Exogenously Applied Potassium Enhanced Morpho-Physiological Growth and Drought Tolerance of Wheat by Alleviating Osmotic Imbalance and Oxidative Damage

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Abstract

Water availability is the most important aspect of plant growth and development, which has an impact on crop yield and productivity. Plants respond to water shortages by altering their physiology, biochemistry, and osmotic balance. This study aimed to evaluate the role of K applications on wheat performance under water stress. The study was comprised of five levels of potassium applications, i) NK = control-no K applied, ii) WA = water applications at the heading stage (50 DAS), iii) K₅₀ = 50 mg L⁻¹, iv) K₁₀₀ = 100 mg L⁻¹, and v) K₁₅₀ = 150 mg L⁻¹, and two levels of water status viz. i) WS₀ = Control-no Water stress and ii) WS₁ = Water stress (50 DAS). Results revealed that plant height, leaf area, and dry weights were decreased by 29.3, 20.8 and 23.3%, respectively, under water stress due to the reduction in the photosynthetic pigments in plants. Alternatively, cell membrane injuries were increased along with the overproduction of malondialdehyde and hydrogen peroxide. However,

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the production of metabolites (soluble proteins and phenolics), and antioxidant enzymes (superoxide dismutase, catalase, protease, and peroxidase) regulate plant defense. The K supplementation ameliorated the harmful effect of water stress as proved by improving plant growth attributes, leaf area and dry weight, and chlorophyll contents. In addition, the production of antioxidant enzymes and metabolites contents were increased under K applications which improved stomatal conductance and photosynthetic rate by 38.4 and 12.8%, respectively resulting in the grain yield increase of 25.3%. Among the different K levels, K applied at 150 mg L⁻¹ was the most effective as compared to 50 mg L⁻¹ and 100 mg L⁻¹, which increased the seed yield by 29.5% as compared to the control. In crux, K applications have an important role to impart water stress tolerance in wheat plants based on the improvements in plant physicochemical activities.

**Keywords:** antioxidant defense, cell membrane stability, lipid peroxidation, potassium applications, water stress tolerance

**Introduction**

One of the main abiotic stresses that has a negative impact on morpho-physiological attributes in plants is the unavailability of water leading to water stress [1]. The food security of millions of people who depend on wheat and other staple crops is seriously jeopardized under water-limited conditions that ultimately showed significant yield reductions [2]. Water deficit may affect plant morphological development, leading to reduce grain yield and yield components in wheat plants [3]. Water stress affects the metabolic pathways of plants by adversely affecting photosynthetic pigments or decreasing production. Wasaya et al. [4] have revealed that water stress led to decrease carbon assimilation, gaseous exchange, plant water status, grain weight, and grain yield in wheat genotypes. Drought also adversely affects plant growth due to loss of turgor, the xylem's ability to provide water to nearby cells is compromised [5]. Nawaz et al. [6] revealed the negative impacts of drought stress, including cell division, transpiration rate, reduced nutrient uptake, and photosynthetic rate of plants. Water stress may induce oxidative damage due to the over-accumulation of oxygen radicals [7] that may lead to reduce photosynthetic efficiency, cell division, seed germination, growth, stem elongation, root proliferation, assimilate translocation [8] water status, and gaseous exchange [9], causing the sterility of pollen grains [10] and yield reduction. The severity of water stress depends upon the duration of the unavailability of water, crop vulnerability during specific developmental stages, and plant species. Water deficit stress at the post-anthesis stage significantly reduced the wheat yield [11]. Therefore, it is an urgent need to increase crop productivity for sustaining food security by minimizing the detrimental effects of drought stress.

It has been reported earlier that the mineral-nutrient status of plants plays a critical role in increasing plant resistance to drought stress [12]. The K uptake in plants is inhibited due to the lower diffusion rate of K in soil under drought-stress conditions [13]. Hence, sufficient availability of K in growth media is very expedient to maintain proper growth under deficit stress.

Potassium (K) is an essential nutrient that is required by plants to complete their life cycle and is the most abundant cation in the plant kingdom [12]. The role of K in the plant developmental process is well known. Potassium has an important role in photosynthesis, phloem transport, stomatal regulation, protein synthesis, enzyme activation, and stress resistance [13] due to the osmotic adjustment, maintenance of cell turgidity, and aquaporins [14]. It helps the plants to generate a solute gradient potential in the cells that helps to uphold the turgidity and increases the cell growth through osmotic adjustment that improves the water stress [15, 16]. Root development and K diffusion rate from soil towards roots are constrained during water stress which ultimately limits K acquisition [14]. Römheld and Kirkby [17] stated that under water deficit conditions, exogenous application of K enhances the root growth and root surface area which ultimately increases the water uptake by the plant. Moreover, plants obtaining K resulted in high leaf water and turgor potential and lower osmotic potential under drought conditions [18, 19]. Potassium supplementation may upregulate plant water status and gas exchange parameters that to improve crop yield [20, 21].

