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Zinc oxide nanoparticles and *Klebsiella* sp. SBP-8 alleviates chromium toxicity in *Brassica juncea* by regulation of antioxidant capacity, osmolyte production, nutritional content and reduction in chromium adsorption

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ABSTRACT

Heavy metals are one of the most damaging environmental toxins that hamper growth of plants. These noxious chemicals include lead (Pb), arsenic (As), nickel (Ni), cadmium (Cd) and chromium (Cr). Chromium is one of the toxic metal which induces various oxidative processes in plants. The emerging role of nanoparticles as pesticides, fertilizers and growth regulators have attracted the attention of various scientists. Current study was conducted to explore the potential of zinc oxide nanoparticles (ZnONPs) alone and in combination with plant growth promoting rhizobacteria (PGPR) *Klebsiella* sp. SBP-8 in Cr stress alleviation in *Brassica juncea* (L.). Chromium stress reduced shoot fresh weight (40%), root fresh weight (28%), shoot dry weight (28%) and root dry weight (34%) in *B. juncea* seedlings. Chromium stressed *B. juncea* plants showed enhanced levels of malondialdehyde (MDA), electrolyte leakage (EL), hydrogen peroxide (H_2O_2) and superoxide ion ($O_2{\bullet}^-$). However, co-supplementation of ZnONPs and *Klebsiella* sp. SBP-8 escalated the activity of antioxidant enzymes i.e., superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) in *B. juncea* grown in normal and Cr-toxic soil. It is further proposed that combined treatment of ZnONPs and *Klebsiella* sp. SBP-8 may be useful for alleviation of other abiotic stresses in plants.

1. Introduction

Various abiotic stresses impose deleterious effects on growth of plants (Akram et al., 2020; Al-Huqail et al., 2020). These abiotic stresses include heavy metals which deteriorates soil structure, thereby leading to decreased growth of plants. Various anthropogenic activities contribute to addition of heavy metals into environment. Heavy metal pollution has increased in our environment due to various agricultural, industrial and mining-related activities (Vareda et al., 2019). This increase in heavy metal toxicity in our environment is a challenging factor for sustenance of ecosystem balance (Xu et al., 2020). The toxicity of heavy metals substantially affects the growth and yield of many crops. Heavy metals alter numerous physiological and biochemical processes in plants (Baweja and Kumar, 2020). Various effective mitigation strategies are required to overcome the hazardous effects of heavy metals (Al-Huqail et al., 2020).

Heavy metals must be eliminated from the human food chain through economically efficient and environmentally sustainable methods (Carolin et al., 2017). Hexavalent chromium (Cr^{6+}), although not essential for plant development, and is recognized as second most resilient element in Earth's crust (Soni et al., 2023). The presence of excessive Cr in soil is a serious threat to various cash crops (Adimalla et al., 2019). Chromium can alter chloroplast membrane structure and hinders electron transport in the mitochondria. This disrupt the light as

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well as dark reaction of the photosynthesis (Ahmad et al., 2020; Sharma et al., 2020; Prasad et al., 2021; Zaheer et al., 2022). Additionally, Cr^{6+} binds to heme group of cytochrome and disrupt electron transfer during light reaction (da Conceicao Gomes et al., 2017). Chromium has the potential to elicit cytotoxic effects in plants, thereby leading to disturbance in nutrient uptake and cell division processes (Prasad et al., 2021). The primary effect of heavy metals on plant is induction of oxidative stress resulting in generation of reactive oxygen species (ROS). Chromium stress generates ROS, which attack cell membrane and cause lipid peroxidation (Mansoor et al., 2023). As a result, MDA content also rise up in plant cell. In severe cases, higher MDA content may cause cell dysfunction or even cell death (Yalcinkaya et al., 2019). Chromium induces detrimental effects on cellular proteins and DNA, causing chromosomal aberrations. Anomalies in chromosome behavior are identified as the primary factor contributing to disturbance in physiological processes in plants (Wakeel et al., 2020). Additionally, Cr disrupts the performance of enzymes crucial for both carbon dioxide fixation and the electron transport system (Wakeel et al., 2020). The cytotoxic effects of Cr negatively impact crucial enzymes necessary for the biosynthesis of chlorophyll (Ashraf et al., 2021). Plants have evolved multiple defense strategies to mitigate the toxic effects of Cr, like improved antioxidant activities and the conjugation of metal ions. Nevertheless, excessive Cr stress can adversely affect various morpho-physio-biochemical processes, leading to hindrance of overall growth and development in plants (Gong et al., 2017). Extended exposure to Cr stress will lead to disturb nutrient absorption and impact metabolic processes in plants, resulting in cellular death (Javed et al., 2021).

Rhizospheric microorganisms with advantageous properties, specifically plant growth-promoting rhizobacteria (PGPR), play a pivotal role in fortifying plants against adverse conditions and fostering overall plant development. PGPR, identified as beneficial microorganisms, establish a presence in the plant rhizosphere, promoting plant growth through various mechanisms (Chamkhi et al., 2022; Nonthakaew et al., 2022). PGPR colonize root zone and makes nutrients available for the plants. Fix nitrogen and solubilize phosphorus in the soil making it available for root absorption. Besides this, PGPR release siderophores and phytohormones which enhanced nutrient availability and thus growth of the plants. Plant growth promoting bacteria also release volatile signaling molecules that enhance plant immunity towards pathogens and also increase plant's enzymes action involved in nutrient uptake, stress tolerance and other metabolic processes (de Andrade et al., 2023). PGPR actively contribute to nitrogen fixation (Smercina et al., 2019), produce siderophores to facilitate iron absorption, synthesize indole-3-acetic acid, and regulate auxin levels by utilizing tryptophan found in root exudates (Keswani et al., 2020; Duca and Glick, 2020). In addition, these microorganisms effectively reduce plant ethylene production by synthesizing ACC deaminase and offer defense against pathogenic attacks. PGPR plays a vital role in improving the bioavailability and mobility of heavy metals in soils. Consequently, this enhances their accessibility to plants, promoting an efficient phytoremediation process (Liu et al., 2022).

The *Klebsiella* sp. strain exhibited a notable capacity for heavy metal removal (Cd, Cr, Pb) in *Vigna radiata* L., leading to enhanced growth and biomass accumulation (Chakraborty et al., 2021). Gupta et al. (2020) documented that the introduction of *Klebsiella* sp. resulted in increased oxidative stress tolerance and improved development of tomato plants subjected to Cr-induced stress. The application of *Klebsiella* sp. also exhibited the role in enhancement of biomass of tomato seedlings grown in Cr-stressed conditions. Additionally, both strains of *Klebsiella* showed increased in activity of antioxidant enzymes (SOD, CAT, POD) associated with reduction of oxidative stress in tomato seedlings (Han et al., 2023). Nanotechnology encompasses the utilization of diverse methodologies and instruments to synthesize nanoparticles, demonstrating potential efficacy in mitigating diverse abiotic stresses and facilitating crop growth improvement. Zinc oxide nanoparticles appears to be a promising strategy in the field of plant science, demonstrating potential benefits for augmenting both plant growth and yield. ZnONPs when applied on suitable concentration can enhanced seed germination. Zinc oxide nanoparticles are also involved to increase chlorophyll content and photosynthesis. Besides this, ZnONPs can boost antioxidant activity, protein synthesis, nutrient uptake and antimicrobial activities inn plants. Hence, their application resulted in healthier seedling growth and development and in the end higher yield. Their stress alleviation property also enables to cultivate on a wider area, especially stress prone environment like heavy metals contaminated sites (Gauba et al., 2023). Depending on the size of ZnONPs and duration of exposure, it exhibits varying effects on plant growth and development. At low concentration and duration, ZnONPs proved beneficial for plants (Thounaojam et al., 2021). Numerous investigations elucidated that the size of NPs, dose and exposure period are pivotal factors that influence their effects on plants. Zinc oxide nanoparticles have been shown in recent studies to mitigate the deleterious effects of cadmium toxicity in *Zea mays* and *Triticum aestivum*. This improvement is linked to a reduction in oxidative damage that the plant tissues' enhanced antioxidant defense system was able to achieve (Khan et al., 2019; Rizwan et al., 2019). Zinc oxide nanoparticles have been applied to *B. juncea*., which has resulted in increased biomass production, improved antioxidant enzyme activity (Geremew et al., 2023), and a crucial contribution to membrane integrity and maintenance (Singh et al., 2021).

Nanoparticles such as silver, iron oxide, titanium dioxide, magnesium oxide etc. are extensively used as pesticides, fertilizers, growth regulators and abiotic stress reliever for plants. Besides, as of our knowledge, role of ZnONPs combined with *Klebsiella* sp. SBP-8 (PGPR) in the mitigation of Cr stress in *B. juncea* is not reported. We selected the most economically valued ornamental *B. juncea* to investigate its response to ZnONPs combined with *Klebsiella* sp. SBP-8 to determine their response towards the changes in morpho-physiological, biochemical attributes and Cr build-up under Cr toxicity. This research was designed to find out the protective role of ZnONPs and *Klebsiella* sp. SBP-8 for reducing the damaging impact of Cr toxicity in *B. juncea*.

