



## SALINITY AND CITRUS ROOTSTOCKS AND INTERSTOCKS

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### ABSTRACT

In comparison to other horticultural crops, Citrus species are classified among very sensitive plants to salinity. One of the methods for increasing salinity tolerance in Citrus is grafting of commercial and sensitive cultivars to salinity on resistant rootstocks to salinity and evaluation the effect of interstocks. In this regard, recognition the reaction of different Citrus species under salinity stress as well as evaluation the effects of different combinations of rootstock and scion and interstock on each other can be effective help on increasing resistance to salinity and will be reduced the difficulties in the way of crop production. Putting an interstock between rootstock and scion not only can be improve growth, longevity, productivity and fruit quality, but also is able to increase tolerance to salinity. Finding Citrus cultivars and species, which could be reduce salinity stress in the role of interstock, is suitable solution for confronting to salinity problems in Citrus growing regions. Accordingly, understanding the basic mechanism of salinity in Citrus and response of different Citrus cultivars and species to salinity stress has particular importance.

### INTRODUCTION

Plants during their growth and development period may be confront with environmental stresses such as drought, heat, UV rays, pollutions, pests and diseases attack and salinity. Among these stresses, salinity is one of the biggest factors limiting plant growth and crop production in the world, which has long been a concern for human [1]. In some areas, salinity is relative to soil quality or the quality of bedrock, but in some areas may be due to irrigation with saline water, poor drainage and excessive application of chemical fertilizers [2]. In saline areas, many amounts of chloride, sulfate, bicarbonate, and sodium, calcium, magnesium, and sometimes nitrate and potassium ions are the most important causes of salinity, which in the saline areas sodium and chloride ions are usually the predominant ions and leads to damages caused by salinity [3]. Soil salinization is a serious problem in low rainfall areas. In the soil of saline areas, the salts concentration naturally is high and irrigation by unsuitable water can be increase more accumulation of salts and is leading to increase soil salinity [4]. In the saline environments, there is a great deal complexity in environmental variations. In these areas, different ions are effective on appearance of salinity conditions and twin effects of these ions to each other can be have synergist and/or antagonist influence in the plant. In high concentration of these ions, necessary nutrient elements for plant is limiting, pH and soil structure is changing and is leading to reduce oxygen amount in root medium or the effects of mechanical damages will occur in the plants. Due to the complexity of salinity agents, adaptation and tolerance conditions of plants to salinity also is complex and variable. Under salinity conditions, some plants biochemical and physiologically are compensated the damages arising from salinity [5]. In relation to salinity, plants generally were classified into two categories halophyte and glycophyte. Halophytes tolerate high salinities and in contrast, glycophytes have low tolerance against salinity. Most horticultural crops due to low tolerance to salinity are in the glycophyte category [6]. Citrus is one of the important horticultural crops, which in viewpoint of production is after Banana in the second world ranking and every year are increasing its area under cultivation and production level in the world [7]. Most Citrus orchards there are in the regions that in these regions due to insufficient or non-regular distribution annual rainfall, irrigation of the trees to produce maximum yield is necessary.

In some of these regions, the soil is so salty that reduces growth and productivity of citrus. In comparison to other horticultural crops, Citrus species are classified among very sensitive plants to salinity. However, salt tolerance is various between different Citrus species. Since the commercial Citrus species are propagated by grafting, scion tolerance to salinity is much depending to rootstock type [8]. Therefore, one of the methods for increasing salinity tolerance in Citrus is grafting of commercial and sensitive cultivars to salinity on resistant rootstocks to salinity and evaluation the effect of interstocks [9]. In this regard, recognition the reaction of different Citrus species under salinity stress as well as evaluation the effects of different combinations of rootstock and scion and interstock on each other can be effective help on increasing resistance to salinity and will be reduced the difficulties in the way of crop production. Citrus growing regions in Iran are divided into two parts, north and south. Except the part of northern orchards, other citrus orchards are located in the areas that in these areas climates and soil are susceptible to salt damages. In these areas, especially in the southern regions of Iran, low rainfall, necessity of irrigation due to hot weather and high evaporation, existence salt elements in water and excessive salts in the soil increase damages related to salinity day by day.

## **SALINITY**

All under cultivation lands, if are irrigated with unsuitable water, have ability to salinization. History shows several societies that their structure has been on the irrigated agriculture have disappeared because of the used lands salinization [10]. For example, history indicates that salinization of arable lands in ancient Mesopotamia in 3700 to 4400 years ago has been due to irrigate by saline water [11]. Salt stress has been an important factor in human history and agricultural systems since ancient to present times, which can be impaired in plant growth and development due to the presence of excessive minerals in the soil [1] or excessive accumulations of salt in the irrigated soil [12]. Salinity problem is one of the most important factors limiting plant growth and crop production in the world [10]. Soil and water salinity has always existed in nature, but the problems arising from salinity are increasing due to improper use of water and soil resources by human [13]. Currently, approximately 40% of the arable lands in the world are confronting to the problem of salinity [14]. According to statistics provided by the Ministry of Jihad-Agriculture, 15% of the total cultivated lands in Iran are salty or are involved in some way with salinity problem. In the under cultivation lands, due to water evaporation from the soil surface, salt is settling in a few cm of the soil surface and farmers are not able to destroy it through more irrigation. This subject has been a basic problem in many arid and semi-arid regions of the world [4]. Salinity problem arises when the concentration of nonessential ions is increasing in the soil than essential ions for plant and the plant is forcing to absorb required and necessary elements from rich environment of unnecessary and often toxic elements. In saline environments, the ions of chloride, sulfate, bicarbonate, sodium, calcium, magnesium, and sometimes nitrate and potassium ions are problematic and chlorides and sulfates are the most important factors in incidence of salinity stress [15].

## **CLASSIFICATION OF PLANTS IN RELATION TO SALINITY**

In relation to salinity, plants are divided into two categories:

### **I. Halophytes plants**

Halophyte plants called the plants that are able to tolerate high concentrations of salt. Halophyte plants are divided into two groups of true halophytes (Euhalophytes) and optional halophytes (Oligohalophytes). Euhalophytes tolerate high salinities and Oligohalophytes tolerate moderate salinity and do not tolerate high salinities. Many halophytes are able to store the absorbed chloride and sodium by root in vacuole of leaf mesophyll cells and thereby prevent adverse effects of the two ions in cytoplasm [16,17].

### **II. Glycophytes plants**

Glycophytes are the plants that do not able to tolerate high salt concentrations and in saline environments severely are placed under stress. Only some of these plants are able to accumulate absorbed sodium and sometimes chloride [18,19] in the vacuole of themselves root cells and then excrete additional ions by expending energy [20].

Generally, the results of investigations show that effect of salinity on plant growth and metabolism is due to reduce osmotic potential arising from salt accumulation [21,22] and ions toxicity [23,24,25,26]. Salinity stress affects different aspects of plant growth and metabolism. In this context, the most important cases that have been evaluated by researchers are photosynthesis [23,27,28,29] nitrate reduction [30,31,32,33] and internal imbalance of plant growth regulators [34,35,36]. Thus, in saline environments, high concentration of ions in root environment affects the nutrition uptake by the root [37,38,39], which these effects is mainly attributed to the high concentrations of chloride and sodium [40].

Reduction of potassium uptake that has role in stomata conductivity in the leaves and activating enzymes and transportation within plant [40,41], reduction of calcium and magnesium uptake [42] and uptake reduction of ammonium and nitrate [43] are among negative effects of salinity stress in relation to nutrients absorption by plants. During period of evolutionary history, many of the native plants of different varieties and families have adapted to salinity. Accordingly, investigators have suggested integrated approach of biological and physical processes in saline soils to prevent excessive expansion of salinity as well as preventing crop reduction and increasing plant fertilization [44,45].

