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The Promotive Effect of Putrescine on Growth, Biochemical Constituents, and Yield of Wheat (*Triticum aestivum* L.) Plants under Water Stress

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Abstract: Drought stress is a significant environmental variable affecting wheat growth and development. Plant stress tolerance is intimately related to growth regulators of plants as polyamines. The study assessed the impact of drought (50% water irrigation and 100% water irrigation), priming of grains in putrescine (0.25, 0.5, and 1 mM), and their interactions on the growth, yield, and physiological attributes of wheat plants. Drought conditions declined plant height, fresh and dry weights, leaves and tillers numbers, and flag leaf area. However, applying putrescine, especially at (1 mM), enhanced wheat growth performance in normal or water-deficit conditions. Drought stress decreased spike length (28.6%), number of spikelets (15.6%), number of grains (30.3%), the weight of the spike (23.5%), and the weight of the grains/spike (37.5%). In addition, drought decreased the contents of chlorophyll a, chlorophyll b, free amino acids, and total phenols, while applying putrescine enhanced wheat plant growth performance in normal or drought conditions. Putrescine at (1 mM) achieved the highest increase in plant height (38.8%), root length (50%), leaves number (166%), tillers number (80%), flag leaf area (70.3%), shoot fresh weight (99.4%), shoot dry weight (98.4%), root fresh weight (97.8%), root dry weight (210%) compared to the untreated plants. Moreover, pretreatment with putrescine improved chlorophyll a (13.3%), chlorophyll b (70.3%), carotenoids (61.8%), soluble sugars (49.1%), amino acids (42.7%), phenols (52.4%), number of spikelets (59.3%), number of grains (81.1%), and weight of spike (45.4%). Moreover, variations in the protein profile of wheat plants were due to drought conditions and putrescine application. In conclusion, priming wheat grains with putrescine effectively induces protective mechanisms against water stress and improves wheat plants' physiological attributes and yield components.

Keywords: polyamine; drought; wheat; pigments; osmolytes; phenols; flavonoids; protein; yield

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1. Introduction

The Gramineae family includes wheat (*Triticum aestivum* L.) crop. Wheat plants are cultivated by humans in widely extended areas [1]. Wheat is considered one of the most economical and valuable grain crops. The primary national goal is wheat breeding by raising the productivity per unit area and expanding the planted area in recently reclaimed lands. Wheat ranks third internationally and is used as a fundamental food grain for rural and urban cultures providing farmers with straw for animal feed [2,3]. In the last decade, about 65 million ha of wheat productivity was influenced by a water shortage [4]. Since wheat can grow in arid and semiarid areas, irrigation management is essential. Reducing water irrigation is an effective means of irrigation management

under water deficit conditions. The irrigation water deficit influences crops' growth and productivity [5–7]. Drought (water deficit) is considered one of the most significant abiotic stresses resulting in remarkable decreases in the growth of plants and productivity [3]. Drought changes widely range from morphological stages to molecular levels in several growth stages [8]. Under drought stress, wheat plants accumulate "Osmolytes" such as sugars and amino acids to manage water absorption [9]. Under a water shortage, sugars can replace water even more than proline which acts as a hydration shell surrounding biomolecules [10]. Proteins are biomolecules of primary importance for all physiological processes in the plant cell [3]. An increase in wheat proteins of shoots cultivated under stress caused by a shortage water was reported. In addition, deficit water irrigation increased phenols and flavonoids under drought stress in wheat plants [11]. Reactive oxygen species, including singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), superoxide anion (O^{2-}), and hydroxyl radical (OH), are produced in stressed plants. These species disrupt enzyme activity and cause oxidative damage, photoinhibition, and disruption to nucleic acids, lipids, and proteins, which results in cell death. [12,13]. Drought conditions inhibit plant growth, morphological attributes, water relations, stomatal closure, membrane stability, and disruptions in carbohydrate lipid and protein metabolism [3,14]. The adjustment of osmotic balance differs from wheat cultivars. Therefore, studying the physiological attributes of the drought tolerance of target wheat plants and their defense mechanisms is necessary.

Priming with growth regulators is one of the most crucial strategies for reducing the negative impacts of drought and enhancing crop growth and survival. Putrescine is a member of the polyamine family, considered a class of growth regulators of plants. All living things include these nitrogenous chemicals, characterized by low molecular masses [15,16]. Applications of polyamines cope with drought effects on crops by increasing free polyamines, regulating their metabolism, and accumulating osmolytes [17]. An earlier investigation by [18] proved that putrescine inhibited the accumulation of drought-induced reactive oxygen species by boosting antioxidation enzyme activities. Similar findings also proved that polyamines enhanced the antioxidant defense system [19]. Putrescine was to improve the chemical constituents under stress and to improve a variety of plant attributes when applied topically [20,21]. However, the question remains: does pretreatment with putrescine increase the tolerance of wheat plants against (water deficit) drought conditions? Therefore, the main aims of this investigation are to assess the harmful effects of drought stress on wheat plants and evaluate the alleviative role of putrescine on growth attributes and biochemical contents of these drought-stressed plants.

2. Materials and Methods

2.1. Experiment Design

A greenhouse experiment was conducted in the winter of 2017–2018 at the Faculty of Science (Girls) at Al-Azhar University, Cairo, Egypt. Agriculture Research Center, Egypt, supplied the grains of wheat (cv. Sods 1), which soaked in putrescine (0.00, 0.25, 0.5, 1 mM) for 12 h. On 21 November, pretreated grains were planted in earthenware pots (No. 50) and 20 kg of sand-filled soil in a completely randomized design with three replicates for each treatment. The soil texture was sandy with a field capacity of 11.5%, pH 8.7, EC 0.35 dm m^{-1}, Na^+ 1.2, Ca^{++} 1.27%, K^+ 0.25, Mg^{++} 0.58, Cl^- 1.7, HCO^{3-} 1.10 meq L^{-1}. Five seedlings were in each pot after full emergence. Potassium sulfate and calcium superphosphate were applied. After 30 and 60 days following seeding, ammonium nitrate was added. Plant irrigation was completed every week with full-water irrigation. At 30 days after planting, the pots were divided into control and drought stress (50% water irrigation) groups. The amount of water irrigation was decided based on the soil field capacity (FC).