2. Materials and methods

Seeds of *B. juncea* were obtained from Punjab seed center, Lahore, dated Aug. 2023. Seeds were surface sterilized through rinsing with 0.5% sodium hypochlorite solution. The experiment was conducted in controlled conditions. Mixed the soil with organic manure in 2:1. Following this, soil was sterilized at 121 °C for half hour. *Klebsiella* sp. SBP strains were obtained from Microbiology Lab, University of Punjab, Lahore, Pakistan. Bacterial inoculation was done according to Penrose and Glick (2003) with few modifications. Fresh culture was taken and added in sterile 0.03 M $MgSO_4$ (0.5 ml) and the solution was diluted to adjust absorbance to 0.15 at 600 nm. Sterile seeds were taken in petri plates and rinsed with bacterial suspension in the sterile 0.03 M $MgSO_4$ (0.15 OD at 600 nm). Following that bacterized seeds were air dried in aseptic conditions for an hour and then sown in plastic pots. For Cr contamination, $K_2Cr_2O_7$ was obtained from Sigma Aldrich and 1 mM solution was applied at ratio of 1 L per pot. Zinc oxide nanoparticles at conc. of 60 ppm were applied through foliar spray. The pots were maintained at 175 μmol m^{-2} s^{-1} and photoperiod of 16 h. One plant from each replicate was harvested after 15 days of treatment (foliar spray) to investigate immediate effect of treatments on morphological and growth attributes of *B. juncea* seedlings.

2.1. Synthesis of ZnO nanoparticles

Zinc sulfate (0.1 M, 2 L) solution was prepared and taken in round bottom flask. Following that the solution was kept in continuous stirring at room temperature. The same volume of sodium hydroxide (0.1 M) solution was prepared and added dropwise at the flow rate of 3 ml min^{-1}, in the round bottom flask having zinc sulfate solution, with continuous stirring. In this way, metal salt was reduced to free metal.

Awaited, for 30 min to ensure completion of the reaction. The precipitates were then obtained and filtered, washed with distilled water. The filtrate was then oven dried (105 °C, 7 h). The dried material was crushed into fine powder and heated at 600 °C in furnace for 5 h. The resulted material was again turned to fine powder.

2.2. Photosynthesis pigment estimation

0.5 g of fresh leaves were ground in liquid nitrogen using a pestle and mortar. After treating samples with 80% acetone, mixture was centrifuged (HERMLE Z 326 K Universal) for 5 min at 4500 rpm at 20 °C. Using a spectrophotometer (Hitachi U-2001 spectrophotometer Tokyo, Japan), the filtrate was measured at wavelengths of 470, 652, 665, and 750 nm in order to estimate the amounts of carotenoids, chlorophyll *a*, and chlorophyll *b*, followed by Vernon (1960).

2.3. Determination of leaf relative water content

For determination of leaf relative water content (LRWC), leaves were obtained from the middle of the branches. With tweezers leaves removed from the stem, to get the (FM) fresh mass of the leaves it immediately weighed. Clausen and Kozlowski (1965). A closed Petri dish was used to get the (TM) turgid mass of leaves, in it leaves were floated in distilled water for 3 h. To get the dry mass (DM), leaves were placed in pre heated oven, at 80 °C, for 48 h (Catsky, 1974a; Turner, 1981). An analytical scale was used having 0.0001 g precision. LRWC was calculated using the values of TM, DM, FM with this equation.

$$LRWC\ (\%) = [(FM - DM) / (TM - DM)] \times 100.$$

2.4. Estimation of malondialdehyde

Heath and Packer (1968) methodology was used to measure malondialdehyde (MDA). For this purpose, potassium phosphate dibasic and monobasic buffer (50 mM, pH 7.8, 25 ml), containing 1% polyethene pyrrole was taken and 0.1 g frozen plant leaf was grinded in mortar at 4 °C. Homogenate was centrifuged at 10,000×*g* for 15 min. After being heated to 100 °C for 15–30 min, the mixture was rapidly cooled in ice bath. A Hitachi U-2001 spectrophotometer (Tokyo, Japan) was used to measure the absorbance of the supernatant at wavelengths of 532 and 600 nm. The MDA content was calculated using the following equation.

$$\Delta\ (A\ 532\ nm - A\ 600\ nm) / 1.56 \times 105 = MDA\ level\ (nmol).$$

2.5. Determination of hydrogen peroxide

Hydrogen peroxide was extracted from plants followed by Patterson et al. (1984). After collection of fresh leaves were frozen in liquid nitrogen and grounded into powder with pestle and mortar, 0.15 g charcoal (to remove any potential interference from other compounds) and 5 ml of 5% trichloroacetic acid, TCA (to stop the reaction and precipitate proteins that might interfere with UV absorption) added in it. This mixture at 4 °C for 20 min centrifuged at 10, 000×*g*. Addition of 17 M ammonia it's pH maintained 8.4 and then filtered. The filtrate was divided into 1 ml aliquots. 8.1 g catalase were added and kept it for 10 min at room temperature. Both type of solution without and with aliquots, 1 ml colorimetric reagent were added. For 10 min, solution was incubated at 30 °C. Spectrophotometer were used to find the absorbance which were 505 nm.

2.6. Determination of electrolyte leakage

For determination of electrolyte leakage (EL), three week older aerial parts of plants were collected. Then with deionized water continuously rinsed and incubated for different periods of time in solution (5 × 10−3% (v/v) Silwet L-77) control with 50 μM MV. A conductor meter was used to check conductivity after the incubation period of time. For removing of electrolytes, it was subjected to autoclave at 120 °C for 30 min. Total percentages of electrolytes were expressed as EL. In triplicate the measurement was determined.

2.7. Determination of antioxidant enzymes

To find the catalase activity, the obtained extract was mixed in every well had 0,001% Antifoam 204 and 100 mM H_2O_2 with (50 mM K_2HPO_4/KH_2PO_4 buffer pH 7) solution. For quantification of the CAT enzymatic activity the coefficient 43.6 [$mM^{-1}*cm^{-1}$] were used. Hydrogen peroxide disappearance was determined by formation of curve of lope during absorbance for 40 min at 30 °C with 240 nm range.

2.8. SOD (superoxide dismutase) activity

In each well (50 mM K_2HPO_4/KH_2PO_4 buffer pH 7.8, 0.1 mM EDTA, 0.05 mM cytochrome *c*, 10 mM xanthine and 0.0002U/mg xanthine oxidase) were mixed with extract Cytochrome C. The inhibition was measured by the representation of the slope of curve. This were recorded by absorbance at 550 nm wavelength, 25 °C for 40 min. In control reaction the xanthine or extract were omitted (Khanom et al., 2000).

2.9. Determination of POD (peroxidase)

To determine POD, 4-methylcatechol used as substrate. The oxidation of 4-methylcatechol by hydrogen peroxide absorption were increased. This measured by spectrophotometer at 420 nm. In the reaction mixture sodium phosphate buffer (pH 7.0), 5 mM H_2O_2, 5 mM 4-methylcatechol, 500 μL of crude extract in a total volume of 3.0 ml at room temperature. One unit of enzyme activity (unit min^{-1} mg^{-1} protein) were defined as 0.01 change in absorbance per minute, under assay conditions (Onsa et al., 2004).

2.10. Determination of ascorbate

For ascorbate determination, ac-dipyridyl were used. For this 500 mg of bulk roots grounded in mortar for fine powder with liquid nitrogen. One mL of 2% metaphosphoric acid added for homogenized frozen powder. After this transferred into homogenized tubes (final volume were 2 ml). This mixture was centrifuged for 15 min at 4 °C at 22000 g. The reaction mixture was contained 0.6 ml of 0.3 M potassium (K) phosphate buffer (pH 7.4), 0.2 ml of sample, 5 mM EDTA and 0.2 ml of Milli Q water. The absorbance was read at 525 nm. A standard curve covering the range of 0–25 nmol ascorbic acid was used. Triplicate measurements were taken for all samples (Mansour et al., 2018).

2.11. Determination of glutathione

The total GSH contents were quantified using Ellman's (1959) technique. The approach is based on the reaction of 5,5-dithiobis-2-nitrobenzoic acid (DTNB) and Ellman's reagent with sulfhydryl compounds, which produces a yellow tint. An aliquot (3 ml) of 4% sulfosalicylic acid was added to 0.5 ml of the filtrate obtained by macerating the leaf tissue in phosphate buffer. The treated combination was properly reacted with the Ellman reagent. The OD readings were taken at 412 nm. The intensity of the yellow color is directly proportional to the GSH concentration.

2.12. Estimation of mineral nutrients

Paul et al. (2017) method was followed for sample digestion using HNO_3 and $HClO_4$ in 9:4 ratio. For the purpose 0.1 g sample taken in 100 ml digestion flask and 10 ml of HNO_3 were added in it and placed for overnight. Following that 8 ml of $HClO_4$ were added and the mixture was heated on hot plate until the fumes production ceases. The digestion is completed when the solution become colorless. Cool the solution and added 20 ml of distilled water. After filtration the solution was used for determination of mineral nutrients using flame photometer (Sherwood model 360).

2.13. Estimation of gene expression

The method of Ali et al. (2022) was followed to quantify the anti-oxidant gene expression level using qRT-PCR. The Qiagen Rneasy Plant Mini kit (Hilden, Germany) was used for RNA isolation from the sample plant and to remove contamination. To do RT-PCR, 1 μg of RNA was reverse transcribed using oligo (dT) primers using a RevertAid First Strand cDNA Synthesis Kit (Fermentas GmbH, St. Leon-Rot, Germany) as per the manufacturer's instructions. Based on earlier research, gene-specific primers were generated and utilized in an RT-PCR reaction to amplify cDNA of target and reference genes of interest. RT-PCR was performed in triplicate on the Thermal Cycler, following the manufacturer's instructions. The PCR amplification settings were 95 °C for 10 min, followed by 40 cycles of 15 s at 95 °C and 60 °C, respectively. Melting curve analysis was used to assess amplification specificity; the curve was run from 55 °C to 95 °C to confirm the amplification of a single amplicon. qRT-PCR data was normalized using cycle threshold (CT) values, and relative expression levels were obtained.

