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Methyl Jasmonate–Nano-Sulfur Interaction Enhances Salinity Tolerance through Coordinated Redox Homeostasis and Proline Metabolism in Mustard

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Abstract

Background and aims Soil salinity disrupts photosynthetic functions through increased oxidative stress. The interaction between phytohormones and nutrient-based nanomaterials offers a promising strategy for enhancing salt tolerance. This study investigated the individual and interactive effects of MeJA, S and nS in mitigating salinity stress in mustard.

Methods Mustard plants were treated with 10 µM MeJA applied individually or in combination with 200 mg S kg^{-1} soil or 200 mg nS kg^{-1} soil under 100 mM NaCl. Antioxidant and proline metabolism, stomatal architecture, cell viability and photosynthesis and growth were evaluated.

Results Salinity increased H_2O_2 and TBARS levels resulting in reduced photosynthesis and plant growth. The combined MeJA and nS application alleviated salinity stress more prominently than their individual applications by reducing H_2O_2 and TBARS levels, enhancing the activities of antioxidant enzymes and AsA–GSH cycle metabolite pools. This coordinated response preserved stomatal integrity and cell viability and increased net photosynthesis and dry matter accumulation. Furthermore, MeJA+nS enhanced proline accumulation through increased activity of pyrroline-5-carboxylate synthetase (P5CS) and γ-glutamyl kinase (GK) and suppressed proline oxidase (PROX) activity.

Conclusion MeJA and nS application together alleviated salinity stress more conspicuously through coordinated regulation of ion homeostasis, antioxidant defense and proline metabolism, maintaining stomatal architecture, photosynthesis and growth. This integrated MeJA plus nS strategy represents a promising nano-enabled approach for maintaining photosynthetic performance and biomass accumulation of mustard under saline conditions.

Keywords: Abiotic stress; plant hormones; nano-nutrients; sulfur assimilation; antioxidant defense.

Introduction

Soil salinity is one of the most detrimental abiotic stresses affecting crop production worldwide and poses an increasing threat to global food security. Climate change, improper irrigation practices, inadequate drainage, and growing freshwater scarcity are accelerating the expansion of salt-affected soils, thereby reducing the productivity of numerous economically important crops (Mukhopadhyay et al., 2021; Oduor et al., 2025). A major consequence of salinity stress is the excessive generation of reactive oxygen species (ROS), including superoxide radicals, hydrogen peroxide, and hydroxyl radicals. The overaccumulation of ROS disrupts cellular redox homeostasis, causing oxidative damage to lipids, proteins, nucleic acids, and membrane systems, and ultimately impairing plant metabolism and physiological functions (Kesawat et al., 2023; Giannakoula, 2025). To counteract ROS-induced cellular damage, plants activate antioxidant defense system, comprising enzymatic antioxidants, such as superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR), and non-enzymatic antioxidants, including ascorbate, glutathione, carotenoids, and phenolic compounds. Among these defense mechanisms, the ascorbate–glutathione (AsA–GSH) cycle plays a central role in maintaining cellular redox

homeostasis and detoxifying ROS. However, prolonged or severe salinity can disrupt this antioxidant defense network, resulting in sustained oxidative stress and cellular damage (Foyer and Kunert, 2024).

Therefore, elucidating the mechanisms underlying salinity tolerance and developing sustainable strategies to mitigate salt-induced damage are essential for improving crop productivity. Maintenance of ionic and osmotic homeostasis is a critical adaptive mechanism that enables plants to withstand salinity stress (Ma et al., 2026; Daraz et al., 2023). In addition to these physiological adjustments, phytohormones play pivotal roles in coordinating plant responses to salinity by regulating ion transport, osmolyte accumulation, antioxidant defense, and the expression of stress-responsive genes. Hormones such as abscisic acid, salicylic acid, ethylene, brassinosteroids, and jasmonates have been widely shown to enhance plant adaptation to saline environments (Fatma et al., 2022; Waheed et al., 2024; Mahajan et al., 2025).

Among these phytohormones, methyl jasmonate (MeJA), a volatile derivative of jasmonic acid, functions as an important signaling molecule involved in plant growth, development, and stress responses (Ruan et al., 2019; Fatma et al., 2021). Although endogenous jasmonates can transiently inhibit growth by redirecting metabolic resources toward stress adaptation, exogenous application of MeJA at appropriate concentrations has been shown to enhance salinity tolerance by strengthening antioxidant capacity, maintaining ionic homeostasis, and activating stress-responsive signaling pathways (Yin et al., 2023; Wang et al., 2023). MeJA has, therefore, emerged as an important lipid-derived signaling molecule that enhances plant tolerance to salinity and other abiotic stresses through coordinated modulation of physiological, biochemical and molecular defense responses (Per et al., 2016; Ali et al., 2020; Fatma et al., 2022; Nie et al., 2025; Lashkari et al., 2025).

As a bioactive derivative of jasmonic acid, MeJA regulates stress adaptation, in part, through the COI1–JAZ–MYC signaling module, which integrates antioxidant defense, osmotic adjustment, secondary metabolism, and the expression of stress-responsive genes (Hasanuzzaman et al., 2022; Ahmad et al., 2023; Sonatan et al., 2026). Earlier studies in *Arabidopsis* demonstrated that jasmonate-induced stress tolerance was associated with enhanced transcript accumulation (Xiang and Oliver, 1998). Exogenous MeJA application has also been reported to improve photosynthetic performance by preserving PSII integrity, enhancing chlorophyll fluorescence and carbon assimilation, maintaining membrane stability, regulating nitrogen metabolism, stimulating antioxidant enzyme activities, and promoting the accumulation of compatible osmolytes such as proline (Sonatan et al., 2026). Furthermore, MeJA contributes to the maintenance of the PSII reaction-centre D1 protein and induces the expression of photosynthesis-associated genes, including *psbA*, *psbB*, and *psbC*, thereby facilitating the repair and preservation of the photosynthetic apparatus, reducing oxidative damage, and ultimately enhancing plant resilience under stress conditions (Wang et al., 2023; Fatma et al., 2021).

Essential nutrients also contribute to maintaining ionic balance and metabolic homeostasis, thereby mitigating salt-induced growth inhibition and enhancing plant resilience to salinity stress (Kaya and Ashraf, 2024; Mir et al., 2024). Among these nutrients, sulfur (S) is an essential macronutrient that enhances plant tolerance to abiotic stresses through its critical roles in protein synthesis, antioxidant metabolism, and redox homeostasis. Sulfate assimilation is initiated by ATP sulfurylase (ATP-S) and ultimately leads to the biosynthesis of sulfur-containing metabolites, including cysteine, methionine, and glutathione, which are indispensable for cellular defense (Alvi et al., 2023; Jena et al., 2025). Glutathione, a major antioxidant and an integral component of the ascorbate–glutathione (AsA–GSH) cycle, plays a central role in maintaining cellular redox homeostasis and protecting the photosynthetic apparatus from oxidative damage. Moreover, adequate S nutrition is closely associated with

enhanced nitrogen assimilation, protein synthesis, and maintenance of photosynthetic efficiency, collectively contributing to improved plant resilience under salinity stress (Jahan et al., 2021a; Mir et al., 2024). However, salinity can impair S assimilation and glutathione biosynthesis, thereby weaken antioxidant defense and increasing oxidative damage. Consequently, improving S availability and utilization has emerged as a promising strategy for enhancing plant tolerance to saline conditions. Nevertheless, the effectiveness of conventional sulfur fertilizers is constrained by slow oxidation and nutrient losses, highlighting the need for more efficient sulfur-delivery systems, including sulfur-based nanomaterials.

Recent advances in agricultural nanotechnology have introduced nano-fertilizers as promising alternatives to conventional fertilizers because of their high surface area, enhanced nutrient-use efficiency, and potential for controlled nutrient release. Among these, nano-sulfur (nS) has attracted considerable attention because of its potential to improve S availability while reducing nutrient losses (Subramanian et al., 2022). Following application to soil, elemental sulfur is oxidised to sulfate, which is subsequently taken up by plant roots via membrane-bound sulfate transporters (SULTRs) in an energy-dependent process and is translocated primarily through the xylem. Nano-sulfur may additionally interact with roots through nanoscale diffusion, potentially facilitating its uptake and distribution within root tissues and other cellular compartments. Following uptake, nS may be oxidized to sulfate, contributing to the synthesis of organic sulfur compounds and thereby increasing S availability for cellular metabolism. The enhanced surface reactivity and bioavailability of nS may improve sulfur utilization and reduce nutrient losses compared with conventional elemental sulfur applications (Sun et al., 2024). Previous studies have demonstrated that nS application can enhance chlorophyll content, nutrient uptake, antioxidant activity, and biomass accumulation under various environmental stress conditions (Khalifa et al., 2024).

During abiotic stress, nS can modulate plant metabolism by influencing oxidative stress, osmotic adjustment, photosynthesis, nutrient homeostasis, and the expression of stress-responsive genes. For instance, in salt-stressed fava bean plants, nS application enhanced the accumulation of sugars, amino acids, proline, glycine betaine, and antioxidants, while reducing H_2O_2 accumulation (Khalifa et al., 2024). These findings suggest that nS can improve osmotic adjustment and alleviate oxidative damage in plants exposed to salinity stress. Evidence from tomato further indicates that nS undergoes nanoscale-specific bioassimilation, resulting in time- and coating-dependent alterations in gene expression and metabolite profiles. The accumulation of nS-associated components in plant tissues was accompanied by metabolic changes linked to stress resistance and defense responses (Wang et al., 2021).

The complementary functions of MeJA and nS may provide a promising strategy for enhancing photosynthetic performance and salinity resilience by coordinately regulating redox homeostasis and osmolyte metabolism. We therefore hypothesized that the combined application of MeJA and nS would provide greater protection against salinity stress than their individual application by enhancing antioxidant metabolism, maintaining ROS homeostasis, and modulating proline metabolism, thereby preserving photosynthetic performance. Accordingly, this study compared the effectiveness of S and nS in alleviating salinity stress and further evaluated the individual and combined effects of MeJA, S and nS on plant growth, photosynthetic performance, antioxidant defense, and proline metabolism in *Brassica juncea* under salt stress. The study provides new insights into the potential interaction between MeJA signaling and nS-mediated sulfur nutrition and highlights their combined application

as a promising strategy for maintaining photosynthetic performance and physiological stability of mustard under salinity stress.

2. Material and methods

2.1. Plant material, growth conditions, and experimental treatments

Seeds of mustard (*Brassica juncea* L.) cv. Pioneer 45S46 were surface-sterilized with 0.01% $HgCl_2$ for 15 min and subsequently rinsed several times with double-distilled water. The sterilized seeds were sown in earthen pots (23 cm diameter) containing 5 kg of reconstituted soil comprising peat, compost and sand in a 4:1:3 (w/w) ratio. Six plants were maintained in each pot. The plants were grown under natural environmental conditions in the net house of the Department of Botany, Aligarh Muslim University, Aligarh, India. The average day and night temperatures were 20 ± 3 °C and 12 ± 2 °C, respectively, with a relative humidity of 60 ± 5%. Photosynthetically active radiation (PAR) was maintained at 750 ± 25 µmol m^{-2} s^{-1} under a photoperiod of approximately 10–12 h.

A preliminary screening experiment was conducted to determine the optimum concentration of nano-sulfur (nS). Plants were treated with 0, 50, 100, 200, and 300 mg nS kg^{-1} soil, and growth, photosynthetic, and oxidative stress parameters were evaluated. Sulfur nanoparticles were obtained from Nanoshel Limited (Cas No. 7704-34-9) as a light-yellow powder with >99.9% purity and a nominal particle size of 20–100 nm. Based on the overall plant response across the measured parameters, 200 mg nS kg^{-1} soil was selected as the optimum concentration for subsequent experiments.

Another experiment was conducted to compare the effects of elemental sulfur (S) and nano-sulfur (nS) on mustard tolerance to salinity stress. The experiment included four treatment groups: (i) untreated control (CK), (ii) 100 mM NaCl, (iii) NaCl combined with elemental nS (200 mg nS kg^{-1} soil) and (iv) NaCl combined with S (200 mg S kg^{-1} soil). The NaCl and elemental S concentrations were selected based on our previously published studies (Masood et al., 2024; Fatma et al., 2014), while the nS concentration was selected based on the results of a preliminary screening experimentation, as mentioned above. Salt treatment commenced 10 d after sowing (DAS) and was administered three times at alternate-day intervals over a period of one week. The untreated control plants received neither NaCl nor S or nS treatment. .

A separate experiment was conducted to investigate the effects of methyl jasmonate (MeJA) alone, and in combination with nS or elemental S on mustard responses with or without NaCl. The experiment comprised nine treatments: (i) control (CK), (ii) 100 mM NaCl, (iii) 10 µM MeJA, (iv) MeJA+ nS, (v) MeJA+ S, (vi) nS + NaCl, (vii) MeJA + NaCl, (viii) MeJA + nS + NaCl, and (ix) MeJA +S +NaCl. All sulfur-based treatments were applied at 200 mg kg^{-1} soil. MeJA was applied as a foliar spray at a concentration of 10 µM. It was obtained from Sigma-Aldrich (Cat. No. 392707) as a pale-yellow liquid with 95% purity. Foliar application commenced at 15 DAS and was repeated three times at alternate-day intervals, with 30 mL of the MeJA solution applied per pot on each application. The NaCl treatment at 100 mM was given as described above for Experiment 2. MeJA concentration was selected based on our previously published studies (Per et al., 2017). Plant responses were assessed at 30 DAS. All treatments were arranged according to a randomized block design with four biological replicates per treatment (n = 4).

