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Research Article

Effect of Saline Water and Spray of 28-Homobrassinolide on Growth of Polyembryonic Mango (*Mangifera indica* L.) Seedling

Arpita M Patel[1]*, Patel CR[2] and Tanvi A Patel[2]

[1]*Department of Fruit Science, ASPEE College of Horticulture, Navsari Agricultural University, Navsari, Gujarat, India*

[2]*Associate Research Scientist (Horticulture), Agriculture Experimental Station, Navsari Agricultural University, Paria, Gujarat, India*

***Corresponding Author:** Arpita M Patel; Department of Fruit Science, ASPEE College of Horticulture, Navsari Agricultural University, Navsari, Gujarat, India.

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Abstract

A study was undertaken to evaluate the effect of saline water and spray of 28-homobrassinolide on growth of polyembryonic mango (*Mangifera indica* L.) seedling during the years 2021-22 and 2022-23 at Agriculture Experimental Station, Navsari Agricultural University, Paria, (Gujarat). The experiment was laid out in Completely Randomized Design with factorial concept comprising of four salinity levels [S1 : Best available water (control), S2: 2 dS m-1, S3: 4 dS m-1, S4: 6 dS m-1] and three spray concentration of 28-homobrassinolide [H1: No spray, (control), H2 : 0.5 ppm and H3 : 1 ppm]. Saline water application and 28-homobrassinolide spray was started at 80 days after sowing up to 240 days. Treatment containing application of water having lowest salinity (best available water) resulted in significantly higher plant height, stem diameter, number of leaves, fresh and dry biomass Along with higher survival at 9 months after sowing during both years and in pooled analysis. Application of saline water having EC 6 dS/m adversely affected the growth of polyembryonic mango seedlings, reduced vigour, biomass and survival at 9 month of sowing. The spray concentrations of 28-homobrassinolide significantly affected the growth of polyembryonic mango seedlings at 9 month of sowing, maximum values for various growth parameters along with vigour, biomass and survival were noted in mango seedlings treated with spray of 1 ppm 28-homobrassinolide during both years and in pooled analysis. The interactions of salinity levels and 28-homobrassinolide spray significantly affect different growth parameters of mango seedlings. Maximum plant height, stem diameter, number of leaves fresh and dry biomass and survival at 9 months after sowing during both the years and in pooled analysis were recorded in treatment combination of best available water along with 1 ppm spray of 28-homobrassinolide. Therefore, it can be concluded that polyembryonic mango seedlings of selected genotype can be grown with saline water irrigation having salinity up to 4 dS/m along with 1 ppm 28-homobrassinolide spray for better growth, biomass and higher survival up to 9 months after sowing.

Keywords: Mango; Salinity; 28-Homobrassinolide; Polyembryonic

Introduction

Mango (*Mangifera indica* L., family Anacardiaceae) is one of the major fruit crops of Asia and has developed its own importance all over the world. Mango is native to South East Asia from where the "common mango" or "Indian mango" has been distributed worldwide to become one of the most widely cultivated fruits in the tropics. Mango fruit is mainly used for fresh consumption, canning, juice and other products. Mango fruit is a rich source of potassium, beta-carotene and antioxidants. Ripe mangoes contain moderate levels of vitamin C, rich in vitamin A, B1 and B2. India is the largest producer of mango in the world. Major mango growing states are Andhra Pradesh, Uttar Pradesh, Karnataka, Bihar, Gujarat and Tamil Nadu in India. Various abiotic stresses like drought, salinity, etc. are becoming serious issues for crop production including mango. Among these stresses, salinity through soil and irrigation water is becoming a major problem in mango cultivation due to their negative effects on plant growth and production [1]. Along the coastal belt of Gujarat, saline water is the major problem and farmers are bound to use saline water for irrigation which affects the growth of plant. Growth rate and size of the plant progressively decreased with increased salinity. Plant height and leaf area are drastically reduced due to salinity. Mango rootstocks and commercial cultivars differ in their response to salinity stress [2]. Polyembryonic genotypes of mango have better salt tolerance potential than monoembryonic genotypes. Identifying the level of salt tolerance in polyembryonic mango genotypes can be helpful for its use as tolerant rootstocks for grafting and subsequent planting under salinity affected areas. Selection of salinity tolerant mango genotype is a potential solution for the salinity problem. There are various methods to minimize the destructive impacts of salinity. Brassinosteroids (BRs) are group of naturally occurring phytohormones with growth promoting nature and are essential for normal growth and development [3]. Brassinosteroids (BRs) are ubiquitous steroid plant growth substances that occupy prominent functions in various biochemical pathways leading to accelerating plant stress responses [4]. BRs regulated stress response owing to a complex progression of biochemical reactions such as activation or repression of key enzymatic reactions, stimulation of protein assimilation, and the assembly of diverse chemical defense materials. Brassinosteroids have a shielding effect against the stresses and thereby enhance tolerance or resistance of the plants against several extreme conditions such as temperature, drought stress, salt stress, toxicity of heavy metals and disease-causing microorganisms [5,6].

Materials and Methods

The present investigation entitled effect of saline water and spray of 28-homobrassinolide on growth of polyembryonic mango (*Mangifera indica* L.) seedling was carried out during the years 2021-22 and 2022-23 at Agriculture Experimental Station, Navsari Agricultural University, Paria, (Gujarat, India). The polyembryonic mango stones were sown in black polythene bags (9 × 11-inch size) containing media mixture of garden soil: biocompost (4:1). Single stone was sown in each bags. The stones were soaked for 30 minutes in solution containing fungicide and then used for sowing in polythene bags containing media. The experiment comprising four salinity levels [S1: Best available water (Control), S2: 2 dS m-1, S3: 4 dS m-1 and S4: 6 dS m-1] along with three spray concentration of 28-homobrassinolide [H1: No spray, (control), H2 : 0.5 ppm and H3 : 1 ppm] which formed twelve treatment combination which were replicated thrice. The Saline water application was started at 80 days after sowing and continued up to 9 months after sowing. Uniform volume of water (500 ml) was applied to all the plants in all treatments when irrigation required to maintain uniformity among salinity treatments. The experiment was set up in a Completely Randomized Design with factorial concept. The standard method of analysis of variance technique appropriate to Completely Randomized Design was used for individual years as well as for pooled analysis over the year described by [5]. The means of all the treatment were compared using Duncan's Multiple Range Test (DMRT).

