



Morphological indicators of salinity stress and their relation with osmolyte associated redox regulation in mango cultivars

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Abstract

Soil salinization is a global issue impeding horticulture production and is approaching an alarming status due to climate change and urbanization. Breeding salt-tolerant rootstock varieties is an ideal strategy to mitigate stress due to salinity in mango and other perennial fruit species. Stress combating strategies employed by seedlings of 7 mango were studied under saline conditions (200 mM NaCl, EC: > 4.0 dSm⁻¹, pH 8.5) in pot experiments. Significantly high accumulation of proline (19.07 µg g⁻¹ FW in Bappakai), glycine betaine (55.11 µg g⁻¹ FW in 13–1), and total sugars (17.33 mg g⁻¹ FW in Kurukkan) were found to be the common mechanism employed by the tolerant cultivars to counter the osmotic stress, under suboptimal conditions. Non-enzymatic antioxidants viz., tannins (17.18 mg g⁻¹), phenols (18.68 mg g⁻¹), and anthocyanins (1.59 mg g⁻¹) were increased in seedling of “13–1”, the salt-resistant cultivar from Israel. Reactive oxygen species (ROS) regulation by increased activity of superoxide dismutase and catalase in the two polyembryonic cultivars of Indian origin (Kurukkan and Nekkare) suggests their potential use as rootstocks to combat oxidative stress. The tolerance index of various cultivars was calculated by averaging the scores of morphological stress indicators, and its correlation with studied parameters suggests that salinity resilience is more tightly linked to enhanced catalase accumulation ($r^2 = 0.8361$) that is reduced ionic stress. This evidence assign the role of osmotic stress alleviation and redox regulation in salt tolerance mechanism operational in native Indian cultivars, Nekkare and Kurukkan at par with known salt tolerant rootstocks.

Keywords Salinity stress · Polyembryonic mango rootstocks · Osmolyte · Non-enzymatic antioxidants · ROS · Correlation

Abbreviations

CAT	Catalase
CRD	Completely randomized design
DMRT	Duncan's multiple range test
EC	Electrical conductivity
FW	Fresh weight
GB	Glycine betaine
H ₂ O ₂	Hydrogen peroxide
HCl	Hydrochloride
NaCl	Sodium chloride
POD	Peroxidase

PPO	Polyphenol oxidase
ROS	Reactive oxygen species
RPM	Revolutions per minute
SOD	Superoxide dismutase
TI	Tolerance index
TSP	Total soluble protein

Introduction

Global extent of salinity and the associated problems are increasing in irrigated arid and semi-arid regions across the world (Wicke et al. 2011; FAO 2021), which imposes substantial costs on food production and environmental sustainability. The deleterious effects of salinity on plant growth are associated with (1) low osmotic potential of the soil thereby causing water stress, (2) nutritional imbalance, (3) specific ion effect (salt stress), and (4) oxidative stress (Arzani and Ashraf 2016). These cause adverse pleiotropic

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effects on plant growth and development at physiological and biochemical levels (Levitt 1980; Munns 2002) as well as, at the molecular level (Winicov 1998; Tester and Davenport 2003). It remains a severe limitation to sustainable crop production in many areas of the top fruit-producing countries like China (Li et al. 2014), India (Singh and Sharma 2017), Brazil (Pessoa et al. 2016), United States (Anonymous 2009), and Spain (Zuazo et al. 2003).

Among fruits, mango is categorized as a highly salt-sensitive species with salt effects at EC lower than 1 ms cm^{-1} (Maas and Grattan 1999). Recent estimates classify nearly 52 M ha of land as salt-affected soils in India including South Asia (Wicke et al. 2011). Sensitivity to saline conditions is characterized in mango by scorched leaf tips and margins, leaf curling, (Maas 1986) and in severe cases reduced growth, abscission of leaves, and death of trees (Jindal et al. 1976). Pot experiment studies showed significant reduction in studied morphological parameters like plant height, stem girth, several leaves, leaf area and specific leaf weight in mango varieties (Deivasigamani et al. 2018). Utilization of salt tolerant rootstock is the strategy for successful fruit production and crop diversification in the problem areas for fruit species like citrus, grapes, mango, olives etc. (Munns 2002). In mango increased soil salinity levels are a serious concern for the establishment of new orchards, cause for tree decline in established orchards, implicated in poor growth, internal fruit breakdown, *Ceratocystis* susceptibility, and fruit productivity. Therefore, in arid and salinity affected areas mango decline and other problems could be attributed to the non-availability of salt-resistant rootstock in mango (Martinez et al. 1999; Schmutz and Ludders 1993). Since information concerning the impact of salinity on fruit yield and quality is meagre, it needs substantiation particularly for Indian varieties of commercial importance and polyembryonic rootstocks (Teaotia and Bhati 1970; Singh and Singh 1976; Durán et al. 2002; Pandey et al. 2014b; Menzel et al. 2017). Polyembryonic cultivar '13/1' was reported to display tolerance to low-quality water, (Kadman et al. 1976; Gazit and Kadman 1983) and is widely used in Israel. South African mango industry continues to utilize 'Sabre' for stabilized fruit production (Menzel et al. 2017). Other rootstocks identified in mango include Gomera-1 and 3, for Osteen Scion variety that is categorized as salt excluders (Zuazo et al. 2003), and *M. zeylanica* that outperformed 13–1 for higher CO_2 assimilation and salt exclusion property (Schmutz 2000). Similarly in India, mango rootstock 'Olour' (Srivastav et al. 2010) and 'Turpentine' were found salinity tolerant (Pandey et al. 2014b). Additionally, irrigation water led to increased Na^+ ion concentrations (Hafez et al. 2011) in the leaves of 'Sukkary' and 'Zebda' mango rootstocks in Egypt. The

