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Research Article

Selenium and potassium supplementation improve nitrogen metabolism, antioxidant activity, and osmolyte production, reducing the growth and photosynthetic inhibition caused by polyethylene glycol (PEG) in wheat

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Abstract

Drought stress has a significant impact on all crops, affecting both their growth and development. In this study, wheat plants were subjected to drought stress induced by 15% of PEG along with or without supplementation of selenium and potassium supplements. Plants subjected to PEG alone showed a decrease in a number of plants morphophysiological and biochemical parameters such as a decline in plant height, dry mass, carotenoids, total chlorophyll, stomatal conductance, net photosynthesis, and intercellular CO_2 levels. Nevertheless, adding selenium and potassium supplements effectively mitigated these decreases. PEG's drought also led to the overproduction of harmful ROS-inducing substances, H_2O_2 and O_2. Certain factors led to a significant instance of lipid peroxidation, which the addition of selenium and potassium effectively reduced and leaded to protection of plants from oxidative stress damage. The combination of selenium and potassium also significantly reduced the protease and lipoxygenase activities. This effect was even more noticeable when dealing with synergic Se+K applications. The levels of enzymatic (CAT, SOD, APX, and GR) and non-enzymatic (AsA, GSH, and tocopherol) antioxidants went up a lot after treatment with PEG. Moreover, the inclusion of selenium and potassium supplements further amplified the increase. Adding selenium and potassium supplements improved the activity of nitrate reductase (NR), an enzyme that breaks down nitrogen. The combination treatment has shown remarkable efficacy in reducing the adverse effects caused by PEG. Furthermore, the research revealed that the inclusion of selenium and potassium supplements led to heightened quantities of phenols and flavonoids. As a result of the drought, there was a limited absorption of mineral ions, such as nitrogen, phosphorus, potassium, and calcium. However, the addition of selenium and potassium supplements significantly mitigated this decline.

Keywords: antioxidant system; nitrogen metabolism; osmoregulation; PEG; selenium; wheat

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Introduction

Plants are vulnerable to a variety of environmental factors, both natural and human-induced, as they are sessile and cannot change their position (Khan *et al.*, 2022). Drought stress not only effect the growth of plants but also decreases its productivity (Ahanger *et al.*, 2014; Hussain *et al.*, 2018). This deficiency of water can

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restrict root development, impede the uptake of vital minerals, disrupt enzyme function, impact photosynthesis, effect the expression of genes, and also negatively impact the process of protein formation (Ahanger *et al*., 2021). A lack of water in plants can result in restricted growth attributes, primarily due to limited growth at the cellular level and the overproduction of ROS (Ahluwalia *et al*., 2021). Due to a deficiency of water, plants face the problem of water usage, resulting in a decline in the functioning of plants and their yield performance (Seleiman *et al*., 2021). To overcome this issue of water scarcity plants have developed certain approaches. These strategies focus on improving the naturally occurring mechanisms that are regulated by molecular changes (Qin *et al*., 2021; Ahanger *et al*., 2021). When plants are exposed to stressful situation, such as water deficit, they display key responses such as their antioxidant system, osmoregulation, as well as accumulation of extracellular metabolites to overcome the negative effects (Begum *et al*., 2020; 2021).

One of the most important macronutrients responsible for the healthy growth and development of plants is Potassium (K) (Ahanger *et al*., 2017a; Song *et al*., 2018; Sardans and Penuelas, 2021). The presence of potassium (K) has an important positive impact on the metabolism in empowering plants to resist various forms of stress (Khan *et al*., 2021; Hasanuzzaman *et al*., 2018). Research conducted by Ahanger *et al*. (2017b) and Xu *et al*. (2020a) has demonstrated that K^+ has a beneficial role in improving nitrogen and carbon metabolism. This exerts a significant influence on the overall development, metabolic processes, genetic activity, and productivity of plants. The impact of potassium (K) on the regulation of tolerance mechanisms for improved stress adaption and tolerance has been assessed, and the role of K in mitigating stress and making the plant tolerant has been confirmed (Zorb *et al*., 2014; Ahanger *et al*., 2017a). Environmental stressors trigger the formation and build-up of ROS in tissues, leading to the activation of potassium-permeable cation channels and subsequent outflow of potassium (K). Depletion of potassium (K) in cells can lead to programmed cell death, as it activates proteases and endonucleases (Demidchik *et al*., 2014). Hence, it is crucial to ensure the ideal concentration of potassium (K) in soils to enhance the development of plants and enhance endurance responses (Khan *et al*., 2022). Selenium (Se) is also an essential beneficial element for humans, plants, and animals, as demonstrated by recent research work carried out by Alyemeni *et al*. (2018), Elkelish *et al*. (2019), and Khan *et al*. (2015). Various research has demonstrated that selenium has the capacity to ease the adverse impacts of cold weather, high temperature, water stress, salinity, and cadmium stress on various crops (Hawrylak-Nowak *et al*., 2010; Djanaguiraman *et al*., 2010; Nawaz *et al*., 2016; Alyemeni *et al*., 2018; Elkelish *et al*., 2019). Selenium (Se) and sulfur (S) have similar chemical properties due to their placement in the same group on the periodic table, leading Se to replace S in compounds containing sulfhydryl groups. This substitution can alter the molecular structure and biological function of these compounds, potentially affecting protein synthesis and enzyme activity (Harbone, 1997). Similar findings were found in another experimental work conducted by Alyemeni *et al*. (2018), it has been noticed that selenium (Se) can improve plant functioning and yield performance. This is accomplished by enhancing the Induction of tolerance mechanisms that aid plants in removing the negative effects of stress. Plants have the ability to take in selenium in various forms, including selenate, selenite, and organic selenium compounds like selenomethionine (SeMet) and selenocysteine (SeCys), which are absorbed through their roots. Nevertheless, selenides and elemental selenium are not absorbed (White and Broadley, 2009; Gupta and Gupta, 2017). Plant absorption of selenium is aided by sulphate transporters with a high affinity for the element (Shinmachi *et al*., 2010; Gigolashvili and Kopriva, 2014; Gupta and Gupta, 2017).

Wheat, scientifically referred to as *Triticum aestivum* L., is cultivated extensively for the purpose of consumption as food and is regarded as an important crop of cereals. Scarcity of water being a global problem, drastically impedes its growth, other essential physiological and biochemical processes of wheat, resulting in a considerable decrease in crop yield worldwide. The current study is designed to understand the impact of adding potassium (K) and selenium (Se) to the system can help alleviate the negative impacts of water deficiency or not.

Materials and Methods

Treatments, experimental design

Wheat seeds underwent sterilization by being treated with a 0.01% solution of $HgCl_2$ and subsequently rinsed with distilled water. Seeds that had undergone sterilization were carefully planted in clay pots filled with sand that had been treated with ethanol to remove any microbes. Prior to planting, every pot was completely soaked with 200 mL of undiluted Hoagland solution.

In the start ten healthy seeds were germinated. Post germination of the seeds, thinning of the sprouted seedlings were brought to five healthy one. Additionally, half of the pots underwent water stress by being treated with a 15% concentration PEG (PEG-6000). A solution of PEG-6000 was created using Hoagland nutritional solution. In addition, the pots were supplemented with either 1 µM Na_2SeO_4 (sodium selenite), 3 mM KNO_3 (potassium nitrate), or a combination of both selenium and potassium, either separately or together with PEG-6000. The experimental treatments can be classified into different categories: (1) Control (2) Adding 1 µM Selenium, (3) Adding 3 mM Potassium, (4) Combining Selenium and Potassium, (5) Using PEG, (6) Combining PEG and Selenium, (7) Combining PEG and Potassium, (8) Combining PEG, Selenium, and Potassium. Pots were kept in growth chamber maintained at day/night temperatures of 25/15 °C, light intensity of 300 mmol /m^2 /s and humidity of 60-70%. Treatments were given every other day. After a period of twenty days of stress, plants treated with Se and K were carefully removed and examined for several criteria as described.

Plant height and dry mass

To ascertain the plant's dry weight, the plant root and shoot tissues were dried at a temperature of 60 °C in an oven for a duration of 72 hours. The height was determined through manual measurement using a scale.

Determination of photosynthetic parameters

The concentrations of carotenoids and total chlorophylls were quantified in recently harvested leaf tissues using the methodology outlined by Arnon (1949). Breifly, leaf tissues were grinded in acetone extract and the absorbance of the acetone extract was quantified at optical density (OD or wavelength) of 480 nm, 645 nm, and 663 nm. The Li-6400, a portable photosynthetic device manufactured by LI-COR Inc. in the United States, was utilised to quantify crucial photosynthetic parameters such the parameters being referred to stomatal conductance (gs), net photosynthetic rate (Pn) and internal CO_2 concentration (Ci). The measurements were obtained from leaves that were fully expanded.

Quantification of soluble sugars, glycine betaine and free proline

The techniques described by Schields and Burnet (1960) for sugar estimation was followed briefly dried leaf powder of wheat (100 mg), was soaked in 80% (v/v) ethanol for 24 h and the absorbance of the solution was measured at 585 nm, Bates *et al.* (1973) protocol was followed for proline estimation by briefly grinding 250 mg of wheat fresh leaves in a solution of 3% sulfosalicyclic acid (w/v) and spectrophotometrically measuring the OD at 520 nm, Grieve and Grattan (1983) protocol was followed . Briefly a finely grounded wheat tissues were diluted in an equal volume of 1M H_2SO_4, followed by distribution into aliquots of 0.5 ml in centrifuge tubes, the tubes were cooled over ice for 1 h, which was followed by mixing 0.2 ml of cold potassium iodide reagent. The mixture was stirred gently and was kept at 4 °C overnight. Next day the mixture was centrifuged at 10,000 rpm for 15 min at 4 °C to get the precipitated per iodide crystals. The crystals were dissolved in 1,2-dichloroethane, and the absorbance was measured at 365 nm after 2 h. Glycine betaine was dissolved in 1 M H_2SO_4 and it was served as a standard.

