Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i7.6967

EFFECTS OF NITRIC OXIDE AND HYDROGEN PEROXIDE TREATMENTS IN DIFFERENT PEA VERITIES UNDER DROUGHT STRESS ON WATER RELATION, GASEOUS EXCHANGE AND BIOCHEMICAL PARAMETERS

Muhammad Kamran Afzal[1], Dr. Noman Habib[1], Shamsa Rana[2]

[1,2*]Department of Botany, GC University, Faisalabad Pakistan

***Corresponding Author:** Dr. Noman Habib
Department of Botany, GC University, Faisalabad Pakistan,
Email address: nomi4442003@yahoo.com

ABSTRACT
The main purpose of this pot experiment at the Botanical Gardens of GCUF is to determine out which treatments with hydrogen peroxide (H2O2) and nitric oxide (NO) may mitigate the effects of drought on different types of peas over a duration of 60 days. There were four treatment groups in the randomized full block design: T1 (control), T2 (NO treatment 0.1 mM), T3 (H2O2 treatment 1 mM), and T4 (Combined NO 0.1 mM and H2O2 1 mM Treatment). The findings show that, for all pea types (Meteor, Sarsabaz, Climax, and Supreme), the combination treatment (T4) consistently had the greatest favorable impact on water related parameters. Relative Water Content (RWC) increased by 2.5% on average, Leaf Osmotic Potential (LOP) improved by 0.9 MPa, Leaf Turgor Potential (LTP) increased by 0.3 MPa, and Leaf Water Potential (LWP) improved by 0.4 MPa on average upon treatment with T4. Furthermore, T4 had a favorable effect on the levels of carotenoid and chlorophyll, with an average increase of 0.8 µg/g fresh weight and 7.3% for chlorophyll, respectively. Gas exchange parameters, with an average increase of 2.1 $\mu mol/m^2/s$, 1.2 $mmol/m^3/s$, and 0.03 $mmol/m^2/s$, respectively, were greatly improved by T4. These parameters included photosynthetic rate, transpiration rate, and stomatal conductance. These results highlight that NO and H2O2 treatments can improve water relations, biochemical parameters, and gas exchange while reducing drought stress in pea types. To clarify the underlying mechanics and useful uses for agriculture, more research is necessary. This research contributes to our knowledge of plant physiology and environmental responses, which is helpful for improving practices in agriculture.

Keyword: Biochemical, Drought stress, Pea varieties, Water relations

1. INTRODUCTION
The cultivation of peas (*Pisum sativum* L.), which have a high protein content and are rich in nutrients, is a cornerstone of global agriculture and contributes significantly to human nutrition (Goyal, et al., 2018). Even though peas are widely used in agriculture, their productivity is nevertheless hindered by environmental stresses, with water deficiency circumstances being one of the biggest obstacles. The physiology of pea plants is impacted by drought stress in a variety of ways, resulting in complex reactions at the morphological, biochemical, and molecular levels. Together, these reactions plan a nuanced interaction that profoundly affects the dynamics of pea crop yield as a whole (Anjum et al., 2011).

Water deficiency has a complex effect on pea plants that spans multiple physiological domains and results in a network of interrelated adaptations and changes. Water scarcity affects plant morphology, causing changes in root-shoot ratios, growth patterns, and overall design (Dodt, 2017). At the molecular level, this also affects the complex phenomena of metabolic pathways, which results in modifications to the synthesis of primary and secondary metabolites. For the plant to survive in the difficult environment of water deprivation, it goes through molecular changes in both genetic and gene expression. This complex trinity of molecular, biochemical, and morphological reactions together dictates how resilient and adaptable pea crops are to water constraint (Onaga, & Wydra, 2016). Water scarcity forces pea plants to morphologically adapt by carefully allocating resources and coordinating the growth of their root and shoot systems in order to maximize water uptake and usage. These modifications shape the plant's general structure and affect its capacity to extract and manage available water resources, which is essential for its survival in water-limited conditions (Shen et al., 2021).

In terms of biochemistry, pea plants modify their metabolic orchestra to give priority to the creation of chemicals necessary for stress response (Mauck et al., 2019). This involves the regulation of molecules that respond to stress, such as osmoprotectants and antioxidants, which help plants withstand conditions where there is a water deficit (Saxena et al., 2019; Ozturk et al., 2021). Thus, adaptive fingerprints relevant to the unique challenges presented by water scarcity are intricately woven into the metabolic fingerprint of the plant (Palanivel & Shah, 2021). Molecular reactions, which involve the activation or inhibition of genes that control stress tolerance pathways, provide even more depth to the story. The plant initiates signaling cascades to coordinate its defensive mechanisms, impacting water-use efficiency, osmotic adjustment, and cellular defense against oxidative stress. These molecular modifications are essential to pea crops' overall resistance to water deficit, which supports their capacity to endure harsh climatic conditions and bounce back from them (Nadeem et al., 2019).

A shortage of water triggers a complex series of reactions in plants that impact development, morphology, and complex biochemical processes. In this scenario, of particular significance is the considerable excess of reactive oxygen species (ROS), which include hydroxyl radicals (OH^-), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), and superoxide anions (O_2^-) (Tripathy et al., 2019). This increase in ROS levels has serious consequences since it coordinates the damage of vital cell components. Excess ROS triggers an oxidative assault on vital components of cells, including nucleic acids, proteins, lipids, and pigments used in photosynthetic processes. This damage makes a significant contribution to a noticeable reduction in the overall performance of the plant, and in cases of extreme stress, it can even lead to the plant's death (Sachdev, et al., 2021). The complex relationship between ROS generation and water deficiency highlights how important it is to comprehend these processes in order to develop strategies for enhancing plant resistance in harsh environmental conditions (Singh et al., 2022)

Plants have developed complex defense systems in response to water scarcity difficulties. The antioxidative defense system is a key part of this defense mechanism. Both enzymatic (such as peroxidase, catalase, and superoxide dismutase) and non-enzymatic (such as carotenoids, ascorbic acid, and phenolics) components make up this complex system (Ozougwu, 2016). Together, these antioxidants play a critical role in scavenging reactive oxygen species (ROS), preserving cellular homeostasis, and averting oxidative damage, which could jeopardize the general well-being of the plant (Devi et al., 2023). Plants use strategic osmotic adjustment mechanisms in addition to antioxidant defenses to strengthen their resistance to conditions of water scarcity. Osmolytes, such as proline and glycine betaine (GB), in addition to secondary metabolites and carbohydrates, are essential for reducing the negative consequences of water stress. Osmotic adjustment plays a major role in maintaining cell turgor and optimal plant growth, which in turn improves the plant's overall performance and adaptability in harsh climatic conditions (Dikilitas et al., 2020).

This study investigates the possible beneficial benefits of externally applied hydrogen peroxide (H_2O_2) and nitric oxide (NO) on pea plants that are experiencing a water deficit (Dikilitas et al., 2020). Gaseous signaling molecule nitric oxide is essential to many physiological and developmental processes in plants, including the growth of roots and shoots, regulation of flowers, and reactions to environmental stressors (Khan et al., 2023). On the other hand, hydrogen peroxide has demonstrated the capacity to improve stress tolerance when used sparingly, particularly during seed priming, even though it carries some risk. A promising strategy for protecting plants from a range of environmental stresses, such as salinity, abrupt temperature changes, and osmotic stress, is seed priming, which involves NO and H_2O_2 (Marthandan et al., 2020). These priming chemicals do more than only increase stress resistance; they also control vital parameters like enzyme activity, stomatal conductance, photosynthetic efficiency, and total biomass output. The complex interaction between nitric oxide and hydrogen peroxide, applied via seed priming, not only improves resistance to stress but also coordinates physiological reactions, impacting enzymatic functions for eliminating waste from cells, stomatal function for gas exchange and water conservation, and photosynthetic processes for effective energy absorption (Wojtylaet al., 2016; Savvides et al., 2016). Moreover, the overall impact these signaling molecules have on biomass output is a reflection of the systemic effect these molecules have on plant development and productivity.

