J. Hortic. Sci.
Vol. 18(1) : 150-161, 2023

https://doi.org/10.24154/jhs.v18i1.2158

Original Research Paper

Physio-biochemical responses of polyembryonic mango (*Mangifera indica* L.) genotypes to varying levels of salinity stress

Nimbolkar P.K.[1*], Kurian M.R.[2], Upreti K.K.[3], Laxman R.H.[3] Shivashankara K.S.[3] and Varalakshmi L.R.[4]

[1]Department of Fruit Science, College of Horticulture and Forestry, Central Agricultural University, Pasighat - 791102 Arunachal Pradesh, India
[2]Division of Fruit Crops, [3]Division of Basic Sciences, [4]Division of Natural Resources, ICAR-Indian Institute of Horticultural Research, Bengaluru - 560089, Karnataka, India
*Corresponding author Email : prashantnimbolkar111@gmail.com

ABSTRACT

Mango genotypes that are salinity tolerant can possibly be used as clonal rootstock for sustained production of salinity sensitive commercial mango cultivars in salt affected soils. Present study was carried out to elucidate the effect of salinity stress induced by salts of NaCl+$CaCl_2$ (1:1) at concentrations of 0, 25, 50 and 100 mM on fifteen polyembryonic mango genotypes. The physio-biochemical parameters such as relative water content, chlorophyll content, epicuticular wax content, water potential (Ψ), carbohydrate content, lipid peroxidation, proline accumulation and antioxidant enzymes were determined at each level of salinity in all genotypes. On the basis of these physio-biochemical changes, the study illustrated that the polyembryonic genotypes, Turpentine, Deorakhio, Olour, Bappakkai, Vattam, Nekkare, Kurukkan, Kensington, Muvandan, EC-95862, Manipur, Sabre, Vellaikolamban, Kitchener and Mylepelian were in the decreasing order in response to salinity stress tolerance.

Keywords : Antioxidant enzymes, lipid peroxidation, proline, RWC, salinity tolerance

INTRODUCTION

Mango (*Mangifera indica* L.) is grown in tropical and sub-tropical parts of the world and in India it occupies around 2.217 million hectares with 18.506 million MT production (Abd-Allatif *et al*., 2015). In this era of climate change, various abiotic stresses such as drought, salinity, high/low temperature are becoming serious issues for crop production acting as a principal cause for crop failure, yield reduction and decline in productivity. Among these stresses, salinity is a matter of great concern which covers nearly 6.74 million hectares of agricultural land in India and it might touch 16.2 million hectares by 2050 (Anonymous, 2015). Area under salt affected soils is increasing steadily due to several factors like insufficient precipitation, deforestation, amount of salt concentration in river basins, poor drainage, increasing rate of evaporation *etc*. Mango being a salt sensitive crop, shows scorching of leaf tips and margins, leaf curling and in severe cases growth reduction, low chlorophyll content, increased abscission of leaves and death of trees, particularly at early stages of growth under salinity stress (Srivastav *et al*., 2007). Though several strategies like leaching, good drainage, application of high-quality irrigation water, tillage and amendments of coarse organic matter *etc*. can be applicable to maintain the soil and plant health under saline condition, these are expensive and temporary. Breeding for resistant genotypes could be laborious and complex due to the polygenic nature of salt resistance. If good-quality water or adequate drainage facilities are not available in salt affected soils, the only option is to introduce the salt tolerant crops to make use of such soils.

Of late, use of salt tolerant rootstocks has gained wide attention in management of salinity and sustained production of different fruit crops like mango, citrus and grape. Though rootstocks have widely been studied for manipulating growth and flowering in mango, there is limited information on its use for alleviating adverse effects of salinity. The rootstocks induce salinity tolerance by restricting the movement and/or avoiding absorption and accumulation of toxic ions from the saline soils by undergoing various physio-biochemical changes. However, evaluation of mango rootstocks

This is an open access article distributed under the terms of Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original author and source are credited.

employing physio-biochemical attributes under imposed salinity and assessing the relative tolerance has drawn little attention. In the present investigation, physio-biochemical response of 15 polyembryonic mango genotypes to varying levels of salinity stress was studied with an objective to identify rootstocks exhibiting better salinity tolerance and their further use in grafting for commercial mango cultivation in salt affected soils.

MATERIALS AND METHODS

Plant material and growing conditions

The present study was conducted at ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru during 2015 to 2017, to study the response of different polyembryonic mango genotypes to salinity stress levels. Five months old seedlings of fifteen genotypes *viz*., EC-95862, Bappakkai, Vellaikolamban, Nekkare, Turpentine, Muvandan, Kurukkan, Kensington, Olour, Manipur, Deorakhio, Vattam, Mylepelian, Sabre and Kitchener, raised in polythene bags filled with 1.5 kg mixture of soil, sand and FYM (1:1:1 v/v), were subjected to salinity stress levels by irrigating with 0 mM, 25 mM, 50 mM and 100 mM solutions of NaCl+$CaCl_2$ (1:1, w/w) at regular interval of four days. When visual symptoms of salinity stress as marginal leaf burning started to appear on leaves after 40 days under 100 mM salinity stress, leaf samples at each level of salinity stress were collected from all genotypes for estimation of physio-biochemical parameters. For estimation of antioxidant activities, the fresh leaf samples were ground in liquid nitrogen and preserved at -80°C. The different physio-biochemical parameters were estimated using procedures as described below.

Relative water content (RWC)

RWC was estimated by following the procedure given by Barrs and Wheather (1962). Uniform leaf discs of one centimeter diameter were cut using cork borer and fresh weight of twenty discs was recorded. These discs were placed in petri plates containing 15-20 mL distilled water. After 4-5 hours, turgid weight of the discs was recorded and kept in hot air oven at 60±5°C temperature until constant dry weight was attained. RWC was expressed in *per cent* by using formula RWC = [(Fresh weight - Dry weight) / (Turgid weight - Dry weight)] x 100.

Carbohydrate content

The carbohydrate content of the leaves was estimated using the anthrone reagent method (Hedge and Hofreiter, 1962). Fresh leaf sample of 100 mg was taken into test tube and hydrolysed with 5 mL of 2.5 N HCl and kept in a boiling water bath for three hours and then cooled to room temperature. Solution was neutralized by adding solid Na_2CO_3 until the effervescence ceased. Total volume was then made up to 50 mL with distilled water and supernatant was collected. To 0.5 mL supernatant, 4 mL of anthrone reagent was added and the solution was heated for 8 minutes in a boiling water bath and cooled rapidly. The resultant green to dark green colour was read at 630 nm using UV-Visible spectrophotometer (T80+ UV/VIS, PG Instruments Ltd. UK). The final values were calculated using following formula and expressed in mg/ g fresh weight of sample

$$\text{Carbohydrate content (mg/g)} = \frac{(OD_{630nm} \times \text{Std. value})}{\text{Aliquot took (ml)}} \times \frac{\text{Total vol. of the extract (ml)}}{\text{Wt. of the sample (g)}} \times \frac{100}{1000}$$

Membrane stability index (MSI)

The MSI was determined through *per cent* electrolyte leakage as discussed by Laxman (2014). Ten leaf discs having 10 mm diameter were taken using cork borer and transferred to test tube containing 30 mL distilled water and incubated for 30 minutes at room temperature and initial electrical conductivity (EC_1) was recorded using conductivity meter (model SYSTRONICS; India). The tubes were incubated at 50°C for half an hour and EC_2 was recorded. The test tubes were placed in hot water bath at 100°C for one hour and EC_3 was noted. The MSI was calculated using the following formula MSI= [1-((EC_2-EC_1)/ EC_3)] ×100.

