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Effect of interaction zinc-salinity of soil on parameters (proline, soluble sugar and protein) in bean (*Vicia faba* L.)

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Abstract

The weakness of agricultural production in Algeria is one of the main constraints to meet the needs of consumption, while soil pollution by heavy metals and salinity becomes a factor of more or less concern for agriculture with detrimental effects on crop production and biodiversity. We are interested in this research work, to study the biochemical behavior of a model plant (*Vicia faba* L.) in the face of metal and salt stress. For this, we studied the effect of four concentrations of zinc sulphate (0, 300, 500 and 700 ppm) combined with 100 and 200 meq.l^{-1} of NaCl for 2 weeks, after 45 days of plant growth. Levels of proline, proteins and soluble sugars were analyzed by spectrophotometry. The results obtained show an increase in the content of proline and soluble sugars as a function of the increasing concentration of zinc and NaCl at leaf and root levels. The levels of proline and soluble sugars in the leaves are much higher than those of the roots. The content of proline and the higher soluble sugars (168.727 mg g^{-1} dry weight and 56.274 mg g^{-1} dry weight respectively) are obtained at the dose of 700 ppm of zinc combined with 200 meq.l^{-1} of NaCl. The results obtained show that the combined effect of zinc and salt results in a significant decrease in the total protein content of the treated plants, the lowest levels of protein in the leaves are obtained at the dose of zinc of 700 ppm added to 200 meq.l^{-1} (33.68 mg.g^{-1} dry weight).

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Introduction

The salinity of soil and irrigation water is a major problem worldwide, according to FAO and the most recent estimates it already affects at least 400 million hectares and seriously threatens an equivalent area (Legros, 2009). Indeed, this constraint is considered as one of the main factors limiting agricultural productivity and development (Baatour *and al*, 2004).

Osmotic adjustment is one of the main adaptive mechanisms of plants with respect to stress which is specifically related to a net increase in the concentration of stressed solute. Soluble sugars have a major role to play in the osmotic adjustment; their synthesis is stimulated by salt stress in many species by either blocking glycolysis or starch hydrolysis (Rathinasabapathi, 2000). The peculiarity in the accumulation of proline by highly stressed plants may be one of the parameters to characterize the development of tolerance to salinity in glycophytes (Paquin and Pelletier, 1987).

To this contaminant is added the heavy metals and their accumulation in the environment, which leads to the disruption of the functioning of natural ecosystems but also agro-systems while high levels of metals in agricultural soils reduce crop yields.

In agriculture, most heavy metals accumulated in soil are absorbed by crop plants. Plants require 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 (Yang *and al.*, 2005). These 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), zinc is particularly important for the metabolism of sugars, proteins and phosphates, and 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 *and 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) . It is in this context that the objective of our research work in the study of the biochemical behavior (proline, soluble sugars, proteins) of the bean (*Vicia faba* L.) grown on a substrate contaminated with zinc and under salt stress.

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 added to NaCl at 100meq L^{-1} and 200 meq. L^{-1} (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.

The parameters analyzed

Content of proline

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

Content of soluble sugars

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

Content of Protein

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

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 proline

The results obtained show a significant increase in the content of proline in the leaves than the roots of plants subjected to Zinc stress at 300, 500,700 ppm combined with 200meq NaCl (129,62; 126,263 and 168,727 $mg.g^{-1}$ dry weight, respectively) compared to those combined with 100 meq NaCl and the control plant 76, 992 $mg.g^{-1}$ dry weight (Figure 1).

Statistical analysis reveals a highly significant effect of proline content in leaves and roots of stressed plants compared to controls.

Table 1. The experimental device.

concentration	Lot 1	Lot 2	Lot3	Lot4	Lot5	Lot6	Lot7
NaCl(meq.l^{-1})	0	100	100	100	200	200	200
Zinc (ppm)	0	300	500	700	300	500	700

Content of soluble sugars

Figure (2) shows the variations in the content of soluble sugars analyzed 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 combined with 200 meq NaCl, the results register a much more gradual increase with a rate of 56.274 $mg.g^{-1}$ dry weight.

Analysis of the variance shows that the presence of zinc combined with NaCl induces a highly significant effect on the accumulation of soluble sugars in the leaves and roots compared to control plants.

Content of protein

The results of Figure (3) show a significant increase in the content of protein compared to the control at 300 ppm zinc combined with 100 meq NaCl (100.29, 130.425 $mg.g^{-1}$ dry weight, respectively), then it drops to 33.68 $mg.g^{-1}$ dry weight of the plants stressed at 700 ppm zinc combined with 200 meq NaCl. Statistical analysis shows that the accumulation of protein is highly significant in the leaves and roots with all zinc treatments combined with NaCl.