Quantification of γ-glutamyl kinase and proline oxidase

The freshly harvested tissues of the shoot were crushed using a cold pestle and mortar in Tris buffer solution with a pH of 7.5. Following the centrifugation procedure, a solid residue was acquired. Subsequently, the pellet was used to evaluate the assays of proline and γ-glutamyl kinase employing the methods described by Hayzer and Leisinger (1980) and Huang and Cavalieri (1979), correspondingly. The sample mixture for γ-GK consisted of the solution containing Tris buffer at a concentration of 50 mM with a pH of 7.0, $MgCl_2$ at a concentration of 20 mM, L-glutamate at a concentration of 50 mM, ATP at a concentration of 10 mM, and hydroxamate-HCl at a concentration of 100 mM., and the enzyme to activate the reaction. The reaction was halted by introducing a stop buffer consisting of $FeCl_3$ and TCA. Subsequently, the optical density was measured at a wavelength of 535 nm. The activity of γ-GK was assessed by measuring the formation of γ-glutamyl hydroxamate. The quantification was performed in units/mg protein/min. The PROX assay utilized a Tris buffer with a pH of 8.5, along with $MgCl_2$, KCN, NADP, 2,6-dichlorophenol indophenol (DCPIP), phenazine methanosulphate, and proline. The measurement of absorbance at a wavelength of 600 nm was monitored over a period of 3 minutes. The reduction in millimoles of DCPIP was employed to measure PROX activity and calculated as units/minute/mg of protein.

Hydrogen peroxide, superoxide and lipid peroxidation estimation

The concentration of H_2O_2 was quantified by following the protocol specified by Velikova *et al.* (2000). The procedure involved extracting 100 mg of freshly harvested leaf tissue using trichloroacetic acid (TCA). The solution was combined with the solution was mixed with a buffer containing potassium phosphate pH of which was adjusted at 7.0, and the OD of potassium iodide was quantified at a wavelength of 390 nm. To determine the amount of O_2^- present, we extracted 100 mg of recently harvested tissue using a potassium phosphate buffer with a concentration of 65 mM and a pH of 7.8. Which was followed by centrifugation of the mixture at a speed of 5000 g. The supernatant underwent a reaction with hydroxylamine hydrochloride at a concentration of 10 mM. After that, sulfanilamide and naphthylamine were added. The mixture was kept at 25 °C for 20 min. The absorbance of the mixture was quantified at an OD of 530 nm, as reported in a previous study by Yang et al. in 2011. The computation required the utilisation of a $NaNO_2$ standard curve.

The quantification of lipid peroxidation in both healthy and stressed plants was performed by using the methodology outlined by Heath and Packer in 1968. To summarise, the tissue that was just acquired was combined with TCA supernatant and subsequently treated with thiobarbituric acid. The resulting malonaldehyde (MDA) was measured employing a spectrophotometer set to wavelengths of 532 nm and 600 nm.

Analysis of lipoxygenase and protease activities

The assays of lipoxygenase were assessed by following the methodology published by Doderer *et al.* (1992), with the fatty acid Linoleic acid served as the substrate. The alteration in optical density was quantified at a wavelength of 234 nm and an extinction coefficient of 25 mM^{-1} cm^{-1} were used for the computation. The quantification of protease activity was performed using the procedure described by Green and Neurath (1954). In summary, we obtained recently harvested tissue and placed it in a cold solution of sodium potassium buffer (50 mM, pH 7.4) that was enriched with PVP. The extract underwent centrifugation at a force equivalent to 5000 g/5 min, referred to as the supernatant was subsequently combined with casein at a temperature of 40 °C. The quantity of tyrosine that was released was then measured using Folin Ciocalteu's reagent in an alkaline solution, and the absorbance was recorded at a wavelength of 660 nm. The quantification of protease activity involved measuring the amount of tyrosine released per milligrams of protein.

Nitrate reductase determination

An assessment was conducted on the enzymatic activity of nitrate reductase (NR) in freshly collected leaf tissue. The tissue, weighing 300 mg, was submerged in a solution of potassium phosphate buffer with a concentration of 100 millimolar and a pH of 7.5, which also included KNO_3 and n-propanol. The tissue was carefully stored in a controlled environment, shielded from any light, and maintained at a constant temperature of 30 °C for a duration of 3 hours. Later on, a 1 mL portion was combined with 1-nephthylethylene diamine dihydrochloride and sulphanilamide. The solution was left undisturbed for twenty minutes and OD was quantified at 540nm as performed by Hashem *et al.* (2016).

Analysis of antioxidant enzyme activity

To acquire antioxidant enzymes, the newly grown shoot tissue was pulverized in a solution containing A mixture comprising 100 mM phosphate buffer the pH was adjusted at 7.8, 1% polyvinyl pyrrolidone, 1 mM EDTA, and 0.1 mM phenylmethylsulfonyl fluoride (PMSF) was prepared. Grinding was performed using a pre-cooled pestle and mortar. The liquid portion of supernatant obtained after subjecting the mixture was centrifuged for 15 min at 12,000 rpm and 4 ºC, the supernatant was collected and analysed for the enzyme analysis. The role of SOD was assessed by quantifying the reduction of nitroblue tetrazolium chloride using photochemical techniques, in which the enzyme functioned as an inhibitor. The measurement was performed at a wavelength of 560 nm, as documented by Bayer and Fridovich (1987). Aebi (1984) protocol was followed to quantify the catalase activity in the samples, which entailed observing the decrease in absorbance at 240 nm over a period of 2 minutes. The analysis of ascorbate peroxidase (APX) activity involved tracking the reduction in H_2O_2 concentration at an OD of 290 nm for a period of 3 minutes (Nakano and Asada, 1981).

Quantification of ascorbate, tocopherol and reduced glutathione

The procedures proposed by Mukherjee and Choudhuri (1983) and Ellman (1959) were followed to measure the concentrations of reduced glutathione (GSH) and ascorbate (AsA). The extraction of tocopherol was performed by mixing ethanol and petroleum ether in a ratio of 1.6:2. Subsequently, the liquid portion was exposed to a 2% solution of 2, 2-dipyridyl in a dimly lit setting. Backer et al. did a study in 1980 where they measured absorbance at a precise wavelength of 520 nm. The calculations were executed utilizing a conventional curve.

Quantification of overall phenolic, flavonoid, and phenylalanine ammonia lyase activity

The Singleton and Rossi (1965) approach was employed to ascertain the overall phenol content. Methanol was employed as a solvent to extract the desiccated powdered plant material. The supernatant obtained was further mixed with the reagent namely Folin–Ciocalteu and the OD was performed at a 765 nm. A conventional gallic acid calibration curve was used for the quantification purposes. In order to quantify flavonoids, the previously described protocol of Zhishen *et al.* (1999) was followed. The plant sample underwent a drying process and subsequently underwent methanol treatment for the purpose of extraction. The extracted solution was quantified at an OD of 510 nm. The calculation employs a standardized curve derived from a traditional methodology. The PAL test was carried out according to the protocol described by Zucker (1965), with the measurement of trans-cinnamic acid synthesis completed at a wavelength of 290 nm.

Estimation of Ca, N, P and K

The nitrogen level was assessed using a modified micro-Kjeldahl method, whereas the phosphorus concentration was quantified using a spectrophotometric technique. Flame photometry was employed to ascertain the amounts of potassium (K) and calcium (Ca) (Khan *et al.*, 2021).

Statistical analysis

The data reflects the average (± standard error) of four replicas. Statistical significance of the data was evaluated employing Duncan's Multiple Range Test, with the least significant difference (LSD) computed at a significance level of $p < 0.05$. The Pearson's correlation analysis and PCA analysis of the complete set of data was also performed by Origin pro 2022 pack at a significance level of $p <0.05$.

Results

The results demonstrating the impacts of drought stress, application of selenium (Se), potassium (K), and the synergistic effect on the height (PH) and dry mass (DW) of plants are presented in table 1. Compared to the untreated group, we observed an important reduction in the morphological parameters such as height and weight, with decreases of 38.94% and 40.81% respectively. When Se and K were used in combination, they caused the most notable increase in both parameters, resulting in a 51.19% increase in height and a 38.40% in mass respectively. When cultivated under normal circumstances, the addition of selenium (Se) and potassium (K) resulting considerable increase in both height and dry weight, in comparison to the control treatment. The treatment that included Se and K exhibited the most notable enhancement, with a 32.64% increase in height and a 32.32% increase in dry mass (Table 1).

