International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(4): 2270-2276

Received: 01-05-2018
Accepted: 05-06-2018

Pranava Pandey
Department of Fruit Science, Bihar Agricultural University, Sabour, Bihar, India

Avnish Kumar Pandey
Department of Fruit Science, ACHF, Navsari Agricultural University, Navsari Gujarat, India

Correspondence
Pranava Pandey
Department of Fruit Science, Bihar Agricultural University, Sabour, Bihar, India

To fix up the physio-chemical bases of tolerance in polyembryonic mango rootstock under salt stress

Pranava Pandey and Avnish Kumar Pandey

Abstract
Polyembryonic mango (*Mangifera indica* L.) rootstocks, 'Kurukkan' and 'Olour' were irrigated with tap water containing 0, 50 and 100 mM NaCl at four days interval for 40 days. NaCl stress reduced net photosynthetic rate (*Pn*), stomatal conductance (*Cs*), trehalose and phenol content in both rootstocks. However, increased the activities of peroxidase (POX), catalase (CAT), superoxide dismutase (SOD), membrane injury index (MII), proline content, total soluble sugar content, in plant tissues compared to what occurred in plants in the absence of NaCl. Salt tolerance mechanism in 'Olour' may be due to higher POX and CAT activities, higher proline and phenol content, lower MII and lower leaf abscission and also higher root Cl^- content in plant tissues while 'Kurukkan' could tolerate high NaCl concentration by maintaining higher net photosynthetic rate and stomatal conductance, higher SOD activity, higher sugar and trehalose content, higher accumulation of root Na^+ in plants. Hence, based on the results it could be said that salt tolerant mechanism occur in both the rootstocks.

Keywords: Catalase, proline, peroxidase, superoxide dismutase

1. Introduction
Mango (*Mangifera indica* L.) is the most common and important fruit of India and is called as "King of fruits" not just for its super flashy yellow color and taste, but also for the array of health benefits it offers. India stands on top position among mango's growing countries and produces 40.48% of the total world mango production (FAO, 2010) [13]. It has been well known that mango fruits are an important source of micronutrients, vitamins and other phytochemicals. Moreover, mango fruits provide energy, dietary fibre, carbohydrates, proteins, fats and phenolic compounds (Tharanathan *et al*. 2006) [34], which are vital to growth, development and health of humans. Each part of a mango tree, such as its leaves, flowers, bark, fruit, pulp, peel and seeds contains essential nutrients that can be utilized.
It is a matter of concern that the productivity of mango orchards in India is low compared to other countries. There are several factors are responsible for the poor productivity of mango orchards, amongst, soil and water salinity are playing key roles. In India, the area affected by salinity is around 6.75 million hectare (CSSRI, 2016) [7] and these areas are suffering with very poor productivity of mango may be due to the sensitivity of this crop to salinity and excess of chloride (Zuazo *et al*. 2003) [38]. Srivastava *et al*. (2010) [32] reported that plants of the mango rootstock 'Olour' showed higher activities of SOD, CAT, and POX when treated with salt than non-salt-treated plants. Sodium ions may be toxic and often induce cellular damage which affects plant growth and development and are not essential for plant growth also (Zhu, 2007) [37]. Hafez *et al*. (2011) [18] reported that the leaves of Sukkary' and 'Zebda' mango rootstocks showed increased Na^+ ion concentrations as the plants aged under saline irrigation water. High salinity was more.

Abbreviation: *Pn*, net photosynthetic rate; *Cs*, stomatal conductance; POX, peroxidase; CAT, catalase; SOD, superoxide dismutase; MII, membrane injury index; mM, mili mole.
Effective than low salinity in this respect. Na^+ ion concentrations in the leaves of 'Zebda' rootstock were higher than in 'Sukkary' mango rootstocks. Pandey *et al*. (2014a) [28] reported that under high salinity-stress seedlings of the mango rootstocks exhibited higher antioxidant enzyme activities, more lipid peroxidation, and higher proline concentrations. Mango plants under NaCl treatment showed poor vegetative growth and seedling mortality (Dubey *et al*. 2006; Srivastavaz *et al*. 2009) [11], high defoliation (Pandey *et al*. 2014b; Dubey *et al*. 2006) [27, 11].

Salinity treatment increased the Na^+ and Cl^- ion concentrations in both leaves and roots of polyembroynic mango seedlings (Kishor *et al*. 2009; Pandey *et al*. 2014a) [20, 28]. Seedlings of polyembryonic mango genotypes 'Kurukkan' and 'Olour' showed more tolerant than 'Kerala 1' and 'Kerala 2' under various levels of NaCl treatment (Dubey *et al*. 2006) [11].

Soil salinity is one of the most important abiotic stresses, while, the mechanism (s) of salt-tolerance are complex and include osmotic adjustments through the accumulation of compatible solutes, lowering the concentrations of toxic ions in the cytoplasm by restricting the influx of Na^+ ions, ion sequestration in the vacuoles, Na^+ ion extrusion, and scavenging of ROS (Mittler, 2002) [25].

With the above investigation in relation to polyembrynic (mango stone contain more than one embryo including the zygotic and nucellar embryos) rootstocks of mango, it may appear that these rootstocks have more salt tolerant behavior. And that's why polyembrynic rootstocks had been taken in this study. At present the traditional approach of using the salts out of the system is increasingly becoming impractical and enhanced adaptation to live with the salts has emerged as the new paradigm. Therefore, in the present study, emphasis had given to find out the rootstock which have more tolerance and fix the basis. Here, physiological and biochemical changes and the uptake of salt ions were studied.

