Enhancing the Growth of Wheat (*Triticum aestivum* L.) Through Putrescine Application Under Drought Stress Conditions

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Abstract- **Drought stress is one of the main factors restricting growth and development in subtropical area where wheat is being grown. Field experiments were conducted in the Aren field -toralbaha district, Lahj governorate, Yemen to study the effects of putrescine application on the morphological and physiological characters of plants under drought conditions. The experiments was arranged using a randomized complete block design with two factors (1) putrescine treatment (0, 1.25, and 2.5 mM) and (2) drought stress (DS) consisting of 50% water irrigation and 100% well watering (WW). One week prior to heading and one week following the anthesis stage, the aerial portions of the plant were sprayed with putrescine. Some characteristics of morphology and physiology were observed and analyzed. The Results indicated that drought stress had a substantial impact on the majority of growth parameters; however, putrescine treatment significantly increased plant growth, with the exception of plant height. More malondialdehyde (MDA) content and less superoxide dismutase (SOD) activity during drought stress. Putrescine, interestingly, was able to raise SOD activity and lower MDA concentration in both conditions. The effects of drought stress were seen in leaf relative water content, total soluble sugar, and proline. The amount of proline, total soluble sugar, and relative water content all rose after putrescine treatment. There was a suggestion that putrescine could enhance the development and resilience of wheat cultivated in drought stress condition.**

Keywords—Drought , Putrescine, Wheat.

I. INTRODUCTION

Wheat (*Triticum aestivum L.*) is one of the most extensively consumed cereal crops in Yemen. Improving wheat yield and planting area will be crucial to reducing the imported wheat. Yemen is a subtropical country with little precipitation in the fall and variable topography, which makes it susceptible to drought stress as wheat grows and develops.

Drought stress one of the most significant factors that negatively impacts plant growth and yield output worldwide is drought [1] Drought stress negatively affects plants, changing their morphology and physiology, which in turn lowers crop growth and agricultural yield [2] . According to [3], drought stress reduces stem elongation, cell division, and proliferation, which lowers plant growth and production. Actually, restricting the amount of water available has a detrimental effect on plant metabolism by disturbing the physiological and biochemical processes because of ionic and osmotic imbalances, which lowers plant development and yield [4].

According to [5], plants exposed to drought stress accumulate osmolytes including sugars and amino acids to control water intake. Sugars can replenish water more effectively than proline, which serves as a hydration shell for biomolecules in water-limited conditions [6]. Plant tolerance to drought stress is studied at various levels, including morphology, physiology, and molecular responses.

Polyamines are known as a group of natural compounds with aliphatic nitrogen structures that may play important roles in many physiological processes such as cell growth, development, and plant response to environmental stress. In addition, polyamines are also known as anti-aging and anti-stress agents due to their acid neutralizing and antioxidant properties [7]. These polyamines are believed to play a vital role in regulating plant defense responses to various environmental stressors. Foliar application of putrescine (a type of polyamine) in wheat under drought stress conditions significantly reduced MDA content, increased total chlorophyll and proline production, and improved growth and all yield parameters [8].

The effect of putrescine application of wheat on improving plant growth and yield under drought stress conditions in Yemen has yet to be investigated. The objective of this study was to investigate the effect of drought stress on morphological and physiological growth characteristics of plants treated with putrescine in Yemen.

II. MATERIALS AND METHODS

A. Plant Materials

The plant material used in this study was a local wheat variety (*Triticum aestivum L.*), provided by Alkoud Agricultural Research in Abyan Governorate, Yemen.

B. Field Experiment

The field experiment carried out during the winter season, from January to March 2024, in Aren field, located in Tor Al-Baha District, Lahj Governorate,Yemen (13° 83' North and 43° 33' East) situated at an altitude of 312 meters above sea level. The site experiences an average annual precipitation of 10 mm, concentrated during the season from June to march year 2024. During the experimental period, the relative humidity ranged from 40% to 55%, with an average of 51%. Daytime temperatures during the study ranged from 25°C to 28°C, , while the site received an average of 10–12 hours of sunlight per day. The physical and chemical properties of the soil were analyzed. The soil texture was sandy with a field capacity of 11.5%, pH of 8.1 and an electrical conductivity of 2.71 dS/m. It also had 420 mg/kg of total nitrogen, 21.60 mg/kg of available phosphorus, 89.83 mg/kg of available potassium, and 1.12% soil organic carbon.

