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Research Article

PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF WHEAT (*TRITICUM AESTIVUM* L.) PLANTS TO POLYAMINES UNDER LEAD STRESS

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ABSTRACT

Objective: The distribution, growth, development and productivity of wheat plants are greatly affected by various abiotic stresses such as lead (Pb) stress which become one of the most abundant toxic metal in the earth crust. Under the three applied polyamine (PAs) applications, the efficiency of wheat plants to tolerate Pb^{2}+ stress in terms of growth and yield characteristics was noticed to varying degrees.

Methods: The current study focused on the impact of 2.0 mM lead (Pb^{2}+) on growth and performance of wheat plants before and after PAs applications. The sterilized seeds were 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).

Results: Point out that, better growth and yield characteristics, chlorophyll "a" (Chl-a), chlorophyll "b" (Chl-b), soluble sugars, indoles, and enzymatic antioxidants (i.e., peroxidase (POX), catalase, ascorbate peroxidase, ascorbate oxidase, polyphenol oxidase, and glutathione reductase) and the enzyme α-amylase contents were obtained with seed soaking in 0.25 mM Spm, 0.50 mM Spd, or 1.0 mM Put than those generated with seed soaking in water under 2.0 mM Pb^{2+} stress. In contrast, the concentration of endogenous Pb^{2+} was significantly reduced.

Conclusion: Among all tested PAs, 1.0 mM Put showed the best results and thus is recommended, as seed soaking, for wheat to grow well under Pb^{2+} stress.

Keywords: *Triticum aestivum*, Lead, Polyamines, Osmoprotectants, Antioxidant enzyme, Growth, Yield.

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops that belong to the family Poaceae and most widely grown for human and animal feed. In Egypt, wheat grains are used principally as human food, as worldwide. Wheat provides 37% of the total calories and 40% of the protein in Egyptian people diet [1]. Abiotic stresses, including metal toxicity, are shown not only to decrease wheat growth and productivity [2]. Lead (Pb) is the second most harmful pollutant after arsenic and recently listed as "the chemical of great concern" according to the new European REACH regulations [3]. It severely affects normal plant metabolism, morph-physiological features, and crop growth and productivity [4,5]. It often leads to diminished growth, reductions in chlorophyll biosynthesis, hormonal imbalance, and induce over-production of reactive oxygen species (ROS) in plants [5-7]. Lead, being non-redox metal, causes ROS production that led to oxidative stress within plant cells [8,9]. Plants normally have three mechanisms of Pb-tolerance, that is, (a) passive mechanisms (plant develops different types of physical barriers against Pb uptake), (b) inducible mechanisms (metal detoxification and its excretion to extra-cellular spaces), and (c) activation of anti-oxidative defense system (which includes both enzymatic and non-enzymatic anti-oxidants) to scavenge ROS [4,9]. Anti-oxidants, both enzymatic such as superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), and ascorbate peroxidase (APX), and non-enzymatic such as reduced glutathione (GSH) and oxidized glutathione (GSSG), are involved in direct and/or indirect detoxification of ROS in plants [10,11]. Except anti-oxidants, plants also accumulate various types of organic compounds or osmolytes proline and soluble sugars to shield essential cellular structures and to maintain cell osmotic potential [5,12].

PAs are low molecular weight, non-protein polycations at physiological pH with a strong binding capacity to negatively charged DNA, RNA, and different protein molecules, and the ability to stabilize membrane structures [13,14]. They regulate many physiological, growth, and developmental processes in abiotic and biotic plant stress responses [15,16]. Since then, a large number of reports have shown the relationship between PA accumulation and the activities of their biosynthetic enzymes during and after stresses [17-20]. The most common PAs found in plants are putrescine (Put), spermidine (Spd), and spermine (Spm). The concentrations of Put and Spd in plants are usually more abundant than Spm, which is often present in a trace amount [21].

In addition, PAs are effective radical scavengers in a number of chemical and enzyme-systems [22]. In engineered plants, tolerance to multiple environmental stresses is enhanced by an overproduction of PAs [18,23]. It was hypothesized that exogenous applications of the three most common PAs (i.e., Spm, Spd, and Put), used as seed soaking, enhance the growth and productivity of wheat plants grown under Pb2+ stress. Therefore, the aim of this study was to evaluate the improvements in growth characteristics, physio-chemical attributes, and yield and its components of wheat plants grown under 2 mM Pb^{2+} stress.

METHODS

Plant material, growing conditions, and treatments seeds of wheat (*T. 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 2 min. They were thoroughly washed several times with distilled water. After air-drying for 2 h, the sterilized seeds were soaked for 8 h at room temperature, either in distilled water (as a control), 0.25 mM Spm, 0.50 mM Spd, or in 1.0 mM Put. The selection of these polyamines (PAs) concentrations was based on the best response through our preliminary studies (data not shown). After air-drying overnight, 20 water- or PAs-treated seeds were sown on 1st of November, 2013 and

December 1, 2014, in each plastic pot (40 cm in diameter, 50 cm depth) that previously filled by 15 kg sand. 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 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 Pb2+-free nutrient solution (control). The second one was irrigated with Pb2+-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 11 h. Half-strength Hoagland's nutrient solution (Hoagland *et al*., 1950) was used for the two experiments. The composition of Hoagland's nutrient solution was KNO3 (1020 mg/L), a $(NO_3)_2.4H_2O$ (492 mg/L), $NH_4H_2PO_4$ (230 mg/L), $MgSO_4.7H_2O$ (420 mg/L), H_3BO_3 (2.86 mg/L), $MnCl_2.4H_2O$ (1.81 mg/L), $H_2MoO_4.H_2O$ (0.09 mg/L), $FeSO_4.7H_2O$ (0.07 mg/L), and $(CHOH)_2(COOH)_2$ (0.02 mg/L). The Pb2+-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 ten in each pot, and 2 mM Pb2+, using PbCl2, was added to the ½-strength Hoagland's nutrient solution. Each pot was supplemented every 3 days with 1 L of Pb2+-containing Hoagland's nutrient solution. The 2 mM Pb2+ dose was also selected based on our preliminary studies (data not shown). This level of Pb2+ was greatly affected wheat seedling growth. The Pb2+ concentration in the medium was maintained at 2 mM 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 7.0, 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 Pb2+-containing nutrient solution was terminated after 60 days from sowing after exposing the seedlings to Pb2+ stress for 60 days/20 irrigations. The 75-day-old plants (at the end of tillering stage) from each treatment were collected for various growth and physio-chemical measurements, while yield and its components assessments were conducted at harvest (ripening) stage. Plant growth, 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 plants were separated into two parts; shoots and roots. Plant height, and root and flag leaf lengths (cm) were measured using a meter scale. Numbers of tillers and leaves per plants were taken. Flag leaf area (cm^2) was assessed using a Planimeter Device Model planix-7. Root size/volume (cm^3) was measured using a gradual cylinder with water, where the difference between water surface in the cylinder after and before submerging a root indicates a root size. The length of shoots was measured using a meter scale. 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, numbers of spikelets per spike and grains per spike were counted, grain weight per plant, and1000-grain weight.

