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Growth, lipid peroxidation, antioxidant enzymes and nutrient accumulation in Amrapali mango (*Mangifera indica* L) grafted on different rootstocks under NaCl stress

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Abstract

Ion (Na^+ and Cl^-) exclusion and upregulating antioxidant activities in mango scion cultivar through grafting on salt tolerant rootstocks could extend the mango cultivation in salt affected area with commercially viable production. Thus, the selection of rootstock-scion combinations with enhanced tolerance to salinity requires a better understanding of rootstock influence on ion exclusion and biochemical changes. To achieve this goal, two polyembryonic, Olour and Kurakkan and one monoembryonic *viz*., non-descript seedling (common mango rootstock) grafted with the scion Amrapali were tested under NaCl stress. Grafted plants were irrigated with water containing 0.0 or 50 mM NaCl at four days interval for 90 days. In the tested rootstock-scion combinations, minimum reduction in plant height and leaf numbers under salinised condition was found in graft with non-descript seedlings and Olour rootstocks, respectively. However, malondialdehyde (MDA) content and catalase (CAT) activity were similar in all rootstock-scion combinations. The higher peroxidase activity (213.43 Ab_{560} U g^{-1} leaf fresh weight) and proline accumulation (215.98 µg g-1 of fresh weight) was observed in plants grafted with Olour rootstock. Graft with non-descript seedlings had reduced accumulation of Na^+ in leaf concentration while graft Olour inhibited the accumulation of leaf Cl^- concentration in the presence of NaCl. Olour seems to be good Cl^- excluder rootstocks while non-descript seedling could effectively exclude Na^+ from leaf tissues of scion cultivar.

Keywords: Glutathione reductase, Nutrients accumulation, Peroxidase, Rootstock, Salinity.
Abbreviations: CAT_Catalase, GR_Glutathion reductase MDA_Malondialdehyde, POX_ Peroxidase, SOD_ Superoxide dismutase.

Introduction

Mango is an important fruit crop, widely grown in subtropical and tropical regions, which experience various soil problems in general, and salinity in particular. About 7% of the world's and 20% of the irrigated land areas are affected by soil salinity (Yamaguchi and Blumwald 2005). Mango is considered to be a salt sensitive crop (Mass 1986) causing scorching of leaf tips and margins and leaf curling (Jindal et al., 1976) resulted inhibition of seedling growth (Dubey et al., 2006; Srivastav et al., 2007). Salinity also reduces chlorophyll contents, CO_2 assimilation and nutrient uptake (Schmutz and Ludders, 1999; Dubey et al., 2006), and ion homeostasis (Duran Zuazo et al., 2004). One of the biochemical changes that could occur in plants under salt stress is the production of reactive oxygen species (ROS), having detrimental effects on normal metabolism through oxidative damage to lipids, proteins, and nucleic acids (Mittler, 2002). The balance between production and removal of ROS is controlled by cellular osmo-protectants (Bohnert and Jensen, 1996) and antioxidant enzyme systems (Apel and Hirt, 2004). In order to prevent oxidative damages, plants evolve complex antioxidant system, including both enzymatic (scavenger enzymes) and non-enzymatic (mainly ascorbate and glutathione) components differentially found in cell compartments (Mittler, 2002). Enzymatic components include superoxide dismutase (SOD) which catalyses the dismutation of superoxide anions to dioxygen and hydrogen peroxide (H_2O_2). HO may be scavenged by a variety of peroxidases including CAT and APX. Therefore, technologies to improve antioxidant enzyme activities and regulate translocation and accumulation of ions under salt stress conditions in mango are of beneficial use. The salt-treated seedlings of mango rootstock Olour have shown higher SOD, CAT and POX activities than untreated plants (Srivastav et al., 2010). For plant growth sodium ions are not essential but sodium content in irrigation water or soil above certain critical level is toxic to plants induces cellular damage and affect plant growth and development (Zhu 2007). Irrigation with saline water has been reported to increase Na^+ content in leaf of Sukkary and Zebda rootstocks of mango with plant ageing (Hafez et al., 2011). Kadman et al. (1976) and Gazit and Kadman (1983) had demonstrated tolerance to saline water in the rootstocks 13/1 and Gomera-1, respectively. Dubey et al. (2006), Dubey et al. (2007) and Srivastav et al. (2009) studied salt tolerance limit of different polyembryonic mango rootstocks and reported that Olour and Kurakkan were tolerant to salt stress. However, the mechanisms of salt tolerance associated with the exclusion of Na^+ and Cl^- ions from leaves involve both the uptake selectivity in roots and also resistance in transferring these ions to the shoot (Maathuis and Amtmann 1999) thus minimize ionic toxicity in leaf tissues of rootstocks of citrus (García-Sánchez et al., 2002), rose (Wahome et al., 2001),