2.2. Measurement of photosynthetic performance, gas exchange parameters and p-SUE

Photosynthetic performance was assessed by measuring leaf gas exchange characteristics using a CID-340 portable Infrared Gas Analyzer (Bio-Science, Camas, WA, USA). Relative chlorophyll content was determined during the early morning with a SPAD-502 DL PLUS chlorophyll meter (Konica Minolta, Japan). Data were

obtained from four independent pots per treatment (n = 4), and fully expanded leaves from six plants per pot were pooled to constitute one biological replicate.

For photosynthetic-sulfur use efficiency (p-SUE), the ratio of net photosynthetic rate to sulfur content on a unit leaf area basis was calculated. A comprehensive description of the experimental procedures is provided in the Supplementary File S1.

2.3. *Measurement of chlorophyll fluorescence parameters.*

Chlorophyll fluorescence was assessed using a Junior PAM fluorometer (Heinz Walz, Germany) to determine the maximum efficiency of photosystem II (Fv/Fm) and the photochemical quenching (qP). Measurements were performed on the second fully expanded leaves immediately below the shoot apex. A detailed description of the fluorescence measurement protocol is provided in the Supplementary File S1

2.4. Measurement of growth attributes

Plants were carefully collected and thoroughly washed with distilled water to remove surface contaminants. Leaf area was measured using a leaf meter (LA211, Systronics, New Delhi, India). Fresh weight was recorded, and samples were oven-dried at 80 °C until constant weight to determine dry weight.

2.5. Determination of oxidative stress markers

Hydrogen peroxide (H_2O_2) content was quantified according to Okuda et al. (1991). Lipid peroxidation was evaluated by measuring thiobarbituric acid reactive substances (TBARS) as described by Dhindsa et al. (1981). Briefly, 400 mg of fresh leaf material was homogenized under chilled conditions in 10% (w/v) trichloroacetic acid containing 0.25% (w/v) thiobarbituric acid. The extract was heated at 95 ºC for 30 min and immediately cooled in an ice bath to terminate the reaction. Following centrifugation at 10,000×g for 10 min, a 1.0 mL aliquot of the clarified extract was combined with 4.0 mL of 20% (w/v) trichloroacetic acid, and the absorbance was measured at 532 nm; background interference was corrected using the corresponding reading at 600 nm. The localization of hydrogen peroxide (H_2O_2) was carried out following the protocol reported by Kumar et al. (2014). A detailed description of the experimental procedure is presented in the Supplementary File S1.

2.6. Determination of Na^+ and Cl^- contents

Na^+ and Cl^- contents in roots and leaves were determined from plant samples digested with a tri-acid mixture of nitric acid, sulfuric acid, and perchloric acid (10:5:4, v/v/v). Na^+ concentration was measured using a flame photometer (Khera-391, Khera Instruments, New Delhi, India), whereas Cl^- was quantified by argentometric titration with 0.02 N $AgNO_3$, using 5% K_2CrO_4 as the indicator. Detailed analytical procedures are provided in the Supplementary File S1.

2.7. Determination of ATP-S activity and the contents of sulfur and cysteine

ATP-sulfurylase (ATP-S) activity was quantified as described by Lappartient and Touraine (1996). Total sulfur concentration in plant tissues was measured using a turbidimetric assay, whereas cysteine (Cys) was quantified according to the procedure established by Gaitonde (1967). A comprehensive description of all analytical methods is presented in the Supplementary File S1.

2.8. Assessment of reduced glutathione (GSH) levels

The level of reduced glutathione (GSH) was determined according to the procedure developed by Griffith(1980). The GSH content was determined by means of the enzyme recycling method in which GSH was oxidized by 5,5'-dithiobis (2-nitrobenzoic acid) and then reduced by glutathione reductase using NADPH as a reductant. Fresh leaf

samples (0.5 g) were frozen in liquid nitrogen and resuspended in 2.0 mL of 5% (w/v) sulfosalicylic acid. The homogenate was centrifuged at 12,000 g for 10 min and the clear supernatant was taken for subsequent assays.

2.9. Antioxidant metabolism

Fresh leaf tissue (0.2 g) was homogenized under chilled conditions in 100 mM potassium phosphate buffer (pH 7.0) supplemented with 0.05% (v/v) Triton X-100 and 1% (w/v) polyvinylpyrrolidone (PVP). The crude homogenate was clarified by centrifugation at 15,000 ×g for 20 min at 4 ℃, and the resulting extract was used for SOD and GR assays. For APX extraction, the same buffer was fortified with 2 mM ascorbic acid (AsA). SOD activity was evaluated according to the methods of Giannopolitis and Ries (1977). APX activity was estimated from the rate of ascorbate oxidation at 290 nm following Nakano and Asada (1981), whereas GR activity was quantified from NADPH consumption at 340 nm in the presence of glutathione, using the procedure of Foyer and Halliwell (1976). DHAR activity was determined from the increase in ascorbate at 256 nm resulting from glutathione-dependent reduction of dehydroascorbate, as described by Foyer et al. (1989). MDHAR activity was analyzed according to the protocol of (Hossain et al., 1984). Complete methodological information is available in Supplementary File S1.

2.10. Assessment of nitrogen (N) assimilation and nitrate reductase (NR) activity

Nitrate reductase (NR) activity was determined according to the method of Kuo et al. (1982) with the modifications reported by Iqbal et al. (2012). To maximize enzyme activity, leaves intended for NR analysis were harvested at midday under maximum light intensity to ensure optimum activation of the enzyme. The leaf content of Nitrogen was determined using the method of Lindner (1944). Further details of the procedure are given in Supplementary File S1.

2.11. Evaluation of proline metabolism and its metabolic enzymes.

Proline content was determined according to the protocol of Bates et al. (1973). Pyrroline-5-carboxylate synthase (P5CS) activity and its associated γ-glutamyl kinase (GK) activity were assayed based on the formation of γ-glutamyl hydroxamate, following the method of Hayzer and Leisinger (1980), with absorbance recorded at 540 nm. Proline oxidase (PROX) activity was estimated using the method of Huang and Cavalieri (1979) with minor modifications, as previously described by Khan et al. (2013). A detailed description of the analytical procedure of above is provided in the Supplementary File S1.

2.12. Histochemical detection of superoxide ion (O_2^-) and H_2O_2

Histochemical visualization of superoxide (O_2^-) and hydrogen peroxide (H_2O_2) in leaf tissues was carried out following the method of Kumar et al. (2014) with slight modifications. Superoxide accumulation was detected using nitro blue tetrazolium (NBT), whereas hydrogen peroxide was localized by staining with 3,3-diaminobenzidine (DAB). The NBT staining reagent was freshly prepared by dissolving 0.2 g NBT in 100 mL of 50 mM sodium phosphate buffer (pH 7.5). For DAB staining, 100 mg of DAB was dissolved in double-distilled water, adjusted to pH 3.8, and stored in an amber-coloured bottle until use. Leaf samples were separately immersed in the NBT and DAB solutions and then maintained overnight at room temperature. Following staining, chlorophyll was removed by immersing the leaves in boiling absolute ethanol for 20 min. The decolourized leaves were subsequently photographed to document the in-situ accumulation of O_2^- and H_2O_2.

2.13. Scanning electron microscopy

Leaf samples for scanning electron microscopy (SEM) analysis were prepared according to the protocol reported by Daud et al. (2009). The upper portions of leaves from different treatment groups were collected at about 10:00 am and immediately immersed in 2.5% glutaraldehyde for fixation. Stomatal micrographs were captured using a Scanning Electron Microscope at a magnification of 3000× (JEOL JSM-6510 LV, Japan) at the University Sophisticated Instrumentation Facility (USIF), Aligarh Muslim University, Aligarh, India.

2.14. Cell viability analysis

Root cell viability and H_2O_2 accumulation were assessed by fluorescence staining with propidium iodide (PI) and 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA), respectively. Fresh root segments were thoroughly washed, sectioned and incubated with 25 μM PI for 30 min in the dark or 20 μM DCFH-DA for 20 min at room temperature. Following staining, root sections were mounted on glass slides and visualized using a confocal laser scanning microscope (CLSM; Zeiss LSM 780, Tokyo, Japan).

2.15. Statistical analysis

Experimental observations were analyzed by One-way analysis of variance (ANOVA) using SPSS statistical software (version 17.0 for Windows; IBM Corp., Armonk, NY, USA). Data are presented as the mean ± standard error (SE) derived from four independent biological replicates (n = 4). Treatment means were compared using the least significant difference (LSD) multiple comparison test, and differences were considered statistically significant at $p < 0.05$. Means assigned the same alphabetical letter did not differ significantly according to the LSD test. To evaluate multivariate patterns and relationships among the physiological and biochemical datasets, principal component analysis (PCA) was carried out with OriginPro software (version 9.8 for Windows; OriginLab Corp., Northampton, MA, USA).

Results

Selection of nano-sulfur concentration

Application of 0, 50, 100, 200 and 300 mg nS kg^{-1} soil differentially influenced photosynthetic performance, growth characteristics, and oxidative stress markers. Notably, 200 mg nS kg^{-1} soil was more effective than other treatments, enhancing chlorophyll content, stomatal conductance, net photosynthetic rate, intercellular CO_2 concentration, leaf area and plant dry weight by 52.98%, 18.67%, 53.22%, 29.82%, 45.58% and 21.43%, respectively, compared with the CK plants. Furthermore, supplementation with 200 mg nS kg^{-1} soil markedly alleviated oxidative damage and reduced the contents of H_2O_2 and TBARS by 52.78% and 55.36%, respectively, compared with the CK (Table S1 and Fig. 1a).

Effect of nano-sulfur and sulfur on photosynthetic performance, plant growth and p-SUE under salt stress

Salt stress severely inhibited plant growth and photosynthetic efficiency, as reflected by decreased plant dry weight, net photosynthetic rate, stomatal conductance, SPAD value and intercellular CO_2 concentration by 50.46%, 43.36%, 24.33%, 37.02%, and 22.87%, respectively, compared with the CK (Table 1). Application of nS and S individually improved all measured growth attributes, with nS exhibiting a comparatively stronger promotive effect than S. Relative to the CK, nS treatment increased plant dry weight, net photosynthetic rate, stomatal conductance, SPAD value and intercellular CO_2 by 40.70%, 28.67%, 32.35%, 37.53%, and 31.67%, respectively. Under NaCl exposure, both nS and S effectively alleviated salt toxicity, although the mitigation was more pronounced in plants treated with nS (Table 1 and Fig. 1b). The nS + NaCl and S + NaCl treatments enhanced plant dry weight by 88.37% and 67.44%, net photosynthetic rate by 66.67% and 44.44%, stomatal conductance

by 30.04% and 24.73%, SPAD value by 50.20% and 38.37%, and intercellular CO_2 by 27.38% and 22.81%, respectively compared with the NaCl-treated plants (Table 1 and Fig. 1b).

Salt stress caused a significant 32.35% reduction in photosynthetic sulfur use efficiency (p-SUE) compared with the CK. In contrast, the individual application of nS and S increased p-SUE by 24.02% and 15.69%, respectively, over the CK plants. Furthermore, under salt stress conditions, supplementation with nS and S significantly improved p-SUE by 39.13% and 28.26%, respectively, relative to NaCl-treated plants (Table 1).

Impact of nano-sulfur and sulfur on oxidative stress markers under salt stress

Salt stress markedly enhanced oxidative stress as reflected by a significant increase of 2.03-fold in H_2O_2 and 1.90-fold in TBARS contents than in CK. Application of nS and S alone reduced H_2O_2 accumulation by 0.46-fold and 0.57-fold, respectively, while TBARS levels declined by 0.50-fold and 0.61-fold compared with the CK (Table 1). Under NaCl stress conditions, both nS and S supplementation effectively alleviated oxidative damage. The ameliorative effect was more pronounced under nS treatment, reducing H_2O_2 and TBARS levels to 0.55-fold and 0.56-fold, respectively. In contrast, S treatment reduced H_2O_2 and TBARS levels to 0.62 and 0.60-fold, respectively, compared to NaCl-treated plants (Table 1).

Table 1 Chlorophyll content (SPAD value), stomatal conductance, net photosynthesis, intercellular CO_2, plant dry weight, content of H_2O_2, and thiobarbituric acid reactive substance (TBARS) of mustard (*Brassica juncea* L. cv. Pioneer-45S46) leaves at 30 days after sowing (DAS). Plants were treated with 200 mg nS kg^{-1} soil, 200 mg S kg^{-1} soil in presence or absence of 100 mM NaCl.