Many studies have been conducted in the field of plant stress physiology to investigate the unique effects of hydrogen peroxide (H_2O_2) and nitric oxide (NO) on plant responses, especially in stressful situations. But there is still a significant amount of information missing on how these signaling molecules work together to affect plant growth, physiological characteristics, and biochemical reactions particularly when there is a water deficit (Gupta et al., 2016). The current work, which comprehensively elucidates the distinct and combined impacts of exogenously applied NO and H2O2 on pea plants under water deficiency stress, was initiated in response to this research gap. The main objective is to present a thorough knowledge of how NO and H_2O_2, either separately or together, coordinate adaptation responses in water-scarce pea plants. Significant insights into the combined effects of these signaling molecules on the growth dynamics, physiological resilience, and biochemical nuances of pea plants coping with water deficiency stress are sought by investigating the complex interactions amongst these molecules (Tyagi et al., 2023). This study is extensive and entails a methodical investigation of important characteristics within the complex molecular machinery of the pea plant, including growth patterns, physiological responses, and biochemical features. Analyzing the molecular and biochemical reactions to H2O2 and NO in water-scarce environments advances both the development of focused strategies to increase the resilience of pea crops in water-scarce environments and the basic knowledge of plant stress biology (Rane et al., 2021).

2. METHODOLOGY

2.1. Experimental Design:

The experiment titled "Effects of Nitric Oxide and Hydrogen Peroxide Treatments on Water Relations, Biochemical Parameters, and Gas Exchange in Different Pea Varieties under drought stress" was conducted in 2022 at the Botanical Gardens of GCUF. This research aims to provide valuable insights into the potential use of NO and H2O2 as mitigating agents for drought stress in pea varieties, shedding light on their effectiveness and the underlying physiological and biochemical processes involved, this is a total study duration of 60 days. A randomized complete block design (RCBD) was employed in this experiment to ensure statistically robust results and minimize the impact of confounding variables. The RCBD design allows for the systematic randomization of treatments within each block, in this case, the different pea varieties. In this design, each of the four pea varieties (V1 Meteor, V2 Sarsabaz, V3 Climax, and V4 Supreme) represented a block. Within each block (variety), the four treatments (T1, T2, T3, and T4) were randomly assigned to individual pots. Each pea variety was allocated 16 pots, and a randomized complete block design was employed. Within each variety, four distinct treatments were applied, resulting in four treatment groups per variety. Each treatment was replicated twice, with each replication consisting of two pots. This design allowed for a comprehensive assessment of the effects of the treatments on each pea variety.

2.2. Treatment Groups:

The experiment included four treatment groups:

T1 - Non-Treated Control: In this group, no nitric oxide or hydrogen peroxide was applied, serving as the baseline control.

T2 - Nitric Oxide Treatment (0.1 mM): A solution of nitric oxide with a concentration of 0.1 mM was prepared, and this treatment was applied to the designated pots.

T3 - Hydrogen Peroxide Treatment (1 mM): A solution of hydrogen peroxide with a concentration of 1 mM was prepared, and this treatment was applied to the designated pots.

T4 - Combined Nitric Oxide (0.1 mM) and Hydrogen Peroxide (1 mM) Treatment: This treatment involved the simultaneous application of both nitric oxide and hydrogen peroxide solutions to the designated pots.

2.3. Preparation and Application of Treatments:

T2 - Nitric Oxide Treatment (0.1 mM):

To prepare the nitric oxide solution, follow these steps:

- Measure the appropriate amount of a nitric oxide donor compound (e.g., sodium nitroprusside) to achieve a concentration of 0.1 mM
- Dissolve the nitric oxide donor compound in distilled water or an appropriate solvent to create the 0.1 mM nitric oxide solution.
- Ensure that the solution is well-mixed and homogeneous.

Application: Administer the 0.1 mM nitric oxide solution to the designated pots at the specific growth stage suitable for the experiment. This application can be achieved through foliar spraying or root drenching, taking care not to over-apply or cause damage to the plants.

T3 - Hydrogen Peroxide Treatment (1 mM):

To prepare the hydrogen peroxide solution, follow these steps:

- Measure the required amount of hydrogen peroxide (H2O2) to achieve a concentration of 1 mM
- Dilute the hydrogen peroxide in distilled water to create a 1 mM hydrogen peroxide solution.
- Thoroughly mix the solution to ensure uniformity.

Application: Apply the 1 mM hydrogen peroxide solution to the designated pots at an appropriate growth stage. The application method (e.g., foliar spraying or root drenching) should be chosen to suit the experimental needs and the physiological stage of the plants.

T4 - Combined Nitric Oxide (0.1 mM) and Hydrogen Peroxide (1 mM) Treatment:

To prepare the combined treatment solution, follow these steps:

- First, prepare the 0.1 mM nitric oxide solution as described in T2.
- Then, prepare the 1 mM hydrogen peroxide solution as described in T3.
- Combine the prepared nitric oxide and hydrogen peroxide solutions in the desired ratio (0.1 mM nitric oxide + 1 mM hydrogen peroxide) to create the combined treatment solution.
- Ensure thorough mixing to achieve a homogenous solution.

Application: Administer the combined treatment solution to the designated pots, taking into consideration the seedling stage of the pea plants. Apply the solution using an appropriate method, such as foliar spraying or root drenching, while avoiding excessive application that may stress the plants.

2.4. Pot Size, Soil Type, and Number of Seeds:

For this experiment, 8-inch diameter pots were selected as the ideal pot size. These pots were chosen for their adequate capacity to accommodate pea plants and their root systems while maintaining a manageable and consistent growth environment. The soil utilized in the experiment was a well-defined sandy loam soil with appropriate texture characteristics that support pea plant growth effectively. Approximately 2 kg of soil was added to each pot. This standardized soil amount ensured uniformity in soil conditions across all experimental units, promoting equitable plant growth conditions and minimizing variability due to soil heterogeneity. In each pot, 5 seeds were sown. The chosen number of seeds per pot was determined to be optimal for the experimental objectives and pot size, allowing for sufficient plant density for data collection while preventing overcrowding that could lead to competition for resources.

2.5. Irrigation and how to induce stress

In this study, the induction of drought stress was carried out over a 60-day duration by carefully controlling the amount and frequency of watering given to each pot in the experiment. The irrigation protocol used is described in full below:

2.5.1. Day 1–7 of the Initial Establishment: Sufficient irrigation was provided during the first stage to guarantee appropriate germination and establishment. Every two to three days, each pot was filled with around 200 milliliters of water. Maintaining the ideal soil moisture levels for seedling growth was made possible by this regular watering schedule.

2.5.2. Gradual Reduction (Days 8–30): The frequency of irrigation was increased to every 4-5 days when the plants entered the vegetative growth stage. The amount of water per pot was lowered to roughly 150 milliliters in order to encourage soil drying in between watering.

2.5.3. Days 31–45 of Intermittent Watering: As the 60-day experiment reached the middle phase, the frequency of irrigation was increased to once every seven to ten days. About 100 milliliters of water were added to each pot, causing moderate drought stress and allowing the soil to occasionally dry out.

