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Exogenous Jasmonic Acid Induces Lead Stress Tolerance in Kidney Bean (*Phaseolus vulgaris* L.) by Changing Amino Acid Profile and Stimulating Antioxidant Defense System

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Abstract

In this study, the impact of different lead (Pb) concentrations (100 and 1000 µM) on growth parameters, photosynthetic pigment content, relative water content (RWC), ion homeostasis, protein and amino acid profile of kidney bean (*Phaseolus vulgaris* L.) plants is investigated. In addition, the changes in antioxidant enzyme activities, including ascorbate peroxidase (ASPX), catalase (CAT), and glutathione-S-transferase (GST) have been determined. Reduced glutathione (GSH) content was also calculated. The potential of jasmonic acid (100 µM) as a stress-signaling molecule in alleviating the drastic effects of lead stress on kidney bean plants has been evaluated. The current study found out that Pb treatment significantly (P<0.05) reduced growth, chlorophyll contents, relative water content, essential and non-essential amino acid levels, and essential ions (Mg^{2+} and Ca^{2+}). The Pb effect was directly related to the applied concentrations. The results showed that JA effectively alleviates the inhibitory effect of Pb on plant growth and chlorophyll contents, probably by reducing the Pb uptake, maintaining ion homeostasis, increasing antioxidant enzyme activity (ASPX, CAT and GST), and metal-binding molecules including GSH and amino acids such as proline and cysteine. To the researchers' knowledge, this study is the first of its kind to unravel the protective role of exogenous JA in alleviating the effects of Pb stress in *Phaseolus vulgaris* L., and the underlying mechanism for the JA-induced stress tolerance.

Keywords: Amino acids, Antioxidant enzymes, Glutathione, Growth, Ion homeostasis, Jasmonate, Kidney beans, Protein.

1. Introduction

Heavy metals constitute a major problem for plants and highly affect their metabolic activities. Lead (Pb) is a very toxic environmental pollutant (Grover *et al*., 2010; Pourrut *et al*., 2011). The toxic effect is a result of lead extracting and melting processes, the use of paints containing lead, gasoline and explosives, and Pb-enriched sewage treatment and disposal (Chany and Ryan, 1994). Pb was found to accumulate in cultivated soils close to industrial areas. It is absorbed by plants and accumulates in different organs (Arshad *et al*., 2008). Pb induces a broad range of morphological, physiological, and biochemical effects that can be toxic on living organisms (Pourrut *et al*., 2011). This metal reduces seed germination, affects plant growth, and root elongation. It also impedes seedling development and chlorophyll production (Sharma and Dubey, 2005; Maestri *et al*., 2010). In addition, Pb phytotoxicity inhibits the activities of enzymes, especially those containing sulfhydryl (-SH) groups, interfering with the mineral nutrition and water balance. The hormonal status and membrane permeability get interrupted. It also induces secondary stresses, similar to those caused by nutritional deficiencies and excessive reactive oxygen species (Krämer and Clemens, 2005; Sharma and Dubey, 2005). These disorganizations disrupt the normal physiological conditions of the plant.

To cope with Pb stress, as well as stresses caused by other metals, plants have several defense strategies that are associated with the cellular-free metal content [eg., cell wall binding, metal exclusion, chelation and sequestration (Hall, 2002)] and to the control and modulation of cellular responses [eg., repair of proteins that have been damaged by stress factors and antioxidative defenses (Hall, 2002)]. The production of chelators and the upcoming trapping of metal complexes are major factors needed to limit free metal concentrations. Glutathione (GSH) is a tripeptide produced in the cell cytoplasm and chloroplasts that scavenge $^1O_2$ and hydrogen peroxide (H_2O_2). Glutathione is oxidized to glutathione disulfide, which functions as a redox regulator and an antioxidant. GSH is a substrate for glutathione-S-transferases (GSTs), which play an important role in the detoxification of xenobiotics. GSH is also a precursor of phytochelatins, which control cellular heavy metal levels, and is involved in controlling gene expression (Sofo *et al*. 2010; Seth *et al*., 2012).

Methyl jasmonate (MeJA) and its free-acid, jasmonic acid (JA), together called jasmonates, are important cellular regulators related to diverse developmental

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activities, including seed germination, root growth, fruit ripening, and senescence (Xang and Hause, 2002, Samota *et al*. 2017). In addition, jasmonates induce defense mechanisms in plants in response to pathogen attacks, wounding, and abiotic stress factors (Javid *et al*., 2011). Jasmonic acid, when added exogenously, stimulates the expression of genes involved in GSH synthesis providing protection against oxidative stress (Xang and Oliver, 1998). Recently, Ali *et al*. (2018) observed that JA could act as a "stress-ameliorating molecule" by improving the tolerance of rapeseed plants to cadmium toxicity.

Although the role of JA in protecting plants against environmental stresses, such as drought, low temperature, salinity and cadmium toxicity, has been extensively studied (Cheong and Choi, 2003; Hassanein *et al*., 2009), Pb-related tolerance strategies in JA-treated are not yet fully- understood. The objectives of this study are to investigate the role of JA acting as a growth regulator in alleviating the harmful effects of Pb on *Phaseolus vulgaris* L. and to determine the changes in the total protein pattern, amino acid profile, antioxidant enzyme activities, GSH level and ion homeostasis in response to JA in both Pb-treated and control plants.

2. Materials and Methods

2.1. Plant Material

The kidney bean (*P. vulgaris* L.) seeds were purchased from Crop Institute, Agricultural Research Center, Giza, Egypt.

