Fruits 73(4), 228–235 | ISSN 0248-1294 print, 1625-967X online | https://doi.org/10.17660/th2018/73.4.4 | © ISHS 2018

Original article

Screening polyembryonic mango accessions for salt tolerance and assessing dynamics of sodium (Na^+), potassium (K^+), and antioxidants

T. Damodaran[1,a], S. Rajan[2], S.K. Jha[1], V.K. Misra[1] and A. Sahu[1]
[1] ICAR-Central Soil Salinity Research Institute, RRS, Lucknow, India
[2] ICAR-Central Institute of Subtropical Horticulture, Lucknow, India

Summary

Introduction – **Salinity is an important abiotic stress limiting mango production worldwide. Salt tolerant polyembryonic rootstock enables mango cultivation in salt-affected and wastelands. The objective of the study was to assess the field tolerance of polyembryonic mango accessions in salt affected sodic soils, through the dynamics of sodium, potassium, antioxidants and associated biochemicals in response to salt stress.** *Materials and methods* – **The rootstocks were collected from seawater inundated zone of the South Andaman Islands and then evaluated at the experimental farm of ICAR-Central Soil Salinity Research Institute, Lucknow, India for a period of two years (2014–2016). The variables plant height, survivability percentage, Na^+/K^+ ratio, phenol and proline contents, superoxide dismutase (SOD) and peroxidase (POD) activities were assessed.** *Results and discussion* – **Among the accessions, 'ML-2' and 'ML-6' showed higher tolerance to salt stress with complete survival (100%). Similarly, the data on plant height were significantly similar to those of the control '13-1'. The data of Na^+/K^+ ratio in leaves and meristem tips indicated an effective Na^+ exclusion mechanism adopted by 'ML-2' and 'ML-16' through increased uptake in K^+. Increased accumulation of proline and phenolics in 'ML-2' and 'ML-6' with the increase in the activities of SOD and POD led them to a better survivability in salt stress conditions.** *Conclusion* – **Our investigation has led to the selection of two salt stress tolerant rootstocks (cvs. 26 ML-2 and ML-6) suitable for mango production in salt affected sodic soils in tropical and sub-tropical conditions.**

Keywords
India, mango, *Mangifera indica*, antioxidant activity, mineral nutrition, phenolics, proline, rootstock selection, salt stress

Résumé

Evaluation d'accessions de manguier polyembryonnaire pour leur tolérance au sel et mesure de leur comportement dynamique vis-à-vis du sodium (Na^+), du potassium (K^+) et des composés antioxydants.

Significance of this study

What is already known on this subject?

- Mango is considered to be salt sensitive; its cultivation is declining among the small scale growers globally due to salinity. Polyembryonic graft compatible rootstock with tolerance to abiotic stress, particularly salt stress, is preferred for propagation on commercial scale.

What are the new findings?

- Post-tsunami collections and evaluation of polyembryony accessions collected from salt water inundated zones have given six accessions showing tolerance under pot experimentation. Their field tolerance and physiology in saline sodic conditions still need to be checked before commercialization.

What is the expected impact on horticulture?

- The production of salt tolerant polyembryony rootstock in tropical and sub-tropical conditions is a good option for commercial growers to improve the mango productivity under salt stress. The accessions identified can serve as a source for breeding programs for abiotic stress tolerance.

Introduction – **La salinité est un stress abiotique sévère limitant la production de mangue dans le monde entier. Un porte-greffe polyembryonnaire tolérant au sel permet au manguier d'être cultivé sur sols salés ou dégradés. L'objectif de l'étude était d'évaluer la tolérance au champ d'accessions de manguier polyembryonnaire sur des sols sodiques affectés par le sel, à travers la dynamique du sodium, du potassium, et des composés biochimiques anti-oxydants et associés en réponse au stress salin.** *Matériel et méthodes* – **Six porte-greffes ont été collectés dans la zone inondée d'eau de mer des îles Andaman du Sud, puis évalués à la ferme expérimentale du l'Institut central de recherche sur la salinité des sols de l'ICAR, à Lucknow en Inde, sur une période de deux ans (2014–2016). Les variables suivantes ont été évaluées: hauteur de la plante, pourcentage de survie, rapport Na^+/K^+, teneurs en composés phénoliques et en proline, et activités de la superoxyde dismutase (SOD) et de la peroxydase (POD).** *Résultats et discussion* – **Parmi les accessions, 'ML-2' et 'ML-6' ont mon-**

[a] Corresponding author: damhort73@gmail.com.

tré une plus grande tolérance au stress salin avec une survie complète (100%). De même, les données sur la hauteur des plantes étaient significativement similaires à celles du témoin '13-1'. Les données relatives au rapport Na^+/K^+ dans les feuilles et les extrémités méristématiques ont indiqué un mécanisme d'exclusion efficace de Na^+ adopté par 'ML-2' et 'ML-16' par augmentation de l'absorption de K^+. L'accumulation de proline et de composés phénoliques dans les cvs. ML-2 et ML-6, correspondant à l'augmentation des activités SOD et POD, a conduit ces accessions à une meilleure capacité de survie en situation de stress salin. *Conclusion* **– Notre étude a conduit à la sélection de deux porte-greffes tolérants au stress salin (cvs. 26 ML-2 et ML-6) recommandables pour la production de mangue sur sols sodiques affectés par le sel dans des conditions tropicales et subtropicales.**

Mots-clés
Inde, manguier, *Mangifera indica*, activité anti-oxydante, nutrition minérale, composés phénoliques, proline, sélection de porte-greffe, stress salin

Introduction

Mango (*Mangifera indica* L.) is mainly grown in semi-arid regions with sub-tropical and tropical climate. Salinity and sodicity are the most significant ecological factor that causes extensive crop yield loss in these regions (Giri *et al.*, 2003). With the development of canal irrigation systems in major river basins, nonetheless the predicted scenario of seepage to the adjacent vulnerable agricultural fields caused accumulation of salts on the surface creating saline sodic soils (Zhu, 2001). Such soils with pH > 8.5 and exchangeable sodium percentage (ESP) > 15 impose serious constraint in growth of mango (Damodaran *et al.*, 2013) by increasing the sodium (Na^+) ion toxicity and creating ionic imbalance in plant tissues (Munns and Tester, 2008). Increasing salinity caused decline in vegetative growth and yield (Bray *et al.*, 2000).