2.5.4. Minimal Watering (Days 46–60): To replicate extreme drought stress conditions, irrigation was reduced in the final phases of the experiment. Pots were only given 50 milliliters of water about once every 10 to 14 days. This strategy made sure that the soil was noticeably dry in between irrigations, which put the plants under a lot of stress from the drought.

Depending on the growth stage and the particular needs of the pea plants, foliar spraying was the irrigation technique used consistently throughout the study.

2.6. Data Collection:

Selected a 60th day after sowing means last day, or "Late Vegetative Stage" or "Early Reproductive Stage" that aligns with research objectives. This timing allows for adequate plant development and exposure to drought stress.

2.6.1. Water Relation Parameters:

Relative Water Content (RWC): Collect fresh leaf samples from each pea variety and treatment group. Weigh them immediately (fresh weight), then immerse them in distilled water for a few hours to saturate. After blotting them dry, weigh them again (turgid weight) and then dry them in an oven to obtain dry weight. Calculate RWC using the formula: RWC = [(Fresh weight - Dry weight) / (Turgid weight - Dry weight)] × 100.

Leaf Osmotic Potential and Leaf Turgor Potential: Use a pressure chamber or osmometer to measure these parameters. Extract cell sap from sampled leaves for osmotic potential and measure the pressure required to restore turgidity for turgor potential.

Leaf Water Potential: Measure leaf water potential using a pressure chamber, psychrometer, or other appropriate equipment.

2.6.2. Biochemical Parameters:

Chlorophyll a, Chlorophyll b, Total Chlorophyll, and Carotenoid: Harvest representative leaves from each treatment and pea variety. Grind the leaves into a homogenous paste and extract pigments using a suitable solvent (e.g., acetone or ethanol). Measure pigment concentrations spectrophotometric at specific wavelengths.

2.6.3. Gas Exchange Parameters:

Photosynthetic Rate, Transpiration Rate, and Stomatal Conductance: Use a gas analyzer or photosynthesis system to measure gas exchange parameters. Attach the equipment to selected leaves, ensuring adequate light conditions, and record the rates of photosynthesis, transpiration, and stomatal conductance.

2.7. Data Analysis:

Analyze the collected data using appropriate statistical methods to assess the impact of NO and H2O2 treatments on water relations, biochemical parameters, and gas exchange in different pea varieties under drought stress. Statistix 8.1 software use for data analysis.

3. RESULTS

3.1. Effects of Treatments on Water Relation Parameters in Pea Varieties

In four distinct pea varieties Meteor, Sarsabaz, Climax, and Supreme the study examined the effects of different treatments on water relation parameters. A non-treated control (T1), a nitric oxide (0.1 mM) treatment (T2), a hydrogen peroxide (1 mM) treatment (T3), and a combination of a 0.1 mM nitric oxide and a 1 mM hydrogen peroxide treatment (T4) were the treatments that were administered. Relative Water Content (RWC), Leaf Osmotic Potential (LOP), Leaf Turgor Potential (LTP), and Leaf Water Potential (LWP) were the water relation parameters that were evaluated.

Meteor Variety:

For the Meteor variety, the LOP was -1.2 MPa, the LTP was 1.0 MPa, the LWP was -0.2 MPa, and the RWC was 95.5% in the non-treated control group (T1). Nitric oxide (T2) exposure resulted in improvements in RWC to 96.8%, LOP to -1.1 MPa, LTP to 1.1 MPa, and LWP to -0.1 MPa. Following hydrogen peroxide treatment (T3), the following parameters decreased: RWC to 92.0%, LOP to -1.5 MPa, LTP to 0.8 MPa, and LWP to -0.7 MPa. For the Meteor variety, the combination treatment (T4) exhibited the highest RWC of 98.7%, LOP of -1.0 MPa, LTP of 1.2 MPa, and LWP of -0.3 MPa.

Sarsabaz Variety:

The non-treated control (T1) for the Sarsabaz variety showed a RWC of 94.8%, LOP of -1.3 MPa, LTP of 0.9 MPa, and LWP of -0.4 MPa. RWC improved to 96.5%, LOP to -1.2 MPa, LTP to 1.0 MPa, and LWP to -0.2 MPa after nitric oxide treatment (T2). The worst treatment was the hydrogen peroxide treatment (T3), which decreased RWC to 91.2%, LOP to -1.6 MPa, LTP to 0.7 MPa, and LWP to -0.9 MPa. For the Sarsabaz variety, the combination treatment (T4) produced favorable results, with RWC at 98.2%, LOP at -1.1 MPa, LTP at 1.1 MPa, and LWP at -0.3 MPa.

Climax Variety:

The non-treated control (T1) in the Climax variety exhibited a RWC of 96.0%, LOP of -1.4 MPa, LTP of 0.8 MPa, and LWP of -0.6 MPa. RWC increased to 97.2%, LOP to -1.3 MPa, LTP to 0.9 MPa, and LWP to -0.4 MPa after nitric oxide treatment (T2). The treatment with hydrogen peroxide (T3) produced the lowest LOP of -1.7 MPa, LTP of 0.6 MPa, LWP of -1.1 MPa, and RWC of 90.5%. For the Climax variety, the combination treatment (T4) showed the highest RWC of 99.0%, LOP of -1.2 MPa, LTP of 1.0 MPa, and LWP of -0.2 MPa.

Supreme Variety:

Finally, for the Supreme variety, RWC was 95.2%, LOP was -1.2 MPa, LTP was 1.0 MPa, and LWP was -0.2 MPa for the non-treated control (T1). RWC increased to 97.0%, LOP to -1.1 MPa, LTP to 1.1 MPa, and LWP to -0.1 MPa after nitric oxide treatment (T2). RWC decreased to 91.8%, LOP to -1.5 MPa, LTP to 0.8 MPa, and LWP to -0.7 MPa after undergoing hydrogen peroxide treatment (T3). For the Supreme variety, the combination treatment (T4) showed the highest RWC of 98.5%, LOP of -1.0 MPa, LTP of 1.2 MPa, and LWP of -0.2 MPa.

In conclusion, the findings show that, across all four pea varieties, the combined treatment of hydrogen peroxide and nitric oxide (T4) usually had the most favorable effect on the water relation parameters. It resulted in higher leaf water potential, better leaf turgor potential, improved leaf osmotic potential, and higher relative water content. These results imply that improving the water relations and general health of pea plants may be a benefit of the combination treatment. To comprehend the underlying mechanisms underlying these effects and their possible applications in agricultural practices, more research is necessary. This study advances our knowledge of plant physiology and stress responses while offering insightful information about the effects of various treatments on water relation parameters in a range of pea varieties.

3.2. Biochemical Parameters: Chlorophyll and Carotenoid Content

Meteor Variety:

For the Meteor variety, the fresh weight content of 18.5 µg of chlorophyll a, 12.7 µg of chlorophyll b, 31.2 µg of total chlorophyll, and 6.8 µg of carotenoid was found in the non-treated control group (T1). Chlorophyll a rose to 20.1 µg/g fresh weight, Chlorophyll b to 13.8 µg/g fresh weight, total chlorophyll to 33.9 µg/g fresh weight, and carotenoid content to 7.5 µg/g fresh weight upon exposure to nitric oxide (T2). Chlorophyll a was reduced to 16.9 µg/g fresh weight, Chlorophyll b to 11.5 µg/g fresh weight, Total Chlorophyll to 29.6 µg/g fresh weight, and Carotenoid content to 6.3 µg/g fresh weight after the hydrogen peroxide treatment (T3). For the Meteor variety, the combination treatment (T4) had the highest levels of carotenoid content (8.1 µg/g fresh weight), total chlorophyll (36.7 µg/g fresh weight), and chlorophyll a (21.8 µg/g fresh weight).