Malondialdehyde content

Leaf tissue weighing 0.25 g was homogenized with 5 mL of distilled water using pestle and mortar. The sample was taken into test tube and 5 mL of thiobarbituric acid (TBA) and 5 mL of trichloroacetic acid (TCA) were added. The contents were heated for 30 minutes in a boiling water bath and later centrifuged at 10000 rpm for 10 minutes. The absorbance of supernatant was read at 532 and 600 nm in UV-Visible spectrophotometer (T80+ UV/VIS, PG Instruments Ltd.). The malondialdehyde (MDA) content was calculated using its extinction coefficient 155 mM cm^{-1} (Heath and Packer, 1968) and formula:

MDA content (nmol g^{-1} FW) = [(A532-A600) ×V×1000/Ɛ] ×W, where Ɛ is the specific extinction coefficient (155 mM cm^{-1}), V is the volume of sample and W is the fresh weight of leaf.

Chlorophyll content

Leaf sample weighing 100 mg was placed into test tube and 10 mL of dimethyl sulphoxide (DMSO) was added. The tubes were then incubated in an oven at temperature 65°C for 3½ hours and the contents filtered. The chlorophyll content was determined using spectrophotometer at wavelengths of 663 and 645 nm, against pure DMSO as blank. The amount of chlorophyll a, chlorophyll b and total chlorophyll in terms of mg g^{-1} fresh weight basis was estimated using the method described by Hiscox and Isrealstam (1979). The values were calculated using formula:

$$\text{Chl a} = \frac{[12.7(A663) - 2.69(A645)] \times V}{W} \times 1000$$

$$\text{Chl b} = \frac{[22.9(A645) - 4.68(A663)] \times V}{W} \times 1000$$

Total chlorophyll = Chl a+Chl b

Where, A = absorbance, V = volume of DMSO solution, W = weight of sample

Epicuticular wax content (ECW)

ECW was estimated using the method described by Ebercon *et al*. (1977) with some modifications. Fully expanded mature leaves were cleaned using cotton to remove dust particles. Three leaf segments (3 cm^2) were immersed in test tube containing 10 mL chloroform and shaken with electronic shaker for 30 seconds. Then chloroform was transferred to another test tube and allowed to evaporate completely. A five mL of acidic $K_2Cr_2O_7$ was added and the solution was heated in boiling water bath for 30 minutes. After cooling, final volume was adjusted to 12 mL with distilled water and optical density was read at 590 nm using UV-VIS spectrophotometer (T80+ UV/VIS, PG Instrument Ltd., UK).

Leaf water potential (ψ)

The Ψ of fully mature and expanded leaf of each genotype was determined instantly after collecting between 11.30 am and 12.30 pm, using pressure bomb apparatus (ARIMAD, v3000 MRC) and expressed in -MPa.

Proline content

Proline content was estimated using rapid colorimetric method (Bates *et al*., 1973). Leaf sample of 0.25 g was extracted by homogenizing in 5 mL of 3% aqueous sulphosalicylic acid followed by centrifugation at 10000 rpm for 10 min at 4^0C Two mL supernatant was collected in test tube, and 2 mL of glacial acetic acid and 2 mL acid ninhydrin were added. The test tubes with sample were heated in the boiling water bath for 1 hour, and then placed in ice bath to terminate the reaction. After addition of 4 mL toluene to the reaction mixture and stirring it for 20-30 seconds using electronic shaker, the toluene layer was separated and warmed at room temperature. The intensity of dark pink color produced was measured at 520 nm with toluene as blank using a UV-visible spectrophotometer (T80+ UV/VIS, PG Instruments Ltd. UK) and expressed in $mg100^{-1}g$ FW.

Antioxidant enzymes activity

Superoxide dismutase (SOD) activity was estimated using the method described by Du and Bramlage (1994). Leaf tissue weighing 0.25 g was homogenized with 5 mL of 0.05 M potassium phosphate buffer (pH 7.8) containing 0.2% PVP, 0.1 mM EDTA and 3 mM $MgCl_2$. Activity was assessed using a reaction mixture containing buffer, 39 mM methionine, 450 µM nitro blue-tetrazolium (NBT), 12 µM riboflavin, 10 mM EDTA and enzyme extract 0.03 mL. The inhibition of photo-oxidation of NBT under fluorescent light was measured at 560 nm and expressed in units of enzyme mg^{-1} of protein. The catalase (CAT) activity was determined using the method described by Masia (1998). Enzyme was extracted from 0.25 g leaf sample using 5 mL of 0.067 M sodium phosphate buffer (pH 7.0) containing 0.2% PVP, 0.1 mM EDTA and 3 mM $MgCl_2$. Activity was measured by the reduction in absorbance at 240 nm in a mixture containing buffer, 0.3 mL H_2O_2 solution and enzyme extract for 5 min at 1 min interval and expressed in units of enzyme $min^{-1}mg^{-1}$of protein. The POX activity was determined as described by Chander (1990). Enzyme was extracted using 5 mL of 0.05 M citrate buffer (pH 6.4) containing 0.2% PVP, 0.1 mM EDTA and 3 mM $MgCl_2$ and the activity was estimated by measuring the H_2O_2 (0.2 mL) dependent oxidation of o-phenylenediamine (0.2 mL) at 450 nm for 5 min at 1 min interval and expressed in unitsμg^{-1} protein. The polyphenol oxidase (PPO) was determined following Selvaraj and Kumar (1995)

using pyrogallol as substrate. Frozen tissue of 0.25 g weight was thoroughly ground with 5 mL of 50 µM citrate buffer (pH 6.8) with 0.2% PVP 0.1 mM and EDTA. The extract was centrifuged at 10000 rpm for 10 minutes and the supernatant was collected. After initiating the reaction by adding pyrogallol, the increase in absorbance was measured at 450 nm for 5 minutes at 1 minute interval and values expressed as OD change $min^{-1}µg^{-1}$ of protein.

Experimental design and statistical analysis

The experiment was laid out in factorial completely randomized design with six plants each genotype under each treatment. The data were analysed by using statistical software SAS 9.3 versionand subjected to the analysis of variance (ANOVA). Significant differences among the genotypes induced by salinity stress were compared using Fisher's test at $P \leq 0.05$. The resulted values mentined in text are per cent increased and decreased over control.

