Current Research in Microbial Sciences 7 (2024) 100299

Contents lists available at ScienceDirect

Current Research in Microbial Sciences

journal homepage: www.sciencedirect.com/journal/current-research-in-microbial-sciences

Paddy seeds bacterization with ACC deaminase producing endophyte *Alcaligenes faecalis* SSP8 regulates physiology, leaves gas exchange parameters, PSII photochemistry and antioxidant enzymes metabolism in NaCl stressed seedlings

Shobhit Raj Vimal [a], Jay Shankar Singh [b], Sheo Mohan Prasad [a,*]

[a] Ranjan Plant Physiology & Biochemistry Laboratory, Department of Botany, University of Allahabad, Prayagraj, 221102, India
[b] Department of Environmental Microbiology, Babasaheb Bhimrao Ambedkar University, Lucknow, 226025, India

ARTICLE INFO

Keywords:
Endophytes
Climate change
NaCl stress
Paddy seedlings
Stress agriculture

ABSTRACT

The endophytic microbes play crucial roles to crop development under stress environmental conditions. In this research, 36 endophytic bacterial strains having diverse morphology were isolated from exotic wild plant *Croton bonplandianus*. The strain SSP8 was selected for experimental study as it efficiently tolerate NaCl (0–1200 mM), produced Indole-3-acetic acid (IAA) (46 μg mL^{-1}) and 1-amino-1-cyclopropane-1-carboxylate (ACC) deaminase (176.70 nmol α-ketobutyrate mg^{-1} protein h^{-1}). The SSP8 was identified as *Alcaligenes faecalis* with 16 S r-RNA gene sequencing and submitted to NCBI-USA with accession number OR225818. The *A. faecalis* SSP8 significantly enhanced paddy seeds germination percentage, seedlings vigour index and vegetative growth parameters under different NaCl (0–180 mM) regimes. The paddy seedlings chlorophyll contents, *Chl-a* fluorescence transient (PSII photochemistry), leaves gas exchange parameters were significantly enhanced in *A. faecalis* SSP8+NaCl (0–180 mM) conditions. The oxidative stress biomarkers and antioxidant enzymes activities were significantly declined in *A. faecalis* SSP8+NaCl (0–180 mM) treated seedlings. In conclusion, based on the above results the paddy seeds bacterization with *A. faecalis* SSP8 could be a bio-prospective tool to alleviate the NaCl stress and enhance the paddy crop agriculture productivity in salt affected marginal soils.

1. Introduction

The soil salinization is exacerbating abiotic soil stress situations that crop plants faces during vegetative growth and flowering stages (Vimal et al., 2024). Among the abiotic stresses, sodium chloride (NaCl) induced soil salinity stress negatively influenced productivity and quality of crops and thereby affecting the food security at worldwide (Hasanuzzaman et al., 2021; Hualpa-Ramirez et al., 2024). Soil salinity affects over 833 million hectares of soils and 10% of cropland worldwide (FAO, 2022). About, 1.5 million hectares of agricultural lands worldwide are affected with the soil salinity problems (FAO, 2017). Soil salinization refers to higher accumulation of water-soluble salts in soils, typically due to various factors such as irrigation practices, poor drainage, arid climates and presence of naturally occurring salt deposits (Hualpa-Ramirez et al., 2024). The salts actively involved in salinization as NaCl composed of Na^+ and Cl^- ions (Vimal et al., 2019; Alhaddad et al., 2024). The NaCl stress has both osmotic (cell dehydration) and toxic (ion accumulation) impacts on plants (Joshi et al., 2022). Excessive soil salinity disturbed soil structure, deteriorated soil fertility, raises soil pH and electrical conductivity (EC), declined soil aeration, water infiltration, leading to soil compactness, etc. (Gill and Tuteja, 2010). Na^+ ions in the soil lead to nutritional imbalance, metabolic problems, and antioxidant enzymatic regulation in plant systems (Ray et al., 2016b; Vimal and Singh, 2020). Through the inhibition of nitrate reductase (NR) function, the uptake and accumulation of greater soil Cl^- disturbs photosynthetic phenomenon (Gill and Tuteja, 2010; Joshi et al., 2022). Higher concentrations of soil Na^+ and Cl^- in the rhizosphere causes competitive inhibition with other important soil plant nutritional ions such as K^+, NO_3^-, $H_2PO_4^-$, etc. for protein transporters binding sites (Hasanuzzaman et al., 2021). The excessive soil Na^+ and Cl^- ions may affects transport proteins in root cells, which in turn affect translocation, deposition and partitioning inside the plant (Joshi et al., 2022; Vimal

* Corresponding author.
E-mail addresses: shobhitraj1512@gmail.com (S.R. Vimal), jayshankar_1@yahoo.co.in (J.S. Singh), profsmprasad@gmail.com (S.M. Prasad).

https://doi.org/10.1016/j.crmicr.2024.100299

Available online 4 November 2024

et al., 2023a). The NaCl stress significantly affects paddy plant cytoplasm equilibrium, ROS production activity, plant growth, ion homeostasis, photosynthesis, PSII photochemistry and mineral availability (Raju and Prasad, 2021; Oviya et al., 2023).

Plants utilize their own self-defence systems against the oxidative damages due to higher NaCl stress (Alhaddad et al., 2024). Among the NaCl stress defensive strategies, the synthesis of antioxidants, antioxidant enzymes regulations, accumulation of compatible osmolytes, modulation of phytohormones, Na^+/Cl^-ions homeostasis may significantly contribute to manage the stress induced toxicity (Gill and Tuteja, 2010; Hualpa-Ramirez et al., 2024). Enzymatic and non-enzymatic components including catalase (CAT), superoxide dismutase (SOD), peroxidase (POX), glutathione reductase (GR) and non-enzymatic antioxidants such as ascorbic acid, tocopherol, carotenoids, etc. can significantly eliminated stress-induced ROS and protect plant cells against the oxidative injuries (Ray et al., 2016 a; b; Vimal et al., 2019). Under NaCl stress affected plant photosynthetic pigments machinery drastically decline photosynthesis and respiration rates, leaves gas exchange parameters as well as plant PSII photochemistry (Vimal et al., 2023a).The researchers are suggesting that more details investigations on understanding the significance of different radicals/signaling molecules ($O_2^{\bullet-}$, H_2O_2, NO, H_2S, etc.) in plants are still required to withstand against different abiotic and biotic stresses due to soil salinity (Taibi et al., 2016; Hasanuzzaman et al., 2021).

Ethylene, a gaseous plant hormone, influence a wide range of plant growth and developmental activities such as seed and root hair germination, elongation, leaves and petals abscission, fruit development and ripening, senescence, etc. (Glick, 2014; Segura and Molina, 2022; Vimal et al., 2023b). An elevated ethylene level may cause a negative effect on plant development as well as activation of stress associated genes expressions (Choudhury et al., 2023). The ethylene level in plants is directly correlated with endogenous ACC (1-amino-cyclopropane-1-carboxylic acid) levels too (Vimal et al., 2023b). The NaCl stress induced production of ethylene is denoted as "stress ethylene" and led to multiple negative impact on paddy plant health in different studies (Vimal and Singh, 2020; Vimal et al., 2023b). The plant growth promontory microorganisms (bacteria and fungi) having ACC deaminase activities significantly decline ethylene level and indicates a stress resistance under various environmental adverse situations including NaCl induced stress (Vimal et al., 2019; Segura and Molina, 2022). More recent studies are required to find out the potential of ACC deaminase producing microbes in management of NaCl mediated abiotic stress in different crops.

Currently the beneficial microbial services offer the most viable and sustainable way to increase the agricultural yields without compromising the soil fertility (Singh et al., 2016; Vimal et al. 2017; Rai et al., 2023). The ability of plant-endophytic microbes to produce plant growth and stress regulators, signalling and bio-active molecules in welfare of agricultural has drawn a lot of attention in recent years (Ray et al., 2016b; Mastan et al., 2020; Jia et al., 2022; Vimal et al., 2023a; Bushra et al., 2024). The plant-growth-promoting endophytic bacteria (PGPEB), residing inside plant tissues and assist in plant growth and development both directly and indirectly via various mechanisms (Oviya et al., 2023). Endophytic bacteria lives in less competitive zones compared to the exophytic or rhizospheric microorganisms, forming a complementary connection with the host plant without exhibiting any deleterious impacts (Alhaddad et al., 2024; Vimal et al., 2024). The endophytes inside the plants are actively involved in the syntheses of multiple stress regulating phytohormones, osmo-protectants, minerals, bio-active compounds and can contributes an induce systemic resistance to plants against various stress environmental conditions (Ray et al., 2016a; b; Choudhury et al., 2023). Therefore, it is assumed that beneficial efficient bacterial endophytes may offers a viable tool to improve the paddy crop plant health and growth as a nature-based solution (NbS) to the soil salinity stress agro-ecosystem. However, the experimental evidences related to above said research assumptions are still in incipient stage. Thus, the present study was conducted to isolate and screen the potential endophytic bacterial strains from naturally growing plants under soil salinity. The new salinity tolerant bacterial endophytic strains can be exploited to develop effective and viable bio-inoculants to enhance the paddy crop production in soil salinity affected areas. Addition of natural ACC deaminase producing NaCl tolerant endophytic bacteria isolated from indigenous soil salinity stressed plants to the paddy seedlings may offers a promising option for next generation sustainable paddy agriculture. The most efficient salinity tolerant endophytic isolate would be selected on the basis of ACC deaminase, IAA production activity and would be identified with 16 S r-RNA gene sequencing methods. There are scanty reports on application of bacterial endophytes to reduce salinity stress, especially on paddy crops. This study will be conducted to examine the efficacy of isolated saline tolerant bacterial endophytes on paddy seedlings under different NaCl (0–180 mM) stresses. The rice paddy seeds would be treated with the isolated saline tolerant bacterial endophytes to examined its impact on paddy seed germination percentage, seedlings vigour index, growth promotion, chlorophyll contents, leaves gas exchange parameters, PSII photochemistry, oxidative stress biomarkers and antioxidant enzymes metabolism.