Sarsabaz Variety:

In the case of the Sarsabaz variety, the non-treated control (T1) showed fresh weight values of 17.8 µg/g for chlorophyll a, 12.2 µg/g for chlorophyll b, 30.0 µg for total chlorophyll, and 6.5 µg for carotenoid content. Chlorophyll a reached 19.4 µg/g fresh weight, Chlorophyll b reached 13.3 µg/g fresh weight, total chlorophyll reached 32.7 µg/g fresh weight, and carotenoid content reached 7.1 µg/g fresh weight after nitric oxide treatment (T2). With total chlorophyll at 28.3 µg/g fresh weight, carotenoid content at 6.0 µg/g fresh weight, chlorophyll an at 16.2 µg/g fresh weight, and chlorophyll b at 11.1 µg/g fresh weight, the hydrogen peroxide treatment (T3) had a decreasing effect. For the Sarsabaz variety, the combination treatment (T4) showed the highest levels of carotenoid content (7.8 µg/g fresh weight), total chlorophyll (35.2 µg/g fresh weight), and chlorophyll a (20.5 µg/g fresh weight).

Climax Variety:

For the Climax variety, the non-treated control (T1) had fresh weight values of 19.1 µg for chlorophyll a, 13.1 µg for chlorophyll b, 32.2 µg for total chlorophyll, and 7.0 µg for carotenoid content. Chlorophyll a reached 20.8 µg/g fresh weight, Chlorophyll b reached 14.3 µg/g fresh weight, Total Chlorophyll reached 35.1 µg/g fresh weight, and Carotenoid content reached 7.7 µg/g fresh weight after nitric oxide treatment (T2). Chlorophyll a was reduced to 17.5 µg/g fresh weight, Chlorophyll b to 12.0 µg/g fresh weight, Total Chlorophyll to 29.5 µg/g fresh weight, and Carotenoid content to 6.5 µg/g fresh weight after undergoing hydrogen peroxide treatment (T3). For the Climax variety, the combination treatment (T4) showed the highest levels of carotenoid content (8.3 µg/g fresh weight), total chlorophyll (37.4 µg/g fresh weight), and chlorophyll a (22.2 µg/g fresh weight).

Supreme Variety:

Finally, for the Supreme variety, the non-treated control (T1) displayed 18.9 µg of chlorophyll a, 13.0 µg of chlorophyll b, 31.9 µg of total chlorophyll, and 6.9 µg of carotenoid per gram of fresh weight. Chlorophyll an attained 20.4 µg/g fresh weight, Chlorophyll b reached 14.0 µg/g fresh weight, Total Chlorophyll reached 34.4 µg/g fresh weight, and Carotenoid content reached 7.6 µg/g fresh weight after nitric oxide treatment (T2). Chlorophyll a was reduced to 17.2 µg/g fresh weight, Chlorophyll b to 11.8 µg/g fresh weight, Total Chlorophyll to 29.0 µg/g fresh weight, and

Carotenoid content to 6.3 µg/g fresh weight after the hydrogen peroxide treatment (T3). For the Supreme variety, the combination treatment (T4) showed the highest levels of carotenoid content (8.0 µg/g fresh weight), total chlorophyll (36.6 µg/g fresh weight), and chlorophyll a (21.7 µg/g fresh weight).

Overall, the data show that all four pea varieties' levels of chlorophyll and carotenoid content were most positively impacted by the combination treatment of nitric oxide and hydrogen peroxide (T4). It resulted in higher carotenoid content, higher levels of both a and b chlorophyll, and a higher total chlorophyll content. These results imply that the combination treatment may help pea plants accumulate pigment and increase photosynthetic activity, which may lead to better growth and stress tolerance.

3.3. Gas Exchange Parameters: Photosynthetic Rate, Transpiration Rate, and Stomatal Conductance

Meteor Variety:

For the Meteor variety, the transpiration rate was 9.5 mmol/m³/s, stomatal conductance was 0.07 mmol/m²/s, and photosynthetic rate was 18.2 µmol/m²/s in the non-treated control group (T1). The photosynthetic rate increased to 19.5 µmol/m³/s, transpiration rate to 10.0 mmol/m³/s, and stomatal conductance to 0.08 mmol/m³/s when exposed to nitric oxide (T2). The photosynthetic rate dropped to 16.5 µmol/m³/s, the transpiration rate to 8.5 mmol/m²/s, and the stomatal conductance to 0.06 mmol/m²/s after the hydrogen peroxide treatment (T3). For the Meteor variety, the combination treatment (T4) gave the best results in terms of photosynthetic rate (21.0 µmol/m³/s), transpiration rate (10.5 mmol/m²/s), and stomatal conductance (0.09 mmol/m²/s).

Sarsabaz Variety:

The non-treated control (T1) for the Sarsabaz variety showed a transpiration rate of 9.2 mmol/m²/s, a photosynthetic rate of 17.8 µmol/m²/s, and a stomatal conductance of 0.06 mmol/m²/s. The photosynthetic rate increased to 19.0 µmol/m³/s, the transpiration rate to 9.7 mmol/m³/s, and the stomatal conductance to 0.07 mmol/m³/s after nitric oxide treatment (T2). With the photosynthetic rate at 16.8 µmol/m³/s, transpiration rate at 8.8 mmol/m²/s, and stomatal conductance at 0.05 mmol/m²/s, the hydrogen peroxide treatment (T3) had a decreasing effect. At 21.2 µmol/m³/s, transpiration rate of 10.8 mmol/m²/s, and stomatal conductance of 0.08 mmol/m²/s, the combination treatment (T4) exhibited the highest values for the Sarsabaz variety.

Climax Variety:

In the Climax variety, the stomatal conductance was 0.05 mmol/m²/s, transpiration was 8.0 mmol/m²/s, and photosynthetic rate was 16.9 µmol/m²/s for the non-treated control (T1). The photosynthetic rate increased to 18.2 µmol/m³/s, transpiration rate to 8.5 mmol/m²/s, and stomatal conductance to 0.06 mmol/m²/s with nitric oxide treatment (T2). The photosynthetic rate dropped to 15.7 µmol/m³/s, the transpiration rate to 7.5 mmol/m²/s, and the stomatal conductance to 0.04 mmol/m²/s after the hydrogen peroxide treatment (T3). At 20.0 µmol/m³/s, transpiration rate of 9.5 mmol/m²/s, and stomatal conductance of 0.07 mmol/m²/s, the combination treatment (T4) demonstrated the highest photosynthetic rate for the Climax variety.

Supreme Variety:

In the case of the Supreme variety, the photosynthetic rate was 17.8 µmol/m²/s, transpiration rate was 8.5 mmol/m²/s, and stomatal conductance was 0.06 mmol/m²/s for the non-treated control (T1). The photosynthetic rate increased to 19.2 µmol/m³/s, transpiration rate to 9.0 mmol/m³/s, and stomatal conductance to 0.07 mmol/m³/s with nitric oxide treatment (T2). The photosynthetic rate dropped to 16.5 µmol/m³/s, the transpiration rate to 7.8 mmol/m²/s, and the stomatal conductance to 0.05 mmol/m²/s after the hydrogen peroxide treatment (T3). For the Supreme variety, the combination treatment (T4) showed the highest transpiration rate of 9.8 mmol/m³/s, stomatal conductance of 0.08 mmol/m²/s, and photosynthetic rate of 20.5 μmol/m²/s.