RESULTS AND DISCUSSION

Relative water content (RWC) and carbohydrate content under salinity stress

The RWC of the mango leaves in the plants subjected to different levels of salinity varied from lower (25 mM) to higher (100 mM) salt concentration and indicated decreasing percentage within the range of (1.97 to 28.31%) over control. Turpentine maintained good amount of RWC (13.91%), while, Mylepelian showed maximum reduction (28.31%) at highest salt concentration (100 mM) over control (Table 1). Data indicated increased carbohydrate content with increased levels of salinity. Carbohydrate content increased from 26.90% to 71.63% with 25 mM and 50 mM salt solutions compared to control. Higher carbohydrate content was observed in genotypes Turpentine (82.87%) followed by Deorakhio (82.39%) and Olour (80.68%) compared to Mylepelian (35.90%), Kitchener (38.15%) and Vellaikolamban (47.26%) over control at 100 mM salt. Quantifying the effects of salinity on RWC is required to know the plant water status under salinity stress condition. Reduction of RWC in leaves of mango genotypes imposed with 25 mM, 50 mM and 100mM salinity levels may be because of osmotic stress and cellular dehydration induced by salt, as pointed out by Lata *et al*. (2011) in mango. Increasing level of salinity stress tended to increase the carbohydrate content in leaves of all the mango genotypes. Among the genotypes, maximum increase in carbohydrate content was found in Turpentine while Mylepelian depicted the

Table 1 : Changes in RWC (%) and carbohydrate content (mg g^{-1} FW) in polyembryonic mango genotypes under varying levels of salinity

Genotype	RWC (%)				Carbohydrate content (mg g^{-1} FW)			
	0 mM	25 mM	50 mM	100 mM	0 mM	25 mM	50 mM	100 mM
EC-95862	84.77^{ef}	80.37^{de}	75.94^{efg}	69.55^{defg}	32.33	45.43^{bcd}	47.67^{cdef}	52.33^{cd}
Vattam	88.60^{ab}	85.39^{ab}	81.20^{abc}	75.04^{ab}	35.33	52.60^{abc}	57.70^{abc}	63.00^{ab}
Vellaikolamban	87.66^{bcd}	81.99^{cde}	77.31^{def}	69.20^{efg}	32.23	43.30^{bcd}	44.17^{ef}	47.47^{de}
Nekkare	84.80^{ef}	81.50^{cde}	77.49^{def}	71.61^{cde}	33.33	49.17^{abc}	53.90^{abcde}	58.33^{bc}
Mylepelian	86.82^{cd}	79.86^{e}	73.10^{g}	62.24^{h}	30.73	39.00^{d}	39.83^{f}	41.77^{e}
Turpentine	87.36^{bcd}	85.63^{a}	81.62^{a}	75.21^{a}	37.37	58.57^{a}	64.13^{a}	68.33^{a}
Sabre	84.44^{f}	79.22^{e}	74.82^{fg}	68.11^{fg}	31.37	43.03^{cd}	44.43^{def}	48.73^{de}
Manipur	88.50^{ab}	83.43^{abcd}	78.97^{abcde}	71.52^{cde}	30.67	43.07^{cd}	44.43^{def}	49.20^{de}
Kitchener	88.28^{bc}	82.18^{bcde}	76.42^{ef}	66.66^{g}	34.17	44.73^{bcd}	45.47^{def}	47.20^{de}
Kensington	89.90^{a}	85.99^{a}	81.80^{a}	74.86^{ab}	31.97	45.10^{bcd}	47.67^{cdef}	53.97^{cd}
Olour	84.89^{ef}	82.30^{bcde}	78.22^{cde}	72.17^{bcd}	33.13	50.23^{abc}	55.77^{abcd}	59.87^{bc}
Kurukkan	84.71^{ef}	81.11^{cde}	77.31^{def}	70.99^{cdef}	31.03	45.77^{bcd}	49.97^{bcdef}	54.30^{cd}
Bappakkai	88.56^{ab}	85.74^{a}	81.39^{ab}	75.17^{a}	35.63	53.33^{ab}	59.87^{ab}	64.07^{ab}
Muvandan	87.28^{bcd}	83.33^{abcd}	78.52^{bcde}	71.78^{cde}	31.53	44.47^{bcd}	46.67^{cdef}	52.97^{cd}
Deorakhio	86.20^{de}	83.75^{abc}	79.68^{abcd}	73.37^{abc}	37.30	57.67^{a}	63.13^{a}	68.03^{a}
S.E.m(±)	0.74	1.56	1.48	1.42	3.26	3.43	3.87	2.59
LSD ($P \leq 0.05$)	1.52	3.21	3.05	2.92	NS	10.04	11.41	7.63

least increase over control. Increased carbohydrate content under salinity might be indicative of stress adaptation as it not only functions as osmoprotectant but also helps in osmotic adjustment, carbon storage and radical scavenging which could be altered under salinity stress. Singh *et al*. (2000) also reported increase in CHO (glucose, fructose, sucrose and fructans) under salinity stress in grape cvs. Perlette, Pusa Seedless and Beuty Seedless.

Amount of malondialdehyde (MDA) and Membrane stability index (MSI) under salinity stress

At lower salt concentration (25 mM), least amount of MDA content was recorded in Turpentine (10.20%), whereas, maximum was recorded in Mylepelian (29.20%) (Table 2). At 50 mM and 100 mM salt stress also, similar trends were observed. MSI reduced with increase in salt concentration (Table 2). In Mylepelian at 25 mM and 50 mM level of salinity stress, MSI was reduced to15% and 25%, respectively, and in Turpentine only 1% and 3%, respectively compared to 0 mM salt stress. The genotypes Turpentine, Deorakhio and Olour shown better membrane stability (16.09%, 19.51% and 19.72% reduction, respectively) while Mylepelian, Kitchener and Vellaikolamban had more reduction (36.75%, 35.88% and 33.80%, respectively) at 100 mM salt concentration when compared with 0 mM concentration. MDA content serves as an indicator for amount of cell damage during osmotic stress induced by salinity. Lower accumulation of MDA content in salt tolerant genotypes may be due to the potential of genotypes to protect cell damage during salinity stress. Oxidative stress, induced by saline condition, leads to the formation of lipid peroxidation in the form of MDA has been used as essential marker or indication for identifying the amount of cell damage (Tayebimeigooni, 2012). Study conducted by Dayal *et al*. (2014) also depicted the increase in accumulation of content in NaCl stressed Amrapali, Kurrukan and Olour mango plants. Higher membrane stability and lower level of MDA in genotypes Turpentine, Deorakhio and Olour might be due to the increased activities of antioxidant enzymes (POX, CAT, SOD and PPO) which serve as a protective mechanism against oxidative stress. The maintenance of membrane stability under salinity stress might be associated to the less MDA accumulation and thereby less membrane injury which can be considered as an effective parameter for finding the tolerance and sensitive nature of plant under salt stress. The results on MSI are in conformity with the findings of Gora *et al*. (2017) where MSI in monoembryonic and polyembryonic cultivars of mango drastically

Table 2 : Changes in MDA (nmoles g^{-1} FW) and MSI (%) in polyembryonic mango genotypes under varying levels of salinity