Mango, being the most preferred fruit crop of the tropics, is constantly being exposed to salinity and drought that makes salt tolerance as the most desirable characteristic for a mango rootstock development (Gazit and Kadman, 1980). Physiological mechanism of salt tolerance suggests displacement of sodium from root cells through maintenance of adequate potassium concentrations and proper sodium/potassium (Na^+/K^+) ratio (Kafkafi and Bernstein, 1997). Further, increase in ability to produce proline, phenolic compounds and antioxidant enzymes are considered to be a well-known adaptive mechanism of salt stress (Thomas *et al.*, 1992). Generally, polyembryonic rootstocks coming from well-adapted local conditions are preferred for producing trees with uniform vigor and tolerance to stress. Use of monoembryonic rootstocks has resulted in orchards with low productivity and poor ability to withstand the salt stress in the Asian sub-continent where mango is said to be originated (Galan Sauco, 2009). Therefore, breeding for salt tolerance in mango is one among the priority research area in the tropics. Considered to be salt sensitive, mango produces severe scorching symptoms on leaves when exposed to high sodium (Na^+) at an early growth stage (Mass, 1986). The problem was further aggravated with the reduction in intake of potassium (K^+) ions due to high Na^+ uptake in sodic soils (Fricke *et al.*, 1996). Little efforts have been attempted in the past to identify salt tolerant polyembryonic genotypes with the ability to maintain higher levels of K^+ in tissues (Al-Karaki, 2000). Certain phenolic compounds, antioxidant enzymes like super-oxy-dismutase (SOD) and peroxidase (POD) are prone to remove the ROS and aid in maintenance of balance between production and removal of reactive oxygen species (ROS) (Apel and Hirt, 2004). Accumulation of amino acids such as proline is also a well-known adaptive mechanism of salt stress (Thomas *et al.*, 1992). Limited research was focused on screening rootstocks with high antioxidant production ability upon exposure to stress as an adaptive mechanism for salt tolerance. Moreover, being a cross-pollinated crop polyembryonic graft compatible rootstock are preferred for propagation on commercial scale (Litz and Gomez, 2002). Therefore, the identification of salt tolerant polyembryonic mango rootstocks in natural population with high K^+ uptake and antioxidant production ability is one such simple and economical method for sustaining crop production in problem soils. The cv. 13-1 is one such rootstock used as a source for salt tolerance. However, its success as rootstock was limited with the choice varieties of the Indian origin due to its poor fruit set percentage under sub-tropical conditions (Ram and Rajan, 2003). Early studies in the sub-continent were carried out by exposing 40 polyembryonic mango accessions from tsunami affected areas of the Andaman Islands to calcareous soils in pot experiment. Preliminary results have indicated that six polyembryonic accessions showing a relative tolerance to salt stress needed further field screening (Damodaran *et al.*, 2013).

The present experiment aimed to i) investigate the ability of the six polyembryonic accessions to salt tolerance under *in-situ* sodic field conditions in comparison with standard rootstock '13-1'; ii) assess the dynamics of Na^+ and K^+ in leaves and meristem tissues; iii) document changes in proline, phenol content and activities of antioxidant enzymes like super-oxy-dismutase and peroxidase, traditionally associated with salt stress.

Materials and methods

Plant materials

The 42 mango accessions collected from post-tsunami salt water inundated areas of Andaman Islands were used for screening in sodic soils of pH 9.51 under pot culture experiments (Damodaran *et al.*, 2013). In our study, six salt tolerant polyembryonic accessions (Table 1) that showed tolerance in pot experiment were selected for further field evaluation in sodic soils with high soil pH. The seeds were sown in nursery beds along with standard check '13-1' after extraction from the pulp and washing twice in tap water during April 2013. In July 2013, a total of 15 nucellar seedlings from each accession showing uniform growth vigor were uprooted from nursery bed and planted in polythene bags of size 12.5 × 15.5 cm filled with 2.5 kg of good quality orchard soil having a pH of 7.45, an electrical conductivity (EC) of 0.22 dS m^{-1} (1:2, w/v, soil water solution) and an organic carbon content of 3.0 g kg^{-1}. The accessions were randomly grouped into three replicates (5 plants per replicate) and used for field experiments in sodic soil.

Soil analysis

The experiment was conducted at Experimental Farm, ICAR-Central Soil Salinity Research Institute, Regional Research Station, Shivri, Lucknow, India. The soil samples (at 0–15 cm; 15–30 cm; and 30–60 cm depth) were collected

Table 1. Post-tsunami collections of polyembryonic mango accessions used in the study.

Accessions	Locations	Latitude	Longitude	Altitude (m)
ML-2	Manjeri, South Andaman	N 11° 32.15"	E 92° 39.03"	67
ML-3	Manjeri, South Andaman	N 11° 32.31"	E 92° 39.03"	63
ML-6	Manjeri, South Andaman	N 11° 32.31"	E 92° 39.03"	63
GPL-1	Guptapara, South Andaman	N 11° 33.62"	E 92° 39.00"	67
GPL-3	Guptapara, South Andaman	N 11° 33.62"	E 92° 39.00"	67
GPL-4	Guptapara, South Andaman	N 11° 33.62"	E 92° 39.00"	67
A13-1	Standard check from Central Institute for Subtropical Horticulture, Rehmenkela, Lucknow, Uttar Pradesh	N 26° 74.10"	E 80° 94.67"	123

Table 2. Mean soil properties of the CSSRI, experimental field of sodic soil used for screening located at Lucknow, Uttar Pradesh. Values are means ± standard deviation (analyses in triplicate). EC: Soil electrical conductivity; SAR: Sodium adsorption ratio.