and grape (Paranychianakis and Angelakis, 2008) and are well known mechanism for developing salt stress resistance. In mango cv Turpentine, strongest reduction in CO_2 assimilation and transpiration and with highest leaf Cl^- accumulation have been reported, while grafted on '13-1' rootstock (Schmutz and Ludders (1999). The mechanisms of resistance against salinity in grafted plants display a great complexity which may be associated with specific interactions between the genotypes of scion and rootstock. Although the exclusion capacity of some mango rootstocks is known to be associated with certain physiological characteristics favourable to salinity resistance (Schmutz and Ludders 1999; Durán Zuazo et al., 2004); biochemical and physiological mechanisms involved with the interaction between genotypes of scion and rootstock are still poorly understood. In the present studies, exposure of Amrapali scion grafted on two salt tolerant polyembryonic rootstocks and one non-descript monoembryonic seedling rootstock (widely used rootstock) was investigated under NaCl stress. Growth, nutrient accumulation, and up regulation of antioxidant system and proline accumulation were the focus of the current studies.

Result

Effect of rootstocks on growth parameters

The data relating to plant growth of mango grafts as influenced by rootstocks under NaCl stress are given in Table 1. Prior to impose the stress, the plant height of tested grafts was statistically similar, however, it was significantly reduced 90DAS under saline conditions, maintaining its highest value with Olour followed by Kurukkan and non-descript rootstocks in comparison to control (non-saline). Compared with respective non-salinised controls, maximum inhibition in plant height was recorded in graft with Kurakkan (24.20%). Furthermore, graft with non-descript seedlings had higher leaves/plant under saline condition which was not differed significantly with Olour. However, graft with Kurakkan had the lowest number of leaves per plant. Scorching of leaf margin of Amrapali scion was visually noticed first on Kurakkan rootstock. Compared with respective non-salinised controls, higher reduction in leaves (36.60%) was found in graft with Kurakkan and least reduction (4.66%) was therein graft with Olour under NaCl stress. Dry weight of shoot and root was also influenced significantly by salinity, rootstocks and their interactions (Table 1). With the imposition of NaCl stress, Amrapali grafts on Olour had the highest root weight which was statistically similar with non-descript rootstocks. Olour contributed highest shoot dry weight even under non-saline conditions too. Furthermore compared with respective non-salinised plants, the maximum reduction in shoot dry weight (42.82%) was found in graft with Kurakkan. However, in the presence of NaCl, graft with Olour had higher root dry weight which did not differ significantly with graft onto non-descript seedling rootstock. Compared with the graft grown in absence of NaCl, the maximum reduction in root dry weight (38.35%) was found in graft with non-descript seedling and the minimum was there in graft with Olour (21.97%) which was non-significantly different with Kurakkan (23.65%).

Effect of rootstocks on antioxidant enzymes activities

Salinity, rootstock and their interections influenced POX , SOD, CAT, and GR activities significantly. In the presence of NaCl, graft with Olour had significantly higher POX activity followed by graft onto Kurakkan and non-descript seedling rootstock (Fig. 1A). Compared with the respective non-salinised controls, highest increase in POX activity (129.63%) was recorded in graft with kurakkan followed by Olour (118.74%). All the rootstock-scion combinations had almost equal SOD activity under non-salinised condition (Fig. 1B). Notwithstanding significantly higher SOD activities was recorded in grafts with non-descript seedling rootstock which was statistically similar with graft onto Kurakkan in the presence of NaCl salt. The lowest activity of SOD was found therein graft with Olour. Compared with the respective non-salinised controls, the highest reduction in SOD activity was recorded in graft with Olour (13.17%) under salinised condition. In the presence of salt, rootstock failed to exert any significant influence on CAT activity (Fig. 1C). It decreased irrespective of rootstocks under salt stress conditions as compared to non-salinised condition. Neverthless higher reduction in CAT activity under salinised condition was recorded in graft with non-descript seedling rootstock (56.57%) followed by graft with Kurakkan (46.20%) as compared to their respective controls. Significantly higher GR activity was recorded in graft with Kurakkan and minimum in graft with non-descript seedling rootstock in the presence of NaCl. However, higher upregulation compared to non-salinised respective controls, was recorded in leaves of Amrapali grafted on Kurakkan rootstock (120.752%) and Olour (91.42%) under salinised condition (Fig. 1D).

Effect of rootstocks on MDA content

Interaction between salinity and rootstock indicated that under both the conditions, all rootstock-scion combinations had almost equal MDA content but higher under NaCl stress (Fig. 2A). However, in comparision with respective non-salinised plants, graft with Kurakkan showed highest increase in MDA content (22.43%) under imposed saline condition.

