J. Basic. Appl. Sci. Res., 2(1)935-938, 2012
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ISSN 2090-4304
Journal of Basic and Applied Scientific Research
www.textroad.com

Assessing the Intensity of Tolerance to Lead and its Effect on Amount of Protein and Proline in Root and Aerial Parts of Two Varieties of Rape Seed (*Brassica Napus L.*)

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ABSTRACT

The present research is devoted to evaluation of the effect of Hoagland nutrient with different Lead concentrations (0, 100, 200, and 400 μM of ($Pb(NO_3)_2$) on protein and proline contents in root and aerial parts of two varieties (Hyola308 and RGS003) of rape seed (*Brassica napus L.*). These varieties were grown using Hoagland solution at laboratory and hydroponics conditions and their intensity of tolerance to Pb^{2+} was subsequently measured. Root length was measured by ruler, while protein and proline concentrations in root and aerial parts were determined using ultraviolet (UV) spectrophotometer (u-2000) method. Statistical analysis of samples was carried out through variance decomposition and F test at probability level of 5% at four replications using SPSS software, and the resulting histograms were drawn using Excel software. The obtained results indicate that increasing Pb^{2+} concentration causes a decrease of root length at both rape varieties. Regarding the average length of the roots, intensity of tolerance to Lead was higher in RGS003 compared to Hyola308. With increasing Pb^{2+} concentration in the nutrient solution, protein and proline concentrations at root increased at both varieties in comparison with control sample. Furthermore, increase in Pb^{2+} concentration in the nutrient gave rise to decrease in protein concentration at aerial parts of Hyola308, whereas it was accompanied by an increase of protein concentration at aerial parts of RGS003 variety.
KEY WORDS: *Lead; Rape seed (Brassica napus L.); Proline; Protein.*

1. INTRODUCTION

Lead is the most toxic metal contaminating the environment. Existing Lead in nature is mostly due to Lead manufacturing industries, dye additives, benzene, insecticides, fertilizers, car exhaust, and soldering. To decrease Lead toxicity, plants possess different mechanisms including production of factors and proteins which adjoin heavy metals, not allowing heavy metals enter the cells, which is accomplished by selecting metal ion transport, and metal exclusion in vacuole [1]. One toxicity clearance mechanisms of heavy metals pertains to decrease in damage to proteins' membrane [2]. In the current research, effect of intensity of tolerance to Lead has been investigated through measuring the protein concentration in two varieties of rape seed.

2. MATERIALS AND METHODS

Seeds of two rape seed varieties prepared from Safiabad center in Dezful city were cultivated three times the required amount at depth of 1.5 cm with the same distances in two separate vessels containing washed sand. The sand was washed by 3% chloridric acid for at least 24 hours and was then frequently washed by tap water and finally by distilled water. This was carried out in order to strengthen the salts mixed with sand. The seeds sprouted at temperature of about 25^{o}C. During appearance of split peas and emergence of the first leaves, which took approximately 7 days, seeded papules were nourished by appropriate amounts of distilled water and Hoagland nutrient solution. Homogeneous seeded papules were selected and transferred by plastic utensil containing 400ml of Hoagland nutrient solution. The utensil were kept at laboratory controlled conditions with average temperature of 25 ± 1 ^{o}C at days and 18 ± 1 ^{o}C at nights, with 14/10 h light cycle. Aerating of the nutrient solution was renewed. At this step, Hoagland nutrient solution was prepared by different Lead concentrations, i.e. 0, 100, 200, and 400 μm/l of Lead nitrate and the prepared solutions were poured into utensil according to their labels.

The transpired water from the plants was daily compensated by distilled water. Cropping was accomplished after 10 days. With the purpose of determining the level of Lead toleration and measuring the concentration of protein and proline in root and aerial parts of two varieties of rape seed, the following statistical completely random design was performed with four replications.

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2.1. Intensity of Tolerance

Intensity of Tolerance (IT) to Lead was computed according to following formula:
IT = Mean length of roots in experimental solvents / Mean length of roots in sample

2.2. Measuring the Protein Concentration

Dry sample of the plant weighing 0.5-1 g was grinded in 5ml of Tris chloridric acid buffer and the resulting samples were next centrifuged with high speed. 0.05 ml of supernatant in centrifuged sample was extracted and 1 ml of reagent was added to it, including 0.5 ml Sodium tartarate 2%, 0.5 ml sulphate 1%, and 0.5 ml Sodium carbonate 10% dissolved in 0.5-normal hydroxide. It was kept in laboratory temperature for 15 minutes and 3 ml of 0.2-normal Fuline reagent was then added to this solution. Subsequently, the resultant solution was maintained for 15 minutes in Ben Marie at 50^{o}C. Absorbance level was measured by Hitachi ultraviolet (UV) spectrophotometer (u-2000) at wavelength of 625 nm. The amount of protein was after that calculated using the following formula [3]:

$$M \quad \frac{C \times 0/005}{W}$$

Where M represents protein amount per gram of plant dry sample, W denotes dry weight of the sample, and C specifies concentration.