2. Materials and methods

2.1. Sampling site and isolation of salt tolerant endophytic bacteria

The exotic *Croton bonplandianum* (Euphorbiaceae) plant samples were collected and reported in our recent finding (Vimal et al., 2023a). The bacterial endophytes were isolated from stem part of *C. bonplandianus* plants for this study. For surface sterilization, plant stems were washed in running tap water and placed in 2% NaClO solution for 10 min and washed four times with 0.02 M sterile potassium phosphate buffer (pH 7.0). Each sterile tissue was homogenized in sterile cool (4 °C) mortar and pestle with sterile MiliQ water. 100 μL of homogenate was taken and serial dilution was made up to 10^{-3}. Each dilution (50 μL) was plated on Nutrient agar medium (g L^{-1}; Peptone (5), NaCl (5), HM peptone B (1.5), yeast extract (1.5), agar (15) at pH (7.2 ± 0.2); Pikovaskaya medium (g L^{-1}; Dextrose (10), $Ca_3(PO_4)_2$ (5), $(NH_4)_2SO_4$ (0.5), NaCl (0.2), $MgSO_4{\cdot}7H_2O$ (0.1), KCl (0.2), yeast extract (0.5), $MnSO_4.H_2O$ (0.002), $FeSO_4{\cdot}7H_2O$ (0.002), agar (15) at pH (7.2 ± 0.2) and King's B medium (g L^{-1}; Protease peptone (20), K_2HPO_4 (1.5), $MgSO_4{\cdot}7H_2O$ (1.5), agar (15) and pH (7.2 ± 0.2) with three replications and incubated for 24–96 h at (28±2 °C). The pure colonies with different morphology were purified with streak plate method and stored at 4 °C for study.

2.2. Auxin indole-3-acetic acid (IAA) production activity of isolates

Indole-3-acetic acid (IAA) production activity was determined according to Vimal et al. (2019). Pure bacterial endophyte strains were inoculated in Nutrient Broth (g L^{-1}; Peptone (5), NaCl (5), HM peptone B (1.5), yeast extract (1.5), agar (15)) at pH (7.2 ± 0.2) and incubated at 28 ± 2 °C for 72 h After 72 h, 2 mL of each culture was pelleted by centrifugation (6000 rpm) and the supernatant was discarded. Cell pellets were washed with 1 mL of phosphate buffer saline (PBS) and re-suspended. After this, about 1 mL of supernatant was transferred to a fresh tube in which 100 μg mL^{-1} of 10 mM ortho-phosphoric acid and 2 mL of Salkowski's reagent were added, and incubated for 30 min in dark at room temperature. Development of pink colour indicated IAA production and quantified at 530 nm using spectrophotometer and calculated by comparing with the standard curve prepared with crude IAA.

2.3. 1-amino-1-cyclopropane-1-carboxylate (ACC) deaminase production activity of isolates

2.3.1. Primary screening

Each of the isolated endophyte strain was inoculated on to the petri plates containing Dworkin and Foster minimal salt medium (g L^{-1}; KH_2PO_4 (4), Na_2HPO_4 (6), $MgSO_4{\cdot}7H_2O$ (0.2), glucose (2), gluconic acid (2), citric acid (2), agar (15) and trace elements mg L^{-1} [$FeSO_4{\cdot}7H_2O$ (1), H_3BO_3 (10), $MnSO_4.H_2O$ (11.19), $ZnSO_4{\cdot}7H_2O$ (124.6), $CuSO_4{\cdot}5H_2O$ (78.22), MoO_3 (10), $FeSO_4{\cdot}7H_2O$ (1000)] at pH 7.2) supplemented with 3 mM ACC as the sole nitrogen source (Dworkin and Foster, 1958). The inoculated plates were incubated at 28±2 °C for 48–98 h The isolates colony appearance on medium found positive for the utilization of ACC and selected for quantitative study.

2.3.2. Secondary screening

Quantitative measurement of ACC deaminase activity of endophytic isolates was carried out according to Honma and Shimomura (1978) with some modifications. The technique was employed to measure the amount of α-ketobutyrate produced when the enzyme ACC deaminase cleaves ACC. Each of the endophyte which was positive for ACC utilization on DF plates was inoculated in DF salt minimal broth medium supplemented with ACC (3 mM) and incubated at 28±2 °C for 48 h in a rotary shaker at 150 rpm. After incubation, sample was centrifuged at 10,000 rpm for 10 min. The collected supernatant was used to quantify the amount of α-ketobutyrate produced by ACC positive endophytic isolates by comparing the absorbance measured at 540 nm with the standard curve of α-ketobutyrate. The ACC deaminase activity was expressed as nmol of α-ketobutyrate mg^{-1} protein h^{-1}.

2.4. Molecular characterization of endophytic bacterial isolates

The potent salt tolerant endophytic bacteria strain was identified by 16 s r-RNA gene sequencing technique. Total genomic-DNA of strain SSP8 was isolated from the log phase culture. The isolated DNA quality was measured with Nano-Drop at 260/280. Total 131 ng of extracted DNA was used for amplification along with 10pM of each primer. The primers 27F (5′ GGA TGA GCC CGC GGC CTA 3′) and 1492R (3′ CGG TGT GTA CAA GGC CCGG 5′) was used in the partial amplification of 16 s r-RNA encoding genes (Sun et al., 2008). Amplification was performed with reaction mixture (Template DNA 1 μL, 2 μL each F and R primer, 4 μL dNTPs (2.5 mM), 10 μL Taq polymerase assay buffer (10X), 1 μL Taq polymerase enzyme (3 U mL^{-1}), water 30 μL and prepare final reaction volume of 50 μL (Vimal et al., 2023a). The PCR conditions for 30 cycles completed in multiple steps as initial denaturation 96 °C (3 min), denaturation at 94 °C (1 min), annealing at 50 °C (1 min) and primer extension at 72 °C (2 min) followed by a final extension at 72 °C (7 min) and hold at 4 °C with thermocycler (Applied Biosystems, USA). Aliquots of the PCR products were analysed in 1.5% (w/v) agarose gel in horizontal gel electrophoresis. The amplified product was purified and sequenced in *ABI 3130xl* Genetic Analyser platform (Applied Biosystems, USA).

2.5. Sequence analyses, submission and phylogenetic tree preparation

The generated sequence was aligned with Clustal Omega Software (EMBL-EBI UK) and CHIMERA was removed with UCSF Chimera 1.1951 and run in nucleotide BLAST (BLASTN) (http://www.ncbi.nlm.ni-h.gov/BLAST) for similarity percentage with other available sequences in database of National Centre of Biotechnology Information (NCBI)-USA. The nucleotide sequence data has been deposited in Gene bank NCBI-USA and accession number was generated.

2.6. Application of isolated salt tolerant A. faecalis SSP8 on paddy plant

2.6.1. Paddy seeds procurement and surface sterilization

For the present study, the paddy crop cultivar (*Oryza sativa* L.) HUR 917 was procured from Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, South Campus (Barkachha), Mirzapur, India. Seeds were neatly washed with demineralized water and treated with 1% NaClO solution for 10 min for surface sterilization.

2.6.2. Development of endophyte free paddy seeds

The endophyte free paddy seeds were developed according to our recent report Vimal et al. (2023a).

2.6.3. Inoculum preparation and paddy seeds treatments

The endophyte SSP8 based inocula was developed according to method of Vimal et al. (2019) with some modifications. The cell counts in inocula was adjusted to 10^9 CFU/mL. The 50 mL of bacterial suspension containing 9×10^8 CFU/mL was mixed with sterile egg cell powder, aseptically. The 4 g/Kg of carboxy methyl cellulose (CMC) powder was used as sticker (Nandkumar et al., 2003). Cell count was maintained at 3×10^8 CFU/mL at time of seed treatments.

2.7. Experimental design

2.7.1. Experiment 1: seed germination and seedlings development

The untreated (control) and treated (*A. faecalis* SSP8) paddy seeds were allowed to germinate in petri dish containing sterilized cotton moistened with DDW at 28±2 °C for 72 h in darkness. The uniformly germinated seeds having equal cotyledon size were sown in sterilized sand containing plastic trays. The seedlings were dark-adapted for 48 h at 28±2 °C. After 48 h, seedlings were transferred in a growth chamber under photon flux density of 300 μmoL photons m^{-2} s^{-1}, 16:8 h light-dark period and relative humidity was maintained 65–70 % at 28 ± 2 °C for 15 days. Seedlings develop till two-leaves stage and were irrigated alternately with sterilized DW and half-strength Hoagland's nutrient solution with different (0, 60, 120, 180 mM) NaCl doses (Hoagland and Arnon, 1950). The seeds germination percentage and seedling vigour index were measured after 15 days of incubation at 28±2 °C.

2.7.2. Experiment 2: seedlings assay and stress conditions

On completion of 15 days, paddy seedlings were uprooted gently and roots were washed with sterile DDW to remove the adhered sand particles and then acclimatized in 50% Hoagland solution supplemented with respective NaCl doses for 24 h at 28±2 °C. For hydroponics experiments, bacterial culture was harvested by centrifugation (8000 × *g* for 10 min, 10 °C), washed once with 0.85% NaCl and re-suspended in 0.01 M $MgSO_4$ (~10^5 C.F.U. mL^{-1}). For control, 0.6 mL of 0.01 M $MgSO_4$ solution was added into the half-strength Hoagland nutrient solution. For treatments, 0.6 mL of bacterial suspension was mixed in 29 mL of half-strength Hoagland nutrient solution with respective NaCl (0, 60, 120, 180 mM) concentrations. After that, three healthy and uniform size seedlings were transferred in black film wrapped glass pot for hydroponics study. The nutrient medium was aerated with sterile air to avoid root anoxia. There were three replicates for each treatment and experiments were conducted three times to examine results reproducibility. After 15 days of treatments, seedlings from each pot were harvested to measure different physiological and biochemical parameters.