Genotype	MDA (nmoles g^{-1} FW)				MSI (%)			
	0 mM	25 mM	50 mM	100 mM	0 mM	25 mM	50 mM	100 mM
EC-95862	25.03^{abcd}	30.24^{bcde}	33.44^{def}	40.52^{c}	89.25	79.29^{bcd}	75.46^{de}	65.38^{d}
Vattam	23.27^{cd}	27.05^{efg}	30.11^{hi}	35.57^{fg}	89.95	86.24^{ab}	80.42^{bc}	71.07^{b}
Vellaikolamban	27.29^{ab}	33.55^{ab}	37.63^{ab}	47.01^{b}	89.87	77.56^{cd}	71.86^{fg}	59.49^{fg}
Nekkare	24.26^{bcd}	28.24^{defg}	31.51^{fgh}	37.12^{ef}	90.23	84.68^{abc}	78.22^{cd}	69.99^{bc}
Mylepelian	27.76^{a}	35.87^{a}	38.75^{a}	51.57^{a}	87.53	73.76^{d}	65.58^{h}	55.36^{g}
Turpentine	23.18^{cd}	25.55^{g}	28.47^{i}	33.89^{g}	90.62	88.85^{a}	87.05^{a}	76.03^{a}
Sabre	26.15^{abc}	32.13^{bc}	35.73^{bcd}	44.62^{b}	89.75	79.10^{bcd}	72.76^{ef}	61.04^{ef}
Manipur	27.38^{a}	33.55^{ab}	37.12^{abc}	45.68^{b}	89.79	79.54^{bcd}	73.09^{ef}	65.19^{de}
Kitchener	25.16^{abcd}	31.87^{bcd}	35.10^{cde}	44.60^{b}	89.67	77.29^{cd}	68.38^{gh}	57.49^{fg}
Kensington	23.44^{cd}	27.87^{efg}	30.71^{ghi}	36.90^{ef}	89.98	80.95^{abcd}	77.19^{cd}	66.75^{cd}
Olour	23.48^{cd}	26.28^{fg}	29.72^{hi}	35.48^{fg}	90.82	88.30^{a}	85.95^{a}	72.91^{ab}
Kurukkan	24.17^{cd}	28.69^{cdefg}	31.42^{fgh}	37.59^{def}	90.25	82.23^{abcd}	78.02^{cd}	68.96^{bcd}
Bappakkai	26.13^{abc}	29.48^{cdef}	33.38^{def}	39.83^{cd}	89.97	86.67^{ab}	83.60^{ab}	71.46^{b}
Muvandan	24.88^{abcd}	29.85^{bcdef}	32.99^{efg}	39.18^{cde}	89.80	80.52^{abcd}	76.08^{de}	65.81^{cd}
Deorakhio	22.90^{d}	25.31^{g}	28.43^{i}	34.15^{g}	90.77	88.65^{a}	86.50^{a}	73.05^{ab}
S.E.m(±)	1.52	1.81	1.21	1.31	0.968	4.23	1.75	2.05
LSD ($P \leq 0.05$)	3.11	3.71	2.48	2.69	NS	8.67	3.59	4.21

decreased with increasing NaCl stress. The maintenance of high MSI in Turpentine, Deorakhio and Olour pointed out their ability to maintain good amount of cell turgidity by improving their RWC and water potential compared to Mylepelian, Vellaikolamban and Kitchener, which depicted more reduction in MSI with increasing levels of salinity.

Leaf chlorophyll status and proline accumulation in response to salinity stress

There was variation in chlorophyll content among all the genotypes under salt stress (Table 3). The reduction in chlorophyll 'a' (2.18% to 31.80%), chlorophyll 'b' (11.01% to 34.85%) and total chlorophyll (4.59% to 32.52%) content among all genotypes which imposed with 25 mM and 50 mM salt concentrations. The genotypes Turpentine (15.32%), Deorakhio (20.87%) and Olour (22.50%) responded with less reduction in chlorophyll 'a' content, while, Mylepelian (51.42%), Kitchener (51.23%) and Vellaikolamban (47.03%) showed more reduction. Similar trend was recorded with relation to chlorophyll 'b' and total chlorophyll content (Table 3) in the same genotypes at higher level of salinity stress over control. It was noticed that the proline content increased with increase in salt concentration from 25 mM to 100 mM and it varied significantly among the genotypes under each level of salinity (Fig. 1). Higher proline accumulation in leaves of genotypes Turpentine (66.40%) and Deorakhio (64.39%) was recorded at 100 mM salt concentration, whereas, genotypes Mylepelian (48.65%) and Kitchener (52.22%) were found to limits the accumulation of proline at the same salinity stress. Decrease in chlorophyll content with increased salinity stress could be due to adverse effect of salinity on chlorophyll metabolism leading to its decline. Another cause for reduction in chlorophyll content could be variation in chlorophyll synthesis linked to the induction of 'chlorophyllase' a chlorophyll degrading enzyme in a plant species under saline conditions (Gunes *et al*., 2007). Proline is considered as a compatible osmolyte involved in carbon and nitrogen storage that helps in stress adaptation. In the present study, the extent of proline accumulation was influenced in mango genotypes by salinity stress. The maximum increased accumulation of proline over control was in cv. Turpentine and this could be an indication for its ability to combat the adverse effect of salinity stress through osmotic adjustment, reactive oxygen species (ROS) scavenging or by enhancing the anti-oxidant activity (Sharma and Dietz, 2006; Patel *et al*., 2011).

Epicuticular wax (ECW) deposition and leaf Ψ under salinity stress

ECW depositions on leaf surface varied across the genotypes at different levels of salt concentration. The ECW content increased as salt stress increased. Higher amount of ECW deposition was recorded in Turpentine at 25 mM and 50 mM, whereas, less content in Mylepelian. At higher salt concentration (100 mM), maximum increase of ECW was observed in Turpentine (91.28%) followed by Deorakhio (89.92%) and Olour (85.33%), whereas, minimum (50.47% to 62.35%) was recorded in Mylepelian, Kitchener and Vellaikolamban (Fig. 2). The Ψ in leaves generally declined with increasing salinity stress (Fig. 3). Among the fifteen genotypes

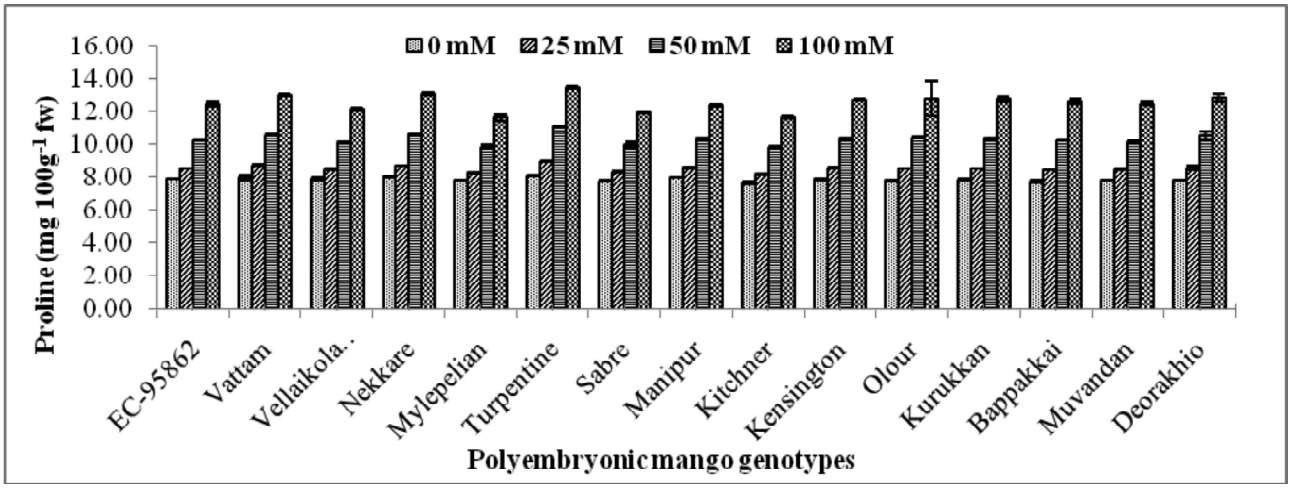

Fig. 1 : Changes in proline content (mg 100g $^{-1}$FW) in polyembryonic mango genotypes under varying levels of salinity

