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Seed germination and seedling growth responses of polyembryonic mango (*Mangifera indica* L.) genotypes to salinity stress

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Abstract

Mango (*Mangifera indica* L.) is a salt sensitive crop particularly at early stages of growth. So, it is essential to study the response of mango seed germination and seedling growth against salinity stress. In this experiment, the effect of salinity (induced by 50 mM NaCl) stress was studied on seed germination, biomass content and growth responses in newly emerged seedlings of sixteen polyembryonic mango genotypes namely EC-95862, Vattam, Vellaikolamban, Nekkare, Mylepelian, Turpentine, Sabre, Manipur, Kitchener, Kensington, Olour, Kurukkan, Bappakkai, Chandrakaran, Muvandan and Deorakhio. Among these genotypes Turpentine (97.50%), Bappakkai (92.50%) and Vattam (80%) showed early and maximum germination after 20 days of sowing while the genotypes, Chandrakaran, Sabre, Kensington and Vellaikolamban depicted 80.00%, 85.00%, 85.00% and 87.50% germination, respectively. Other genotypes had late germination under 50 mM salinity stress (35 days of sowing). The germinated mango seedlings were exhibited significant decline in plant height, numbers of leaf per plant, number seedlings per seed, stem diameter (mm), root length (cm), fresh and dry weight of shoot (g), fresh and dry weight of root (g) under saline conditions, with plant biomass being most sensitive to salinity. With respect to growth behavior, the genotypes Turpentine, Deorakhio and Olour showed a minimum reduction in biomass under salinity and Kitchener, Mylepelian and Chandrakaran, the maximum.

Keywords: Seed germination, salt stress, polyembryonic mango seedlings, salinity tolerance, growth behavior

Introduction

Mango is one of the major fruit crops, extensively grown in tropical and subtropical parts of the world. In India, mango is grown on 2.217 million hectares area with a production of 8.506 million MT (Anonymous, Although India occupied first position in mango production its low productivity is a matter of concern. Around the world various abiotic stresses like drought, salinity, *etc*. are becoming serious issues for crop production. Among these stresses, salinity through soil and irrigation water is becoming a major problem in horticulture production system because of their negative effects on plant growth and productivity (Qin *et al*., 2010) [18]. Being a salt sensitive crop, mango suffers under salinity stress during early stages of growth (Dubey *et al*., 2006) [9]. India's 6.74 million hectares of agricultural land is salt affected and it is estimated that by 2050 it will go up to 16.2 million hectare (Anonymous, 2015b) [4]. Several strategies such as leaching, good drainage, application of high-quality irrigation water, tillage and amendments of coarse organic matter such as straw, corn stalks, sawdust, wood shavings *etc*. are being adopted with varied success to maintain the soil and plant health under saline condition (Rakshit *et al*., 2010) [19], but these strategies are laborious, cost intensive, temporary and complex. The only viable option to mitigate salt stress in mango is the use of salt tolerant rootstock. Rootstocks play an important role in enhancing mango production and productivity of commercial scion varieties. Polyembryonic mango genotypes produce zygotic seedlings and nucellar seedlings which arise from zygotic embryo and nucellar embryo, respectively. Kadman *et al*., (1976) [14] mentioned that the polyembryonic genotypes of mango have better salt tolerance potential than monoembryonic genotypes. Identifying the level of salt tolerance in polyembryonic mango genotypes will be helpful to use them as tolerant rootstocks for grafting of choice cultivars susceptible to salinity. Studies on rootstocks screening for salinity stress has been done in fruit crops like citrus (Pedroso, 2014) [17] grape and mango (Pandey, 2014) [8]. However, studies on seed germination and screening of mango genotypes at initial growth stage for their potential use as rootstock for salinity tolerance are lacking. Keeping in a view these facts, we investigated the effects of NaCl stress on seed germination and early growth in sixteen polyembryonic mango genotypes.

Materials and Methods

The present study was carried out at ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru Karnataka, India (12.97°N and 77.56°E) during the years 2015-2016 and 2016-2017. The stones of sixteen polyembryonic mango genotypes namely EC-95862, Vattam, Vellaikolamban, Nekkare, Mylepelian, Turpentine, Sabre, Manipur, Kitchener, Kensington, Olour, Bappakkai, Kurukkan, Chandrakaran, Muvandan and Deorakhio were extracted from fully riped fruits. The husks of mango stones were removed and kernels were used for studying seed germination. Ten kernels of each genotype were sown in 250 g Fermented Cocopeat and kept in germination chamber at 23 ± 2 ºC and R.H. 80% to maintain the congenial environment for germination. Salinity level of 0 mM and 50 mM of NaCl concentrations were imposed at regular intervals. The seed germination percentage was calculated at an interval of 10 days after sowing using the formula-:

$$\text{Seed Germination} = \frac{\text{Number of Seeds Germinated}}{\text{Total Number of Seeds}} \times 100$$