2.2. Growth Conditions and Treatments

The present study was conducted in the greenhouse of Botany Department, Faculty of Science, Ain Shams University. Healthy kidney bean (*P. vulgaris*) seeds of a matching size were chosen and surface-sterilized in 0.0 5% (w/v) sodium hypochlorite solution, and were repeatedly washed with distilled water. Ten seeds were scattered in each pot at a depth of 3 cm. Five plastic pots (25-cm deep and 40-cm diameter) were used for each treatment. Each pot contained 14 kg of a blend of clay and sand (2:1 w/w). The pots were kept in a greenhouse under normal conditions (the mean light and dark temperatures were 24°C and 12°C ± 3°C, respectively). Two-week-old seedlings were irrigated with the specific lead nitrate [$Pb(NO_3)_2$] concentrations at 70 % of the soil water-holding capacity, and two concentrations of $Pb(NO_3)_2$ were used; 100 and 1,000 µM. After two days, the plants grown on each $Pb(NO_3)_2$ concentration were separated into two groups. The first group was sprayed with 100 µM of JA in 0.01% Tween 20 and the second group was sprayed with water to serve as the control. After two days of JA treatment, growth parameters, including shoot and root lengths, mean leaf area per plant, number of leaves per plant, and fresh and dry weights (FW and DW, respectively) of shoots and roots, the relative water content (RWC), photosynthetic pigments and mineral ions, including zinc (Zn), calcium (Ca) and magnesium (Mg), were measured. In addition, changes in the antioxidant enzyme activities (ascorbate peroxidase (APX), catalase (CAT) and GST) were measured. The GSH level and amino acid and protein profiles were also determined.

2.3. Determination of the Relative Water Content (RWC)

The RWC was calculated in 2-cm fresh leaf discs, excluding the midribs. Discs were weighed quickly and immediately left to float for twenty-four hours in the dark on deionized water in Petri dishes to saturate them. The water adhering to the discs was blotted away, and the turgor mass was noted. The dry masses of the discs were determined by dehydrating them at 80°C for forty-eight hours (Fariduddin *et al*., 2009). The RWC was calculated using the formula below:

$$RWC = \frac{\text{Fresh mass} - \text{dry mass}}{\text{Turgor mass} - \text{dry mass}} \times 100$$

2.4. Photosynthetic Pigments

The concentrations of photosynthetic pigments, chlorophyll a (chl a), chlorophyll b (chl b) and carotenoids, were measured spectrophotometrically as described by Metzner *et al*. (1965). One gram of fresh leaves was homogenized in 85 % (v/v) aqueous acetone. The extinction was measured against a blank of pure 85 % aqueous acetone at three wavelengths (663, 644 and 452.5 nm). The concentrations of the pigments (chl a, chl b and carotenoids) were calculated as µg ml^{-1} according to the following equations:

$$\text{Chl a} = 10.3\ E_{663} - 0.918\ E_{644}$$

$$\text{Chl b} = 19.7\ E_{644} - 3.87\ E_{663}$$

$$\text{Carotenoids} = 4.2\ E_{452.5} - (0.0264 \text{ chl a} + 0.4260 \text{ chl b})$$

The pigment contents were calculated as µg g^{-1} FW of leaves.

2.5. Antioxidant Enzyme Activities

The APX (EC 1.11.1.11) activity level was determined according to Asada (1992) by measuring the decrease in optical density at 290 nm as a result of the oxidation of acyl-CoA synthase using a Spectronic 601 UV spectro-photometer. The reaction mixture (3 mL) contained 50 mM ascorbic acid and 0.1 mM Ethylenediamine tetraacetic acid (EDTA) and 0.1 mL enzyme extract. The reaction was started by adding H_2O_2 to a final 1.5-mM concentration. The non-enzyme extract mixture was used as the blank. The assay was carried out according to Prochazkova *et al*. (2001). The enzyme activity was expressed as unit h^{-1} g^{-1} FW.

The CAT (EC 1.11.1.6) activity was determined by measuring the initial rate of disappearance of H_2O_2 (Aebi, 1983). The reaction mixture (3 mL) contained 10 mM of potassium phosphate buffer (pH 7) and 0.1 mL of enzyme extract. The reaction was started by adding 0.035 mL of 3 % H_2O_2. The decline in optical density at 240 nm was monitored. The non-enzyme reaction mixture was used as the blank. The CAT activity was expressed as unit h^{-1} g^{-1} FW.

The GST (EC 2.5.1.13) activity was measured according to Vontas *et al*. (2000) by observing the conjugation of 1-chloro, 2,4-dinitrobenzene (CDNB) with reduced GSH. This is indicated by an increase in the absorbance at 340 nm. One unit of enzyme conjugates 10 nmol of CDNB with reduced GSH per minute at 25°C. The reaction mixture contained 980 µl PBS (pH 6.5), 10 µL of 100 mM CDNB and 10 µL of 100 mM GSH. The GST activity was calculated using the extinction coefficient of CDNB: 0.0096 $\mu M^{-1}cm^{-1}$.

2.6. Protein Extraction and Quantification

Leaf tissue (0.5 g from each treatment) was ground on ice using a mortar and pestle with 5 mL of 10 mM potassium phosphate buffer (pH 7.0) containing 4 % (w/v) polyvinylpyrrolidone. The crude extract was centrifuged at 12,000 ×g for thirty minutes at 4°C, and the supernatant was used. The amount of protein in the extract was determined using Bradford's method (Bradford, 1976).