Table 3 : Changes in chlorophyll 'a', chlorophyll 'b' and total chlorophyll (mgg^{-1} FW) in polyembryonic mango genotypes under varying levels of salinity

Genotype	Chlorophyll 'a'				Chlorophyll 'b'				Total chlorophyll			
	0 mM	25 mM	50 mM	100 mM	0 mM	25 mM	50 mM	100 mM	0 mM	25 mM	50 mM	100 mM
EC-95862	1.71	1.55^{c}	1.40^{bcde}	1.10^{cdef}	0.88	0.67^{abc}	0.64	0.50	2.59	2.22^{cde}	2.04^{cdef}	1.60^{cdefg}
Vattam	1.67	1.58^{c}	1.46^{bcde}	1.25^{bcdef}	0.69	0.56^{c}	0.54	0.47	2.36	2.14^{def}	2.01^{cdef}	1.72^{cde}
Vellaikolamban	1.54	1.34^{d}	1.14^{e}	0.82^{f}	0.89	0.63^{abc}	0.61	0.49	2.43	1.97^{f}	1.75^{f}	1.31^{fg}
Nekkare	1.94	1.81^{b}	1.68^{abc}	1.46^{abc}	0.77	0.61^{bc}	0.60	0.51	2.71	2.42^{bc}	2.28^{bc}	1.97^{abc}
Mylepelian	2.00	1.65^{bc}	1.37^{cde}	0.97^{def}	0.62	0.41^{d}	0.40	0.31	2.62	2.06^{ef}	1.77^{f}	1.28^{g}
Turpentine	2.10	2.05^{a}	1.97^{a}	1.77^{a}	0.79	0.70^{ab}	0.69	0.59	2.88	2.75^{a}	2.66^{a}	2.37^{a}
Sabre	1.77	1.56^{c}	1.34^{de}	0.99^{cdef}	0.92	0.66^{abc}	0.63	0.52	2.69	2.22^{cde}	1.97^{def}	1.51^{defg}
Manipur	1.70	1.51^{cd}	1.32^{de}	1.02^{cdef}	0.86	0.64^{abc}	0.60	0.49	2.56	2.15^{def}	1.92^{ef}	1.51^{defg}
Kitchener	1.84	1.56^{c}	1.30^{de}	0.90^{ef}	0.90	0.63^{abc}	0.60	0.47	2.74	2.19^{cdef}	1.90^{ef}	1.37^{efg}
Kensington	2.02	1.84^{b}	1.72^{ab}	1.38^{abcd}	0.97	0.75^{a}	0.72	0.59	2.99	2.59^{ab}	2.44^{ab}	1.97^{abc}
Olour	1.75	1.68^{bc}	1.61^{bcd}	1.36^{abcde}	0.78	0.66^{abc}	0.66	0.57	2.54	2.35^{cd}	2.27^{bcd}	1.93^{bcd}
Kurukkan	2.26	2.07^{a}	1.95^{a}	1.65^{ab}	0.88	0.70^{ab}	0.67	0.56	3.14	2.76^{a}	2.62^{a}	2.20^{ab}
Bappakkai	1.74	1.65^{bc}	1.56^{bcd}	1.33^{abcde}	0.77	0.63^{abc}	0.63	0.54	2.51	2.28^{cde}	2.19^{bcde}	1.87^{bcd}
Muvandan	1.72	1.56^{c}	1.42^{bcde}	1.13^{cdef}	0.94	0.72^{ab}	0.69	0.57	2.66	2.28^{cde}	2.11^{cde}	1.70^{cdef}
Deorakhio	1.54	1.48^{cd}	1.43^{bcde}	1.22^{bcdef}	0.79	0.68^{abc}	0.68	0.59	2.33	2.17^{def}	2.11^{cde}	1.81^{bcd}
S.E.m(±)	0.408	0.100	0.159	0.227	0.224	0.067	0.11	0.141	0.337	0.115	0.152	0.203
LSD ($P \leq 0.05$)	NS	0.205	0.326	0.465	NS	0.136	NS	NS	NS	0.234	0.310	0.415

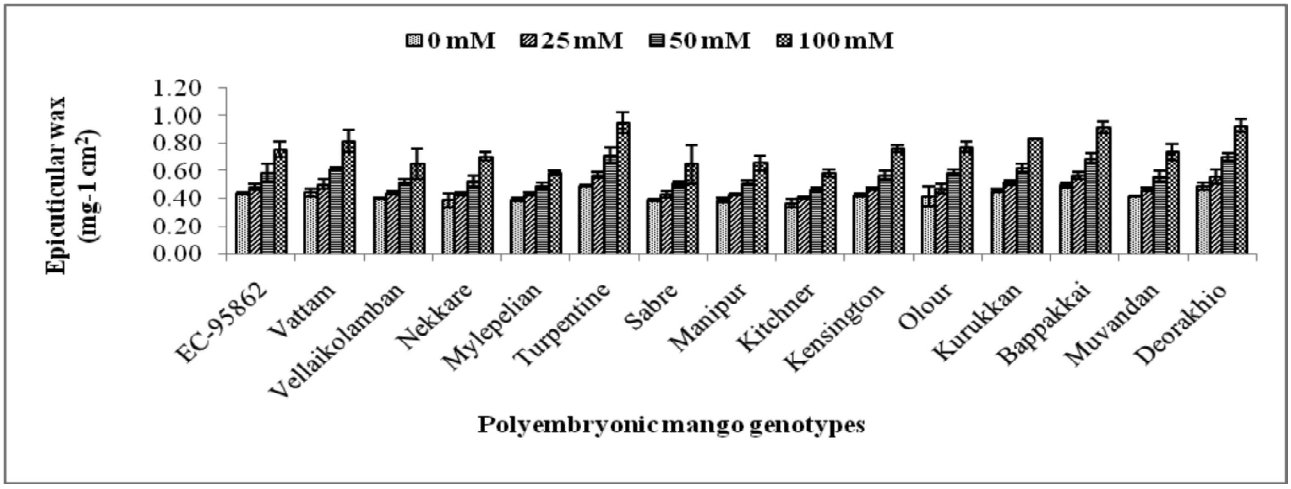

Fig. 2 : Changes in epicuticular wax content ($mg^{-1}cm^2$) of polyembryonic mango genotypes under varying levels of salinity

