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Bacillus megaterium JPR68 modulates soil nitrogen uptake and suppress collar rot in Bhut Jolokia by triggering systemic resistance

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Abstract

Background and aims:

Bhut Jolokia cultivation is constrained by collar rot and fungal diseases, and the role of PGPR in enhancing nitrogen uptake remains unclear. This study investigated the effects of *Bacillus megaterium* JPR68 on plant growth, oxidative stress regulation, and metabolism, and examined PR gene expression and nitrogen assimilation under *Rhizoctonia solani* stress, along with its impact on yield and soil health.

Methods

The study combined *in vitro* and *in vivo* (pot and field) experiments to assess BmJPR68 in Bhut Jolokia. *In vitro* assays evaluated seed dormancy, germination, and vigor, while greenhouse and field trials measured growth and yield. ROS localization, antioxidant enzymes, PR gene expression, and nitrogen assimilation were analyzed under pathogen stress. Fruit bioactives, capsaicinoids, fatty acids, nutrient content, total NPK uptake, and soil nutrients were quantified.

Results

Bacillus megaterium JPR68 (BmJPR68) associated with enhanced nitrogen assimilation in Bhut Jolokia by modulating internal signaling and transport systems, improving plant architecture and fruit yield. Treated plants showed upregulation of *NR, NiR, NRT1.1, NRT1.2, NRT2.1, NRT2.2*, and *GSH* genes. GC-MS identified thirteen fatty metabolites, while improved nitrogen assimilation and collar rot resistance contributed to higher productivity.

Conclusions

The findings indicate that pre-treatment of Bhut Jolokia plants with BmJPR68 significantly enhances chili yield and capsaicin accumulation. Field trials validated these results, demonstrating improved plant growth, higher yield attributes, and elevated soil macro- and micronutrients. This study provides an effective and sustainable strategy for managing collar rot disease without relying on chemical fertilizers, while maintaining productivity through optimized plant architecture.

Introduction

Bhut Jolokia, also known as Ghost chilli or pepper (*Capsicum chinense* Jacq.), is a spice crop that is both endemic and economically important to Northeast India. It is well-known for its high pungency (capsaicinoids), medicinal properties, and large-scale consumption. However, this crop is affected by various diseases including collar rot, wilt, fruit rot, root rot, and damping-off. Notably, collar rot disease caused by *Rhizoctonia solani* (Rs) is currently posing a serious challenge, leading to an estimated yield loss of 8–72% (Talukdar et al. 2015; Gogoi et al. 2026). Recent studies have emphasized the use of plant growth-promoting rhizobacteria (PGPR) to improve plant growth and suppress fungal diseases (Saikia et al. 2005; Swetha et al. 2025). PGPR can induce systemic resistance (ISR), particularly through root pre-inoculation and protect against various pathogens (Kim and Jeun, 2006; Pieterse et al. 2014; Grover et al. 2021; Gogoi et al. 2025). ISR triggers plant immunity by activating pathogenesis-related (PR) genes and proteins, which interfere with the fungal cell wall via. the jasmonic acid (JA) and ethylene (ET) pathways during infection (Gogoi et al. 2022; Lazarus and Easwaran, 2024; Rakhonde et al. 2025). These PR genes and proteins not only affect the pathogen directly but also produce signaling molecules that regulate plant defense-related pathways (Saikia et al. 2006). It is widely recognized that plants respond to pathogen stresses by generating reactive oxygen species (ROS) (Siddika et al. 2024). The production of ROS induces oxidative stress in plants and can damage key cellular components, including DNA, proteins, and membrane structures (Cheeseman, 2007; Kartashov et al. 2008).

Numerous studies have shown that PGPR-induced plant growth can increase nitrogen (N) uptake by plant roots (Adesemoye et al. 2008). PGPR can influence nitrogen (N) metabolism in plants, including the biosynthesis of essential N compounds (Di Benedetto, 2017). The process of nitrogen assimilation in plants begins with the uptake of inorganic nitrate (NO_3^-), which is subsequently reduced to nitrite (NO_2^-) and ammonium (NH_4^+) by enzymes such as nitrate reductase (NR) and nitrite reductase (NiR) (Akhtar et al. 2024). Biotic stress can influence nitrogen uptake and enzyme activity in plants via PGPR by modulating the expression of nitrogen metabolism transporters and enzymes (Madhusudhan and Sudhakar, 2024). PGPR are capable of biological nitrogen fixation (BNF), which can enhance nitrogen nutrition in plants (Ladha et al. 2016). We hypothesized that PGPR may promote Bhut Jolokia growth by upregulating nitrogen metabolism and boosting plant growth efficiency.

In our previous studies, *Bacillus megaterium* JPR68 (BmJPR68) currently known as *Priestia megaterium* (prior to 2020) was identified as a potential ISR-inducing PGPR in Bhut Jolokia for the control of collar rot disease (Gogoi et al. 2024, 2025). This ISR-causing strain resulted in increased activity of defense-related enzymes and the modulation of defense-related genes following challenge inoculation with *R. solani* (Gogoi et al. 2024). In continuation of our earlier work, the present study aims to (i) investigate the growth promotion, reduction of oxidative damage and major metabolic regulation of Bhut Jolokia by *B. megaterium* (BmJPR68), (ii) investigate gene expression and protein accumulation related to pathogenesis, along with nitrogen assimilation in plants subjected to *R. solani* stress, and (iii) evaluate the efficacy of this strain in improving both plant and soil health, as well as in boosting crop yield under field conditions. The results demonstrate that BmJPR68 may serve as a promising strain to improve the cultivation of Bhut Jolokia in Northeast India. To the best of our knowledge, this is the first report investigating the molecular mechanisms (including enzymes, genes, proteins and nitrate assimilation) involved in enhancing antipathogenic resistance in Bhut Jolokia through rhizobacteria under *R. solani* stress.

Materials and methods

Seed dormancy comparison and seedling investigations

In the seed dormancy comparison study, fresh seeds were initially soaked in bacterial inoculum for 2 h, after which they were allowed to germinate at 30°C in Murashige and Skoog (MS) media. Water-soaked seeds were served as a mock (control). The assays were conducted three times, with each repetition involving 5 seeds per plate and 10 seeds per glass bottle.

Stress induction and induced systemic resistance (ISR) against R. solani

Plants were pre-treated with *Bacillus megaterium* (BmJPR68), followed by infection using a mycelial suspension of *Rhizoctonia solani* (Rs), as described earlier (Gogoi et al. 2024; 2025). Inoculated plants were maintained under a transparent netted greenhouse cover to ensure high humidity, and a photoperiod of 13.5 hours at a temperature of 30 ± 2°C was observed in the greenhouse conditions. The pot experiment was carried out by using complete randomized design (CRD) with four treatments: mock (untreated/water treated plants), Rs (*R. solani* treated plants), BmJPR68 (*B. megaterium* treated plants), and BmJPR68 + Rs (plants pre-treated with *B. megaterium* BmJPR68, followed by a challenged inoculation with *R. solani* after 7-days). Each treatment consisted of 5 replicates. For phenotypic characterization, the plants were allowed to grow for 6 months, then harvested and analyzed. In mock plants were treated with water only. For the analysis of gene expression and transcriptomics, leaves of Bhut Jolokia from both infected and uninfected plants were collected, rapidly frozen using liquid nitrogen, and stored at -80°C until further use. The plants inoculated with the pathogen exhibited disease symptoms, which were scored for disease severity (Gogoi et al. 2024) at three distinct time intervals: the initial assessment of disease severity was taken 15-days post *R. solani* inoculation, followed by subsequent evaluations at 30 and 60 days after inoculation. Additionally, the survival rate of the plants was recorded throughout the experimental period.

Histochemical observation of ROS

In situ detection of O_2^- and H_2O_2 accumulation was carried out using nitro blue tetrazolium (NBT) and 3,3-diaminobenzidine (DAB) histochemical staining (Liu et al. 2007). Leaves after different treatment were detached from plants and submerged in NBT solution (1 mg mL^{-1} NBT in 10 mM sodium potassium phosphate buffer pH 7.8) and DAB solution (1 mg mL^{-1}, pH 5.5). After leaves were stained for 40 min (NBT) or 2 h (DAB), the leaves were de-stained by 75% (v/v) ethyl alcohol in an 85°C water bath to remove the chlorophyll completely. The samples were photographed after cooling.

The estimation of H_2O_2 was assayed according to Yu et al. (2003). The rate of O_2^- generation was determined following Yang et al. (2011) with a few modifications. Leaves were ground with 5 mL 0.1% TCA (w/v). The mixture was centrifuged at 10,000 x *g* for 15 min at 4°C. A 0.5 mL supernatant was taken and mixed with 1 mL of 1 M KI, followed by mixing of 0.5 mL 10 mM K_2HPO_4 buffer (pH 7.0). Reaction mixture was subjected to dark conditions for 60 min. The absorbance of the samples was measured at 390 nm. H_2O_2 amount was calculated with a standard curve (Önder et al. 2020). The rate of O_2^- generation was calculated using the hydroxylamine oxidation method (Wang and Luo 1990). 100 mg of leaf tissue was homogenized with 1 mL of 50 mM phosphate buffer (pH 7.8) in a chilled mortar. The mixture was centrifuged at 10,000 rpm for 15 min at 4°C and 0.5 mL of the supernatant was mixed with 0.5 mL of 50 mM phosphate buffer (pH 7.8) and 1 mL of 1 mM hydroxylamine chloride. The solution was incubated for 1 h at 25°C. Subsequently, 1 mL of 17 mM p-aminobenzene sulfonic acid and 1 mL of 7 mM α-naphthylamine were added and incubated for 20 min at 25°C. The absorbance was measured at 530 nm. The malondialdehyde (MDA) was measured using the method described earlier (Zhang et al. 2018; Kim et al. 2016).

RNA extraction and gene expression analysis

For real-time PCR analysis, total RNA was extracted with Trizol (Invitrogen) followed by ethanol precipitation. The cDNA was synthesized using cDNA synthesis kit (Thermo Scientific, USA), as per the manufacturer's protocol. Gene expression analysis was performed using PowerUp SYBR Green qPCR mastermix (Invitrogen) in a StepOnePlus™ qPCR system (QuantStudio5 Dx, Thermo Fisher Scientific). The relative mRNA levels of desired genes were measured by the threshold cycle (Ct). Gene expression was calculated with the $2^{-\Delta\Delta Ct}$ representing the x-fold difference from the calibrator with actin as internal standard (Gogoi et al. 2024). All gene-specific primers used in this study are shown in the Table S1. All experiments were carried out thrice with 3-replications.

Immunoblot analysis

Immunoblot analysis
Total protein was extracted from 100 mg of ground frozen leaf tissue of Bhut Jolokia plants mixed with 100 µl RIPA buffer containing protease inhibitor. Subsequently, the sample was subjected to sonication and vortexing for 3 cycles, followed by centrifugation at 10,000 *g* for 15 min at 4˚C. The supernatant was store at -80 ˚C for protein detection. Protein concentration was measured with Pierce BCA protein assay Kit (Invitrogen), according to the manufacturer's instructions. Samples (100 mg protein per lane) were loaded and separated by SDS-PAGE (Laemmli, 1970) and transferred to nitrocellulose membrane. Membranes were blocked for 2 h at room temperature with blocking buffer. The primary antibodies for targeted protein used were PR10A (PA5-98368, Invitrogen), Catalase1 (PA5-98626, Invitrogen), WRKY47 (PA5-144455, Invitrogen), PDF 2.2 (PA5-144381, Invitrogen) against Actin (Mouse monoclonal, Merck, A0480-25UL). For, Catalase 1 and WRKY47, we employed SDS-PAGE with a 10% gel to separate the proteins and subsequently transferred the proteins to a nitrocellulose membrane using a wet transfer technique (Idea Scientific). For determination of PR10A and PDF 2.2, the proteins were separated by 12% SDS-PAGE. Blots were incubated with antibodies specific to actin. The blot was washed three times with wash buffer for 5 min to eliminate excess antibodies

(Corpas et al. 1998). Goat anti-rabbit IgG, HRP conjugated (12–348, Merck) served as the secondary antibody for all targeted proteins, while Rabbit anti-mouse IgH (H&L), HRP conjugated (AS09627, Merck) was used as a secondary antibody for actin, followed by washing as described above. The antigen-antibody complex was examined with Clarity Western ECL Chemiluminescent Substrate (Bio-Rad, 1705061). Uncropped and original immunoblotting images were provided in Fig. S1 (a-d).

Activities of N metabolism enzymes

The four enzymes viz., NR, NiR, GS, GR, GOGAT, and GDH, were assayed in freshly harvested flag leaf at flowering stages. The protein concentration was determined using the BCA protein estimation kit (Thermo Fisher Scientific) from all of the enzyme extracts as instructed by the manufacturer. All the assays were carried out with five replications. The specific activity of each enzyme was expressed as µmol of product generated per mg protein. Leaf sample (0.5 g) was homogenized in pre-cold mortar pestle using extraction buffer which contained 1 mL of 50 mM K-P buffer (pH 7.0) including 100 mM KCl, 1 mM AsA, 5 mM β-mercaptoethanol, and 10% (w/v) glycerol, and subjected for centrifugation (10,000× *g*, 15 min). Supernatants were used for assessing enzyme activity at 4°C using a protein estimation kit (Thermo Fisher Scientific). Bovine serum albumin (BSA) was used as standard. Nitrate reductase (NR) activity was assayed (Lea et al. 2006) and expressed as nmol N dioxide (NO_2) min^{-1} mg^{-1} protein. Nitrite reductase (NiR) was used to measure NiR activity (Hageman, 1984). Glutamine synthetase (GS) activity was assessed by the quantification of G-glutamylhydroxamate (G-GHA) formation (O'Neal and Joy,1973) according to Shah et al. (2017). GR activity was measured using extinction coefficient of 6.2 mM^{-1} cm^{-1} (Halliwell and Foyer, 1978). The glutamate synthase (GOGAT) extraction buffer contained 10 mmol L^{-1} Tris-HCl (pH 7.6), 1 mmol L^{-1} $MgCl_2$, 1 mmol L^{-1} EDTA, and 1 mmol L^{-1} mercaptoethanol. The standard assay mixture consisted of 40 mmol L^{-1} potassium phosphate buffer (pH 7.5), 10 mmol L^{-1} L-glutamine, 10 mmol L^{-1} 2-oxoglutarate, 0.14 mmol L^{-1} NADH, and crude enzyme (final volume 3 mL). Absorbance was recorded at 340 nm for 3–4 min at room temperature (25°C). Absorbance (340 nm min^{-1}) was calculated from the initial linear portion of the curve. Glutamate dehydrogenase (GDH) was used for the measurement of GDH (Gupta and Prasad 2022). Nitrate content was determined by nitration of salicylic acid. Briefly, leaves were ground in liquid nitrogen and resuspended in 20 mM HEPES (pH 8.0). After centrifugation at 10,000 ×*g* for 10 min at 4ºC, aliquots of 5 mL of supernatant were mixed with 45 mL of 5% (v/v) salicylic acid in sulfuric acid for 20 min. The solution was neutralized by slowly adding 950 mL of NaOH (2N). Absorbance was determined at 410 nm and the values obtained were compared with those of a standard curve constructed using KNO_3 and normalized by protein content (Frungillo et al. 2014).