beneficial role of Gomera-3 on fruit yield, growth, and mineral nutrition of mango cv. 'Keitt' was recorded (Zuazo and Tarifa 2006) in comparison to rootstocks like 13–1, Gomera 1, and Turpentine. Iyer (2001) emphasized the importance of *M. zeylanica* as a rootstock for saline soils, and (Dinesh et al. 2015) emphasized the use of indigenous cultivars as they would have inbuilt genes which would probably express in varied stress environments. Developing or identifying suitable rootstock harbouring resilience associated traits such as high water use efficiency, drought tolerance, and salt tolerance is important so that the identified rootstock can simultaneously tolerate multiple stresses (Singh and Sharma 2017). The main objectives of the present study were to assess the potential of indigenous polyembryonic cultivars under simulated saline conditions in pot experiments and decipher the tolerance mechanism by corroborating biochemical and enzymatic assays in comparison to '13–1'. Screening of young rootstock seedlings in pot culture studies can indicate stress symptom manifestation in the leaves as root studies are not feasible for large mango canopies. The biochemical and enzymatic responses will further be correlated with recorded morphological symptoms of salinity stress to ascertain most important factor attributing to enhanced tolerance mango cultivars. Focussed research in this area of mango rootstock development, utilizing correlation of biochemical parameters with morpho-physiological responses is needed so that the problem of salinity stress could be mitigated using native germplasm and replicated in rootstock-scion interaction studies.

Materials and methods

Plant material and salt stress induction

Salinity screening pot experiments were conducted on seedlings of seven different mango cultivars viz., polyembryonic cultivars 13–1, Nekkare, Kurukkan, Bappakai, Chandrakaran, Goa and one monoembryonic mango cultivar i.e., Amrapali (scion variety) during April to December 2019; at the net house in ICAR-Central Institute for Subtropical Horticulture, Lucknow, situated at 26.88° N , 80.76° E . All the cultivars originated from India except polyembryonic cultivar 13–1, which is from Israel. The stones of all these cultivars were washed in running tap water and sown under net house conditions immediately after extraction from the fruit during June–July 2018. Seedlings of same age (approximately 10 months-old) were further selected based on their vigour and leaf size, and transplanted in 30 cm diameter of poly-bag (50 microns, capacity: 12 kg) each containing 10.0 kg of potting mixture in the ratio of 1:1:1 (w/w/w) mix of soil, sand,

and well-rotted farmyard manure. All the seedlings were saturated with tap water and properly drained at the start of the experiment set up (before salinity stress imposition). Three seedlings were used in the study, per cultivar for control and stress separately. Salinity stress was imposed by weekly application of 500 ml NaCl (200 mM) to one set of pots (3 replicates per cultivar) to imitate salinity environment (EC: $> 4.0 \text{ dSm}^{-1}$ and $\text{pH} > 8.5$). Changes in the soil pH, soil electrical conductivity (EC in dSm^{-1}), and soil moisture status (gravimetric method) were monitored periodically during the progression of stress and was determined using pH meter (Labman Scientific Instruments, India) and EC meter (Labman Scientific Instruments, India), respectively. Saline solution was reapplied during the experiment to maintain the salinity conditions in the polybags, control plants were also maintained as a check for comparison in all cultivars by applying tap water (pH neutral, $\text{EC} < 2 \text{ dSm}^{-1}$).

Experimental design and sampling data

All plants were arranged in a completely randomized design (CRD) with three replicates, and data for morphological characteristics were recorded up to 60 days, when sensitive cultivars displayed extreme stress symptoms and died.

Plant sample extraction

The leaves were taken from the 3rd and 4th leaf of the plant apex for analysis of biochemical parameters at the start of salinity stress (C) and 21 days after stress (T). One gram of leaf tissue was homogenized with 10 ml of 80% methanol. The homogenate was centrifuged at 6000 rpm for 10 min. The extraction was repeated with 80% methanol. The residue was dissolved in a known volume of distilled water. The supernatant was stored at $-40 \text{ }^\circ\text{C}$ and used for biochemical estimations. All the chemicals used for the biochemical and enzymatic analysis were from Sigma Aldrich and Himedia.

Estimation of osmolytes

Proline (PRO)

The Proline content was estimated by the method of Bates et al. (1973) using aqueous sulfosalicylic acid and absorbance at 520 nm. The standard Proline solutions (0.2–1 mL) corresponding to 2.0–10 μg concentration were also treated as above. All the tests were carried out in triplicates and readings were tabulated.

Glycine betaine (GB)

The amount of Glycine Betaine was estimated by Grieve and Grattan (1983) using cold Potassium Iodide reagent and absorbance were measured at 365 nm. The standard Glycine Betaine solutions (0.2–1 mL) corresponding to 2.0–10 μg concentration were used to tabulate and determine the GB content.

Total soluble sugar

For the estimation of total soluble sugars, the method reported by Yemm and Willis (1954) using cold anthrone solution was followed. The total sugar content was determined by taking the absorbance at 620 nm using glucose as a standard and was expressed as mg per mg of dry weight.

Estimation of non-enzymatic antioxidant

Total phenol content

The amount of total phenol content was estimated by Malik and Singh (1980). Phenol reacts with phosphomolybdic acid in Folin–Ciocalteu reagent to produce a blue colored complex in alkaline medium, which can be estimated spectrophotometrically at 650 nm. The standard Catechol solutions (0.2–1 mL) corresponding to 2.0–10 μg concentration were used for deriving concentrations.

