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Effect of Salt Stress and Bentonite on the Germination and Proline Content of *Vicia faba* L. Plant var. 'Semilla violeta' and 'Reine mora'

[1]H.A. Reguieg Yssaad, [2]A. Latigui, [1]T. Nouri and [1]L. Bessafi
[1]Laboratoire de la Biodiversité et Conservation des eaux et des sols Université de Mostaganem, Algeria
[2]Faculty of Nature and Life Sciences, University Ibn Khaldoun, Tiaret, Algeria

Corresponding Author: Ahmed Latigui, Faculty of Nature and Life Sciences, University Ibn Khaldoun, Tiaret, Algeria

ABSTRACT

Salinity is considered the most important abiotic stress, which limits growth and productivity of plants and degrades agricultural soils in arid and semi arid areas. The study was conducted on *Vicia faba* L. 'Semilla violeta' and 'Reine mora'. Sowing was carried on plastic pots containing sandy substrates of bentonite 3, 5, 7 and 10% which corresponded salinity doses of NaCl, $MgCl_2$ and $MgSO_4$ 20, 40 and 60 mmol L^{-1}, respectively. Saline effect was investigated on germination and proline content. Results showed that the addition of 5% of bentonite improved the germination rate and 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

INTRODUCTION

Agricultural Soils of arid and semi arid areas, where irrigation is employed salinity is increased (Djerroudi-Zidane *et al.*, 2010). Abiotic stresses factors such as drought, heat and soil salinity cause significant crop yield losses (Ashraf and Akhtar, 2004) by limiting plant growth on diverse crop species (Basal and Hemphill, 2006; Abbruzzese *et al.*, 2009; Misra *et al.*, 2006). Plant salt-tolerance expressed by morpho-physiological characteristics (Pessarakli, 2002), molecular/biochemical (Ozturk *et al.*, 2002), mechanisms that are induced under these stress conditions and delays germination and seedling emergence (Ashraf and Ahmad, 2000).

The plant response to a salinity depends on the variety (Radhouane, 2008; Benmahioul *et al.*, 2009), salt concentration on substrate, growing conditions as temperature and light and growth stages (Kaymakanova, 2009; Peel *et al.*, 2004). Identification of salt tolerant varieties would, certainly improve crop production in irrigated areas. The primary effects of high levels of salt are caused by ion imbalance and hyperosmotic stress on plants and their effects involve oxidative damage to enzymatic proteins and membrane integrity (Zhu, 2000).

A saline soil is currently facing at an irreversible ecological damage characterized by a transition from semi arid to arid areas (Belkhodja and Bidai, 2004). In this sense, it is better improve crop management of cultivated saline areas based on a profound knowledge of biological interactions. Because, under extreme 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 cultivated soils development by introducing bentonite and rich clay which increases cations exchange on soil. Halilat and Tessier (2006) had ensured that the contribution of the bentonite in sandy soil improves its physical properties and water retention (El-Diwani *et al.*, 2012). The addition of clay would improve the agricultural potential and increase reserves and capacity of water and minerals (Costa *et al.*, 2004). Because, reduction and accumulation of bentonite in the cytoplasm obtain osmotic adjustment (Misra et Gupta, 2005; Girija *et al.*, 2002) and on realization of various stages plant of seed germination (Thakur and Sharma, 2005). The presence of abiotic stress, such as salinity causes an accumulation of proline (Djerroudi-Zidane *et al.*, 2010). It allows protection membranes and enzyme systems, especially in young plant organ (Islam *et al.*, 2009). Proline seems to play a role in maintaining pressure cytosol and vacuole pH regulation (Ottow *et al.*, 2005).

The objective of this study consists to evaluate 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.

MATERIALS AND METHODS

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

Substrate: 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. Then, it was 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 using silver nitrate. The bentonite was 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 L. The bottom of each pot was lined with a layer by gravel to ensure good leaching.

Plant materials and cultural methods: *Vicia faba* L. 'Reine mora' and 'Semilla violeta' have been introduced from Spain and have used as a model for toxicological studies for diverse stress (Marcato-Romain *et al.*, 2009). The broad bean has rapid growth, large biomass and susceptible nutrient deficiency (Loss *et al.*, 1997). The seeds were disinfected with bleach to 8% for 10 min 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 pot^{-1}. Plants were watered three times a week, twice with deionized water and once in the nutrient Hoagland solution diluted to 1 ppt.

