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Ameliorative Effect of P and Ca, Mg, K Addition on Buckwheat in the Presence of Heavy Metal Stress

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Abstract

As an important agricultural plant, buckwheat (*Fagopyrum esculentum*) was used in this study. In order to examine the negative effect of heavy metal pollution, As, Cd and Pb were added to growth medium. On the other hand, for reducing the stress effect of metals P and Ca, Mg, K was also included. Obtained results showed that P was more effective than Ca, Mg and K addition for reducing the metal stress. It was observed that P addition aids to settle the decreased growth related parameters caused by heavy metal stress. Besides, P has balancing effect on the increased MDA and proline contents. It can be concluded that, although using Ca, Mg and K addition to fertilizers can reduce the damage caused by heavy metal pollution, P addition would be more effective and adequate to achieve efficient results.

Keywords: *Fagopyrum esculentum*, pollution, proline, MDA, photosynthetic pigments.

1. Introduction

One of the most crucial environmental problems of the present day is heavy metal pollution. Their potential harm to humans, animals, and plants is being studied widely. It is known that metal pollution has mutagenic, cytotoxic, and carcinogenic effects [1]. The damage caused by the pollutants affects the plants by their capacity to adapt. Their growth process is the first parameter that is affected by the environmental conditions. Thus, these variables are crucial for the agriculture plants. Since the heavy metal accumulation can be observed both in roots and shoots, studying on the plant tissue has drawn an attention to understand the damage [2].

Metals are grouped as essential and nonessential micronutrients for plants. Essential metals are critical for growth mechanisms, plays a role in redox reactions and takes part in several metabolic processes. Passive diffusion via cell membrane or active transport against the concentration gradient is the transfer mechanisms of these elements [3]. Some of these essential metals are Fe, Zn, Cu, Ni and Co. On the other hand, nonessential metals like Hg, As, Pb and Cd have highly toxic effects on plants, specifically on their growth metabolism [4]. The main reason for this toxicity is related to these metals' chemical similarity with certain elements. They tend to replace some essential elements and interrupt the protein and enzyme mechanisms. They also cause an increase of free radical formation and ROS (reactive oxygen species), resulting in oxidative stress in plants [5].

Due to the increased pollution, the number of stress factors that effects plants' growth process also has been increased. The damage caused by these factors varies on the plants' capacity to adapt as well as the environmental conditions. These variables are crucial for the agriculture plants. Heavy metals one of the most important pollutants for plants. Thus, investigating the toxic effect of heavy metals on the consumption crops is crucial. Wheat is a vital crop for human and animal consumption, and it provides a number of micronutrients as well as certain proteins and carbohydrates [6]. When the heavy metal accumulation is high, the consumption of wheat can cause health issues for the consumers [7].

In this study, as an important agricultural plant buckwheat *(Fagopyrum esculentum, L.)* was used. Buckwheat is known to be highly fibrous, containing several flavonoids and tannins, vitamins (B1, B2, B6) as well as amino acids and lipids [8].

The aim of the study was to investigate and compare the ameliorative effect of P and Ca, Mg, K addition in the presence of heavy metal stress (As, Cd and Pb) on buckwheat.

2. Materials and Methods

2.2 Plant material and growth conditions

The surface of the buckwheat seeds was disinfected with sodium hypochlorite (30%) for 10 min, followed by excessive sterile water rinsing. Approximately 30 to 35 seeds were planted into plastic trays covered with filter paper and cotton-containing half-strength Hoagland's

solution. They were let to grow for 10 days in a growth chamber at 23±2 °C with 16h light: 8h dark photo-cycle. By the end of the 10th day, half-strength Hoagland's solutions containing each 2.5 ppm Cd, As, Pb (B; BS for shoot, BR for root); 2.5 ppm Cd, As, Pb with P (C; CS for shoot, CR for root); and 2.5 ppm Cd, As, Pb with Ca, K and Mg (D; DS for shoot, DR for root) were prepared and 20 buckwheat seeds were planted to these solutions. For the control group (A; AS for shoot, AR for root), only half-strength Hoagland's solution was used. All four groups were grown in the growth chamber with the same physical parameters for another 10 days and shoot and root tissues were then freshly used in the experiments.