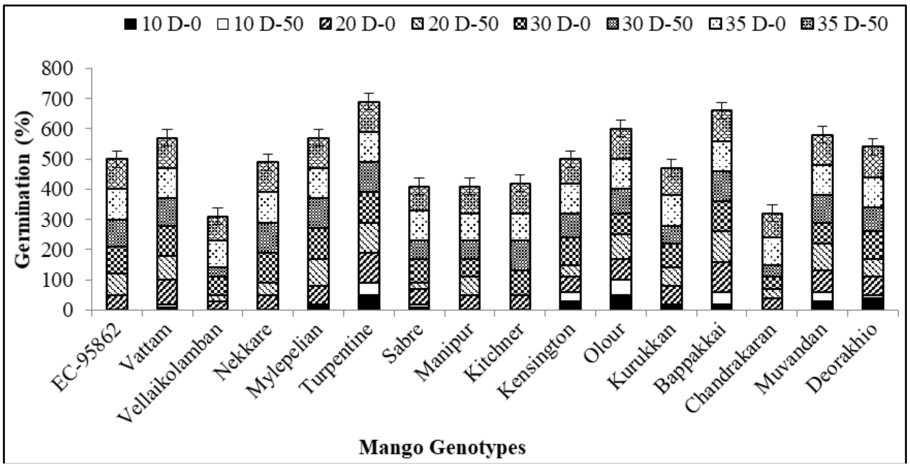
After 35 days when the mango kernels showed >80% germination, the germinated seedlings were used for recording the growth observations after uprooting them from the germination medium. The seedling height and root length were measured using measuring scale and expressed in cm; number of leaves per plant and seedlings per seed were counted manually; stem diameter (mm) was measured using 'Vernier calipers'; and fresh and dry weights of shoot (g) and root (g) were recorded with weighing balance. The germination percentage for both years of study and average value of both years were used for interpretation of results. The changes in growth parameters were interpreted in terms of percent increase or decrease over control. The experiment was laid out in Factorial Completely Randomized Design (FCRD) with 10 numbers of the mango seeds in each of the 16 genotypes. The statistical software SAS 9.3 version and analysis of variance (ANOVA) were used for analysis and interpretation of data generated during the present study. Significant differences among the genotypes induced by salinity stress were compared using Fisher's test at $P \leq 0.05$. The correlation analysis among the all growth parameters were done using data analysis tool of Microsoft Office Excel-2010 and significant level were depicted on the basis of Pearson's correlation coefficient 'r' (Critical Values) table.

Results and Discussion

The mango seeds imposed with 0 mM and 50 mM salinity level, depicted the variation in number of days required for germination and germination percentage. The seeds of Turpentine and Olour genotypes showed 50% germination after ten days of sowing, while no germination was recorded in genotypes EC-95862, Manipur and Kitchener even though imposed with 0 mM salinity. The 50 mM salinity treated seeds of genotypes, Olour, Turpentine and Mylepelian showed early and maximum germination with 45.00%, 37.50% and 37.50%, respectively compared to other genotypes whereas 0.00% germination was observed in EC-95862, Vellaikolamban, Kitchener, Kurukkan and Chandrakaran after 10 days of sowing (Fig. 1&2). After 35 days of sowing under 0 mM and 50 mM salinity stress, almost all genotypes showed more than 75% germination (Fig. 1&2). So, our findings confirms that mango seeds of the selected mango genotypes can germinate under 0 mM and 50 mM NaCl salinity level with 'Fermented Cocopeat' as growing medium and congenial environmental conditions. The contrary results to our study about the adverse effect of salinity on germination were registered in orthodox seeds of Maize (Aliu *et al*., 2015) [1], *Cucumis melo* (Sohrabikertabad *et al*., 2013) [22] and Barley (El Goumi *et al*., 2014) [10] in which it's pointed out that germination rate was low under salinity stress. The mango seeds are however recalcitrant type, sometimes showing vivipary also and sustain their germination viability up to four weeks with its endogenous moisture content. Mango seeds are bigger and restrain enough moisture to germinate by itself that can avoid the demand of water from external source for germination. This could be the probable reason for mango seed germination to be unaffected when supplied with saline water in our study. The 'Fermented Cocopeat' used in the experiment as germination medium retains good amount of moisture that extended the interval for application of salt water. El-Desouky and Atawia (1998) [11] illustrated in his study on citrus that the salinity reduced the seed germination and delay in germination were rootstock specific.