The combined treatment of nitric oxide and hydrogen peroxide (T4) in all four pea varieties generally had the most favorable effect on gas exchange parameters, such as photosynthetic rate, transpiration rate, and stomatal conductance, according to the results. Increased transpiration rates, higher photosynthetic activity, and improved stomatal conductance were the results of this treatment. Based on these results, it appears that the combination treatment may help pea plants' overall physiological activity, which may lead to increased growth and stress tolerance. To investigate the mechanisms underlying these effects and their useful applications in agriculture, more research is necessary. This study advances our knowledge of plant physiology and environmental responses while offering insightful information about how various treatments affect gas exchange parameters.

Table 1. Effect of different treatments of Nitric Oxide (0.1 mM) and Hydrogen Peroxide on water relation parameter of different varieties of pea under drought stress

Pea Variety 1 (Meteor)				
Treatment	Relative Water Content (%)	Leaf Osmotic Potential (MPa)	Leaf Turgor Potential (MPa)	Leaf Water Potential (MPa)
T1	95.5	-1.2	1.0	-0.2
T2	96.8	-1.1	1.1	-0.1
T3	92.0	-1.5	0.8	-0.7
T4	98.7	-1.0	1.2	-0.3
Pea Variety 2 (Sarsabaz)				
T1	94.8	-1.3	0.9	-0.4
T2	96.5	-1.2	1.0	-0.2
T3	91.2	-1.6	0.7	-0.9
T4	98.2	-1.1	1.1	-0.3
Pea Variety 3 (Climax)				
T1	96.0	-1.4	0.8	-0.6
T2	97.2	-1.3	0.9	-0.4
T3	90.5	-1.7	0.6	-1.1
T4	99.0	-1.2	1.0	-0.2
Pea Variety 4 (Supreme)				
T1	95.2	-1.2	1.0	-0.2
T2	97.0	-1.1	1.1	-0.1
T3	91.8	-1.5	0.8	-0.7
T4	98.5	-1.0	1.2	-0.2

Table 2. Effect of different treatments of Nitric Oxide (0.1 mM) and Hydrogen Peroxide on Biochemical parameters of different varieties of pea under drought stress

Pea Variety 1 (Meteor)				
Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carotenoid
T1	18.5	12.7	31.2	6.8
T2	20.1	13.8	33.9	7.5
T3	16.9	11.5	29.6	6.3
T4	21.8	14.9	36.7	8.1
Pea Variety 2 (Sarsabaz)				
T1	17.8	12.2	30.0	6.5
T2	19.4	13.3	32.7	7.1
T3	16.2	11.1	28.3	6.0
T4	20.5	14.0	35.2	7.8
Pea Variety 3 (Climax)				
T1	19.1	13.1	32.2	7.0

T2	20.8	14.3	35.1	7.7
T3	17.5	12.0	29.5	6.5
T4	22.2	15.2	37.4	8.3
Pea Variety 4 (Supreme)				
T1	18.9	13.0	31.9	6.9
T2	20.4	14.0	34.4	7.6
T3	17.2	11.8	29.0	6.3
T4	21.7	14.9	36.6	8.0

Chlorophyll and Carotenoid Content (µg/g fresh weight)

Table 3. Effect of different treatments of Nitric Oxide (0.1 mM) and Hydrogen Peroxide on Gas exchange parameters of different varieties of pea under drought stress

a Variety 1 (Meteor)			
Treatment	Photosynthetic Rate	Transpiration Rate	Stomatal Conductance
T1	18.2	9.5	0.07
T2	19.5	10.0	0.08
T3	16.5	8.5	0.06
T4	21.0	10.5	0.09
Pea Variety 2 (Sarsabaz)			
T1	17.8	9.2	0.06
T2	19.0	9.7	0.07
T3	16.8	8.8	0.05
T4	21.2	10.8	0.08
Pea Variety 3 (Climax)			
T1	16.9	8.0	0.05
T2	18.2	8.5	0.06
T3	15.7	7.5	0.04
T4	20.0	9.5	0.07
Pea Variety 4 (Supreme)			
T1	17.8	8.5	0.06
T2	19.2	9.0	0.07
T3	16.5	7.8	0.05
T4	20.5	9.8	0.08

Photosynthetic Rate ($\mu mol/m^2/s$), Transpiration Rate ($mmol/m^2/s$), Stomatal Conductance ($mmol/m^2/s$)

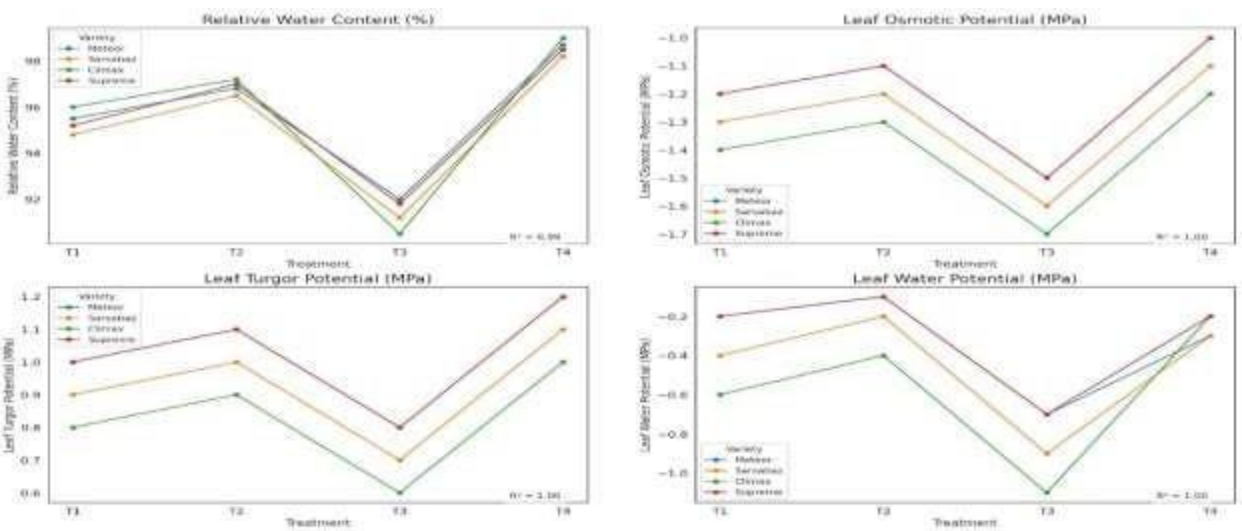

Fig 1. Response of treatments on water relation parameters in Pea varieties

The (Fig 1) shown effects of four treatments (T1, T2, T3, T4) on important water relation parameters in four pea varieties (Meteor, Sarsabaz, Climax, Supreme) are shown in these line graphs. Relative Water Content, Leaf Osmotic Potential, Leaf Turgor Potential, and Leaf Water Potential are some of the parameters. Every color denotes a distinct variation, allowing for an easy visual comparison of various treatments. Grasp the physiological adaptations of these kinds under varying situations requires a grasp of the models' fit, which is indicated by the R-squared values.

