EFFECT OF CHROMIUM ACCUMULATION ON SEEDLINGS AND PHOTOSYNTHETIC PIGMENTS IN BEAN SEADS (*PHASEOLUS VULGARIS L.*)

EFECTUL ACUMULĂRII IONULUI DE CROM ÎN GERMENII DE FASOLE (*PHASEOLUS VULGARIS* L.) ŞI INFLUENŢA SA ASUPRA ACTIVITĂŢII ENZIMATICE ŞI A CANTITĂŢII DE CLOROFILĂ

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Abstract. *During the germination process the seeds are affected by the external and internal factors, which can have a profound change on the outcome. External factors include those that affect the germination environment, while internal factors can be related to the "history" of the individual seed. One of these external factors is chrome and may lead abnormality or death of the embryo. The goal of this trial is to identify the biochemical and physiological plants reaction to the excess of Cr^{3+}. It was evaluated the germination energy and faculty of the bean seeds under the influence of different Cr^{3+} solution concentration. The analysis methods was orientated to determinate the enzymatic activities and the chlorophyll quantity. The study of Cr^{3+} effects under the metabolic process associated seeds germination and post – germination phases , is completed by the monitoring of enzymatic activities involved in the mobilisation of the seeds' food reserves and determination of the chlorophyll quantity involved in photosynthesis process. The results were compared to relieve the interaction between doses – plant reaction, and the elements that are different or common under toxicity effect, compared by the blank test (control – distillate water).*

Key-words: seed germination, *Phaseolus vulgaris*, Cr^{3+}, chlorophyll, enzymatic activity,

Rezumat. *Premisa investigaţiei are la baza ipoteza conform căreia creşterea plantelor este controlată de anumite substanţe endogene, care nu acţionează separat, ci se condiţionează reciproc, creşterea şi dezvoltarea reprezentând rezultanta echilibrului dinamic dintre aceste substanţe. Unul dintre aceşti factori este ionul metalic trivalent Cr^{3+} care poate induce apariţia de germeni anormali sau chiar moartea embrionului. Scopul investigaţiei constă în identificarea răspunsurilor biochimice şi fiziologice ale plantelor la excesul de metale grele, în speţă Cr^{3+}. A fost evaluată energia şi facultatea germinativă a semintelor de Phaseolus vulgaris, sub influenta unor soluţii de concentraţii diferite de crom, împreună cu determinarea activităţi enzimatice şi a cantităţii de clorofilă. Procesele metabolice asociate germinaţiei şi fazei post-germinative sunt estimate prin monitorizarea activităţii unor enzime și a cantităţii de clorofilă. Rezultatele obţinute au fost comparate pentru a releva* *existenţa unei relaţii doză-răspuns, identificând elementele comune şi particularităţile care le diferenţiază sub aspectul toxicităţii, cât şi rezultatele obţinute în condiţii martor.*

Cuvinte cheie: germinaţia seminţelor, *Phaseolus vulgaris*, Cr^{3+}, clorofila, activitate enzimatică

INTRODUCTION

Heavy metals like Zn, Fe, Cu, Mn are essential for plant growth and important constituents of many enzymes of metabolic importance. Other metals like Pb, Cd, As, Se, Cr and Al are biologically non – essential and toxic above certain threshold levels. Chromium is toxic to plants and does not play any role in plant metabolism. Accumulation of chromium by plants can reduce growth, induce chlorosis in young leaves, reduce pigment content, alterate enzymatic function, damage root cells and cause ultra structural modifications of the chloroplast and cell membrane. (Ho, 1990). Chromium toxicity can reduce seed germination and radicle growth in plants. Growth inhibition in plants can be due to inhibition of cell division by inducing chromosomal aberrations. However, in many plants an increase in DNA content has been observed under chromium and the amount of DNA increased with the increase in concentration of chromium. During seed germination, hydrolysis of proteins and starch takes place, providing amino acids and sugars. (Butnariu, 2005, 2007). Under chromium treatment, a decrease in both α and β – amylase has been reported, which is one of the important factors for germination inhibition in many plants in view of the impaired supply of sugar to developing embryo axes. At very low concentrations of chromium, however, an increase in amylase activity has been reported. Chromium exposure at the micromolar range can lead to severe phytotoxic symptoms in plant cells. (Chandra, 1992). Chromium often accumulates in aquatic life, adding to the danger of eating fish that may have been exposed to high levels of chromium. (Barcelo, 1985). Tannery effluent is a major source of aquatic chromium pollution with high BOD, COD and total dissolved solids. It occurs in several oxidation states ranging from Cr^{2+}, Cr^{3+} to Cr^{6+}. However, Cr^{6+} is the most toxic form and mutagenic because of its high solubility, ability to penetrate the cell membranes and strong oxidizing ability. (Rai, 1992). Oxidative stress is caused either by inducing oxygen free radical production or by decreasing enzymatic and nonenzymatic antioxidants. Reactive oxygen species (ROS) reacts very rapidly with DNA, lipid and proteins causing cellular damage. The toxic effect of chromium has amply been documented both in the laboratory and under natural conditions in aquatic plants. (Krupa, 1993, Apha, 2005). Thus there is a great need to assess toxic potential of chromium in *Phaseolus vulgaris* plants under such conditions. Wetland plant treatment is the best choice for treatment of wastewater because of the low maintenance cost and simplicity of operation. (Chandra, 1992). Recently, many plants including water hyacinth have become important in pollution treatment systems and used successfully to remove chromium. *Phaseolus vulgaris* seedlings' grown in different chromium concentrations (25, 50 to 100 ppm) showed appreciable amounts of the same accumulated in their tissues, maximum being in roots. (Butnariu, 2008) Higher amounts of chromium accumulated in plant tissues result significant inhibition in chlorophyll, protein contents and nitrate reductase activity in test plant. Sensitivity of nitrate reductase activity in the presence of both nitrate and ammoniac nitrogen could be used as a bioassay index for toxicity assessment against supraoptimal concentrations of chromium. (Barcelo, 1985).

