Effect of Salt Stress and Bentonite on the Germination and Proline content of the 'Semilla *Violeta'* and *'Reine mora'* broad bean (*Vicia faba* L.*)*

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Abstract

Salinity is considered as the most important abiotic factor. It limits growth and productivity of plants and degrades agricultural soils in arid and semi arid area. The study was conducted on *Vicia faba L.'Semilla violeta'* and '*Reine mora'*. Sowing was realized in plastic pots containing sandy substrates of bentonite 3, 5, 7, and 10% associated with abiotic stresses of salinity corresponding to doses of NaCl, $MgCl_2$ and $MgSO_4$ 20, 40, and 60 mmol.L^{-1} respectively. The effect of these constraints on germination and proline content was investigated. The results showed that the addition of 5% of bentonite has improved the rate and germination time. However, the combined effect of salinity and bentonite showed a relatively increase of proline in '*Reine mora'* than those of *'Semilla violeta'*.

Key words: : Bentonite, salinity, *Vicia faba,* germination, proline.

1. Introduction

The soils of arid and semi arid bioclimatic area, where the practice of agriculture uses irrigation, suffer from salinity (Djerroudi-Zidane and *al.*, 2010).Abiotic stress factors such as drought, heat and soil salinity cause significant agricultural yield losses (Ashraf and Akhtar, 2004) and limit the growth and productivity factors of different plant species (Basal and Hemphill, 2006, Abbruzzese and *al.,* (2009) and Misra and *al.*, (2006). Salt-tolerance is, also expressed by morpho-physiological characteristics (Pessarakli, 2002), molecular/biochemical (Ozturk and *al.*, 2002); mechanisms that are induced under these stress conditions and delays germination and seedling emergence (Ashraf and Ahmad, 2000).

The response to salt plant species depends on the variety (Radhouane, 2008; Benmahioul and *al.*, 2009), on the salt concentration of the substrate, growing conditions and physiological stages (Kaymakanova, 2009, Michael and *al.*, 2004). Identification of salt tolerant varieties would, certainly improve production in areas at risk or irrigated with brackish water. It would present, then an interest in breeding improvement. The primary effects of high levels of salt are caused by ion imbalance and hyperosmotic stress in plants. Secondary effects involve oxidative damage to enzymatic proteins and membrane integrity (Zhu, 2000).

A saline soil is currently facing at an irreversible ecological evolution characterized by a transition from semi arid to arid covering large areas (Belkhodja and Bidai 2004).For this purpose, it is better to opt for sound management of cultivated land based on a profound knowledge of biological interactions using available natural resources. Because, under extreme

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salinity conditions, the rehabilitation of saline soils requires the implementation of multidisciplinary studies strategies for understanding the response of plants (Belkhodja and Benkablia, 2000).

This perspective integrates a cultivated soils development by introducing bentonite and rich clay. This action would lead to increased cations exchange capacity. Halilat and Tessier (2006) had ensured that the contribution of the bentonite in sandy soil improves its physical properties and water retention (El-Diwani and *al.,* 2012). The addition of clay would improve the agricultural potential and increase reserves and capacity of water and minerals (Costa and *al.*, 2004).

The objective of this work consists to test four salt concentrations 0, 20, 40 and 60 mmol.L^{-1} composed by NaCl, $MgCl_2$and $MgSO_4$. Each of these concentrations was used with rates of bentonite 3, 5, 7 and 10%; a total of 16 different substrates. Because, production and accumulation of bentonite in the cytoplasm obtain osmotic adjustment (Misra and Gupta, 2005, Girija *and al.*, (2002) and on realization of various stages plant of seed germination (Thakur and Sharma, 2005).Moreover, the presence of abiotic stress causes an accumulation of proline (Djerroud-Zidane and *al.*, 2010) and allows protecting membranes and enzyme systems, especially in young plant organ (Muzahidul and *al.*, 2009). Proline seems to play a role in maintaining pressure cytosol and vacuole pH regulation (Ottow *and al.,* 2005).

