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# PLANT GROWTH PROMOTING PSEUDOMONAS ENHANCES SALINITY TOLERANCE IN CARROT (*DAUCUS CAROTA* L.) THROUGH MORPHOLOGICAL AND BIOCHEMICAL MODULATION ACROSS DIVERSE GENOTYPES

**Tahir Shahzad[1], Anum Sajid[2], Rabia Shaheen Niazi[3], Sobia Niaz[*4], Nabiha Asghar[5], Dr. Muhammad Iqbal Hussain[6], Dr. Zill-E-Huma[7], Dr. Abid Ejaz[8], Dr. Mian Jahan Zaib Rasheed[9]**

*[1,2,7,8,9]Department of Botany, University of Sargodha, Sargodha, Punjab, Pakistan*
*[3]Department of Biological Sciences, Thal University of Bhakkar, Punjab, Pakistan*
*[*4]Department of Botany, Bahauddin Zakariya University, Multan, Pakistan*
*[5]Soil and Water Testing Laboratory, Chiniot, Punjab, Pakistan*
*[6]Scientific Officer (Lab), Soil and Water Testing Laboratory, Chiniot, Punjab, Pakistan*

[1]tahirshahzad174@gmail.com, [2]anumqu11@gmail.com, [3]rabia.shaheen@tu.edu.pk, [*4]sobasco786@gmail.com, [5]nabihaasghar103@gmail.com, [6]driqbal7878@gmail.com, [7]humashah690@gmail.com, [8]abid155yahoo.com, [9]jahanzaibrasheedgc@gmail.com

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**Corresponding Author: ***
**Sobia Niaz**

**Abstract**

*Salinity is a key abiotic restriction on carrot (Daucus carota L.) productivity worldwide. Plant growth-promoting rhizobacteria (PGPR) are an environmentally friendly way to improve crop tolerance to salinity. This study examined the morphological and biochemical responses of 30 carrot genotypes to control ($T_0$), salt stress ($T_1$), and salt stress combined with Pseudomonas bioinoculation ($T_2$). The experiment was conducted using a randomized complete block design with three replications. Morphological features such as root length, root weight, and root diameter were assessed, as well as biochemical measures such as total soluble sugars (TSS), reducing sugars (RS), phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO), and peroxidase (POD). Salinity significantly ($P < 0.001$) decreased root growth, PAL, PPO, and decreasing sugar levels, but raised TSS and POD activities, indicating osmotic correction and activation of antioxidant defence systems. Bioinoculation with Pseudomonas partially restored root growth and antioxidant enzyme activities while reducing oxidative stress under saline conditions. Significant genotype-treatment interactions were found for morphological features and PPO activity, indicating genetic heterogeneity in salinity tolerance. Pearson correlation study found a substantial positive relationship between root weight and diameter ($r = 0.942$). PCA explained 51.85% of total variation, with root morphological features playing the most important role in genotype discrimination, while hierarchical cluster analysis classified the examined genotypes as salt-tolerant or salt-sensitive. Genotypes 9, 11, 21, 26, 28, and 30 consistently showed superior growth in saline circumstances, indicating that they could be useful genetic resources for*

*breeding salt-tolerant carrot cultivars. These data show that Pseudomonas bioinoculation efficiently reduces salinity-induced damage and offers a potential technique for long-term carrot production in salt-affected soils.*

## Introduction

Vitamins, minerals, dietary fibre, and bioactive compounds are some of the nutrients that vegetables offer and are important for human health (Xu et al., 2023; Wallace et al., 2020). Carrot, *Daucus carota* L., belongs to the Apiaceae family and is one of the most economically important vegetable crops both due to its nutritional value and worldwide consumption (Ahmad et al., 2019; Paparella et al., 2024). Carrot contains high levels of provitamin A carotenoids (α-carotene and β-carotene) along with good amounts of vitamins, minerals, dietary fibre and antioxidants, which play a major role in human nutrition (Ikram et al., 2024).

It is estimated that the production of carrots in the world is more than 38 million tons per year, of which China accounts for the largest share, followed by the European Union, Uzbekistan, Russia and the USA (Paparella et al., 2024). Carrot is an important winter vegetable crop in Pakistan, harvested from 13.9 thousand hectares with an annual output of 242.3 thousand tonnes. Sheikhupura and Kasur are identified as the major production areas, while the contribution from Punjab is almost 68% of the country's production (Farwan, 2022). Despite favourable climatic conditions, carrot productivity in Pakistan is still low, as it is facing limited genetic resources, poor nutrient management, and rising environmental stresses such as soil salinity (Simon et al., 2021).

Salinity is a major abiotic constraint on crop production globally. Nearly 20–23% of irrigated agricultural land is affected by salinity, which is further increasing as a result of inadequate irrigation, drainage systems, and climate change (Majeed & Muhammad, 2019). It is estimated that 2.2-6.5 million hectares of crop land are salt affected in Pakistan, which is one of the serious problems for the sustainable cultivation of crops (Caro, 2021). Plants suffer from poor establishment, lower chlorophyll levels, decreased photosynthesis, and decreased biomass under salinity due to the effects of osmotic stress, ion toxicity, nutrient imbalance, and oxidative damage (Hussain et al., 2019). Carrot is moderately sensitive to salinity, and the improvement of its tolerance has been one of the important research goals.

Plant growth-promoting rhizobacteria (PGPR) have emerged as eco-friendly and sustainable strategies to overcome salt stress in crops. Pseudomonas species are among these beneficial microorganisms that have great potential in plant growth promotion by producing phytohormones, solubilizing phosphate minerals, biological nitrogen fixation, enhancing nutrient uptake and regulating stress-responsive mechanisms (Chandran et al., 2021). Furthermore, Pseudomonas activates antioxidant defense systems and regulates ionic balance and osmotic adjustment, which decreases the negative effects of salinity on plant growth (Yasmin et al., 2020).

The advantages of PGPR under saline conditions have been documented, but data on the interaction of PGPR and various carrot genotypes in saline conditions are still scarce. Thus, the present study was undertaken to investigate the morphological and biochemical response of carrot genotypes to salinity stress and to further investigate the influence of Pseudomonas bioinoculation in alleviating salinity-induced damage. The results will help to formulate sustainable solutions to enhance carrot productivity in salt-affected soils.

## Materials and Methods

### Plant Material

A total of 29 carrot (*Daucus carota* L.) genotypes were collected from Gene Bank, Department of Botany, University of Sargodha, Pakistan as experimental material.

**Experimental Site and Design**
The experiment was carried out at the University of Sargodha, Punjab, Pakistan, during 2019-2020. The pot experiment was set up in a randomized complete block design (RCBD) with three replications.

**Treatments**
Three treatments were used:
- $T_0$: Control
- $T_1$: Salt stress

$T_2$ is the combined effect of salt stress and Pseudomonas bioinoculation.

**Bioinoculum Preparation and Application**
The bioinoculum was obtained from the University of Wah, Pakistan and was based on Pseudomonas bacterium. Maize straw was used as a carrier that was mixed with the bacterial consortium, and 100 g of the bioinoculum was added to each pot 15 days after germination.

**Crop establishment and management**
The seeds were sown in October 2019, and irrigation was performed approximately 21 days after sowing. Weed and pest control were carried out as per the standard agronomic practices during the season. The roots of carrots were collected in January 2020 for morphological and biochemical analysis.

**Morphological Parameters**
**Root Length**
The length of roots was measured from crown to root tip on a measuring scale (cm).

**Root Weight**
The fresh weight of the roots (g) was obtained with an electronic balance.

**Root Diameter**
A digital Vernier calliper was used to measure the root diameter (cm).

**Leaf Area**
Leaf area ($cm^2$) was measured using a leaf area meter.

**Biochemical Analysis**
**Sample Preparation**
Fresh leaves were cut and broken in liquid nitrogen and crushed in 0.02 M phosphate buffer (pH 7.0). The homogenate was spun at 12,000 rpm for 10 min, and the supernatant was subjected to biochemical analyses.

**Total Soluble Sugars**
The soluble sugar content was estimated by the Yemm and Willis (1954) method.

**Reducing Sugars**
Muhammad and Tabassum (2007) estimated a reduction in sugar content.

**Phenylalanine ammonia lyase (PAL) activity**
The spectrophotometric measurement of absorbance at 290 nm was used to determine PAL activity.

**Polyphenol Oxidase (PPO) Activity**
The activities of PPO were measured at 280 nm using the procedure described by Decker (1977).

**Peroxidase Activity**
Absorbance at 470 nm was used to measure peroxidase activity (David and Murray 1965).

**Statistical Analysis**
Analysis of variance (ANOVA) was used to analyse data in Statistica, XLSTAT, Statistix and Microsoft Excel. Treatment means were compared to determine the effect of genotype, salinity and Pseudomonas bioinoculation on morphological and biochemical parameters.

**Results**
**Descriptive statistics of morphological and biochemical traits**
The morphological and biochemical traits of the plants under control ($T_0$), salt stress ($T_1$) and salt stress and Pseudomonas bioinoculation ($T_2$) exhibited descriptive statistics, as shown in Table 1. The control treatment had the highest mean root weight (10.29 g), root diameter (11.12 cm),

root length (6.67 cm), PAL activity (0.824) and PPO activity (1.443).

