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Biochemical responses of monoembryonic and polyembryonic seedlings of mango rootstocks under salt stress conditions

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Abstract
The present investigation was carried out to study the biochemical response of monoembryonic and polyembryonic rootstocks seedlings to saline irrigation water. Two rootstocks, Dashehari and Bappakai seedlings were subjected to different NaCl concentration (0, 15.8, 31.6, 37.48 and 74.97 g NaCl/10 kg pot soil). The result indicated that proline content was increased with enhanced salinity level and recorded the highest 0.042 and 0.033 µg/mg with 74.9 g NaCl salinity level in monoembryonic and polyembryonic rootstocks respectively, while total chlorophyll and carotenoids were decreased with increasing salinity level. Reducing sugar and total sugar increased from 0.003 to 0.010 percent in monoembryonic and 0.003 to 0.014 percent in polyembryonic with increasing salinity level from 0 to 74.9 g per pots, whereas starch content decreased in both type embryonic rootstocks. The polyembryonic seedlings was found more tolerant to NaCl by responding increased proline and sugar content, with low decline in chlorophyll and carotenoid content. Thus, polyembryonic seedlings proved to be the more adaptable rootstock to saline conditions.

Keywords: Monoembryonic, polyembryonic, rootstocks Dashehari, Bappakai

Introduction
The mango (*Mangifera indica* L.) is one of the most important fruit crops at the global level, especially in Asia. Its popularity and importance realized by the fact that it is often referred as ‘King of fruits’ in the tropical world (Singh, 1996) [43]. Among fruits, mango fruit is the richest source of vitamin A (4800 IU) and also contain amino acids, carbohydrates, fatty acids, minerals, organic acids and proteins (Ara *et al.*, 2014; Shobana and Rajalakshmi 2010; Mathew 1983 and Albert 1984) [6, 41, 28, 2]. India is the leading producer of mango in the world, contributing more than 40.48% of the total world production (FAO, 2010).

Mango occupies the first position of fruit acreage, but it is well admitted that our present level of production is not sufficient enough. Successful mango cultivation is beset with many intricate problems like biotic and abiotic stresses. Plants are frequently exposed to a plethora of stress conditions such as salinity, drought, heat, flooding and heavy metal toxicity etc., where the various anthropogenic activities have accentuated the existing stress factors (Allakhverdiev *et al.,* 2000; Siringam *et al.,* 2012) [3, 44]. Among these stresses, salinity stress is a serious problem in mango cultivation worldwide (Yildirim *et al.*, 2009; Qin *et al.,* 2010) [49, 33].

Salt concentrations in irrigation water affects physiological and biochemical processes negatively including water relations and gas exchange attributes (Maeda and Nakazawa, 2008) [27], nutritional imbalance (Yang *et al.,* 2008) [48], disturbing the membranes stability (Dogan *et al.,* 2010) [14]. It has adverse effect on leaf chlorophyll and relative water content (RWC), as reported in *S. argentea* seedlings due to salt stress (Qin *et al.,* 2010). The high salt concentrations cause a great reduction in growth parameters such as fresh and dry weights of shoots and roots (Balal *et al.,* 2011) [10].

Mango is well known very sensitive crop to saline conditions (Maas, 1986), leading to scorched leaf tips and margins, leaf curling, and in severe cases reduced growth, abscission of leaves, and death of trees (Gora *et al*., 2017 and Jindal *et al*., 1976) [18], information concerning the salt tolerance of mango rootstocks is lacking, particularly on the impact of salinity on fruit yield (Ayers and Westcot 1989; Maas and Grattan, 1999). Mango seeds can be classified into two groups, monoembryonic and polyembryonic, based on their mode of reproduction.

Monoembryonic mango seed contain a single zygotic embryo, and hence only one seedling per seed, that is probable hybrid origin (Litz *et al.,* 1984, and Patena *et al*., 2002) [26, 31]. Polyembryonic mango seeds can contain one or more embryos; one is usually, but not always zygotic (Litz 1984 and Dominguez *et al*., 2004) [26]. Adventitious embryos develop from the nucellus, a maternal tissue surrounding the embryo sac, and consequently the seedlings of polyembryonic mangoes are usually very similar to the maternal parent.