2. Materials and Methods

2.1 Plant Material

Mature fruits of 'Olour' and 'Kurukkan' polyembryonic mango genotypes were harvested. And after ripening, stones of the fruit were taking out from the pulp and washed thoroughly with running tap water and sown in the nursery beds immediately in July 2011. The seedlings were allowed to grow until 15th January 2012. In February 2012, uniform seedlings (n = 40 per genotype) were selected on the basis of vigour, leaf size and leaf shape and then transplanted in plastic pots (30 cm diameter), containing 8.0 kg of a 1:1:1 (w/w) mixture of loamy soil, sand and well-rotted farm yard manure (FYM). The mixture used in pots had $EC_{(1:2)}$ of 0.13 dS m^{-1}, a $pH_{(1:2)}$ of 7.10, a cation exchange capacity (CEC) 10.71 cmol kg^{-1}, and organic carbon 0.46%. All seedlings were irrigated by normal tap water (EC = 0.10 dS m^{-1}), and after establishment they were fertilised with 30 g of a mixture consisting of 1:1:1 (w/w/w) urea, single super phosphate, and potassium sulphate and allowed to grow for 30 days until the start of the NaCl treatments. Seedlings were raised under day-time temperatures of 24º -33 ºC, night-time temperatures of 14º-21 ºC, and a relative humidity (RH) of 65-92%, under natural light conditions with a 58 h photoperiod and an altitude of 228.61 m above mean sea level. The experiment was conducted at experimental farm of Fruits and Horticultural Technology, IARI, New Delhi.

2.2 Salt Treatments

A pot experiment was conducted by applying three levels of NaCl [0.0 mM NaCl (the tap water control), 50 or 100 mM NaCl] to two different mango rootstock genotypes ('Olour', 'Kurukkan') at 4 days intervals for 40 days. The quantity of NaCl applied was calculated on the basis of the calibration graph developed by Dubey *et al*. (2006) [11] to achieve a soil salinity value of approximately 4.0 dS m^{-1}. The level of salinity was selected to reflect the occurrence of saline soil in fruit-growing areas.

The seedlings (n = 10 per treatment per genotype) were then irrigated with 50 or 100mM NaCl, while control plants (n = 10 per genotype) were irrigated with tap water (EC = 0.10 dS m^{-1}) at 4 days intervals for 40 days by considering the loss of moisture from each pot calculated by directly weighing each the pot.

2.3 Physiological Parameters

Rate of photosynthesis and stomatal conductance was measured on leaf using portable Infrared Gas Analyzer (IRGA LI-COR 6400 Model). The photosynthetic rate was determined in the upper most fully expanded leaf between 10 am and 11.30 am by providing artificial light source of light intensity 1000 CO_2 µmol $m^{-2}S^{-1}$. The net photosynthetic rate was expressed as µmoles CO_2 m^{-2} s^1.

The membrane injury index of leaf was examined by using the method described by Deshmukh *et al*. (1991) [10]. Accurately weighed 0.5 g of freshly sampled leaf material and immersed in a test tube containing 10 ml of double distilled water. These tubes were incubated at 45 ^{0}C for 30 minutes in a hot water bath. Thereafter, electrical conductivity of the incubated solution (C_1) was measured with the help of a conductivity meter (Systronics India Ltd., Mumbai, India). These tubes were then incubated in hot water bath (100 ^{0}C) for a period of 10 minutes. The incubated solution was cooled down to the room temperature and electrical conductivity (C_2) was measured. The membrane injury index of leaf was calculated according to the following formula:

$$MII = (C_1/C_2) \times 100$$

2.4 Biochemical Parameters

2.4.1 Trehalose Estimation

Trehalose concentrations were estimated according to the method of Brin (1966) [5] and Ferreira *et al*. (1997) [14]. Trehalose was extracted from 10 mg dry weight of each leaf samples with 2.0 ml of 0.5 M trichloroacetic acid, at 0 ^{0}C for 20 minutes. After that the mixture was centri fused at 5000 x g and the supernatant was collected. For estimation of trehalose, 0.3 ml of extracted solution was mixed with 3.0 ml of anthrone reagent keeping the tube in ice. After that, the tubes were heated at 100 ^{0}C for 10 minutes in water bath and then the tubes were quickly transferred to dark for 20 minutes. The absorbance of solution was taken at 620 nm on UV-VIS double beam PC 8 scanning Auto cell spectrophotometer, UVD 3200 (Labomed, INC, USA) and the values of trehalose content were estimated using known standards.

2.4.2 Total Soluble Sugars estimation (TSS)

Fresh leaf sample was boiled and the supernatant decanted into a beaker. The extraction was repeated four times (three times with 20 ml of 80% (v/v) ethanol in water and finally with 20 ml of distillated water) by boiling the sample for 4-5 min and decanting the supernatant. For clarification, 50 ml aliquot of the sugar extract was evaporated in a water bath. Subsequently, the sample was treated with 1 ml saturated solution of lead acetate to precipitate the colloidal substances. It was then filtered into a 50 ml volumetric flask and made up to the volume. An aliquot of this solution was used for determining the total sugar by athrone reagent method (Sadasivam and Manickam 1992) [29].

2.4.3 Antioxidant enzymes activity

2.4.3.1 Preparation of crude enzyme extract

Fresh leaf samples (n = 10 leaves) were collected from each of the ten plants of each genotype in each NaCl treatment after 30 days, placed in an ice box and washed immediately with tap water, followed by distilled water. A 1.0 g leaf sample from each plant was weighed and homogenized in a pre-chilled mortar and pestle by adding 5 ml of pre-chilled 50 mM phosphate buffer, pH 7.0. Each homogenate was placed in a centrifuge tube and centrifuged at 15,000 x *g* for 20 min at 4 ºC. The supernatant was sieved through two layers of muslin and stored in a refrigerator. This was used as the crude enzyme extract with which to measure three key antioxidant enzyme activities.