Preparation of solution

Preparation saline water

For Water with different levels of salinity was prepared by dissolving (NaCl) in best available water (BAW) as per the respective treatments and desired salinity level adjusted with the help of EC meter.

Preparation of 28-homobrassinolide

The product available in market with 0.04% 28-homobrassinolide was used for the experiment during both years. A solution with 0.5 ppm 28-homobrassinolide was prepared by adding 1.25 ml of commercial product in one litre of water. Similarly, the solution containing 1.0 ppm 28-homobrassinolide was prepared by adding 2.50 ml of commercial product in one litre of water.

Time of application

Observations recorded

Plant Height (cm)

The height of five selected seedlings was measured from surface of soil media to the growing tip of seedlings with the help of measuring scale at monthly interval starting from 4 to 9 months after sowing and the mean data were calculated and expressed in centimetre.

Stem diameter (mm)

Stem diameter of the five observational seedlings were measured with the help of Vernier calipers at 3 cm from the base at monthly interval starting from 4 to 9 months after sowing. The mean was worked out and recorded as stem diameter.

Number of leaves

Fully expanded leaves on each five selected seedlings were counted at monthly interval starting from 4 to 9 months after sowing and the mean data were calculated for statistical analysis.

Leaf area (cm^2)

To measure the leaf area, one fully expanded leaf was taken from the middle portion of non-tagged seedling and the leaf area was measured by using leaf area meter at monthly interval starting from 4 to 9 months after sowing and the mean data were used for statistical analysis.

Fresh weight of shoot (g)

The fresh weight of shoot of five selected seedlings was recorded at the end of experimental period of 9 months in each treatment. For taking fresh weight, the polyethylene bags were carefully removed by cutting with the blade. The soil balls of the plants were dipped for 10 minutes in bucket containing tap wat er to remove soil adhered with the roots. Again, the roots were further dipped in clean tap water to completely remove the soil adhered with them. The water on the surface of exposed roots was blot with soft paper towel to remove any free surface moisture. The shoots of these plants were separated with the secateurs from the point where the shoots and roots are clearly distinguished and the shoot portion was weighed immediately on an electronic weighing balance and mean fresh weight of shoot was recorded in each treatment. The separated shoot portion was tagged before recording the fresh weight.

Fresh weight of root (g)

While taking the observation on fresh weight of shoot, the separated root portion of five tagged plants were also tagged individually and immediately used for taking observation on fresh weight of root, weighed individually on an electronic weighing balance and average fresh weight of root was recorded in each treatment.

Dry weight of shoot (g)

The same tagged plants that used for recording of fresh weight were then used for the dry weight purpose. The shoots of each tagged plants were chopped individually and kept in separate properly labelled punched brown paper bag and then placed in hot air oven. The shoots were dried in oven at 65 ºC till the constant dry weight was attained. Then the dried shoots were weighed separately on an electronic weighing balance and average weight was calculated.

Dry weight of root (g)

The root portion of each of the five selected plants used for recording fresh weight of roots were chopped individually and placed in properly labelled punched brown paper bags, then oven dried at 65 oC temperature till a constant weight was attained. After 3 days, the dry weight of roots was measured with the help of electronic weighing balance and the average dry weight of root was computed and recorded for each treatment.

Relative water content

For the estimation of relative water content, fully mature leaf was used from randomly selected plants in each treatment at the end of experiment. The leaf was detached, kept in a polythene bag, sealed and immediately brought to laboratory. Then the disc of 1.5 cm diameter was cut from the leaf excluding mid rib, and fresh weight of these leaf discs was recorded immediately using electronic digital balance. Then the leaf discs were kept in deionised water in covered petri dish for two hours to record turgid leaf weight. Then the leaf discs were oven dried at 65º C for overnight and dry weight was recorded. The relative water content of leaves was calculated by using following formula.

$$\text{Relative Water Content (\%)} = \frac{\text{Fresh weight (g)} - \text{Dry weight (g)}}{\text{Turgid weight (g)} - \text{Dry weight (g)}} \times 100$$

Survival (%)

At the end of experimental period of 9 months after sowing, the survived seedlings in each treatment were counted and the survival percentage was calculated for each treatment using following formula:

$$\text{Survival (\%)} = \frac{\text{No.of survived seedling}}{\text{Total number of seedlings}} \times 100$$

Results and Discussion

Plant Height (cm)

Effect of salinity

Significantly higher plant height (58.79, 61.13 and 69.96 cm) during individual year and in pooled analysis, respectively at 9 MAS was noted in treatment S1 (control). Lower plant height at 9 (46.99, 45.9 and 46.49 cm) MAS during both years and pooled analysis, respectively was noticed in treatment S4 (6 dS m-1). Lower plant height in treatments with higher salinity levels might be due to buildup of higher concentrations of Na+ and Cl- salts in soil solution that hindered the absorption of other essential minerals and resulted in nutritional imbalance in plants. It is also possible that continuous uptake of (Na+ and Cl -) ions at higher concentrations increased their buildup in plants upto toxic level that adversely affect physiological, biochemical and other metabolic plant processes and lead to stunted growth [6]. Salinity related growth reduction in terms of plant height and leaf area was noted in grape [7].

Effect of 28-Homobrassinolide spray

The higher plant height was noted in treatment H1 i.e. 1 ppm 28-homobrassinolide spray at 9 (56.64, 57.47 and 57.06 cm) MAS in individual years and in pooled, respectively. Minimum plant height at 9 (50.23, 50.56 and 50.39 cm) MAS during individual years as well as in pooled analysis, respectively was observed in treatment H0 (control). Brassinosteroids plays an important role in cell elongation by promoting transverse orientation of microtubules [8], Therefore, exogenous brassinolide application might have accelerating effect on cell elongation and cell division and thereby resulted as higher plant height in treatments of BR.