2.7. Protein Electrophoresis

One-dimensional SDS-PAGE was carried out according to the method described by Studier (1973) in a linear polyacrylamide resolving gel (12 %) with a stacking gel (4 %). The samples were loaded into the wells and electrophoresed at 100 V until the dye front reached the bottom of the gel. The gel was removed from the plates and shaken in staining solution (400 mL methanol, 100 mL glacial acetic acid, 500 mL distilled water and 1 g Coomassie Brilliant Blue R-250) for two hours, and was then transferred to a destaining solution (400 mL methanol, 100 mL glacial acetic acid and 500 mL distilled water) until protein bands appeared.

2.8. Amino Acid Analysis

The samples were dried, defatted and weighed to 100 mg in screw-capped tubes. Then, 5 mL of 6.0 N HCl was added. The hydrolysis tubes were attached to a system, which allowed the connection of nitrogen and vacuum lines without disturbing the samples. The tubes were placed in an oven at 110°C for twenty-four hours. The tubes were then opened, and the content of each tube was filtered and evaporated for dryness in a rotary evaporator. A suitable volume of sodium citrate buffer (pH 2.2) was added to each dried film of the hydrolyzed samples. After dissolving all of the soluble materials completely, the samples were then filtered using a 0.2-µm membrane filter, and were then ready for analysis (Baxter, 1996). The system used for the analysis was a high-performance Amino Acid Analyzer, Biochrom 20 (Auto Sampler Version) from Pharmacia Biotech, constructed at The National Center for Radiation Research and Technology (NCRRT). The chromatogram analysis was performed using an Ezchem^{TM} Chromatography Data system's tutorial and user's guide, version 6.7.

2.9. Reduced Glutathione Content

The reduced glutathione (GSH) content was extracted and determined by the method of Tanaka *et al*. (1985). Data are expressed as µg g^{-1} FW.

2.10. Macro and Micro Minerals

The macro minerals Mg^{2+} and Ca^{2+}, and the micro mineral Zn^{2+} were extracted from dried roots and shoots (including stems and leaves) according to Chapman and Pratt (1978), and were measured using an atomic absorption spectrometer in terms of mg kg^{-1} DW of the sample for Zn^{2+} and g kg^{-1} DW for Mg^{2+} and Ca^{2+}.

2.11. Pb Accumulation

The plant samples were digested with HNO_3 (65 % Merck supra pure) and $HCIO_4$ (65 % Merck supra pure) in 5:1 ratio until a transparent solution was obtained (Allen *et al*., 1986; Markert, 1996). The Pb concentration was determined using a Perkin Elmer 4300 DV Inductive Coupled Plasma and expressed as mg kg^{-1} DW.

2.12. Statistical Analyses

The experimental design was a complete random block. According to Snedecor and Cochran (1990), the averages of data were statistically analyzed using a two-way analysis of variance. Significant values were determined according to the least significant difference ($P < 0.05$) using the STAT-ITCF program (Foucart, 1982).

3. Results

3.1. Growth Parameters

Lead significantly reduced all the detected growth parameters in the kidney beans (*Phaseolus vulgaris* L.) compared to the untreated control plants (Table 1). The decrease was directly proportional to the applied Pb concentration. Maximum inhibition in growth was observed in plants that received 1000 µM Pb $(NO_3)_2$ and was calculated by 43.5 %, 52 %, 60.4 %, 72.4 %, 25 % and 75.4 % below the control value in the shoot length, root length, fresh weight of shoot and root, dry weight of shoot and root, respectively. Treatment with JA caused a substantial increase in these traits under normal as well as Pb-stress conditions. Jasmonic acid completely overcame the inhibitory effect of 100 µM Pb $(NO_3)_2$ on length, the fresh and dry weights of shoot.

Table 1. Effect of Pb $(NO_3)_2$ treatments (100 and 1000 µM) in control plants or in plants treated with JA (100µM) on growth parameters of kidney bean (*Phaseolus vulgaris* L.) plants.

Treatment	Pb $(No_3)_2$ (µM)	Shoot length (cm)	Root length (cm)	Fresh weight of shoot (g)	Fresh weight of root (g)	Dry weight of shoot (g)	Dry weight of root (g)
Reference	0	11.32±0.57	15±0.60	2.8±0.30	1.107±0.123	0.2±0.035	0.134±0.016
Controls	100	9.5±0.50	12.5±0.40	1.9±0.20	0.77±0.16	0.175±0.015	0.05±0.013
	1000	6.4±0.80	7.2±0.30	1.11±0.21	0.305±0.004	0.15±0.012	0.033±0.002
Jasmonic acid (100 µM)	0	14.0±0.50	21±1.00	4.19±0.15	2.85±0.05	0.35±0.02	0.2±0.05
	100	12.5±1.2	13±0.50	3.77±0.08	0.97±0.05	0.3±0.04	0.1±0.002
	1000	9.75±0.35	10±0.40	2.267±0.363	0.617±0.096	0.2±0.033	0.075±0.004
LSD at 0.05		2.19	1.789	0.729	0.292	0.097	N.S.

N.S. = Not significant; Data expressed as mean of ten samples ± SD.

3.2. Relative Water Content (RWC)

Changes in RWC in response to Pb toxicity in the presence or absence of JA treatment were shown in Figure 1. Pb toxicity significantly reduced RWC of kidney bean leaves. The reduction was evaluated by 20 % and 25 % in plants treated with 100 and 1000 µM of Pb $(NO_3)_2$, respectively. Leaf RWC increased in plants treated with JA and subjected to 0, 100 and 1000 µM of Pb $(NO_3)_2$ by 18.9 %, 23.1 % and 7.5 %, respectively compared to the untreated plants that received the same amounts of Pb $(NO_3)_2$. The percentage of RWC in plants sprayed with JA and subjected to 100 µM of Pb $(NO_3)_2$ was much higher than that of the untreated plants grown under normal growth conditions.