The polyembryonic mango has a greater salt tolerance than mono embryonic population (Kadman *et al.,* 1976) [24]. Poly embryonic mango mostly have poor fruit quality and have little commercial value but they have potential to be used as a root stock for saline/alkaline soil or when irrigation water contains excess sodium or other soluble injurious ions for plants. The salt tolerant rootstocks M-13-1 and Gomera-1, bears capacity to restrict the uptake and transport of Cl^- and Na^+ ions from the rootstock to the above ground parts (Duran *et al.*, 2003) [15]. Salinization of agricultural land is increasing and in many areas salinity management is critical for the successful crop production. In this view, identification of salt tolerant/resistant, high yielding and quality of fruits rootstocks of mango is important for future use. Hence, the present investigation was undertaken to understand the performance of monoembryonic and polyembryonic mango seedlings under salt stress condition.

Material and Methods

An experiment was conducted under net house condition (50 % light intensity) at Plant Physiology Laboratory, Division of Crop Production, Central Institute for Subtropical Horticulture, Rehmankhera, Lucknow. The treatments comprised with 5 (five) treatments *viz.*, control as no salt (T_0), 15.8 g NaCl (T_1), 31.6 g NaCl (T_2), 37.487 g NaCl (T_3) and 74.97 g NaCl/10 kg pot soil (T_4) on monoembryonic and polyembryonic mango seedlings in complete randomized design. Ten plants were randomly selected from nursery for biochemical observations after the application of treatments to the tagged plants. Chlorophyll content of leaf (a, b and total) were estimated as suggested by Arnon (1949) and Proline content was estimated as per the method described by Bates et al. (1973) Starch concentration was measured after the leaf had been washed with ethanol. Starch was dispersed in alkaline solution and hydrolysed with amyloglucosidase (Novo Nordisk Bioindustries Ltd, Bagsvard, Denmark), then its concentration was determined by spectrophotometry using a glucose analysis kit (Boehringer Mannheim Corp./Diffchamb, Lyon, France). Sugars were extracted with 80% ethanol and were estimated by the method of Dey and Harborne (1990). Total carotenoids were estimated by the colorimetric method on the spectrophotometer (Model: Jasco V-670 UV-VIS-NIR spectrophotometer, Japan) as suggested by Roy (1973) [36]. Data were analyzed statistically and compared treatment means at the 5 % level of significance (Panse and Shukatme, 1985) [30].

Result and Discussion

The result of mono and polyembryonic seedlings under salt conditions were elucidated in the table 1 and 2 with regards to biochemical parameters *viz.* proline, chlorophyll, reducing sugar, total sugar and starch.

Proline (µg/mg FW)

In polyembryonic seedlings, proline content was found at higher level than monoembryonic seedlings. Proline content gradually increased (µg/mg FW) in treatments with increased intensity of salinity stress. Among the five salinity levels, highest proline content was recorded in T_4 treatment (0.041 & 0.033) in polyembryonic and monoembryonic rootstocks, respectively.

It has been extensively reported that proline content increases due to salinity in the citrus and different plants. (James *et al*. 2002; Roussos & Pontikis 2007) [21, 35]. Reports indicate the proline contents provides also rapid mechanism for maintaining turgor and affects the solubility of various proteins (Abraham *et al*., 2003) [1] and protects them against denaturation under saline condition (Jantaro *et al*., 2003; Tonon *et al*., 2004) [12, 45]

More proline accumulates under salt stress in both leaf and root tissue (Aziz *et al.*1999) and Putatively protects against the osmotic potential generated by salt (Watanabe *et al*. 2000, Chen *et al*. 2007) [46, 12]. The similar result observed by Hsu *et al.* 2003; Seki *et al*. (2007). Jiping Liu (1997) [20, 39, 23] in response to drought and salinity stress, many plant species accumulate high levels of proline, which is thought to function in stress adaptation.