Interaction effect

Treatment combination containing best available water and 1 ppm 28-homobrassinolide spray (S1H3) resulted in maximum plant height at 9 (60.09, 63.09 and 59.96 cm) MAS during both years and pooled data respectively. The minimum plant height at 9 (46.99, 45.99 and 46.49 cm) MAS during individual year and in pooled data respectively at was observed in treatment combination of 6 dS m-1 along with no spray (S1H3). Higher plant height under the treatments involving salinity levels along with 28-homobrassinolide spray might be due to BRs can play vital roles in promoting growth and development of plants exposed to saline conditions by modulating a number of metabolic phenomena affecting a plant's tolerance to salt-stress [9]. [10] revealed that under high salinity conditions spraying with 1 to 2 mg l−1 BL significantly increased the plant height.

Stem diameter (mm)

Among the salinity levels, higher stem diameter at 9 (10.90, 10.84 and 10.87 mm) MAS during both years and pooled analysis, respectively was registered in treatment S1 (control) while lowest stem diameter was observed with treatment S4 (6 dS m-1) at 9 (8.71, 8.86 and 8.79 mm) MAS during both experimental years as well as pooled analysis. Lower stem diameter under higher salinity level might be due to disruption in cell division and elongation caused by low osmotic potential in plant cells under salt stress condition [11]. The occurrence of damage in middle lamella under salinity stress might have lead to lower stem diameter. The graded levels of NaCl salt affected the plant height, stem diameter, number of leaves, leaf area and survival of mango [12]. The findings of this study are in line with research in mango [13].

Spray of 1 ppm 28-Homobrassinolide accounted highest stem diameter at 9 (10.62, 10.43 and 10.52 mm) MAS during 1st year, 2nd year and pooled respectively and lower stem diameter at 9 (8.89, 8.95 and 8.92 mm) MAS during both experimental years as well as pooled analysis, respectively was observed in treatment without spray (H0). Application of 28-HBR enhances the processes of cell elongation and cell multiplication in the new vegetative shoots and thereby might be influenced its dimensions [14,15,16] Similar results were also recorded by [17] in mango; [8] in melon and [18] in apricot.

The treatment combination containing best available water and spray of 1 ppm 28-homobrassinolide (S1H3) resulted in highest stem diameter at 9 (11.69, 11.36 and 11.53 mm) MAS during individual year and pooled, respectively. The stem diameter was observed lowest in treatment combination of 6 dS m-1 salinity along with no spray (S1H3) at 9 (7.63, 7.41 and 7.52 mm) MAS in both years as well as pooled analysis, respectively. Brassinolids control a wide range of biological activities that enhance the stress tolerance of plants and cell elongation [19] and regulates growth, biomass, and metabolism [20] which might be correlated with higher stem diameter in HBR spray.

Number of leaves

Effect of salinity

The higher number of leaves at 9 (33.01,35.10 and 34.05) MAS in both years and in pooled analysis was observed for plants under treatment S1 (best available water). Significantly lower number of leaves at 9 (19.96, 18.09 and 19.02) MAS during both experimental years as well as in pooled analysis was noted with treatment S4 (6 dS m-1 salinity). The plants raised under higher salinity might have faced nutrient toxicities primarily due to excess accumulations of Na+ and Cl- ions [21] as well as deficiencies of essential nutrient elements which resulted in osmotic stress in plants and thereby normal processes of cell multiplication affected. Under such circumstances, it is possible that the new leaf initiation process slowed down in plants raised with high saline water and reflected as decreased number of leaves per plant. It is also possible that the plants were not able to fulfil the demand of growing points of seedlings for essential nutrient elements and photosynthetic food material so forced to extract from older leaves to supply towards new leaves. As the seedlings under S4 shed their leaves pre-maturely, the number of leaves per plant reduced. Similar results were also reported in mango [22- 25], ber [26] grape [7] and guava [27].

Effect of 28-Homobrassinolide spray

The treatment H3 (1 ppm spray) recorded highest number of leaves at 9 (28.00, 27.31 and 27.65) MAS during individual years and in pooled, respectively. The lowest number of leaves at 9 (24.43, 24.63 and 24.53) MAS during both studied years and pooled, respectively was noticed in plants without spray (H0). Exogenous application of 28-Homobrassinolide increases photosynthetic activity and the antioxidant defence system [28]. Relative to control, the treatments with brassinolide triggered enhancement in plant growth and demonstrated the ability of brassinolide to alleviate saline stress on plant growth [10].

Interaction effect

The treatment combination of S1H3 resulted in highest number of leaves at 9 (33.35, 37.96 and 35.65) MAS during both the years as well as in pooled findings, respectively. Lowest leaf numbers at 9 (19.96, 18.09 and 19.02) MAS during individual years as well as pooled findings, respectively was observed in treatment combination of S4H1 (6 dS m-1 + no spray). Spray of brassinolide might have decreased the undesirable impacts of salinity on morphological attributes. [29] reported that application of 24-Epibrassinolide had a defensive role on cell ultra-structure in salt-affected leaves, which additionally prohibited nuclei and chloroplast deprivation and hence better photosynthesis results.

Leaf area (cm^2)

Effect of salinity

Considerably the higher leaf area at 9 MAS (41.44, 43.98 and 42.71 cm^2) during individual years and pooled analysis, respectively was recorded in control (S_1) whereas, Leaf area was found lowest in treatment S_4 treatment at 9 MAS (35.81, 35.64 and 35.73 cm^2) during individual years and in pooled data, respectively. Leaves are imperative places of essential biochemical pathways. In this experiment, lower leaf area under different concentrations of salinity could be due to the reduced development and differentiation of tissues, shrinkage of the cell contents, unbalanced nutrition, damage of membrane and disturbed 'avoidance mechanism' [30]. The photosynthetic capacity of leaves might be also decreased as the salinity of water increased up to 6 dS m^{-1} leading to lower production of photosynthetic food material required to maintain the growth, thereby possibly plants forced to reduce the leaf size. Decrease in leaf area may be due to reduced net assimilation rate, a reduction of mesophyll cell size and/or a decrease in palisade cell layer in the leaves. The remarkable effects of salinity including declined vegetative features such as plant height and leaf area also recorded in grape [7].