Effect of rootstocks on proline accumulation

Salinity, rootstock and their interaction influenced proline content significantly (Fig, 2B). The maximum proline content was recorded in graft with Olour followed by non-descript seedling rootstock in the presence of salt the. The higher increase in proline content under salinised condition was recorded in graft with Olour rootstock (79.12%) as compared to respective control and the minimum increase was found in graft with Kurakkan (33.00%) as compared to their respective controls.

Effect of rootstocks on nutrients accumulation

Salinity, rootstock and their interections influenced tissues Na^+ concentration significantly (Table 2). Na^+ concentration increased significantly under NaCl stress in all the rootstock-scion combinations as compared to respective non-salinised controls. For root and stem Na^+ concentrations, all the rootstock-scion combinations had similar Na^+ concnetrations under either of the conditions except stem Na^+ concentration under non-salinised conditon in which graft with non-descript seedling rootstock had significantly higher Na^+ concentration in stem tissues. Compared to the respective non-salinised controls, the maximum incease in root Na^+ (53.10%) was recorded in graft with non-descript rootstock. While for stem Na^+ concentration, graft either with Olour or Kurakkan had

Table 1 Effect of NaCl stress on plant height, numbers of leaf, and dry weight of shoot and roots of Amrapali mango grafted on three rootstocks 20 days prior and 90 days after imposing NaCl stress.

Treatments	Plant height (cm) (20 DPS)	Plant height (cm) (90 DAS)	Initial numbers of leaves (20 DPS)	Final Numbers of leaves (90 DAS)	Dry weight (g)	
					Shoot	Root
Non-saline						
Non-descript	36.18 ± 3.15^{a}	43.39 ±3.18^{bc}	17.00 ± 0.70^{a}	18.80 ± 0.71^{a}	9.09 ± 0.44^{a}	6.70 ± 0.65^{a}
Olour	39.04 ± 4.54^{a}	50.57 ± 4.42^{a}	9.60 ± 0.23^{cb}	15.22 ± 0.12^{b}	10.45 ± 0.44^{a}	5.87 ± 0.35^{ba}
Kurukkan	37.61 ± 4.68^{a}	43.87 ± 2.97^{b}	7.00 ± 0.36^{c}	15.14 ± 0.83^{b}	9.85 ± 0.39^{a}	3.72 ± 0.45^{dc}
Saline						
Non-descript	34.36 ± 4.84^{a}	37.54 ± 1.86^{d}	19.80 ± 0.56^{a}	15.15±0.76^{b}	8.62 ± 0.92^{a}	4.13 ± 0.51^{dc}
Olour	36.99 ± 3.77^{a}	41.67 ± 5.19^{c}	12.00 ± 0.66^{b}	14.51±0.51^{b}	9.71 ± 0.70^{a}	4.58 ± 0.57^{bc}
Kurukkan	35.17 ± 3.24^{a}	39.90 ± 2.85^{e}	9.00 ± 0.26^{cb}	9.60±0.11^{c}	5.63 ± 0.94^{b}	2.84 ± 0.27^{d}
LSD ($P \leq 0.05$)						
NaCl (S)	NS	1.42	1.34	0.87	0.76	0.56
Rootstock (R)	1.87	1.75	1.64	1.06	0.93	0.69
S X R	NS	3.73	3.49	2.26	1.78	1.47

Each data represent the mean value of ten samples ± standard deviation. Means with the same letters are not significantly differed at $P \leq 0.05$ (Tukey's honest significance test). DPS; Days prior to salinisation, DAIS; days after salinisation.