3.3. Photosynthetic Pigments

Different levels of lead stress significantly reduced both chlorophyll a and b contents, with chlorophyll b being the most affected as indicated by the increased chl a/b ratio compared to the control value. In contrast, carotenoids significantly increased by 92.3 % and 154.1 % over the control value in plants treated with 100 and 1000 µM of Pb $(NO_3)_2$, respectively (Table 2).

Jasmonic acid (100 µM) significantly increased chlorophyll a, b, carotenoids and the total pigment content compared to the untreated control plants. Their values in JA-treated plants were calculated by 2.68, 1.92, 4.24 and 2.7-fold of the control plants grown under normal conditions, respectively. In addition, JA completely alleviated the inhibitory effect of 100 µM of Pb stress on chlorophyll a and b. Interestingly, the total photosynthetic pigment content was significantly higher in the plants treated with different levels of Pb $(NO_3)_2$ alone or in combination with JA compared to the untreated control plants.

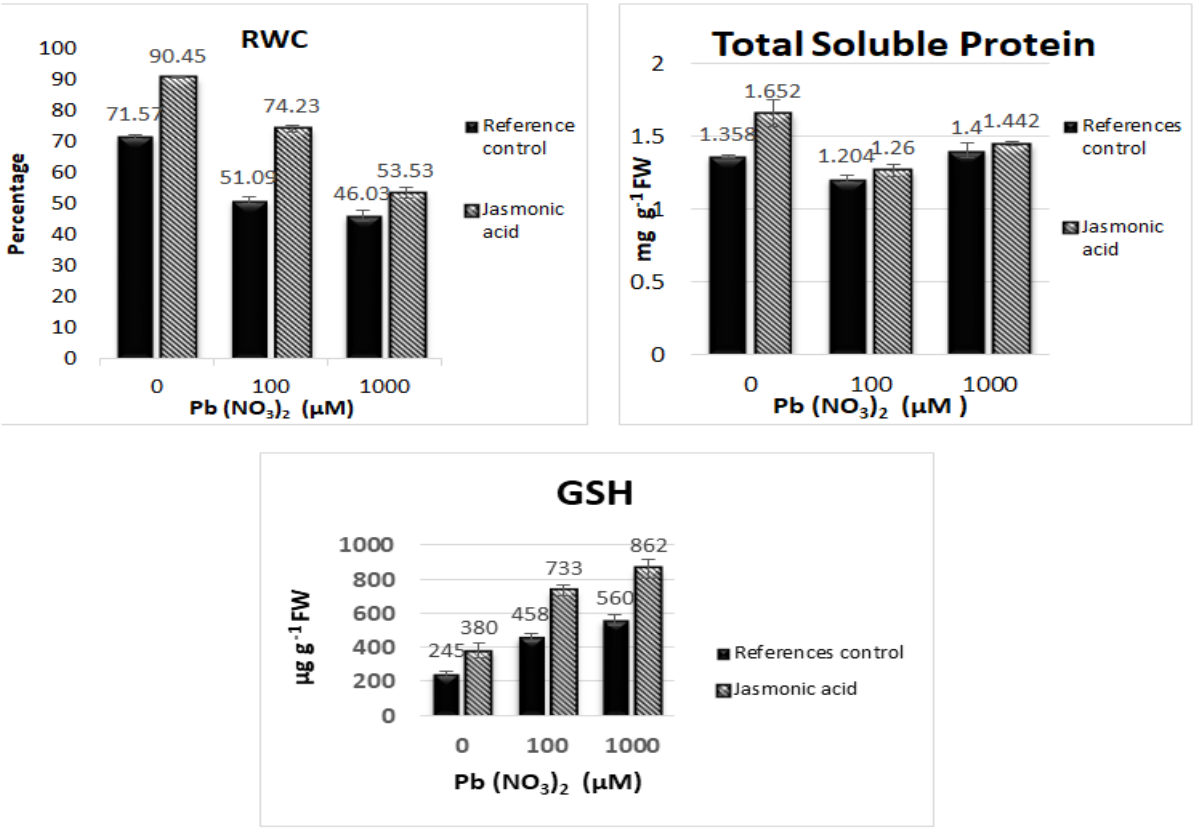

Figure 1. Effect of Pb $(NO_3)_2$ treatment (100 and 1000 µM) on control plants or plants treated with JA in terms of relative water content (RWC), total soluble protein, and glutathione content of *Phaseolus vulgaris* L. plants. Data presented as mean ± SD.

Table 2. Effect of Pb $(NO_3)_2$ treatments (100 and 1000 µM) in control plants or in plants treated with JA (100µM) on photosynthetic pigments content (µg g^{-1} FW) of kidney bean (*Phaseolus vulgaris* L.) leaves.

Treatment	Pb $(NO_3)_2$(µM)	Chlorophyll a	Chlorophyll b	Carotenoids	Chl a/Chl b	Total pigments
Reference	0	334.594±9.144	203.32±2.38	107.015±3.25	1.646±0.064	644.929±10.014
Controls	100	316.957±0.703	125.752±1.688	205.824±1.934	2.520±0.028	648.533±0.457
	1000	283.003±6.797	126.897±2.197	272.162±0.508	2.230±0.092	682.061±4.093
Jasmonic acid (100 µM)	0	897.164±8.136	389.694±0.994	453.891±3.768	2.302±0.027	1740.748±3.375
	100	500.439±1.342	205.847±1.247	312.695±2.975	2.431±0.021	1018.981±3.070
	1000	304.22±2.01	127.635±0.665	255.253±0.912	2.384±0.028	687.107±2.258
LSD at 0.05		17.895	5.082	7.800	0.169	15.052

Data expressed as mean of five samples ± SD.