Effect of 28-Homobrassinolide spray

Significantly higher leaf area at 9 MAS (41.58, 41.31 and 41.44 cm^2) during both the years along with pooled analysis, respectively was observed in 1 ppm spray and significantly lower leaf area at 9 MAS (36.42, 37.13 and 36.77 cm^2) during both years and pooled findings, respectively was noted in treatment H_1 (no spray). Brassinolides accelerates cell division and expansion in the apical meristem, which leads to improvement in leaf expansion [31]. Brassinolides are known to activate the key enzymes of photosynthesis such as rubisco [32] and catalase activity [33]. These steroidal hormones are also known to regulate gene expression and thus mediate growth activity [34]. The increased cell division and cell elongation due to enhanced functional metabolism by the BRs might be the reason for enhancement in the growth of plants under treated condition [14]. Brassinosteroids application enhanced leaf area and biomass production in through increased nitrate reductase activity, relative water content, chlorophyll content, water uptake, nitrogen assimilation rate and ultimately photosynthesis was explained by [35] and [36] Improved growth also reported by [37] due to higher photosynthetic rate in tomato plants upon BRS application.

Interaction effect

The higher leaf area at 9 MAS (45.16, 48.04 and 46.60 cm^2) during both trial years as well as pooled analysis, respectively in treatment combination of best available water along with 1 ppm spray (S_1H_3). Lower leaf area at 9 MAS (30.26, 34.28 and 32.27 cm^2) during both years and in pooled, respectively was noted in treatment combination S_4H_1 (6 dS m^{-1} + no spray). Brassinolide can play vital roles in promoting growth and development of plants exposed to saline conditions by modulating a number of metabolic phenomena affecting a plant's tolerance to salt-stress [38]. Salinie water application drastically repressed all morphological attributes of grapevine seedlings. Conversely, when brassinolide was sprayed, the undesirable impacts of salinity on morphological attributes were decreased. Under high salinity level (3000 mg l−1), spraying with 1 to 2 mg l−1 BL significantly increased the leaf area plant−1 in grapes [10]. Strawberry plants are relatively sensitive to salt stress and it has been shown to reduce leaf number and leaf area. However, this reduction in growth was alleviated with foliar application of 0.5 and 1 ppm 24-EBL which resulted in significant increases in growth parameters of plants grown at 35 mM NaCl compared to the control plants.

Fresh weight of shoot and root (g)

Effect of salinity

The fresh weight of shoot and root was notably affected due to application of water having different salinity. Highest fresh weight of shoot (48.86, 51.20 and 50.02 g) and root (32.68, 35.08 and 34.05 g) during individual year and in pooled, respectively was noticed in treatment S1. Considerably lower fresh weight of shoot (34.22, 36.40 and 35.31 g) and root (28.55, 30.22 and 29.38 g) was noted in treatment S4 (6 dS m-1salinity). The probable reasons behind reduction in fresh weight may be due to decreased osmotic potential in growing media which restricts the uptake of water by plants from the media. As the salt concentration of irrigation water increases, the nutrient imbalance and hyper-osmotic stress plays a major role in disturbing the cellular functions of plant. Thus, all the physiological processes associated with growth of plants will be adversely affected [39] resulting in poor growth of plants in terms of reduced height, number of leaves, leaf area and relative water content. Similar results were also obtained in mango [40] grape [7], ber [41] and pomegranate [42].

Effect of 28-Homobrassinolide spray

Significantly higher fresh weight of shoot (45.21, 48.87 and 47.04g) and root (33.26, 35.26 and 34.47g) during both the years along with pooled analysis, respectively was recorded in treatment under 1 ppm 28-Homobrassinolide spray. Lowest fresh weight of shoot (38.58, 38.10 and 38.34g) and root (29.48, 29.48 and 29.48g) was observed in treatment H1 (no spray). The fresh weight of shoot and root was increased with BRs spray might be due to its effect on physiological processes, including enhancing photoprotection and improving photosynthetic efficiency, improving antioxidant capacity and reducing ROS production and improving mineral assimilation [43,44] The increase in weight of cucumber plants sprayed with BRs may be due to the positive effect on accumulation of various osmolytes or through higher cell division and enlargement.

Interaction effect

Fresh weight of shoot and root affected significantly due to the interaction effects of different salinity levels and 28-Homobrassinolide spray concentrations. During individual experimental year and also in pooled, the treatment combination of S1H3 resulted in highest fresh weight of shoot (53.05, 57.74 and 55.40 g) and root (35.07, 38.74 and 37.74g) which was also at par with the treatment combinations of S2H2 and S2H3 for fresh weight of shoot. Lowest fresh weight of shoot (32.83, 35.39 and 34.11g) and root (25.26, 26.26 and 25.76g) during both year and in pooled analysis, respectively was observed in treatment combination of S4H1. Brassinolides control a wide range of biological activities that enhance the stress tolerance of plants and cell elongation and genetically enhancing BR biosynthesis [19] regulates growth, biomass, and metabolism [20]. Exogenous application of BRs elevates growth and plant biomass under stress conditions thus results obtained in this study were in coherence with [20,45-47].

Dry Weight of Shoot and Root (g)

Effect of salinity

Effect of different levels of salinity have considerable imact on dry weight of shoot and root. The result reflects that mango seedlings under treatment S1 had significantly higher dry weight of shoot (17.72, 20.28 and 19.00 g) and root (13.51, 15.21 and 14.36g) during both years of experiment as well as in pooled analysis, respectively. Lower dry weight of shoot (9.19, 11.19 and 10.19g) and root (11.21, 12.32 and 11.76g) were noted in treatment S4 during individual years of experiment and in pooled analysis, respectively. The dry weight of plants can be correlated with fresh weight of plants and it can be accepted that the plants that have lower fresh weight have also lower dry weight. In this experiment, the plants grown with best available water recorded highest fresh weight of shoot and root. Therefore, it is acceptable that these plants also have higher dry weight of shoot and root. The dry weight of shoot and root was significantly reduced as the salinity level of water increased which is correlated with fresh weight of plants under. Increasing salinity has an inverse relationship with stomata conductance and net photosynthetic rate leading to reduction in assimilation and dry matter production. The results reported in mango [38,48], grape [7], ber [41], cashew [49] also supports the findings of present experiment.

Effect of 28-Homobrassinolide spray

The plants from treatment H3 resulted highest dry weight of shoot (16.22, 19.01 and 17.61g) and root (13.64, 15.46 and 14.55g) during both years and in pooled analysis, respectively. The lowest dry weight of shoot (11.93, 12.43 and 12.18g) and root (11.14, 12.31 and 11.72g) was noted in treatment H1. When strawberry plants treated with BB16 or EP24, greater dry weights, surface areas and lengths, as well as greater crown diameters were observed. These results are consistent with previous reports of other authors who observed an increase in root branching, total length and area in Silene vulgaris plants subjected to moderate drought stress [50,51].