2.8. Effect of isolate A. faecalis SSP8 and NaCl on paddy seeds germination, seedlings physiology & biochemistry

2.8.1. Evaluation of seeds treatments on seeds germination percentage and seedlings vigour index

The control and *A. faecalis* SSP8 treated seeds were plated equidistantly on different petri plates containing sterilized moistened

absorbent cotton (three layers) to examine seeds germination percentage. The germinated seeds sown in sterilized sand were used for seedling vigour index before hydroponics Experiment II. Percent seed germination was count after 6 days and, mean root and shoot lengths for seedlings vigour were measured and calculated after 15 days of incubation.

$$\text{Germination (\%)} = \frac{\text{Numberofseedsgerminated}}{\text{TotalNumberofseedsplated}} \times 100 \quad (1)$$

$$\text{VigorIndex[VI]} = [(\text{MeanRootLength} + \text{MeanShootLength}) \times \text{Germination\%}] \quad (2)$$

2.8.2. Measurement of seedlings growth

The fresh weight of treated and untreated samples was recorded using a single pan digital balance (Model CA 223, Contech, India). The shoot and root length were measured using meter scale. Seedlings were further oven dried at 65 °C for 72 h for the dry weight measurement.

2.8.3. Effect of A. faecalis SSP8 and NaCl on chlorophyll contents

Chlorophyll a (*Chl* a), chlorophyll *b* (*Chl* b), *Chl* a/b ratio, total chlorophyll (*Chl a + b*) and carotenoids were examined spectrophotometrically with modified methods of Wellburn (1994). Fully expanded leaves 0.5 (g) samples were dipped overnight (12 h) in 85% acetone for the extraction of chlorophyll pigments. The supernatant taken was centrifuged (6000 rpm, 5 min) at 4 °C (Remi CPR-30 Plus) and diluted with the same concentration of acetone for spectrometric measurements. The pigment contents were calculated at absorbance at 452.5, 644, 663 nm alongside blank of untainted acetone (85%). *Chl* a, b and total chlorophyll and carotenoids were estimated according to Vimal et al. (2023a).

2.8.4. Estimation of oxidative stress biomarkers

Superoxide radical (SOR; $O_2^{\bullet-}$) activity was measured according to method of Elstner and Heupel (1976). NO_2^- formation was monitored from hydroxylamine in presence of $O_2^{\bullet-}$. The absorbance of the coloured aqueous phase was recorded at 530 nm. The content of $O_2^{\bullet-}$ was quantified from a standard curve prepared by graded solution of $NaNO_2$.

Hydrogen peroxide (H_2O_2) content was determined by the method of Velikova et al. (2000). 150 mg leaves sample were homogenized with 5 mL 0.1 % (w/v) tri-choloro-acetic acid (TCA) in ice bath. The homogenate was centrifuged (12,000 rpm, 15 min) and 0.5 mL of the supernatant was added to 0.5 mL 10 mM potassium phosphate buffer (pH 7.0) and 1 mL 1 M KI solution. The H_2O_2 concentration was estimated based on the absorbance of the reaction mixture at 390 nm using a standard curve of H_2O_2.

Malondialdehyde (MDA), a decomposition product from the peroxidation of polyunsaturated fatty acids, was estimated according to the method of Hodges et al. (1999). 500 mg fresh leaves sample were homogenized and extracted at 4 °C in 5 mL 0.05 M PBS buffer (pH 7.8). The extract and 2.5 mL thio-barbituric acid (TBA) were mixed and heated at 100 °C for 15 min, then quickly cooled in an ice bath. After centrifuging (4800 rpm, 10 min), absorbance was measured at 450, 532 and 600 nm respectively. MDA contents was calculated by using an extinction coefficient of 155 $mM^{-1}cm^{-1}$.

2.8.5. Determination of leaves gas exchange parameters

Photosynthetic rate (A), sub-stomatal CO_2 (Ci), stomatal conductance (gs) and transpiration rate (E) were recorded using Portable Photosynthesis System (Model LCi-SD, UK). All measurements were done under PAR of 1200 ± 5 µmol m^{-2} s^{-1}, relative humidity 65–70 °C, and atmospheric CO_2 of 290±2 ppm at 28±2 °C.

2.8.6. Measurement of Chl a fluorescence transient

Chlorophyll a fluorescence transient was studied in 30-min dark-adapted leaves using a hand-held leaf fluorometer (FluorPen FP 100, Photon System Instruments, Czech Republic) and different parameters pertaining to PSII performance. The following parameters of PS II photochemistry were calculated as per the given formulae of Strasser et al. (2000). Subsequently different parameters like yields or flux ratios; φP_0 (maximum quantum yield of primary photochemistry equivalent to the Fv/Fm); φE_0 (quantum yield of electron transport); Φ_0 (probability that a trapped exciton moves an electron into the electron transport chain beyond QA), and PIABS (performance index), specific energy fluxes per reaction center (RC) i.e. ABS/RC (electron transport flux/RC); TR_0/RC (trapped energy flux per RC); ET_0/RC (absorbance flux per RC), and DI_0/RC (dissipated energy flux per RC) were determined.

2.8.7. Antioxidant enzymes activities

CAT (EC 1.11.3.6) activity was measured in the terms of decrease in absorbance due to breakdown of H_2O_2 which was recorded spectrophotometrically at 240 nm and quantified by using the extinction coefficient 39.4 $mM^{-1}cm^{-1}$as given by Aebi (1984). One unit (U) of CAT activity is defined as 1 nmol of H_2O_2 dissociated min^{-1}.

SOD (EC 1.15.1.1) activity was assayed according to the method given by Giannopolitis and Reis (1977). The photochemical reduction of NBT (formation of purple formazan) was deter-mined spectrophotometrically at 560 nm and compared with the blank devoid of the enzyme extract. One unit (U) of SOD activity is determined as the amount of enzyme required to cause 50% inhibition in NBT reduction.

POX (EC 1.11.1.7) activity was assayed by monitoring the increase in absorbance due to the oxidation of guaiacol at 470 nm and calculated by using an extinction coefficient of 25.5 $mM^{-1}cm^{-1}$ as proposed by Zhang (1992). One unit (U) of POD activity is the amount of enzyme oxidising 1 nmol of guaiacol min^{-1}.

APX (EC 1.11.1.11) activity was assayed according to Nakano and Asada (1981). The reaction was initiated by addition of H_2O_2 and ascorbate oxidation measured at 290 nm for 3 min. Enzyme activity was quantified using the molar extinction coefficient for ascorbate (2.8 mM^{-1} cm^{-1}). One unit of APX is determined as 1 mM mL^{-1} ascorbate oxidized per min.

Protein concentration in paddy plant was determined according to Bradford (1976) using bovine serum albumin (BSA) as standard.

2.9. Statistical analyses

Results were statistically analysed by analysis of variance (ANOVA). There were three replicates ($n = 3$) for each independent experiment. Duncan's multiple range test was applied for mean separation for significant differences amongst treatments at $P < 0.05$ significance level. The relationship between paddy plant growth parameters, total chlorophyll contents, leaves gas exchange parameters, oxidative biomarkers and antioxidant enzymes activities were examined by Pearson's correlation (two-tailed) analysis. All statistical analyses were performed using SPSS (Version 20: IBM, Armonk, NY, USA).

3. Results

3.1. Isolation, PGP attributes and molecular characterization of endophytic bacterial isolates

Total, 36 endophytic bacterial strains with diverge morphology were isolated and purified from stem part of selected plant. Ten strains exhibited NaCl tolerance level of >1100 mM concentration. Eight strains efficiently produced IAA (>25 µg mL^{-1}) and among them three strains produced ACCD activity of 276.70, 255.52 and 125.50 nmol α-ketobutyrate mg^{-1} protein h^{-1}, respectively. One most efficient strain SSP8 produced IAA (46 µg mL^{-1}) and ACCD activity (176.70 nmol α-ketobutyrate mg^{-1} protein h^{-1}) and exhibited NaCl tolerance (0–1200 mM) was selected for further experimental study. Strain SSP8 was genomically identified as *Alcaligenes faecalis* with 16 S r-RNA gene sequencing and nucleotide sequence BLAST (BLASTn) in NCBI-USA.

Table 1
Effect of *A. faecalis* SSP8 and NaCl on seeds germination and seedlings vigour index. The values given are means of three independent experiments ± SE. $N = 24$ (8 treatments × 3 replicates).

Treatments	Ck	*Alcaligenes faecalis* SSP8	60 mM NaCl	60 mM NaCl + *A. faecalis* SSP	120 mM NaCl	120 mM NaCl + *A. faecalis* SSP8	180 mM NaCl	180 mM NaCl + *A. faecalis* SSP8	F-value	P-value
Germination %	86.6 ± 6.66^{ab}	96.6 ± 3.33^{b}	80.0 ± 5.55^{bc}	90.0 ± 5.55^{ab}	76.6 ± 3.33^{bc}	86.6 ± 3.33^{ab}	70.0 ± 1.00^{c}	83.3 ± 3.33^{abc}	*3.500*	*<0.10*
Vigour Index	937.55 ±52.82^{b}	1032.11 ±90.42^{a}	805.88 ±70.14b^{cd}	947.77±80.37^{bc}	666.00 ±18.78^{cd}	795.00±72.66^{bc}	612.22 ±52.22^{d}	838.85±41.47^{bc}	*6.321*	*<0.001*

Different letters indicate significant difference between treatments for each parameter ($P < 0.05$) according to DMRT.
Ck = Control (0 mM); F = ANOVA value; P = Significant level.