### **EFFECTS OF SALINITY ON THE PLANTS**

Salinity is affecting on various aspects of plant metabolism and is leading to the changes in plant physiology and morphology [46]. Salt accumulation phenomenon is limited to the cells that are dividing and severely respiring. These cells mainly are meristematic cells and in viewpoint of ions uptake are very active [47]. Symptoms of salinity stress are similar to the symptoms of drought stress, with the difference that the plants affected by salinity usually are not wilting [48]. Salinity stress has complex effects on physiological process of plant and reduction of growth rate is the most important plant reaction to salinity stress. Three main limitations for plants growth in salinity conditions are consisting:

- Collide ionic balance and reduce the availability of nutrients
- Toxic effects of ions
- Osmotic effects and reduce water amount [49].

Under salinity conditions, despite existence of mineral elements in soil, plant may be deficient in some essential elements. In this condition, usually decrease solubility of the elements such as Copper, Iron, Manganese and Zinc and increase solubility of the elements such as Boron and Molybdenum [10]. In the saline soils, due to Sodium competition, uptake of the elements such as Calcium, Magnesium and Potassium is reducing [50]. It has reported that in saline condition, uptake of nitrate and ammonium ions reduce and finally the plant affect by nitrogen deficit. This subject leads to reduce synthesis of amino acids and protein. In addition, nitrate reduction in roots is difficulty under salinity stress [43]. Reducing Magnesium uptake leads to reduce activity of many enzymes as well as causes to reduce chlorophyll synthesis [51]. Under salinity stress, excessive accumulation of the ions such as Sodium and Chloride causes to toxicity of them. Under these conditions, reduction of carbohydrates decrease and amount of starch is going down. Also, due to disorder in protein synthesis or increasing hydrolysis of proteins, amount of soluble proteins decreases [52]. The involved enzymes in biochemical process of photosynthesis such phosphoenolpyruvate (PEP) carboxylase, ribulose-bisphosphate (RuBP) as well as the enzymes of pentose-phosphate and glycolysis paths affect by ionic toxicity [53]. Salinity condition is leading to produce free radicals within cell, and unsaturated fatty acids in the membranes of intracellular are oxidized and membrane structure is impaired [54]. Osmotic effects are one of the other effects of salinity stress, which during it, water relations in the plant is impaired. In this relation occur three important changes in water relations of plant that are consisting: osmotic change, turgidity reduction and reduction of elasticity properties of cell wall [8]. These changes are resulting from the reduction in water content of the plant and water deficiency affects plant activity via influence on opening of stomata [55].

### **PLANT RESISTANCE TO SALINITY**

Under salinity stress, plant must be resist against potential reduction of water around the root, existence of toxic ions and disorder in uptake of necessary mineral elements. Accordingly, resistance to salinity is considered as ability of a plant in growth and completion its life cycle in the presence of high concentrations of salt especially sodium chloride [29]. Tolerance to salinity mostly is depending to physiological and anatomical aspects of plant and different plants use various procedures against salinity. Stabilization of ions concentration in the cytoplasm is one of the most important factors in salinity tolerance. Glycophyte plants prevent uptake of salt ions through some mechanisms and thereby prevent salt accumulation in their cells. Whereas, halophyte plants by salt accumulation are leading to constant ions concentration in the cytoplasm [44]. For example, in the glycophytes such as Maize [56], Sorghum [57], Bean [13], Wheat and Barley [44] tolerance to salinity is relation to absence entry permission of sodium ion into the cytoplasm. However many halophytes did not prevent to entry of sodium ion into the cytoplasm but they are stabilizing ion concentration of tissues in very high salinity [58]. Ions transportation into or out of the cytoplasm is the very important factor, which will regulate and stabilize the concentration of ions. This operation is doing by  $H^+$ -ATPase pumps within plasma membrane and tonoplast [59]. Restore the absorbed sodium and chloride to the root is the other mechanisms to salinity tolerance. In some plants, when sodium and chloride ions are absorbed from root and entered to xylem, the parenchyma cells selective absorb and restore them via phloem to root. Sodium reabsorption and transfer it to the root is found in species that have a low tolerance to salinity [60].

Salt discharge through salt glands is the other salinity tolerance mechanisms that are observing in some halophyte plants. In these plants, there are particular secretory structures on leaf surface, which have no vascular connection and additional salt excrete through them. These particular structures have a big role in ion regulation in leaf cells and are the most important mechanisms that halophyte plants cope with increasing salinity by it [61]. Carnify also is a type of salinity resistance mechanism that is found in many halophytes. In this case, there is high proportion of vacuole in the cells in comparison with cytoplasm. In this position cell that has too much water in its vacuoles and dilute salt concentration and excessive saline mineral do not damages [52]. In many plants, under various stresses including salinity, different materials accumulate that are compatible with the cytoplasm and are leading to osmotic adjustment [62]. This process also is one of the other procedures of plant resistance to salinity. Inorganic ions such as sodium, calcium and potassium, organic compounds including reducing sugars, some amino acids especially proline and fourth type amines combinations are involve in osmotic regulation [63]. Increasing proline and Glycine Betaine is a highlight and physiologic response in some higher plants under salinity stress. There are many reports that the addition of proline and Glycine Betaine to plant growth medium has been caused to decrease or neutralize the effects of salinity [64]. Proline accumulation under stress condition can be result of reducing protein synthesis or increasing protein catabolism [65,66]. In general, it can said that proline is enzymes guard; regulator of osmotic pressure between cytoplasm and vacuole; regulator of cytosol pH as well as a resistant nitrogen-carbon compound for quick catch up stresses effects [67,68,69,70].

### **SALINITY EFFECT ON CITRUS**

Citrus is among important horticultural crops in the world, which in terms of international trade is placed in second ranking after Banana [71]. Citrus with tolerance ability  $EC=1.1-3.2 \text{ ds.m}^{-1}$  is among sensitive plants to salinity so that in moderate and high salinity condition severely is damaging. However these plants continue to exist up to  $EC=16.0 \text{ ds.m}^{-1}$  [72,73]. Numerous studies have showed that citrus show tolerance to salinity by various mechanisms, which these mechanisms are consisting application of rootstocks, different combinations of scion and rootstock and interstocks. Application of proper rootstock or suitable scion and rootstock combinations as well as interstocks can be prevent from entrance of toxicity levels of saline elements into plant [74]. Various rootstock and scion combinations are the result of human choice than 1500 years ago. In particular, in a recent century were made many selection in citrus and human has produced citrus in places other than their natural origin. In the past, generally, citrus were grown in the areas that salinity of irrigation water was low. For example in Australia, which water salinity has been lower than  $0.5 \text{ ds.m}^{-1}$ . Now in the condition that salinity of irrigation water is higher, citrus is growing until salinity of  $1.5 \text{ ds.m}^{-1}$ , which this is equal to 7 mM concentration of chloride [75]. Soil salinity may be multiplied between irrigations depending on soil type, irrigation water and irrigation period [76]. For this reason, breeding and continuous identification of rootstocks or combinations of rootstock and scion is very necessary for growing and maintaining of citrus in the media that salinity is increasing.