Treatment	Chlorophyll content (SPAD value)	Stomatal conductance (mmol CO_2 m^{-2} s^{-1})	Net photosynthesis (µmol CO_2 m^{-2} s^{-1})	Intercellular CO_2 conc. (µmol CO_2 mol^{-1})	Plant dry weight ((g $plant^{-1}$)	Photosynthetic-SUE (gm^{-2})	H_2O_2 content (nmol g^{-1}FW)	TBARS content (nmol g^{-1}FW)
CK	38.9± 1.48^{c}	374 ± 14.25^{c}	14.3 ± 0.54^{c}	341 ± 12.99^{c}	0.86 ± 0.03^{c}	20.4 ± 0.77^{c}	15.3 ± 0.58^{cd}	4.56 ± 0.17^{d}
NaCl	24.5± 0.93^{f}	283 ± 10.78^{f}	8.1 ± 0.30^{e}	263 ±10.02^{f}	0.43 ± 0.01^{e}	13.8 ± 0.52^{e}	31.1 ± 1.18^{a}	8.65 ± 0.32^{a}
nS	53.5± 2.03^{a}	495 ± 18.86^{a}	18.4 ± 0.70^{a}	449 ± 17.11^{a}	1.21 ± 0.04^{a}	25.3 ± 0.96^{a}	7.1 ± 0.27^{f}	2.31 ± 0.09^{ef}
S	48.7± 1.85^{ab}	455 ± 17.34^{b}	17.1 ± 0.65^{b}	412 ± 15.70^{b}	1.13 ± 0.04^{b}	23.6 ± 0.89^{b}	8.8 ± 0.32^{e}	2.79 ± 0.10^{e}
nS + NaCl	36.8± 1.40^{cd}	368 ± 14.02^{cd}	13.5 ± 0.51^{cd}	335 ± 12.76^{d}	0.81 ± 0.03^{e}	19.2 ± 0.73^{c}	17.3 ± 0.65^{c}	4.94 ± 0.18^{c}
S+ NaCl	33.9± 1.29^{e}	353 ± 13.45^{e}	11.7 ± 0.44^{d}	323 ± 12.31^{e}	0.72 ± 0.02^{d}	17.7 ± 0.67^{d}	19.4 ± 0.73^{b}	5.21 ± 0.19^{b}

294 Data are presented as means ± SE (n=4). Data followed by the same letter are not significantly different by LSD test at $p < 0.05$.

S-assimilation mediated salt stress mitigation by nano-sulfur and sulfur

Salt stress markedly disrupted S metabolism in plants, resulting in significant increases in ATP-S activity, cysteine and GSH content by 16.67%, 19.97%, and 20.45%, respectively, whereas the S content declined by 34.35% compared with the CK (Table 2). Application of nS exhibited a more pronounced effect, enhancing ATP-S activity, cysteine, and GSH content by 86.36%, 71.81%, and 54.54%, respectively, while increasing S content by 67.07% compared with the CK plants. Application of S improved sulfur metabolism, increasing ATP-S activity and contents of cysteine and GSH by 76.52%, 58.05%, and 46.74%, respectively, while S content increased by 56.10% compared with the CK. Under NaCl stress, the nS treatment provided maximum protection, increased ATP-S activity, cysteine and GSH content by 72.08%, 52.73% and 39.05%, respectively, while S content increased by 46.44%. In contrast, S treatment increased ATP-S activity, cysteine and GSH content by 62.99%, 44.48% and 32.28%, respectively, while S content increased by 30.96% compared with NaCl-stressed plants (Table 2).

Table 2 Activity of ATP- sulfurylase (ATP-S), Content of sulfur (S), cysteine (Cys) and GSH in mustard (*Brassica juncea* L. cv. Pioneer-45S46) leaves at 30 days after sowing (DAS). Plants were treated with 200 mg nS kg^{-1} soil, 200 mg S kg^{-1} soil in presence or absence of 100 mM NaCl.

Treatment	ATP- S activity (U mg^{-1} protein min^{-1})	Sulfur content (mg g^{-1} DW)	Cysteine content (nmol g^{-1} FW)	GSH content (nmol g^{-1} FW)
CK	1.32 ± 0.05^{e}	4.92 ± 0.18^{c}	5.96 ± 0.22^{e}	311.3 ± 11.8^{f}
NaCl	1.54 ± 0.05^{d}	3.23 ± 0.12^{f}	7.15 ± 0.27^{d}	375.2 ± 14.3^{e}
nS	2.46 ± 0.09^{c}	8.22 ± 0.31^{a}	10.24 ± 0.40^{bc}	481.4 ± 18.3^{bc}
S	2.33 ± 0.08^{cd}	7.68 ± 0.29^{ab}	9.42 ± 0.36c	457.1 ± 17.4^{d}
nS+NaCl	2.65 ± 0.08^{a}	4.73 ± 0.18^{d}	10.92 ± 0.35^{a}	521.7 ± 19.8^{a}
S+NaCl	2.51 ± 0.07^{ab}	4.23 ± 0.16^{e}	10.33 ± 0.32^{b}	496.3 ± 18.9^{b}

Data are presented as means ± SE (n=4). Data followed by the same letter are not significantly different by LSD test at $p < 0.05$.

Effect of MeJA and nS on photosynthetic attributes under NaCl stress

NaCl stress markedly impaired photosynthetic performance in mustard plants, significantly reducing chlorophyll content (SPAD value), stomatal conductance (gs), net photosynthetic rate (Pn), intercellular CO_2 concentration (Ci), PSII efficiency (Fv/Fm) photochemical quenching (qP) and plant dry weight by 32.45%, 26.98%, 36.91%, 32.88%, 26.42% 29.18% and 36%, respectively, as compared with the CK. Under non-stress conditions, MeJA alone improved SPAD value, gs, Pn, Ci, Fv/Fm, qP and plant dry weight by 16.49%, 22.07%, 18.62%, 14.29%, 27.74%, 16.33% and 21.74%, respectively, compared with the CK. In contrast, the combined application of MeJA and nS or S enhanced these physiological attributes to a greater extent with MeJA plus nS treatment than MeJA plus S. MeJA+ nS treatment resulted in increased SPAD value, gs, Pn, Ci, Fv/Fm, qP and plant dry weight by 44.41%, 31.75%, 48.99%, 24.93%, 14.25% 21.44% and 58.70% respectively, compared with the CK. Under NaCl stress, the individual application of MeJA or nS mitigated the adverse effects of salinity on photosynthetic performance. Application of nS to plants under salinity stress resulted in increased, SPAD value, gs, Pn, Ci, Fv/Fm, qP and plant dry weight by 27.17%, 25.36%, 51.19%, 40.41%, 30.77%, 29.10% and 34.48%, while MeJA increased these parameters by 15.75%, 18.48%, 41.67%, 33.47, 25.21, 23.44 and 22.41%, respectively, relative to NaCl-treated plants. The combined application of MeJA with nS or S produced more substantial effects and enhanced photosynthetic performance under NaCl stress. Application of MeJA + nS under NaCl treatment more prominently exhibited ameliorative effect than MeJA + S, increasing SPAD value, gs, Pn, Ci, Fv/Fm, qP and plant dry weight by 43.70%, 34.78%, 50%, 47.76%, 35.21% 40.23% and 48.28%, respectively, relative to NaCl-treated plants. Thus, MeJA plus nS demonstrated the highest efficacy in restoring photosynthetic performance under NaCl stress. (Table 3 and Fig. 1c).



Fig. 1 Response of mustard (*Brassica juncea* L. cv. Pioneer 45S46) to NaCl stress, nano-sulfur (nS), sulfur (S) and methyl jasmonate (MeJA). Plants treated with 0, 50, 100, 200 and 300 mg nS kg^{-1} soi (**a**). Plants treated with 100 mM NaCl, 200 mg nS kg^{-1} soil, 200 mg S kg^{-1} soil and their combinations with NaCl (**b**) and plants treated with CK, 100 mM NaCl, 10 µM MeJA, MeJA+nS, MeJA+S, nS+NaCl, MeJA+NaCl, MeJA+nS+NaCl and MeJA+S+NaCl (**c**), at 30 days after sowing (DAS).

MeJA and nS mediated regulation of stomatal responses

NaCl stress severely disrupted stomatal aperture compared to CK plants (Fig. 2a, b). Under non-stress conditions, MeJA alone maintained relatively normal stomatal morphology and aperture (Fig. 2c). The combined application of MeJA and nS more effectively alleviated the adverse effects of salinity on stomatal architecture and aperture

than MeJA alone. Moreover, treatments such as MeJA + nS and MeJA+ S preserved stomatal architecture and aperture, thereby facilitating enhanced gas exchange and maintaining physiological activity (Fig. 2d, e). Notably, MeJA+nS showed a more pronounced positive response than the other treatments, indicating its superior ability to preserve stomatal integrity under salinity stress (Fig. 2f).

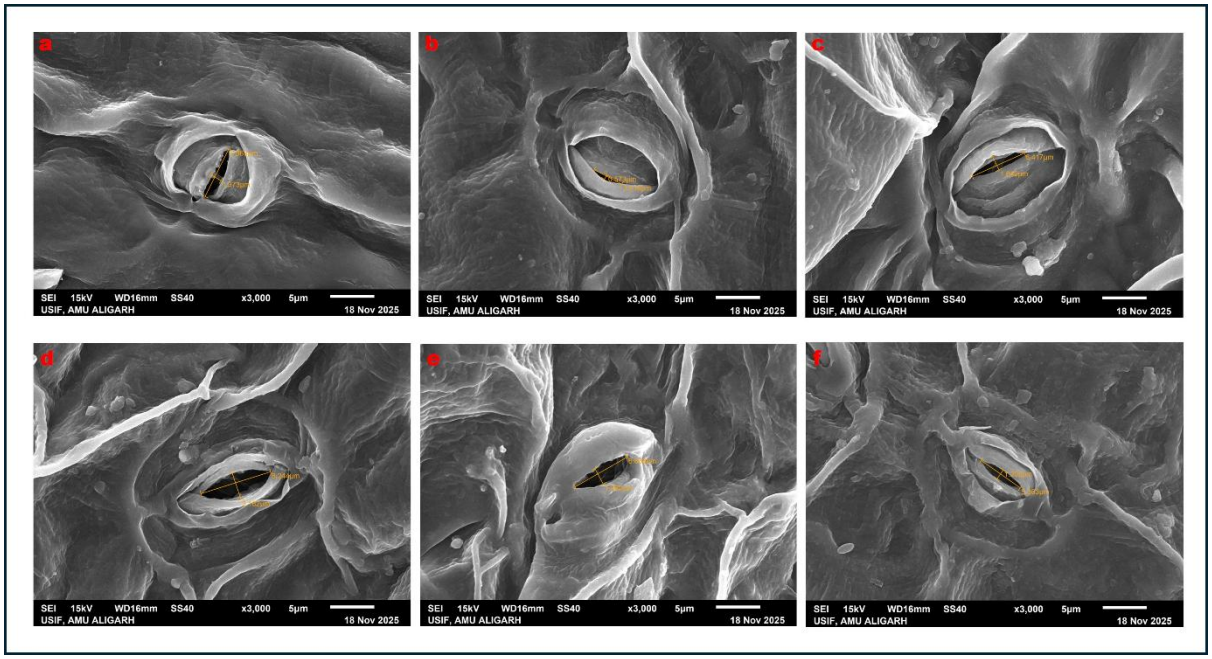


Fig. 2 Stomatal behaviour of mustard (*Brassica juncea* L. cv. Pioneer 45S46) under CK (**a**), 100 mM NaCl (**b**), 10 µM MeJA (**c**), MeJA+ 200 mg nS kg^{-1} soil (**d**), MeJA+ 200 mg S kg^{-1} soil (**e**) and MeJA+nS+NaCl (**f**) at 30 days after sowing (DAS). The stomatal aperture was observed under a scanning electron microscope at 3000× magnification. CK: control; NaCl: sodium chloride; MeJA: methyl jasmonate; nS: nano sulfur; S: Sulfur.

Influence of MeJA and nS supplementation on oxidative stress under NaCl stress

NaCl stress induced oxidative damage in mustard plants, as evidenced by significant increases in H_2O_2 and TBARS levels by 1.59 and 1.63-fold, respectively, compared with the CK. Under non-stress conditions, MeJA alone reduced the accumulation of these oxidative stress markers, and decreased H_2O_2 and TBARS contents by 0.83- and 0.88-fold, respectively, compared with the CK. , The combined application of MeJA with nS or S effectively reduced the accumulation of these oxidative stress markers, with the MeJA+ nS treatment produced the greatest reduction, lowering H_2O_2 and TBARS contents to 0.38 and 0.32-fold, respectively, compared with the CK (Table 3). Under NaCl stress, individual application of nS or MeJA effectively mitigated oxidative damage, with nS produced the greater reduction in H_2O_2 and TBARS contents by 0.69 and 0.82-fold, respectively, whereas MeJA reduced these parameters by 0.76 and 0.86-fold, respectively. Furthermore, the combined application of MeJA with nS or S exerted a stronger protective effect against salinity induced oxidative stress. Among all salinity-treated groups, the MeJA+ nS treatment was the most effective in decreasing H_2O_2 and TBARS levels to 0.63 and 0.73-fold, respectively, compared with NaCl-stressed plants. This demonstrates the superior capacity of the combined treatment of MeJA + nS to alleviate oxidative damage and preserve the cellular integrity under NaCl stress (Table 3).

