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Effect of Salt Stress on Antioxidant Enzyme Activities and Nutrient Ion Concentrations in Mango Rootstocks

P. Deivasigamani[1*], R.M. Vijayakumar [1] and R. Sivakumar[2]
[1]Department of Fruit Crops,
Tamil Nadu Agricultural University, Coimbatore
[2]Regional Research Station, Paiyur

A pot culture experiment was conducted during 2015 – 2016 at the College Orchard, TNAU, Coimbatore for identification of salt-tolerant rootstocks in mango. The experiment was carried out with two factors (rootstocks and salinity levels) under Completely Randomized Design and replicated thrice. The mango rootstocks *viz*., Alphonso, Banganapalli, Sendhuram, Neelum and Bangalora were used and three different levels of salt concentration (25 mM NaCl, 50 mM NaCl and 75 mM NaCl) along with control were imposed. The salinity levels were imposed to the seedlings grown in polybags up to 90 days at an interval of 2 -3 days depending upon moisture availability. Alphonso rootstock registered the highest values of catalase and peroxidase (10.03 µg H_2O_2 g^{-1} min^{-1} and 1.34 change in OD at 430 nm min^{-1} g^{-1} respectively) followed by Neelum at 75 mM NaCl salinity level. Higher accumulation of K^+ ions in leaf (0.72 %) and root (1.21 %) were registered by Alphonso rootstock at 75 mM NaCl. The accumulation of Na^+ ions which is supposed to be harmful, was found to be more in leaf (0.39 %) and root (0.41 %) tissues of Bangalora rootstocks at 75 mM NaCl whereas the same was found to be the least in leaf (0.23 %) and root (0.25 %) tissues of Alphonso rootstocks. Based on the antioxidant enzyme activities and nutrient ion concentrations it is concluded that Alphonso rootstocks were found to be the best among the rootstocks studied followed by Neelum under salinity stress.

Key words: Catalase, Peroxidase, Potassium, Sodium, Alphonso mango

Mango (*Mangifera indica* L.) is an important fruit crop grown in tropical and subtropical regions. Gupta and Sen (2003) reported that, soil was the major factor for influencing plant growth in mango. Soil having pH 7.86, EC 0.49 mmhos/cm, ESP 11.9 and irrigation water having EC 0.50 mmhos/cm, pH 7.46 are found to be most suitable for initial establishment of mango seedlings. Hence, establishment of mango orchards in areas having soil and/or water EC beyond this limit is difficult. Instead of going for soil reclamation for tackling the problem of soil salinity, it would be appropriate to develop salt tolerant rootstocks (Dubey *et al.,* 2006). In the present study, attempts were made to study the effect of salt stress on antioxidant enzyme activities and nutrient ion concentrations in mango rootstocks.

Material and Methods

A pot culture experiment was conducted with four levels of NaCl, *i.e*., 0.0 mM NaCl, 25, 50 and 75 mM NaCl on five different mango rootstocks, namely, Alphonso, Banganapalli, Sendhuram, Neelum and Bangalora. Fully matured stones from healthy mango fruits were collected and utilized for raising the seedlings. The mango seedlings were transplanted from raised nursery beds and planted in polybags of size 40 x 30 cm. The bags were filled with potting mixture containing sand, soil and FYM (1:2:1).The control plants were irrigated with water without any added NaCl (EC = 1.6 dSm^{-1} and pH- 6.8). The salinity levels were imposed to the seedlings grown in polybags upto 90 days at an interval of 2 -3 days depending upon moisture availability. The antioxidant activity and nutrient ion concentrations were measured after 90 days of salt stress treatments. The details of rootstock and salinity levels are as follows:

Rootstocks	Salt levels
T_1 - Alphonso	S_1 - 0 mM NaCl (Control)
T_2 - Banganapalli	S_2 - 25 mM NaCl
T_3 - Sendhuram	S_3 - 50 mM NaCl
T_4 - Neelum	S_4 - 75 mM NaCl
T_5 - Bangalora	

Catalase activity was determined by titration method using potassium permanganate (Gopalachari, 1963) expressed as µg H_2O_2 g^{-1} min^{-1}. Peroxidase content of the leaf sample was estimated by the method of Gjessing and Sumner (1943) and expressed Change in OD at 430 nm min^{-1} g^{-1}. The total potassium (K^+) and sodium (Na^+) ion concentrations in the leaves and roots were estimated using 5:2 (v/v) H_2SO_4: $HClO_4$ diacid-digested samples and flame photometer (Jackson, 1973). The experiment was carried out with two factors (rootstocks and salinity levels) under Completely Randomized Design and replicated thrice. The obtained data were statistically analysed using LSD at 0.05.