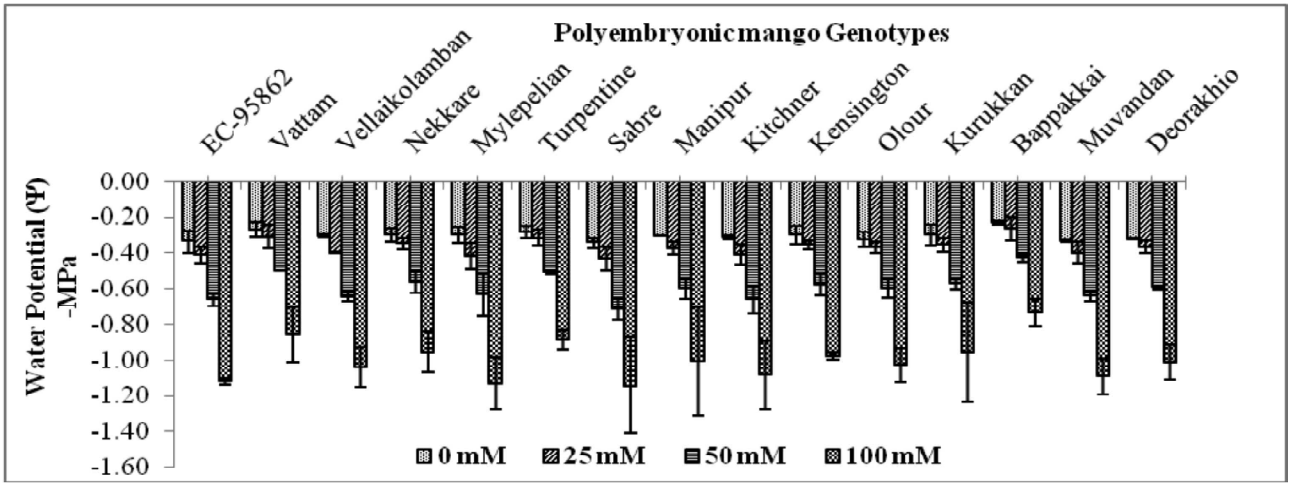

Fig. 3 : Changes in Ψ (-MPa) in leaves of polyembryonic mango genotypes under varying levels of salinity

it was varying from (-0.267 to -0.433 MPa) in 25 mM and (-0.427 to 0.717 MPa) in 50 mM salinity stress. The genotypes Turpentine, Deorakhio and Olour showed less reduction in Ψ (212.94% to 215.31%) while Mylepelian, Kitchener and Vellaikolamban exhibited more reduction (276.67% to 239.13%) in Ψ at higher level of salt concentration (100 mM) over control. The epicuticular wax serves as protective layer and helps in regulating the gas exchange, leaf temperature and light reflectance properties under abiotic stress conditions which imparts tolerance (Mansour, 2007). ECW deposition during the stress condition is important for balancing the transpiration and overheating of leaves which affect the photosynthesis. Though non-significant differences among the genotypes with respect to ECW, the rate of deposition on the cuticular surface was found to increase with increase in salinity stress. This adaptive mechanism can relate to the tolerance of genotypes *viz.*, Turpentine, Deorakhio and Olour, which exhibited the maximum increased ECW content over control in comparison to other rootstocks (Mylepelian, Kitchener and Vellaikolamban). Leaf Ψ became more negative as level of salinity increased. High osmotic pressure induced by salinity stress adversely affects the ability of plant cells to uptake water and nutrients. The study of Fozouni *et al*. (2012) has recently confirmed that leaf Ψ reduced under salinity stress in grape cv. Dastarchin and red Sultana. Maintenance of high water potential under salinity stress is evidence for salt tolerance nature of Turpentine, Deorakhio and Olour. This ability might be generated through multi-factors like greater maintenance of RWC and MSI, higher accumulation of proline *etc*.

Antioxidant enzymes activity

Peroxidase (POX) activity increased as the level of salt oncentration increased compared to control plants (Fig. 4). It was in the ranged 6.00% to 14.04% under 25 mM and 19.77% to 37.82% in 50 mM treated plants compare to control. When treated with 100 mM salt concentration, the genotypes Turpentine, Deorakhio and Olour had higher activity of POX (89.23%, 85.02% and 83.85%, respectively) over control. However, a least increase in POX activity was recorded in Mylepelian, Kitchener and Vellaikolamban (46.19%, 51.10% and 56.66%, respectively). There was significant variation in terms of SOD activity, among the genotypes under different levels of salinity (0 mM, 25 mM, 50 mM and 100 mM). Activity of SOD was in the range of 14.24 to 18.35 units mg^{-1} of protein under control (0 mM), (Fig. 5). Maximum increase in SOD activity was recorded in Turpentine (22.22%) and minimum in Mylepelian (11.06%) at 100 mM level. Salinity at each level influenced the CAT activity in all genotypes (Fig. 6). CAT activity increased at 25 mM and 50 mM from (2.82% to 4.05%) in Mylepelian, whereas, in Turpentine it increased from 13.73% to 24.71%. Higher CAT activity was recorded in Turpentine (40.35%), Deorakhio (36.46%) and Olour (34.59%) against 9.73% in Mylepelian at 100 mM salt concentration. Activity of PPO increased with increase in salt concentration (Fig. 7). Maximum increase in PPO activity (122.05%, 105.49% and 105.22%) was noted at 100 mM salinity stress in the leaves of Turpentine, Deorakhio and Olour genotypes respectively, while, minimum increase (38.57%, 48.01% and 50.43%) was recorded in Mylepelian, Kitchener and Vellaikolamban, respectively. Similar trend of marginal increase in PPO activity was examined in 25 mM and 50 mM salinity stress-imposed plants. Generally, stress condition triggers generation of ROS such as O_2-, OH, $_1O^2$ and H_2O_2 which translocate the stress signals to different plant parts activating enzymatic and non-enzymatic antioxidant machineries, which subsequently stand against this adverse condition. POX, SOD, CAT and PPO are part enzymatic antioxidants defense system which has crucial role for mitigating the adverse effects of biotic and abiotic stresses. SOD an antioxidant enzyme, plays important role in dismutation of superoxide anion radicals and scavenging activity of cell against ROS under stress

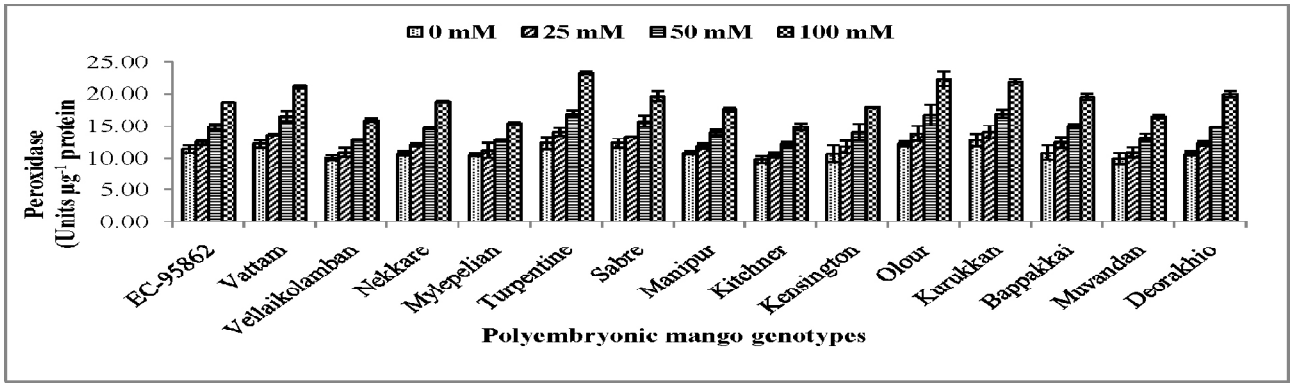

Fig. 4. Changes in POX activity of polyembryonic mango genotypes under varying levels of salinity