2.14. Statistical analysis

CoStat software was used for analysis of variance (ANOVA). For mean compare, Tukey's HSD test was performed at $p < 0.05$ significance level. Graphs were made using MS excel. Pearson's correlation and principle component analysis were performed using R-software.

3. Results

3.1. Characterization of zinc oxide nanoparticles

Ultraviolet–visible spectroscopy, FTIR Spectroscopy, XRD, SEM and EDX techniques were entertained for characterization of prepared NPs.

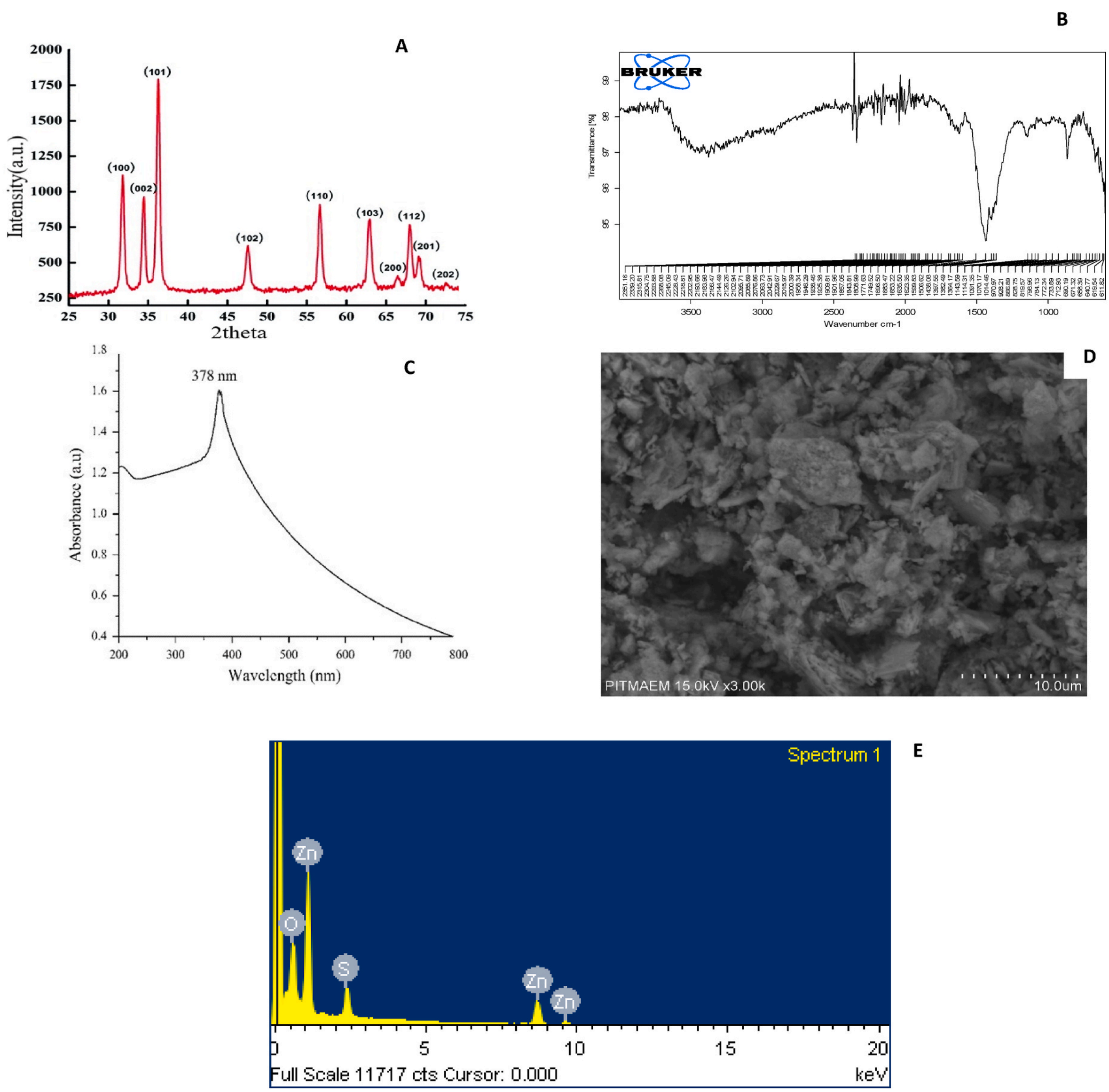

Fig. 1. XRD pattern of zinc oxide nanoparticle (A), FTIR spectra of zinc oxide nanoparticle (B), UV-VIS spectra of zinc oxide nanoparticle (C), SEM micrograph of zinc oxide nanoparticle (D), EDX graph of zinc oxide nanoparticle (E).

3.1.1. X-ray diffractogram

XRD pattrens shown in Fig. 1A represents the crystallization of ZnONPs. The diffraction in the figure support the synthesis of ZnONPs. Definite broadening of line in XRD peaks depicts that the prepared sample is of nanoscale. When compared with standard database, the obtained results depict the formation of ZnONPs as shown by the peaks at 32.00, 34.20, 36.93, 47.51, 57.12, and 69.40 corresponding to ZnONPs (100), (002), (101), (102), (110), and (201). ZnONPs average crystal size was 30.83 nm. XRD peaks does not have any characteristic peak other than ZnONPs thus reflected that the prepared powder was free of impurities.

3.1.2. Fourier transform infrared spectroscopy (FTIR)

FTIR is employed to identify the functional group of synthesized NPs. These measurements are also carried out to identify biomolecules bound specifically to the surface of NPs. This analysis also provide evidence for successful transfer of plant's extract functional group during the NP green synthesis. The study of physisorption of molecules at NPs surface can be also employed using this method. Further, this IR spectra of suspension also revealed info regarding specific surface area, surface charge density and the concentration of the NPs. In this study, FTIR spectra for ZnONP production was manifested with peak at 453 cm^{-1}. This validate the ZnONP synthesis, as by average metal-oxygen vibration in FTIR spectra for the production of metallic-NPs lies between 400 and 700 cm^{-1} (Fig. 1B).

3.1.3. UV-VIS spectra

Nanoparticles size play key role in changing their properties. UV-VIS spectroscopy is widely used to examine optical properties of nanosized particles. In a wavelength scan measurement using UV-VIS spectrophotometer, the presence of ZnO was detected at 378 nm, within range of 200–800 nm, as shown in Fig. 1C. The significant sharp absorption of ZnO depicted the monodispersed nature of NPs.

3.1.4. Energy Dispersive X-ray analysis (EDX)

EDX technique is used for elemental analysis and chemical characterization of a sample. Each element has has a unique sets of peaks on X ray spectrum. The characterization capabilities of EDX relies on this fundamental principle. Energy dispersive spectrometer was employed to estimate the number and energy of X ray emitted from a specimen. EDX analysis also allows to measure elemental composition of the specimen. EDX (Fig. 1D) confirms the presence of zinc and oxygen.

3.2. Effect of ZnONPs and Klebsiella sp. SBP-8 on growth characteristics of B. juncea

Cr stress significantly impeded growth attributes (shoot fresh and dry weight, root fresh and dry weight). ZnONPs, *Klebsiella* sp. SBP-8 and Zn treatment effectively enhanced growth under Cr stress and non-stress conditions. Maximum growth was observed in plants treated with ZnONPs alone as depicted by shoot fresh weight (18 g), shoot dry weight (3.18 g), root fresh weight (11.28 g) and root dry weight (2.10 g), shown in Table 1.

3.3. Effect of ZnONPs and Klebsiella sp. SBP-8 on photosynthetic pigments of B. juncea

Cr toxicity significantly decreased Chl *a*, Chl *b*, total chl and carotenoids in *B. juncea* seedlings grown in Cr-contaminated pots. ZnONPs, *Klebsiella* sp. SBP-8 and Zn treatments alone and in combination effectively improved photosynthetic pigments in control as well as Cr-stressed plants (Table 2).

Table 1
Effect of ZnONPs and *Klebsiella* sp. SBP-8 on shoot fresh weight, root fresh weight, shoot dry weight and root dry weight of *B. juncea* seedlings under Cr stressed conditions.

Treatments	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
C	15 ± 1.03 ab	2.34 ± 0.07bc	7.45 ± 0.05 b	1.15 ± 0.02 b
Cr	09 ± 0.78c	1.67 ± 0.08e	5.34 ± 0.04c	0.76 ± 0.45 d
ZnONPs	18 ± 0.92a	3.18 ± 0.23a	11.28 ± 0.31a	2.10 ± 0.39a
PGPB	17 ± 1.13 ab	2.86 ± 0.38 ab	10.47 ± 0.16 ab	1.67 ± 0.06 ab
Zn	16 ± 0.67 ab	2.45 ± 0.17 b	10.67 ± 0.19 ab	1.48 ± 0.39 ab
ZnONP + Cr	13 ± 1.18bc	2.18 ± 0.21c	7.19 ± 0.23bc	1.03 ± 0.46bc
PGPB + Cr	12 ± 1.27bc	1.98 ± 0.17 d	6.93 ± 0.08bc	0.87 ± 0.28cd
Zn + PGPB + Cr	11 ± 0.56bc	2.09 ± 0.29cd	6.89 ± 0.13bc	0.98 ± 0.19c
ZnONP + PGPB + Cr	14 ± 1.07 b	2.17 ± 0.16cd	7.48 ± 0.15 b	1.12 ± 0.39bc

Data are presented as means of 5 independent replicates ± S.E (standard error). Different letters on the bar graphs indicate that mean values of the treatments are significantly different at $p \leq 0.05$ (Tukey's test).

Table 2
Effect of ZnONPs and *Klebsiella* sp. SBP-8 on photosynthetic pigments of *B. juncea* seedlings under Cr stressed conditions.

