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Biofertilizers Alleviate Salinity Stress in Medicinally Important Plant - *Catharanthus roseus* (L.) G. Don by Enhancing Morphological and Photosynthetic Attributes

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ABSTRACT
Salinity stress is the major growth limiting factor in most economically important crops as it adversely declines the yield and productivity of plants. *Catharanthus roseus* is an essential medicinal plant for the drug industry, and its productivity is highly affected by salt stress. The present study investigates the potential role of plant growth-promoting bacteria (PGPB) and arbuscular mycorrhizal fungal (AMF) consortium in alleviating salinity stress in *C. roseus*. We analyzed growth parameters and photosynthetic attributes (chlorophyll pigment and gas exchange parameters) in salinity stress conditions and with the application of PGPB and AMF. *Catharanthus roseus* showed a significant decline in shoot height, root height, fresh weight, dry weight, specific leaf area, and relative water content by 17.79, 36.80, 17.90, 65.55, 48.35, and 43.29%, respectively, under salinity stress. However, mycorrhizal inoculation improved the above growth parameters by 35.57, 36.60, 44.51, 231.48, 22.87 and 18.50%, respectively. Electrolytic leakage increased by 45.09% under salinity stress but in contrast, it is decreased by 17.02% under mycorrhizal inoculation as compare to control treatment. Photosynthetic attributes viz. photosynthetic rate (Pn), stomatal conductance (C), and transpiration rate (E) also showed a significant decline in salinity treatment by 42.57, 51.96 and 31.98%, respectively, as compared to control treatment. In comparison, mycorrhizal inoculation increased these photosynthetic attributes by 225.13, 204.72 and 159.51%, respectively. The present research suggested that phosphate solubilizing bacteria and mycorrhizal application play the most effective role in inducing salinity stress tolerance in *C. roseus* and, therefore, can be considered a cost-effective and sustainable approach to provide tolerance against salinity stress.

Key words: Plant growth promoting bacteria; Arbuscular mycorrhizal fungi; chlorophyll pigment; Photosynthetic rate; Stomatal conductance; Transpiration rate

INTRODUCTION

Edaphic salinity is a key abiotic stress factor impacting global agriculture resulting in reduced crop productivity. Even the most conservative estimates indicate that around 20% of all the global arable land is affected by soil salinity (Pitman and Läuchli 2002). Globally, the agriculture industry suffers a loss of around 27.3 billion US$ due to soil salinity (Qadir et al. 2014). Reduced productivity is not the only detrimental impact of soil salinity, but also leads to destabilization of soil structure, soil erosion, poor hydrological and nutrient cycling, reduced water availability to plants, reduced soil fertility, ionic imbalance of soil leading to ion toxicity, and loss of soil buffering ability against contaminants (FAO 2021). Therefore, understanding the impact of soil salinity is critical from a plant growth perspective and in terms of maintaining soil health and fertility.

Soil salinity is one of the major abiotic stresses leading to reduced plant growth and productivity by causing osmotic stress, cellular ion toxicity, and reactive oxygen species (ROS) formation leading to oxidative stress and reduced nutrient mobilization (Kumar et al. 2020). Reducing the leaf surface area is one of the plants' earliest and most visible manifestations of salinity stress that negatively affect photosynthesis (Parida and Das 2005). Salinity stress damages the photosynthetic machinery of the plants via inhibition of chlorophyll biosynthesis, decreasing the CO_2 availability due to the stomatal closure and damaging the photosynthetic apparatus causing a gross reduction in the photosynthetic efficacy (Zahra et al. 2022).

Over the years, several strategies have been

employed to reduce the drastic effects of salt stress, such as transgenic crops, conventional breeding of resistant varieties, and interspecific hybridization. Nevertheless, these have limitations due to the genetic and physiological complexity of the salt tolerance trait, and these techniques are long-drawn and costly (Flowers 2004). There has been an increased dependence on chemical fertilizers to cope with the low yield in salinity stress (Machado and Serralheiro 2017). Several environmental issues have been raised regarding the use of inorganic fertilizers because of their rising cost, associated environmental pollution, and declining soil fertility. Therefore, identifying a sustainable approach for improving crop yield is essential to mitigate stress conditions (Abdel Latef et al. 2020).

The role of plant growth regulators in alleviating different abiotic stress has gained attention globally. They promote plant growth by accumulating sufficient nutrients, reducing plant pathogens, and increasing phytohormone production (Shrivastava and Kumar 2015). Several plant growth-promoting bacteria (PGPB) are recognized, which aid in improving specific nutrient availability. These free-living aerobic bacteria (e.g., *Azotobacter chroococcum*) promote plant growth by fixing nitrogen and producing phytohormones under saline conditions (Van Oosten et al. 2018). *Bacillus amyloliquefaciens,* a phosphate solubilizing PGPB, enhance tolerance to abiotic stress by providing several benefits to plants increasing nutrient uptake, chlorophyll content, shoot and root growth, and increasing proline content (Adesemoye and Kloepper 2009, Kim et al. 2017). Potassium availability is another critical factor required for the growth and development of plants. Potassium solubilizing PGPB, like *Enterobacter asburiae* enhances plant growth by increasing the available potassium in the soil and secreting auxins (Zhang and Kong 2014). Arbuscular mycorrhizae fungi (AMF), an essential component of the soil microbial community, help to tolerate various abiotic stress (Shrivastava and Kumar 2015). PGPBs, thus are a sustainable and viable approach for improving plant growth and alleviating salt stress.