Table 3 Chlorophyll content (SPAD value), stomatal conductance, net photosynthesis, intercellular CO_2, PSII efficiency, photochemical quenching, plant dry weight, content of H_2O_2, and thiobarbituric acid reactive substance (TBARS) of mustard (*Brassica juncea* L. cv. Poineer-45S46) leaves at 30 days after sowing (DAS). Plants were treated with 10 µM methyl jasmonate (MeJA), 200 mg nS kg^{-1} soil, 200 mg S kg^{-1} soil with combinations, in the presence or absence of 100 mM NaCl.

Treatment	Chlorophyll content (SPAD value)	Stomatal conductance (mmol CO_2 m^{-2} s^{-1})	Net photosynthesis (µmol CO_2 m^{-2} s^{-1})	Intercellular CO_2 conc. (µmol CO_2 mol^{-1})	PSII efficiency (Fv/Fm)	Photochemical quenching (qP)	Plant dry weight (g $plant^{-1}$)	H_2O_2 content ($nmolg^{-1}FW$)	TBARS content ($nmolg^{-1}FW$)
CK	39.6 ± 1.50^{cd}	358 ± 13.64^{c}	14.5 ± 0.55^{cd}	364 ± 13.87^{d}	0.667 ± 0.025^{d}	0.698 ± 0.026^{d}	0.92 ± 0.03^{d}	21.7 ± 0.82^{e}	3.45 ± 0.13^{f}
NaCl	25.4 ± 0.96^{h}	276 ± 10.52^{h}	8.4 ± 0.16^{h}	245 ± 9.33^{h}	0.468 ± 0.017^{h}	0.512 ± 0.019^{h}	0.58 ± 0.58^{h}	34.6 ± 1.31^{a}	5.64 ± 0.21^{a}
MeJA	45.8 ± 1.74^{c}	437 ± 16.65^{c}	17.6 ± 0.67^{c}	416 ± 15.85^{c}	0.852 ± 0.032^{c}	0.812 ± 0.030^{c}	1.12 ± 1.12^{c}	18.2 ± 0.69^{ef}	3.05 ± 0.11^{g}
MeJA+nS	54.3 ± 2.06^{a}	498 ± 18.98^{a}	22.2 ± 0.84^{a}	456 ± 17.38^{a}	0.882± 0.033^{a}	0.886 ± 0.033^{a}	1.46 ± 1.46^{a}	8.3 ± 0.31^{h}	1.12 ± 0.04^{i}
MeJA+S	48.9 ± 1.86^{b}	467 ± 17.80^{b}	20.1 ± 0.76^{b}	424 ± 16.16^{b}	0.834 ± 0.031^{b}	0.846 ± 0.032^{b}	1.33 ± 1.33^{b}	11.8 ± 0.44^{g}	1.84 ± 0.07^{h}
nS+NaCl	31.8 ± 1.21^{f}	346 ± 13.18^{f}	12.7 ± 0.48^{g}	344 ± 13.11^{ef}	0.612 ± 0.023^{f}	0.661 ± 0.025^{f}	0.78 ± 0.78^{f}	24.1 ± 0.91^{c}	4.65 ± 0.17^{c}
MeJA+NaCl	29.4 ± 1.12^{fg}	327 ± 12.46^{g}	11.9 ± 0.45^{gh}	327 ± 12.46^{g}	0.586 ± 0.022^{g}	0.632 ± 0.024^{g}	0.71 ± 0.71^{fg}	26.5 ± 1.01^{b}	4.88 ± 0.18^{b}
MeJA+nS+NaCl	36.5 ± 1.39^{d}	372 ± 14.18^{d}	14.1 ± 0.53^{e}	362 ± 13.79^{d}	0.661 ± 0.025^{de}	0.692± 0.026^{d}	0.86 ± 0.86^{de}	22.1 ± 0.84^{d}	4.12 ± 0.15^{e}
MeJA+S+NaCl	34.2 ± 1.30^{e}	354 ± 13.49b^{e}	13.3 ± 0.50^{f}	346 ± 13.18^{e}	0.628 ± 0.023^{e}	0.674 ± 0.025^{e}	0.81 ± 0.81^{e}	23.9 ± 0.91^{cd}	4.33 ± 0.16^{d}

366 Data are presented as means ± SE (n = 4). Measurements sharing the same letter are not significantly different at $p < 0.05$ using the LSD test.

Histochemical detection of ROS in response to MeJA and nS under NaCl stress

NBT and DAB staining were employed to visualize the accumulation of ROS in leaves. The intensity of NBT and DAB staining reflects the levels of ($O_2^{\bullet-}$) and (H_2O_2) respectively, in tissues subjected to different treatments. Leaves exposed to NaCl stress exhibited pronounced blue and brown precipitates, indicating elevated accumulation of $O_2^{\bullet-}$ and H_2O_2 compared to CK (Fig. 3a-b and Fig. 4a-b). MeJA alone noticeably reduced the intensity of NBT and DAB staining and the number of colored patches compared with NaCl-stressed leaves, indicating a lowered basal accumulation of ROS (Fig. 3c and Fig. 4c). The combined application of MeJA with nS further reduced staining intensity, with MeJA+ nS produced a more pronounced reduction in blue and brown precipitates than MeJA alone, suggesting enhanced suppression of ROS accumulation (Fig. 3d and Fig. 4d). Under NaCl stress, the combined MeJA+ nS treatment exhibited a pronounced reduction in blue and brown precipitates, with substantially fewer and less intense precipitates than those observed under NaCl stress alone, indicating a strong reduction of $O_2^{\bullet-}$ and H_2O_2 accumulation under salinity (Fig. 3f and Fig. 4f).

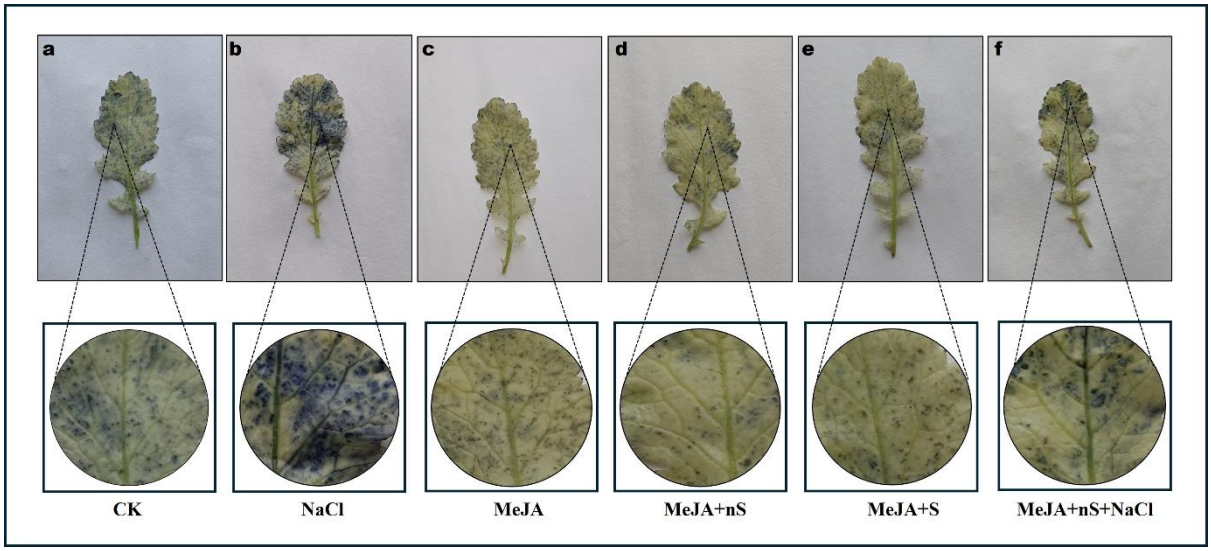


Fig. 3 Histochemical staining method of NBT showing visualization of superoxide anion visualization in mustard (*Brassica juncea* L. cv. Pioneer-45S46) leaves at 30 days after sowing (DAS). Leaves were represented as under CK (**a**), 100 mM NaCl (**b**), 10 µM MeJA (**c**), MeJA+ 200 mg nS kg^{-1} soil (**d**), MeJA+200 mg S kg^{-1} soil (**e**) and MeJA+nS+NaCl (**f**). NBT: nitro blue tetrazolium; CK: control; NaCl: sodium chloride; MeJA: methyl jasmonate; nS: nano sulfur; S: Sulfur.

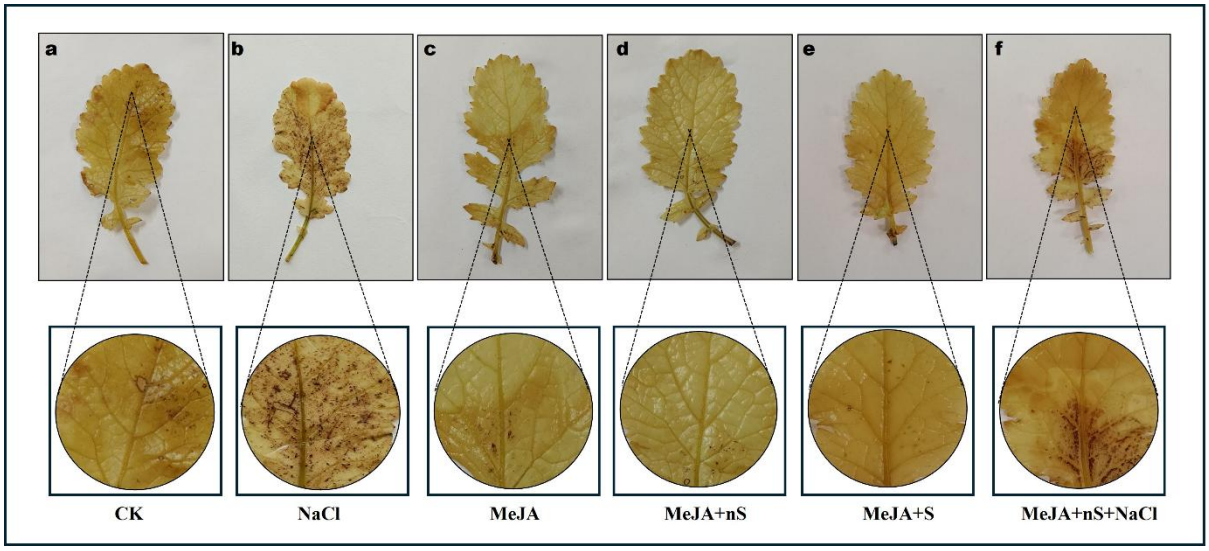


Fig. 4 DAB staining showing the visualization of hydrogen peroxide localization of mustard (*Brassica juncea* L. cv. Pioneer-45S46) leaves at 30 days after sowing (DAS). Leaves were represented as under CK (**a**), 100 mM NaCl (**b**), 10 µM MeJA (**c**), MeJA+ 200 mg nS kg^{-1} soil (**d**), MeJA+200 mg S kg^{-1} soil (**e**) and MeJA+nS+NaCl (f). DAB: 3, 3-diaminobenzidine; CK: control; NaCl: sodium chloride; MeJA: methyl jasmonate; nS: nano sulfur; S: Sulfur

Effect of MeJA and nS treatments on NaCl induced cell death

Confocal microscopy revealed pronounced root cell death under NaCl stress, as evidenced by intense red PI fluorescence resulting from its binding to nucleic acids in membrane-compromised cells (Fig. 5a-b). MeJA alone supplementation markedly reduced PI fluorescence, indicating improved membrane integrity and reduced cellular damage (Fig. 5c), while as the combined MeJA+ nS treatment further reduced PI fluorescence under non-stress conditions, exhibiting the weakest fluorescence signal and thereby indicating greater preservation of root cell membrane integrity. Under NaCl stress, the MeJA+ nS treatment produced a pronounced reduction in PI fluorescence, with substantially fewer intensely fluorescent regions, indicated that the combined treatment effectively limited membrane disruption and root cell death (Fig. 5f). Root ROS accumulation was further examined using DCFH-DA, a fluorescent probe for H_2O_2 detection. NaCl exposure substantially increased DCFH-DA fluorescence, indicating enhanced ROS accumulation in root tissues (Fig. 5g-h). MeJA alone markedly suppressed ROS-associated fluorescence, suggesting a lower level of ROS accumulation. The MeJA+nS treatment produced the weakest florescence, demonstrating reduced oxidative damage in roots (Fig. 5j). Notably, under NaCl stress, the MeJA+nS+NaCl treatment exhibited the lowest DCFH-DA fluorescence among the salinity treatments, demonstrating a pronounced reduction in ROS accumulation and consequently, lowered oxidative damage in root tissues (Fig. 5l).