Salt stress reduced root weight (9.05 g), root diameter (10.95 cm), root length (5.80 cm), PAL activity (0.737), and PPO activity (1.257). The bioinoculations (0.788 and 1.363) in saline conditions showed some degree of improvement in PAL and PPO activity over saline stress. The salt-stressed plants had the highest level of TSS (0.779), and the control plants had the highest level of RSS (0.042). Under salt stress, peroxidase activity increased (0.292), while under the other combinations, peroxidase activity decreased with salt plus bioinoculation (0.255). Standard errors and standard deviations showed that there was an acceptable amount of variation among the observations within each treatment.

**Table 1: Descriptive Statistics of Morphological and Biochemical Parameters**

| Statistics | Treatment | RW | RD | TSS | RS | RL | PAL | PPO | POD |
|---|---|---|---|---|---|---|---|---|---|
| **Minimum** | Control (T0) | 7.72 | 7.42 | 0.689 | 0.039 | 6.1 | 0.81 | 1.41 | 0.215 |
| | Salt (T1) | 7.07 | 8.31 | 0.713 | 0.026 | 5.5 | 0.73 | 1.21 | 0.275 |
| | Salt + BI (T2) | 5.67 | 6.73 | 0.681 | 0.033 | 4.5 | 0.777 | 1.33 | 0.245 |
| **Mean** | Control (T0) | 10.288 | 11.122 | 0.758 | 0.042 | 6.667 | 0.824 | 1.443 | 0.225 |
| | Salt (T1) | 9.053 | 10.953 | 0.779 | 0.029 | 5.8 | 0.737 | 1.257 | 0.292 |
| | Salt + BI (T2) | 7.517 | 9.098 | 0.758 | 0.036 | 4.933 | 0.788 | 1.363 | 0.255 |
| **Standard Error** | Control (T0) | 0.925 | 1.078 | 0.029 | 0.001 | 0.318 | 0.008 | 0.024 | 0.006 |
| | Salt (T1) | 0.727 | 0.923 | 0.028 | 0.001 | 0.153 | 0.004 | 0.024 | 0.009 |
| | Salt + BI (T2) | 0.638 | 0.704 | 0.033 | 0.001 | 0.233 | 0.006 | 0.018 | 0.006 |
| **Standard Deviation** | Control (T0) | 2.265 | 2.639 | 0.071 | 0.002 | 0.551 | 0.013 | 0.042 | 0.01 |
| | Salt (T1) | 1.782 | 2.262 | 0.068 | 0.003 | 0.265 | 0.007 | 0.042 | 0.015 |
| | Salt + BI (T2) | 1.563 | 1.724 | 0.081 | 0.002 | 0.404 | 0.01 | 0.031 | 0.01 |
| **Maximum** | Control (T0) | 13.45 | 14.53 | 0.827 | 0.044 | 7.2 | 0.837 | 1.49 | 0.235 |
| | Salt (T1) | 11.76 | 14.56 | 0.843 | 0.033 | 6 | 0.743 | 1.29 | 0.305 |
| | Salt + BI (T2) | 9.92 | 11 | 0.833 | 0.039 | 5.3 | 0.797 | 1.39 | 0.265 |

**Pearson Correlation Analysis among Morphological and Biochemical Traits**

Table 2 shows that the morphological traits were positively correlated with each other, as revealed by Pearson correlation analysis. The root diameter had a very strong positive correlation with the root weight (r = 0.942); the length of roots had a moderate positive correlation with the diameter of roots (r = 0.578) and root weight (r = 0.456). There were weak negative correlations between total soluble sugars and root length (r = -0.134), root weight (r = -0.003) and root diameter (r = -0.063). Root weight (r = −0.385) and root diameter (r = −0.333) were negatively correlated with PPO activity, while PAL activity (r = 0.349) was positively correlated with PPO activity. POD activity showed a weak positive association with root length (r = 0.221), root weight (r = 0.111), root diameter (r = 0.129), and total soluble sugars (r = 0.053), whereas it was negatively correlated with reducing sugars (r = −0.336), PAL (r = −0.161), and PPO (r = −0.213). The relationship between these attributes was generally best with regard to root weight to root diameter

Table 2: Pearson correlation between Traits under Study

| | RL | RW | RD | TSS | RS | PAL | PPO | POD |
|---|---|---|---|---|---|---|---|---|
| RL | 1 | | | | | | | |
| RW | 0.456320 | 1 | | | | | | |
| RD | 0.578483 | 0.941783 | 1 | | | | | |
| TSS | -0.133879 | -0.003397 | -0.062516 | 1 | | | | |
| RS | 0.062223 | 0.173638 | 0.181749 | -0.043181 | 1 | | | |
| PAL | -0.025296 | -0.053572 | -0.072039 | -0.067230 | 0.136817 | 1 | | |
| PPO | -0.029707 | -0.384884 | -0.332942 | -0.074122 | -0.032251 | 0.349162 | 1 | |
| POD | 0.220624 | 0.110517 | 0.129004 | 0.053120 | -0.335533 | -0.161343 | -0.212988 | 1 |

**Two-way ANOVA of Biochemical Traits**

The two-way analysis of variance showed highly significant ($P < 0.001$) differences between the genotypes in all biochemical traits studied, such as total soluble sugars, reducing sugars, PAL, PPO and POD (Table 3). Additionally, treatment significantly affected all biochemical parameters; reduction sugars (-), PAL (-), PPO (-) and POD (-) were found to be highly significant ($P < 0.001$), and total soluble sugars were significant ($P < 0.05$). The genotype × treatment interaction was significant only for PPO ($P < 0.01$), while the other interactions, total soluble sugars, reducing sugars, PAL, and POD, were not significant. These results suggest that biochemical responses were mainly determined by the genotype and treatment, whereas genotype-specific treatment responses were observed primarily for PPO activity.

**Two-way ANOVA of Morphological Traits**

The genotype made highly significant ($P < 0.001$) contributions to the variations in root length, root weight and root diameter, as revealed by the analysis of variance (Table 4). Treatment also had highly significant effects ($P < 0.001$) on all morphological traits. In addition, the cross-treatment interaction was very significant for root length ($P < 0.001$), significant for root weight ($P < 0.01$), and significant for root diameter ($P < 0.05$). These findings show a high level of genetic variability among carrot genotypes and that their morphological reactions to salinity and bioinoculation were quite different.

Table 3: Mean squares of biochemical traits

| SOV | df | TSS | RS | PAL | PPO | POD | |
|---|---|---|---|---|---|---|---|
| Genotype | 29 | 0.117929*** | 0.001905*** | 0.070826*** | 0.647487*** | 0.046677*** | |
| Treatment | 2 | 0.014857* | 0.007481*** | 0.107631*** | 0.664484*** | 0.055909*** | |
| Genotype× Treatment | 58 | 0.003843 | 0.000352 | 0.000338 | 0.001747** | 0.004569 | |
| Error | 178 | 0.003799 | 0.000362 | 0.000242 | 0.000958 | 0.004556 | |

Significance levels: *P < 0.05; **P < 0.01; ***P < 0.001; values without asterisks are not significant (P ≥ 0.05).

Table 4: Mean squares of morphological traits

| SOV | df | RL | RW | RD | |
|---|---|---|---|---|---|
| Genotype | 29 | 50.597100*** | 226.872138*** | 94.991898*** | |
| Treatment | 2 | 198.922111*** | 652.431880*** | 428.086164*** | |
| Genotype× Treatment | 58 | 6.438854*** | 28.558600** | 13.318130* | |
| Error | 178 | 0.815752 | 16.660148 | 9.458360 | |

Significance levels: *P < 0.05; **P < 0.01; ***P < 0.001; values without asterisks are not significant (P ≥ 0.05).

**The effect of treatments on reducing sugars (RS)**
The results of the boxplot indicated that the reduction in sugar (RS) content was different among the treatments. Salt stress ($T_1$) significantly reduced the RS content in comparison to the control treatment ($T_0$), which had the highest median and mean values. The salt + bioinoculation treatment ($T_2$) showed a partial recovery of the RS from $T_1$ but was lower than the control. The control treatment had greater variability and two high outliers compared to $T_1$ and $T_2$, which had relatively narrower distributions (Figure 1a).

**Effect of Treatments on Root Length (RL)**
Salinity treatments caused a progressive reduction in the length of the root. The salt stress caused a reduction in root growth compared to the highest root length obtained for the control treatment, which had the highest median and mean root length. The root length was also lower in the plants treated with bioinoculation and salt than in the control. The control treatment had more variability in distribution than the stress treatments (Figure 1b).

**Effect of Treatments on Root Weight (RW)**
Root weight increased under the control and decreased with salt stress. There was the lowest median and mean in the salt + bioinoculation treatment. The range of values was also the greatest for the control treatment, suggesting that there was more variation among the genotypes, and the salt-stressed treatments had relatively smaller ranges of values (Figure 2a).

**Effect of Treatments on Root Diameter (RD)**
Salt stress reduced the root diameter to a maximum under the control treatment. Salt + bioinoculation resulted in the lowest median diameter. Boxplots showed that there was high variation between each treatment, but there was generally a decrease in root diameter in the saline treatment (Figure 2b).