2.2. Morphological Growth and Yield Traits

At the vegetative stage, after 65 days of cultivation, samples were collected from each treatment to measure growth characteristics (height of plant (cm), length of root (cm), number of tillers/plant, number of leaves/plants, area of flag leaf (cm^2), weights of fresh and dry both shoot and root (g) per plant). At harvest, samples were collected to yield components: spike length/plant, spikelets number/plant, the weight of spike/plant, grains number/spike, and weight of grains/spike.

2.3. Leaf Pigments

Chlorophyll a, Chlorophyll b, and carotenoids were measured in wheat leaf tissue samples in acetone (85%), illustrated by [22]. The extraction of (0.1 g) wheat leaves that were still fresh were in acetone. Centrifuging homogenized plant material at 1006× *g* produced a supernatant containing (10 mL) of 85% acetone. Spectrophotometer (VEB Carl Zeiss) was used to the absorbances at 663, 644, and 452 nm against blank acetone. The pigment fraction concentrations (carotenoids, chlorophyll a, and b) were mg/g fresh weight.

2.4. Total Soluble Sugars (TSS)

The amount of total soluble sugars was measured in an ethanolic extract of fresh wheat plant flag leaves using [23]. For 10 min in a boiling water bath, combine 0.1 mL of ethanol extract with 3 mL of fresh anthrone [(150 mg) of anthrone + (100 mL) of 72% H_2SO_4], let the mixture cool, and read the result at 625 nm using a spectrophotometer. To decide TSS, the glucose standard curve was employed.

2.5. Total Free Amino Acids

Fresh leaves of the wheat plant were extracted in ethanol (80%) for free amino acids determination [24] ninhydrin technique. Following centrifugation, one mL of the extract was mixed with 0.5 mL of buffer, which had 27 g of sodium acetate, 20 mL of distilled water, 5 mL) of CH_3COOH (glacial), and 1.5 mL of distilled water (pH = 5.4) with 490 ppm of NaCN. The setting is 75 mL. After that, add 0.5 mL of the ninhydrin solution, which is made up of (200 mg) of ninhydrin, (10 mg) of cadmium acetate in 0.2 mL of acetic acid (glacial), 0.8 mL of distilled water, and 10 mL from acetone (50%). The previously combined solution was submerged in water for five minutes or until a purple hue appeared. Once cooled, 5 mL of 50% isopropanol was added. A VEB Carl Zeiss spectrophotometer was to read the absorbance at 570 nm. The L-glutamate standard curve prepared to determine free amino acids (mg g^{-1} FW).

2.6. Free Proline

Free proline was measured using the procedure illustrated by [25]. About 0.5 g of leaf fresh weight was grounded in 10 mL of aqueous sulfosalicylic acid (3%). Two mL of the extract were mixed with two ml of the acid ninhydrin reagent and kept at 100 °C for an hour. The reaction mixture of proline was extracted with 4 mL of toluene. Toluene was removed from the aqueous phase at room temperature. The absorbance was conducted on a spectrophotometer at 520 nm. A standard curve for L-proline was to assay proline content. The proline expressed as µmol g^{-1} FW (fresh weight).

2.7. Total Phenolic Content

The colorimetric method was used to find the contents of total phenolic compounds of fresh leaves [26,27]. In a boiling water bath for 10 min, the extract (1 mL) was mixed with (10 drops) of strong hydrochloric acid and allowed to cool. Then, (1.5 mL) of sodium carbonate (14%) and (1 mL) of Folin–Ciocalteau reagent were combined. After thoroughly shaking the mixture, (5 mL) of distilled water was added. The combination was then placed in a bath of boiling water for 5 min. Using the pyrogallol standard curve, the data were reported as (mg g^{-1} FW) after the absorbance at 650 nm was measured.

2.8. Total Flavonoids

The colorimetric method was used to detect the total flavonoid content of wheat flag leaves [28]. The sample (0.5 g) was combined with (10 mL) of 80% ethanolic alcohol. The supernatant was collected after centrifugation at (2500× *g*) for 10 min, and extraction was carried out once more. Supernatants were concentrated in water at 45 °C to (5 mL). The proper dilutions of the sample extracts (2 mL) were reacted with (0.2 mL) of sodium nitrite 5% and, after 5 min, (0.2 mL) of 10% $AlCl_3$ formed a flavonoid–aluminum complex. Immediately after, 510 nm absorbance was evaluated using the catechin standard.

2.9. Protein Profile

Liquid nitrogen (N_2) was used to rapidly freeze 0.2 g of fresh leaves to create a protein extract, which was then used for protein profiling on an SDS-polyacrylamide gel [29]. Molecular weight markers (11–180 kDa) determined the molecular weights (Mwt) of the isolated proteins.

2.10. Statistical Analysis

One-way analysis of variance (ANOVA) was used to statistically analyze data via Minitab® 18.1 Statistical Data Document [30]. To compare means, the Tukey scale test was calculated at a 5% probability level. The data were presented as mean ± error of standard (n = 3).

