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Response of *Triticum aestivum* (L.) Plants Grown Under Cadmium Stress to Polyamines Pretreatments

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Abstract: The role of exogenously-applied polyamines [i.e., spermine (Spm), spermidine (Spd) and putrescine (Put)] in the improvement of cadmium (Cd^{2+}) tolerance in wheat plants, and their effects on growth, yield and its components and changes in the osmoprotectant and endogenous Cd^{2+} concentrations and the contents of some nutrients in plants grown under 2.0 mM Cd^{2+} stress were assessed. The efficiency of wheat plants to tolerate Cd^{2+} stress in terms of growth and yield characteristics was noticed to varying degrees with the three applied polyamines. The reasonable growth of Cd^{2+}-stressed seedlings and consequently acceptable grain yield was correlated with the improvements in the concentrations of osmoprotectants and tissue health in terms of relative water content (RWC) and membrane stability index (MSI), and reductions in electrolyte leakage (EL) and tissue Cd^{2+} concentration. Results show that, seed soaking in 0.25 mM Spm, 0.50 mM Spd or 1.0 mM Put generated significant better growth and yield characteristics, MSI, RWC, leaf photosynthetic pigment and osmoprotectant concentrations, and nutrient contents than seed soaking with water under 2.0 mM Cd^{2+} stress. In contrast, the Cd^{2+} concentration and EL were significantly reduced. However, the Cd^{2+}-free control was the best treatment when compared to the all other stressed treatments. Seed soaking in 1.0 mM Put was the best, generating wheat plants that most tolerant to Cd^{2+} stress than those generated from the other two polyamines. Therefore, this study recommend to use the 1.0 mM Put, as seed soaking treatment for wheat to grow well under Cd^{2+} stress.

Keywords: Wheat, Cadmium, Polyamines, Osmoprotectants, Growth, Yield

1. Introduction

Worldwide, wheat (*Triticum aestivum* L.) is one of the most important cereal crops used principally as human food. It provides 37% of the total calories and 40% of the protein in the Egyptian people diet [1]. Recently, a great attention of several Egyptian investigators has been directed to increase the productivity of wheat to minimize the gap between the production and consumption. This may be achieved through the increase of unit land area productivity and the increment of cultivated area. Increase of wheat yield per unit of area can be achieved by using the high yielding bred varieties and applying the optimum cultural practices [2].

Cadmium (Cd^{2+}) is believed as one of the most important contaminant in the ecosphere. The main sources of Cd^{2+} in environment are mining and smelting of Cd^{2+}-containing ores, municipal wastes, pesticides, trace emissions, burning of fossil fuels and phosphorus fertilizers [3, 4]. It has been reported previously that toxic metals have the ability to contaminate various ecosystems via different sources including industrial wastewater disposal, agricultural chemicals, mining, geogenic, etc. [5]. Cadmium is not an essential nutrient and it is one of the heavy metals that are known to generate toxicity even at a very low concentration. It accumulates in plants during growth in edible parts, thereby, endangering crop yields and their qualities, causing a potential hazard to human and animal health. It causes enzyme inactivation and damages cells by acting as antimetabolite or forms precipitates or chelates with a number of essential metabolites [6]. With no biological function but extremely toxic, soil Cd^{2+} pollution can result in various problems. Such problems are inhibition of plant growth, photosynthesis and ATPase of plasma membrane, retardation of chlorophyll biosynthesis, reduction of nutrient uptake, alteration of water balance and ion homeostasis, reduction of various enzymes activities and stimulation of stomatal closure. These problems cause poor quality of products, loss of yield, and ultimately metal toxicity to animals and humans [7–10].

Several treatments have been applied to alleviate plant Cd^{2+} toxicity [11–14]. Polyamines (PAs) are lightweight-molecular molecules that are present in all living organisms. They are known to be essential for growth and development in prokaryotes and eukaryotes especially in plants. Putrescine (Put), spermidine (Spd) and spermine (Spm) are the major PAs in plants. Put and Spd are usually more abundant than Spm, which is often present at trace amounts [15]. These PAs are not only involved in numerous cellular and molecular processes, but also in their responses to environmental stresses such as heavy metals stresses [16, 17]. They are effective radical scavengers in a number of chemical and enzyme-systems [18]. In engineered plants, tolerance to multiple environmental stresses is enhanced by an overproduction of PAs [19, 20].