2. Materials and Method

The trial was shared between biology laboratory and environmentally controlled greenhouse located at the University of Oran, Algeria (35°38'N, 0°36'O).

2.1 Substrate: was composed by sand and bentonite. The first one was recovered from the sea side. It was washed with tap water and sieved to a diameter of 2 mm. It was then, dipped in a solution of HCl diluted at 1/5 and washed several times with distilled water. It has been disinfected with bleach at 25%. The desalination was made by silver nitrate. The bentonite has been, previously granulated using a mill and sieved to electrical sieve of 2 mm to obtain a fine powder facilitating its disposal mixture. The amount of bentonite added in the treated soil corresponded to a percentage of dry weight of soil. The pots used had a diameter of 15 cm and a volume of 1.5 liter. The bottom of each pot was lined with a layer by gravel to ensure good leaching.

2.2 Plant materials and cultural methods:

Vicia faba L. '*Reine mora* 'and '*Semilla violeta'* introduced from Spain were chosen because of their use in the laboratory as a model plant for toxicological studies of different stress (Marcato-Romain and *al.*, 2009). The broad bean has many interests such as rapid growth, large biomass, sensitivity to metals and large cell size (Loss and *al.*, 1997).The seeds were disinfected with bleach to 8% for 10 minutes and rinsed several times with distilled water to remove all traces of chlorine.

Sowing took place on 02-02-2011 in pots filled with one kilogram of each substrate used. The Irrigation was performed with distilled water. The water holding capacity was determined by the difference between the quantity of water supplied by irrigation and that recovered after 24 h.

Reserve easily water used corresponded between 30 and 60% of the water retention capacity. That means 40 and 80 ml per pot. Plants were watered three times a week, twice with deionized water and once in the nutrient Hoagland solution diluted to 1 ppt.

2.3 Experimental Device Statistical analysis:

Four doses of bentonite were tested 3, 5, 7 and 10%. The substrates were made up by 3 salts Na Cl, $MgCl_2$ and $MgSO_4$. Each dose of bentonite was contained in 16 pots for four salt concentrations. The substrates controls were irrigated with deionized water during the period of application of stress. After 50 days of germination, the application of stress was conducted once during the last week before collection of plant in the substrates for analysis.

Table1: Composition of the salt solution ($mmol.L^{-1}$).

NaCl + $MgCl_2$ + $MgSO_4$	**NaCl**	**$MgCl_2$**	**$MgSO_4$**
20	10	5	5
40	20	10	10
60	30	15	15

The proline was analyzed by the method of Bergman and Loxley (1970). The optical density was measured using a spectrophotometer at 505 nm.
The experimental design was adopted at two factors, bentonite with 4 doses 3, 5, 7 and 10%, and the other salinity with 4 doses 0, 20, 40 and 60 $mmol.L^{-1}$ (Table 1).
The data obtained were subjected to an ANOVA analysis with two factors at randomization fixed classification. The averages were compared using the Student's method and Fisher. They are made by the software STATBOX 6.40 and confirmed by Statistical 7.