2.3. Measuring the Proline Concentration

Wet sample of the plant weighing 1g was grinded in 10ml of sulfosalisilic acid and the resulting samples were next filtered. Afterwards, 2 ml of ninhydrin reagent and 2 ml of pure acitic acid were added to it and the test tubes were kept in Ben Marie at 100^{o}C for 1 hour. Test tubes were then put into ice bath for 30 minutes. Then, 4 ml toluene was added to each test tube and they were shaken completely. Subsequently, absorbance value of the top color layer, containing toluene and proline, was measured by Hitachi ultraviolet (UV) spectrophotometer (u-2000) at wavelength of 520 nm [4]. Variance analysis and F test of the obtained results were fulfilled at probability level of 5% using SPSS software, and the resulting histograms were drawn using Excel software.

4. Conclusion

In order to determine the effect of Lead on intensity of tolerance, amount of protein and proline was measured in root and aerial parts of two varieties of raped seed, namely Hyola308 and RGS003, which led to following results.

The results obtained from this research indicate that regarding root length, both varieties had a significant decrease at level of $p<5\%$ compared to control sample when Lead concentration increased in nutrient solution (Table 1).

Table 1. Measuring the root length in two varieties of rape seed (Hyola308 and RGS003) after 10 days of growth in Hoagland nutrient solutions with different Lead concentrations.

Lead concentration in nutrient solution (µm/l)	Hyola308	RGS
0	5.5	3
100	4.5	3.5
200	4	2.5
400	3	1.5

Regarding average percent of root lengths, it was reveled that intensity of tolerance to Lead is higher in RGS, compared to Hyola308 (Table 2).

Table 2. Measuring the percentage of root tolerance intensity in two varieties of rape seed (Hyola308 and RGS003) after growth in Hoagland nutrient solutions with different Lead concentrations.

Lead concentration in nutrient solution (µm/l)	Hyola308	RGS
100	58	96
200	50.81	60.01
400	39.33	58.66

Furthermore, increase of protein amount in roots of both varieties was significant at level of $p<5\%$ compared to control sample when Lead concentration in the nutrient solution increased (Table 3). It is obvious that protein concentration was higher in root of Hyola308 compared to that of RGS.

Additionally, amount of protein in aerial parts of Hyola308 decreased significantly at level of p<5% compared to control sample at all Lead treatments (Table 4).

Table 3. Protein concentration in roots of two varieties of rape seed (Hyola308 and RGS003) after 10 days of growth in Hoagland nutrient solutions with different Lead concentrations.

Lead concentration in nutrient solution (µm/l)	Hyola308	RGS
0	58	110
100	200	180
200	390	320
400	500	400

Table 4. Protein concentration in aerial parts of two varieties of rape seed (Hyola308 and RGS003) after 10 days of growth in Hoagland nutrient solutions with different Lead concentrations.

Lead concentration in nutrient solution (µm/l)	Hyola308	RGS
0	320	380
100	330	200
200	340	180
400	350	150

On the other hand, although increase was observed in protein amount in aerial parts of Hyola308 by increasing lead concentration in nutrient solution, this increase was not statistically significant at level of p<5% compared to control sample (Table 3). Comparison of averages showed that increase of protein amount was higher in root of Hyola308, compared to that of RGS.