3. Results

3.1. Morphological Characteristics

3.1.1. Plant Height, Root Length, Leaves Number, Flag Leaf Area, and Tillers Number

The presented data in Table 1 clarified how various morphological characteristics of wheat plants (plant height, root length, number of leaves, flag leaf area, and number of tillers) respond to putrescine, drought, and their interactions. Most characteristics of wheat plants were improved using putrescine at several concentrations (0.25, 0.5, and 1 mM) under typical conditions. On the other side, wheat plants grown under drought conditions showed reductions in plant height, number of leaves, flag leaf area, and tillers number by 18.33%, 35.72%, 23.28%, and 16.65%, respectively, except root length, which enhanced about 33.33%. Grain priming in putrescine at different concentrations improved wheat plants cultivated under drought conditions in comparison with the untreated ones. Putrescine at 1 mM significantly increased plant height, root length, leaves number, flag leaf area, and tillers number of drought-stressed wheat plants by 38.78%, 50%, 166.67%, 70.25%, and 79.96%, respectively, compared with untreated plants.

Table 1. The response of some growth characteristics of wheat plants to putrescine, drought stress, and their combination. Values depict the mean and standard error. Different letters indicate significance between means.

Treatments		Plant Height (cm)	Root Length (cm)	Leaves Number	Flag Leaf Area (cm^2)	Tillers Number
Irrigation	**Putrescine**					
Control	0.0 mM	60 ± 0.58 e	6.00 ± 0.1 d	4.67 ± 0.33 de	14.5 ± 0.35 cde	2.00 ± 0.1 bc
	0.25 mM	72 ± 1.15 a	7.33 ± 0.67 cd	8.33 ± 0.33 abc	18.1 ± 0.64 bc	2.33 ± 0.33 bc
	0.50 mM	74 ± 0.58 a	8.67 ± 0.33 bc	9.33 ± 0.33 ab	19.7 ± 1.11 ab	3.00 ± 0.1 b
	1.0 mM	71 ± 0.58 ab	9.67 ± 0.33 abc	10.33 ± 0.67 a	23.3 ± 1.92 a	4.67 ± 0.33 a
Drought	0.0 mM	49 ± 0.58 f	8.00 ± 0.1 bcd	3.00 ± 0.1 e	11.2 ± 0.73 e	1.67 ± 0.33 c
	0.25 mM	64 ± 0.58 d	9.00± 0.1 bc	5.67 ± 0.33 d	12.8 ± 0.24 de	2.0 0± 0.1 bc
	0.50 mM	66 ± 0.58 cd	10.0 ± 1.0 ab	6.67 ± 0.33 cd	15.8 ± 0.61 bcd	2.67 ± 0.33 bc
	1.0 mM	68 ± 0.58 bc	12.0 ± 0.58 a	8.00 ± 0.57 bc	19.0 ± 0.13 ab	3.00 ± 0.1 b

3.1.2. Weights of Fresh and Dry of Both Shoot and Root

The obtained results in Table 2 displayed the impact of putrescine, drought, and their interactions on weights of fresh and dry matter for shoots and roots. Individual application of putrescine at various levels (0.25, 0.5, and 1 mM) increased the mentioned parameters. The most effective treatment was putrescine at (1 mM), which increased shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight by 61.62%, 62.03%, 43.9%, and 90.66%, respectively. In the case of drought, the weight of fresh shoot, dry shoot, fresh root, and dry root of wheat were significantly inhibited by about 45.37%, 45.03%, 42.28%, and 49.56%, respectively. Application of the tested concentrations of putrescine on drought-stressed wheat plants led to marked enhancements in the fresh and dry weights for shoots and roots compared to the untreated ones. Putrescine at (1 mM) significantly enhanced the fresh and dry weights of shoot and root, by 99.43%, 98.43%, 97.8%, and 210.17%, respectively.

Table 2. The response of shoot and root weights of the wheat plant to putrescine, drought stress, and their combination. Values depict the mean and standard error. Different letters indicate different significance between means.

Treatments		Shoot Fresh Weight (g)	Shoot Dry Weight (g)	Root Fresh Weight (g)	Root Dry Weight (g)
Irrigation	**Putrescine**				
Control	0.0 mM	6.3 ± 0.13 cd	1.51 ± 0.03 cd	0.82 ± 0.08 bcd	0.27 ± 0.02 b
	0.25 mM	7.7 ± 0.49 bc	1.85 ± 0.12 bc	0.83 ± 0.03 bcd	0.30 ± 0.02 b
	0.50 mM	9.3 ± 1.01 ab	2.22 ± 0.24 ab	1.03 ± 0.03 ab	0.42 ± 0.03 a
	1.0 mM	10.2 ± 0.35 a	2.45 ± 0.08 a	1.18 ± 0.06 a	0.52 ± 0.02 a
Drought	0.0 mM	3.4 ± 0.04 e	0.83 ± 0.01 e	0.47 ± 0.02 e	0.14 ± 0.03 c
	0.25 mM	5.2 ± 0.35 de	1.25 ± 0.09 de	0.62 ± 0.01 de	0.23 ± 0.01 bc
	0.50 mM	6.4 ± 0.11 cd	1.54 ± 0.03 cd	0.77 ± 0.03 cd	0.27 ± 0.03 b
	1.0 mM	6.9 ± 0.53 cd	1.65 ± 0.13 cd	0.94 ± 0.07 bc	± 0.03 a

3.2. Photosynthetic Pigments

Efficacy of putrescine, drought, and their interactions on some pigments (chlorophylls and carotenoids) of wheat leaves (Figure 1). Under normal conditions, priming wheat plants with different concentrations (0.25, 0.5, and 1 mM) of putrescine resulted in significant enhancements in chlorophyll a, chlorophyll b, and carotenoids. Putrescine at 0.5 mM recorded the highest treatment via boosting chlorophyll a, chlorophyll b, and carotenoids of wheat leaves by 16.67%, 32.97%, and 55.17%, respectively. However, water deficit stress led to significant suppression in chlorophyll a (about 10.7%) and chlorophyll b (about 26.6%) and insignificant increases in carotenoid contents in the tested plants. Concerning the interaction between putrescine and drought, putrescine at 0.25, 0.5, and 1 mM significantly promoted chlorophylls and carotenoids in drought-stressed wheat plants.