Discussion

The results show that under the action of zinc and salinity the bean synthesizes and accumulates larger amounts of proline in the aerial part of the plants, particularly in the leaves compared to the control.

These results are similar with to those obtained by other authors on different species, in fact many studies indicate that proline migrates to the leaves and is located under salt stress in eggplant (JOSHI,1984), the Cotton (BOUTELIER, 1986), The vine (IMAMULHUQ and LARHER, 1985) and The Bean (AIT SADI,1990).

some authors such as SINGH and *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 *and al*, 2008), this ability of plants to the synthesis and accumulation of proline is not specific only to halophytes (HU and *al.*, 1992), it is also specific for numerous glycophytes such as tomato (HERNANDEZ *and al.*, 2000), barley (HASSANI *and al.*, 2008).

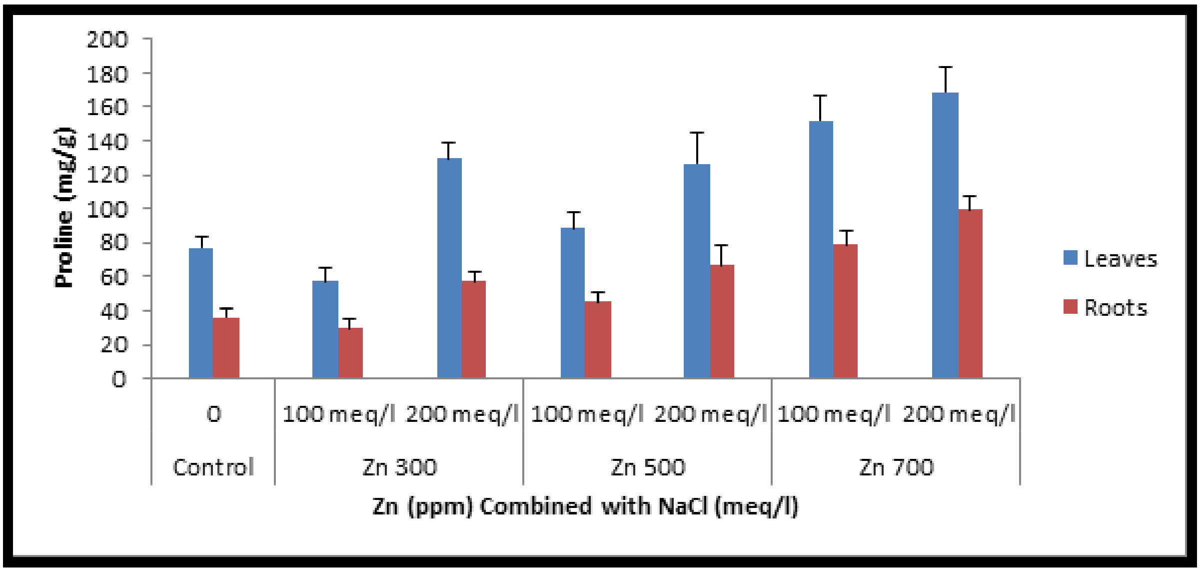

Fig. 1. Proline content (mg.g^{-1} dry weight) of the leaves and roots of Broad bean (Vicia faba L.) stressed to Zinc (ppm) Combined with NaCl (meq/l).