Interaction effect

The treatment combination of S1H3 registered higher dry weight of shoot (20.94, 25.94 and 23.44g) and root (15.93, 19.13 and 17.53) during both the studied years along with pooled analysis, respectively which was at par with S1H2. Lower dry weight of shoot (7.88, 9.21 and 8.54g) and root (10.07, 12.07 and 11.07) was noted in treatment combination of S4H1. Brassinoids control a wide range of biological activities that enhance cell elongation as well as stress tolerance of plants and brassinolide biosynthesis regulates growth, biomass and metabolism of plants [20]. Exogenous application of BRs elevates growth and thereby plant biomass under stress conditions. Increment in root surface of strawberry plants exposed to saline stress after treatment with BB16 was reported.

Relative water content

Effect of salinity

Significantly higher relative water content of leaves (90.81, 92.63 and 91.72%) during both experimental years and pooled analysis was noted in plants from treatment S_1 (best available water). Lower relative water content in leaves (83.24, 84.08 and 83.66%) was recorded in plants from treatment S_4 (6 dS m^{-1}). Higher relative water content in leaves of plants under treatment of lower salinity is probably due to uninterrupted absorption of water necessary to maintain turgidity of cells and thereby various physiological activities in leaves. On the other hand, plants irrigated with high salinity water might caused osmotic stress, leading to restricted absorption of water which resulted in cellular dehydration. Osmotic regulation is an indication of response to osmotic stress and when there is a water limitation caused by salinity stress, osmotic potential is declined and this in turn causes the reduction of RWC of the leaves. Similar results were obtained by [26] in citrus and [7] in grape.

Effect of 28-homobrassinolide spray

Higher relative water content (91.98, 91.56 and 91.77%) during individual year as well as pooled analysis, respectively was spotted in treatment H_3 while, lower relative water content (82.33, 85.21 and 83.77%) was found in plants without spray (H_1). Brassinolide regulates water uptake and preservation of plant water potential, which leads to improving relative water content [27] and/or reduced Na+ accumulation and improved K+ uptake resulted in the avoidance of osmotic and ionic upset to the plant [52]. These findings are in conformity with results obtained by [10] in grape.

Interaction effect

The treatment combination of (S_1H_3-best available water and spray of 1 ppm 28-homobrassinolide) resulted in significantly higher relative water content (98.86, 99.92 and 99.39%) during both experimental years along with pooled analysis, respectively. The lower relative water content (76.50, 79.98 and 77.74%) was observed in treatment combination of S_4H_1 involving 6 dS m^{-1} salinity level without spray of 28-HBR. Leaf relative water content is considered as an alternative measure of plant water status, reflecting the metabolic activity in plant tissues. Lack of available water causes a decrease in cell turgor and water potential of the plants ultimately affecting the plant growth. The decrease in relative water content under salinity stress has already been reported [48]. Brassinolide are known to improve water relations such as increase in relative water content and water uptake, leaf water potential, water use efficiency, stomatal conductance and thus the transpiration rate in stressed plants [53]. This decrease could be attributed to root systems which are not able to compensate for water lost by transpiration through a reduction of the absorbing surface. Most probably 24-Epibrassinolide treatment elevated leaf relative water content (LRWC) to a level higher than the non-treated salt stressed plants. Increased LRWC by BRs application have been reported for Fragaria ananassa [54] under salinity stress; Brassica juncea [36] under cadmium stress and Vignia radiata under aluminium stress.

Survival percentage

Effect of salinity

The result indicated that different levels of saline irrigation water significantly influenced the survival percentage of polyembryonic mango seedlings at 9 MAS. Significantly higher survival percentage (99.95, 99.91 and 99.93%) was in the plants under treatment S1 (Control) and was also at par with treatments S2 and S3. The survival of plants decreased as the salinity of water increased and significantly lower survival percentage (92.01, 93.12 and 92.56%) was recorded in treatment S4. One of the major causes of growth reduction in salt stressed plants is the specific ion toxicity (mainly Na+and Cl-). The linear increase in Na+content and decreased values of K+ can be correlated with decreased growth of plants under high salinity conditions. Salinity in root growing media higher than the tolerance limit of particular plant species disrupts osmotic, ionic and nutritional balance of plants, eventually leading to death [55] High concentrations of sodium salts in the soil cause ionic and hormonal alterations in the plants [56], which aggravate over time and may lead to plant death, mainly due to the toxicity caused by sodium ion. [48] also reported that increase in salinity decreased the rate of survivability of mango.

Effect of 28-homobrassinolide spray

The data concerned with influence of different concentrations of 28-HBR spray on survival percentage of mango seedlings was found non-significant.

Plant height (cm) at 9 MAS												
28-HBR Spray	2021-22			Mean	2022-23			Mean	Pooled			Mean
(ppm) Salinity levels	H_1: Cont	H_2: 0.5	H_3: 1		H_1: Cont	H_2: 0.5	H_3: 1		H_1: Cont	H_2: 0.5	H_3: 1	
S_1: BAW	56.35ab	59.94a	60.09a	58.79a	59.35abc	60.94ab	63.09a	61.13a	57.85ab	60.44ab	61.59a	59.96a
S_2: 2 dS/m	54.72abc	56.77ab	59.37a	56.95a	54.72cde	56.77bcd	60.04abc	57.17a	54.72bcd	56.77abc	59.70ab	57.06a
S_3: 4 dS/m	46.30e	47.01de	55.00abc	49.44b	49.30ef	48.35f	55.00cde	50.88b	47.80e	47.68e	55.00bcd	50.16b
S_4: 6 dS/m	38.87f	49.99cde	52.11bcd	46.99b	37.87g	48.65f	51.78def	46.10b	38.37f	49.32de	51.94cde	46.54b
Mean	49.06b	53.43ab	56.64a		50.23b	53.68ab	57.47a		49.68b	53.55ab	57.06a	
	S	H	S × H		S	H	S × H		S	H	S × H	
SEm ± CD at 5 % CV (%)	1.07	0.92	1.85		1.05	0.91	1.82		1.03	0.90	1.79	
	3.11	2.69	5.39		3.07	2.67	5.32		3.02	2.62	5.23	
	6.03				5.87				5.81			

Table 1: Effect of salinity levels and 28-homobrassinolide spray on plant height (cm) of polyembryonic mango seedlings at 9 MAS. Values in a column bearing different superscripts are significantly different at 0.05 level.