2.3 Growth analysis

At the beginning of the experiment and at the 10th day random seedlings were collected from each group and their shoot and root lengths were measured. Fresh weight of the plants was also calculated in order to observe the weight change of each treatment group.

2.4 Photosynthetic pigment measurement

The concentration of photosynthetic pigments was determined by using the method of Lichtenthaler and Wellburn [9]. DMSO extracts of the fresh shoots were homogenized and centrifuged at 4000 rpm for 10 min to remove the cell debris or any residual solids. The color intensity of the clear supernatant was measured at 665, 645 and 470 nm (TECAN, Männedorf, Switzerland) for chlorophyll a, chlorophyll b and carotenoids, respectively.

2.5 Determination of MDA concentration

MDA concentration of the samples was determined spectrophotometrically as described by Heath and Packer [10]. 500 mg sample was homogenized in 3 ml of 0.5% TBA containing 20% TCA (W/V). After homogenization, samples were incubated at 95°C for 30 min and then cooled in the ice bath. Afterwards, samples were centrifuged at 10000 rpm for 15 min, and the absorbance of the resulting supernatant was measured at 532 and 600 nm (TECAN, Männedorf, Switzerland). In order to calculate the results, the non-specific absorbance at 600 nm was subtracted from the 532 nm absorbance.

2.6 Determination of proline content

Proline content was determined by using the modified method of Bates [11]. 0.5 g of the shoot and root tissues from the all four groups were homogenized in 1 mL of 5% sulfosalicylic acid solution (PowerGenTM Model 125, FisherBrand, USA). The homogenate was then centrifuged at 13000 rpm for 10 min. 1 mL ninhydrin solution was prepared by dissolving 1.25 g ninhydrin in 30 mL of glacial acetic acid and 20 mL of 6 M orthophosphoric acid in order to prepare to acidic ninhydrin solution. 1 mL of each supernatant was then added into a test tube with the prepared glacial acetic acid- ninhydrin solution and the tubes were incubated in a water bath for 1 h at 95 ºC, and then allowed to cool to room temperature. 2 mL toluene was added to each tube and mixed with vortexed for 20 seconds. After leaving the test tubes for 10 minutes to allow the separation of toluene and aqueous phase, the toluene phase was collected and its absorbance was measured at 520 nm (TECAN, Männedorf, Switzerland). The concentration of proline, which is expressed as µmol/g FW, was then calculated from a proline standard curve.

2.7 Statistical Analysis

The significance of the difference between mean values obtained from three independent experiments was determined by two-way analysis of variance (ANOVA) (Tukey's multiple comparisons test) by using GraphPad Prism 6.0 (La Jolla, CA, USA). The data were considered statistically significant at $p < 0.05$.

3. Results

3.1 Growth analysis

At the end of the 10th day, the shoot lengths were measured and 42.73% of a decrease was observed when BS was compared with the control group (AS). When BS was compared with CS and DS, 43.38% and 28.67% of an increase was observed respectively as a result of P and Ca, Mg, K addition (Figure 1A).

Same comparisons were made for the root lengths. It was shown that metal treatment (BR) caused 44.68% of a decrease when measured against the AR group. The comparison of BR against CR showed 39.42% of an increase, while BR-DR comparison showed no significant difference (Figure 1B).

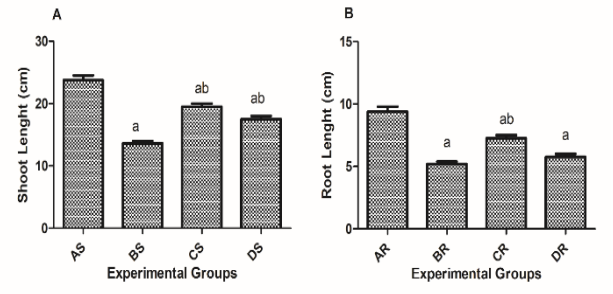

Figure 1. Comparison of the shoot and root lengths of the experimental groups. Figure 1A shows the shoot length change of the experimental groups while Figure 1B shows the root length change. (a: significance according to A, b: significance according to B).

Fresh weight calculations of the buckwheat seeds were also examined and it was seen that weight of group B was decreased 38.28% compared to control group (A). P addition (C) caused 29.11% increase in the weight, while Ca, Mg, K addition (D) caused no significant difference (Figure 2).