Determination of glutathione

The levels of oxidized, reduced, and total glutathione (GSH + GSSG) were estimated (Smith, 1985). Leaf tissue (1g) was homogenized in 10 mL of 5% (w/v) sulfosalicylic acid and centrifuged at 10,000×*g* for 25 min at 4°C. The supernatant was collected for glutathione analysis. To determine GSH + GSSG, 0.1 mL of 0.5 M potassium phosphate buffer (pH 7.5), 0.5 mL of 0.1 M sodium phosphate buffer (pH 7.5) containing 5 mM EDTA, 0.1 mL of 2 mM NADPH, 0.1 mL of glutathione reductase, 0.15 mL of 0.6 mM DTNB, and 0.05 mL of the supernatant were added to a cuvette. The mixture was thoroughly mixed before adding the supernatant, which initiated the reaction. A blank was prepared without the supernatant. The reduction of DTNB was monitored by measuring absorbance at 412 nm for 3 min. The GSH + GSSG content was determined using a standard curve of GSH (200–400 ng) plotted against the rate of absorbance increase at 412 nm. To determine oxidized glutathione (GSSG), 1.5 mL of 0.5 M potassium phosphate buffer (pH 7.5) and 0.2 mL of 4-vinyl pyridine were added to 1 mL of the supernatant, allowing the reaction to proceed for 1 h to remove reduced glutathione (GSH). The GSSG content was then measured using the same procedure as for total glutathione, using a GSSG standard curve (50–200 ng). The GSH content was calculated by subtracting the GSSG content from the total glutathione (Kumar, 2021).

Determination of bioactive compounds in Bhut Jolokia fruits

The total phenolic content of the extract was determined by the Folin-Ciocalteu method (Kim et al. 2003). The total flavonoid content was determined following a method as described by Park et al. (2008). The standard curve for total flavonoids was made using a Quercetin standard solution (0 to 100 mg L^{-1}). The total flavonoids were expressed as mg of Quercetin equivalents per gram (g) of dried fraction. Antioxidant potential from stress-treated plants fruit samples measured *in vitro* through DPPH (2, 2-diphenyl-1- picrylhydrazyl) assay (Brand-Williams et al. 1995). The stock solution was prepared by dissolving 24 mg of DPPH in 100 mL methanol and stored at -20°C until required. The scavenging activity was estimated using the percentage of DPPH radical scavenged according to the following equation:

Radical scavenging activity (%) = [(Ao – As)/Ao]×100 (Ao is absorbance of control blank, and As is absorbance of sample extract).

The assay for superoxide anion radical scavenging activity (SRSA) was facilitated by riboflavin-light-NBT system method. Briefly, 1 mL of the sample was taken at different concentrations (25 to 500 µg mL^{-1}) and mixed with 0.5 mL of phosphate buffer (50 mM, pH 7.6), 0.3 mL riboflavin (50 mM), 0.25 mL PMS (20 mM), and 0.1 mL NBT (0.5 mM). The reaction was started by illuminating the reaction mixture using a fluorescent lamp. After 20 min of incubation, the absorbance was recorded at 560 nm. Estimation of ascorbic acid was carried out following standard protocols (Bates et al. 1973). The extractive ability to scavenge hydroxyl radicals was assessed (Halliwell and Gutteridge,1989). The ABTS radical scavenging assay was determined (Re et al. 1999). The level of β-carotene was assessed using the techniques as described by Kumar (2021). The concentration of crude fruit protein was measured using the BCA protein estimation kit (Thermo Fisher Scientific) as per the manufacturer's instructions, with BSA used as the standard.

Analysis of macro and micro nutrient contents of Bhut Jolokia

Na, K, Ca, Mg, Fe, Cu, Zn, and Mn were determined using AAS (Analytikjena, Germany) while P was assessed through UV Photometry (Analytik Jena). For the analysis, the 0.5 g of each sample put in a dry flask individually, 5 mL of HNO_3 was added to each sample and mixed. Then 4 mL of 33% H_2O_2 was gently mixed after being added. It was heated on a hot plate inside a fume hood, generating a vigorous effervescence. Once brown fumes became less dense (15–20 min), the solution was allowed to cool. A faintly yellow liquid and a small amount of white solid remained in suspension. The solution was filtered, rinsed with 5 mL of (1:1) HCl (density 1.18 g mL^{-1}), and brought to a final volume of 25 mL with distilled water (Pequerul et al.1993). The TC/TN Analyzer (Primacs Series, Skalar, Netherlands) was used to measure the C and N content.

Quantification of capsaicinoid by UHPLC

Freshly harvested Bhut Jolokia fruits (5.0 g) of different treatments (Mock, Rs, BmJPR68, BmJPR68 + Rs) were lyophilized, crushed in a mortar-pestle, and then 2 mL HPLC-grade methanol (Al Othman et al. 2011) was added. It was heated at 70ºC for 3 h with occasional shaking, then the mixture was cooled at room temperature, centrifuged at 5000 rpm and the supernatant was separated. The remaining biomass was extracted similarly another two times (1.5 mL × 2). The supernatants were pooled together and filtered through a 0.45 µm syringe filter for UHPLC-PDA analysis (Ultimate 3000, Thermo Fisher Scientific). The analysis was performed under the following conditions: (a) analytical C18 column (2.1 × 100 mm, particle size 1.8 µm); (b) mobile phase: acetonitrile (ACN)/water added with 0.1% formic acid; (c) solvent program; 0 min, 10% ACN/water; 10 min, 90% ACN/water; 12 min, 90% ACN/water; 14 min, 10% ACN/water; 16 min, 10% ACN/water; (d) detection: 280 nm; (e) flow rate 1.0 mL/min; (f) injection volume: 20 µL. The major capsaicinoids in the UHPLC profile were identified through a comparison with liquid chromatography-mass spectrometry (LC-MS) data. The identity of capsaicin was further confirmed through an authentic standard. The absolute quantification of capsaicin was done in the samples (µg g^{-1} FW) through the standard graph prepared using an external standard. Other major capsaicinoids (dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin) possess aromatic chromophore that is identical to capsaicin and they were semi-quantified using the standard graph prepared for it.

Profiling of fatty metabolites in Bhut Jolokia samples

Extraction of fatty metabolites

Fresh Bhut Jolokia fruit samples (Mock, Rs, BmJPR68, BmJPR68 + Rs), 5.0 g each, were oven dried at 60°C, coarsely ground in a domestic grinder, and subjected to solvent extraction individually using *n*-hexane (125 mL) in a Soxhlet apparatus. Further, *n*-hexane was evaporated in rotary evaporator to obtain deep red resinous extracts (223–351 mg).

Preparation of methyl esters

The prepared fatty metabolite-rich extracts (50 ± 5 mg) were individually added with 0.5 mL of 2.0 M ethanolic KOH and heated at 60°C for 1.5 h in stirring condition. Then, the reaction mixture was cooled at room temperature and acidified (pH 2–3) by 1.0 M HCl. It was extracted with *n*-hexane (1 mL × 3). The pooled organic layers were passed through anhydrous sodium sulfate and concentrated to dryness to yield 25.0–33.0 mg hydrolyzed product. Further, they were individually subjected to methylation by adding 1.0 mL HPLC-grade methanol and a catalytic amount of concentrated sulfuric acid which was heated at 60°C in stirring condition for 3 h. Methanol was removed and the mixture was added with 0.5 mL of distilled water and extracted with *n*-hexane (0.5 mL × 3). The hexane layers were processed similarly to obtain 15.0–20.0 mg red waxy semi-solid which were further analyzed in GC-MS.

Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analysis was performed using an Agilent 8890 gas chromatograph coupled with an Agilent 7010B triple quadrupole mass spectrometer and an HP-5MS capillary column (30 m × 0.32 mm × 0.25 µm). Helium was employed as the carrier gas at a flow rate of 1.5 mL/min. The injector temperature was set at 250°C. The oven temperature program, lasting 30 min, began with an initial hold at 50°C for 1.0 min, followed by a ramp at 10°C/min to 200°C, holding for 2 min. The temperature was then increased at 15°C/min to 305°C, where it was held for 5 min. Samples were dissolved in HPLC grade ethyl acetate and 0.5 µL was injected using a PAL3 RSI 85 autosampler with a split ratio of 10:1. Data were processed using Agilent MassHunter Qualitative Analysis 10.0 software, integrated with the NIST 2017 mass spectral library. The retention index (RI) values were calculated using a series of linear alkanes (C8-C24). The 'area sum %' of the individual peaks was represented as the 'relative %' of the identified metabolites.

Field experiment and physico-chemical characterization of soil

Field trial was conducted in a complete randomized block design (CRBD) for the four treatments with ten replications- viz., i. control or mock (water treated plants); ii. *Rhizoctonia solani* (Rs) treated plant; iii. *Bacillus megaterium* (BmJPR68) treated plant; iv. *B. megaterium*, pre-treated followed by a challenged inoculation with *R. solani* (BmJPR68 + Rs) treated plant. The field trials were repeated twice (2023 and 2024) for proper validation. Plant materials were grown under standard agronomic conditions at the experimental field of CSIR-NEIST, Jorhat, Assam, India, with an average annual rainfall of about 600 mm, 60–80% relative humidity, and 30/25°C temperature (max-min). The size of experimental plots was 760.5 cm^2 divided into 4 rows (each separated by 62 cm) in each block (45 cm × 702 cm) with replicate. An upland crop field (12×20 m^2) was prepared for Bhut Jolokia cultivation. The preparation of the field included clearing weeds and soil preparation so that soil conditions were suitable for proper plant growth and development. Seven kilograms of compost were applied to each plot as green manure. Each block was further partitioned into four sub-plots (5×4 m^2) by earth embankments.

The width of the shoulder on each earth embankment was 0.2 m. Then each of the small subplots was mixed properly with compost and allowed for the whole system to come to a semi-dry condition. Each experimental unit was arranged with 0.05 to 0.06 m row spacing and 0.1 m distance between plants. The distance between the plots and blocks was maintained at 1.5 and 2.0 m, respectively, to avoid bacterial contamination in order to manage the experimental plots.

Following the harvesting of plants and fruits, soil samples were collected from each plot at depths of 0 to 15 cm to assess the effects of different organic inputs on organic carbon, available nitrogen, available phosphorus, and available potassium. Soil sampling was done to check the chemical and nutritional composition of the soil *viz.*, soil texture, pH (in H_2O), electrical conductivity (EC), maximum water holding capacity (MWHC). To determine pH and EC, distilled water (DW) was thoroughly mixed with soil at a 1:1 ratio, kept overnight, and then pH and EC were measured with a PCSTestr 35 multi-parameter. Extractable cations like Cu, Fe, Zn, and Mn were determined by atomic absorption spectrophotometer (AAS) (Brar et al., 2024). N and C were determined using a total TC/TN analyzer, while P was determined through Photometry (Analytik Jena), and K was measured using AAS. The soil physico-chemical properties and Pearson correlation analysis in experimental field are shown in Table S2 and Fig. S4.

Biocontrol agent and pathogen inoculation

To prepare inoculum, *Bacillus megaterium* JPR68 (Bm JPR68) strain was grown in nutrient broth for 24 h at 30 ± 2 ^{o}C in a shaker incubator at 150 rpm. Cell suspensions contained about 3×10^8 CFU mL^{-1}, which equates to 0.5 at 600 nm. Fully grown *Rhizoctonia solani* (Rs) from Petri plate culture was scraped, mixed in sterile distilled water (SDW), and filtered using muslin cloth, resulting in a final *R. solani* suspension of ~ 6×10^7 CFU mL^{-1} (approx.) that was used for the study (Goudjal et al. 2014). The inoculated plants were scored for disease severity using the 0–5 scale (Pandey et al. 2003) at three time intervals: first observation for disease severity was taken at 7 days post- *R. solani* inoculation, followed by observations on the 15th and 30th days after inoculation. The disease severity was also employed to determine the percentage disease index (PDI) and the area under disease progress curve (AUDPC) based on the methods of Campbell and Madden (1990), Johnson and Wilcoxson (1980), and Van der Aplank (1963), respectively.

$$\mathrm{PDI} = \frac{\mathrm{SumofallratingX100}}{\mathrm{TotalnoofobservationXMaximumratinggrade}}$$

$$\mathrm{AUDPC} = \sum_{i=1}^{n-1}\left(\frac{X_{i+1}+X_i}{2}X(t_{i+1}-t_i)\right)$$

Where X_i is PDI at the ith observation, t_i is the time (in days after inoculation) at the ith observation, and n is the total number of observations.

Agronomic and plot yield parameters

Germinated seeds of Bhut Jolokia were sown in the field at the same time, and the plantlets were examined daily. At the fruiting stage (75 days post-planting), plant height, the count of primary branches, secondary branches, and leaf area were measured manually. The height of the plant and stem girth were measured 60 days post-transplanting (DAP). The Biovolume index (BI) was determined (Parkash et al. 2024).

Biovolume index (BI) = Plant height (cm)× Stem diameter (cm)

The yield of fruit per plant was assessed from every treatment. To assess the yield, the overall weight of fruit from each plant was determined. The fresh/dry weight of the plant was taken at the time of harvest. A random selection of fruits from each individual plant was collected. The fruit samples were dehydrated at room temperature (30 ∘C) to reduce their natural moisture content. Subsequently, the weights of these dried fruit samples were measured to determine the fruit weight per plant. Likewise, additional parameters of plant growth such as height, number of branches, and leaf count were measured manually during the fruiting phase (75 days post-planting).

