1.017	0.896	0.274
4	0.902	0.493	0.730	1.061	0.539	1.085
6	1.219	0.450	0.381	1.239	1.365	0.620
8	1.928	0.857	0.943	4.925	0.986	0.555

Per cent Ca ⁺⁺ content in aerial parts						
Control	3.700	3.800	2.933	3.700	3.800	2.933
2	3.600	3.417	2.700	3.300	2.700	2.300
4	3.300	3.400	2.500	2.700	2.500	2.100
6	2.900	2.500	3.200	2.350	2.200	2.100
8	2.600	2.300	2.500	1.200	1.600	2.300

Per cent Ca^{++} content in roots						
Control	2.400	2.400	1.300	2.400	2.400	1.300
2	2.200	3.000	1.900	1.700	2.000	1.800
4	1.700	1.700	1.800	1.950	2.100	1.500
6	2.800	1.200	1.600	1.700	1.600	1.500
8	2.000	1.300	2.000	1.600	1.200	2.200

Per cent Mg^{++} content in aerial parts						
Control	1.120	1.080	1.200	1.120	1.080	1.200
2	0.840	0.660	0.480	0.960	1.380	0.600
4	0.660	0.660	0.600	0.780	0.900	0.660
6	0.490	0.720	0.480	0.720	0.420	0.540
8	0.240	0.540	0.600	0.300	0.300	0.480

Per cent Mg^{++} content in roots						
Control	1.780	3.240	1.140	1.780	3.240	1.140
2	2.700	1.740	2.460	1.930	1.560	0.540
4	1.320	0.720	3.300	2.400	2.770	1.140
6	1.870	1.080	2.640	1.700	0.960	1.140
8	0.480	3.540	4.500	1.380	3.240	3.360

Per cent Cl^- content in aerial parts						
Control	0.500	0.380	0.280	0.500	0.380	0.280
2	0.780	0.440	0.440	0.560	0.560	0.560
4	0.880	0.500	0.560	0.720	0.660	0.720
6	0.880	0.500	0.620	0.780	0.780	0.720
8	1.340	0.720	0.840	0.840	0.960	1.120

Per cent Cl^- content in roots						
Control	0.280	0.840	0.340	0.280	0.840	0.340
2	0.340	0.620	0.280	0.440	0.500	0.380
4	0.380	0.780	0.340	0.880	0.780	0.780
6	0.500	0.880	0.280	0.380	0.440	0.220
8	0.160	0.880	0.340	0.340	0.620	0.280

Per cent SO_4^{--} content in aerial parts						
Control	0.018	0.036	0.018	0.018	0.036	0.018
2	0.018	0.026	0.031	0.018	0.018	0.048
4	0.022	0.022	0.032	0.021	0.027	0.061
6	0.022	0.023	0.027	0.029	0.034	0.034
8	0.022	0.027	0.033	0.027	0.027	0.045

Per cent SO_4^{--} content in roots						
Control	0.014	0.015	0.013	0.014	0.015	0.013
2	0.013	0.012	0.019	0.012	0.013	0.040
4	0.019	0.014	0.015	0.009	0.015	0.029
6	0.015	0.015	0.013	0.007	0.021	0.017
8	0.016	0.013	0.019	0.007	0.029	0.034

ABSTRACT

- a) Title of Thesis : Screening of *Citrus* rootstocks for salt tolerance at seedling and callus level
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- c) Title of degree : Doctor of Philosophy
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- f) Year of award of degree : 2000
- g) Major subject : Plant Breeding
- h) Total number of pages in thesis : 132 + xvi
- i) Number of words in the Abstract : Approximate 350

The present study was planned to screen citrus rootstocks for salt tolerance under *in vivo* and *in vitro* conditions, to study the changes in physiological and morphological characters in *citrus* rootstocks as affected by salinity and to find out association between *in vivo* and *in vitro* screening for tolerance to salinity.

In first experiment, seeds of three rootstocks viz., rough lemon, Rangpur lime and trifoliate orange were sown *in vivo* at control, 2, 4, 6 and 8 dS/m of Cl⁻ dominated salinity and *in vitro* at control, 0.05, 0.10, 0.15 and 0.20 M of NaCl in growth medium. In second experiment, eight months old seedlings of these rootstocks were planted in two type of

salinity i.e. Cl^- dominated or SO_4^- dominated type at control, 2, 4, 6 and 8 dS/m. After eight months of growth under salt stress, the dried samples of aerial parts and roots were analysed for Na^+ , K^+ , Mg^{++} , Ca^{++} , Cl^- and SO_4^- . In third, experiment, calli of these rootstocks were put in stress medium at control, 0.05, 0.10, 0.15 and 0.20 M NaCl, for six weeks and dried calli were analysed for the elements mentioned above.

Salinity reduced and delayed the seed germination, but less affected rootstock was Rangpur lime. Increase in salinity reduced seedling growth, but minimum reduction occurred in Rangpur lime and maximum in trifoliate orange.

Seedling mortality occurred at an early age in Cl^- dominated salinity and trifoliate orange was the most affected rootstock. Increase in salinity reduced the plant growth, but least reductions occurred in Rangpur lime. Na^+ and Cl^- increased with increase in salinity, but less increase was observed in Rangpur lime. K^+ and Mg^{++} content decreased with increase in salinity. Rangpur lime maintained these ions at moderate salinity. Rangpur lime also exhibited less increase in Na: K ratio at low salinity.

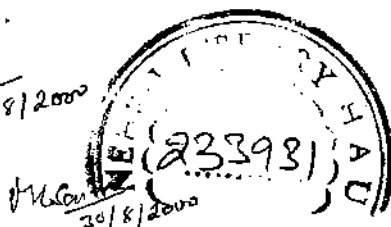
Callus growth was reduced in saline medium, but less reduction occurred in Rangpur lime, but reduction was severe in trifoliate orange. Na^+ and Cl^- , Na: K ratio and per cent browning of calli increased with increased salinity, but increase was less in Rangpur lime than trifoliate orange and rough lemon. Rangpur lime callus also maintained K^+ and Mg^{++} at moderate salt concentration.

Seed germination, Na:K ratio and Cl^- conc. in aerial parts of seedlings and calli were found to be better criteria for screening salt tolerant genotypes. All the techniques studied were found effective for screening of rootstocks for salinity tolerance.

Major Advisor

M. Saini
30/8/2000

Head of the Department



Signature of student

Bairi