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Impact of nitric oxide on sunflower growth and drought tolerance mechanisms

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Abstract: Sunflower (*Helianthus annuus* L.), a globally significant oilseed crop, faces substantial yield losses due to drought stress, a major environmental constraint. In this study, the effects of nitric oxide (NO) to increase drought tolerance in four sunflower genotypes (resistant Irtysh, RAR 56 and sensitive Zarya, RAR 133) showing different stress responses were investigated. Conducted in a controlled hydroponic system, the experiment applied 100 μM NO under 12% polyethylene glycol (PEG)-induced drought, assessing growth, physiological, and biochemical parameters. PEG alone reduced shoot and root growth, relative water content (RWC), and ion levels (K, Ca, Mg, Na), while increasing oxidative stress markers (malondialdehyde (MDA), H_2O_2, •OH) and electrolyte leakage, particularly in sensitive genotypes. NO application, both alone and with PEG, significantly mitigated these effects, enhancing root fresh weight, RWC, and antioxidant enzyme activities (superoxide dismutase (SOD), peroxidase (POX), catalase (CAT) and glutathione reductase (GR)), while reducing reactive oxygen species (ROS) and lipid peroxidation. Resistant genotypes (Irtysh, RAR 56) exhibited superior stress amelioration. These findings highlight NO's role as a signalling molecule in augmenting drought resilience through genotype-specific mechanisms. The differential responses among genotypes suggest opportunities for identifying genetic markers associated with NO-mediated drought tolerance, which could guide marker-assisted breeding programs. Additionally, integrating these insights with genomic editing techniques may accelerate the development of drought-resistant sunflower cultivars tailored for water-scarce regions. Future research should optimise NO delivery methods and evaluate field-scale efficacy to advance sustainable sunflower production in water-limited environments.

Keywords: antioxidant enzyme activities; elemental content; *Helianthus annuus*; relative water content; water scarcity

Oilseeds are essential to global agriculture, significantly contributing to vegetable oil production. Among them, sunflower (*Helianthus annuus* L.) is a leading oilseed crop, valued for its high oil content, adaptability, and yield potential (Khan et al. 2015; BYSD 2023). Ranking third globally in oilseed production, about 90% of sunflower seeds are used for oil extraction. Turkey and Kazakhstan stand as the sixth and twelfth largest producers, respectively (FAO 2023). Sunflower cultivation is critical in water-scarce regions, where resilience to environmental stressors like drought is vital for sustainable agriculture. Drought stress, a major constraint, severely impacts sunflower yields, particularly during water-dependent growth stages.

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Plants respond to drought stress by closing stomata to reduce transpiration, which conserves water but hampers photosynthesis. This disrupts carbon dioxide assimilation and the electron transport chain in chloroplasts, leading to excessive reactive oxygen species (ROS) production – superoxide anions (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and singlet oxygen ($^1O_2$) – causing oxidative stress. This damages cellular structures and reduces biomass.

Understanding plant defences and enhancing drought resilience are the key to sustainable crop production. Recent studies highlight nitric oxide (NO) as a crucial signalling molecule that mitigates drought effects in plants (Hamurcu et al. 2020; Pandey et al. 2023). It activates antioxidant pathways, facilitates signal transduction, and reduces ROS-induced damage, including lipid peroxidation, which is a primary cause of cellular damage (Lau et al. 2021). Several studies have reported a significant reduction in malondialdehyde (MDA) and H_2O_2 levels after NO treatment in drought-stressed plants (Majeed et al. 2020; Pandey et al. 2023; Lei et al. 2025). These reductions were attributed to the enhanced antioxidant defence mechanisms of NO with increased activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and peroxidase (POX) (Rezayian et al. 2020). Though there are different studies on observing the effects of NO on drought stress in different crops, studies on sunflower are limited. Thus, to fill the gap, this study investigates the role of NO in improving drought tolerance of four sunflower genotypes with varying drought responses: tolerant genotypes, RAR 56 and Irtysh and sensitive genotypes, RAR 133 and Zarya. By evaluating physiological and biochemical changes under drought conditions, the study seeks to understand how NO counteracts stress and mitigates yield losses due to water scarcity in sunflower genotypes. These insights aim to advance sustainable sunflower farming in water-limited environments.

MATERIAL AND METHODS

Plant material and growth conditions. Four sunflower (*Helianthus annuus* L.) genotypes [commercial (Irtysh, Zarya), and pure (RAR 56, RAR 133)] from Selçuk University and Kazakh National Agrarian Research University with varying drought responses were employed in this study. Drought tolerance level of these genotypes was observed in a 14-day preliminary trial where plants were grown in different drought-stressed conditions (4, 8, 10, 12, 16, and 20% polyethylene glycol (PEG) 6000) along with a control (1/5 strength Hoagland solution, pH = 6.0) (data not shown). Among all the treatments, 12% PEG was the dose with the most pronounced stress response, and it best separated the differences between genotypes. Moreover, among all the genotypes, while Zarya and RAR 133 showed signs of desiccation and had low dry weights, RAR 56 and Irtysh genotypes demonstrated high dry weights on the 14th day of 12% PEG application. Thus, in this experiment, seeds of drought-tolerant genotypes, RAR 56 and Irtysh and drought-susceptible genotypes, Zarya and RAR 133, were first treated with 5% sodium hypochlorite for 10 min and then sterilised by washing 3 times with de-ionised water (dI H_2O). After waiting in de-ionised water for 2 h, they were placed in the dark in petri dishes containing moist filter paper until germination (4–7 days). Further, the seedlings were transferred to 3-litre pots containing 1/5 Hoagland solution (pH 6.0) and grown in a controlled hydroponic system with 45–55% humidity, a 16-h light/8-h dark cycle, 21 ± 1 °C temperature, and 10 000 lux per day light intensity. At three-four leaves stages, four different treatments were initiated including control (1/5 strength Hoagland solution), NO [100 µM NO in the form of sodium nitroprusside (SNP)], drought stress (12% PEG 6000), and PEG + NO (12% PEG 6000 with 100 µM NO in the form of SNP) that continued for 14 days. While 12% PEG 6000 was identified as the appropriate dose to observe drought stress symptoms in sunflower genotypes in our preliminary experiment, 100 µM NO dose has been accepted as the appropriate dose for sunflower growth in several previous studies (Laspina et al. 2005; Yadav et al. 2010). The nutrient solution was renewed every 3 days to maintain the nutrient balance of the plants and to ensure optimum growth conditions. This study, which investigated the effects of NO application on sunflower genotypes under drought conditions, was carried out in a total of 48 pots with 4 applications × 4 genotypes × 3 replicates (with 3 plants per replicate). Moreover, all the analyses and measurements were conducted in triplicates.