The study was conducted under two different irrigation conditions using a randomized complete block design (RCBD) with three replications. The experiment involved two factors: putrescine concentration (0, 1.25, and 2.5 mM) and two irrigation regimes, fully watered and drought-stressed (50% of field capacity).

The experimental plots, each measuring 1.25 × 4.00 meters, consisted of six units. Each plot contained five rows, with a spacing of 25 cm between rows. The experimental plots ,was fertilized based on standard wheat cultivation. A total of 6 g of nitrogen (equivalent to 120 kg/ha) was applied in three split doses including, 2.4 g during soil preparation, 1.8 g at the tillering stage (20–25 days after sowing), and 1.8 g at the booting stage (45–50 days after sowing), using urea (46% N). Phosphorus was applied as a basal dose at 3 g per plot (equivalent to 60 kg/ha) using diammonium phosphate (DAP, 46% P_2O_5), and potassium was also applied basally at 2 g per plot (equivalent to 40 kg/ha) using muriate of potash (MOP, 60% K_2O). Zinc sulfate (25% Zn) was added at 0.5 g per plot (equivalent to 10 kg/ha) to address potential micronutrient deficiencies, while 5 kg of farmyard manure (equivalent to 10 tons/ha) was incorporated into the soil two weeks before sowing to enhance organic matter content. Each fertilizer application was followed by irrigation to ensure proper nutrient uptake and minimize losses. The field plots were prepared under controlled conditions. To maintain weed-free conditions, manual weeding was performed every two weeks.The moist regime was maintained by irrigating the plots regularly based on the field capacity of soil. Soil moisture was monitored weekly using soil moisture sensors to ensure consistency in the control plots.

Irrigation was provided weekly under full irrigation conditions. Thirty days after planting, the plots were divided into fully irrigated and drought-stressed groups (50% irrigation). Water irrigation was controlled by measuring field capacity. However, Putrescine was applied by spraying 300 ml of solution onto the aerial parts of the plants one week before heading and one week after anthesis.

C. Morphological parameters

Growth characteristics, including plant height, flag leaf area, and total dry weight, were measured using five randomly selected samples from each experimental unit. Plant height was recorded using standard techniques, while the flag leaf area was measured after anthesis. When the experiment was finished, the entire plant was weighed using a standard electronic balance to determine its fresh weight. The plant was then dried in an oven set at 80C0 for 48 hours, and its dry weight and total dry weight were then determined by weighing the dried plant.

D. Physiological Parameters

The physiological parameters assessed after putrescine application included total chlorophyll, proline, total soluble sugars in leaves, superoxide dismutase (SOD) activity, malondialdehyde (MDA) content, and relative water content (RWC).

The procedure of [9] was used to determine the content of chlorophyll a, b, and total of chlorophyll at absorbance (645 and 663 nm) using a Spectrophotometer.

Chlorophyll content were determined by the following formulas:

Chlorophyll a = 12.7(A663) - 2.69(A645) * V/1000 * W (1)

Chlorophyll b = 22.9(A645) - 4.68(A663) * V/1000 * W (2)

chlorophyll Total = 20.2(A645)-8.02(A663) *V/1000*W (3)

Where:
A: Absorbance of the sample (chlorophyll a peak).
V: Volume of the extract (in mL).
W: Fresh weight of the leaf sample (in grams).

Proline content was analyzed using the method of [10]. The fresh leaves were mixed in 3% of sulfosalicylic acid, then were centrifuged for 15-20 minutes at 3,000 g for 15-20 minutes. The supernatant was mixed with acid ninhydrin and glacial acetic acid, heated in a water bath for an hour, and then cooled in an ice bath. Absorbance was measured at 520 nm, and proline concentration was expressed as mg of proline per gram of fresh leaf weight.

Total soluble sugars were measured using the method of [11], with glucose standard solutions used for reference, and results expressed as mg/g of dry weight.

SOD enzyme activity was evaluated according to [12], with measurements taken at 0, 30, 60, 90, and 120 minutes after exposing the reaction mixtures to fluorescent light. Absorbance was measured at 560 nm using a visible spectrophotometer, and SOD activity was calculated using the formula:

SOD unit = (Control Tangent – Sample Tangent) / (0.5 × Control Tangent) (4)

MDA content was determined following the procedure of [13] from fresh leaves. Absorbance readings were taken at 532 nm and 520 nm, and MDA content was calculated using the formula:

MDA(nmol/g)=({(A532-A520)/ε}×10^6)/FreshWeight(g) (1)

where $\varepsilon = 155\ L\ mmol^{-1}\ cm^{-1}$.