Soil depth (cm)	pH	EC (dS m^{-1})	Sodium (Meq L^{-1})	Carbonate (Meq L^{-1})	Bicarbonate (Meq L^{-1})	Ca (Meq L^{-1})	Mg (Meq L^{-1})	SAR
0–15	9.13 ± 0.19	0.686 ± 0.104	7.38 ± 0.79	4.00 ± 0.50	0.00 ± 0.00	1.50 ± 0.50	0.00 ± 0.00	8.52 ± 0.90
15–30	9.49 ± 0.16	0.748 ± 0.026	10.47 ± 0.72	3.50 ± 0.30	2.00 ± 0.50	1.00 ± 0.20	0.00 ± 0.00	14.81 ± 0.84
30–60	9.90 ± 0.18	0.834 ± 0.029	11.30 ± 0.60	3.00 ± 0.50	1.00 ± 0.50	1.00 ± 0.40	0.00 ± 0.00	15.98 ± 0.98

from 12 locations of the experimental field where the screening was to be conducted. Soil analysis for each parameter was carried out in triplicate and reported as mean value with standard deviation (SD). The samples were analyzed for pH, EC, calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}) carbonate (CO_3^{2-}) and bicarbonate (HCO_3^{-}) in the saturated extracts of the soil. The pH of the soil extract was determined potentio-metrically by a ORION ion analyzer (5 star series) using a pH electrode calibrated with pH buffers of 7.0 and 10.0. Carbonate (CO_3^{2-}) and bicarbonate (HCO_3^{-}) were determined by titrimetric method (acid-base titration) (Richards, 1954). Calcium (Ca^{2+}) and magnesium (Mg^{2+}) were determined by versenate method (EDTA titration) (Chang and Bray, 1951). Sodium (Na^{+}) was determined by flame photometer (Richards, 1954) while sodium adsorption ratio (SAR) was determined by the following generic equation:

$$\text{SAR} = \frac{Na}{\sqrt{(Ca + Mg)/2}}$$

The final soil data of the selected block are presented in Table 2. The field was divided into blocks with similar soil pH level based on soil analysis. The homogenous block with no significant difference in soil pH was selected for the experiment. The soil pH ranged from 9.13 in the surface to 9.90 at the depth of 30–60 cm. The SAR increased from 8.81 at 0–15 cm to 16.03 at 30–60 cm. Sodium was predominantly found in the soil in the form of carbonate and bi-carbonate at all depths. The seedlings from polybags were planted in the experimental field of sodic soil during July 2014.

Plant survival and growth parameters

Observations on survival percentage (M) were recorded at intervals of 6, 12, 18 and 24 months after planting (MAP) to ascertain the response of the different accessions to sodicity and characterize the period of tolerance.

$$Survival\ \%\ (S) = \frac{Number\ of\ plants\ survived}{Total\ number\ of\ plants} \times 100$$

Plant height was recorded at the time of planting in experimental field and at the end of the 1st and 2nd year after planting (YAP).

Na^+ and K^+ contents

The leaf (3rd leaf from terminal portion of seedling) and meristem tip samples were collected at time of planting and at the end of first and second year after planting for analyzing the content of Na^+ and K^+ by extracting the oven-dried (65 °C) samples in 100 mmol m^{-3} acetic acid kept in a water bath for 2 h at 90 °C. Na^+ and K^+ content in the extract were determined using a flame photometer. The ratio Na^+/K^+ was estimated after analysis of Na^+ and K^+. Samples were collected from all five plants in each replicate to ascertain the exact role of Na^+ and K^+ in relation to salt tolerance. The mean of five samples was used as the value of one replicate for statistical analysis.

Proline and phenol contents

The proline content in matured leaves was measured by rapid colorimetric method suggested by Bates *et al.* (1973). Proline was extracted from 0.5 g of leaf samples homogenized in 3% sulphosalicylic acid (10 mL) and centrifuged at 10,000 ×*g* for 10 min. Free proline content was determined from standard curve using analytical grade L-proline (E-Merck, Mumbai) and calculated on fresh weight basis. Phenol content was estimated as per the procedure given by Zieslin and Ben-Zaken (1993), and expressed as catechol equivalent g^{-1} protein.

Antioxidant enzyme assay

Leaves were sampled at the time of planting and at the end of the 1st and 2nd YAP for analyzing the enzyme activity. Enzyme extractions were carried out at 4 °C, freeze-dried leaves were frozen in liquid nitrogen and ground with an ice-cold pestle and mortar, and then extracted in 100 mM potassium phosphate buffer (pH 7.5) containing 1 mM EDTA. The homogenates were centrifuged at 20,000 rpm for 30 min at 4 °C. The supernatant filtered through two layered cheese cloth were used for the assays of enzymatic activity.

The superoxide dismutase (SOD) activity assay was determined according to the method El-Moshaty *et al.* (1993). The reaction mixture (3 mL) contained 50 mM phosphate buffer (pH 7.0), 75 µM EDTA, riboflavin and methionine. Riboflavin was added at the last minute to initiate the reaction. The mixture was shaken thoroughly and placed under 15 W fluorescent bulbs for 10 min. The tubes were then covered with a black cloth and the absorbance was measured at 560 nm on a spectrophotometer (VISISCAN 167, Systronics India Ltd.).

The peroxidase (POD) activity assay was carried out using the reaction mixture (3 mL) consisted of (0.25%) v/v guaiacol in 10 mM potassium phosphate buffer (pH 6.9) containing 10 mM hydrogen peroxide (Hammerschmidt *et al.*, 1982). The enzyme activity was expressed as number of absorbance units g^{-1} fresh weight basis (FW).