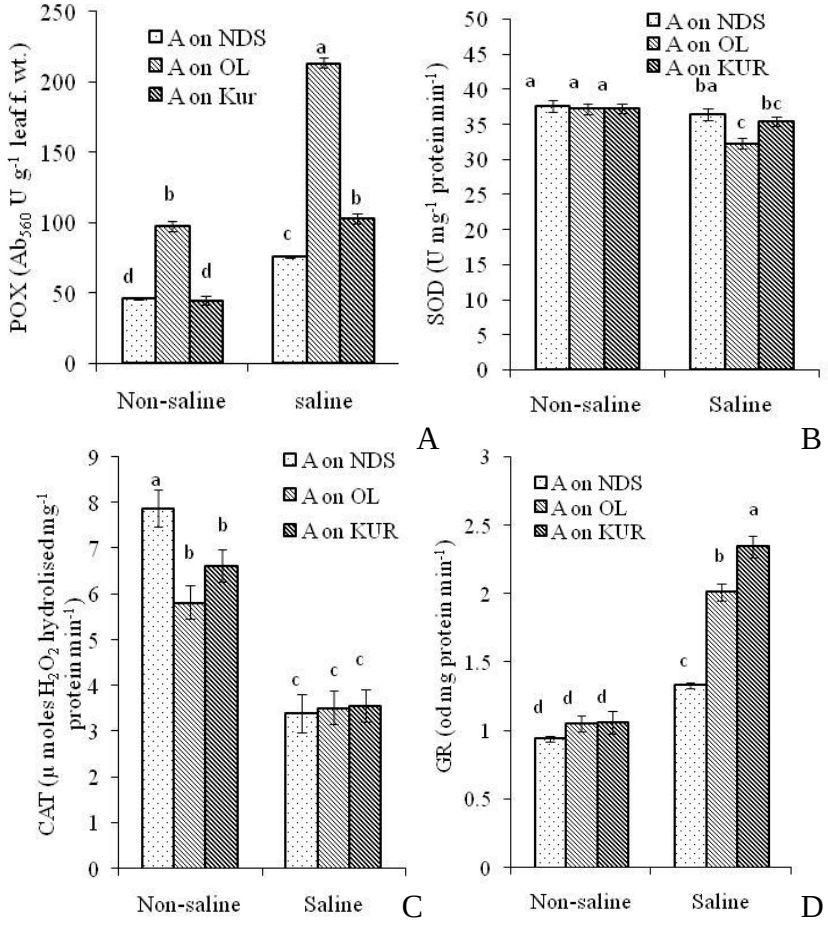

Fig 1. Effect of NaCl stress on leaf peroxidase (POX) (A), superoxide dismutase (SOD) (B), catalase (CAT) (C) and glutathione reductase (GR) (D) enzymes activities in leaves of Amrapali mango grafted on three rootstocks. Vertical bar represent mean of ten samples ± SD. Means with the same letters are not significantly differed at $P \leq 0.05$ (Tukey's honest significance test).
A; Amrapali, NDS; non-descript seedling, OL; Olour (polyembryonic), KUR; Kurakkan (polyembryonic).

Table 2 Effect of NaCl stress on tissues Na and Cl concentrations in roots, leaves, and stems of Amrapali mango grafted on three rootstocks 90 days after imposing NaCl stress.

Treatments	Na (%)			Cl (%)		
	Root	Stem	Leaf	Root	Stem	Leaf
Non-saline						
Non-descript	0.22 ± 0.01^{b}	0.23 ± 0.01^{b}	0.15 ± 0.01^{c}	1.25 ± 0.02^{c}	1.34 ± 0.05^{c}	1.44 ± 0.03^{d}
Olour	0.23 ± 0.01^{b}	0.20 ± 0.01^{c}	0.14 ± 0.01^{c}	1.12 ± 0.05^{d}	1.08 ± 0.05^{d}	1.11 ± 0.06^{e}
Kurukkan	0.25 ± 0.01^{b}	0.20 ± 0.01^{c}	0.15 ± 0.01^{c}	0.82 ± 0.06^{e}	0.83 ± 0.07^{e}	1.09 ± 0.05^{e}
Saline						
Non-descript	0.35 ± 0.02^{a}	0.26 ± 0.02^{a}	0.23 ± 0.01^{b}	1.40 ± 0.02^{b}	1.59 ± 0.06^{b}	2.00 ± 0.12^{b}
Olour	0.33 ± 0.01^{a}	0.28 ± 0.02^{a}	0.25 ± 0.02^{ba}	1.66 ± 0.04^{a}	1.63 ± 0.07^{b}	1.62 ± 0.07^{c}
Kurukkan	0.34 ± 0.02^{a}	0.28 ± 0.02^{a}	0.26 ± 0.01^{a}	1.56 ± 0.06^{a}	1.83 ± 0.05^{a}	2.44 ± 0.05^{a}
LSD ($P \leq 0.05$)						
NaCl (S)	0.01	0.01	0.01	0.04	0.05	0.06
Rootstock (R)	0.01	0.01	0.01	0.05	0.06	0.08
S X R	0.03	0.02	0.02	0.10	0.12	0.17

Each data represent the mean value of ten samples ± standard deviation. Means with the same letters are not significantly differed at $P \leq 0.05$ (Tukey's honest significance test).