3.4. Antioxidant Enzymes Activity

Data presented in Table 3 show that Pb-stress significantly increased the activity of catalase, ascorbate peroxidase, and glutathione transferase enzymes compared to the unstressed control plants. The increase in antioxidant enzymes activity was directly proportional to the applied concentration of Pb $(NO_3)_2$, and was calculated in the plants that received 1000 µM of Pb $(NO_3)_2$ by 1.9, 1.7 and 6.4-fold of the untreated control values in catalase, ascorbate peroxidase, and glutathione transferase, respectively.

Table 3. Effect of Pb$(NO_3)_2$ treatments (100 and 1000 µM) in control plants or in plants treated with JA (100µM) on antioxidant enzymes (catalase and ascorbate peroxidase) and glutathione transferase activities of kidney bean (*Phaseolus vulgaris* L.) plants.

Treatment	Pb $(No_3)_2$ (µM)	Catalase (CAT) Unit $h^{-1}g^{-1}$ FW	Ascorbate peroxidase (APX) Unit $h^{-1}g^{-1}$ FW	Glutathione transferase (GST) mM g^{-1}FW
Reference	0	2.74±0.14	9.8±0.022	451.03±6.44
Controls	100	5.02±0.15	9.83±0.122	1114.33±20.59
	1000	5.2±0.0	16.82±0.375	2895.83±00
Jasmonic	0	3.65±0.26	1.54±0.01	1650.62±0.29
acid (100	100	6.5±0.12	17.07±0.253	2587.77±00
µM)	1000	6.82±0.06	35.46±0.171	3968.75±33.33
LSD at 0.05		0.00	0.624	18.85

Data expressed as mean of three samples ± SD.

Spraying the kidney bean plants with jasmonic acid increased the level of catalase and glutathione transferase activities in the control plants as well as in the plants treated with 100 and 1000 µM of Pb $(NO_3)_2$. On the other hand, JA treatment significantly decreased the APX activity in control plants (Table 3), whereas its activity levels increased in JA-treated plants grown under 100 and 1000 µM of Pb $(NO_3)_2$ stress compared to the plants treated with Pb alone.

3.5. Protein Analysis

The total soluble protein increased in kidney bean plants sprayed with JA under normal and Pb-stress conditions compared to untreated plants grown under the same conditions (Figure 1).

Total protein was extracted from the control group and each treatment. Equal amount of protein was loaded in each lane of the SDS-polyacrylamide gel. The effect of lead nitrate (100 and 1000 µM) on the protein profiles of kidney beans in the absence or presence of jasmonic acid are shown in Figure 2. The soluble protein profiles of the control and all treatments included six common major bands and several minor bands. The main polypeptide bands are located between 10 and 200 KDa.

The electrophoretic analysis of protein patterns of the control and both concentrations of Pb (100 and 1000 µM) showed that the polypeptides with molecular weights ranging from 10 to 200 KDa were all obvious in the control (Figure 2). Lead stress increased the accumulation of certain protein bands, and completely inhibited or caused the *de novo* synthesis of others, compared with the control plants grown under normal conditions. In this respect, the 20 and 70 KDa bands were more intense accumulating in plants grown on 100 µM Pb, compared with the control. On the other hand, three protein bands with molecular weights 150, 40 and 25 KDa were completely inhibited in response to the 100 µM of Pb. This number increased to 6 (Molecular weight: 150, 120, 110, 100, 40 and 25 KDa) in the plants that received 1000 µM of Pb. A new protein band with the molecular weight 50 KDa was detected in the Pb-stressed plants, but was not found in the control plants (Figure 2)

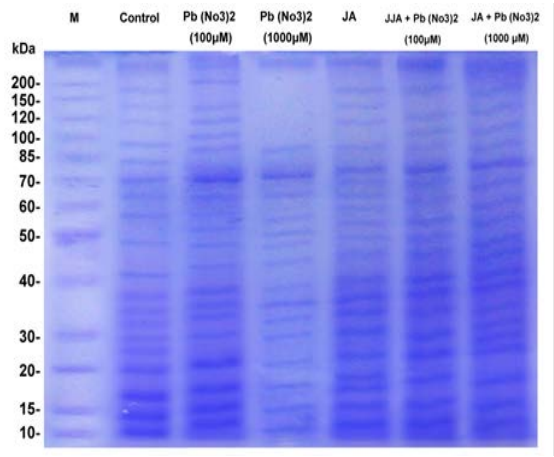

Figure 2. SDS-PAGE protein profiles of *Phaseolus vulgaris* L. treated with different concentrations and combinations of Pb $(NO_3)_2$ and JA.

Jasmonic acid caused a dual effect on the protein profile of kidney bean plants. It restored the stress inhibited proteins in the 100 to 200 KDa region, and induced the synthesis of two new protein bands in the range of (40-70 KDa).