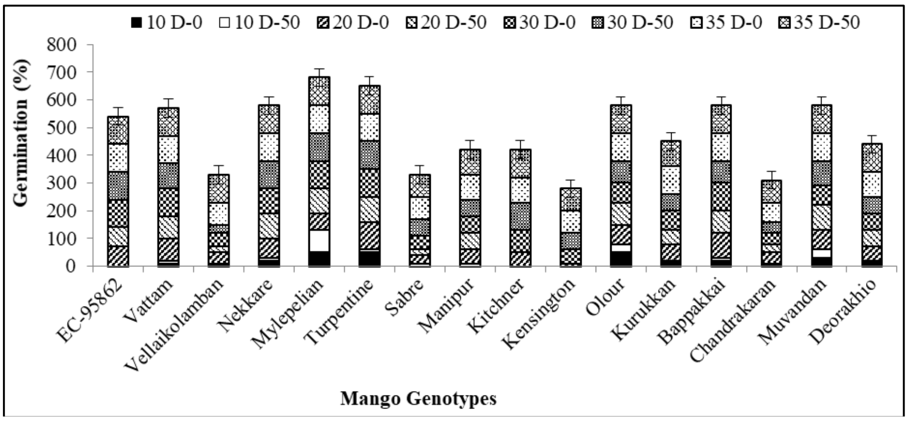
After germination the newly emerged mango seedlings had shown the marginal differences in biomass content and growth behavior under salinity stress. The maximum reduction plant height was noted in the genotypes Chandrakaran (9.94%) followed by Mylepelian (8.33%), Kitchener (6.82%) and Vellaikolamban (5.96%) whereas, the genotypes Turpentine (0.15%) Deorakhio (0.83%), Olour (0.91%) and Bappakkai (1.80%) showed minimum reduction in 50 mM treated seedlings over control (Fig. 3). The salinity induces osmotic stress which causes the negative effect on protein synthesis, enzymatic activities and hormonal imbalance; collectively inhibiting the plant growth (Mazher, 2007) [15]. Study conducted by Tsai *et al*. (2015) [23] and Sharma *et al*. (2013) [21] also revealed that salinity stress adversely affected the plant growth in Pink wax apple and *Citrus jambhiri*. With regards to the number of leaves, seedlings showed marginal reduction in the number of leaves per plant at 50 mM (Fig. 4). Least reduction in number of leaves in salinity stressed plants over control were recorded in Turpentine (0.48%), Deorakhio (1.30%) and Olour(2.19%) while higher percent of reduction were noticed in Chandrakaran (15.48%), Mylepelian (13.88%) and Kitchener (11.36%). Stress condition causes the imbalance in ion homeostasis and slows down the supply of growth promoting hormones and nutrients which might be responsible for growth inhibition and emergence of new leaves. The study by Amira *et al*., 2011 suggested that leaf number decreased after imposition of salinity stress in *Vicia faba* genotypes.

Results presented in the Fig. 5 showed that there was variation in the number polyembryonic seedlings under control as well as salinity stressed plants. The maximum average number of seedlings per seed was observed in the genotype Deorakhio (3.43) followed by Turpentine (3.14) and Muvandan (2.43) when imposed with 50 mM while same trend was noticed under 0 mM stressed plants (Deorakhio - 3.71, Turpentine -3.43 and Muvandan -2.71). Least number of polymbryonic seedlings was detected in the genotypes, Chandrakaran (1.14), Sabre (1.29) and Vellaikolamban (1.43) in 50mM NaCl treated seedlings. Reduction in root length and stem diameter was noticed in salt treated seedlings compared to control. Root length quantifies the growth of newly emerged seedlings which can be affecting under salinity stress. The Fig. 6 demonstrated that genotypes, Turpentine (0.63%), Deorakhio (0.72%) and Olour (1.37%) had minimum reduction in root length while maximum reduction was observed in Chandrakaran (11.05%), Mylepelian (9.44%) and Kitchener (8.92%). Salinity around the root zone slows down the water absorption capacity of roots by reducing the water potential. These results are in agreement with that of Camlica and Yaldiz (2017) [7] who found that root length decreased when *Ocimum basilicum* plants were subjected to salinity stress. The results shown in Fig. 7 confirms that genotypes, Turpentine, Deorakhio and Olour showed less reduction in stem diameter 2.45%, 3.01% and 4.93%, respectively over control under salinity while seedlings of Chandrakaran (17.57%), Mylepelian (14.15%) and Kitchener (13.44%) had more reduction in stem diameter over control, but there was not much differences in stem diameter among the seedlings of sixteen genotypes. These findings agreed with what Roy *et al*. (2015) [20] who concluded that graded levels of NaCl salt affect the plant height, stem diameter, number of leaves, leaf area and survivability of mango. The reduction in stem girth might be due to disruption in cell division and elongation caused by low osmotic potential in plant cells under stress condition Bartels and Sunkar (2005) [5]. The shoot and root biomass were adversely affected by salinity treatment. The fresh weight of shoot and root under 50 mM NaCl salinity level was reduced by 16.11% and 39.33% in seedlings of Chandrakaran whereas only 1.89% (shoot) and 2.42% (root) reduction was recorded in Turpentine (Fig. 8&9). The reduction in dry weight of shoot (16.06%) and root (29.38%) were observed in Chandrakaran seedlings while in seedlings of Turpentine it was around (0.68%) and root (2.06%) (Fig.10&11). Reduction in shoot and root biomass was related to the growth reduction caused by the adverse effect of salinity. Study conducted by El-Hammady *et al*. (1995) revealed that salinity treatments affected the fresh and dry weight of citrus rootstocks. The decrease in shoot and root biomass of mango and citrus rootstocks under osmotic stress was also reported by Dayal *et al*. (2014) [8] and Balal *et al*. (2012) [5], respectively. The correlation analysis among the growth parameters under salinity stress (Table 1) showed that the plant height was significantly correlated to the stem diameter, fresh and dry weight of shoot and roots. The stem diameter showed positive correlation with root length, fresh and dry weight of shoot and root, respectively. The fresh and dry weight of shoot and root correlated to almost all growth parameters except root length. Number of seedlings also showed positive correlation to stem diameter and plant biomas (fresh and dry weight). This showed that seedling growth under salinity stress is induced by different plant parts and their biomass production and it can be genotype specific. The significant correlation among plant height, fresh and dry weight of shoot and root portions under salinity stress was noted by Homma (2016) [13] in potato genotypes.