It was hypothesized that exogenous application of PAs (e.g., Spm, Spd and Put), used as seed soaking, improves wheat plants growth and productivity when grown under Cd^{2+} stress. Therefore, the aim of the present study was to assess the improvements in growth characteristics, physio-chemical attributes, and yield and its components of wheat plants grown under 2 mM Cd^{2+} stress.

2. Materials and Methods

2.1. Plant Material, Growing Conditions and Treatments

Seeds of wheat (*Triticum aestivum* L., cv. Sakha 93) were obtained from the Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. They were surface sterilized with a 1% sodium hypochlorite solution for two minutes and thoroughly washed several times with distilled water, and left to air-dry. The sterilized seeds were then soaked for 8 h at room temperature, either in distilled water (as a control), 0.25 mM spermine (Spm), 0.50 mM spermidine (Spd) or in 1.0 mM putrescine (Put). The selection of these polyamine concentrations was based on the best response through our preliminary studies (data not shown). After air-drying overnight, 20 water- or plyamine-treated seeds were sown, on 1st of November, 2013 and 1st of December, 2014 in each plastic pot (40 cm in diameter, 50 cm depth), previously filled by 15 kg sand for each pot. Sand soil used in this study was washed in each of the two seasons with commercial HCl (10% conc.) for 24 h to remove all anions and cations, then was washed with distilled water for several times to remove the excess of acid. Pots (n = 120 for five treatments) were arranged for growing plants in an open greenhouse at the Experimental Farm of the Faculty of Agriculture, Dar El-Ramad, Fayoum, Egypt. The water-soaked seeds were divided into two groups for two treatments. One of them was irrigated with Cd^{2+}-free nutrient solution (control). The second one was irrigated with Cd^{2+}-containing nutrient solution as in the other three treatments. The average day and night temperatures were 19 ± 3°C and 10 ± 2°C, respectively. The relative humidity ranged from 62.0% to 65.1%, and day-length ranged from 10 to 11h. Half-strength Hoagland's nutrient solution as shown in Table 1 [21] was used for the two experiments. The $CdCl_2$-free nutrient solution was supplied every 2 days to all pots up to complete emergence. Excess solution was drained through holes in the base of the pots. At this stage (15 days after sowing), seedlings were thinned to 10 in each pot, and 2 mM Cd^{2+}, using $CdCl_2$, was added to the ½-strength Hoagland's nutrient solution. Each pot was supplemented every 3 days with 1 L of Cd^{2+}-containing Hoagland's nutrient solution. The 2 mM Cd^{2+} dose was also selected based on our preliminary studies (data not shown). This dose was greatly affected wheat seedling growth. The Cd^{2+} concentration in the medium was maintained at 2 mM by using an inductively coupled plasma atomic emission spectrometry (ICP- AES, IRIS-Advantype, Thermo, USA). Initial soil pH was 5.5, but it was corrected to 6.8 by adding 3 g of $CaCO_3$ per pot. The pH of the nutrient solution was adjusted to 7, with diluted HCl or NaOH. The experimental layout was completely randomized design with 24 replicates/pots (each pot represents one replicate) for each of the five treatments (5 treatments × 24 replicates/pots × 10 seedlings in each pot = 1200 seedlings for each experiment). The experiment was continued up to harvest, but the irrigation with Cd^{2+}-containing nutrient solution was terminated after 60 days from sowing after exposing the seedlings to Cd^{2+} stress for 60 days/20 irrigations. The 75-day-old seedlings from each treatment were collected for various growth and physio-chemical measurements, while yield and its components assessments were conducted at harvest stage.

Table 1. *Contents of the Hoagland–Arnon nutritive solution.*

Components	Concentrations (mg L^{-1})
KNO_3	1020.00
$Ca(NO_3)_2$	492.00
$NH_4H_2PO_4$	230.00
$MgSO_4.7H_2O$	420.00
H_3BO_3	2.86
$MnCl_2.4H_2O$	1.81
$H_2MoO_4.H_2O$	0.09
$FeSO_4.7H_2O$	0.07
$(CHOH)_2(COOH)_2$	0.02

2.2. Plant Growth, and Yield and Its Components Assessments

The 75-day-old wheat plants were removed from their pots and moved smoothly to remove the adhering sand particles by dipped them in a bucket filled with water. The length of shoots was measured by using a meter scale and leaves number per plant were counted. Plants were weighed to record their fresh weights, then placed in an oven at 70°C to reach a constant dry weights (DW).