Total chlorophyll

Total chlorophyll content was reduced in all treatments grown under salt stress conditions. Chlorophyll contents were decreased with increasing salinity level and maximum inhibiting effect was recorded in (T_4) in both the rootstocks. Maximum salinity level (T_4) resulted in chlorophyll minimum content of 1.23 µg/mg fw in poly and 0.87 µg/mg fw in monoembryonic rootstocks.

Similar finding were reported by Gu *et al.* 2004 that decrease in chlorophyll contents due to salinity. Most studies show that salinity adversely affects chlorophyll content (Meloni *et al. 2003*) [29].

As the results it is evident that chlorophyll contents in pea were decreased with the increasing salinity level and maximum inhibiting effect was recorded at high salt stress (75 mM) (Shahid *et al*., 2012) [9]. The lower chlorophyll content at higher salinity level also indicates stress and damaged the photosynthetic apparatus and decline the level of photosynthetic pigments may be attributed to salinity induced inhibition of chlorophyll biosynthesis (Khan, 2006 and Anstasia *et al*. 2013) that may be caused by the induced nutrients deficiency.

Carotenoids

Carotenoid concentration decreased with increasing NaCl concentration. Carotenoid level in the treatments was lower than the control value (0.179 & 0.237 mg/g fw, respectively), when the plants were grown under salinity stress and found lowest 0.14 and 0.193 mg/g in mono & polyembryonic rootstocks respectively. Similar findings were reported by Singh *et al*., 2008; Saikachout *et al.,* 2013 and Sairam *et al*., 2002 have also suggested that carotenoids may be one of the required factor salt tolerances in crop plants and therefore, carotenoids content may be helpful to differentiate between salt sensitive and salt tolerance in plants.

Reducing sugars and total sugar

Salt intensity is increased in reducing sugar and total sugar in all the monoembryonic and polyembryonic seedlings. The maximum reducing sugar and total sugar concentration was recorded under higher salt intensity level as compared to control seedlings. Reducing sugar in both monoembryonic and polyembryonic seedling as accumulated (T_0 to T_4) were 0.003, 0.004, 0.005, 0.008, 0.014 and 0.003, 0.003, 0.0040, 0.006, 0.007, respectively. The lowest quantity of total sugar was observed in control conditions in both monoembryonic and polyembryonic seedlings as compared to the treated ones. Total sugar concentration significantly increased with salinity stress.

The similar result has been reported by Balal *et al.* (2011) [10]. Salt stress caused an increase in total soluble sugars in all the citrus rootstocks. The maximum total soluble sugars concentration was recorded under higher salinity treatment (90 m*M*) as compared to control. More accumulation of total sugar is a common phenomenon under stress condition (William *et al*. 2000; Garg *et al.* 2002) [47, 17] reported an increase in total soluble sugar with progressive increase in salinity which have an important role in osmoregulation (Mohanty *et al*., 2002)

Starch (%)

Starch content was found higher in polyembryonic seedlings as compared to monoembryonic seedlings. Starch content maximum recorded in T_4 level i.e., 0.021 and 0.019 % in poly and mono embryonic rootstocks of mango, respectively. The starch content was negatively correlated with increased intensity of salinity stress as visualized in both monoembryonic and polyembryonic seedlings.

A depletion of leaf carbohydrate concentration when citrus plants were grown under stress has been observed (Arbona *et al*. 2005) [7]. Another similar finding was observed (Perez *et al*. 2007) [32] a large reduction on leaf starch concentration in salinized plant.