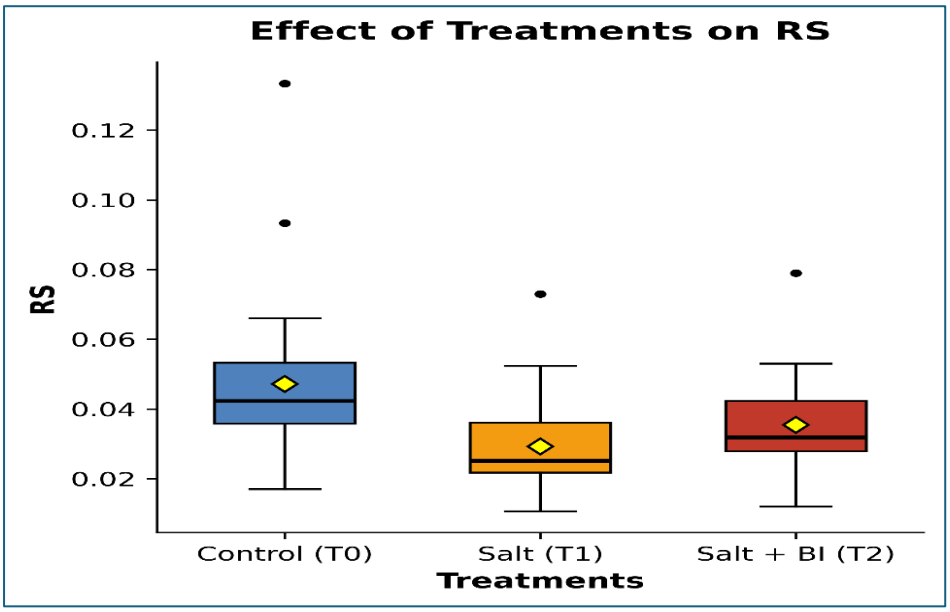
**Effect of Treatments on Phenylalanine Ammonia-Lyase (PAL)**
Under salt stress, PAL activity was reduced in comparison to the control. An increase in PAL activity compared with salt stress alone was observed after bioinoculation under saline conditions, which shows partial recovery of enzyme activity. Moderate variation in PAL values was observed among the treatments, and the distribution was relatively uniform (Figure 3a).

**Effect of Treatments on Total Soluble Sugars (TSS)**
The highest TSSC was observed under salt stress, which shows the adaptation of the plants to salt stress in the form of osmotic adjustment. The medians were the same for the control and salt + bioinoculation treatments. There was little difference in the variability of TSS among the treatments, but salt stress had a slightly higher central tendency (Figure 3b).

**Effect of Treatments on Polyphenol Oxidase (PPO)**
**The** PPO activity was greatest in the control and decreased under salt stress. Under saline conditions, bioinoculation partially recovered the PPO activity, and the value was between the control and saline treatments. There were moderate ranges of variation and no obvious outliers among treatments in the boxplots (Figure 4a).

**Effect of treatments on peroxidase (POD)**
The activity of peroxidase was stimulated in cells subjected to salt stress compared to the control, indicating the activation of antioxidant defense mechanisms. The salt + bioinoculation treatment was found to have lower POD activity compared to salt stress alone, but levels remained slightly higher than the control. Moderate variability of the distributions among treatments with no extreme outliers' Figure 4b.

a




b

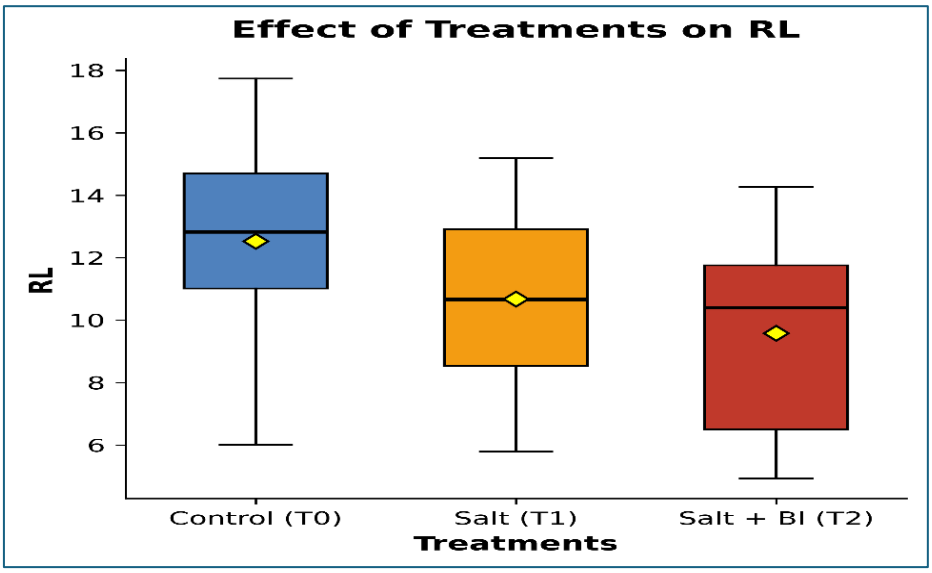



Figure (1a-1b): Effect of Control (T0), Salt (T1), and Salt + BI (T2) treatments on 1) Reducing sugars b) root length (RL).

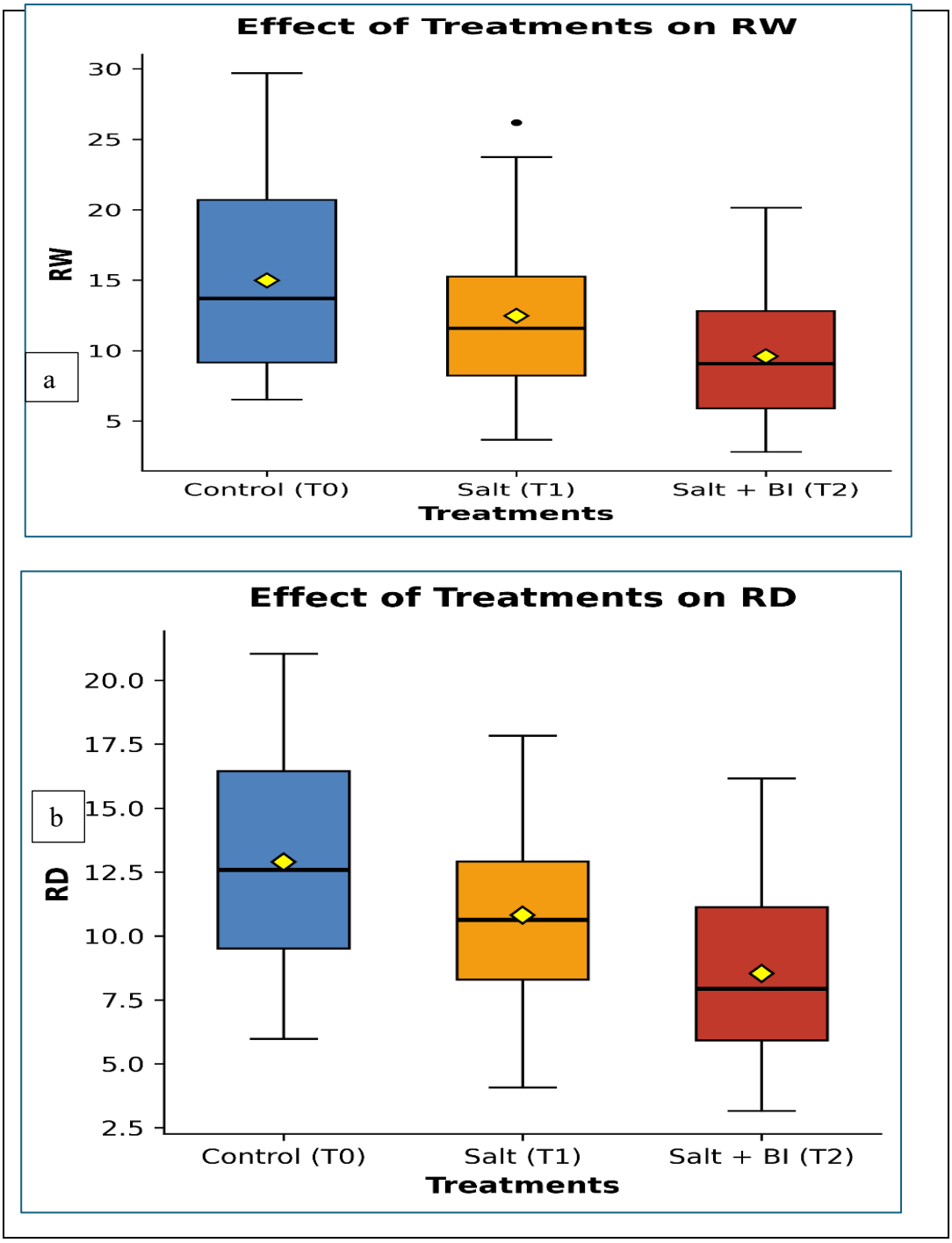



Figure (2a-2b): Effect of Control (T0), Salt (T1), and Salt + BI (T2) treatments on 1) Root weight b) root diameter