### **CITRUS ROOTSTOCKS AND SALINITY**

Studies about citrus rootstocks have done in relation to application of seedling and grafting rootstocks. These studies have performed to evaluate cases such as: seedling emergence, plant morality, shoot dead, plant total weight, root, shoot and leaf weight of seedling, fibrous root length and dry weight, stem length, weight of terminal parts of plant, plant weight, stem and trunk diameter, trunk circumference, tree canopy volume, stems and leaves number, leaf necrotic (leaf scald percent), leaf abscission, leaf area, yield amount and physiological properties under salinity stress [71]. The presence resources indicate significant difference in viewpoint of resistance to salinity in Citrus genus and related species, but just as Maas [8] has reported there is no complete union between rootstocks. For example, there are differences between relative resistance of Rough lemon and Sweet orange in relation to amount of chloride in the leaf [73,77]. Grieve and Walker [73] believes difference between rootstocks may be due to genetic variations or experiment conditions (for example Australian and American Rough lemon). It is clear that reevaluation of these experiments in order to assess and create citrus rootstocks in saline condition may be distinguished some non-uniformity [78]. Research groups during in recent decades via screening and selection of resistance to salinity have reported and screened many seedling, hybrid and grafted rootstocks as following Kirkpatrick and Bitters [79] number of 29 rootstocks; Peynado and Yong [80] number of 15 rootstocks; Wutscher [81] number of 15 rootstocks; Cooper and Gorton [82] number of 30 rootstocks; Cooper and Shull [83] number of 67 rootstocks; Cooper [84] number of 50 rootstocks; Ream and Furr [85] number of 60 rootstocks; Peynado and Sluis [86] number of 20 rootstocks; Grieve and Walker [73] number of 12 rootstocks; Sykes [87] number of 25 rootstocks; Gallasch and Dalton [88] number of 31 rootstocks and Chen [89] number of 27 rootstocks of citrus.

Although screening many combinations of rootstock and scion is an admirable work in order to evaluate the media with various salinity but has practically many difficulties [90] and in fact, it is need to the method that reliability predicts rootstock and scion combination as mature tree under salinity stress. Despite performance of extensive experiments, have reported especial rootstocks like Cleopatra mandarin and Rangpur lime as tolerant rootstocks to salinity [71]. Treating the plants by various salt compounds leads to the problems relation to genotype comparison and arranging the appeared symptoms in them. In the performed experiments by Lioyd *et al.* [91], the appeared symptoms in rootstocks in reaction to salinity were observed in the middle germination and during the first 10-15 weeks of plant growth. They used also 6-24 months old seedlings in some of the treatments. These plants were treated by salt for several weeks and months. The evaluated results indicated that small young plants had high uniformity and such deduced that citrus plant age is very important factor in reaction of plant to salinity because the older plants (2-years-old) showed various reaction to salinity than the younger plants (a few months) [92].

## SODIUM AND CHLORIDE ACCUMULATION

In the wide range of plants, salt excretion is one of the mechanisms of salinity tolerance, which in fact is ability of limiting uptake or transport of salt from root to shoots [93]. Many efforts have performed to explain mechanism of prevention from salt entrance into plant or tissue, which these efforts mainly have performed on Soya bean and maize. The presence reports indicate that in Soya bean salt was excreted in internal cells of root cortex, i.e. where excretion is including reuptake of sodium from xylem sap in the area close to the root tip. Citrus alike Soya bean has excretion mechanism of sodium and chloride via prevention of them entrance into tissue [9]. Citrus rootstocks have different ability in limiting of chloride absorption and in particular via limiting or preventing chloride entrance into the cells, which these mechanisms still have not completely known. Citrus rootstocks have significant influences on accumulation of  $\text{Cl}^-$  and  $\text{Na}^+$  or both ions in foliage of the grafted and non-grafted trees. The range of  $\text{Cl}^-$  concentration can be reach up to tenfold between minimum and maximum efficiency of  $\text{Cl}^-$  regulators [80,82]. Although citrus rootstocks indicate various abilities to prevent  $\text{Na}^+$  entrance into foliage but accumulation of  $\text{Na}^+$  in the leaf is very lower than  $\text{Cl}^-$  [79,82]. Maas [8] the best  $\text{Cl}^-$  repulsive has introduced Sun queen mandarin, Grapefruit, Cleopatra mandarin, Chinese box orange and Rangpur lime respectively but other investigators the best  $\text{Na}^+$  repulsive have reported Sour orange, Cleopatra mandarin, Rusk citrange, Rough lemon and Rangpur lime respectively [71]. Trifoliate sour orange (*Poncirus trifoliata*) has introduced as weak repulsive for  $\text{Cl}^-$  [80], however is an effective repulsive for  $\text{Na}^+$  in low salinity [94]. Rangpur lime rootstock is limiting entrance of  $\text{Cl}^-$  to scion, which may be due to be its hybrid [95,96]. Therefore, Trifoliate sour orange rootstock is limiting entrance of  $\text{Na}^+$  to scion, which also may be due to be its hybrid [97]. These cases show that repulsive of  $\text{Cl}^-$  and  $\text{Na}^+$  is an inherited trait. Walker and Douglas [92] reported that difference between rootstocks is basis on amount of salt concentration in the leaf and in small amount on salt of stem that emphasize on difference of total transportation from root to foliage i.e. different rootstocks have various ability to salt repulsive.

Absorption and transmittal of  $\text{Cl}^-$ ,  $\text{Na}^+$  and  $\text{K}^+$  in relation to increase salinity is various. Treating by salt increased water amount in leaf, stem and root of Etrog citron but decreased water content in leaves, stem and roots of Kharnakhata and Rangpur lime. Due to these variations and difference in water content of leaf (60-69% fresh weight), stem (54-63%) and root (70-80%) was compared ions concentration in the tissues basis on tissue water in terms of mM. In other study, interaction between rootstock and scion was evaluated in dealing with salinity. In this study was used the grafted Navel orange and Clementine mandarin (*Citrus clementina*) on Cleopatra mandarin and Troyer citrange rootstocks. These plants were treated by nutrient solutions containing 0 to 60  $\text{mM.L}^{-1}$  NaCl under greenhouse conditions for 14 weeks. Reduction of relative growth created by salt was relative to scion, while leaves abscission was depending on rootstock type.  $\text{Cl}^-$  accumulation in the leaf's scions on Cleopatra mandarin rootstock was less than Troyer citrange rootstock.  $\text{Cl}^-$  distribution in the whole plant demonstrated that Clementine has lower ability in transfer of  $\text{Cl}^-$  from root to leaves, yet sodium content in the scions on Troyer citrange rootstock was lower than Cleopatra mandarin. Sodium distribution in the whole plant indicated that Troyer citrange decreases transfer of  $\text{Na}^+$  to scion, but there were a few differences in the all rootstock and scion combinations in viewpoint of phosphorus. As a conclusion, investigators announced that salinity strongly decreases nitrogen amount in the leaves of the grafted plants on Troyer citrange rootstock, but it had a few influence on the grafted plants on Cleopatra mandarin rootstock. In the same study, nitrogen accumulation pattern showed negative correlation with  $\text{Cl}^-$  accumulation. In addition, salinity caused to reduce  $\text{K}^+$ ,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in the leaf and root of rootstocks, scions and all combinations of scion and rootstock [98].

Results of several studies emphasize on this opinion that there are apparent differences between Mandarin and Troyer citrange rootstocks in limiting entrance of  $\text{Cl}^-$  [96].

Walker and Douglas [92] indicated that there is accurate relevance between accumulation of  $\text{Cl}^-$  and damage severity in the leaf. Behboudian *et al.* [99] reported that leaf tissues of Valencia orange, Taylor lemon and Ellendale tangor scions had similar concentrations of  $\text{Cl}^-$  after treating by salt and they concluded that the influence of rootstock on  $\text{Cl}^-$  accumulation in shoots is very higher than scion effect.