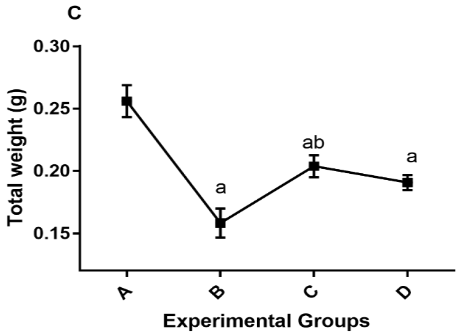

Figure 2. Total fresh weight comparison among the experimental groups. (a: significance according to A, b: significance according to B).

3.2 Photosynthetic pigment measurement

When the B, C, and D groups compared with the control group, chlorophyll a, chlorophyll b, total chlorophyll and total carotenoid levels decreased for 75.84%, 59.03%, 70.24% and 75.25% respectively. When compared with the group B, P addition caused an increase of 168.48%, 65.70%, 103.15% and 175.44% for these pigments respectively. On the other hand, Ca, K, Mg addition caused no significant difference for the same comparison

Table 1. Photosynthetic pigment content (mg/g) of the experimental groups (a: significance according to A, b: significance according to B).

	Chl. a	Chl. b	Total Chl.	Total carotenoid
A	4,362+0,29	3,729+0,07	8,091+0,22	2,171+0,02
B	0,879+0,08 a	1,528+0,10 a	2,407+0,19 a	0,560+0,09 a
C	2,359+0,38 ab	2,532+0,21 ab	4,892+0,59 ab	1,797+0,01 ab
D	1,149+0,02 a	1,318+0,18 a	2,468+0,15 a	0,813+0,11 a

3.2 MDA concentration

MDA concentration was determined spectrophotometrically and results showed that MDA levels of the metal group (BS) increased 52% compared to the control group's (A) shoot results. When the shoots' MDA concentrations of the BS, CS, and DS groups were compared among, it was found that P addition (CS) to the metal stressed buckwheat seeds caused 19.2% and Ca, Mg, K addition (DS) caused 16.10% decrease when compared with BS (Figure 3A). MDA levels of the roots were also observed and found that metal stress (BR) caused 96% increase compared to AR group. Roots' MDA comparison among the BR, CR, and DR showed that there is a 22% decrease for CR (P addition) and 16.14% for DR (Ca, Mg, K addition) compared to BR (Figure 3B).

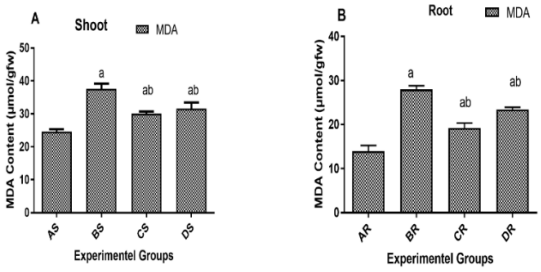

Figure 3. Changes in the MDA levels of the experimental groups. Figure 3A shows the MDA levels of the shoots while Figure 3B shows MDA levels of the roots. (a: significance according to A, b: significance according to B).

3.3 Proline Content

After 10 days of the metal treatment of the buckwheat seeds, 101.01% of an increase was measured for the proline content of the BS shoots compared to the control group. On the other hand, P addition caused 22.35% and Ca, Mg, K addition caused 16.07% of a decrease of the proline content when compared with BS group (Figure 4A).

Similar results were obtained for the root proline content measurements. While 101.2% of an increase was measured for BR as compared to the AR group, P addition (CR) caused 22.75% and Ca, Mg, K addition (DR) caused 17% of a decrease of the proline content as compared with BR group (Figure 4B).

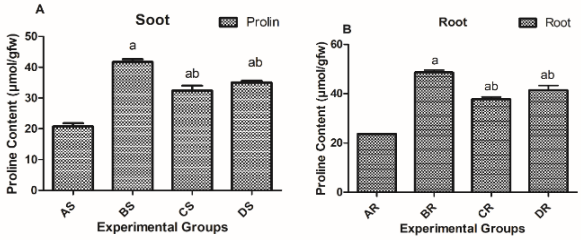

Figure 4. Proline content of the experimental groups. Figure 4A shows the proline level changes of the shoots while Figure 4B shows proline level changes of the roots. (a: significance according to A, b: significance according to B).