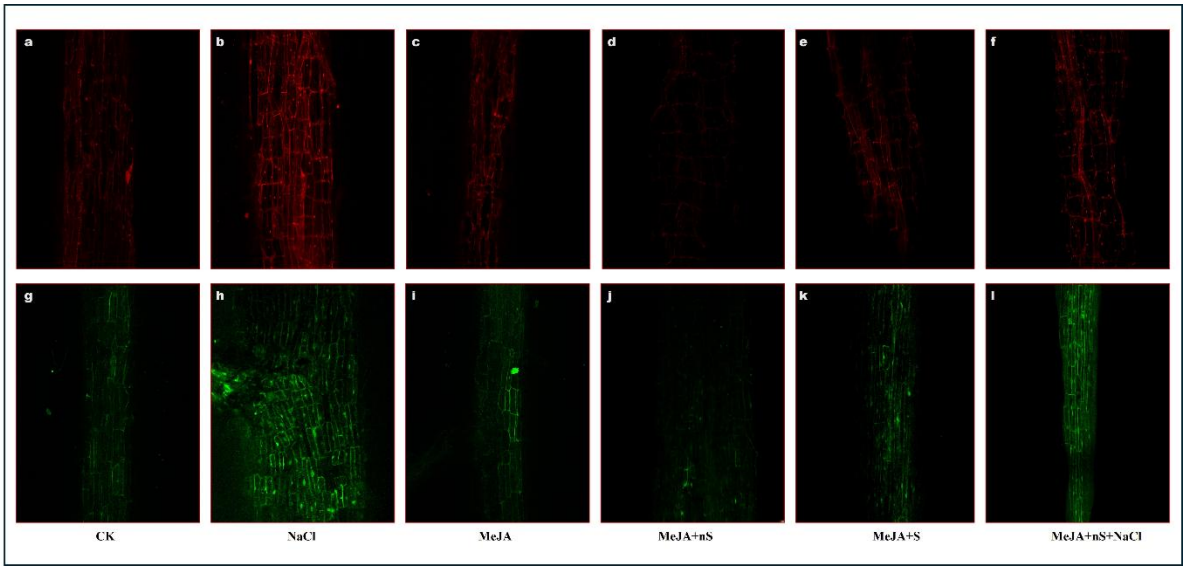


Fig. **5** Confocal microscopy revealed propidium iodide (PI) fluorescence, strong red fluorescence indicates loss of membrane integrity and reduced cell viability. CK (**a**), 100 mM NaCl (**b**), 10 µM MeJA (**c**), 10 µM MeJA + 200 mg nS kg^{-1} soil (**d**), 10 µM MeJA+200 mg S kg^{-1} soil (**e**) and MeJA+nS+NaCl (**f**). Strong green fluorescence denotes enhanced ROS generation, CK (**g**), 100 mM NaCl (**h**), 10 µM MeJA (**i**), 10 µM MeJA + 200 mg nS kg^{-1} soil (**j**), 10 µM MeJA+200 mg S kg^{-1} soil (**k**) and MeJA+nS+NaCl (**l**). CK: control; NaCl: sodium chloride; MeJA: methyl jasmonate; nS: nano sulfur; S: Sulfur.

MeJA and nS enhance ion homeostasis in salinity

Salinity stress markedly increased Na^+ and Cl^- accumulation in both roots and leaves compared with the CK (Table 4). Under non-saline conditions, application of MeJA alone reduced root Na^+ and Cl^- by 16.99% and 11.20%, whereas leaf Na^+ and Cl^- accumulation was reduced by 20.31% and 15.42%. The combined application of MeJA + nS produced a greater impacts and reduced root Na^+ and Cl^- by 32.35% and 23.53%, respectively, while leaf Na^+ and Cl^- contents were reduced by 38.41% and 35.36%, respectively, relative to the CK. Under NaCl stress, individual application of nS or MeJA reduced Na^+ and Cl^- accumulation in both roots and leaves; with nS+NaCl being more effective than MeJA. The nS treatment reduced root Na^+ and Cl^- contents by 49.66% and 37.89% and leaf Na^+ and Cl^- contents by 42.94% and 28.40%, respectively, whereas MeJA could reduce these parameters by 44.04%%, 30.48%, 37.82% and 21.28%, respectively, as compared to the NaCl-treated plants. Among all NaCl-stressed treatments, the combined MeJA+ nS treatment exhibited the greatest reduction in ionic accumulation, decreasing root Na^+ and Cl^- by 61.43% and 54.26%, respectively, and leaf Na^+ and Cl^- by 56.45% and 53.17% compared with NaCl alone (Table 4), indicating that the combined treatment most effectively restricted toxic ion accumulation under salinity stress.

Table 4 Na^+ and Cl^- contents in the roots and leaves of mustard plants (*Brassica juncea* L. cv. Poineer-45S46) at 30 days after sowing (DAS). Plants were treated with 10 µM methyl jasmonate (MeJA), 200 mg nS kg^{-1} soil, 200 mg S kg^{-1} soil with combinations, in presence or absence of 100 mM NaCl.

Treatment	Roots		Leaves	
	Na^+	Cl^-	Na^+	Cl^-
	(mg g^{-1} DW)		(mg g^{-1} DW)	
CK	9.18 + 0.34^{d}	7.74 + 0.29^{e}	8.41 + 0.32^{d}	6.77 + 0.25^{e}
NaCl	26.34 + 1.00^{a}	18.21 + 0.69^{a}	21.31 + 0.81^{a}	15.46 + 0.58^{a}
MeJA	7.62 + 0.29^{e}	6.34 + 0.24^{ef}	6.12 + 0.23^{e}	5.43 + 0.20^{e}
MeJA+ nS	6.21 + 0.23^{g}	5.46 + 0.20^{f}	4.97 + 0.18^{f}	4.15 + 0.15^{fg}
MeJA+ S	7.13 + 0.27^{ef}	6.17 + 0.23^{g}	5.18 + 0.19^{ef}	5.02 + 0.19^{f}
nS + NaCl	13.26 + 0.50^{bc}	11.31 + 0.43^{c}	12.16 + 0.46^{bc}	11.07 + 0.42^{c}
MeJA + NaCl	14.74 + 0.56^{b}	12.66 + 0.48^{b}	13.25 + 0.50^{b}	12.17 + 0.46^{b}
MeJA+ nS + NaCl	10.16 + 0.38^{d}	8.33 + 0.31^{d}	9.28 + 0.35^{d}	7.24 + 0.27^{d}
MeJA + S + NaCl	10.81 + 0.41^{c}	9.12 + 0.34^{cd}	10.12 + 0.38^{c}	8.06 + 0.30^{cd}

Data are presented as mean ± SE (n=4). Measurements sharing the same letters are not significantly different at $p < 0.05$ using the LSD test.

MeJA and nS mediated enhancement of nitrogen metabolism under salinity stress

NaCl stress substantially disrupted nitrogen metabolism in mustard plants, as evidenced by a 30.32% reduction in NR activity and a 51.63% decline in N content compared with the CK (Fig. 6a, b). Under non-saline conditions, application of MeJA alone enhanced NR activity and N content by 15.48% and 26.98%, respectively, compared with the CK. The combined application of MeJA with nS or S enhanced nitrogen metabolism, with MeJA + nS showing the greatest improvement, increasing NR activity and N content by 43.55% and 66.05%, respectively, relative to the CK. Under NaCl stress, the individual application of nS or MeJA improved nitrogen metabolism, exhibiting increased NR activity and N content by 32.41% and 51.92% with nS and 21.76% and 45.19% with MeJA, respectively. The combined application of MeJA with nS or S further improved nitrogen metabolism under NaCl stress, with MeJA+ nS showed greatest increase in NR activity and N content by 40.74% and 88.46%, respectively, compared with NaCl-treated plants (Fig 6a, b).

Regulation of proline metabolism under salinity stress by MeJA and nS

NaCl stress induced proline metabolism by 1.30-fold, indicating activation of osmotic adjustment relative to the CK. Under non-stress conditions, MeJA alone increased proline content by 1.79-fold relative to the CK, whereas the combined application of MeJA with nS or S produced a more pronounced enhancement. MeJA+ nS treatment showed the greatest increase of 2.19-fold compared with the CK (Fig. 6c).. Under NaCl stress, individual application of nS or MeJA improved proline accumulation. nS treatment increased proline content by 1.37 -fold,

whereas MeJA+NaCl increased it by 1.24-fold, relative to NaCl-treated plants. Notably, the MeJA+ nS + NaCl treatment exhibited the strongest response and increased proline content by 1.98-fold compared with NaCl-treated plants (Fig. 6c).

Salinity stress markedly altered the activities of key enzymes involved in proline metabolism. Relative to the CK, NaCl treatment significantly enhanced the activities of pyrroline-5-carboxylate synthetase (P5CS) and γ-glutamyl kinase (GK) by 1.25 and 1.75-fold, respectively, while PROX activity declined to 0.73-fold, indicating a shift towards proline biosynthesis under NaCl stress. Under non-saline conditions, exogenous application of MeJA alone significantly stimulated P5CS and GK activities by 1.37 and 1.83 -fold, respectively, while PROX activity declined to 0.56-fold. The combined application of MeJA+ nS elicited the greatest enhancement, increasing P5CS and GK activities by 2.07- and 2.47-fold, respectively, and reducing the PROX activity to 0.23-fold, relative to the CK (Fig. 6d, e, f). Under NaCl stress, supplementation with MeJA or nS further promoted the activities of P5CS and GK, with the nS+NaCl treatment increased these activities by 1.41 and 1.16-fold, respectively, while suppressed PROX activity by 0.61-fold relative to NaCl alone. Among all stressed treatments, the combined MeJA+nS+NaCl treatment produced the strongest response, increased P5CS and GK activities by 1.86- and 1.69-fold, respectively, and decreased PROX activity by 0.19-fold compared with NaCl-treated plants (Fig. 6d, e, f). These coordinated changes in the proline metabolic pathway are consistent with enhanced proline biosynthesis and reduced proline degradation, thereby contributing to improved osmotic adjustment and increased salinity tolerance.

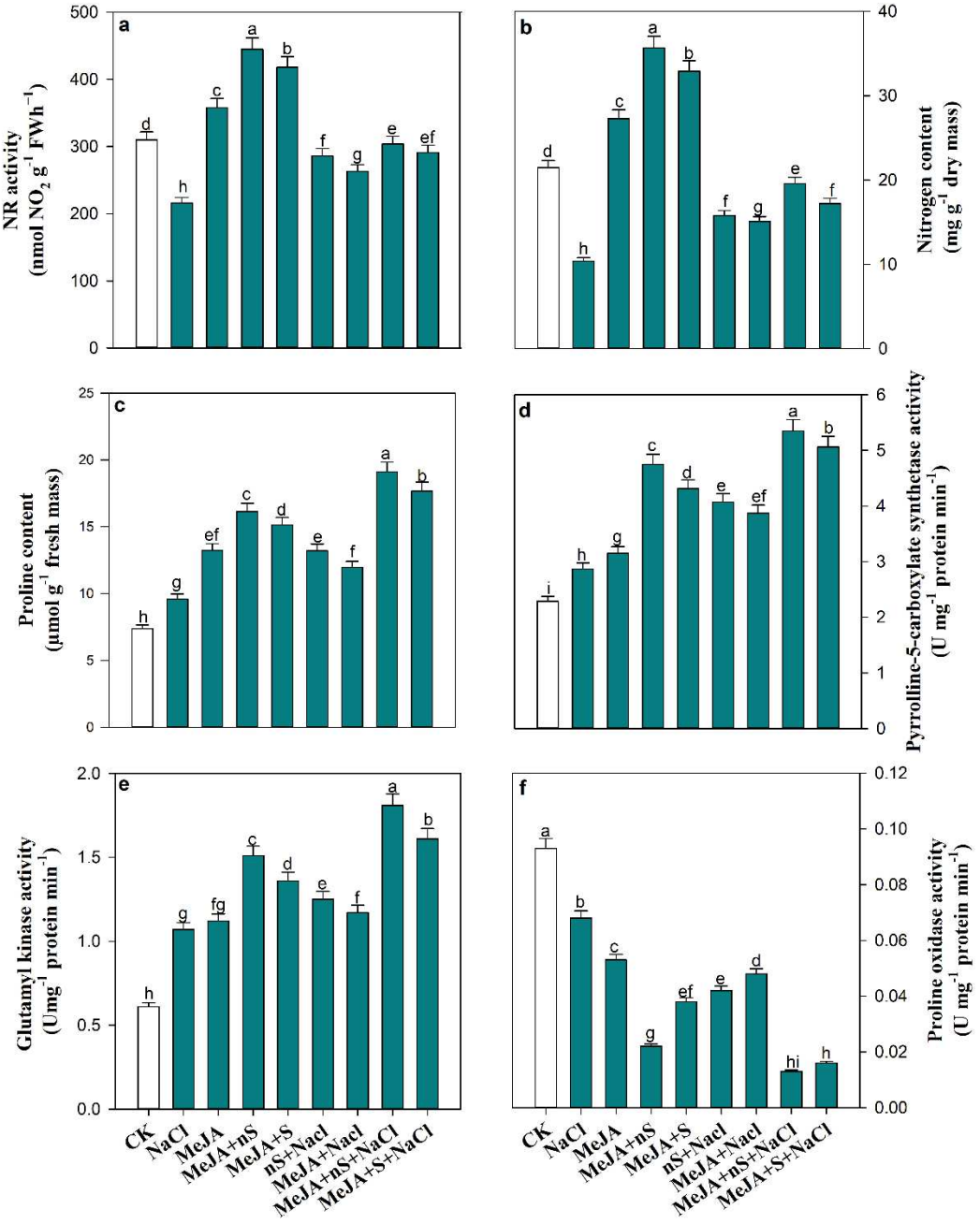


Fig. 6 Activity of nitrate reductase (NR) (**a**), nitrogen content (**b**), content of proline (**c**), activity of P5CS (**d**), GK (**e**) and PROX (**f**) in mustard (*Brassica juncea* L. cv. Pioneer-45S46) leaves at 30 days after sowing (DAS). Plants were treated with 10 µM (MeJA), 200 mg nS kg^{-1} soil (nS) and 200 mg S kg^{-1} soil in presence or absence of 100 mM NaCl. Data are presented as means ± SE (n=4). Data followed by same letter are not significantly different by LSD test at $p < 0.05$. MeJA: methyl jasmonate; nS: nano sulfur; S: sulfur; NR: nitrate reductase; P5CS: pyrroline-5-carboxylate synthetase; GK: Glutamyl kinase; PROX: Proline oxidase.