Measurement of nutrient content in plant and soil

Plant samples (leaves, stems, and roots) were collected, cleaned, air-dried in the shade, then crushed in an electric grinder and stored in paper bags for chemical analysis over both years. Both pre and post-inoculated plant samples (leaf, stem and roots) were harvested and cleaned,

followed by measuring the fresh weight (FW) of each plant individually, and placed in a hot oven at 60 ˚C for drying. The dry weight (DW) of the plant was measured once it reached a stable weight after drying. Dried material was ground in a mortar pestle and stored in paper bags for chemical analysis. The major nutrients, viz. total N, P and K were estimated and expressed in mg g^{-1} (Table 5.4). After harvesting of Bhut Jolokia plants, soil samples of each plot were collected at a depth of 0–15 cm to assess the presence of macro and micro nutrients in the soil. N, P, K, and micronutrients like Cu, Fe, Zn and Mn measured using AAS (Brar et al. 2024; Lindsay and Norvell, 1978).

Results

BmJPR68 regulates seed dormancy

A seed dormancy experiment was conducted to evaluate the efficacy of BmJPR68 as a seed coating to improve seed germination, vigor, storability, and facilitating root colonization for protection against soil-borne diseases. In our earlier study, we found that BmJPR68 application enhanced germination in both water-treated and pathogen-exposed seeds (Gogoi et al. 2024). In consistent with these findings, the effect of BmJPR68 treatment on the 6-months seed dormancy of seeds that were treated with water and infected by the pathogen, Rs was investigated in this study. It was observed that treatment with Rs significantly reduced seed viability, resulting in minimal germination after 4 weeks of culture. On the other hand, seeds treated with BmJPR68 or those subjected to BmJPR68 + Rs exhibited a higher germination rate in a shorter cultivation time compared to the mock (treatment with water only). However, seeds treated with BmJPR68 + Rs exhibited a marginally reduced germination rate compared to those treated with BmJPR68. After 5 months of tracking growth parameters, seedling height, root length, and biomass showed a decline in seedlings affected by pathogens (Table 1). For BmJPR68 pre-treated seeds, the length and weight of the seedlings were significantly greater than those of the mock and Rs. Notably, seeds pre-treated with BmJPR68 under

pathogen stress exhibited growth parameters very similar to those of the mock group. These results collectively demonstrated that BmJPR68 application enhanced seed viability, preserved seed dormancy, and promoted overall seedling growth and development.

BmJPR68 for plant growth promotion (PGP) and induced systemic resistance (ISR) against collar rot disease

The influence of BmJPR68 on pathogen stress tolerance in Bhut Jolokia plants grown in pots was assessed by evaluating various growth parameters (Fig. 1b-d). Under Rs stress, the quantity of shoots and roots, as well as the total fresh and dry mass, were significantly reduced in comparison to the mock treatment (control) (Fig. 2a-j). However, treatment with BmJPR68 significantly improved several growth parameters- including plant height, stem diameter, root length, fruit length and width, and the fresh and dry mass of roots and shoots in comparison to mock treatment (Fig. 2a-m). Moreover, yield- related parameters such as the number of leaves, branches, flowers, and fruits, along with the weight of 10 fruit, and total yield per plant, were significantly higher ($p \leq 0.05$) in plants treated with BmJPR68 than in the mock group. In our previous study, inoculation of Bhut Jolokia plants with BmJPR68, plant roots confirmed that the strains were colonized and associated with roots, which led to the development of microcolonies (Gogoi et al. 2024). Microcolony developed by each bacterial inoculated plant's root surface normally occurs with effective colonization.

Plants subjected to Rs stress showed early signs of collar rot infection, which progressed to wilting symptoms within 30 days, with most plants dying by the 7th week of the growth period (Fig. 1b). In contrast, plants treated with BmJPR68 under Rs stress (BmJPR68 + Rs) revealed better growth and yield performance than the mock group (Fig. 1–2). The disease severity index (DSI) was significantly higher in Rs treated plants (86.36%) compared to BmJPR68 + Rs treated plants (9.6%). Additionally, the survival rate was lowest in the Rs treatment group (Table 2). These findings indicated that BmJPR68 effectively enhanced pathogen resistance and improved growth parameters in Bhut Jolokia under collar rot stress (Fig. 1–2).

Histological observation of ROS (O_2^-, H_2O_2) and MDA accumulation in leaf cells

The NBT and DAB staining were employed to evaluate ROS accumulation in leaf tissues of Bhut Jolokia. The blue coloration generated by the reduction of NBT was examined under a microscope to assess the relative levels of superoxide anions. The accumulation and subsequent cell death were clearly evident in plants treated Rs and BmJPR68 + Rs. However, a high concentration of superoxide ions observed in Rs treated plants showed a significant reduction in the BmJPR68 + Rs treatment. Leaf tissues from the mock and BmJPR68 treatments remained unstained. Additionally, DAB staining assessed the accumulation of H_2O_2 in leaf tissues, as shown by a corresponding brown precipitate. H_2O_2 plays a role in the regulation of stomatal opening and closure by elicitors (Lee et al. 2020). A strong brown deposit was seen in the leaves treated with Rs, which was relatively reduced in BmJPR68 + Rs treated leaves. These observations were quantified by measuring the total staining area (%) and the levels of O_2 or H_2O_2 contents, which were consistent with the histological findings (Fig. 3a-f). For instance, the NBT- stained area was as follows: BmJPR68 ≈ mock (30.0-31.6%) < BmJPR68 + Rs (82.0%) < Rs (90.6%). A similar pattern was observed for the DAB staining area with BmJPR68 ≈ mock (13.3–16.6%) < BmJPR68 + Rs (35.0%) < Rs (92.0%). A similar trend was also observed in malondialdehyde (MDA) accumulation, a biomarker of lipid peroxidation: BmJPR68 ≈ mock < BmJPR68 + Rs < Rs. These results indicate that pretreatment with BmJPR68 significantly inhibited oxidative stress in Bhut Jolokia plants infected with Rs.

Activity of antioxidant enzymes, pathogenesis-related (PR) genes in induced plants

Induced plants were assessed for antioxidative enzymes such as lipoxygenase (LOX), catalase (CAT), β-glucanase, and proline levels. The highest levels of activity were observed in BmJPR68 + Rs, in comparison to BmJPR68 and mock (control) plants (Fig. 4a-d). Additionally, LOX, β-glucanase, CAT, and proline levels were significantly elevated ($p \leq 0.05$) in Rs inoculated plants when compared to mock plants. In Rs treated plants, LOX and CAT activity were significantly increased at 0.41 U^{-1}mg protein^{-1} min and 14.19 U^{-1} mg protein^{-1}min, respectively, compared to mock plants (0.13 U^{-1}mg protein^{-1}min and 5.76 U^{-1}mg protein^{-1}min).

Notably, in proline, BmJPR68 + Rs treated plants exhibited the highest activity at 8.53 µmoles g^{-1} FW, whereas Rs treated plants showed 6.75 µmoles g^{-1} FW) (Fig. 4d). However, no difference was noted between the mock plants and those treated with BmJPR68. Bhut Jolokia immunization of with BmJPR68 triggered systemic resistance to Rs, linked to an enhancement in the activity levels of defense-related enzymes.

In continuation of our previous work, we examined the expression patterns of the defense marker genes to determine whether BmJPR68 reduced the pathogen infection in plants via differential gene regulation. The transcript levels of the *PR1, PR3, LOX3, CAT, TPX* and *CRT* exhibited similar increasing trend in both the Rs stressed and BmJPR68 + Rs plants (Fig. 4e-j). We determined how the expression of these genes varies in the presence of BmJPR68 during the infection of Bhut Jolokia plants with *R. solani* infection. *LOX3* expression was significantly ($p \leq 0.05$) elevated in *R. solani* infected seedlings relative to the mock (Fig. 4e). On the other hand, expression of *LOX3* was increased by 12.9-fold in plants infected with Rs. However, only the plants inoculation with BmJPR68 showed expression align to the mock. This aligns with the earlier report (Gogoi et al. 2024; 2025). Additional, plants treated solely with BmJPR68 alone did not influence *CAT* expression, while treatment with BmJPR68 + Rs boosted its expression by 27.6 fold (Fig. 4f). The transcript levels of *PR1* and *PR3* were significantly elevated in BmJPR68 + Rs plants by 5-fold and 4.8-fold, respectively as compared to mock treatments (1.2 fold and 1.1 fold) (Fig. 4g-h). Only Rs stressed plants exhibited a 4.7-fold and 3.6-fold increase, respectively, when compared to BmJPR68 treated plants (1.8 fold and 1.7 fold). The *CRT* transcript level was higher in BmJPR68 + Rs treated plants, reaching 13.1-fold, while the fungus treated plant exhibited only a 7-fold increase (Fig. 4i). The expression level of *TPX* in Rs was induced 10-fold compared to the mock (1.2 fold), and was further enhanced by BmJPR68 + Rs treatment to a 10.6-fold increase (Fig. 4j). The expression patterns in this transcript indicated an early activation of the defense mediated by BmJPR68 following *R. solani* infection. The plants subjected to the pathogen for gene expression analysis in this experiment were those that were significantly affected by disease. Overall, the above transcript expression patterns mentioned above indicated that multiple genes were differentially regulated by JA/ET through early activation of BmJPR68 during interaction with pathogen and could be play a role in managing the *R. solani* infection process.

Accumulation of PR-proteins in Bhut Jolokia plants infected with R. solani

To investigate the accumulation pattern of pathogenesis-related proteins (PR-proteins) during pathogen interactions with the pathogen, the expression levels of specific proteins, i.e., catalase1, WRKY47, PR10A, and PDF1.2 were examined under four different treatments *viz*., mock (water-treated plants), Rs (plants treated with pathogen), BmJPR68 (plants treated with bacteria, BmJPR68), and BmJPR68 + Rs (plants treated with both bacteria and the pathogen). Protein extracts were subjected to electrophoresis and then transferred to nitrocellulose membranes, followed by probing with specific antisera targeting different PR-proteins. Protein extracts from leaves of *Arabidopsis thaliana* L. (Ecotype Columbia-O) served as positive controls. A distinct pattern of PR-protein accumulation was observed in Bhut Jolokia plants inoculated with both Rs and BmJPR68 + Rs. Notably, when compared with the positive control, the accumulation of Catalase1 protein was found to be at greater levels in the BmJPR68 + Rs treatment than in mock plants. PR10A was expressed at higher levels in Rs treated and BmJPR68 + Rs treated plants, but, mock and BmJPR68 treated plant leave extracts also contained very low levels of these proteins or didn't contain altogether. Consistent with the accumulation pattern, WRKY47 and PDF1.2 exhibited elevated expression levels in BmJPR68 + Rs treated plants compared to Rs treated plants (Fig. 4k). These findings showed minimal accumulation of PR proteins in mock and BmJPR68 treated plants compared to those treated with Rs. However, plants treated with BmJPR68 + Rs exhibited the highest levels of protein expression.

Activities of the nitrogen metabolism-associated enzymes under pathogen stress

Nitrogen metabolism is known to reduce the production of reactive oxygen species (ROS) and maintain various physiological processes, including hormone homeostasis and cellular metabolism levels. The enzymes involved in nitrogen metabolism, namely NR, NiR, GS, GR, GOGAT, GSSG, GSH and GDH, were assayed in freshly harvested mature leaves. The protein was measured from all of the enzyme extracts. All the assays were performed in 10 replicates. Activity of the enzyme had been defined as µmol of product formed per mg protein. In shoots, nitrate was reduced to nitrite by the cytosolic enzyme NR. BmJPR68 + Rs plants exhibited the highest NR activity, while mock plants showed the lowest, irrespective of N level and plant tissue. To understand the cumulative modulation of nitrate transport and reduction, we also measured nitrate content in shoots. We compared them with mock plants known to have low nitrate content levels in Rs treated plants. To investigate whether NO also regulates this rate-limiting step in nitrogen assimilation, the NR activity was measured in leaves and compared with mock plant. The plants treated with BmJPR68 showed significantly decreased NR activity, whereas those treated with BmJPR68 + Rs demonstrated increased NR activity. Nevertheless, Rs significantly diminished NR activity, and both treatments exhibited a similar pattern in which a higher N level leads to increased NR activity (Fig. 5). This may be due to the plants probably being in an adaptive phase under stress. These findings indicate an inhibitory effect of Rs on NR activity. Like NR activity, NiR activity also showed a significantly higher in Rs and BmJPR68 + Rs treated plants, while a reduction in NiR activity was observed in mock plants (Fig. 5a-b). Accordingly, the nitrate levels in induced plants were measured, and a significantly elevated N content was recorded in BmJPR68 + Rs plants as compared to the Rs stressed plants alone (Fig. 5c). For GS and GR activity, the four treatments showed a significant difference in shoots, with the highest in mock as compared to Rs stressed plants (Fig. 5d-e). The GOGAT activity was similar in mock and Rs stress plants (Fig. 5f). In general, GDH had similar trends to the above in the mentioned four treatments. Additionally, the difference was also visible between Rs and mock plants. Similarly, there was no significant difference noted in glutamate dehydrogenase (GDH) activity in the mock plants (Fig. 5g). Different treatments had significantly influenced the endogenous GSH level. The Rs stress also had a significant effect on the GSH level. Under Rs stress, the oxidized form of glutathione (GSSG) markedly increased to 32.7- 44.37 µmoles g^{-1} FW, while the BmJPR68 inoculation of GSH significantly reduced the GSSG levels in the Rs plants (Fig. 5h). After BmJPR68 treatment, glutathione content increased markedly by 31.15 µmoles g^{-1} FW increased after Rs stress, in comparison to the mock plants. The endogenous GSH content rose when BmJPR68 was inoculated on Rs stress plants (Fig. 5i). The GSH+GSSG levels were lower in the Rs treated plants, while BmJPR68 + Rs significantly increased (Fig. 5j). The differences between the Rs treated and mock plants were not significant for majority of the tissue's enzymatic activities, suggesting that BmJPR68 maintains the function of N-assimilating enzymes by alleviating the adverse effects of pathogen stress on N metabolism.