At harvest stage (150 days after sowing), spikes of plants in all pots were collected to determine yield and its components through assessing the spike length, number of grains per spike, grains weight per plant and 1000-grain weight.

2.3. Determination of Leaf Photosynthetic Pigments

Total chlorophylls and total carotenoids were extracted from leaves in 80% acetone, and their concentrations were analyzed spectrophotometrically by the method of [22].

2.4. Determination of Membrane Stability Index (MSI), Relative Water Content (RWC) and Electrolyte Leakage (EL)

Estimation of MSI was done as described in [11]. Duplicate 0.2 g samples of leaf tissue were placed in test tubes containing 10 ml of double-distilled water. One sample was heated at 40°C in a water bath for 30 min, and the solution electrical conductivity was taken (EC_1). The second sample was boiled at 100°C for 10 min, and the conductivity was also measured (EC_2). The MSI was calculated using the following formula: MSI (%) = [1 − (C1/ C2)] × 100

Fresh 2 cm-diameter fully-expanded leaf discs were taken to estimate the RWC after the exclusion of midribs. The fresh weight of discs (FW) were recorded and immediately immersed in double-distilled water in Petri dishes for 24 h, in the dark, to reach the saturation and the turgid weight (TW) was measured. After dehydration of the discs at 70°C for 48 h, the dry weight (DW) was recorded. The RWC was then calculated using the formula in [23]: RWC (%) = [(FM − DM) / (TM − DM)] × 100

The technique in [24] is based on the increase of cellular membrane permeability and concomitantly greater electrolyte diffusion out of cells when tissue is injured by a stress. The leaf EL was determined using twenty leaf discs that were placed in a boiling tube containing 10 ml deionized water and the electrical conductivity (EC_1) was recorded. The tube contents were heated to 45°C − 55°C for 30 min in a water bath and the electrical conductivity (EC_2) was taken. The tube contents were then boiled at 100°C for 10 min and the electrical conductivity (EC_3) was written. The EL was calculated using the following formula: EL (%) = $[(EC_2 - EC_1) / EC_3] \times 100$

2.5. Determination of Macronutrients and Cadmium (Cd^{2+})

The wet digestion of 0.1 g of fine dried material of plants was conducted using sulphuric and perchloric acid mixture as mentioned in [25]. Phosphorus (%) was colorimetrically determined using chlorostannus molybdo-phosphoric blue color method in sulphuric acid system as described in [26]. Potassium (%) was determined using a Perkin-Elmer, Flam photometer [27]. Nitrogen (%) was determined in powdery dried material of plants by Orange G dye, colorimetric method described in [28]. The powdery dried plant samples were ashed at 500°C for 12 h to determine the Cd^{2+} ion concentration. The ashed samples were dissolved in 3.3% HNO_3 (v/v). The concentration of Cd^{2+} was measured by inductively coupled plasma optical emission spectroscopy (ICP-OES, Varian, and Australia). Measurement of Cd^{2+} in plants were checked against certificated Cd^{2+} value in different reference plant materials obtained from the National Institute of Standards and Technology (Gaithersburg, USA).

2.6. Determinations of Free Proline, Total Free Amino Acids and Protein Concentrations

Free proline of plant samples was extracted by sulfosalicylic acid (3%), and its concentration was then determined (mg g^{-1} dry weight) colorimetrically using acid ninhydrin reagent as outlined by [29].

Total free amino acids were extracted from plants using 80% ethanol, and their concentrations were then determined (mg g^{-1} dry weight) colorimetrically using ninhydrin reagent as mentioned by [30].

Protein concentration was calculated (mg g^{-1} dry material) by multiplying nitrogen concentration by 6.25.

2.7. Statistical Analysis

All obtained data were statistically analyzed by the technique of ANOVA for the completely randomized design using MSTAT-C (Michogen, USA), and LSD at 5% level of probability was used to test the differences between treatment means.