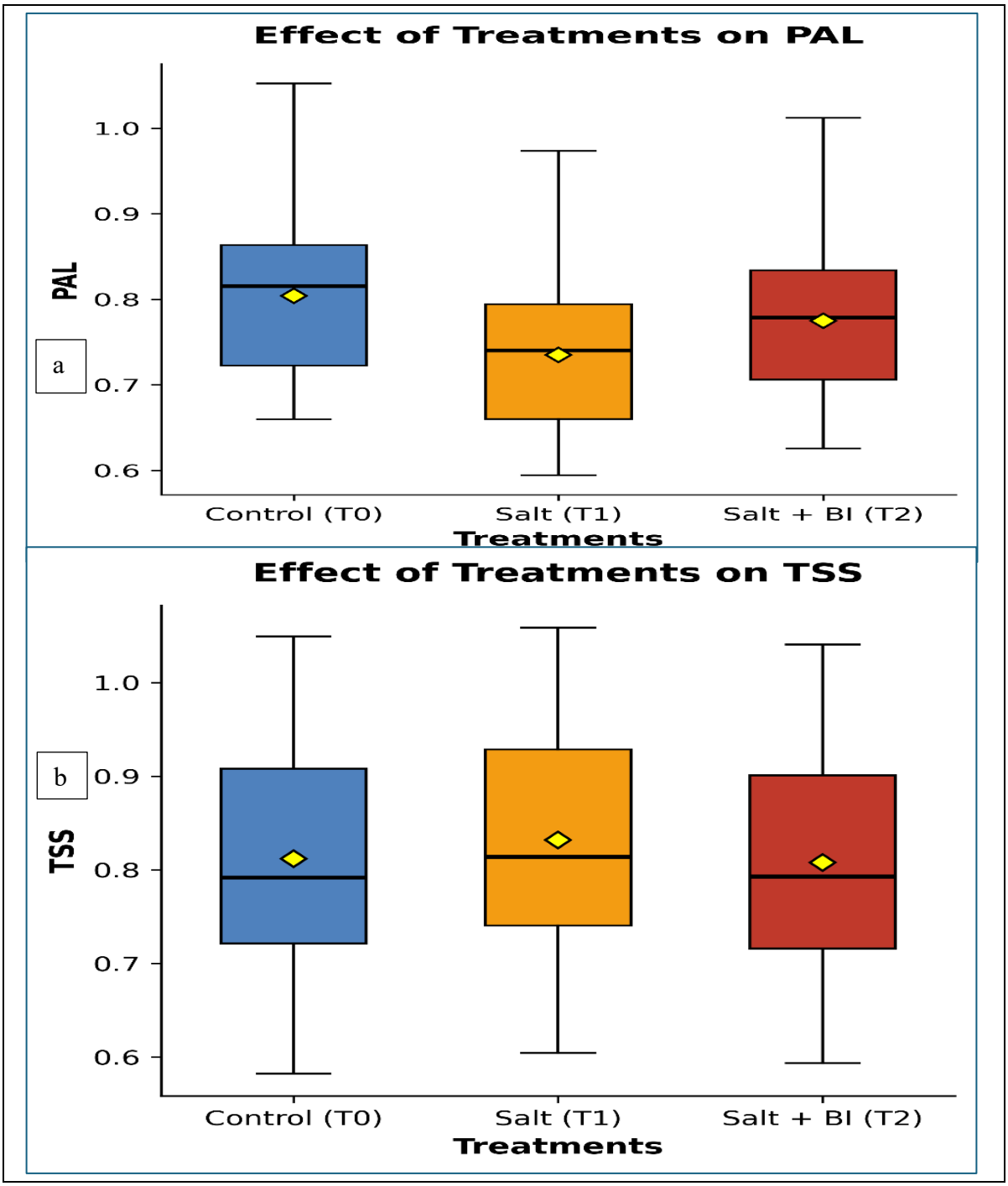



Figure (3a-3b): Effect of Control (T0), Salt (T1), and Salt + BI (T2) treatments on 1) Phenylalanine Ammonia-Lyase b) Total Soluble Sugars

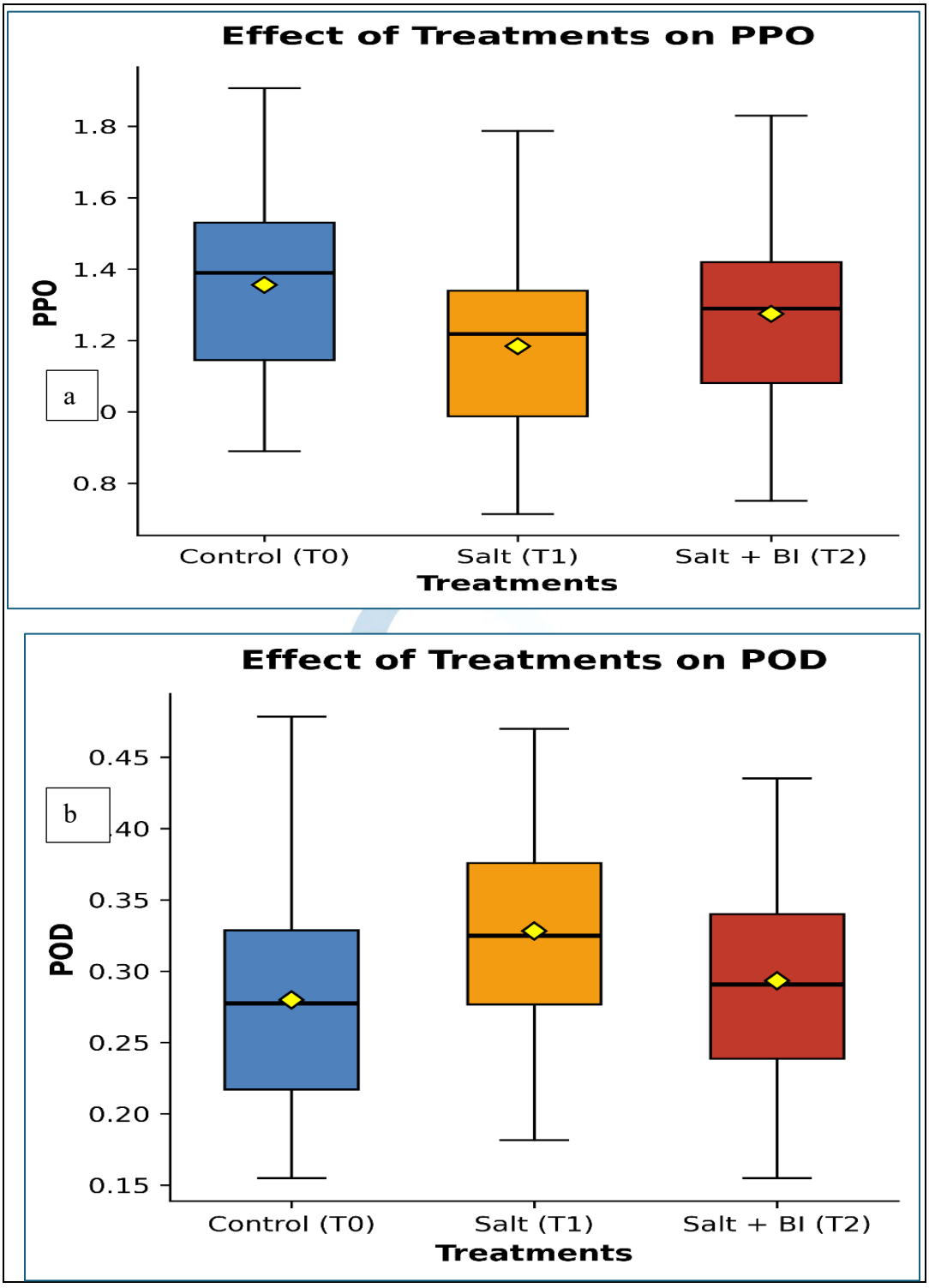



Figure (3a-3b): Effect of Control (T0), Salt (T1), and Salt + BI (T2) treatments on 1) Polyphenol Oxidase b) PeroxidaseGenotypic Variation in Phenylalanine Ammonia-Lyase (PAL) Activity

The 30 genotypes of carrot differed in their PAL activity under the three treatments. The activity of PAL ranged between approximately 0.69 and 1.05 under the control treatment ($T_0$), while it was reduced to 0.62–0.98 under salt stress ($T_1$). Under saline conditions ($T_2$), partial restoration of PAL activity was observed, ranging from approximately 0.67 to 1.01. Under salt stress, the majority of genotypes showed lower PAL activity than the control, while several genotypes showed higher PAL activity after bioinoculation. The effects of salinity on PAL activity were partially mitigated by Pseudomonas inoculation; however, this effect was observed depending on the genotype (Figure 4a).

**Polymorphism of Polyphenol Oxidase (PPO) Activity**

There was also a significant genotype difference in PPO activity. The PPO activity under the control treatment varied between 0.90 and 1.90, and for the salt stress treatment, this range was 0.72–1.80. In all most genotypes, the activity of PPO increased after bioinoculation, ranging from approximately 0.75 to 1.84. PPO activity was markedly increased in several genotypes but slightly decreased in a few others when cultivated in $T_2$ in comparison to $T_1$. Overall, the activity of PPO was found to be highest in the control treatment, lowest in the salt stress treatment and slightly higher in the bioinoculation treatment (Figure 4b).