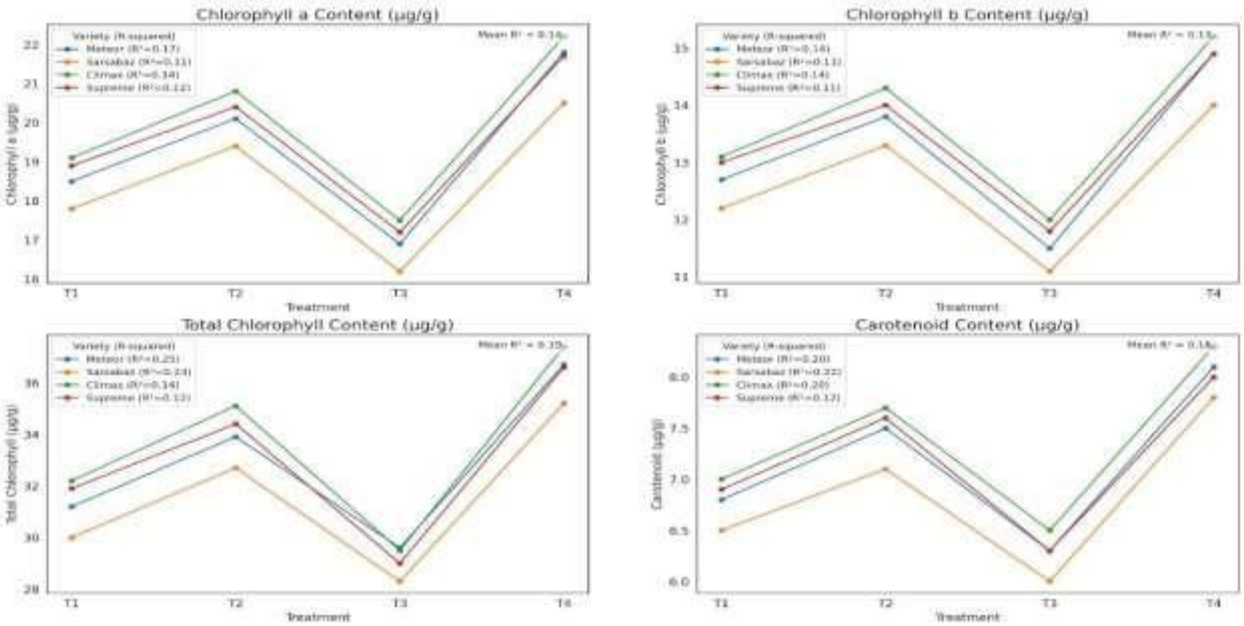

Fig 2. Differential response of pea varieties to oxidative treatments: A biochemical profiling

Fig 2 shown that four distinct pea types (Meteor, Sarsabaz, Climax, and Supreme) are stressed by drought, and this series of line graphs shows that various treatments including hydrogen peroxide and nitric oxide (0.1 mM) affect important biochemical markers. Chlorophyll a, b, total chlorophyll, and carotenoid content all given in μg/g of fresh weight—are among the parameters that are measured. Every graph (T1, T2, T3, T4) illustrates a different biochemical parameter and shows how each pea variety responds to the treatments. The legend includes the R-squared values for each variety, which show how much of the variance is explained by the treatments for each parameter.

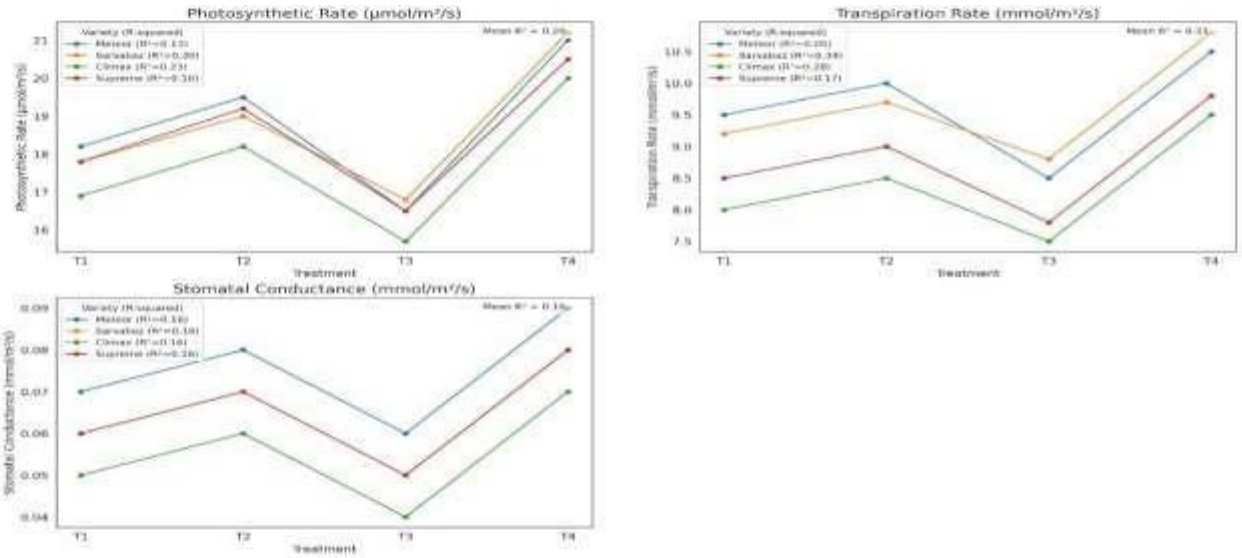

Fig 3. Response of Pea varieties to drought stress treatments: A gas exchange analysis

The effects of treating four types of peas under drought stress with hydrogen peroxide and nitric oxide (0.1 mM) on critical gas exchange parameters, such as photosynthetic rate, transpiration rate, and stomatal conductance, are shown in a series of line plots (Fig. 3). The Meteor, Sarsabaz, Climax, and Supreme pea varieties' performances are contrasted in each plot throughout the course of four distinct treatments (T1 to T4). The units of measurement for photosynthetic rate, transpiration rate, and stomatal conductance are μmol/m³/s, mmol/m³/s, and mmol/m³/s, respectively. A quantitative indicator of treatment efficiency is provided by the R-squared values given in each plot, which show the variance in these parameters explained by the treatments for each variety.

4. DISCUSSIONS

4.1. Effects of Treatments on Water Relation Parameters in Pea Varieties

This study's findings provided important light on how various treatments affect the water relation parameters of four different pea varieties: Meteor, Sarsabaz, Climax, and Supreme. The hydration position and overall condition of these pea plants are affected differently by the treatments, which include a nitric oxide treatment, hydrogen peroxide treatment, nitric oxide treatment, and a combined nitric oxide and hydrogen peroxide treatment. It is clear from looking at the water relation parameters that, among the four pea varieties, the combination treatment of nitric oxide and hydrogen peroxide (T4) generally had the most positive impact on these parameters. Greater Leaf Turgor Potential (LTP), less negative Leaf Water Potential (LWP), less negative Leaf Osmotic Potential (LOP), and increased Relative Water Content (RWC) were the outcomes of this treatment. These results imply that T4 efficiently increases cell turgor, decreases osmotic stress, and increases water retention all essential for the best possible growth and development of plants (Osman, 2015). The beneficial effects of T4 on water relation parameters are consistent with earlier studies that have demonstrated the function of hydrogen peroxide and nitric oxide in controlling plant water balance (Rai et al., 2020). The enhanced water relations seen under T4 may be attributed to nitric oxide, which is known to take part in a number of physiological processes, such as stomatal regulation and water uptake (Habib et al., 2020). Asgher et al. (2017) suggest that hydrogen peroxide, when managed at suitable concentrations, may function as a molecule that signals in stress responses, which might be the reason for its beneficial impacts when paired with nitric oxide. However, it is imperative to recognize that water relations in some pea varieties like Sarsabaz and Supreme were negatively impacted by the hydrogen peroxide treatment (T3). This response variation demonstrates the sensitivity different pea varieties are to oxidative stress and underscores the significance of implementing plant species genetic diversity consideration when implementing such treatments (Araújo, 2015; Bagheri et al., 2023). These findings have applications in agriculture, where crop productivity and stress tolerance depend on effective water management. In summary, this study has clarified how different treatments affect the water relation parameters in distinct pea varieties. Nitric oxide and hydrogen peroxide treatment (T4) in combination appears to be a viable method for improving water relations in pea plants.