Table 1. Effect of selenium (1 μM Na2SeO4) and potassium (3 mM K2O) individually and combinedly on the plant height, plant dry weight, nitrogen, phosphorous, potassium, calcium, total phenols, total flavonoids and activity of phenyl alanine ammonia lyase (PAL), in osmotic stressed the presented data is the mean (± standard error) of four independent samples, and any significant variations at a significance level of P < 0.05 are denoted by different letters

	Control	**Se**	**K**	**Se + K**	**PEG (15%)**	**PEG + Se**	**PEG + K**	**PEG + Se + K**
Plant height (cm)	24.6 ±1.31d	27.1 ±1.78c	29.51 ±1.98b	32.63 ±2.11a	15.02 ±1.00h	17.35 ±1.12g	18.83 ±1.17f	22.71 ±1.19e
Plant dry weight (gm plant^{-1})	1.454 ±0.06d	1.627 ±0.073c	1.793 ±0.82b	1.924 ±0.098a	0.8605 ±0.0025h	0.9323 ±0.0042g	0.9988 ±0.0072f	1.191 ±0.034e
Nitrogen (mg^{-1} DW)	30.23 ±2.2d	34.54 ±2.4c	38.98 ±3.2b	43.11 ±3.5a	15.81 ±1.01h	17.88 ±1.12g	24.34 ±2.1f	28.09 ±2.4e
Phosphorous (mg^{-1} DW)	16.4 ±1.01d	18.07 ±1.5c	21.28 ±1.8b	22.44 ±1.9a	7.08 ±0.56h	8.03 ±0.68g	11.33 ±0.87f	13.74 ±0.93e
Potassium (mg^{-1} DW)	20.13 ±1.3d	23.21 ±1.5c	29.29 ±2.3b	33.18 ±2.7a	10.38 ±0.92h	12.39 ±0.98g	15.97 ±1.2f	18.55 ±1.5e
Calcium (mg^{-1} DW)	13.09 ±0.89d	14.63 ±0.94c	16.58 ±1.1b	17.95 ±1.5a	6.01 ±0.28h	7.38 ±0.29g	8.98 ±0.32f	10.18 ±0.88e
Total phenol (mg^{-1} DW)	2.20 ±0.14f	2.65 ±0.16d	2.98 ±0.19c	3.31 ±0.201b	2.41 ±0.18e	3.09 ±0.19c	3.41 ±0.21b	3.76 ±0.23a
Total flavonoids (mg^{-1} DW)	1.019 ±0.071f	1.319 ±0.085d	1.559 ±0.088c	1.767 ±0.10b	1.219 ±0.082e	1.566 ±0.086c	1.809 ±0.12b	2.222 ±0.14a
PAL activity (nmol trans cinnamic acid min^{-1} mg^{-1} protein)	23.55 ±1.6f	35.43 ±2.4d	43.44 ±3.1c	57.99 ±3.8b	27.96 ±1.4e	44.48 ±3.3c	61.55 ±4.2b	72.33 ±4.8a

The stress induced by PEG resulted in a decrease in the K, Ca, P and N concentrations. Supplementation with Se and K resulted to an increase in the concentrations of diverse necessary nutrients. The total amount of Se increased by 14.25%, 10.18%, 15.30%, and 12.45% respectively, while the content of K increased by 28.94%, 29.75%, 45.50%, and 26.66% correspondingly. Upon combining Se and K, there was a substantial increase in content, specifically by 42.60%, 36.82%, 64.82%, and 37.12% accordingly. The application of specific components to young plants that experienced stress greatly diminished the decrease in several crucial nutrients.

The seedlings that received both selenium (Se) and potassium (K) treatments exhibited the highest percentages of relief: 77.67% for nitrogen (N), 94.06% for phosphorus (P), 78.70% for potassium (K), and 69.38% for calcium (Ca). This was in contrast to the seedlings that solely underwent drought stress (Table 1).

The drought stress led resulted in a notable increase in the accumulation of total phenols (9.54%) and total flavonoids (19.62%), as well as an elevation of 18.72% An increase in PAL activity, in comparison to the control, was noted. Furthermore, the effects were augmented by the application of Se and/or K (Table 1). The plants subjected to PEG + Se + K treatment displayed a significant increase in flavonoids, phenols, and PAL activity related to the control group. The phenols experienced a substantial increase of 70.90%, the flavonoids saw a considerable increase of 118.05%, and the PAL activity showed a remarkable increase of 207.13%. Under typical circumstances, the inclusion of selenium (Se) and potassium (K) significantly augmented the quantities of phenols, flavonoids, and PAL activity, as demonstrated in Table 1.

As shown in the Figure 1 that the influence of, selenium (Se), and potassium (K) treatment on the amounts of total chlorophyll and carotenoids in wheat plant under drought condition has been increased. The incorporation of Se resulted in an impressive spike of 23.79% in total chlorophyll and a significant rise of 9.00% in carotenoids related to the control. The addition of K resulted in a significant boost of 51.42% in total chlorophyll and a noteworthy rise of 15.60% in carotenoids related to the control. The addition of Se and K resulted in a substantial increase of 64.62 and 31.12% in the total chlorophyll and carotenoid contents, respectively, in comparison to the control. Application of PEG to create drought stress led to a notable decrease of 41.22% and 28.95% in total chlorophylls and carotenoids, respectively, related to the untreated control treatment. The seedlings that received treatment with PEG + Se + K exhibited the most significant enhancement, leading to a major improvement of 67.91% and 39.26% (Figure 1A and B). The levels of Pn, Ci, and gS showed a substantial rise of 22.34%, 20.65%, and 18.39% respectively due to the presence of Se, when compared to the control group. The percentages exhibited notable increments, with values of 50.03%, 28.70%, and 23.29% for one set of factors, and 67.96%, 42.59%, and 41.58% for another group of factors (Figure 2A and C). As a result of the drought, there was a substantial decline in photosynthesis rate (Pn), intercellular carbon dioxide concentration (Ci), and stomatal conductance (gS). However, the addition of selenium (Se) and potassium (K) supplements significantly decreased the decline (Figure 2A-C).

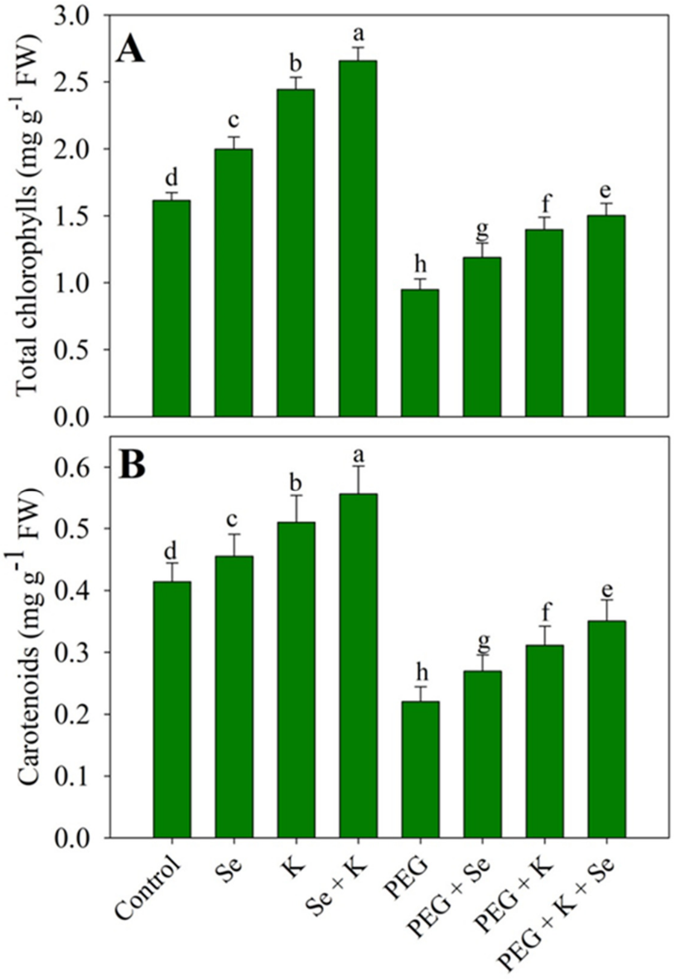

Figure 1. The levels of total chlorophyll and carotenoids in wheat (*Triticum aestivum* L) plants subjected to osmotic stress induced by PEG, with and without Se and K supplementation

The presented data is the mean (± standard error) of four independent samples, and any significant variations at a significance level of $P < 0.05$ are denoted by different letters.