Stem diameter (mm) at 9 MAS												
28-HBR Spray	**2021-22**			**Mean**	**2022-23**			**Mean**	**Pooled**			**Mean**
(ppm) Salinity levels	**H_1: Cont**	**H_2: 0.5**	**H_3: 1**		**H_1: Cont**	**H_2: 0.5**	**H_3: 1**		**H_1: Cont**	**H_2: 0.5**	**H_3: 1**	
S_1: BAW	10.25^{bc}	10.75^{bc}	11.69^{a}	10.90^{a}	10.37^{b}	10.21^{bc}	11.36^{a}	10.65^{a}	10.31^{b}	10.48^{b}	11.53^{a}	10.77^{a}
S_2: 2 dS/m	9.90^{bc}	10.03^{bc}	10.83^{b}	10.25^{a}	10.19^{cd}	10.05^{bc}	10.10^{bc}	10.12^{ab}	10.05^{c}	10.04^{bc}	10.47^{b}	10.18^{b}
S_3: 4 dS/m	7.80^{e}	9.97^{bc}	10.12^{bc}	9.30^{b}	9.06^{ef}	9.90^{bcd}	10.07^{bc}	9.68^{b}	8.00^{e}	9.94^{bc}	10.10^{bc}	9.49^{c}
S_4: 6 dS/m	7.73^{e}	9.18^{d}	9.85^{c}	8.91^{b}	7.41^{f}	9.03^{de}	10.16^{bc}	8.86^{b}	7.57^{e}	9.10^{d}	10.01^{bc}	8.89^{c}
Mean	8.89^{b}	9.85^{a}	10.62^{a}		9.26^{b}	9.80^{ab}	10.43^{a}		9.09^{c}	9.89^{b}	10.53^{a}	
	S	H	S×H		S	H	S×H		S	H	S×H	
SEm ±CD at 5 %CV (%)	0.18	0.15	0.30		0.23	0.20	0.39		0.14	0.12	0.24	
	0.51	0.44	0.89		0.66	0.57	1.14		0.41	0.36	0.71	
	5.34				6.93				4.31			

Table 2: Effect of salinity levels and 28-homobrassinolide spray on stem diameter (mm) in leaf of polyembryonic mango seedlings at 9 MAS. Values in a column bearing different superscripts are significantly different at 0.05 level.

Number of leaves at 9 MAS												
28-BR Spray(ppm)	**2021-22**			**Mean**	**2022-23**			**Mean**	**Pooled**			**Mean**
Salinity levels	**H_1: Cont**	**H_2: 0.5**	**H_3: 1**		**H_1: Cont**	**H_2: 0.5**	**H_3: 1**		**H_1: Cont**	**H_2: 0.5**	**H_3: 1**	
S_1: BAW	32.52^{ab}	33.16^{a}	33.35^{a}	33.01^{a}	32.29^{bcd}	35.05^{b}	37.96^{a}	35.10^{a}	32.40^{bc}	34.10^{ab}	35.65^{a}	34.05^{a}
S_2: 2 dS/m	28.83^{c}	29.34^{c}	29.89^{bc}	29.35^{b}	30.42^{cd}	29.75^{d}	33.10^{bc}	31.09^{b}	29.62^{d}	29.55^{d}	31.49^{cd}	30.22^{b}
S_3: 4 dS/m	20.16^{d}	22.43^{d}	26.76^{c}	23.11^{c}	18.41^{f}	22.08^{e}	19.59^{ef}	20.03^{c}	19.29^{gh}	22.25^{ef}	23.17^{e}	21.57^{c}
S_4: 6 dS/m	16.21^{e}	21.66^{d}	21.99^{d}	19.96^{d}	17.39^{f}	18.28^{f}	18.60^{f}	18.09^{c}	16.80^{h}	19.97^{g}	20.29^{g}	19.02^{d}
Mean	24.43^{b}	26.65^{ab}	28.00^{a}		24.63^{a}	26.29^{a}	27.31^{a}		24.53^{b}	26.47^{ab}	27.65^{a}	
	S	H	S×H		S	H	S×H		S	H	S×H	
SEm ± CD at 5 % CV (%)	0.58	0.50	1.00		0.54	0.47	0.94		0.46	0.40	0.80	
	1.68	1.46	2.91		1.58	1.37	2.74		1.35	1.17	NS	
	6.56				6.24				5.26			

Table 3: Effect of salinity levels and 28-homobrassinolide spray on number of leaves of polyembryonic mango seedlings at 9 MAS. Values in a column bearing different superscripts are significantly different at 0.05 level.

Leaf area (cm^2) at 9 MAS												
28-HBRSpray	**2021-22**			**Mean**	**2022-23**			**Mean**	**Pooled**			**Mean**
(ppm) Salinity levels	**H_1: Cont**	**H_2: 0.5**	**H_3: 1**		**H_1: Cont**	**H_2: 0.5**	**H_3: 1**		**H_1: Cont**	**H_2: 0.5**	**H_3: 1**	
S_1: BAW	39.92bc	39.24bc	45.16a	41.44a	41.62bc	42.28b	48.04a	43.98a	40.77bc	40.76bc	46.60a	42.71a
S_2: 2 dS/m	37.46bc	38.78bc	41.01b	39.08ab	37.36de	39.62bcd	42.32b	39.77b	37.41de	39.20cd	41.67b	39.43b
S_3: 4 dS/m	37.03bc	38.59bc	40.43bc	38.68ab	35.23e	40.47bcd	36.76de	37.49bc	36.13e	39.53bcd	38.60cd	38.09b
S_4: 6 dS/m	30.26d	36.48c	40.71b	35.81b	34.28e	34.52e	38.12cde	35.64c	32.27f	35.50e	39.41bcd	35.73c
Mean	36.42b	38.52ab	41.58a		37.13b	39.22ab	41.31a		36.77b	38.87ab	41.44a	
	S	H	S × H		S	H	S × H		S	H	S × H	
SEm ±	0.69	0.60	1.20		0.73	0.63	1.26		0.41	0.35	0.70	
CD at 5 %	2.02	1.75	3.50		2.12	1.84	3.68		1.18	1.02	2.05	
CV (%)	5.37				5.56				3.12			

Table 4: Effect of salinity levels and 28-homobrassinolide spray on leaf arae (cm^2) of polyembryonic mango seedlings at 9 MAS.