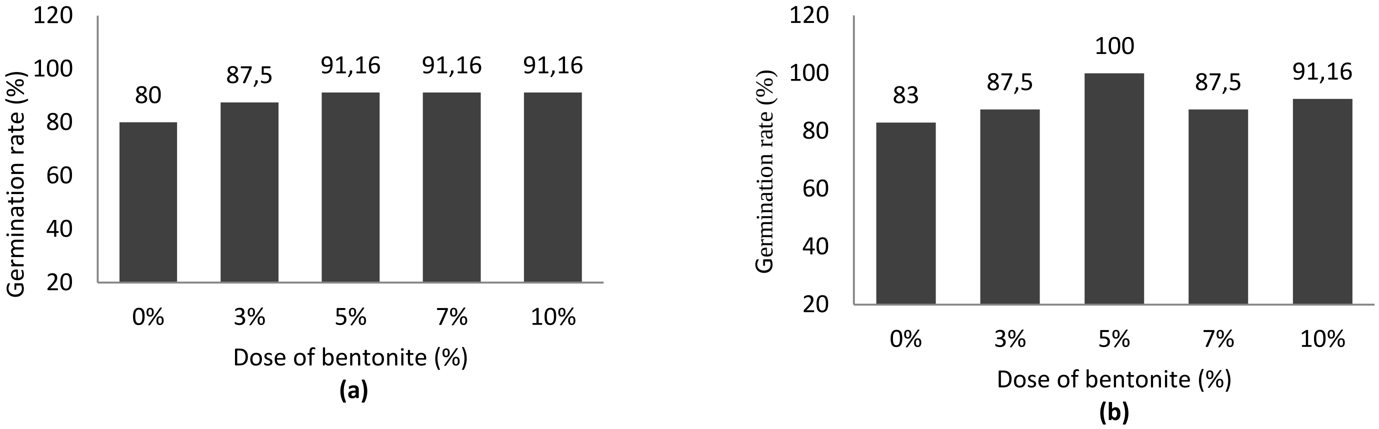
3. Results and Discussion

3.1 Effect of bentonite on the rate and duration of germination

The lowest rate 80 % of germination of '*Semilla violeta*' (fig. 1a) was obtained in the control substrate without bentonite. However, higher rates 91.16% were obtained in substrates with 5, 7 and 10% of bentonite. The same observation was made for '*Reine mora*'**(**fig.1 b) where it was found that 100% germination was obtained in substrates treated with 5% of bentonite; an increase of 20% compared to control. Sobhan and *al*., (2010);Serhat-Ouadabas and Mut, (2007) showed that the introduction of Bentonite in substrate contributed significantly to the improvement of the physico-chemical properties of soil by increasing water supplies necessary for germination, Cation Exchange Capacitance (CEC) (Cravero and *al*., 2000) and soil microflora (Yoshida et *al*., 2004). Furthermore, we observed that the doses of 5 and 10% of bentonite caused a relatively high germination, respectively with 100 and 91.16 % (Fig.1b) compared to the control substrate.
'*Semilla violeta'* had a tolerance criterion regarding to the high content of clay up to 10% bentonite. However, '*Reine mora*'***recorded*** 100 % of germination (fig.1 b) in the sandy substrate enriched to 5% bentonite. For both varieties (fig. 1), bentonite had further improved the rate and

germination time compared to the sandy substrate. According to Benkhelifa (2007), bentonite of the area study appreciably increases the water content of the substrate. From Heller and *al.* (2004), germination is characterized by a stabilization of the hydration and the respiratory activity at a high level. This one succeeds the phase of imbibitions corresponding to strong hydration. Imbibitions depend inevitably on the quantity and water chemical quality. The duration of germination 7 days of '*Semilla violeta'* (fig.2 a) was relatively long in the substrate without bentonite regarding to other substrates. The smallest duration 3 days was obtained in the substrate to 5% bentonite. Because, according to McKinstry and Anderson (2003), the addition of bentonite increased organic matter and decreased pH, exchangeable sodium, CEC, and percent clay; favorable factors of germination.