Relative Water Content (RWC) was measured according to [14], which calculates the water status of plant tissue by comparing fresh and dry weights.

Physiological analyses were carried out at the Laboratory of faculty agriculture Lahj university, Laboratory of faculty science university of Aden and Yemen Standardization Metrology & Quality Control Organization-Aden-Yemen.

E. Statistical Analysis

Data was statistically analyzed using SPSS software ver. 16. The significance of treatment effect to the observed parameters was tested using F test at $\alpha = 0.05$. Duncan

Multiple Range Test (DMRT) at α= 0.05 was used to discriminate the means when the F test indicated a significant effect of treatments.

III. RESULTS

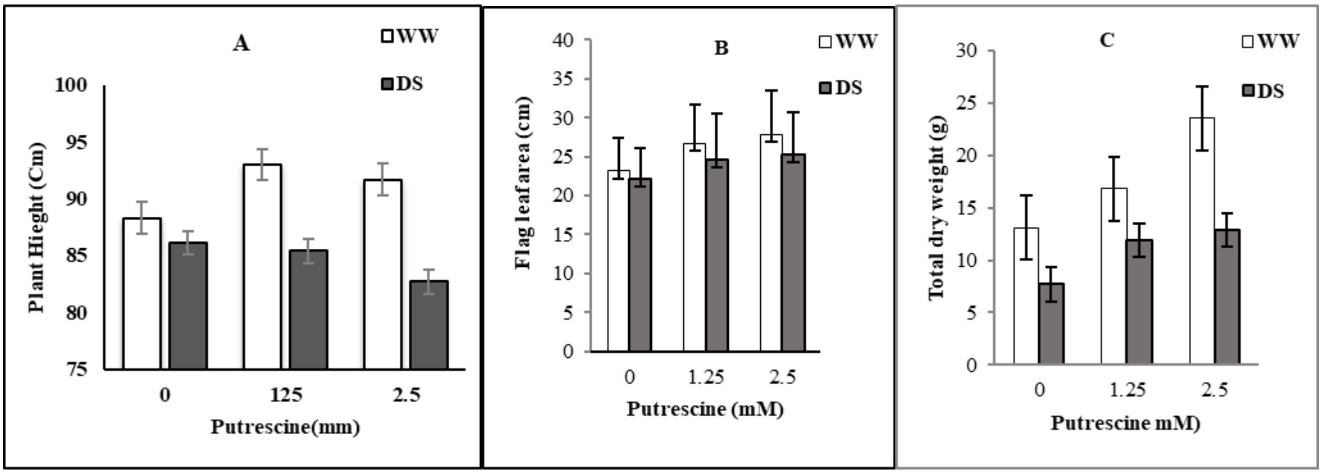

Fig.1 Mean of plant height (A), flag leaf area (B) and total dry weight (C) of wheat grown at drought stress condition under putrescine application. ww=well watering, Ds=drought stress. Bars were standard error.

A. *The Effects of Putrescine Application on the Morphological characters Under Drought Stress*

Plant growth as notably impacted by drought stress. The morphological traits of plants, such as plant height, flag leaf area, and total dry weight, were significantly reduced under drought conditions compared to well-watered plants, as shown in Figure 1. However, putrescine application at various concentrations improved most of these traits. At a concentration of 1.25 mM, putrescine significantly increased plant height, flag leaf area, and total dry weight compared to untreated plants. Without putrescine, no significant difference in plant height was observed among drought-stressed plants (Figure 1A), but putrescine application influenced plant height differently under the two water conditions. While putrescine increased plant height under well-watered conditions, it had no effect under drought stress. Similarly, flag leaf area and total dry weight were significantly reduced in plants exposed to drought, but both parameters increased with higher putrescine concentrations under both conditions (Figure 1B and 1C). Interestingly, putrescine restored flag leaf area and total dry weight to levels comparable to those under well-watered conditions when it was not applied. However, the application of putrescine under drought stress did not raise these traits to the same level as when putrescine was applied in favorable water conditions. No significant effect was found between the two concentrations of putrescine on flag leaf area and total dry weight in drought-stressed plants.