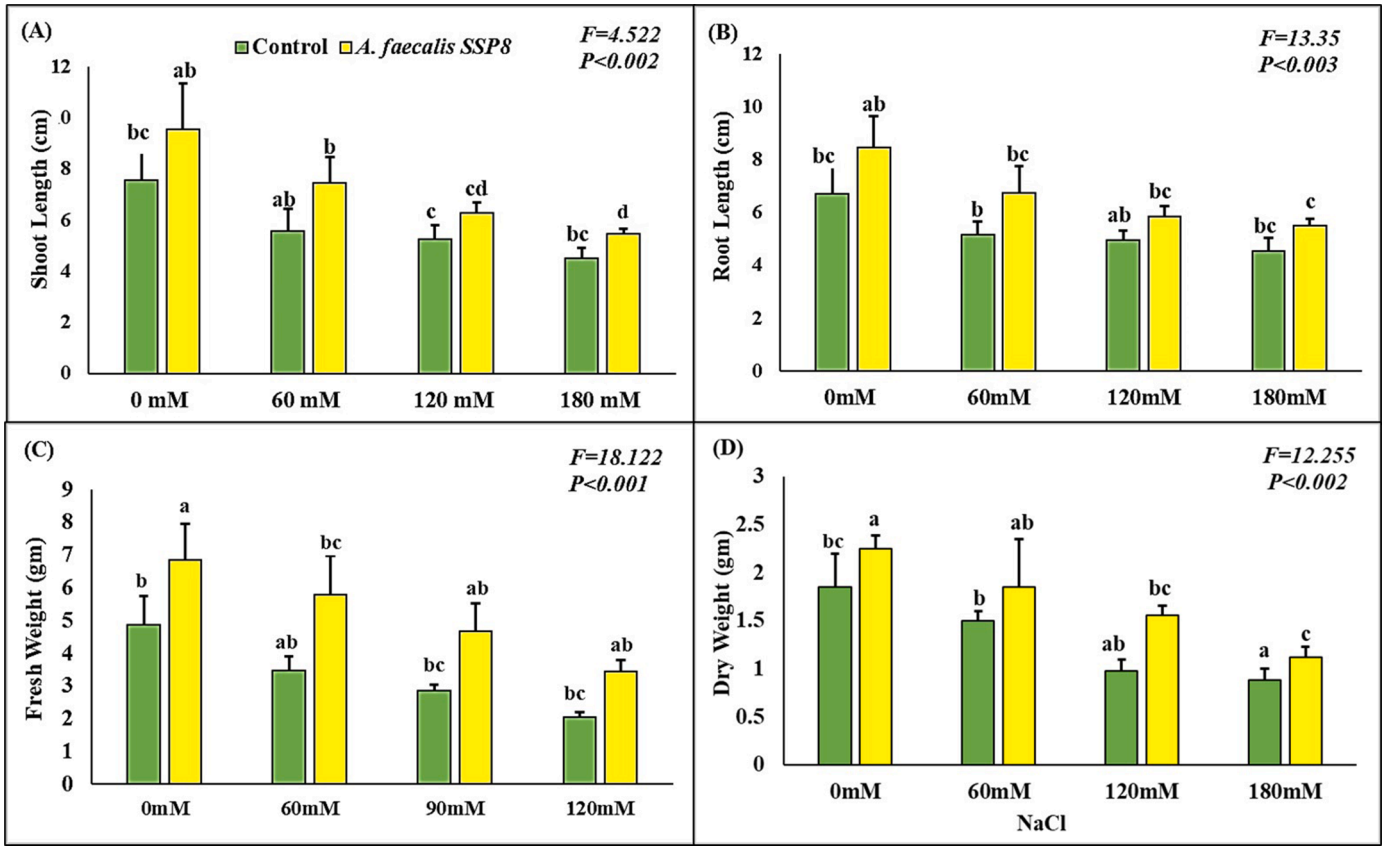

Fig. 1. Effect of *A. faecalis* SSP8 strain SSA4 and NaCl on paddy seedlings vegetative growth parameters (A) Shoot length (B) Root length (C) Fresh weight and (D) Dry weight exposed to NaCl (0–180 mM) stress. Results presented are means ± standard error of three replicates ($n = 3$). Different letters assigned to the bars testimony significant ($P < 0.05$) difference as per DMRT analysis of one-way ANOVA.

SSP8 exhibited closest relationship with other *A. faecalis* strains and shows similarity of 99.76%. The sequence has been submitted in Gene Bank NCBI-USA as *A. faecalis* SSP8 with accession number OR 225,818.

3.2. Effect of A. faecalis SSP8 and NaCl on seeds germination percentage and seedlings vigor index

The paddy seeds treatment results showed that the *A. faecalis* SSP8 significantly ($P < 0.005$) improved seeds germination percentage and vigour index compared to respective control (Table 1). NaCl treatments caused grater toxicity and declined seeds germination percentage (7.62%, 11.54%, 19.16%) as well as seedlings vigour index (14.04%, 28.96%, 53.13%) at (60, 120 and 180 mM) compared to control (0mM NaCl) seedlings. The *A. faecalis* SSP8 inoculation potentially enhanced seeds germination percentage (11.54%, 12.51%, 13.05% and 19.0%) and seedlings vigour index (10.08%, 17.60, 19.36% and 36.36%) at (0, 60, 120 and 180 mM + *A. faecalis* SSP8) NaCl doses, respectively.

3.3. Effect of A. faecalis SSP8 and NaCl on seedlings growth parameters and chlorophylls contents

The study indicated a significant ($P < 0.005$) improvement in all the vegetative plant growth parameters evaluated in *A. faecalis* SSP8 treated paddy seedlings over control plants irrespective of the different NaCl doses (Fig.1). Plant growth parameters as shoot length declined (26.09%, 30.46%, 40.13%), root length (23.36%, 26.33%, 32.29%), fresh weight (28.39%, 41.35%, 57.81%) and dry weight (18.91%, 34.05%, 52.43%) at (60 mM, 120 mM and 180 mM NaCl) treatments compared to control (0mM NaCl) seedlings. *A. faecalis* SSP8 in association of NaCl improved shoot length (26.49%, 25.44%, 19.61%, 13.93%), root length (36.16%, 31.06%, 18.18%, 15.38%), fresh weight (61.52%, 43.67%, 28.77%, 24.29%) and dry weight (48.64%, 33.34%, 27.86%, 25.00%) at (0, 60, 120 and 180 mM + *A. faecalis* SSP8) doses, respectively.

The chlorophyll contents in *A. faecalis* SSP8 treated and untreated paddy seedlings significantly decreased with enhancement of NaCl doses

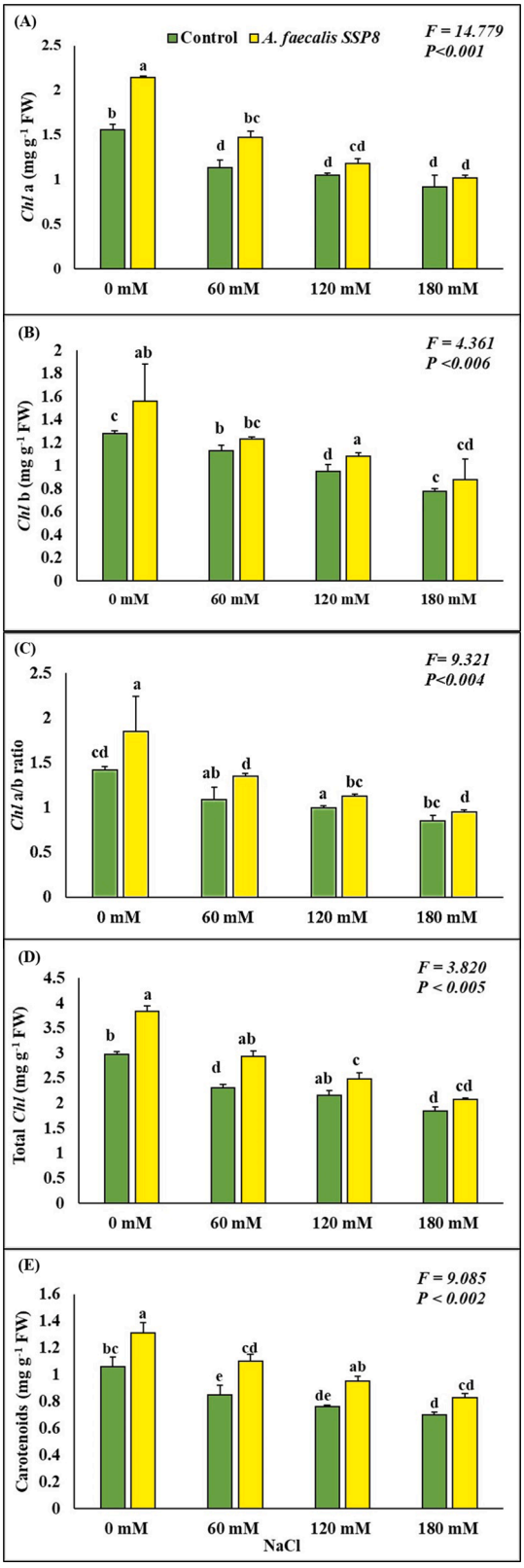

Fig. 2. Effect of *A. faecalis* SSP8 strain SSA4 and NaCl on paddy seedlings chlorophyll contents (A) Chl *a* (B) Chl *b* (C) Chl *a/b* ratio (D) Total Chl and (E) Carotenoids exposed to NaCl (0–180 mM) stress. Results presented are means ± standard error of three replicates ($n = 3$). Different letters assigned to the bars testimony significant ($P < 0.05$) difference as per DMRT analysis of one-way ANOVA.

Table 2
Effect of *A. faecalis* SSP8 and NaCl on leaf gas exchange parameters in paddy seedlings exposed to NaCl (0–180 mM) treatments. The values given are means of three independent experiments ± SE. $N = 24$ (8 treatments × 3 replicates).