### INFLUENCE OF DIFFERENT SALTS

Some researchers believe adding calcium as  $\text{CaSO}_4$  or  $\text{Ca}(\text{NO}_3)_2$  to saline solution of root medium may be improved the effects of salinity in citrus. Leaf abscission increased in the grafted Navel orange on Cleopatra mandarin rootstock by adding  $\text{Ca}^{2+}$  to medium but decreased in the grafted Navel orange on Troyer citrange rootstock in the same conditions [100,101]. In the other study, adding  $2 \text{ mmol.L}^{-1} \text{Ca}^{2+}$  to medium had no change on leaf and shoot growth and did not change in the accumulated level of  $\text{Cl}^-$ ,  $\text{Na}^+$  and  $\text{K}^+$  in the leaves of all the applied rootstocks that were under  $50\text{-}100 \text{ mmol NaCl.L}^{-1}$  salinity for 6 weeks [98]. Growth of Trifoliate sour orange decreased by using  $\text{NaHCO}_3$  more than  $\text{Na}_2\text{SO}_4$  in the same concentration of  $\text{Na}^+$  [102]. Salem and El-Khorieby [103] found that the arrangement of the applied salts with equal concentrations in growth reduction of the tested rootstocks is  $\text{NaCl} > \text{KCl} > \text{CaCl}_2$ . Moreover, the salts of KCl and NaCl are significantly leading to reduce growth of the grafted citrus trees but in the many cases,  $\text{NaNO}_3$  has lower effects [104]. Nitrogen deduction in the leaves and fibrous roots are in the close relationship with  $\text{Cl}^-$  accumulation in them [105]. Cleopatra mandarin rootstock is a restrictive of entrance of  $\text{Cl}^-$  to plant. In the grafted plants on Cleopatra mandarin rootstock, nitrogen amount in the leaves and fibrous roots decreased lower than the grafted plants on Troyer citrange rootstock [106]. Reduction of nitrogen uptake by the plants under salinity stress may be due to inhibitory effect of  $\text{Cl}^-$  on absorption of  $\text{NO}_3^-$  [105,107]. Inhibitory effect of  $\text{Cl}^-$  on absorption of nitrate may be due to interaction between these ions in the location of ion transportation and also the citrus roots may be differently operate in distinction between the available ions and absorption mechanisms for some ions may be the more specialized than other ions. Other possibility is that high concentration of intracellular of  $\text{Cl}^-$  causes to reduce evacuation flow of nitrate and its absorption and in various forms,  $\text{Cl}^-$  regulates amount of nitrate absorption. For this reason, high level of nitrogen in the restrictive rootstocks of  $\text{Cl}^-$  entrance is due to low concentration of  $\text{Cl}^-$  [105].

### SCION INFLUENCE

The performed studies about multiplex combinations of rootstock and scion have showed that salinity has influence on their growth and performance and influence of rootstock is higher than scion [108]. However, there are many reports that indicate that scion is effective on plant growth. When the scion effects clearly appear that, the used rootstock is poor in viewpoint of ions excretion or prevention of entering those [91]. In this relation, some investigators believe that the rootstocks with excretion ability of extra  $\text{Cl}^-$  ion have more effects than scion in non-accumulation of chloride in the scion [74,99]. As chloride accumulation in the leaves as influenced by rootstock and scion,  $\text{Na}^+$  levels as influenced by rootstock [98] and scion [108]. Banuls *et al.* [98] evaluated the effect of scion on accumulation of  $\text{Cl}^-$  and  $\text{Na}^+$  in different combinations of rootstock and scion. They studied the scions of Navel orange and Clementine on Cleopatra mandarin and Troyer citrange rootstocks. These plants were treated by NaCl concentrations between  $0\text{-}60 \text{ mmol.L}^{-1}$ . The obtained results indicated that reduction of growth in high salinities is depending to reduction of water potential in the scion than accumulation of  $\text{Cl}^-$  and  $\text{Na}^+$  in the scion. Growth reduction in many cases is due to the changes caused by salt in the water relations of leaf, which is causing to reduce photosynthesis [92]. It has been suggested that osmotic regulation and turgescence keeping can be as important factor in determination of photosynthesis rate in the scion and be responsive of scion growth reduction under salinity stress [77]. Walker and Douglas [92] found that leaf water potential of Valencia orange trees is various by influenced of rootstock type. Whereas, leaf's cells of Valencia orange have high turgescence, so their growth rate is higher on the rootstock that have lower ability to limit salt entrance. This subject can be indicative the observations that in them, growth reduction of plants under salinity stress is depending on ability of rootstock to limit salt [8]. Moreover, Behboudian *et al.* [99] observed that the different grafted citrus cultivars on the same rootstocks under salinity stress showed significant difference in leaf turgescence potential. Sodium amount in the leaves of non-grafted Rough lemon has been very lower than the leaves of grafted Valencia orange on Rough lemon. In contrast, grafting Valencia on Rough lemon did not cause any change in amount of  $\text{Cl}^-$ . Grafting scions of lemon cultivars on Sour orange and Alemow caused to various effects in leaf  $\text{Na}^+$  and  $\text{Cl}^-$  amounts [109]. It is clear that accumulation of  $\text{Na}^+$  and  $\text{Cl}^-$  in the leaves may influenced by rootstock, scion or combination of rootstock and scion. Accumulation of  $\text{Cl}^-$  in the leaves has very close correlation with leaf damage (abscission percent) in Navel orange and Clementine mandarin [104].

Generally,  $\text{Na}^+$  distribution in different parts of plants indicate that some combinations of rootstock and scion lead to limit of  $\text{Na}^+$  transportation from root to shoots and leaf. For example, grafting Clementine on Troyer citrange rootstock than other rootstocks, has tendency to keep  $\text{Na}^+$  in different parts of rootstock such as fibrous and primary roots, but has less tendency to accumulate  $\text{Na}^+$  in different parts of scion such as leaf and stem. Generally, it is concluding that limiting sodium transport is not only depending to scion, but it seems in this regard the rootstocks also are effective [99]. Banuls and Primo-Millo [104] reported that the rootstock is effective on accumulation of  $\text{Cl}^-$  in the scion's leaves, but the grafted scions on Cleopatra mandarin and Clementine rootstocks had lower  $\text{Cl}^-$  than the grafted scions on Navel orange rootstock. This subject shows in some rootstock and scion combinations, the scion has important effect in accumulation of  $\text{Cl}^-$  in the leaf's tissues. This effect may be due to reduce ability in transport  $\text{Cl}^-$  from rootstock to scion, which it has observed in combination of Clementine on Cleopatra mandarin rootstock. This information reveals that there is an effective barrier in the roots of Cleopatra mandarin, which limit  $\text{Cl}^-$  entrance into roots and also there is ability to limit its transportation from root to foliage by Clementine scion, so that  $\text{Cl}^-$  accumulation occur in the rootstock roots and stem.

### INTERSTOCK INFLUENCE

According to the existing reports, interstocks have tremendous influence on accumulation of  $\text{Cl}^-$  and  $\text{Na}^+$  ions in the leaves and therefore, reduce the effects of salinity stress. In the study, Verna lemon was grafted on three rootstocks including Sour orange, Cleopatra mandarin and Alemow by using Sun Queen orange and the grafted plants were treated by different levels of salinity up to  $\text{NaCl}$  42.5 mM. The results showed that in application of interstock, chloride accumulation was very much narrower than non-application if interstock. These investigators believe that low amounts of  $\text{Cl}^-$  in the leaves of Verna lemon on Sour orange rootstock has been due to interstock existence than rootstock. Because, they indicated in the previous studies that rootstock and scion combinations of Sour orange accumulate high levels of  $\text{Cl}^-$  in themselves leaves and amount of  $\text{Na}^+$  had no change more than 8  $\text{mM.kg}^{-1}$  DM. It is clear that to determine effect of interstock is needed further experiments that should conduct in various designs. Obviously, this method can found profitable application of interstocks and their potential to create citrus resistant to salinity in the target areas [109,110].