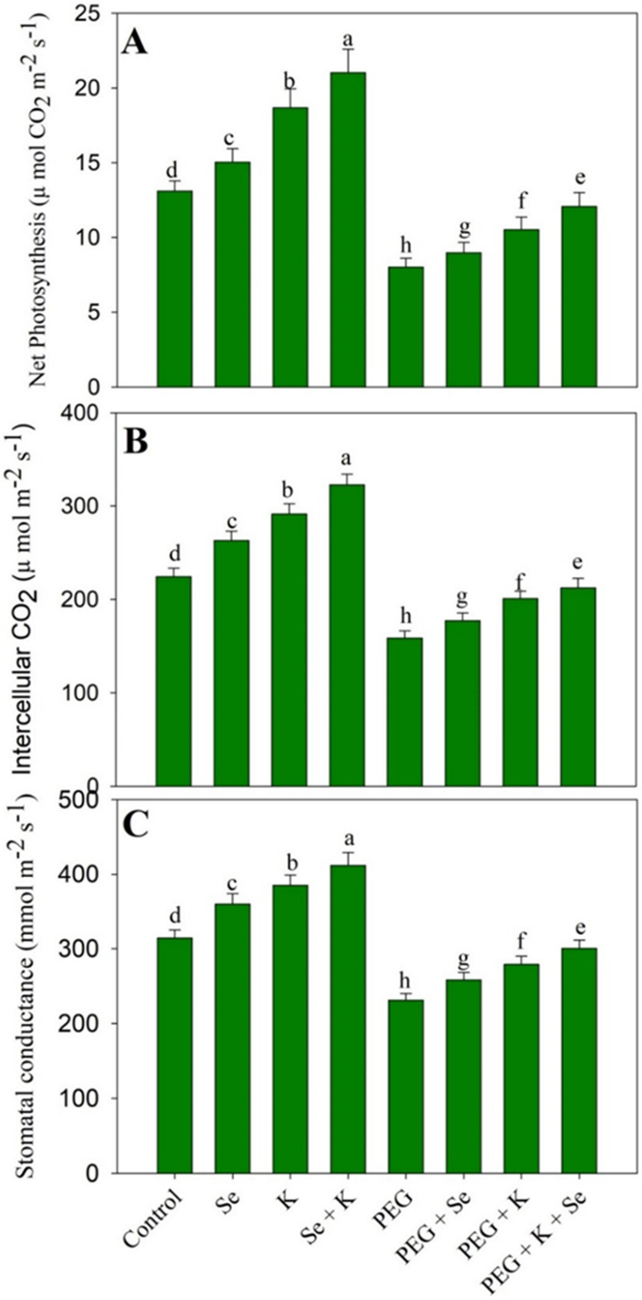

Figure 2. The net photosynthesis, intercellular CO_2 concentration, and stomatal conductance in wheat (*Triticum aestivum* L.) plants cultivated under PEG-induced osmotic stress, with and without Se and K supplementation

The presented data is the mean (± standard error) of four independent samples, and any significant variations at a significance level of $P < 0.05$ are denoted by different letters

Due to the effects of drought stress, an increase in H_2O_2 and O_2^- was observed. The concentrations of hydrogen peroxide (H_2O_2) and superoxide (O_2^-) exhibited a substantial rise of 185.55% and 192.55% respectively, compared to the control., as a result of the impact of drought stress. Administering either Selenium (Se) or Potassium (K), or a combination of both, resulted in a reduction in H_2O_2 and O_2^- concentrations. The combined supplementation of Se and K resulted in a substantial decrease, specifically 47.03% and 45.04% respectively. Upon the addition of Se and K to PEG-challenged seedlings, there was a notable reduction of 46.27% and 53.18% in H_2O_2 and O_2^- levels, respectively, compared to seedlings that were not supplemented in any way (Figure 3A and B). Following the application of Polyethylene glycol (PEG), there was a significant increase in lipid peroxidation (188.87%), protease activity (46.45%), and lipoxygenase activity (142.98%) in comparison to the untreated control plants (Figure 4). The addition of Selenium (Se) and Potassium (K) led to a significant reduction in protease, lipid peroxidation, and lipoxygenase activities. Application of Se and/or K to plants experiencing PEG-induced stress resulted in a notable reduction in lipid peroxidation, protease activity, and lipoxygenase levels. The decreases observed were 54.65%, 24.58%, and 48.99% correspondingly, in comparison to PEG stressed plants that did not receive Se + K supplementation (Figure 4).

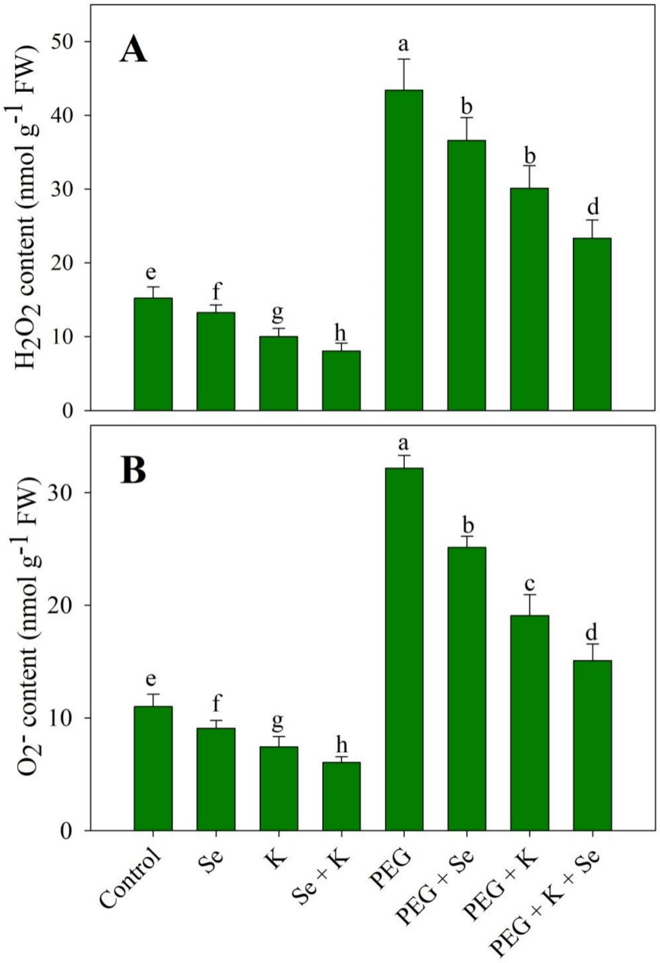

Figure 3. Hydrogen peroxide and superoxide contents in wheat (*Triticum aestivum* L) plants cultivated under PEG-induced osmotic stress, with and without Se and K supplementation

The presented data is the mean (± standard error) of four independent samples, and any significant variations at a significance level of $P < 0.05$ are denoted by different letters.

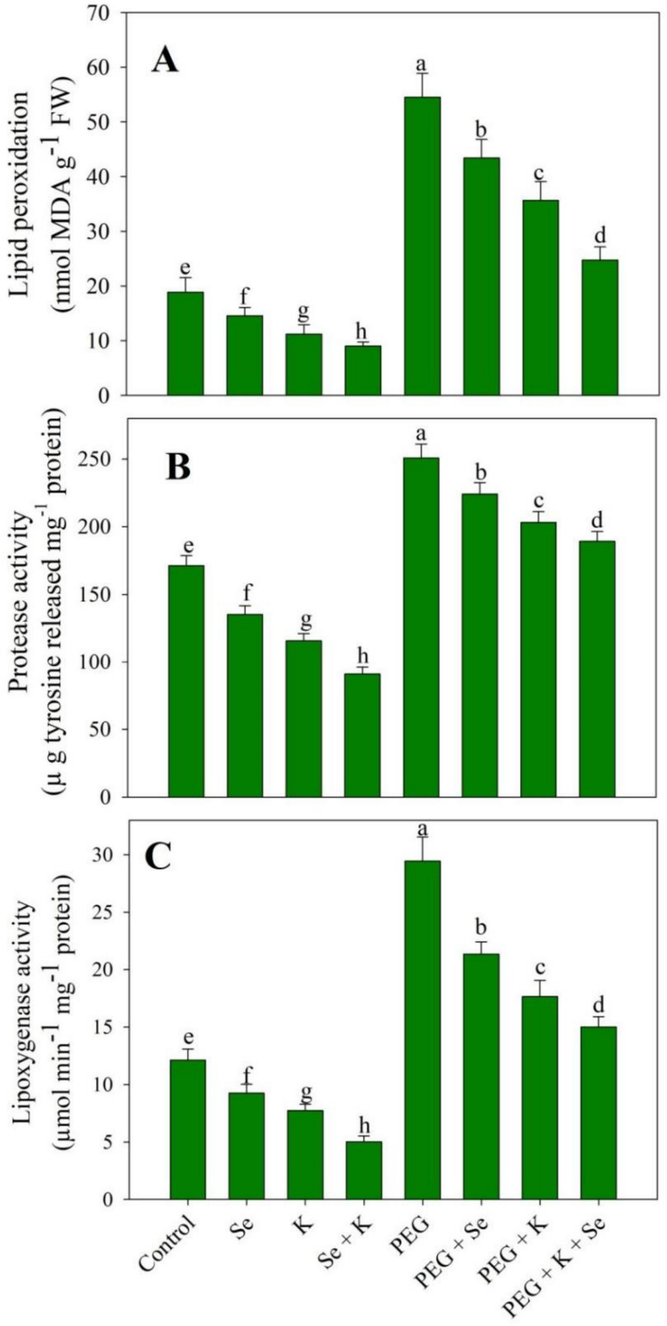

Figure 4. Lipid peroxidation, activity of protease and lipoxygenase in wheat (*Triticum aestivum* L) plants cultivated under PEG-induced osmotic stress, with and without Se and K supplementation

The presented data is the mean (± standard error) of four independent samples, and any significant variations at a significance level of $P < 0.05$ are denoted by different letters.

The inclusion of selenium (Se) and potassium (K), both individually moreover, when used together, there was a substantial enhancement in the efficacy of antioxidant enzymes. The plants subjected to Se + K treatment shown a significant increase of 178.73, 83.88, 94.26 and 85.40 percent were observed in SOD, CAT, APX, and GR respectively, in comparison to untreated control group. The levels of SOD, CAT, APX, and GR were seen to increase significantly by 38.93%, 19.60%, 22.92%, and 20.19% correspondingly, as a result of drought stress. When plants experiencing PEG-induced stress were supplemented with additional Selenium (Se) and/or Potassium (K), their activity levels were further augmented. Consequently, the levels of APX, SOD, GR and CAT showed a notable rise in comparison to the control group (Figure 5A-D). PEG-induced stress led to a considerable rise in 13.82% in AsA levels, 12.70% in GSH levels, and 23.84% in tocopherol levels. The addition of Selenium (Se) and Potassium (K) had a discernible positive effect, resulting in a substantial rise of 46.89%, 43.75%, and 90.72% in Ascorbic Acid (AsA), Glutathione (GSH), and tocopherol levels respectively, as compared to the control group. The plants that were exposed to a mixture of PEG, Se, and K showed a substantial increase in Ascorbic Acid (AsA) by 66.46%, Glutathione (GSH) by 53.70%, and tocopherol by 119.20% compared to the control group (Figure 6A-C).