2.4.3.2 Superoxide dismutase (SOD)

The activity of superoxide dismutase in fresh leaf sample was determined according to Fridovich (1975) [15]. The assay is based on the ability of SOD to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). The reaction mixture was prepared in tubes consisting of 0.2 ml of 200 mM methionine, 0.2 ml of 1.5 mM EDTA, 0.2 ml of 1.125 mM NBT, 0.2 ml off 75 mM ribloflavin, 0-10 µl of enzyme extract and phosphate buffer (50 mM; pH 7.8) to make the final volume of the reaction mixture to 3 ml. Rivoflavin was added as the last component and the tubes were shaken well. The reaction was started for a specified time of 15 minutes by keeping the tubes 30 cm below a light bank consisting of two 15 Watt fluorescent lamps. After 15 minutes light was switched off and the tubes were immediately covered with a black cloth in order to stop the reaction and absorbance of the mixture was then read at 560 nm wavelength on UV-VIS double beam PC 8 scanning Auto cell spectrophotometer, UVD 3200 (Labomed, INC, USA). A complete reaction mixture containing 0 µl of enzyme extract, developing maximum colour served as a control. A non-irradiated complete reaction mixture with no colour development served as blank.

2.4.3.3 Catalase

The method suggested by Luck (1975) [22] was followed to estimate the catalase activity in the fresh leaf sample. The assay was based on the estimation of residual hydrogen peroxide (H_2O_2) by oxidation with potassium permanganate ($KMnO_4$) by titration. The reaction mixture was prepared in tubes by adding 3 ml of phosphate buffer (0.1M; pH 7.0), 2 ml of 5.0 mM H_2O_2 and 1.0 ml of crude enzyme extract. The reaction mixture was incubated for exactly 1 minute at 20ºC followed by addition of 10 ml of 0.35 M H_2SO_4 in order to stop the reaction. A blank was prepared by adding enzyme extract to an acidified solution of reaction mixture at zero time. The residual H_2O_2 was estimated by titrating the reaction mixture against $KMnO_4$ (0.01M) till the appearance of faint pink colour persisting atleast for 15 seconds. The catalase activity was expressed as µ moles H_2O_2 hydrolyzed $min^{-1}mg^{-1}$ protein.

2.4.3.4 Peroxidase

The activity of peroxidase in fresh leaf sample was determined using method proposed by Thomas *et al*. (1981) [35]. The assay utilizes guiacol as the enzyme substrate. The reaction mixture was prepared in tubes by adding 3 ml of phosphate buffer (0.1 M; pH 7.0), 30 µl of H_2O_2 (20 mM), 50 µl of guiacol (20 mM) as enzyme substrate and 50 µl of crude enzyme extract. The reaction mixture was incubated in cuvette for exactly 10 minute at 20 ºC temperature. The absorbance was read at 436 nm wavelength on UV-VIS double beam PC 8 scanning Auto cell spectrophotometer, UVD 3200 (Labomed, INC, USA). The increase in absorbance was recorded at 30 second intervals till the constant reading was obtained. POX activity was expressed in A_{436} unit min^{-1} g^{-1} leaf fresh weight (FW).

2.4.4 Proline

The proline content in fresh leaves of each treatment was estimated by rapid colorimetric method as suggested by Bates *et al*. (1973) [3]. Each fresh leaf sample (0.5 g) was homogenized in 5.0 ml of 3% (w/v) sulpho-salicylic acid in pre-chilled mortar and pestle. Then, it was centrifuged at 10,000 x g for 10 minutes at 4 ºC. The supernatant was diluted to 10 ml with double-distilled water and from it 0.1 ml was taken in test tube and made up to 1.0 ml volume with double-distilled water. This was mixed with 5 ml of acid ninhydrin reagent and 5 ml of glacial acetic acid for one hour at 100 ºC in hot water bath. Thereafter, the reaction was terminated by keeping the solution on ice bath. Then, 4 ml toluene was added and mixed vigorously with the help of a vortex stirrer for 20-30 seconds. The chromophore containing toluene layer (light pink) was aspirated from the aqueous phase, warmed to room temperature and then absorbance was read at 520 nm on UV-VIS double beam PC 8 scanning Auto cell spectrophotometer, UVD 3200 (Labomed, INC, USA) using pure toluene as a blank. The proline concentration in the samples was determined from a standard curve prepared by using analytical grade proline (SRL, Chem Co. Mumbai)

2.4.5 Total phenols

Total phenols in fresh leaves were quantified according to Malik and Singh (1980) [24]. One gram fresh leaf sample of each treatment were homogenized in mortar and pestle after adding 80% ethanol (v/v). Then, it was centrifuged at 10,000 x g for 20 minutes and the supernatant was filtered using Whatman No. 42 filter paper. The residue was extracted five times with 80% ethanol. The collected supernatants were evaporated to dryness in a water bath (68 ^{0}C). Residues were dissolved in 10 ml of distilled water. Out of which 0.1 ml was taken and total volume was made up to 3.0 ml with double-distilled water. Then freshly prepared 0.5 ml Folin-Ciocalteau reagent was added. After 3 minutes, 2 ml of 20% sodium carbonate (Na_2CO_3) was added in each tube, mixed thoroughly and placed on a hot water bath (58 ºC) exactly for 1 minute. Then it was cooled to room temperature and absorbance was measured against blank at 750 nm using UV-VIS double beam PC 8 scanning Auto cell spectrophotometer, UVD 3200 (Labomed, INC, USA).