Although the impact of water stress on wheat has long been discussed, however, the reports on the effect of potassium supplementation to reduce the oxidative stress caused by water stress based on plant physiology, metabolites, and antioxidant enzymes in wheat need to be explored. This study was done to determine whether K can mitigate the negative effects of water stress based on morpho-physiological characteristics and grain yield of wheat plants. It was hypothesized that foliar application of K improves the drought resistance in wheat by improving photosynthetic rate, antioxidant potential, and osmotic adjustment.

**Experimental**

A wire-house study was laid out to assess the response of K supplementation on the performance of wheat under a water-limited environment. The wheat variety (Glaxay-2013) was used, and the
sowing was done in plastic pots of size (25 × 30 cm) which were filled with silica-sand (6 kg/pot) during the Rabi season (2018-19) in wire-house at the Department of Agronomy, University of Agriculture, Faisalabad. Firstly, the sand was dried in direct sunlight, and sieved to avoid any other impurities like stones, or any plant parts remaining. The field capacity (FC) of the sand was measured according to the gravimetric method.

To measure FC, 100g of sand was saturated by adding 25 ml of water to reach the saturation point. The saturated sand was oven-dried at (90°C) for four days till it attains constant weight and calculated the FC. In each pot, 12 seeds of wheat were sown in the sand at adequate depth for better germination and only eight seedlings were maintained by thinning 15 DAS at 5 leaf stage [22, 23]. Plant nutrition was fulfilled by applying seedlings were maintained by thinning 15 DAS at 5 leaf stage [22, 23]. Plant nutrition was fulfilled by applying a full-strength Hoagland’s solution in the pot along with stage [22, 23]. Water stress was applied at inflorescence emergence [22, 23]. Water stress was maintained in 15 pots) and the other half-grown with normal watering (100% FC was maintained in 15 pots) until maturity. Field capacity was maintained by weighing each pot, and water was added on a weight basis twice a day.

Observations

Leaf Pigments

Wheat samples were used to measure the amount of chlorophyll using the Arnon [24] and Davies [25] techniques. For this calculation, 0.5 g of fresh leaves of wheat were ground into pieces of 0.5 (cm). The extract was made at a temperature of 10°C overnight by using 5 mL acetone solution (80%). The extract was centrifuged for five minutes at 14000 × g. Spectrophotometer was used to measure the absorbance at 645, 652, and 663 nm.

Water Relation Parameters

The relative water content (RWC) was measured following the mechanism given by Ahmad et al. [26], while leaf water potential (LWP) was measured by collecting the leaf samples randomly from each treatment. Leaves were plucked with a pair of scissors in the early morning before high sunshine to decrease the chance of excess water loss. The fully matured top 3rd leaf from every plant in each biological was taken for measurement. The water potential of the leaf was measured by the pressure bomb chamber apparatus used for leaf water potential. Leaf samples for osmotic potential were kept at -20°C in the freezer for 15 days. After 15 days leaves were defrosted, and crushed by a glass rod. Sap extracted from the leave was sucked by a syringe and kept in Eppendorf tubes. Samples were directly run in Cryoscopic Osmometer to determine the leaf osmotic potential of wheat samples (Wescor 5500). After getting leaf water and osmotic potential, the pressure potential (ψp) was calculated using Equation (1):

\[ ψp = ψw – ψs \] (1)

The RWC in leaf was measured by taking three healthy leaves from each treatment and fresh weight (FW) was taken with digital balance (Shimadzu-AW-320, Kyoto, Japan). The test tubes were filled with distilled water and soaked the samples. After 24 hours, the samples got out of test tubes, and water was wiped off using tissue paper to get soaked/turgid weight (TW). The samples were dried in an oven for almost 72 h at 65°C to get the dry weight (DW). Then the RWC was measured according to the procedure defined by Mullan and Pietragalla [27].

Gas Exchange Parameters

The samples for the gas exchange attributes were taken from 9:00 am to 11:00 am in direct sunlight, at the reproductive growth stage (61 DAS). Gas exchange attributes including transpiration rate (Tr), stomatal conductance (Gs), photosynthetic rate (Pn), and intercellular CO₂ concentration (Ci) were recorded by using an infrared gas analyzer (CI-340 portable, Hoddesdon, England) as followed from Ahmad et al. [26].