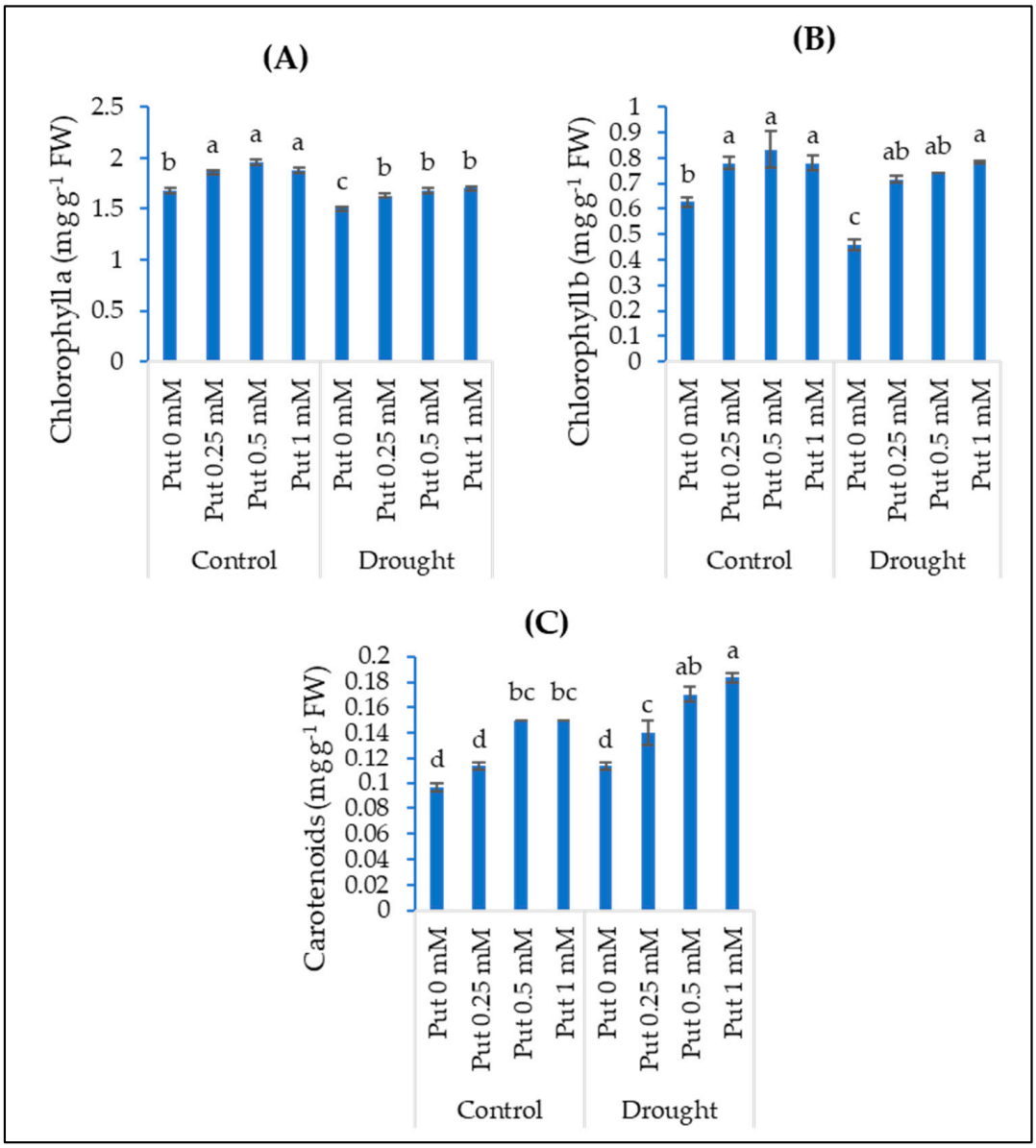

Figure 1. Change of chlorophyll a (**A**), chlorophyll b (**B**), and carotenoids (**C**) contents in wheat plants pretreated with putrescine, drought stressor, and their combination. Each bar shows mean ± standard error. Different letters indicate different significance between means.

3.3. Osmolytes (Soluble Sugars, Free Amino Acids, Free Proline)

The results in Figure 2 illustrated the efficacy of putrescine, drought, and their interactions on the amount of free amino acids, total soluble sugars, and proline of wheat plants. Individual treatment of putrescine at 0.25, 0.5, and 1 mM increased the contents of sugars, amino acids, and free proline. Putrescine at (1 mM) increased the mentioned parameters by 84.59%, 24.68%, and 56.37%, respectively. In contrast, drought stress caused significant enhancement in soluble sugars content (32.81%), inhibition in free amino acids content (7.47%), and non-significant enhancement in proline content (14.89%) compared with un-stressed plants. Regarding the role of putrescine on drought-stressed wheat plants, the tested concentrations reinforced the levels of total soluble sugars, free amino acids, and free proline. Putrescine at (1 mM) recorded the highest increase in sugars and amino acids by 49.07% and 42.69%, respectively, while 0.5 mM recorded the highest proline contents increase by 115.74%.