2.5 Tissue ions analysis

The Na^+ ion content in leaves and roots were estimated using tissue samples digested with a 9:4 (v/v) mix of HNO_3:$HClO_4$ and a microprocessor based flame photometer (Flame Photometer-128; Systronics, New Delhi, India) through the method of Jackson (1980). Cl^- ion content in mango leaves and roots were measured using mercuric (II) thiocyanate, as suggested by Adriano and Doner (1982) [1]. The extraction of Cl^- ions from leaves and roots used a 1:100 (w/v) mix of 0.1 M sodium nitrate (Gaines *et al*. 1984) [16].

2.6 Statistical Analysis

The experiment was conducted in Factorial Complete Randomized Design (FCRD) with five replications. Each

replication consisted of two plants. Data were submitted to analysis of variance (ANOVA) with SPSS 16.0 package (*SPSS*, Chicago, IL, USA). Treatment mean differences were separated by the least significant difference ($LSD_{0.05}$) test if F tests were significant ($P \leq 0.05$) using Duncan's multiple range test (DMRT).

3. Results

3.1 Physiological Parameters

For membrane injury index (MII), salinity differs significantly (Fig.1). Irrespective of rootstocks, MII increased with increasing level of NaCl concentrations. At higher salinity level (100 mM), 'Kurukkan' (99.67%) had higher MII than 'Olour' (92.00%) as compared to respective controls.

Salinity affects photosynthetic rate significantly too. Maximum reduction in photosynthetic rate was noticed at higher salinity level in both rootstocks. Compared with respective control, higher reduction in photosynthetic rate was recorded in 'Olour' (76.44%) as compared to 'Kurukkan' rootstock (57.86%) at higher salinity level.

In case of stomatal conductance, both rootstocks showed almost equal stomatal conductance in absence of NaCl salt. However, salinity reduced stomatal conductance at varying level in all rootstocks. The higher reduction in stomatal conductance was recorded in 'Olour' (57.14%) as compared to 'Kurukkan' (20.00%) at higher NaCl concentration. Similar trend was also observed at lower salinity level.

3.2 Biochemical Parameters

Effect of salinity on rootstocks showed that under control condition 'Kurukkan' had higher proxidase activity over 'Olour' rootstock while in presence of NaCl, the higher peroxidase activity was recorded (95.00% higher than respective control) in 'Olour' at higher level of NaCl concentration as compared to 'Kurukkan' (52.58% higher than respective control).

Effect of salinity on rootstocks showed that higher increase in catalase activity by 34.07% was recorded in 'Olour' as compared to respective control at 100mM NaCl concentration. And at higher salinity level, 'Kurukkan' had higher increase in SOD activities (36.63% more than control) compared to 'Olour' (14.21% more than control).

In case of proline content significant ($P \leq 0.05$) increase in proline accumulation in both the rootstocks under treatment compared with the respective control. Higher increase in proline accumulation by 61.73% was recorded in 'Olour' as to 'Kurukkan' (40.36%) when seedlings were irrigated with water containing 100mM NaCl.

Trehalose content increased with increasing level of NaCl concentration in both rootstocks. In presence of NaCl, the trehalose content recorded higher (379.04% higher than control) in 'Kurukkan' at higher salinity level as compared to 'Olour' (101.03% higher than control).

In control condition, significantly lower phenol content was recorded in 'Kurukkan'. However, in presence of salt, phenol content recorded maximum (25.28% higher than control) in 'Olour' at higher salinity level followed as of 'Kurukkan' (15.44% higher than control).

At higher salinity level, the total soluble sugars content in leaf tissues increased higher in 'Kurukkan' (29.98% higher than control) compared to 'Olour' (16.29% higher than control) while in case of root tissues total soluble sugars content increased higher in 'Olour' (47.28% higher than control) compared to 'Kurukkan' (28.25% higher than control).

In case of leaf abscission, significantly lower abscission was recorded in 'Olour' in control while maximum leaf abscission was recorded in 'Kurukkan' (17.29%) at higher salinity level compared with respective control.

3.3 Tissue Nutrients Accumulation

Salinity effect indicated that sodium accumulation in leaf and root tissues increased with increasing level of NaCl concentration in both the rootstocks. In absence of NaCl, significantly higher Na^+ concentration was recorded in leaf and root tissues of 'Kurukkan'. However, at higher salinity level, the Na^+ concentration increased higher (62.50%) in leaf tissues of 'Olour' and root tissues (52.63%) of 'Kurukkan' as compared to respective controls.

Chloride accumulation in leaf and root tissues also increased with increasing level of NaCl concentration in both rootstocks. In control condition, Cl^- accumulation in leaf tissues was statistically similar while in case of root tissues 'Kurukkan' had significantly higher accumulation. The maximum Cl^- accumulation in leaf and root tissues by 43.10% in 'Kurukkan' and 40.43% in 'Olour' genotypes was recorded at higher salinity level as compared to respective controls.

4. Discussion

Data pertaining to effect of salinity on MII indicated that at higher salinity level, higher MII was found in 'Kurukkan' than 'Olour' rootstock compared to respective controls. The higher MII in 'Kurukkan' may be due to higher accumulation of Cl^- in their leaves resulting in more damage to cells. NaCl also enhanced the free radical scavenging capacity of treated plants, including higher levels of superoxide dismutase, catalase, and peroxidase. 'Olour' rootstock had shown higher level of activity of catalase, and peroxidase enzyme may leads to less damage of membrane. These enzymes are also linked to the MII and reductions in photosynthetic activity, as reported by Deshmukh *et al*. (2006) [9].

The higher reduction in photosynthetic rate and stomatal conductance was recorded in 'Olour' rootstock at higher salinity level suggest that the negative effects of salt stress on photosynthesis may be due to the higher uptake of Na^+ in leaf tissues of 'Olour'. These findings corroborated with Kaya *et al*. (2001) [19] and Sharma *et al*. (2011) [30] findings that showed photosynthetic capacity suppressed by salinity.