In addition to the cash crops, the commercial cultivation of important medicinal plants is necessary due to their high demand in both developed and developing countries due to the presence of different lifesaving compounds. It has become crucial to combat factors limiting their growth and physiological attributes in large-scale production (Ghorbanpour and Varma 2017). Exposure to highly saline soils causes a severe decline in the productivity and yield of medicinal plants with a simultaneous reduction in the content of active chemical components in the plants (Mondal and Kaur 2017). *C. roseus* (L.) G. Don. is a highly exploited and important industrial medicinal plant that belongs to the family Apocynaceae and contains vinblastine and vincristine, two commercially important anticancerous alkaloids. These alkaloids are highly valuable as they are used in leukemia chemotherapy and in treating Hodgkin's disease (Jaleel et al. 2007). Most studies on *C. roseus* have focused mainly on testing its salinity tolerance, the effect of salinity on its growth and physiology, and various strategies for ameliorating salinity stress in this plant. Limited research is available on beneficial microbial strains in mitigating salinity stress in *C. roseus.* Probably, no such study has been carried out till date which focused on the application of these microbial strains, viz. nitrogen-fixing bacteria (*A. chroococcum)*, phosphate solubilizing bacteria (*B. amyloliquefaciens*), potassium solubilizing bacteria (*E. esburia*) and mycorrhizal consortium containing *Funneliformis mosseae*, *Rhizophagus intraradices,* and *Gigaspora species* collectively under salinity stress in *C. roseus.* Thus, comprehensive research is required in this area to evaluate their application in *C. roseus* under salinity stress conditions. We hypothesize that the inoculation with different biofertilizers under salinity stress conditions will improve the growth and photosynthetic attributes during salinity stress in *C. roseus*. Hence, the objectives of the present study were (i) to check the effect of salinity stress on growth parameters, chlorophyll pigments, and gas exchange parameters, (ii) to explore the effect of the growth-promoting bacteria and mycorrhizal consortium in inducing salinity stress tolerance in *C. roseus.*

MATERIALS AND METHODS

Study region and plant material

This study was conducted in the botanical garden of the Department of Botany, University of Delhi, India (28°41'21.92"N and 77°12'37.21"E) and at an average elevation of 213 m for 120 days. The

experiment was conducted from December to March 2020-21. Seeds of *C. roseus* (IC49581) were procured from Germplasm Evaluation Division, Indian Council of Agricultural Research (ICAR) - National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India. Before use, the seeds were surface sterilized with 1% sodium hypochlorite solution for 15 minutes and then washed consecutively with double-distilled water several times. Germination experiments viz., percentage seed germination and the mean germination time were determined with the seeds pre-soaked in double distilled water between the folds of germination paper under dark conditions at 25 °C.

Study design, salinity dose, and biofertilizer amendments

Twelve treatments were designed for this study to determine the efficacy of various biofertilizer amendments to ameliorate salinity stress conditions viz., 1) control (No treatment); 2) salt stress (4.5dS/m); 3) BioN; 4) BioN + Salt stress; 5) BioP; 6) BioP + Salt stress; 7) BioK; 8) BioK + Salt stress; 9) BioNPK; 10) BioNPK+ Salt stress; 11) Mycorrhizae, 12) Mycorrhizae + salt stress. To determine the salinity dose, we surveyed six commercial *C. roseus* growing fields in Southern India, viz., two locations each in Tamil Nadu, Karnataka, and Maharashtra. This region was specifically selected since the maximum commercial production for *C. roseus* in India is from these regions. The highest natural salinity value obtained (4.5 dS/m) from the survey was used as the salinity dose in this study, found in soil samples from Karnataka.

The biofertilizers were obtained in powder form from the Division of Microbiology, ICAR, New Delhi. The biofertilizers selected consisted of Nitrogen-fixing bacteria (*A. chroococcum*) (BioN), Phosphate solubilizing bacteria (*B. amyloliquefaciens*) (BioP), Potassium solubilizing bacteria (*E. esburia*) (BioK) all of which contained 10^8 cfu g^{-1} in a charcoal-based carrier. The mycorrhizal strain had a consortium of *F. mosseae*, *R. intraradices,* and *Gigaspora* species with approximately 90 – 100 propagules g^{-1} soil.