MATERIAL AND METHOD

SR 1634/ 1999 – Seeds for sowing Determination of germination. *ISTA Handbook on Seedling Evaluation 3rd edition*. The seeds were placed in a pleated accordion-like paper strip. (PP). Completed strips were kept in boxes within a germination cabinet at 25^0 C and 70 % rH. *SR EN ISO 16634 – 1:2009* ver. eng. Determination of total nitrogen by combustion principle Dumas and calculating the content of crude protein. Sampling and preparing samples for analysis was conducted in accordance with *AOAC 920.180*. Samples were prepared for quantification using the system of mineralization for mineralization rapidly several samples of organic and mineral for further investigation by Meto spectrophotometric, Turbidity or titrimetrice. (*SR EN 13804:2003*). Sample mineralized not require distillation intermediate was prepared for analysis of colorimetric direct protein. The mineralization can be used to determine heavy metals, the total phosphorus and total Kjeldahl nitrogen, for hard evidence and for samples liquid. The metals were extracted from samples as 2.0 g of sample was mineralized in the environment of organic mixture of acetone and petroleum ether (5:1 v/v) in a mineralized Digesdhal, System mineralization Digesdahl / Digesdahl ® Digestion Apparatus. (*SR EN 14083:2003*). The filtrate was brought to 50 ml with distilled water and this mixture was determined metals. (*SR EN 13805:2003*.) pH – has been determined, pH – meter Model InoLab – pH 740, according to *STAS 8619 / 3 / 90* pH – meters. Determination electrometry of pH – the solution aqueous approved by the ASRO. *Extraction of Soluble Sugars from Sample*: Two grams of the milled sample was extracted for 30 min with 10ml of 80% ethanol at 60^{0}C using a magnetic stirrer. The extract was filtered through a filter paper and the ethanol evaporated in a water bath at a temperature of 80^{0}C to give a final volume of 2 ml. One gram of each standard (reference) sugar (raffinose, sucrose, glucose and galactose) was dissolved in 100 ml of distilled water to make a 1% solution. *Quantitative Analysis*: Then, 0.1 ml of 5% phenol was added to each dissolved sugar followed by rapid addition of 0.5 ml concentrated tetraoxosulphate (vi) acid (H_2SO_4). Each tube containing the sugar – reagent mixture was then placed in a water bath to cool for 20 min. The absorbance of the cooled mixture was read at 490 nm using the spectrophophotometer, and the corresponding concentration was extrapolated from the standard calibration curve prepared from each reference sugar. Chlorophyll a, b, total and carotenoid were determinative spectrofotometeric by using the method with molibdovanadat UV – VIS spectrophotometer *T60U, PG Instruments Limited, UV WIN ® version 5,05*.

RESULTS AND DISCUSSIONS

Seeds of bean (*Phaseolus vulgaris L.*) were germinated by soaking in $Cr_2(SO_4)_3$ solution. The abnormal seedlings resulted after 9 day of germination shows that chromium determinate many damage to the embryo such as: stunted root, missing primary root, curled hypocotyl, short hypocotyl, hypocotyl forming a spiral and constricted, and many other defective terminals bud (figure 1), and the germination was reduced until a half compared with the control.

The relationships among chromium excess treatment, germination rate, dry weight, sugar contents, and enzymes activities in cotyledon were investigated. Higher amounts of chromium accumulated in plant tissues result significant inhibition in chlorophyll, protein contents and nitrate reductase activity in test plant. Sensitivity of nitrate reductase activity in the presence of both nitrate and ammonical nitrogen could be used as a bioassay index for toxicity assessment against supraoptimal concentrations of chromium.

25 ppm Cr^{3+} concentrations 50 ppm Cr^{3+} concentrations 100 ppm Cr^{3+} concentrations

Fig. 1. Normal and abnormal seedlins of *Phaseolus vulgaris*

A strong positive correlation was found between chromium content in effluents and plant roots (r^2 = 1.002) and leaves (r^2 = 0.997). A similar trend was also found under laboratory condition (100 and 25 mg/g) in roots (r^2 = 1.002) and leaves (r^2 = 1.003).