Statistical analysis

All plant growth data were assessed in a randomized block design with three replicates ($n=5$ per replicate). The data of survival percentage were subjected to arcsine transformation and the transformed values were used for analysis. The differences among treatments were analyzed through one way variance analysis (ANOVA), the Duncan test ($P<0.05$) was used for separation of means. Data were analyzed using SAS 9.2 statistical software.

Results and discussion

Effect of salt stress on survival and growth of the seedlings

Salt stress in sodic soils reduced the survival rate of accessions over the period of time (Figure 1). However, significantly higher survival rate was shown by the cvs. ML-2, ML-6 and the standard check '13-1' during the experiment period. Significant decrease in survival rate at all stages (6, 12, 18 and 24 MAP) of observations was registered in the cvs. ML-3, GPL-1, GPL-3 and GPL-4. At 24 MAP under salt stress, the leaves developed scorching injury followed by necrosis leading to seedling mortality (morphological observations, data not shown). Continuous exposure of the wheat plants to high pH and SAR (Sodium Absorption Ratio) in the soil under *in situ* over a longer period result in mortality of plants (Mishra *et al.*, 2017). Garg and Malhotra (2008) also observed that elevated sodium concentration and high pH in field conditions impart adverse changes in soil physical properties leading to plant mortality in *Nigella sativa,* a medicinal plant.

Salt stress reduced the growth of the seedlings with the increase in the duration of exposure. Significant decrease in height (Figure 2) was noticed in the cvs. ML-3, GPL-1, GPL-3 and GPL-4 at 1 year after planting (YAP). Stunted growth and plant mortality are the most common effect of salinity in mango (Gupta and Sen, 2003). Similar observations of stunted growth in mango seedlings screened for salinity tolerance were also made by Srivastava *et al.* (2009). It has been

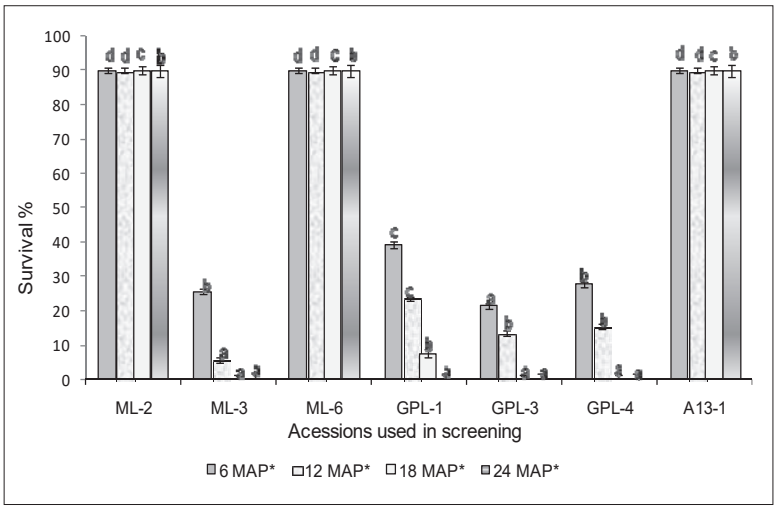

Figure 1. Effect of sodicity on survival percentage of seven polyembryonic mango accessions. Bar values are the means of three replicates, the error bars represent standard errors of the means ($n=5$). Means of different accessions followed by the same letter are not significantly different according to Duncan's multiple range test at $P=0.05$. MAP: Months after planting.

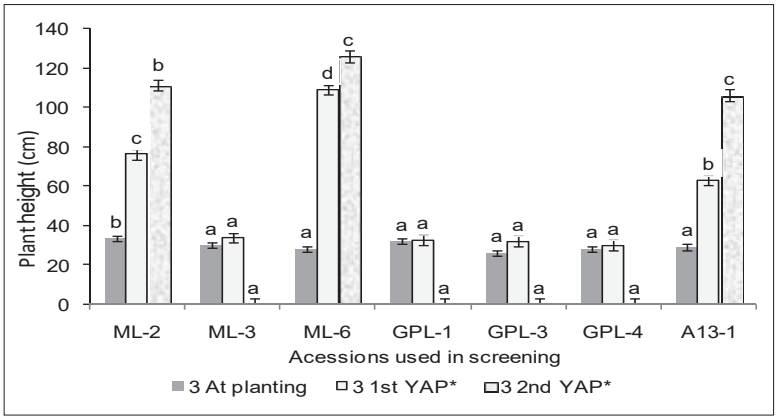

Figure 2. Effect of sodicity on growth (plant height) of seven polyembryonic mango accessions. Bar values are the means of three replicates, the error bars represent standard errors of the means ($n=5$). Means of different accessions followed by the same letter are not significantly different according to Duncan's multiple range test at $P=0.05$. YAP: Year after planting.

reported that salinity causes lower water potential in roots causing reduction in growth rate (Munns, 2002).

However, the cvs. ML-6, ML-2 and 13-1 showed a significant increase in plant growth during the experimental period. Higher plant height and number of leaves were recorded in the local salt tolerant rootstock Zebda and Sukkary of mango when compared with '13-1' under saline conditions of Egypt (Shaban, 2010). Long-term exposure to stress conditions lead to better selection of tolerant rootstocks in perennial crops (Munns *et al*., 1995). Earlier work (Damodaran *et al*., 2013) on salinity screening in mango in pot studies showed that long-duration screening for a period of 240 days aided in eliminating rootstocks that failed to survive the salt injury after 60 days after planting in pots. In the current study, the accessions ('ML-3', 'GPL-1', 'GPL-3' and 'GPL-4') that showed tolerance till 240 days in pot culture studies earlier, when screened for a period of two years in field conditions became susceptible to salt stress. However, the tolerant 'ML-2', 'ML-6' survived without any stress symptoms during the entire experimental period. At 2nd YAP the plant height in tolerant accessions ranged from 111.00 to 125.67 cm which had no significant difference with the height of the tolerant control cv. 13-1 (106.00 cm).