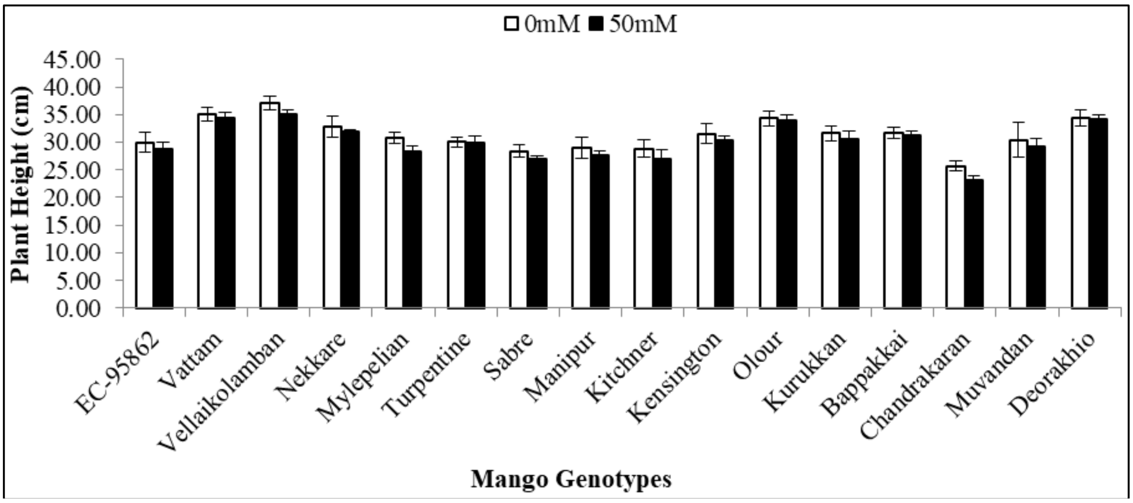
Note: (D-days; 0-0 mM; 50-50 mM)

Fig 1: Seed germination responses of polyembryonic mango genotypes under control and saline (2015-2016).



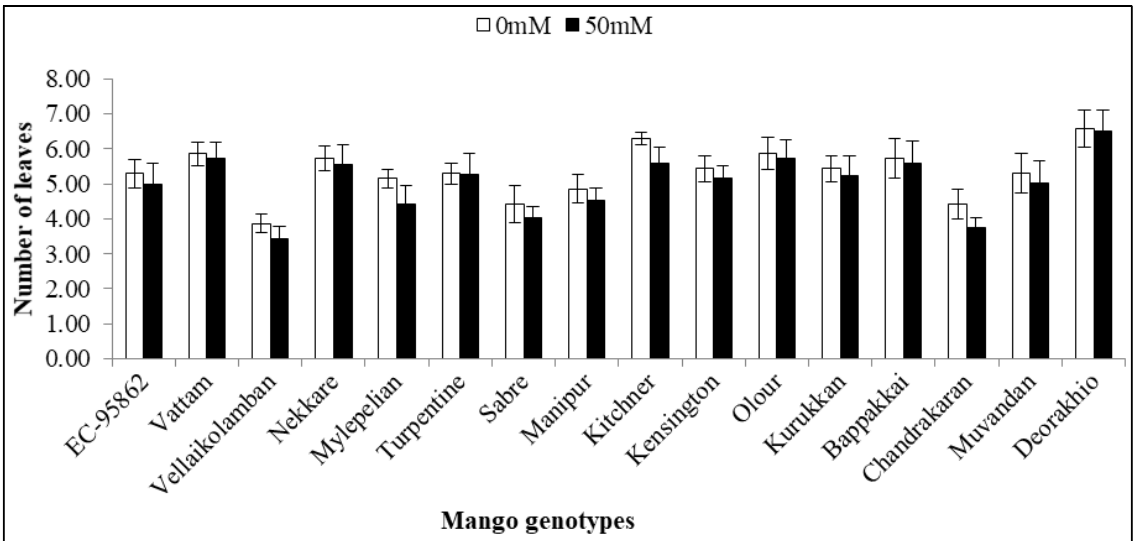
Note: (D-days; 0-0 mM; 50-50 mM)

Fig 2: Seed germination responses of polyembryonic mango genotypes under control and saline conditions (2016-2017).