In present study, POX and CAT activities increased in both rootstocks under salt stress and recorded higher in leaves of 'Olour' at higher level (100 mM) of NaCl concentration; however, SOD activity increased higher in 'Kurukkan'. SOD, POD and CAT are usually considered as the key components of antioxidant defense of the plants (Xue *et al*. 2008) [36]. The higher POX and CAT activity in 'Olour' genotype showed that these cells had higher capacity for catalyzing H_2O_2 to H_2O and O_2 as envisaged by lower inhibition in growth (data not shown) and cellular damage. While lower SOD activity in 'Olour' may be an adaptive response to prevent potential damage of tissues. It indicated that salt-stressed plants exhibited specific responses of SOD, POD and CAT activities. The distinction in antioxidant enzyme activities between leaves might be associated with the marked differences among the types of cellular metabolism existing in the leaves (Srivastav *et al*. 2010; Cavalcanti *et al*. 2007) [32, 6].

In case of proline concentration in leaf, significantly higher was measured in 'Olour' at higher salinity level. Proline concentrations have also been reported to increase under NaCl stress in *Morus Alba* (Ahmad *et al*. 2007) [2] and in wheat (Tatar and Gevrek, 2008) [33], that suggests a protective role

for proline in avoiding the negative effects of salt stress in mango.

Trehalose content increased with increasing level of NaCl concentration in both rootstocks. The trehalosse content recorded higher in 'Kurukkan' at 100 mM NaCl concentration. Trehalose appears to improve water retention in plant cells. It also protects cell membranes and enzymes (Muller *et al*. 1995) [26] even though the mechanism of action is poorly understood. Similar findings were reported by El-Bashiti *et al*. (2005) [12] in wheat under salt or drought stress conditions.

Phenol content recorded higher in 'Olour' at higher salinity level. The accumulation of phenols may be due to the complex or a set operation of three factors including decreased conversion of phenols to quinines by lower activity of polyphenol oxidase, high oxidation of carbohydrate via the pentose phosphate shiikimate pathway and low utilization of phenols for lignin formation. Indeed, phenolic compounds participate in the defense against ROS which are inevitably produced when aerobic or photosynthetic metabolism is impaired by environmental stresses (Ksouri *et al*. 2007) [21].

In present study, carbohydrate content increased and found higher in leaf tissues of 'Kurukkan' at 100mM NaCl. The greater carbohydrate accumulation in leaves than in roots under stressed conditions may be associated with decrease in translocation from leaves to roots. It is interesting to observe that even in stressed conditions, soluble carbohydrate content was higher in leaves, contributing therefore to water status maintenance in roots.

Interaction between salinity and rootstocks also affected leaf abscission significantly. The higher leaf abscission was recorded in 'Kurukkan' at higher salinity level compared control. Leaf abscission increased mainly due to a cellular intoxication by Cl^- ions and when a certain level of leaf Cl^- concentration is achieved, ACC content (the direct precursor of ethylene hormone) and leaf ethylene production are triggered and massive leaf abscission occurs (Gomez-Cadenas *et al*. 1998) [17].

At higher salinity level, the Na^+ accumulation increased higher in leaf tissues of 'Olour' and root tissues of 'Kurukkan'; however, Cl^- accumulation increased higher in leaf tissues of 'Kurukkan' and root tissues of 'Olour' as compared to respective controls. These results revealed that 'Olour' had a greater ability to restrict Cl^- ions translocation to leaf tissues while 'Kurukkan' had a greater ability to restrict Na^+ ions translocation from root to leaves which may attributed to less injury or mortality of seedlings of 'Olour'. These findings are also correlated with Olive (Chartzoulakis *et al*. 2002) [8] and Rangpur lime (Maas 1993) [23] crop under salt stress.

5. Conclusion

Based on the above findings, it could be said that salt tolerance mechanism in 'Olour' may be due to higher POX and CAT activities, higher proline and phenol content as well as higher root Cl^- content while in 'Kurukkan' due to by maintaining higher SOD activity, higher photosynthetic rate, higher sugar and trehalose content as well as higher accumulation of root Na^+. In this way the results indicated that 'Olour' may have more tolerance behavior as antioxidant enzymes and proline considered as key components. Moreover, this study provides information on physiological and biochemical bases of salt tolerance in the mango rootstocks. It may strengthen our understanding about the mechanisms by which salinity affects plant growth and development.

6. Acknowledgement

The research programme was funded by Division of Fruits and Horticultural Technology, Indian Agricultural Research Institute, New Delhi.

Table 1: Effects of NaCl stress on Na, and Cl content (dry weight basis) in leaves and root tissues of rootstocks

Treatment	Na (%)		Cl (%)	
	Leaf	Root	Leaf	Root
Kurukkan				
Control	0.20^{b}	0.19^{bc}	1.16^{a}	1.35^{c}
50 mM	0.23^{cd}	0.27^{d}	1.51^{c}	1.54^{d}
100 mM	0.25^{de}	0.29^{d}	1.66^{d}	1.69^{e}
Olour				
Control	0.16^{a}	0.14^{a}	1.20^{a}	0.94^{a}
50 mM	0.22^{c}	0.17^{ab}	1.43^{b}	1.28^{b}
100 mM	0.26^{e}	0.21^{c}	1.52^{c}	1.32^{bc}
LSD *(P ≤ 0.05)*	0.02	0.03	0.05	0.06

Means followed by the same letter (s) within treatment are not significantly different at $P \leq 0.05$ for F test using Duncan's multiple range test (DMRT).

