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Screening of Citrus Rootstocks for Salinity Tolerance Based on Initial Growth Attributes and Leaves Proline Content

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Abstract - Citrus fruit being a major horticultural crop consumed globally, is severely affected by issues related to biotic and abiotic stresses. Following stress effects, a research study was carried out to evaluate the morphological and physiological responses of citrus rootstocks to different levels of salinity stress. The seedlings of citrus rootstocks (*'Troyer' citrange*, *Citrus volckameriana* and Sour Orange (*Citrus aurantium*)) were treated with different salinity levels (0, 50, 100 and 150 *mM* NaCl). Salinity stress at the level of 150 *mM* NaCl, reduced plant height, number of leaves plant^{-1}, stem thickness, leaf area, chlorophyll content, fresh and dry weight of both shoot and root, survival percentage significantly. In contrast, maximum toxicity symptoms and highest content of proline accumulation in the leaves were observed in plants subjected with 150 m*M* NaCl. Moreover, the tallest plants, highest number of leaves plant^{-1}, stem thickness, chlorophyll content, leaf area, fresh and dry shoot weight, root weight, survival percentage, minimum toxicity symptoms and maximum proline accumulation were recorded in Sour orange rootstock compared to *'Troyer' citrange* and *Citrus volckamariana*. From the research findings it could be concluded that growth performance of all citrus rootstocks diminished with the increased salinity levels from 0 to 150 *mM* NaCl. The rootstock Sour orange was found tolerant to saline condition while '*Troyer*'*citrange* proved to be highly sensitive under saline condition.

Keywords: Citrus rootstocks, Growth and Yield, Nutritional imbalances, Physiological disturbances, Salinity stress

I. INTRODUCTION

Biotic and abiotic stresses have become a serious issue all over the world, affecting plant growth and productivity. Abiotic stress causes a serious crop loss worldwide, contributing to the production decline of major crops by 50%. Moreover, soil salinity has become one of the major environmental factors affecting many crop plants' growth and productivity. The reduction in arable land due to salinization is in direct relation with the needs of the increasing population which is at an increasing rate (Sudhir and Murthy, 2004). The deleterious effect of high salinity damages is noticed at germination, seedling stage, and other stages of plants life that lead to a significant decrease in growth, yield, and finally death of the plants. About 19.5% of total irrigated lands and 2.1% of total cultivated drylands are salt-affected throughout the world (FAO, 2016). A major portion of saltaffected Pakistani soils is sodic (>60%) with excessive Na+ salts causing more salinity stress (Plaut, 1993).

Citrus is one of the most important members of the Rutaceae family considered a major household item in the world of the fruit juice industry. The genus citrus consists of different species like mandarin, oranges, grapefruit, lemon, and lime with small categories as tangerine, pummelos, and tangelos, widely grown in the subtropical and tropical regions of the world (Chaudhary *et al*., 1989). It is one of the well-known fruits for their refreshing fragrance, providing an adequate amount of Vitamin C and phytochemicals like

carotenoids, limonoids, flavanones, and Vitamin B complex that greatly pays off against cardiovascular and degenerative diseases, obesity, cancer, thrombosis, and atherosclerosis (Iglesias *et al*., 2007; Ladaniya, 2008). For a particular area, while selecting fruit plants, rootstocks should be given careful consideration on which scion varieties are to be grafted or budded. Rootstocks affect the vigor, productivity, longevity, quality, and resistance to different diseases, insects, and pests of a scion variety. Rootstock should be adaptable to various soil and climatic conditions and resistant to different diseases and insect pests. Sour orange (*Citrus aurantium* L.) seedling is a universal rootstock for citrus and is extensively used in the Mediterranean region due to its extended deep root system. It is also highly drought tolerant. *'Troyer' citrange* (*Poncirus trifoliata × Citrus sinensis)* reasonably vigorous rootstocks, resistant to *Phytophthora parasitica*,nematodes, and tristeza virus, with good cold tolerance and is sensitive to salinity. *Citrus volckamariana* rootstock is vigorous, less drought-tolerant, adopts a wide range of soil conditions, tolerates moderate saline conditions, and shows resistance to frost conditions (Anonymous, 2018).

Citrus is considered the top-ranked fruit of world production and is produced commercially in more than 50 countries. In the world's top producing countries like Pakistan, Florida, and Spain citrus production is mostly affected by saline conditions due to agricultural practices like poor water systems (Garcia *et al*.,2002). Citrus plants are considered to be sensitive to saline condition (Al-Yassin,2005) due to the specific toxicity of Cl^- and/or Na^+ and to the osmotic effect caused by the high concentration of salts (Garcia-Sanchez *et al*.,2000) and plants face physiological disturbances and reduction in growth even at low to moderate exposure of salts. Semi-arid areas are preferable to citrus cultivation and in these areas soils and water contain many soluble salts like chlorides and sulfates which disturb the nutritional balance of plants resulting reduction in the growth and yield of citrus crops. The exposure of citrus to salinity causes serious physiological dysfunctions such as reduced leaf area, chlorotic or necrotic patches on leaves, delayed development, growth inhibition, and a limitation in development (Khoshbakht*et al*., 2018). The major saline ions Na^+ and Cl^- can affect the nutrient uptake through competitive interactions or by affecting the ion selectivity of membranes. In terms of reduced photosynthesis and transpiration rates, sodium concentration is considered to be the most influential factor (Behboudian*et al*.,1986).