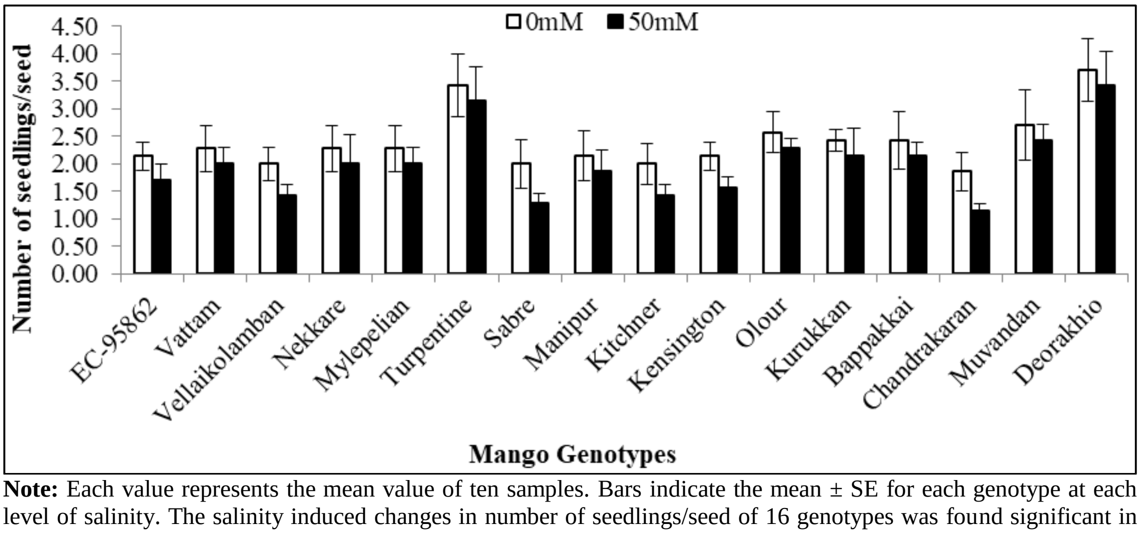
Note: Each value represents the mean value of ten samples. Bars indicate the mean ± SE for each genotype at each level of salinity. Effect of salinity levels on plant height of 16 mango rootstock seedlings was found significant at *P*≤0.05 using Fisher's Least Significant Difference. (LSD at 5%= 0mM-4.450, 50mM-2.886)

Fig 3: Effect of salinity on plant height of polyembryonic mango genotypes under saline condition.



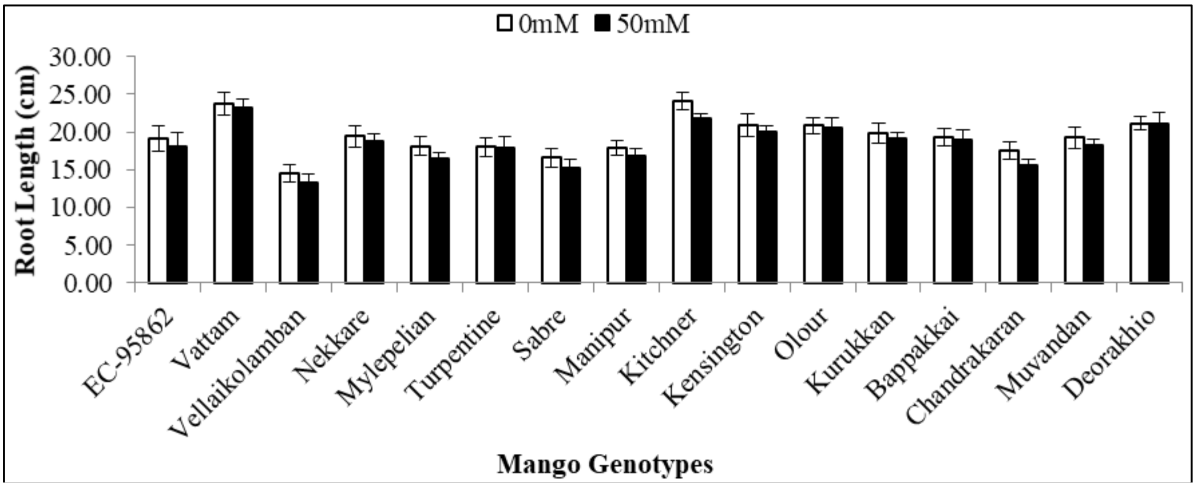
Note: Each value represents the mean value of ten samples. Bars indicate the mean ± SE for each genotype at each level of salinity. The salinity induced changes in number of leaves of 16 genotypes was found significant at *P*≤0.05 using Fisher's Least Significant Difference. (LSD at 5%= 0mM-1.122, 50mM-1.435)

Fig 4: Effect of salinity on number of leaves in polyembryonic mango genotypes under saline condition.