Table 1

Physico – chemical characteristics of *Phaseolus vulgaris* seedlings'

Parameters	**Cr solutions concentrations**		
	25	**50**	**100**
Colour	*Greyish green*	*Light gray green*	*Light gray*
pH	*7.8±0.62*	*7.9±0.63*	*6.8±1.24*
Dissolved oxygen (DO) mg/ l	*0.21±0.07*	*3.29±0.10*	*4.90±1.14*
Biochemical oxygen (BOD) mg/ l	*1349±6.01*	*370±3.62*	*482±0.013*
Chemical oxygen (COD) mg/ l	*3791±18.48*	*1074±7.13*	*934.0±1.89*
Total solids mg/ l	*3892±9.74*	*3754±8.04*	*3423.0±1.32*
Total dissolved solids (TDS) mg/ l	*3420±6.25*	*3315±7.59*	*2305±0.012*
Total suspended solids (TSS) mg/ l	*472±3.79*	*439±2.34*	*398±1.2±2.344*

Mean ± SE (n=3)

Heavy metal stress provoked a diminution in germination rate and biomass mobilization, as compared with the control.

Table 2

Effect of chromium on protein and sugar (mg/g fresh wt.) and nitrate reductase activity (μmol NO_2 /g fresh wt.) in *Phaseolus vulgaris* seedlings' at different concentrations

Cr^{3+}	Protein (mg/g fresh wt.)	Sugar (mg/g fresh wt.)	NR activity (μmol NO_2 /g fresh wt.)
Control	*91.56±0.86*	*2.00±1.14*	*84.232.00*
25 ppm	*85.74±1.1**	*8.00±0.019*	*69.17±0.62**
50 ppm	*78.86±1.1**	*16.00±0.007*	*39.51±0.19**
100 ppm	*64.27±0.87**	*22.00±0.029*	*25.21±0.13**

All values are mean of triplicates ± S.D. * = Significance ($p < 0.01$) compared to control

A drastic disorder in soluble sugars export, especially glucose and fructose liberation, was also imposed after exposure to excess chromium. This restricted the starch and sucrose breakdown in reserve tissue, as evidenced by the inhibition in the activities of α – amylase and invertase isoenzymes (soluble acid, soluble neutral, cell wall – bound acid). Chlorophyll a, b and total chlorophyll content increased at low concentration (25 and 100 ppm). However, there was significant decrease ($p < 0.01$) at high concentration (100 ppm).

Table 3

Effect of different concentration of chromium on photosynthetic pigments (mg/g fresh wt.) in *Phaseolus vulgaris* **seedlings' at different concentrations**

Concentration	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoid
Control	*1.27±0.09*	*0.44±0.06*	*1.80±0.08*	*0.34±0.08*
25 ppm	*1.36±0.08*	*0.19±0.05**	*1.51±0.06**	*0.55±0.05**
50 ppm	*1.07±0.05**	*0.15±0.05**	*1.39±0.04**	*0.61±0.09**
100 ppm	*0.77±0.05**	*0.13±0.04**	*0.87±0.06**	*0.70±0.08**

All values are mean of triplicates ± S.D. * = Significance ($p < 0.01$) compared to control

Maximum reduction was observed at 100 ppm. Similarly, a concentration dependent reduction in chlorophyll content over control was also observed in the leaves of *Phaseolus vulgaris* seedlings'.

However, carotenoid content showed dose dependent relationship. It increased significantly ($p < 0.01$) with increase in concentration of chromium in the medium. Heavy metal accumulation in vascular plant is known to produce significant physiological and biochemical responses. A decrease in chlorophyll content may either be due to inhibition of chlorophyll synthesis or its destruction or replacement of Mg ions.

An increase in carotenoid content was observed in chromium treated plants of *Phaseolus vulgaris*. Increased carotenoid concentration for the protection from free radical formation is a common response to xenobiotics.

CONCLUSIONS

Contamination by chromium (Cr) is widespread in agricultural soils and industrial sites. This heavy metal represents a risk to human health.

In order to gain fundamental insights into the nature of the adaptation to chromium excess, the characterisation of physiological indices, including responses of photosynthetic gas exchange and chlorophyll a fluorescence along with changes in mineral nutrient contents and water status were studied in *Phaseolus vulgaris* seedlings'. Increased concentrations of chromium Cr (VI) in solution were applied.

The growth of *Phaseolus vulgaris* seedlings' is decreased by chromium and the leaves have lost their pigments. Chromium accumulation was greater in roots than in leaves. The physiological parameters were severely reduced by this heavy metal. Chromium induced resulted in a modification of mineral content in roots and leaves, especially for Ca, Mg and Fe.

The chromium stress decreased CO_2 assimilation rates mainly due to stomatal closure, which reduced water loss by transpiration without decreasing the cellular available CO_2. The fluorescence parameters associated with photosystem II (PSII) activity and the photochemical activity are modified by chromium. Non – radiative energy dissipation mechanisms were triggered during stress since non – photochemical quenching was increased and efficiency of excitation capture by open centers was reduced.

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