Table 1: Changing in biochemical molecule with salt regimes

Treatments	Proline (µg/mg Fw)		Total Chlorophyll (mg/g Fw)		Carotenoids(mg/g Fw)	
	Monoembryonic	Polyembryonic	Monoembryonic	Polyembryonic	Monoembryonic	Polyembryonic
T_0	0.005	0.006	1.482	1.848	0.179	0.237
T_1	0.008	0. 011	1.384	1. 774	0.172	0. 228
T_2	0.010	0.020	1.225	1.366	0.168	0.215
T_3	0.015	0. 025	1.113	1. 352	0.140	0. 200
T_4	0.033	0.042	0.866	1. 235	0. 128	0.193
Mean	0. 01	0.01	0.51	0. 63	0. 07	0.09
SEm+	0. 000	0.000	0.005	0. 008	0.002	0. 002
CD at 5 %	0. 001	0. 001	0.016	0.025	0. 006	0.006
CV	2.77	2.17	0.85	1.09	2.66	1.77

Table 2: Changing in biochemical molecule with salt regimes

Treatments	Reducing sugar (%)		Total sugar (%)		Starch (%)	
	Monoembryonic	Polyembryonic	Monoembryonic	Polyembryonic	Monoembryonic	Polyembryonic
T_0	0.003	0.003	0.393	0.471	0.031	0.034
T_1	0.004	0.005	0.456	0.558	0. 028	0. 031
T_2	0.006	0.005	0.464	0.558	0.024	0.027
T_3	0.007	0. 008	0.562	0.623	0.021	0. 024
T_4	0. 010	0.014	0. 635	0.743	0. 019	0.021
Mean	0.002	0.003	0.210	0.250	0. 01	0. 01
SEm+	0. 0002	0. 0001	0. 003	0. 011	0.0001	0..001
CD at 5 %	0.0005	0.0004	0.008	0. 032	0. 002	0.002
CV	5. 03	3.96	1. 02	3. 65	4.12	3.76

Conclusion

The current study indicates that increasing NaCl concentration exhibited inhibitory effect on total chlorophyll, carotenoid, reducing sugar, total sugar and starch except proline content in both monoembryonic and polyembryonic seedlings of mango. However, polyembryonic were found more tolerant to salt stress as compared to monoembryonic. Therefore, mango cultivar should be grafted with polyembryonic seedlings under such saline condition to grow successful cultivation mango.

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References

1. Abraham EG, Rigo G, Szekely Vagy R, Koncz C, Szabados L. Light dependent induction of proline biosynthesis by abscisic acid and salt stress is inhibited by brass inosteriod in *Arabidopsis, Plant Mol. Biol.* 2003; 51:363-372.
2. Albert Hill F. Economic botany, Tata Mcgraw Hill Publishing Co. Ltd., Bombay. 1984; 2:337.
3. Allakhverdiev SI, Sakamoto A, Nishijama Y, Inaba M, Murata N. Ionic and osmotic effects of NaCl induced inactivation of photosystems I and II in Synechococcus sp. Plant Physiol. 2003; 123:1047-1056.
4. Anastasia E, Giannakoula IF. The effect of water stress and salinity stress on growth and physiology of tomato (*Lycopersicon esculentum* Mill.). Arch. Bio. Sci., Belgrade. 2013; 65:611-620.
5. Anonymous. 2010. www.agriexchange.apeda.gov.in/Market%20Profile/one/MANGO.aspx.
6. Ara R, Motalab M, Uddin MN, Fakhruddin ANM, Saha BK. Nutritional evaluation of different mango varieties available in Bangladesh. Int. Food Res. J. 2014; 21:2169-2174.
7. Arbona V, Iglesias DJ, Jacas J, Primo-Millo E, Talon M, Gomez-Cadenas A. Hydrogel substrate amendment alleviates drought effect on young citrus plant. Plant and Soil. 2005; 270:73-82.
8. Arnon DI. Copper enzymes in isolated chloroplast: Polyphenol oxidase in Beta vulgaris. Plant Physiol. 1949; 24:1-15.