Although *Citrus* species are classified as salt-sensitive (Mass1990,1993), there is great variation in the ability of citrus plants to tolerate salinity depending upon rootstock (Zekri and Parsons,1992) and scion (Lloyd *et al*.,1989,1990; Nieves *et al*.,1990). The tolerance of the different species of *Citrus* can be determined by their capacity to exclude the potentially toxic Na^+ and Cl^- ions (Storey,1995).

Several approaches are used to mitigate the adverse effects of soil and irrigation water salinity but, a more permanent solution to this problem keeping in view the increasing utmost food demand of the world would be the use of salt-tolerant rootstocks. This study was aimed to investigate the performance of citrus rootstocks in terms of salinity tolerance; to find out the minimum level of salinity for better growth of citrus rootstock; to evaluate minimum toxicity symptoms of salinity stress on different citrus rootstocks.

II. MATERIALS AND METHODS

Experimental site

The experiment was conducted in a controlled condition in a Screen House at Horticulture Section, Agriculture Research Institute (ARI), Tarnab Peshawar, in 2019. The site is in the valley of Peshawar, Khyber Pakhtunkhwa, Pakistan. Peshawar is located at 34.01° N latitude, 71.35° E longitude at an altitude of 350 m above sea level in with sub-tropical climate (Ahmad *et al*., 2019; Jan *et al*., 2009). Peshawar is located about 1600 km north of the Indian Ocean (Alam*et al*., 2020). Both the summer and winter weather are extreme (Basit *et al*., 2019), characterized by severe winter and prolonged hot summer, where the average minimum temperature during winter is 5°C and maximum up to 47 °C during summer. The wettest month (with the highest rainfall) is March (78 mm), and driest month (with the lowest rainfall) is June (7 mm) approximately (Sajid *et al*., 2020).

Treatments and Experimental Design

The research study was comprised of two factors i.e., three citrus rootstocks (*'Troyer' citrange*, *Citrus volckamariana* and Sour Orange) and four different concentration of salinity solution (Control (0 *mM* NaCl), low (50 *mM* NaCl), Moderate (100 *mM* NaCl) and high (150 *mM* NaCl)). The experiment was laid out in Completely Randomized Design (CRD) with two factors, repeated three times.

Plant materials and Growth conditions

Seeds of three Citrus rootstocks like *'Troyer' citrange*, *Citrus volckamariana* and Sour Orange were obtained from Progeny block located at Tarnab Agriculture Research Institute Peshawar Pakistan and were sown on flat trays under screen house condition at first week of February. After germination,

uniform sized plants from all the rootstocks were selected and transplanted to larger plastic bags of size 9 x 6 cm^2 maintaining proper moisture condition for better establishment by frequently irrigation. These rootstock seedlings were subjected with different concentration of sodium chloride (NaCl) with irrigation water after twenty days of transplantation.

Soil Properties

Soil used for experimental purpose in plastic bags, was taken randomly and were tested at soil lab, ARI Tarnab Peshawar before transplantation of citrus seedling. The soil samples were found silty loam indicating total soil N (0.44%), available K (1.80%), available P (5.40) and organic matter (0.89%), respectively. The soil pH and electrical conductivity were 8.0 and 0.20, respectively (Table 1).

Preparations of NaCl solution

To prepare different levels of salinity i.e., 50, 100, and 150*mM* NaCl atomic mass of NaCl were multiplied with different salinity levels then divided with thousand and results were obtained in grams i.e., 2.94 g, 5.85 g, and 8.77 g, then each level was dissolved in one liter of water. The electric conductivity (E.C) of the media was determined before treatment application by taking random samples from the seedling transplantation media.

Table 1. *Soil analysis of experimental samples*

Soil analysis	**Results**
Soil texture	Silt Loam
pH	8.0
Electric conductivity	0.20 dSm^{-1}
Organic matter	0.89%
Nitrogen (N)	0.44%
Phosphorus (P)	5.4%
Potassium(K)	1.80%

Growth measurements Plant height

The height of randomly selected plants from each treatment was measured using the measuring tape and their average was calculated.

Number of leaves plant [1]

The number of leaves per plant was counted carefully after application of treatment and their mean were taken.

Stem thickness (mm)

Stem thickness of randomly selected plants from each treatment in every replication was measured by using digital Vernier caliper and the average was computed.

Single leaf area (cm^2)

Four leaves were randomly selected from all treatments of all replications and their areas were found through the graph paper method, then average leaf area per single leaf was obtained and recorded.