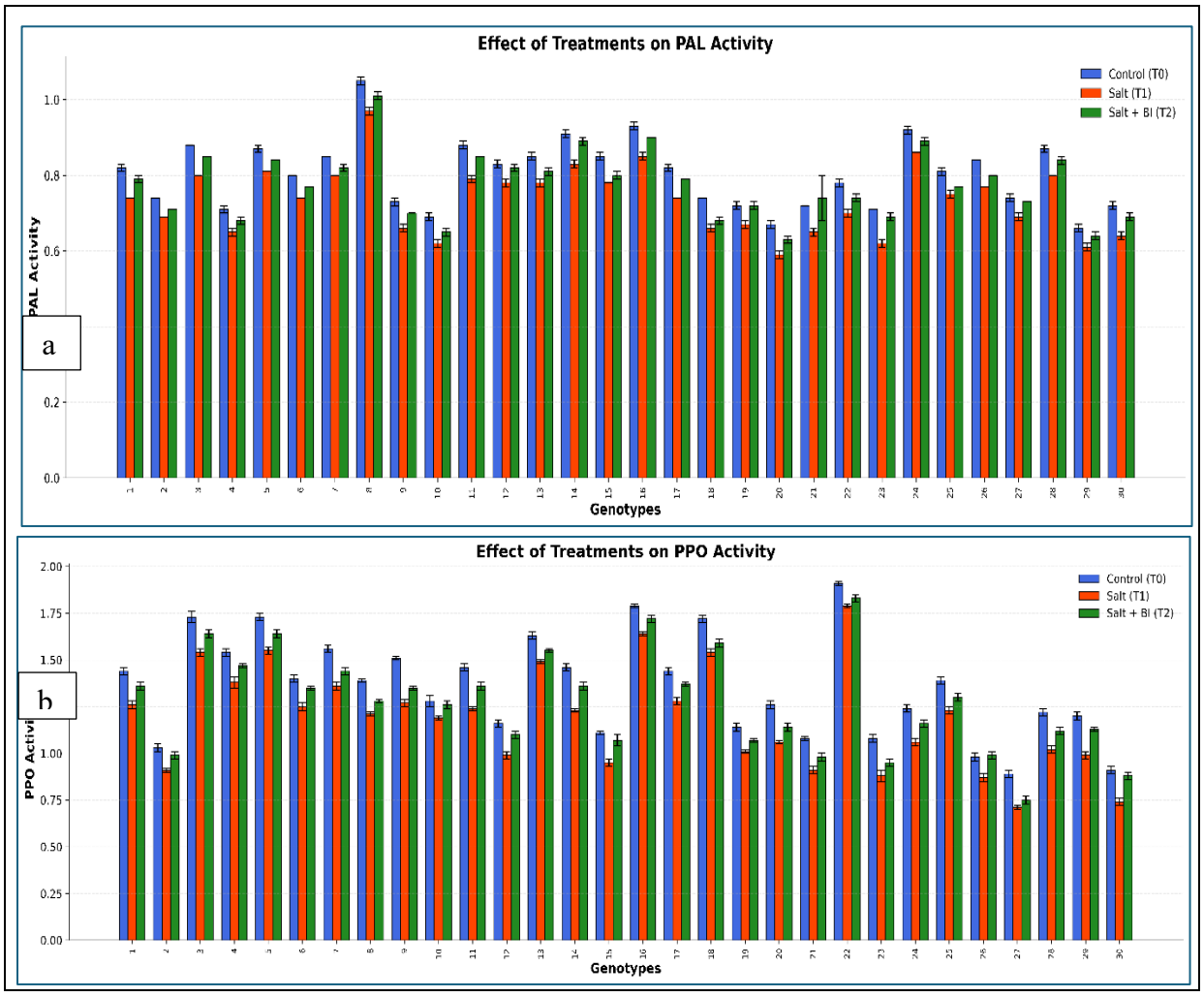


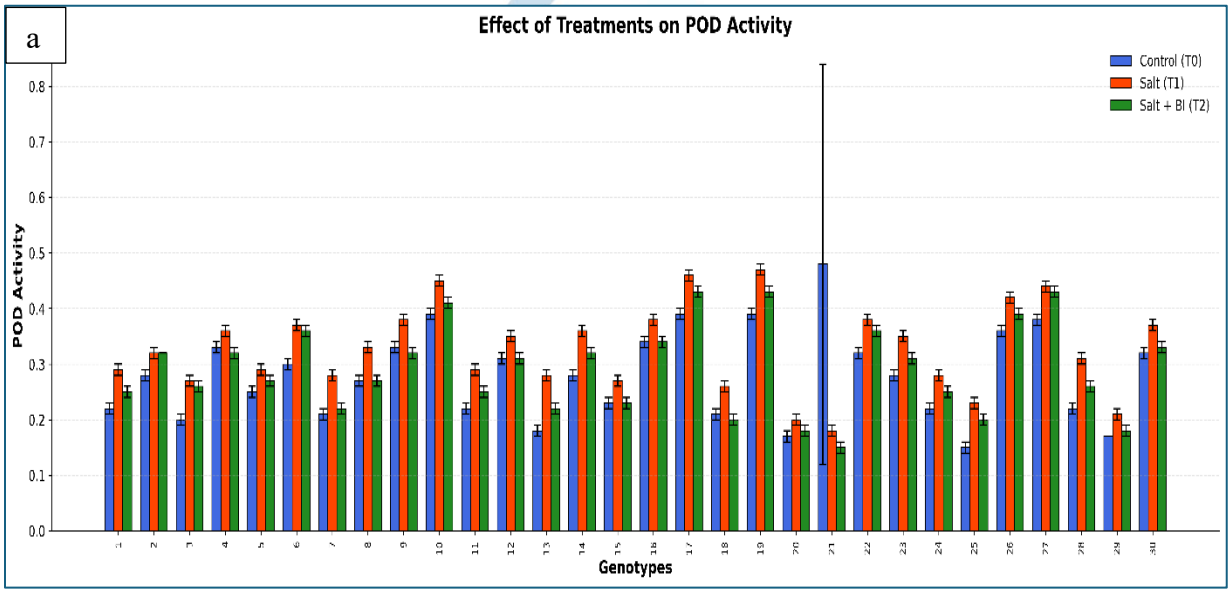

**Figure (4a-4b):** ***Effect of treatments on a) polyphenol oxidase b) root length in different genotypes.***

**Genotypic variation in the activity of peroxidase (POD)**

There was a significant difference in the POD activity in the carrot genotypes in the three treatments (Figure 5a). The activity of POD was found to be approximately 0.15 to 0.48 under the control treatment, and one genotype had a comparatively higher value under the control treatment. POD activity was higher under salt stress, ranging from approximately 0.18 to 0.47. The activity of POD was slightly affected by the bioinoculation treatment (0.15–0.44) compared with salt stress alone. While salinity induced higher POD activity in all the genotypes, the increase was not as high in the case of bioinoculation, suggesting less oxidative stress.

**Effect of Treatments on Root Length**

The root length also showed significant differences between the 30 genotypes in the three treatment groups (Control, Salt and Salt + Bi). The root length of the control (T0) was generally higher than that observed under the other salinity levels, while salt stress (T1) tended to significantly decrease root length for most of the genotypes. In several genotypes, the inhibitory effect of salinity was partially removed by the Salt + Bi treatment (T2), but root length in most cases was still less than that in the control. Under control conditions, root lengths of 28 and 3 were the longest, and those of 1 were the shortest under all conditions. Of the several genotypes, 9, 10, 27 and 30 showed relatively high root lengths under salinity stress, indicating their tolerance. Bi under saline conditions increased root growth in many genotypes, especially genotypes 22, 28, 29 and 30, signifying the beneficial effect of Bi in reducing the growth inhibition effect caused by salinity.