Influence of MeJA and nS supplementation on the antioxidant system under NaCl stress

NaCl stress markedly altered the AsA–GSH antioxidant system in mustard plants, significantly increased DHAR and MDHAR activities by 1.26 and 1.64-fold, respectively, and increased AsA and DHA contents by

1.40 and 1.45-fold , respectively, compared with the CK (Fig. 7). Under non-stress conditions, MeJA alone enhanced the AsA–GSH cycle, and increased DHAR and MDHAR activities and AsA and DHA contents by 1.54, 1.92, 1.57 and 1.76-fold, respectively, compared with the CK. The combined application of MeJA with nS or S further stimulated the AsA–GSH cycle, with MeJA+nS showed the strongest response, as reflected by increased activity of DHAR and MDHAR by 2.07 and 2.61-fold, and AsA and DHA content by3.03 and 2.7-fold, respectively, compared with the CK. Under NaCl stress, individual application of nS or MeJA further enhanced the AsA–GSH antioxidant system. The nS+NaCl treatment increased activity of DHAR and MDHAR, and content of AsA and DHA by 1.41, 1.37, 1.60 and 1.59-fold, respectively, compared to NaCl treated plants. The combined application of MeJA with nS or S further enhanced these responses under salinity stress. Among the treatments, MeJA+nS+NaCl elicited the strongest response, increasing activity of DHAR and MDHAR, and content of AsA and DHA by 1.97, 1.98, 2.27 and 2.08-fold, respectively, compared with NaCl-treated plants (Fig. 7). A similar enhancement was observed in the enzymatic components of the antioxidant defense system. NaCl exposure increased SOD, APX and GR activities and GSH content by 1.39, 1.54, 1.12 and 1.19-fold, respectively, compared with the CK (Fig. 8). Under non-stress conditions, MeJA alone enhanced antioxidant defense, and increased SOD, APX and GR activities and GSH content by 1.44, 1.63, 1.42 and 1.26-fold, respectively, compared with the CK. The combined application of MeJA with nS or S produced a stronger response, with MeJA+nS elicited the greatest enhancement, increasing SOD, APX and GR activities and GSH content by 2.37, 2.19, 1.88-and 1.58-fold, respectively, compared with the CK. . Under NaCl stress, individual application of nS or MeJA also enhanced antioxidant defense. The nS+NaCl treatment increased SOD, APX and GR activities and GSH content by 1.38, 1.27, 1.48 and 1.21-fold, respectively, whereas MeJA+NaCl increased these parameters by 1.23, 1.20, 1.36 and 1.17-fold, respectively, relative to NaCl-treated plants. The combined application of MeJA with nS or S further enhanced antioxidant defense under salinity stress. Among the treatments, MeJA+nS+NaCl produced the strongest response, increased SOD, APX and GR activities and GSH

content by 2.23, 1.56, 1.91 and 1.41-fold respectively, compared with NaCl-treated plants (Fig. 8).

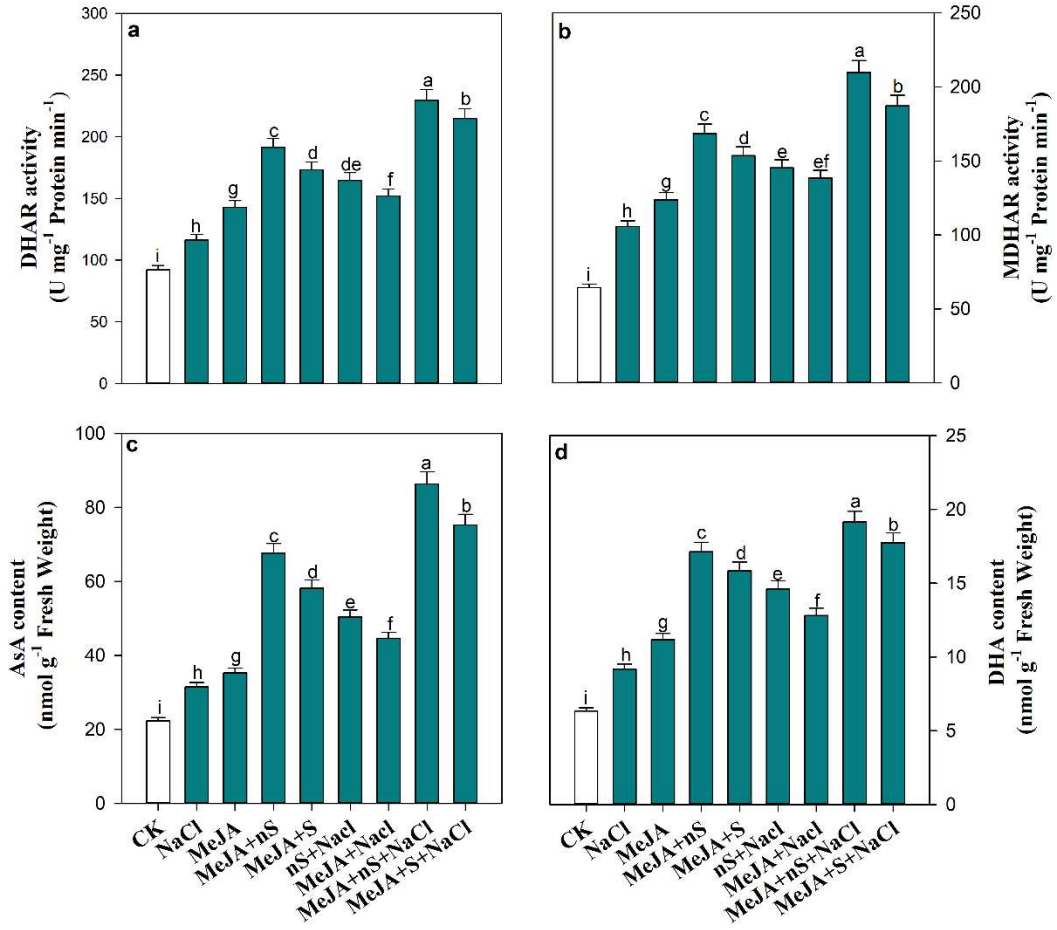


Fig. 7 Activity of dehydroascorbate reductase (DHAR) (**a**), monodehydroascorbate reductase (MDHAR) (**b**), and contents of ascorbate (AsA) (**c**), and dehydroascorbate (DHA) (**d**) in mustard (*Brassica juncea* L. cv. Pioneer-45S46) leaves at 30 days after sowing (DAS). Plants were treated with 10 µM (MeJA), 200 mg nS kg^{-1} soil and 200 mg S kg^{-1} soil in presence or absence of 100 mM NaCl. Data are presented as means ± SE (n=4). Data followed by same letter are not significantly different by LSD test at $p < 0.05$. MeJA: methyl jasmonate; nS: nano sulfur; S: sulfur.

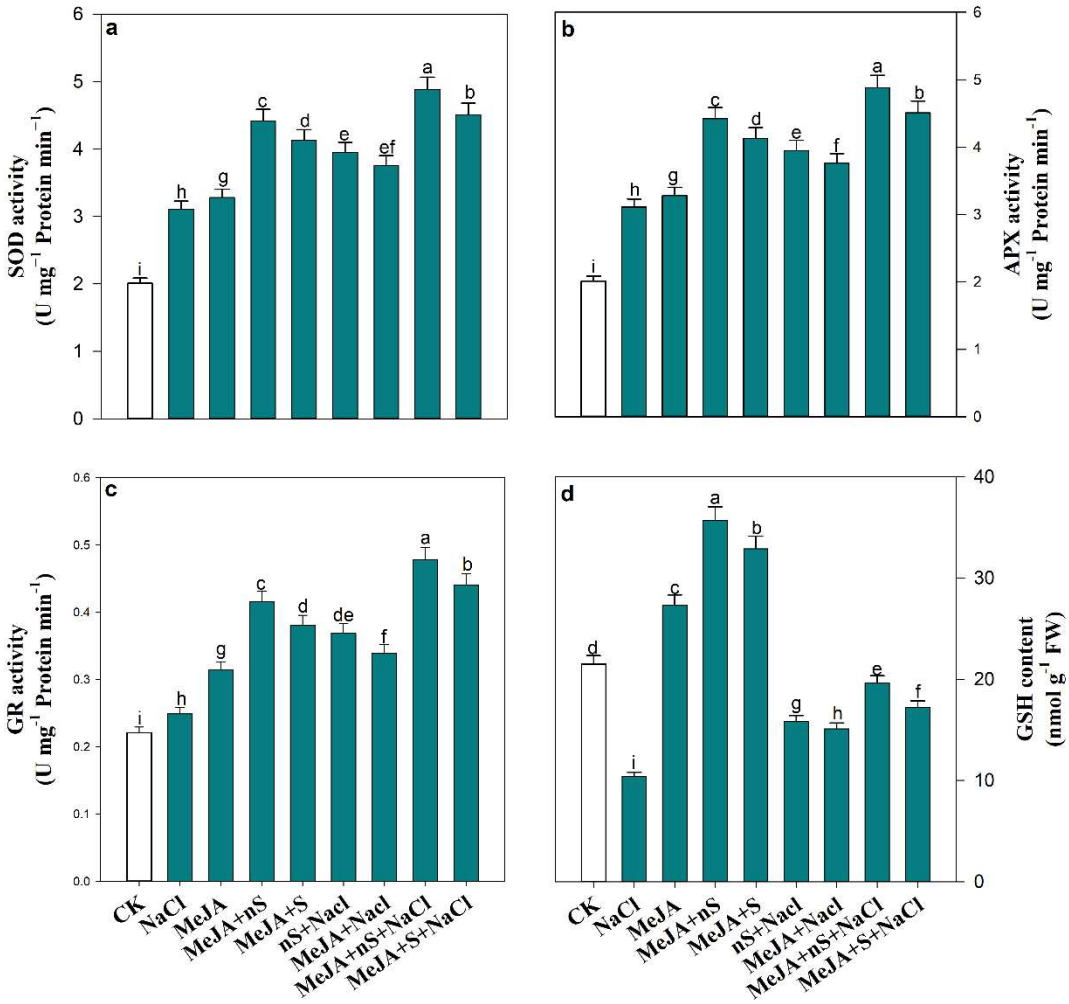


Fig. 8 Activity of superoxide dismutase (SOD) (**a**), ascorbate peroxidase (APX) (**b**), glutathione reductase (GR) (**c**). and content of reduced glutathione (GSH) (**d**) in mustard (*Brassica juncea* L. cv. Pioneer-45S46) leaves at 30 days after sowing (DAS). Plants were treated with 10 µM (MeJA), 200 mg nS kg^{-1} soil and 200 mg S kg^{-1} soil in presence or absence of 100 mM NaCl. Data are presented as means ± SE (n=4). Data followed by same letter are not significantly different by LSD test at $p < 0.05$. MeJA: methyl jasmonate; nS: nano sulfur; S: sulfur; SOD: superoxide dismutase; APX: ascorbate peroxidase; GR: glutathione reductase: GSH: reduced glutathione.