Values in a column bearing different superscripts are significantly different at 0.05 level.

Fresh weight of shoot (g) 9 MAS												
28-HBRSpray	**2021-22**			**Mean**	**2022-23**			**Mean**	**Pooled**			**Mean**
(ppm) Salinity levels	**H_1: Cont**	**H_2: 0.5**	**H_3: 1**		**H_1: Cont**	**H_2: 0.5**	**H_3: 1**		**H_1: Cont**	**H_2: 0.5**	**H_3: 1**	
S_1: BAW	43.53b	49.99a	53.05a	48.86a	42.25bcd	53.60a	57.74a	51.20a	42.89b	51.79a	55.40a	50.02a
S_2: 2 dS/m	41.53bc	43.61b	52.97a	46.03a	43.26bc	44.33b	54.00a	47.20a	42.39b	43.97b	53.49a	46.62a
S_3: 4 dS/m	36.44def	38.71cde	39.50bcd	38.21b	36.49e	38.32cde	46.15b	40.32b	36.46cd	38.52c	42.83b	39.28b
S_4: 6 dS/m	32.83f	34.50f	35.33def	34.22b	35.39e	36.22e	37.59de	36.40b	34.11d	35.36d	36.46d	35.31c
Mean	38.58b	41.70ab	45.21a		39.35b	43.12b	48.87a		38.96b	42.41b	47.04a	
	S	H	S × H		S	H	S × H		S	H	S × H	
SEm ± CD at 5 % CV (%)	0.80	0.69	1.38		0.96	0.83	1.66		0.72	0.62	1.24	
	3.14	2.72	5.45		3.78	3.27	6.55		2.11	1.82	3.65	
	5.70				6.55				5.03			

Table 5: Effect of salinity levels and 28-homobrassinolide spray on fresh weight of shoot (g) of polyembryonic mango seedlings at 9 MAS.

Values in a column bearing different superscripts are significantly different at 0.05 level.

Fresh weight of root (g) 9 MAS												
28-HBR Spray	**2021-22**			**Mean**	**2022-23**			**Mean**	**Pooled**			**Mean**
(ppm) Salinity levels	**H_1: Cont**	**H_2: 0.5**	**H_3: 1**		**H_1: Cont**	**H_2: 0.5**	**H_3: 1**		**H_1: Cont**	**H_2: 0.5**	**H_3: 1**	
S_1: BAW	32.03b	30.29bc	36.74a	33.02a	34.23bc	31.62cde	38.74a	34.86a	33.13b	30.95bc	37.74a	33.94a
S_2: 2 dS/m	29.95bc	30.00bc	33.00ab	32.32a	31.62cde	31.33de	33.33ab	32.09ab	30.79bc	30.66bc	33.16a	31.54a
S_3: 4 dS/m	28.34cd	32.45b	31.88b	30.88ab	28.68ef	32.38cd	32.54cd	31.20b	28.51cd	32.41b	32.21b	31.04b
S_4: 6 dS/m	25.26d	29.97bc	30.43bc	28.55b	26.26f	31.30de	33.09cd	30.22b	25.76d	30.63bc	31.76b	29.38b
Mean	28.90b	30.67a	33.01a		30.20b	31.66b	34.43a		29.55	31.17	33.72	
	S	H	S × H		S	H	S × H		S	H	S × H	
SEm ±CD at 5 % CV (%)	0.52	0.45	0.90		0.46	0.40	0.79		0.36	0.31	0.63	
	2.06	1.78	3.56		1.80	1.56	3.12		1.43	1.24	2.48	
	5.06				4.26				3.44			

Table 6: Effect of salinity levels and 28-homobrassinolide spray on Fresh weight of root (g) of polyembryonic mango seedlings at 9 MAS. Values in a column bearing different superscripts are significantly different at 0.05 level.

Dry weight of shoot (g) 9 MAS												
28-HBRSpray	**2021-22**			**Mean**	**2022-23**			**Mean**	**Pooled**			**Mean**
(ppm) Salinity levels	**H_1: Cont**	**H_2: 0.5**	**H_3: 1**		**H_1: Cont**	**H_2: 0.5**	**H_3: 1**		**H_1: Cont**	**H_2: 0.5**	**H_3: 1**	
S_1: BAW	15.13cd	17.09bc	20.94a	17.72a	15.79de	19.09bc	25.94a	20.28a	15.46cd	18.09b	23.44a	19.00a
S_2: 2 dS/m	13.60de	17.89b	16.36bc	15.95a	13.26efg	18.08bc	20.19b	17.18b	13.43de	17.99b	18.27b	16.56b
S_3: 4 dS/m	11.13f	11.66ef	16.37bc	13.05b	12.13fg	14.32def	16.70cd	14.39c	11.63e	12.99e	16.54bc	13.72c
S_4: 6 dS/m	7.88g	8.51g	11.20g	9.19c	9.21h	11.17gh	13.20fg	11.19d	8.54f	9.84f	12.20e	10.19d
Mean	11.93b	13.78b	16.22a		12.60c	15.67b	19.01a		12.27c	14.73b	17.61a	
	S	H	S × H		S	H	S × H		S	H	S × H	
SEm ±CD at 5 %CV (%)	0.44	0.38	0.76		0.47	0.41	0.81		0.35	0.30	0.60	
	1.74	1.51	3.02		1.86	1.61	3.22		1.37	1.18	2.37	
	9.46				8.94				6.98			

Table 7: Effect of salinity levels and 28-homobrassinolide spray on Dry weight of shoot (g) of polyembryonic mango seedlings at 9 MAS. Values in a column bearing different superscripts are significantly different at 0.05 level.