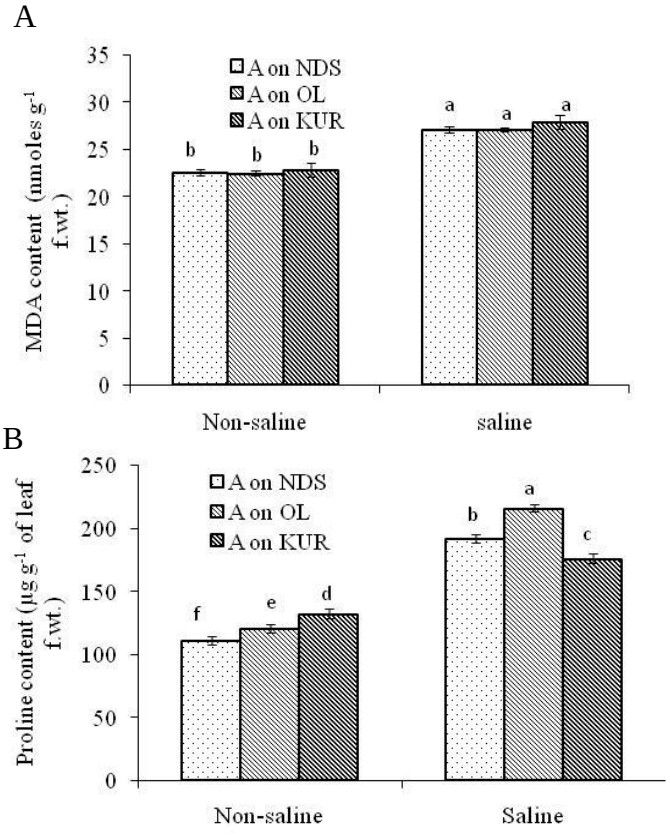

Fig 2. Effect of NaCl stress on MDA (A) and proline contents (B) in leaves of Amrapali mango grafted on three rootstocks. Vertical bar represent mean of ten samples ± SD. Means with the same letters are not significantly differed at $P \leq 0.05$ (Tukey's honest significance test). A; Amrapali, NDS; non-descript seedling, OL; Olour (polyembryonic), KUR; Kurakkan (polyembryonic)

increased maximum concentration (40.0%). Neverthless, higher increased in leaf Na^+ concentration (78.60%) was recorded in graft with Olour followed by graft with Kurakkan (73.33%) as compared to respective non-salinised controls. The minimum increase (53.33%) in leaf Na^+ concentration was recorded in graft with non-descript rootstock. Leaf Cl^- concentration increased significantly in all the rootstock-scion combinations under salinised condition (Table 2). Notwithstanding significantly higher leaf Cl^- , under salinised condition, was recorded in graft with Kurakkan and minimum in graft with Olour. Compared with the respective non-salinised controls, maximum increased in leaf Cl^- (123.85%) was also recorded in graft with Kurakkan and minimum increase (19.11%) was found in graft with Olour under salinised condition. The maximum increase in root Cl^- (90.24%) and stem Cl^- (120.50%) was recorded in graft with Kurakkan under salinised condition as compared to respective non-salinised controls. Salinity, rootstock and their interactions influenced tissues K^+ concentration significantly (Table 3). Root and stem K^+ concentrations decreased significantly under NaCl stress in all the rootstock-scion combinations as compared to respective non-salinised controls while, leaf K^+ concentration decreased significantly only in graft with Kurakkan under saline condtion. In the presence of NaCl, leaf K^+ concnetration was not affected significantly in other graft combinations. Compared with non-salinised control plants, leaf Ca^{2+} concentration did not differ significantly in any of the rootstock-scion combinations under salinised condition (Table 3). Under salinised condition, graft with non-descript seedling rootstock had higher leaf Ca^{2+} concentration which was non significantly different with graft onto Kurakkan. Compared with the respective non-salinised controls, maximum decrease in leaf Ca^{2+} (18.18%) was recorded in graft with Kurakkan and minimum decrease (7.42%) was found in graft with non-descript seedling rootstock under salinised condition. Significantly lower root Ca^{2+} concentration in graft with non-descript seedling rootstock was recorded under salinised condition while no effect on root Ca^{2+} was noticed in other graft combinations. Furthermore, stem Ca^{2+} concentration decreased significantly in graft with non-descript and Olour rootstock under salinised condition.

Discussion

We have previously described that polyembryonic rootstocks Olour and Kurakkan are salt tolerant (Dubey et al., 2006, Dubey et al., 2007, Srivastav et al., 2009). Keeping in view the previous work, the relative ability of these salt tolerant polyembryonic rootstocks was tested during course of present studies with non-descript seedling rootstock graft with Amrapali scion under saline irrigation. Under imposed NaCl stress, Olour and non-descript seedling rootstocks exhibited the more salt tolerance than Kurakkan by showing lowest

Table 3. Effect of NaCl stress on tissues K and Ca concentrations in roots, leaves, and stems of Amrapali mango grafted on three rootstocks 90 days after imposing NaCl stress.