Growth parameters and K, Ca, Mg and Na analysis in plant samples. At the end of 14 days, root and shoot samples were harvested when plants started to show morphological responses to drought stress. The morphological symptoms included reduction in leaf size, shape, area, and shoot length, along with a decrease in root density and root length. Growth

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parameters, including root-shoot length and fresh weights, were measured immediately post-harvest and dry weights were weighed after drying the samples in an oven at 70 °C for 72 hours. For elemental analysis, harvested samples were dried at 70 °C for 72 h and pulverised. Further, 0.3 g of dried samples were digested with 5 mL HNO_3 at 210 °C and 1.38 MPa using microwave digestion, diluted to 25 mL with deionized water, filtered, and analysed for K, Mg, Ca, and Na via ICP-AES (Varian, Vista, Palo Alto, CA, USA) (Khan et al. 2022).

Relative water content (RWC). Lateral leaf tips were harvested, weighed fresh (FW), rehydrated in deionised water for 6 h to get turgid weight (TW), and then oven-dried at 70 °C for 72 h to measure dry weight (DW). RWC was calculated as: RWC = [(FW – DW)/(TW – DW)] × 100. As leaf tips are generally the most sensitive areas to drought stress, tip sections were preferred to accurately reflect the effect of stress on RWC calculations.

Determination of proline content. For proline analysis, 0.1 g fresh leaf sample was used, and free proline content was measured as absorbance at 520 nm from toluene-extracted liquid, and proline concentration was calculated via a calibration curve as µmol proline/g fresh weight (Bates et al. 1973; Khan et al. 2021).

Determination of cell membrane permeability (electrolyte leakage). Cell membrane permeability was measured as electrolyte leakage (Dionisio-Sese & Tobita 1998). Leaf samples (100 mg), cut into 5-mm pieces, were incubated in 10 mL deionised water at 32 °C for 2 h and the initial conductivity (EC1) was recorded. After autoclaving at 121 °C for 20 min and cooling to 25 °C, the final conductivity (EC2) was measured. Further, leakage was calculated as: ES = (EC1/EC2) × 100.

Measurements of stress markers: MDA, OH and H_2O_2 content. Lipid peroxidation in leaf samples was quantified as MDA content via thiobarbituric acid reactive substances (TBAR) reaction by measuring absorbance at 532–600 nm (Hamurcu et al. 2020; Rao & Sresty 2000). •OH were quantified by deoxyribose competition in a Fe^{3+}/ascorbate/EDTA/H_2O_2 system (Kim & Minamikawa 1997). For H_2O_2, 0.1 g leaf sample was homogenised in 1 mL 0.1% trichloroacetic acid (TCA), centrifuged at 12 000 rpm for 15 min with 0.5 mL supernatant mixed with 0.5 mL phosphate buffer (10 mM, pH 7.0) and 1 mL 1 M KI, and absorbance was measured at 390 nm, and calculated as µmol/g fresh weight (Terzi et al. 2014).

Enzyme extractions and assays. For the extraction of antioxidant enzymes, leaf samples that were flash-frozen using liquid nitrogen and stored at –80 °C were used. One gram of frozen leaf sample was homogenised in liquid nitrogen with 2% (w/v) polyvinylpolypyrrolidone (PVPP), one mM disodium-EDTA, and 50 mM sodium phosphate buffer (pH 7.8), filtered, and centrifuged at 14 000 rpm for 30 min at 4 °C. The resulting supernatant was retained for enzyme activity assays, and the entire extraction process was conducted at 4 °C. The method of Bradford (1976) was used to estimate the total soluble protein contents of the enzyme extracts employing bovine serum albumin as a standard. Further, SOD activity was measured at 560 nm via 50% nitro blue tetrazolium (NBT) reduction inhibition following the method of Beauchamp and Fridovich (1971) and expressed as U/mg protein. POX activity was assessed at 465 nm by diaminobenzidine (DAB) oxidation with H_2O_2 (Herzog & Fahimi 1973), in mol/mL/min. CAT activity was determined at 240 nm by H_2O_2 decomposition (Bergmeyer 1974), in mol/min. GR activity was quantified at 340 nm by nicotinamide adenine dinucleotide phosphate (NADPH) oxidation with glutathione disulfide (Foyer & Halliwell 1976), in mmol/mL/min.

Statistical analysis. Two-way analysis of variance (ANOVA) was conducted using Minitab 19 (Minitab Inc., State College, USA). Further, Tukey's multiple comparison test was applied to determine the differences between group means.

RESULTS

Growth parameters. NO treatment under controlled conditions improved growth parameters (shoot/root length, fresh/dry weights) in four sunflower genotypes (Figure 1), with Zarya showing the greatest increase (2.3-fold root fresh weight; Figure 1D). PEG alone reduced growth across all genotypes, most notably in Zarya's shoot fresh weight (91% decrease; Figure 1C). PEG + NO treatment lowered growth compared to control, with Zarya's shoot fresh weight dropping by 79% (Figure 1C), but enhanced growth relative to PEG alone, with Irtysh exhibiting the largest gain (1.5-fold root fresh weight; Figure 1D).

Na, K, Ca and Mg contents. Statistical analysis showed that PEG + NO treatment significantly altered shoot K levels in Zarya and RAR 133, Ca levels in Zarya under control + NO, and Na levels in RAR 56

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under PEG + NO. PEG alone reduced K, Ca, Mg, and Na concentrations across genotypes, with RAR 56 showing the largest K drop. PEG + NO increased K in Zarya and RAR 133 (Figure 2A), slightly raised Ca (1%) in RAR 133 (Figure 2B), and boosted Mg in RAR 133, though others declined (Figure 2C). Na decreased with PEG but increased with PEG + NO, notably fourfold in RAR 56 (Figure 2D).

Relative water content. RWC indicates drought stress resistance (Khoyerdi et al. 2016). Control + NO treatment raised RWC in Irtysh and Zarya, suggesting NO enhances water uptake, but lowered it in RAR 56 and RAR 133, indicating genotype-specific responses. PEG and PEG + NO reduced RWC across all genotypes vs. control, with RAR 133 showing the largest drop. Compared to PEG alone, PEG + NO improved RWC, with a peak 7% rise in RAR 133 (Figure 3).

Proline content. Control + NO reduced proline in Irtysh, Zarya, and RAR 133 vs. control, but increased it 21% in drought-resistant RAR 56. PEG alone raised proline in Irtysh, Zarya, and RAR 56, but decreased it 16% in drought-sensitive RAR 133. PEG + NO boosted proline 31% in resistant Irtysh vs. control, while others declined; RAR 56 showed the largest drop vs. PEG alone. Significant differences were found in Irtysh and Zarya under PEG, and Zarya under PEG + NO (Figure 4).