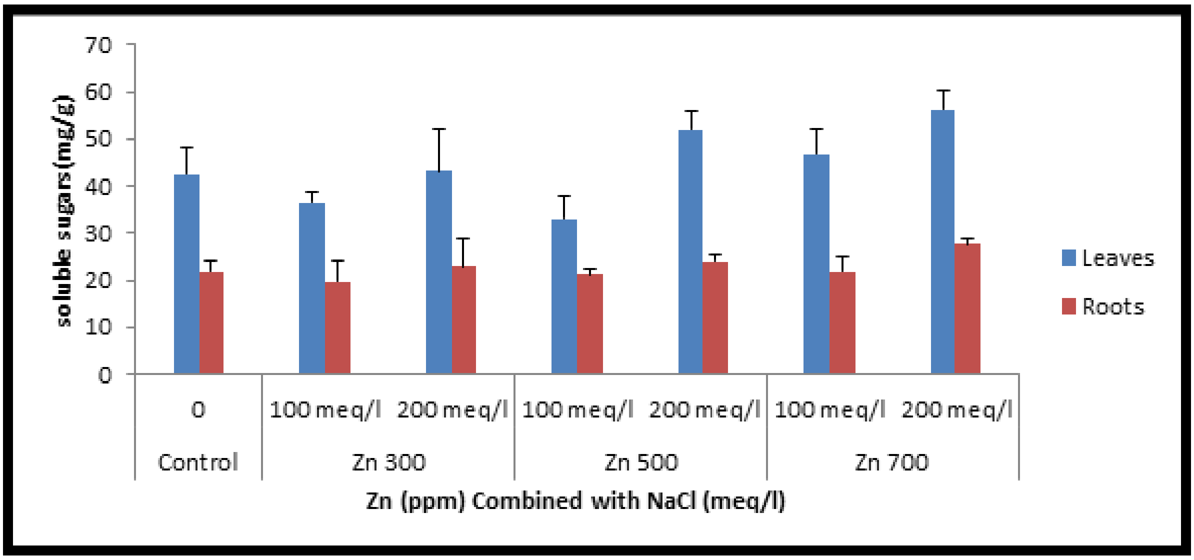

Fig. 2. Soluble sugars content (mg.g^{-1} dry weight) of the leaves and roots of Broad bean (Vicia faba L.) stressed to Zinc (ppm) Combined with NaCl (meq/l).

The accumulation of soluble sugars observed at the leaf level is among the most observed phenomena in the response to stress, COSTA and SPITZ (1997) then DUBEY and SINGH (1999) suggest that this accumulation has been reported in response to different environmental stresses and especially metals. According to Bouzoubaa *and al.*, (2001), the accumulation of soluble sugars could have an osmotic role preventing dehydration of the cells and the maintaining of the equilibrium of the osmotic force.

In addition, this study shows that the protein content decreases with the increase of zinc concentrations combined with salinity, this decrease has already been reported by SOUSSI *and al.*, (1998) on chickpea, by PARIDA *and al.*,(2004) on Bruguiera parviflora and by GABALLAH and GOMAA, (2007) on two varieties of beans, Giza blanka (salt tolerance) and Giza (salt-sensitive).

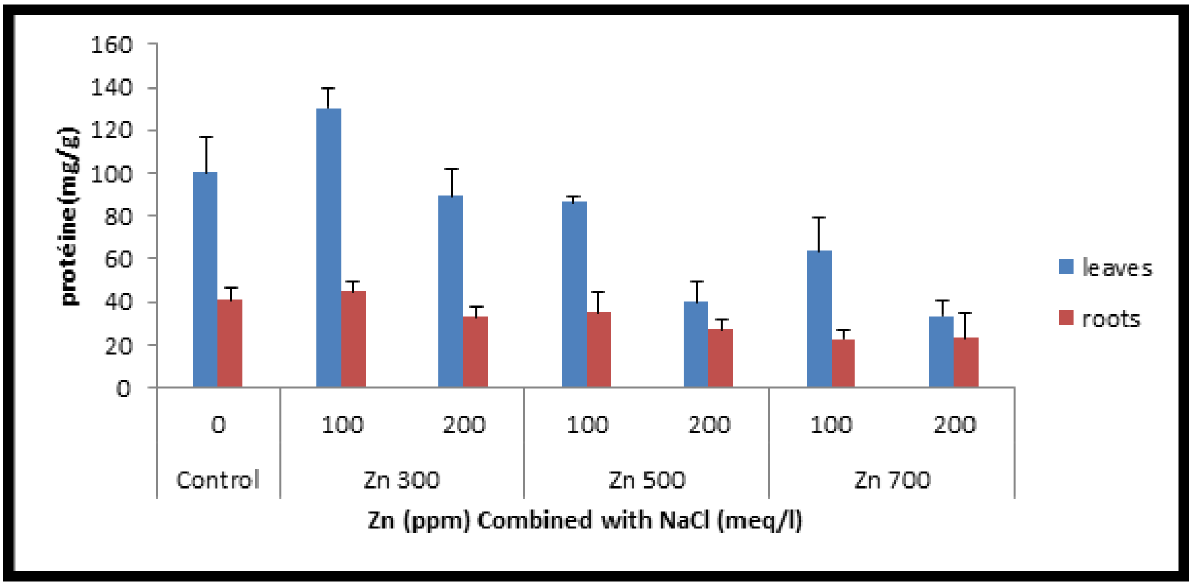

Fig. 3. Protéine content (mg.g^{-1} fresh weight) of the leaves and roots of broad bean (Vicia faba L.) stressed to Zinc (ppm) Combined with NaCl (meq/l).

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

Conclusion

The response of (*Vicia faba* L.) to salt and 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 concentrations combined with NaCl.

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