Treatments	K (%)			Ca (%)		
	Root	Stem	Leaf	Root	Stem	Leaf
Non-saline						
Non-descript	0.22 ± 0.02^{b}	0.32 ± 0.02^{a}	0.20 ± 0.01^{bc}	8.37 ± 0.30^{a}	7.04 ± 0.31^{a}	4.58 ± 0.27^{a}
Olour	0.24 ± 0.02^{ba}	0.29 ± 0.02^{b}	0.22 ± 0.01^{ba}	8.48 ± 0.23^{a}	6.80 ± 0.23^{ba}	3.05 ± 0.18^{dc}
Kurukkan	0.26 ± 0.01^{a}	0.32 ± 0.01^{a}	0.24 ± 0.02^{a}	7.58 ± 0.21^{ba}	6.35 ± 0.19^{bac}	4.29 ± 0.30^{ba}
Saline						
Non-descript	0.17 ± 0.01^{d}	0.23 ± 0.02^{c}	0.19 ± 0.01^{c}	7.13 ± 0.10^{b}	6.12 ± 0.23^{bc}	4.24 ± 0.21^{ba}
Olour	0.21 ± 0.01^{bc}	0.20 ± 0.01^{c}	0.19 ± 0.01^{bc}	7.65 ± 0.36^{ba}	5.52 ± 0.15^{c}	2.67 ± 0.28^{d}
Kurukkan	0.19 ± 0.02^{dc}	0.22 ± 0.01^{c}	0.18 ± 0.01^{c}	7.44 ± 0.21^{ba}	7.04 ± 0.52^{a}	3.51 ± 0.47^{bc}
LSD ($P \leq 0.05$)						
NaCl (S)	0.01	0.01	0.01	0.41	0.32	0.32
Rootstock (R)	0.02	0.01	0.01	0.50	0.39	0.39
S X R	0.03	0.03	0.03	1.06	0.85	0.83

Each data represent the mean value of ten samples ± standard deviation. Means with the same letters are not significantly differed at $P \leq 0.05$ (Tukey's honest significance test).

growth inhibition in plant height, leaf numbers and shoot dry weight of Amrapali plants tested in the present study. Beside pronounced growth inhibition of scion, first symptom of leaf burning, relatively more defoliation, and scorching was also observed in graft onto Kurakkan indicated that increase in leaf burning and defoliation was associated with leaf Cl^- build up rather than Na^+ accumulation as Amrapali onto Kurakkan transport more Cl^- in leaves. The growth reduction in mango grafts due to higher Cl^- uptake rather than limited exclusion of Na^+ has also been reported by Schmutz and Ludders (1993). Lower inhibition of growth and defoliation on Olour and/or non-descript seedling rootstock might be associated with lower leaf Cl^- concentration, higher proline content, and more upregulated POX activity under stress in leaves of Amrapali grafted onto these rootstocks. GR, APX, CAT, POX and SOD are involved in the scavenging of the products of oxidative stress, such as hydrogen peroxide generated in the chloroplast (Kraus et al., 1995; Jagtap and Bhargava, 1995) and thus help in ameliorating the adverse effects of oxidative stress. Superoxide is scavenged by SOD to produce H_2O_2, which is subsequently eliminated mainly by APX and CAT (Gueta-Dahais et al., 1997). But in the present studies, SOD and CAT activities decreased in leaves of Amrapali grafted either onto Olour or Kurakkan rootstocks under NaCl stress but no change in SOD activity was found in graft with non-descript rootstock, however, CAT activity decreased to the maximum extent with this rootstock. Contrary to SOD and CAT activities, POX and GR activities showed the increase in salt treated Amrapali plants on all the rootstocks and increase of GR was high on Kurakkan and Olour rootstock, while POX increased maximum on Olour. Similarly Almansa et al. (2002) also reported decrease in SOD activity in *Citrus limonum* leaves on *C. reticulata* and *C. microphylla* rootstocks. In present studies, POX activity increased under salinised condition in all the rootstock scion combinations, however, higher activity of POX in graft onto Olour indicated that these cells had higher capacity for catalyzing $2H_2O_2 \Rightarrow 2H_2O$ and O_2 as envisaged by minimum inhibition in growth and least defoliation. These are in agreement with the findings of Quiroga et al. (2000); Lee et al. (2001); Cherian and Reddy (2003); Madhvi et al. (2004); Gratao et al. (2005) who had also suggested its role in plant tolerance. Glutathione reductase plays an important role in cell defense against reactive oxygen plant metabolism by sustaining reduced status of an important antioxidant glutathione (Seo et al., 2006). Our results indicated an increase in GR activity under NaCl stress in all rootstock-scion combinations corroborates with the findings of Koca et al. (2007) in *Sesamum indicum*. Increase in the glutathione reductase activity in plants resulted in accumulation of glutathione (GSH) levels and ultimately confers the tolerance in plants. This is well correlated in the present study with the higher increase in the activity of glutathione reductase (GR) in leaves of Amrapali grafted on Kurakkan and Olour rootstocks. MDA is the result of lipid peroxidation and its content has been often used as an indicator of the extent of lipid peroxidation (Zhang et al., 2008; Zhu et al., 2008). In the present study too higher lipid peroxidation measured as MDA content was observed in NaCl stressed plants irrespective of rootstocks studied. Nevertheless, all rootstock-scion combinations had similar MDA content in the leaves of Amrapali, but foliar scorching, defoliation and growth inhibition was more pronounced in the plants grafted on Kurakkan indicating ion toxicity and nutritional imbalance might be more injurious rather than oxidative damage in mango. Proline is the key osmolytes contributing towards osmotic adjustment (Yoshiba et al., 1997; Mundree et al., 2002). In the present studies, leaf proline content increased under NaCl stress in all rootstock-scion combinations. Nevertheless this increase was relatively much higher in grafts either with Olour or non-descript seedling rootstock than Kurakkan suggesting more stress tolerance of former rootstocks. In organisms ranging from bacteria to higher plants, the strong correlation exists between increased cellular proline levels and the capacity to survive under drought and salt stress (Ahmad and Jhon, 2005). Higher level of proline in the leaves of Amrapali grafted onto Olour and /or non-descript rootstock might be the possible salt tolerance in mango. Salt tolerance has been associated with the ability to restrict the uptake and/or translocation of Na^+ and Cl^- ions from the roots to shoot. We observed decrease in transport of Cl^- and Na^+ from the roots of Olour to scion of Amrapali and decreased Na^+ transport from roots of Kurakkan and non-descript seedling rootstocks to the scion of Amrapali. Since both Na^+ and Cl^- concentration was higher in roots of Olour and only Na^+ concentration was higher in roots of Kurakkan and non-descript rootstocks. It could be inferred that Kurakkan and non-descript seedling rootstocks were not able to restrict transport of Cl^- from roots to leaves of Amrapali as Cl^- concentration in leaves, was higher in this graft combination than roots. This suggested that Cl^- exclusion mechanism exist in Olour rootstock. The existence of similar mechanism during the salt stress has also been reported in Olive (Tattini et al., 1995) and Rangpur lime (Mass, 1993).