BmJPR68 regulates transcript levels of Bhut Jolokia shoots involved in nitrogen uptake/assimilation

Nitrogen assimilation commences with the uptake of nitrate through both low and high-affinity transport systems, where the *NRT1.1* and *NRT2.1* transporter genes play key roles (Akhtar et al. 2024). We assessed the expression of these genes in shoots of Bhut Jolokia plants. The gene expression profiles of genes associated with N-uptake are shown in Fig. 5k-r, where the results are shown as relative fold changes in treated plants compared to mock (control) plants. Following 30 days of pathogen treatment, the expression of genes, viz. *NR, NiR, NRT1.1, NRT1.2, NRT2.1, NRT2.2* were up-regulated when compared to the corresponding mock plants.

In BmJPR68 + Rs, the expression levels of NR and NiR were significantly increased compared to mock plants, by 8.3-fold and 6-fold, respectively (Fig. 5k-l). The NR transcript levels in the BmJPR68 treated plant (1.2-fold) were not significantly different from the mock plant (1.1-fold) (Fig. 5k). In the Rs treatment plants, *NiR* levels were found to be 0.9-fold, while mock plants displayed a similar expression with 1.1-fold (Fig. 5l). The transcript level of *NRT1.1* was found to be elevated in BmJPR68 + Rs plants by 3.8-fold, but notably, the transcript level significantly dropped in only Rs treated plant by 0.9-fold (Fig. 5m). Plants treated with BmJPR68 displayed no any significant differences when compared to the mock (water treated) plants. Similar expression was recorded in genes *NRT1.2*, *NRT2.1*, and *NRT2.2*, where plants treated with BmJPR68 + Rs exhibited higher expression relative to the mock plants, with increased of 7.6-fold, 9.1-fold, and 2.7-fold, respectively (Fig. 5n-p). The transcript level of the gene *GSH* in Rs treated plants was upregulated by 5.2-fold compared to a 1.5-fold increase in the mock treatment (Fig. 5q). The *GS* gene was significantly upregulated in BmJPR68 + Rs with 2-fold compared to mock (1-fold) plants (Fig. 5r). These findings suggest that BmJPR68 elevated the levels of genes and enzymes associated with N metabolism, leading to a switch from high- to low-affinity nitrate transport. Once taken up into the root via shoot, nitrate is primarily transported to the shoots where it is assimilated at the expense of photosynthetic reducing power.

Bioactive compounds and element analysis in Bhut Jolokia fruits

Antioxidant compounds (total phenolics and flavonoids), antioxidant potential (DPPH, SRSA, HRSA, ABTS radical scavenging assay), and *β*-carotene levels of induced Bhut Jolokia fruits showed significant differences across the treatments (Fig. 5s-z). Since the role of phenolic compounds as natural antioxidants linked to chili quality, the total phenolic (TPC) contents in Bhut Jolokia extracts were assessed. TPC contents ranged between 68.4–83.6 mg GAE g^{1} of the fresh weight (FW) Bhut Jolokia sample. The TPC in both Rs and BmJPR68 + Rs, plant fruits were significantly higher, measuring 82.2 and 83.3 mg GAE g^{-1} FW, compared to mock fruits (68.4 mg GAE g^{-1} FW). However, only the Bhut Jolokia fruits treated with BmJPR68 exhibited no significant difference compared to the mock fruits (Fig. 5s). The TFC level in the chili extracts treatments varied between 8.4 to13.4 mg QE g^{-1} FW (Fig. 5t). The TFC content of BmJPR68 + Rs fruits had 13.4 mg GAE g^{-1} FW, which was significantly ($p \leq 0.05$) greater TFC compared to the mock fruits. The antioxidant activities in different Bhut Jolokia treatments were determined by DPPH, SRSA, HRSA, and ABTS assays. Chilies treated with BmJPR68 + Rs exhibit the highest DPPH inhibition (24.7%), in contrast to the mock treatment at 10.0% (Fig. 5u-w). Only pathogen stressed fruits showed significantly higher DPPH level compared to the BmJPR68 treated chillies, recording 20.0% and 12.8% respectively ($p \leq 0.05$). No significant difference was found between the DPPH activity of mock and BmJPR68 treated Bhut Jolokia. Similar results also observed in SRSA, HRSA, and ABTS (Fig. 5v-x). Ascorbic acid levels were significantly increased under pathogen stress (Fig. 5y). Conversely, β carotene levels exhibited a decrease of 1.41 µg g^{-1} FW in Rs treated fruits compared to mock fruits (2.1 µg g^{-1} FW).

Fruits of Bhut Jolokia subjected to various treatments were analyzed using atomic absorption spectroscopy (AAS) to quantify the content of nine different elements (Table 3). The concentrations of macro elements like Ca, K, Mg, and Na were expressed in mg g^{-1}. The highest Na concentration among the treatments was observed in Rs treated fruits at 4.17 mg g^{-1}, compared to BmJPR68 treated and control (mock) fruits, which had concentrations of 3.85–4.1 mg g^{-1} respectively. However, Mg content was similar in both BmJPR68 and mock-treated plants i.e., 0.063–0.064 mg g^{-1}, with minor differences observed in fruits treated with the pathogen. The highest levels of K, Ca, and Mn were found in plants treated with BmJPR68, measuring 2.9 mg g^{-1}, 7.25 mg g^{-1}, and 0.036 mg g^{-1} respectively. Thus, microelements in Bhut Jolokia samples were also observed, and the lowest Fe content was recorded in fruits treated with BmJPR68 + Rs, showing 0.063 mg g^{-1} compared to the mock fruits (0.1mg g^{-1}). The highest Mn content was found in BmJPR68 treated plants at 0.036 mg g^{-1}, while the lowest Cu content was detected in Rs treated fruits (0.0057 mg g^{-1}). The distribution of these elements across Bhut Jolokia samples showed slight differences. The total carbon and total nitrogen contents in the fruits were measured, the highest carbon content was found in fruits from BmJPR68 + Rs treated plant, which had 24.06 mg g^{-1}, while the lowest was in the pathogen treated fruits, i.e. 12.57 mg g^{-1}. The highest total nitrogen content found was in BmJPR68 + Rs treated fruits at 4.24 mg g^{-1}, with no significant differences observed in mock (water treatment), Rs (pathogen treatment), and BmJPR68 treated fruits.

Quantification of capsaicinoids in the fruits

The major capsaicinoids, *viz.* capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin and homodihydrocapsaicin, were quantified in both the mock and treated fruits using UHPLC (Table 4). Capsaicin, the primary and most pungent capsaicinoid was observed to drastically reduce in the infected fruits (Rs: 249.43 µg g^{-1}), compared to the mock plant (1659.18 µg g^{-1}). Most significantly, treatment with the rhizobacteria, BmJPR68 could elevate the capsaicin content in both mock or infected fruits, producing 2066.32 and 1886.45 µg g^{-1} in BmJPR68 and BmJPR68 + Rs fruit samples, respectively. The level of dihydrocapsaicin (mock: 801.8 µg g^{-1}), significantly decreased in the infected fruits (Rs: 221.29 µg g^{-1}), whereas it was nearly maintained in BmJPR68 + Rs (790.56 µg g^{-1}), but increased by ~ 2.9 times in BmJPR68 (2287.96 µg g^{-1}). Other minor capsaicinoids largely exhibited a similar trend, significantly decreasing in the infected fruits which improved upon BmJPR68 treatment (Fig. S2).

Analysis of fatty metabolites in the fruits

GC-MS analysis identified twelve fatty acids (in the form of methyl ester) and one long-chain hydrocarbon in the Bhut Jolokia extracts. The constituents were characterized by the mass spectral library hits and their retention index values (RIs) (Fig. S3). The relative abundance of individual constituents has been recorded in the Table 5. The fatty acids including myristic acid, 13-methyltetradecanoic acid, pentadecanoic acid, 14-methylpentadec-9-enoic acid, 14-methylpentadecanoic acid, palmitoleic acid, palmitic acid, margaric acid, linoleic acid, oleic acid, stearic acid and arachidic acid were identified in all the samples. The qualitative GC-MS profile was largely comparable among the mock, infected and treated samples. Palmitic acid (22.9–25.1%), linoleic acid (20.7–43.6%) and oleic acid (15.2–20.1%) were the dominant the fatty acids present, together sharing 64.6–83.4% of the total relative area. They were followed by myristic acid (1.1–3.8%), 13-methyltetradecanoic acid (0.7–4.6%), 14-methylpentadec-9-enoic acid (2.9–6.3%), 14-methylpentadecanoic acid (1.6–3.1%), palmitoleic acid (1.9–5.7%) and stearic acid (2.9–4.1%). The other constituents were found in minor abundance (< 2.0%). The findings indicated that *R. solani* infection did not significantly affect the qualitative fatty acid profile in Bhut Jolokia.

Plant growth promoting and induced systemic resistance in field condition

In field conditions, BmJPR68 significantly ($p \leq 0.05$) improved various plant growth parameters like plant height, number of branches, stem diameter, leaf per plant, root length, flowers per plant, and biomass (both fresh and dry weight) of Bhut Jolokia. Out of the four treatments, BmJPR68 demonstrated the most significant growth metrics, achieving a plant height of 90.5 cm, total branches numbering 30.6 per plant, stem diameter of 1.28 cm, total leaf count of 295 per plant, root length of 48.82 cm, total flowers at 69 per plant, fresh weight of 591 g, and dry weight of 357 g (across ten replicates). These results were significantly better than the mock (water-treated) plants (Fig. 6). The effect of BmJPR68 was notably more prominent throughout the field trial. In this study, applying BmJPR68 to the soil resulted in enhanced growth for Bhut Jolokia plants. However, plants inoculated with pathogen (Rs) showed the lowest measurements across all growth factors: plant height 47.3 cm, branches per plant 15, leaves per plant 42, root length 24.2 cm, and total flower count. The

fresh and dry weights for each plant were 222.2 g and 34.5 g, respectively, which were significantly less than the weights of water-treated plants, which were i.e. 511.6 g and 149 g, respectively (Fig. 6). Notably, the plants treated with BmJPR68 + Rs showed significantly ($p \leq 0.05$) improved Bhut Jolokia plant growth, demonstrating a height of 83 cm, 28 branches per plant, 292 leaves per plant, a root length of 43.92 cm, 53 flowers per plant, and fresh and dry weights of 591.2 g and 187 g, respectively, in contrast to the water-treated plant group. The plants treated with BmJPR68 showed a significantly higher biovolume index than the mock plants (Table 6). However, the plant treated with BmJPR68 + Rs showed an increase in biovolume index in comparison to the mock plant. The results confirm that in the field, BmJPR68 promotes growth and enhances resistance to collar rot caused by *R. solani* when applied as a single inoculum. The results indicated that BmJPR68 shows significantly improves plant growth and can be suggested as a growth-promoting rhizobacteria for Bhut Jolokia.

In general, BmJPR68 significantly reduces the collar rot disease (Gogoi et al. 2024). BmJPR68 showed a significant effect on collar rot disease during the 3rd, 6th, and 8th weeks after *R. solani* inoculation. During the first week of observation, the PDI for collar rot disease in all examined plants was 0, indicating that none of the plants exhibited any signs of the disease. Starting from the third week, the observations revealed a growing intensity of collar rot in the plants. Upon examining the disease, it became clear that the overall PDI showed a consistent weekly increase. Throughout this study, plant growth was monitored and assessed on a weekly basis until reaching maturity. The inoculation of the pathogen (Rs) on BmJPR68 pre-treated plants (BmJPR68 + Rs) resulted in a significant reduction ($p \leq 0.05$) of up to 28.41% in PDI. Plants inoculated with Rs exhibited the highest PDI at 64.6%. The average PDI was not found in control plants (those treated with water), and in BmJPR68 treated plants (Table 6). The results indicated a 28.4% reduction in disease, and no disease symptoms were observed in collar rot infection due to BmJPR68-triggered resistance in Bhut Jolokia plants during the entire study duration (Table 5.3). In plants treated with mock (water) and BmJPR68, the area under the disease progress curve (AUDPC) was not observed, whereas those treated with Rs recorded the highest AUDPC value of 1994.5. The plants treated with BmJPR68 + Rs exhibited a significantly reduced ($p \leq 0.05$) AUDPC of 871.95 in comparison to the plants treated with Rs alone. This suggests that BmJPR68 effectively controlled collar rot disease in Bhut Jolokia plants.

Bhut Jolokia yield attributes under field conditions

Compared to seedlings treated with water, those treated with BmJPR68 yielded more fruits per plant when grown in soil, as indicated by the data Fig. 6i-q. Among the all treatments, only BmJPR68-treated plants produced the highest fruit count per plant (29.3 nos.), while pathogen-treated plants (Rs) exhibited the lowest fruit count per plant (11.9 nos.). However, the BmJPR68 + Rs treated plants showed significantly greater results compared to the mock plant. Thus, the impact of BmJPR68 treatments was markedly greater under Rs stress in the field experiment. The yield and, consequently, the returns will rise with the quantity of fruits produced by each plant. Consequently, the yield and the number of fruits per plant are directly connected. The plants that treated with BmJPR68 through soil application yielded more fruit in the current studies. This could be a result of the availability of the ideal nutrient levels for plants to attain various stages of growth. According to Fig. 6i-k, it can be inferred that BmJPR68 applications to the soil resulted in increased ripe fruit weight (g) in Bhut Jolokia plants compared to the control (mock plant). Of the four treatments, the plants treated with BmJPR68 produced the heaviest ripe fruit (276.8 g), while those treated with BmJPR68 + Rs yielded 171.8 g, and the Rs treated plants had the least fruit weight at 17.5 g. Additionally, in comparison to the mock plant fruits, the dry weight of the fruits from the BmJPR68-treated plant was markedly greater at 103.94 g (Fig. 6l). The Rs treated plant had a lower dry weight of fruits (8.28 g). Throughout the trial, the effect of BmJPR68 treatments was significantly higher. According to the results of the present studies indicate that the treatment of Bhut Jolokia plants with BmJPR68 substantially increased the weight of their mature fruit. Additionally, ten randomly chosen fruit weights were collected from each treatment, showing that fruits from BmJPR68 treated plants were weightier than those from mock plants, while reduced fruit weights were observed in Rs treated plants. The data presented in Fig. 6m revealed that treatments with BmJPR68 resulted in an increase of fruit length (cm) in comparison to the mock (4 cm). Among the various treatments, the longest fruit length of 5.6 cm was recorded only in BmJPR68, while the shortest was observed in Rs treated fruits at 3.3 cm. Likewise, it can be inferred that BmJPR68 treatments significantly enhanced fruit width (cm) compared to mock (Fig. 6n). The highest fruit width of 4.01 cm was recorded in only plants treated with BmJPR68, while the smallest was observed in pathogen-treated plants (2.5 cm). Additionally, it was found that the fruits from BmJPR68-inoculated plants exhibited a maximum pericarp thickness of 1.55 mm and a minimum of 1.06 mm for Rs (Fig. 6o). The greater value of peri-carp thickness of BmJPR68 was demonstrated in comparison to the mock plant. The data analysis presented in Fig. 6p indicated that BmJPR68 treatments enhanced the seed count per fruit when compared to the untreated control. Of all the treatments, only fruits treated with BmJPR68 had up to ≈21 seeds per fruit, whereas those treated with Rs had a minimum weight of 5.25 g.