B. *The effects of putrescine application under drought stress on the physiological characteristics*

Water shortage (50% of field capacity) negatively impacted several physiological traits in this study. Total chlorophyll content decreased under drought conditions. plants subjected to drought stress had significantly lower total chlorophyll levels than those under well-watered conditions (control), as shown in Figure 2A. However, putrescine application significantly increased total chlorophyll content compared to the control, regardless of water condition. The highest improve was recorded in plants treated with 1.25 mM of putrescine. Although overall chlorophyll levels were low, the treatments showed significant differences in response to drought stress.

Wheat ability to sustain growth under drought stress is attributed to the efficient synthesis of sugars, polysaccharides, and other osmolytes like proline. In this study, drought stress increased proline content compared to well-watered control plants (Figure 2B). Proline levels varied

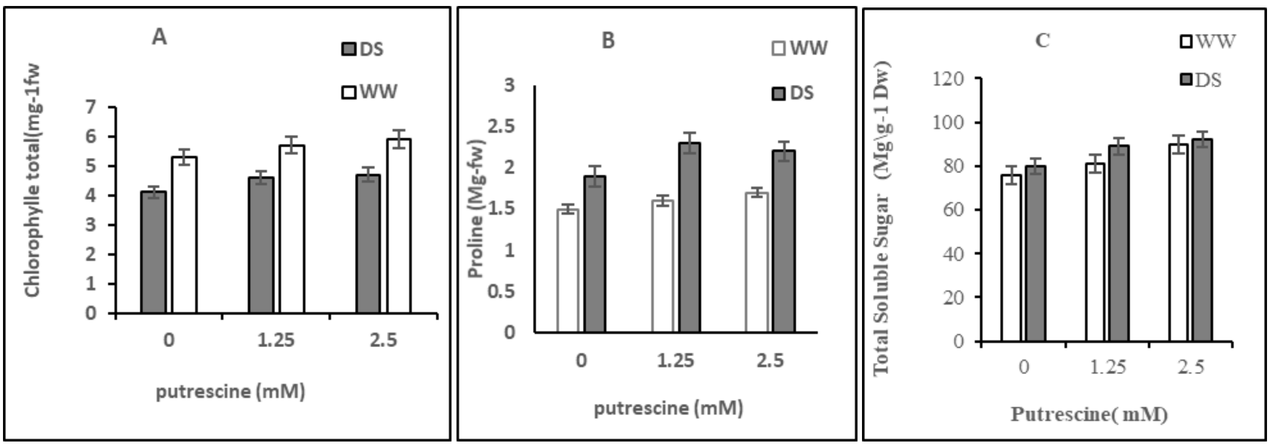

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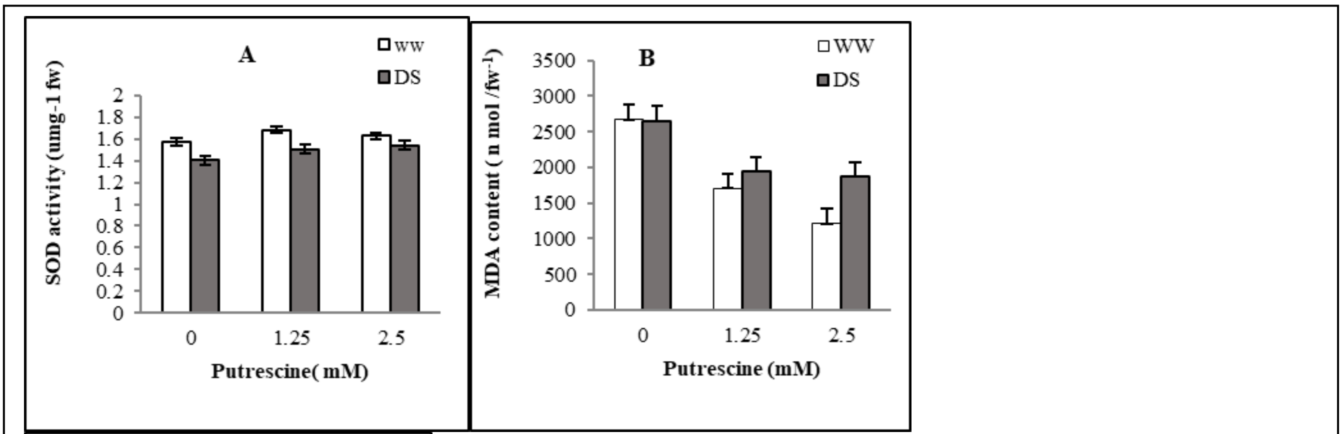
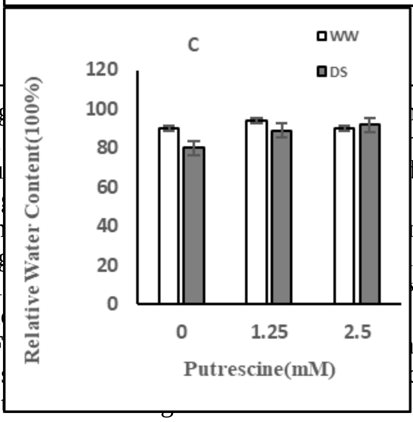
Fig 2. Mean of Chlorophylle total (A), Proline (B) and Total Soluble Sugar (C) of wheat grown at drought stress condition under putrescine application. ww=well watering, Ds=drought stress. Bars were standard error.