Treatments	Photosynthetic rate (A) (μ mol CO_2 m^{-2} s^{-1})	Sub-stomatal CO_2 (Ci) (μmol CO_2 mol^{-1} air)	Stomatal conductance (gs) (m mol H_2O m^{-2} s^{-1})	Transpiration rate (E) (m mol H_2O m^{-2} s^{-1})
Ck	8.45±0.45^{a}	7.61 ±0.47^{ab}	0.25±0.012^{ab}	3.78±0.42^{ab}
Ck + *A. faecalis* SSP8	9.06±0.31^{a}	8.86 ±0.43^{b}	0.27±0.017^{b}	4.20±0.36^{b}
60 mM	5.84±0.45^{bc}	6.58 ±0.46^{bc}	0.17±0.014^{de}	2.85±0.15^{cd}
60 mM + *A. faecalis* SSP8	6.97±0.28^{b}	7.73 ±0.39^{a}	0.22±0.012^{cd}	3.68±0.20^{abc}
120 mM	5.45±0.58^{c}	5.05 ±0.09^{d}	0.16±0.008^{de}	2.26±0.29^{de}
120mM+ *A. faecalis* SSP8	6.18±0.62^{bc}	5.93 ±0.15^{cd}	0.19±0.018^{bc}	3.18±0.13^{bc}
180 mM	4.96±0.26^{c}	4.99 ±0.25^{d}	0.14±0.017^{e}	1.90±0.17^{e}
180 mM + *A. faecalis* SSP8	6.06±0.29^{bc}	5.68 ±0.31^{cd}	0.18 ±0.014^{cde}	2.88±0.17^{cd}
F value	*F = 11.400*	*F = 14.714*	*F = 9.648*	*F = 8.934*
P value	*P < 0.001*	*P < 0.002*	*P < 0.001*	*P < 0.002*

Different letters indicate significant difference between treatments for each parameter ($P < 0.05$) according to DMRT.
Ck = Control (0 mM); *F* = ANOVA value; *P* = Significant level.

in paddy seedlings (Fig.2). NaCl treatment declined Chl *a* (27.56%, 32.69%, 41.02%), Chl *b* (19.53%, 25.78%, 39.06%), Chl *a/b* ratio (23.23%, 29.57%, 40.14%), total *Chl* (22.48%, 27,51%, 38.25%), and carotenoid contents (19.81%, 28.30%, 33.96%) at (60, 120 and 180 mM) doses compared to control (0mM NaCl) seedlings. The *A. faecalis* SSP8 treated paddy seedlings significantly improved Chl *a* (37.17%, 30.08%, 12.38%, 10.86%), Chl *b* (21.87%, 19.41%, 13.68%, 12.82%), Chl *a/b* ratio (30.28%, 23.85%, 13.00%, 11.76%), total Chl (28.85%, 27.27%, 14.81%, 13.04%) and carotenoids (34.73%, 29.41%, 25.00% and 18.57%) under increased level of NaCl (0, 60, 120 and 180 mM + *A. faecalis* SSP8) treatments, respectively.

3.4. Effect of A. faecalis SSP8 and NaCl on leaves gas exchange parameters and PSII photochemistry

The paddy seedlings leaves gas exchange parameters were significantly declined in cumulative NaCl stress conditions (Table 2). The parameters as A (22.14%, 36.69%, 50.20%), Ci (13.53%, 33.63%, 34.42%), gs (26.08%, 34.78%, 39.13%) and E (24.40%, 40.21%, 49.73%) declined in NaCl (60, 120, 180 mM) treated seedlings compared to control (0mM NaCl) seedlings. In *A. faecalis* SSP8 inoculated seedlings exhibited a significant improvement in A (21.61%, 20.17%, 13.39%, 9.07%), Ci (19.18%, 18.23%, 17.42%, 13.82%), gs (34.78%, 29.41%, 26.66%, 21.42%) and E (11.11%, 29.12%, 40.70%, 51.57%) under increased level of NaCl (0, 60, 120 and 180 mM + *A. faecalis* SSP8) treatments, respectively.

The leaves PS II photochemistry includes JIP-test or *Chl* a fluorescence kinetics presented in Fig.3. All the value obtained for PSII were normalized with 1.0 and presented in radar graph format. Drastic changes were seen in the JIP test parameters for seedlings exposed to lower to higher NaCl stress. On different NaCl doses (60 mM, 120 mM and 180 mM), the value of fluorescence kinetics parameters as φP_0, φE_0, Φ_0 and PI_{ABS} of PSII was severely affected and declined and specific energy fluxes per reaction centre parameters as ABS/RC, TR_0/RC, ET_0/

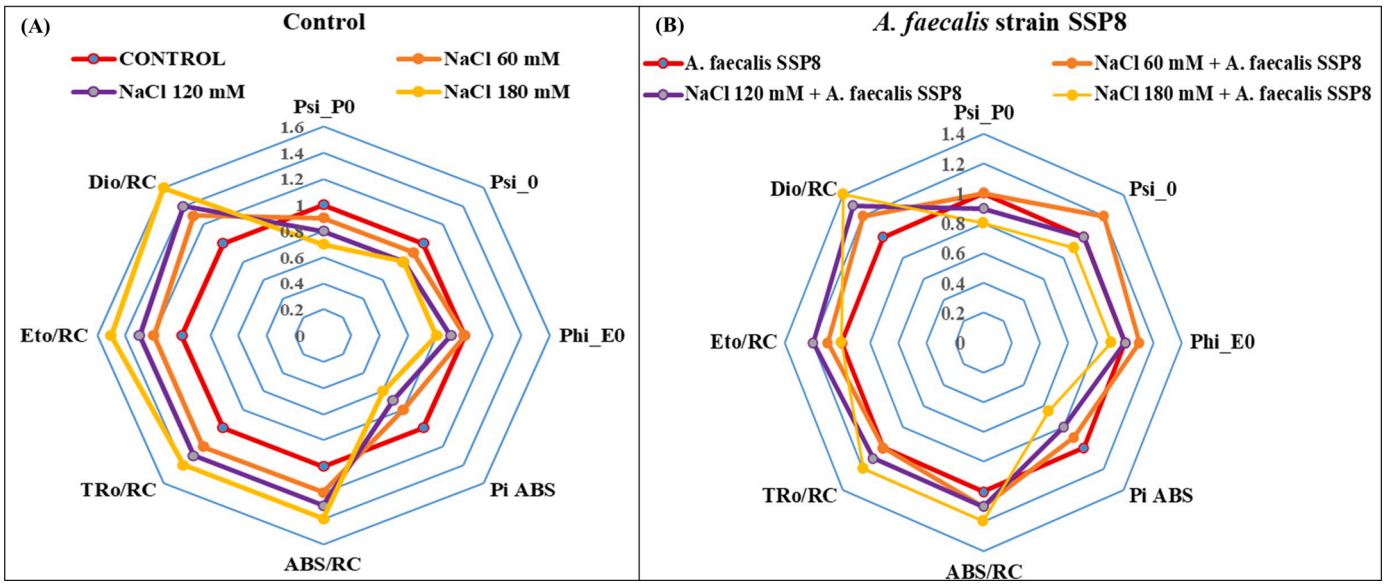

Fig. 3. Effect of *A. faecalis* SSP8 strain SSA4 and NaCl on paddy seedlings Chlorophyll a kinetics on flux ratios and specific energy fluxes (per reaction centre-RC) and performance index in (A) NaCl stress (0–180 mM) (B) *A. faecalis* SSP8 strain SSA4 exposed to different (0–180 mM) NaCl. Measurements were performed randomly on the second leaf of paddy seedlings repeated three times.

Table 3
Effect *A. faecalis* SSP8 and NaCl on oxidative stress biomarkers in paddy seedlings exposed to NaCl (0–180 mM) treatments. The values given are means of three independent experiments ± SE. $N = 24$ (8 treatments × 3 replicates).

Treatments	SOR (n mol g^{-1} FW)	H_2O_2 (n mol g^{-1} FW)	MDA (n mol g^{-1} FW)
Ck	177.86 ± 4.39^{d}	201.91 ± 4.76^{ef}	8.08 ± 1.78^{cd}
Ck + *A. faecalis* SSP8	155.87 ± 6.24^{e}	190.93 ± 3.22^{f}	6.53 ± 1.25^{c}
60 mM	226.18 ± 6.89^{b}	236.07 ± 6.92^{bc}	12.72 ± 1.86^{ab}
60 mM + *A. faecalis* SSP8	201.14 ± 7.50^{c}	207.40 ± 3.39^{def}	9.80 ± 1.64^{bcd}
120 mM	236.50 ± 5.05^{ab}	246.66 ± 6.60^{ab}	13.50 ± 1.59^{ab}
120 mM + *A. faecalis* SSP8	202.96 ± 3.61^{c}	212.89 ± 5.31^{de}	10.83 ± 1.48^{bc}
180 mM	242.30 ± 8.20^{a}	225.09 ± 7.61^{a}	15.56 ± 1.04^{a}
180 mM + *A. faecalis* SSP8	200.81 ± 2.74^{c}	222.49 ± 4.86^{cd}	12.55 ± 1.20^{ab}
F value	*F = 39.664*	*F = 16.417*	*F = 6.662*
P level	*P < 0.001*	*P < 0.001*	*P < 0.002*

Different letters indicate significant difference between treatments for each parameter ($P < 0.05$) according to DMRT.
Ck = Control; *F* = ANOVA value; *P* = Significant level.

RC and DI_0/RC were showing enhancement due to stress toxicity (Fig.3A). However, seedlings treated with *A. faecalis* SSP8 significantly enhanced fluorescence kinetics parameters as well as declined specific energy fluxes per reaction center parameters at NaCl (0–180 mM) toxicity (Fig.3B).

3.5. Effect of A. faecalis SSP8 and NaCl on oxidative stress biomarkers and antioxidants enzymes activities

The oxidative stress biomarkers exhibited a significant decline in *A. faecalis* SSP8 inoculated treatments irrespective of NaCl stress conditions (Table 3). The NaCl stressed paddy seedlings recorded an increase in (27.16%, 32.85%, 36.23%) SOR, (16.91%, 22.16%, 26.33%) in H_2O_2 and (48.77%, 57.89%, 81.98%) in MDA contents at 60 mM, 120 mM and 180 mM NaCl doses, compared to control (0mM NaCl) seedlings. The seedlings treated with *A. faecalis* SSP8 significantly declined SOR (6.74%, 11.07%, 14.18%, 17.12%), H_2O_2 (5.43%, 12.14%, 13.69%, 26.08%) and MDA (23.62%, 22.95%, 19.77%, 19.34%) at (0, 60, 120 and 180 mM NaCl + *A. faecalis* SSP8), compared to respective control seedlings.