Tannin content

The amount of tannin content was estimated by Price and Butler (1977) using vanillin hydrochloride method and absorbance at 500 nm in a double beam spectrophotometer. The standard catechin solution (0.2–1 mL) corresponding to 2.0–10 μg concentration was also used.

Anthocyanin content

Total anthocyanin content was estimated by the method described by Ranganna (1997) using ethanolic HCl and the absorbance measured at 535 nm using double beam spectrophotometer result was expressed as mg of total Anthocyanin content per 100 mg of fresh weight.

Preparation of crude enzyme extraction and assays

The 4th fully expanded leaf from the plant apical portion was used for the enzyme extraction and samples were stored at $-80 \text{ }^\circ\text{C}$ after harvesting. About 1 g of the leaf tissue was homogenized in 5 mL of 0.1 M phosphate

buffer and centrifuged at 6000 rpm for 10 min. The supernatant was used for further enzymatic assay activity.

Total antioxidant activity and ROS scavenging enzyme activity

SOD (EC 1.15.1.1) activity was determined according to the method of Kakkar et al. (1984), by spectrophotometric determination at 560 nm. Catalase activity was estimated by using the method of Luck (1974) based on the UV absorption of hydrogen peroxide at 240 nm. PPO determination was done by estimation of Catechol oxidase and laccase activities spectrophotometrically at 495 nm (Esterbauer et al. 1997). Peroxidase activity was estimated by the method described by Reddy et al. (1995) through absorbance at 430 nm.

Estimation of tolerance index

Salinity stress symptoms were assigned score from 1 to 10 as given below:

No symptoms-10; Wavy leaf margin-9; Yellowing-8; Flaccid/ wavy leaf margin -7; Burning initiated in leaves-6; Yellowing and Burning of leaves-5; Wavy leaf margin and yellowing spot with Burning-4; Burning of all leaves-3; Leaf Dropping and Burning of all leaves-2; Complete wilting of plant-1.

These scores were used to calculate the tolerance index for each cultivar using the following formula:

$$\text{Stress tolerance index (TI)} = \text{MIc} \times \text{MIs} / \overline{\text{MIc}}^2 \text{ (Fernandez 1992)}$$

where MIc and MIs are the morphological indicators of stress symptoms under control and stress conditions for individual cultivars, respectively. MIc is the mean of the morphological index under control conditions for all the cultivars.

Statistical analysis

The experiment was conducted in a complete randomized block design with three replications. All data were subjected to analysis of variance (ANOVA) to calculate F values using SPSS 22.0 followed by Duncan's multiple range test (DMRT). *P* values ≤ 0.05 were considered as significant. Box and Whisker plots illustrating the variation of the PPO and POD enzyme activity under control and salinity stress were drawn using Past 3.11 (Hammer et al. 2001). The relationship between Tolerance Index with that of various osmolytes and antioxidants was determined through correlation studies using Microsoft Excel (2007).

Results

Accumulation of compatible organic solutes and osmolytes

The experimental materials were exposed to soil salinity stress and utilized to estimate the accumulation of organic osmolytes (Fig. 1) for comparison with control. All the compatible solutes showed an increased accumulation under suboptimal conditions which was statistically significant ($P \leq 0.05$) (21 days after stress), the increasing trend was highest for Bappakai (19.07 $\mu\text{g g}^{-1}$ FW) followed by 13–1 (16.19 $\mu\text{g g}^{-1}$ FW) (Fig. 1a). High proline content in these cultivars indicates enhanced proline synthesis under a salinized environment, coupled with lower proline degradation providing osmoprotection of tissues. Furthermore, salinity induced enhanced accumulation of glycine betaine in seedling of Amrapali (56.8 $\mu\text{g g}^{-1}$ FW) and 13–1 (55.11 $\mu\text{g g}^{-1}$ FW) (Fig. 1b) and total sugars in Kurukkan (17.33 mg g^{-1} FW) (Fig. 1c), suggests central role of GB for overcoming osmotic and ion related stress-induced by enhanced salinity in 13–1, a tolerant type from Israel.

Stress-induced production of non-enzymatic antioxidants

Increased concentrations of phenol, tannin, and anthocyanin showed the effect of salinity led accumulation in leaves as presented in Table 2. Constitutive high phenolic content was recorded in 13–1 (16.18 mg g^{-1}) which also displayed a maximum amount of accumulation due to increased salinity (18.68 mg g^{-1}). Significant variations were recorded for all the studied cultivars for tannin accumulation, wherein seedlings of 13–1 (17.18 mg g^{-1}), Chandrakaran (15.60 mg g^{-1}), and Amrapali (15.58 mg g^{-1}) outperformed other cultivars under saline conditions. The high endogenous phenolic and tannin content in seedling 13–1, may be attributed to its salt-tolerant nature Anthocyanin accumulation, showed a significant increase (DMRT categorization) in salinized conditions in all cultivars, wherein, seedlings of Bappakai (1.71 mg g^{-1}), Chandrakaran (1.61 mg g^{-1}), 13–1 (1.59 mg g^{-1}), and Nekkare (1.55 mg g^{-1}) had highest concentrations.

Regulation of enzymatic antioxidants

Enzymatic antioxidant activities were estimated to ascertain their role in salinity stress response in the studied samples.