Sodium (Na+) and potassium (K+) accumulation

The salt stress in sodic soils caused a significant variation in the accumulation of sodium and potassium in leaves (Table 3) and growing meristem (Table 4) among the seven accessions used for screening. At planting, no significant variations in Na^+ and K^+ uptake among the accessions were observed. However, at the 1st YAP, the cvs. ML-2, ML-6 and 13-1 showed a significant decrease of the Na^+/K^+ ratio in leaves and growing meristem compared to others (cvs. ML-3, GPL-1, GPL-3 and GPL-4). The Na^+/K^+ ratio of the cvs. ML-2 and ML-6 were similar to that of the tolerant control '13-1'. This is in agreement with the earlier finding (Wolf *et al*., 1991) where low Na^+/K^+ ratio in tolerant plants was reported which was considered as a significant salt tolerant adaptation. This is further supported by the earlier findings (Duran Zuazo *et al*., 2003), where 'Gomera 1' rootstock of mango showed more salinity tolerance than '13-1' with lower Na^+/K^+ ratio in the leaves and stem than '13-1'. Under saline conditions, the ability of the plant to prevent salt from reaching the toxic levels in the leaves is one such indicator that distinguishes the salt tolerant and sensitive accession as reported in the studies of rice (Yeo *et al*., 1991).

Indeed, the accessions 'ML-2', 'ML-6' and '13-1' showed significant increase of potassium accumulation (1.660, 1.680 and 1.710 meq L^{-1}, respectively) in leaves and the growing meristem (1.390, 1.559 and 1.264 meq L^{-1}, respectively). Decreased Na^+ and increased K^+ uptake was reported as part of a mechanism involved in changing a genotype to become tolerant to salt stress (Watad, 1991). This suggests that salt tolerant plants possess enhanced ability to accumulate K^+ and restrict the Na^+ in the photosynthesizing and meristematic cells, what is in agreement with earlier observations (Ashraf, 2004). Our results also showed that the cvs. ML-3, GPL-1, GPL-3 and GPL-4 accumulated more Na^+ than K^+ in leaves and meristem tips. Notably the values of Na^+ in the leaves and meristem of the cvs. ML-3, GPL-1, GPL-3 and GPL-4 are quite significantly higher compared with those in cvs. ML-2, ML-6 and 13-1. At the 2nd YAP estimations of Na^+ and K^+ could

Table 3. Changes in sodium (Na^+) and potassium (K^+) accumulation in leaves (in Meq L^{-1}) and in the Na^+/K^+ ratio of seven polyembryonic mango accessions. Values are means of three replicates ($n = 5$). YAP: Year after planting.

Polyembryonic mango accessions	At planting			1st YAP			2nd YAP		
	Na^+	K^+	Na^+/K^+	Na^+	K^+	Na^+/K^+	Na^+	K^+	Na^+/K^+
ML-2	0.211 a	0.987 c	0.214 a	1.243 bc	1.660 b	0.748 a	0.640 a	1.532 a	0.418 a
GPL-3	0.289 a	1.102 c	0.262 a	0.657 a	0.207 a	3.177 b	**	**	**
ML-3	0.179 a	0.688 a	0.260 a	0.690 a	0.460 a	1.500 a	**	**	**
GPL-1	0.275 a	0.942 bc	0.292 a	0.840 ab	0.280 a	3.000 b	**	**	**
ML-6	0.324 a	1.026 c	0.316 a	1.343 c	1.680 b	0.800 a	0.583 a	1.653 a	0.353 a
GPL-4	0.224 a	0.783 ab	0.286 a	0.657 a	0.207 a	3.177 b	**	**	**
13-1 (check)	0.228 a	0.988 c	0.231 a	1.400 c	1.710 b	0.818 a	0.471 a	1.427 a	0.330 a

Means in the columns followed by the same letter are not significantly different according to Duncan's multiple range test at $P = 0.05$.
**: Plants attained mortality during the 2nd YAP.

Table 4. Changes in sodium (Na^+) and potassium (K^+) contents (in Meq L^{-1}) and Na^+/K^+ ratio in the meristem tips of seven polyembryonic mango accessions. Values are means of three replicates ($n = 5$). YAP: Year after planting.

Polyembryonic mango accessions	At planting			1st YAP			2nd YAP		
	Na^+	K^+	Na^+/K^+	Na^+	K^+	Na^+/K^+	Na^+	K^+	Na^+/K^+
ML-2	0.120 a	0.954 a	0.126 a	0.510 a	1.390 b	0.367 a	0.640 b	1.532 a	0.417 b
GPL-3	0.160 bc	1.102 a	0.145 a	1.680 b	0.210 a	8.000 b	0.000 a	**	**
ML-3	0.130 ab	0.966 a	0.135 a	1.240 b	0.198 a	6.263 b	0.000 a	**	**
GPL-1	0.125 ab	1.012 a	0.123 a	1.890 b	0.244 a	7.746 b	0.000 a	**	**
ML-6	0.140 ab	1.059 a	0.132 a	0.468 a	1.559 b	0.300 a	0.583 b	1.653 a	0.353 a
GPL-4	0.242 d	1.450 a	0.167 a	2.440 c	0.450 a	5.422 b	0.000 a	**	**
13-1 (check)	0.178 c	1.264 a	0.140 a	0.378 a	1.264 b	0.299 a	0.471 b	1.427 a	0.330 a

Means in the columns followed by the same letter are not significantly different according to Duncan's multiple range test at $P = 0.05$.
**: Plants attained mortality during 2nd YAP.

not be made in the cvs. ML-3, GPL-1, GPL-3 and GPL-4 due to complete mortality of the plants. The accumulation of Na^+ ions in leaf tissues causes reduction in plant growth by decreasing the intake of K^+ in tomato (Turhen and Seniz, 2010), as Na^+ competes with K^+ for major binding sites in many key metabolic processes in the cytoplasm (Garg and Malhotra, 2008). The observations under study and similar findings demonstrated the potential of the cvs. ML-2, ML-6 and 13-1 to hold K^+ has been crucial for inducing salt tolerance.