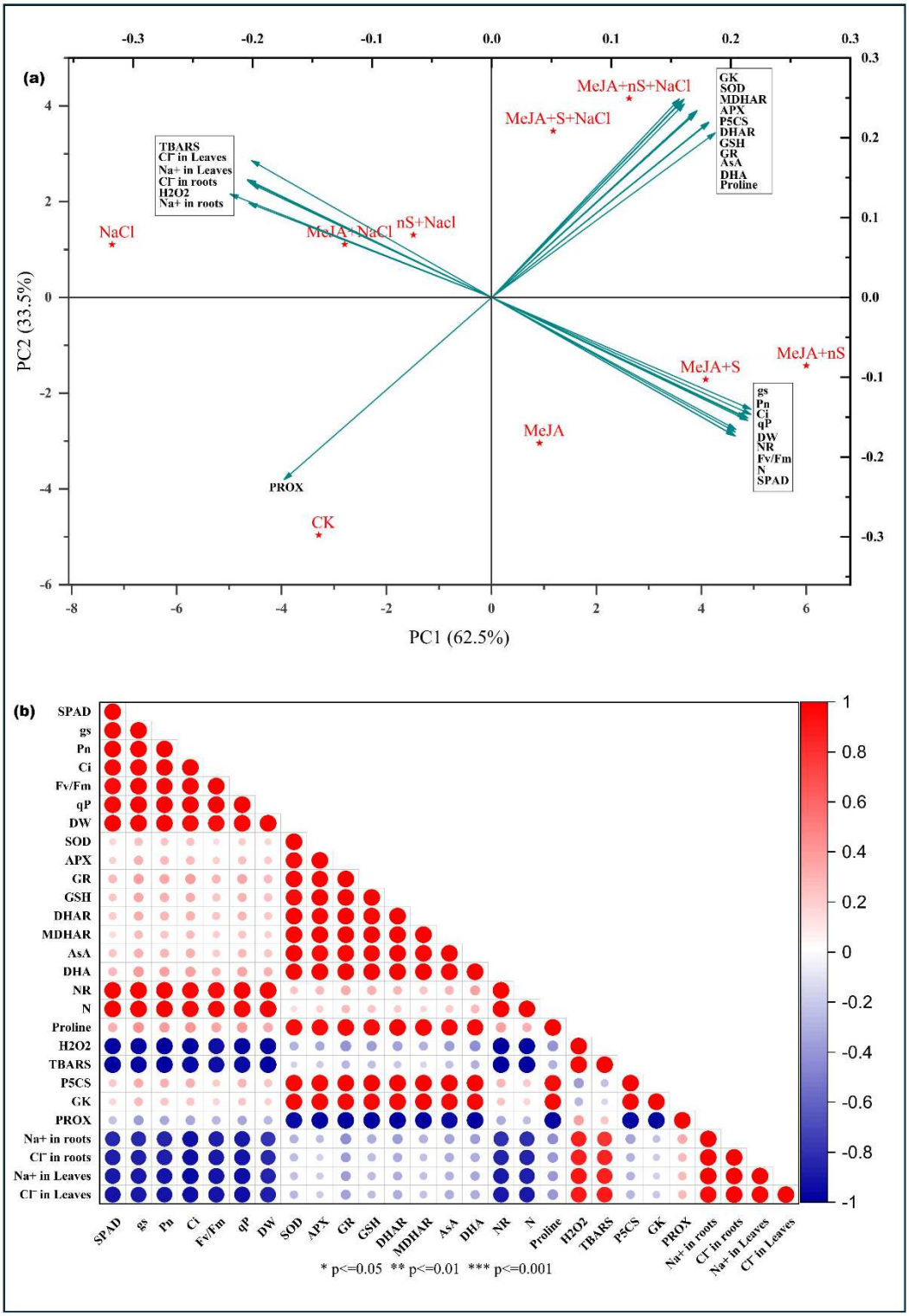
Principal component analysis

Principal component analysis (PCA) illustrates the relationships among physiological, biochemical, and oxidative stress-related traits in mustard plants. The first two principal components explained 96.0% of the total variation, with PC1 and PC2 accounting for (62.5%) and (33.5%), respectively. NaCl, MeJA+NaCl, and nS+NaCl treatments were positioned predominantly on the negative side of PC1 and associated with higher oxidative stress markers and Na^+/Cl^- accumulation. In contrast, MeJA+nS and MeJA+S treatments were associated with photosynthetic attributes, growth, and nitrogen status, whereas MeJA+nS+NaCl and MeJA+S+NaCl clustered in the positive quadrants and showed strong associations with antioxidant defense components, including SOD, APX, GR, DHAR, MDHAR, GSH, AsA, DHA, and proline. The CK treatment was positioned opposite to several stress-associated variables, while PROX was oriented toward the CK region. Overall, the PCA demonstrates that

MeJA and nS combinations shifted the physiological profile away from salt-induced ionic and oxidative stress toward enhanced photosynthetic, growth performance, nitrogen metabolism, osmoprotective metabolism and antioxidant protection, thereby enhancing salinity tolerance in *Brassica juncea* (Fig. 9a).

Pearson correlation

Pearson correlation analysis revealed strong positive correlations among photosynthetic performance, antioxidant metabolism, osmoprotection and ion homeostasis. SPAD, gs, Pn, Ci, Fv/Fm qP and DW. These parameters were also positively associated with nitrate reductase (NR) activity and nitrogen (N) content. Antioxidant defense components, including SOD, APX, GR, GSH, DHAR, MDHAR, AsA and DHA, exhibited strong positive interrelationships and were positively associated with proline accumulation and proline biosynthetic traits (P5CS and GK). In contrast, H_2O_2 and TBARS showed strong negative correlations with photosynthetic performance, NR activity, N content and DW, whereas root and leaf Na^+ and Cl^- accumulation displayed strong negative associations with these traits. Proline and its biosynthetic components were generally associated positively with antioxidant defense, while PROX exhibited an opposing correlation pattern.. The strong correlations among antioxidant capacity, nitrogen metabolism, proline metabolism, photosynthetic and growth attributes demonstrated that MeJA and nS work together to maintain redox and ionic balance, thereby increasing the ability to withstand salt stress. From the findings, it can be concluded that the relationships obtained in the investigated systems are statistically significant ($p<0.05$, $p<0.01$, $p<0.001$). In this regard, it can be stated that MeJA and nS have a mutually enhancing effect in improving salinity tolerance by regulating distinct protective mechanisms (Fig. 9b).



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556 **Fig. 9** Biplots of principal component analysis (PCA, a) and the Pearson correlation coefficients (b) between
557 different variables in mustard (*Brassica juncea* L. cv. Pioneer 45S46) grown with or without salt stress (100 mM
558 NaCl) and treated with methyl jasmonate (MeJA; 10 µM), nano-sulfur (200 mg nS kg^{-1} soil) and sulfur (200 mg
559 S kg^{-1} soil). SOD, superoxide dismutase; APX, ascorbate peroxidase; GR, glutathione reductase; GSH, reduced
560 glutathione; DHAR, dehydroascorbate reductase; MDHAR, monodehydroascorbate reductase; ASA, ascorbate;

DHA, dehydroascorbate; NR, nitrate reductase; N, nitrogen content; P5CS, pyrroline-5-carboxylate synthetase; GK, glutamyl kinase; PROX, proline oxidase

Discussion

Salinity is a major abiotic stressor that severely limits crop productivity by disrupting photosynthesis, nutrient homeostasis, and cellular redox balance (Ahmed et al., 2024). In the present study, NaCl stress markedly increased oxidative damage while reducing photosynthetic efficiency and p-SUE, indicating substantial physiological and metabolic impairment. These effects are primarily associated with salt induced osmotic stress and ionic toxicity. Salinity stress triggers ROS mediated signaling, which contributes to ion homeostasis through vacuolar Na^+ sequestration, tonoplast Na^+ leak regulation, and SOS1-mediated Na+ extrusion (Bazihizina et al., 2022). Salinity induced osmotic and ionic disturbances can impair chloroplast function, restrict carbon assimilation, and stimulate reactive oxygen species (ROS) production

(Alharbi et al., 2022). The reductions in SPAD value, stomatal conductance photosynthesis indicate that both stomatal and non-stomatal limitations contributed to the decline in photosynthetic performance. Furthermore, salinity induced degradation of chlorophyll pigments and disruption of thylakoid membrane integrity reported under salt stress may likely have contributed to impaired carbon fixation and reduced biomass accumulation. The report is consistent with the previous findings reported by Wang et al. (2024) and Dhokne et al. (2022).

Nano-sulfur effectively alleviated the detrimental effects of salinity and consistently outperformed bulk sulfur, highlighting the advantages of nano-enabled sulfur delivery. Its high surface area and enhanced reactivity facilitated sulfur uptake and assimilation, promoting the biosynthesis of sulfur-containing metabolites essential for stress adaptation (Sun et al., 2024). Sulfur functions as a vital macronutrient by supporting S assimilation and stimulating the production of Cys and GSH, two key metabolites involved in maintaining cellular redox homeostasis and detoxifying ROS. Enhanced S availability has been shown to promote the accumulation of antioxidant compounds, including ascorbate, phenolics, and tocopherols, and to improve ion homeostasis by reducing Na^+ toxicity and increasing K^+ and Ca^{2+} uptake, thereby sustaining photosynthetic activity and plant growth under saline conditions (Jahan et al., 2021b). Consistent with these mechanisms, nS-treated plants maintained higher chlorophyll content, stomatal conductance, photosynthetic rate, and biomass under salt stress. The marked increase in p-SUE further indicates more effective utilization of absorbed S for photosynthetic metabolism. Salinity disrupts sulfur homeostasis, causing tissue S depletion despite compensatory upregulation of the assimilation pathway, while the reduced S pool limits sustained synthesis of Cys and GSH. Thus, enhancing S supply offers a route to strengthen antioxidant defense under salinity; the restoration of p-SUE by nS in the present study suggests improved S metabolism, which likely contributes to chloroplast protection and enhanced photosynthetic performance. These findings support the report of Alrabie et al. (2025). Comparable improvements in nutrient use efficiency following nano-fertilizer application under abiotic stress have also been documented by Shoukat et al. (2025), supporting the superior effectiveness of nano-enabled nutrient management

The enhanced performance of nS was further supported by increased ATP-S activity and greater accumulation of S and Cys. ATP-S catalyzes the first committed step of sulfate assimilation and thereby plays a central role in regulating Cys and GSH biosynthesis (Yuan et al., 2021; De Bont et al., 2022). Thus, the increase in ATP-S activity suggests enhanced sulfur assimilation rather than merely increased sulfur uptake. The greater accumulation of Cys provides an important metabolic precursor for GSH biosynthesis, thereby strengthening cellular redox regulation and antioxidant capacity. Collectively, these coordinated responses suggest that nS

enhances both sulfur assimilation and its subsequent metabolic utilization, contributing to the maintenance of photosynthetic function and promotion of plant growth under saline conditions.

Salinity-induced stomatal closure restricts CO_2 diffusion into leaves and can impair PSII function, resulting in reduced photosynthetic efficiency and plant growth (Zahra et al., 2022). In the present study, NaCl stress caused pronounced stomatal deformation, accompanied by substantial reductions in photosynthetic performance and chlorophyll fluorescence characteristics. These adverse effects were most effectively alleviated by the combined application of MeJA and nS, suggesting a complementary interaction between jasmonate-mediated stress signaling and sulfur-dependent redox regulation. MeJA may enhance stress signaling and contribute to the maintenance of chlorophyll stability, PSII integrity, and photosynthetic activity under salinity (Deb et al., 2026). Meanwhile, nS improved sulfur acquisition and assimilation, thereby supporting GSH-mediated antioxidant defense and reducing ROS-induced damage to chlorophyll and PSII components (Ahmadi et al., 2018; Per et al., 2016). Importantly, the changes in plant biomass provide a growth-related indication of these physiological responses; reduced dry matter accumulation under salinity is consistent with impaired photosynthetic carbon assimilation. Conversely, the improved dry weight under MeJA+nS treatment suggests that maintaining photosynthetic activity and redox homeostasis may have supported assimilate production and biomass formation under saline conditions (Ahmed et al., 2026). The combined regulation of stomatal function, PSII stability, and ROS homeostasis may, therefore, contribute to sustained photosynthetic performance and plant growth under salinity stress. Previous studies have demonstrated that exogenous MeJA alleviates salt-induced inhibition of photosynthesis and growth in *Brassica napus* by improving physiological and photosynthetic performance (Ahmadi et al., 2018). Similarly, exogenous MeJA has been reported to protect PSII function and maintain redox homeostasis in pea exposed to cadmium stress (Manzoor et al., 2022). More recently, MeJA application was shown to enhance gas exchange, chlorophyll content, and chlorophyll fluorescence in basil plants subjected to salinity stress, further demonstrating its protective role in maintaining photosynthetic function under saline conditions (Farhadi et al., 2026). Taken together, these findings support the potential of MeJA+nS treatment to coordinate hormonal signaling and sulfur-mediated antioxidant defense, thereby improving photosynthetic stability and stress tolerance under salinity.

Oxidative stress is a major consequence of salinity, as evidenced by the significant accumulation of H_2O_2 and TBARS in NaCl-treated plants. Excessive ROS accumulation can damage membrane lipids, proteins, and chloroplast structures, resulting in membrane destabilization and disruption of cellular metabolism (Ma et al., 2025). In the present study, nS markedly reduced both H_2O_2 and TBARS levels, indicating attenuation of oxidative damage. This protective effect was likely associated with enhanced sulfur assimilation and increased availability of Cys for GSH biosynthesis. Improved sulfur metabolism may, in turn, strengthen the antioxidant capacity of plants, thereby protecting photosynthetic machinery and supporting carbon assimilation under saline conditions. These findings suggest that enhanced sulfur assimilation and utilization represent important mechanisms underlying the beneficial effects of nS on photosynthetic performance and salinity tolerance.

Maintenance of cellular redox homeostasis is fundamental to plant adaptation to salinity stress. In the present study, NaCl treatment markedly increased ROS accumulation, as indicated by intense DAB and NBT staining, elevated H_2O_2 and TBARS contents, and enhanced propidium iodide fluorescence in both leaves and roots. These responses indicate substantial oxidative stress, membrane damage, and loss of cellular integrity. In contrast, the

combined application of MeJA and nS markedly reduced ROS accumulation and membrane injury, demonstrating enhanced protection against salinity-induced oxidative stress. The effectiveness of MeJA+nS may be attributed to the complementary actions of jasmonate-mediated stress signaling and sulfur-dependent antioxidant defense. MeJA enhanced stress-responsive antioxidant mechanisms, whereas nS increased sulfur assimilation and supported Cys- and GSH-dependent ROS detoxification. The resulting improvement in redox homeostasis may help preserve membrane integrity and cellular function under salinity. These observations are consistent with previous reports demonstrating that excessive ROS accumulation disrupts membrane integrity and accelerates cellular dysfunction under saline conditions (Alrabie et al., 2025; Basu and Kumar, 2024).