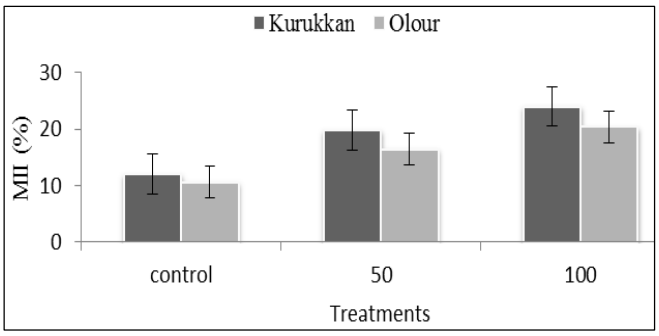

Fig 1: Effect of NaCl treatment (mM) on membrane injury index (MII) of rootstocks

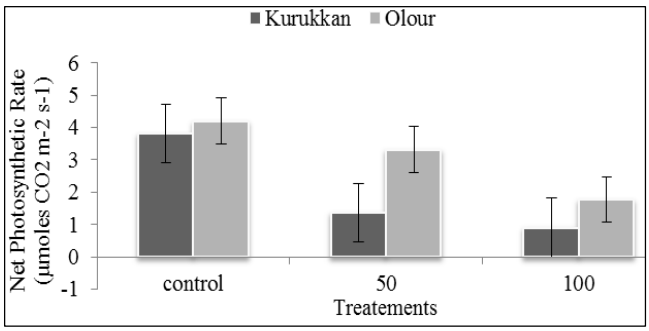

Fig 2: Effect of NaCl treatment (mM) on net photosynthetic rate (*Pn*) of rootstocks

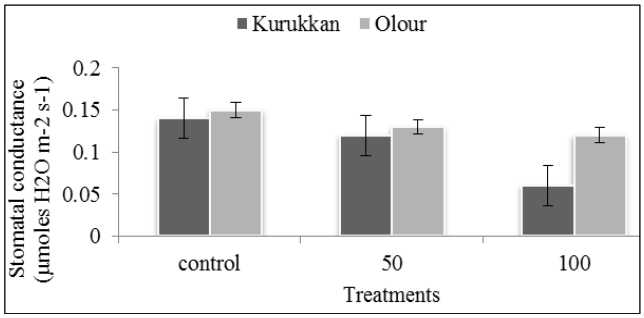

Fig 3: Effect of NaCl treatment (mM) on stomatal conductance (*Cs*) of rootstocks

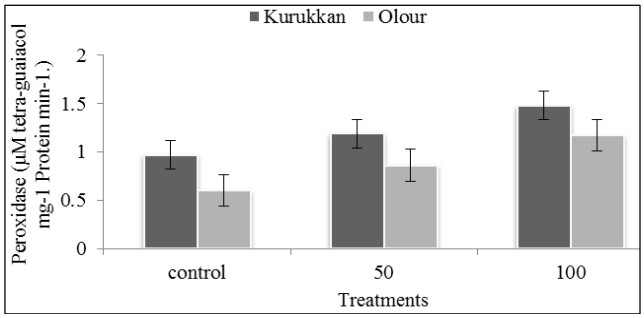

Fig 4: Effect of NaCl treatment (mM) on Peroxidase (POX) of rootstocks

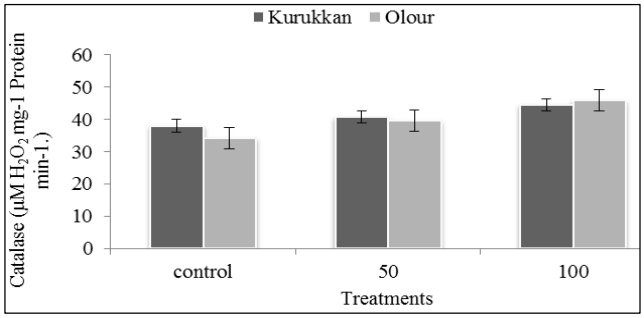

Fig 5: Effect of NaCl treatment (mM) on Catase (CAT) of rootstocks

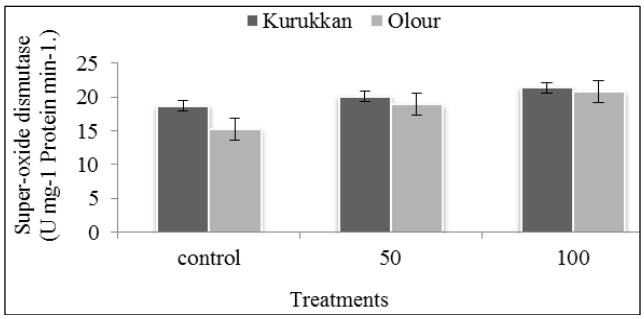

Fig 6: Effect of NaCl treatment (mM) on super-oxide dismutase (SOD) of rootstocks

