



EFFECT OF ZINC ON PARAMETERS (PROTEIN, SOLUBLE SUGAR AND PROLINE) IN BEAN (*VICIA FABAL*.)

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Abstract

The use of solid urban waste as fertilizer causes soil and plant contamination by heavy metals. Heavy metals, such as Cu, Zn, Pb, Cd, Cr... pose particularly serious problems for agriculture with adverse effects on crop production and biodiversity.

In our study, we are interested in discovering the biochemical behavior of a model plant *Vicia faba* L. in the face of metallic stress. For this, we studied the effect of four concentrations of zinc sulphate (0, 300, 500 and 700 ppm) for 2 weeks after 45 days of growth of the plant. Levels of protein, proline and soluble sugars were analyzed spectrophotometry.

The results show a significant reduction in protein levels according to the progressive increase of the metal (Zinc), with the observation that the contents in the leaves are higher than those of the roots, noting that the lowest values are obtained at the dose of 700 ppm zinc (56.503 mg g⁻¹ dry weight).

On the other hand, the same dose of Zinc leads to a significant increasing accumulation of the proline and soluble sugar content relative to the control according to the increasing concentration of metal (zinc) at leaf levels much more than roots. The content of proline and the higher soluble sugars (182.977 mg g⁻¹ dry weight and 70.859 mg g⁻¹ dry weight respectively) are obtained at a dose of 700 ppm zinc.

Key words: “*Vicia faba* L.”, “heavy metals”; “Zinc (Zn)”; “biochemical behavior”

Introduction

Human activities have considerably increased the release of various molecules into the environment, some of which are toxic to all living organisms (microorganisms, plants and living things). Of the molecules introduced, heavy metals play a significant role (Toppi and Gabbrielli, 1999).

The contribution of metals to the soil is usually done either by atmospheric deposition, or by direct application to the soil, or indirectly through vegetation, materials (fertilizers, phytosanitary, organic amendments, sludge treatment plant including ...) metal concentrates (Alloway, 1990; Adriano, 2001).

Agricultural intensification is usually accompanied by a significant increase in fertilization, which is defined as all the amendments (fertilizer and chemical fertilizers)

made to the land to provide the plants with nutrient supplements necessary for their growth to improve and increase crop yield and quality (Anafide, 2006).

Agricultural activities are the source of many pollutants scattered throughout the environment. Metallic trace elements (ETMs), including metals and metalloids, are among these pollutants at risk of priority concern because they are very toxic and non-degradable elements. The soils have been contaminated by metal pollutants whose concentrations have steadily increased during the 20th century causing toxic effects in living beings and disrupting the functioning of ecosystems (Nriagu and Pacyna, 1988).

Most soil-accumulated MTEs are absorbed by crop plants. Plants need certain metal ions for their growth and development, such as copper, zinc, manganese, iron or cobalt, which are used in certain enzymes or as co-factors (Clarkson, 1995; Grotz *et al.*, 1998). These

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elements, necessary in small quantities, prove however toxic even lethal when they are present in strong concentration.

Zinc is part of a very large number of enzymes (more than 300), within which it fulfills three types of functions: catalytic, co-catalytic or structural. Zinc is particularly important for the metabolism of sugars, proteins and phosphates. In addition, zinc also influences the integrity and permeability of membranes, and allows the protection of lipids and membrane proteins from oxidative stress (Marschner, 2011).

But a high dose of zinc in plants often takes the form of growth retardation and wilting of the aerial parts (Broadley *et al.*, 2007). Another common symptom is chlorosis, which causes yellowing of the leaves between the veins. Excess zinc disrupts cell function by degrading chloroplasts and the absorption of minerals such as phosphorus, magnesium, and manganese, disrupting chlorophyll synthesis (Boawn and Rasmussen, 1971, Carroll and Loneragan, 1968).

Proline is an amino acid often considered a biomarker of stress (Szabados and Savouré, 2009; Djerroudi-Zidane *et al.*, 2010). According to Mile *et al.* (2002), proline accumulation is one of the adaptive strategies activated by the plant in the face of environmental constraints. According to another point of view, the accumulation of proline is not a reaction to adaptation to stress, but rather the sign of a metabolic disturbance (Cheikh M'hamed *et al.*, 2007).

Soluble sugars play a central role in the structure, metabolism and function of plants. They are more involved in many stress response mechanisms, biotic or abiotic (Ramel, 2009).