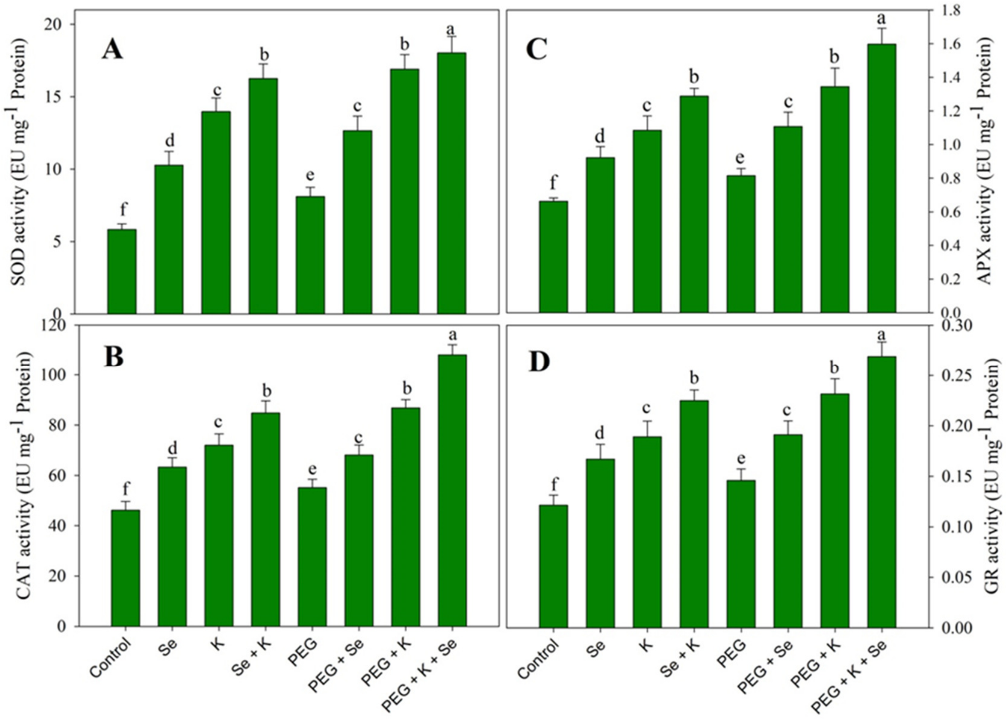

Figure 5. Activity of superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase in wheat (*Triticum aestivum* L) plants cultivated under PEG-induced osmotic stress, with and without Se and K supplementation

The presented data is the mean (± standard error) of four independent samples, and any significant variations at a significance level of P < 0.05 are denoted by different letters.

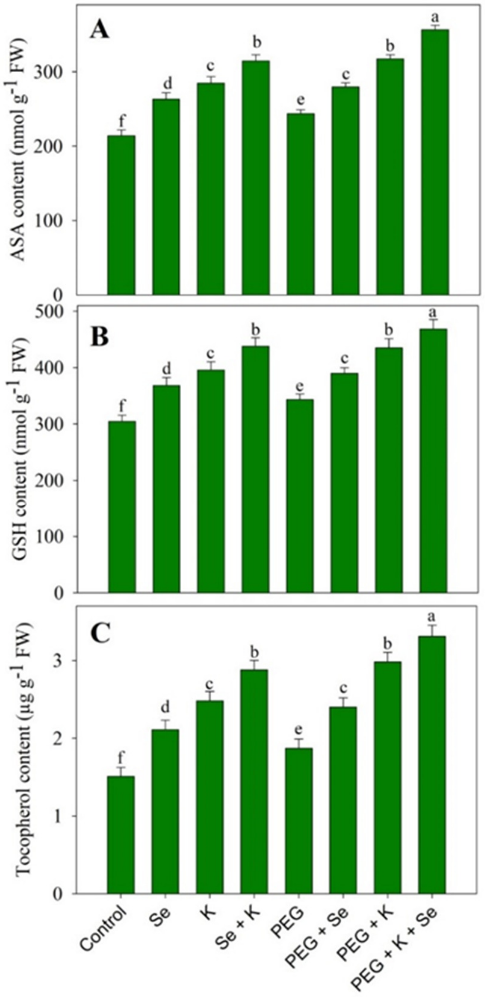

Figure 6. Content of ascorbic acid, reduced glutathione and tocopherol in wheat (*Triticum aestivum* L) plants cultivated under PEG-induced osmotic stress, with and without Se and K supplementation
The presented data is the mean (± standard error) of four independent samples, and any significant variations at a significance level of $P < 0.05$ are denoted by different letters.

The use of PEG treatment resulted in a considerable increase of 28.32% in proline levels, 24.66% in sugar levels, and 17.27% in glycine betaine levels under drought stress (Figure 7A-C). The levels of sugar, glycine betaine and proline in seedlings rose further when they were subjected to polyethylene glycol (PEG) stress, particularly in the presence of selenium (Se) and/or potassium (K). The observed rise in this study was really noteworthy. The levels of proline grew by 201.25%, sugars climbed by 84.17%, and glycine betaine increased by 109.25% compared to seedlings that were solely exposed to PEG stress (Figure 7A-C). Following PEG treatment, the γ-GK activity exhibited a 3.72% increase, whereas the PROX activity showed a 6.48% drop. The plants subjected to selenium (Se) and/or potassium (K) treatment exhibited a significant increase in the activity of γ-GK and a decrease in the activity of PROX. When comparing the control group, the activity of γ-GK exhibited a substantial rise of 87.08%, but the activity of PROX demonstrated a notable drop of 57.70% in plants that were treated with PEG + Se + K (Figure 8A and B).

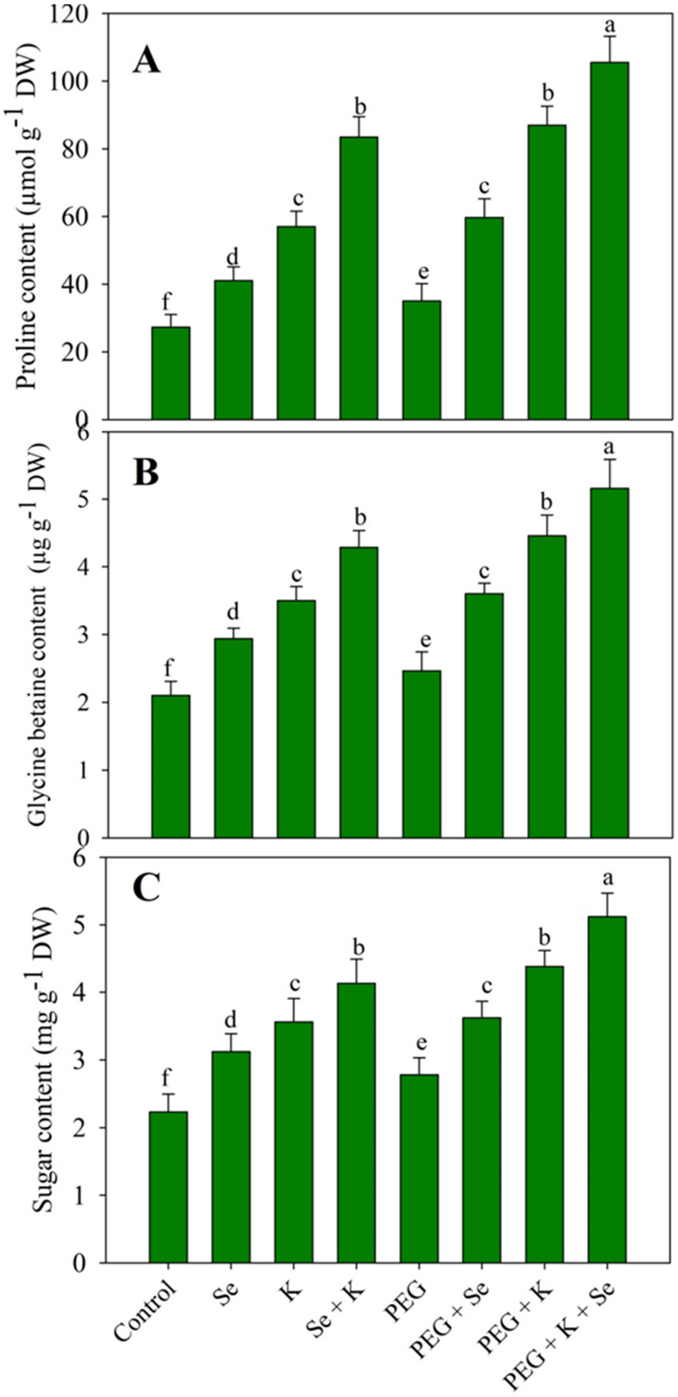

Figure 7. Content of proline, glycine betaine, sugars and free amino acids in wheat (*Triticum aestivum* L) plants cultivated under PEG-induced osmotic stress, with and without Se and K supplementation

The presented data is the mean (± standard error) of four independent samples, and any significant variations at a significance level of $P < 0.05$ are denoted by different letters.