Seeds were inoculated with the biofertilizers dissolved in sugar solution (200g biofertilizers dissolved in 100g L^{-1} of household sugar solution). The surface sterilized seeds were adequately coated with this slurry and allowed to dry under room conditions before sowing. Each earthen pot had an average diameter of 30 cm and was filled with 5 kg of autoclaved (121°C, 15 psi, 1 hour) garden soil. The control pot and the pots without salinity dose were irrigated to field capacity with groundwater (Electrical conductivity – 360 µS/cm). While the pots marked for salinity stress were irrigated with 45 mM NaCl solution until the soil EC came to 4.5 dS/m, which was measured with the help of an electrical conductivity tester (Gro Line Soil EC Tester, Hanna Instruments, USA).

The soil used in this study was sandy loam having a pH of 7.7. The electrical conductivity of the soil was 460 µS/cm. The chemical properties were: organic carbon 1.92%, total Kjeldahl nitrogen 0.28%, available phosphorus 0.17%, exchangeable sodium 0.012%, and exchangeable potassium 0.024%.

Estimation of morphological features and leaf characteristics

All the morphological parameters were determined 120 days after sowing (DAS). The leaf area was determined using a portable laser leaf area meter (CID Bio-science CI-202, USA). The leaves were oven dried at 80°C for dry weight and then computing specific leaf area. Shoot height and root height were determined using a standard meter scale. The fresh and dry weights were determined immediately post-harvesting in three replicates for each treatment and oven dried at 80°C in a hot-air oven for 48 hrs.

Leaf relative water content was determined for each treatment by taking 3-5 excised mature leaves. The leaf lamina was punched with the help of a cork borer to create leaf discs (10 mm). After taking the fresh weight of the leaf discs, they were placed immediately in distilled water for 4 hrs at 20°C to avoid respiratory losses (Barrs and Weatherley 1962). The, turgid weight was taken to calculate water uptake after blotting them carefully. The dry weight of the leaf disc was determined by drying the leaf tissue at 70°C for 48 hrs.

Leaves were appropriately washed with distilled water to remove surface contamination and then placed in individual stopper vials containing 10ml of distilled water to determine electrolyte leakage (Lutts et al. 1996). These samples were placed in a shaker at 100 rpm for 24 hrs at room temperature of 25°C. After incubation, the electrical conductivity (EC) of the bathing solution was recorded. The same

samples were then placed at 120°C for 20 min, and the second reading (EC) was taken after cooling the solution at room temperature.

Estimation of photosynthetic pigments and leaf gas exchange parameters

Chlorophyll a, b, and carotenoids were determined using the 80 % acetone extraction method. Fresh leaf samples (0.5 g) from each treatment were grounded in a chilled mortar containing liquid nitrogen. To this powdered maceration, 5 ml of 80 % chilled acetone and a pinch of $CaCO_3$ were added. The leaf extract was centrifuged at 4°C for 15 min at 10,000 g. The supernatant was collected in another centrifuge tube, and chlorophyll-a, -b, and carotenoids were determined by taking absorbance in a UV-Visible spectrophotometer (DU 730, Beckman Coulter) at 663nm, 646nm, and 470nm, respectively. The concentration of photosynthetic pigments (mg g^{-1} FW) were calculated using (Wellburn 1994) equations.

Gas exchange parameters like photosynthetic rate (Pn), transpiration Rate (E), and stomatal conductance (C) were measured on fully expanded leaves between 0900 hrs to 1200 hrs using a handheld IRGA-based photosynthetic system (CID, Bioscience, CI-340, USA) under ambient sunlight and temperature conditions. The system was operated in open mode, with the measurement time for each leaf being 30 seconds. The IRGA sampling duration was 1 sec, the gas flow rate was 0.3 L min^{-1}, and the chamber area was 11.1 cm^2, respectively.

Statistical analysis

Multiple sample t-test was conducted between the saline and non-saline treatments of the six biofertilizer amendments (Control, BioN, BioP, BioK, BioNPK, and Mycorrhizae) using GraphPad Prism ver. 9.4.1 (GraphStats, USA). A two-way analysis of variance (ANOVA) was performed to study the interaction between salinity and various biofertilizer amendments using IBM SPSS ver. 21. Principal component analysis (PCA) was performed for factor reduction and determination of the key parameters influencing the growth and physiology of *C. roseus* under different treatments. A Two-tailed Pearson's correlation was used to determine the degree and directionality of the interaction between all parameters. The correlation and PCA analysis were performed separately for salinity and non-salinity treatments. PCA and correlation analysis were analyzed using PAST software ver. 4.11. The data has been presented as the means of three replications with the standard deviation.

RESULTS

Effects of PGPB and the mycorrhizal consortium on growth and morphological parameters of plant

Growth parameters viz. shoot height, root height, fresh weight, dry weight, specific leaf area (Table 1), and relative water content (Fig. 2) showed a significant decline in salinity stress treatment. The mycorrhizal inoculation without salinity treatment showed the highest value for all the growth parameters. The study showed a decline in shoot height, root height, fresh weight, dry weight, specific leaf area, and relative water content in salt stress treatment with 17.79, 36.80, 17.90, 65.55, 48.35 and 43.29%, respectively compared to control treatment. The mycorrhizal inoculations increased the growth parameters by 35.57, 36.60, 44.51, 231.48, 22.87 and 18.50%, respectively. Electrolyte leakage (EL) (Fig.1) was maximum (45.09%) in salinity stress, and a decline of 17.02% was observed in mycorrhizal inoculation compared to control values.