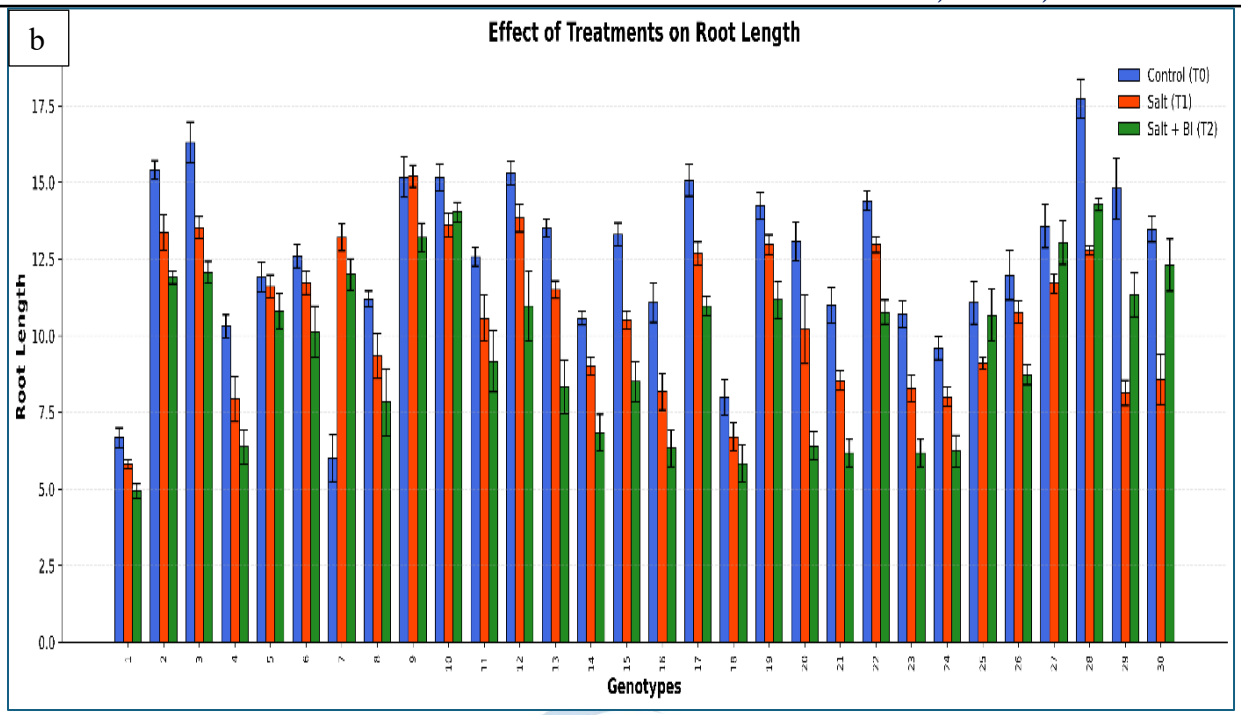



**Figure (5a-5b): Effect of treatments on a) phenylalanine ammonia-lyase b) polyphenol oxidase (PPO) activity in different genotypes.**



**Effect of Treatments on Root Weight**

There was significant genotypic variation in root weight as influenced by various treatments. The root biomass was the highest in control plants, and in most of the genotypes, salt stress significantly reduced root weight. The extent of reduction varied, however, between the different genotypes, suggesting differential responses to salinity.

Genotypes 11, 12, 21, 26, 28 and 30 had relatively greater root biomass than the rest of the genotypes. Bi supplementation partially restored biomass accumulation under salt stress in several genotypes, as indicated by the increase in root weight in comparison with salt stress alone. However, the root weight of the salt + Bi treatment was still lower than that of the control, showing that Bi reduced but did not completely reverse the negative impact of salinity (Figure 6a).

**Effect of Treatments on Root Diameter**

There was also a significant response to salinity and Bi application on the root diameter. Mostly, the highest diameter root was obtained under control conditions, and salt stress resulted in a significant reduction for the majority of genotypes. Under saline conditions, bi application had a positive effect on root diameter, although the effect differed among the genotypes. The root diameters under stress conditions were relatively large for genotypes 9, 11, 21, 26, 28 and 30, indicating high adaptation to salinity. Genotypes 4, 6, 18 and 24 had the highest decrease in root diameter after salinity treatment. Overall, salinity had a negative impact on root thickness, while Bi supplementation enhanced root development (Figure 6b).

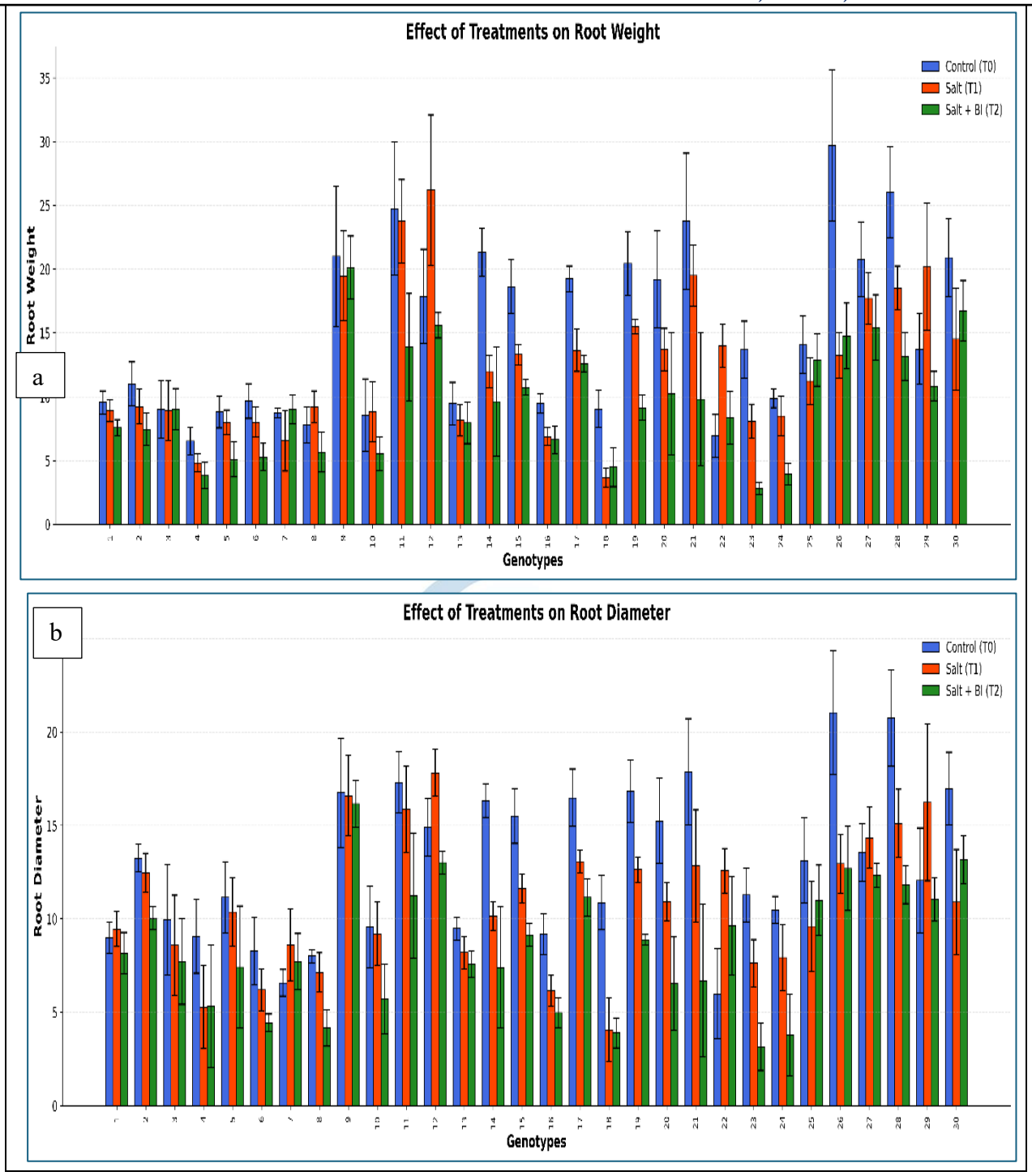



**Figure (6a-6b): Effect of treatments on a) root diameter b) root weight in different genotypes.**

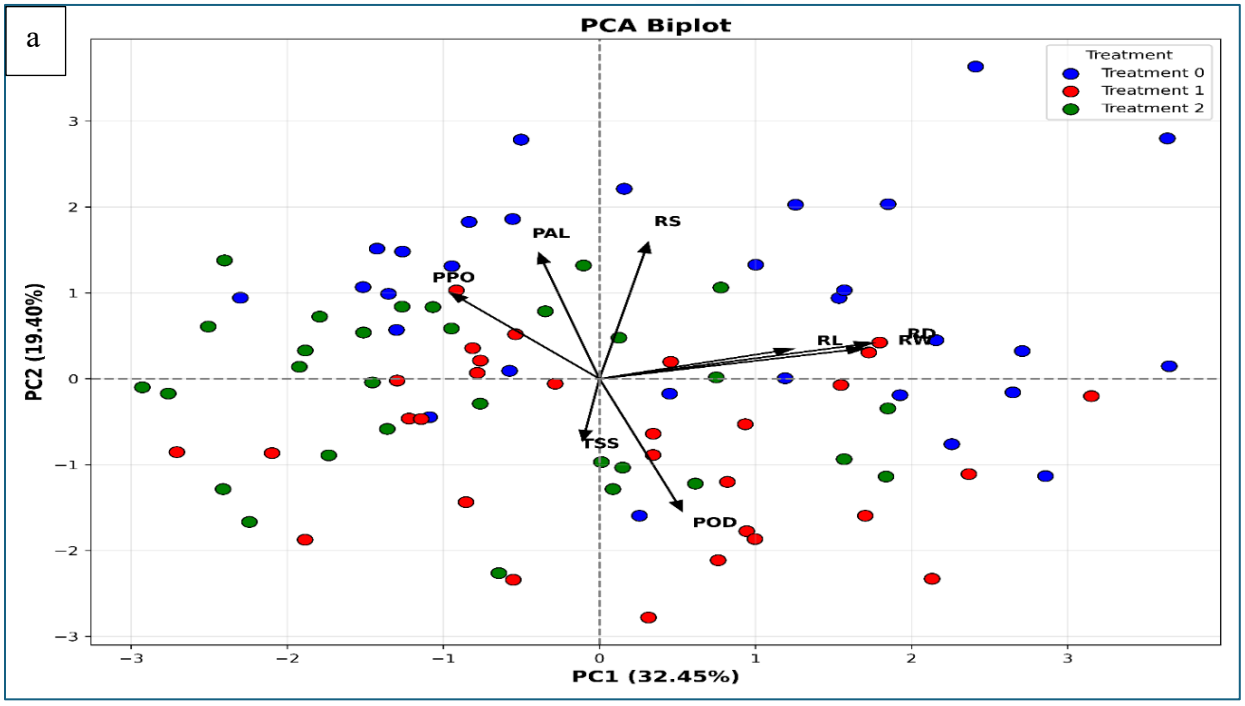
**Principal Component Analysis (PCA)**

PCA explained 51.85% of the total variance, with the first principal component (PC1) accounting for 32.45% and the second principal component (PC2) accounting for 19.40%. The PCA biplot separated the measured traits based on the contribution to the total variation. Root length (RL), root width (RW), and root diameter (RD) had high positive loadings on PC1 and were highly correlated with each other, suggesting a