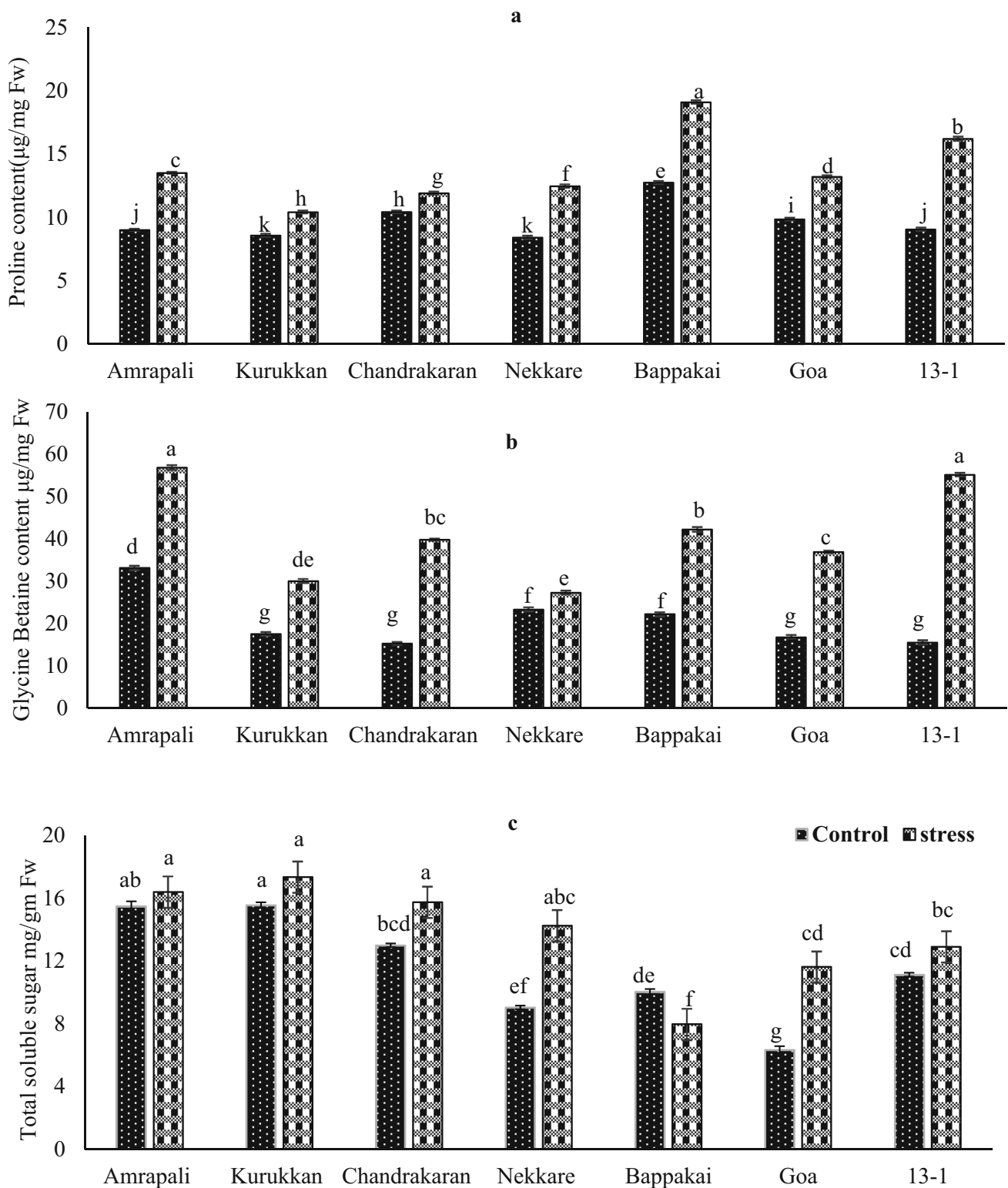


Fig. 1 Enhanced accumulation of (a) Proline (b) Glycine betaine (c) Total Soluble Sugar under salinity stress

SOD The seven cultivars showed significant variations in native SOD activity as recorded under control conditions (Table 2). Constitutive higher SOD activity was recorded

for seedlings 13-1 ($18.45 \text{ U min}^{-1} \text{ mg}^{-1} \text{ TSP}$) and Amrapali ($21.70 \text{ U min}^{-1} \text{ mg}^{-1} \text{ TSP}$) on day 21 of experiment under control conditions. However, stress

imposition (NaCl treatment) elicited enhanced SOD activity in Kurukkan ($16.97 \text{ U min}^{-1} \text{ mg}^{-1} \text{ TSP}$), Nekkare ($15.02 \text{ U min}^{-1} \text{ mg}^{-1} \text{ TSP}$), Bappakai ($22.11 \text{ U min}^{-1} \text{ mg}^{-1} \text{ TSP}$), Goa ($19.20 \text{ U min}^{-1} \text{ mg}^{-1} \text{ TSP}$) and 13–1 ($24.94 \text{ U min}^{-1} \text{ mg}^{-1} \text{ TSP}$). Interestingly SOD activity was reduced for Amrapali and Chandrakaran, the two most susceptible cultivars as per morphological stress indicators.

CAT The effect of salinity stress on catalase activity in seedlings of seven cultivars showed significant variations for CAT activity which showed genotypic variations under controlled and salt stress environments (200 mM NaCl). Amrapali (monoembryonic scion variety), Chandrakaran, and Goa (rootstock polyembryonic cultivar) recorded significantly reduced CAT activity at 21 days sampling on salinity imposition. However, Nekkare ($24.02 \mu\text{M H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1} \text{ TSP}$), Kurukkan ($17.72 \mu\text{M H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1} \text{ TP}$), and 13–1 ($16.23 \mu\text{M H}_2\text{O}_2 \text{ min}^{-1} \text{ mg}^{-1} \text{ TSP}$) recorded significant enhancement, thereby highlighting the role of CAT in salinity stress response (Table 2).