Treatments	Chl a	Chl b	Total Chl content	Carotenoids
C	1.25 ± 0.03bc	0.34 ± 0.014 b	1.59 ± 0.02 b	3.45 ± 0.12 ab
Cr	0.75 ± 0.02de	0.19 ± 0.024 d	0.94 ± 0.15 d	1.87 ± 0.08 d
ZnONPs	1.28 ± 0.12 b	0.56 ± 0.033a	1.84 ± 0.24a	4.35 ± 0.05a
PGPB	1.17 ± 0.17c	0.47 ± 0.057 ab	1.64 ± 0.27 ab	3.67 ± 0.07 ab
Zn	1.38 ± 0.24a	0.32 ± 0.085bc	1.70 ± 0.18 ab	2.89 ± 0.15c
ZnONP + Cr	0.68 ± 0.07de	0.25 ± 0.054bc	0.93 ± 0.17 d	3.06 ± 0.17bc
PGPB + Cr	0.97 ± 0.18 d	0.24 ± 0.078bc	1.21 ± 0.09cd	2.18 ± 0.08cd
Zn + PGPB + Cr	1.18 ± 0.28cd	0.21 ± 0.064c	1.39 ± 0.12c	2.97 ± 0.14bc
ZnONP + PGPB + Cr	1.21 ± 0.13bc	0.28 ± 0.095bc	1.49 ± 0.16bc	3.15 ± 0.06 b

Data are presented as means of 5 independent replicates ± S.E (standard error). Different letters on the bar graphs indicate that mean values of the treatments are significantly different at $p \leq 0.05$ (Tukey's test).

3.4. Effect of ZnONPs and Klebsiella sp. SBP-8 on leaf relative water content, membrane stability index, net photosynthetic rate and transpiration rate

Chromium stress significantly reduced LRWC, MSI and Tr in *B. junea* seedlings. In case of *B. juncea* seedlings grown in non-toxic conditions, application of ZnONPs improved LRWC by 6.25% as compared to Zn-treatment. Co-supplementation of ZnONPs and *Klebsiella* sp. SBP-8 increased LRWC and MSI by 34.02% and 35.89%, respectively, in comparison with Zn and *Klebsiella* sp. SBP-8 treated *B. juncea* seedlings grown in Cr-contaminated soil. Likewise, in case of *B. juncea* seedlings grown in Cr-contaminated soil, co-application of ZnONPs and *Klebsiella* sp. SBP-8 significantly increased transpiration rate (44.71%) in *B. juncea* seedlings in comparison with Cr-only treatment (Fig. 2).

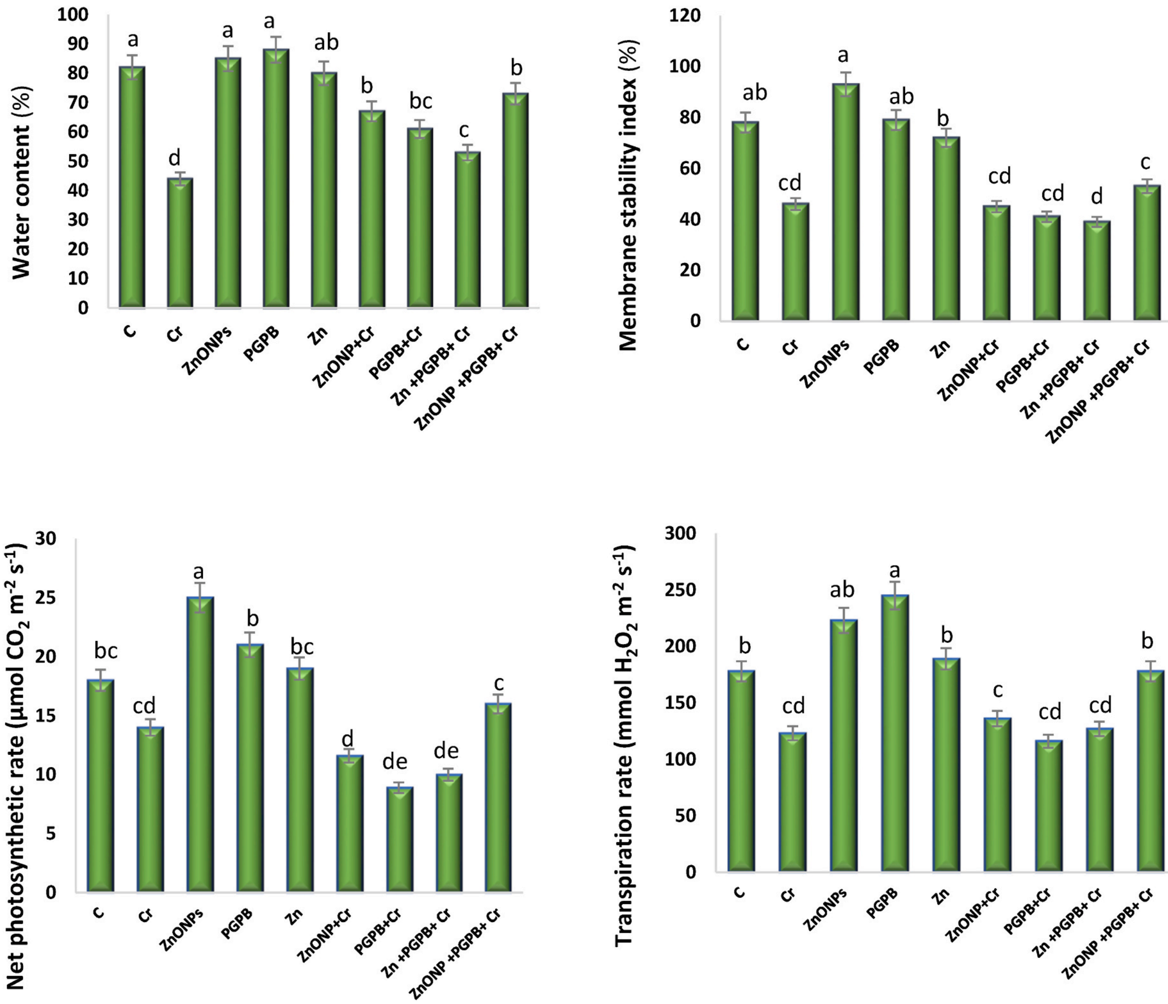

Fig. 2. Effect of ZnONPs and *Klebsiella* sp. SBP-8 on relative water content, membrane stability index, net photosynthetic rate and transpiration rate of *B. juncea* seedlings under Cr stressed conditions. Data are presented as means of 5 independent replicates ± S.E (standard error). Different letters on the bar graphs indicate that mean values of the treatments are significantly different at $p \leq 0.05$ (Tukey's test).

3.5. Effect of ZnONPs and Klebsiella sp. SBP-8 on malondialdehyde content, hydrogen peroxide, electrolyte leakage and superoxide anionic content of B. juncea seedlings

Chromium stress significantly increased MDA content in *B. juncea* seedlings as compared to control. Application of ZnONPs and *Klebsiella* sp. SBP-8 reduced MDA content in *B. juncea* seedlings as compared to control. In case of Cr-toxic potted soil, co-treatment of ZnONPs and *Klebsiella* sp. SBP-8 significantly reduced MDA content (8.65%) in *B. juncea* seedlings as compared to alone treatment of ZnONPs. Likewise, combined supplementation of ZnONPs and PGPR significantly reduced H_2O_2, EL and $O_2\bullet-$ in *B. juncea* seedlings as compared to Cr-treatment (Fig. 3).

3.6. Effect of ZnONPs and Klebsiella sp. SBP-8 on antioxidant enzyme activity of B. Junea seedlings

As far as the SOD activity is concerned, Cr stress significantly increased SOD activity in comparison with control treatment. Application of ZnONPs, *Klebsiella* sp. SBP-8 and Zn improved SOD activity in *B. juncea* seedlings as compared to control treatment. In Cr-contaminated pots, co-application of ZnONPs and *Klebsiella* sp. SBP-8 increased SOD enzyme activity by 21.76% as compared to seedlings grown in Cr-only treatment. Similarly in case of activity of CAT enzyme, co-supplementation of ZnONPs and *Klebsiella* sp. SBP-8 increased CAT activity by 44.75% as compared to alone treatment of ZnONPs in *B. juncea* seedlings grown in Cr-contaminated soil. Application of ZnONPs and *Klebsiella* sp. SBP-8, alone or in combination, improved activity of POD enzyme in *B. juncea* seedlings as compared to control treatment (Fig. 4).

3.7. Effect of ZnONPs and Klebsiella sp. SBP-8 on non-enzymatic antioxidant of B. Junea seedlings

Application of ZnONPs, alone or in combination with *Klebsiella* sp. SBP-8, significantly enhanced ASA content in *B. juncea* seedlings grown in normal and Cr-contaminated soil. Application of ZnONPs increase ASA content by 41.04% in *B. juncea* seedlings as compared to Zn-treatment in normal conditions. In case of Cr-toxificated potted soil, co-active application of ZnONPs and *Klebsiella* sp. SBP-8 improved ASA content by 71.67% in *B. juncea* seedlings in comparison with alone treatment of ZnONPs. In case of GSH content, application of ZnONPs enhanced GSH content by 76.31% in *B. juncea* seedlings as compared to control treatment. Synergistic application of ZnONPs and *Klebsiella* sp. SBP-8 enhanced GSH content by 29.03% as compared to ZnONPs treated *B. juncea* seedlings grown in Cr-contaminated soil (Fig. 5).