strong positive correlation between root growth traits. PC2 was statistically positively correlated with root surface area (RS), while PPO activity was mainly associated with the lower right quadrant, suggesting an inverse correlation with the traits located in the upper quadrants. PAL and PPO had clustered together in the negative part of PC1, indicating that there was a positive relationship between these antioxidant enzymes. The TSS was situated near the origin, which suggests that it made a relatively small contribution to the total variation compared to the other measured traits. There was clearly much overlap between the three treatments, but a few control samples were more concentrated towards the positive side of PC1, suggesting a generally good performance for root growth.

**Hierarchical Cluster Analysis**

The 30 genotypes were divided into two broad categories by hierarchical cluster analysis, indicating a large genetic difference among the genotypes regarding their response to salinity and the application of Bi. The first major cluster was formed by the genotypes having relatively similar root growth characteristics and generally poor performance in saline conditions, while the second cluster consisted of genotypes that had comparatively superior root traits and higher tolerance to stress. Within each of the main clusters, several smaller subclusters were identified, which showed different levels of similarity between individual genotypes. The clustering pattern was in good agreement with the PCA results, in which the genotypes with similar root traits were grouped together. The separation of distinct clusters indicates that great genetic diversity among the evaluated genotypes exists and will be valuable for germplasm selection for future salt-tolerant breeding programs (Figure 7b).



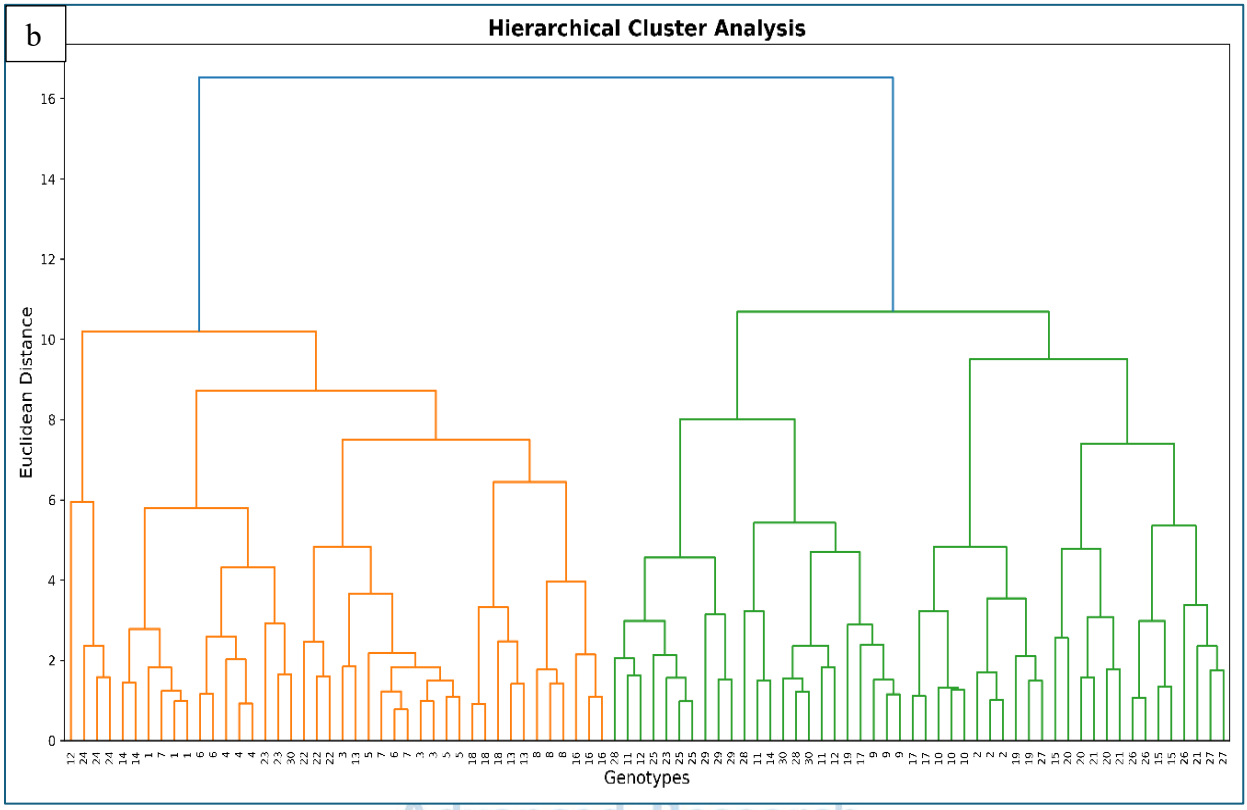



**Figure (7a-7b): PCA biplot showing the distribution of genotypes b) Hierarchical cluster under Control (T0), Salt (T1), and Salt + BI (T2) treatments based on growth and biochemical traits.**

## Discussion

### Root Length Effect of Salinity and Pseudomonas Bioinoculation

The present study revealed that salinity significantly decreased the root length of most carrot genotypes, while Pseudomonas bioinoculation partially enhanced root growth in saline conditions. However, root length under Salt + BI was still lower than that of the control treatment. Highly significant genotypic variations were also noted in which genotypes 9, 10, 27, 28 and 30 remained comparatively better salt tolerant with a longer root length under salinity compared to other genotypes. The results were similar to those reported by other studies, which found that salinity stress and/or ion toxicity limit root elongation in carrot and other crops (Zamani et al., 2024; Rahnama et al., 2019; Huqe et al., 2021). In a similar manner, PGPR inoculation resulted in enhanced root growth (Sonia et al., 2024; Alam et al., 2021).

Reduced water uptake, ionic toxicity, nutrient imbalance and cell division and elongation inhibition are all possible causes for salinity-induced reductions in root length (Balasubramaniam et al., 2023). Conversely, Pseudomonas stimulates root development by producing phytohormones, increasing nutrient availability, decreasing stress-induced ethylene by ACC deaminase activity, and strengthening antioxidant defense systems (Yasmeen et al., 2021; Sharma et al., 2025). The partial recovery of root length noted in the present work may be attributed to these mechanisms that enhance the growth of roots and allow the plants to survive in saline conditions.

**Effect of Salinity and Pseudomonas Bioinoculation on Root Weight**

The present study showed that salinity significantly affected the root weight of most carrot genotypes, while a positive effect of Pseudomonas bioinoculation was observed under saline conditions on the root biomass. However, the root weight of the salt + BI treatment was still less than that of the control treatment. Marked genotypic variation was found, where genotypes 11, 12, 21, 26, 28 and 30 showed comparatively more biomass of roots under stress. The results are in line with earlier studies showing that salinity is reported to cause a decline in root biomass due to decreased water absorption, photosynthesis, and uptake of nutrients (Zhao et al., 2021; Mbarki et al., 2020). Similar results after PGPR inoculation have been reported by Sandhu et al. (2023) and Dugasa et al. (2019).

The decline in root biomass under salinity is primarily attributed to osmotic stress, a decrease in carbon assimilation, nutrient imbalance and an increase in energy expenditure for stress adaptation. Conversely, Pseudomonas has a beneficial role in increasing biomass by enhancing nutrient uptake, producing more phytohormones, stimulating root growth and increasing photosynthetic efficiency (Awais et al., 2023; Balasubramaniam et al., 2023; El Sabagh et al., 2021). Partial recovery of root weight in the Salt + BI treatment was probably due to these physiological effects.

**Effect of Salinity and Pseudomonas Bioinoculation on Root Diameter**

In the present study, root diameter was significantly decreased in saline conditions, and bioinoculation with Pseudomonas alleviated this reduction. Under salt stress, relatively high root diameter was found in genotypes 9, 11, 21, 26, 28 and 30, which were better adapted. The results are consistent with previous studies that reported that salinity resulted in a reduction in radial root growth by interfering with root development and vascular differentiation (Shelden & Munns, 2023; Kurczyńska et al., 2025).

This decrease in the diameter of the root is correlated with a decrease in the activity of the cambium, a decrease in the expansion of cortical cells and oxidative damage due to excessive salinity. Bioinoculation was likely to have enhanced the thickness of the roots by increasing nutrient supply, improving hormonal balance and hence antioxidant protection in the roots, resulting in the maintenance of the structural integrity of the roots under saline conditions (Kumar et al., 2022; Castiglione et al., 2021).