Phenol and proline contents

Under the two years of screening period, salt stress caused significant increases of phenol and proline concentrations in the accessions 'ML-2', 'ML-6' and '13-1' compared with other accessions (Table 5). However, at planting no significant difference in phenol and proline concentrations among the accessions was observed. At the 1st YAP we have found that the phenol content in leaves increased significantly in 'ML-2' (233.51 mg g^{-1}), 'ML-6' (235.61 mg g^{-1}) and '13-1' (235.15 mg g^{-1}), that recorded higher survival percentage compared to the susceptible accessions ('GPL-3', 'ML-3', 'GPL-1' and 'GPL-4'). Similarly, a significant increase in the proline accumulation was observed in 'ML-2', 'ML-6' and '13-1' compared to others at one YAP. The proline content ranged from 1.67 mg g^{-1} to 1.74 mg g^{-1} in the accessions with higher survival percentage while it ranged from 0.356 mg g^{-1} to 0.776 mg g^{-1} in the susceptible accessions at one YAP.

At the 2nd YAP, no observations on phenol and proline content could be recorded in the susceptible accessions, as they failed to survive. However, the tolerant accessions ('ML-2', 'ML-6' and '13-1') did not show any significant difference in the phenol and proline content. It has been reported that accumulation of osmolytes like proline increase the synthesis of phenols, a well-known adaptive mechanism in perennials against salt stress (Reuveni *et al.*, 1991). Similarly, trials in Egypt on mango rootstocks confirm the higher accumulation of proline content in the leaves of 'Sukkary' (more resistant to salinity) than in 'Zebda' (salt sensitive) (Hafez *et al.*, 2011). Higher proline accumulation (43.79 µg g^{-1}) and phenol content were observed in the tolerant rootstock of mango seedlings when subjected to sodium chloride stress (Pandey, 2015). Based on our results and the reports from other workers, it was observed that rootstocks which exhibited higher survival percentage and tolerated salt stress showed higher proline and phenol contents in the leaves compared to the susceptible accessions.

Effect of antioxidant enzymes

Plants have developed several antioxidant systems to reduce the higher ROS production. SOD is one such antioxidant enzyme that catalyzes the conversion of superoxide radical to molecular oxygen and H_2O_2. In the present investigation, the initial data at planting (Table 6) revealed that the activity of SOD and POD was not significantly affected by the salt stress in sodic soil due to the limited exposure to stress. However, a significant increase in SOD and POD was observed in the cvs. ML-2, ML-6 and 13-1 relative to the other accessions at one YAP.

The SOD in the accessions with high survivability rate (cvs. ML-2, ML-6 and 13-1) ranged from 48.833 to 50.690 units g^{-1} tissue, while for the accessions with low survivability rate (cvs. GPL-3, ML-3, GPL-1 and GPL-4) it ranged from

Table 5. Changes in phenol and proline contents (in mg g^{-1}) in leaves of seven polyembryonic mango accessions. Values are means of three replicates ($n = 5$). YAP: Year after planting.

Polyembryonic mango accessions	Phenolics			Proline		
	At planting	1st YAP	2nd YAP	At planting	1st YAP	2nd YAP
ML-2	120.533 a	233.510 d	263.880 a	0.177 ab	1.670 c	1.640 a
GPL-3	122.933 a	151.400 b	**	0.197 ab	0.716 b	**
ML-3	117.907 a	172.250 c	**	0.180 ab	0.586 b	**
GPL-1	128.567 a	181.406 c	**	0.150 a	0.776 b	**
ML-6	121.633 a	235.613 d	256.040 a	0.156 a	1.676 c	1.590 a
GPL-4	131.400 a	138.916 a	**	0.213 b	0.356 a	**
13-1 (check)	128.833 a	235.153 d	267.470 a	0.187 ab	1.746 c	1.637 a

Means in the columns followed by the same letter are not significantly different according to Duncan's multiple range test at $P = 0.05$.
**: Plants attained mortality during the 2nd YAP.

Table 6. Changes in SOD and POD enzyme activities (in absorbance units min^{-1} g^{-1} FW) in the leaves of seven polyembryonic mango accessions. Values are means of three replicates ($n = 5$). YAP: Year after planting.

Polyembryonic mango accessions	SOD			POD		
	At planting	1st YAP	2nd YAP	At planting	1st YAP	2nd YAP
ML-2	31.500 a	48.833 c	66.021 a	2.252 ab	4.653 c	5.009 a
GPL-3	35.443 a	34.956 ab	**	2.560 ab	2.471 ab	**
ML-3	35.426 a	36.980 b	**	2.117 a	2.034 a	**
GPL-1	33.436 a	33.870 ab	**	2.224 ab	2.977 b	**
ML-6	34.393 a	49.090 c	66.556 a	2.140 ab	4.791 c	5.153 a
GPL-4	32.733 a	32.216 a	**	2.550 ab	2.264 a	**
13-1 (check)	33.900 a	50.690 c	68.246 a	2.612 b	4.773 c	5.148 a

Means in the columns followed by the same letter are not significantly different according to Duncan's multiple range test at $P = 0.05$.
**: Plants attained mortality during the 2nd YAP.