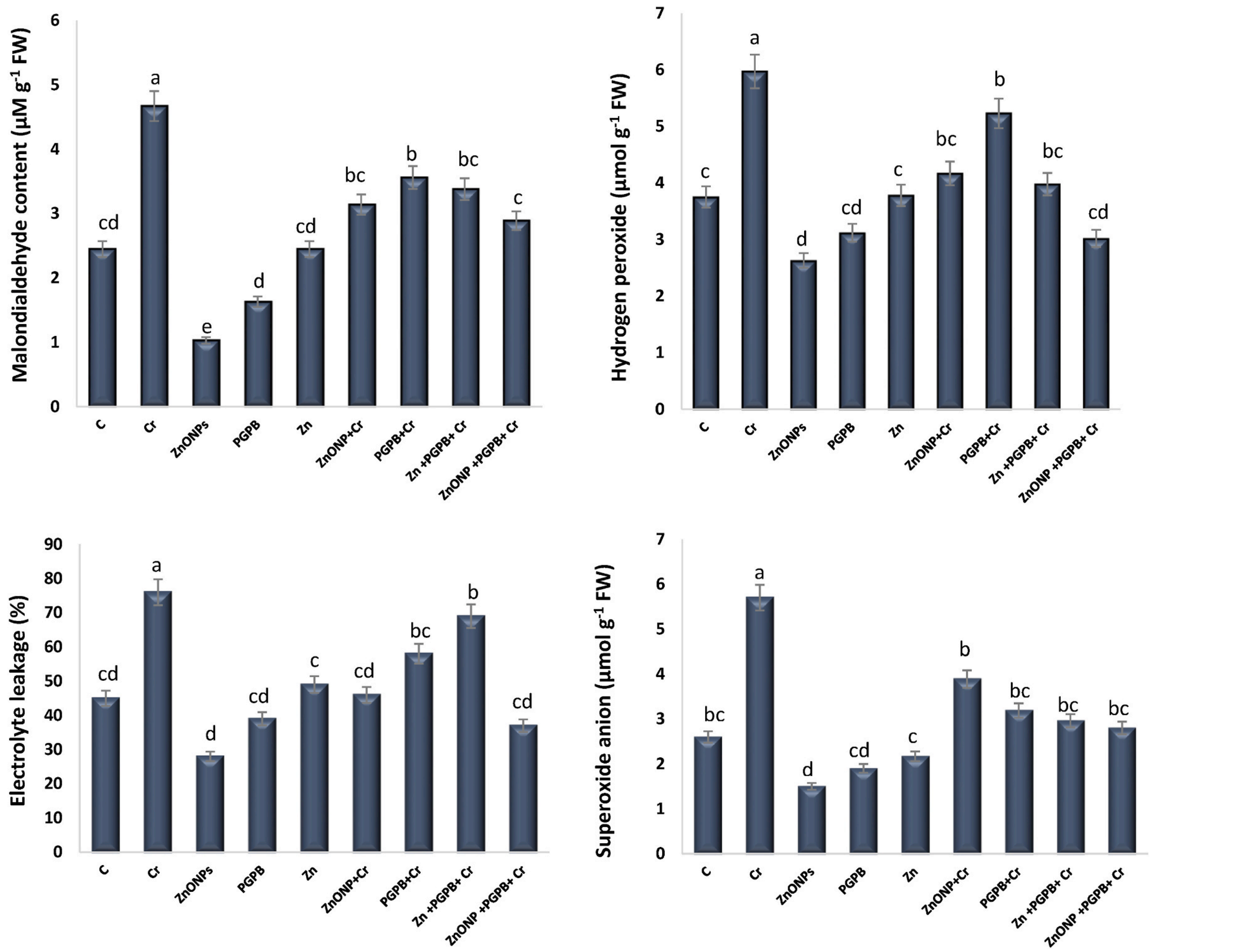

Fig. 3. Effect of ZnONPs and *Klebsiella* sp. SBP-8 on malondialdehyde content, hydrogen peroxide, electrolyte leakage and superoxide anionic content of *B. juncea* seedlings under Cr stressed conditions. Data are presented as means of 5 independent replicates ± S.E (standard error). Different letters on the bar graphs indicate that mean values of the treatments are significantly different at $p \leq 0.05$ (Tukey's test).

3.8. Effect of ZnONPs and Klebsiella sp. SBP-8 on nutritional content of B. juncea

Cr stress significantly reduced mineral nutrients (Zn^{2+}, K^{+}, Mo^{2+}, Mg^{2+} and Ca^{2+}) in *B. juncea*. ZnONPs, *Klebsiella* sp. SBP-8 and Zn treatment effectively enhanced nutritional content under Cr stress and non-stress conditions. Maximum values for these mineral nutrients were observed in plants treated with ZnONPs alone (Table 4).

3.9. Effect of ZnONPs and Klebsiella sp. SBP-8 on chromium uptake of B. juncea

Cr uptake in stressed plants was 9549 µg g^{-1} DW of root and 514 µg g^{-1} DW of shoot. The translocation factor for this group of plants was 0.05. ZnONPs, *Klebsiella* sp. SBP-8 and Zn treatment tends to decrease Cr uptake in the roots and its successive translocation to the shoot (Table 3).

3.10. Effect of ZnONPs and Klebsiella sp. SBP-8 on expression of stress responsive genes of B. Junea seedlings

Fig. 6 explains the effect of ZnONPs and *Klebsiella* sp. SBP-8 on the expression of stress responsive genes in *B. juncea* seedlings. Application of ZnONPs and *Klebsiella* sp. SBP-8 enhanced SOD expression in normal and Cr-contaminated conditions. In case of *B. juncea* seedlings grown in Cr-contaminated soil, co-application of ZnONPs and *Klebsiella* sp. SBP-8 enhanced SOD expression by 57.69% as compared to individual application of ZnONPs.

CAT expression increased in *B. juncea* seedlings supplemented with ZnONPs and *Klebsiella* sp. SBP-8 in normal and Cr-contaminated soil. Treatment of ZnONPs enhanced CAT expression by 26.92 and 42.44% as compared to *Klebsiella* sp. SBP-8 and Zn, respectively, in *B. juncea* seedlings grown in non-contaminated soil. Synergistic application of ZnONPs and *Klebsiella* sp. SBP-8 esclated CAT expression by 17% as compared to ZnONPs treated *B. juncea* seedlings grown in normal and Cr-contaminated soil. Likewise, POD and APX expression increased significantly in *B. juncea* seedlings synergistically treated with ZnONPs and *Klebsiella* sp. SBP-8 as compared to Cr-only treatment.

3.11. Pearson's correlation and Principle Component Analysis (PCA)

Pearson's correlation and principle component analysis were performed to estimate the relations among different attributes studied and also between the individual treatment. Pearson's correlation in Fig. 7 revealed that all the studied attributes can be divided in two groups, based on their relation to each other, either positive or negative. In group I, growth parameters (SFW, RFW, RFW and RDW), physiological attributes (chl *a,* Chl *b,* total chl, carotenoids, Pn, Tr and LRWC), nutritional contents (Zn, K, Mo, Mg & Ca ions) and Cr translocation factor (TF) were in significantly positive correlation with each other. Similarly, in group II, oxidative stress markers (MDA, EL, superoxide ions and H_2O_2), and Cr concentration in root and shoot of *B. juncea* were

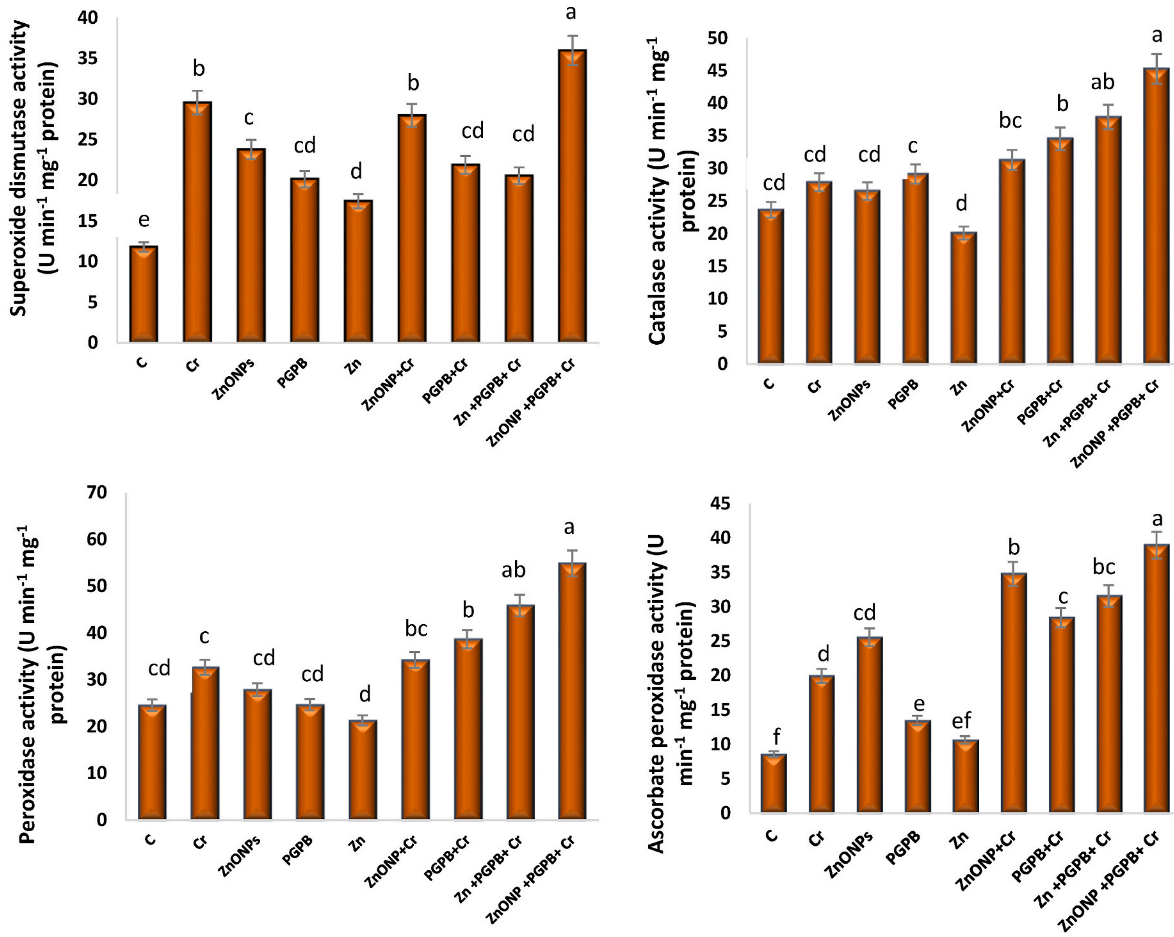

Fig. 4. Effect of ZnONPs and *Klebsiella* sp. SBP-8 on SOD, CAT, POD and APX activity of *B. junea* seedlings under Cr stressed conditions. Data are presented as means of 5 independent replicates ± S.E (standard error). Different letters on the bar graphs indicate that mean values of the treatments are significantly different at $p \leq 0.05$ (Tukey's test).