Chlorophyll content (SPAD)

Chlorophyll content was determined in the fresh leaf samples using a Minolta SPAD chlorophyll meter. The results were presented as SPAD units.

Toxicity symptoms

Toxicity symptoms like leaf tip burning, defoliation, yellowing, etc., particularly in the leaves were observed visually and data was taken accordingly in each treatment of every replication. Each data was generated according to the 0 and 5 scales of Goell (1969).

Survival percentage (%)

The survival percentage of citrus rootstocks was recorded at the end of the experiment and the values were recorded in percentage by using the following formula.

$$Survival percentage = \frac{\text{Number of plants survived after treatment application}}{\text{Total number of plants used}}$$

Fresh weight and dry weight of shoots

All the shoots were detached and were weighed with the help of a digital electronic balance. The same shoot was then oven-dried at 80 ^0C for 48 hours for measuring the dry weight.

Fresh weight and dry weight of roots

The roots were detached, then washed with tap water and weighed with the help of digital electronic balance. The same roots were then oven-dried at 80 ^{0}C for 48 hours for measuring the dry weight.

Proline content analysis

Proline was determined through the method described by Bates *et al*., (1973). 0.2 g of fresh and young tips from each sample of leaves were taken and dip into liquid nitrogen for 2-3 minutes, then through a tissue miser crashed the tissues and homogenized with 4 ml of 3% sulfosalicylic acid for five minutes at room temperature, the homogenate was then centrifuged at 3000. After that, they were filtered through Whatman-2 filter paper and again mixed the supernatant with 4 ml of 3% sulfosalicylic acid. The filtrates were then reacting with 2 cm^3 acid ninhydrins in a test tube in a boiling water bath for one hour. The reaction was terminated in an ice bath. After extracting the reaction mixture with 4 cm^3 toluene tubes were cool down to room temperature. The data were expressed on an mg/g dry weight basis.

Statistical analysis

Data analysis of the studied parameters was done by using analysis of variance (ANOVA) technique by using Statistical software i.e., STATISTIX 8.1 and LSD test was done at 1% probability level for mean comparison of different treatments.

Table 2. *Plant height, number of leaves plant^{-1}, stem thickness, leaf area, chlorophyll content and toxicity of citrus rootstock seedlings as influenced by different salinity concentrations.*

Salinity level (S)	Plant height (cm)	No. of leaves plant^{-1}	Stem thickness (cm)	Leaf Area (cm^2)	Chlorophyll content (SPAD)	Toxicity symptom (%)
Control	54.55±4.79A	35.50±2.50A	4.95±0.38A	12.06±1.76A	51.88±2.19A	0.53±0.08D
Low (50mM))	47.66±5.62B	32.78±2.00B	4.48±0.28B	10.98±1.72B	47.66±2.62B	0.96±0.13C
Moderate(100mM))	39.78±5.85C	27.89±2.31C	3.67±0.46C	9.94±1.40C	41.55±2.73C	1.72±0.56B
High (150mM))	30.88±5.99D	20.00±1.00D	2.83±0.32D	8.83±1.29D	33.88±3.12D	2.37±0.77A
Rootstocks (R)						
Troyer Citrange	8.47±0.95B	27.89±5.23A	3.86±0.86B	8.47±0.95C	44.83±7.63A	1.91±1.08A
Citrus volckamariana	10.64±1.22C	26.66±5.33B	3.61±0.76B	10.64±1.22B	40.25±7.08B	1.29±0.65B
Sour orange	12.24±1.43A	34.22±3.28A	4.47±0.80A	12.24±1.43A	46.16±5.64A	0.98±0.39C
Factors						
Salinity (S)	**	**	**	**	**	**
Rootstocks (R)	**	**	**	**	**	**
S ×R	ns	ns	Ns	ns	ns	Fig 1**

Means followed by different letters shows significantly difference at P≤0.01; ns: Non significant; *:P≤0.05 ;**: P≤0.01

III. Results and Discussion

Plant height (cm)

Salinity treatments significantly influenced plant height of citrus rootstocks (Table 2). According to results, it was revealed that application of salinity treatment progressively reduced plant height by 12.6, 27.0 and 43.3% respectively, over control treatment with 50,100 and 150 m*M* NaCl application. In all rootstocks, the plant height (44.51 and 15.03%) was found higher in Sour orange rootstock as compared to *'Troyer' citrange* and *Citrus volckamariana* rootstock. Application of different salinity treatments exhibited reduction in plant height of citrus rootstocks, taller plants was observed in control treatment and dwarf plants were noticed in plants subjected with high saline treatment (150 m*M* NaCl)-which might be due to fact that NaCl affects the permeability of the plasma membrane and increases influx of external ions and efflux of cytosolic solutes in plant cells (Allen *et al*., 1995). Secondly, NaCl causes hardening of the cell wall (Nabil and Coudret, 1995) and a decrease in water conductance of the plasma membrane causing a reduction in plant height (Cramer, 1992). Our results conform with the findings of Bernardo *et al*... (2006), who observed a significant reduction in seed germination, plant height and shoot dry weight with increasing salinity-induced stress in cowpea cultivars. It might be due to reduction of cell elongation and