Nutrient content, total NPK uptake and carbon (C) content of induced Bhut Jolokia plants

The nutrient levels in the leaves, stems, and roots of Bhut Jolokia plants were greatly influenced by the BmJPR68 treatment. Compared to the mock, the plants treated with BmJPR68 exhibited the highest total nitrogen content (118.2, 27.5, and 51.03 mg g^{-1} for the leaf, stem, and root, respectively). Similarly, BmJPR68 treated plants had higher P contents in their leaves, stems, and roots (2.96, 2.79, and 5.86 mg g^{-1}) than mock plants. A comparable pattern was noted in K content as well. On the other hand, plants treated with Rs had the lowest levels of N (82.13, 20.1, and 41.35 mg g^{-1}in the leaf, stem, and root, respectively), along with reduced quantities of P and K. The highest total NPK uptake per plant occurred in the BmJPR68 treatment (64.39 mg g^{-1} per plant), while the lowest was in Rs treated plants (43.56 mg g^{-1} per plant). Notably, in comparison to mock plants, those plants treated with BmJPR68 + Rs exhibited an elevated NPK content. Overall, as shown in Table 7, the BmJPR68 treatment significantly enhanced the total NPK uptake in Bhut Jolokia plants, while the Rs treated plants exhibited the lowest absorption levels. Moreover, the organic carbon content in leaves, stems, and roots of Bhut Jolokia plants was higher in only BmJPR68 treated plants as compared to mock ones (Table 8). However, the BmJPR68 + Rs treated plants showed only a slight increase in carbon content when compared to the mock. These results suggest that the application of BmJPR68 improved nutrient levels in the plant and enriched macronutrient properties of the soil.

Organic carbon content, available macronutrients and micronutrients of soil

After the harvesting of Bhut Jolokia crop, soil samples were collected from each plot at a depth of 0–15 cm to assess the effect of different organic inputs on organic carbon, available nitrogen (N), phosphorus (P), potassium (K), and the micronutrients copper (Cu), iron (Fe), zinc (Zn), and manganese (Mn) using an AAS (Lindsay and Norvell 1978). The BmJPR68 treatment raised the total NPK concentration by 6.95, 2.01, and 0.82 mg g^{-1}, respectively, while the levels of Cu, Fe, Zn, and Mn were lower in soils treated with BmJPR68, i.e. 0.0035, 6.28, 0.078, and 0.15 mg g^{-1}, respectively. On the other hand, plants treated with Rs exhibited marginally increased levels of heavy metals and decreased the levels of NPK, measuring 0.68 and 0.56 mg g^{-1}, when compared to the soil prior to inoculation. Soil organic carbon levels were influenced by different organic inputs, with a significant increase observed in BmJPR68-treated soil, while the lowest levels were recorded in Rs treated soils (Table 8). Additionally, organic carbon contents of the soil were found to be higher following inoculation of BmJPR68 + Rs. Significant differences were also observed in the combined data on treatment effect and interactions. The results suggest that the BmJPR68 treatment considerably enhanced the availability of both macronutrients and micronutrients in the soil.

Pearson's Correlation among the variables of plants and principal component analysis (PCA) of soil after BmJPR68 inoculation

The scatter matrix revealed a noteworthy positive correlation among plant height, the number of branches number, total leaf count, plant width, root length, fresh weight, dry weight, biovolume index, the quantity of fruits and flowers, fruit weight per plant, length of fruit, width of fruit, pericarp thickness, and seed count per fruit. It showed a significant negative correlation with soil pH, indicating that as the bacterial population increased. Similarly, BmJPR68 exhibited a strong positive correlation with the parameters of plant growth (Fig. 7a). Principal component analysis also revealed a positive and significant correlation with soil NPK and extractable cations like Zn, Cu, Fe, and Mn, whereas a negative correlation was observed with Fe content (Fig. 7b).

Discussion

Rhizospheric microbes play a key role in promoting vegetative growth, development and reducing pathogen invasion through soil and foliar attacks (Rizvi et al. 2024). Plant growth-promoting rhizobacteria are well-recognized for preventing phytopathogen entry by reinforcing mechanical tissues such as the cell wall, callose, and lignin deposition and stimulating the production of defense-related enzymes and reactive oxygen species (ROS), ultimately leading to induced systemic resistance (Yadav et al. 2021; Sadhana et al. 2024). The oxidative burst, characterized by the rapid production of H_2O_2 and O_2^-, indicated an early defense response in plants against various pathogens (Lee et al. 2020). In this support, we observed histochemical analysis of DAB and NBT staining in Bhut Jolokia leaves during *R. solani* infection, where the accumulation of H_2O_2 and O_2^-, was visible as dark brown and blue coloration on the leaf surface post-inoculation. These precipitates appeared more distinct in leaves challenged by pathogens, particularly in those treated with BmJPR68 + Rs in comparison to untreated and mock plants. Though the role of ROS in defense systems is well-documented, the processes underlying ROS generation and the ROS-regulated signaling pathways in host plants remain only partially understood (Ali et al. 2018). To counteract excessive ROS, plants establish an antioxidant defense mechanism (Kapoor et al. 2019). The findings of this study align with earlier results, showing increased levels of antioxidant defense enzymes such as LOX, CAT, β-glucanase, and proline following host-pathogen interactions. Additionally, defense-related genes like *PR1, PR3, LOX, TPX*, and *CRT* were upregulated in response to pathogen infection compared to mock plants. The present study also examines the PR-related protein in plants that have been induced following pathogen infection. The accumulation of these PR-proteins was greater in the pathogen (Rs) and BmJPR68 + Rs treated plants compared to the mock plants, which is the first report regarding Bhut Jolokia plants.

The BmJPR68 primed with Bhut Jolokia plants exhibited a significant reduction in lesion development and disease severity, in both *in vivo* and *in vitro* conditions. The findings indicate that ISR demonstrated resilience, with collar rot lesion development being significantly reduced in plants treated with BmJPR68 *+* Rs infection compared with those treated solely with Rs. In this study, the decline in enzyme activities involved in nitrogen metabolism under pathogen stress from *R. solani* could explain for the observed reductions. Ability of plants to assimilate nitrogen, synthesize proteins, and regulate overall nitrogen metabolism depends profoundly on the activity of key enzymes associated with these processes (Zayed et al. 2023). Consequently, the reactions catalyzed by these enzymes are vital for plant growth and development (Gaudinier et al. 2018; Rizwan et al. 2022). Our findings confirmed that under pathogen stress, the activities of NR, NiR, GS, GOGAT, and GDH in Bhut Jolokia plants significantly declined (Fig. 5a, b, e, f, g). This reduction in enzyme activity likely leads to decreased protein synthesis due to impaired nitrogen assimilation (Frungillo et al. 2014). The lack of substrates triggers a cascade of events that further reduce the activity of specific enzymes involved in nitrogen metabolism (Ashraf et al. 2018; Fagard et al. 2014). However, it has been shown that under stressful conditions, plant biostimulants can boost the activity of key enzymes involved in nitrogen metabolism (Hao et al. 2024). The present study highlights that applying BmJPR68 to Bhut Jolokia plants under stress significantly enhanced the activity of enzymes related to nitrogen metabolism. These findings indicate that BmJPR68 inoculation may help maintain consistent nitrogen assimilation by upregulating essential enzyme activities, thereby alleviating the negative effects of *R. solani* stress on nitrogen metabolism. The increased activity of these important enzymes and genes after BmJPR68 inoculation likely aids in enhanced protein synthesis.

The present study also examines the bioactive compounds of fruits from induced plants *i.e.*, phenolic, flavonoids, DPPH, SRSA, HRSA, ABTS ascorbic acid and *β* carotene that possess antioxidant capabilities against free radicals and ROS. However, soluble extracts exhibited a higher content of phenolics and flavonoids than the bound counterpart (da Silva et al. 2024). The highest quercetin, ascorbic acid, and *β*-carotene contents were observed in the fruits, whereas BmJPR68-treated plants have been shown to possess more antioxidant bioactive compounds such as *β*-carotene, quercetin, and other antioxidant activities that are effective scavenging ROS and free radicals. A higher content of phenols and flavonoids, along with higher ascorbic acid content as a non-enzymatic antioxidant system, participates in cellular ROS scavenging (Kozlov et al. 2024). Capsaicin and its related compounds are known as capsaicinoids which are phenolic compounds found in the fruits of the Capsicum genus (Srivastava et al. 2024). Capsaicin and dihydrocapsaicin are the predominant capsaicinoids found in hot peppers (Mahmood et al. 2021). The present investigation reveals that the levels of capsaicinoids (capsaicin, dihydeocapsaicin, homocapsaicin, homodihydrocapsaicin, and nordihydrocapsaicin) were elevated in Bhut Jolokia fruits treated with BmJPR68 compared to the mock, along with their rapid identification and separation from Bhut Jolokia. In our study on capsaicinoid accumulation in the Bhut Jolokia plant via induced systemic resistance (ISR), we explored the influence of phenolic intermediates on capsaicinoid biosynthesis. For instance, it has been shown that 8-methylnonenoic

acid plays a significant regulatory role in the capsaicinoid biosynthesis pathway (Narasimha et al. 2006). Capsaicinoid accumulation coincides with the reduction of flavonoids and the increased accumulation of lignin-like substances (Materska and Perucka, 2005). Additionally, lower pungency levels do not correspond to higher flavonoid concentrations compared to those with higher pungency. The biosynthesis of flavonoids may occur alongside capsaicinoid synthesis within the phenylpropanoid pathway, where each regulates the synthesis of the other in distinct ways.

Volatile organic compounds (VOCs) are closely associated with the flavor and fragrance of food, and their analysis is crucial for assessing food quality, authenticity, purity, and origin (Ko et al. 2014). Methyl and ethyl esters impart strong fruity notes to foods, while terpenes contribute woody, floral, fruity, and spicy aromas. Several aliphatic esters have been identified in the *C. chinense* Jacq. variety (Pino et al. 2011; Murakami et al. 2019). It has been established that esters, particularly straight-chain esters, are typically synthesized from fatty acids through oxidation, while branched saturated and unsaturated esters can originate from the metabolism of amino acids.

Promoting seedling growth is essential to achieve optimal plant development in field conditions, making PGPR treatment a significant agricultural method (Cumming, 2009). Numerous PGPRs are known for boosting plant growth via different mechanisms, including improved nutrient uptake, hormone synthesis, and pathogen suppression (Saikia et al. 2018). To achieve these effects, PGPR must effectively colonize plant roots, which requires establishing sufficient bacterial populations to exert beneficial impacts (Abou Jaoudé et al. 2024). Although PGPR treatments have shown promising results in controlling plant pathogens in laboratory and greenhouse studies, but field results have been inconsistent (Saharan and Nehra, 2011). However, in this study, BmJPR68 showed significant effectiveness in controlling collar rot, one of the most destructive diseases of Bhut Jolokia. These findings could facilitate the development of effective and economically viable management strategies against collar rot under field conditions. The agronomic benefits of BmJPR68 were further confirmed by improved plant growth characteristics, such as increased branch and leaf production, which contribute to the overall health and productivity of Bhut Jolokia plants. Enhancing the development of stems and roots, aided by BmJPR68, was crucial for the nutrient absorption and the distribution of nutrients in reproductive organs, resulting in increased fruit yield. The weight of the 10 fruits of the treated plants was significantly higher than the control, suggesting that BmJPR68 had a positive effect on the development of the fruit. This increase in fruit weight can be attributed to the improved root system development, which enhances nutrient uptake and ultimately increases crop yield (Cumming, 2009). The findings of this research underscore the potential of BmJPR68 to improve fruit weight, thereby enhancing agricultural productivity and sustainability. The yield of Bhut Jolokia plants is closely linked to vegetative growth, as indicated by increased plant biomass, including leaf, root, and stem weight. The presence of BmJPR68 as a biological agent during the vegetative growth phase appears to enhance Bhut Jolokia yields, suggesting that using BmJPR68 could reduce the reliance on inorganic fertilizers. The highest total NPK uptake by plants was recorded in BmJPR68 treated soil, displaying a significant increase in comparison to mock treatment. These results are in agreement with the study of Alwan et al. (2024), which also reported increased nutrient uptake by chilli plants following foliar treatment with PGPR. A significant increase in soil organic carbon was observed with the application of organic management practices, particularly BmJPR68. This increase in organic carbon is likely due to the addition of organic matter, which enhances the soil carbon levels by providing a source of carbon and energy for microorganisms, thus promoting their rapid proliferation in the soil (Dang et al. 2024). Additionally, the rise in organic carbon could be attributed to increased root biomass and plant residues, along with the application of organic manures (Dhaliwal et al. 2024). In contrast, the lower organic carbon content in Rs treatment could be due to a reduced amount of added nutrients compared to other treatments. The available N, P, and K content increased due to the addition of various organic amendments in combination with the recommended nutrient doses and PGPR application. Furthermore, the highest percent increases in micronutrients such as Fe, Mn, Zn, and Cu were recorded in the Rs treatment. The increase in micronutrient content in Bhut Jolokia could be linked to BmJPR68 application, which supplied a balanced and sufficient quantity of nutrients at critical growth stages like flowering and fruiting. These results align with the findings of Yildirim et al. (2011), who reported that both macronutrient and micronutrient uptake in plants was significantly enhanced by PGPR application. Similarly, Mia et al. (2010) demonstrated that PGPR promotes nutrient absorption in wheat from the rhizospheric soil. Our study demonstrated that BmJPR68 promoted the growth of young Bhut Jolokia plants and effectively managed collar rot disease in field conditions, a major problem for this crop. To our knowledge, this is the first report on the effectiveness of BmJPR68 in managing collar rot in Bhut Jolokia in field conditions.