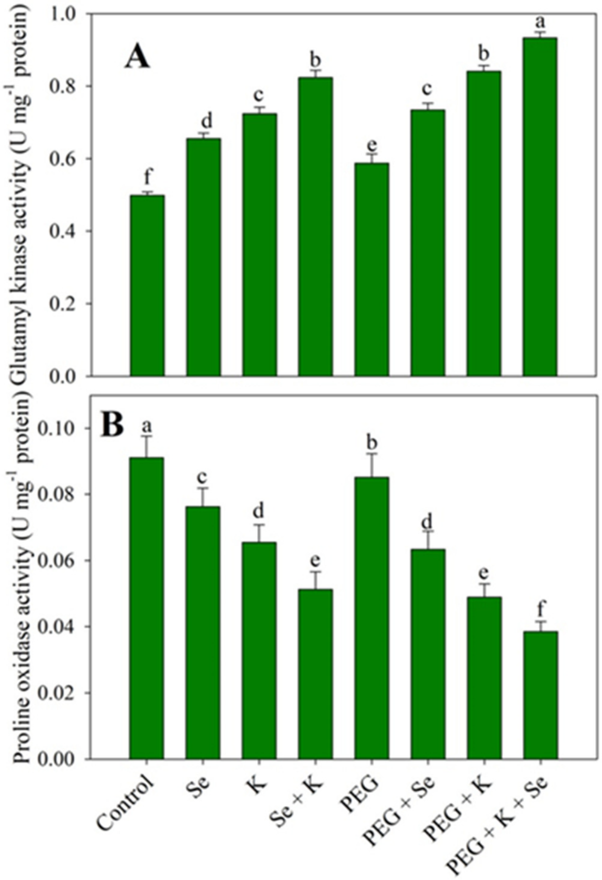

Figure 8. Activity of glutamyl kinase and proline oxidasein wheat (*Triticum aestivum* L) plants cultivated under PEG-induced osmotic stress, with and without Se and K supplementation

The presented data is the mean (± standard error) of four independent samples, and any significant variations at a significance level of $P < 0.05$ are denoted by different letters.

The activity of NR dropped by 48.24% as a result of PEG-induced drought stress, in comparison to the control. The addition of selenium (Se) and/or potassium (K) significantly boosted the activity of NR, leading to a substantial increase of 58.83% with the Se + K therapy compared to the control. The activities of NR in plants subjected to PEG + Se + K treatment were markedly diminished, exhibiting a decrease of 68.17% in comparison to untreated groups (Figure 9A-C).

The primary component Biplot analysis (PCA) indicated significant correlations between multiple variables under PEG-induced drought stress, as well as the potential for synergistic effects of K^+ and Se supplementation. The variables that showed a strong correlation were organized into quadrants. The biplot demonstrated a variation of 81.07%, with PC1 accounting for 49.37% and PC2 accounting for 31.71% (Figure 10). There were good relationships observed between the antioxidants, morphological traits, pigmented content, and appropriate solutes of wheat plants. The calculation of plant biomass was performed using Pearson's correlation analysis to compare antioxidants and biochemical properties (Figure 11). The presence of chlorophyll was observed to have a substantial positive impact on the height and biomass of plants. Enhancing these characteristics (Figure 8) leads to a substantial rise in the production of plant biomass. Strong associations were seen between stem length (SL), fruit weight (FW), and root length (RL) and the levels of carotenoids, relative water content (RWC), total soluble sugar, and chlorophyll content. The rise in biomass (stem length, root length, and fresh weight) and chlorophyll content resulted in heightened production of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), flavonoids, total soluble solids

(TSS), and proline. Figure 11 revealed a notable adverse correlation between H_2O_2 and O_2– as well as all plant biomass indicators. The correlation between total soluble sugars, antioxidants, total protein, O_2^-, antioxidant enzymes, and radical scavenging capacity, however, was found to be considerably negative. Reduced levels of antioxidants lead to a decrease in the amount of plant biomass under specific circumstances.

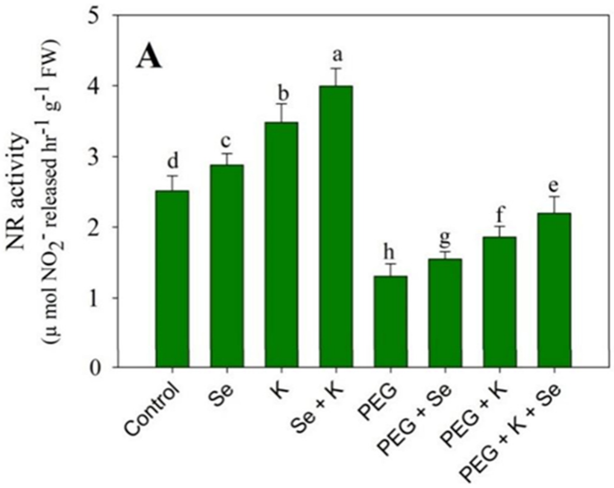

Figure 9. Activity of nitrate reductase in wheat (*Triticum aestivum* L) plants cultivated under PEG-induced osmotic stress, with and without Se and K supplementation

The presented data is the mean (± standard error) of four independent samples, and any significant variations at a significance level of $P < 0.05$ are denoted by different letters.

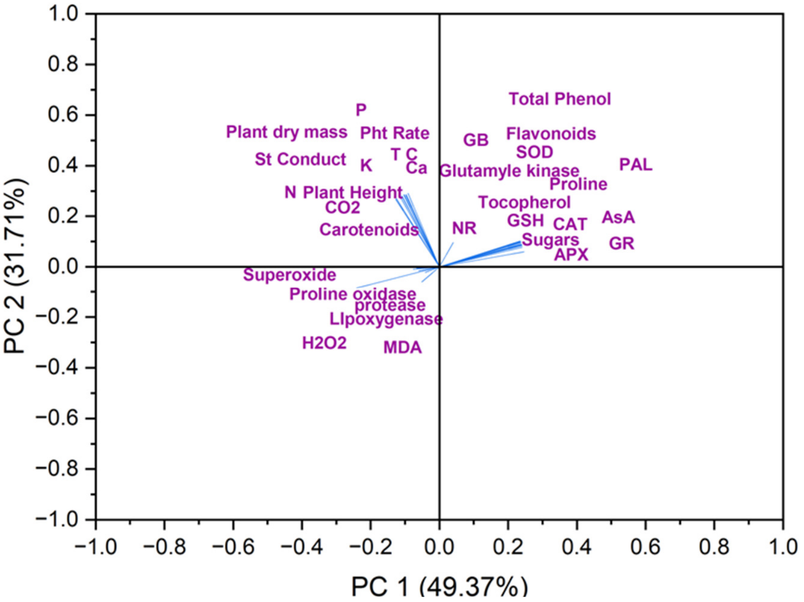

Figure 10. Shows the PCA analysis of different treatments in wheat (*Triticum aestivum* L) plants grown under PEG induced osmotic stress with and without Se and K supplementation

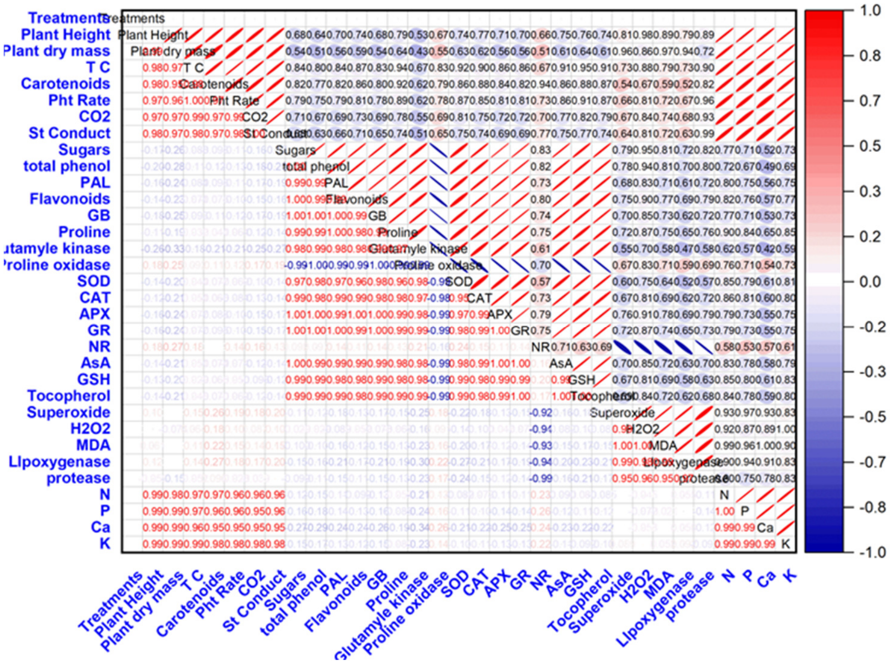

Figure 11. Shows the Pearson's correlation analysis of different treatments of in wheat (*Triticum aestivum* L) plants grown under PEG induced osmotic stress with and without Se and K supplementation

