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CONTRIBUTION TO THE STUDY OF THE BIOCHEMICAL PARAMETERS OF *VICIA FABA* L. CULTIVATED UNDER SALINITY CONDITIONS AND IN THE PRESENCE OF BENTONITE

Bachir Bouiadjra Mohammed El Amine[1*], Ghellai Malika[2] and Belkhodja Moulay[1]

[1*]Laboratory of Plant Physiology, University of Oran 1Ahmed Ben Bella, Oran, Algeria.
[2]Ahmed Zabana Relizane University Center, Algeria.

Abstract

The present work based on the study of biochemical parameters in the roots and leaves of *Vicia faba* L. cultivated in different doses of the two types of bentonite (3%, 5%, 7% and 10%) one from Mostaganem and the other of Meghnia, under different saline concentrations (100 and 200) Meq.L^{-1} of NaCl; a study on the content of leaves and roots of this plant in total soluble sugars, and an evaluation of the chlorophyll contents. Concerning soluble sugars we noticed a strong accumulation under the effect of the salt constraints; this accumulation remains clearly higher in the leaves compared to the roots. Relative to the chlorophyll content, it was conducted to properly determine the combined action of bentonite and salinity. The completion of this work allowed us to mention that the doses of 3 and 5% present an improvement in the parameters studied with regard to salinity.

Key words : *Vicia faba* L., bentonite, salinity, NaCl, soluble sugars.

Introduction

Salinity is a growing ecological problem all over the world, particularly the Mediterranean basin and North Africa, this phenomenon and considered as a most important abiotic factor which limits the growth and the productivity of plants (Khan and Panda., 2008) The decrease in water resources in these regions (Muns *et al.,* 2006) and the extension of irrigated areas triggers the salinization of the soil (Ben Naceur et *al* 2001; Rochdi *et al.,* 2005; Araujo *et al.,* 2006). One of the major mechanisms of adaptation to ionic and osmotic stresses is expressed by the plant's capacity to accumulate at the symplasmic and active level of ions such as K + and Na + (Wang *et al.,* 2002; Parida and Das., 2005; Munns and Tester., 2008). It is in this perspective that the action of developing cultivated soils is integrated by introducing bentonite, it contributes to the increase in the nitrogen content assimilable in the soil, the application of bentonite also improves the chemical parameters of sandy soils, increases agricultural production and economy of water and nutrients (Menouare., 2015).

***Author for correspondence :** E-mail: aminebachirb@yahoo.fr

The objective of our work is to highlight the influence of a salt constraint represented by different concentrations of NaCl (0, 100, 200 Meq.L^{-1}) associated with the few doses of two types of bentonite (3%, 5%, 7%, 10 %) on the biochemical parameters of a legume species, the broad bean (*Vicia faba* L) in terms of total soluble sugar content and the content of Chlorophyll.

Materials and Methods

Plant material and growing medium

The plant material used is a bean legume (*Vicia faba* L.). The variety selected is a commercial variety commonly cultivated by farmers in the region.

The substrate used corresponds to a mixture of sand with two types of bentonite from two regions; mostaganem, and maghnia (cities of western Algeria). The prepared Substrate consists of two volumes of sand with one volume of Peat. Four doses of bentonite are used: 3%, 5%, 7% and 10%.

We applied a salt stress of different doses of NaCl 0, 200 and 400 Meq.L^{-1} for each group of plants transplanted in the mentioned substrates of bentonites.

Parameters analyzed

Determination of total soluble sugars

Total soluble sugars (sucrose, glucose, fructose, their methyl derivatives and polysaccharides) are measured by the Dubois *et al*., (1956) method.

It consists of taking 100 mg of plant material (leaf + root), in test tubes, adding 3 ml of 80% ethanol to extract the sugar, then leaving at room temperature for 48 hours. At the time of dosing, the tubes are placed in the oven at 80°C to evaporate the alcohol. In each tube we add 20mL of distilled water to the extract.

In glass tubes, 2 ml of the solution to be analyzed are added, 1 ml of 5% phenol is added; 5mL of concentrated sulfuric acid is quickly added while avoiding pouring acid against the walls of the tube. An orange-yellow solution is obtained on the surface; it is passed through a vortex shaker to homogenize the solution. The tubes are left for 10 min and placed in a water bath for 10 to 20 min at a temperature of 30°C.

Dosage of Chlorophyll

The pigment extraction was carried out according to the method of Lichtenthaler (1987).