### DISTRIBUTION OF $\text{Na}^+$ AND $\text{Cl}^-$ IN PLANT STRUCTURAL COMPONENTS

Distribution of  $\text{Na}^+$  and  $\text{Cl}^-$  in the parts of plant determines as a percentage of total plant content by combination of rootstock and scion and ion type. For example, 50-75 percent  $\text{Na}^+$  was observed in the leaf of the plants that were treated by 60 mM  $\text{NaCl}$ , but there was 35-60 percent  $\text{Cl}^-$  in their leaves [98]. In the plant such as Trifoliolate orange that is the most important restrictive of  $\text{Na}^+$  entrance, percentage of  $\text{Na}^+$  between leaves, stem and root was 0.3, 31 and 66 percent respectively, whereas amount of  $\text{K}^+$  in the leaf was equal 36 percent of total potassium of plant [111]. In relation of Distribution of  $\text{Na}^+$  and  $\text{Cl}^-$  in the different parts of plant,  $\text{Na}^+$  and  $\text{Cl}^-$  levels in the root tissue has lower changes than stem and leaf tissues [112]. Distribution of  $\text{Na}^+$  and  $\text{Cl}^-$  are usually influenced by  $\text{Ca}^{2+}$  concentration in saline medium. Calcium nitrate and calcium sulfate reduce accumulation of  $\text{Na}^+$  and  $\text{Cl}^-$  in the leaf [98,100,104], whereas calcium chloride may increase  $\text{Cl}^-$  in the leaf [101]. It has suggested that calcium-induced changes in distribution of  $\text{Na}^+$  and  $\text{Cl}^-$  may depend on composition of rootstock, scion and ion type [98].

### STRUCTURAL RELATIONS AND ROOT FUNCTIONS

#### ROOT MORPHOGENESIS

According to Castle [113], root morphogenesis, indicate two forms for citrus root. The first form is including the lattice of lateral fine roots (which are producer of materials for dense part and fibrous root) and the second form is including the roots that mainly are vertical and lower branching. Different rootstocks usually are various in viewpoint of vertical and lateral growth of their roots, so that low-growth rootstocks have fine and low-depth root system [114]. Soil texture type has more influence on root growth. Root growth in the sandy soils is very strong but in the clay soils, root may placed in soil surface influenced by non-permeable layers. Difference in soil texture between liquid and sandy medium has usually indicated that there are large effects in root morphology and its reaction to salinity [115].

#### ROOT ANATOMY

Fibrous roots in citrus have an epidermis cell layer, which placed on hypodermis and are including two types cell: One Moriband cell that are long and the other passage cell that are short [116]. Existence a lot of  $\text{K}^+$  has confirmed in the passages cell. Hypodermis is a thick layer of large cells, which have large vacuoles and thin cell wall and place before root tip.

Hypodermis differentiation is leading to improve chemical components of cell walls and dead of large cells. Some cells die 40-50 mm above root tip (cap), but other cells along with passage cells are metabolically active. There is connection pores via plasmodesmata between epidermis and passage cells, but effect of this cell walls and their interconnectivity is obscure on permeability of epidermis and hypodermis against ions and water [117]. One of the important cases of root system is the cyclical nature of the growth pattern and sleep during the winter season. This subject studied by Castel [113]. During the slow growth, stopping activity of meristem cells and be corky of fibrous roots may be cause to granulate entire root and among white roots, but effect of winter sleep is not yet clear exactly on activities root cells and especially uptake of  $\text{Na}^+$  and  $\text{Cl}^-$  [71].

### **MOBILIZATION AND ABSORPTION OF $\text{Na}^+$ AND $\text{Cl}^-$ IN ROOT AND ITS EXTRA-CELLULAR DISTRIBUTION**

In the rootstocks that have different ability in viewpoint of salinity resistance, in whole root, stable balance levels of  $\text{Na}^+$  and  $\text{Cl}^-$  are the same. The concentration of these ions increase in low salinity over time (for example 25 mM NaCl), but then remain constant at higher concentrations [92]. Measurement of chloride and sodium ions density in fibrous roots of Rangpur lime and Etrog citron in different times is indicator the difference between type of the compressed ion, rootstock type and different parts of root. Terminal part of root (2-12 mm from root tip) collects lower  $\text{Na}^+$  and  $\text{Cl}^-$  than farther area from root tip. In fact, at a distance of 40-50 mm from the root tip, the amount of  $\text{Na}^+$  and  $\text{Cl}^-$  in the salt resistant rootstocks (Rangpur lime) quickly reaches to equilibrium level, except  $\text{Cl}^-$  that in pericycle region, which equilibrium phase is relatively slow. It seems absorption of root and filling of xylem by  $\text{K}^+$  is not in a direction, but it is likely that the accumulation of these ions in vacuoles of root cells is competitive [92].

### **ROOT HYDRAULIC CONDUCTIVITY**

Root hydraulic conductance is various between citrus cultivars [10] and salinity stress affect it. This case has been observed in Washington navel orange [118], Cleopatra mandarin and Trifoliate orange [111] and Sour orange [119]. Root hydraulic conductance in non-saline conditions is lower in the salt resistant cultivars. For example, root hydraulic conductivity in Sour orange and Cleopatra mandarin is lower than sensitive rootstocks such as Milam lemon and Trifoliate orange [119]. The rootstocks with stronger hydraulic conductance have the more subtle roots under salinity stress and root weight/root length ratio is low [120]. It is expected that in the rootstocks with low root hydraulic conductivity, the roots are thicker and root weight/root length ratio increases [119]. NaCl reduces root hydraulic conductivity because root diameter increases and the number of fine roots reduce. Another factor that causes to reduce citrus root hydraulic conductivity is increasing corky in root system [121].

### **ROUTE OF WATER AND IONS ABSORPTION**

Corky hypoderm in citrus is the first main barrier to mass movement of water and ions in the root. Radial movement of water via hypoderm of citrus roots is depending on hydrophilic properties of micro-pores, corky layers as well as number and permeability of passage cell in the root [122]. In the experiment, dead of epidermis cells and expanding of casparian strip and corky layers in the hypodermis cells of onion root and increasing root age caused to reduce permeability of plasma membrane of root cell between 0.45 to 5.3 percent [123]. A similar reduction in plasma membrane permeability of citrus are expected depending on soil solution. In the passage, cells may be flow water more than ion into root because membranes permeability to water is greater than ions. In salinity stress, citrus roots are corky to close of root tip, which this subject causes to reduce water absorption [124,125]. Thus, salinity may be change the permeability of hypoderm in citrus root. In the comparative study that was performed on citrus rootstocks in sandy and liquid media for 6 months, was observed higher levels of  $\text{Na}^+$  and  $\text{Cl}^-$  in the leaves of the planted rootstocks in the liquid medium than the planted rootstocks in the sandy medium. The grown plants in the sandy medium had more fibrous and branching roots, while the grown plants in the liquid medium had a large number of long root and lower lateral roots, which it seems these roots, are not as good as corky [126]. Endoderm is the second barrier for mass movement of water and ions in the wide of root. At this location, is occurring the secondary corky and or putting corky layers in the walls of endoderm and is causing to closure plasmodesmata contact ways with other cells. In fact, it is inferred that passage cells in endoderm have very important role in regulation of water and ions entrance to xylem. On the other hand, membrane permeability may be different in various times, because growth of citrus root is a successive series of growth flow and corky root extension rate will vary with amount of root activity [127]. There is a little information about the depth of water movement and absorption of ions in citrus root. It has reported that salinity decreased the number of roots with white tip as well as lateral roots in seedy Valencia orange.

In addition, it caused to reduce the ratio of non-corky part/corky parts in root system. Appearance of lateral roots leads to non-union of endoderm and perhaps may cause to eliminate linkage of hypodermis tissue and thus, it is possible to create the pores for entrance into apoplast [128]. In citrus due to have fibrous root with large branches, may create many real or potential points for water leakage into apoplast [129].