**Effect of Salinity and Pseudomonas Bioinoculation on Reducing Sugars (RS)**

The present study indicated that reducing the amount of sugar significantly reduced under salt stress, while the bioinoculation of Pseudomonas partially restored sugar levels but did not reach the control level. The results showed that bacterial inoculation had a positive impact on metabolic performance under stress and that salinity had a negative effect on carbohydrate metabolism. Shiyab et al. (2019) and Yadav et al. (2021) reported similar decreases in reducing sugars under salty conditions for carrots and other crops. Similarly, Mellidou et al. (2021) and Ilyas et al. (2020) reported similar improvements in plant growth and carbohydrate metabolism under salinity in PGPR.

The decrease in reducing sugars under salinity can be explained by the effects on a plant's capacity to photosynthesize (Sami et al., 2021), its ability to metabolize carbohydrates and its need to utilize sugars for stress adaptation. The partial recovery after Pseudomonas inoculation may be attributed to the ability to recover nutrients, increase photosynthetic activity, produce phytohormones, and enhance antioxidant defense, thereby maintaining carbohydrate metabolism and improving salinity tolerance (Ma et al., 2020).

**Effect of Salinity and Pseudomonas Bioinoculation on Total Soluble Sugars (TSS)**

Total soluble sugars showed a trend towards increase under salt stress, suggesting an osmotic adjustment to salinity. The TSS was slightly decreased by the combined effect of

bioinoculation and salt stress compared to salt stress alone, which indicates that bacterial treatment reduced the stress and kept the sugar concentrations near the control. Zhou et al. (2022) and Mehrabi et al. (2024) reported similar increases in soluble sugars under saline conditions.

Soluble sugars accumulate in cells as a protective mechanism to help preserve cellular osmotic balance, stabilize membranes and prevent dehydration of cells caused by salt. Increased water uptake, nutrient acquisition and ionic balance under saline conditions as a result of Pseudomonas inoculation is reflected in the slightly lower TSS after Pseudomonas inoculation, indicating less stress than without Pseudomonas inoculation.

**Effect of Salinity and Pseudomonas Bioinoculation on Phenylalanine Ammonia-Lyase (PAL)**

The present study showed that the enzyme activity of PAL decreased under salt stress and that the bioinoculation of Pseudomonas partially recovered enzyme activity under saline conditions. The activity of PAL in plants under salt+BI did not achieve the same level as that in the control, but the improvement suggests that bacterial inoculation can have a beneficial effect on the plant defense system. Singh et al. (2022), Gupta et al. (2022), and Singh et al. (2024) also reported similar reductions in PAL in several crop plants under salinity. Similarly, increased PAL activity after PGPR inoculation was reported by Rahimi et al. (2020) and Jayapala et al. (2019).

PAL is the main regulatory enzyme in the phenylpropanoid pathway involved in the synthesis of phenolic compounds that play a role in plant defense. The reduction in PAL activity with salinity could be due to oxidative stress, metabolic disruption, and enzyme inhibition due to high salt levels. After Pseudomonas inoculation, the improvement may be attributed to the ability to enhance nutrient uptake, synthesis of phytohormones and activation of antioxidant defense systems, which all contribute to protecting cellular metabolism and promoting phenolic biosynthesis under saline conditions.

**Phenology of Salinity and Pseudomonas Bioinoculation on Polyphenol Oxidase (PPO)**

Pseudomonas bioinoculation significantly reduced the PPO activity under salt stress, which was partially restored under the bioinoculation treatment, while the activity was the highest under the control treatment. The differential biochemical response to salinity was suggested by the large genotype by genotype variation. The reduction in PPO activity under salt stress has been reported in other plants, such as carrots, by Nowicki et al. (2025) and Mohamed et al. (2021). It has also been reported that PGPR can enhance antioxidant enzyme activities in saline environments (Ha-Tran et al., 2021; Shultana et al., 2022).

PPO is also an important part of plant defense, as it oxidizes phenolic compounds and prevents oxidative damage when stress occurs. Low PPO activity with salinity may be due to a salinity-induced disruption of cellular metabolism and a low level of enzyme synthesis. The increase in antioxidant activity of beneficial bacteria after Pseudomonas treatment indicates that these bacteria were able to improve antioxidant activity and stability of membranes and decrease oxidative injury, which resulted in improved physiological performance under salt stress.

**Effect of Salinity and Pseudomonas Bioinoculation on Peroxidase (POD)**

Under salt stress, POD activity was enhanced in the present study compared with that under control conditions, while bioinoculation with Pseudomonas slightly decreased POD activity to levels higher than those under control conditions. This response demonstrates the activation of antioxidant defense mechanisms under saline conditions and decreased oxidative stress after bacterial inoculation. These levels of salinity-induced increases in POD activity were also reported by Venkatachalam et al. (2025) and Dasgupta et al. (2023).

Peroxidase is an important antioxidant enzyme that can mobilize hydrogen peroxide produced under the condition of oxidative stress induced by salt stress. The rise in POD activity with salinity indicates higher activity to detoxify reactive oxygen species (ROS) to preserve cell components. When the plants were bioinoculated, the comparatively low POD activity indicated that Pseudomonas decreased oxidative damage by fixing the ionic balance, nutrient uptake and antioxidant efficiency, which reduced the need for additional POD activity to maintain cellular homeostasis.

**Principal Component Analysis (PCA) Effect**

The total variance of the evaluated carrot genotypes was explained by 51.85%, which was covered by PC1 and PC2 with 32.45% and 19.40% of the total variance, respectively. Root length, root weight and root diameter were significantly correlated with PC1, which shows that root system development primarily drove genotypic variation between the different treatments. The high level of correlation between these traits indicates that they were similarly affected by salinity and bioinoculation. Under abiotic stress, Asharp et al. (2024) and Li et al. (2021) reported similar PCA patterns for carrot and other crops, with a large proportion of the phenotypic variability explained by root-related traits.

The morphological and biochemical traits were separated in the PCA biplot, suggesting that they are involved in stress adaptation in different ways. The grouping of root traits is associated with a high positive correlation among these traits and their high importance in sustaining plant growth under saline conditions, while biochemical traits are considered physiological defense strategies activated due to stress. Thus, PCA was useful in effectively summarizing the complex relationships between the measured traits and identifying that root length, root weight and root diameter are reliable and could be used as a reliable selection criterion in carrot breeding programs for salt tolerance (Guo et al., 2024; Yang et al., 2022).

**Effect of Hierarchical Cluster Analysis (HCA)**

Thirty carrot genotypes were classified into two major groups with hierarchical cluster analysis reflecting significant genetic diversity in their salinity and Pseudomonas bioinoculation response. Genotypes having the same morphological and biochemical features were clustered together, while all the salt-tolerant genotypes were grouped distinctly from the more sensitive genotypes. The clustering pattern was very similar to the PCA results and was used to confirm the consistency of the results of multivariate analysis in distinguishing the genotypes according to their stress tolerance. Similar clustering patterns have been reported using morphological and physiological traits in carrots (Mohi-Ud-Din et al., 2021; Balko et al., 2023).

The results demonstrated some genetic variation among the carrot varieties regarding salinity tolerance and adaptation mechanisms, which are evident in clusters. The tolerant cluster of genotypes is likely to have better ion homeostasis, osmotic adjustment, antioxidant defense mechanisms, and root development than the susceptible cluster of genotypes. Thus, hierarchical cluster analysis can be useful for germplasm characterization and can be employed in future breeding programs to select successful germplasms for the development of salt-tolerant carrot cultivars (Wu et al., 2025; Kumar et al., 2024).

**Conclusion**

Salinity significantly reduced carrot growth by reducing root length, weight, diameter, PAL activity, PPO activity, and sugar content, while increasing total soluble sugar accumulation and peroxidase activity as adaptive responses to osmotic and oxidative stress. Pseudomonas bioinoculation helped to minimize these negative effects by boosting root development, restoring antioxidant enzyme activity, and lowering stress-induced oxidative damage, although full recovery to control levels was not attained. Significant genotypic variance revealed significant genetic variety in salinity tolerance across the tested

carrot germplasm. Genotypes 9, 11, 21, 26, 28, and 30 consistently demonstrated superior morphological performance in saline circumstances, making them excellent candidates for producing salt-tolerant carrot cultivars. Multivariate analysis showed root length, root weight, and root diameter as reliable salinity tolerance selection variables, while PCA and hierarchical clustering effectively distinguished between tolerant and susceptible genotypes. Overall, combining salt-tolerant germplasm with Pseudomonas-based bioinoculation is an environmentally sustainable technique for increasing carrot productivity in salt-affected agricultural soils while also providing useful resources for future breeding initiatives.

**Declarations**

**Consent for publication**

All subjects gave their "informed consent" for the publication of details within the text ("informed consent") to be published in the above Journal and Article. Written "informed consent" was obtained from all authors for the publication of this manuscript.

**Availability of data and materials**

The data generated are provided within the manuscript and will be available from the author upon reasonable request.

**Competing Interest**

All authors declare that there are no competing interests.

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Not applicable

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