Determination of leaf photosynthetic pigments chlorophylls a and b was extracted from leaves in 80% acetone, and their concentrations were analyzed spectrophotometrically by the method of [24].

Assays of enzymatic activities

Alpha-amylase activity determination

The activity of α-amylase was determined using the method described by Petrova and Bolotina [25] as follows: Half gram of fresh leaves of each treatment was homogenized in polytron with 4 ml phosphate buffer (pH 6.0). Extract was centrifuged for 15 min at 400 rpm. The activity of α-amylase was determined in the supernatant using soluble starch as a substrate and 0.1 ml of the extract. Enzyme activity was expressed (nm min^{-1} g^{-1} fresh leaves) as changes in the optical density (O.D.) at 620 nm.

Assays of antioxidant enzymes activities

CAT

CAT activity was determined by the method of Beer and Sizer [26] as follows: Half gram of fresh leaves of each treatment was homogenized in polytron with 4 ml phosphate buffer (pH 7.0). Extract was centrifuged at 400 rpm for 15 min. CAT activity was measured in the supernatant using 1.9 ml of reagent grade water, 1.0 ml of H_2O_2 as substrate, and 0.1 ml of extract. Enzyme activity was expressed (nm min^{-1} g^{-1} fresh leaves) as changes in the O.D. at 240 nm.

SOD

Activity of SOD was assayed by a photochemical method described by Cakmak and Marshner [27] and based on a SOD-inhibitable reduction of nitro blue tetrazolium (NBT) chloride by superoxide radicals. Assays were carried out under illumination in growth chamber. For the SOD assay, the reaction medium (5 ml) was consisted of 50 mM phosphate buffer (pH 7.6), 0.1 mM sodium Ethylenediaminetetraacetic acid (Na-EDTA), enzyme extracts (50–150 µl), 50 mM Na_2CO_3 (pH 10.2), 12 mM L-methionine, 75 µM p-NBT chloride, and finally 2 µM riboflavin was added in glass vials and the reaction has been initiated by turning the lights on. The light intensity was about 700 µE $m^{-2}s^{-1}$ and the assay was lasted 15 min. The amount of enzyme extract that caused a 50% decrease in the SOD-inhibitable NBT reduction was defined as a unit at 560 nm.

Peroxidase (POX)

Peroxidase activity was determined as outlined by Maehly and Chance [28] as follows: Half gram of fresh leaves of each treatment was homogenized in polytron with 4 ml phosphate buffer (pH 6.0). Extract was centrifuged at 400 rpm for 15 min. The activity of enzyme was measured in the supernatant using reaction mixture consisted of 1.5 ml of phosphate buffer, 1.5 ml of H_2O_2 (20 volume), and 1.5 ml of 0.04 M catechol solution as substrate and 0.1 ml of the extract. Enzyme activity was expressed (nm min^{-1} g^{-1} fresh leaves) as changes in the O.D. at 470 nm.

Glutathione reductase (GR)

The activity of GR was measured in the samples that were prepared from 0.3 g of leaves homogenized under ice-cold conditions in 3 ml of extraction buffer containing 50 mM Tris–HCl buffer (pH 7.6), and 1 mM EDTA. The GR activity was measured by following the decrease in absorbance at 340 nm (expressed as nm min^{-1} g^{-1} fresh leaves) due to NADPH oxidation. The reaction mixture was contained leaf extract, 1 mM EDTA, 0.5 mM GSSG, 0.15 mM NADPH, 50 mM Tris–HCl buffer (pH 7.5), and 3 mM $MgCl_2$ [29].

Polyphenol oxidase (PPOX)

PPOX activity was determined using the method described by Taneja and Sachar [30] as follows: Half gram of fresh leaves of each treatment was homogenized in polytron with 4 ml phosphate buffer (pH 6.0). The reaction mixture was contained 2 ml of 1% catechol solution as substrate, 0.2 ml of enzyme extract, and rest of 0.05 M sodium phosphate buffer (pH 6.8) in volume of 4 ml. The enzyme activity was expressed (nm min^{-1} g^{-1} fresh leaves) as changes in the O.D. at 430 nm.

Ascorbic acid oxidase (AAO)

AAO activity was determined by the method mentioned by Dawson and Magee [31] as follows: Half gram of fresh leaves of each treatment was homogenized in polytron with 4 ml phosphate buffer (pH 6.2). The sample cuvette contained 1.0 ml of sodium phosphate buffer (pH 6.2), 0.2 ml of ascorbic acid 10^{-3} molar as substrate, 0.1 ml of enzyme extract, and

1.7 ml of distilled water. Enzyme activity was expressed (nm/5 min/0.5 g fresh weight [FW] of leaves) as changes in the O.D. at 265 nm.

Determination of hydrogen peroxide (H_2O_2)

The H_2O_2 concentration was assessed using frozen leaf samples [32]. Samples (1.0 g) were ground in an ice bath with 5 ml 0.1% (w/v) trichloroacetic acid. The homogenates were centrifuged at 12,000× *g* for 15 min. Then, 0.5 ml of the supernatant was added to 0.5 ml of potassium phosphate buffer (10 mM, pH 7). The absorbance of the supernatant was measured at 390 nm. The concentration of H_2O_2 was determined from a standard curve prepared with known concentrations of H_2O_2 and expressed as μM mg^{-1} FW.

Determination of lead (Pb^{2+}) concentration

The powdery dried plant samples and grains were ashed at 500°C for 12 h for determination of Pb concentration. The ashed samples were dissolved in 3.3% HNO_3 (v/v). The concentration of Pb was measured by inductively coupled plasma optical emission spectroscopy (ICP-OES, Varian, and Australia). The Pb measurements in plant materials were checked against certificated Pb values in different reference plant materials obtained from the National Institute of Standards and Technology (Gaithersburg, USA). The Pb contents were calculated by multiplying the dry weight (DW) values of roots or shoots with their Pb concentration values.