PPO and POD Box and whisker plot displayed enhanced PPO activity due to salinity stress on day 0 itself for all the cultivars. While under control conditions majority of cultivars group below the median, wherein interquartile range was least for control samples on days 7 and 21, the interquartile range was enhanced due to the compromised environment, on days 7 and 21 indicating variable response of cultivars. Lengthening of whiskers and box denotes more variability in PPO activity due to salinity stress (Fig. 2a). Similarly, peroxidase activity in all cultivars was most variable at the start of the experiment as seen by a big box and wide whiskers, the experiment progression up to 21 days displayed plateauing of POD activity as per small box (Fig. 2b). On day 7 and 21 the plot displays a big box with smaller whisker showing more skewness, implying most cultivars to have lesser POD activity than the median range. Overall, under control conditions, the enzyme activities (PPO and POD) displayed variability at the start of the experiment (day 0, implying constitutive enzyme activity), due to the genetic nature of the cultivar, which plateaued on the progression of experiment, while under salinized conditions reverse trend followed that is, variability in enzyme activity enhanced due to stressed environment.

Assessment of salinity tolerance index and its relation with the biochemical parameters

Morphological manifestation of the salinity stress symptoms in poly-bag experiments were recorded in leaves of control and stressed seedlings and used for assigning scores for morphological stress indicators from 1 to 10. Progression of stress symptoms in different varieties was recorded at an interval of 21 days and was used to arrive at the mean

of morphological indicators (score) for control and stress plants (triplicate) upto 60 days respectively. As observed in Fig. 3A seedlings of “Amrapali” which is a monoembryonic commercial variety displayed susceptibility symptoms and “Kurukkan”, an indigenous polyembryonic cultivar displays salinity tolerance. Higher values of MIC for control seedlings indicate absence of salinity stress indicators. While higher values of MIs in seedlings 13-1 (9.00), Nekkare (8.08), and Kurukkan (8.58) denote higher Tolerance Index in comparison to MIs in Amrapali (5.17), while lower value recorded in the seedling Goa (MIs 4.00/TI 0.41) and Amrapali (MIs 5.17/TI 0.55) indicates poor tolerance to salinity (Table 1). Relation of tolerance index with osmolyte and antioxidant parameters indicated the strong positive correlation between TI and Catalase activity ($r^2 = + 0.8361$, $p = 0.02$ significant at 5 df) (Table 2; Fig. 3B), while in case of other biochemical parameters like Phenol, Proline, Glycine betaine, Tannin, and Polyphenol oxidase, the correlation was non-significant (Fig. 3B). Therefore, it could be postulated by the correlation estimates that Catalase is the most common antioxidant enzyme responsible for imparting salinity tolerance in mango cultivars.

Discussion

Salt tolerance in higher plants is regulated by several physiological and biochemical processes and the present study has evaluated the response of important seedlings of polyembryonic mango cultivars to saline conditions through estimations of known parameters to summarise their individual and cumulative effects. Salt-induced problems are known to affect mango and avocado, in as low as 20 mM NaCl (EC: 2 mS cm^{-1}) (Ebert et al., 2002). Salinized soils induce trapping of available water by the salt ions, thereby inducing the plants to accumulate organic (compatible) and inorganic osmolytes for maintaining the water potential. Accumulation of organic osmolytes and compatible solutes is the most commonly known method for adapting to salinized conditions (Rhodes et al. 2002). These compatible solutes include mainly proline (Mundree et al. 2002), (GB) (Khan et al. 1998), and sugars (Kerepesi and Galiba 2000). Previously, an increased level of proline in polyembryonic cultivar “Olour” was implicated in less membrane damage of mango leaves, due to its osmoprotectant nature (Srivastav et al. 2010). Similarly, our results of significantly increased proline accumulation in all seedlings under imposed salinity showed conformity with the findings of (Skorupa et al. 2019) in sugar beet, wheat (Tatar and Gevrek 2008), melon (Akrami and Arzani 2018), pomegranate (Saed 2005), and in *Morus alba* (Ahmad et al. 2007) besides others. Proline is key osmolyte