Osmolytes and Metabolites

The Folin Ciocalteu method determines phenolic content from the extraction of wheat leaf as defined by Singleton and Rossi [28] at 62 DAS. The wheat samples (200 µL) were kept in test tubes and then 1.0 mL of Folin Ciocalteu reagent and 7.5% of sodium carbonate were mixed gently to keep them for 30 minutes. Perkin-Elmer λ15 UV-vis spectrophotometer, Norwalk, CT at 700 nm was used to note the absorbance. Lowry et al. [29] described the method to measure total soluble proteins (TSP). The 1 mL of wheat leaf extract was taken in a test tube from each biological replicate, while the blank contained 1 mL of phosphate buffer and alkaline solution (1 mL) was added to each test tube at pH 7.0, and mixed
thoroughly with all the reagents, and permitted to keep at room temperature for 10 minutes. The 0.5 mL of Folin Phenol reagent was added, thoroughly mixed, and kept for incubation for 30 minutes at room temperature. Spectrophotometer (Hitachi, 220, Japan) was used to measure the absorbance at 620 nm.

**Oxidative Metabolism**

The sampling was done at 62 DAS for the measurement of oxidative metabolism including stress indicators, malondialdehyde (MDA) content, hydrogen peroxide (H$_2$O$_2$) activity, electrolyte leakage, and membrane stability, while antioxidants activity was also measured in response to these stress indicators. Among the antioxidants, superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) were also measured. The MDA content was assessed using the technique of Dhindsa et al. [30] by using thiobarbituric acid (TBA) which indicates membrane damage. In a glass test tube, the plant extract (0.5 mL) was gently mixed with 5% TCA (4 mL) having 5% TBA, and it was heated for 30 minutes at 95°C and the reaction was completed on ice. The centrifugation was done for 10 min at 12,000g and observed the absorbance at 532 and 660 nm by using a spectrophotometer (Hitachi, 220, Japan).

Potassium iodide was used to determine H$_2$O$_2$ content by following the method of Velikova et al. [31]. Precisely, 500 μL of supernatant was thoroughly mixed with 10 mM K$_2$HPO$_4$ buffer (pH 7.0; 500 μL), then added the 1 M of KI solution (1 mL), and the absorbance was examined in the reaction mixture at 390 nm. An analytical grade H$_2$O$_2$ standard curve (Sigma-Aldrich) was used to calculate the H$_2$O$_2$ content. To determine cell membrane injury and cell membrane stability, the electrical conductivity of wheat leaves leachates was measured at room temperature after soaking in the distilled water overnight at 100°C [32]. The sample leaves were cut into pieces in the tubes and filled with 10 mL distilled water in two groups, one of them kept at room temperature overnight. The second set was placed at 100°C in a water bath for 15 minutes, and the electric conductivity of C$_1$ and C$_2$ were measured. The CMS and CMI were calculated by using equations 2 and 3 given below:

\[
\text{Cell membrane stability (CMS)} = \left[1 - \frac{(C1/C2)}{100}\right] \times 100
\]  

(2)

\[
\text{Cell membrane injury (\%)} = 100 - \text{CMS}
\]  

(3)

A 0.1 M of phosphate buffer, 20 mM guaiacol, and 40 mM H$_2$O$_2$ freshly prepared solutions were thoroughly mixed with a ratio of 8:1:1 to measure the activity of peroxidase as defined by Liu et al. [33]. A master mixture of 100 μL was taken in a 96-well plate and mixed with sample extract (100 μL). With or without the addition of enzyme extract in the microplate reader, the absorbance was observed at 470 nm for 3 minutes.

The catalase was measured according to Liu et al. [33]. According to this procedure, a 100 μL of H$_2$O$_2$ (5.9 mM: 35% pure) freshly made mixture was added with enzyme extract (100 μL). The H$_2$O$_2$ was vanished using a microplate reader and the absorbance was measured for 3 min at 240 nm. Kumari et al. [34] defined the process to measure superoxide dismutase by using riboflavin (50 μL), methionine (100 μL), phosphate buffer (250 μL) of 7.5 pH, and nitro-blue tetrazolium (50 μL). A microplate reader was used to measure the absorbance at 560 nm and expressed as a unit per mg of protein. The enzymatic content that was used to inhibit the nitro-blue tetrazolium (50%) photoreduction defines the one unit of superoxide dismutase.

**Growth Parameters**

The sample collection of the measurement of plant growth by calculating root and shoot length, fresh and dry weights at 75 DAS. In each biological replicate, the randomly tagged plants’ root and shoot lengths were measured using a meter rod. The root length was taken by uprooting the tagged plants, while root and shoot fresh and dry weights were measured by an electric balance (Kern 440–49A, Balingen, Germany). The samples were sun-dried before measuring dry weight, then heated at 65°C in an oven (Memmert110, Schawabach, Germany), and dry weight was calculated with digital balance (Shimadzu AW-320, Kyoto, Japan).