32.210 to 36.980 units g^{-1} tissue. Similar observations were also noticed in the POD activity among the seven accessions screened in the study. At the 2nd YAP, no observations with respect to SOD and POD could be made in the cvs. GPL-3, ML-3, GPL-1 and GPL-4 due to plant mortality under salt stress. However, no significant SOD and POD activity was noticed among 'ML-2', 'ML-6' and '13-1' plants that survived. The increase in SOD activity with the period of study in tolerant accessions suggested the enhancement of the ROS scavenging defense mechanism as demonstrated earlier by Winston (1990). Fletcher *et al*. (2000) found that POD catalyzed hydrogen peroxide free radical dependent oxidation during salt stress and inhibited lipid peroxidation, what may cause delay in senescence of tolerant species.

Similar findings (Parida *et al*., 2004) also revealed the role of antioxidant enzymes (SOD and POD) in inducing salt tolerance in mangroves. This was further supported by recent results (AbdAllatif *et al*., 2015) showing that POD and SOD activity increased rapidly in the salt-tolerant mango 'Zebda' compared to others used in screening. These results indicate that higher antioxidant enzymatic activities are found to exist in the accessions that exhibit tolerance to sodicity and salinity, which is an interesting indicator for identifying tolerant accessions.

Conclusion

Six polyembryony mango rootstocks were screened for field tolerance to sodicity (salt stress) along with one tolerant check cv. 13-1. The cvs. ML-2 and ML-6 showed high tolerance to sodicity, exhibiting significantly higher survival rate and plant height than the others used in the study. Furthermore, the tolerant accessions ('ML-2' and 'ML-6') were shown to possess a higher K^+ ion accumulating ability in their leaves and meristem tips that caused significant reduction in the Na^+/K^+ ratio.

Moreover, the high phenolic and proline compounds and much activated antioxidant enzyme activity of the tolerant accessions confirmed the role of antioxidants in imparting tolerance to salt stress. Though '13-1' was observed to explicit sodicity tolerance, the poor fruit set percentage of this cultivar under sub-continent conditions as reported earlier limited its utility in this region. Thus, the accessions 'ML-2' and 'ML-6' should be considered as better salt tolerant rootstocks of polyembryonic mango for the tropics and sub-tropics.

Acknowledgments

Authors greatly acknowledge the Director, ICAR-Central Soil Salinity Research Institute, Karnal for support towards conducting the above experiment in the six plots of the experimental farm.

References

AbdAllatif, A.M., El Kheshin, M.A., and Rashedy, A.A. (2015). Antioxidant potential of some mango (*Mangifera indica* L.) cultivars growing under salinity stress. Egypt. J. Hort. *42*, 654–665.

Al-Karaki, G.N. (2006). Nursery inoculation of tomato with arbuscular mycorrhizal fungi and subsequent performance under irrigation with saline water. Sci. Hortic. *109*, 1–7. https://doi.org/10.1016/j.scienta.2006.02.019.

Apel, K., and Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. *55*, 373–399. https://doi.org/10.1146/annurev.arplant.55.031903.141701.

Ashraf, M. (2004). Some important physiological selection criteria for salt tolerance in plants. Flora *199*, 361–376. https://doi.org/10.1078/0367-2530-00165.

Bates, L.S., Waldren, R.P., and Tear, I.D. (1973). Rapid determination of free proline for water stress studies. Plant Soil 39, 205–207. https://doi.org/10.1007/BF00018060.

Bray, E.A., Bailey-Serres, J., and Weretilnyk, E. (2000). Responses to abiotic stresses. In Biochemistry and Molecular Biology of Plants, W. Gruissem, B. Buchannan, and R. Jones, eds. (Rockville, MD: American Society of Plant Physiologists), p. 1158–1249.

Chang, K.L., and Bray, R.H. (1951). Determination of calcium and magnesium in soil and plant materials. Soil Sci. *72*, 449–458. https://doi.org/10.1097/00010694-195112000-00005.

Damodaran, T., Shailendra, R., Kumar, R., Sharma, D.K., Misra, V.K., Jha, S.K., and Rai, R.B. (2013). Post-tsunami collection of polyembryonic mango diversity from Andaman islands and their *ex situ* reaction to high sodium in sodic soil. J. Appl. Hortic. *15*(1), 21–25.

Durán Zuazo, V.H., Martínez-Raya, A., and Aguilar Ruiz, J. (2003). Salt tolerance of mango rootstocks (*Mangifera indica* L. cv. Osteen). Spanish J. Ag. Res. *1*(1), 67–78.

El-Moshaty, F.I.B., Pike, S.M., Novacky, A.J., and Sehgal, O.P. (1993). Lipid peroxidation and superoxide production in cowpea (*Vigna unguiculata*) leaves infected with tobacco ringspot virus or southern bean mosaic virus. Physiol. Mol. Plant Path. *43*, 109–119. https://doi.org/10.1006/pmpp.1993.1044.

Fletcher, R., Gilley, A., Davis, T., and Sankhla, N. (2000). Triazoles as plant growth regulators and stress protectants. Hortic. Rev. *24*, 55–138.

Fricke, W., Leigh, R.A., and Tomos, A.D. (1996). The intercellular distribution of vacuolar solutes in the epidermis and mesophyll of barley leaves changes in response to NaCl. J. Exp. Bot. *47*, 1413–1426. https://doi.org/10.1093/jxb/47.9.1413.

Galán Saúco, V. (2009). Physiological disorders. In The Mango, Botany, Production and Uses (2nd edn), R. Litz, ed. (CAB International), p. 303–316. https://doi.org/10.1079/9781845934897.0303.

Garg, V.K., and Malhotra, S. (2008). Response of *Nigella sativa* L. to fertilizers under sodic soil condition. J. Med. Arom. Plant Sci. *30*, 122–125.

Gazit, S., and Kadman, A. (1980). 13-1 Mango rootstock selection. Hort. Sci. *57*, 81–87.

Giri, B., Kapoor, R., and Mukerji, K.G. (2007). Improved tolerance of *Acacia niloticato* salt stress by arbuscular mycorrhiza, *Glomus fasciculatum* may be partly related to elevated K/Na ratios in root and shoot tissues. Microb. Ecol. *54*, 753–760. https://doi.org/10.1007/s00248-007-9239-9.