100 mg of fresh foliar sample were kept in 10 ml 95% acetone and the tube was allowed to keep in dark at 4^0C for 48 hours. The optical density (OD) was taken at 663 and 647 nm. The chlorophyll contents a and b was calculated by the following relations:

Chlorophyll a (µg / ml) = (12.25 OD 663) - (2.79 OD 647)

Chlorophyll b (µg / ml) = (7.15 OD 647) – (5.10 OD 663)

Total Chlorophyll (µg / ml) = Chl a + Chl b

Results

It is noted that the plants treated with 100 Meq.L^{-1} of NaCl and in the absence of bentonite record higher contents of soluble sugars whatever for the leaves and for the roots unlike the plants which were watered by the nutritive solution and those treated at 200 Meq.L^{-1} where the contents were less important than the first.

It is mentioned that there is a very important difference when comparing the accumulation of soluble sugars in the leaves registering higher values than in the roots.

Under the combined action of salinity and bentonite, our results show that with the addition of different concentrations of maghnia or mostaganem bentonites the values of the soluble sugar content have undergone a remarkable increase by adding to our growing medium and by combining bentonite with different doses of NaCl salt.

Total soluble sugar content

Witness; In the presence of salinity and in the absence of two types of bentonite

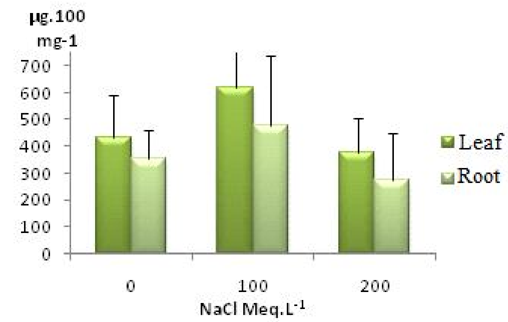

Fig. 1: Soluble sugar content in the leaves and roots of the *Vicia faba* L. bean grown in the absence of bentonite and stressed by salinity.

Maghnia bentonite 3% Dose

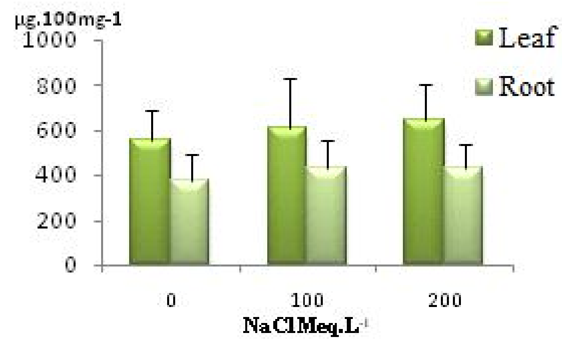

Fig. 2: Soluble sugar content in the leaves and roots of the *Vicia faba* L. bean grown in the presence of 3% Maghnia bentonite and stressed by salinity.

Mostaganem bentonite 3% Dose

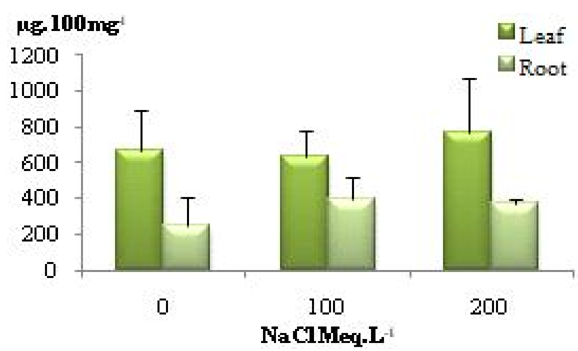

Fig. 3: Soluble sugar content in the leaves and roots of the *Vicia faba* L. bean grown in the presence of 3% Mostaganem bentonite and stressed by salinity.

Maghnia bentonite 5% Dose

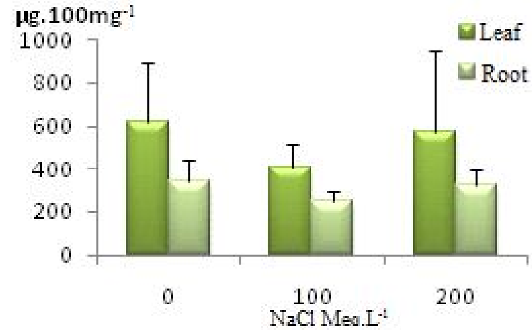

Fig. 4: Soluble sugar content in the leaves and roots of the *Vicia faba* L. bean grown in the presence of 5% Maghnia bentonite and stressed by salinity.