cell division (Loreto *et al*., 2003; Ndayiragije and Lutts, 2006). Camara-Zapata *et al*... (2004) also observed a decrease in growth rate in citrus rootstocks due to high osmolality in the external medium. Further presence of high salts in the root zone compels the plants to use more energy to extract water from the soil solution interpreting a drought-like effect that ultimately affected plant growth and development. These results in conformity with the findings of Ibrahim *et al*.,(2018*); Grieve* and Walker (1983); and Hassan and Galal (1989) who reported that increasing salinity noticeably reduced plant height. Various selection techniques have been used to screen citrus rootstocks for salinity tolerance. The difference in plant vigor is measured by difference in plant height, leaf expansion and abscission and chloride accumulation in the leaves (Shannon and Grieve, 1999). **Number of leaves plant ^{1}**

The analysis of data revealed that application of salinity treatment significantly reduced number of leaves per plant (Table 2). Plants subjected with 150m*M* NaCl treatments produced minimum number of leaves per plant over control treatment, showing a reduction of (43.66%). Regarding rootstock, the number of leaves plant^{-1} (22.69 and 28.30%) was observed higher in Sour orange over *'Troyer' citrange* and *Citrus volckamariana* rootstocks. Application of salinity treatment progressively reduced number of leaves per plants which might be due to harmful effects of salts present in the root zone which increase sensitivity to water shortage as a result slows down growth of the plants (Andersen and Brodbeck, 1988). These results are in conformity with the findings of Hassine and Lutts (2010) reported that plants used more energy to extract water from saline solution, resulting in poor turgor pressure. Tuteja (2007) reported that accumulation of excessive salts in the root rhizosphere produce osmotic stress condition that decrease the uptake of water and nutrients to the roots and results the inhibition of cell elongation and shoot expansion. Similarly, Hu and Schmidhalter (2005) reported that osmotic stress produced by saline condition resulted competition of Na and Cl ions with other essential elements like K^{+}, Ca^{+2} and NO^{-3} as results reduction in plant growth occurs. Derbew (2006) stated that decrease in the number of leaves observed were not only related to the growth inhibiting effects of salt, but also to the injurious effects of salt due to defoliation of the damaged leaves. The present results are in line with Romero- Aranda *et al*. (1998) and Dong *et al*. (2007) who stated that salinity affects the number of leaves plant^{-1}and cause reduction in number of leaves.

Stem thickness (mm)

The average stem thickness was significant influenced by salinity treatment and rootstock, while interaction of both factors for stem thickness was found non-significant (Table 2). The application of salinity treatment decreased the stem thickness linearly compared to control treatment. Plants supplemented with increasing level of salinity treatment decrease stem thickness by 10.10, 25.85 and 42.85% over control treatment. Similarly, sour orange rootstock attained maximum stem thickness compared to those observed in *Citrus volckamariana*rootstock by 23.82%.

The results revealed that stem thickness decreased with increasing NaCl levels in the growth medium in all citrus rootstocks. Anjum *et al*. (2001) stated that higher soil salinity decreased stem thickness and its magnitude varied among the rootstocks. The plant stem thickness is among the commonly used indicator to estimate response to abiotic stress including salinity (Babu *et al*., 2012). Salinity increases the accumulation of Na^{+} and Cl^{-} in plant shoots and roots resulting in K deficiency and change in K-channel activity (Kanai *et al.,* 2011). The above results are in in agreement with Perez- Perez *et al*. (2007) who reported that plants exposed to salinity stress closes its stomata as results less water loss occur through transpiration. These changes led to lowered photosynthetic rate which is the main energy providing source of the plant. Naeem *et al*. (2020) reported that simulation of plants to salinity stress above 100m*M* exhibited reduction in morphological attributes like plant height, number of compound leaves, stem thickness and leaf area *of* tomato.

Leaf area (cm^{2})

The application of salinity treatment and rootstock showed a significant influence on leaf area, whereas their interaction had non-significant influence on leaf area (Table 1). The application of saline treatment at 50,100 and 150 m*M* NaCl decreased the leaf area of citrus from 12.06±1.76 cm^{2} in control to 10.98±1.72 cm^{2}, 9.94±1.40 cm^{2} and 8.83±1.29cm^{2}, respectively. Regarding citrus rootstock, Sour orange rootstock produced higher leaf area (15.03 and 44.51%) compared to *Citrus volckamariana* and *'Troyer' citrange* rootstock. In the present study, exposure of citrus seedling to increasing salinity stress condition progressively decreased leaf area which might be because high salinity condition disturbs the physiological and biochemical activities as result leaf area decrease and defoliation occur (Craine, 2005; Munns *et al*., 2006; Dong *et al*., 2007). The leaf area depends on the cell elongation and cell division in leaf tissue (Lauchli and Epstein, 1990). Kashem*et al*. (2000) observed the detrimental effect of high saline condition and reported that exposure of plants to high salinity condition significantly reduced leaf area which might be the adverse effect of salinity stress on nitrogen availability as result reduction in