3. Results

3.1. Effect of Seed Soaking in Different Polyamines on Vegetative Growth of Wheat Plants Grown Under Cadmium Stress

Cd^{2+}-treated plants represented significant reductions in the growth characteristics (i.e., plant height, number of leaves per plant, plant fresh weight and plant dry weight) compared to the controls over two studied seasons (Table 2). These reductions were 58.4, 57.2, 61.6 and 72.6%, respectively in 2013/2014 season and were 55.0, 55.0, 67.7 and 70.6%, respectively in 2014/2015 season. Under Cd^{2+} stress, Spm-, Spd- or Put-pretreated plants showed significant increases in the mentioned growth characteristics compared to the corresponding untreated plants in both growing seasons. The Put was observed to be the best pretreatment, alleviating the deleterious effects of Cd^{2+} stress and significantly increased these growth traits compared to Cd^{2+}-stressed plants. The increases were 89.1, 83.5, 82.7 and 165.4%, respectively for the first season, and were 55.3, 84.8, 120.5 and 135.0%, respectively for the second season.

Table 2. *Effect of seed soaking in polyamines [spermine (Sp), spermidine (Spd) and putrescine (Put)] on vegetative growth of wheat plants grown under cadmium stress.*

Treatments	Plant height (cm)	No. of leaves plant^{-1}	Plant fresh weight (g)	Plant dry weight (g)
2013/2014				
Control	30.8a	4.67a	15.52a	3.91a
Cd^{2+}	12.8d	2.00d	5.96d	1.07d
Cd^{2+} + Sp	22.8c	3.30c	8.84c	1.97c
Cd^{2+} + Spd	22.8c	3.36c	9.26c	2.19c
Cd^{2+} + Put	24.2b	3.67b	10.89b	2.84b
2014/2015				
Control	33.3a	4.53a	15.84a	0.68a
Cd^{2+}	15.0d	2.04d	5.11d	0.20d
Cd^{2+} + Sp	22.2c	3.38c	9.70c	0.37c
Cd^{2+} + Spd	22.6c	3.45c	10.13c	0.38c
Cd^{2+} + Put	23.3b	3.77b	11.27b	0.47b

3.2. Effect of Seed Soaking in Different Polyamines on Yield and Its Components of Wheat Plants Grown Under Cadmium Stress

Data in Table 3 reveal that Cd^{2+}-treated plants showed significant reductions in the yield and its components (i.e., spike length, number of grains per spike, grains weight per plant and 1000-grain weight) compared to the controls over both 2013/2014 and 2014/2015 seasons. These reductions were 65.9, 45.1, 62.7 and 50.9%, respectively in the first season and were 62.2, 44.1, 66.8 and 50.0%, respectively in the second season. Under Cd^{2+} stress, Spm-, Spd- or Put-pretreated plants exhibited significant increases in the spike length, number of grains per spike, grains weight per plant and 1000-grain weight compared to the corresponding untreated plants in both growing seasons. The Put was noticed to be the efficient pretreatment, mitigating the injurious effects of Cd^{2+} stress and significantly increased these yield characteristics compared to Cd^{2+}-stressed plants. The increases were 75.8, 33.1, 85.1 and 55.6%, respectively for the 1st season, and were 70.4, 34.0, 90.5 and 58.3%, respectively for the 2nd season.

Table 3. *Effect of seed soaking in polyamines [spermine (Sp), spermidine (Spd) and putrescine (Put)] on yield and its components of wheat plants grown under cadmium stress.*

Treatments	spike length (cm)	No. of grains spike^{-1}	1000-grain weight (g)	Grains weight plant^{-1} (g)
2013/2014				
Control	7.63a	23.67a	25.16a	1.10a
Cd^{2+}	2.60d	13.00d	9.41d	0.54d
Cd^{2+} + Sp	3.57c	16.20c	15.12c	0.78c
Cd^{2+} + Spd	3.97c	16.67c	14.29c	0.81bc
Cd^{2+} + Put	4.57b	17.25b	17.36b	0.84b
2014/2015				
Control	7.40a	26.33a	28.55a	1.20a
Cd^{2+}	2.80d	14.67d	9.46d	0.60c
Cd^{2+} + Sp	3.30c	18.50c	17.68c	0.90b
Cd^{2+} + Spd	4.03c	18.87c	16.03c	0.91b
Cd^{2+} + Put	4.77b	19.73b	18.12b	0.95b

3.3. Effect of Seed Soaking in Different Polyamines on Pigments and Tissue Health of Wheat Plants Grown Under Cadmium Stress