Dry weight of root (g) 9 MAS												
28-HBRSpray	**2021-22**			**Mean**	**2022-23**			**Mean**	**Pooled**			**Mean**
(ppm)Salinity levels	**H_1: Cont**	**H_2: 0.5**	**H_3: 1**		**H_1: Cont**	**H_2: 0.5**	**H_3: 1**		**H_1: Cont**	**H_2: 0.5**	**H_3: 1**	
S_1: BAW	10.62ef	13.98b	15.93a	13.51a	11.95d	14.55bc	19.13a	15.21a	11.29e	14.26bc	17.53a	14.36a
S_2: 2 dS/m	11.17def	11.90cde	13.50bc	12.19ab	12.54cd	13.20cd	15.57b	13.77ab	11.85e	12.55de	14.54b	12.98ab
S_3: 4 dS/m	12.68bcd	12.47bcd	13.53bc	12.89a	12.36cd	12.80cd	14.53bc	13.23ab	12.52cde	12.64cde	14.03bcd	13.06ab
S_4: 6 dS/m	10.07f	11.94cde	11.61def	11.21b	12.07cd	12.27cd	12.61cd	12.32b	11.07e	12.10e	12.11e	11.76b
Mean	11.14b	12.57ab	13.64a		12.23b	13.21b	15.46a		11.68b	12.88b	14.55a	
	S	H	S × H		S	H	S × H		S	H	S × H	
SEm ±CD at 5 %CV (%)	0.30	0.26	0.51		0.43	0.37	0.74		0.29	0.25	0.51	
	1.17	1.01	2.03		1.68	1.46	2.91		1.16	1.01	2.02	
	7.13				9.36				6.77			

Table 8: Effect of salinity levels and 28-homobrassinolide spray on Dry weight of root (g) of polyembryonic mango seedlings at 9 MAS.

Values in a column bearing different superscripts are significantly different at 0.05 level.

Relative water content (%) 9 MAS												
8-HBRSpray	**2021-22**			**Mean**	**2022-23**			**Mean**	**Pooled**			**Mean**
(ppm)Salinity levels	**H_1: Cont**	**H_2: 0.5**	**H_3: 1**		**H_1: Cont**	**H_2: 0.5**	**H_3: 1**		**H_1: Cont**	**H_2: 0.5**	**H_3: 1**	
S_1: BAW	89.27b	84.30b	98.86a	90.81a	90.37bc	87.58bc	99.92a	92.63a	89.82b	85.94b	99.39a	91.72a
S_2: 2 dS/m	88.83b	91.27ab	91.77ab	90.62a	92.25b	94.29ab	87.25bc	91.26ab	90.54b	92.78ab	89.51b	90.94ab
S_3: 4 dS/m	74.73c	89.57b	89.90b	84.73a	79.25d	89.14bc	89.79bc	86.06ab	76.99c	89.35b	89.85b	85.40ab
S_4: 6 dS/m	76.50c	85.83b	87.40b	83.24a	78.98d	83.99cd	89.26bc	84.08b	77.74c	84.91bc	88.33b	83.66b
Mean	82.33b	87.74ab	91.98a		85.21a	88.75a	91.56a		83.77b	88.25ab	91.77a	
	S	H	S × H		S	H	S × H		S	H	S × H	
SEm ±CD at 5 % CV (%)	1.49	1.29	2.58		1.36	1.17	2.35		1.34	1.16	2.33	
	5.89	5.11	10.22		5.36	4.64	9.29		3.94	3.42	6.83	
	5.12				4.59				4.58			

Table 9: Effect of salinity levels and 28-homobrassinolide spray on Relative water content (%) of polyembryonic mango seedlings at 9 MAS.

Values in a column bearing different superscripts are significantly different at 0.05 level.

Survival (%) at 9 MAS												
28-HBR Spray	**2021-22**			**Mean**	**2022-23**			**Mean**	**Pooled**			**Mean**
(ppm)Salinity levels	**H_1: Cont**	**H_2: 0.5**	**H_3: 1**		**H_1: Cont**	**H_2: 0.5**	**H_3: 1**		**H_1: Cont**	**H_2: 0.5**	**H_3: 1**	
S_1: BAW	99.94	99.94	99.94	99.95a	99.86	99.87	99.99	99.91a	99.90	99.92	99.96	99.93a
S_2: 2 dS/m	98.47	99.58	99.91	99.32ab	98.58	99.56	99.89	99.35a	98.53	99.57	99.90	99.33a
S_3: 4 dS/m	94.70	95.96	97.36	96.01ab	94.62	95.96	96.23	95.60a	94.66	95.96	96.79	95.80a
S_4: 6 dS/m	91.01	90.71	94.29	92.01b	91.34	92.38	95.63	93.12a	91.18	91.55	94.96	92.56a
Mean	96.03	96.56	97.88		96.12	96.94	97.93		90.07	96.75	97.91	
	S	H	S × H		S	H	S × H		S	H	S × H	
SEm ±CD at 5 %CV (%)	1.40	1.21	2.42		1.42	1.23	2.47		1.38	1.20	2.39	
	5.52	NS	NS		5.63	NS	NS		4.05	NS	NS	
	4.32				4.40				4.28			

Table 10: Effect of salinity levels and 28-homobrassinolide spray on Survival (%) of polyembryonic mango seedlings at 9 MAS.

Values in a column bearing different superscripts are significantly different at 0.05 level.

Interaction effect

The interaction effects of salinity level and 28- Homobrassinolide spray concentrations were found non-significant regarding survival percentage of polyembryonic mango seedlings.

Conclusion

From the results of two years study, it can be concluded that different salinity levels and spray concentration of 28-Homobrassinolide significantly influenced the growth of polyembryonic mango seedlings. Application of water having EC less than 2 dS m-1 resulted in superiour growth of polyembryonic mango seedlings with greater biomass and higher survival. While, application of water having 6 dS m-1 salinity reduced the growth of mango seedlings, adversely affecte the plant biomass with minimum survival after 9 months of sowing. Spray of 28-homobrassinolide at 1 ppm promoted the growth of polyembryonic mango seedlings and also recorded higher biomass after 9 months of sowing. Polyembryonic mango seedlings of selected genotype can be grown with saline irrigation having salinity up to 4 dS m-1 along with 1 ppm 28-homobrassinolide spray for better growth, biomass and higher survival up to 9 months after sowing.

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