photosynthesis rate and hence decrease leaf area. Similarly, Yasar *et al.* (2006) demonstrated that the surplus of sodium and chlorine ions changes the metabolic activities of the cell wall and limits the cell wall elasticity, thus reducing the turgor pressure competence in the cell. Thus, leaf expansion is a salt sensitive process (Najila*et al*., 2008) and the leaf area is seriously affected by the salinity stress (Babu *et al.,* 2012). The present results are in close conformity with Azab and Hegazy (1995) who reported that growth parameters like plant height and leaf area were decreased with application of salinity. Naeem *et al*. (2020) reported that plants exposed to high salinity stress above 100 mM progressively reduced leaf area of tomato plant.

Chlorophyll content (SPAD)

The analysis of results revealed that application of salinity treatment at 50,100 and 150m*M* NaCl showed 8.85, 24.86 and 53.12% reduction in chlorophyll content over control treatment. Among citrus rootstocks, maximum chlorophyll content (46.16±5.64SPAD) was observed in Sour orange rootstock, while *Citrus volckamariana* rootstock attained minimum chlorophyll content (40.25±7.08SPAD) (Table 2). Salinity stress adversely affected the chlorophyll content of citrus rootstock which might be due to rapid maturation and senescence of the leaves caused by plants supplemented with high salinity stress (Yeo *et al*., 1991). The present results are in harmony with those reported by Nieves *et al*. (1991) who stated that increasing salinity decreased leaf chlorophyll content. Similarly, Murkute*et al*. (2005) and Bihnert*et al*. (1995) reported that salinity stress reduces the activity of specific enzymes that plays a vital role in chlorophyll biosynthesis and the reduction in magnesium, iron, and manganese. They also reported that salinity condition produces water imbalance condition in the rootzone between the apoplast and symplast that leads to turgor decrease, which in turn may cause growth reduction. The exposure of citrus rootstock to moderate and high salinity stress significantly reduced chlorophyll content. Citrus rootstock supplemented with high salinity conditions results reduction in chlorophyll content compared to saline tolerant rootstock that show increase or unchanged chlorophyll content (Stepien and Johonson, 2009; Ashraf and Harris, 2013). Applied salinity stress significantly decreased the leaf chlorophyll content of tomato which might be due to the harmful effect of salinity stress at higher concentration which causes the induction of early maturing of leaves and diminished chlorophyll pigments that finally cause reduction in chlorophyll content (Kashem*et al*. (2000) reported that reduction in chlorophyll content under salinity stress simulated plants might be attributed due to the fact that high concentration of saline solution causes the early maturation of leaves and reduces the chlorophyll pigment that eventually decreased chlorophyll content.

Toxicity symptoms (%)

Salinity treatment, rootstock and their interaction influenced toxicity symptoms of citrus significantly (Table 2). The toxicity symptoms (tip burning and defoliation of leaves) (2.73±0.77%) were found maximum in plants supplemented with salinity treatment at 150 m*M* NaCl over control treatment (0.58±0.08%). Regarding citrus rootstocks, minimum toxicity (0.98±0.39%) symptoms was observed in sour orange rootstocks followed by *Citrus volckamariana* (1.29±0.65%), while *'Troyer' citrange* rootstock attained (1.91±1.08%) toxicity symptoms. The combination of salinity treatment and citrus rootstocks shows that maximum toxicity symptoms (3.38) were noted in *'Troyer' citrange* rootstock at 150 *mM* NaCl, while minimum (0.43) toxicity symptoms were recorded at sour orange rootstock at control treatment (Figure 1).

Application of salinity treatment progressively stimulated high toxicity symptoms in citrus rootstocks (tip burning and defoliation of leaves) compared to control treatment.

Our findings are also supported by Banuls and Primo-Millo (1995) reported that the first symptom of leaves was a progressive burn of leaf tips followed by leaf abscission in citrus. Moreover, the application of saline solution to the seedling plants adversely influencing its growth and physiology even at low concentration. Salinity stimulates the accumulation of toxic ions like sodium and Chlorine in plant tissue and produce differential symptoms related to cellular toxicity and chlorosis, leaf tip burns and defoliation (Levy and Syvertsen, 2004). Mer *et al*. (2000) reported that exposure of plants to salt stress caused dieback in young shoots of the six rootstocks. Akbar *et al.* (2001) reported that significant leaf drops and toxicity symptoms including leaf burning, leaf defoliation and yellowing were observed in citrus rootstocks like *'Troyer' citrange* and *Citrus volckamariana* at maximum salinity levels (150 m*M*). The present results are agreed with Lopez- CLiment*et al*. (2008) who reported that leaf injury is the first adverse effect of salt toxicity in citrus followed by leaf abscission. In our study maximum toxicity symptoms of salinity were recorded in *'Troyer' citrange* compared to Sour orange and *Citrus volckamariana* rootstocks. Our results are supported by findings of Shamhevet (1990) reported that *'Troyer' citrange* was found more sensitive to saline condition compared to other rootstocks. Similar results were also supported by Levy and Bañuls*et al.* (1990), who also observed the detrimental effect of

salinity and reported that salt stress disturb the photosynthesis process and cause severe leaf damage and abscission. Moreover, detrimental effect of NaCl is mostly linked with accumulation of high chloride ions in plant tissue (Walker *et al*., 1984).