The treatments results on paddy seedlings antioxidants enzymes activities are represented in Fig.4. The activity of CAT (48.37%, 135.34%, 168.98%), SOD (30.11%, 40.20%, 52.69%) and POX (21.22%, 25.61%, 34.54%) were significantly enhanced in NaCl treated seedlings (60, 120 and 180 mM) compared to control (0 mM NaCl) seedlings. The *A. faecalis* SSP8 inoculation significantly declined CAT (18.80%, 25.88%, 25.86%), SOD (13.74%, 16.16%, 19.88%) and POX (14.45%, 15.75%, 16.50%) under increased level of NaCl (60, 120 and 180 mM + *A. faecalis* SSP8) treatments, respectively. However, APX activity was enhanced (69.38%, 112%, 176.54%) at NaCl (60, 120 and 180 mM) treated seedlings compared to control (0 mM NaCl) seedlings. This APX activity was further enhanced by (15.18%, 14.55%, 10.48%) in *A. faecalis* SSP8 inoculated seedlings at NaCl (60, 120 and 180 mM + *A. faecalis* SSP8) treated seedlings compared to respective control seedlings.

4. Discussion

Microorganisms residing inside plant tissues often considered as endophytes provide beneficial support with multiple mechanisms to their host throughout lifetime (Choudhury et al., 2023; Vimal et al., 2024). Apart from inducing resistance against biotic stress, endophytes have also been found efficient in inducing resistance against abiotic stress (Ray et al., 2016 a; b). Endophytes are reported to produce ACC deaminase enzyme that modulates the ethylene level to an equilibrium which is optimum for growth promotion, thereby declining the saline stress toxicity and facilitate plant health development even under different environmental stress (Glick 2014; Segura and Molina, 2022). Paddy crop is staple food and cash crop highly susceptible for soil salinity and severely affect with NaCl toxicity around the globe (Vimal et al., 2019; FAO, 2022). Previous studies have discovered that the bacteria isolated from stress soils are more efficient at improving plant tolerance to stress environment than bacteria isolated from non-stress soils indicates the potential of indigenous microbiota (Alhaddad et al., 2024). Hence, the present study is conducted to examine potential of indigenous endophytic microbe *Alcaligenes faecalis* strain SSP8 isolated from naturally growing *C. bonplandianus* plants in paddy crop seedlings. Strain SSP8 tolerate (>1200 mM) NaCl and produces IAA (46 µg mL^{-1}) and ACC deaminase (176.70 nmol α-ketobutyrate mg^{-1} protein h^{-1}). Although *Alcaligenes* sp. has been reported to manages plant health under abiotic and biotic stress environments (Ray et al., 2016a; Jia et al.,

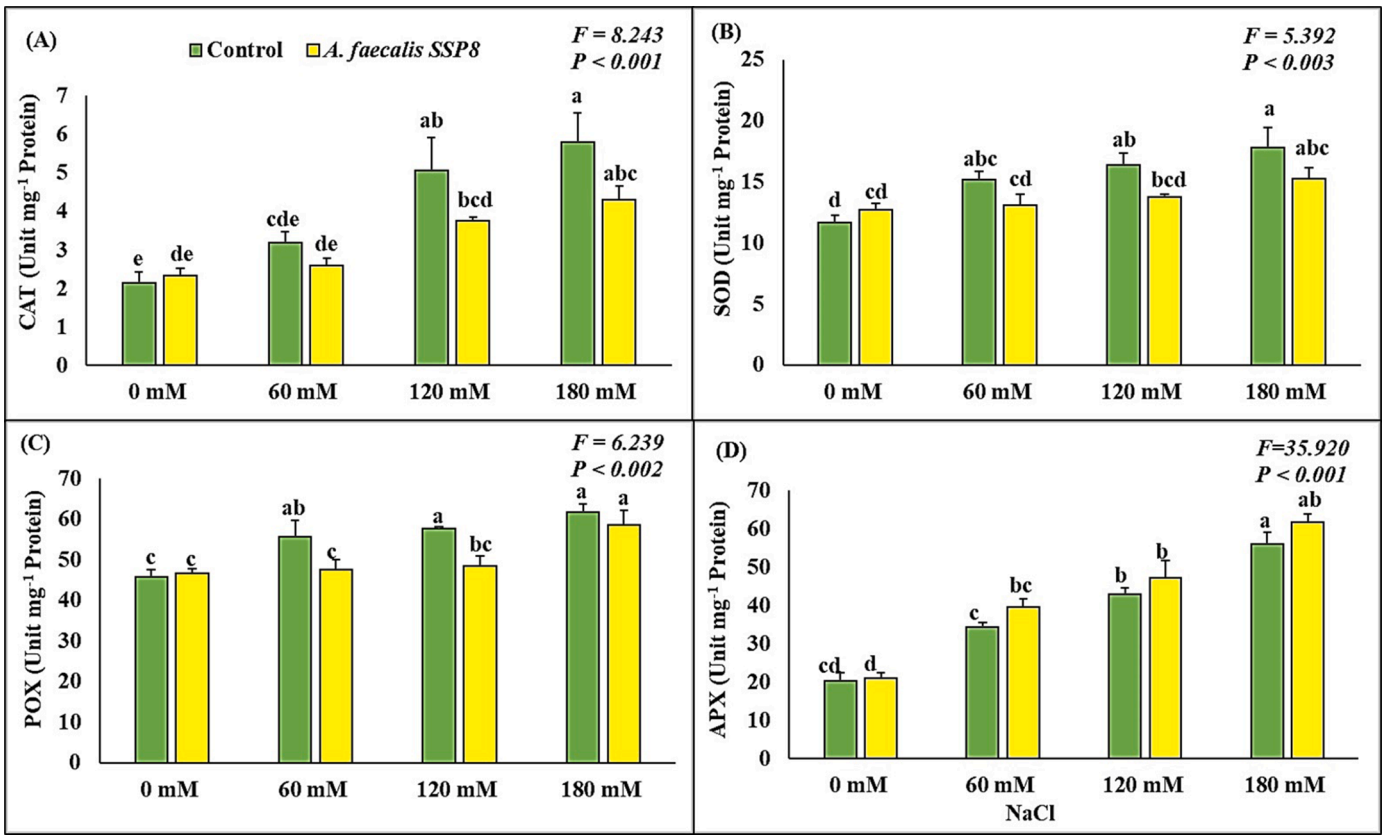

Fig. 4. Effect of *A. faecalis* SSP8 strain SSA4 and NaCl on paddy seedlings antioxidant enzymes parameters (A) CAT (B) SOD (C) POX and (D) APX exposed to NaCl (0–180 mM) stress. Results presented are means ± standard error of three replicates ($n = 3$). Different letters assigned to the bars testimony significant ($P < 0.05$) difference as per DMRT analysis of one-way ANOVA.

2022). However, *A. faecalis* play in paddy plant physiological and biochemical attributes, leaves gas exchange paraments, PSII photochemistry, oxidative biomarkers and antioxidants metabolism in NaCl stress environments in paddy seedlings have not been previously investigated.

NaCl stress significantly declined seed germination, plant physiological and morphological parameters beyond tolerable limit (Joshi et al., 2022; Choudhury et al., 2023). Paddy crop is highly sensitive to excessive NaCl concentration in different soils and irrigation water (Bal et al., 2013). In the present study, paddy seeds bacterized with *A. faecalis* strain SSP8 based bioinocula significantly enhanced seeds germination percentage at 5 days of interval and seedlings vigour index at 15 days of time under different NaCl doses ranges from 0 to 180 mM (Table 1). Paddy seedlings growth parameters as shoot length, root length, fresh weight and dry weight were significantly improved in *A. faecalis* strain SSP8 inoculated seedlings under NaCl stress compared to non-salinized control. NaCl tolerant endophyte *A. faecalis* strain SSP8 play pivotal role in paddy seedlings growth management in different regimes of NaCl toxicity. These results are linked with the findings of Vimal et al. (2019), reported ACC deaminase producing *Curtobacterium albidum* SRV4 in management of paddy crop health under NaCl stress. Similarly, ACC deaminase producing PGPR- *Bacillus amyloliquefaciens* enhanced seed germination and plant vigour index under abiotic stress in pearl millets (Murali et al., 2021). Mastan et al. (2020) isolated and reported plant probiotic native bacterial endophyte *Alcaligenes faecali* CFRB1 in enhancement of plant growth parameters, severity of nematode infection, root rot and metabolites *forskolin* production in medicinal herb *Coleus forskohlii* plant.

Chlorophyll pigments (*Chl* a; b; *Chl* a/b ratio; Total *Chl* and Carotenoids) plays a leading role in photosynthesis cycle and led to plant health and development (Oviya et al., 2023; Bushra et al., 2024). The declined in chlorophyll contents and leaf gas exchange parameters (A, Ci, gs and E) in paddy seedlings due to NaCl stress toxicity was in conformity to our previous research investigation (Vimal et al., 2023a). The rice paddy seeds treatments with *A. faecalis* SSP8 potentially restore chlorophyll contents to initiate leaf gas exchange parameters under NaCl stress toxicity (Table 2 and 3). Mastan et al. (2020) reported analogous findings for *Chl* contents with *A. faecalis* inoculation in *C. forskohlii*. The *A. faecalis* inoculation exhibited significant enhancement in *chl* contents and leaves gas exchange parameters in club root under biological stress (Jia et al., 2022). Our study is positively correlated with previous studies suggesting the potential of *A. faecalis* SSP8 in restoration of *Chl* pigments, *Chl* a florescence and leaf gas exchange parameters as photosynthetic rate, stomatal conductance, sub-stomatal conductance and transpiration rate management under NaCl stress toxicity.