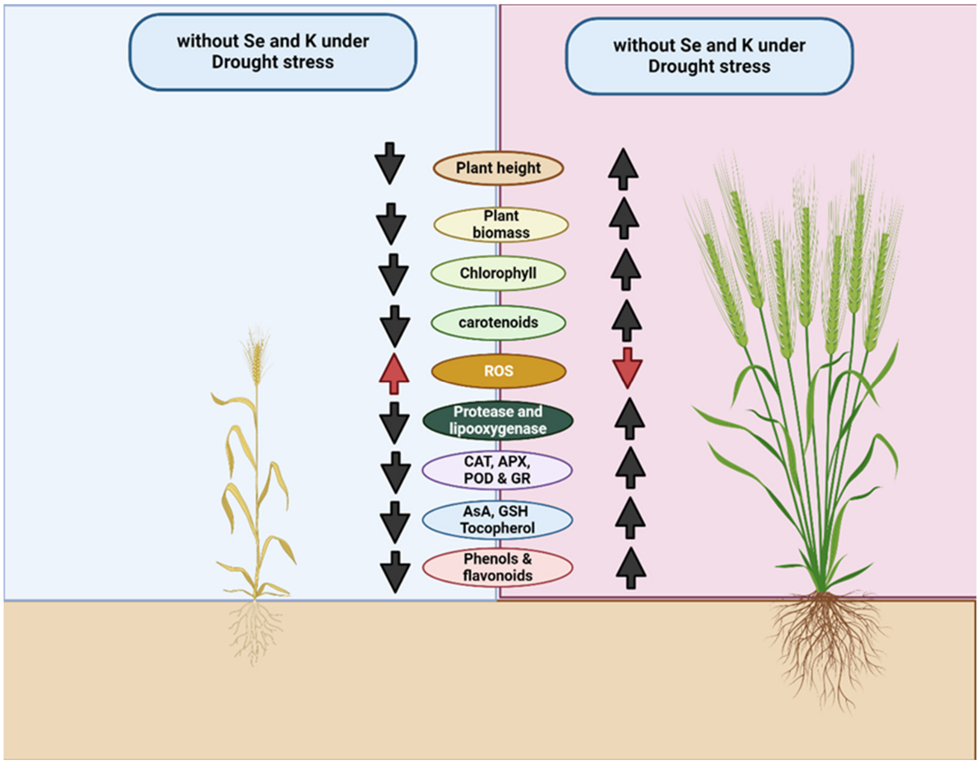

Figure 12. Shows the schematic diagram of the results of the whole experimental work

Discussion

Drought stress has become a critical global issue, threatening water availability and agricultural productivity. During the contemporary period, alterations in the global climate have led to significant changes in the distribution of rainfall. As a result, many regions around the world are currently facing severe water shortages. To mitigate the adverse effects of drought stress, various methods have been implemented, combining innovative management practices with biotechnological interventions. The present experiment investigated the impacts of separately investigating Selenium (Se) and Potassium (K), as well as their combined effects, under PED induced drought stress. The growth and biomass production underwent a substantial decline due to the effects of drought stress. Prior studies have also documented a decline in growth, encompassing both height and dry weight, as a result of water deprivation stress in various crops (Jatav *et al.*, 2014; Ghouri *et al.*, 2021). The decline in growth is attributed to the inhibition of the cell cycle caused by water scarcity, hence constraining the cell's ability to proliferate and reproduce (Schuppler *et al.*, 1998; Setter and Flannigan, 2001; Sutuliene *et al.*, 2022). Research conducted by Jatav *et al.* (2014) and Shrivastava *et al.* (2016) has shown that K supplementation can improved the plant growth attributes. Scientific research has demonstrated that the addition of Selenium (Se) has been proven to enhance the growth of wheat (Elkelish *et al.*, 2019) and garden cress (Khosravi *et al.*, 2022). Studies have demonstrated that the inclusion of specific components such as potassium (K) and selenium (Se) can reduce the harmful effects of water on the growth of plants and the generation of dry mass (Ahanger *et al.*, 2015; Yao *et al.*, 2009). The enhanced growth and subsequent reduction of decline caused by water deficiency can be ascribed to the increased preservation of tissue water content, chlorophyll synthesis, and photosynthesis, which greatly influences the metabolism. The addition of K and Se resulted in a substantial increase in the concentrations of chlorophyll and carotenoids. This phenomenon was particularly pronounced in plants that got both nutrients concurrently. Drought stress impaired the photosynthetic pigments, as well as the process of photosynthesis. Dalal and Tripathi (2012) conducted study which found that stressors can impede pigment synthesis by influencing the activity of enzymes involved in chlorophyll formation. The introduction of potassium (K) and selenium (Se) may have had a beneficial impact on the enzymes involved in the synthesis of chlorophyll. This could have resulted in an elevation of chlorophyll concentrations and a significant alleviation of the negative impacts of drought. In addition, the utilization of potassium (K) and selenium (Se) resulted in an increase in photosynthesis rate, cellular CO2, and stomatal conductance. Prior studies have demonstrated that the utilisation of potassium (K) and selenium (Se) can augment the concentrations of chlorophyll and carotenoids. Drought can have a substantial impact on plant photosynthesis by affecting different elements, including the production of chlorophyll, the functioning of PSII, and the stability of the thylakoid D1 protein (Batra *et al.*, 2014). A study conducted by Abid *et al.* (2018) shown that drought stress leads to a substantial decrease in photosynthesis and stomatal conductance in wheat plants. During difficult conditions, the efficiency of photosynthesis decreases, leading to a decrease in the activity of Rubisco and a considerable drop in nitrogen uptake (Khan *et al.*, 2015; Xu *et al.*, 2020b).

PEG application was also found associated in reducing the enzymatic activity, nitrogen metabolism, resulting in a noticeable increase in the absorption and utilisation of nitrogen under drought stress. Nevertheless, the plants that were exposed to Se and/or K^+ exhibited a noteworthy rise in the activity of NR, indicating a rapid uptake of the accumulated nitrogen and so facilitating the conversion of nitrogen into amino acids (Shrivastava *et al.*, 2016; Ahanger *et al.*, 2017b). Enzymes in the Nitrogen assimilatory pathway in plants play a crucial function, particularly in catalysing the step that limits the rate of the process (Shrivastava *et al.*, 2016). Dellero (2020) suggested that enhanced nitrogen absorption and assimilation directly affect nitrogen utilisation efficiency. The drought-induced reduction in enzyme activity directly affects N assimilation, which is strongly associated with decreased N absorption (Ahanger *et al.*, 2017b). The addition of selenium (Se) and potassium (K), either separately or in combination, had a significant effect on the uptake of nitrogen (N) and the activity of assimilatory enzymes. This had a significant positive impact on mitigating the adverse consequences of drought. A study conducted by Khosravi et al. in 2022 revealed that the presence of Selenium (Se) greatly increased the enzymatic activity of Nitrate Reductase (NR) in garden cress. Prior studies have documented enhanced nitrogen uptake and utilisation resulting from the inclusion of potassium (K) supplements (Ahanger *et al.*, 2017b; Xu *et al.*, 2020a). Xu *et al.* (2020a) found that the utilisation of K improves the performance of transporters involved in the uptake of N. The adequate presence of nitrogen has been discovered to have a vital impact on several biological processes, including photosynthesis, enzyme function, and stress tolerance (Iqbal *et al.*, 2015). Studies have demonstrated that a decrease in the absorption of nitrogen significantly impacts the functioning of Rubisco and causes changes in the characteristics of photosynthesis, both in terms of stomatal and non-stomatal aspects (Iqbal *et al.*, 2015). The presence of selenium (Se) and/or potassium (K) in the mixture can significantly enhance the absorption and utilisation of nitrogen (N), guaranteeing an ample supply of N for the synthesis of Rubisco, optimal functioning of enzymes, defence against light-induced damage, and the development of stress-resistant amino acids.

Furthermore, the utilisation of Se and/or K led to a notable increase in the synthesis of suitable solutes such as proline, sugars, and glycine betaine, as well as an acceleration in nitrogen metabolism. Plants can protect themselves from harmful effects of stresses by ensuring they have an adequate amount of osmolytes. Chen and Murata (2011), noted that these osmolytes exert both particular and wide-ranging impacts on the plant. The excessive accumulation of sugar impedes the activity of Rubisco (Sivakumar *et al.*, 2002) and controls crucial biological processes including as flowering, germination, delayed senescence and photosynthesis. Furthermore, research has demonstrated that it can influence hormone levels, hence enhancing an individual's capacity to manage stress (Sami et al., 2016). Glycine betaine is essential for safeguarding the structure and function of large molecules, maintaining the integrity of cell membranes, and assisting in the removal of ROS (Giri, 2011; Annunziata *et al.*, 2019). Specific chemicals, such as sugars, can impact the signalling pathways in plants. These substances can interact with different channels to create a complex network that regulates plant responses (Chinnusamy *et al.*, 2004; Rosa *et al.*, 2009). Sivakumar *et al.* (2000) found that Proline can efficiently inhibit the oxygenase activity of Rubisco in plants experiencing stress. Furthermore, it acts as a scavenger for reactive oxygen species (ROS), enhances the antioxidant system, and protects important macromolecules such as enzymes (Hayat *et al.*, 2012; Ahanger *et al.*, 2014; Meena *et al.*, 2019; Ghosh *et al.*, 2022). This study suggests that the enhanced accumulation of osmolytes in plants exposed to selenium (Se) and/or potassium (K) may have played a role in reducing stress by eliminating excessive reactive oxygen species (ROS), safeguarding photosynthesis, and maintaining optimal enzyme activity. Elkelish *et al.* (2019) and Alyemeni *et al.* (2018) have previously shown that the inclusion of selenium (Se) and potassium (K) can enhance the levels of osmolytes in various crop plants.