Conclusions

This study demonstrates that the application of BmJPR68 activates the antioxidant defense system specifically LOX, CAT, *β*-glucanase, proline, reduces ROS levels, and enhances the activity of nitrogen metabolism-related enzymes and genes in Bhut Jolokia plants under *R. solani* stress. This is the first report showing that ISR triggered by BmJPR68 increases pungency (capsaicinoid content) and the accumulation of bioactive compounds, including pathogenesis-related (PR) proteins, in Bhut Jolokia in response to biotic stress. Notably, BmJPR68 also elevated the pungency of the fruit under pathogen stress. Moreover, our research emphasizes the need for deeper investigation into the molecular mechanisms underlying BmJPR68, particularly its role in nutrient uptake to improve plant resilience against different pathogens. Field application of BmJPR68 on Bhut Jolokia significantly improved plant growth, yield parameters, soil organic carbon levels, and availability of macro- and micronutrients compared to the control treatment. Its application led to a significantly improvement in both plant growth and soil health. Therefore, to increase Bhut Jolokia productivity and maintain soil health, the proposed nutrient management module incorporating BmJPR68 can be recommended as an effective approach for suppressing collar rot disease under field conditions. These results suggest that BmJPR68 offers a sustainable and cost-effective strategy to reduce crop losses and increase yield under pathogen stress. The development and application of eco-friendly technologies like biofertilizers are vital for addressing global warming, and this study provides valuable insights into improving crop productivity through sustainable means.

Declarations

Declaration of competing interest

The authors declare that they have no knowledge of any competing financial or personal relationships that could appear to influence the work reported in this paper. The authors further state that they possess no conflicting interests.

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Data availability

The data supporting this study are available within the article and its supplementary materials.

CRediT authorship contribution statement

Priyanka Gogoi: Methodology, Data curation, Formal analysis, Visualization, Validation, Software, Writing-original draft, writing review & editing; Parthiv Kar: Data curation; Saikat Haldar: Visualization, Formal analysis; Writing review & editing; Ratul Saikia: Conceptualization, Project administration, Funding acquisition, Supervision, Writing review & editing.

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Tables

Table 1 Influence on plant length and biomass of seedling by *Bacillus megaterium* JPR68 under pathogen stress. Seedlings remained to germinating at 30 °C in MS media. Water-soaked seeds were served as a mock; pathogen or *R. solani* treated served as Rs. Each experiment was repeated three times, and in every repeat 10 seeds were used in glass bottles. Different lowercase letters on the bars indicated a significant difference, as determined by Tukey test ($p \leq 0.05$). All data points were means ± SD (n = 3). Mean values and SDs at least for three biological replicates are shown.

Treatment	Seedlings length (cm)		Seedlings weight (g)	
	Root	Shoot	Fresh	Dry
Mock	11.4±3.97^{ab}	11.6±3.84 ^{ab}	2.113±0.62 ^{ab}	0.1766±0.04 ^{ab}
Rs	8.4±3.4^{b}	5.2±0.83 ^{c}	1.648±0.34^{b}	0.1242±0.01 ^{b}
BmJPR68	16.6±2.4^{a}	15.4±1.14 ^{a}	2.908±0.61^{a}	0.232±0.05 ^{a}
BmJPR68+Rs	8.6±5.54^{b}	9.4±1.34 ^{b}	2.7±0.56 ^{a}	0.202±0.07 ^{ab}

Table 2 Disease severity index and survival rate of Bhut Jolokia plant in different treatment under pot experiment. Different lowercase letters on the bars indicated a significant difference, as determined by Tukey test ($p \leq 0.05$). All data points were Means ± SD (n = 5). Mean values and SDs for five biological replicates. (Mock- water treated; Rs- pathogen *R. solani* treated plants).

-

Treatment	DSI (%)	Survival (%)
Mock	NA	100[a]
Rs	86.36±1.4[a]	32.4[b]
BmJPR68	NA	100[a]
BmJPR68+Rs	9.6±0.5[b]	98.2[ab]

Table 3 Macro and micro element contents of induced Bhut Jolokia. Results are expressed as mean ± SD (n = 3). Letter represents significance among the treatments (Tukey test).

Treatment	Na	K	Ca	Mg	Fe	Cu	Zn	Mn	P
Mock	**mg g^{-1}**								
	4.12±0.04[a]	2.37±0.018[c]	0.24±0.01[b]	0.064±0.001[a]	0.1±0.002[a]	0.0079±0.0002[a]	0.071±0.006[a]	0.029±0.009[b]	2.37±0
Rs	4.17±0.01[a]	1.99±.01[d]	0.3±0.017[b]	0.061±0.001[d]	0.086±0.002[b]	0.0057±0.007[b]	0.027±0.0005[d]	0.017±0.001[d]	1.99±.(
BmJPR68	3.85±0.02[b]	2.9±0.011[a]	7.25±0.272[a]	0.063±0.01[c]	0.074±0.005[c]	0.0076±.004[a]	0.058±0.0004[b]	0.036±0.003[a]	2.9±0.(
BmJPR68+ Rs	3.8±0.04[b]	2.7±0.03[b]	0.18±0.024[b]	0.064± 0.001[b]	0.06±0.001[d]	0.0078±0.002[a]	0.035±0.0004[c]	0.023±0.002[c]	2.7±0.(

Table 4 Various capsaicinoids concentrations in induced Bhut Jolokia fruits under pathogen stress. Results are expressed as mean ± SD (n = 3). Letter represents significance among the treatments (Tukey test) (FW-Fresh weight; ND-not detected).

Treatment	Absolute	Semi-quantification			
	Capsaicin	Dihydrocapsaicin	Nordihydro capsaicin	Homocapsaicin	Homodihydr ocapsaicin
	µg g^{-1} of FW				
Mock	1659.18±18.7[c]	801.8±10.4[b]	153.54±9.48[a]	80.82±10.7[a]	54.19±0[a]
Rs	249.43±1.83[d]	221.29±1.22[c]	51.8±0.91[c]	24.32±1.2[c]	ND
BmJPR68	2066.32±2.44[a]	2287.96±2.4[a]	118.48±1.53[b]	41.21±1.8[bc]	19.35±0.3[c]
BmJPR68+Rs	1886.45±40.0[b]	790.56±4.8[b]	147.48±0.30[a]	50.08±1.5[b]	48.79±0.91[b]

Table 5 The identified fatty metabolites (esterified) through GCMS in the extracted Bhut Jolokia samples along with their Match, R Match, retention indices (RI), and relative percentage values (Mock-water treated, Rs-pathogen treated, BmJPR68-bacteria treated, BmJPR68+Rs-bacteria and pathogen treated plant fruits); ND- not detected; NR- not recorded.

R_t	Constituents	Relative % (GC-MS)							
		Match	R Match	RI_{Lib}	RI_{Calc}	Mock	Rs	BmJPR68	BmJPR68+Rs
14.85	Myristic acid, methyl ester	895	915	1725	1728	1.09	1.33	2.25	3.77
15.53	13-Methyltetradecanoic acid, methyl ester	869	904	1779	1789	0.72	0.65	2.11	4.61
15.92	Pentadecanoic acid, methyl ester	933	934	1820	1822	1.28	0.69	1.12	1.81
16.40	14-Methylpentadec-9-enoic acid, methyl ester	892	910	NR	1862	5.50	2.84	6.33	5.79
16.60	14-Methylpentadecanoic acid, methyl ester	850	871	1883	1878	3.01	1.61	2.15	3.05
16.79	Palmitoleic acid, methyl ester	919	922	1898	1894	2.36	1.92	3.28	5.72
17.05	Palmitic acid, methyl ester	926	928	1926	1915	24.85	22.85	24.48	25.10
18.46	Margaric acid, methyl ester	883	900	2028	2033	1.17	0.54	0.95	1.29
19.31	Linoleic acid, methyl ester	961	962	2092	2107	38.28	43.59	30.76	20.74
19.38	Oleic acid, methyl ester	857	893	2091	2114	15.16	16.94	20.07	18.74
19.65	Stearic acid, methyl ester	924	929	2128	2137	3.47	4.07	3.48	2.86
21.43	Arachidic acid, methyl ester	874	942	2329	2334	ND	ND	0.31	0.27
24.76	*n*-Heptacosane	813	865	2700	ND	1.24	0.75	0.52	0.46

Table 6 Effect of *Bacillus megaterium* JPR68 on biovolume index of Bhut Jolokia plant under field conditions and plant disease index (PDI) and the area under disease progress curve (AUDPC) for both untreated and treated plants. Different lowercase letters on the bars indicated a significant difference, as determined by Tukey test ($p \leq 0.05$). All data points were means ± SD (n = 3). Mean values and SDs at least for three biological replicates are shown. (Mock- water treated; Rs- pathogen *R. solani* treated plants).

Treatment	Biovolume index (BI)	PDI	AUDPC
Mock	71.3 ± 25.6^{b}	0	0
Rs	24.5 ± 8.45^{c}	64.6^{a}	1994.5^{a}
BmJPR68	116.29 ± 17.66^{a}	0	0
BmJPR68+Rs	91.725 ± 23.1^{b}	28.413^{b}	871.95^{b}

Table 7 Effect of BmJPR68 on N, P and K content in Bhut Jolokia plants. Different lowercase letters on the bars indicated a significant difference, as determined by Tukey test ($p \leq 0.05$). All data points were means ± SD (n = 3). Mean values and SDs at least for three biological replicates are shown.

Treatment	N			P			K		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
	mg g^{-1}								
Mock	86.8 ± 3.72^{c}	24.3 ± 1.8^{a}	43.8 ± 1.49^{b}	2.01 ± 0.024^{d}	2.6 ± 0.03^{a}	$5.15 \pm 0.002^{b}5$	27.22 ± 0.98^{a}	23.54 ± 4.7^{a}	0.78±0
Rs	82.13 ± 1.6^{c}	20.1 ± 0.4^{b}	41.35 ± 1.9^{b}	2.0 ± 0.0049^{c}	2.29 ± 0.0028^{b}	4.49 ± 0.005^{d}	39.091 ± 6.24^{a}	17.16 ± 3.7^{a}	11.02±
BmJPR68	118.26 ± 1.64^{a}	27.5 ± 2.2^{a}	51.03 ± 1.26^{a}	2.96 ± 0.09^{b}	2.79 ± 0.23^{a}	$.86 \pm 0.004^{a}$	27.93 ± 4.9^{a}	26.75 ± 5.06^{a}	4.115±
BmJPR68+Rs	100.26 ± 0.39^{b}	23.7 ± 0.95^{ab}	48.6 ± 2.32^{a}	3.19 ± 0.019^{a}	1.47 ± 0.007^{b}	4.65 ± 0.002^{c}	28.779 ± 4.7^{a}	16.49 ± 3.9^{a}	3.337±

Table 8 Effect of BmJPR68 on macro and micronutrients levels in soil.Different lowercase letters on the bars indicated a significant difference, as determined by Tukey test ($p \leq 0.05$). All data points were means ± SD (n = 3). Mean values and SDs at least for three biological replicates are shown.

Soil treatment	Macronutrient			Micronutrient				Organic Carbon
	N	P	K	Cu	Zn	Mn	Fe	
	mg g^{-1}							
Mock	2.74±0.48^{b}	0.43±0.001^{d}	0.659±0.01^{b}	0.0037±0.009^{b}	0.059±0.008^{d}	0.53±0.006^{c}	4.19±0.027^{c}	9.97±0.88^{b}
Rs	2.2±0.06^{b}	0.68±0.002^{c}	0.56±0.03^{b}	0.0048±0.0045^{b}	0.071±0.001^{c}	0.97±0.01^{c}	3.99±0.035^{d}	12.61±0.84^{b}
BmJPR68	6.95±0.71^{a}	2.01±0.004^{b}	0.82±0.078^{b}	0.0035±0.009^{b}	0.078±0.002^{b}	0.15±0.005^{b}	6.28±0.012^{a}	14.985±1.4^{a}
BmJPR68+Rs	7.77±0.087^{a}	1.8±0.002^{a}	0.82±0.07^{a}	0.0092±0.0022^{a}	0.099±0.002^{a}	0.146±0.003^{a}	5.62±0.035^{b}	23.8±1.4^{a}

Figures



Figure 1

Phenotypic assessment of Bhut Jolokia plant following *R. solani* stress. (a) *In vitro*disease induction in Bhut Jolokia plant, scale bar, 1cm, (b) *In vivo* (pot condition) in Bhut Jolokia plant following pathogen stress. Scale bar, 5 cm. (c) yield of Bhut Jolokia in different treatments for each plant, (d) morphology of fruits randomly collected from each treatment (10 samples: fruit length, width, thickness). scale bar, 2 cm.