In this context, our work aims to study the effect of a metal (Zinc) on the content of some biochemical parameters (proteins, soluble sugars and proline) of the bean (*Vicia faba* L.).

Materials and Methods

Plant material

The plant material used was the bean (*Vicia faba* L., variety Aguadulce), The seeds of *Vicia faba*, are rinsed with water and then plunged for fifteen minutes in a solution of hypochlorite of sodium at 12% diluted half to eliminate any possible fungal contamination. After several rinses with water to remove the remains of hypochlorite of sodium.

Methods of culture

The Seeds are germinated in alveoli containing

compost for 15 days in a greenhouse to select seedlings of the same size and growth rate for transplanting. Then, the seedlings are replanted in plastic pots of 3kg (20 cm diameter and 30 cm high). The bottom of each pot has been lined with a layer of gravel to ensure good drainage, on this layer is deposited a gauze band to retain the substrate, it is filled with a mixture of sand and industrial compost at respective proportions of (2v / 1v). A watering every three days is performed with the nutrient solution of Hoagland (1938) to 60% of the capacity of retention of the substrate.

Application of stress

Vicia Faba plants aged 45 days are irrigated with a solution of zinc sulphate ($ZnSO_4$) at concentrations of 300 ppm, 500 ppm, and 700 ppm (Table 1).

After two weeks of stress, the plants are separated, the leaves and roots separated and dried for 24 hours at 80°C. Then the dry samples are crushed and put into closed vials using a stopper.

Table 1: The experimental device

Concentration	Lot 1	Lot 2	Lot3	Lot4
Zinc (ppm)	0	300	500	700

2. The parameters analyzed

Content of Protein

The levels of protein are determined by the use of Bradford method, (1976).

Content of soluble sugars

The levels of soluble sugars are determined by the use of the technique of Dubois *et al.*, (1956).

Content of proline

The technique of Monneveux and Nemmar, (1986) was used to determine the content of proline.

Statistical analyzes

Statistical analyzes based on the average comparison tests, using 4 repetitions for each dose, were applied using STAT-BOX 6.4 software using the ANOVA variances analysis calling for the Newman Keuls test with a threshold $p = 5\%$.

Results

Content of protein

The results of Fig. 1 show that the protein content obtained for the leaves of plants treated with 300 ppm of zinc increases slightly compared to that of the leaves of the control plants (128.922, 100.293 mg.g⁻¹ dry weight, respectively) then it drops to 56.503 mg.g⁻¹ dry weight of

stressed plants at 700 ppm zinc (Fig. 1).

Statistical analysis shows that protein accumulation is highly significant in leaves and roots with all zinc treatments.

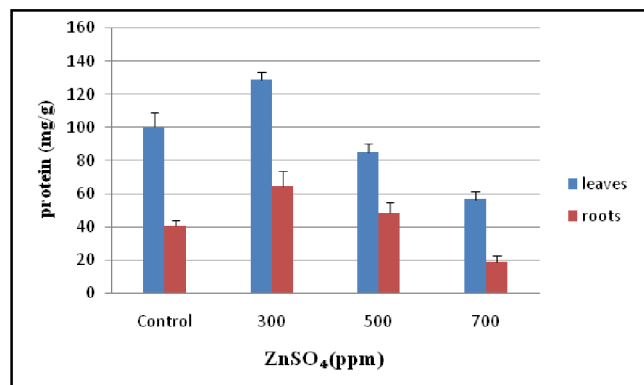


Fig. 1. Protéine content (mg.g⁻¹ fresh weight) of the leaves and roots of broad bean (*Vicia Faba L.*) stressed to Zinc (ppm)

Content of soluble sugars

The results of the effect of different zinc treatments on soluble sugar contents fig. 2 show a decrease in the leaves and roots with a higher rate for the leaves compared to the roots.

On the other hand, for the leaves of plants stressed at 700 ppm zinc, the results show a gradual increase much more with a rate of 70.859 mg.g⁻¹ dry weight.

Variance analysis shows that the presence of zinc induces a highly significant effect ($p = 0$) on the accumulation of soluble sugar in leaves and roots compared to control plants.