Gupta, N.K., and Sen, N.L. (2003). Studies on initial establishment of mango seedling in saline environment. South Indian Hortic. *51*, 106–109.

Hafez, O.M., Saleh, M.A., Ellil, A., and Kassab. O.M. (2011). Impact of ascorbic acid in salt tolerant of some mango rootstock seedlings. J. Appl. Sci. Res. *7*(11), 1492–1500.

Hammerschmidt, R., Nuckles, E.M., and Kuc, J. (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiol. Plant Path. *20*, 73–82. https://doi.org/10.1016/0048-4059(82)90025-X.

Kafkafi, U., and Bernstein, N. (1996). Root growth under salinity stress. In Plant Roots: The Hidden Half, Y. Waisel, A. Eshel, and A. Kafkafi, eds. (New York: Marcel Dekker, Inc.), p. 435–451.

Litz, R.E., and Gomez-Lim, M.A. (2002). Genetic transformation of mango. In Transgenic Plants and Crops, G. Khachatourians, A. McHughern, R. Scorza, W.K. Nip, and Y.H. Hui, eds. (New York: CRC Press), p. 421–436.

Mass, E.V. (1986). Applied Agricultural Research *1*(1) (New York: Springer-Verlag).

Mishra, V.K., Srivastava, S., Jha, S.K., Sharma, D.K., Damodaran, T., Singh, Y.P., and Nayak, A.K. (2017). Temperature induced changes in wheat (*Triticum aestivum*) growth and yield under salt affected environment of Indo-gangetic plains. Arid Land Res. Mgt. https://doi.org/10.1080/15324982.2017.1298684.

Munns, R. (2002). Comparative physiology of salt and water stress. Plant Cell Environ. *25*, 239–250. https://doi.org/10.1046/j.0016-8025.2001.00808.x.

Munns, R., and Tester, M. (2008). Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. *59,* 651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911.

Munns, R., Schachtman, D.P., and Condon, A.G. (1995). The significance of a two-phase growth response to salinity in wheat and barley. Australian J. Plant Physiol. *22*, 561–569. https://doi.org/10.1071/PP9950561.

Pandey, P., Singh, A.K., Dubey, A.K., and Dahuja, A. (2015). Biochemical and salt ion uptake responses of seven mango (*Mangifera indica* L.) rootstocks to NaCl stress, J. Hortic. Sci. Biotechnol. *89*(4), 367–372. https://doi.org/10.1080/14620316.2014.11513094.

Parida, A.K., Das, A.B., and Mohanty, P. (2004). Defense potentials to NaCl in a mangrove, *Bruguiera parviflora*: differential changes of isoforms of some antioxidative enzymes. J. Plant Physiol. *161*, 531–542. https://doi.org/10.1078/0176-1617-01084.

Ram, S., and Rajan, S. (2003). Status report on genetic resources of mango in Asia-Pacific Region (New Delhi: International Plant Genetic Resource Institute, ICAR), pp. 196.

Reuveni, M., Lerner, H.R., and Poljakoff-Mayber, A. (1991). Salinity induced changes in hexokinase activity of carrot cells in suspension culture. Life Sci. Adv. Plant Physiol. *10*, 13–19.

Richards, L.A. (1954). Diagnosis and improvement of saline and alkali soils. Soil Sci. *78*, 154. https://doi.org/10.1097/00010694-195408000-00012.

Shaban, A.E.A. (2010). Comparative study on some polyembryonic Mango rootstock. Agri. Environ. Sci. *7*, 527–534.

Srivastav, M., Dubey, A.K., Singh, A.K., Singh, R., Pandey, R.N., and Deshmukh, P.S. (2009). Effect of salt stress on mortality, reduction in root growth and distribution of mineral nutrients in Kurukkan mango at nursery stage. Indian J. Hortic. *66*, 28–34.

Thomas, J.C., McElwain, E.F., and Bohnert, H.J. (1992). Convergent induction of osmotic stress-responses. Plant Physiol. *100*, 416–423. https://doi.org/10.1104/pp.100.1.416.

Turhan, A., and Seniz, V. (2010). Salt tolerance of some tomato genotypes grown in Turkey. J. Food Agric. Environ. *8*, 332–339.

Watad, A.E.A., Reuveni, M., Bressan, R.A., and Hasegawa, P.M. (1991). Enhanced net K^+ uptake capacity of NaCl-adapted cells. Plant Physiol. *95*, 1265–1269. https://doi.org/10.1104/pp.95.4.1265.

Winston, G.W. (1990). Physiochemical basis of free radical formation in cells: Production and defenses. In Stress Response in Plants – Adaptation and Acclimatization Mechanisms, R.G. Alscher, and J.R. Cumming, eds. (Wiley-Liss Inc., U.K.), p. 57–86.

Wolf, O., Munns, R., Tonnet, M.L., and Jeschke, W.D. (1991). The role of the stem in the partitioning of Na^+ and K^+ in salt-treated barley. J. Ex. Bot. *42*, 697–704. https://doi.org/10.1093/jxb/42.6.697.

Yeo, A.R., Lee, K.S., Izard, P., Boursier, P.J., and Flowers, T.J. (1991). Short and long-term effects of salinity on leaf growth in rice (*Oryza sativa* L.). J. Ex. Bot. *42,* 881–889. https://doi.org/10.1093/jxb/42.7.881.

Zhu, J.K. (2001). Genetic analysis of plant salt tolerance using *Arabidopsis thaliana*. Plant Physiol. *124*, 941–948. https://doi.org/10.1104/pp.124.3.941.

Zieslin, N., and Ben-Zaken, R. (1993). Peroxidase activity and presence of phenolic substances in peduncles of rose flowers. Plant Physiol. Biochem. *31*, 333–339.

Received: Aug. 16, 2017
Accepted: May 22, 2018