4. Discussion

Metals are absorbed by the plant roots from the soil, and only some of them are used in their growth mechanism. These metals are essential elements for the plants. However, other heavy metals like Cd and Pb are not essential for the plant growth and causes toxicity even in small doses [12].

Growth rate change is one of the immediate response of the plants against the increased stress. Growth reduction can be caused by the reduced cell division rate or decreased photosynthesis, respiration or protein synthesis

levels [13]. According to the results obtained from the conducted experiments; 2,5 ppm Cd, As and Pb stress caused a decrease in root and shoot length and total plant weight as compared to the control group (Fig 1A,1B,2C). P addition to the metal stress caused 43.38% of an increase in plant shoots (Fig 1A).

Antioxidant defense mechanisms of the plants play a crucial role in the protection of organelles such as mitochondria and chloroplast. Stress factors such as heavy metal exposure increase ROS and this leads membrane function disorders [14]. ROS cause oxidative damage by affecting the metabolism and affecting the lipids and proteins. MDA is produced as a result of lipid peroxidation. Thus, MDA level can be used as an indicator of oxidative stress [15]. Conducted experiments showed that as a result of metal toxicity, MDA and proline content of the shoots increased by 52% and %101,01 respectively as compared to the control group. On the other hand, P addition to the metal stress decreased the MDA content by 19,2% and proline content decreased by %22.75 (Fig 3A,3B,4A,4B).

Several factors might affect the movement of non-essential metals through the soil. Some of these factors can be counted as pH, soil structure, level of precipitation, metal concentration and amount of P fertilizer supplementation. Level of As carriage can be counted as low-level carriage. This level depends on the existence of positive load of the soil due to the Fe and Al presence. Conducted studies showed that P addition increases the As the movement of the soil [16]. P is also crucial for phosphorylation reactions, an essential part of the cell membrane and indispensable part of the nucleic acids. It also reduces the heavy metal toxification by subtilizing the metals. Observed ameliorative effect of P on the conducted experiments can be explained by these crucial properties.

There is a number of essential nutrients for plants in the soil. Some of these are N, P, Ca and Mg. N is mainly taken as nitrate and ammonium by plants. The main source of Mg, K and Ca is the decay of the rocks. However, since Ca is easily drained, lack of Ca can come up in the soil by time. Mg is known to substitute with other cations. Its substitution rate is known to be K> $NH4^+$ > Ca > Na [17]. As a crucial essential element, K takes part in enzyme activation and protein synthesis regulation. K plays a role in heavy metal toxicity reduction by reducing the oxidative stress caused by the existence of heavy metals in the plants [18]. Just like K, Ca also takes part in enzyme activation and metabolism regulation. It is also known that Ca shows chemical similarities to Cd such as binding sides, thus the existence of Cd, Ca is replaced with it by plants [19]. Ca causes a decrease in the heavy metal intake, so that toxicity also decreases. It also takes a role in the defense compound expression as well as taking part in the defense mechanisms against the stress. Mg plays a crucial role in chlorophyll biosynthesis and since it proliferates the antioxidant enzyme activity, heavy metal toxicity decreases in the presence of Mg [20]. According to obtained results, MDA and proline content were increased at the plant shoots in the presence of heavy metals. Against this metal toxicity, K, Ca and Mg addition caused 16.10% and 16.07% of reduction of MDA and proline content levels respectively (Fig 3A, 3B, 4A, 4B).

To conclude, obtained results pointed that P addition was more effective than Ca, Mg and K addition in the presence of heavy metal stress. It was shown that decreased parameters of the growth rate under the stress conditions were more balanced in the presence of P. Measured parameters of MDA and proline content was also similar to growth-related parameters. It was also observed that although Ca, Mg and K addition was also relatively ameliorative, P would be a better choice to achieve efficient results in order to reduce metal stress-related negative effects.

References

1. More, T.G.; Rajput, R.A.; Bandela, N.N. Impact of heavy metals on DNA content in the whole body of freshwater bivalve, *Environmental Science and Pollution Research.* 2003; 22, 605–616.

2. Rattan, R.K.; Datta, S.P.; Chhonkar, P.K.;Suribabu, K.; Singh, A.K. Long term of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater. *Agriculture, Ecosystems & Environment*. 2005;109, 310–322.