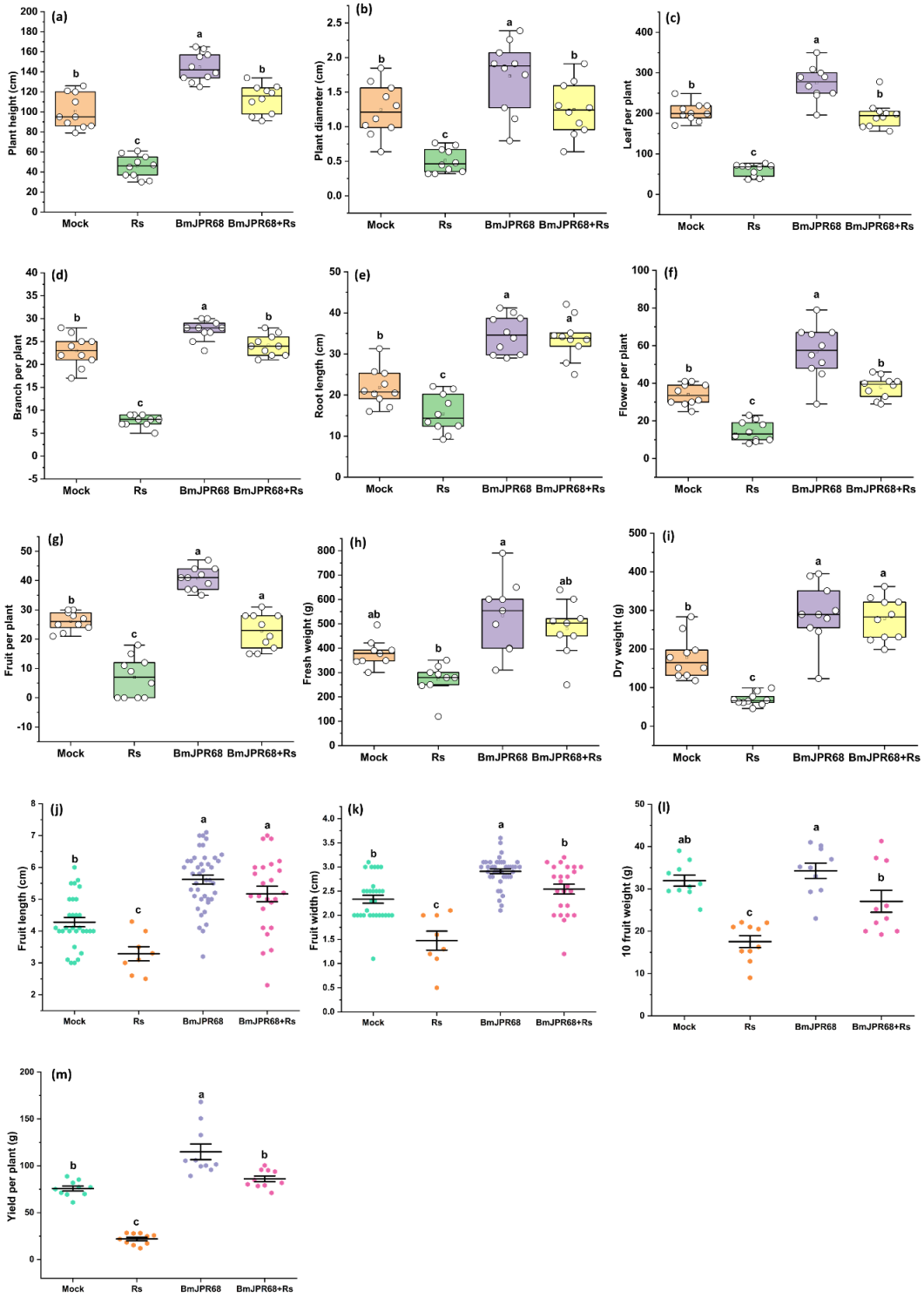

Figure 2

Effects on different physiological parameters on Bhut Jolokia plants subjected to the pathogen stress. Traits associated with yield - (a)plant height, (b) diameter of main stem, (c) number of leaves, (d) counts of branches, (e) roots length, (f) quantity of flowers, (g) counts of fruit, (h) fresh weight of plant, (i) plant dry weight. Statistical analysis of fruit morphology including (j) fruit length, (k) fruit width, (l) weight for 10 fruits, and (m) total yield per plant. Values are means ± SDs (n = 10 independent fruit). Different letters represent significant differences at $p \leq 0.05$ determined by Tukey's multiple comparisons test.

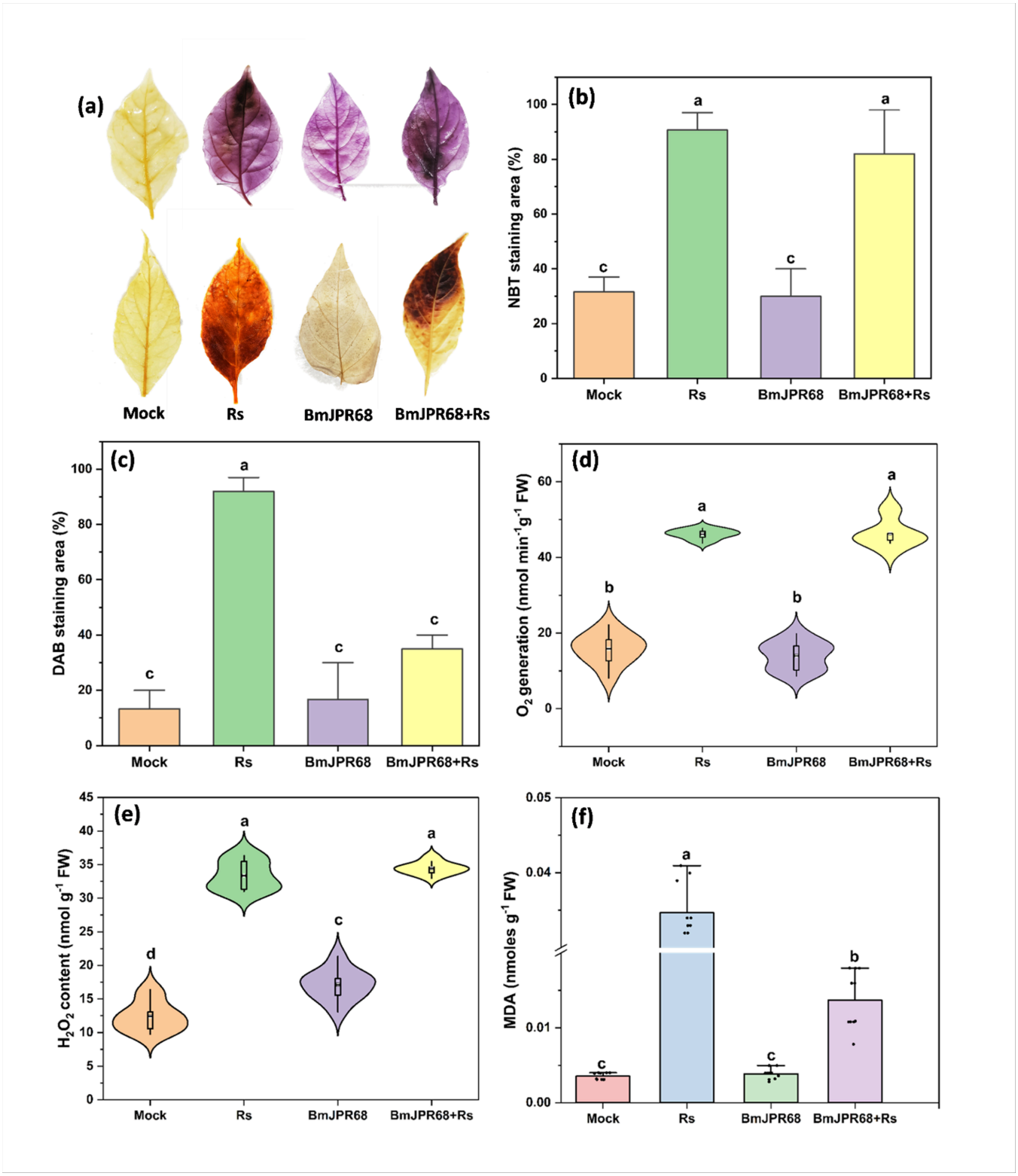

Figure 3

Effect of physiological indexes of ROS accumulation and MDA production in Bhut Jolokia plants following *R. solani*inoculation. (a) Visualization of leaf with NBT and DAB staining. (b) NBT staining area (%) of Bhut Jolokia leaves characterized after 3-days of inoculation with *R. solani*. (c) DAB staining area (%) of Bhut Jolokia leaves charactered after 3 days of inoculation with *R. solani* after. (d) Superoxide anion production in chilli leaves; (e-f) Hydrogen peroxide and malondialdehyde (MDA) production in Bhut Jolokia leaves. Mock (water treated plant); Rs (pathogen treatment); BmJPR68 (*B. megaterium* treated); BmJPR68+Rs (BmJPR68 pretreated seed then 30 days after inoculation with the pathogen, *R. solani*. Different lowercase letters on the bars indicated a significant difference, as determined by Tukey's multiple comparisons test ($p \leq 0.05$). All data points were means ± SD (n = 10). Mean values and SDs at least for three biological replicates are shown.

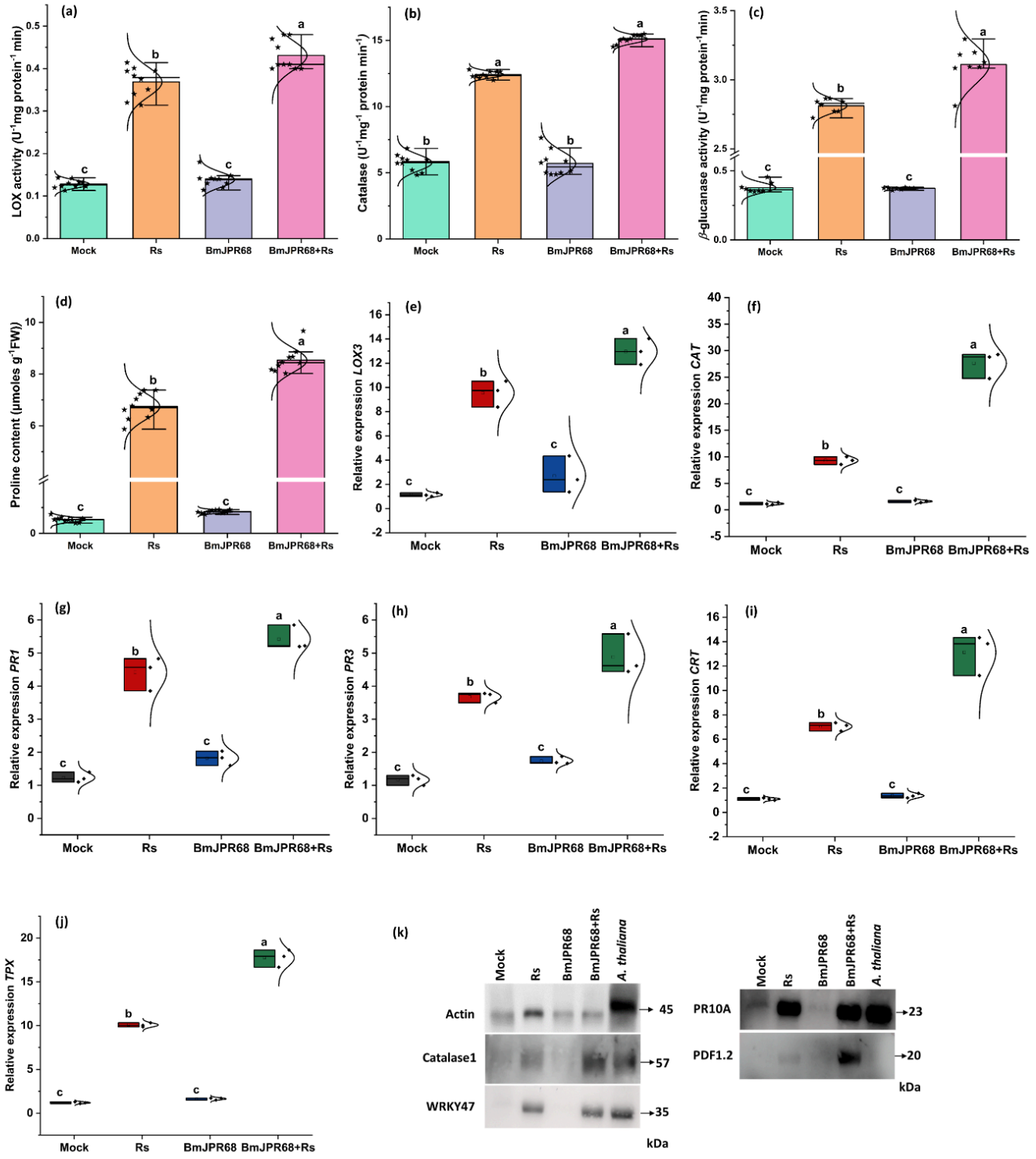

Figure 4

Induction activity of antioxidant enzyme involved in plant defense, transcript levels of pathogenesis-related (PR) genes, and western blot analyses of pathogenesis-related (PR) protein associated with plant defense. Twelve samples were analyzed for each replication, and each treatment consisted of five replications. (a) lipoxygenase (LOX) activity, (b) catalase, (c) β- glucanase activity, (d) proline activity of Bhut Jolokia. Treatments: Mock- uninoculated (water treatment) plants, Rs- only pathogen, *R. solani* treated plants, BmJPR68- *B. megaterium* JPR68 treated plants, BmJPR68 + Rs - BmJPR68 pre-treated plant then 7-day after challenged inoculation with the pathogen, *R. solani*. Transcript levels of pathogenesis-related (PR) genes involved in plant defense through ISR in Bhut Jolokia under *R. solani* (Rs) stress. The expression analysis of (e) *PR1*, (f) *PR3*, (g)*LOX3*, (h) *CAT*, (i) *TPX* and (j) *CRT.*Transcript level of BmJPR68 pretreated Bhut Jolokia plant then days after challenged inoculation with the pathogen. Uninoculated plants were used as a mock. Data were normalized to the Actin housekeeping genes. (k) targeted protein band of Catalase1, WRKY47, PR10A and PDF1.2 under pathogen stress, each lane was loaded with 100 mg of protein. *Arabidopsis thaliana* L. Ecotype Columbia-O, was used as positive control. Protein size given in kilodalton (kDa). Each gel was blotted and

reacted with a different stress-specific protein antibody; Values are mean with SD of three independent experiments, each including ten biological replicates. Statistical significance was determined by Two-way ANOVA followed by Tukey's post-test, $p \leq 0.05$. Means with the same letter are not significantly different.