Determination of total soluble sugars

Soluble sugars were extracted from dried leaf tissue with 80% ethanol. One gram of the dried tissues was homogenized with 80% ethanol then put in a boiling water bath for 15 min. After cooling, the extract was filtered and the filtrate was oven dried at 60°C and then dissolved in a known volume of water to be ready for soluble sugars determination. The soluble sugars concentrations were determined by the anthrone sulfuric acid method described by Scott and Melvin [33]. Briefly, 1 ml of the extract was mixed with 9 ml of anthrone sulfuric acid reagent in a test tube and heated at 100°C for 7 min. The absorbance was read spectrophotometrically (Shimadzu, RF-5301PC, Japan) at 620 nm, against a blank containing only distilled water and anthrone reagent. All data were calculated as mg g^{-1} DW of leaves.

Determination of total indoles

Total indoles of roots and shoots of plant samples were determined (mg g^{-1} DW) using *p*-dimethylaminobenzaldehyde reagent according to the colorimetric method of Larson *et al*. [34] and the modification of Selim *et al*. [35].

Determination of water use efficiency (WUE_G)

WUE was calculated by dividing the grain yield (g pot^{-1}) by the total amount of water added to each pot. Therefore, WUE for grain yield (WUE_G) was calculated from grain yield according to Stanhill [36] as following:

$$WUE_G = \text{Grain yield (ton)}/\text{Total water used (gallon)}.$$

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.

RESULTS

Effect of seed soaking in different polyamines on vegetative growth of wheat plants are grown under lead stress.

Data presented in Tables 1 and 2 show that presence of $PbCl_2$ (2 mM) in the growth medium resulted in a marked decrease in growth traits (i.e., plant height, No. of tillers $plant^{-1}$, No. of leaves $plant^{-1}$, flag leaf length, flag leaf area $plant^{-1}$, root size, root length, shoot FW and DW, and root FW and DW) of wheat plants compared to the controls (without Pb treatment). However, treating wheat plants subjected to Pb at same concentration with spermine (Spm), Spd or Put, either by seed soaking or by foliar spraying, significantly improved the aforementioned growth characteristics. In general, seed soaking treatments were found to be more effective than foliar spray treatments. In addition, it has been found that seed soaking in Put was found to be the best treatment under the stress of Pb. This treatment significantly increased plant height, No. of tillers $plant^{-1}$, No. of leaves $plant^{-1}$, flag leaf length, flag leaf area $plant^{-1}$, root volume, root length, shoot FW and DW, and root FW and DW under Pb stress by 59.6%, 54.6%, 77.7%, 73.1%, 79.5%, 95.7%, 60.0%, 96.6%, 78.0%, and 190.3%, respectively, compared to the Pb-stressed controls in 2013/2014 season. In addition, these growth characteristics were increased by Put application for seeds under Pb stress by 39.1%, 69.6%, 78.3%, 91.1%, 71.0%, 105.9%, 48.6%, 111.9%, 183.8%, 87.3%, and 25.8%, respectively, compared to the Pb-stressed controls in 2014/2015 season.

Leaf photosynthetic pigments

The presence of Pb (2 mM $PbCl_2$) in the growth medium resulted in a marked decrease in the concentrations of leaf photosynthetic pigments

Table 1: Effect of Pas, Spm, Spd, or Put on some growth characteristics of wheat plants grown under lead (Pb) stress

Pb+PAs treatments		Parameters				
Soaking in PAs	**Spraying with PAs**	**Plant height (cm)**	**No. of tillers $plant^{-1}$**	**No. of leaves $plant^{-1}$**	**Flag leaf length (cm)**	**Flag leaf area $plant^{-1}$ (cm^2)**
2013/2014 season						
Control*		30.83	2.33	4.67	9.90	19.93
Pb (only)		15.77	1.08	2.11	4.90	10.33
-	Pb+Spm	18.50	1.33	3.33	7.70	13.27
-	Pb+Spd	19.03	1.35	3.37	7.78	14.39
-	Pb+Put	23.97	1.35	3.41	8.10	17.77
Pb+Spm		21.43	1.60	3.68	8.09	15.17
Pb+Spd		21.90	1.65	3.70	8.15	16.23
Pb+Put		25.17	1.67	3.75	8.18	18.54
$LSD_{0.05}$		1.20	0.05	0.16	0.09	1.70
2014/2015 season						
Control*		33.27	2.67	4.53	9.80	17.67
Pb (only)		17.67	1.12	2.12	4.27	8.77
-	Pb+Spm	21.40	1.42	3.46	6.20	10.57
-	Pb+Spd	21.60	1.53	3.52	6.53	11.20
-	Pb+Put	22.90	1.73	3.63	7.20	13.83
Pb+Spm		22.93	1.67	3.57	7.43	13.63
Pb+Spd		22.93	1.73	3.69	7.63	13.97
Pb+Put		24.57	1.90	3.78	8.16	15.00
$LSD_{0.05}$		0.18	0.05	0.16	0.09	1.26

*Control means plants without any treatments; Pb or PAs. PAS: Polyamines, Spm: Spermine, Spd: Spermidine, Put: Putrescine

(chlorophyll "a" [Chl-a], chlorophyll "b" [Chl-b] of wheat plants compared to the controls (without Pb treatment), as shown in Table 3. Pb, was found to be more toxic at the same concentration on wheat plants, showing lower leaf pigment concentrations. However, treating wheat plants grown under Pb stress with Spm, Spd or Put, either by seed soaking or by foliar application, significantly improved the concentrations of leaf photosynthetic pigments. In general, seed soaking treatments were better than foliar spray treatments under heavy metal stress. In addition, it has been found that seed soaking using Spd was found to be the best treatment, generating best values of chlorophylls and carotenoids under the stress of Pb. This treatment significantly increased Chl. "a" and "b" under Pb stress by 76.5 and 127.3%, respectively, compared to the Pb-stressed controls in 2013/2014 season. In addition, these pigments Chl. "a" and "b" were increased by Spd application for seeds under Pb stress by 94.4 and 73.3%, respectively, compared to the Pb-stressed controls in 2014/2015 season.