9. Balal RM, Khan MM, Shahid MA, Mattson NS, Abbas T, Ashfaq M, *et al*. Comparative studies on the Physiobiochemical, enzymatic, and ionic modifications in salt tolerant and salt sensitive Citrus rootstocks under NaCl stress. J Am. Soc. Hor. Sci. 2012; 137:1-10.
10. Balal RM, Yasin M, Muhammad A, Khan M, Jaskani MJ, Ashfaq M. Influence of salt stress on growth and biochemical parameters of citrus rootstocks. Pak. J Bot. 2011; 43(4):2135-2141
11. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water stress studies. Plant Soil Environ. 1973; 39:205-07.
12. Chen Z, Cuin TA, Zhou M, Twomey A, Naidu BP, Shabala S. Compatible solute accumulation and stressmitigating effects in barley genotypes contrasting in their salt tolerance. J Exp. Bot. 2007; 58:4245-4255.
13. Dey PM, Harborne JB. Methods in Plant Biochemistry. Hort Sci. 1990; 19:715-717.
14. Dogan M, Tipirdamaz R, Demir Y. Salt resistance of tomato species grown in sand culture. Plant Soil Environ. 2010; 56:499-507.
15. Duran Zuazo VH, Martínez-Raya1 A, Aguilar Ruiz J. Salt tolerance of mango rootstocks (*Magnifera indica* L. cv. Osteen). Spanish J Agri. *Res.* 2003; 1:67-78
16. Duran VH, Martínez-Raya A, Aguilar J. Salt tolerance of mango rootstocks (*Mangifera indica* L. cv. Osteen). Spanish J Agri. Res. 2003; 1:67-78.
17. Garg AK, Ownes JK, Wu RJ. Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stress. Proceeding of the National Academy of Sciences of the United States of America. 2002; 99:15898-15903.
18. Gora JS, Singh VK, Sarolia DK, Kumar Kamlesh Rajkumar, Bhati V. Performance of mango (*Mangifera indica* l.) monoembryonic and polyembryonic seedlings under salt stress condition. Int. J Curr. Microbiol. App. Sci. 2017; 6:3051-3056.
19. Gu RS, Fonseca L, Puskas L, Hackler A, Zvara D, Dudits, *et al*. Transcript identification and profiling during salt stress and recovery of Populus euphratica. Tree Physiol. 2004; 24:265-276.
20. Hsu SY, Hsu YT, Kao CH. The effect of polyethylene glycol on proline accumulation in rice leaves. Biol. Plant. 2003; 46:73-78.
21. James RA, Rivelli AR, Munns R, Caemmerer SV. Factors affecting Co_2 assimilation, leaf injury and growth in salt stressed durum wheat. Funct. Plant Biol. 2002; 29:1393-1403.
22. Jantaro SP, Maenpaa Mulo P, Incharoensakdi A. Content and biosynthesis of polyamines in salt and osmotically stressed cell of Synechocystis sp. PCC 6803. FEMS Microbiol. Lett. 2003; 228:129-35.
23. Jiping L, Zhu JK. Proline accumulation and salt-stress-induced gene expression in a salt-hypersensitive mutant of arabidopsis. Plant Physiol. 1997; 114:591-596.
24. Kadman A, Gazit S, Ziv G. Selection of mango rootstock for adverse water and soil conditions in arid areas. Acta Horti. 1976; 57:81-88.
25. Khan NA. NaCl inhibit chlorophyll synthesis and associated changes in ethylene evolution and anti oxidative enzyme activities in wheat. Biol. Plantarum. N 2006; 47:437-440.
26. Litz RE. *In vitro* somatic embryogenesis from nucellar callus of monoembryonic *Mangifera indica* L. Hort. Sci. 1984; 19:715-717.
27. Maeda Y, Nakazawa R. Effects of the timing of calcium application on the alleviation of salt stress in the maize, tall fescue, and reed canary grass seedlings. Biol Plant. 2008; 52:153-156.
28. Mathew KM. The Flora of the Tamil Nadu Carnatic. 1983; 3:266.
29. Meloni DA, Oliva MA, Martinez CA, Cambraia J. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environ. Exp. Bot. 2003; 49:69-76.