4.2. Biochemical Parameters: Chlorophyll and Carotenoid Content

The impact of different treatments on two important biochemical parameters chlorophyll and carotenoid content was examined in this study for four different pea varieties: Meteor, Sarsabaz, Climax, and Supreme. These included a control group that received no treatment, treatments with nitric oxide, hydrogen peroxide, and a combination of nitric oxide and hydrogen peroxide. According to Anwar et al. (2023), the Meteor variety showed the greatest improvement in total chlorophyll, carotenoid content, chlorophyll a, and chlorophyll b in the combination treatment (T4), indicating its potential to support photosynthetic pigments that are essential for maintaining photosynthesis and plant growth. In the same way, T4 proved useful in increasing pigment content in the Sarsabaz variety, offsetting the oxidative effects seen in T3. This underscores the sensitivity of pigment accumulation in Sarsabaz to oxidative and nitrosative stressors, aligning with previous

research (Tang et al., 2021).
Nitric oxide treatment (T2) was found to be beneficial in increasing pigment levels in the Climax variety, whereas hydrogen peroxide treatment (T3) had the opposite effect and resulted in decreased pigment content. In terms of encouraging pigment accumulation, the combination treatment (T4) performed better than the other treatments once more. According to these results, nitric oxide treatment is especially beneficial for enhancing pigment synthesis in Climax varieties, whereas hydrogen peroxide may prevent pigment accumulation in this particular variety (Roychoudhury, 2021). Last but not least, T4 consistently had a positive impact on the levels of carotenoid and chlorophyll in the Supreme variety, exactly as it performed in the other varieties. According to Su et al. (2015), the findings demonstrate how T4 may promote pigment accumulation in Supreme, which would enhance photosynthetic activity and stress tolerance.

4.3. Gas Exchange Parameters: Photosynthetic Rate, Transpiration Rate, and Stomatal Conductance

Due to their investigation of gas exchange parameters such as photosynthetic rate (PR), transpiration rate (TR), and stomatal conductance (SC), researchers have gained a great deal of insight into how four distinct pea varieties Meteor, Sarsabaz, Climax, and Supreme respond to different treatments. These comprised an untreated control, a treatment with hydrogen peroxide, a treatment with nitric oxide and hydrogen peroxide combined, and a control with nitric oxide. The results demonstrate the diverse effects of these treatments on the physiological processes vital to plant growth and development. Based on the analysis of the gas exchange parameters, the combination treatment of hydrogen peroxide and nitric oxide (T4) showed the best results out of the four types of peas. This treatment caused to increases in transpiration rate, stomatal conductance, and photosynthetic rate. These findings indicate that T4 effectively promotes photosynthesis, increasing carbon uptake, while also supporting the regulation of transpiration and stomatal activity (Éva et al., 2019).
The favorable impact of T4 on gas exchange parameters is consistent with earlier studies focusing on the function of nitric oxide and hydrogen peroxide as signaling molecules in controlling stomatal behavior and photosynthesis (Adamipour et al., 2019). Particularly nitric oxide has been related to the regulation of stomatal aperture, which affects gas exchange rates directly (De et al., 2020). The combination of these signaling molecules may increase photosynthetic activity and improve water-use efficiency in pea plants, as suggested by the synergistic effects seen under T4. Still, it is important to recognize that different pea varieties responded differently to treatments. The Sarsabaz variety, for example, showed a significant increase in both Photosynthetic Rate and Transpiration Rate under T4, suggesting that it is a good candidate for this combined treatment. Li et al. (2017) reported that the Climax variety showed increased stomatal conductance, but a less visible response in photosynthetic rate and transpiration rate.
These findings have significant practical implications for agriculture. Optimizing gas exchange parameters can improve crop productivity and resilience to stress, especially when done with treatments like T4. Increased yield and decreased susceptibility to environmental stressors, like drought, can be achieved through enhanced photosynthetic activity and efficient water use (Ullah et al., 2018). To sum up, this study has shed important light on the way various treatments affect the gas exchange parameters in distinct pea varieties. Nitric oxide and hydrogen peroxide treatment (T4) appears to be a viable method for increasing photosynthetic rate, transpiration rate, and stomatal conductance, all of which contribute to the overall performance of plants.

CONCLUSION

Finally, the extensive research into the way various treatments impact gas exchange, water relations, and biochemical parameters in different pea varieties shows that the combination treatment of hydrogen peroxide and nitric oxide (T4) is the best option. T4 continuously showed the best results across the board, with higher gas exchange rates, higher chlorophyll and carotenoid content, and improved water relation parameters. These results highlight the T4 treatment's capacity to enhance photosynthetic activity, physiological function, and broad terms plant health in pea plants. In order to optimize pea cultivation and raise crop yields, we advise farmers in Pakistan to take into consideration adopting this ensuring T4 treatment.

Acknowledgments
The work presented in this manuscript is a part of the Ph.D. thesis of Mr. Muhammad Kamran Afzal, Reg. No. 2017-GCUF-02547, a Ph.D. scholar at Government College University Faisalabad, Pakistan.

Author Contribution
Dr Noman Habib: Conceptualizing, experimental design, supervision, project administration, and funding acquisition; **Muhammad Kamran Afzal:** Experiment performance, draft writing, analysis, data curation, investigation, and methodology; **Shamsa Rana** original draft writing, visualization, statistical analysis, methodology, investigation, software, review, and editing.

REFERENCES
1. Anjum, S. A., Xie, X., Wang, L. C., Saleem, M. F., Man, C., & Lei, W. (2011). Morphological, physiological and biochemical responses of plants to drought stress. African Journal of Agricultural Research, 6(9), 2026-2032.
2. Devi, P., Dey, S. R., Saini, L., Kumar, P., Panigrahi, S., & Dwivedi, P. (2023). Toward Sustainable Agriculture: Strategies Involving Phytoprotectants Against Reactive Oxygen Species. In Reactive Oxygen Species: Prospects in Plant Metabolism (pp. 229-247). Singapore: Springer Nature Singapore.
3. Dikilitas, M., Simsek, E., & Roychoudhury, A. (2020). Role of proline and glycine betaine in overcoming abiotic stresses. Protective Chemical Agents in the Amelioration of Plant Abiotic Stress: Biochemical and Molecular Perspectives, 1-23.
4. Dodt, M. G. (2017). Characterisation of root architectural responses of mungbean to water deficit (Doctoral dissertation, Queensland University of Technology).
5. Goyal, M., Singh, J., Kumr, P., & Sirohi, A. (2018). Pulses for human nutritional security. Pulse Improvement: Physiological, Molecular and Genetic Perspectives, 1-11.
6. Gupta, K., Sengupta, A., Chakraborty, M., & Gupta, B. (2016). Hydrogen peroxide and polyamines act as double-edged swords in plant abiotic stress responses. Frontiers in Plant Science, 7, 1343.
7. Khan, M., Ali, S., Al Azzawi, T. N. I., & Yun, B. W. (2023). Nitric Oxide Acts as a Key Signaling Molecule in Plant Development under Stressful Conditions. International Journal of Molecular Sciences, 24(5), 4782.
8. Marthandan, V., Geetha, R., Kumutha, K., Renganathan, V. G., Karthikeyan, A., & Ramalingam, J. (2020). Seed priming: A feasible strategy to enhance drought tolerance in crop plants. International Journal of Molecular Sciences, 21(21), 8258.
9. Mauck, K. E., Kenney, J., & Chesnais, Q. (2019). Progress and challenges in identifying molecular mechanisms underlying host and vector manipulation by plant viruses. Current Opinion in Insect Science, 33, 7-18.
10. Nadeem, M., Li, J., Yahya, M., Sher, A., Ma, C., Wang, X., & Qiu, L. (2019). Research progress and perspective on drought stress in legumes: A review. International Journal of Molecular Sciences, 20(10), 2541.
11. Onaga, G., & Wydra, K. (2016). Advances in plant tolerance to abiotic stresses. Plant Genomics, 10(9), 229-272.
12. Ozougwu, J. C. (2016). The role of reactive oxygen species and antioxidants in oxidative stress. International Journal of Research, 1(8).