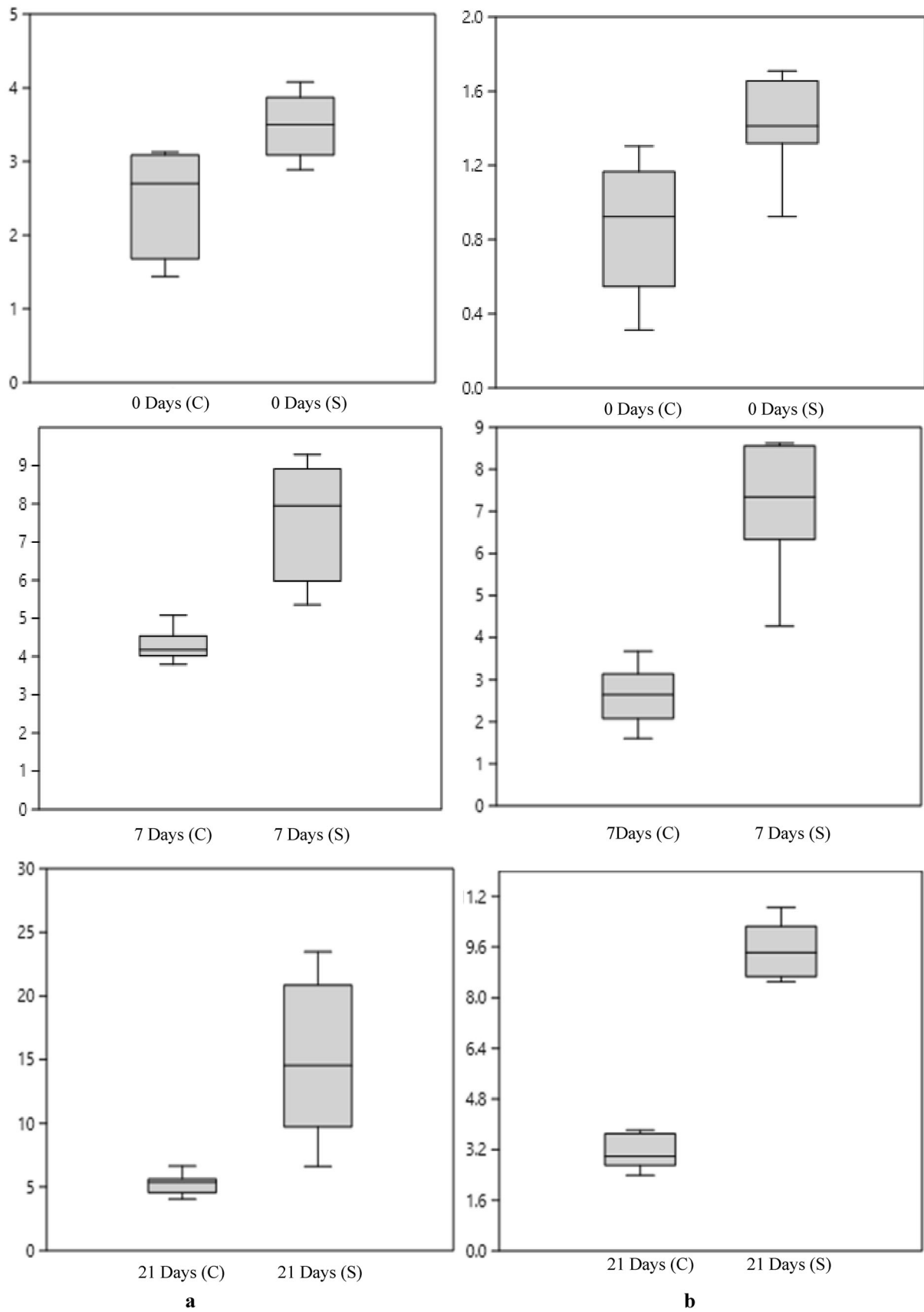


Fig. 2 Box plot illustrating variations in **a** PPO **b** POD due to salinity stress

related to modifications in carbohydrate metabolism (Bartels and Sunkar 2005), and it accumulates in larger amounts than other amino acids in salt-stressed plants (Ali-Dinar et al. 1999). Its accumulation under stress conditions may either be caused by induction or activation of enzymes of proline biosynthesis or a decreased proline oxidation to glutamate, decreased utilization of proline in protein synthesis, and enhanced protein turnover (Delauney and Verma 1993). Other studies have concluded that proline can quench OH radicals under abiotic stress (Smirnoff and Cumbes 1989). Another solute that was produced in a significantly higher amount under stress is glycine betaine (Fig. 1b) which functions as an osmoprotectant and has a crucial role in plant adaptation to osmotic stress through stabilization of the tertiary structure of proteins (Zhu 2002; Munns and Tester 2008). It is one of the most abundant quaternary ammonium compounds produced in higher plants under stressful environments (Yang et al. 2003), and

Table 1 Estimates of Salinity Tolerance index of mango cultivars through stress symptoms evaluation of seedlings

Seedlings of cultivars	Mic	MIs	TI
Amrapali	9.67 ± 0.23	5.17 ± 0.42	0.55
Kurukkan	9.33 ± 0.25	8.58 ± 0.28	0.87
Chandrakaran	9.00 ± 0.11	5.33 ± 0.47	0.52
Nekkare	10.00 ± 0.17	8.08 ± 0.38	0.88
Bappakai	10.00 ± 0.15	4.25 ± 0.48	0.46
Goa	9.33 ± 0.23	4.00 ± 0.49	0.41
13-1	9.67 ± 0.23	9.00 ± 0.27	0.95

Where *Mic* morphological Indicators under control condition, *MIs* morphological indicators under salinity stress, *TI* tolerance index (Fernandez 1992) calculated by formula $MIC^c * MIs / MIC^2$

functions as an enzyme and membrane protectant and stabilizes the PSII protein pigment complexes under stressful conditions (Papageorgiou and Murata 1995). Effect of GB on enhancing salt tolerance of mango seedlings “El-Gahrawey” cultivar grown under salinity stress was demonstrated with Sodium Silicate Pentahydrate