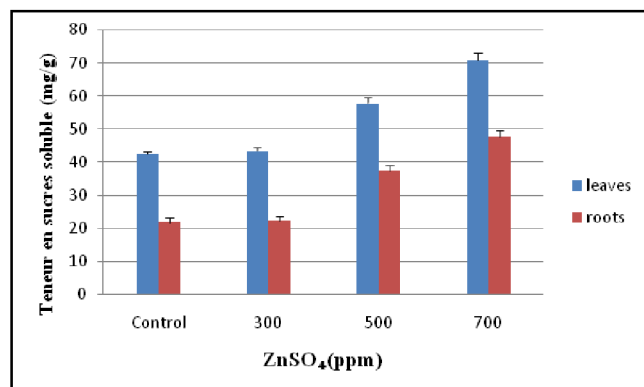


Fig. 2. Soluble sugars content (mg.g⁻¹ dry weight) of the leaves and roots of Broad bean (*Vicia Faba L.*) stressed to Zinc (ppm).

Content of proline

The results of fig. 3 show that the accumulation of proline is greater in the leaves than in the roots of the

bean. The accumulation of proline in the leaves and roots increases gradually with increasing doses of zinc.

The highest proline content in the leaves and roots is obtained at a dose of 700 ppm zinc.

Statistical analysis shows a highly significant ($p = 0$) effect on proline accumulation in the leaves and roots of the plant for all applied doses of zinc.

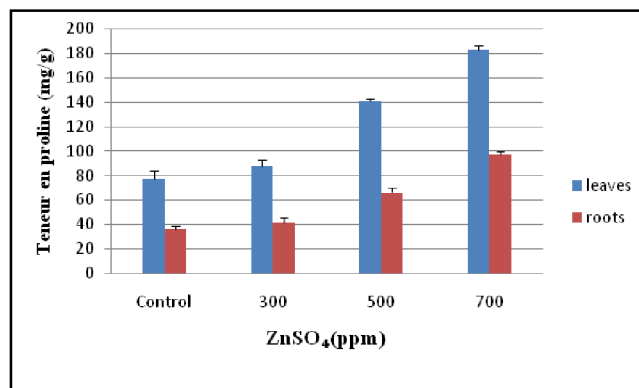


Fig. 3. Proline content (mg.g⁻¹ dry weight) of the leaves and roots of Broad bean (*Vicia Faba L.*) stressed to Zinc (ppm).

Discussion

Generally, the metals exert a depressive or even inhibitory effect on the development of the plant. The main results obtained show that the protein content decreases with the increase in zinc concentrations, this decrease has already been reported by Soussi *et al.*, (1998) on chickpea, by Parida *et al.* (2004) on *Bruguiera parviflora* and by Gaballah and Gomaa (2007) on two varieties of beans, Giza blanka (salt tolerance) and Giza (salt-sensitive).

The decrease in protein content could also be due to a low availability of amino acids and the denaturation of the enzymes involved in the synthesis of amino acids and proteins (Popova *et al.*, 1995).

In addition, the soluble sugar contents are high in the leaves and roots of the bean under metallic stress compared to the plant control. These results are in agreement with those of Hajihashemi *et al.*, (2006), where the accumulation of soluble sugars observed at the leaf level is one of the most observed phenomena in the stress response.

Costa and Spitz (1997) then Dubey and Singh (1999) suggest that this accumulation has been reported in response to different environmental stresses and particularly metals. According to Bouzoubaa *et al.*, (2001), the accumulation of soluble sugars could have an osmotic role preventing dehydration of cells and maintaining the balance of osmotic force.

The same effects are recorded for proline where there is remarkable accumulation with respect to the increase in zinc doses. This accumulation is greater in the leaves than in the roots, which could explain its synthesis at the leaf level and then its migration to the roots (Djerroudi-Zidane *et al.*, 2010).

Some authors like Singh *et al.*, 1973 believe that the amounts of proline accumulated could be related to the level of stress tolerance but rather as a sign of a metabolic disturbance (Cheikh M'hamed *et al.*, 2008), this ability of plants to the synthesis and accumulation of proline is not specific only to halophytes (HU *et al.*, 1992), it is also specific for numerous glycophytes such as tomato (Hernandez *et al.*, 2000), barley (hassani *et al.*, 2008).

Conclusion

The response of (*Vicia faba* L.) to metal stress resulted in a very large accumulation of soluble sugars and proline in the stressed bean compared with the unstressed plant, while there is a proportional decrease in protein with the increased in Zinc.

Finally, our results show a significant effect on the biochemical parameters of the bean (*Vicia faba* L.).

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