## STEM AND LEAVES

Salinity is causing to reduce shoot/root ratio in seedy citrus rootstock. Relative enhancement in root volume (absorption level) than shoot volume (consumption location) increases the concentration of  $\text{Na}^+$  and  $\text{Cl}^-$  in the leaf's cells. Conversely, increasing of citrus leaves carnify under salinity stress, slightly lead to reduce the effects of high amounts of  $\text{Na}^+$  and  $\text{Cl}^-$  in the leaves via diluting.  $\text{NaCl}$  increase carnify rate. This phenomenon is due to increase the number of spongy mesophyll cells the rate of three times and leads to increase leaf thickness [101]. Movement of  $\text{Na}^+$  and accumulation of  $\text{Cl}^-$  in citrus leaf is depending on salinity level, rootstock and scion combination and ionic combination and components of soil or medium. In citrus seedlings,  $\text{Na}^+$  and  $\text{Cl}^-$  may rapidly reach to equilibrium level, while in citrus trees that have neutralizing capacity, this time it may take 4-5 months [118].  $\text{Na}^+$  and  $\text{Cl}^-$  have trend to accumulate in the leaves, so that there is non-uniform leaner relation between their concentration in the leaf and salt concentration in out medium especially in the salinity sensitive rootstocks [130,131]. In the salt sensitive rootstocks such as Etrog citron not only accumulation rate of  $\text{Na}^+$  and  $\text{Cl}^-$  is higher [132], but duration of the period in which the concentration of these ions reaches to equilibrium level has been higher than the salinity resistant rootstocks such as Rangpur lime [126].  $\text{Na}^+$  and particularly  $\text{Cl}^-$  concentration in the older leaves at the bottom is greater than apical young leaves [111] and especially in the leaves of the main stem is higher than subsidiary shoots [130]. Furthermore, the concentration of  $\text{Cl}^-$  in different parts of stem from lower parts to upper parts of Trifoliolate orange almost is uniform (trifoliolate orange is a weak restrictive of  $\text{Cl}^-$  entrance). However, the concentration of  $\text{Cl}^-$  in the upper parts of Cleopatra mandarin and Rangpur lime, which are strong restrictive of salt entrance, is less than lower parts. Conversely, amount of  $\text{Na}^+$  decrease along the main stem of Trifoliolate orange (trifoliolate orange is a strong restrictive of  $\text{Na}^+$  entrance) and reach to amount of control plants. This reduction amount in sweet orange and Rangpur lime is very less [73]. The made changes by  $\text{NaCl}$  in leaf  $\text{K}^+$  amount vary in different rootstocks [92]. Citrus leaves do not show large changes for  $\text{K}^+$  in during period of salinity, although Trifoliolate orange, which is restrictive of  $\text{Na}^+$  entrance, tends to show an increase for leaf  $\text{K}^+$  [111]. Potassium of stem remains relatively constant in a range of different salinities and or mild reduction occurs in Pineapple orange on Cleopatra mandarin and Trifoliolate orange rootstocks [111], and or decreases in Etrog citron, Kharnakhata and Rangpur lime [92]. Whereas, in the some cases has been found that leaf  $\text{K}^+$  concentration decreased from lower shoots toward upper shoots and also stem  $\text{K}^+$  concentration decreased from bottom of stem toward upper parts [73]. Selective absorption of  $\text{K}^+$  than  $\text{Na}^+$  has been shown in leaf of sour orange and sweet orange [133]. Sodium absorption by leaf has decreased by  $\text{Ca}^{2+}$  [129].  $\text{Cl}^-$  absorption in the leaf of sweet orange has decreased by the anions such as  $\text{NO}_3^-$  and may adjust by  $\text{K}^+$  or  $\text{Na}^+$  [134].

## FRUITS

Common indexes to determine the harvest time of citrus fruit are physical properties, fruit size, peel color and flavor quality that are influenced under salinity stress. Flowering intensity, fruit set and final number of fruits in 24-years-old Washington navel orange on sweet orange rootstock decreased by treating them with 20 mM  $\text{NaCl}$  for five years. In these trees, final number of fruit decreased to 38 percent in comparison with the treated trees by 5 mM  $\text{NaCl}$  [118]. Salinity reduced primary growth of sweet orange fruits grafted on the treated sweet orange rootstock by high levels of  $\text{NaCl}$ , but due to delay in fruit maturity (25 days), fruit growth continued, so that the fruits were similar in viewpoint of size in both treatments at complete maturity. Researchers have suggested that because salt leads to fall leaf, consequently the stored carbohydrate decrease as well as flowering and fruit set will decrease [98]. Reduction of fruit yield in sweet orange [135,136], Grapefruit [75] and Lemon [109] substantial has been relative to the little number of fruits than small size of fruits. leaf  $\text{Cl}^-$  (as dry weight percent) in Marsh seedless grapefruit and Washington navel orange on three rootstocks showed high correlation ( $r=0.898$ ) with  $\text{Cl}^-$  of fruit juice [137]. It is concluded that  $\text{Cl}^-$  transition occurs simultaneously to leaves and fruits. Chloride concentration in fruit juice of Marsh seedless grapefruit, Washington navel orange [137] and Shamouti orange [138] has been between 1 to 9 mM and  $\text{Na}^+$  concentration in fruit juice between 0.05 to 0.5 mM. The concentration of  $\text{Na}^+$  and  $\text{Cl}^-$  in pulp of fruit almost is lower than its concentration in the leaves [139]. Concentration of  $\text{Na}^+$  and  $\text{Cl}^-$  in the pulp of citrus fruit is lower than sugar concentration. In the other study, were evaluated physical properties of juice of Verna lemon fruit juice under saline irrigation water and rootstock type. The used rootstocks were Sour orange, Cleopatra mandarin and Alemow. Salinity and rootstock affected quality of fruit juice.

Sour orange and Cleopatra mandarin rootstocks were better than Alemow, because Verna lemon fruits on these rootstocks were very marketable in relation to soluble sugar and acidity than Alemow [109]. Generally, can concluded that there is significant interaction between salinity and other factors such as rootstock and scion in viewpoint of fruit or fruit juice quality and the damage in different cultivars will connect to properties of rootstock and scion and amount of salinity stress [109,140].

### CITRUS ROOTSTOCKS

Many cultivars that is selected for optimal quality fruit production have not suitable roots and it is necessary that these cultivars be grafted on other rootstocks until produce satisfactory plants. There are rootstocks for many plant species, which the grafted cultivars on them can tolerate unfavorable conditions such as heavy and moist soil and or resist against pathogens and terricolous pest [141]. Selection of proper rootstock and also scion from the best commercial cultivars that be compatible to the rootstocks and be desirable from the viewpoint of yield, quality, marketability, resistance to plant pests and diseases and compatible to unfavorable conditions of climate, soil and water is one of the most important horticultural aspects in citrus growing. The important citrus rootstocks can be noted Rough lemon (*Citrus jambhiri* Lush), Volkamer lemon (*C. volkameriana* Ten. and Pasq), Alemow (*C. macrophylla* wester), Rangpur lime (*C. limonia* Osbeck), Sour orange (*C. aurantium* L.), Cleopatra mandarin (*C. reshni* Hort. ex. Tanaka), Trifoliolate orange (*P. trifoliata* [L.] Raf.), Citrange (*C. sinensis* × *P. trifoliata*), Citromelo (*P. trifoliata* × *C. paradisi*) and Sweet orange [142]. In Iran is also used other rootstocks such as Mexican lime (*C. aurantium* Swingle), Sweet lime (*C. limettioides* Tanaka) and Bakraei (*C. limettioides* × *C. reticulata* Blanco) in the southern regions. To select suitable rootstock should be considered compatibility of rootstock and scion, rootstock growth rate, resistance of rootstock to diseases, effect of rootstock on yield, resistance to cold, nutrient absorption and fruiting alternation [141,142].