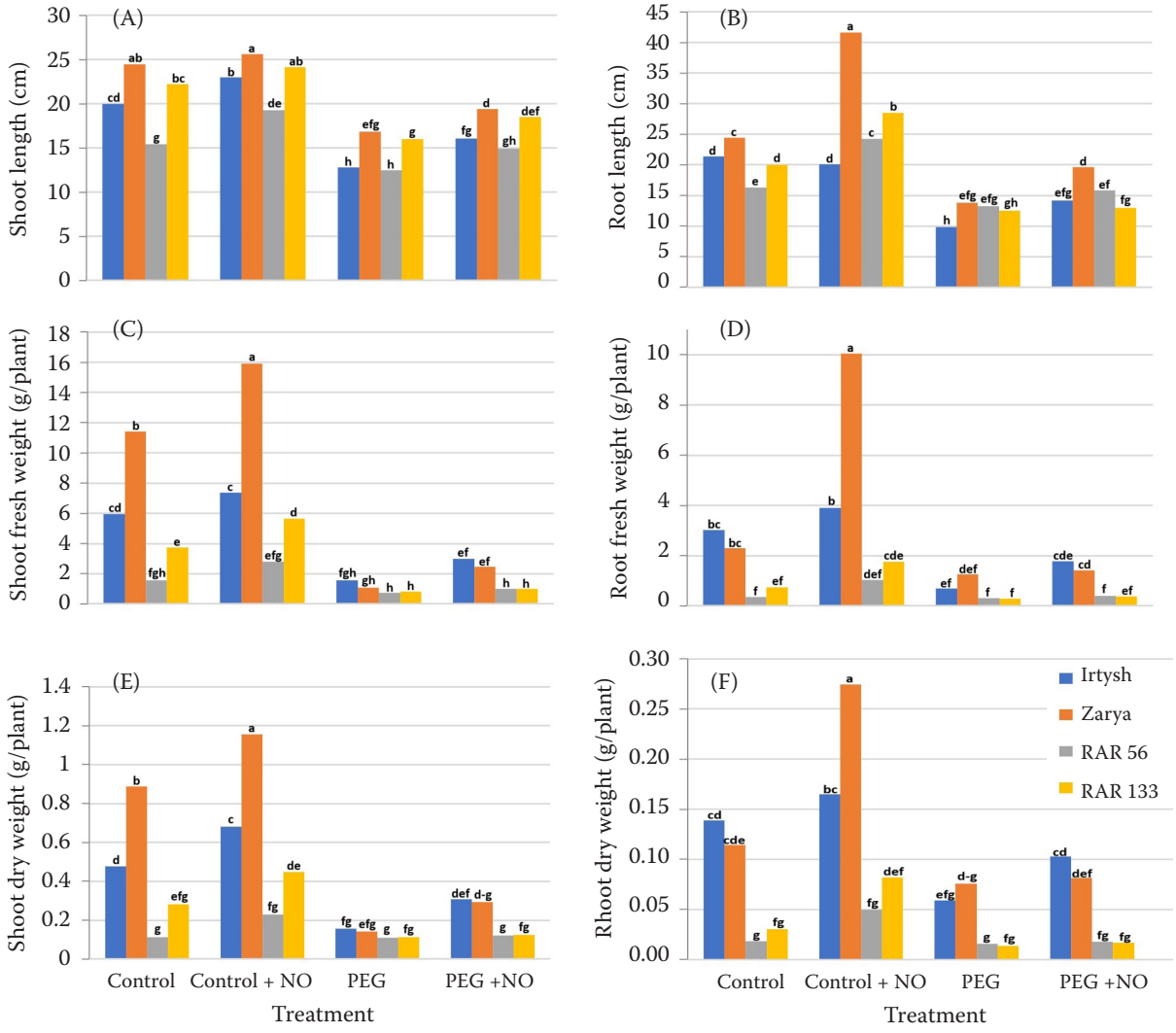

Figure 1. Changes in growth parameters of four sunflower genotypes under control, control + nitric oxide (NO), polyethylene glycol (PEG), and PEG + NO treatments: shoot length (A), root length (B), shoot fresh weight (C), root fresh weight (D), shoot dry weight (E), root dry weight (F)

Different lowercase letters show significant differences between genotypes under various treatments at $P < 0.01$; genotypes with different letters differ significantly

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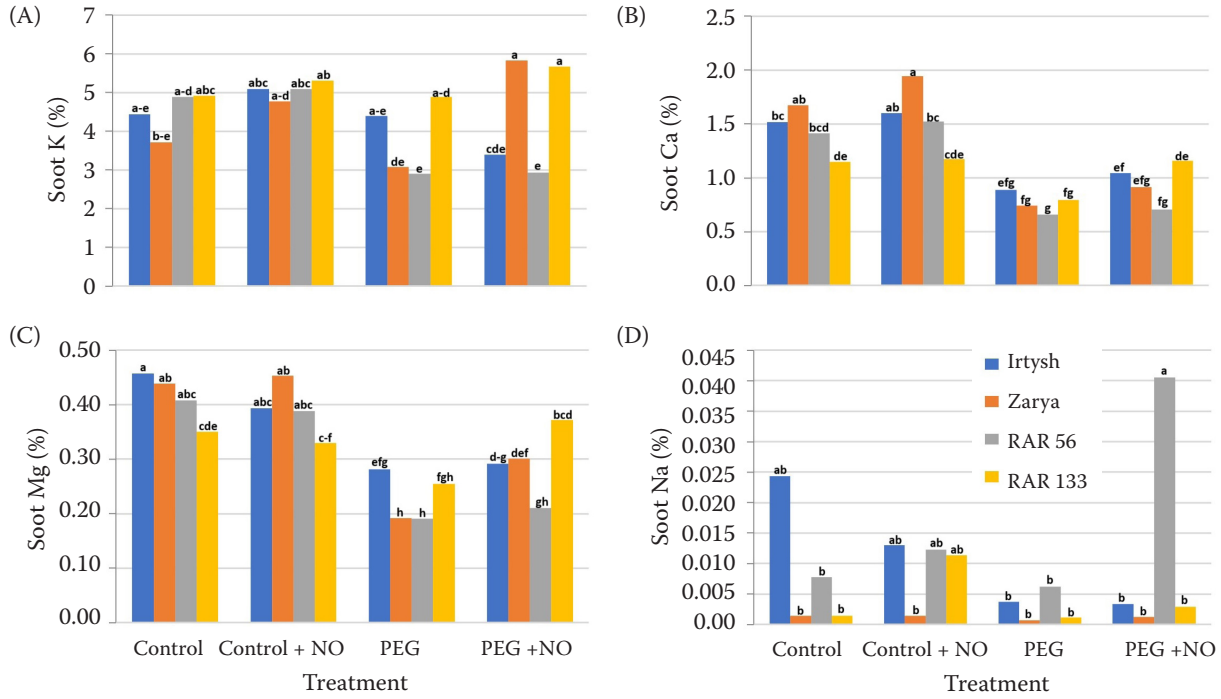