3. Stroiński, A.Some physiological and biochemical aspects of plant resistance to cadmium effect. I. Antioxidative system. *Acta Physiologiae Plantarum*. 1999; 21, 175–188.

4. Rai, V.; Vajpayee, P.; Singh, S.N.; Mehrotra, S. Effect of chromium accumulation on photosynthetic pigments, oxidative stress defense system, nitrate reduction, proline level and eugenol content of Ocimumtenuiflorum L. *Plant Science*. 2004; 167(5), 1159–1169.

5. Dietz, K.J.; Baier, M.; Krämer, U. Free Radicals and Reactive Oxygen Species as Mediators of Heavy Metal Toxicity in Plants. *Heavy Metal Stress in Plants, Springer Berlin Heidelberg, Berlin, Heidelberg*. 1999; 73–97.

6. Singh, A.;Sharma, R.K.; Agrawal, M.; Marshall, F.M. Health risk assessment of heavy metals via dietary intake of foodstuffs from the wastewater irrigated site of a dry tropical area of India, *Food Chemical Toxicology*. 2010; 48, 611–619.

7. Khan, S.; Cao, Q.; Zheng, Y.M.; Huang, Y.Z.; Zhu, Y.G. Health risk of heavy metals in contaminated soils and food crops irrigated with waste water in Beijing. *China Environmental Pollution*. 2008; 152, 686–692.

8. Kreft I.; Fabjan N.; Yasumoto K., Rutin content in buckwheat (*Fagopyrum esculentum Moench*) food materials and products. *Food Chemistry*. 2006; 98, 508–512.

9. Lichtenthaler, K.; Welburn, A.R. Determination of total carotenoids and chlorophylls A and B of leaf extracts in different solvents. *Biochemical Society Transactions*. 1983; 11, 591-592.

10. Heath, R. L.; Packer, L. Photo peroxidation in isolated chloroplasts.

Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics.*1968; 125,189-198.

11. Bates, L.S.; Waldren, R.P.;Teare, I.D. Rapid determination of free proline for water-stress studies. *Plant Soil.* 1973; 39, 205–207.

12. Peralta-Videa, J.R.; Lopez, M.L.; Narayan, M.; Saupe, G.; Gardea-Torresdey, J. The biochemistry of environmental heavy metal uptake by plants: implications for the food chain, *The International Journal of Biochemistry & Cell Biology*. 2009; 41, 1665–1677.

13. Armendariz, A.L.; Talano, M.A.; Travaglia, C.; Reinoso, H.; Wevar-Oller, A.L.; Agostini, E. Arsenic toxicity in soybean seedlings and their attenuation mechanisms. *Plant Physiology and Biochemistry*. 2016; 98, 119–127.

14. Gill, S.S.; Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*. 2010; 48, 909–930.

15. Chaoui, A.; El Ferjani, E. Heavy metal-induced oxidative damage is reduced by β-estradiol application in lentil seedlings. *Plant Growth Regulation*. 2014; 74, 1–9.

16. Campos V., Arsenic in groundwater affected by phosphate fertilizers at São Paulo. *Environmental Geology*. 2002; 42, 83–87.

17. Peñalosa, J.M.; Cáceres, M.D.; Sarro, M.J. Nutrition of bean plants in sand culture: Influence of calcium/potassium ratio in the nutrient solution. *Journal of Plant Nutrition*. 1995; 18, 2023–2032.

18. Shen, W.; Nada, K.; Tachibana, S. Involvement of polyamines in the chilling tolerance of cucumber cultivars. *Plant Physiology*. 2000; 124, 431–440.

19. Lauer Júnior, C.M.; Bonatto, D.; Mielniczki-Pereira, A.A.; Zilles Schuch, A.; Dias, J.F.; Yoneama, M.-L.; Henriques, J.A.P. The Pmr1 protein, the major yeast Ca2+- ATPase in the Golgi, regulates intracellular levels of the cadmium ion. *FEMS Microbiology Letters*. 2008; 285, 79–88.

20. Chou, T.S.; Chao, Y.Y.; Huang, W.D.; Hong, C.Y.; Kao, C.H. Effect of magnesium deficiency on antioxidant status and cadmium toxicity in rice seedlings. *Journal of Plant Physiology*. 2011; 168, 1021–1030.