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Impact of salinity on the growth, physiology, and biochemical parameters of mango (*Mangifera indica* L.) genotypes

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Abstract

Salinity is a main abiotic factor reduces agricultural productivity, especially during the early stages of growth. The present research was conducted in a polyhouse located in North farm at Karunya Institute of Technology and Sciences, Coimbatore, aimed at assessing salt tolerance of five local Mango (*Mangifera indica* L.) genotypes at the seedling stage (3-month-old) at various salinity levels (Control, 25, 50, 75, and 85 mM NaCl). Genotypes are Mundapa, Sakkarai kutty, Muvandan, Chengavarkai, Vellari. The experiment conducted using Two factorial Completely Randomized Design with three replications. There were significant differences in growth, physiological, and biochemical characteristics such as plant height, number of leaves, leaf length, leaf area, stem diameter, relative water content, chlorophyll content, proline between genotypes, salinity levels and their interactions. G₃ exhibited from other genotypes in terms of plant height (51.77 cm), number of leaves (14.37), leaf area (80.64 cm²), relative water content (64.65%), chlorophyll content (1.18), and proline accumulation (167.10) during stress conditions. Significant genotype × salinity interaction observed, with control treatment (S₁) record maximum values, particularly in G₃S₁, for plant height (54.36 cm), number of leaves (17.95), leaf area (105.31 cm²), relative water content (85.61%), and chlorophyll content (1.64), while severe salinity (S₅) resulted minimum values in G₂S₅, with plant height (32.74 cm), number of leaves (5.94), leaf area (21.32 cm²), relative water content (28.13%), chlorophyll content (0.24). In contrast, G₂ recorded the lowest values, showing a high sensitivity to salinity. Increasing salinity levels reduced growth characteristics, relative water content, chlorophyll content, whereas proline concentration gradually increased, indicating stress resistance. Findings indicate G₃ Muvandan had more tolerance mechanisms in Coimbatore, such as osmotic adjustment and ion management, allowing it to perform well in saline conditions. As a result, G₃ is a suitable genotype for cultivation in salt-affected areas and could be used in breeding efforts to improve salinity resistance.

Keywords: *Mangifera indica* L, genotypic variation, relative water content, chlorophyll content, proline, salinity tolerance

1. Introduction

Mango (*Mangifera indica* L.), a member of the *Anacardiaceae* family, is an important tropical and subtropical fruit crop farmed in more than 100 countries worldwide (Purseglove, 1972) [20]. This fruit crop is native to southern Asia and India, having been grown for many generations in the Indo-Burma region (Kwee and Chang, 1985) [12]. Its mouthwatering taste and alluring flavour had been earned it the title of "King of fruits." Vitamins A and C, carotenes, flavonoids, sterols, aromatic acids, essential oils, fatty acids, and phenolics are all abundant in mangos. According to Pandey and Dinesh (2010) [18], it is a nutritious fruit that contains the majority of the important nutrients required by the body. Mangoes were produced on 2.4 million hectares of land in India, producing 21.79 million MT (Anonymous 2024) [1]. Despite holding the top spot in mango production, India's low productivity is a cause for worry. Abiotic stressors such as salt and drought are becoming major problems for agricultural productivity worldwide. Due to its detrimental impact on plant growth and productivity, soil and irrigation water salinity is one of the stresses that is becoming a significant issue in horticultural production systems (Qin *et al.*, 2010) [21]. India is predicted to produce more than 223.98 lakh tonnes of mangoes, with a productivity of around 22.39 MT and an area of about 2.4 million hectares (Anonymous, 2024) [1].

Mangoes are grown on 145, 886 hectares of land in Tamil Nadu, with an average yield of 6.5 MT/ha (NHB, 2024) [15]. India exports around 32, 104 metric tonnes of fruits worth Rs. 420 crores, mostly to the United Arab Emirates, the United Kingdom, the United States, Kuwait, and Qatar (APEDA, 2023) [12].

One of the most harmful abiotic factors that lowers crop productivity globally is salinity in the soil. According to reports (Bajpai *et al.*, 2017; Deivasigamani *et al.*, 2018) [13, 7], the majority of mango cultivars are extremely sensitive to soil or water salinity, especially during the seedling stage. This can lead to poor seedling growth, scorching of the tips and margins of the leaves, and curling of the leaves, which in extreme cases can result in plant death. To use polyembryonic mango genotypes as tolerant rootstocks for grafting of preferred cultivars vulnerable to salinity, it will be useful to determine their degree of salt tolerance. Research on salinity screening for seedlings. Fruit crops such as citrus, grape, and mango have been stressed (Pedroso, 2014; Pandey, 2014) [19, 17].