**Yield Attributes**

Yield attributes such as hundred-seed weight and seed yield plant$^{-1}$ were taken at 142 DAS. Digital balance was used to measure hundred-seed weight. The crop was manually harvested at maturity when the whole plant color turned golden brown. The spikes were threshed to separate awns and grains, and then grain yield plant$^{-1}$ was measured using an electric balance.

**Statistical Analysis**

The data analysis was done by using Statistix 10.1. (Analytical Software, Statistix, Tallahassee, FL, USA, 1985-2003) through the analysis of variance (ANOVA) technique [35]. Tukey’s HSD (honestly significant difference) was used to compare treatment means at a 5% probability level, while graphics were made using Sigma-Plot 10.0, while correlation matrix was done by using R (version 4.1.2).

**Results**

Results have revealed that water stress significantly affected the morpho-physiological, biochemical, and yield attributes (Tables 1-3; Figs 1-4). Whereas, foliar supplementation of K has shown a key role to impart the harmful effect of water stress in wheat plants.
Chlorophyll Pigments

Water stress showed a significant ($p \leq 0.05$) impact on photosynthetic pigments in wheat plants. However, K applications played an ameliorative role under water-limited conditions to support wheat plants by sustaining chlorophyll pigments (Table 1). The interaction water stress × K application was significant for chlorophyll a, and non-significant for chlorophyll b and total chlorophyll in wheat leaves. As compared to control-no drought, the water stress reduced the chlorophyll a, chlorophyll b, and total chlorophyll content in wheat by 33, 26 and 34%, respectively. On the other hand, K supplementation improved chlorophyll content in wheat leaves under water stress as chlorophyll a, chlorophyll b, and total chlorophyll content increased by 30, 29, and 14%, respectively, as compared to control-no K. However, the maximum improvement in chlorophyll pigments was observed when K was applied at 100 mg L$^{-1}$ (Table 2).

Gas Exchange Attributes

Water stress showed a significant ($p \leq 0.05$) effect on the gas exchange attributes. However, foliage applications of K under water-limited conditions have also shown a significant impact on the gas exchange attributes in wheat plants (Table 2). The interaction water stress × K applications was significant for transpiration rate (Tr), while a non-significant effect was observed for stomatal conductance (Gs), photosynthetic rate (Pn), and intercellular CO$_2$ concentration (Ci) in wheat. Nonetheless, water stress reduced the stomatal conductance, transpiration rate, photosynthetic rate, and intercellular CO$_2$ concentration in wheat by 38, 46, 38, and 20%, respectively, as compared to control-no drought. However, K applications improved the gas exchange attributes under water stress as stomatal conductance, transpiration rate, photosynthetic rate, and intercellular CO$_2$ concentration in wheat increased by 38, 42 12, and 10%, respectively, over control (Table 2).

### Table 1. Impact of potassium applications on chlorophyll content, total soluble sugars, phenolic content, protease, and cell membrane injury in wheat under water stress.

<table>
<thead>
<tr>
<th>Water stress (W)</th>
<th>Potassium Applications (K)</th>
<th>Chl a (mg g$^{-1}$ FW)</th>
<th>Chl b (mg g$^{-1}$ FW)</th>
<th>Total Chl (mg g$^{-1}$ FW)</th>
<th>Total soluble proteins (mg g$^{-1}$ FW)</th>
<th>Phenolic content (mg g$^{-1}$)</th>
<th>Protease (Unit g$^{-1}$ FW)</th>
<th>Cell membrane injury (CMI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>NK</td>
<td>2.18 c</td>
<td>0.63 cd</td>
<td>3.01 c</td>
<td>2.60 c</td>
<td>18.26 f</td>
<td>13.57 d</td>
<td>24.06 de</td>
</tr>
<tr>
<td></td>
<td>WA</td>
<td>2.21 c</td>
<td>0.65 c</td>
<td>3.04 c</td>
<td>2.63 c</td>
<td>18.73 f</td>
<td>13.74 d</td>
<td>22.17 e</td>
</tr>
<tr>
<td></td>
<td>K$_{50}$</td>
<td>2.34 b</td>
<td>0.74 b</td>
<td>3.31 b</td>
<td>3.37 b</td>
<td>24.91 e</td>
<td>15.80 c</td>
<td>19.86 f</td>
</tr>
<tr>
<td></td>
<td>K$_{100}$</td>
<td>2.59 a</td>
<td>0.83 a</td>
<td>3.552 a</td>
<td>3.84 a</td>
<td>30.13 cd</td>
<td>19.51 b</td>
<td>15.21 g</td>
</tr>
<tr>
<td></td>
<td>K$_{150}$</td>
<td>2.45 b</td>
<td>0.77 ab</td>
<td>3.42 ab</td>
<td>3.72 a</td>
<td>27.96 de</td>
<td>17.16 c</td>
<td>17.29 g</td>
</tr>
<tr>
<td>Water stress</td>
<td>NK</td>
<td>1.12 f</td>
<td>0.43 e</td>
<td>1.95 f</td>
<td>2.05 f</td>
<td>27.50 de</td>
<td>16.48 c</td>
<td>35.83 a</td>
</tr>
<tr>
<td></td>
<td>WA</td>
<td>1.15 f</td>
<td>.45 e</td>
<td>1.97 f</td>
<td>2.09 f</td>
<td>29.16 d</td>
<td>17.02 c</td>
<td>34.26 a</td>
</tr>
<tr>
<td></td>
<td>K$_{50}$</td>
<td>2.34 b</td>
<td>0.57 d</td>
<td>2.19 e</td>
<td>2.36 e</td>
<td>33.70 bc</td>
<td>19.17 b</td>
<td>30.93 b</td>
</tr>
<tr>
<td></td>
<td>K$_{100}$</td>
<td>2.10 c</td>
<td>0.64 cd</td>
<td>2.38 d</td>
<td>2.55 cd</td>
<td>39.13 a</td>
<td>24.25 a</td>
<td>24.75 d</td>
</tr>
<tr>
<td></td>
<td>K$_{150}$</td>
<td>1.97 d</td>
<td>0.61 cd</td>
<td>2.29 dc</td>
<td>2.44 dc</td>
<td>36.86 ab</td>
<td>20.33 b</td>
<td>27.78 c</td>
</tr>
<tr>
<td>LSD ($p \leq 0.05$)</td>
<td>0.119</td>
<td>0.081</td>
<td>0.177</td>
<td>0.151</td>
<td>3.99</td>
<td>1.64</td>
<td>2.29</td>
<td></td>
</tr>
</tbody>
</table>