Figure 2. Changes in element concentrations of four sunflower genotypes under control, control + nitric oxide (NO), polyethylene glycol (PEG), and PEG + NO treatments: shoot K (A), shoot Ca (B), shoot Mg (C), shoot Na (D)
Different lowercase letters show significant differences between genotypes under various treatments at $P < 0.01$; genotypes with different letters differ significantly

Cell membrane permeability (electrolyte leakage). Electrolyte leakage, indicating membrane integrity, varied by treatment in sunflower genotypes. Control + NO increased the leakage in sensitive Zarya and RAR 133 but decreased it in resistant Irtysh and RAR 56 vs. control. PEG raised leakage in Irtysh and RAR 133 but lowered it in Zarya and RAR 56. PEG + NO reduced leakage in Irtysh and

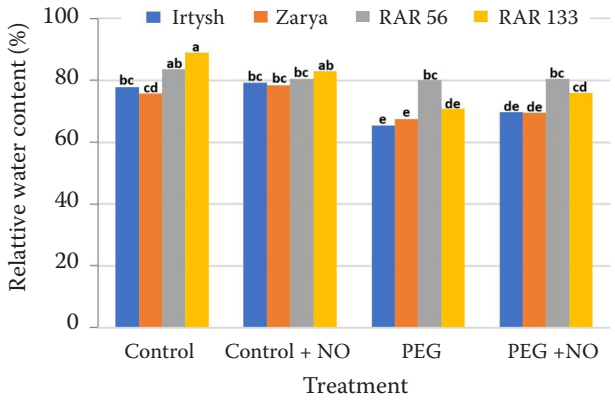

Figure 3. Changes in relative water content (%) of four sunflower genotypes under control, control + nitric oxide (NO), polyethylene glycol (PEG), and PEG + NO treatments
Different lowercase letters show significant differences between genotypes under various treatments at $P < 0.01$; genotypes with different letters differ significantly

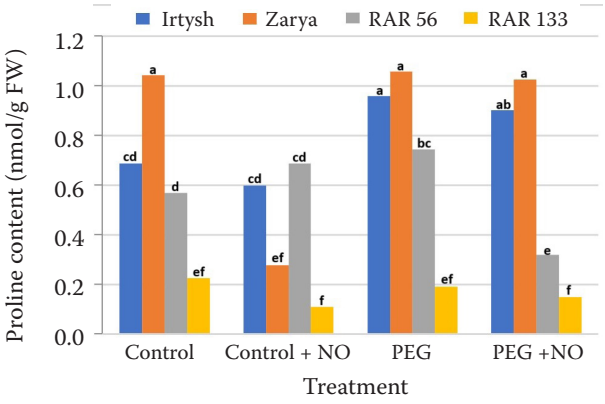

Figure 4. Changes in proline content (nmol/g FW) of four sunflower genotypes under control, control + nitric oxide (NO), polyethylene glycol (PEG), and PEG + NO treatments
Different lowercase letters show significant differences between genotypes under various treatments at $P < 0.01$; genotypes with different letters differ significantly

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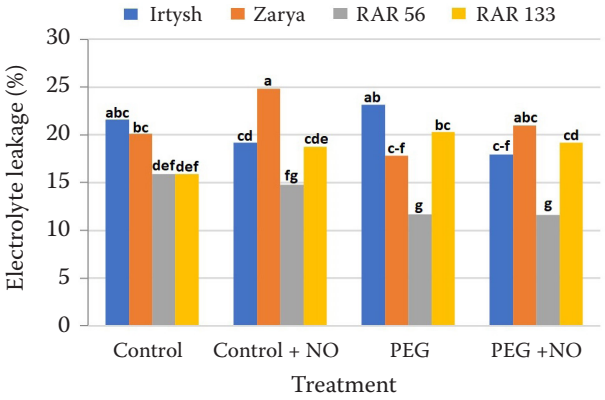

Figure 5. Changes in electrolyte leakage values (%) of four sunflower genotypes under control, control + nitric oxide (NO), polyethylene glycol (PEG), and PEG + NO treatments
Different lowercase letters show significant differences between genotypes under various treatments at $P < 0.01$; genotypes with different letters differ significantly

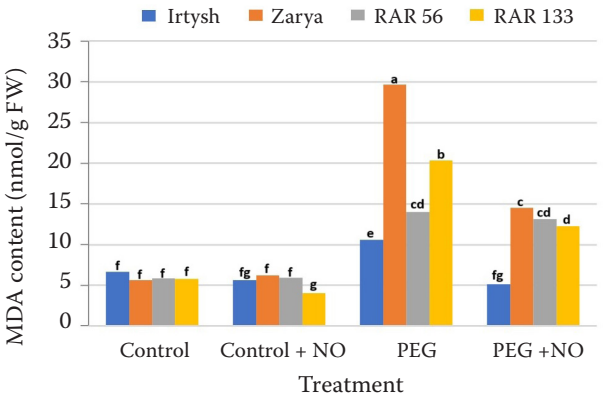

Figure 6. Changes in MDA content (nmol/g FW) of four sunflower genotypes under control, control + nitric oxide (NO), polyethylene glycol (PEG), and PEG + NO treatments
Different lowercase letters show significant differences between genotypes under various treatments at $P < 0.01$; genotypes with different letters differ significantly

RAR 56 but increased it in Zarya and RAR 133 vs. control. Compared to PEG alone, PEG + NO lowered leakage in Irtysh, RAR 56, and RAR 133, but raised it 18% in Zarya. Significant difference noted in Zarya under control + NO (Figure 5).

Malondialdehyde (MDA) content. MDA levels, reflecting lipid peroxidation, varied across treatments in sunflower genotypes. Control + NO lowered MDA in Irtysh and RAR 133 but raised it in Zarya and RAR 56 vs. control. PEG increased MDA in all genotypes, with a fourfold spike in sensitive Zarya. PEG + NO reduced MDA 23% in resistant Irtysh vs. control, but increased it in others, with a 1.5-fold rise in Zarya. Compared to PEG alone, PEG + NO decreased MDA in all genotypes, with a 51% drop in Zarya. Significant difference in Zarya's MDA noted under PEG (Figure 6).