Chlorophyll a fluorescence transient Photosystem II (PSII) photochemistry is the most sensitive photochemical processes interrupted by NaCl stress toxicity (Jia et al., 2022; Yang et al., 2023). The fluorescence kinetics parameters as Phi_P_0, Phi_E_0, Psi_$_0$ and PI_{ABS} of PSII was significantly declined in NaCl treated paddy seedling and find restored in *A. faecalis* + NaCl treated seedlings at different NaCl doses compared to respective control seedlings. The primary cause of fluorescence kinetics parameters declining values may be the slowing of the electron transport from PS II to PS I due to saline stress (Singh et al., 2015). The higher NaCl concentration led to induced photoinhibition on the donor side of the PSII complex as well as decline in size and number of active reaction centres (Taibi et al., 2016; Yang et al., 2023). Decline in number of active reaction centres further intensified the load on remaining reaction centres (Kondamudi et al., 2016). This load resulted in enhancement of energy flux per reaction centre parameters as ABS/RC, TR_0/RC, ET_0/RC and DI_0/RC (Raju and Prasad, 2021). However, paddy seedlings treated with *A. faecalis* SSP8 exhibited different PGP activities

Table 4
Pearson's correlation (2-tailed) between paddy seeds germination percentage, vigour index, seedlings Total *Chl* contents, leaves gas exchange parameters, oxidative stress biomarkers and antioxidants enzymes activities in *A. faecalis* SSP8 and NaCl (0–180 mM) treatments. $N = 24$ (8 treatments × 3 replicates).

Parameters	Germination %	Vigour Index	Total Chl ab	Carotenoid	A	Ci	gs	E	SOR	H_2O_2	MDA	CAT	SOD	POX	APX
Germination %	1.000														
Vigour Index	0.739**	1.000													
Total Chl *ab* (mg g^{-1} FW)	0.496*	0.561*	1.000												
Carotenoid (mg g^{-1} FW)	0.437*	0.323^{NS}	0.011^{NS}	1.000											
A (μ mol CO_2 m^{-2} s^{-1})	0.692**	0.773**	0.366^{NS}	0.389^{NS}	1.000										
Ci (μmol CO_2 mol^{-1} air)	0.676**	0.721**	0.422*	0.450*	0.824**	1.000									
gs (m mol H_2O m^{-2} s^{-1})	0.659**	0.668**	0.309^{NS}	0.499*	0.813**	0.723**	1.000								
E (m mol H_2O m^{-2} s^{-1})	0.751**	0.751**	0.450*	0.426*	0.805**	0.755**	0.807**	1.000							
SOR (n mol g^{-1} FW)	−0.713**	−0.821*	−0.407*	−0.527**	−0.864**	−0.812**	−0.838**	−0.817**	1.000						
H_2O_2 (n mol g^{-1} FW)	−0.804**	−0.787**	-0.384^{NS}	−0.588**	−0.794**	−0.795**	−0.832**	−817**	0.901*	1.000					
MDA (n mol g^{-1} FW)	−0.701**	−0.641**	−0.461**	-0.314^{NS}	−0.877**	−0.789**	−0.819**	−0.815**	0.809**	0.809**	1.000				
CAT (Unit mg^{-1} protein)	−0.491*	−623**	-0.282^{NS}	−0.410*	−0.704**	−0.762**	−0.632*	−0.660*	0.724*	0.782**	0.721**	1.000			
SOD (Unit mg^{-1} protein)	−0.578**	−0.570*	-0.174^{NS}	−0.383	−0.715*	−0.677**	−0.644**	−0.721*	0.780**	0.830**	0.777*	0.822**	1.000		
POX (Unit mg^{-1} protein)	−0.602**	−0.658**	-0.241^{NS}	−0.545*	−0.646*	−0.628**	−0.671**	−0.719*	0.651**	0.741**	0.667*	0.611**	0.638**	1.000	
APX (Unit mg^{-1} protein)	−0.416**	−0.582**	-0.250^{NS}	−0.291*	−0.742**	−0.705**	−0.685**	−0.640**	0.599**	0.570**	0.661*	0.681**	0.567**	0.688**	1.000

NS=Not significant;.
* Significant at $P < 0.05$.
** Significant at $P < 0.01$ level.

and potentially declined NaCl stress toxicity and efficiently recovered and regulated PSII photochemistry parameters at different NaCl (0–180 mM) concentrations.

Oxidative biomarkers allow for the assessment of the degree of oxidation processes within the cell (Velikova et al., 2000; Oviya et al., 2023). Different ROS are constantly generated and scavenged in non-saline environments to maintain the equilibrium of cell cytoplasm for plant cellular needs. The Superoxide radical ($O_2^{\cdot-}$), H_2O_2, and contents of end product of lipid peroxidation-MDA which are normally present in plant tissues in relatively low amount (Vimal et al., 2023a; Bushra et al., 2024). Disturbances in NaCl stress induced ionic equilibrium led to harmful oxygen byproducts ($O_2^{\cdot-}$), H_2O_2 formation. Over accumulation of hydroxyl radicals enhanced Cu and Fe availability for Fenton reactions (Raju and Prasad, 2021). NaCl stress induces peroxidation of lipids as elevated MDA contents, oxidation of proteins, nucleic acids and essential macromolecules in cell (Hasanuzzaman et al., 2021; Hualpa-Ramirez et al., 2024). Different studies depict the rise in oxidative stress biomarkers in plants under NaCl stress (Singh et al., 2015; Joshi et al., 2022). *A. faecalis* SSP8 efficiently tolerate NaCl (>1200 mM) exhibiting stress regulating activities, declined the oxidative biomarkers activities in at different NaCl (0–180 mM) concentration compared to respective paddy crop seedlings. Our study is supported by studies on different PGP bacteria shown the potential to radically decrease oxidative stress markers activity in different plants (Rashid et al., 2021; Vimal et al., 2023a; Oviya et al., 2023).

Plants suffers oxidative damages due to the generation of undue ROS in reaction to osmotic and ionic stresses caused by NaCl (Vimal et al., 2019; Hualpa-Ramirez et al., 2024). However, plants have a well-established antioxidant defence system that detoxifies the generated ROS or keeps the balance between ROS generation and breakdown to combat the oxidative damage caused by salt (Gill and Tuteja 2010; Joshi et al., 2022). To counteract the oxidative damage brought on by NaCl, plants however, have a well-established antioxidant defence system that either detoxifies the produced ROS or maintains the balance between ROS formation and scavenging (Taibi et al., 2016; Vimal et al., 2017). In our study, increase in antioxidant enzymes as CAT, SOD, POX and APX exhibited increase in stress level and accomplish ROS formation-scavenging activities supported with previous studies on different plants (Ray et al., 2016b; Vimal et al., 2019). The reduction in ROS have been associated with a decline in antioxidant enzymes activities (Bushra et al., 2024). Similarly, our research findings showed significantly declined in CAT, SOD, POX level in *A. faecalis* + NaCl treated paddy seedlings compared to respective controls (Fig. 4). Different PGP microbes efficiently decline stress toxicity and maintain antioxidant defence systems to promote plant health under stress environment (Choudhury et al., 2023). These antioxidant enzymes activities are negatively correlated with plant health parameters and leaves gas exchange parameters indicates positive enhancement in plant health under saline stress environment (Table 4). Thus, multiple previous studies support our overall findings about the beneficial and more efficient role of endophytic bacteria *A. faecalis* in salinity stress management in paddy crop seedlings.

5. Conclusion

To meet the continuous rising food demand for future generations, there is urgent need of use of native stress supportive microbes based green natural solutions for paddy crop cultivation. Our results provide a beneficial aspect of indigenous NaCl tolerant, ACC deaminase producing endophytic microbe *A. faecalis* SSP8, isolated from naturally grown stressed plant, in paddy seedlings growth promotion and stress management activities. The paddy seeds bacterized with *A. faecalis* SSP8 significantly enhanced paddy seedlings physiology and biochemistry under various doses of NaCl (0–180 mM) in medium. Based on the above findings, this study recommended that bacterization of *A. faecalis* SSP8 have significant impact on paddy growth due to its PGP attributes under NaCl stress. A positive corelation between growth, *Chl* contents and leaves gas exchange parameters and negative corelation between all these parameters and oxidative stress biomarkers and antioxidant enzymes activities confirmed that an adverse impact of higher dose of NaCl on paddy seedlings counteracted by NaCl tolerant *A. faecalis* SSP8. Thus, understanding the mechanisms underlying plant stress responses and *A. faecalis* SSP8 role in developing strategies to mitigate negative effects are crucial for paddy seedlings development under NaCl stress. However, in-depth studies are required to support *A. faecalis* SSP8 application in sustainable paddy crop agriculture.

CRediT authorship contribution statement

Shobhit Raj Vimal: Conceptualization, Perform Experiment, Data analysis, Writing - original draft. **Jay Shankar Singh:** Review & editing. **Sheo Mohan Prasad:** Supervision.

Funding statement

SRV is financially supported in the form of Dr. D.S. Kothari Postdoctoral Fellowship (Award letter No.F.4–2/2006 (BSR)/BL/20–21/393 (September 14, 2021) 90th List) University Grants Commission, New Delhi, India. SMP is thankful to CSIR, New Delhi, India for awarding Emeritus Scientist scheme (21/1166/24/EMR-II).

Data Availability Statement

The datasets presented in this study can be found in online repository. The names of the repository and accession number can be found below:

NCBI, USA (Accession: OP:225818).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

SRV is thankful to University Grants Commission, New Delhi, India for providing Dr. D.S. Kothari Postdoctoral Fellowship.

Data availability

Data will be made available on request.

References

Abei, H., 1984. Catalase in vitro. Methods Enzymol 105, 121–126.

Alhaddad, F.A., Bitaar, Z.M., Abu-Dieyeh, M.H., 2024. Diversity, characterization, and potential applications of bacterial endophytes isolated from the halophyte *Limonium axillare*. J. Plant Grow. Regul. https://doi.org/10.1007/s00344-024-11254-6.

Bradford, M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding (PDF). Anal. Biochem. 72, 248–254.