Mostaganem bentonite 5% Dose

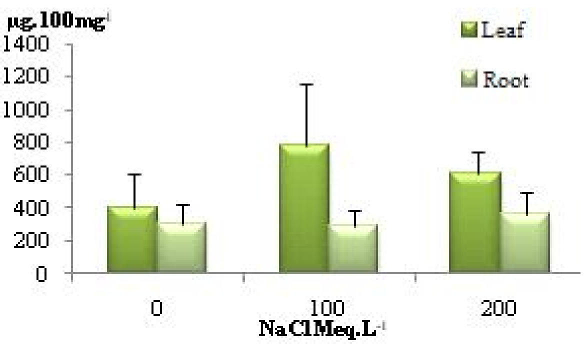

Fig. 5: Soluble sugar content in the leaves and roots of the *Vicia faba* L. bean grown in the presence of 3% Mostaganem bentonite and stressed by salinity.

Maghnia bentonite 7% Dose

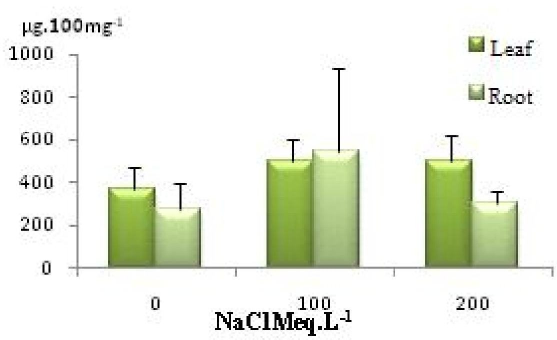

Fig. 6: Soluble sugar content in the leaves and roots of the *Vicia faba* L. bean grown in the presence of 7% Maghnia bentonite and stressed by salinity.

The combination of the different doses of mostaganem bentonite with or without salt in the growing medium recorded relatively higher levels of soluble sugars for both the leaves and the roots than that of maghnia.

It is noted that the chlorophyll content of the leaves of plants cultivated in different substrates combined with

Mostaganem bentonite 7% Dose

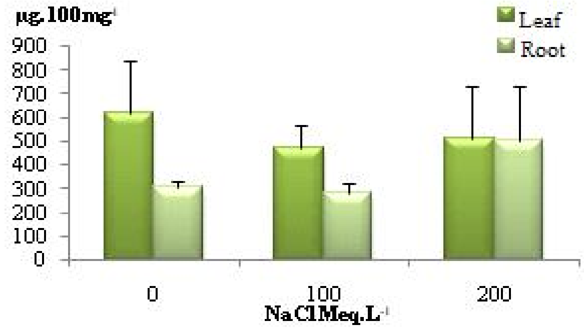

Fig. 7: Soluble sugar content in the leaves and roots of the *Vicia faba* L. bean grown in the presence of 7% Mostaganem bentonite and stressed by salinity.

Maghnia bentonite 10% Dose

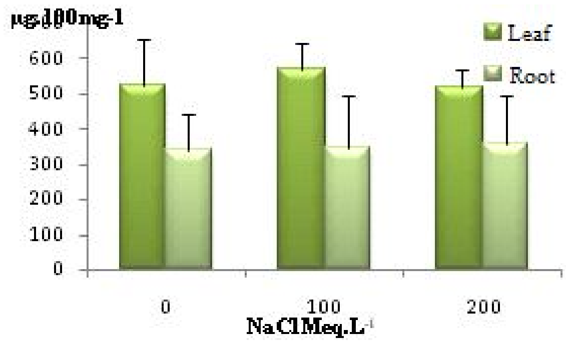

Fig. 8: Soluble sugar content in the leaves and roots of the *Vicia faba* L. bean grown. in the presence of 7% Maghnia bentonite and stressed by salinity.

Motaganem bentonite 10% Dose

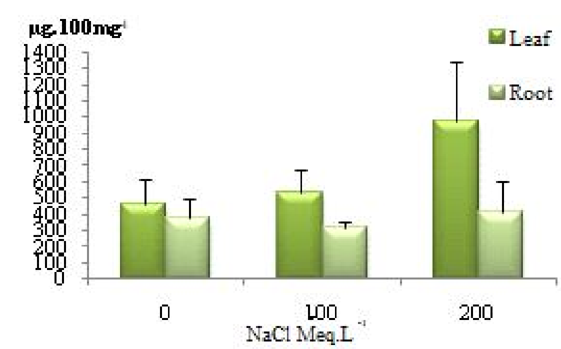

Fig. 9: Soluble sugar content in the leaves and roots of the *Vicia faba* L. bean grown in the presence of 7% Mostaganem bentonite and stressed by salinity.

bentonite at different doses marked a drop by increasing the doses of bentonite obtained from the two stations studied and in the absence of saline stress, on the other hand for the results obtained in the other treatments having a combined action of different doses of bentonite with the salinity, values higher than those of the controls are noted.