The two main stresses that salinity induces in plants are osmotic stress, which reduces plant water intake, and ionic stress, which is mostly caused by the detrimental effects of sodium and chloride ions on plant cells (Munns and Tester, 2008) [14]. Some mango seedlings did not grow well in salinity-prone locations. Salinity is one of the primary abiotic stressors that restricts plant development and productivity, posing a major threat to agriculture's long-term viability. Yamaguchi and Blumwald (2005) [23] estimate that salinity presently affects 20% of the world's irrigated land.

However, there was insufficient research on the screening of mango genotypes throughout the early stages of growth for their possible application as rootstock for saline resistance. In light of these facts, we examined how NaCl stress affected early growth in five genotypes of polyembryonic mangos at the seedling stage. This research focused on evaluating morpho-physiological responses, including stress indices, shoot and root growth and chlorophyll content. The results helped to choose tolerant genotypes that can be employed in salt-affected regions or incorporated into breeding programs to create cultivars that can withstand salt stress. The growth performance of various mango genotypes under different salinity levels at the seedling stage was examined.

2. Materials and Methods

The current study was carried out at the North Farm, School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Coimbatore, Tamil Nadu, between 2025 and 2026. At a height of 467 meters above mean sea level, the experimental location is situated at 10°56'25.79' N latitude and 76°44'32.31' E longitude. The area has a tropical climate zone. Meteorological data collected throughout the trial period

2.1 Plant Materials

2.1.1 Genotypes used

This study included the five mango genotypes G₁-Mundapa, G₂-Chengavarkai, G₃-Muvandan, G₄-Sarkarai Kuty, and G₅-Vellari was used for screening the salinity tolerance at the seedling stage.

2.2 Experimental Procedure

Seedlings were raised in nursery beds and transplanted into polybags (36 × 21 cm) filled with about 5 kg of soil. The

plants were kept in consistent circumstances, with regular irrigation and weeding. Salinity treatments were applied when seedlings reached three months of age and had three to four completely grown leaves.

2.2.1 Imposed Salinity Stress

Salinity stress was induced by using varying levels of sodium chloride (NaCl) solution. Plants were irrigated on alternate days using 1litre of solution per plant. Treatments included varying NaCl concentrations (control, 25 mM, 45 mM, 65 mM, and 85 mM). The electrical conductivity (EC) ranged from 1.6 to 7.91 dsm⁻¹, whereas the pH remained at 7.8 to avoid confounding nutrient availability.

2.2.2 Experimentation Design

This experiment was organised in a Factorial Completely Randomized Design (FCRD) with Factor 1: genotypes at 5 levels and Factor 2: Salinity Levels at 5 levels. A total of 25 treatment combinations were kept with 3 replications. The plants were kept in a semi-protected structure to prevent saline dilution from precipitation while still allowing for adequate light and aeration.

2.3 Observations Recorded

2.3.1 Morphological parameters

2.3.1.1 Plant Height (cm)

Plant height was measured from the plant's base to the tip of the apical bud using a measuring scale and expressed in centimetres.

2.3.1.2 Number of leaves per plant (No.)

The number of fully grown leaves per plant was manually counted during the observation.

2.3.1.3 Length of leaves (cm)

The length of the third leaf from the top was measured from its base to the tip and given in cm.

2.3.1.4 Leaf width (cm)

The leaf width of the treated plants was measured on a meter scale and expressed in centimeters (cm), including the leaf stem.

2.3.1.5 Stem diameter (mm)

The stem diameter of a germinated seedling was measured using a 'Vernier caliper' and expressed in millimeters (mm). The stem diameter was measured after leaving one centimeter from the root starting point.

2.3.2 Physiological parameters

2.3.2.1 Leaf area (cm²)

Leaf Area Meter (LICOR, 3000) is used for estimating leaf area and expressed in cm².

2.3.2.3 Relative water content (RWC)

The Barrs and Weatherley (1962) [4] method was used to determine relative water content (RWC, %). Fresh leaf discs were weighed to determine fresh weight (FW), floated in distilled water to determine turgid weight (TW), and then oven-dried at 80 °C for 48 hours to determine dry weight (DW). RWC was computed as:

$$RWC(\%) = \frac{FW - DW}{TW - DW} \times 100$$

2.3.2.4 Chlorophyll

A 250 mg fresh leaf sample was weighed and macerated with 10 mL of 80% acetone. The contents were centrifuged at 3000 rpm for ten minutes. The supernatant was extracted

and diluted to 25 ml with 80% acetone. The optical density was calculated at 645 and 663 nanometers. Yoshida (1972)^[30] estimated the chlorophyll content of leaves and expressed it in mg g^{-1} .

$$\text{Total chlorophyll} = (8.02 \times \text{OD at 663}) + (20.2 \times \text{OD at 645}) \times \frac{V}{1000 \times W}$$

OD-Optical Density; V-Final volume of supernatant solution (25 mL)

W-Weight of the leaf sample collected in grams (0.25 g).