30. Panse VG, Sukhatme PV. Statistical Methods for Agricultural Workers. Indian Council of Agricultural Research Publication, 1985, 87-89.
31. Patena, *et al*. Plant Cell Tissue Organ Cult. 2002; 79(1):101-104.
32. Perez JG, Botia P, Sanchez FG. Leaf water relation and net gas exchange responses of salinized Carrizo citrange Seedling during stress drought stress and recovery. Annuals of Bot. 2007; 100:335-345.
33. Qin J, Dong WY, He KN, Yu Y, Tan GD, Han L, *et al*. NaCl salinity-induced changes in water status, ion contents and photosyn thetic properties of *Shepherdia argentea* (Pursh) Nutt. Seedlings. Plant Soil Environ. 2010; 56:325-332.
34. Rivera DM, Manzanilla-Ramira MA, Robles-Gonzales M, Gomez Lim MA. Plant Cell Tiss. Org. Cult. 2004; 79:101-104.
35. Roussos PA, Pontikis CA. Changes of free, soluble conjugated and bound polyamine titers of jajuba explants under sodium chloride salinity *In vitro*. J Plant Physiol. 2007; 64:895-903.
36. Roy SK. A simple and rapid method for estimation of total carotenoid pigments in mango. J Food Sci. Technol. 1973; 10:45-46.
37. Saikhachout S, Hamza KJ, Karray N. Salt-induced changes in antioxidative enzyme activities in shoot tissue of two *Atriplex verities*. Not Bot Horti Agrobo. 2013; 41:115-121.
38. Sairam RK, Veerabhadra RK, Srivastava GC. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidative activity and osmolyte concentration. Plant sci. 2002; 163:1037-1046.
39. Seki M, Umezawa T, Urano K, Shinozaki K. Regulatory metabolic networks in drought stress responses. Curr. Opin. Plant Biol. 2007; 10:296-302.
40. Shahid MA, Balal RM, Pervez MA, Abbas T, Ashfaq M, Ghazanfar U, *et al*. Differential response of pea (*Pisum sativum* L.) genotypes to salt stress in relation to the growth, physiological attributes antioxidant activity and organic solutes. Aust. J Crop Sci. 2012; 6:828-838.
41. Shobana V, Rajalakshmi K. Quantitative analysis of primary metabolites in *Mangifera indica* (*Unripe mango*) Rasayan J Chem. 2010; 3:597-599.
42. Singh AK, Singh RA, Kumar S. Influence of salinity on seedling growth and metabolism in maize genotype. Ind. J Plant Physiol. 2008; 13:95-99.
43. Singh H. Mango. ICAR, New Delhi, India, 1996.
44. Siringam K, Juntawong N, Chaum S, Boriboonkaset T, Kirdmanee C. Salt tolerance enhancement in indica rice (*Oryza sativa* L. spp. indica) seedlings using exogenous sucrose supplementation. Plant Omics J. 2012; 5:52-59.
45. Tonon GC, Kevers O, Faivre-Rampant M, Graziani, Gaspar T. Effect of NaCl and mannitol iso-osmotic stresses on proline and free polyamine levels in

embryogenic Fraxinus angustifolia callus. J Plant Physiol. 2004; 161:701-8.

46. Watanabe A, Kojima K, Ide Y, Sasaki S. Effects of saline and osmotic stress on proline and sugar accumulation in *Populus euphratica in vitro*. Plant Cell Tissue & Organ Culture. 2000; 63:199-206.
47. Williams LER, Lemonie, Saucer N. Sugars transporters in higher plants: a diversity of roles and complex regulation trends. Plant Sci. 2000; 5:283-290.
48. Yang CW, Wang P, Li CY, Shi DC, Wang DL. Comparison of effects of salt and alkali stresses on the growth and photosynthesis of wheat. Photosynthetica. 2008; 46:107-114.
49. Yildirim E, Karlidag H, Turan M. Mitigation of salt stress in strawberry by foliar K, Ca and Mg nutrient supply. Plant Soil Environ. 2009; 55:213-221.