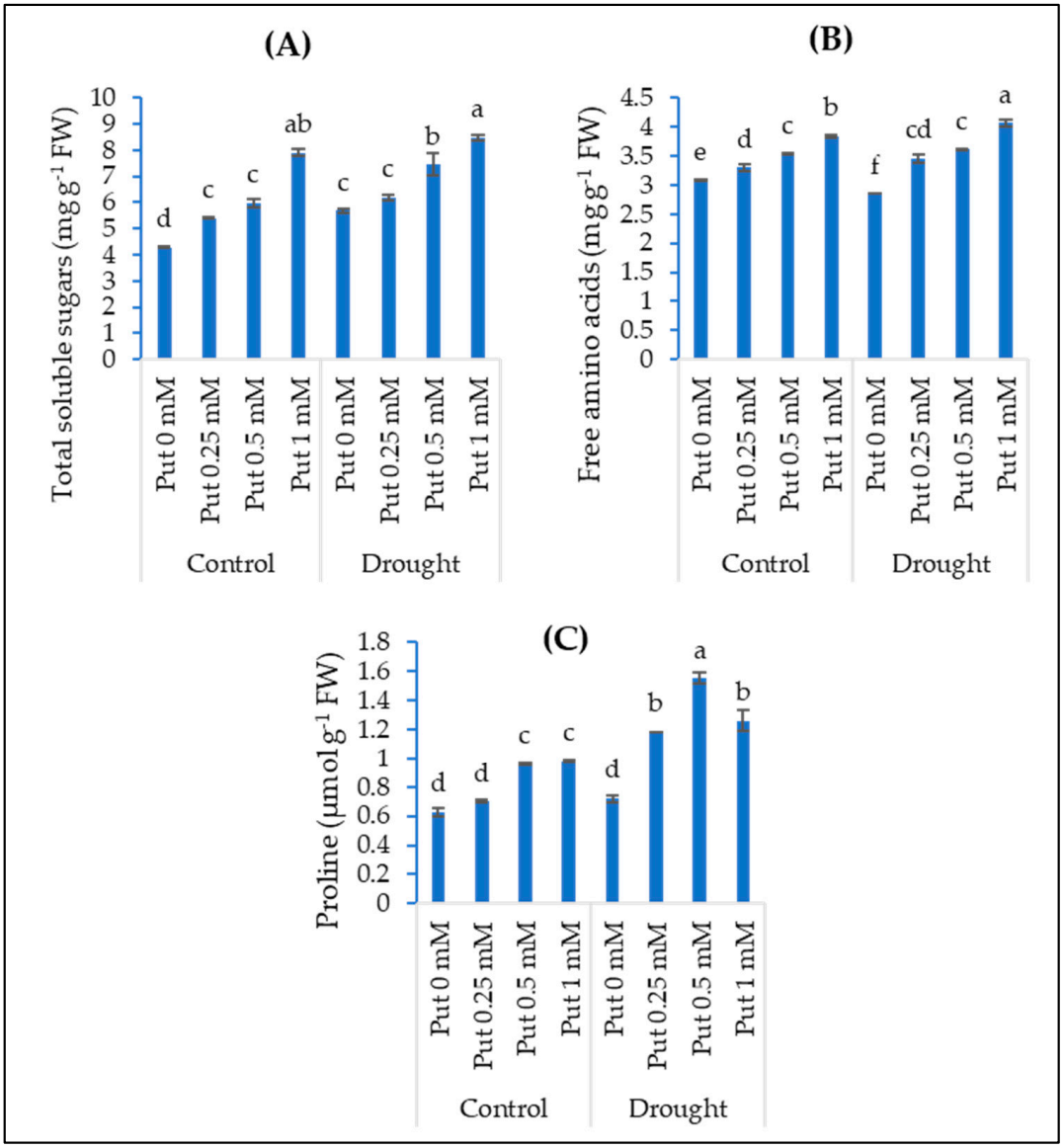

Figure 2. Change in soluble sugars (**A**), free amino acids (**B**), and free proline levels (**C**) in wheat plants pretreated with putrescine, drought stressor, and their combination. Each bar shows mean ± standard error. Different letters indicate different significance between means.

3.4. Phenols and Flavonoids

The efficacy of various levels (0.25, 0.5, and 1 mM) of putrescine, drought condition, and their interactions on wheat plants was seen in Figure 3. In normal conditions, treating wheat plants with putrescine at (1 mM) enhanced the highest phenols and flavonoid contents by 25.1% and 23.49%, respectively. Under drought conditions, the phenol contents of wheat plants decreased insignificantly, while flavonoid contents significantly increased by 36.99%. In the case of wheat plants that are grown under drought conditions, the application of putrescine at 0.25, 0.5, and 1 mM promoted phenols amount to 13.74%, 35.63%, and 52.37% while inhibiting flavonoids contents by 28.47%, 25.18%, and 19.71%, respectively.