in significantly positive correlation with each other. Fig. 7 also revealed that under Cr stress, uptake of Cr through roots and then its translocation to shoots increased the Cr level in *B. juncea* plants which ultimately resulted in reduced growth, photosynthesis, transpiration rate and various other physiological attributes discussed previously. Further, Fig. 7 suggested that increased Cr translocation towards shoot enhanced growth and physiological activities in *B. juncea* plants. This might be due the fact that, Cr translocated in the shoot might be sequestered, volatilized into less toxic form, conjugated with metal chelators or stored safely such that it can't further harm to cell functioning.

PCA plots to assess the influence of Cr level in the root and shoot of *B. juncea* are shown in Figs. 8 and 9. In Fig. 9, dots (•) with numbers 1, 2, 3 up to 9 represent treatments applied during experiment, where 1; control, 2; Cr, 3; ZnONPs, 4; PGPR, 5; Zn, 6; 2 + 3, 7; 2 + 4, 8; 2 + 4 + 5 and 9; 2 + 3 + 4. All the treatments, including control, applied in this study were successfully distributed in first two components (PC1 and PC2). Where, Cr-stress (2) is separated from are other treatments including control. This clearly suggested that Cr-stress poses significant impact on growth physiological and biochemical attributes of *B. juncea* studied in this research. Fig. 8 depicted that all the variables (100% of the dataset) were distributed in total of 8 principle components (PCs). In Figs. 8 and 9, among all the principle components of *B. juncea*, PC1 and PC2 exhibited the highest contribution (84%) of total variance in the dataset. Out of which PC1 contributed 63.22% and PC2 contributed 20.80%. PCA was performed to validate the results Pearson's correlation test. The first group of variables positively correlated with PC1 includes nutritional ions, photosynthetic attributes, and growth indices. While a significant negative correlation was found among PC1 and the variables aligned with PC2 such as oxidative stress markers (MDA, EL, H_2O_2 and superoxide ions) and root and shoot Cr level. In Fig. 9, most variables are equidistant from the axis' origin point, it indicates that these contribute equally to the variation recorded by the principal components. This also suggests that these variables are highly interconnected.

4. Discussion

Heavy metal stressed imperiled plants are exposed to endless generation of ROS resulting in reduction in growth and yield. Inappropriate ROS production leads to damaging cellular effects, which disturbs cellular integrity (Mansoor et al., 2023). Chromium stress is involved in augmentation of ROS production, which disturbs the growth and metabolic processes (Singh et al., 2022). Various plant growth regulators are reconnoitered as phyto-stimulants to reverse the toxic effect of Cr toxicity through enhanced activity of enzymatic and non-enzymatic antioxidants (Didi et al., 2022). Current research was designed to explore the efficacy of ZnONPs and *Klebsiella* sp. SBP-8 in mitigation of Cr stress in *B. juncea* seedlings.

During the current research, Cr stress reduced fresh weight, dry weight, root length, shoot length of *B. juncea* seedlings (Table 1). This reduction in growth is due to overproduction of ROS, and reduced relative water content (Huang et al., 2019). Various researchers have reported that PGPR reverse the toxic effect of heavy metal stress and restore growth in plants grown in contaminated soil (Wang et al., 2022). Singh et al. (2015) reported that *Klebsiella* sp. SBP-8 is involved in

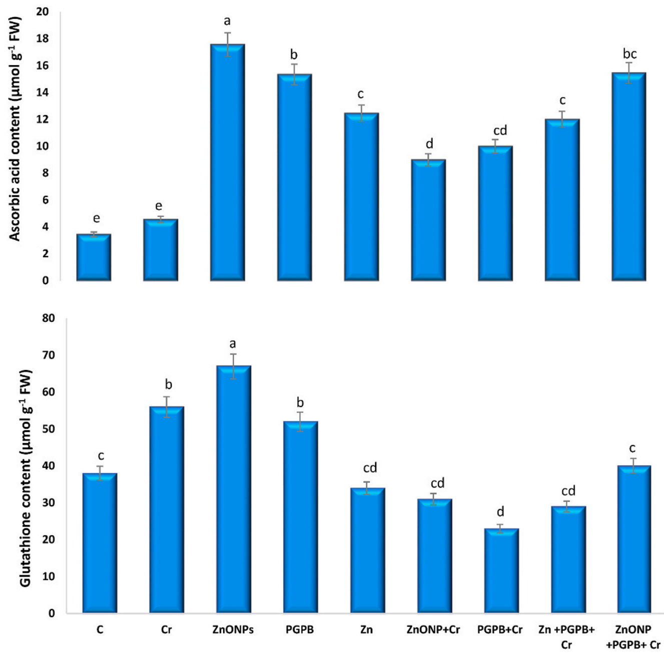

Fig. 5. Effect of ZnONPs and *Klebsiella* sp. SBP-8 on ascorbate and glutathione content of *B. junea* seedlings under Cr stressed conditions. Data are presented as means of 5 independent replicates ± S.E (standard error). Different letters on the bar graphs indicate that mean values of the treatments are significantly different at p ≤ 0.05 (Tukey's test).

Table 3
Effect of ZnONPs and *Klebsiella* sp. SBP-8 on Cr uptake of *B. juncea* seedlings under Cr stressed conditions.

Treatments	Root (µg g^{-1} DW)	Shoot (µg g^{-1} DW)	Translocation factor
C	ND	ND	ND
Cr	9549 ± 38 b	514 ± 28.18a	0.05 ± 0.001bc
ZnONPs	0.27 ± 0.07e	0.18 ± 0.03 d	0.48 ± 0.012a
PGPB	0.49 ± 0.06de	0.21 ± 0.01cd	0.45 ± 0.024 ab
Zn	0.89 ± 0.05 d	0.39 ± 0.07cd	0.43 ± 0.027 b
ZnONP + Cr	10378 ± 28a	489 ± 32.19 ab	0.04 ± 0.017bc
PGPB + Cr	9189 ± 24bc	381 ± 15.28 b	0.04 ± 0.018bc
Zn + PGPB + Cr	8278 ± 12c	284 ± 09.23bc	0.03 ± 0.028c
ZnONP + PGPB + Cr	7829 ± 18cd	176 ± 10.29c	0.02 ± 0.019c

Data are presented as means of 5 independent replicates ± S.E (standard error). Different letters on the bar graphs indicate that mean values of the treatments are significantly different at p ≤ 0.05 (Tukey's test).

Table 4
Effect of ZnONPs and *Klebsiella* sp. SBP-8 on nutritional content of *B. juncea* seedlings under Cr stressed conditions.

Treatments	Nutritional Content				
	Zn^{+2} (meq. g^{-1} DW) 24-35	K^{+2} (meq. g^{-1} DW) 20-60	MO^{+2} (meq. g^{-1} DW) 25-48	Mg^{+2} (meq. g^{-1} DW) 30-45	Ca^{+2} (meq. g^{-1} DW) 10-30
C	28 ± 1.04 ab	23 ± 1.07bc	26 ± 1.87c	31 ± 2.05 b	12 ± 0.87cd
Cr	17 ± 0.95cd	15 ± 0.94 d	18 ± 1.05de	24 ± 1.75 d	8 ± 0.54e
ZnONPs	35 ± 1.28a	34 ± 1.85a	38 ± 2.14a	46 ± 2.89a	24 ± 1.64a
PGPB	25 ± 1.38bc	27 ± 1.27 ab	33 ± 2.02 b	39 ± 2.75 ab	21 ± 1.53 ab
Zn	34 ± 1.08a	25 ± 1.06 b	30 ± 1.98bc	37 ± 2.56 ab	18 ± 1.93 b
ZnONP + Cr	21 ± 1.35c	17 ± 0.84cd	23 ± 1.05cd	28 ± 2.08bc	11 ± 0.72 d
PGPB + Cr	24 ± 0.84bc	18 ± 1.34cd	19 ± 1.04 d	32 ± 2.45c	15 ± 0.64c
Zn + PGPB + Cr	28 ± 1.86 ab	19 ± 1.07cd	21 ± 1.28 d	36 ± 2.54 ab	13 ± 0.84cd
ZnONP + PGPB + Cr	23 ± 1.74bc	21 ± 1.64c	25 ± 1.64cd	29 ± 2.14bc	17 ± 0.61bc

Data are presented as means of 5 independent replicates ± S.E (standard error). Different letters on the bar graphs indicate that mean values of the treatments are significantly different at p ≤ 0.05 (Tukey's test).

systemic tolerance against salt stress in *Triticum aestivum*. Kusale et al. (2021) reported the luxurious growth in maize and wheat due to enhanced production of indole-3-acetic acid (IAA), 1 aminocyclopropane-1-carboxylate deaminase (ACCD), exopolysaccharides (EPS) and various osmolyte, when plants were inoculated with PGPR. Gupta et al. (2020) elaborated the role of another strain *Klebsiella* sp. CPSB4 in Cr stress alleviation in tomato seedlings. This Cr-induced stress tolerance was credited to enhanced activity of SOD, CAT and POD in tomato seedlings. We also found that *Klebsiella* sp. SBP-8 reduced Cr stress in *B. juncea* seedlings via enhanced activity of antioxidant enzymes i.e., SOD, CAT, APX and POD.