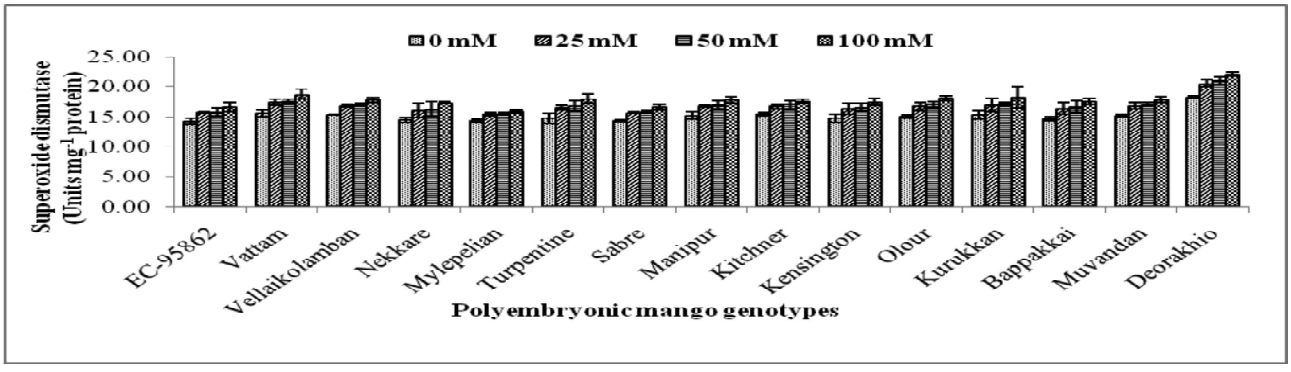

Fig. 5 : Changes in SOD activity of polyembryonic mango genotypes under varying levels of salinity

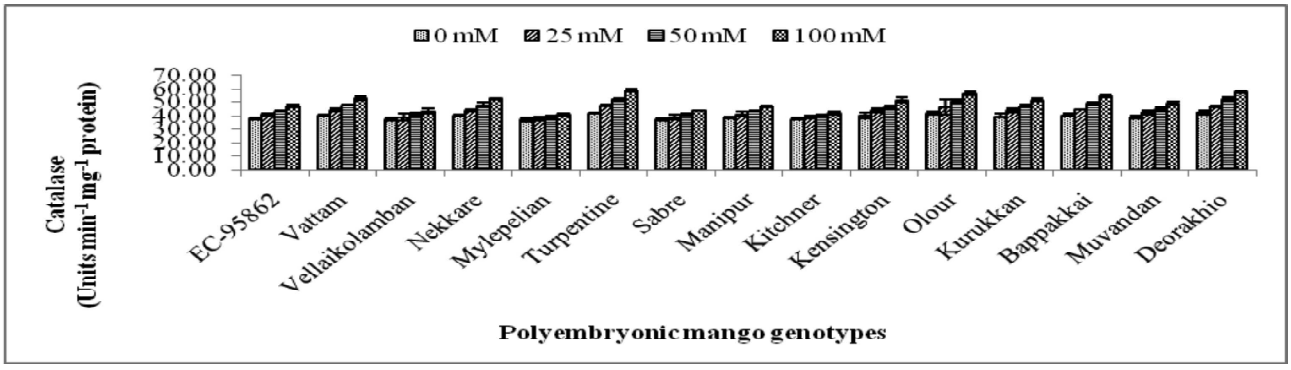

Fig. 6 : Changes in CAT activity of polyembryonic mango genotypes under varying levels of salinity

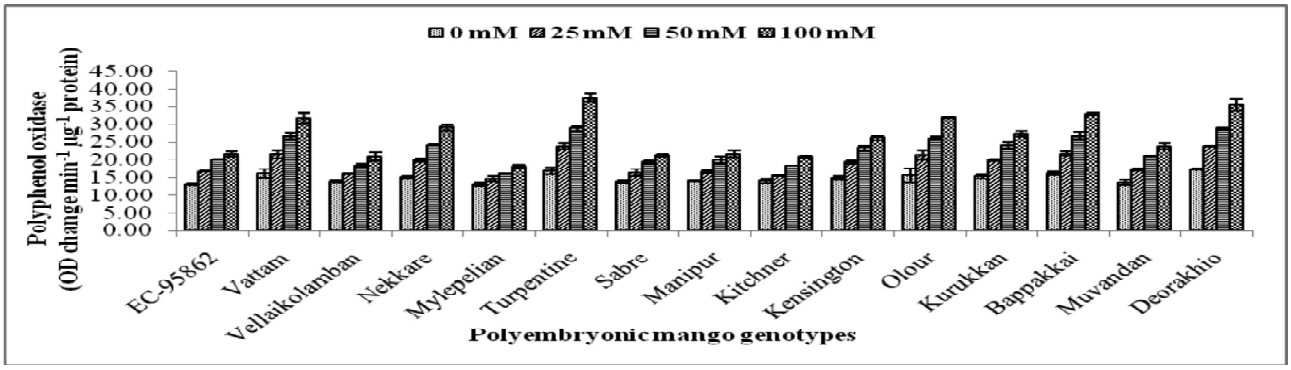

Fig. 7 : Changes in PPO activity of polyembryonic mango genotypes under varying levels of salinity

conditions. The increase in POX activity under salinity induced stress in rootstock-scion combinations of mango genotypes also reported by Dayal *et al*. (2014). The results are also in support of the findings of Rahnama and Ebrahimzadeh (2005) where the activity of SOD increased in salt tolerant cultivars of the potato. Increase in CAT activity plays a defence role against the accumulation of H_2O_2 under stressed condition by overcoming potential damage to leaf tissues. The genotypes depicted the higher percent of CAT activity under increasing salinity stress found better tolerance nature. Results yielded similar findings as that of Pandey *et al*. (2014) who showed increase in CAT activity with higher level of salinity in seven mango rootstocks. The higher activity of PPO also found under salinity stress which helps to imparts defense mechanism against salinity stress in mango (Abd-Allatif, 2015). With consideration to overall enzymatic study the activity of CAT, SOD, POX and PPO increased with salinity stress among all the mango genotypes. Maximum *per cent* increase over control was observed in seedlings of tolerant genotypes such as Turpentine, Deorakhio and Olour over the sensitive ones (Mylepelian, Kitchener and Vellaikolamban), in which minimum activities of the same antioxidant enzymes were noticed.