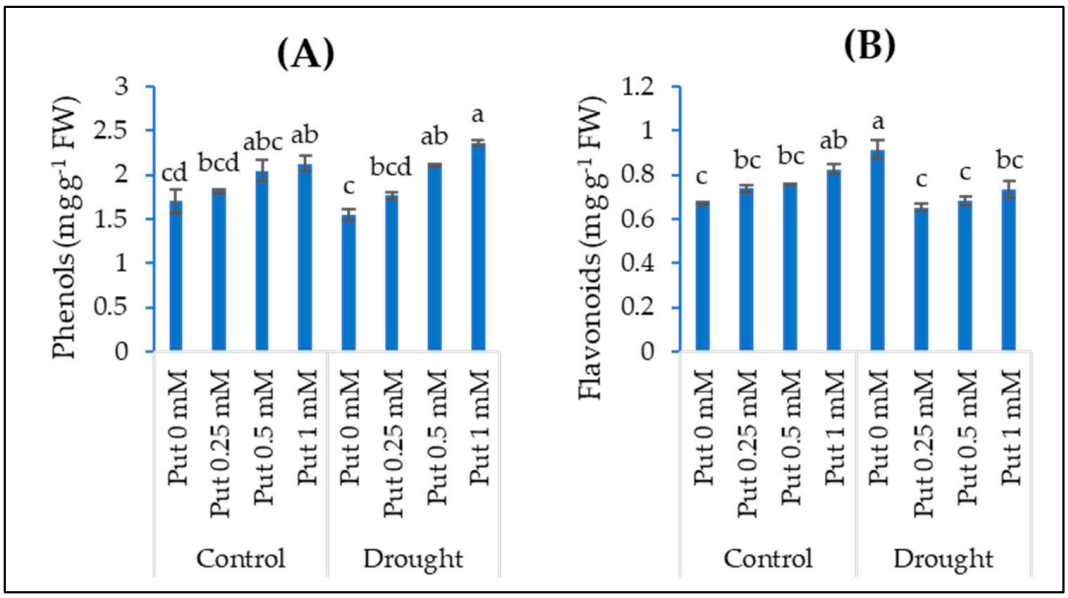

Figure 3. Change in total phenols (**A**) and total flavonoid (**B**) levels in wheat plants pretreated with putrescine, drought stressor, and their combination. Each bar shows mean ± standard error. Different letters indicate different significance between means.

3.5. Profile of Protein

Analysis of the protein pattern of wheat plants showed variations in the resulting bands shown in Figure 4 and Table 3. Results revealed 34 polypeptide bands with various molecular weights between 11 kDa and 180 kDa with a polymorphic rate of 26.47%. Concerning the stabilization of protein, there are 23 monomorphic bands. Water deficit stress led to variations in the profile of protein by inducing the synthesis or disappearance of some bands. Water deficit stress synthesized a unique band (54 KDa) while disappearing protein bands at 52 and 20 KDa. Furthermore, drought conditions and putrescine enhanced polymorphic polypeptides with 36 and 16 KDa regarding control plants. Moreover, under drought stress, the putrescine at (1 mM) formed the polymorphic polypeptide 110, 32, and 26 KDa in water deficit-stressed plants. Under drought stress, only putrescine at 0.5 mM promoted the appearance of the unique band at 64 KDa in water deficit-stressed plants and polymorphic bands 110, 63, 32, and 26. Under drought stress, only putrescine at 0.25 mM stimulated the appearance of the polymorphic bands at 46 and 63 KDa in water deficit-stressed plants.

Table 3. Change in protein pattern in putrescine-pretreated wheat plants under water deficit condition. L1, control; L2, 0.25 mM putrescine; L3, 0.5 mM putrescine; L4, 1 mM putrescine; L5, water deficit conditions; L6, water deficit condition combined with 0.25 mM putrescine; L7, water deficit condition combined with 0.5 mM putrescine; L8, water deficit condition combined with 1 mM putrescine.

No.	MW	L1	L2	L3	L4	L5	L6	L7	L8
1	180	+	+	+	+	+	+	+	+
2	135	+	+	+	+	+	+	+	+
3	118	+	+	+	+	+	+	+	+
4	110	-	-	-	-	-	-	+	+
5	100	+	+	+	+	+	+	+	+
6	80	+	+	+	+	+	+	+	+
7	70	+	+	+	+	+	+	+	+

Table 3. *Cont.*

No.	MW	L1	L2	L3	L4	L5	L6	L7	L8
8	66	+	+	+	+	+	+	+	+
9	65	+	+	+	+	+	+	+	+
10	64	-	-	-	-	-	-	+	-
11	63	-	-	+	-	-	+	+	-
12	61	+	+	+	+	+	+	+	+
13	60	+	+	+	+	+	+	+	+
14	54	-	-	-	-	+	-	-	-
15	52	+	+	+	+	-	-	-	-
16	48	+	+	+	+	+	+	+	+
17	46	-	-	+	-	-	+	-	-
18	37	+	+	+	+	+	+	+	+
19	36	-	+	+	+	+	+	+	+
20	34	+	+	+	+	+	+	+	+
21	33	+	+	+	+	+	+	+	+
22	32	-	-	-	-	-	-	+	+
23	31	+	+	+	+	+	+	+	+
24	30	+	+	+	+	+	+	+	+
25	28	+	+	+	+	+	+	+	+
26	26	-	-	-	-	-	-	+	+
27	23	+	+	+	+	+	+	+	+
28	21	+	+	+	+	+	+	+	+
29	20	+	-	-	-	-	-	-	-
30	18	+	+	+	+	+	+	+	+
31	17	+	+	+	+	+	+	+	+
32	16	-	+	+	+	+	+	+	+
33	15	+	+	+	+	+	+	+	+
34	12	+	+	+	+	+	+	+	+
Total		25	26	28	26	25	27	31	28

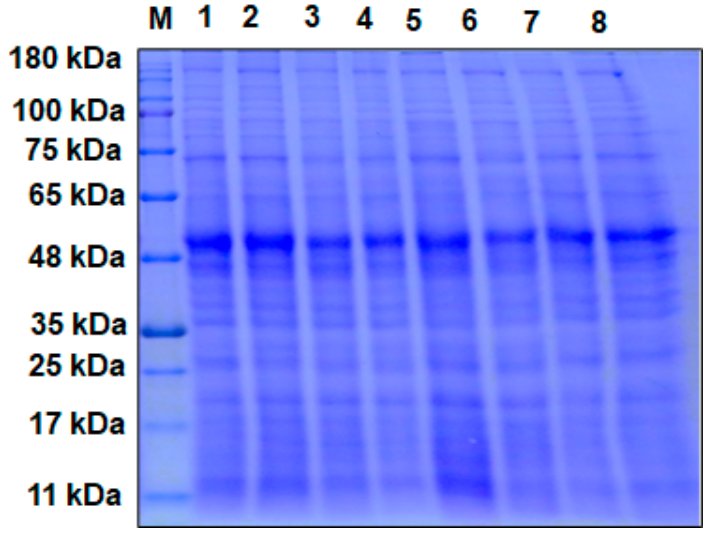

Figure 4. Change in protein pattern in putrescine-pretreated wheat plants under water deficit condition. L1, control; L2, 0.25 mM putrescine; L3, 0.5 mM putrescine; L4, 1 mM putrescine; L5, water deficit conditions; L6, water deficit condition combined with 0.25 mM putrescine; L7, water deficit condition combined with 0.5 mM putrescine; L8, water deficit condition combined with 1 mM putrescine.