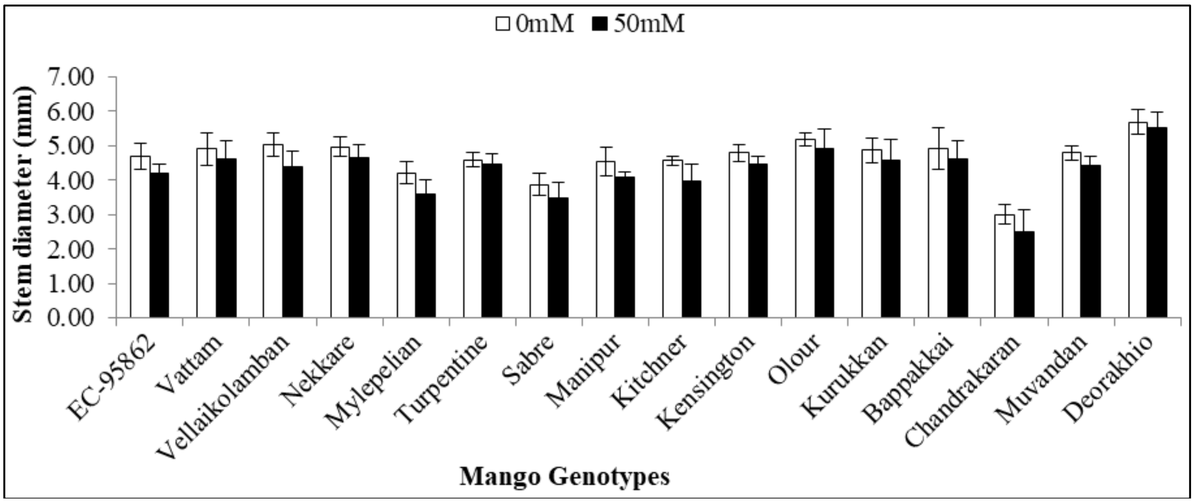
Note: Each value represents the mean value of ten samples. Bars indicate the mean ± SE for each genotype at each level of salinity. The salinity induced changes in number of seedlings/seed of 16 genotypes was found significant in treated plant and non-significant in control seedlings at *P*≤0.05 using Fisher's Least Significant Difference. (LSD at 5%= 0mM-NS, 50mM-0.978)

Fig 5: Effect of salinity on number of seedlings per seed of polyembryonic mango genotypes under saline condition.



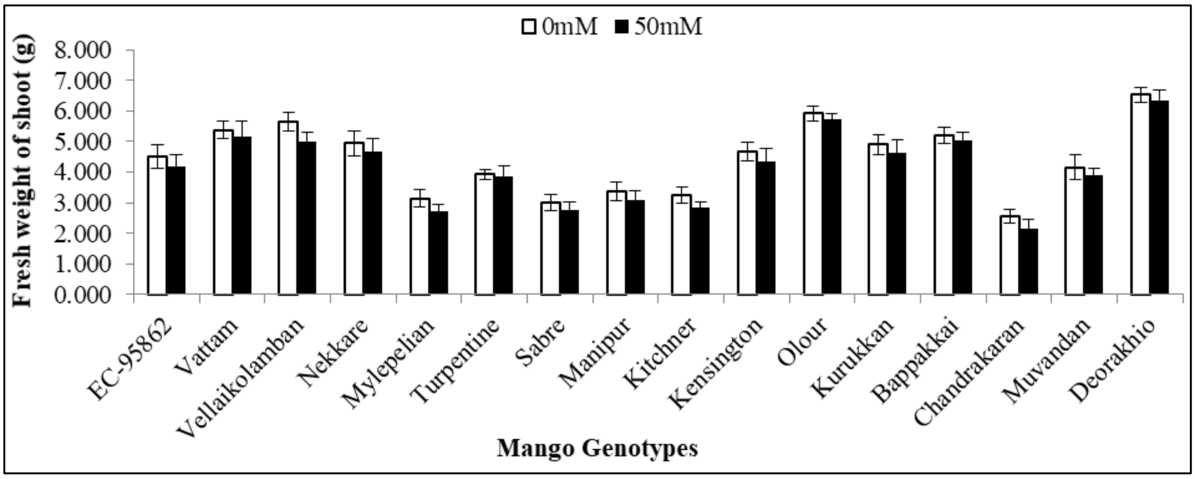
Note: Each value represents the mean value of ten samples. Bars indicate the mean ± SE for each genotype at each level of salinity. The salinity induced changes in root length of 16 genotypes found significant at $P \leq 0.05$ using Fisher's Least Significant Difference. (LSD at 5%= 0mM-3.552, 50mM-3.254)

Fig 6: Effect of salinity on root length of polyembryonic mango genotypes under saline condition.