Effect of PGPB and the mycorrhizal consortium on chlorophyll content and photosynthetic attributes

A significant decline in various gas exchange parameters like Pn, C, E, and chlorophyll pigments was observed under salinity stress (Fig. 2). Mycorrhizal treatment and bacterial inoculations improved gas exchange parameters (Pn, C, and E) and chlorophyll pigments under salinity stress. In the case of photosynthetic pigments, the amount of chlorophyll-a and b was highest in mycorrhizal treatment without salinity stress. The percentage decline in salinity stress treatment for Pn, C, and E was 42.57, 51.96 and 31.98%, respectively, while mycorrhizal inoculation augmented the values by 225.13, 204.72 and 159.51%, respectively as compared to the control values.

Statistical analysis

The two-way ANOVA showed a significant difference between the means in treatments, salinity,

Table 1. Effect of bio-fertilizer treatments under salinity stress (4.5 dS m^{-1}) condition on the shoot length, root length, plant fresh weight, plant dry weight, and specific leaf area in 120 days old *Catharanthus roseus* plants

Treatments	Shoot Length (cm)	Root Length (cm)	Plant Fresh Weight (g)	Plant Dry Weight (g)	Specific Leaf Area ($m^2 Kg^{-1}$)
Control	45.29±1.09	17.30±0.41	34.18±1.07	2.71±0.42	22.61±0.74
Salt Stress	37.23±1.95	11.27±0.53	28.05±1.28	0.93±0.19	13.41±0.52
Bio N	56.86±1.50	21.43±0.97	44.82±0.88	7.90±0.82	24.57±0.72
S + Bio N	49.59±0.48	18.30±0.41	38.40±0.87	3.94±0.25	20.58±1.13
Bio P	59.37±0.50	22.07±0.82	48.17±0.69	8.79±0.57	26.04±1.50
S + Bio P	48.20±0.07	19.83±0.40	41.32±1.41	4.69±0.30	21.68±0.92
Bio K	48.73±1.68	22.03±0.25	40.76±0.47	5.15±0.12	23.76±2.21
S + Bio K	46.75±0.43	17.57±0.39	38.49±0.44	3.79±0.37	21.76±0.09
Bio NPK	52.25±0.45	22.47±0.87	46.12±1.36	7.28±0.30	24.15±0.75
S+Bio NPK	48.73±0.55	21.20±0.86	43.58±0.61	6.63±0.34	23.38±0.67
Myco	61.40±1.67	23.63±0.95	49.38±0.52	8.98±0.70	26.92±0.57
S+Myco	48.90±1.17	22.00±1.07	46.15±0.83	6.09±0.18	22.06±1.89

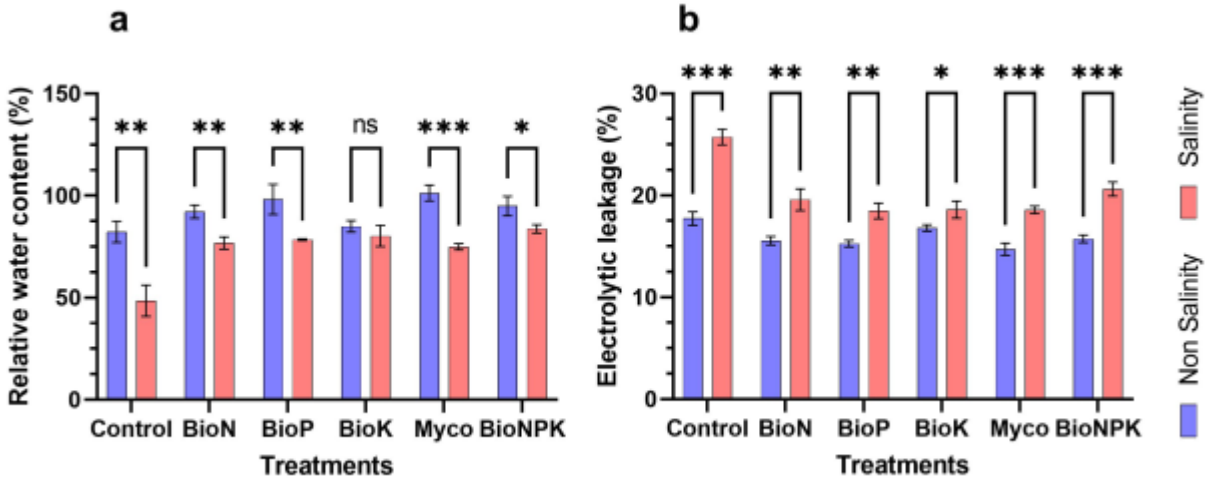

Figure 1. a) Percentage relative water content and b) electrolytic leakage estimates of *C. roseus* under different biofertilizer amendments and salinity doses. The results indicate the mean and standard deviation. The data was analyzed using a multiple-sample t-test at a significance level of $p<0.05$. ns: non-significant, *$p<0.05$, **$p<0.01$, and ***$p<0.001$