3.6. Yield Characteristics

The results obtained in Table 4 clarified the efficacy of putrescine application, drought conditions, and their interactions with wheat plants. Utilizing putrescine at 0.25, 0.5, and 1 mM has promotive impacts on yield attributes of the grown plants under normal conditions. In the case of spike length, putrescine at 0.5 mM was the most effective treatment, while 0.25- and 0.5-mM concentrations recorded the same significant value in the number of spikelets. Furthermore, putrescine at (1 mM) recorded the highest increases in the number of grains, the weight of spike, and the weight of grains/spike, reaching 48.7%, 45.76%, and 94.03%, respectively. On the contrary, growing wheat plants in water deficit conditions led to significant decreases in most yield parameters; spike length by 28.58%, number of spikelets by 15.63%, number of grains by 30.26%, the weight of spike by 23.45%, and weight of (grains/spike) by 37.45%. Under drought stress, grains priming with putrescine (0.25, 0.5, and 1 mM), enhanced spike length (about 36%, 18%, and 32%), number of spikelets (about 33%, 33%, and 59%), number of grains (about 1.9%, 47%, and 81%), the weight of the spike (about 34%, 37%, and 45%), and the weight of the grains/spike (about 36%, 61%, and 48%), respectively.

Table 4. The response of yield components of the wheat plant to putrescine, drought stress, and their combination. Values depict the mean and standard error. Different letters indicate different significance between means.

Treatments		Spike Length	Number of Spikelets	Number of Grains	Weight of Spike (g)	Weight of Grains/Spike (g)
Irrigation	**Putrescine**					
Control	0.0 mM	11.67 ± 0.33 a	10.67 ± 0.33 bc	25.33 ± 0.67 c	1.18 ± 0.02 b	0.68 ± 0.04 cd
	0.25 mM	12.17 ± 0.60 a	14 ± 0.57 a	27.67 ± 1.45 bc	1.57 ± 0.02 a	0.92 ± 0.10 bc
	0.5 mM	12.67 ± 0.67 a	14 ± 1.15 a	31 ± 0.57 b	1.69 ± 0.08 a	1.11 ± 0.07 ab
	1.0 mM	11 ± 0.57 ab	13.33 ± 0.33 a	37.67 ± 1.45 a	1.72 ± 0.01 a	1.31 ± 0.10 a
Drought	0.0 mM	8.33 ± 0.33 b	9 ± 0.1 c	17.67 ± 0.33 d	0.90 ± 0.01 c	0.42 ± 0.03 d
	0.25 mM	11.33 ± 0.33 a	12 ± 0.1 ab	18 ± 0.57 d	1.21 ± 0.02 b	0.58 ± 0.03 d
	0.5 mM	9.83 ± 0.17 ab	12 ± 0.1 ab	26 ± 1 c	1.24 ± 0.04 b	0.68 ± 0.03 cd
	1.0 mM	11 ± 1.15 ab	14.33 ± 0.33 a	32 ± 0.57 b	1.31 ± 0.02 b	0.63 ± 0.03 cd

4. Discussion

Water deficit is a problem that mainly and directly threatens agriculture, as it negatively affects the growth and production of crops due to the imbalance between the number of reactive oxygen species and their antioxidants [31]. It is necessary to enhance the plant tolerance against the environmental stresses to survive. A possible approach is the application of polyamine, putrescine, or its derivatives, such as Spermine and Spermidine. Putrescine pre-treatment proved to have an effective, positive, and protective role in wheat plants against oxidative stress conditions, particularly drought [32].

Plant organs are highly affected to respond to abiotic stresses through changes in plant growth regulation. In agreement with our findings, a recent study by [33] showed that shoot and root as well as leaf area lengths were inhibited due to drought. Moreover, drought stress decreased Leaf areas compared with un-stressed maize plants [19] and growth traits (plant height, the number of leaves, and the leaf area) of cotton plants [34]. The inhibition in the earlier growth parameters could be related to the inhibition in cell turgor, which suppresses cell elongation and development [35]. Regarding putrescine application, a study by [36] described enhancements in plant height and leaf areas of wheat plants under normal conditions. Putrescine as a polyamine is considered a class of plant growth regulators [37]. Concerning the interaction between drought and putrescine impact, similarly, putrescine improved plant lengths and leaf areas of drought-stressed wheat plants [36]. Recently, putrescine at different concentrations could recover Korean ginseng from stress conditions by enhancing most morphological growth attributes [38]. Overall,

putrescine has a role in various hormonal pathways, scavenging reactive oxygen species, and adjusting osmotic balance [39,40].

In line with our results, drought stress decreased shoot and root weights (fresh and dry) for broccoli plants [33]. A study [19] submitted that fresh and dry weights of maize plants were significantly decreased due to exposure to drought conditions. Alfalfa plants exposed to drought stress decreased fresh and dry mass of shoots and roots compared with un-stressed plants [41]. In an earlier study, putrescine increased the shoot and root weights in wheat [42] and geranium plants [16]. Putrescine had a beneficial impact on the morphological growth aspects [43]. In this respect, putrescine treatments enhanced the growth parameters of cotton plants compared with untreated plants [44]. Polyamines had a role in various physiological activities, including embryonic development, root growth, organogenesis, floral differentiation, fruit ripening or apoptosis, and plant defense responses. Its cationic nature explains most of its biological activity [45–48].