*Corresponding author's email: sinrassiga@gmail.com

Results and Discussion

Antioxidant enzyme activities

The five rootstocks showed significant variations in catalase activity under different levels of salinity (Fig 1). At 75 mM NaCl, the higher catalase activity (10.03 µg H_2O_2 g^{-1} min^{-1}), was recorded in Alphonso (T_1S_4) , whereas the lower catalase activity (17.17 µg H_2O_2 g^{-1} min^{-1}) was recorded in Bangalora (T_5S_4) which was followed by T_3S_4 (15.13 µg H_2O_2 g^{-1} min^{-1}) in Sendhuram. The result of the experiment revealed that significant differences were observed in the interaction effect between rootstocks and salt levels in peroxidase activity (Fig. 2). At higher salt levels (75 mM NaCl), the maximum peroxidase activity (1.34 change in OD at 430 nm min^{-1} g^{-1}) was recorded in Alphonso (T_1S_4), whereas the minimum peroxidase activity (0.66 change in OD at 430 nm min^{-1} g^{-1}) was recorded in Bangalora (T_5S_4). The up-regulation of catalase activity may be an adaptive response to overcome any potential damage to leaf tissues by preventing the accumulation of toxic levels of H_2O_2 produced during metabolism. Similar trend of higher levels of catalase and peroxidase in higher salt concentrations was also observed in mango by Pandey *et al.* (2014) and Abd Allatif *et al.* (2015).

Table 1. Effect of salt stress on leaf potassium content (%) of mango rootstocks

Treatments	S_1	S_2	S_3	S_4	Mean
T_1	1.66	1.47	1.20	0.72	1.26
T_2	1.59	1.33	0.93	0.42	1.07
T_3	1.59	1.28	0.82	0.35	1.01
T_4	1.63	1.42	1.01	0.51	1.14
T_5	1.59	1.18	0.61	0.31	0.92
Mean	1.61	1.34	0.91	0.46	1.08

Source	Rootstocks (T)	Salt levels (S)	Interaction (T× S)
S.Ed	0.01	0.01	0.01
C.D (0.05)	0.02	0.02	0.03*

Nutrient ion concentrations

There was significant reduction in leaf and root potassium content in leaves due to imposed salt stress in all the rootstocks and the reduction was higher at higher levels of salt stress.

Table 2. Effect of salt stress on root potassium content (%) of mango rootstocks

Treatments	S_1	S_2	S_3	S_4	Mean
T_1	1.70	1.48	1.38	1.21	1.44
T_2	1.70	1.25	0.90	0.62	1.12
T_3	1.67	1.22	0.81	0.56	1.06
T_4	1.67	1.31	1.02	0.81	1.20
T_5	1.63	1.13	0.72	0.43	0.98
Mean	1.67	1.28	0.97	0.73	1.16

Source	Rootstocks (T)	Salt levels (S)	Interaction (T× S)
S.Ed	0.01	0.01	0.01
C.D (0.05)	0.02	0.02	0.03*

The interaction effect showed that at 50 mM NaCl, the highest leaf potassium content (1.20 %) was recorded in Alphonso (T_1S_3), followed by T_4S_3 (1.01 %) and the lowest leaf potassium content (0.61%) was recorded in Bangalora (T_5S_3). At 75 mM NaCl, the maximum leaf potassium content (0.72 %) was recorded in Alphonso (T_1S_4), followed by T_4S_4 (0.51 %) and the minimum leaf potassium content (0.31%) was recorded in Bangalora (T_5S_4). In the case of root potassium content, the interaction effect showed that at 50 mM NaCl, the maximum content (1.38 %) was recorded in Alphonso (T_1S_3), followed by R_4S_3 (1.02 %) and the minimum content (0.72%) was recorded in Bangalora (T_5S_3). At 75 mM NaCl, the maximum root potassium content (1.21 %) was recorded in Alphonso (T_1S_4), followed by T_4S_4 (0.81 %) and the minimum root potassium content (0.43%) was recorded in Bangalora (T_5S_4). Increased potassium uptake has made a positive impact by reducing the toxic ions entering into the plant system from the soil solution, and such exclusion mechanism can be an added advantage to the tolerant variety. It is well documented that a greater degree of salt tolerance in plants is associated with a more efficient system for the selective uptake of K^+ over Na^+ (Noble and Rogers, 1992).