Hydroxyl radical (•OH). Control + NO reduced •OH scavenging activity in all sunflower genotypes vs. control. PEG increased it in all, with the greatest

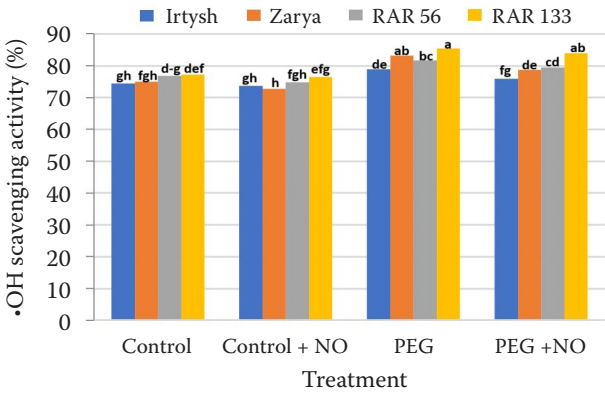

Figure 7. Changes in hydroxyl radical (•OH) scavenging activity values (%) of four sunflower genotypes under control, control + nitric oxide (NO), polyethylene glycol (PEG), and PEG + NO treatments
Different lowercase letters show significant differences between genotypes under various treatments at $P < 0.01$; genotypes with different letters differ significantly

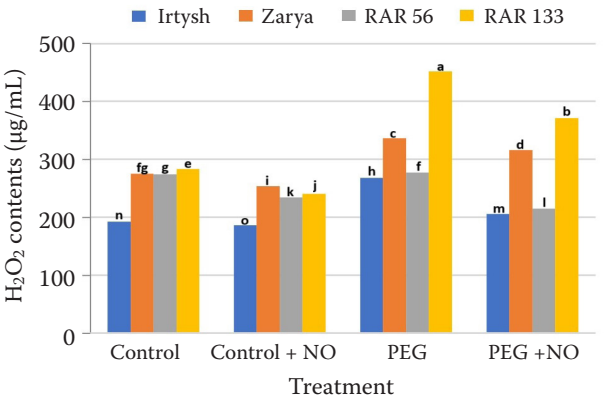

Figure 8. Changes in hydrogen peroxide (H_2O_2) contents (µg/mL) of four sunflower genotypes under control, control + nitric oxide (NO), polyethylene glycol (PEG), and PEG + NO treatments
Different lowercase letters show significant differences between genotypes under various treatments at $P < 0.01$; genotypes with different letters differ significantly

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rises in sensitive RAR 133 and Zarya. PEG + NO also elevated •OH scavenging vs. control, peaking at 9% in RAR 133, but slightly lowered it vs. PEG alone, with a 5% drop in Zarya. Significant difference noted in RAR 133 under PEG (Figure 7). PEG and PEG + NO mitigate oxidative stress and boost defence, though NO's effect varies by genotype and environment, showing less pronounced increases than PEG alone.

Hydrogen peroxide (H_2O_2). Under control + NO treatment, H_2O_2 content decreased in all sunflower genotypes compared to the control. PEG treatment increased H_2O_2 levels across all genotypes, with a 59% rise in drought-sensitive RAR 133. PEG + NO treatment elevated H_2O_2 relative to the control, except in resistant RAR 56 (22% reduction). Compared to PEG alone, PEG + NO reduced H_2O_2 in all genotypes, with a 23% decrease in resistant Irtysh. Statistical analysis confirmed that significant H_2O_2 increases in RAR 133 under PEG (Figure 8). PEG + NO treatment effectively mitigates oxidative stress, particularly in commercial sunflower lines, with genotype-specific responses.

Superoxide dismutase (SOD), peroxidase (POX), catalase (CAT) and glutathione reductase (GR) enzyme activities. SOD activity in sunflower genotypes increased under control + NO, PEG, and PEG + NO treatments compared to the control, with the drought-sensitive Zarya genotype showing the largest rises (45%, 104%, 78%, respectively). PEG + NO reduced SOD activity compared to PEG alone, with a 16% drop in Zarya. Statistical analysis confirmed a significant SOD increase in RAR 56 under PEG (Figure 9A).

POX activity increased in all sunflower genotypes under control + NO, with resistant RAR 56 showing the largest rise (108%). PEG and PEG + NO treatments further elevated POX activity, with RAR 56 exhibiting 5-fold and 11-fold increases, respectively. PEG + NO vs. PEG alone boosted POX activity, with a 105% rise in RAR 56. Statistical significance was confirmed, notably in RAR 56 under PEG + NO (Figure 9B).

CAT activity rose under control + NO in Zarya and RAR 56 but dropped in others. PEG increased CAT by 45% in Zarya, while others declined. PEG +

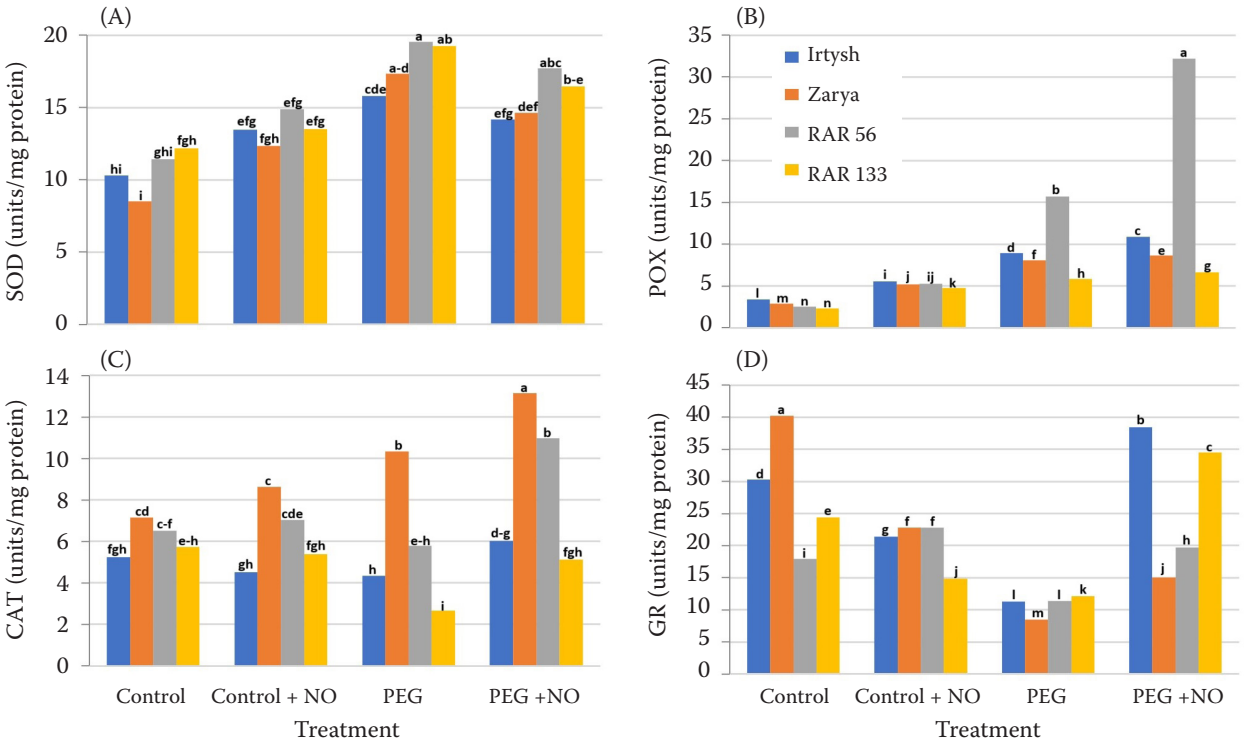

Figure 9. Changes in enzyme activities (units/mg protein) of four sunflower genotypes under control, control + nitric oxide (NO), polyethylene glycol (PEG), and PEG + NO treatments: superoxide dismutase (SOD) (A), peroxidase (POX) (B), catalase (CAT) (C), glutathione reductase (GR) (D)

Different lowercase letters show significant differences between genotypes under various treatments at $P < 0.01$; genotypes with different letters differ significantly

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NO reduced CAT by 10% in RAR 133 but increased it elsewhere, with a 92% rise in RAR 133 vs. PEG alone. Significant changes were noted, especially in Zarya under PEG + NO (Figure 9C).