2.3.3 Biochemical parameters

2.3.3.1 Proline

Proline content was determined using a fast colorimetric approach (Bates *et al.*, 1973)^[5]. A leaf sample (0.25 g) was homogenized in 5ml of 3% aqueous sulphosalicylic acid, then centrifuged at 10000 rpm for 10 minutes at 4 °C. Two millimeters of supernatant were collected in a test tube, and

2 mm of glacial acetic acid and acid ninhydrin were added. The test tubes were heated in a boiling water bath for 1 hour before being placed in an ice bath to terminate the reaction. The toluene layer was separated by adding 4 mL of toluene to the reaction mixture and stirring it for 20-30 seconds with an electric shaker. The intensity of the dark pink colour produced was measured at 520 nm with toluene as a blank using a UV-Visible spectrophotometer (T80 + UV/VIS, PG Instruments Ltd., UK), and the proline content was estimated using the formula below and represented in mg 100g^{-1} fresh weight.

$$\text{Proline content } \mu\text{Mg}^{-1} = \frac{\text{value from graph } (\mu\text{g}) \times 4 \text{ ml toluene}}{115.5} \times \frac{5}{\text{weight of sample (g)}}$$

2.4 Statistical analysis

The traditional methods of Gomez and Gomez (1984)^[9] were employed for statistical analysis of the data, and essential differences were estimated at the five percentage probability level, where the treatment differences were substantial after estimating the analysis of variation.

3. Results and Discussion

3.1 Plant height

The results for plant height revealed the impact of salinity stress on genotypic performance and their interactions (Table 1). The genotypes and salt levels both had a substantial effect on plant height. Among the genotypes, G_3 had the highest plant height (51.77 cm), followed by G_1 , and G_2 had the lowest (37.90 cm), showing poor performance under the experimental conditions. Salinity stress induced a progressive decrease in plant height. Plant height was maximum under control conditions S_1 (0 mM, 48.78 cm), and lowest at S_5 (85 mM, 41.35 cm). This decrease in plant height at higher salinity levels may be attributed to osmotic stress, which inhibits water intake, as well as the toxic effects of excess salts, which hinder cell division and elongation. The interaction of genotypes and salinity levels revealed that G_3S_1 (54.36 cm) had the highest plant height, and G_2S_5 (32.74 cm) had the lowest. This obviously implies that G_3 is more resistant to salt stress, whereas G_2 is more susceptible. Overall, the decrease in plant height under salinity stress indicates that increased salt concentrations have a negative impact on plant growth, while tolerant genotypes can maintain better growth than sensitive genotypes.

The reduction in plant height under saline conditions could be related to osmotic stress and ionic toxicity, which limit water uptake and produce nutrient acquisition imbalances. High concentrations of Na^+ and Cl^- ions disrupt cellular metabolism, resulting in decreased cell division and elongation, ultimately limiting plant growth. Furthermore, salinity stress can disrupt hormonal balance, lowering auxin and gibberellin activity, which are required for stem elongation. G_3 had greater performance, indicating a better capacity for osmotic adjustment, ion homeostasis, and

turgor pressure maintenance, allowing for continued development under stressful environments. The experiment findings are consistent with studies by Srivastav *et al.* (2007)^[27], Roy *et al.* (2014)^[28], and Pandey *et al.* (2014)^[17] on mango growth parameters.

3.2 Number of leaves

The data in Table 1, shows how different genotypes reacted to varying salt concentration regarding leaf production. Both genotypes and the level of salinity significantly influenced the final leaf count. G_3 outperformed the others with an average of (14.37) leaves, while G_2 exhibited the poorest growth with the lowest leaf count (10.72), indicating poor growth performance. Salinity stress caused a gradual and persistent decline in the number of leaves, from S_1 (16.14) in control conditions to S_5 (7.75) at maximum salinity. This decline likely stems from salt induced interference with cell division and leaf development, representing a survival mechanism where the plant limits its surface area to minimize water loss during stress.