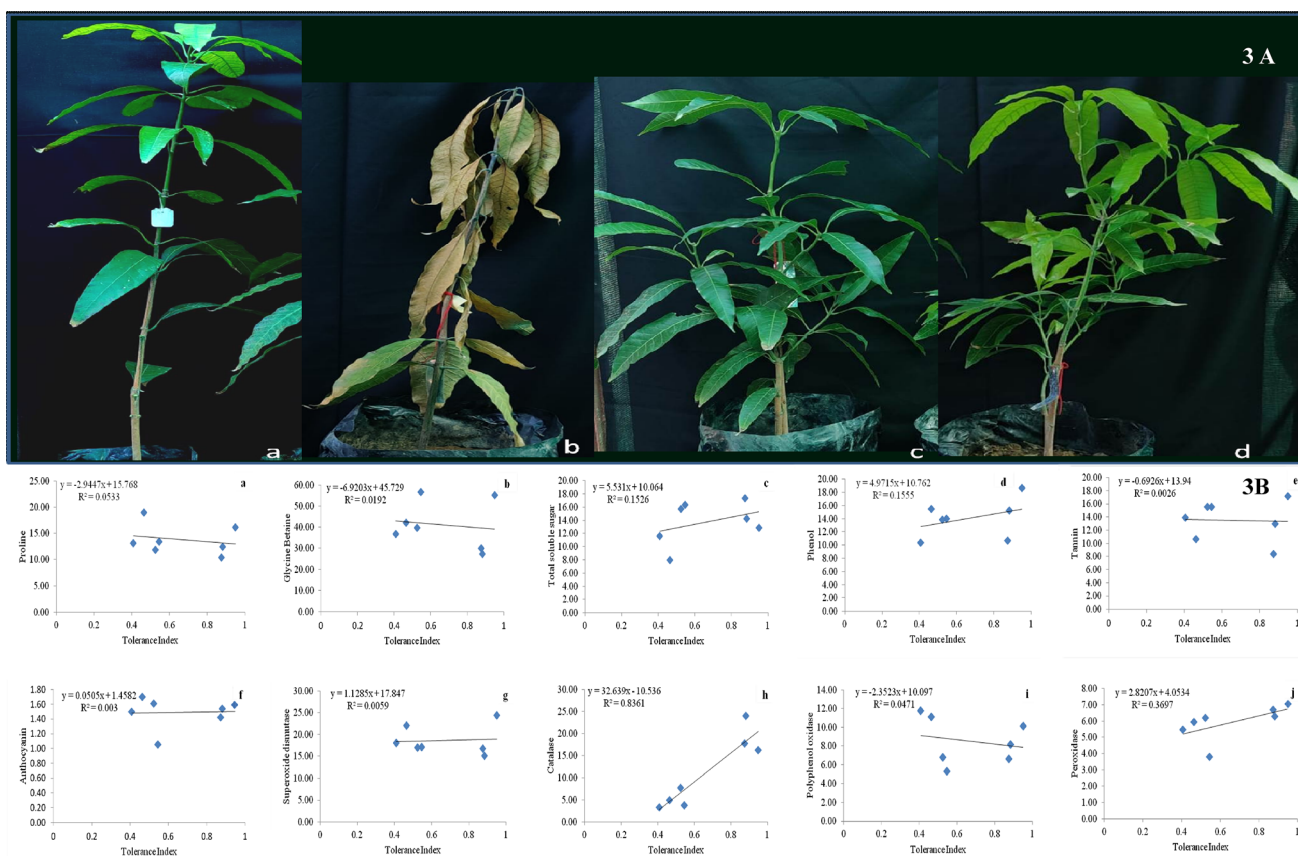


Fig. 3 A Morphological manifestation of salinity stress symptoms on mango seedlings 60 DAS (a) Amrapali control, (b) Amrapali stress, (c) Kurukkan control, (d) Kurukkan stress (DAS: Days after stress); **3 B** Correlation between tolerance index (TI) and (a) Proline

(b) Glycine betaine (c) Total Soluble Sugar (d) Phenol (e) Tannin (f) Anthocyanin (g) Superoxide Dismutase (h) Catalase (i) Polyphenol oxidase (j) Peroxidase

Table 2 Tolerance index (TI), osmolyte and antioxidant content of mango seedlings under salinity stress

Seedling of cultivars	TI	Pro ($\mu\text{g g}^{-1}$ FW)	GB ($\mu\text{g g}^{-1}$ FW)	TSS (mg g^{-1} FW)	Ph (mg g^{-1} FW)	Ta (mg g^{-1} FW)	SOD (Unit/ min)	CAT (Unit/ min)	PPO (Unit/ min)	POD (Unit/ min)
Amrapali	0.55	13.49 \pm 0.08	56.80 \pm 0.57	16.38 \pm 0.13	13.99 \pm 0.36	15.58 \pm 0.16	17.07 \pm 0.45	3.77 \pm 0.12	5.31 \pm 0.43	3.81 \pm 0.50
Kurukkan	0.87	10.40 \pm 0.11	29.99 \pm 0.46	17.33 \pm 0.15	10.68 \pm 0.17	8.39 \pm 0.19	16.71 \pm 0.33	17.73 \pm 0.22	6.61 \pm 0.57	6.71 \pm 0.70
Chandrakaran	0.52	11.89 \pm 0.12	39.76 \pm 0.24	15.73 \pm 0.20	13.85 \pm 0.18	15.60 \pm 0.15	16.93 \pm 0.33	7.79 \pm 0.00	6.75 \pm 0.71	6.20 \pm 0.68
Nekkare	0.88	12.45 \pm 0.13	27.25 \pm 0.45	14.24 \pm 0.17	15.29 \pm 0.22	13.02 \pm 0.13	15.02 \pm 0.35	24.03 \pm 0.27	8.13 \pm 0.81	6.29 \pm 0.71
Bappakai	0.46	19.07 \pm 0.13	42.17 \pm 0.54	7.96 \pm 0.83	15.53 \pm 0.19	10.63 \pm 0.98	22.06 \pm 0.12	5.00 \pm 0.15	11.07 \pm 0.99	5.96 \pm 0.69
Goa	0.41	13.19 \pm 0.11	36.87 \pm 0.29	11.61 \pm 0.10	10.41 \pm 0.29	13.96 \pm 0.23	18.00 \pm 0.36	3.38 \pm 0.12	11.75 \pm 1.07	5.47 \pm 0.64
13-1	0.95	16.19 \pm 0.15	55.11 \pm 0.40	12.89 \pm 0.34	18.68 \pm 0.48	17.18 \pm 0.36	24.39 \pm 0.33	16.23 \pm 0.27	10.13 \pm 0.86	7.04 \pm 0.73
Correlation coefficient		0.0533	0.0192	0.1526	0.1555	0.0026	0.0059	0.8361*	0.0471	0.3697