Furthermore, Ahmad et al. (2014), Ahanger and Agarwal (2017a, b), and Shrivastava et al. (2016) have also found that the use of Se and K treatment has positive effects on the osmolyte content in agricultural plants. However, the interaction effects have not been detected yet and so require additional investigation. The enhanced buildup of proline, resulting from the addition of selenium and/or potassium, was attributed the regulation of enzymes attributed to its breaking down and synthesize. The presence of either Se or K had a notable influence on the activity of γ-GK, significantly augmenting it, whereas PROX was suppressed. Consequently, these organisms exhibited an increased buildup of proline. The regulation of proline buildup is achieved by modifying the activity of enzymes involved in its degradation, as demonstrated by the investigations carried out by Khan *et al.* (2015) and Elkelish *et al.* (2019). Nevertheless, the precise mechanisms have not yet been disclosed.

Applying Se and/or K substantially decreased the generation of reactive oxygen species (ROS), such as H_2O_2 and O_{2-}, hence mitigating the elevated lipid peroxidation induced by dryness. Prior studies have demonstrated that *Oryza sativa, Solanum lycopersicum, Beta vulgaris,* and *Pisum sativum exhibit* heightened generation of reactive oxygen species (ROS) when subjected to water deprivation. As a result, there is an increase in the oxidation of lipids in the cell membrane, which subsequently impacts their overall structure and functional stability. Plants subjected to selenium (Se) and/or potassium (K) treatment exhibit a reduction in the buildup of reactive oxygen species (ROS), indicating that Se and K have a protective function in mitigating oxidative harm induced by dryness. It is crucial to prevent the accumulation of reactive oxygen species (ROS) in order to preserve the integrity and functionality of the membrane, as well as safeguard vital metabolic processes such as photosynthesis. Plants that show decreased buildup of ROS indicate improved structural and functional integrity of their photosynthetic apparatus (Fatma *et al*., 2016). Recent research has shown that the use of selenium (Se) in water-stressed cucumber and potassium (K) in barley has effectively decreased the generation of ROS, resulting in improved membrane integrity. Upon the application of selenium (Se) and/or potassium (K) to plants, a notable reduction in oxidative damage was observed. This decline was associated with a significant drop in the activity of lipoxygenase and protease enzymes. The occurrence of dryness increased the function of lipoxygenase and protease, both of which were notably suppressed in plants subjected to selenium (Se) and/or potassium (K). Environmental conditions can enhance the activity of specific enzymes, leading to an intensified oxidative effect. Lipoxygenases, a type of enzyme, are involved in the process of oxidising polyunsaturated fatty acids, which can have an impact on the stability of cell membranes (Shrestha *et al*., 2021). Zhang *et al*. (2021) conducted a recent study that revealed the impact of salinity and drought on the expression of lipoxygenase genes in foxtail plants. The study demonstrated that these two factors influence the genes in different manners. These findings indicate that these genes have a pivotal function in adapting to stressful circumstances. Proteases possess the capacity to degrade defective proteins inside cells. Proteases play a crucial role in safeguarding cells against the buildup of broken down, impaired, and unfolded proteins that arise from reactive oxygen species (ROS) induced by stress. Furthermore, they contribute to the liberation of amino acids and the synthesis of substances that facilitate the process of transmitting signals.

Plants bolster their innate antioxidant defence mechanism to counteract the detrimental effects of oxidative repercussions caused by stress. The antioxidant system plays a crucial role in protecting important macromolecules and metabolic pathways from damage induced by an excess of reactive oxygen species (ROS). In the current investigation, the inclusion of Se and/or K resulted in a significant enhancement in the efficiency of antioxidant enzymes and the levels of non-enzymatic antioxidants. Antioxidants possess the ability to specifically target and neutralise particular reactive oxygen species (ROS). For example, SOD helps to remove superoxide radicals, while CAT or the ascorbate-glutathione (AsA-GSH) cycle can neutralise hydrogen peroxide. The inclusion of selenium (Se) and/or potassium (K) led to an augmentation of the activity of the examined antioxidant enzymes, along with an elevation in the concentrations of non-enzymatic antioxidants. This behaviour was most noticeable when the simultaneous application of both Selenium (Se) and Potassium (K) took place.

Prior research has demonstrated that the individual administration of selenium (Se) and potassium (K) can augment the effectiveness of antioxidant enzymes, leading to enhanced defence mechanisms in plants against oxidative harm inflicted by stress on their membranes and photosynthetic system. Studies have demonstrated that incorporating selenium (Se) into cucumber plants could potentially alleviate the negative impacts of water scarcity. This is accomplished by enhancing the efficiency of the antioxidant system, which safeguards crucial macromolecular structures from harm (Jozwiak and Politycka, 2019). A recent study by Bahar *et al*. (2021) has shown a significant discovery concerning the improvement of photosynthetic processes and potato yields by employing potassium (K). This is accomplished by enhancing the activity of antioxidant enzymes. Moreover, the increased concentrations of AsA, GSH, and tocopherol in plants treated with Se and/or K demonstrate their significant role in bolstering the plant's defence mechanisms against the harmful effects of drought stress. The AsA, GSH, and tocopherol play a vital role in neutralising dangerous reactive oxygen species (ROS), maintaining a balanced redox state, safeguarding enzymes, and acting as electron donors in the enzymatic elimination of ROS (Ahanger *et al*., 2018). Tocopherol in chloroplasts has a vital function in counteracting the harmful effects of reactive oxygen species (ROS), such as singlet oxygen ($1O_2$) and hydroxyl radical (OH). Moreover, it has a vital function in preventing the spread of lipid peroxidation by efficiently eliminating the lipid peroxyl radical found in the thylakoid membranes (Munné-Bosch, 2005). Tocopherol exhibits the capacity to form coordination complexes with various antioxidants and can also engage with phytohormones such as ethylene, salicylic acid, jasmonic acid, and other similar compounds. This interaction elicits a stress response, which subsequently facilitates a more efficient mitigation of detrimental consequences (Hasanuzzaman *et al*., 2014).

Ascorbic acid (AsA) provides protection to organelles and cells against reactive oxygen species (ROS). Additionally, it exerts a substantial influence on the control of cellular division and growth, functioning as an essential coenzyme for enzymes. Moreover, it serves a pivotal role in protecting photosynthesis, producing hormones, and restoring antioxidants. GSH plays a crucial role in protecting the cell membrane by preserving α-tocopherol and zeaxanthin in their reduced states. Furthermore, it has a pivotal role in regulating the activity of essential enzymes involved in the AsA-GSH cycle and glyoxylase cycle, hence mitigating the harmful effects of oxidative stress (Hasanuzzaman *et al*., 2017). The addition of selenium (Se) and/or potassium (K) supplements may have enhanced the efficiency of the antioxidant system, resulting in improved growth, photosynthesis, and enzyme protection. Consequently, this may have contributed to a reduction in stress levels.

The plants exposed to selenium (Se) and/or potassium (K) exhibited enhanced strategies to endure the harmful consequences of drought. This was achieved by significantly increasing the production of phenols and flavonoids. Phenolic compounds have a vital role in essential biological activities such as cell division, photosynthesis, hormone regulation, and reproduction. The significance of these processes has been emphasised in multiple research (Ahmad *et al*., 2018; Sharma *et al*., 2019a, b; Samec *et al*., 2021; Jan *et al*., 2021). Plants exhibit an augmented synthesis of polyphenols, such as phenolics and flavonoids, in response to stress. They can mitigate the adverse effects of stress by regulating reactive oxygen species (ROS) and preventing photoinhibition. (Sharma *et al*., 2019b; Bistgani *et al*., 2019; Ahanger *et al*., 2019 a,b; Li *et al*., 2021). Prior studies have demonstrated that increased concentrations of phenols and flavonoids can mitigate the detrimental impact of stress on metabolic pathways, particularly photosynthesis, resulting in enhanced growth (Ahmad *et al*., 2018; Ahanger *et al*., 2021; Soliman et al., 2019, 2020). Studies have shown that plants with higher amounts of flavonoids display improved ability to withstand stress due to hormonal processes and the control of important pathways (Jan *et al*., 2021). Secondary metabolites are activated when the antioxidant defence system becomes ineffective in removing ROS (Fini *et al*., 2011; Ahmad *et al*., 2018; Ahanger *et al*., 2018). The increased accumulation of secondary compounds in plants is mainly ascribed to the augmentation of enzyme activities, as evidenced by Sharma *et al*. (2016, 2019b). In the current investigation, the incorporation of selenium (Se) and potassium (K) resulted in a noteworthy enhancement in the activity of phenylalanine ammonia-lyase (PAL), leading to an augmented synthesis of secondary metabolites. Plants that have higher concentrations of secondary metabolites have a notable enhancement in their total antioxidant activity (Ahanger and Agarwal, 2017a, b).

Conclusions

The occurrence of drought had a detrimental effect on the development and process of photosynthesis in wheat. It caused harm to the membranes through oxidative damage and also reduced the absorption and incorporation of minerals. The addition of selenium (Se) and/or potassium (K) has been shown to improve growth and mineral absorption by boosting the activation of tolerance mechanisms. These strategies encompass bolstering the antioxidant system, augmenting the formation of osmolytes, and facilitating the accumulation of secondary metabolites. Upon the addition of selenium (Se) and potassium (K), there was a noticeable decrease in lipid peroxidation, protease activity, and lipoxygenase activity. Furthermore, there was a notable enhancement in nitrogen (N) metabolism. Additional inquiry is necessary in order to comprehensively comprehend the effects at the molecular level.

Authors' Contributions

The author read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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