3.6. Amino Acids Profile

Data presented in Table 4 show that the total amino acid content significantly decreased in response to Pb stress (100 and 1000 µM). On the other hand, treatment with JA significantly increased the total amino acids in plants under normal or stress conditions compared to the corresponding control. Essential amino acids including threonine, valine, methionine, isoleucine, leucine, phenylalanine and lysine significantly decreased in the plants treated with Pb $(NO_3)_2$ (100 and 1000 µM), compared to the control plants. The amounts of essential amino acids increased in response to 100 µM JA treatment whether the plants were grown under normal conditions or subjected to Pb-stress (except valine and isoleucine where JA treatment decreased their amounts under normal growth condition). Most of the non- essential amino acids followed the same pattern in response to Pb stress, with few exceptions. For example, the levels of Asp, Pro, and Arg were significantly increased in the plants treated with different concentrations of Pb and Glu level increased dramatically in the 100 µM Pb $(NO_3)_2$ -treated plants compared to the control plants. The amounts of Pro were 2 and 3.25-fold of the control value in the plants treated with 100 and 1000 µM of Pb $(NO_3)_2$, respectively. JA treatment significantly increased all the detected non-essential amino acids in the plants grown under normal conditions or subjected to Pb stress compared to the corresponding control value (except Tyr and Arg which decreased in response to JA below the corresponding control values) (Table 4). The amounts of Glu, Pro, Gly, and Cys in JA treated plants were 1.45, 2.25, 1.5 and 7-fold of those in the case of the untreated control plants.

Table 4. Effect of Pb $(NO_3)_2$ treatments (100 and 1000 µM) in control plants or in plants treated with JA (100µM) on amino acids (mg g^{-1} DW) profile of kidney bean (*Phaseolus vulgaris* L.) leaves.

Amino acids \ Treatment	Control	100 µM Pb $(NO_3)_2$	1000 µM Pb $(NO_3)_2$	Jasmonic acid (100 µM)	Jasmonic acid (100 µM)+ 100 µM Pb $(NO_3)_2$	Jasmonic acid (100 µM)+ 1000 µM Pb $(NO_3)_2$	LSD at 0.05
Essential amino acids							
Threonine (Thr)	0.65±0.05	0.5±0.02	0.35±0.08	0.7±0.02	0.6±0.03	0.5±0.05	0.218
Valine (Val)	0.7±0.2	0.35±0.01	0.35±0.02	0.6±0.02	0.5±0.01	0.7±0.031	0.097
Methionine(Met)	0.05±0.00	0.05±0.00	0.05±0.00	0.1±0.00	0.05±0.00	0.3±0.05	0.097
Isoleucine (Ile)	0.65±0.02	0.4±0.01	0.35±0.00	0.5±0.02	0.5±0.01	1.0±0.03	0.022
Leucine (Leu)	2.85±0.05	1.65±0.1	1.75±0.2	2.7±0.31	2.2±0.1	1.1±0.05	0.169
Phenylalanine(Phe)	0.7±0.01	0.55±0.05	0.55±0.01	0.8±0.04	0.8±0.01	0.9±0.05	0.138
Lysine (Lys)	0.9±0.05	0.65±0.05	0.55±0.04	1.1±0.1	1.0±0.05	0.8±0.1	0.138
Non-essential amino acids							
Aspartic acid (Asp)	0.75±0.01	1.5±0.03	1.6±0.05	1.2±0.15	1.8±0.04	1.9±0.07	0.218
Serine (Ser)	0.65±0.02	0.5±0.00	0.35±0.01	0.8±0.03	0.6±0.02	0.6±0.04	0.097
Glutamic acid (Glu)	0.55±0.02	1.5±0.05	0.35±0.00	0.8±0.00	1.7±0.1	0.6±0.03	0.097
Proline (Pro)	0.4±0.1	0.8±0.08	1.3±0.15	0.9±0.01	2.0±0.2	2.7±0.1	0.377
Glycine (Gly)	0.6±0.2	0.5±0.01	0.25±0.02	0.9±0.12	0.65±0.01	0.4±0.02	0.258
Alanine (Ala)	0.5±0.01	0.25±0.01	0.25±0.01	0.6±0.02	0.4±0.01	0.3±0.02	0.097
Cysteine (Cys)	0.15±0.03	0.11±0.00	0.05±0.01	1.05±0.05	0.5±0.04	0.4±0.02	0.258
Tyrosine (Tyr)	0.7±0.05	0.25±0.02	0.25±0.01	0.4±0.1	0.2±0.05	0.6±0.07	0.169
Histidine (His)	0.9±0.02	0.65±0.05	0.6±0.02	0.9±0.04	0.9±0.05	1.0±0.07	0.169
Arginine (Arg)	0.9±0.03	1.1±0.05	1.5±0.04	0.8±0.03	0.5±0.01	0.4±0.1	0.195
Total	12.6	11.31	10.45	14.85	14.9	14.2	0.12

Data expressed as mean of three samples ± SD.

3.7. Glutathione (GSH) Content

GSH significantly increased in response to Pb stress (Figure 1). Additional amounts of GSH were accumulated in response to JA treatment. In this respect, the GSH content was found to be higher in the case of plants treated with JA compared to the control ones (treated or untreated with Pb $(NO_3)_2$) (Figure 1).The increase in the GSH content in the JA-treated plants was estimated at 55.1 %, 60 % and 53.9 % in the plants treated with 0,100 and 1000 µM of $Pb(NO_3)_2$, respectively in comparison with the plants that are not treated with JA and grown under the same Pb-stress condition.

3.8. Mineral Ions and Pb2+ Accumulation

Kidney bean plants subjected to heavy metal stress (100 and 1000 µM of Pb $(NO_3)_2$) had significantly lower amounts of zinc, magnesium, and calcium in their shoots and roots compared to the control plants grown under normal conditions (Table 5). The levels of decrease were much more pronounced in response to the 1000 µM of Pb $(NO_3)_2$ and were evaluated as 34.6 %, 20.1 %, and 42 % below the control value in the case of shoot and 23.6 %, 5.5 %, and 42 % in the case of root in Zn, Mg, and Ca, respectively. Exogenous application of JA to heavy metal-stressed plants partially alleviated the inhibitory effect of Pb on Mg and Ca in the shoots and roots. In contrast, JA significantly decreased the Zn amounts in the control plants and those grown under different levels of Pb stress compared to the untreated plants grown under the same condition.