A decrease in K^+ concentration in salt treated plants is likely an important growth limiting factor (Marschner, 1986) because this element plays an essential role in many plant processes. In our study, NaCl treated plants had decreased leaf K^+ concentration in the leaves of Amrapali while grafted on Kurakkan, however, no change in leaf K^+ concentration was observed with non-descript seedling or Olour rootstock. In graft onto non-descript seedling rootstock; the decrease in K^+ concentration in roots may be attributed to an exchange between Na^+ and K^+ in the basal stem and further release of K^+ into xylem. Similar findings were also reported by Walker, (1986) in citrus. The maximum and minimum reduction in leaf Ca^{2+} were found in graft with Kurakkan and non-descript seedling rootstocks. However, no change in Ca^{2+} concentration in root tissues in graft either onto Kurakkan or Olour was observed. Although all rootstock scion-combinations had higher Ca^{2+} in root tissues than leaf indicates that Ca^{2+} translocation was inhibited in all combinations. Further, variation in Ca^{2+} concentration in different plant parts on various rootstocks indicates that Ca^{2+}uptake dependent on rootstock in mango. Similar findings were also reported in other plant species (Ruiz et al., 1997; Mass and Grieve, 1987) under NaCl stress.

Materials and Methods

Plant materials

One-and-half year old Amrapali plants grafted onto two polyembryonic rootstock (Olour and Kurakkan) and one monoembryonic non-descript seedlings were used in this studies. These grafted plants were then shifted in plastic pots (30 cm diameter) each containing 8.0 kg of a 1:1:1 (w/w) mixture of soil, sand and well rotted farm yard manure (FYM). The garden soil had $EC_{(1:2)}$ of 0.15 dS m^{-1}, $pH_{(1:2)}$ of 7.14, cation exchange capacity (CEC) 10.72 cmol kg^{-1}, and organic carbon content 4.8 g kg^{-1}. One month after transplanting, 20 g mixture of urea, single super phosphate and potassium sulphate in the ratio of 1:2:1 was applied to each pot. The experiments were conducted during 2010-2012 in polyhouse under the following conditions: day temperature, 24-31^{o}C; night temperature, 14-19^{o}C; relative humidity (RH), 65-95%; and a photoperiod of 6-9 hours.

Saline treatment

Grafted plants of Amrapali were irrigated for 60 days with tap water until the beginning of the experiments with NaCl. The plants were then irrigated with tap water or water containing 50 mM NaCl at four days intervals after considering the moisture loss as measured by direct weighing of the pots. The treatments were maintained for 90 days.

Growth parameters

Plant heights and the numbers of leaves per plant were recorded 20 days prior to the start of saline treatment and at the end of the experiment. After 90 days of salinisation, all plants were uprooted and washed carefully in running tap water and then 0.1 N HCl, followed by two washing with distilled water and then blotted on absorbent paper to remove surface moisture. Thereafter, the plants were placed in a hot air oven at 60 ^{0}C ± 1 ^{0}C for 36 h until constant weight to record the dry weights (DWs) of shoot (stem plus leaf) and roots.