The relationship between genotypes and salinity showed that the G_3S_1 combination produced the highest leaf count (17.95), whereas G_2S_5 produced the fewest (5.94). These results highlight that G_3 maintains superior leaf production even under pressure, while G_2 is particularly sensitive to saline conditions. Essentially, increasing salt levels hinder leaf development, but hardy genotypes can sustain more growth than weaker ones. This reduction in leaf count under stress is often tied to inhibited cell expansion, early leaf aging, and the shedding of older leaves due to toxic ion buildup and nutrient imbalance. High levels of Na^+ and Cl^- disrupt internal metabolism, which restricts the resources available for generating new leaves.

Furthermore, Salt stress can impair photosynthetic function and shift hormonal levels, specifically by reducing cytokinin activity, which is required for leaf initiation and development. The success of G_3 suggests an ability to maintain internal balance, manage ion levels and keep metabolic processes, which allows it to continue producing leaves in harsh environments. These observations align with earlier research conducted by Pandey *et al.* (2014)^[17],

Perica *et al.* (2008)^[26], and Rahim *et al.* (2021)^[25].

3.3 Leaf length

Based on the data in Table 1, both the specific genotype and salt levels played a major role in determining leaf length. Among those genotypes, G₃ exhibits the most substantial leaf length at (23.21 cm), while G₅ produced the shortest at (18.88 cm), highlighting clear differences in how these plants grow. As salt concentrations increased, leaf length showed a steady decline, dropping from average of 22.71 cm at control S₁ (0 mM) to 17.85 cm at S₅. Looking at the interaction between factors showed that G₃S₁ (24.84 cm) achieved the maximum length, while G₂S₅ (13.42 cm) had the lowest.

This shortening of leaves under salt stress is likely a consequence of osmotic pressure, which restricts water absorption and cell turgor, restricting cell elongation and expansion. Toxic ions, such as Na⁺ and Cl⁻, can interfere with regular metabolism and enzyme function, further checking leaf development. Salinity also tends to throw hormones out of balance, specifically by lowering auxin and gibberellin levels, both of which are vital for elongation. The fact that G₃ maintained longer leaves suggests it is better at maintained longer leaves suggests it is better at managing osmotic adjustments and ion levels, allowing it to keep its cells hydrated and growing even in salty soil. These findings are similar to the results reported by Yao *et al.* (2023)^[29] in their research on berry leaves.

3.4 Leaf width

Data from Table 1, demonstrates that leaf width fluctuated significantly based on the plant genotypes and salinity levels (Table 1). Genotype G₃ produced the widest leaves at an average of (7.14 cm), while G₂ produced the narrowest leaves (6.03 cm), marking a distinct difference in how these plants develop. As salt concentrations increased, leaf breadth showed a steady decline, falling from 7.22 cm in the control at S₁ (0 mM) to 5.83 cm at highest level S₅. The interaction between the factors showed that G₃S₁ (7.56 cm) reached the maximum width, while G₂S₅ (5.13 cm) dropped to a minimum. This reduction in leaf width is likely caused by salt induced stress, which limits cell expansion and reduces the water availability for growth. In contrast, the more resilient genotypes managed to preserve larger leaf dimensions even under these conditions.

The presence of too much Na⁺ and Cl⁻ ions can lead to ionic toxicity, which interferes with metabolic processes and stunts leaf development. Salinity also affects hormonal regulation, particularly by decreasing the activity of growth-promoting hormones like auxins and cytokinin that are essential for widening the leaf surface. The significantly better leaf width seen in G₃ indicates a strong capacity for osmotic regulation and ion imbalance, which protects physiological functions and allow the plants to continue growing despite in environmental stress. This similar study carried out by Yao, *et al.* (2023)^[29].

3.5 Leaf area

Leaf area varied dramatically among genotypes and salt levels. G₃ had the biggest leaf area (80.64 cm²), whereas G₂ had the lowest (53.99 cm²), demonstrating varying growth capacities (Table 1).

As salinity increased, leaf area decreased dramatically, from 100.58 cm² at S₁ (0 mM) to 35.25 cm² at S₅ (85 mM). G₃S₁ had the highest leaf area (105.31 cm²), whereas G₂S₅ had the lowest (21.32 cm²) based on the interaction effect. This loss under salinity stress could be attributed to restricted leaf expansion and decreased water availability, whereas resistant genotypes were able to maintain larger leaf area even under stressful circumstances. Pandey *et al.* (2014)^[17] and Khayyat *et al.* (2014)^[11] both reported similar findings in mango.