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to… in drought-stressed plants compared to controls. Putrescine treatment further increased total soluble sugar levels as concentrations increased. Our findings suggest that putrescine at 2.5 mM resulted in the highest sugar increase, indicating its potential role in alleviating drought stress.

This study observed different responses in wheat plants grown under different conditions. Drought stress adversely affected most physiological traits, as plants modify their metabolism in response to stress, particularly by altering their antioxidant systems. In this study, drought stress reduced SOD activity compared to well-watered plants, except for those treated with 2.5 mM of putrescine (Figure 3A). However, plants treated with putrescine showed a significant increase in SOD activity under both conditions, confirming that putrescine's effect was not dependent on water availability. Putrescine was able to raise SOD activity under drought stress to levels comparable to those seen in well-watered plants without putrescine. No significant difference in SOD activity was found between plants treated with 1.25 mM and 2.5 mM concentrations, indicating that 1.25 mM was the optimal concentration for increasing SOD activity.

Malondialdehyde (MDA) content, a marker for lipid peroxidation and membrane deterioration during stress, was higher in plants under drought stress than in those under well-watered conditions (Figure 3B). However, putrescine application significantly decreased MDA content in plants under both conditions, indicating that its effect was not influenced by water limitation. Putrescine treatment at 1.25 mM reduced MDA levels in drought-stressed plants to match those observed under well-watered conditions when putrescine was applied.

The plants appears different physiological responses to drought stress conditions. The Leaf water content was affected by drought stress. Maintaining adequate water status is crucial for plants, and the RWC under drought stress was significantly lower than that of well-watered plants (Figure 3C). However, putrescine application resulted in increased RWC under drought stress conditions, indicating that it helped improve the plants water retention ability

IV. DISCUSSION

Water stress negatively affects the growth and development of crops, including wheat, as it disrupts metabolic processes in plants [15]. Enhancing plant resistance to environmental stresses is crucial for their survival. The exogenous application of putrescine has shown a positive,

protective role in wheat against oxidative stress, particularly drought stress [16].

The other two growth parameters, flag leaf area and total dry weight, were reduced at drought stress. This reduction in plant growth caused by drought stress that alters cell division that affect the leaf size and weight [17] . Putrescine application only increase leaf area of plant grown at drought stress, which was indicated that effect of putrescine treatment on leaf area was influenced by water states condition. Foliar treatment with putrescine appreciably improved the drought stress tolerant, as evident from, total dry weight and leaf area. [18] reported that application of putrescine significantly increases fresh and dry weight of plant, plant height and leaf area under drought stress and well watering condition. However, polyamins are considered to be regulators of plant growth and development specifically affected cell division and differentiation [8]. Putrescine treatment increased plant dry weight and leaf area at drought stress confirm that putrescine was able to improve the level of drought tolerance for plant under drought stress condition. There was no significant difference in leaf area between plant treated with 1.25 and 2.5mM putrescine indicated that putrescine concentration of 1.25 mM was the optimum concentration to improve this traits. Putrescine treatment was not affected most of plant growth due to reduced source capacity under drought stress.

The most significant difference between the two conditions was water availability, suggesting that variations in plant growth under these conditions were primarily due to differences in irrigation. In this experiment, drought stress clearly impacted plant growth.