Chromium stress results in over-production of ROS that leads to oxidative damage (Srivastava et al., 2021; Wakeel et al., 2020). Over-generation of ROS disturbs membrane structure due to enhanced lipid peroxidation resulting in cellular death (Hasanuzzaman et al., 2020). Sharma et al. (2022) also reported enhanced production of ROS in *B. juncea* seedlings grown in Cr-stressed conditions. Current research also reported that Cr stress resulted in oxidative stress due to enhanced accumulation of MDA, EL, ROS and $O_2•^-$ in *B. juncea* seedlings. However, supplementation of ZnONPs and *Klebsiella* sp. SBP-8 reduced ROS production in *B. juncea* seedlings grown in Cr-contaminated potted soil. This decrease in ROS production leads to reactive species homeostasis in *B. juncea* seedlings, thereby, resulting in sustenance of normal growth and metabolic reactions. Our results are similar to Gupta et al. (2020) who reported that inoculation of *Klebsiella* sp. resulted in reduced lipid peroxidation in *Helianthus annuus* seedling exposed to Cr-contaminated soil. This reduction in MDA level might be correlated to escalated activity of antioxidative enzymes (Liao et al., 2019). Increase in MDA content due to Cr stress also augments EL in exposed seedlings, resulting in cellular damage. Current research findings are analogous to various researcher's findings that Cr stress increased EL in exposed cauliflower seedlings (Ahmad et al., 2020). Concomitantly, application of PGPR and ZnONPs alleviated Cr stress due to reduction in oxidative stress markers (MDA, EL, ROS and $O_2•^-$) and enhanced accumulation of antioxidative enzymes in *B. juncea* seedlings. Research has demonstrated that the application of ZnONPs mitigates the deleterious consequences caused by arsenic in wheat varieties. Furthermore, ZnONPs regulate wheat plants' biochemical activities, mainly by slowing down arsenic absorption (Ahmad et al., 2020).

Ascorbate-glutathione pathway is crucial for accomplishment of metabolomics in plants facing oxidative stress. This is of prime significance involved in modulation of enzymatic and non-enzymatic antioxidants (Alam et al., 2020). These modulations in activities of antioxidant enzymes are correlated to ROS scavenging capacity of plants, thereby, relieving the plants from oxidative stress (Das and Roychoudhury, 2014). This study revealed that Cr stress enhanced the activity of antioxidative defensive system in *B. juncea* seedlings. Researchers reported that Cr stress enhanced the activity of antioxidant enzymes in *Trigonella corniculata* (Aslam et al., 2023), *Capsicum annum* (Nemat et al., 2020), *Solanum lycopersicum* (Alam et al., 2023). APX is a potential enzyme for AsA-GSH cycle, involved in detoxification of ROS. In addition, AsA is also an important metabolite involved in AsA-GSH cycle (Niu et al., 2023). In case of *B. juncea* seedlings grown in Cr-contaminated soil, APX and AsA increased by the coactive application of ZnONPs and *Klebsiella* sp. SBP-8 as compared to Cr-only treatment.

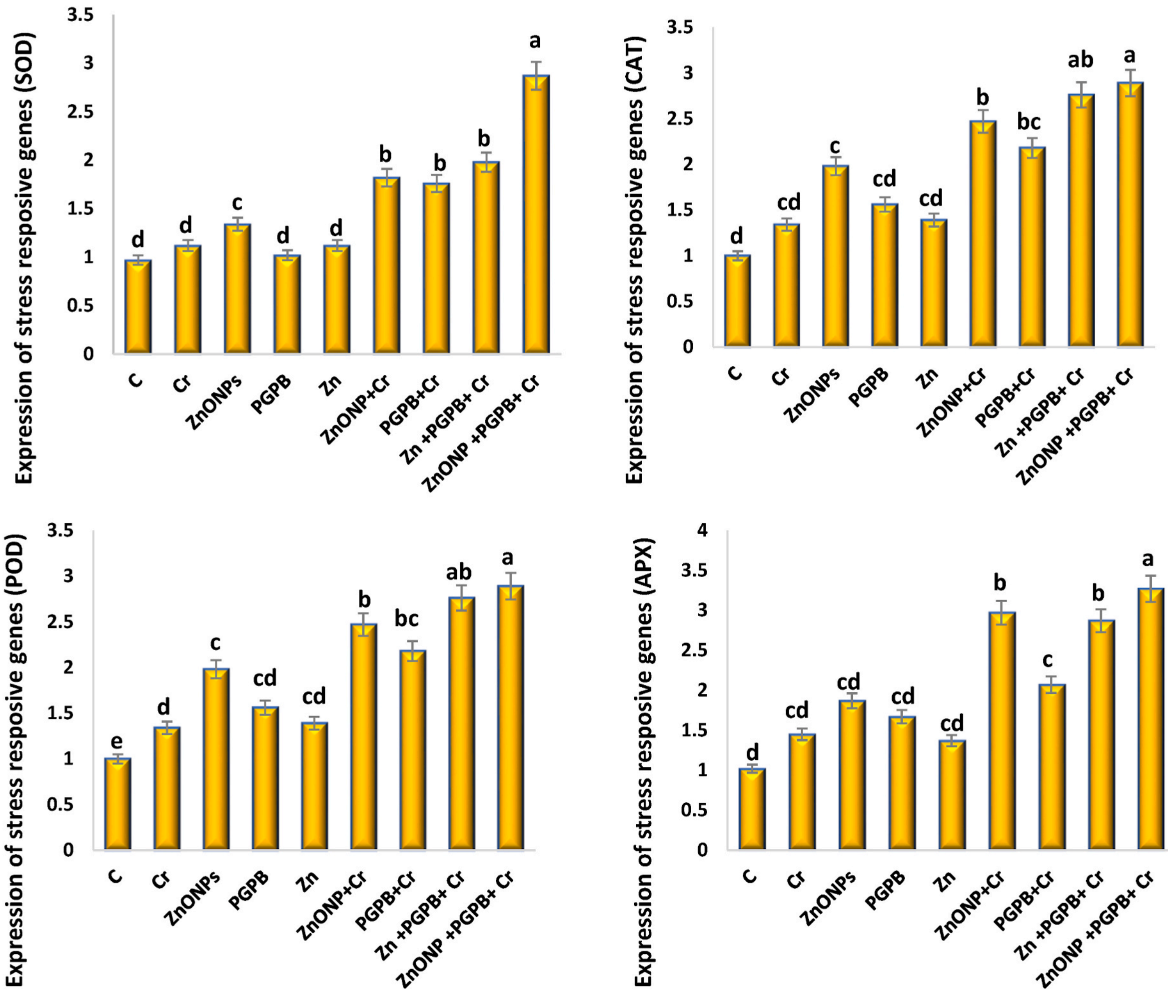

Fig. 6. Effect of ZnONPs and *Klebsiella* sp. SBP-8 on expression of stress responsive genes in *B. junea* seedlings under Cr stressed conditions. Data are presented as means of 5 independent replicates ± S.E (standard error). Different letters on the bar graphs indicate that mean values of the treatments are significantly different at $p \leq 0.05$ (Tukey's test).

Current research explained the efficacy of ZnONPs and *Klebsiella* sp. SBP-8 in mitigation of Cr toxicity in *B. juncea* seedlings through changes in activity of antioxidant enzymes (Fig. 4). Activity of SOD enzyme is involved in conversion of $O_2^{\bullet-}$ to H_2O_2, which results in reduction of $O_2^{\bullet-}$ content (Stephenie et al., 2020). During the current study, ZnONPs and *Klebsiella* sp. SBP-8 enhanced the activity of SOD enzyme in *B. juncea* seedlings grown in normal and Cr-contaminated soil. Various researchers have reported the individual application of ZnONPs and PGPR in augmentation of activity of antioxidant enzymes (Azarin et al., 2022; Yang et al., 2021). Additionally, decrease in H_2O_2 content in *B. juncea* seedlings might be correlated with detoxification of H_2O_2 content through elevation of activity of antioxidant enzymes. Current research also reported augmentation in activity of antioxidant enzymes, which are involved H_2O_2 to H_2O and O_2 resulting in ROS homeostasis in *B. juncea* seedlings. Elevated expression level of the genes for CAT, POD, SOD and APX are depicted by using qRT-PCR (Fig. 6). Coactive application of ZnONPs and *Klebsiella* sp. SBP-8 enhanced the activity of antioxidant enzymes, confirming the attribute towards detoxification of ROS and accomplishment of plant ability to survive in Cr stressed conditions.