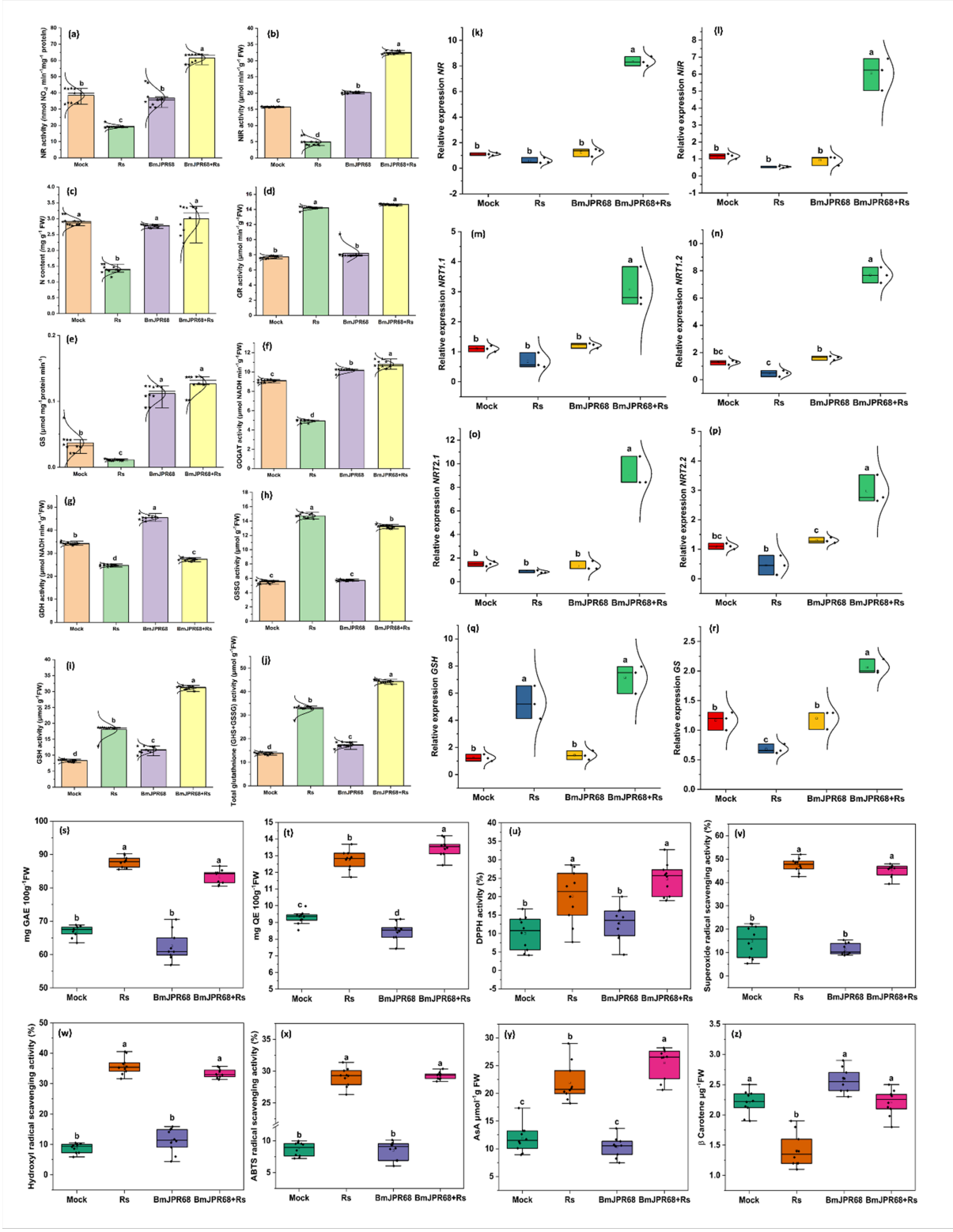

Figure 5

Levels of various enzymes related to N metabolism in shoots, including NR, NiR, N content, GR, GS, GOGAT, GDH, GSSG, GSH, and total glutathione, were assessed in Bhut Jolokia plants under pathogen stress, as well as the impact on bioactive compound activity in Bhut Jolokia fruits exposed to *R. solani* pathogen stress. Treatments: Mock- water treated plants, Rs- pathogen, *R. solani* treated plants, BmJPR68- *B. megaterium* JPR68 treated plants, BmJPR68 + Rs: BmJPR68 pretreated plant 7-days after challenged inoculation with the pathogen, *R. solani* (Rs). Actin housekeeping genes used as endogenous control. Data represent means ± SD from three independent experiments (n=3) with ten biological replicates. Statistical significance was determined by Two-way ANOVA followed by Tukey's post test, $p \leq 0.05$. Means with the same letter are not significantly different. Relative expression levels of key genes related to

nitrogen assimilation. NRT marker genes nitrate reductase (*NR*), nitrite reductase (*NiR*), *NRT1.1*, *NRT1.2*, *NRT2.1*, *NRT2.2*, glutamine synthetase (*GS*), and glutamate synthase (*GOGAT*), Reduced glutathione (GSH) after treatment with BmJPR68 and its combination with pathogen *R. solani*. Expression values were calculated relative to the expression of plants grown in mock (water treated plants) under normalization with Actin gene ($2^{-\Delta\Delta CT}$). Values are mean with SD of three independent experiments, each including three biological replicates. (s) Phenol, (t) Flavonoids, (u) DPPH, (v) Superoxide radical scavenging activity, (w)Hydroxyl radical scavenging activity, (x)ABTS radical scavenging activity, (y)Ascorbic acid, and (z) *β* carotene. Values are mean with SD of three independent experiments, each including ten biological replicates. Statistical significance was determined by Two-way ANOVA followed by Tukey's post-test, $p \leq 0.05$. Means with the same letter are not significantly different.

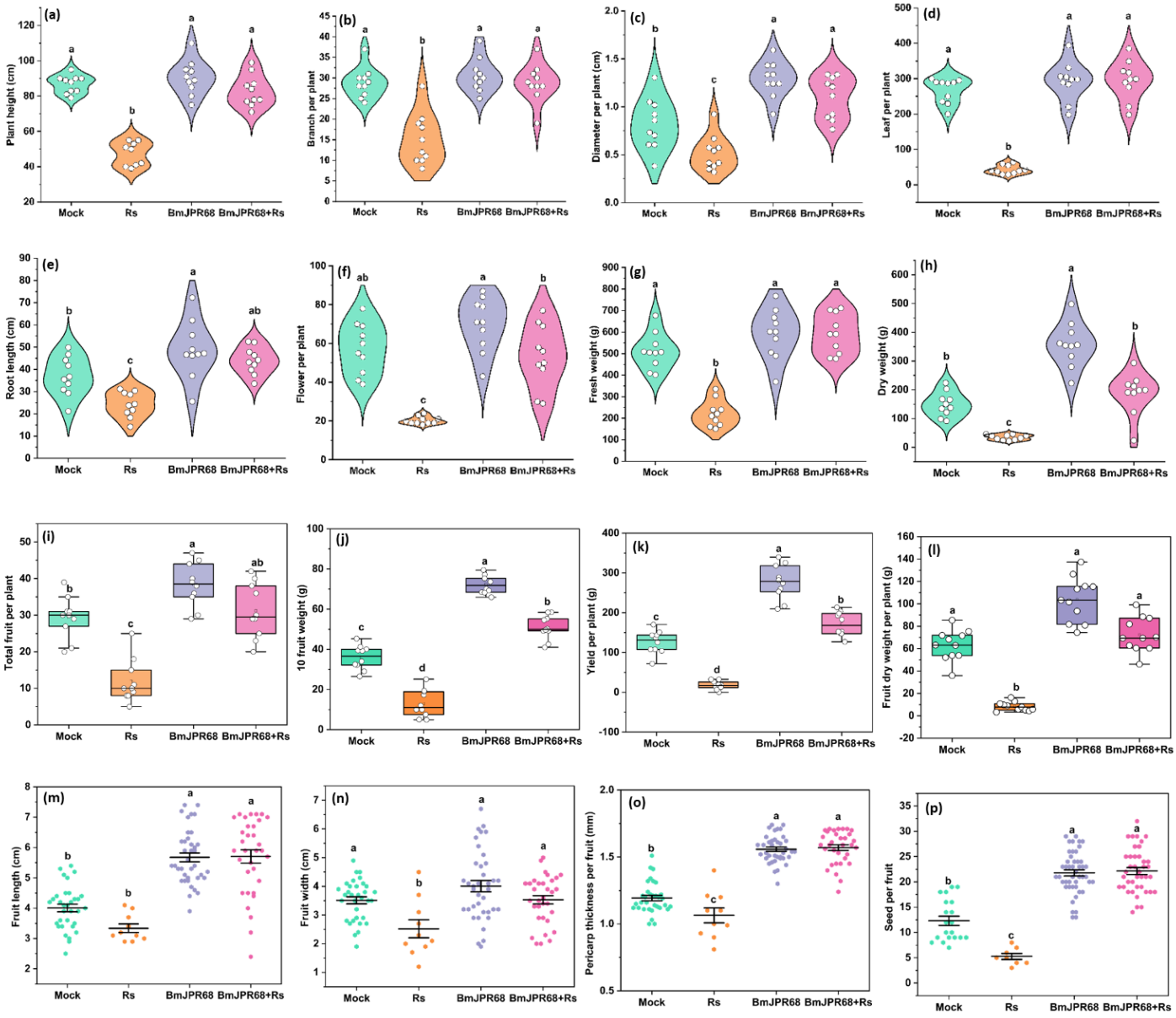

Figure 6

Effect of *Bacillus megaterium* JPR68 (BmJPR68) on growth enhancement and yield of Bhut Jolokia plants under *R. solani* (Rs) infection in field conditions. (a) plant height; (b) branch per plant; (c) stem diameter of per plant; (d) leave per plant; (e) root length; (f) flower per plant; (g) fresh weight of plant; (h) dry weight of plant; (i) total fruit per plant; (j) weight of 10 fruits; (k) yield per plant; (l) fruit dry weight per plant; (m) fruit length; (n) fruit width per plant; (o) pericarp thickness; (p) seed per fruit. Measurements were taken at the apex of all plants subjected to four treatments. Measurements were taken on the top of all plants in all four treatments. Graph represents the average of 10 biological replicates ± SD. Statistical significance was tested by Two-way ANOVA followed by Tukey's post-test. Different letters represent significant differences at $p \leq 0.05$ determined by Tukey's multiple comparisons test.

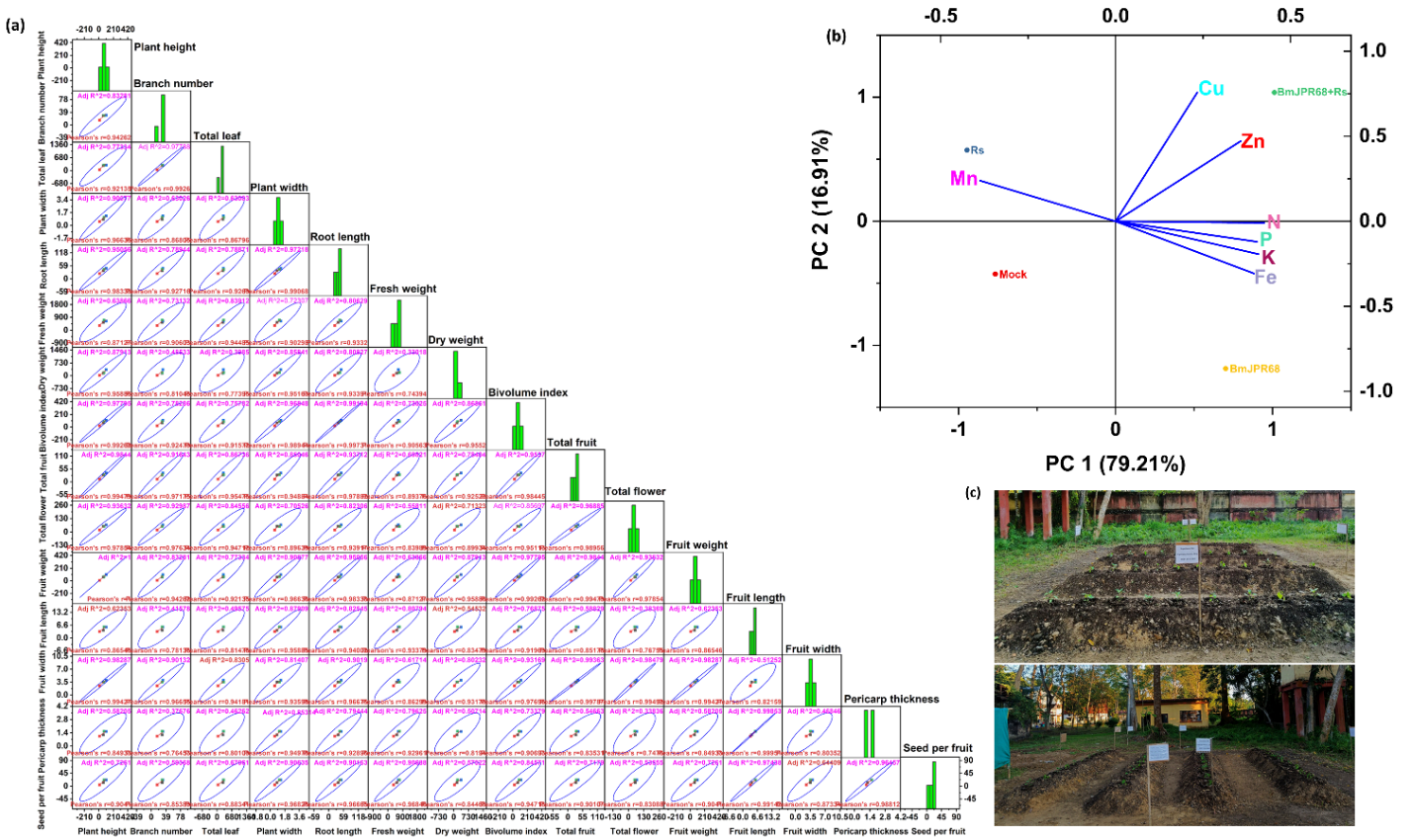

Figure 7

Scatter matrix plot and principal component analysis of (PCA) plants and soil following BmJPR68 treatment in field condition. Scatter matrix plot of Pearson's correlation analysis of different parameters of plants after BmJPR68 treatment, whereas the fifteen parameters are showing significant observations that all the treatments had a positive impact on the increase of yield and mitigation of biotic stress and the increase in fecundity; (b) Principal component loading plots and scores of PCA of different parameters of soil indicate BmJPR68 treatment; (c) a view of field at CSIR-NEIST, Jorhat, Assam.

Supplementary Files

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