Chlorophyll content

Witness; In the presence of two types of bentonite and in the absence of salinity

Maghnia bentonite

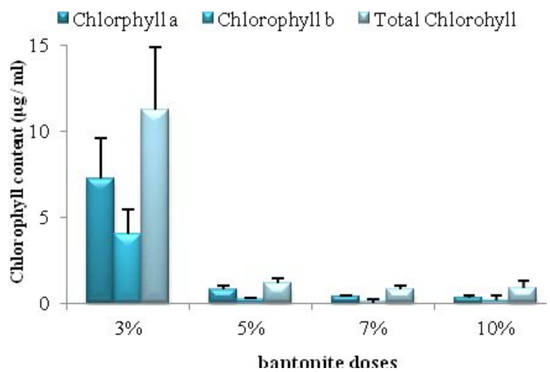

Fig. 10: Chlorophyll content in the leaves of the *Vicia faba* L. bean grown in the presence of Maghnia bentonite and in the absence of salt stress.

Mostaganem bentonite

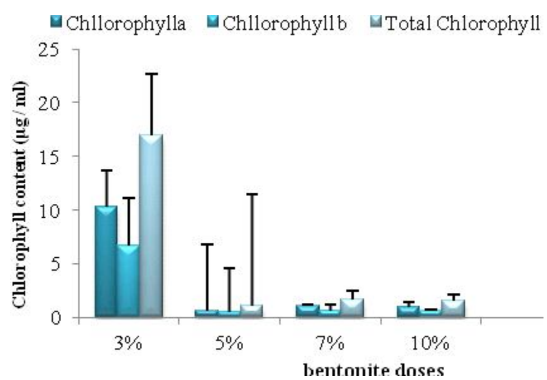

Fig. 11: Chlorophyll content in the leaves of the *Vicia faba* L. bean grown in the presence of Maghnia bentonite and in the absence of salt stress.

Maghnia bentonite + 100 Meq.L^{-1} NaCl

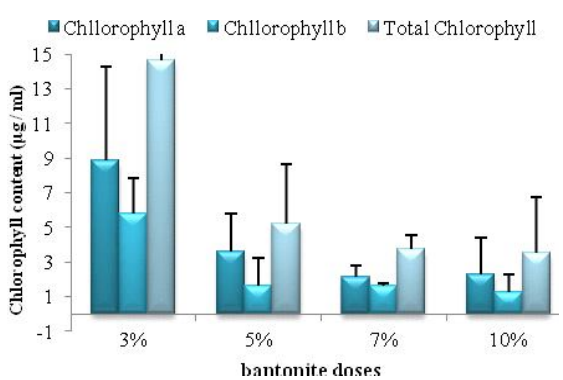

Fig. 12 : Chlorophyll content in the leaves of the *Vicia faba* L. bean cultivated in the presence of Maghnia bentonite under saline stress at 100 Meq.L^{-1} NaCl.

Mostaganem bentonite + 100 Meq.L^{-1} NaCl

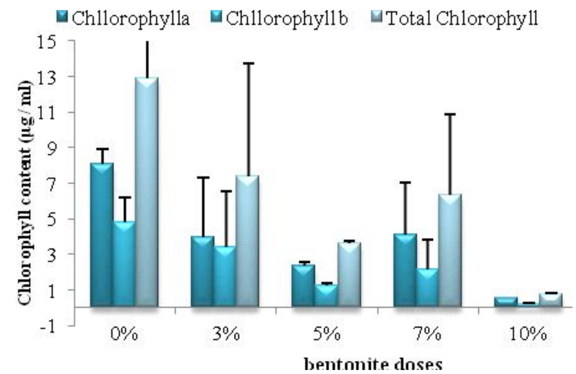

Fig. 13: Chlorophyll content in the leaves of the *Vicia faba* L. bean cultivated in the presence of Mostaganem bentonite under saline stress at 100 Meq.L^{-1} NaCl.