Concentrations of osmoprotectants, soluble sugars, and indoles

Data shown in Table 4 exhibit that the Pb^{2+}-treated plants showed significant increases in the concentrations of soluble sugars, indoles, compared to the corresponding untreated control plants over two studied seasons. Under Pb^{2}stress, the Spm-, Spd-, or Put-pretreated plants showed significant increase in soluble sugars and indoles, concentrations compared to the corresponding PAs-untreated plants over both growing seasons. In general, it has been found that seed soaking in Put (for soluble sugars and indoles) was found to be the best

Table 2: Effect of PAs, Spm, Spd, or Put on some growth characteristics of wheat plants grown under lead (Pb) stress

Pb+PAs treatments		Parameters					
Soaking in PAs	Spraying with PAs	Root system size (cm^3)	Root length (cm)	Fresh weight (g)		Dry weight (g)	
				Shoots	Roots	Shoots	Roots
2013/2014 season							
Control*		3.87	10.67	9.35	6.17	1.51	2.40
Pb (only)		1.63	6.33	3.24	3.05	0.69	0.62
-	Pb+Spm	2.15	8.17	4.80	3.68	0.76	1.18
-	Pb+Spd	2.37	9.00	5.03	4.37	0.93	1.26
-	Pb+Put	3.10	9.23	6.08	5.06	1.16	1.68
Pb+Spm	-	2. 71	8.23	4.35	4.40	0.86	1.23
Pb+Spd	-	2. 88	9.20	4.72	5.16	0.03	1.45
Pb+Put	-	3.19	10.13	6.37	5.43	1.27	1.80
$LSD_{0.05}$		0.17	0.16	0.19	0.53	0.18	0.19
2014/2015 season							
Control*		3.37	11.33	9.70	6.14	1.58	2.36
Pb (only)		1.53	6.90	3.12	2.10	0.71	1.51
-	Pb+Spm	2.11	9.08	4.77	4.37	0.83	1.24
-	Pb+Spd	2.42	9.43	5.08	4.75	1.01	1.35
-	Pb+Put	2.93	10.05	6.17	4.80	1.19	1.60
Pb+Spm	-	2.67	9.63	5.27	5.13	0.93	1.33
Pb+Spd	-	2.83	9.74	5.51	5.17	1.16	1.46
Pb+Put	-	3.15	10.25	6.61	5.96	1.33	1.90
$LSD_{0.05}$		0.19	0.15	0.17	0.49	0.16	0.14

*Control means plants without any treatments; Pb or PAs. PAS: Polyamines, Spm: Spermine, Spd: Spermidine, Put: Putrescine

Table 3: Effect of PAs, Spm, Spd, or Put on leaf pigments and Pb contents of wheat plants grown under lead (Pb) stress

Pb+PAs treatments		Parameters				
Soaking in PAs	Spraying with PAs	Chlorophyll "a" (mg g^{-1} fresh weight)	Chloroph yll "b" (mg g^{-1} fresh weight)	Pb		Translocation index
				Content (mg g^{-1} DW)		
				Shoots	Roots	
2013/2014 season						
Control*		0.25	0.19	0.54	0.31	27.27
Pb (only)		0.17	0.11	2.867	25.89	35.08
-	Pb+Spm	0.27	0.21	2.437	24.45	22.10
-	Pb+Spd	0.29	0.24	2.157	20.86	23.50
-	Pb+Put	0.26	0.22	2.273	24.20	23.21
Pb+Spm	-	0.28	0.22	2.3.83	24.30	23.72
Pb+Spd	-	0.30	0.25	2.116	20.64	24.69
Pb+Put	-	0.26	0.23	2.236	23.89	23.89
$LSD_{0.05}$		0.01	0.02	0.321	0.33	4.15
2014/2015 season						
Control*		0.28	0.12	0.431	0.818	16.81
Pb (only)		0.18	0.15	2.879	25.82	25.71
-	Pb+Spm	0.30	0.22	2.683	24.18	33.77
-	Pb+Spd	0. 33	0.25	2.305	20.44	35.51
-	Pb+Put	0.29	0.23	2.622	23.97	33.79
Pb+Spm	-	0.32	0.23	2.610	24.02	34.36
Pb+Spd	-	0.35	0.26	2.246	20.20	37.37
Pb+Put	-	0.30	0.24	2.679	23.81	33.33
$LSD_{0.05}$		0.01	0.02	0.318	0.34	3.11

*Control means plants without any treatments; Pb or PAs. PAS: Polyamines, Spm: Spermine, Spd: Spermidine, Put: Putrescine

treatment under the stress of Pb. This treatment Put (for soluble sugars and indoles) significant reductions in the soluble sugars under the stress of Pb. by 11.9% in 2013/2014 season, and by 5.9 % in 2014/2015, respectively, and significantly increased in the indoles by 52.16% in 2013/2014 season, and by 70.11% in 2014/2015, respectively.

Effect of seed soaking or foliar spraying of different PAs on antioxidative defense system of wheat plants grown under lead (Pb) stress

Enzymatic antioxidants activity

Data presented in Tables 4 and 5 show that the presence of Pb in the growth medium resulted in significant decreases in the activities of enzymatic antioxidants (i.e., peroxidase [POX], CAT, APX ascorbate oxidase [ASOX], PPOX, and GR) and the enzyme α-amylase, while resulted in significant increase in the activity of SOD in wheat plants compared to the controls (without Pb treatment). There are fluctuations in the activities of these enzymes which induced under the stress of Pb at same concentration.

However, treating wheat plants subjected to Pb with Spm, Spd or Put, either by seed soaking or by foliar spraying, significantly improved the activities of the all aforementioned enzymes, except for the activity of SOD, which fluctuated either in increase, decrease, or in constant state. In general, the all data of all tested enzyme activities cannot to identify

Table 4: Effect of PAs, Spm, Spd, or Put on the leaf contents of osmoprotectants and enzyme (SOD, POX, and CAT) activities of wheat plants grown under lead (Pb) stress