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

Table 1: Composition of the saline solutions (mM 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).

Statistical analysis: All data were analyzed 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.

RESULTS AND DISCUSSION

Effect of bentonite on the rate and duration of germination: The lowest rate 80% of germination of 'Semilla violeta' was obtained in the control substrate without bentonite. However, higher rates were obtained in substrates with 5, 7 and 10% of bentonite (Fig. 1a). The same observation was made for 'Reine mora' where it was found that 100% germination was obtained in substrates treated with 5% of bentonite; an increase of 20% compared to control (Fig. 1b). Ardakani *et al.* (2010), Cravero *et al.* (2000), Odabas and Mut (2007) and Yoshida *et al.* (2004) showed that the introduction of Bentonite in substrate contributed significantly to the improvement of the soil physicochemical properties by increasing water availability for germination, the Cation Exchange Capacitance (CEC) and soil microflora. 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' variety had a tolerance criterion regarding to the high content of clay up to 10% bentonite. However, 'Reine mora' recorded 100 % of germination (Fig. 1b) in the sandy substrate enriched to 5% bentonite. For both varieties, bentonite had further improved the rate and germination time compared to the sandy substrate (Fig. 1). According to Benkhelifa (2007), bentonite of the area study appreciably increases the water content of the substrate. From Heller *et al.* (2004), germination is characterized by a stabilization of the hydration of seed 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 7 days duration of germination for 'Semilla violeta' was relatively longer in the substrate without bentonite regarding to other substrates (Fig. 2a). The smallest duration (3 days) was obtained in the substrate to 5% bentonite. According to McKinstry and Anderson (2003), the addition of bentonite increased organic matter and decreased pH, exchangeable sodium, CEC and percent clay.

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 was shorter in all substrates with bentonite.

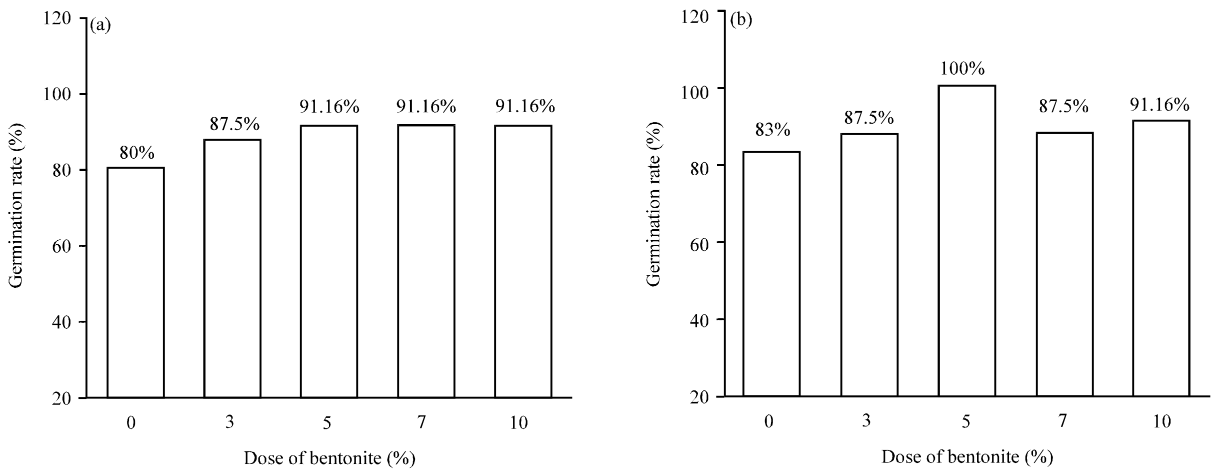

Fig. 1(a-b): Effects of sandy substrate amended with bentonite on Germination rate (%) of *Vicia faba* Plant varieties (a). Semilla violeta and (b) Reine mora