Maghnia bentonite + 200 Meq.L^{-1} NaCl

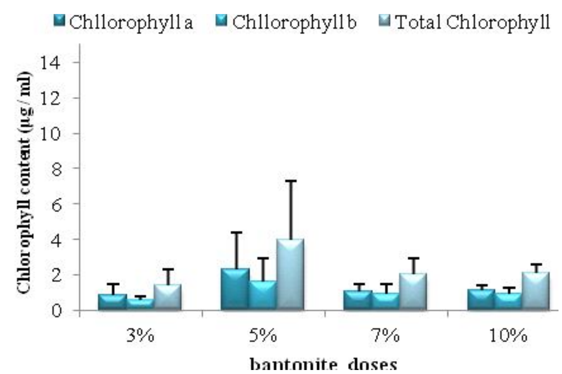

Fig. 14: Chlorophyll content in the leaves of the *Vicia faba* L. bean cultivated in the presence of Maghnia bentonite under saline stress at 200 Meq.L^{-1} NaCl.

Mostaganem bentonite + 200 Meq.L^{-1} NaCl

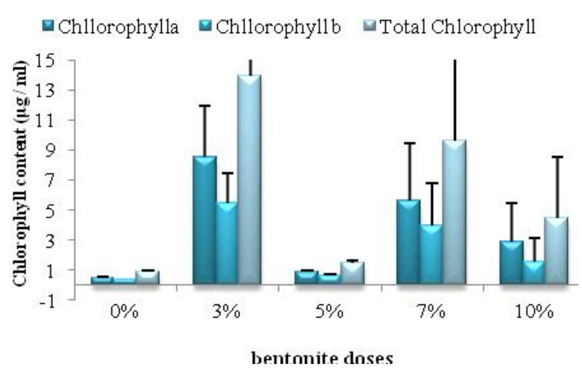

Fig. 15: Chlorophyll content in the leaves of the *Vicia faba* L. bean cultivated in the presence of Mostaganem bentonite under saline stress at 200 Meq.L^{-1} NaCl.

We note in the same way, a maximum content of chlorophylls a, b and total in the leaves of plants cultivated in the substrate having a dose of 3% of bentonite by applying the different parameters for the two types of bentonite except that of Maghnia under stress saline 200 Meq.L^{-1}.

We also add the presence of a high content of chlorophyll a compared to chlorophyll b for all types of treatments.

Discussion

Our results obtained on the total soluble sugar contents seem to be similar with those obtained for other species of glycophytes studied elsewhere by marking an accumulation of this element by applying stress (Laredj, 2013), our results coincide with others studies showing the positive effect of bentonite on the morphological and biometric characteristics of the bean. (Bachir bouiadjra; 2010).

In light of these results, we note the positive effect of bentonite from Mostaganem and Meghnia on the adaptation of plants to the constraints of salinity. The montmorillonite contained in the two natural substances used helps to improve the physicochemical properties of sandy soils (Haliat and Tessier 2006) which translates into an improvement in the water characteristics of the plants (Benkhlifa 2007, Bachir Bouiadjra 2010). The increase in salts in the soil causes a decrease in chlorophyll pigments (Agatian *et al.,* 2000). In the presence of bentonite the chlorophyll levels improve which proves good nutrition of the plant due to the richness of the bentonite of the mineral elements (Achour and youcef., 2003)

Conclusion

At the end of our work which aimed to study the tolerance of *Vicia faba* L. cultivated on soil amended with different doses of the two types of bentonites (3%, 5%, 7%, 10%), one of Mostaganem and the other of Maghnia to salinity, by applying different concentrations of salts (NaCl) at 100 and 200 Meq.L^{-1}, in order to determine the effect of saline stress on two biochemical parameters, namely the accumulation total soluble sugars and chlorophyll content; a, b and total; it appears that this species responds differently to the different doses applied depending on the organ considered and the amount of Bentonite added.

The results obtained show us that the addition of bentonite improves the accumulation of total soluble sugars in the leaves and roots of this legume in different proportions in the face of the applied salt stress; it is a form of adaptation of this glycophyte since bentonite plays an important role in the water balance of this species

The quantity of chlorophyll pigments on the bean leaves seems to be influenced by the different regimes imposed.

With the addition of doses of Mostaganem and Meghnia bentonites to growing media, chlorophyll tenures improve in the presence of salinity.

The presence of bentonite in small doses of 3% allowed the leaves to keep a certain balance of the contents of Chlorophylls a, b and total.

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