13. Ozturk, M., Turkyilmaz Unal, B., García-Caparrós, P., Khursheed, A., Gul, A., & Hasanuzzaman, M. (2021). Osmoregulation and its actions during the drought stress in plants. Physiologia Plantarum, 172(2), 1321-1335.
14. Palanivel, H., & Shah, S. (2021). Unlocking the inherent potential of plant genetic resources: Food security and climate adaptation strategy in Fiji and the Pacific. Environment, Development and Sustainability, 23(10), 14264-14323.
15. Rane, J., Singh, A. K., Kumar, M., Boraiah, K. M., Meena, K. K., Pradhan, A., & Prasad, P. V. (2021). The adaptation and tolerance of major cereals and legumes to important abiotic stresses. International Journal of Molecular Sciences, 22(23), 12970.
16. Sachdev, S., Ansari, S. A., Ansari, M. I., Fujita, M., & Hasanuzzaman, M. (2021). Abiotic stress and reactive oxygen species: Generation, signaling, and defense mechanisms. Antioxidants, 10(2), 277.
17. Savvides, A., Ali, S., Tester, M., & Fotopoulos, V. (2016). Chemical priming of plants against multiple abiotic stresses: Mission possible? Trends in Plant Science, 21(4), 329-340.
18. Saxena, R., Kumar, M., & Tomar, R. S. (2019). Plant responses and resilience towards drought and salinity stress. Plant Arch, 19(Suppl 2), 50-58.
19. Shen, J., Wang, L., Wang, X., Jin, K., & Xiong, C. (2021). Interplay between root structure and function in enhancing efficiency of nitrogen and phosphorus acquisition. The Root Systems in Sustainable Agricultural Intensification, 121-157.
20. Singh, A., Mehta, S., Yadav, S., Nagar, G., Ghosh, R., Roy, A., ... & Singh, I. K. (2022). How to cope with the challenges of environmental stresses in the era of global climate change: An update on ROS stave off in plants. International Journal of Molecular Sciences, 23(4), 1995.
21. Tripathy, S., & Mohanty, P. K. (2017). Reactive oxygen species (ROS) are boon or bane. International Journal of Pharmaceutical Sciences and Research, 8(1), 1.
22. Tyagi, A., Ali, S., Ramakrishna, G., Singh, A., Park, S., Mahmoudi, H., & Bae, H. (2023). Revisiting the role of polyamines in plant growth and abiotic stress resilience: Mechanisms, crosstalk, and future perspectives. Journal of Plant Growth Regulation, 42(8), 5074-5098.
23. Wojtyla, Ł., Lechowska, K., Kubala, S., & Garnczarska, M. (2016). Different modes of hydrogen peroxide action during seed germination. Frontiers in Plant Science, 7, 66.
24. Osman, H. S. (2015). Enhancing antioxidant–yield relationship of pea plant under drought at different growth stages by exogenously applied glycine betaine and proline. Annals of Agricultural Sciences, 60(2), 389-402.
25. Rai, K. K., Rai, N., Aamir, M., Tripathi, D., & Rai, S. P. (2020). Interactive role of salicylic acid and nitric oxide on transcriptional reprogramming for high temperature tolerance in Lablab purpureus L.: Structural and functional insights using computational approaches. Journal of biotechnology, 309, 113-130.
26. Habib, N., Ali, Q., Ali, S., Javed, M. T., Zulqurnain Haider, M., Perveen, R., ... & Bin-Jumah, M. (2020). Use of nitric oxide and hydrogen peroxide for better yield of wheat (Triticum aestivum L.) under water deficit conditions: growth, osmoregulation, and antioxidative defense mechanism. Plants, 9(2), 285.
27. Asgher, M., Per, T. S., Masood, A., Fatma, M., Freschi, L., Corpas, F. J., & Khan, N. A. (2017). Nitric oxide signaling and its crosstalk with other plant growth regulators in plant responses to abiotic stress. Environmental Science and Pollution Research, 24, 2273-2285.
28. Araújo, S. S., Beebe, S., Crespi, M., Delbreil, B., González, E. M., Gruber, V., ... & Patto, M. C. V. (2015). Abiotic stress responses in legumes: strategies used to cope with environmental challenges. Critical Reviews in Plant Sciences, 34(1-3), 237-280.
29. Bagheri, M., Santos, C. S., Rubiales, D., & Vasconcelos, M. W. (2023). Challenges in pea breeding for tolerance to drought: Status and prospects. Annals of Applied Biology, 183(2), 108-120.

30. Anwar, T., Munwwar, F., Qureshi, H., Siddiqi, E. H., Hanif, A., Anwaar, S., ... & Kamal, A. (2023). Synergistic effect of biochar-based compounds from vegetable wastes and gibberellic acid on wheat growth under salinity stress. Scientific Reports, 13(1), 19024.
31. Roychoudhury, A., Banerjee, A., & Lahiri, V. (2015). Metabolic and molecular-genetic regulation of proline signaling and itscross-talk with major effectors mediates abiotic stress tolerance in plants. Turkish Journal of Botany, 39(6), 887-910.
32. Tang, Z., Ju, Y., Dai, X., Ni, N., Liu, Y., Zhang, D., ... & Gu, P. (2021). HO-1-mediated ferroptosis as a target for protection against retinal pigment epithelium degeneration. Redox biology, 43, 101971.
33. Su, F., Jacquard, C., Villaume, S., Michel, J., Rabenoelina, F., Clément, C., ... & Vaillant-Gaveau, N. (2015). Burkholderia phytofirmans PsJN reduces impact of freezing temperatures on photosynthesis in Arabidopsis thaliana. Frontiers in plant science, 6, 810.
34. Éva, C., Oszvald, M., & Tamás, L. (2019). Current and possible approaches for improving photosynthetic efficiency. Plant Science, 280, 433-440.
35. Adamipour, N., Khosh-Khui, M., Salehi, H., Razi, H., Karami, A., & Moghadam, A. (2020). Regulation of stomatal aperture in response to drought stress mediating with polyamines, nitric oxide synthase and hydrogen peroxide in Rosa canina L. Plant Signaling & Behavior, 15(9), 1790844.
36. De Sousa, L. F., de Menezes-Silva, P. E., Lourenço, L. L., Galmés, J., Guimarães, A. C., da Silva, A. F., ... & Farnese, F. D. S. (2020). Improving water use efficiency by changing hydraulic and stomatal characteristics in soybean exposed to drought: the involvement of nitric oxide. Physiologia plantarum, 168(3), 576-589.
37. Li, Y., Li, H., Li, Y., & Zhang, S. (2017). Improving water-use efficiency by decreasing stomatal conductance and transpiration rate to maintain higher ear photosynthetic rate in drought-resistant wheat. The crop journal, 5(3), 231-239.
38. Ullah, H., Santiago-Arenas, R., Ferdous, Z., Attia, A., & Datta, A. (2019). Improving water use efficiency, nitrogen use efficiency, and radiation use efficiency in field crops under drought stress: A review. Advances in agronomy, 156, 109-157.