Pb+PAs treatments		Parameters				
Soaking in PAs	Spraying with PAs	Soluble sugars (mg g^{-1} DW)	Indoles (mg g^{-1} DW)	SOD (Units g^{-1} FW)	POX (nm min^{-1} g^{-1})	CAT (nm min^{-1} g^{-1})
2013/2014 season						
Control*		48.45	13.78	0.15	0.36	0.20
Pb (only)		69.46	23.12	0.24	0.24	0.15
-	Pb+Spm	50.16	29.32	0.10	0.44	0.25
-	Pb+Spd	52.03	29.92	0.20	0.48	0.22
-	Pb+Put	58.95	33.35	0.16	0.44	0.26
Pb+Spm	-	53.91	30.82	0.12	0.59	0.29
Pb+Spd	-	56.89	32.03	0.23	0.53	0.27
Pb+Put	-	61.18	35.18	0.17	0.61	0.30
$LSD_{0.05}$		3.49	0.30	0.02	0.06	0.04
2014/2015 season						
Control*		49.15	15.00	0.19	0.28	0.16
Pb (only)		64.39	20.27	0.28	0.22	0.13
-	Pb+Spm	45.94	29.74	0.15	0.31	0.21
-	Pb+Spd	49.08	30.22	0.22	0.34	0.20
-	Pb+Put	55.10	34.43	0.17	0.37	0.24
Pb+Spm	-	53.08	30.84	0.17	0.54	0.26
Pb+Spd	-	55.84	31.57	0.24	0.49	0.25
Pb+Put	-	60.59	35.90	0.18	0.51	0.28
$LSD_{0.05}$		3.49	0.31	0.02	0.05	0.04

*Control means plants without any treatments; Pb or PAs. PAS: Polyamines, Spm: Spermine, Spd: Spermidine, Put: Putrescine, SOD: Superoxide dismutase, POX: Peroxidase, CAT: Catalase

Table 5: Effect of PAs, Spm, Spd, or Put on the leaf contents of hydrogen peroxide and enzyme (ASOX, PPOX, GR, and α-amylase) activities of wheat plants grown under lead (Pb) stress

Pb+PAs treatments		Parameters				
Soaking in PAs	Spraying with PAs	H_2O_2 (μM mg^{-1} FW)	ASOX (Units g^{-1} FW)	PPOX (Units g^{-1} FW)	GR (nm min^{-1} g^{-1})	α-amylase (nm min^{-1} g^{-1})
2013/2014 season						
Control*		28.4	0.11	0.16	0.68	0.19
Pb (only)		37.3	0.09	0.13	0.58	0.15
-	Pb+Spm	33.1	0.20	0.20	0.70	0.34
-	Pb+Spd	30.7	0.23	0.22	0.64	0.27
-	Pb+Put	33.5	0.24	0.22	0.59	0.29
Pb+Spm		31.5	0.24	0.26	0.76	0.36
Pb+Spd		29.5	0.24	0.26	0.67	0.33
Pb+Put		31.8	0.28	0.28	0.63	0.38
$LSD_{0.05}$		1.95	0.04	0.04	0.04	0.06
2014/2015 season						
Control*		25.5	0.11	0.17	0.71	0.19
Pb (only)		34.6	0.09	0.11	0.60	0.17
-	Pb+Spm	30.8	0.20	0.22	0.73	0.33
-	Pb+Spd	28.7	0.23	0.23	0.66	0.33
-	Pb+Put	31.2	0.24	0.27	0.63	0.36
Pb+Spm		30.1	0.24	0.27	0.74	0.36
Pb+Spd		28.4	0.24	0.26	0.68	0.35
Pb+Put		30.5	0.28	0.31	0.65	0.35
$LSD_{0.05}$		1.93	0.03	0.03	0.05	0.07

*Control means plants without any treatments; Pb or PAs. PAS: Polyamines, Spm: Spermine, Spd: Spermidine, Put: Putrescine, ASOX: Ascorbate oxidase, PPOX: Polyphenol oxidase, GR: Glutathione reductase

whether a treatment or other to be the best. These results are in a parallel line in both growing seasons. However, most of the enzymatic activity is higher. It has been found that seed soaking in Put (for CAT by 100.0 and 115.4, AAO [ASOX] by 211.1, and 211.1) or Spd (for SOD significantly decreased by 4.2, and 8.0, peroxidase [POX] significantly increased by 120.8, and 122.7, and PPOX by 100.0 and seed soaking in Put in the 2nd season by 181.8), and Spm (for GR significantly increased by 31.0 and 23.3 and α-amylase 140.0 and 111.8) application under Pb stress, respectively, in 2013/2014 and 2014/2015 seasons.

Hydrogen peroxide (H_2O_2)

Data in Table 5 show that hydrogen peroxide (H_2O_2) content, of Pb^{2+}-treated plants were significantly increased compared to those of the control plants. These results were recorded over both 2013/2014 and 2014/2015 growing seasons for only H_2O_2. Under Pb^{2+} stress, the Spm-, Spd-, or Put-pretreated plants revealed significant reductions in the H_2O_2 (over two growing seasons), compared to the corresponding Pb^{2+}-treated plants. However, treating wheat plants subjected to Pb with Spm, Spd, or Put, either by seed soaking or by foliar spraying, revealed significant reductions in the H_2O_2 (over two growing seasons), compared to the corresponding Pb^{2+}-treated plants, which fluctuated in decrease. In general, the all data of the H_2O_2 cannot to identify whether a treatment or other to be the best. These results are in a parallel line in both growing seasons. Best deficiency of the H_2O_2 by 14.47 and11.84% over both 2013/2014 and 2014/2015 growing seasons, respectively.

Yield and its components

Data in Table 6 show that Pb^{2+}-treated wheat plants exhibited significant reductions in their yield and its components (i.e., spike length, number of spikelets $spike^{-1}$, number of grains per spike, grains weight per plant, and 1000-grain weight) compared to those of the control plants over both 2013/2014 and 2014/2015 seasons. Under Pb^{2+} stress, the Spm-, Spd-, or Put-pretreated plants showed significant increases in the spike length, number of spikelets per spike, number of grains per spike, grains weight per plant, and 1000-grain weight compared to the corresponding untreated plants in both growing seasons. Except for the parameter 1000-grain weight that correlated with Spd, the Put was noticed to be the effective pretreatment, mitigating the injurious effects of Pb^{2+} stress and significantly increased these yield characteristics compared to Pb^{2+} stressed plants. The increases were 75.8, 46.5, 32.7, 174.3, and 55.6%, respectively, for the 1st season under the stress of Pb, respectively, compared to the controls, and were 70.4, 46.7, 34.5, 102.1, and 58.3%, respectively, for the 2nd season under the stress of Pb, respectively, compared to the controls.