Fresh and dry weight of root and shoot (g)

The mean values of fresh and dry root weight (Table 3) showed that the fresh root weight and dry root weight of citrus rootstock decreased significantly when application of salinity treatment increased from 0 to 150 m*M* NaCl). The highest value of fresh root weight (8.92±0.63g) and dry root weight (3.88± 0.42g) were recorded in control compared to fresh root weight (5.18± 0.65g) and dry root weight (1.88±0.35g) of plants subjected with 150 m*M* NaCl). Among citrus rootstock, the higher value of fresh root weight (7.97±1.40g) and dry root weight (3.46±0.78g) were attained by Sour orange followed by *Citrus volckamariana* (6.81±1.42g and 2.87±0.75g), while *'Troyer' citrange* gave lower value of fresh root weight (6.66± 1.41g) and dry root weight (2.56±0.74g). The application of various salinity treatment significantly influenced fresh and dry shoot weight of citrus rootstocks (Table 3). However, increasing the application of salinity levels significantly decreased fresh and dry weight of shoot over control treatment, and the lowest fresh shoot weight (5.63±0.77g) and dry shoot weight (1.96±0.35g) were observed in plants supplemented with 150 m*M* NaCl. While the highest fresh shoot weight (9.11±0.79g) and dry shoot weight (4.06±0.36g) were recorded in control treatment (0 m*M* NaCl). Regarding citrus rootstock, Sour orange rootstock attained maximum fresh shoot weight (8.51 ±1.29g) and dry shoot weight (3.65±0.80g) when compared with *Citrus volckamariana* (7.18±1.26g and 6.91±1.35g) and *'Troyer' citrange* (6.91± 1.35g and 2.70 ±0.77g).

It is obvious from our findings that salinity levels affected the vegetative growth adversely, which might be due to the fact that salinity disturbs the physiological functions of a plant such as gas exchange, affecting stomatal conductance, photosynthetic and transpiration rate. Moreover, the accumulation of high salts in the root zone bound the plants to use more energy to extract water from the soil solution rendering a drought like effect which ultimately affected plant growth and development. Loreto *et al*. (2003) reported that growth like fresh and dry weights of root decreased with salinity. Plants growing under saline condition are stunted due to reduction of cell elongation and cell division because auxins synthesis is retarded by salinity stress. In our study we observed that increase in salinity levels cause a decrease in fresh root weight which are in a harmony with the findings of Perez-Torner*et al*. (2009). Potassium is one of the essential elements required for plants growth and development and plays a vital role in the maintenance of osmotic balance, regulation of enzymatic activity, protein synthesis, neutralization of negatively charged proteins and the movement of stomata (Wu *et al*., 1996). The functionality of many cytosolic enzymes in plant cells depends on a certain balance of sodium and potassium ion (Mahajan *et al*., 2008). Salinity condition in the root zone produce competition of salt ions uptake (Na^+ and Cl^-) with other nutrient ions i.e. Ca^{2+}, K^{++}, N and P as result whole plant growth is affected (Grattan and Grieve, 1999). Salinity adversely affect membrane function and growth of the root as it induces calcium deficiency (Cramer *et al*., 1988). These findings are in a harmony with the results of Anjum *et al*. (2001) who found that with increasing salinity levels all growth parameters were reduced. In our study, the application of saline solution significantly reduced fresh weight of shoot and root in all studied rootstocks, *'Troyer' citrange* was found to be more adversely affected compared to other rootstocks. These results are in accordance with the findings of Aljuburi (1996) observed that higher concentration of salinity levels significantly reduced the growth attributes of Valencia orange and Sour orange seedlings.

Survival percentage (%)

Survival percentage of citrus was significantly influenced by salinity treatments, rootstocks, and their interaction (Table 3). The survival percentage (100%) of citrus seedling was higher in non-saline condition 0 m*M* NaCl and 50 m*M* NaCl. Whereas the survival percentage (86.66±9.81%) was lower in plants were subjected to 150 m*M* NaCl treated plants. Similarly, highest survival percentage (100%) was recorded in sour orange compared to *Citrus volckamariana* (95.83± 7.21) and *'Troyer' citrange* (90.83± 9.82). The interactive result between salinity levels and citrus rootstocks shows that maximum survival percentage (100%) was noted in sour orange grown under non saline condition, while minimum survival percentage (76.66%) was recorded in *'Troyer' citrange* supplemented with 150 *mM* NaCl (Figure 1). The above results show that salinity have negative effects on citrus rootstocks due to which their survival percentage become less with increasing in salinity levels. Sattar *et al*. (2010) reported that survival percentage of cotton seedlings was significantly reduced by all the concentrations of the salt solutions.