GR activity increased in RAR 56 under control + NO but decreased in others. PEG reduced GR across all genotypes. PEG + NO lowered GR by 62% in Zarya but raised it elsewhere, with a 2.4-fold increase in Irtysh vs. PEG alone (Figure 9D).

Principal component analysis of proline, EC, MDA, ROS and antioxidant enzymes. Principal component analysis (PCA) for Irtysh genotype (PC1: 59.6%, PC2: 30.4%; 90% variance) showed that control and NO treatments had similar profiles, while PEG increased EC, MDA, and H_2O_2, indicating oxidative stress and ion imbalance. PEG + NO reduced stress by boosting CAT, GR, proline, and POX levels, aiding osmotic balance and defence (Figure 10A).

For Zarya (PC1: 71.5%, PC2: 21.5%; 93% variance), control and NO showed low antioxidant activity and EC. PEG elevated MDA, H_2O_2, and •OH, reflecting oxidative stress and damage. PEG + NO enhanced

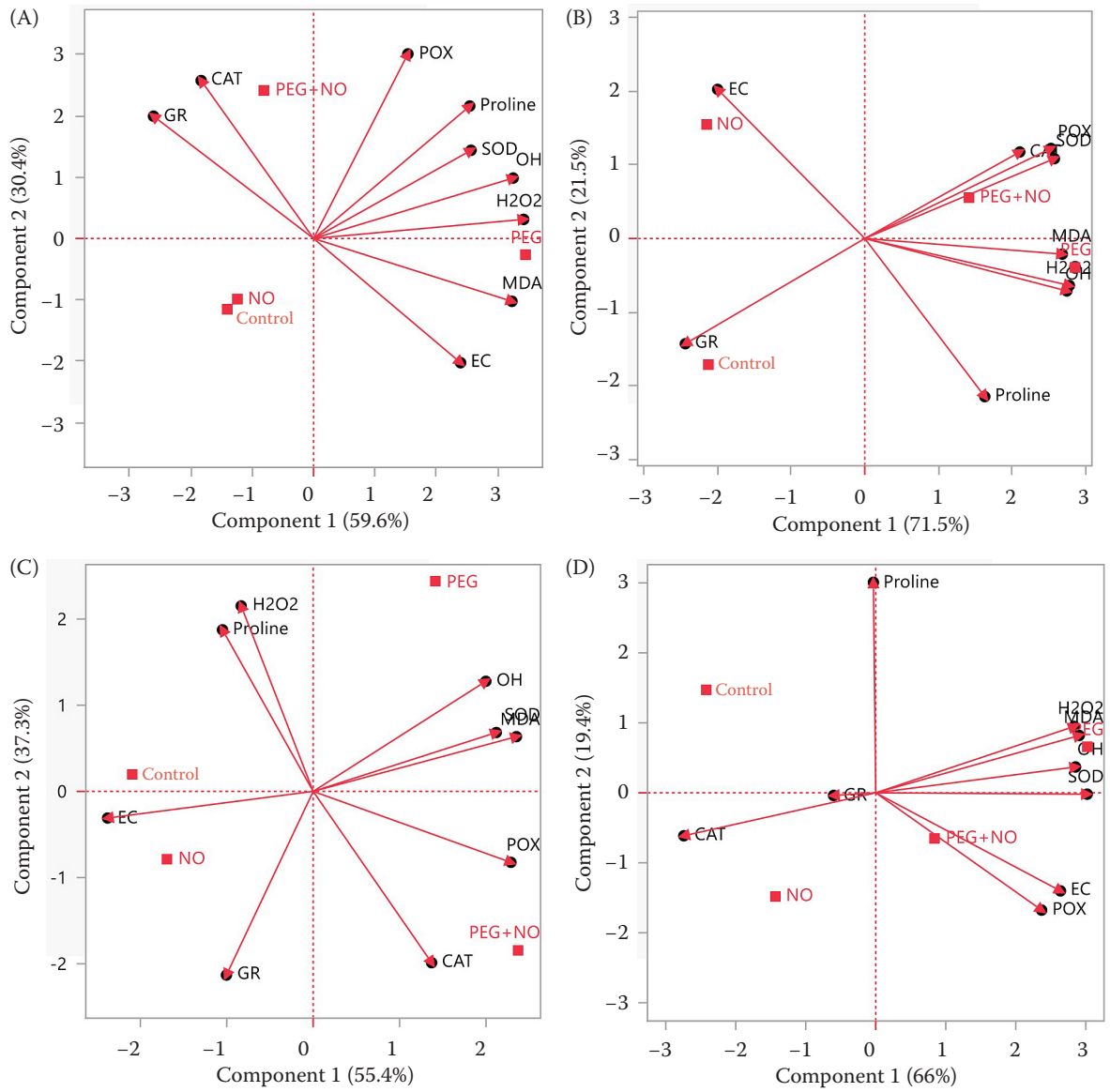

Figure 10. Combined principal component analysis results of the studied traits [proline; electrical conductivity (EC); malondialdehyde (MDA); hydrogen peroxide (H_2O_2), hydroxyl (•OH); superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and glutathione reductase (GR)] of four sunflower genotypes: Irtysh (A), Zarya (B), RAR 56 (C), RAR 133 (D) under control, control + NO, PEG, and PEG + NO treatments

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POX, SOD, and CAT, reducing stress, with proline aiding osmotic regulation (Figure 10B).

In RAR 56 (PC1: 55.4%, PC2: 37.3%; 92.7% variance), control and NO had low EC and GR. PEG increased H_2O_2, proline, •OH, and MDA, signalling stress and damage. PEG + NO improved POX and CAT, mitigating effects, with proline indicating osmotic disruption eased by PEG + NO (Figure 10C).

For RAR 133 (PC1: 66%, PC2: 19.4%; 85.4% variance), control showed low stress markers, NO boosted antioxidants. PEG raised MDA, H_2O_2, and •OH, showing damage. PEG + NO increased EC and POX for defence, with proline highlighting osmotic imbalance reduced by PEG + NO (Figure 10D).