Bushra, S., Faizan, S., Badar, A., Kumari, R., Pandey, E., 2024. Application of *Rhizobium* alleviates the cadmium stress-induced damage to growth, photosynthetic efficiency, cell viability and yield by up-regulating antioxidants in *Cicer arietinum*. L. J. Plant Grow. Regul. https://doi.org/10.1007/s00344-024-11243-9.

Choudhury, A.R., Trivedi, P., Choi, J., Madhaiyan, M., Park, J.H., Choi, W., et al., 2023. Inoculation of ACC deaminase-producing endophytic bacteria down-regulates ethylene-induced pathogenesis-related signalling in red pepper (*Capsicum annuum* L.) under salt stress. Physiol. Plant 175 (2), e13909.

Dworkin, M., Foster, J., 1958. Experiments with some microorganisms which utilize ethane and hydrogen. J. Bacteriol. 75, 592–603.

Elstner, E.F., Heupel, A., 1976. Inhibition of nitrite formation from hydroxylammonium chloride: a simple assay for superoxide dismutase. Analyt. Biochem. 70, 616–620.

FAO, 2022. In: Halt soil salinization, boost soil productivity – proceedings of the global symposium on salt-affected soils. https://doi.org/10.4060/cb9565en, 20-22 October 2021. Rome.

Giannopolitis, C.N., Ries, S.K., 1977. Superoxide dismutase. I. Occurrence in higher plants. Plant Physiol 59, 309–314.
Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48, 909–930.
Glick, B.R., 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol. Res. 169, 30–39.
Hasanuzzaman, M., Raihan, M.R.H., Masud, A.A.C., Rahman, K., Nowroz, F., Rahman, M., et al., 2021. Regulation of reactive oxygen species and antioxidant defense in plants under salinity. Int. J. Mol. Sci. 22, 9326.
Hoagland, D.R., Arnon, D.I., 1950. The water culture method for growing plants without soil. California Agricultural Experiment Station 347, 1–39.
Hodges, D.M., DeLong, J.M., Forney, C.F., Prange, R.K., 1999. Improving the thio-barbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207, 604–611.
Honma, M., Shimomura, T., 1978. Metabolism of 1-aminocyclopropane-1-carboxylic acid. Agric. Biol. Chem. 42, 1825–1831.
Hualpa-Ramirez, E., Carrasco-Lozano, E.C., Madrid-Espinoza, J., Tejos, R., Ruiz-Lara, S., Stange, C., et al., 2024. Stress salinity in plants: new strategies to cope with in the foreseeable scenario. Plant Physiol. Biochem. 208, 108507.
Jia, R., Chen, J., Hu, L., Liu, X., Xiao, K., Wang, Y., 2022. *Alcaligenes faecalis* Juj3 alleviates *Plasmodiophora brassicae* stress to cabbage *via* promoting growth and inducing resistance. Front. Sustain. Food Syst. 6, 942409.
Joshi, S., Nath, J., Singh, A.K., Pareek, A., Joshi, R., 2022. Ion transporters and their regulatory signal transduction mechanisms for salinity tolerance in plants. Physiol. Plant 174 (3), e13702.
Kondamudi, R., Swamy, K.N., Rao, Y.V., Kiran, T.V., Suman, K., Rao, D.S., et al., 2016. Gas exchange, carbon balance and stomatal traits in wild and cultivated rice (*Oryza sativa* L.) genotypes. Acta Physiol. Plant 38, 160.
Mastan, A., Rane, D., Dastager, S.G., Shivegowda, C., Babu, V., 2020. Plant probiotic bacterial endophyte, *Alcaligenes faecalis*, modulates plant growth and forskolin biosynthesis in *Coleus forskohlii*. Probiot. Antimicro. Prot. 12, 481–493.
Murali, M., Singh, S.B., Gowtham, H.G., Shilpa, N., Prasad, M., Aiyaz, M., et al., 2021. Induction of drought tolerance in *Pennisetum glaucum* by ACC deaminase producing PGPR-*Bacillus amyloliquefaciens* through antioxidant defense system. Microbiol. Res. 253, 126891.
Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22, 867–880.
Nandkumar, R., Babu, S., Viswanathan, R., Sheela, J., Rahuchander, T., Samiyappan, R., 2003. A new bio-formulation containing plant-growth-promoting rhizobacterial mixture for the management of sheath blight and enhanced grain yield in rice. BioControl 46, 493–510.
Oviya, G., Rangasamy, A., Ariyan, M., Krishnamoorthy, R., Senthilkumar, M., Gopal, N. O., et al., 2023. Halotolerant nodule rhizobial and passenger endophytes alleviates salinity stress in groundnut (*Arachis hypogaea* L.). J. Plant Grow. Regul. 42, 6620–6635.
Rai, A., Singh, V.K., Sharma, N.K., Singh, J.S., Singh, V.K., Dwivedi, B.S., et al., 2023. Effect of fluorescent *Pseudomonas* on plant growth promotion of *Aloe vera*. J. Plant Nutri. 47, 1089–1100.
Raju, A.D., Prasad, S.M., 2021. Hydrogen sulfide implications on easing NaCl induced toxicity in eggplant and tomato. Plant Physiol. Biochem. 164, 173–184.
Rashid, U., Yasmin, H., Hassan, M.N., Naz, R., Nosheen, A., Sajjad, M., et al., 2021. Drought-tolerant *Bacillus megaterium* isolated from semi-arid conditions induces systemic tolerance of wheat under drought conditions. Plant Cell Rep 41, 549–569.
Ray, S., Singh, S., Sarma, B.K., Singh, H.B., 2016a. Endophytic *Alcaligenes* isolated from horticultural and medicinal crops promotes growth in Okra (*Abelmoschus esculentus*). J Plant Grow. Regul. 35, 401–412.
Ray, S., Singh, V., Singh, S., Sarma, B.K., Singh, H.B., 2016b. Biochemical and histochemical analyses revealing endophytic *Alcaligenes faecalis* mediated suppression of oxidative stress in *Abelmoschus esculentus* challenged with *Sclerotium rolfsii*. Plant Physiol. Biochem. 109, 430–441.
Segura, A., Molina, L., 2022. Plant salt tolerance: ACC deaminase-producing endophytes change plant proteomic profiles. Environ. Microbiol. 8, 3310–3312.
Singh, J.S., Kaushal, S., Kumar, A., Vimal, S.R., Gupta, V.K., 2016. Book review: microbial inoculants in sustainable agricultural productivity-vol. II: functional application. Front. Microbiol. 7, 1–2.
Singh, M., Singh, V.P., Dubey, G., Prasad, S.M., 2015. Exogenous proline application ameliorates toxic effects of arsenate in *Solanum melongena*. L. seedlings. Ecotox. Environ. Safet. 117, 164–173.
Strasser, R.J., Srivastava, A., Tsimilli-Michael, M., 2000. The fluorescence transient as a tool to characterise and screen photosynthetic samples. In: Yunus, M., Pathre, U., Mohanty, P. (Eds.), Probing Photosynthesis: Mechanisms, Regulation and Adaptation. Taylor and Francis, London, pp. 445–483.
Sun, L., Qiu, F., Zhang, X., Dai, X., Dong, X., Song, W., 2008. Endophytic bacterial diversity in rice (*Oryza sativa* L.) roots estimated by 16S r-DNA sequence analysis. Microbial Ecol 55, 415–424.
Taibi, K., Taibi, F., Abderrahim, L.A., Ennajah, A., Belkhodja, M., Mulet, J., 2016. Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defence systems in *Phaseolus vulgaris* L. South Afr. J. Bot. 105, 306–312.
Velikova, V., Yordanov, I., Edreva, A., 2000. Oxidative stress and some antioxidant system in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Sci 151, 59–66.
Vimal, S.R., Patel, V.K., Singh, J.S., 2019. Plant growth promoting *Curtobacterium albidum* strain SRV4: an agriculturally important microbe to alleviate salinity stress in paddy plants. Ecol. Indic. 105, 553–562.
Vimal, S.R., Singh, J.S., 2020. Microbial services to nurture plant health under stress environments. In: Singh, J.S., Vimal, S.R. (Eds.), Microbial Services in Restoration Ecology. Elsevier-USA, pp. 157–179. https://doi.org/10.1016/B978-0-12-819978-7.00011-7.
Vimal, S.R., Singh, J.S., Arora, N.K., Singh, S., 2017. Soil-plant-microbe interactions in stressed agriculture management: a review. Pedosphere 27, 177–192.
Vimal, S.R., Singh, J.S., Kumar, A., Prasad, S.M., 2023b. Plant genotype-microbiome engineering as nature-based solution (NbS) for regeneration of stressed agriculture: a review. Scient. Hortic. 321, 112258.
Vimal, S.R., Singh, J.S., Prasad, S.M., 2023a. Prospective of Indole-3-acteic acid (IAA) and endophyte microbe *Bacillus subtilis* strain SSA4 in paddy seedlings development and ascorbate-glutathione (AsA-GSH) cycle regulation to mitigate NaCl toxicity. Mol. Biotechnol. https://doi.org/10.1007/s12033-023-00743-w.
Vimal, S.R., Singh, J.S., Prasad, S.M., 2024. Plant endomicrobiome services in global food security and agricultural sustainability. In: Kumar, A. (Ed.), Advances in Plant Microbiome Research For Climate Resilient agriculture: Toward Sustainable Farming. Apple Academic Press, Inc. Co-published with CRC Press (Taylor & Francis).
Wellburn, A.R., 1994. The spectral determination of Chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol. 144, 307–313.
Yang, K., Huang, Y., Yang, J., Yu, L., Hu, Z., Sun, W., et al., 2023. The determiner of photosynthetic acclimation induced by biochemical limitation under elevated CO_2 in japonica rice. J. Plant Physiol. 280, 153889.
Zhang, X.Z., 1992. The measurement and mechanism of lipid peroxidation and SOD, POD and CAT activities in biological system. In: Zhang, X.Z. (Ed.), Research Methodology Crop Physiology. Agriculture Press, Beijing, pp. 208–211.