Proline content (mg/g)

The accumulation of proline content of leaves was significantly influenced by salinity treatment, citrus rootstocks, and their interaction (Table 3). The accumulation of proline content in the leaves was

progressively increased from 0.37± 0.05 to 1.55±0.29 mg/g when salinity treatment was increased from 0 to 150 *mM* NaCl, respectively. Similarly, the highest proline accumulation of leaves (1.275 mg/g) was recorded in sour orange than *Citrus volckamariana* (0.99± 0.41 mg/g) and *'Troyer' citrange* (0.90± 0.36mg/g). The highest value of proline accumulation (1.953 mg/g) was observed from sour orange rootstock subjected with 150*mM*NaCl, while lowest accumulation of proline content (0.328 mg/g) was recorded at *'Troyer' citrange* rootstock in untreated plants (control) (Figure 1).

Proline is well known amino acid which is typically stores in plants when plants are exposed to environmental stresses (Kavi-Kishor *et al*., 2005). The accumulation of proline in plants grown under salinity stress is a common stress indicator and is associated with salt stress tolerance of different plant species (Liu and Zhu, 1997). The physiological activity of plants is maintained by accumulation of high concentration of proline when plants are exposed to high salinity condition coupled with Na+ which enhance the formation of osmoregulants as factor of environmental stress tolerant and favors the uptake of water from saline medium. In addition to acts as a compatible osmolyte, proline plays a protective role against salt stress in plants. It can act as an enzyme protectant, free radical scavenger, cytosolic pH buffer stabilizer for subcellular structures and cell redox balancer (Verbruggen and Hermans, 2008). Maggio *et al*. (2002) reported that proline acts as a signaling molecule that initiates adaptation to the stress. Ueda *et al*. (2004) stated that osmotic adjustment under saline conditions is due to accumulation of solutes and proline in plant tissue. Abraham *et al*. (2003) reported that there is inherited difference in the accumulation of proline in several genotypes of plant species and this osmolyte is widely accumulated in higher plants, than other amino acids under salt stress conditions. The present study also confirms that proline accumulation varies in different citrus rootstocks. Results shows that tolerant citrus rootstock like sour orange maintained higher proline accumulation under various levels of salinity compared to *Citrus volckamariana* and *'Troyer' citrange*. The present results are supported by Balal*et al*. (2011) who reported that maximum proline accumulation was recorded at higher salinity level i.e., 90 *mM* followed by 60 *mM* and 30 *mM,* respectively.

Table 3. *Fresh and dry weight of shoot and root, survival percentage and accumulation of proline content of citrus rootstock seedlings as influenced by different salinity concentrations.*

Salinity level (S)	**Fresh root weight (g)**	**Dry root weight (g)**	**Fresh shoot weight (g)**	**Dry shoot weight (g)**	**Plant Survival (%)**	**Proline content (mg/g)**
Control (0)	8.92±0.63^{A}	3.88±0.42^{A}	9.11±0.79^{A}	4.06±0.39^{A}	100±0.00^{A}	0.37±0.05^{D}
Low (50 *mM*)	7.92±0.53^{B}	3.43±0.34^{A}	8.26±0.56^{B}	3.57±0.40^{A}	100±0.00^{A}	0.95±0.007^{C}
Moderate(100*mM*)	6.57±0.51^{C}	2.67±0.39^{B}	7.14±0.66^{C}	2.86±0.42^{B}	95.55±6.28^{B}	1.35±0.20^{B}
High (150 *mM*)	5.18±0.65^{D}	1.88±0.35^{C}	5.63+0.77^{D}	1.96±0.35^{C}	86.66±9.81^{C}	1.55±0.29^{A}
Rootstocks (R)						
Troyer Citrange	6.66±1.41^{B}	2.56±0.74^{B}	6.91±1.35^{B}	2.70±0.77^{B}	90.83±9.82^{C}	0.90±0.36^{C}
Citrus volckamariana	6.81±1.42^{B}	2.87±0.75^{AB}	7.18±1.26^{B}	2.98±0.79^{B}	95.83±7.21^{B}	0.99±0.41^{B}
Sour orange	7.97±1.40^{A}	3.46±0.78^{A}	8.51±1.29^{A}	3.65±0.80^{A}	100±0.00^{A}	1.27±0.57^{A}
Factors						
Salinity (S)	*	*	*	*	*	*
Rootstocks (R)	*	*	*	*	*	*
S× R	Ns	ns	ns	ns	Fig 1**	Fig 1**

Means followed by different letters shows significantly difference at P≤0.01; Ns:Non significant ; *:P≤0.05 ;**: P≤0.01

IV. Conclusions

In the light of above presented results, it was observed that different salinity levels show a detrimental influence on all the growth attributes of citrus rootstock. Increasing soil salinity levels from 50 m*M* to 150 *mM* NaCl attained reduction in vegetative growth (plant height, number of leaves, stem thickness, chlorophyll content, leaf area, root and shoot fresh weight, root, and shoot dry weight) and rise in

physiological attributes like proline accumulation in leaves. The maximum value of growth attributes, less toxicity symptoms, high proline accumulation and survival percentage were recorded in sour orange rootstocks compared to other citrus rootstocks Among the citrus rootstocks, sour orange give best results regarding growth performance under saline condition, while *'Troyer' citrange* was found the least tolerant rootstock. Abiotic stress tolerance in grass.