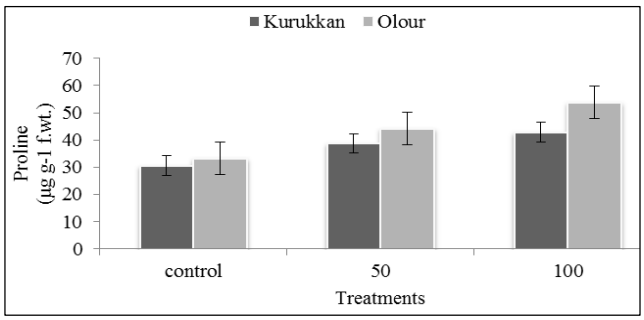

Fig 7: Effect of NaCl treatment (mM) on proline content of rootstocks

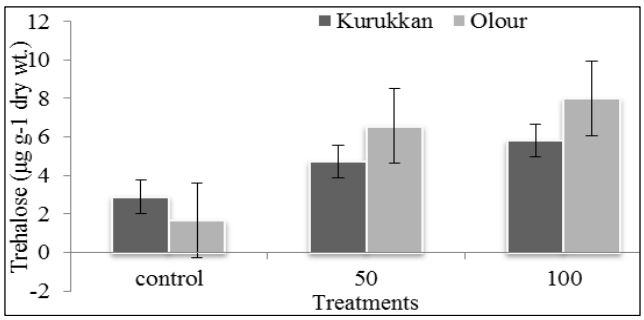

Fig 8: Effect of NaCl treatment (mM) on trehalose content of rootstocks

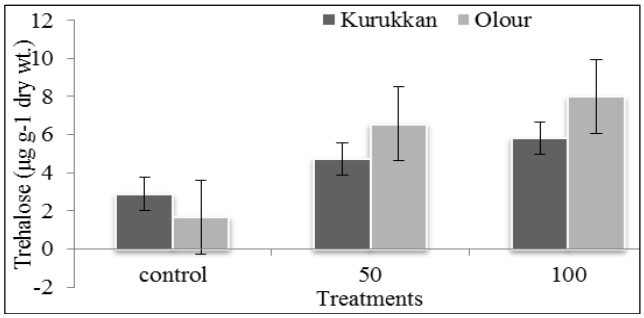

Fig 9: Effect of NaCl treatment (mM) on total phenol content of rootstocks

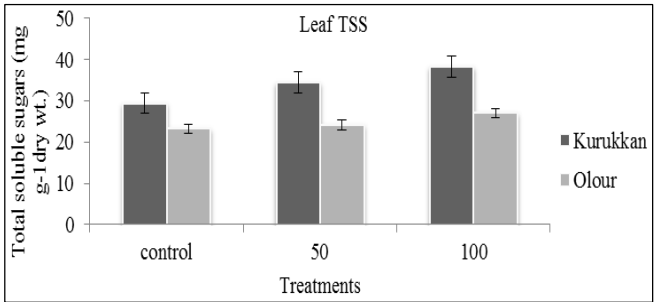

Fig 10: Effect of NaCl treatment (mM) on total soluble sugar (TSS) of rootstocks leaf

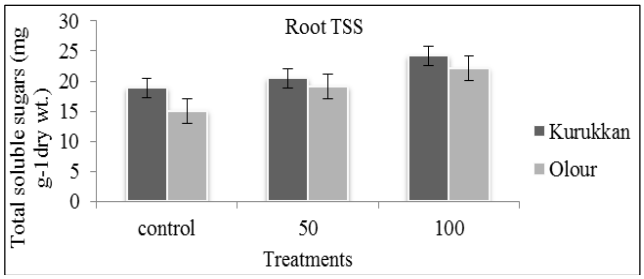

Fig 11: Effect of NaCl treatment (mM) on total soluble sugar (TSS) of rootstocks root

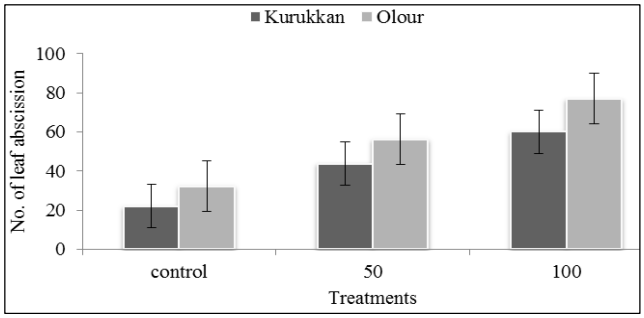

Fig 12: Effect of NaCl treatment (mM) on leaf abscission of rootstocks

Vertical bars (Fig. 1-12) indicate ± SE (n = 10). LSD ($P \leq 0.05$) values for the interactions between salinity and rootstock were MII = 0.96; *Pn* = 0.35; *Cs* = 0.004; POX = 0.02; CAT = 1.52; SOD = 0.29; Proline = 2.46; Trehalose = 0.71; Total phenol = 0.62; TSS leaf = 0.51; TSS root = 0.47 and Leaf abscission = 2.83

7. References

1. Adriano DC, Doner HE. Bromine, chlorine and fluorine. In: Page AL, Miller RH, Keeney DR. (Eds.). Methods of soil analysis part II. Chemical and biological properties. ASA and SSSA, Madison, 1982, 461-462
2. Ahmad P, Sharma S, Srivastava PS. *In vitro* selection of NaHCO3-tolerant cultivars of *Morus Alba* (Local and