Leaf area is an important factor in determining photosynthetic capacity and reducing it has a direct impact on biomass accumulation and plant productivity. Salinity stress also increases leaf senescence and abscission, lowering the effective photosynthetic surface area. Additionally, hormonal imbalance, particularly a drop in the activity of auxin and cytokinin, can lead to restricted leaf expansion under stress. The fact that G₃ was able to keep a much larger leaf surface area shows its strong ability to adjust osmotic levels and manage ions. This helps the plant maintain efficient photosynthesis over time, which is a crucial characteristic for salinity tolerance.

3.6 Stem diameter

Both the genotype and the amount of salt present influenced stem thickness. G₃ showed the greatest stem diameter (18.79 cm), while G₂ had the smallest (17.31 cm), indicating a clear gap growth difference (Table 1). As salinity levels increased, there was a steady drop in stem thickness, falling from an average of 18.49 cm in control S₁ (0 mM) to 17.33 cm at the highest level S₅. When the interaction effect revealed that G₃S₁ (18.96 cm) reached the greatest value, and G₂S₅ (16.14 cm) recorded the lowest. This shrinking of the stems is likely due to the negative impact of salt on tissue formation and overall development, though more resilient plants were able to maintain thicker stems in stressed condition.

Under salinity stress, stem diameter decreases due to reduced cambial activity, slower cell division, and secondary growth, all of which are predominantly driven by osmotic stress and ionic toxicity. Excessive accumulation of Na⁺ and Cl⁻ ions disrupt the plants ability to maintain nutritional equilibrium, compromising the intake of essential nutrients such as potassium and calcium, which are essential for cell wall growth and structure. Furthermore, salt stress can interfere with hormone levels, particularly by lowering gibberellin activity, which is necessary for stem growth. G₃ significantly maintained a thicker stem suggests it has a better ability to manage the internal chemistry and metabolism, allowing for long-term structural growth under stress. These research findings were done by Roy *et al.*, (2014)^[28] in mango.

Table 1: Effect of salinity level on growth parameters (plant height, number of leaves, leaf length, leaf width, leaf area and stem diameter) of five genotypes at seedling stage.