POD converts H_2O_2 into H_2O and lignin. Conversely, elevated H_2O_2 activity in current study was possibly due to Cr stress attenuation, while POD acted on this and moderated H_2O_2 level in *Klebsiella* sp. SBP-8 inoculated plants in addition to FeONPs. Unsaturated fatty acids in the cell membrane persuade MDA synthesis under stress conditions. Hence, the level of MDA in cell reflects oxidative damage of cell. Increased lipid peroxidation resulted in increased MDA content in Cr stressed plants. FeONPs applied plants had reduced MDA level perhaps due to reduced oxidative injury in those plants by the action of FeONPs supplements. Unsaturated fatty acids in the cell membrane persuade MDA synthesis under stress conditions. Hence, the level of MDA reflects oxidative damage of cell.

Current research also focused on the modulations of non-enzymatic antioxidants (AsA, GSH) in *B. juncea* seedlings grown in normal and Cr-polluted soil. These non-enzymatic antioxidants are crucial in quenching ROS and are involved in redox homeostasis in plants facing stressed conditions (Qadir et al., 2022). Furthermore, AsA and GSH are also involved in regulation of various basic physiological process in plants i.e., hormone synthesis, cell differentiation and division, pollen growth etc (Ishikawa et al., 2018). Current study showed augmented level of AsA and GSH in *B. juncea* seedlings grown in Cr-contaminated soil, which are alliance to the results observed in tomato seedlings exposed to Cr-stress (Raja et al., 2023). Furthermore, co-supplementation of ZnONPs and *Klebsiella* sp. SBP-8, alone or in combination, increase AsA and GSH content in *B. juncea* seedlings grown in normal and Cr-contaminated soil. These results are similar to the finding of Sharma et al. (2022).

ZnONPs and Klebisella sp. SBP-8 improved nutrient use efficiency of *B. juncea* under Cr-stress as observed in current study. Applying ZnO-NPs to soya bean and maize improved Zn uptake and plant growth,

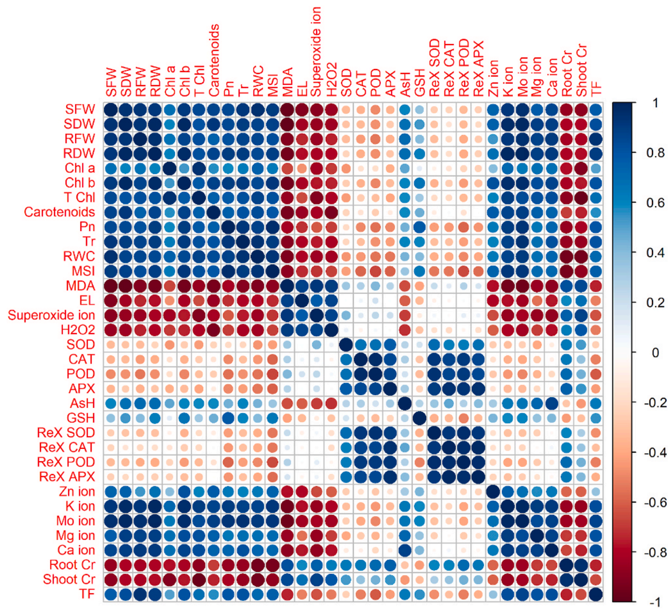

Fig. 7. Pearson's correlation for all studied parameters of *B. juncea* subjected to ZnONPs, *Klebsiella* sp. SBP-8 and Zn treatment under control and Cr stress. (Various abbreviations used are as follows, SFW; shoot fresh weight, SDW; shoot dry weight, RFW; root fresh weight, RDW; root dry weight, T Chl; total chlorophyll, Pn; net photosynthesis rate, Tr; transpiration rate, RCW; relative water content, MSI; membrane stability index, MDA; malondialdehyde, EL; electrolytic leakage, H_2O_2; hydrogen peroxide, SOD; superoxide dismutase, CAT; catalase, POD; peroxidase, APX; ascorbate peroxidase, AsH; ascorbic acid, GSH; glutathione, ReX; relative expression, Zn; zinc, K; potassium, Mo; molybdenum, Mg; magnesium, Ca; calcium, Cr; chromium, TF; translocation factor).

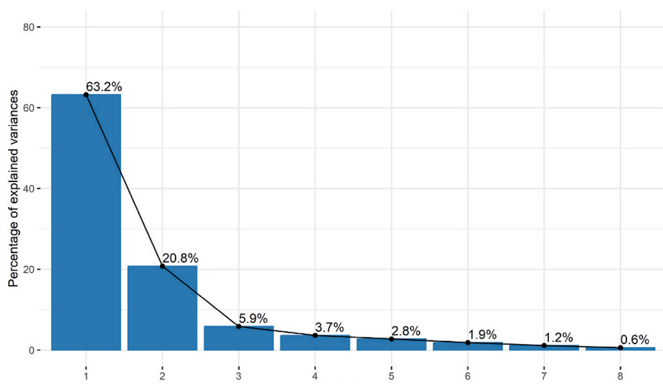

Fig. 8. Principle components with percentage distribution of all studied variables/parameters.

respectively (Subbaiah et al., 2016). Similarly, Tarafdar et al. (2014) found that Zn nano-fertilizer boosted plant growth and yield in pearl millet. In another study, Raliya et al. (2016) described those treating legumes (*Vigna radiate*) with ZnO NPs increased soil phosphorus (P) availability and crop P uptake by enhancing enzyme activity, including phosphatase and phytase. Peralta-Videa et al. (2014) found that medium and high ZnO-NP treatment boosted micronutrient molybdenum (Mo) accumulation in nodules while interfering with Fe, Cu, and K accumulation. These findings fairly support the results obtained in current study.

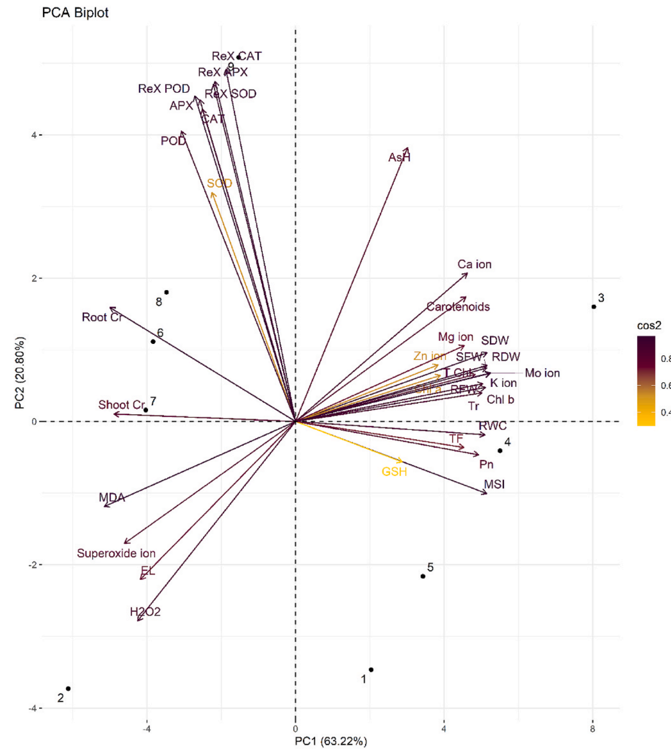

Fig. 9. Principle component analysis (Biplot; dots (•) representing individual treatment and arrows representing parameters) for all studied parameters of *B. juncea* subjected to ZnONPs, *Klebsiella* sp. SBP-8 and Zn treatment under control and Cr stress. (Various abbreviations used are same as in Fig. 7, dots (•) with numbers 1, 2, 3 up to 9 represent treatments applied during experiment, where 1; control, 2; Cr, 3; ZnONPs, 4; PGPB, 5; Zn, 6; 2 + 3, 7; 2 + 4, 8; 2 + 4 + 5 and 9; 2 + 3 + 4).

5. Conclusion

Chromium (Cr) is one of the toxic metals which induces various oxidative processes in plants. Use of nanoparticles for crop development got much attention. Various nanoparticles are being used as pesticides, fertilizers, growth regulator and stress reliever. Zinc oxide nanoparticles (ZnONPs) alone and in combination with PGPR (plant growth promoting rhizobacteria) are least explored in agriculture. In this study, the role of ZnONPs and *Klebsiella* sp. SBP-8 in mitigation of Cr stress in *Brassica juncea* was investigated. Chromium stress (1 mM) reduced growth, physiological and biochemical attributes of *B. juncea* plants. Chromium stress significantly increased MDA, EL and ROS (H_2O_2 and superoxide ions) in *B. juncea* seedlings. However, co-supplementation of ZnONPs and *Klebsiella* sp. SBP-8 escalated the activity of antioxidant enzymes i.e., superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX). Additionally, ZnONPs and *Klebsiella* sp. SBP-8 also increased CAT, POD, APX and SOD gene expression in *B. juncea* grown in normal and Cr-toxic soil. In this way, ZnONPs along with *Klebsiella* sp. SBP-8 induced stress tolerance in *B. juncea* plants and significantly improved their growth and development in control and stress conditions. It is further proposed that combined treatment of ZnONPs and *Klebsiella* sp. SBP-8 may prove useful for the alleviation of numerous abiotic stresses in plants.

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CRediT authorship contribution statement

Anis Ali Shah: Validation, Supervision. **Sadia Zafar:** Methodology. **Sheeraz Usman:** Writing – review & editing, Writing – original draft. **Sumera Javad:** Formal analysis. **Zaib-un-Nisa:** Methodology. **Muhammad Aslam:** Formal analysis. **Zahra Noreen:** Writing – original draft. **Hosam O. Elansary:** Resources, Investigation. **Khalid F. Almutairi:** Resources, Investigation. **Aqeel Ahmad:** Writing – review & editing.

Declaration of competing interest

All the authors declare no conflict of interest.

Data availability

Data will be made available on request.

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