DISCUSSION

Drought stress, a physiological condition arising from insufficient water availability, significantly impacts plant function (Yang et al. 2021; Khan et al. 2024). It reduces plant water potential, turgor, stomatal conductance, and photosynthesis, while inducing ROS accumulation and oxidative damage to cellular components (Seleiman et al. 2021; Bandurska 2022), impairing the metabolism, growth, and yield. Previous studies report decreased biomass, shoot/root length, and dry weight in sunflower genotypes under drought (Hussain et al. 2018; Shehzad et al. 2018, 2020; Almeida et al. 2020). PEG-induced drought in this study reduced shoot/root length and fresh/dry weights may be due to altered membrane permeability, alongside ROS overproduction. However, NO application in this study counteracted these reductions, likely due to its growth-promoting properties and ability to penetrate cell membranes (Farouk & Al-Ghamdi 2021). Literature suggests that NO redirects energy and carbohydrate reserves toward the root system, enhancing root volume and improving plant survival under drought by bolstering water retention (Elsheery et al. 2020). Our results align with this, demonstrating that control + NO and PEG + NO treatments markedly increased root fresh and dry weights, with effects varying by genotype (Figure 11). NO facilitated the accumulation of osmoprotectants, enhancing root water-holding capacity and promoting root growth, thereby supporting water balance under stress conditions (Amnan et al. 2021).

Drought stress alters K, Mg, Ca, and Na levels in sunflower genotypes, reflecting coping mechanisms. K supports water balance, enzyme activity, and ion homeostasis, enhancing drought tolerance via osmotic adjustment and stomatal regulation. PEG + NO treatment increased K in Zarya and RAR 133, consistent with NO boosting root development and K uptake (Liang et al. 2018). NO-mediated K transport regulation via abscisic acid (ABA) signalling (Chen et al. 2013) likely improved cellular K^+ levels in RAR 133, aiding water balance and resilience.

Mg, vital for chlorophyll and photosynthesis, increased in RAR 133 under PEG + NO, suggesting NO enhances Mg bioavailability, optimising energy metabolism and stress defence (Liu et al. 2013). Ca, a key for cell wall integrity and signalling, rose in RAR 133 with PEG + NO, aiding stress tolerance and water balance. Na surged fourfold in resistant RAR 56 under PEG + NO, indicating NO-driven osmotic regulation for improved water retention and drought tolerance (Hamurcu et al. 2020). PEG + NO boosted K, Mg, Ca, and Na, reflecting NO's role in enhancing antioxidant defences and ion homeostasis, with genotype-specific responses – RAR 56 showed high Na accumulation, while Zarya and RAR 133 had milder increases – highlighting differential resilience (Pandey et al. 2023). NO optimises nutrient use, supporting osmolarity and energy production under drought.

Drought stress reduces RWC in sunflower, as seen with PEG treatment, but NO (via SNP) mitigated this, enhancing the water uptake and transport by regulating stomata, boosting root growth, and promoting osmolyte accumulation for turgor maintenance (Sarazin et al. 2017; Hussain et al. 2024). NO also restored K^+ and Ca^{2+} channel function for ion balance (Khoshbakht et al. 2018). Drought stress elevates proline production, a key osmoprotectant that stabilises membranes (Sahay et al. 2019). In this study, the resistant Irtysh genotype exhibited the highest proline accumulation under PEG-induced drought compared to the control, reflecting its superior membrane integrity and drought tolerance. This corroborates reports of increased proline in sunflower under drought (Shehzad et al. 2020). PEG + NO treatment further increased proline in Irtysh but reduced it in other genotypes, consistent with findings in safflower, where NO decreased proline under drought (Chavoushi et al. 2019). These results highlight proline's role in physiological adaptation to drought, modulated by NO in a genotype-specific manner. In EL, the control + NO treatment resulted in high electrolyte leakage values as compared to control in the case of the two

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sensitive genotypes, Zarya and RAR 133. This may seem unexpected at first glance, as NO is generally known to promote growth in plants. However, this finding highlights that the effect of NO on plants may vary depending on the genotype, application dose and current physiological conditions. In Zarya and RAR 133 genotypes, NO application might have potentially increased the oxidative stress. Although NO is useful as a signalling molecule at low doses, it can trigger the production of reactive nitrogen species (RNS) at high amounts or under certain conditions, causing damage to cell membranes. This situation can be more pronounced in sensitive genotypes because these genotypes have less adequate defence mechanisms to cope with oxidative stress than the resistant ones. Therefore, the increased leakage value in Zarya and RAR 133 may reflect the sensitivity of these genotypes to NO. On the other hand, the decrease in electrolyte leakage in the control + NO group in the resistant genotypes Irtysh and RAR 56 indicates that these varieties can detoxify NO more effectively, or the applied NO has a protective effect in these genotypes. PEG increased EL in susceptible RAR 133, indicating membrane damage (Baghery et al.