Table 3. Effect of salt stress on leaf sodium content (%) of mango rootstocks

Treatments	S_1	S_2	S_3	S_4	Mean
T_1	0.15	0.18	0.22	0.23	0.19
T_2	0.14	0.19	0.23	0.27	0.20
T_3	0.18	0.24	0.30	0.33	0.26
T_4	0.16	0.22	0.24	0.27	0.22
T_5	0.18	0.28	0.33	0.39	0.29
Mean	0.16	0.22	0.26	0.29	0.23

Source	Rootstocks (T)	Salt levels (S)	Interaction (T× S)
S.Ed	0.01	0.01	0.01
C.D(0.05)	0.02	0.02	0.04*

Table 4. Effect of salt stress on root sodium content (%) of mango rootstocks

Treatments	S_1	S_2	S_3	S_4	Mean
T_1	0.17	0.20	0.24	0.25	0.22
T_2	0.16	0.21	0.25	0.29	0.23
T_3	0.20	0.26	0.32	0.35	0.28
T_4	0.18	0.24	0.26	0.29	0.24
T_5	0.20	0.30	0.35	0.41	0.31
Mean	0.18	0.24	0.28	0.32	0.26

Source	Rootstocks (T)	Salt levels (S)	Interaction (T× S)
S.Ed	0.01	0.01	0.01
C.D(0.05)	0.02	0.02	0.03*

The leaf and root sodium content exhibited significant differences by different combinations of rootstocks and salt levels (Table 3 and 4). Sodium

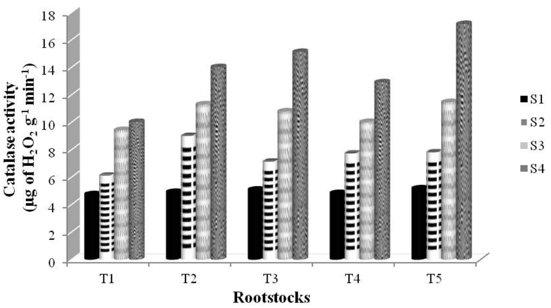

Fig. 1. Effect of salt stress on catalase activity (µg H_2O_2 g^{-1} min^{-1}) of mango root stocks

ion concentrations in leaf tissues increased with increasing salinity in all rootstocks. The interaction effect showed that at 50 mM NaCl, the higher leaf sodium content (0.33 %) was recorded in Bangalora (T_5S_3), which was on par with T_3S_3 (0.30 %) and the lower leaf sodium content (0.22 %) was recorded in Alphonso (T_1S_3). At 75 mM NaCl, the highest leaf sodium content (0.39 %) was recorded in Bangalora (T_5S_4), followed by T_3S_4 (0.33 %) and the least leaf

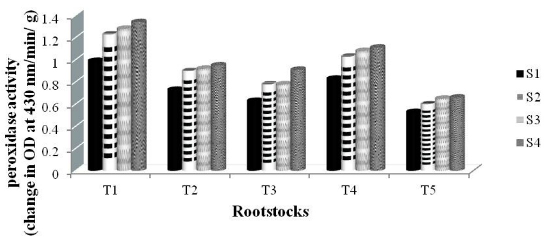

Fig. 2. Effect of salt stress on peroxidase activity (change in OD at 430 nm /min/ g) of mango root stocks

S1 - 0 mM NaCl (Control); S2 - 25 mM NaCl; S3 - 50 mM NaCl; S4 - 75 mM NaC T1 - Alphonso; T2 - Banganapalli; T3 - Sendhuram; T4 - Neelum; T5 – Bangalora

sodium content (0.23%) was recorded in Alphonso (T_1S_4). The mean root sodium content ranged from 0.31 % (T_5) to 0.22 % (T_1).The mean sodium content in various salt concentrations was also significantly different from each other, where it ranged from 0.18 % (S_1) to 0.32 % (S_4). The interaction effect showed that at 50 mM NaCl, the highest root sodium content (0.35 %) was recorded in Bangalora (T_5S_3), followed by T_3S_3 (0.32 %) and the least root sodium content (0.24 %) was recorded in Alphonso (T_1S_3), which was on par with T_4S_3 (0.26 %). At 75 mM NaCl, the higher root sodium content (0.41 %) was recorded in Bangalora (T_5S_4), followed by T_3S_4 (0.35 %) and the lower root sodium content (0.25%) was recorded in Alphonso (T_1S_4).

Based on the present findings, it was concluded that under high salinity stress, the seedlings of salt-tolerant mango rootstocks exhibited lower levels of Na^+ concentrations. Moreover, salt stress increased the concentration of Na^+ concomitant with a decrease in K^+ accumulation in comparatively salt-sensitive rootstocks. Thus, in addition to the toxic effects caused by high concentrations of Na^+ in plant tissue, the salinity-induced changes on uptake and utilization of mineral nutrients contributed to the reduction in plant growth. Furthermore, the tolerance expressed against salinity by Alphonso and Neelum rootstocks may be due to impediment of the uptake of Na+ ions. Similar observations of sodium exclusion by tolerant cultivars/rootstocks were made by Schmutz and Ludders (1999) in mango, Perica *et al.* (2008) in olive, Hajiboland *et al.* (2014) in pistachio seedlings.

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