and the interaction between treatments and salinity (Table 2). Two-tailed Karl Pearson's correlation was performed (Fig. 3) for all the variables under (a) non-saline and (b) saline treatments separately ($p < 0.05$). In non-saline treatments, root length (RL) showed no correlation with shoot height SH and RWC, while saline treatments showed a significant positive correlation with SH and RWC. Photosynthetic rate (Pn) showed a significant positive correlation with SH, Specific leaf area (SLA), and RWC in non-saline treatments, while it showed no correlation with these parameters in saline treatments. Chl-b showed a significant positive correlation with Chl-a, Pn, E, and C in non-saline treatments. Principal component analysis (PCA) was performed to study the association of the respective treatments with growth and photosynthetic parameters under saline and non-saline conditions (Fig. 4). The principal components having an Eigenvalue greater than one were considered significant (Zuur et al. 2007). For non-saline treatments, we found principal components 1 and 2 significant (Eigenvalue > 1), which explained

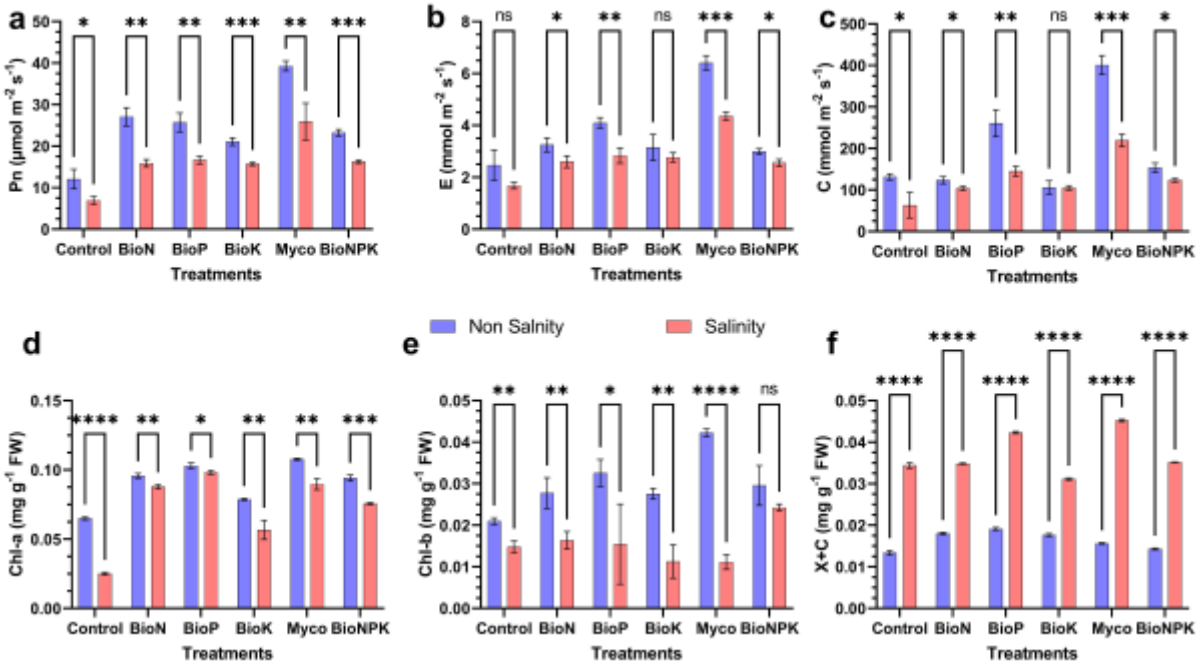

Figure 2. Photosynthetic performance and pigment concentration in *C. roseus* plants supplemented with various biofertilizer amendments under salinity stress. a) Rate of photosynthesis; b) transpiration rate; c) stomatal conductivity; d) chlorophyll a concentration; e) chlorophyll b concentration; and f) xanthophyll and carotenoids concentration. The results indicate mean and standard deviation. The data was analyzed using a multiple sample t-test at a significance level of $p<0.05$. ns: non-significant, *$p<0.05$, **$p<0.01$, ***$p<0.001$, and ****$p<0.0001$

Table 2. Two-way ANOVA results for treatments, salinity, and interaction between treatment and salinity for all the dependent variables

Parameters	**Treatments**	**Salinity**	**Treatment×Salinity**
DF	5	1	5
Pn	96.73***	192.14***	4.79*
E	89.43***	90.42***	7.07***
C	390.70***	435.56***	33.31***
Chl-a	140.45***	149.75***	24.36***
Chl-b	5.86***	134.24***	9.47***
X+C	501.37***	34535.72***	404.62***
SH	123.92***	385.47***	19.89***
RH	86.06***	168.10***	8.57***
FW	159.16***	145.01***	5.13*
DW	97.47***	196.05***	10.9***
SLA	117.77***	98.49***	2.61*
EL	50.09***	403.29***	15.77***
RWC	24.60***	155.17***	8.25***