Biochemical parameters

Enzyme extractions and assays

The leaf samples from each treatment were collected freshly in ice box 50 days after salt treatments and washed immediately with tap water followed by distilled water. For protein enzymes extractions, leaf (1.0 g) were homogenized in pre-chilled mortar and pestle by adding 5 ml chilled phosphate buffer (50 mM; pH 7.0) and the homogenate was centrifuged at 15,000 x g for 20 minutes at 4^{o}C. The supernatant so obtained was sieved through two layers of muslin cloth and stored in refrigerator which was used as extract for the estimation of following antioxidant enzymes. The supernatants were used for the determination of protein content and activity of SOD, CAT, POX and GR enzymes. Soluble protein content of the samples was estimated according to Lowry et al., 1951 using bovine serum albumin egg standard. The assay is based on the biuret reaction of protein with alkaline cupric tartarate, forming Cu^{2+}- protein complex. Superoxide dismutase (SOD) activity in leaf sample was determined 50 days after salinization according to Fridovich (1975). The assay is based on the ability of SOD to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) at 560 nm. SOD activity was expressed in units mg^{-1} protein min^{-1}. Catalase (CAT) activity was determined according to the method of Luck (1965). The assay is based on the estimation of residual hydrogen peroxide (H_2O_2) by oxidation with potassium permanganate ($KMnO_4$) by titration. The catalase activity was expressed as µ moles H_2O_2 hydrolyzed mg^{-1} protein min^{-1}. Peroxidase (POX) activity in leaf was assayed by the method of Thomas et al. (1981). The enzyme activity was expressed in A_{436} units min^{-1} g^{-1} fresh leaf weight. Observed change in absorbance at 436 nm converted into micromoles hydrogen peroxide decomposed using an extinction coefficient of 25.5 per mM per cm. Glutathion reductase (GR) activity was determined according to Smith et al. (1988). Observed change in absorbance at 412 nm were converted into millimoles of DTNB reduced using extinction coefficient of 14.15 per mM per cm.

Malondialdehyde (MDA) determination

The lipid peroxidation level was determined 50 days after salinization in terms of malondialdehyde (MDA) content according to Dhindsa et al., 1981 except for minor modifications during homogenization. The malondialdehyde (MDA) content was calculated using its extinction coefficient 155 mM^{-1} cm^{-1} (Heath and Packer, 1968).

Proline analysis

The proline content in matured leaves was estimated by rapid colorimetric method as suggested by Bates et al. (1973) 50 days after salinization using analytical grade proline (SRL, Chem Co., Mumbai, India) as a standard. The chromophore-containing toluene layer (light pink) was aspirated from the aqueous phase and its absorbance was read at 520 nm using spectrophotometer, (UVD-3200 Labomed, Inc., Culver city, CA, USA) with toluene as a blank. The proline concentration in the samples was determined from a standarad curve prepared by using analytical grade proline (SRL, Chem Co. Mumbai) and calculated on fresh weight basis according to the standard formulae.

Tissue nutrient analysis

The total Na, K and Ca contents of plant leaves, stem and roots were estimated in diacid (9:4; HNO_3:$HClO_4$ v/v) digested plant sample by using a microprocessor-based flame photometer (Flame Photometer-128, Systronics India,Ahemdabad, India) according to Jackson (1980). However, Cl^- extraction from plant leaves, stem and roots was done using 0.1 M sodium nitrate in 1:100 (w/v) ratios (Gaines et al., 1984) and was quantified by mercuric (II) thiocyanate method as suggested by Adriano and Doner (1982).

Statistical analysis

The experiment was conducted in a factorial complete randomized design (FCRD) with ten replications. Data were analyzed using the SAS (9.2) package (SAS institute INC. Cary, NC, USA) to calculate F values followed by Tukey's honest significance test. P values ≤ 0.05 were considered as significant.

Conclusion

The graft combination tested in the present studies suggested that lipid peroxidation in Amrapali leaves appeared to be scion dependent. Moreover, difference in GR and peroxidase activities in different rootstock scion combinations indicated the significant role in upregulating GR and peroxidase activities. The salt tolerance has been observed to be associated with the ability to restrict the uptake and/or translocation of Na^+ and Cl^- ions from the roots to shoot. We observed a decrease in transport of Cl^- and Na^+ from the roots of Olour to scion of Amrapali and decreased Na^+ transport from the roots of Kurakkan and non-descript seedling rootstocks to the scion of Amrapali suggesting existence of Cl^- exclusion mechanism exist in Olour rootstock. It could also be said that in mango, ion toxicity is more injurious than oxidative damage as MDA contents in all rootstocks was found almost equal but more injury symptoms were noticed on Kurakkan rootstocks where Cl^- build was higher. Based on the overall performance and leaf scorching, it could be said that Olour might be used as mango rootstocks in areas where irrigation water contains NaCl salts.

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