NK = control-no potassium applied, WA = Water application, K$_{50}$ = Potassium application at 50 mg L$^{-1}$, K$_{100}$ = Potassium application at 100 mg L$^{-1}$, K$_{150}$ = Potassium application at 150 mg L$^{-1}$, Values sharing same case letter or without lettering for a parameter do not differ significantly ($p \leq 0.05$) by the LSD test.
parameters in wheat plants. However, foliage applied K was imperative to sustain crop performance by sustaining growth and yield parameters under water conditions (Table 3). The interaction water stress × K application was significant for leaf area and shoot fresh weight, while the effect was non-significant for the plant height, leaf dry weight, shoot dry weight, hundred seed weight, and seed yield of the wheat plant. Nonetheless, water stress reduced the plant height, leaf area, leaf dry weight, shoot fresh weight, shoot dry weight, hundred seed weight, and seed yield in wheat as compared to control by 29, 21, 23, 30, 10, 7, and 7%, respectively (Table 3). On the other hand, K applications improved growth and yield attributes under water stress as the plant height, leaf area, leaf dry weight, shoot fresh weight, shoot dry weight, 100-seed weight, and seed yield increased by 25, 12, 16, 6, 11, 18, and 25%, respectively over control.

Table 3. Impact of potassium applications on growth and yield attributes in wheat under water stress.

<table>
<thead>
<tr>
<th>Water stress (W)</th>
<th>Potassium applications (K)</th>
<th>Plant height (cm)</th>
<th>Leaf area</th>
<th>Leaf dry weight (g)</th>
<th>Shoot fresh weight (g)</th>
<th>Shoot dry weight (g)</th>
<th>100-seed weight (g)</th>
<th>Seed yield (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>NK</td>
<td>31.44 de</td>
<td>6.37 b</td>
<td>0.048 bc</td>
<td>3.72 c</td>
<td>0.60 cde</td>
<td>3.67 ef</td>
<td>3.40 e</td>
</tr>
<tr>
<td></td>
<td>WA</td>
<td>33.30 cd</td>
<td>6.40 b</td>
<td>0.050 b</td>
<td>3.73 c</td>
<td>0.63 bcd</td>
<td>3.85 de</td>
<td>3.43 e</td>
</tr>
<tr>
<td></td>
<td>K50</td>
<td>34.65 c</td>
<td>6.75 a</td>
<td>0.053 a</td>
<td>3.87 b</td>
<td>0.65 b</td>
<td>4.17 bc</td>
<td>4.16 b</td>
</tr>
<tr>
<td></td>
<td>K100</td>
<td>41.34 a</td>
<td>6.99 a</td>
<td>0.056 a</td>
<td>4.15 a</td>
<td>0.71 a</td>
<td>4.46 a</td>
<td>4.39 a</td>
</tr>
<tr>
<td></td>
<td>K150</td>
<td>37.93 b</td>
<td>6.79 a</td>
<td>0.054 a</td>
<td>4.03 a</td>
<td>0.68 ab</td>
<td>4.27 ab</td>
<td>4.28 ab</td>
</tr>
<tr>
<td>Water stress</td>
<td>NK</td>
<td>21.12 h</td>
<td>4.78 e</td>
<td>0.035 f</td>
<td>2.64 e</td>
<td>0.56 e</td>
<td>3.39 g</td>
<td>3.17 f</td>
</tr>
<tr>
<td></td>
<td>WA</td>
<td>22.62 h</td>
<td>4.81 e</td>
<td>0.037 f</td>
<td>2.65 e</td>
<td>0.56 e</td>
<td>3.57 fg</td>
<td>3.20 f</td>
</tr>
<tr>
<td></td>
<td>K50</td>
<td>25.62 g</td>
<td>5.37 d</td>
<td>0.040 e</td>
<td>2.70 de</td>
<td>0.59 de</td>
<td>3.84 de</td>
<td>3.77 d</td>
</tr>
<tr>
<td></td>
<td>K100</td>
<td>29.63 ef</td>
<td>5.92 c</td>
<td>0.045 cd</td>
<td>2.83 d</td>
<td>0.64 bc</td>
<td>4.16 bc</td>
<td>4.12 bc</td>
</tr>
<tr>
<td></td>
<td>K150</td>
<td>27.30 fg</td>
<td>5.47 d</td>
<td>0.042 de</td>
<td>2.75 de</td>
<td>0.60 cde</td>
<td>3.99 cd</td>
<td>3.98 c</td>
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<tr>
<td>LSD (p≤0.05)</td>
<td></td>
<td>2.390</td>
<td>0.316</td>
<td>3.270</td>
<td>0.127</td>
<td>0.051</td>
<td>0.217</td>
<td>0.18</td>
</tr>
</tbody>
</table>