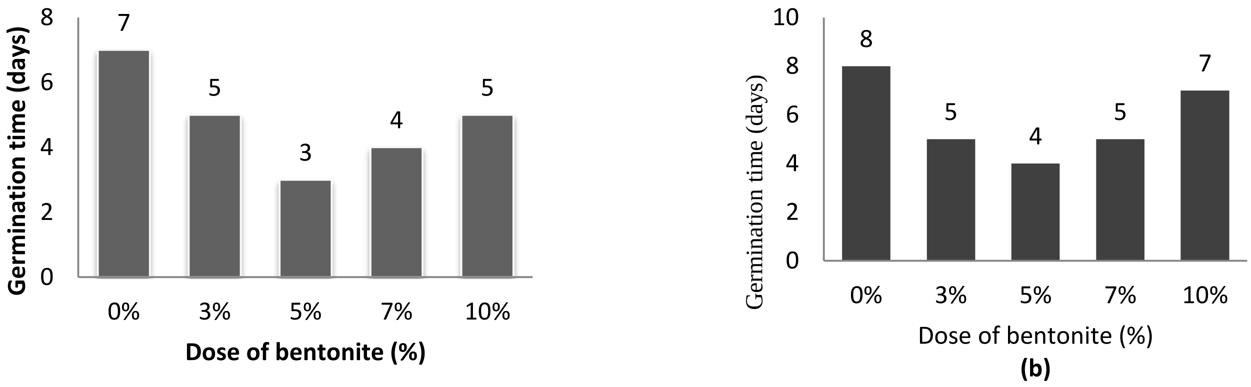
The longest duration 8 days of germination for '*Reine mora'* was also, obtained in unamended substrate bentonite (Fig.2b) and smallest in the treated substrate to 5% bentonite. Therefore, and compared to control soil, germination time is short in all substrates enriched with bentonite.



(a)

(b)

Figure 1(a-b): Effects of sandy substrate amended with bentonite on Germination rate (%) of Vicia faba Plant varieties (a).Semilla violeta and (b) Reine mora.



(b)

Figure 2(a-b): Time of germination (days) of '*Semilla violeta*'(a) and '*Reine mora'* (b) in a sandy substrate amended with bentonite.

3.2 Effect on the proline content

The ANOVA results revealed very highly significant effect of bentonite factor (P>0.001) on the content of proline of '*Semilla violeta and 'Reine mora'* aerial parts (P> 0.001). Nevertheless, the results (Figs 3, 4) show that its accumulation in the aerial part for both varieties is very significant compared to roots.

'*Reine mora'* (figs. 3, 4) accumulated more proline than 'Semilla violeta'. This was due to their varietal differences. Because, it gave each its own characteristics, allowing it to react to salt stress and adapt to new situations.

Proline content (fig. 4 a) in the aerial part of *'Reine mora'* reached its maximum 100.37 and 134.07 µmol/100 mg of dry matter at a dose of 60 mmol.L^{-1} in sandy substrates treated at 7 and 10% bentonite respectively. This high accumulation was due, first at the high salt dose and secondly at the nature of the sodium clay rich in sodium. It provoked, and increased osmotic pressure in the soil solution.

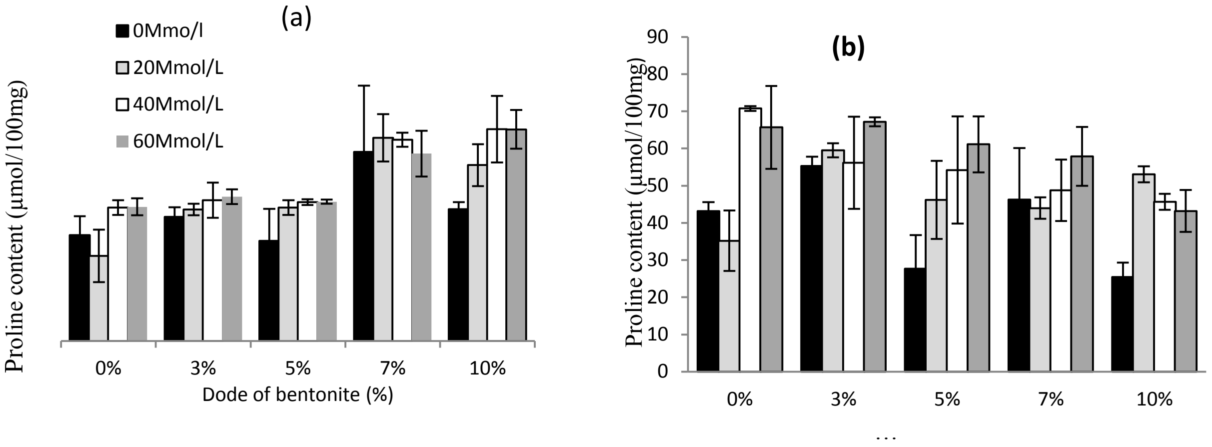


Figure 3: Combined effect of salinity and bentonite on the proline content in the aerial (a) and roots root part (b) of '*Semilla violeta'*.