*$p<0.05$, **$p<0.01$, ***$p<0.001$, DF= Degree of freedom, Pn= Photosynthetic rate, E= Transpiration rate, C= Stomatal conductance, chl-a= Chlorophyll-a, chl-b= Chlorophyll-b, X+C = Xanthophylls and Carotenoids, SH= Shoot height, RH= Root height, FW= Fresh weight, DW= Dry weight, SLA= Specific leaf area, EL= Electrolyte leakage, and RWC= Relative water content

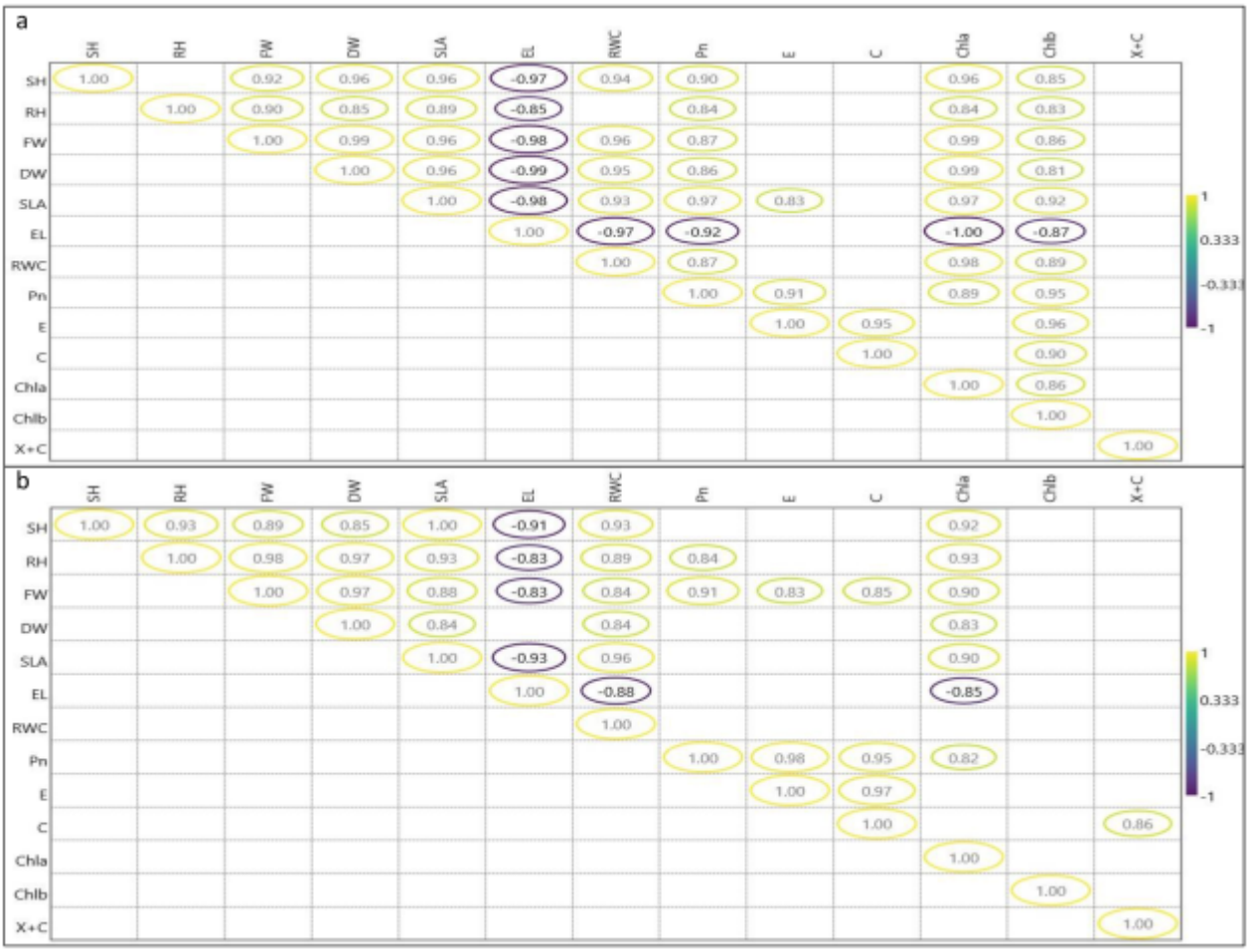

Figure 3. Two-way Pearson's correlation analyses between growth and photosynthetic parameters under a) non-salinity dose and b) salinity dose. The values are indicative of Pearson's correlation coefficient (r). Only significant values ($p<0.05$) are indicated. The values encircled in yellow color denote positive correlation, and purple denote negative correlation

a cumulative 93.03 % of the total variance in the data. Principal component 1 (PC1) showed 82.54 % variance, and (PC2) showed 10.49 % of the total variance. From the biplot (Fig. 4), it is evident that all the parameters except EL showed a positive correlation. The parameters like RWC, Pn, C, E, Chl-b, and all the growth parameters were strongly influenced by mycorrhizal treatment. While in saline treatments, two principal components, PC1 and PC2, were significant and explained a cumulative of 90.57% of the total variance. PC1 and PC2 showed 74.43 and 16.14% of the total variance. From the biplot (Fig. 4c), we observed that EL was strongly associated with the salinity stress treatment. In comparison, all the growth and photosynthetic attributes were strongly influenced by the S+BioP and S+Myco treatments.