NK = control-no potassium applied, WA = Water application, K50 = Potassium application at 50 mg L⁻¹, K100 = Potassium application at 100 mg L⁻¹, K150 = Potassium application at 150 mg L⁻¹; Values sharing same case letter or without lettering for a parameter do not differ significantly (p≤0.05) by the LSD test.
Stress Indicators

Water stress showed a significant effect on stress indicators, including malondialdehyde (MDA), hydrogen peroxide (H$_{2}$O$_{2}$), and cell membrane injury. However, exogenous application of K at the onset of stress showed an ameliorative role to support wheat plants (Table 1; Fig. 1). The interaction water stress × K application was significant for MDA, while nonsignificant for H$_{2}$O$_{2}$, and cell membrane injury in wheat leaves. Nonetheless, as compared to the control, the water stress increased the malondialdehyde, hydrogen peroxide, and cell membrane injury in wheat leaves by 391, 87, and 56%, respectively. On the contrary, applications of K showed a significant reduction in stress indicators as MDA, H$_{2}$O$_{2}$, and cell membrane injury decreased by 16, 19, and 21%, respectively, as compared to no K applied.

Metabolites

Plant metabolites including total protein and phenolic contents were significantly affected by water stress and K applications in wheat plants (Table 1). The water stress × K application interaction was significant for total protein content, while non-significant for phenolic content in wheat plant leaves. Nonetheless, water stress reduced the total protein content by 29% and increased the phenolic content by 37% in wheat leaves as compared to control-no drought. Conversely, exogenous applications of K increased plant metabolite contents under water stress as total protein and phenolic contents increased by 40, and 40%, respectively, in wheat as compared to control-no K applied.
Fig. 3. Impact of potassium application (NK-control-no potassium applied, WA-water application, K50-potassium supplementation at 50 mg L⁻¹, K100-potassium supplementation at 100 mg L⁻¹, and K150-potassium supplementation at 150 mg L⁻¹) on peroxidase a), superoxide dismutase b), catalase c), and protease d) in wheat under water stress conditions (W0-Control-no water stress and W1-water stress). Values represent the average of three replicates per treatment ± SE. Lettering is showing the difference among treatment means, values sharing the same case letter or without lettering for a parameter do not differ significantly (p ≤ 0.05) by the LSD test.

Fig. 4a) Correlation of potassium supplementation with morphological, gas exchange, and biochemical traits under normal conditions in wheat. PH = Plant height, LA = Leaf area, Pn = Photosynthetic rate, Gs = Stomatal conductance, WP = Water potential, RWC = Relative water content, Chl a = Chlorophyll a, Chl b = Chlorophyll b, T chl = Total chlorophyll, TSP = Total soluble proteins, SOD = Superoxide dismutase, Prot = Protease, CAT = Catalase, POD = Peroxidase, TPh = Total phenolics, H₂O₂ = Hydrogen peroxide, MDA = Malondialdehyde, CMI = Cell membrane injury.
Antioxidant Enzymes

Water stress has significantly affected antioxidant activities, including protease (Prot), POD, CAT, and SOD in wheat plants. However, supplementation of K improved the activities of antioxidants (Fig. 3). The interaction water stress × K application was significant for SOD, while non-significant for Protease, POD, and CAT in wheat plant leaves. Nonetheless, water stress improved the activities of Protease, POD, CAT, and SOD in wheat leaves as compared to control-no drought. Potassium applications improved the activities of antioxidants under water stress as protease, peroxidase, catalase, and superoxide dismutase increased by 29, 9, 32, and 35%, respectively, in wheat as compared to control.