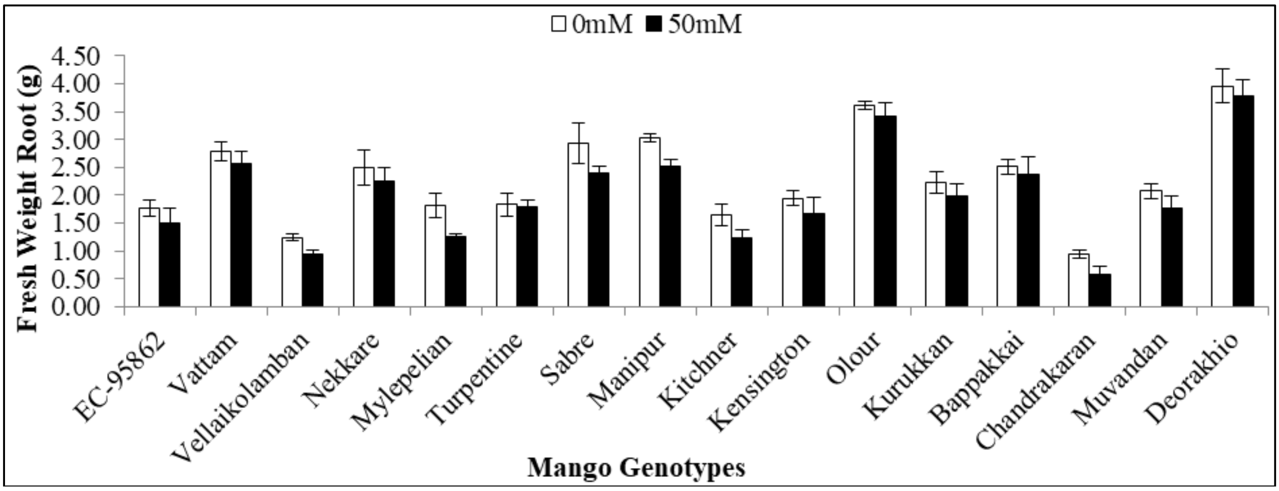
Note: Each value represents the mean value of ten samples. Bars indicate the mean ± SE for each genotype at each level of salinity. The salinity induced changes in stem diameter of 16 genotypes found significant at $P \leq 0.05$ using Fisher's Least Significant Difference. (LSD at 5%= 0 mM-0.945, 50 mM-1.233)

Fig 7: Effect of salinity on stem diameter of polyembryonic mango genotypes under saline condition.



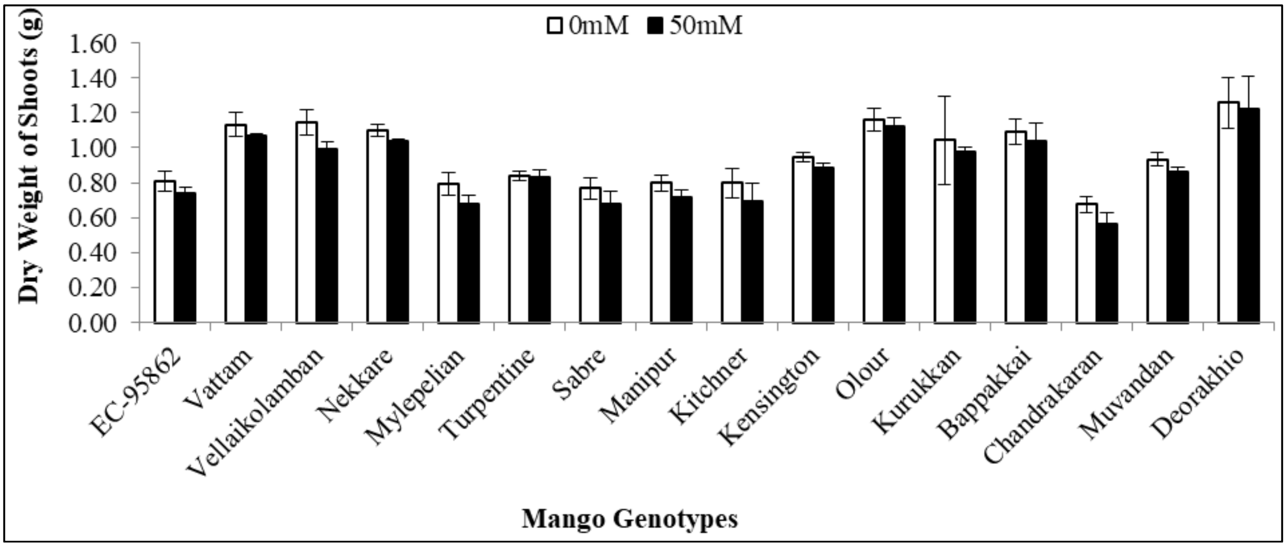
Note: Each value represents the mean value of ten samples. Bars indicate the mean ± SE for each genotype at each level of salinity. The salinity induced changes in fresh weight of shoots of 16 genotypes found significant at $P \leq 0.05$ using Fisher's Least Significant Difference. (LSD at 5%= 0mM-0.834, 50mM-0.915)

Fig 8: Effect of salinity on fresh weight of shoots in polyembryonic mango genotypes under saline condition.