Treatments	Plant height (cm)	No of leaves (No)	Leaf length (cm)	Leaf width (cm)	Leaf area (cm ²)	Stem diameter (cm)
Genotypes						
G ₁	48.17 ^b	12.54 ^b	21.43 ^b	6.86 ^b	73.92 ^b	18.21 ^b
G ₂	37.90 ^c	10.72 ^e	19.51 ^c	6.03 ^c	53.99 ^e	17.31 ^e
G ₃	51.77 ^a	14.37 ^a	23.21 ^a	7.14 ^a	80.64 ^a	18.79 ^a
G ₄	45.51 ^c	12.17 ^c	19.49 ^c	6.47 ^c	65.72 ^c	17.92 ^c
G ₅	41.63 ^d	11.50 ^d	18.88 ^d	6.20 ^d	60.01 ^d	17.69 ^d
SE (d)	0.164	0.073	0.164	0.073	0.31	0.026
CD (5%)	0.33	0.147	0.33	0.147	0.623	0.051
Salt concentration						
S ₁	48.78 ^a	16.14 ^a	22.71 ^a	7.22 ^a	100.58 ^a	18.49 ^a
S ₂	47.01 ^b	14.69 ^b	21.46 ^b	6.92 ^b	88.06 ^b	18.35 ^b
S ₃	44.84 ^c	12.85 ^c	20.70 ^c	6.49 ^c	64.09 ^c	18.03 ^c
S ₄	43.00 ^d	9.86 ^d	19.79 ^d	6.25 ^d	46.29 ^d	17.70 ^d
S ₅	41.35 ^e	7.75 ^e	17.85 ^e	5.83 ^e	35.25 ^e	17.33 ^e
SE (d)	0.164	0.073	0.164	0.073	0.31	0.026
CD (5%)	0.33	0.147	0.33	0.147	0.623	0.051
Interaction (Genotypes x Salt concentration)						
G ₁ S ₁	51.74 ^c	16.37 ^b	23.36 ^{ab}	7.35 ^{ab}	103.30 ^b	18.54 ^{ef}
G ₁ S ₂	49.23 ^e	14.53 ^e	22.42 ^{def}	7.28 ^{ab}	92.31 ^f	18.51 ^f
G ₁ S ₃	47.58 ^f	13.17 ^g	21.37 ^{gh}	6.65 ^{def}	74.52 ^k	18.23 ^h
G ₁ S ₄	46.63 ^g	10.43 ^k	20.48 ^{ijk}	6.68 ^{def}	55.14 ⁿ	17.95 ⁱ
G ₁ S ₅	45.68 ^h	8.21 ⁿ	19.53 ^{lm}	6.37 ^{fgh}	44.34 ^q	17.83 ^j
G ₂ S ₁	43.19 ^j	14.93 ^d	22.59 ^{cde}	6.79 ^{dc}	94.42 ^e	18.13 ^h
G ₂ S ₂	41.21 ^l	12.75 ^h	21.67 ^{gh}	6.47 ^{efg}	78.45 ^j	17.88 ^{ij}
G ₂ S ₃	37.83 ⁿ	11.25 ^j	20.54 ^{ij}	5.96 ^{ijk}	47.26 ^p	17.35 ^l
G ₂ S ₄	34.54 ^o	8.74 ^m	19.33 ^{mn}	5.83 ^{jk}	28.52 ^t	17.06 ^m
G ₂ S ₅	32.74 ^p	5.94 ^q	13.42 ^q	5.13 ^l	21.32 ^v	16.14 ^o
G ₃ S ₁	54.36 ^a	17.95 ^a	24.84 ^a	7.56 ^a	105.31 ^a	18.96 ^a
G ₃ S ₂	53.15 ^b	17.95 ^a	23.29 ^{bc}	7.35 ^{ab}	98.67 ^d	18.87 ^{ab}
G ₃ S ₃	51.48 ^c	14.76 ^{de}	23.25 ^{bc}	7.19 ^{bc}	81.52 ⁱ	18.79 ^{bc}
G ₃ S ₄	50.74 ^d	11.52 ^j	22.84 ^{bcd}	6.94 ^{cd}	65.46 ^l	18.72 ^{cd}
G ₃ S ₅	49.13 ^e	9.67 ^l	21.86 ^{efg}	6.68 ^{def}	52.24 ^o	18.65 ^{de}
G ₄ S ₁	48.94 ^e	15.82 ^c	21.72 ^{fgh}	7.21 ^{bc}	101.35 ^c	18.49 ^f
G ₄ S ₂	47.32 ^{fg}	14.63 ^{de}	20.21 ^{kl}	6.94 ^{cd}	87.53 ^g	18.36 ^g
G ₄ S ₃	45.72 ^h	12.92 ^{gh}	19.13 ^{mn}	6.42 ^{fg}	61.32 ^m	17.98 ⁱ
G ₄ S ₄	43.65 ^{ij}	9.68 ^l	18.78 ⁿ	6.07 ^{hij}	44.56 ^q	17.53 ^k
G ₄ S ₅	41.95 ^k	7.84 ^o	17.65 ^o	5.71 ^k	33.84 ^s	17.24 ^l
G ₅ S ₁	45.68 ^h	15.64 ^c	21.08 ^{hi}	7.23 ^{bc}	98.55 ^d	18.37 ^g
G ₅ S ₂	44.15 ⁱ	13.62 ^f	19.75 ^{klm}	6.58 ^{cf}	83.34 ^h	18.17 ^h
G ₅ S ₃	41.63 ^{kl}	12.17 ⁱ	19.23 ^{mn}	6.24 ^{ghi}	55.86 ⁿ	17.83 ^j
G ₅ S ₄	39.46 ^m	8.96 ^m	17.52 ^{op}	5.73 ^k	37.81 ^r	17.25 ^l
G ₅ S ₅	37.27 ⁿ	7.13 ^p	16.82 ^p	5.26 ^l	24.53 ^u	16.83 ⁿ
SE (d)	0.367	0.163	0.367	0.163	0.694	0.057
CD (5%)	0.738	0.328	0.738	0.328	1.394	0.115

Notes: SE d: Standard error of difference, CD (p = 0.05): Critical difference at 5% level of significance. G: Genotype, S: Salinity level, G × S: Interaction effect. G₁-Mundapa, G₂-Chengavarkai, G₃-Muvandan, G₄-Sarkarai Kutty, and G₅-Vellari.

3.7 Relative water content

In Table 2, genotypes showed considerable differences in relative water content. The highest value was reported in G₃ (64.65%), while the lowest was in G₂ (49.50%), showing disparities in their ability to retain water inside the tissues. This shows that certain genotypes are more suited to maintaining internal moisture under a variety of situations. As saline levels increased, the relative water content decreased dramatically. The values decreased from 76.80% at S₁ (control) to 36.96% at S₅ (85 mM), indicating that increasing salt concentrations influence water absorption and retention in plants. This decrease reflects the difficulty

that plants face in absorbing water when the surrounding medium contains increased salt levels.