### SALINITY AND SEED GERMINATION

Decreasing water potential and ionic toxicity are important factors that affect germination in saline environment [143,144]. Water is the most important factor for initiation of germination process and primary survival of seedling after emergence [145]. Since water potential is depending on salts amount, under high salinity stress, salts accumulation is leading to more negative of soil osmotic potential and is causing to reduce water entrance to seed and germination does not occur [146,147]. Water existence is necessary to perform hydrolytic processes and during these processes, storage materials including lipids, proteins and carbohydrates are converted to simple materials and for use are transferred to embryonic axis [145]. Reduction of water potential in salinity conditions cause to increase abscisic acid (ABA) production. ABA is a natural antagonist for gibberellins [145] and is able to prevent from them effects by inhibition of activity of effective enzymes in gibberellins biosynthesis path [148]. Gibberellins play most roles in controlling and accelerating of germination processes [145]. High levels of ABA in seed case to increase seed sensitiveness than reduction of water potential and this subject also lead to reduce seed germination [149]. Reduction of seed respiration rate also can be one of the bad effects of salinity on seed germination. Whereas, respiration enhancement is the most important metabolical change from initiation of seed germination processes [145], high concentration of ions arising from high salinity damages to electron transition system on the mitochondria membranes and cause to reduce respiration [29]. Increasing seed respiration after dewatering stage, provide the require energy for germination activities [145].

### REACTION OF DIFFERENT CITRUS CULTIVARS TO SALINITY

Citrus trees with tolerance ability  $EC=1.1-3.2 \text{ ds.m}^{-1}$  are among sensitive plants to salinity, so that in moderate and high salinity condition severely is damaging, but these plants continue to exist up to  $EC=16.0 \text{ ds.m}^{-1}$ . In these trees, salt injury usually appears in the form of scald and leaves abscission. These effects are associated with accumulation of sodium and chloride ions as far as toxicity [73,150]. Numerous studies have showed that citrus show tolerance to salinity by various mechanisms, which these mechanisms are consisting application of rootstocks, different combinations of scion and rootstock and interstocks. The performed investigations show that salinity sensitiveness or resistance rate vary between different citrus cultivars. Alike many other woody plants, citrus trees can be accumulate chloride or sodium or both ions as far as toxicity in their tissues.

Oppenheimer [151] is the first person that has reported the negative effects of saline water on growth and development of citrus rootstocks. He reported that the amount of chloride in the grafted trees on Sour orange rootstock has been lower than the grafted trees on Sweet lime rootstock. Cooper and Gorton [82] according to toxicity symptoms of boron and chloride concentration in the leaf, reported that Cleopatra mandarin and Rangpur lime are resistant and Sour orange, Rough lemon and Sweet lime are sensitive rootstocks. Cooper [152] has reported that Cleopatra mandarin repels chloride and accumulates sodium in its tissues.

Zekri [153] reported that sour orange accumulates both chloride and sodium ions in its leaves. Many plants resist under salinity conditions by un-absorption of salt or un-transition of salt from root to shoot. For example in the Soya bean has been distinguished that salinity resistance mechanism is in the root and the cells of root bark are able to repulse of additional salt [154]. It was reported that many citrus rootstocks are able to repulse chloride and sodium in their roots, but this property has not completely been known. It was reported that soil pH also could be affect absorption rate of salts and salinity effects via influence on root cells membrane permeability [105]. Walker and Douglas [92] in the study on the effect of salinity on absorption and distribution of chloride, sodium and potassium ions in citrus seedlings have reported that chloride repulse ability varied between the different evaluated rootstocks. They have indicated that shoot growth was proportionate to amount of chloride and sodium accumulation and shoots growth has decreased by increasing amount of these ions. In this report emphasized that Rangpur lime absorbed chloride very lower than Etrog citron. Ruiz *et al.* [155] performed the study on the effects of salinity on relative growth rate, fresh and dry weight of leaf and absorption rate of mineral elements in citrus. They treated four citrus rootstocks including Cleopatra mandarin, Carrizo citrange, Sour orange and Alemow by water containing 0, 10, 20, 40 and 80 mM NaCl for 40 or 60 days and reported that after 60 days, salinity had significant influence on amount of Cl, Na, K, Ca, Mg, P, Fe, Mn and Zn. On this principle has been emphasized that under salinity stress not only plant growth affect due to osmotic effects and toxicity of sodium and chloride ions, but in this relation, mineral imbalance involved and lead to reduce growth. Zekri and Parsons [119] planted seedlings of Sour orange and Cleopatra mandarin in the sandy medium in greenhouse and irrigated by Hogland solution and gradually added NaCl to the solution until finally osmotic potential reach to -0.1, -0.2 and -0.35 MP. After 6 months, he reported that there was no significant difference between both rootstocks in viewpoint of growth, stomatal conductance, and evapotranspiration rate and root hydraulic conductivity, but each above factors decreased by increasing amount of salinity. Root and shoot dry weight, cross-sections of stem, total leaf area and roots length dropped 44-55% under low salinity stress (-0.1 MP). Significant differences were observed between chlorophyll content and chloride accumulation amount in the leaves of both rootstocks that Sour orange rootstock had many more chloride in its leaves than Cleopatra mandarin. Reduction amount of chlorophyll in the leaves of Sour orange and Cleopatra mandarin were 56% and 11% respectively.

Behboudian *et al.* [99] in the study on effects in salinity on some citrus rootstocks and their combination with some scions reported that rootstock highly affects chloride accumulation in the leaves of scion. In addition, they reported that salinity caused to reduce potassium amount in the root of all rootstocks and the shoots of some scions. Bar *et al.* [156] reported that citrus trees are sensitive to high boron and chloride in irrigation water. They planted one-year-old grafted trees on Troyer citrange and Cleopatra mandarin rootstocks within 8-liters pots in the greenhouse and irrigated by nutrient solutions containing 2, 16 and 48 mM chloride for 3 months. They observed when chloride concentration in the irrigation water was 2 mM, its amount in the grafted plants leaf on Troyer citrange rootstock was 4 times of the grafted plants leaf on Cleopatra mandarin, but when concentration of chloride in irrigation water was 16 mM, this portion reached to 7 times. In 48 mM concentration of chloride in irrigation water, toxic symptoms were observed only in the grafted plants leaf on Troyer citrange rootstock.

Cooper *et al.* [157] reported that rootstock type controls chloride absorption amount in citrus. Some citrus rootstocks tolerate high levels of chloride due to have ability to chloride repulsion. Hewitt *et al.* [158] found that citrus response to salinity is so fast, which after 3-4 weeks irrigating by saline water can understand how to react. Furr and Ream [159] explained that the trait of salinity tolerance in citrus is quantitative and the derived offspring from two salinity resistant parents will had high resistance to salinity and only a few number of the derived offspring from one salinity resistant parent and one salinity sensitive parent will be resistant to salinity.