The lead (Pb) concentrations of grains and WUE

Data in Table 7 show that Pb^{2+}-treated plants exhibited significant increases in Pb concentrations of grains compared to the controls over both 2013/2014 and 2014/2015 seasons. The increases in Pb concentrations were 97.4%, respectively, in the first season under the stress of Pb, respectively, compared to the controls, and were 97.8%, respectively, in the second season under the stress of Pb, respectively, compared to the controls. Under Pb^{2+}- stress, the Spm-, Spd-, or Put-pretreated plants showed significant decreases in Pb concentrations of grains. The Spd, used for seed soaking, was noticed to be the efficient pretreatment, mitigating the injurious effects of Pb^{2+}- stress and significantly decreased Pb concentrations in grains compared to Pb^{2} -stressed plants that not applied with PAs.

WUE is the ability of the crop to produce biomass per unit of water transpired (Liang *et al*., 2006) or the efficiency for producing dry matter per unit absorbed water, and the ability to allocate an increased proportion of the biomass into grains (Manivannan *et al*., 2007). Water scarcity is a major limiting factor in agricultural production all over the world (Liu *et al*., 2005; Shao *et al*., 2008). The values of WUE_G in the Pb^{2+}treated plants were significantly lower than that of the control. These decreases in WUE_G might probably due to the decreases in grain yield of wheat plants (Table 7). Under Pb^{2+} stress, Spm-, Spd-, or Put-pretreated plants mitigated the harmful effect of waste water stress on WUE_G of wheat plants. The improvement of WUE in non-stressed or stressed wheat plants under PAs treatment might be due to the increases in grain yields of wheat plants.

Furthermore, the increases in WUE_G values were higher in The Put treatment that was noticed to be the effective pretreatment, mitigating the injurious effects of Pb^{2+} stress and significantly increased the yield characteristics than those of the others. Yield is a result of the integration of metabolic reactions in plants; consequently any factor that influences this metabolic activity at any period of plant growth can affect the yield (Ibrahim and Aldesuquy, 2003).

Lead concentrations and tolerance index

Data shown in Table 7 exhibit that Pb^{2+}-treated wheat plants showed significant increases in Pb^{2+} concentration compared to the untreated

Table 6: Effect of PAs, Spm, Spd, or Put on grain yield and its components of wheat plants grown under lead (Pb) stress

Pb+PAs treatments		Parameters				
Soaking in PAs	**Spraying with PAs**	**Spike length (cm)**	**No. of spikelets $spike^{-1}$**	**No. of grains $spike^{-1}$**	**1000-grain weight (g)**	**Grains weight $plant^{-1}$ (g)**
2013/2014 season						
Control*		7.63	11.33	23.67	25.16	1.10
Pb (only)		3.03	7.35	15.33	8.29	0.64
-	Pb+Spm	3.80	8.67	15.73	16.34	0.82
-	Pb+Spd	4.73	8.91	16.36	17.55	0.85
-	Pb+Put	5.27	9.67	17.30	14.45	0.91
Pb+Spm	-	4.10	8.80	15.97	16.72	0.83
Pb+Spd	-	4.77	9.12	17.24	17.64	0.90
Pb+Put	-	6.00	10.06	18.00	14.84	0.95
$LSD_{0.05}$		0.52	0.37	0.27	3.50	0.05
2014/2015 season						
Control*		7.40	11.00	26.33	28.55	1.20
Pb (only)		2.97	8.00	16.67	10.32	0.65
-	Pb+Spm	3.40	9.00	19.25	18.71	1.02
-	Pb+Spd	4.05	9.42	19.58	19.38	1.03
-	Pb+Put	4.20	10.12	20.67	15.45	1.09
Pb+Spm	-	3.83	9.23	20.10	18.95	1.05
Pb+Spd	-	4.10	9.67	20.50	19.80	1.08
Pb+Put	-	5.73	10.33	21.33	15.81	1.12
$LSD_{0.05}$		0.70	0.38	0.27	3.50	0.06

*Control means plants without any treatments; Pb or PAs. PAs: Polyamines, Spm: Spermine, Spd: Spermidine, Put: Putrescine

Table 7: Effect of Pas, Spm, Spd, or Put on grain Cd content (quality) and water use efficiency of wheat plants grown under lead (Pb) stress

Pb+PAs treatments		Parameters	
Soaking in Pas	**Spraying with PAs**	**Grain Pb (mg kg^{-1} DW)**	**(WUE) $_G$ (L g^{-1})**
2013/2014 season			
Control*		0.07	9.16
Pb (only)		2.70	5.17
-	Pb+Spm	2.00	7.90
-	Pb+Spd	1.55	7.68
-	Pb+Put	1.91	7.61
Pb+Spm	-	1.95	8.05
Pb+Spd	-	1.43	7.98
Pb+Put	-	1.82	7.70
$LSD_{0.05}$		0.19	0.51
2014/2015 season			
Control*		0.06	9.14
Pb (only)		2.72	5.19
-	Pb+Spm	2.04	7.86
-	Pb+Spd	1.58	7.64
-	Pb+Put	1.96	7.57
Pb+Spm	-	1.98	8.00
Pb+Spd	-	1.46	7.95
Pb+Put	-	1.83	7.69
$LSD_{0.05}$		0.19	0.50

*Control means plants without any treatments; Pb or PAs. PAs: Polyamines, Spm: Spermine, Spd: Spermidine, Put: Putrescine

Table 8: Effect of PAs, Spm, Spd, and Put on Pb tolerance index (%) and content (total amount) of Pb in roots of wheat plants grown under heavy metal stress

Treatments			Parameters	
Heavy metals	**PAs**		**Tolerance index (%)**	
	Soaking	**Spray**	**shoots**	**roots**
2013/2014 season				
Control (without any treatments)			-	-
Pd	-	-	86.71	25.83
	-	Spm	95.54	49.17
	-	Spd	119.18	52.50
	-	Put	151.6	70.00
	Spm	-	109.82	51.25
	Spd	-	132.94	60.42
	Put	-	167.53	75.00
$LSD_{0.05}$			13.14	12.23
2014/2015 season				
Control (without any treatments)			-	-
Pb	-	-	87.51	23.98
	-	Spm	101.57	52.54
	-	Spd	124.80	57.20
	-	Put	148.04	67.80
	Spm	-	113.76	56.36
	Spd	-	142.55	61.86
	Put	-	165.39	80.51
$LSD_{0.05}$			13.18	11.25

*Control means plants without any treatments; Pb or PAs. PAs: Polyamines, Spm: Spermine, Spd: Spermidine, Put: Putrescine

(not received Pb) control plants over both 2013/2014 and 2014/2015 seasons. Under Pb $^{2+}$stress, the Spm-, Spd-, or Put-pretreated plants showed significant decrease in the Pb^{2+} concentration. Among all PAs, the best treatment that was most effective in preventing absorption and translocation of Pb was Spd treatment.