Phitoclatins (PCs) are Glutyton-derived peptides with general structure of the form Gly(y-Glu-Gys) composed of 2-11 units which are produced in plants when they are exposed to stress of heavy metals. Phitoclatins have a crucial role in hemostasys of meta-ions metabolism. Several metal ions, including Cu(II), Pb(II), and Cd(II), induce the synthesis of phitoclatins in plants. Moreover, through their study of Lead effect on three species Pisum sutivum, Vicia faba, and phaseolus, researchers have shown that Lead results in drastic increase of thiol peptides, phitoclatins, and homophitoclatins in P. sutivum roots about 4500 $SHg^{-1}FW$ nmol, which has moderate intensity of tolerance to Lead. On the other hand, phitoclatins concentration in V. faba roots with higher intensity of tolerance compared to P. sutivum was much lower. In addition, P. vulgaris had the lowest intensity of tolerance, but produced moderate amount of phitoclatins [5]. Finding of the researchers is consistent with the results obtained from the current research. In other words, Hyola308 had lower intensity of tolerance compared to RGS; nevertheless, increase of protein amount in its aerial parts showed a significant decrease compared to control sample by increasing Lead concentration in nutrient solution, which is consistent with the results found by other researchers. They have declared that in contrast to amount of N-amino acid and N-protein in root of the plant Silene vulgaris exposed to copper, the amount of these substances has a reduction in aerial parts of the plant by increasing Copper concentration. In other words, Copper has negative effect on metabolism of N-amino acids and proteins of the plant's aerial parts. Also, Lead relates to expression of nucleic acids, i.e. it causes chromatin aggregation and stabilization of double-helix DNA and consequently prevents from transcription and translation processes. On the other hand, the results obtained from this research indicate that the amount of proline in root has a significant increase at level of p<5% by increasing Lead concentration in nutrient solution in both varieties, compared to control sample (Table 5).

Table 5. Proline concentration in roots of two varieties of rape seed (Hyola308 and RGS003) after 10 days of growth in Hoagland nutrient solutions with different Lead concentrations.

Lead concentration in nutrient solution (µm/l)	Hyola308	RGS
0	6	5
100	6.5	7
200	8	9
400	9	10

Increase in Lead concentration in the nutrient solution followed by a significant increase at level of 5% (Fig. 5). Nevertheless, compared to control sample, increase in amount of proline in aerial parts of both varieties of rape seed was not significant at level of 5% (Table 6).

Table 6. Proline concentration in aerial parts of two varieties of rape seed (Hyola308 and RGS003) after 10 days of growth in Hoagland nutrient solutions with different Lead concentrations.

Lead concentration in nutrient solution (µm/l)	Hyola308	RGS
0	6.5	6
100	6	5
200	7.3	7
400	9.5	9

In addition, increase percentage of proline amount was higher in root and aerial parts of RGS, in comparison with Hyola308, and the amount of proline increased in rots of two varieties more than that in their aerial parts.

Proline plays an essential role in reduction of the stresses caused by environment metals, including stresses of heavy metals, in plants and micro-organisms. It perhaps has an anti-oxidant role in the cells under stress [6]. Besides, producing proline is the mechanism of most plants and algae in response to heavy metals, and proline accumulation in the plants under stress has relationship with decrease in membrane damage [7]. Furthermore researcher have concluded, through over-expression of Δ'-pirolin-5-carboxilas synthetase which increases proline production, that producing proline in transgenic algae is 80% higher than its wild type and growth rate of transgenic algae is more than its wild type. Also in the present study, increase percentage of proline amount was higher in RGS, compared o Hyola308. Considering these observations, it seems that Hyola308 is more tolerable toward Lead, compared to PF. This is in accordance with lower decrease percentage of root growth in RGS and consequently higher intensity of tolerance to Lead in this variety, compared to Hyola308.

Based upon the results obtained in the present study, it seems that rape seed plant, especially RGS variety, has a potential capacity so as to detoxify the soil contaminated by Lead.

Acknowledgement

We thanks very much from research assistant of Young Researchers Club of Islamic Azad University (Ahvaz Branch) that provide finance of this research project.

REFERENCES

1. Hu,S.,Lau,K.w.k., ana Wu,M.(2001). Cadmium sequestration in Chlamydomonas reinhardtii. Plant Sci.161,987-996
2. Verma,D.P.S(1999). Osmotic stress tolerance in plant: Role of proline and sulfur metabolism. In Molecular Responses to cold Drought, Heat and Salt Stress in Higer Plant , K.Shinozaki and K.Yamaguchi-shinozaki,eds, pp.153-168
3. lowary,O.H.,Rosebrough, N.J.,Far,A.l.,Randall,R.j.,1951.protein measurement with the folin phnenol reagent, J.,Biol.Chem., 193:256-275.
4. Bates, L.S.,Waldern,R.P.,Treare,I.D.,1973.Rapid determination of free proline for water stress studies, plant and soil., 39:205-207.
5. Piechalak, A., Tomaszewska, B., anuta Baralkiewicz, d., Maleckaa.,2002.Accumulation and detoxification of lead ions in legumes. Phytochemistry., 60: 153-162.
6. Siripornadulsil, s., Traina, S. Verma, D.S. and Sayre,R.t., 2002. Molecular Mechanisms of proline- Mediated Tolerance to Toxic Heavy Metals in Transgenic Microalgae. The Plant Cell.,14:2837-2847.
7. Wierzbicka, M., 1987. Lead translocation and location Allium cepa roots., Can. J. Bot., 65:1851- 1860.