Data in Table 4 exhibit that leaf photosynthetic pigments and tissue health of Cd^{2+}-treated plants were significantly decreased compared to those of the controls over both growing seasons. The reductions in total chlorophyll concentration (Chls), total carotenoids concentration (Car), membrane stability index (MSI) and relative water content (RWC) were 31.9, 53.3, 29.4 and 22.2%, respectively in 2013/2014 season and were 40.4, 52.9, 29.7 and 22.2%, respectively in 2014/2015 season. In contrast, the electrolyte leakage (EL) was behaved the reverse trend, where it increased by 193.1% in the first season and by 137.2% in the second season compared to the control. Under Cd^{2+} stress, Spm-, Spd- or Put-pretreated plants showed significant increases in the Chls, Car, MSI and RWC, and revealed significant reductions in the EL compared to the corresponding untreated plants in both growing seasons. The Put was found to be the best pretreatment, overcoming the adverse effects of Cd^{2+} stress and significantly increased the Chls, Car, MSI and RWC compared to Cd^{2+}-stressed plants.

The increases were 59.4, 142.9, 12.9 and 5.0%, respectively for the 1st season, and were 80.6, 162.5, 10.9 and 3.6%, respectively for the 2nd season. While, El was reduced by Put pretreatment by 57.8 and 53.7% in both growing seasons, respectively.

Table 4. *Effect of seed soaking in polyamines [spermine (Sp), spermidine (Spd) and putrescine (Put)] on leaf photosynthetic pigments, membrane stability index (MSI%), relative water content (RWC%) and electrolyte leakage (EL%) of wheat plants grown under cadmium stress.*

Treatments	Total chorophylls (mg g^{-1} FW)	Total carotenioides (mg g^{-1} FW)	MSI (%)	RWC (%)	EL (%)
2013/2014					
Control	0.94c	0.15c	67.8a	90.2a	4.47b
Cd^{2+}	0.64d	0.07d	47.9c	70.2d	13.16a
Cd^{2+} + Sp	1.02b	0.17b	54.8b	77.9bc	5.44b
Cd^{2+} + Spd	1.10a	0.19a	55.2b	80.0b	5.49b
Cd^{2+} + Put	1.02b	0.17b	54.1b	73.7cd	5.55b
2014/2015					
Control	1.04c	0.17d	69.4a	90.1a	5.97b
Cd^{2+}	0.62d	0.08e	48.8c	70.1c	14.16a
Cd^{2+} + Sp	1.10b	0.20c	55.7b	78.9b	6.40b
Cd^{2+} + Spd	1.20a	0.22a	54.5b	81.0b	6.48b
Cd^{2+} + Put	1.12b	0.21b	54.1b	72.6c	6.56b

3.4. Effect of Seed Soaking in Different Polyamines on Nutritional Status and Cadmium Concentration of Wheat Plants Grown Under Cadmium Stress

Data in Table 5 exhibit that Cd^{2+}-treated plants showed significant decreases in the contents of nitrogen (N), phosphorus (P) and potassium (K), and revealed significant increases in Cd^{2+} ion concentrations compared to the controls over both 2013/2014 and 2014/2015 seasons. The reductions in N, P and K contents were 10.6, 62.3 and 50.5%, respectively and the increase in Cd^{2+} concentration was 16150% in the 1st season and was 10.5, 56.3 and 52.5%, respectively and the increase in Cd^{2+} concentration was 16.54% in the 2nd season. Under Cd^{2+} stress, Spm-, Spd- or Put-pretreated plants showed significant increases in the N, P and K contents, and revealed significant reduction in the Cd^{2+} concentration compared to the corresponding untreated plants in both growing seasons. The Spd was found to be the best pretreatment in this concern.