Zekri [160] in evaluation of salinity effects on seed germination, vegetative growth and mineral elements of some citrus rootstocks found that under salinity stress, seed germination and seedling growth delay in some citrus such as Citranges, Citromeloes, Cleopatra mandarin, Rough lemon, Pineapple orange, Sour orange and Volkamer lemon, but the amount of delay varies depending on the rootstock type. Adding NaCl to medium caused to increase amount of Na, Cl, N, P and K and reduction of Ca and Mg in the shoots of often rootstocks. In addition, they reported that salt tolerance in seed germination stage could not be a good indicator for seedling resistance in the next growth stages. Ayers and Hayvard [143] reported that soil salinity affects seed germination via reduction of water absorption and enhancement of ions uptake in the level of toxicity. Some plants such as Alfalfa and Sugar beet may be sensitive to salinity during seed germination stage, but later shown good resistance to salinity. Hassan and Galal [161] in the study on salinity resistance of five citrus rootstocks observed that salinity resistance are higher in the rootstocks of Cleopatra mandarin, Sour orange, *Citrus amblycarpa*, Volkamer lemon and Rangpur lime respectively. In a study, changes of microelements concentration under salinity stress was evaluated in the root of some citrus species including Bakraei, Volkamer lemon, Sour orange, Sweet lime and Mexican lime and was observed that in the control treatment there was significant difference between species in viewpoint of microelements concentration.

In fact, influence of rootstock type was significant on absorption of microelements. Under salinity stress, iron concentration decreased in the root of all species except Mexican lime and Sour orange and zinc concentration increased in the root of all species except Bakraei. Besides, manganese concentration increased in the root of all species and copper concentration decreased only in the root of Bakraei. Salinity increased chloride concentration in the root of all species and caused to reduce boron concentration in the root of all species except Bakraei. Volkamer lemon and Bakraei had good ability to accumulate chloride in their roots [162].

Nieves *et al.* [109] to assess effects of rootstock under salinity stress, Verna and Fino lemons budded on Sour orange and Alemow rootstocks and irrigated by water containing 40 and 80 mM NaCl and reported that sodium and chloride amounts in the leaves of Fino lemon was more than Verna lemon, but its amount varied depending on rootstock type. Chlorophyll content in the leaves of Fino lemon on each type of rootstock was very lower than Verna lemon. Garcia-Sanchez *et al.* [9] assessed the effect of salinity on Sun burst mandarin scion by influenced different rootstocks and reported that after 6 weeks using various salinity levels (30, 60 and 90 mM), scion growth decreased on both rootstocks of Cleopatra mandarin and Carrizo citrange. Due to salinity, potassium amount decreased in the roots of both rootstocks and nitrogen amount increased. Because of salinity, nitrogen and calcium amount only increased in the scion leaf on Cleopatra mandarin and amount of magnesium and potassium decreased. In addition, they reported that amount of chloride and sodium was very lower in the scion leaf on Cleopatra mandarin rootstock than Carrizo citrange.

Levy and Lifshitz [163] in evaluation the effects of salinity on some citrus cultivars found that in the all salinity levels, Cleopatra mandarin and Sour orange were the best and the weakest chloride repulsive respectively. Fernandez-Ballester *et al.* [164] reported that due to salinity, osmotic potential and water content decreased in the leaf of Sour orange and Alemow and amount of growth reduction in Alemow was very higher than Sour orange. Cerezo *et al.* [165] evaluated the influence of chloride on nitrate absorption by citrus roots and found that due to salinity, amount of nitrate absorption decreased and this reduction had very close relationship with chloride amount. On this principle, has been explained that chloride had antagonistic property in absorption with nitrate. Aboutalebi *et al.* [166] examined the effect of salinity on the concentration of macro elements and sodium in the root of five citrus rootstocks and explained that sodium concentration increased in the root of all rootstocks, but this enhancement in the root of Volkamer lemon and Bakraei was more than other rootstocks.

Fernandez-Ballester *et al.* [167] in relation to the effect of salinity on ions absorption in citrus seedlings found that salinity decreased leaf dry weight of Alemow very higher than Sour orange, but reduction of root dry weight in Sour orange was very higher than Alemow. Besides, they reported that sodium and chloride absorption amount in Sour orange was very more than Alemow. Mademba-Sy *et al.* [168] during the experiment evaluated the accumulation of proline in citrus leaf under salinity stress and reported that amount of proline accumulation varied depending on species type and rootstock type had very influence on increasing proline amount in the scion leaves. Bar *et al.* [168] assessed the effect of chloride in the irrigation water on Cleopatra mandarin (resistant to salinity) and Troyer citrange (sensitive to salinity). They and found that putrecin amount enhanced by increasing chloride amount and the amount of spermin decreased in the leaf and damage arising from chloride toxicity was proportionate to rise of putrecin and to go done of spermin level. In this report, adding nitrate to irrigation water caused to reduce chloride accumulation in the leaf of both rootstocks.

Aboutalebi *et al.* [170] evaluated the effect of rootstock type and NaCl on the concentration of sodium, potassium and chloride ions in the shoot of Kinnow mandarin scion on four rootstocks including Bakraei, Mexican lime, Volkamer lemon and Sour orange. They found that the concentration of potassium, sodium and chloride ions in the control treatment had significant difference with other salinity levels. Salinity increased sodium and chloride concentration in the root and shoots, but enhancement rate varied depending on rootstock type. The lowest chloride and sodium concentration was observed in the shoot of Kinnow scion on Mexican lime rootstocks. Because of salinity, potassium concentration decreased in the scion shoot on Bakraei rootstock and increased on the other rootstocks. According to their results, Mexican lime and somewhat Bakraei had good ability to induce tolerance to salinity in Kinnow mandarin.

#### **APPLICATION OF INTERSTOCK TO REDUCE SENSITIVITY TO SALINITY IN CITRUS**

Application of interstock is customary as the factor to remove incompatibility in propagation of horticultural plants. Interstock is used as a bridge in remedy of the damages that causes to the separation of trunk from root. In some plants such as apple and pear, interstock leads to dwarfing, high growth or low growth.

In order to remove incompatibility between rootstock and scion, the variety of Sweet orange called Pera was produced on two incompatible rootstocks including Swingle Citromelo and Volkamer lemon by using three different interstocks and incompatibility disappeared in the all rootstock and scion combinations [171]. Gil-Izquierdo *et al.* [172] reported that application of interstock causes to improve sap flow and amelioration of flavenoids contents in Lemons.

Centuries that ability of some dwarfing clones as interstock between high growth scion and rootstock has been known to produce dwarf and premature fruit trees. One of the first evidence of the application of such an approach has been in the UK in 1681, which Paradise apple was used as interstock in order to premature the apple on the ornamental apple. In some cases, effect of interstock can be due to the entry of an additional graft site that may probably limit transfer. Complete connection of graft location has been recognized as dwarfing creative agent in Sour orange by Mexican lime interstock. Converse of dwarfing situation in apple (due to the effect of M9 interstock), Mexican lime interstock is high growth. Furthermore, there is evidence indicating that appearing interstock effects directly is due to the interstock piece, not the anomalies of graft location.

In fact, rootstock selection is root system selection for scion. Root system is responsible for water and nutrients absorption; provision of reserve the produced carbohydrates in the leaves and scion compatibility to soil and water conditions and resistance to some diseases. Rootstock affects more than 20 horticultural traits such as resistance to pests and diseases.

Citrus tolerance to abiotic stresses is depending on different factors such as rootstock type, nitrogen fertilizer, soil and climate condition [9,173,174]. Besides, putting an interstock between rootstock and scion not only can be improve growth, longevity, productivity and fruit quality [172], but also is able to increase tolerance to salinity [175]. In the salinity tolerance mechanisms, interstock limits absorption and transport of chloride ion to shoots through reduction of shoot/root ratio as well as reduction of leaf transpiration. In Verna lemon trees on Sour orange rootstock, which Valencia or Castellano oranges were used between scion and rootstock as interstock, not only tolerance to salinity was observed, but also amount of salinity tolerance was depending on interstock cultivar [175]. Gimeno *et al.* [176] reported despite that the application of interstock in citrus trees can be increase tolerance to salinity, but application of Valencia and Castellano orange interstocks could not induce tolerance to waterlogging stress in Verna lemon trees on Sour orange rootstock.

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