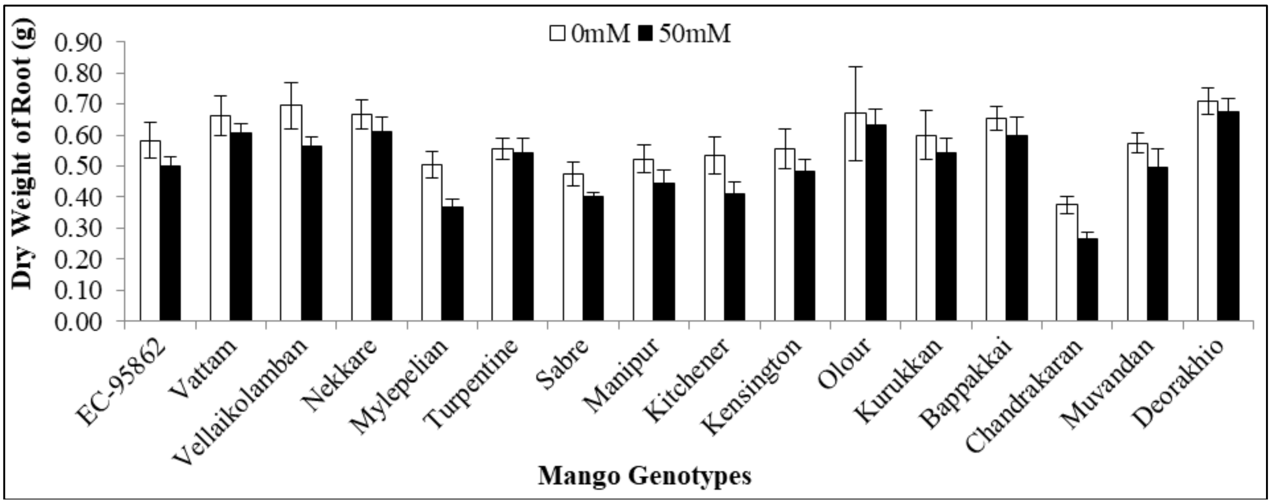
Note: Each value represents the mean value of ten samples. Bars indicate the mean ± SE for each genotype at each level of salinity. The salinity induced changes in fresh weight roots of 16 genotypes found significant at $P \leq 0.05$ using Fisher's Least Significant Difference. (LSD at 5%= 0mM-0.534, 50mM-0.601)

Fig 9: Effect of salinity on fresh weight of roots of polyembryonic mango genotypes under saline condition.



Note: Each value represents the mean value of ten samples. Bars indicate the mean ± SE for each genotype at each level of salinity. The salinity induced changes in dry weight of shoots of 16 genotypes found significant at $P \leq 0.05$ using Fisher's Least Significant Difference. (LSD at 5%= 0mM-0.254, 50mM-0.190)

Fig 10: Effect of salinity on dry weight of shoots in polyembryonic mango genotypes under saline condition.



Note: Each value represents the mean value of ten samples. Bars indicate the mean ± SE for each genotype at each level of salinity. The salinity induced changes in dry weight of root of 16 genotypes found significant at $P \leq 0.05$ using Fisher's Least Significant Difference. (LSD at 5%= 0mM-0.177, 50mM-0.113)

Fig 11: Effect of salinity on dry weight of roots in polyembryonic mango genotypes under saline condition.

Table 1: Correlation among the plant height, stem diameter, fresh weight of shoot, dry weight of shoot, fresh weight of root dry weight of root, root length, number of seedlings in sixteen polyembryonic manog genotypes under salinity stress.

	PH	**SD**	**RL**	**FWS**	**DWS**	**FWR**	**DWR**	**NS**
PH	1							
SD	0.862**	1						
RL	0.400 ns	0.554*	1					
FWS	0.923**	0.880**	0.440 ns	1				
DWS	0.913**	0.863**	0.462 ns	0.985**	1			
FWR	0.909 **	0.907**	0.477 ns	0.959**	0.949**	1		
DWR	0.921**	0.924**	0.468 ns	0.958**	0.951**	0.919**	1	
NS	0.449 ns	0.605*	0.377 ns	0.579*	0.587*	0.571*	0.636**	1

Note: ** indicates the values are significant at ($p<0.01$) level; * indicates the values are significant at ($p<0.05$) level; ns: indicates non significant values. PH - plant height, SD – Stem diameter, RL – Root length, FWS –Fresh weight of shoot, DWS – Dry weight of shoot, FWR – Fresh weight of root, DWR – Dry weight of root, NS-Number of seedlings.

Conclusion

This preliminary study of screening sixteen mango genotypes under saline condition showed that mango seeds can germinate in 50 mM saline irrigation water and use of 'Fermented Coco peat' as rooting media under controlled environmental conditions is favorable for germination. On the other hand, newly germinated seedlings differ in their growth habit under salinity that is genotype specific. On the basis of growth behavior, this study predicts that genotypes Turpentine, Deorakhio, Olour and Bappakkai performed better under salinity stress whilst Chandrakaran, Mylepelian and Kitchener and Vellaikolamban suffered more.

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