DISCUSSION

The present study indicated a significant reduction in all the morphological parameters (shoot height, root height, fresh weight, dry weight, specific leaf area, and relative water content) under saline conditions. The significant decline in growth parameters due to soil salinity affects the plant's growth and development in a toxic way by inducing ionic and osmotic stress in the plant, which leads to decreased productivity. This osmotic and ionic stress reduces the plant's water use efficacy and disrupts the osmotic balance, negatively affecting plant growth and metabolism (Ben-Laouane et al. 2020). Root growth was reduced during salt stress as the water absorption capacity of the plant was disrupted due to osmotic imbalance (García-Caparrós and Lao

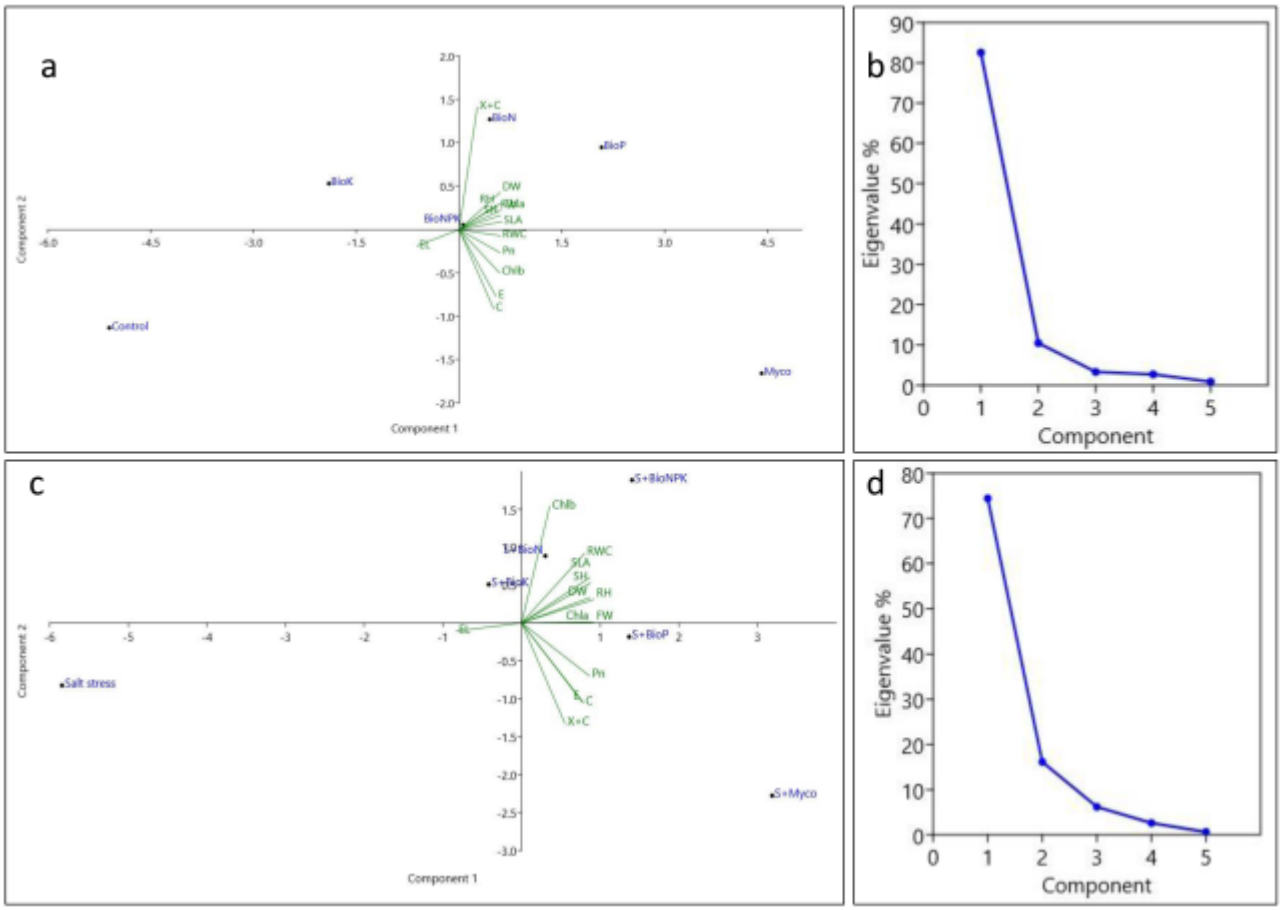

Figure 4. Results of PCA analysis for salinity treatments and biofertilizer amendments on various morpho-physiological parameters. a) and c) biplot representing all parameters as loading vectors and biofertilizer amendments as principal component scores under non-salinity and salinity, respectively. b) and d) scree plot representing the percentage relative Eigenvalue of each component under non-salinity and salinity doses, respectively. SH= Shoot height, RH= Root height, FW= Fresh weight, DW= Dry weight, SLA= Specific leaf area, EL= Electrolyte leakage, RWC= Relative water content, Pn= Photosynthetic rate, E= Transpiration rate, C= Stomatal conductance, chl-a= Chlorophyll-a, chl-b= Chlorophyll-b, X+C = Xanthophylls and Carotenoids