Table 5. *Effect of seed soaking in polyamines [spermine (Sp), spermidine (Spd) and putrescine (Put)] on the contents of some macro-nutrients and cadmium (Cd^{2+}) of wheat plants grown under cadmium stress.*

Treatments	Plant N (%)	Plant P (%)	Plant K (%)	Plant Cd^{2+} (mg kg^{-1} DW)
2013/2014				
Control	3.96a	0.77a	6.79a	0.12d
Cd^{2+}	3.54d	0.29d	3.36d	19.5a
Cd^{2+} + Sp	3.67c	0.60b	6.24b	17.0b
Cd^{2+} + Spd	3.75c	0.65b	6.48ab	13.5c
Cd^{2+} + Put	3.90a	0.51c	5.81c	16.3b
2014/2015				
Control	4.00a	0.71a	6.99a	0.13e
Cd^{2+}	3.58c	0.31d	3.32d	21.0a
Cd^{2+} + Sp	3.70b	0.54c	6.27c	17.5b
Cd^{2+} + Spd	3.78b	0.63b	6.61b	13.8d
Cd^{2+} + Put	3.95a	0.49c	5.99c	16.3c

3.5. Effect of Seed Soaking in Different Polyamines on Proteins, Free Proline and Free Amino Acids Concentrations in Wheat Plants Grown Under Cadmium Stress

Cd^{2+}-treated plants showed significant reductions in the concentration of protein, while revealed significant increases in the concentrations of free proline and total free amino acids compared to the controls over two studied seasons (Table 6). The reduction in protein concentration was 21.5%, and the increases in free proline and total free amino acids concentrations were 32.0 and 43.6%, respectively in 2013/2014 season and the reduction in protein concentration was 22.6%, and the increases in free proline and total free amino acids concentrations were 25.0 and 46.3%, respectively in 2014/2015 season. Under Cd^{2+} stress, Spm-, Spd- or Put-pretreated plants showed significant increase in protein concentration and further increases in free proline and total free amino acids concentrations compared to the corresponding untreated plants in both growing seasons. In general, the Put was found to be the best pretreatment, alleviating the deleterious effects of Cd^{2+} stress and significantly increased the concentrations of protein, free proline and total free amino acids compared to Cd^{2+}-stressed plants.

Table 6. *Effect of seed soaking in polyamines [spermine (Sp), spermidine (Spd) and putrescine (Put)] on the concentrations of protein, free proline and free amino acids of wheat plants grown under cadmium stress.*

Treatments	Average total protein in plant (mg g^{-1} DW)	Average free proline in plant (mg g^{-1} DW)	Average total free amino acids in plant (mg g^{-1} DW)
2013/2014			
Control	13.0a	0.25d	2.34d
Cd^{2+}	10.2c	0.33c	3.36a
Cd^{2+} + Sp	12.0b	0.46ab	2.96c
Cd^{2+} + Spd	12.2b	0.49a	3.25ab
Cd^{2+} + Put	12.7a	0.44b	3.18b
2014/2015			
Control	13.3a	0.28c	2.42c
Cd^{2+}	10.3c	0.35b	3.54a
Cd^{2+} + Sp	12.2b	0.52a	2.77b
Cd^{2+} + Spd	12.3b	0.50a	3.40a
Cd^{2+} + Put	12.8a	0.48a	3.36a

4. Discussion

A pronounced reduction in wheat plant growth characteristics (i.e., plant height, number of leaves per plant, and plant fresh and dry weights; Table 1) due to the presence of cadmium (Cd^{2+}; 2 mM) in the growth medium was observed. This decrease in wheat growth, and consequently the reduction of plant vigor and the inhibition of plant growth may be attributed to Cd^{2+}-induced physiological disruptions [14, 31, 32]. It has been concluded that inhibition in cell division and cell elongation rate, and reduction in the plant biomass production are imposed by the presence of Cd^{2+} ions in wheat growing medium. This mainly occurs due to an everlasting inhibition of proton pump responsible for these processes [33]. Wheat plant growth reduction was associated with Cd^{2+}-induced increase in endogenous Cd^{2+} ion concentration (Table 5), reduction in leaf photosynthetic pigments and loss in plant tissue health in terms of decreases in relative water content (RWC) and membrane stability index (MSI), and increase in electrolyte leakage (EL; Table 4). These results indicate that wheat plants had reduced Cd^{2+} stress-tolerance, particularly at the level of 2 mM. This reduction in wheat Cd^{2+} stress-tolerance may be attributed to the increased Cd^{2+} absorption by roots and its translocation to the shoots in abundant amounts, affecting adversely the cell physiological and biochemical processes through Cd^{2+} oxidative damage to cellular components [14]. To alleviate and repair the damage caused by Cd^{2+}-induced reactive oxygen species (ROS), the exogenous application of polyamines (PAs) found, in this study, to enable wheat plants to develop different cellular defense strategies against Cd^{2+}-induced oxidative stress such as the increase in antioxidants (i.e., carotenoids; Table 4) and osmoprotectants (i.e., free proline and total free amino acids; Table 6).