Correlation Matrix

Results obtained from Pearson’s correlation revealed the correlation among many different parameters analyzed in this study. Water stress has shown a negative correlation with the chlorophyll content, photosynthetic rate, stomatal conductance, and water potential in the wheat plant. In addition, water stress had a negative relation with plant growth, and seed yield parameters, while, it showed a negative correlation with stress indicators (MDA content, H$_2$O$_2$, and electrolyte leakage) under water stress. Furthermore, the correlation matrix revealed a positive correlation between the accumulation of MDA content with the activities of H$_2$O$_2$ and cell membrane leakage (Fig. 4(a-b)) along with a strong negative correlation with photosynthesis, chlorophyll contents, osmolyte production, antioxidant activities, and seed yield.

Discussion

Abiotic stresses, particularly water stress inevitably provoked perturbations in various physiological processes, including chlorophyll pigments, affecting the growth and development of plants, thus, resulting in significant yield decreases in crop plants [36]. The results of this study revealed the harmful effects of water stress plant growth and biomass accumulation due to impaired photosynthesis and stomatal conductance (Tables 1-3; Figs 1-4). However, the application of potassium played an important role to impart negative
impacts of water stress in wheat as the foliar application of nutrients/chemicals readily absorbed and available to regulate plant metabolism [37]. Thus, K applications may upregulate plant morphological attributes leading to improve crop yield and yield attributes [38]. The current experiment established the role of K to alleviate the negative effects of water stress in wheat cultivar ‘Galaxy-2013’ by upregulating carbon assimilation, plant water status, metabolite contents, and antioxidant enzyme activities (Tables 1-3; Figs 1-4).

The results have shown that water stress at the heading stage impaired the plant growth and development as plant height and plant biomass production decreased under unfavorable conditions caused by water stress owing to a decrease in plant water status and chlorophyll pigments, directly affecting the photosynthetic efficiency and stomatal conductance (Table 2). Water stress inevitably reduced carbon assimilation, water potential, and pressure potential leading to reduce cell division and plant growth. The results of previous studies have revealed that water stress may drastically affect wheat productivity [39], especially at the sensitive stage of plants [40, 41, 42]. However, exogenous application of K played an ameliorating role to alleviate the oxidative stress under water-stressed conditions, and the effectiveness depends upon the K concentration used and crop growth stage. Ali et al. [43] have also revealed that the impact of exogenously applied nutrients depends upon the right concentration and stage of the crop. Our study revealed that at the onset of stress, K supplementation ameliorated the deleterious impacts of drought stress and improved crop growth, including, leaf dry weight, leaf area, plant height, and shoot fresh and dry weight at all concentrations of K applied. However, the maximum improvement in crop growth attributes was observed at K100 followed by K150 and K50.

Water stress harmfully affected the accumulation of photosynthetic pigments which directly affected photosynthetic activity and plant growth (Fig. 2). Balqees and Ali [44] revealed that water stress resulted in impair leaf chlorophyll content which might decrease due to the reduced protein contents in water-stressed plants. However, results have revealed the role of K supplementation to improve the chlorophyll in wheat cultivar ‘Galaxy-2013’ which led to improved carbon assimilation to support plant growth under water stress (Fig. 1). This could be attributed to the maintenance of photosynthetic pigments, and reduced oxidative damage caused by water stress (Fig. 1) [45, 46]. Similar to this, prior research revealed that K supplementation improved the plant water status, photosynthetic pigments, and leaf gaseous exchange characteristics, ultimately improving crop yield [42]. Results have revealed that K applications mediated the osmotic adjustment which proved to be a key characteristic linked to maintaining high turgor potential and water retention in response to water stress. However, K acted as a prime osmoticum in wheat plants, playing an important role to adjust under water stress. Egilla et al. [47] revealed that K applications upregulated the osmotic potential of crops improving the cell turgidity, leaf water status, plant photosynthetic rate, and stomatal conductance to enhance the ability against water stress.