Plants treated with Pb $(NO_3)_2$ accumulated significantly higher amounts of Pb in their shoots and roots compared to the untreated plants. JA significantly decreased the amounts of accumulated Pb by 89 % and 95 % in the shoots, and by 48.4 % and 92.4 % in the roots of plants treated with JA + 100 and 1000 of Pb $(NO_3)_2$, respectively below those of plants treated with the same concentration of Pb $(NO_3)_2$ alone.

Table 5. Effect of Pb $(NO_3)_2$ treatments (100 and 1000 µM) in control plants or in plants treated with JA (100µM) on mineral ions content of kidney bean (*Phaseolus vulgaris* L.) plants.

Treatment	Pb $(No_3)_2$ (µM)	Zn^{2+} (mg Kg^{-1} DW)		Mg^{2+} (g Kg^{-1} DW)		Ca^{2+} (g Kg^{-1} DW)		Pb^{2+} (mg Kg^{-1} DW)	
		Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Reference Controls	0	58.325±0.665	46.575±1.875	9.32±0.24	5.11±0.12	25.44±0.56	15.64±0.64	0.025±0.001	2.35±0.23
	100	43.575±0.025	40.825±0.835	8.02±0.03	6.97±0.15	22.83±0.48	9.07±0.01	10.725±0.735	35.25±0.48
	1000	38.075±0.075	35.575±0.095	7.45±0.11	4.83±0.02	14.75±0.15	9.07±0.07	214.5±1.62	462.75±1.88
Jasmonic acid (100 µM)	0	51.575±1.305	40.325±0.345	11.45±0.44	7.38±0.38	28.09±0.09	16.45±0.24	0.825±0.051	1.175±0.165
	100	44.352±0.472	31.075±0.075	9.96±0.46	7.35±0.02	24.27±0.39	13.15±0.16	1.1825±0.027	18.175±0.185
	1000	37.575±0.535	30.825±0.125	8.13±0.10	7.72±0.01	19.1±0.71	11.89±0.11	10.725±0.055	35.25±0.47
LSD at 0.05		2.051	2.627	0.877	0.534	1.395	0.898	2.239	2.547

Data expressed as mean of three samples ± SD.

4. Discussion

Lead (Pb) is an example of a hazardous heavy metal that is not essential for cell metabolism, and can be easily absorbed and accumulated in different plant tissues and organs (Nas and Ali, 2018). This study found out that under Pb stress [100 and 1000 µM of Pb $(NO_3)_2$], all the detected growth parameters, chlorophyll a and b contents, were significantly ($P < 0.05$) reduced in the kidney beans (*Phaseolus vulgaris* L.) compared to the untreated control plants. The decrease was directly proportional to the applied Pb concentration. Similar results were obtained from some other studies using different concentrations of lead: root, shoot and leaf growth; fresh and dry weights are significantly decreased in *Pisum sativum* (Çimrin *et al*., 2007) and in tomato (Opeolu *et al*., 2010).

Active Pb ions can cause toxic effects and damage the photosynthetic systems in plant tissues (Yang *et al*., 2015); plant development is thus affected (Zhou *et al*. 2018). The Chlorophyll content in leaves is proved to be very sensitive to the alteration in plant oxidative status. Therefore, changes at chlorophyll level are observed in plants subjected to stress conditions (Sivaci *et al*., 2004). JA foliar spray significantly increased all the measured growth traits of kidney bean plants (including; shoot and root length, fresh and dry weights) as well as chlorophyll a and b contents compared to the untreated plants. JA completely overcame the harmful effects of up to 100 µM of Pb $(NO_3)_2$ on growth and chlorophylls. Similar results were obtained by Piotrowska *et al*. (2009) who found that chlorophyll content decreased in *Wolffia arrhiza* grown under heavy metal stress conditions compared to the control, but the exogenous application of low concentration of JA increased the chlorophyll content. Our results suggest that the application of 100 µM JA has a positive impact on photosynthetic apparatus in kidney bean plants grown under Pb stress conditions. Owing to the photosynthetic pigments' function of protection from oxidative stress and heavy metals, the increase in their content positively affects the ability of plants to adapt to environmental pollution.

On the other hand, carotenoids levels significantly ($P<0.05$) increased under Pb stress, and additional amounts accumulated when the plants were treated with JA. Carotenoids are known to serve as antioxidants due to their ability to scavenge free radicals, and to reduce cell membrane damage caused by heavy metal poisoning (Czerpak *et al*., 2006). The increased level of carotenoids induced by Pb stress treatment may be an adaptive response in the kidney bean plants under stress conditions.

Under Pb stress, RWC was markedly reduced in the kidney bean leaves compared to the control. Kastori *et al*. (1992) confirmed that excess lead damages the plant root system, and reduces water uptake resulting in an inadequate water supply to the plant shoot system (Kastori *et al*., 1992). The exogenous application of JA somehow reverses the harmful effects of Pb on RWC, JA at 100 µM increased the RWC of plants treated with 100 µM of Pb compared to the untreated control. These results show that jasmonic acid might act as a protector of the dehydration process under both control and metal stress conditions via increasing the RWC, as an attempt to protect plants against adverse conditions.