From the concentrations of Pb, translocation and tolerance indices were obtained (Table 8), from which there is an inverse correlation between them. In addition, with a heavy metal, the translocation index values were significantly reduced, while tolerance index values were significantly increased with PA applications, used as seed soaking or foliar spray. For tolerance index, the best treatment was seed soaking in Put. This treatment increased tolerance index under Pb stress by 93.2 and 190.4% in shoots and roots, respectively, in 2013/2014 season, and by 89.0 and 235.7% in shoot and roots, respectively, in 2014/2015.

DISCUSSION

It is clear from the present data that lead had inhibitory effect on morphological parameters of wheat plants. Shoot length was decreased by Pb application. This reduction may be attributed to a reduction of meristematic cells in the shoot region by the accumulation of Pb [37]. In addition, Pb decreased the root length of wheat plants. This reduction in root length may be due to an inhibition of cell division in meristematic zone of root system. It is well known that Pb is a powerful inhibitor of root growth and accumulates largely in the roots [38,39]. These results correlate with another study in which root length of wheat was reduced due to the heavy metal Pb. It inhibits the growth of plants by alteration in enzyme activity and induction of oxidative stress [37]. It also reduced the root and shoot FWs of wheat in this experiment. These results correlate to earlier study in which high concentration of Pb decreased the root and shoot FWs in wheat plants [40]. Furthermore, Pb is very toxic heavy metal which has detrimental effects on yield and biomass characteristics such as fresh shoot and root weights, dry weights of wheat due to changes in metabolism and physiology of plants. To alleviate and repair the damage caused by ROS due to the presence of heavy metal in plant growth medium, exogenous application of antioxidants such as PAs enables crop plants to develop a complex antioxidant defense system to increase the cellular defense strategy against Pb-induced oxidative stress. Exogenous application of Spm, Spd, or Put decreased the deleterious effects of Pb^{2+} and significantly enhanced wheat plant growth characteristics, as shown in Tables 2 and 3. These improved growths of wheat plants grown under Pb^{2+} stress may be attributed to the reduced concentrations of endogenous Pb^{2+} ions (Table 2). It has been reported that PAs are involved in various biochemical and physiological processes related to plant growth and development [41], which could explain the prevention role of Spm, Spd, or Put in Pb-induced plant growth inhibition.

The decrease observed in photosynthetic activity is often a more sensitive measure than is pigment content. Significant decreases in photosynthetic pigments, particularly chlorophylls because of Pb^{2+} were observed in common bean, cucumber, and wheat plants due to that Pb^{2+} prevents the incorporation of iron (Fe) in phytoporphyrin ring of chlorophyll molecule [42]. The increase in carotene contents in plants under Pb^{2+} stress by PAs application in the present study (Table 4) considers as an additional strategy along with the strategy adopted by plants through the antioxidative defense system to alleviate the toxic effects of free radicals generated under heavy metal toxicity as previously reported by Singh *et al*., Azooz *et al*. [43,44]. It was demonstrated also that plant treatment with PAs prevented the loss in contents of chlorophyll, stabilization of membranes, and delaying the senescence [32,45]**.** Probably, the exogenous application of PAs prevents the chlorophyll loss, protecting the thylakoids membrane structure due to their cation characteristics. It is possible that the exogenous PAs applications induce physiological effect by means of a non-specific influence on the plasmalema. Researchers have proclaimed that Spd, as a best treatment, could regulate the structures and functions of the photosynthetic apparatus through its interaction with the thylakoid membrane [46], which might be related to the increase of chlorophyll contents in plants.

There is no special transport channel for non-essential element, such as Pb within plants. Non-essential metal elements were transported into plants through transporters and channels for essential elements such as Ca and K [47]. Wheat plants accumulated high amounts of Pb in roots than shoots. Such a correlation was observed in the case of Pb

which is immobile. A significantly higher concentration of Pb in barley roots than in straw was observed by Tlustos *et al*. [48]. Root growth is more sensitive to heavy metals than shoot growth. To characterize metal tolerance in plants, one of the most common parameters used is the tolerance index [49]. The ameliorating effect of PAs on heavy metals by inducing the synthesis of specific proteins may be attributed to the role of PAs in increasing the tolerance of wheat plants to heavy metals [50]. The experimental results that exogenous application of PAs plays an important role in increasing the tolerance of wheat plants to Pb treatment by decreasing the accumulation of Pb contents in roots and consequently in shoots as compared with their corresponding control values. This repairing effect induced by exogenous application of PAs may be due to that PAs: (i) Increase the production of phytochelatins particularly in root; (ii) increase the cell wall and vacuolar storage of these heavy metals; (iii) increase the detoxification of heavy metals by increasing the accumulation of these metals in trichomes of leaves and peduncles of wheat plants [51]; and (iv) act as an efficient antioxidants and free radical scavengers under these stresses [52] and increase the root exudates into the soil (biosphere).

Exposure to heavy metals is often accomplished by synthesis and accumulation of various metabolites, including some amino acids, particularly free proline, and soluble carbohydrates [53] in response to metal stress such as Pb^{2+} stress can be elucidated by the degradation of certain proteins and/or by the *de novo* synthesis of amino acids [54]. Many studies showed that PAs are involved in defense mechanisms during the biotic and abiotic stresses [55,56]. The increase in soluble sugars and total indoles as a result of Pb^{2+} stress is noticed in the present study; however, Spm, Spd, or Put application reduced the endogenous concentrations of Pb in wheat plants, showing additional mechanisms by which these PAs reduces the adverse effects of Pb^{2+} stress. El-Bassiouny *et al*. found that application of PAs treatment had favorable effect on the synthesis and accumulation of carbohydrates in leaves of wheat plants [57]. In most cases, plants sprayed with PAs at the different concentrations had higher contents of total carbohydrates in their leaves, compared to the untreated control. With the application of Spd, leaves accumulated more compatible osmolytes such as soluble sugars (i.e., glucose and fructose), sugar alcohol, sorbitol, and proline as a response [58].