Plant appear differ in their responses to water stress depend on the severity, period of stress, and plant species. Figure 1A shows that drought stress had no significant effect on plant height in the absence of putrescine treatment. This finding was intriguing, as drought stress accelerated plant growth, resulting in an increase in plant height to levels similar to well-watered conditions. Drought stress has a significant impact on the rate of plant development, which may initially increase before declining [19]. Putrescine application increased plant height under well-watered conditions but inhibited growth under drought stress. [20] found that wheat showed significant reductions in shoot length when treated with different concentrations of putrescine, compared to controls. He suggested that polyamines may play an essential role in inducing cell division but not cell elongation.

Although water shortages caused a significant decrease in total chlorophyll content, exogenous putrescine improved chlorophyll retention [21]. The delay in chlorophyll degradation with putrescine treatment may result from its stabilizing effect on thylakoid membranes, as shown in studies on photosystem complexes [22]. These findings align with [8], who observed that putrescine application increased chlorophyll a and b content. The increase in photosynthetic pigments can be attributed to putrescine’s anti-senescence properties [23].

In the present investigation, proline contents were increased in plant grown under drought stress spraying with putrescine particlary at 1.25Mm. This findings agree with the results of [24], which found increased in the levels of proline in due to exposure to drought. It also observed that Putrescine treatment increased proline content, in [20]. Furthermore, an earlier study portrayed enhancements in the levels of soluble sugars, free amino acids, and free proline of cotton plants in response to putrescine applications [25]. Proline is a low molecular weight osmotic regulating molecule that regulates redox potentials, scavenges hydroxyl radicals, reduces oxidative damage, and stabilizes cell membranes under stress conditions [26]. [27] reported that, Drought significantly induced accumulation of free proline as we noted nearly 1.7-times higher accumulation of this osmoprotectant in plants compared to control and Putrescine significantly enhanced further Proline accumulation under drought much higher than untreated plant.

The increase in total soluble sugar in drought-stressed plants may be due to the higher demand for sugar during stress for respiration and its role as a signaling molecule during stress. The accumulation of sugars under drought stress helps maintain water relations through osmotic adjustment, particularly in terms of RWC. [28] also reported increased proline and total soluble sugar levels in barley under water stress. Additionally, total soluble sugars may serve as a defense mechanism, helping plants control photosynthetic activity and balance reactive oxygen species [29]. Therefore, total soluble sugar content could be a useful criterion for selecting drought-tolerant genotypes. Earlier research found that drought conditions increased total sugars and proline in cotton plants [30].

In this study, MDA levels, which indicate lipid peroxidation and membrane damage, were higher in plants under drought stress than in well-watered plants. However, putrescine application significantly reduced MDA levels under both conditions. This finding is consistent with [21], who observed that putrescine reduced MDA accumulation. Polyamines help maintain membrane integrity during drought by reducing electrolyte leakage and lipid peroxidation [31]. Polyamines, being cationic compounds, can interact with anionic membrane components such as phospholipids, maintaining membrane structure and preventing degradation [32]. Additionally, no significant differences in MDA content were found between plants treated with 1.25 mM and 2.5 mM putrescine. These results align with previous studies, which found no significant correlation between drought stress and MDA levels in cotton seedlings grown under field conditions [33]. Thus, SOD activity may play a role in mitigating the damaging effects of drought stress.

Wheat grown under drought stress showed reduced SOD activity compared to well-watered plants. However, plants treated with putrescine exhibited significantly higher SOD activity under both conditions. [21] reported that putrescine application reduced oxidative damage by lowering MDA levels and increasing SOD activity under drought stress. Putrescine may bind to antioxidant enzymes like SOD or conjugate with small antioxidant molecules, allowing them to reach affected areas within cells [34].

Drought stress also affected water status, as shown by the decrease in RWC , indicating their greater susceptibility to drought. RWC decreased due to reduced root water absorption, disrupted stomatal control, and overall water shortage [35]. A reduction in RWC is a clear indicator of drought stress, as it reflects the plant's water status. This reduction may be due to increased membrane permeability and lower water supply [36]. However, putrescine treatment improved RWC levels in treated plants under water stress compared to untreated plants [21]. The result of this study are consistent with [37], who found that exogenous polyamine application increased leaf water potential and RWC in rice plants. Putrescine played a crucial role in maintaining better water status and enhancing resistance to water stress.

CONCLUSION

Most of growth and physiological characters of Wheat grown in the experiment was affected by the drought stress. Based on the growth and physiological responses of wheat , putrescine treatment was a potential compound that could be used to improve drought tolerance. The most effective putrescine concentration was1.25 mM. Putrescine application could be a starting point to increase Wheat production under drought stress condition.

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