Drought stress negatively affects the photosynthetic pigments in wheat plants. It reduces the photosynthetic pigments and transpiration and, thus, negatively affects plant productivity [49,50]. In agreement with our results, drought-stressed alfalfa plants declined the contents of chlorophyll a and chlorophyll b compared to un-stressed plants [43]. Additionally, drought conditions caused significant reductions in chlorophyll content [51]. In an earlier study, the contents of leaf pigments of cotton plants as chlorophyll a, chlorophyll b, and total chlorophylls were decreased due to drought stress exposure [44]. In a recent study, such enhancement in carotenoids might improve the antioxidant activity that acts as non-enzymatic antioxidants produced under unfavorable conditions [3], while treating wheat plants with various concentrations of putrescine augmented the contents of chlorophyll a, chlorophyll b, and carotenoids [41]. Putrescine treatment promoted chlorophyll pigments in geranium foliage. The author suggested that such an increment in photosynthetic pigments might be due to the activity of putrescine as anti-senescence [16]. The application of putrescine increased the levels of photosynthetic pigments in several studies [52,53]. On cotton plants, the authors attributed the enhancements of the photosynthetic pigments to the positive impact of polyamines in keeping thylakoid and chloroplast structures [38,44].

One of several mechanisms in the plant to mitigate and counteract the harmful effects of stress is osmoprotectants accumulation, which reduces oxidative damage and enhances plant tolerance. In an earlier study on the impact of drought, the total sugars and proline of cotton plants were enhanced when plants were grown under drought conditions [34]. Quinoa plants subjected to water deficit resulted in marked increases in the contents of total soluble sugars and free proline [54]. Moreover, Free proline contents were increased in common beans after polyethylene glycol (drought inducer) treatment [55]. Our findings agree with the results of [51], which found a reduction in the levels of free amino acids in wheat plants due to exposure to drought. Putrescine enhanced soluble sugars, proline, and amino acids in wheat plants [42]. 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 [44]. Several investigations showed that putrescine was included in various physiological and metabolic processes [56–58]. Recently, total soluble sugars and free proline in stressed Korean ginseng plants were enhanced after the putrescine application [38]. 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 [15,59–61]. Increased total free amino acids may control osmotic adjustment, safeguard cellular macromolecules, store nitrogen, and preserve cellular pH [62].

Plant cells use flavonoids and phenolics as non-enzymatic antioxidants to scavenge reactive oxygen species produced due to stress and increase plant tolerance [63]. A study by [64] documented increases in the contents of flavonoids in marigold plants grown under water stress, while putrescine treatments increased total phenols content in cotton plants [44] and *Thymus vulgaris* plants [35]. Moreover, maize plants increased total phenolic compounds and flavonoids in response to polyamine treatment compared to the con-

trol [62]. The promotion of flavonoids level under water stress conditions was previously documented in several studies [3,51,65]. In drought-stressed maize plants, the contents of phenolic compounds and flavonoids accumulated due to polyamine treatment [62]. Flavonoids are poly-phenolic compounds having an antioxidant ability in plants [64]. Different putrescine concentrations increased the total soluble phenolics in drought-stressed thyme plants compared to untreated plants [35]. This response may be due to the catalytic putrescine effect on photosynthesis, the main source of phenolic compounds via the shikimic acid pathway [66–68].

Regarding the protein pattern, our findings predicted nine stress-responsive genes related to putrescine action in response to water deficit conditions. Put-induced proteins act as a defense line to alleviate the damage effects of drought stress. The synthesis of new proteins might be due to the effectiveness of putrescine in stimulating vital physiological processes, such as nitrogen metabolism and polyamine synthesis [69]. Further, variation in protein expression in wheat plants growing under water deficit stress might be due to PAs protecting the Rubisco protein in wheat [70].

At yield and its components, water deficit application significantly reduced the yield attributes of tomatoes [71]. Similarly, drought stress reduced the yield components of cotton (e.g., open bolls number, seed cotton yield/plant, fiber length, and fiber strength) [34]. In agreement with our findings, the results of [72] documented significant decreases in yield components (grain yields, length of spike, number of grains, and spikelet per spike) of wheat plants under drought conditions. Water deficiency adversely affects the plant growth and productivity of crops. The suppression in growth and yield may be related to the overproduction of reactive oxygen species that caused damage to membranes and cell components [73,74]. Results of [75] documented that putrescine (1.25 and 2.5 mM) enhanced growth, development, and all yield attributes of wheat crops. The application of putrescine led to improvements in the yield of wheat and cotton [36,44]. These improvements are due to enhancing photosynthetic pigments, sugars, amino acids, and proline. Under drought stress, polyamines (spermine, spermidine, and putrescine) on wheat plants enhanced the filling of grains and the weight of grains [76] as well as yield and its components (number of totals, open and closed bolls, boll weight, seed yield/plant, and seed index) of stressed cotton plants [77].

5. Conclusions

The current study concluded how wheat plants respond to exposure to water deficiency conditions (50% water irrigation based on field capacity) and putrescine (0.25, 0.5, and 1 mM). Exposure to drought stress caused harmful effects on growth parameters, yield, and metabolic activities via increasing oxidative stress. Meanwhile, putrescine treatment clarified improvements in the performance of morphology, pigments, proline, amino acids, sugars, phenolics, flavonoids, and yield aspects, which help wheat plants in facing drought stress conditions. It is worth noting that putrescine (1 mM) was the most effective among the tested concentrations. Based on its pivotal role, putrescine is one of the safe solutions for plants to cope with water stress conditions. Grain priming in putrescine is a promising technique to save water irrigation and induces drought defense mechanisms.

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