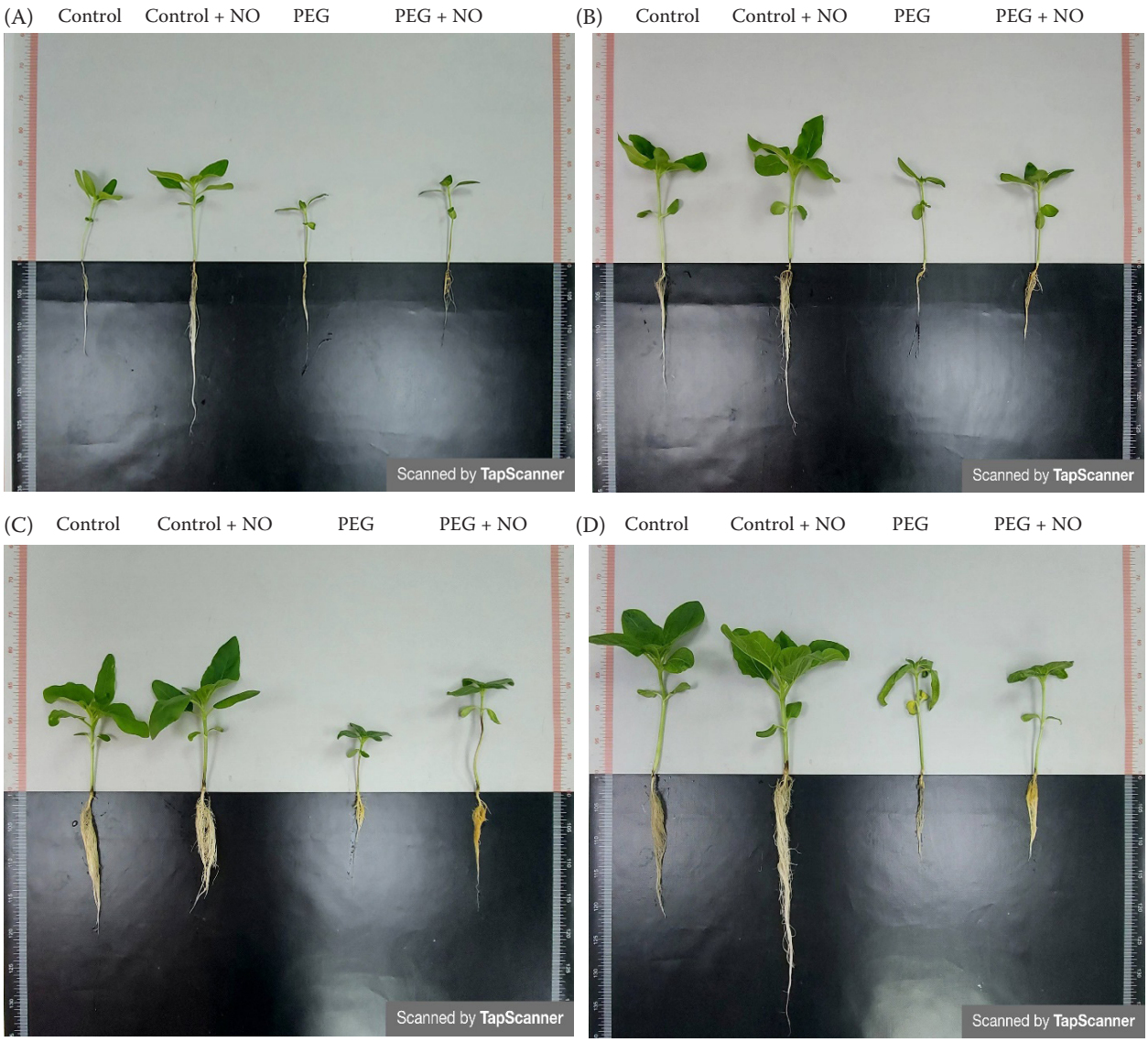

Figure 11. Effect of four different treatments including control (1/5 strength Hoagland solution), NO [100 µM nitric oxide in the form of sodium nitroprusside (SNP)], drought stress (12% PEG 6000), and PEG + NO (12% PEG 6000 with 100 µM nitric oxide in the form of SNP) on growth of: drought resistant RAR 56 (A), drought sensitive RAR 133 (B), drought resistant Irtysh (C), drought sensitive Zarya (D) genotypes

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2023), while PEG + NO lowered EL, suggesting NO enhances membrane integrity (Ekinci et al. 2020). PEG elevated MDA levels in Zarya, reflecting lipid peroxidation (Baghery et al. 2023), but PEG + NO reduced MDA in Zarya and Irtysh, linked to lower H_2O_2 and higher antioxidant activity. Literature confirms that NO curbs lipid peroxidation in sunflower under drought (Shehzad et al. 2023). EL and MDA are interconnected indicators of drought stress. Increased EL reflects membrane destabilisation, correlating with elevated MDA as a biochemical marker of lipid peroxidation. Both parameters, alongside H_2O_2 and •OH radicals, underscore oxidative stress (Hasanuzzaman et al. 2017). In this study, PEG-induced rises in H_2O_2 and MDA were attenuated by NO, highlighting its protective role in cellular defence and stress tolerance through ROS scavenging and membrane stabilisation. Drought stress increases H_2O_2, worsening oxidative damage (Hasanuzzaman et al. 2017). NO reduces ROS by scavenging or boosting antioxidant enzymes (Arora & Bhatla 2017). PEG enhanced H_2O_2 (59% in RAR 133) and •OH (11% in RAR 133 and Zarya), with MDA surging fourfold in Zarya. PEG + NO lowered H_2O_2 by 22% in RAR 56 and •OH scavenging by 5% in Zarya, reducing stress (Shehzad et al. 2023; Hussain et al. 2024).

SOD activity increased by 104% in Zarya under PEG, countering oxidative stress, and NO further enhanced it, aligning with higher H_2O_2 content (Chavoushi et al. 2019). POX in RAR 56 increased five-fold with PEG and eleven fold with PEG + NO, reducing H_2O_2 by 22% (Shehzad et al. 2023). CAT decreased in RAR 133 under PEG but increased by 84% in Zarya with PEG + NO, mitigating ROS (Chavoushi et al. 2019). GR fell 79% in Zarya with PEG but increased 41% in Irtysh with PEG + NO, bolstering redox balance.

NO enhances SOD, POX, CAT, and GR, reducing ROS and MDA, with sensitive genotypes (Zarya, RAR 133) showing more damage and resistant ones (RAR 56, Irtysh) better defenses. NO improves tolerance, especially in sensitive genotypes, highlighting its role in drought protection and crop resilience.

CONCLUSION

This study robustly demonstrates that NO application significantly enhances drought tolerance in sunflower (*Helianthus annuus* L.), a key oilseed crop, by mitigating the diverse effects of water scarcity. PEG treatment alone markedly reduced growth parameters (shoot and root length, fresh and dry weights), RWC, and ion homeostasis (K, Ca, Mg, Na), while elevating oxidative stress markers – MDA, H_2O_2, and •OH. These impacts were most severe in drought-sensitive genotypes Zarya and RAR 133, highlighting their susceptibility to water deficit.

NO application, both alone (control + NO) and with PEG (PEG + NO), substantially counteracted these effects. It improved growth, notably increasing root fresh weight, and enhanced water retention through osmoprotectant accumulation and root development. NO also enhanced cellular defences by upregulating antioxidant enzymes – SOD, POX, CAT, and GR – effectively lowering ROS levels and lipid peroxidation, with a significant MDA reduction in Zarya under PEG + NO. Additionally, NO improved ion regulation, elevating K, Mg, Ca, and Na concentrations, thus supporting osmotic balance and metabolic stability. Drought-resistant genotypes Irtysh and RAR 56 exhibited greater resilience, while NO notably bolstered tolerance in sensitive genotypes.

PCA confirmed these genotype-specific responses, underscoring NO's role as a versatile signalling molecule that mitigates drought-induced damage and enhances adaptation. This positions NO as a promising approach for sustainable sunflower cultivation in water-scarce regions. The observed genotypic variation in NO-mediated drought tolerance highlights the potential for identifying key genetic loci through genomic studies, enabling marker-assisted selection for enhanced stress resilience. Furthermore, integrating NO response mechanisms into breeding programs could facilitate the development of sunflower varieties with superior drought tolerance, bolstering global food security. Future research should refine NO application methods, evaluate long-term field performance, and explore its integration into breeding programs to develop drought-tolerant sunflower varieties, ensuring agricultural resilience amid intensifying climate challenges.

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