Sujanpuri) in response to morphological and biochemical parameters. Horticultural Sci. 2007; 34:115-123.
3. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant and Soil. 1973; 39:205-207.
4. Biochemical and salt ion uptake responses of seven mango (*Mangifera indica* L.) rootstocks to NaCl stress, J Hort Sci & Biotech, 89(4), 367-372.
5. Brin M. Transketolase: clinical aspects (ed.), In: Colo wick SP. and Kaplan NO. (Eds.), Methods in Enzymology. 1966; 9:506-514.
6. Cavalcanti FR, Lima JP, Ferreira-Silva SL, Viégas RA, Silveira JA. Roots and leaves display contrasting oxidative response during salt stress and recovery in cowpea. J Plant Physiology. 2007; 164:591-600.
7. CSSRI. Central soil salinity research institute database, Karnal, India, 2016.
8. Chartzoulakis K, Loupassaki M, Bertaki M, Roulakis I. Effects of NaCl salinity on growth, ion content and CO2 assimilation rate of six olive cultivars. Scientia Hort. 2002; 96:235-247.
9. Deshmukh PS, Sairam RK, Kushwaha SR, Singh T, Chaudhary HB, Almeselmani M. Physiogenetic approaches for increasing wheat (*Triticum aestivum*) productivity under rice-wheat cropping system. Ind J Agri Sci. 2006; 76:667-669.
10. Deshmukh PS, Sairam RK, Shukla DS. Measurement of ion leakage as a screening technique for drought resistance in wheat genotypes. Ind J Plant Physiol. 1991; 34:89-91.
11. Dubey AK, Srivastav M, Singh R, Pandey RN, Deshmukh PS. Response of Mango (*Mangifera indica* L.) Genotypes to graded levels of salt stress. Ind J Agril Sci. 2006; 76(11):670-672.
12. El-bashiti T, Hamamci H, Oktem HA, Yucel M. Biochemical analysis of trehalose and its metabolizing enzymes in wheat under abiotic stress conditions. Plant Sci. 2005; 169:47-54.
13. FAO. Food and Agriculture Organization of the United Nations Database, 2010.
14. Ferreira JC, Paschodin VMF, Panek AD, Trugo LC. Comparison of three different methods for trehalose determination in yeast extracts. Fd Chem. 1997; 60:251-254.
15. Fridovich I. Superoxide dismutase. Ann Rev Biochem. 1975; 44:147-159.
16. Gaines TP, Parker BM, Gascho GL. Automated determination of chloride in soil and plant tissue by sodium nitrate extraction. Agron J, 1984; 76:371-374.
17. Gomez-Cadenas A, Tadeo FR, Primo-Millo E, Talon M. Involvement of abscisic acid and ethylene in the response of citrus seedlings to salt shock. Physiol Plant. 1998; 103:475-484
18. Hafez OM, Saleh MA, Ellil A, Kassabo M. Impact of ascorbic acid in salt tolerant of some mango rootstock seedlings. J Appl Sci Res. 2011; 7:1492-1500.
19. Kaya C, Kirnak H, Higgs D. Enhancement of growth and normal growth parameters by foliar application of potassium and phosphorus in tomato cultivars grown at high (NaCl) salinity. J Plant Nutr. 2001; 24:357-367.
20. Kishor A, Srivastav M, Dubey AK, Singh AK, Sairam RK, Pandey RN *et al*. Paclobutrazol minimizes the effects of salt stress in mango (*Mangifera indica* L.) J Hort. Sci. & Biotech. 2009; 84(4):459-465.
21. Ksouri R, Megidiche W, Debez A, Falleh H, Grignon C, Abdelly C. Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritima*. Plant Physiol Biochem. 2007; 45:244-249.
22. Luck H. Catalase estimation-In methods in enzymatic ananlysis, Ed. By Bergmeyer HU, Academic Press, New York. 1975; 2:885.
23. Maas EV**.** Salinity and citriculture. Tree Physiol. 1993; 12:195-216.
24. Malik CP, Singh MB. Plants enzymology and histoenzymology. Kalyani Publishers, New Delhi, 1980; 286.
25. Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 2002; 7:405-410.
26. Muller J, Boller T, Wiemken A. Trehalose and trehalase in plants: Recent developments. Plant Sci. 1995; 112:1-9.
27. Pandey P, Singh AK, Dubey AK, Awasthi OP. Effect of salinity stress on growth and nutrient uptake in polyembryonic mango rootstocks. Indian J Hort. 2014b; 71(1):28-34.
28. Pandey P, Singh AK, Dubey AK, Dahuja A. Biochemical and salt ion uptake responses of seven mango (*Mangifera indica* L.) rootstocks to NaCl stress. J Hort Sci & Biotech. 2014a; 89(4):367-372.
29. Sadasivam S, Manickam A. In: Biochemical Methods for Agricultural Sci. Wiley Eastern Limited, New Delhi, 1992, 11-12.
30. Sharma DK, Dubey AK, Srivastav M, Singh AK, Sairam RK, Pandey RN *et al*. Effect of putrescine and paclobutrazol on growth, physiochemical parameters, and nutrient acquisition of salt-sensitive citrus rootstock Karna khatta (*Citrus karna* Raf.) under NaCl stress. Plant Growth Regul. 2011; 30:301-311.
31. Srivastava M, Dubey AK, Singh AK, Singh R, Pandey RN, Deshmukh PS. Effect of salt stress on mortality, reduction in root growth and distribution of mineral nutrients in Kurukkan mango at nursery stage. Ind J Hort. 2009; 66(1):28-34.
32. Srivastav M, Kishor A, Dahuja A, Sharma RR. Effect of paclobutrazol and salinity onion leakage, proline content and activities of antioxidant enzymes in mango (*Mangifera indica* L.) Scientia Hort. 2010; 125:785-788.
33. Tatar O, Gevrek MN. Influence of water stress on proline accumulation, lipid peroxidation and water content of wheat. Asian J Plant Sci. 2008; 7:409-412.
34. Tharanathan RN, Yashoda HN, Prabha TN. Mango (*Mangifera indica* L.) The king of fruits- A review. Food Reviews International. 2006; 22:95-123.
35. Thomas RL, Jen JJ, Morr CV. Changes in soluble and bound peroxidase. IAA oxidase during tomato fruit development. J Fd Sci. 1981; 47:158-161.
36. Xue FF, Liu ZP, Mehta SK, Zhao GM. Protective role of calcium against NaCl toxicity in Jerusalem artichoke by up-regulation of antioxidant enzyme. Pedophere. 2008; 18:766-774.
37. Zhu JK. Plant salt stress. In: Encyclopedia of Life Sci. (ELS). Wiley, *Chichester*, 2007, 1-3.
38. Zuazo VHD, Raya AM, Ruiz JA. Salt tolerance of mango rootstocks (*Magnifera indica* L. cv. Osteen). Spanish J Agril Res. 2003; 1:67-78.