Antioxidant enzymes are very good biochemical markers of stress and increasing their activities could be a potential to alleviate the oxidative stress-induced by Pb stress and/or the external supports such as antioxidants. Under Pb stress, PAs induced the increased activities of all enzymes tested and this study, which may be attributed to the antioxidative characteristics of PAs against the generation of ROS, and plants try to force this by stimulation of antioxidants defense system (Li *et al*., 2011) [59]. Overproduction of ROS is a common consequence of different stress factors, including Pb. To maintain metabolic functions under stress conditions, the balance between generation and degradation of ROS is required, otherwise oxidative injuries may occur. The level of ROS in plant tissues is controlled by an antioxidant system that consists of antioxidant enzymes (SOD, CAT, PPOX, ASOX, POX, and GR) and non-enzymatic low molecular weight antioxidants such as GSH, proline, carotenoids, and tocopherols [60]. Therefore, it was expected that the exposure of wheat plants to Pb stress enhanced the activities of antioxidant enzymes, but the reverse state was the obtained result except for SOD. Therefore, the improved values of enzyme activities were recorded in the plants subjected to simultaneous stress of Pb with Spm, Spd, or Put, either by seed soaking or by foliar spraying. The above-mentioned enzymatic components play a relevant role in mitigating heavy metal stress. Several studies have revealed that treatment of heavy metal enhances ROS formation, and thus, substantial increase in the activities of SOD, CAT, and APX was observed (Bashri and Prasad, 2015) [61]. The enzyme SOD is the first line of defense to counter superoxide (O_2^-) radical. It catalyzes the conversion of $O_2^{-\bullet}$ to H_2O_2 that is subsequently converted to H_2O by enzyme POX [62]. This explanation may be elucidating that the activity of only SOD was increased under Pb stress before PAs treatments. The CAT scavenges H_2O_2 by converting it to H_2O and finally O_2, and POX reduces H_2O_2 using several reductants, such as ascorbate, guaiacol, and phenolic compounds [63]. The GR maintains the pool of GSH in the reduced state, which, in turn, reduces dehydroascorbate to ascorbate. Increased expression of GR enhances tolerance to oxidative stress [64]. Several mechanisms have been suggested to explain the increased oxidant resistance attributed to PAs, that is, PAs could act as direct radical scavengers [65]. They could bind to antioxidant enzymes or be conjugated to antioxidant molecules and allow them to permeate to the sites of oxidative stress [66], or may interact with membranes stabilizing molecular complexes of thylakoid membranes [67]. Plasma membrane function may be rapidly affected by heavy metals [68]. Application of PAs showed more increase in enzymatic activities as a reported synergy between antioxidant enzymes and PAs in protective mechanism [69]. The antioxidant protection exerted by exogenous Spm on wheat leaves seemed to be related to a protection of the membrane integrity through lipid stabilization and avoidance of leakage of solutes in Pb^{2+}-induced oxidative damage. On the other hand, Kurepa *et al*. reported that paraquat resistance did not necessarily correlate with increased PAs content [70]. Lovaas suggested that the antioxidative effect of PAs is due to a combination of their anion and cation-binding properties [71]. The binding of PAs to anions (phospholipid membranes and nucleic acids) contributes to a high local concentration at particular sites prone to oxidations, whereas the binding to cations efficiently prevents the site specific generation of "active oxygen" (hydroxyl radicals and singlet oxygen).

Hydrogen peroxide (H_2O_2) plays significant signals for the induction and regulation of stress enzymes such as APX [72]. In the present study, exposure to Pb resulted in increase of H_2O_2 level in wheat plants. This increase of H_2O_2 level is associated with the activation of ROS-producing enzymes and the inactivation of antioxidant enzymes [73]. The increased H_2O_2 levels can be indirectly originated from a decreased H_2O_2-scavenging rate and/or an increased H_2O_2 production by cell enzymatic and non-enzymatic processes. Exogenous PAs treatment increased protein concentration and decreased H_2O_2 level in wheat leaves and ameliorated the harmful effect induced by Pb. Under stress, production of free radicals such as H_2O_2 conjugates to protein, destructing their structures, while the increase in plant protein with PAs application may be attributed to the antioxidative role of PAs in alleviating, to some extent, the generation of H_2O_2 under stress [74].

The Pb^{2+}-treated wheat plants exhibited significant reductions in their yield and its components. These reductions can be attributed to the decrease in total cumulative leaf area, photosynthetic pigments, polysaccharides, and nitrogenous compounds (protein) in leaves and consequently in wheat yielded grains [75]. These results were in a good agreement with those obtained by Mallan and Farrant [76]. Decreases in yield and yield components in different crops under similar conditions have also been reported by many workers [76,77].

It has been shown in the present study that exogenous PAs applications alleviated the adverse effects of Pb^{2+}- stress on yield and yield components of wheat plants and increased them. These increases in yield production by PAs may be due to the increase in longevity of leaves which perhaps contributed to grain filling by enhancing the duration of photosynthates supply to grains [78]. This phenomenon was manifested particularly when it was found that, there was a positive correlation between phloem area in both flag leaf and peduncle of main shoot of wheat plants which accelerate rapid translocation of photo-assimilates from source (i.e., flag leaf) to sink (i.e., grain in spike) [79]. In this respect, PAs play very important roles in many physiological processes (related to yield quality) such as reproductive organ development, tuberization, floral initiation, and fruit development and ripening [80].

WUE is the ability of the crop to produce biomass per unit of water transpired [81] or the efficiency for producing dry matter per unit absorbed water, and the ability to allocate an increased proportion of the biomass into grains [82]. Water scarcity is a major limiting factor in agricultural production all over the world [83,84]. The values of WUE_G

in the Pb^{2+}-treated plants were significantly lower than that of the control. These decreases in WUE_G might probably due to the decreases in grain yield of wheat plants (Table 7). Under Pb^{2+} stress, Spm-, Spd-, or Put-pretreated plants mitigated the harmful effect of waste water stress on WUE_G of wheat plants. The improvement of WUE in non-stressed or stressed wheat plants under PAs treatment might be due to the increases in grain yields of wheat plants. Furthermore, the increases in WUE_G values were higher in the Put treatment that was noticed to be the effective pretreatment, mitigating the injurious effects of Pb^{2+} stress and significantly increased the yield characteristics than those of the others. Yield is a result of the integration of metabolic reactions in plants; consequently, any factor that influences this metabolic activity at any period of plant growth can affect the yield [85].

CONCLUSION

Among all tested PAs, 1.0 mM Put showed the best results and thus is recommended, as seed soaking, for wheat to grow well under Pb^{2+} stress.

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