In the present study, exogenous application of spermine (Spm), spermidine (Spd) or putrescine (Put) decreased the deleterious effects of Cd^{2+} and significantly enhanced wheat plant growth characteristics and yield components (Tables 2 and 3). These improved growth and yield of wheat plants grown under Cd^{2+} stress may be attributed to the reduced concentrations of endogenous Cd^{2+} ions (Table 5) and the increased nutrient contents including K^{+}. It has been suggested that K^{+} participates in sustaining membrane integrity [34] and maintained cells in a turgid status, and consequently reduced ion leakage from cells under Cd^{2+} stress (Table 4). In addition, K leads to an increase in chlorophyll fluorescence which can serve as an indicator of the stress induced by alterations in the balance of endogenous hormones [35]. In [36] it has been reported that Cd^{2+} can negatively alter water relations by annoying water balance through its effect on stomatal conductance, water transport and cell wall elasticity. These Cd^{2+} effects disused the plant tissues their health in terms of reduced RWC under Cd^{2+} stress as shown in the present study (Table 4). However, PAs modulated Cd^{2+} stress effect on RWC through reducing endogenous Cd^{2+} ion concentration (Table 5) and/or through their role in water integrity that was shown in [37]. There is a suggestion that exogenous application of PAs are represented to stabilize plant cell membranes and protect them from damage under stress conditions [38, 39]. The reference [40] reported that Cd^{2+} stress disorganizes the plant water relations, and Cd^{2+} negative effect can be observed in the water uptake, transport and transpiration in plants. Cell membrane integrity was assessed indirectly by evaluating the electrical conductivity of solution that represents the inorganic ions leakage from cells, which increased significantly under Cd^{2+} stress (Table 4). This result has shown to confirm that Cd^{2+} toxicity in wheat leaf was linked to free radical processes in membrane components, leading to increase in membrane permeability due to the alterations occurred in its stability [41]. With inducing the formation of disulfide links, Cd^{2+} binds to sulfhydryl groups and destabilizes the membrane system, leading to the distortion in the structure and function of membrane ion channels [42].

Under Cd^{2+} stress, results of the present study showed increased concentrations of proline and total free amino acids as osmoprotectants in the same time in which protein concentration was reduced (Table 6). The accumulation of free amino acids including proline in response to metal stress such as Cd^{2+} stress can be elucidated by the degradation of certain proteins and/or by the *de novo* synthesis of amino acids [43]. Free proline, as one of the amino acids, is probably one of the most common metabolites synthesized in response to stress as a part of a general adaptation syndrome to unfavorable environmental conditions. Accumulation of fluid compatible osmoregulation, chelation and detoxification of metals, protection of enzymes, regulating cytosolic acidity are of the functions of proline that stabilize the machinery of protein synthesis and trapping of ROS [44]. Many studies showed that PAs are involved in defence mechanisms during the botic and abiotic stresses [45, 46]. The increase in proline and total free amino acids as a result of Cd^{2+} stress is noticed in the present study, however, Spm, Spd or Put reduced their concentrations in wheat plants (Table 6), showing additional mechanisms by which these PAs reduces the adverse effects of Cd^{2+} stress.

5. Conclusion

Results of the present study shows that the efficiency of wheat plants to tolerate 2.0 mM Cd^{2+} stress in terms of growth and yield characteristics was noticed to varying degrees with the three applied PAs (Spm, Spd and Put). The reasonable growth of Cd^{2+}-stressed seedlings and consequently acceptable grain yield was correlated with the improvements in the concentrations of osmoprotectants (free proline and free amino acids) and tissue health in terms of relative water content (RWC) and membrane stability index (MSI), and reductions in electrolyte leakage (EL) and tissue Cd^{2+} concentration. Seed soaking in 1.0 mM Put was the best treatment, generating most tolerant wheat plants to Cd^{2+} stress than those generated from the other two polyamines; 0.25 mM Spm or 0.50 mM Spd. Therefore, this study recommend to use the 1.0 mM Put, as seed soaking treatment for wheat to grow well under Cd^{2+} stress.

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