2018). Another response of plants to salt stress was reduced plant weight and leaf area, which was also observed in our results due to decreased cell suspension (Munns and Tester 2008, Zhang and Shi 2013). Several reports have indicated a significant decline in the growth and yield of the other crop plants similar to our results due to the toxicity caused by salt stress (Francois et al. 1994). Yadav et al. (2013) indicated enhanced growth attributes like shoot height, leaf area, and plant biomass under mycorrhizal inoculation in *Glycyrrhiza glabra*, a potential medicinal plant as AMF induces phosphorus solubilization and uptake, which results in the proper functioning of various physiological processes. AMF augments plant growth under salinity stress by increasing nutrient uptake, maintaining ion homeostasis, accumulation of osmolytes, and increasing photosynthetic rate and antioxidant activities (Dastogeer et al. 2020). Research on sweet basil found improved growth and morphology under mycorrhizal inoculations with salt stress as mycorrhizal strains are effective in enhancing nutrient and water uptake by the plants (Zuccarini and Okurowska 2008), which is in line with our findings. Mycorrhizal strain augments overall plant growth and ameliorates salinity stress by maintaining the osmotic balance of the plant, phytohormone production, and limiting the ROS content in plants by enhancing the antioxidant content during stress conditions (Khalloufi et al.

2017).

Salinity stress augmented the electrolytic leakage, and hence, a significant increment in EL was observed in our results under salinity stress in *C. roseus* leaves, thus, damaging membrane stability. Several researchers have reported a similar increase in electrolyte leakage during salt stress in the case of cucumber, lettuce, spinach, and common purslane (Hnilièková et al. 2019, Alsuvaid and Demir 2022). Results of the present study indicated a significant reduction in photosynthetic pigments like chl-a and chl-b during salt stress due to pigment degradation during salinity stress. It has been observed that sodium ion toxicity causes a reduction in the formation of these pigments by disrupting the enzymatic machinery required for their production with reduced leaf area, leading to a decline in the photosynthetic rate of the plant under salinity stress (Cantabella et al. 2017, Abdel Latef et al. 2020). A significant decline in various gas exchange parameters like Pn, C, and E were also observed under salinity stress. Salinity stress causes the closure of stomata and thus limits gas exchange in leaves which hampers the photosynthetic system in plants. Sodium ions accumulate in the cell's cytoplasm, disrupting the photosynthetic electron transport activities during salt stress, which further reduces the photosynthesis process (Sudhir and Murthy 2004). These findings are similar to previous studies on rice, eggplant, and ryegrass (Özdemir et al. 2004, Ding et al. 2012, Wu et al. 2017). Our results suggest that mycorrhizal strain improves chlorophyll concentration under salt stress in *C. roseus*. Our result agrees with similar studies on maize plants (Sheng et al. 2008). Wang et al. (2022) have indicated that mycorrhizal inoculations effectively improved the photosynthetic ability of plants by increasing stomatal conductance and photosynthetic rate under salt stress in agreement with our results. Mycorrhizae are known to improve water content in plants, thus, induce higher water use efficacy (Elhindi et al. 2017). This indirectly improves photosystem II's efficiency and other gas exchange parameters, increasing plants' photosynthetic rate (Colla et al. 2007). Mycorrhizal inoculation enhanced the chlorophyll pigment content by increasing magnesium uptake under salinity stress in *Sesbania sesban,* an important medicinal plant of the Fabaceae family (Abd Allah et al. 2015).

CONCLUSION

The major limitation in the commercial cultivation of *C. roseus* is the soil salinity stress which drastically declines its growth and productivity. The results of the present study demonstrated the crucial role of PGPB and mycorrhizal consortium in the augmentation of plant growth and biomass under salinity stress conditions. From this investigation, we concluded that mycorrhizal consortium and BioP proved to be the most effective among all other inoculations in ameliorating the detrimental effects of salinity stress. Salinity stress significantly declined plant growth, biomass, photosynthetic pigments, gas exchange parameters, and increased electrolyte leakage. Our results further confirmed the potential role of PGPB and mycorrhizal consortium in inducing salt tolerance to the plant by various mechanisms like increasing net photosynthetic rate, relative water content, and nutrient uptake, thus, enhancing the overall yield of the plant in a cost-effective, sustainable and eco-friendly manner in *C. roseus*. These results provided an important basis for biofertilizer inoculations in other medicinal plants suffering from salinity stress. Further research is required to test these biofertilizer strains in the field-growing areas to ascertain their efficacy. Their application is beneficial in providing salt stress tolerance to the plant and increasing overall growth and productivity.

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