The interaction between genotypes and salt levels followed a similar pattern. The highest value was observed in G₃S₁ (85.61%), while the lowest was discovered in G₂S₅ (28.13%). This suggests that G₃ is better able to maintain water balance under stress, but G₂ is more susceptible to saline environments. The decrease in RWC under salinity stress is primarily caused by the soil solution's higher osmotic potential, which limits water availability to plants and inhibits water uptake by roots. The presence of greater RWC in G₃ indicates better osmotic adjustment, probably due to the buildup of suitable solutes and improved root water absorption capacity. This feature is critical for maintaining physiological functions under stress. These studies were previously conducted by Khayyat *et al.*, (2014) ^[11] in pomegranate and Nimbolkar *et al.*, (2023) ^[16] in mango.

3.8 Chlorophyll content

According to the data in Table 2, there were notable variations in chlorophyll levels among the different genotypes. The G₃ exhibited the highest concentration at (1.18), whereas G₂ (0.72) recorded the lowest. These figures highlight the varying capacities of these genotypes to maintain their photosynthetic pigments, showing differences in their ability to sustain photosynthetic pigments. Specifically, concentrations dropped from a peak as salinity levels increased, there was a continuous drop in chlorophyll concentration. The values decreased from a peak of 1.41 at S₁ (0 mM) to a low of 0.52 at S₅ (85 mM), This trend suggests that higher salt concentrations negatively impact both the stability of these pigments and the overall photosynthetic efficiency of the plants.

The relationship between genotypes and salinity levels followed a similar trend. G₃S₁ had the most chlorophyll (1.64), while G₂S₅ had the least (0.24). This suggests that G₃ is better at retaining chlorophyll under stress, whereas G₂ is more severely damaged by salt. The decrease in chlorophyll content under salt stress could be related to chlorophyll breakdown, damage to chloroplast ultrastructure, and inhibition of chlorophyll production enzymes. Furthermore, salinity-induced nutritional imbalance, particularly reduced nitrogen and magnesium intake, inhibits pigment production. G₃ has the ability to retain increased chlorophyll content, which supports improved photosynthetic apparatus protection and prolonged photosynthetic effectiveness in stressful conditions. A similar study was conducted by Nimbolkar *et al.*, (2023) ^[16] and Morsy (2003) ^[13].

3.9 Proline content

Proline content ranged between genotypes shown (Table 2), with G₃ having the highest value (167.10) and G₂ having the lowest (119.04). This demonstrates heterogeneity in the genotypes' ability to respond to stress circumstances. The values increased from 123.42 at S₁ (0 mM) to 162.14 at S₅ (85 mM), indicating that proline accumulation is related to stress response in plants.

The interaction between genotypes and salt levels revealed that G₃S₅ (191.2) had the highest proline content, whereas G₂S₁ (103.6) had the lowest. This suggests that G₃ has a greater ability to collect proline under stress, whereas G₂ has a weaker reaction. Under salt stress, proline accumulation is critical for osmotic correction, protein and membrane

stability, and ROS scavenging. It also aids in maintaining cellular redox balance and safeguarding metabolic activities under stress. The greater concentration of proline in G₃ indicates a more efficient stress-responsive biochemical pathway, which contributes to its tolerance. Similar studies carried out by Pandey *et al.*, (2014) [17], Nimbolkar *et al.*, (2023) [16].

Table 2: The effect of Genotypes and Salinity concentration on relative water content (RWC), chlorophyll content and proline.