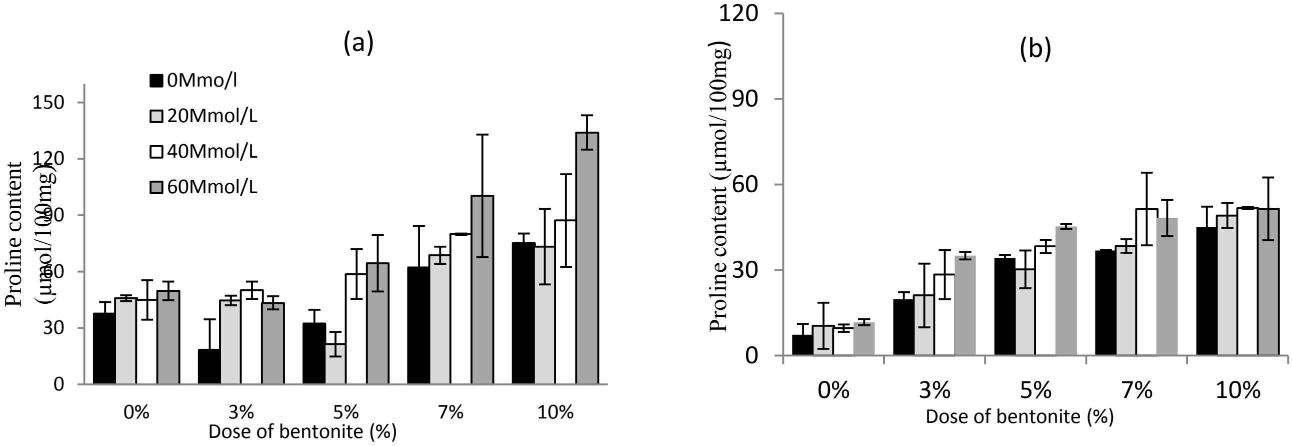


Figure 4: Combined effect of salinity and bentonite on the proline content in the aerial (a) and roots part (b) of '*Reine mora'*.

This explained the high concentration of proline in response to salt stress. The results were consistent with those of El **Midaoui** et ***al.*, (2007).**They confirmed that a major physiological traits of tolerance to environmental stresses is osmotic adjustment. It was achieved through an accumulation of osmoregulatory compounds leading to a reduction of osmotic potential, allowing the maintain a ceofturg or potential **(Shukdeb, 2010)** and **Ingweye** and ***al.*, (2010).**

Unlike the sandy substrates treated at 7 and 10% bentonite, proline content was lower in soils amended with 5% bentonite. This explained the regulatory effect of the osmotic pressure at this dose of bentonite.

According to the results, the dose of saline 20 mmol.L-^{1} did not trigger any salt stress. Because according to **Kasmi** and ***al.*, (2012)**, **Choi** et ***al.*, (2011)**, **Latigui** and ***al.* (2011)** and **Latigui** and **Dellal (2009)**, the threshold of electrical conductivity should not exceed 3.5 µS.cm-1, especially for vegetable crops.

Conclusions

The doses of 5 to 10% bentonite gave relatively the best rates and germination time for both varieties. The substrates composed by 7 to 10% of bentonite added to 60 mmol.L-1 of salt gave the highest amounts of proline in the aerial part and those of roots for two varieties. However, the amount found on *'Reine mora'* was greater than that found on *'Semilla violeta'*. This latter variety had high resistance to stress caused by the relatively large amount production of proline.

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