Excess Pb in cells damages plants either directly or indirectly by increasing the oxidative load caused by ROS formation (Kumar *et al*., 2011). Generally, the alleviation of oxidative stress is attributed to the increase in enzyme activity and the scavenging of ROS formed in response to the stressful condition (Foyer and Noctor, 2005). The present results show that Pb-stress significantly ($P<0.05$) increased the activity of catalase, ascorbate peroxidase, and glutathione-*S*-transferase as well as the glutathione content in the kidney bean plants. The results proved that the plants treated with Pb and JA have significantly increased the ASPX, GST activities and glutathione content [ascorbate - glutathione (Asc–GSH) cycle components] compared to the plants treated with Pb alone. The ASC–GSH cycle serves in the removal of H_2O_2, which is inevitably formed as a by-product of the normal metabolism or as a consequence of environmental stress factors (Latowski *et al*., 2010). The stimulation of Asc–GSH cycle in addition to the catalase activity in response to exogenous JA treatment proved that JA help kidney bean plants in upgrading their antioxidant capacity to scavenge more free radicals.

Lead stress increased the accumulation of two protein bands (Mwt: 20 and 70 KDa), and completely inhibited six protein bands (Mwt: 150, 120, 110, 100, 40 and 25 KDa) and caused the *de novo* synthesis of a new protein band with the molecular weight of 50 KDa compared to the control plants grown under normal conditions (Figure 2). In this respect, Kumar *et al*. (2011) detected the up-regulation of fourteen proteins in *Catharanthus roseus* in response to the Pb treatment, among which are the two HSP 70s and HSP 20. HSPs functions as molecular chaperones, the proteins which are involved in the "house-keeping" inside the cell (Sørensen *et al*., 2003). The detected up-regulated and *de-novo* synthesized proteins in response to the Pb stress are expected to help in managing the cellular activities in the plants under oxidative stress. On the other hand, the detected inhibition in other proteins indicated that Pb could increase the degradation of certain proteins. This degradation might be attributed to the binding of metals to the sulphydryl groups in these proteins, leading to the disruption of structure and / or promotion of DNA damage due to ROS accumulation (Tomas *et al*., 2014). Jasmonic acid application restored the stress-inhibited proteins and induced the synthesis of two new protein bands in the range of (70-40 KDa). Several studies reported that JA induces the accumulation of a specific set of proteins, which are called jasmonate-induced proteins (JIP). These proteins are divided into five groups on the basis of their function, that is, stress and defense, photosynthesis, carbohydrates and energy production, protein metabolism, and secondary metabolites (Sharma *et al*. 2013, Farooq *et al*. 2016).

JA showed a potential to restore the accumulation of most amino acid levels that decreased in response to the Pb $(NO_3)_2$ treatment, and significantly ($P<0.05$) increased the accumulation of other amino acids including glutamic acid, glycine, cysteine and proline (Table 5). For proline in particular, the data suggest that metal-induced proline plays a vital role in metal-stress defense. Strong experimental evidence, including work with transgenic plants and algae, indicates that proline can act as an antioxidant, metal-binding and signaling molecule (Emamverdian *et al*., 2015). Histidine, cysteine, and other

amino acids are known as a potent chelators of heavy metal ions. Their accumulation should be considered as a positive response to heavy metal stress and not as a consequence of metabolic dysregulation (Sharma and Dietz, 2006). In addition, glycine, cysteine, and glutamine availability play a key role in the GSH content (Droux, 2004).

Pb stress significantly (P<0.05) reduced the amounts of Mg^{2+} and Ca^{2+} ions, and increased the level of Pb accumulated in kidney bean shoots and roots compared to the control. The detected reduction in Ca^{2+} might be ascribed to the fact that the absorption of lead by roots occurs via the apoplastic pathway or via the Ca^{2+}-permeable channels (Pourrut *et al*., 2011).

Meanwhile, at a concentration of 100 µM, JA was able to restore the decrease in Mg^{2+} and Ca^{2+} contents that took place due to the treatment with 100 µM of Pb $(NO_3)_2$ in both the plant shoot and root. Mg^{2+} plays an important role in many metabolic processes such as being a cofactor of enzyme activity with ATP, the central atom in chlorophyll, and a stabilizer for ribosomal structure (Tanoi and Kobayashi, 2015). The stimulatory effect of JA on the chlorophyll a and b contents in plants grown under Pb stress could be attributed to its effects on chlorophyll biosynthetic pathway and ameliorating the inhibitory effects of Pb stress on the Mg ion content in plant cells.

In addition, JA significantly (P<0.05) reduced the amounts of Pb accumulated in shoots and roots of plants grown under Pb stress compared to the untreated plants grown under the same stress conditions. Reduction in heavy-metal uptake in response to exogenous application of stress hormones might be caused by the reduced transpiration rate and symplastic loading of heavy metal into xylem (Lux *et al*., 2011). The protective role of 100 µM JA against Pb toxicity in the kidney bean plants could be attributed to the JA-induced inhibition of Pb bioaccumulation which allows plant cells to maintain precise homeostatic regulation of intracellular heavy-metal levels.

Conclusion

Lead strongly inhibits kidney bean growth and chlorophyll and water content. The negative effects that lead was found to have on kidney bean plants might be attributed to the impaired uptake of macronutrients (Mg^{2+} and Ca^{2+}), inhibition of essential and non-essential amino acid levels, and the accumulation of high amounts of Pb in plant shoots and roots.

JA at 100 µM was shown to effectively protect kidney bean plants from hazardous effects resulting from Pb exposure. The mechanism of JA-induced stress tolerance in kidney beans was proved to be related to the blockade of heavy metal entry to the cell, and the stimulation of the antioxidant defense mechanisms to reduce the oxidative damage induced by Pb treatment. However, further investigations are still needed to fully explore the interaction between heavy metals and jasmonic acid.

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