The reduced Na^+ and Cl^- accumulation in the combined MeJA+ nS treated plants suggests improved ionic homeostasis as a potential mechanism underlying their enhanced salinity tolerance. Excessive Na^+ and Cl^- accumulation disrupts cellular ion balance and water relations, ultimately impairing photosynthetic function and growth (Karimi et al., 2022). Mechanistically, jasmonate signaling may contribute to ionic regulation by modulating the expression and activity of ion transport systems involved in Na^+ exclusion, K^+ retention and long-distance ion transport. Supporting this interpretation, Hussain et al. (2022) demonstrated that exogenous MeJA alleviated salinity induced ionic disturbances in rice, improving K^+ accumulation and the K^+/Na^+ ratio along with differential expression of ion transporter genes. In parallel, nS may support sulfur assimilation and the synthesis of cysteine and glutathione, thereby contributing to cellular redox regulation and protection against oxidative damage (de Bont et al., 2022). These complementary effects may have helped preserve cellular integrity and guard cell function, providing a physiological explanation for the corresponding enhancement in stomatal conductance. The associated responses in intercellular CO_2 concentration and net photosynthesis further support the maintenance of gas exchange under salinity (Boulal et al., 2022). Collectively, the results suggest that MeJA and nS may contribute complementary effects on ion homeostasis and sulfur dependent redox protection, although their precise molecular interaction requires further investigation.

The enhanced salt tolerance conferred by the combined application of MeJA and nS was closely associated with coordinated activation of enzymatic and non-enzymatic antioxidant defenses. Although salinity itself induced antioxidant enzymes as part of an adaptive response, MeJA+nS treatment further enhanced the activities of SOD, APX, GR, DHAR, and MDHAR, accompanied by increased levels of AsA and DHA. These coordinated changes indicate enhanced functioning of the AsA–GSH cycle, which plays a central role in maintaining cellular redox homeostasis and facilitating ROS detoxification. The improved antioxidant capacity may be associated with enhanced sulfur assimilation, which provides sulfur-containing metabolites required for efficient ROS scavenging and protection of photosynthetic machinery (Mohi-Ud-Din et al., 2021). Consequently, the strengthened antioxidant defense likely restricted H_2O_2 accumulation and lipid peroxidation, thereby contributing to the preservation of membrane stability under salinity stress (Fig. 10). Similar findings were reported by Per et al. (2016), who demonstrated that MeJA-mediated stimulation of sulfur assimilation and glutathione metabolism enhanced antioxidant defense and helped preserve photosynthetic function under abiotic stress. MeJA acts as an important stress-signaling molecule that coordinates antioxidant responses with sulfur metabolism. Furthermore, the observed increase in endogenous jasmonate levels may indicate positive feedback within the stress-signaling network, potentially reinforcing downstream defense responses. Collectively, these findings highlight the potential role of MeJA in coordinating sulfur metabolism, antioxidant defense, and photosynthetic protection during salinity stress (Sehar et al., 2023).

Histochemical and confocal analyses further corroborated the biochemical responses, demonstrating that NaCl stress markedly enhanced ROS accumulation and compromised root cellular integrity. Increased NBT and DAB staining, together with stronger DCFH-DA and propidium iodide (PI) fluorescence, indicated elevated accumulation of H_2O_2 and superoxide, increased oxidative stress, and membrane damage under salinity. MeJA markedly attenuated these responses, whereas the combined MeJA+nS treatment markedly reduced staining intensity and ROS associated fluorescence in roots, accompanied by weaker propidium iodide fluorescence, reduced oxidative damage and improved membrane integrity. These responses suggested that MeJA-mediated stress signaling, combined with nS supported redox metabolism, enhanced ROS detoxification and preserved cellular stability. Previous studies have similarly linked jasmonate-mediated stress responses and sulfur metabolism with improved antioxidant defence under adverse conditions (Manzoor et al., 2025). Collectively, these findings indicate that combined MeJA+nS application effectively alleviated salinity induced oxidative injury in mustard. Salinity also caused a substantial decline in nitrate reductase activity, reflecting impaired nitrogen assimilation, which can restrict amino acid and protein biosynthesis (Hamidian et al., 2023; Beisekova et al., 2026). This observation is consistent with previous findings that salinity suppresses nitrogen uptake and assimilation in mustard, including reductions in NR activity and the expression of key nitrogen-assimilatory genes (Majid et al., 2021). Importantly, the stronger response observed following MeJA+nS treatment suggests a functional interaction between sulfur nutrition and nitrogen assimilation. Sulfur availability is closely interconnected with nitrogen metabolism, and sulfur deprivation has been shown to substantially reduce NR activity, whereas sulfur supplementation can restore it (Ristova and Kopriva, 2022). Therefore, the improved nitrogen status observed in MeJA+nS-treated plants under salinity may reflect the complementary effects of jasmonate-mediated stress regulation and enhanced sulfur availability, collectively supporting nitrate assimilation and maintenance of nitrogen homeostasis (Fig. 10).

Similarly, the combined treatment significantly increased proline accumulation, which contributes to osmotic adjustment, membrane stabilization, ROS scavenging, and protection of cellular proteins under stress (Jurkoniene et al., 2023). The enhanced proline accumulation observed following MeJA+nS treatment is consistent with previous reports showing that jasmonates stimulate proline biosynthesis, while adequate sulfur nutrition supports amino acid metabolism under stress conditions (Hamidian et al., 2023; Mulaudzi et al., 2023). Thus, the coordinated regulation of ROS detoxification, nitrogen assimilation, sulfur metabolism, and proline accumulation by MeJA+nS may substantially contribute to maintaining cellular homeostasis and enhancing salinity tolerance (Fig. 10).

At the metabolic level, MeJA+nS strongly enhanced proline accumulation through increased P5CS and GK activities, accompanied by suppression of PROX activity. In contrast, salinity alone induced a moderate shift toward proline biosynthesis. The coordinated regulation of these enzymes indicates a metabolic reprogramming toward enhanced proline synthesis and reduced degradation, thereby promoting osmotic adjustment and protecting cellular components against salt-induced oxidative damage. This response is consistent with the established role of jasmonates in regulating stress-responsive genes involved in proline biosynthesis (Delgado et al., 2021). Thus, the coordinated regulation of sulfur assimilation and proline metabolism may provide an additional adaptive mechanism that strengthens cellular homeostasis, preserves metabolic function, and enhances salinity tolerance in *Brassica juncea* (Fig. 10).

Multivariate analyses provided further evidence that salinity tolerance in *Brassica juncea* is governed by coordinated interactions among ionic homeostasis, redox regulation, photosynthesis, nitrogen metabolism, plant dry weight and osmoprotection. The positive associations of photosynthetic and nitrogen-related traits with these protective responses, together with their negative relationships with oxidative markers and ionic accumulation in the Pearson correlation network, further indicate that maintenance of photosynthetic competence was closely linked to effective redox and ionic homeostasis. In particular, the coordinated association of P5CS and GK with proline, accompanied by their negative relationship with PROX, supports enhanced proline accumulation through simultaneous stimulation of biosynthesis and restriction of degradation. Collectively, these multivariate patterns indicate that MeJA and nS were associated with a coordinated physiological response rather than mitigation of salinity through a single protective pathway. Their combined application promoted a shift from ionic imbalance and oxidative injury toward enhanced antioxidant defense, osmotic adjustment, nitrogen metabolism, and photosynthetic stability. This integrated response provides a systems-level basis for the enhanced salinity acclimation observed in *Brassica juncea*.

Collectively, the present findings demonstrate that the combined application of nS and MeJA alleviates salinity stress through complementary and coordinated physiological and biochemical mechanisms. nS enhanced sulfur assimilation, ATP-S activity, cysteine accumulation, and p-SUE, thereby strengthening GSH-dependent redox regulation and supporting photosynthetic performance. Concurrently, MeJA activated antioxidant defense, nitrogen metabolism, and osmotic adjustment through stress-responsive signaling pathways. The integration of these processes effectively reduced oxidative damage, preserved membrane integrity and cellular viability, and maintained metabolic homeostasis under saline conditions (Fig. 10). Overall, these findings highlight the combined application of MeJA and nS as a promising strategy for enhancing salinity tolerance in mustard through coordinated regulation of sulfur metabolism, antioxidant defense, nitrogen assimilation, osmotic adjustment, and stress-responsive signaling.

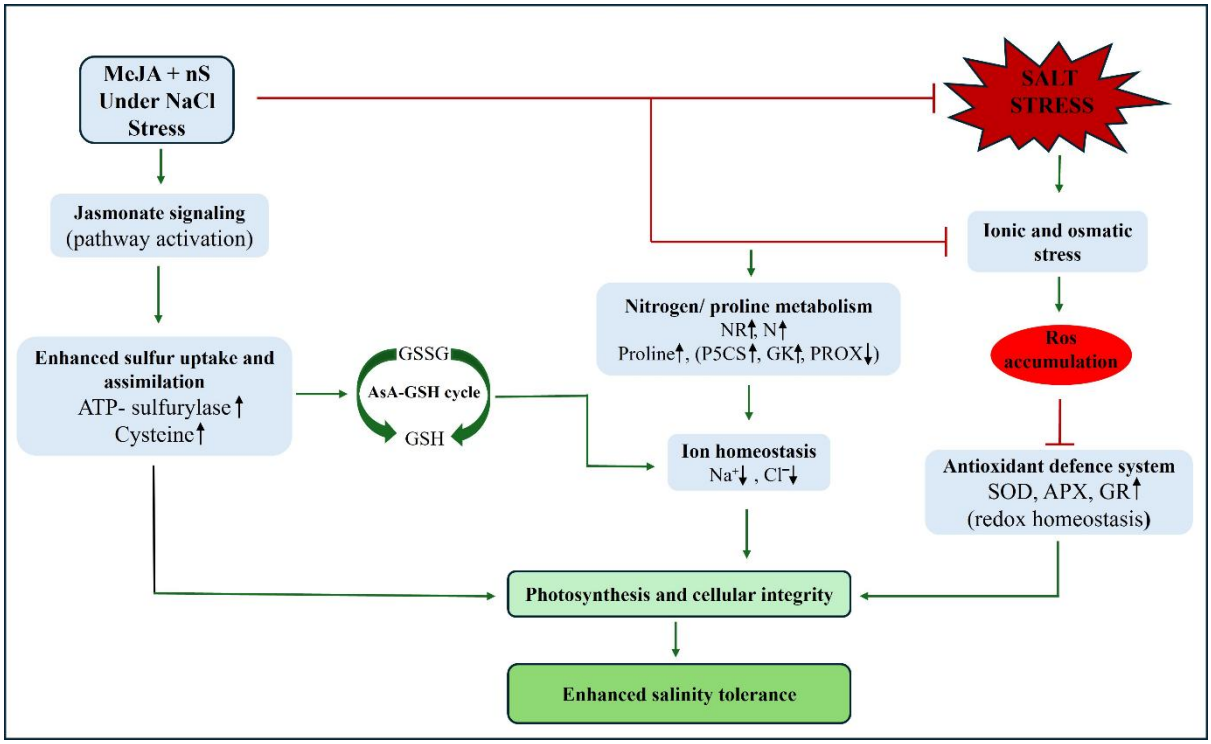


Fig. 10 Schematic representation of the proposed mechanism by which methyl jasmonate (MeJA) and nano-sulfur (nS) synergistically alleviate NaCl induced stress. MeJA mediated signaling and nS enhanced sulfur assimilation jointly stimulate nitrogen metabolism, AsA-GSH antioxidant system and regulating proline metabolism and ion homeostasis leading to improved ROS scavenging, osmotic adjustment, membrane stability, photosynthesis, and ultimately enhanced salinity tolerance.

Conclusion

Salinity markedly impaired the growth and physiological performance of *Brassica juncea* by disrupting photosynthesis, sulfur and nitrogen metabolism, antioxidant capacity, and proline metabolism, while promoting ROS accumulation and oxidative damage. Although individual applications of MeJA or sulfur partially alleviated salt-induced injury, their combined application, particularly MeJA+nS, provided the most pronounced protection. The MeJA+nS treatment maintained ion homeostasis, enhanced sulfur assimilation, p-SUE, antioxidant defense, and the AsA–GSH cycle, while also improving NR activity and proline metabolism through stimulation of P5CS and GK activities and suppression of PROX activity. These coordinated responses promoted proline accumulation and osmotic adjustment, thereby strengthening cellular redox homeostasis. Consequently, oxidative damage was reduced, membrane integrity and stomatal function were preserved, and photosynthetic efficiency and plant growth were sustained under saline conditions.

Overall, the present findings demonstrate that the MeJA–nS interaction enhances salinity tolerance in mustard through integrated regulation of sulfur metabolism, antioxidant defense, nitrogen assimilation, proline metabolism, and osmotic adjustment. The coordinated improvement of photosynthetic protection, redox homeostasis, metabolic stability and biomass accumulation highlights the potential of MeJA+nS application as a sustainable strategy for improving mustard resilience and productivity in saline environments.

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Declarations

Conflicts of Interest: The authors declare no conflicts of interest.

Data availability statement: The data supporting the findings of this study are available on request from the corresponding author upon reasonable request.

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