CONCLUSION

The polyembryonic mango genotypes under varying levels of salinity depicted that Turpentine, Olour, Deorakhio, Bappakkai and Vattam were found to respond with less reduction in RWC, chlorophyll 'a'and total chlorophyll content, and with more increase in leaf epicuticular wax, carbohydrate, proline, CAT, SOD, POX and PPO and less increase in lipid peroxidation (MDA). However, there were no significant differences in chlorophyll 'b' content and leaf Ψ at each level of salinity across the all genotypes. Physio-biochemical parameters such as proline content, antioxidant enzymes activity RWC and leaf ECW were the prominent biochemical markers for assessment of the salt tolerance of mango genotypes. Based on the overall performance pertaining to physio-biochemical changes under different levels of salinity, the genotypes in decreasing order of salt tolerance are Turpentine, Deorakhio, Olour, Bappakkai, Vattam, Nekkare, Kurukkan, Kensington, Muvandan, EC-95862, Manipur, Sabre, Vellaikolamban, Kitchener and Mylepelian. Among these genotypes, Turpentine,

Deorakhio, Olour and Bappakkai were performed better in response to their physio-biochemical behavior at higher level of salinity which could be potential rootstocks for salt sensitive commercial mango cultivars.

ACKNOWLEDGEMENT

Authors are thankful to the Director, ICAR-Indian Institute of Horticultural Research, Bengaluru, India for providing the requisite facilities during the course of research work. First author would like to thank UGC, New Delhi for granting Fellowship for pursuance of Ph.D. research work.

REFERENCES

Abd-Allatif, A.M., El Kheshin M.A., Rashedy, A. A. 2015. Antioxidant potential of some mango (*Mangifera indica* L.) cultivars growing under salinity stress. *Egypt J. Hortic.*, **42**(2): 654-665.

Anonymous. 2015. CSSRI Vision 2050, Indian Council of Agricultural Research (ICAR)-Central Soil Salinity Research Institute (CSSRI), Karnal-132001, India, pp. 1-48.

Barrs, H. D. and Wheatherl, P. E. 1962. A re-examination of relative turgidity for estimating water deficit in leaves. *Aust. J. Biol. Sci.*, **15**: 413-428.

Bates, L.S., Waldren, R. P. and Teare, I.D. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil.*, **39**: 205-207.

Chander, S. 1990. Enzymatic associations with resistance to rust and powdery mildew in peas. *Indian J. Hortic.*, **47**: 341-345.

Dayal, V., Dubey, A. K., Awasthi, O. P., Pandey R. and Dahuja, A. 2014. Growth, lipid peroxidation, antioxidant enzymes and nutrient accumulation in Amrapali mango (*Mangifera indica* L.) grafted on different rootstocks under NaCl stress. *Plant Know J.*, **3**(1):15-22.

Du, Z. and Bramlage, W. J. 1994. SOD activities in senescing apple fruit (*Malus domestica* Borkh.). *J. of Food Sci.*, **59**: 581-584.

Ebercon, A., Blue, A. and Jorden, W. 1977. A rapid colourimetric method for epicuticular wax content of sorghum leaves. *Crop Sci.*, **17**: 179-180.

Fozouni, M., Abbaspou, N. D. and Baneh, H. 2012. Leaf water potential, photosynthetic pigments and compatible solutes alterations in four grape cultivars under salinity. *Vitis.*, **51**(4): 147-152.

Gora, J. S., Singh, V. K., Sarolia, D., Kamlesh, K., Rajkumar and Bhati, V. 2017. Performance of Mango (*Mangifera indica* L.) monoembryonic and polyembryonic seedlings under salt stress condition. Int. *J. Curr. Microbiol. Appl. Sci.*, **6**(6): 3051-3056.

Gunes, A., Inal, A., Bagci, E.G. and Pilbeam, D.J. 2007. Silicon-mediated changes of some physiological and enzymatic parameters symptomatic for ixidative stress in spinach and tomato grown in sodic-B toxic soil. *Plant and Soil*. **290**: 103-114.

Hedge, J. E. and Hofreiter, B.T. 1962. *In Book: Carbohydrate Chemistry, 17 (Eds. Whistler R.L. and Be Miller,* J.N.), Academic Press, New York.

Heath, R. L. and Packer, L. 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys*., **125**: 189-198.

Hiscox, J. D. and Isrealstam, G.F. 1979 A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.*, **57**: 1332-1334.

Lata, K., Srivastav, M., Dubey, A.K., Singh, A.K. and Sairam, R.K. 2011. Effect of polyamines on seedlings of two mango (*Mangifera indica* L.) rootstocks under salt stress. *Indian J. Plant Physiol.*, **16**(3-4): 258-267.

Laxman, R. H., Rao, N. K., Biradar, G., Sunoj, V. S. J., Shivashankara, K. S., Pavithra, C.B., Dhanyalakshmi, K. H., Manasa, K. M., Bhatt, R. M., Sadashiva, A. T. and Christopher, M. G. 2014. Antioxidant enzymes activity and physiological response of tomato (*Lycopersicon esculentum* M.) genotypes under mild temperature stress. *Indian J. Plant Physiol.*, **19**(2): 161-164.

Mansour, A. M., Jennifer, R. W. and Robert, S. H. 2007. The impact of epicuticular wax on gas exchange and photoinhibition in *Leucadendron lanigerum*, Proteaceae. *Acta Ecol.*, **31**: 93-101.

Masia, A. 1998. SOD and CAT activities in apple during ripening and post-harvest and with special reference to ethylene. *Physiol. Plant.*, **104**: 668-672.

Pandey, P., Singh, A. K., Dubey, A.K. and Dahuja, A. 2014. Biochemical and salt ion uptake responses of seven mango (*Mangifera indica* L.) rootstocks to NaCl stress. *J. Hortic. Sci. Biotech.*, **89**(4): 367-372.

Patel, S.K., Dubey, A. K., Srivastav, M., Singh, A. K., Dahuja A. and Pandey R. N. 2011. Effect of NaCl in the irrigation water on growth, anti-oxidant enzyme activities, and nutrient uptake in five citrus rootstocks. *J. Hortic. Sci. Biotech.*, **86**(2): 189-195.

Rahnama, H. and Ebrahimzadeh, H. 2005. The effect of NaCl on antioxidant enzyme activities in potato seedlings. *Biol. Plant.*, **49**(1): 93-97.

Selvaraj, Y. and Kumar, R. 1995. Enzymatic regulation in ripening mango fruit. *Indian J. Hortic.*, **51**: 316-323.

Sharma, S. S. and Dietz, K. J. 2006. The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. *J. Exp. Bot.*, **57**: 711-726.

Singh, S. K., Sharma, H. C., Goswami, A. M., Datta, S. P. and Singh, S. P. 2000. *In vitro* growth and leaf composition of grapevine cultivars as affected by sodium chloride. *Biol. Plant.*, **43**: 283-286.

Srivastav, M., Dubey, A.K., Pandey, R.N. and Deshmukh, P.S. 2007. Effect of soil salinity on survival, growth and chlorophyll contents of 'Kurukkan' mango (*Mangifera indica*). *Indian J. Agric. Sci.*, **77**: 685-688.

Tayebimeigooni, A., Yahya, A., Maziah, M., Ahmad, S. and Zakaria, W. 2012. Leaf water status, proline content, lipid peroxidation and accumulation of hydrogen peroxide in salinized chinese kale (*Brassica alboglabra*). *J. Food Agric. Environ.*, **10**(2): 371-374.

(Received : 24.03.2022; Revised : 30.05.2023; Accepted 31.05.2023)