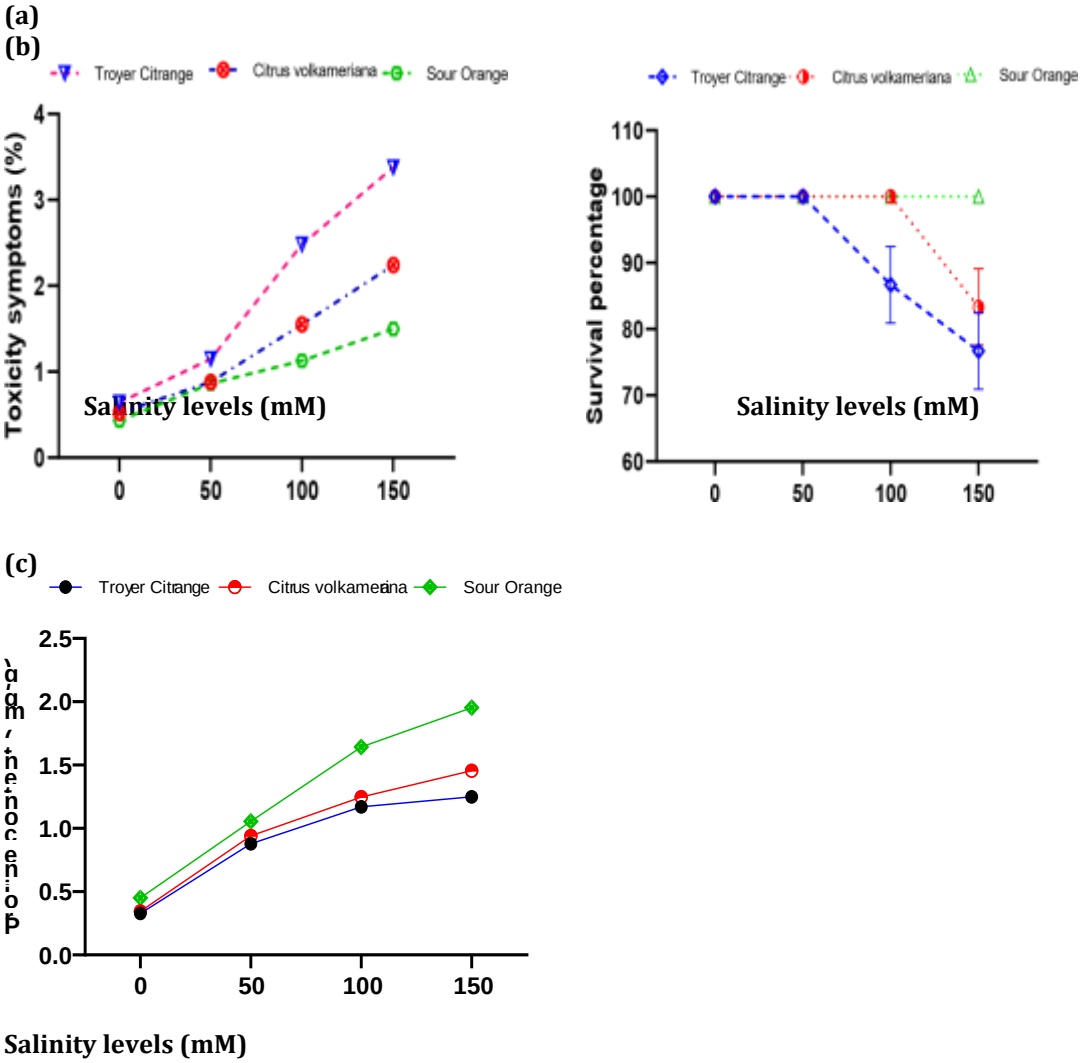

Figure 1. *Toxicity symptom, survival percentage and proline content as influenced by salinity levels and various citrus rootstocks.*

Author Contributions

The following statements shows the contribution of authors in different ways;

"Conceptualization, M.S. and AU.; methodology, AU. and M.S.; software, I.U.; validation, A.B. and M.S.; formal analysis, I.U. and Q.S.A.; investigation, M.S., and AU; Resources; B.H., K.N., N.A., I.A., M.M., data curation, I.U. and A.B.; writing-original draft preparation, A.B.; writing-review and editing, A.B. and I.U.; visualization, M.S. and A.B.; supervision, M.S. All authors have read and agreed to the final version of the manuscript."

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Conflicts of Interest

The authors declare non availability of conflict of interest.

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