Treatments	Relative water content (%)	Chlorophyll	Proline (μmol g ⁻¹ FW)
Genotypes			
G ₁	58.10 ^b	1.07 ^b	151.54 ^b
G ₂	49.50 ^c	0.72 ^c	119.04 ^c
G ₃	64.65 ^a	1.18 ^a	167.10 ^a
G ₄	54.99 ^c	0.94 ^c	138.10 ^c
G ₅	52.26 ^d	0.79 ^d	129.72 ^d
SE (d)	0.354	0.018	0.84
CD (5%)	0.711	0.037	1.687
Salt concentration			
S ₁	76.80 ^a	1.41 ^a	123.42 ^c
S ₂	69.19 ^b	1.23 ^b	128.26 ^d
S ₃	52.92 ^c	0.88 ^c	141.64 ^c
S ₄	43.64 ^d	0.66 ^d	150.04 ^b
S ₅	36.96 ^e	0.52 ^e	162.14 ^a
SE (d)	0.354	0.018	0.84
CD (5%)	0.711	0.037	1.687
Interaction (Genotypes x Salt concentration)			
G ₁ S ₁	77.31 ^b	1.47 ^b	132.2 ^{ij}
G ₁ S ₂	69.50 ^c	1.38 ^{cd}	136.3 ^b
G ₁ S ₃	55.86 ⁱ	1.03 ^{gh}	150.4 ^{ef}
G ₁ S ₄	47.55 ^l	0.82 ^j	160.3 ^d
G ₁ S ₅	40.32 ^o	0.67 ^{kl}	178.5 ^b
G ₂ S ₁	72.31 ^d	1.24 ^e	103.6 ^o
G ₂ S ₂	65.45 ^e	1.04 ^g	109.2 ⁿ
G ₂ S ₃	47.67 ^l	0.68 ^{kl}	120.3 ^l
G ₂ S ₄	33.97 ^q	0.44 ^{mn}	126.4 ^k
G ₂ S ₅	28.13 ^r	0.24 ^o	135.7 ^{hi}
G ₃ S ₁	85.61 ^a	1.64 ^a	145.8 ^g
G ₃ S ₂	77.15 ^b	1.45 ^{bc}	152.7 ^c
G ₃ S ₃	61.43 ^h	1.14 ^f	167.4 ^c
G ₃ S ₄	53.86 ^j	0.95 ^{hi}	178.4 ^b
G ₃ S ₅	45.23 ^m	0.75 ^{jk}	191.2 ^a
G ₄ S ₁	75.25 ^c	1.40 ^{bcd}	121.3 ^l
G ₄ S ₂	67.42 ^f	1.25 ^e	125.4 ^k
G ₄ S ₃	51.37 ^k	0.94 ⁱ	139.3 ^h
G ₄ S ₄	43.23 ⁿ	0.61 ^l	147.6 ^{fg}
G ₄ S ₅	37.68 ^p	0.52 ^m	156.9 ^d
G ₅ S ₁	73.52 ^d	1.34 ^d	114.2 ^m
G ₅ S ₂	66.46 ^{fg}	1.06 ^{fg}	117.7 ^{lm}
G ₅ S ₃	48.27 ^l	0.65 ^l	130.8 ⁱ
G ₅ S ₄	39.60 ^o	0.49 ^{mn}	137.5 ^h
G ₅ S ₅	33.46 ^q	0.42 ⁿ	148.4 ^{fg}
SE (d)	0.792	0.041	1.878
CD (5%)	1.591	0.082	3.722

Notes: SE d: Standard error of difference; CD (p = 0.05): Critical difference at 5% level of significance. G: Genotype; S: Salinity level; G × S: Interaction effect. G₁-Mundapa, G₂-Chengavarkai, G₃-Muvandan, G₄-Sarkarai Kutty, and G₅-Vellari.

4. Conclusion

This research study clearly demonstrated that salt stress has a major impact on growth, physiological, and biochemical characteristics at the seedling stage. Increasing salinity (0 to 85 mM NaCl) with different polyembryonic genotypes, Mundapa, Sakkarai Kutty, Muvandan, G₁-Mundapa, G₂-

Chengavarkai, G₃-Muvandan, G₄-Sarkarai Kutty, and G₅-Vellari. Chengavarkai, and Vellari resulted in a significant decrease in plant height, number of leaves, leaf length and width, leaf area, relative water content, and chlorophyll content, showing that osmotic stress and ionic toxicity had harmed plant growth and metabolism. In contrast, proline accumulation increased gradually under saline conditions, demonstrating its protective role in osmotic adjustment and lowering levels of stress. Among the five genotypes examined, G₃-Muvandan consistently performed better than the others by sustaining higher growth, water status, and chlorophyll content, as well as increased proline accumulation under stress conditions and showing superior salinity tolerance. Among the studied genotypes, G₂-Chengavarkai consistently underperformed across the majority of the metrics, which highlights a susceptibility to salt stress. G₃S₁ showed peak performance, reaching a maximum plant height (54.36 cm), leaf count (17.95), leaf area (105.31 cm²), relative water content (85.61%), and chlorophyll content (1.64). Severe salt stress (S₅) reduced all parameters, with the lowest values recorded in the G₂S₅ combination, including plant height (32.74 cm), leaf number (5.94), leaf area (21.32 cm²), relative water content (28.13%), and chlorophyll content (0.24). However, proline accumulation increased under stress, with the greatest value seen in G₃S₅ (191.2), indicating a significant adaptation response of the tolerant genotype to high salinity levels. The observed genotypic variance emphasizes the importance of physiological and biochemical variables in salt tolerance in more resilient genotypes. Overall, the findings indicate that seedling stage screening is an effective and dependable technique for identifying salinity tolerant genotypes. The genotype G₃ Muvandan is an appropriate strategy for agriculture in salt affected areas and could be a valuable genetic resource in breeding projects aiming at increasing salinity resistance.

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6. Competing Interest

Authors declare that there are no conflicting interests associated with this manuscript.

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