Whereas Correlation coefficient significant at $p = 0.02$, (df 5) for tolerance index versus. Biochemical parameters; pro- proline, GB glycine betaine, TSS total soluble sugar, Ph phenol content, Ta tannin, An anthocyanin, SOD superoxide dismutase, CAT catalase, PPO polyphenol oxidase, POD peroxidase

(Doaa and Shalan 2020). Furthermore, GB led stress tolerance was associated through increased CAT and SOD activities along with the reduction of cell membrane damage, lipid peroxidation and ion homeostasis pathway (Alasvandyari et al. 2017). GB is known to be synthesized in chloroplast for the protection of thylakoid membranes; thereby helping to maintain photosynthetic efficiency (Dikilitas et al. 2020). The importance of Total Sugar content for salinity tolerance in mango rootstocks cultivars was reportedly due to regulation of photosynthesis, respiration, and carbohydrate metabolism (Allatif et al. 2015), as recorded in our studies also.

Tannin and phenols, a group of secondary metabolites implicated in plant protection and have been recognized for their antioxidant properties, being involved in eliciting the proper response in plants during biotic and abiotic factors (Kiani et al. 2021). These non-enzymatic antioxidants can mediate the scavenging of harmful reactive oxygen species (ROS) in plants under different abiotic stressors by stimulating phenyl propanoid pathway and redox mechanism (Samec et al. 2021). Furthermore, enhanced anthocyanin accumulation as stress response can be corroborated by similar findings in *Arabidopsis* and *Solanum* which was further confirmed by transcriptomic and metabolomic studies (Ben-Abdallah et al. 2016). All the four enzymatic antioxidants in our study elicited increased production and are inferred as the stress response. The increase in the concentration of PPO and POD activity in all seven rootstocks under stress conditions supports the findings of (Pandey et al. 2014a). However, in the same study, Amrapali (raised on Olour rootstock) was reported to have decreased SOD and CAT activity, thereby implying the superiority of Kurukkan, Nekkare, and 13–1 as rootstocks, as they possess additional attributes to counter enhanced salinity through the efficient removal of free radicals and facilitation of normal membrane functioning (Srivastav et al. 2010). Enhanced expression of the POD enzymes results in the increased production of lignin and the associated protective compounds like quinones which ultimately work for reducing the damage induced by oxidative stress (Shigeto et al. 2015).

Some important morphological salinity stress symptoms recorded in the mango seedlings included leaf curling, browning, scorching, etc. as earlier reported by (Maas 1986). Salinity tolerance has been correlated with mean productivity, yield indices, and yield stability in wheat (Singh et al. 2015). In mango, lenticel discoloration was found correlated with PPO, POD, and lipoxygenase (Prasad et al. 2020). Along the same lines, even though enhanced accumulation of osmolytes like proline and glycine betaine was recorded in the polyembryonic cultivars, it was found to be poorly related with morphological indicators of salinity tolerance. The correlation studies indicated that

tolerance mechanism depicted by high score of morphological indicators under salinity stress (MIs) to be correlated with catalase ($r^2 = + 0.8361$), which may be recommended as a strong enzymatic biomarker for tuning redox regulation in mango. Based on tolerance index exhibited by seedlings tested under salinized conditions, the mango cultivars can be graded as sensitive TI: 0.41–0.55, (Goa > Bappakai > Chandrakaran > Amrapali) and tolerant TI: 0.87–0.95, (13–1 > Nekkare > Kurukkan).

Conclusion

Based on the present study, it could be inferred that the salinity stress response can be attributed to the enhanced accumulation of compatible solutes which was statistically significant ($P \leq 0.05$) in most of the cultivars. Both enzymatic and nonenzymatic antioxidants were found overexpressed in the salinity tolerant cultivars viz., Kurukkan, Nekkare, and 13–1. Efficient antioxidative defence i.e., enhanced production of a full set of antioxidant enzymes (SOD, CAT, PPO, and POD) and enhanced synthesis of secondary metabolites anthocyanins, tannins, and phenols under stress conditions may protect cells from ion-induced oxidative damage by binding the ions and thereby showing reduced toxicity on cell organelles. Correlation studies reveal operation of antioxidant enzyme (CAT) as a critical biomarker, which is undoubtedly supported by osmoprotection and ion regulation dynamics. Enhanced levels of osmoprotectants and ROS antioxidants could be probable mechanisms of salinity resilience in the tolerant cultivars viz., Nekkare and Kurukkan. These could be used as candidate salinity tolerant rootstock cultivars with osmoprotection and detoxification mechanisms and should be promoted as potential rootstocks of native Indian origin for their utilization in nursery industry. The research findings provide new leads which need to be assessed by a transcriptome and/or proteome profiling of the identified contrasting salinity response cultivar say, tolerant cultivar (Nekkare or Kurukkan) against the susceptible cultivar (Amrapali) in large salinity screen pot experiments and validation through real time PCR assays.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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