The present study revealed that K supplementation at booting showed significant improvements in the production of osmolytes/metabolites, such as phenolic and protein contents with more improvement at K spray at 150 mg L-1 in wheat under drought conditions (Fig. 1) as these osmolytes act as a scavenger for ROS and maintain osmotic homeostasis in plants [48]. The K applications have improved the accumulations and activities of osmolytes/metabolites in plants to ameliorate the harmful effects of water stress showing a positive correlation between osmolyte production and K applications [49, 50, 51]. In addition, the foliar spray of K was very effective as it was readily absorbed, translocated, and accumulated where they participate in cellular activities and enzyme activation. Furthermore, a high concentration of phenolics in plants can detoxify reactive oxygen species (ROS) that helped to improve water stress tolerance in plants [52]. The role of compatible solutes/osmolytes has been well-established/well-recognized due to due to its abilities, including cell membrane stabilization, enzyme protection, acting as osmoticum for cellular turgidity, and detoxification of ROS [53].

Water stress caused the over-production of ROS which ultimately affected the plant growth and metabolism. Nevertheless, K induced mechanism resulted in reducing the damages caused by the over-production of oxygen radicals [17, 54]. This study found that water stress decreased the amount of soluble protein, possibly as a result of increased oxidative damage which is consistent with earlier findings that water stress might stimulate soluble protein degradation through unnecessary ROS generation (Fig. 2; Table 1). The foliar applications of K under water stress improved the concentration of total phenolics and soluble protein in wheat plants, signifying the important role of K applications to attenuate water stress by maintaining the higher osmotic balance of plants. The production of MDA is known to be an index of the severity of oxidative stress caused by stressful conditions [55]. In addition, the stability and integrity of the membrane may act as an index of water stress tolerance in plants as the stability of the membrane is prone to water deficit conditions [56]. The production of MDA dramatically increased under water stress in wheat cultivar ‘Galaxy-2013’, eventually increasing H2O2 and lipid peroxidation (Fig. 2), leading to increase cell membrane injury [57, 58]. Results have demonstrated the role of K to augment the water stress tolerance, and this improvement may be ascribed to the deemed role of K leading to the osmotic adjustment and stability of cell membranes. The optimum dose of K significantly increased plant life in water-stressed situations through root extension and membrane stability and integrity improvement. The results have revealed an important
relationship between the activities of antioxidant enzymes and oxidative stress under water stress [39, 48]. The upregulation of enzymatic antioxidants alleviates the effects of oxidative damages caused by hydrogen peroxide as CAT converts molecular O$_2^-$ into H$_2$O$_2$, and this H$_2$O$_2$ was further reduced by the ameliorative role of superoxide-dismutase and converts it into oxygen and water [59, 60]. Furthermore, K supplementation abridged oxidative damage under water stress due to the upregulation of the antioxidant defense system that includes the activities of CAT, Protease, SOD, and POD, reducing MDA accumulation, electrolyte leakage, and H$_2$O$_2$. The role of K to impart abiotic stresses lies in its activity to reduce the concentration of nicotinamide adenine dinucleotide phosphate oxidases to retain the activities of photosynthetic electron transport [13, 61]. The production of enzymatic and non-enzymatic antioxidants under water stress, and K applications may ameliorate the negative impact of lipid peroxidation, H$_2$O$_2$, and membrane leakiness in wheat plants, which may lead to improve the antioxidant-based plant defense system [62] including phenolics, soluble proteins, superoxide dismutase, catalase, ascorbate peroxidase, protease, and peroxidase [58, 63].

Water stress has deleterious impacts on plant growth and yield as water limitations at either growth stage lead to reduce the crop yield and yield-related attributes (Table 3) which is also supported by Waraich et al. [15]. Results have revealed that wheat yield was significantly affected by water stress relative to control, as hundred seed weight decreased by 18% which ultimately lead to a decrease in crop yield (Table 3). On the other hand, exogenous applications of K improved the grain yield under water stress compared to control i.e., no potassium application treatment. Anees et al. [64] and Shahzad et al. [65] have also reported a positive correlation between K applications and grain yield attributes under water stress. Improvements in grain yield under potassium applications may be due to an improvement in photosynthetic rate and plant water status, leading to improve yield attributes, including the number of grains and hundred-grain weight which is also in line with Raza et al. [65], Shah et al. [66], and Ahmad et al. [20].

Conclusions

Potassium supplementations improved the tolerance of water stress in wheat plants by improving plant growth, dry matter accumulation, and grain yield. Potassium application played an important role to improve crop growth and yield in wheat plants grown under water stress conditions as compared to control-no K applied, with more improvement at K$_{100}$ (potassium applied at 100 mg L$^{-1}$) followed by K$_{150}$ (potassium applied at 150 mg L$^{-1}$) and K$_{50}$ (potassium applied at 50 mg L$^{-1}$) respectively. Water stress-induced oxidative damages were also reduced under potassium applications by upregulating plant metabolites and antioxidant enzyme activities. The results are providing a clear understanding for researchers of how K can impart water stress tolerance on physicochemical mechanisms in wheat “Glassay-2013” that could be useful for further investigations at the cellular level.

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Conflict of Interest

The authors declare no conflict of interest.

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