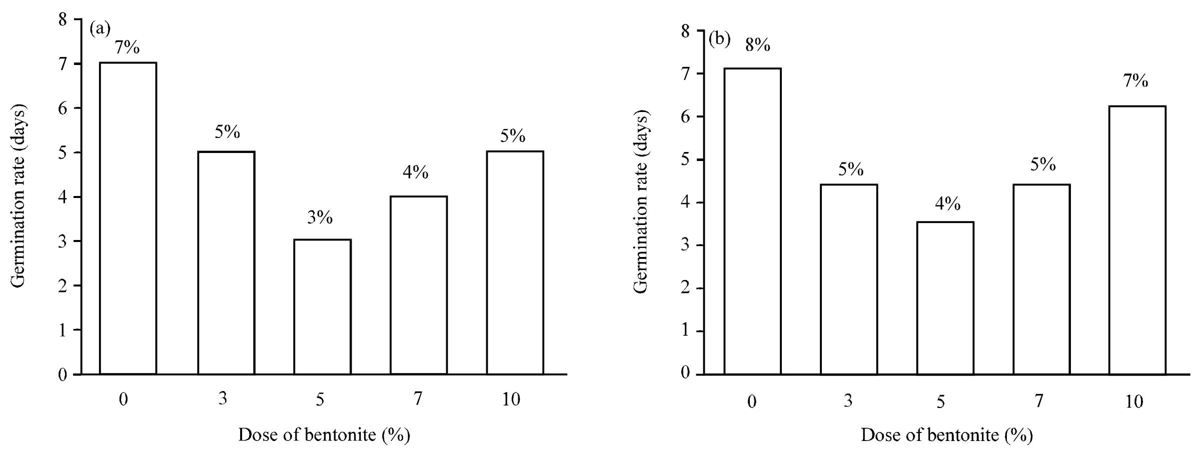

Fig. 2(a-b): Effects of sandy substrate amended with bentonite on time of germination (days) of *Vicia faba* plant varieties (a) Semilla violeta and (b) Reine mora

Effect on the proline content: The ANOVA results revealed very highly significant effects of bentonite factor ($p>0.001$) on the proline content for 'Semilla violeta and 'Reine mora' above ground biomass ($p>0.001$). Nevertheless, the results (Fig. 3 and 4 show that its accumulation in the shoots part for both varieties is very significant compared to roots.

The accumulated of the proline in *Vicia faba* was more in var, 'Reine mora' than the var. 'Semilla violeta' (Fig. 3 and 4) accumulated more proline than 'Semilla violeta'. This was due to the differences between the two varieties. Because, it gave each its own characteristics, allowing it to react to salt stress and adapt to these new situations.

Proline content in the aerial part of 'Reine mora' reached its maximum 100.37 and 134.07 μmol 100 mg^{-1} of dry matter at a dose of 60 mmol L^{-1} in sandy substrates treated at 7 and 10% bentonite, respectively (Fig. 4a). 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

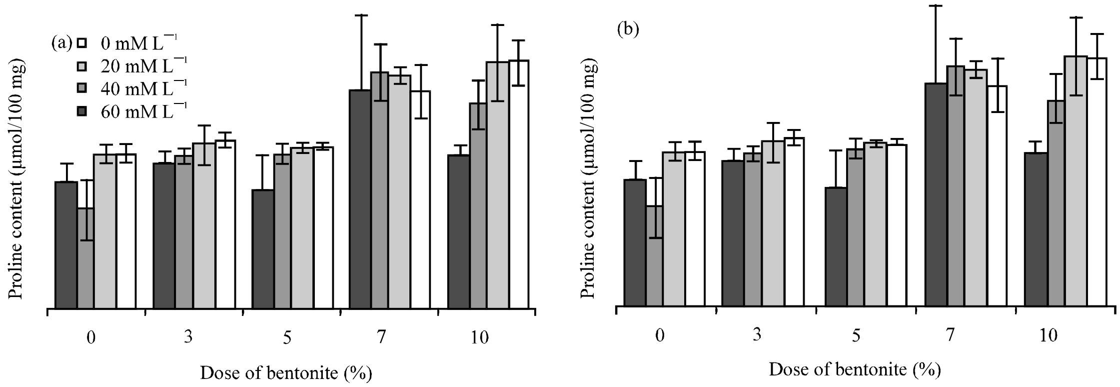

Fig. 3(a-b): Combined effect of salinity and bentonite on the proline content in (a) Aerial and (b) Root part of *Vicia faba* var. Semilla violeta

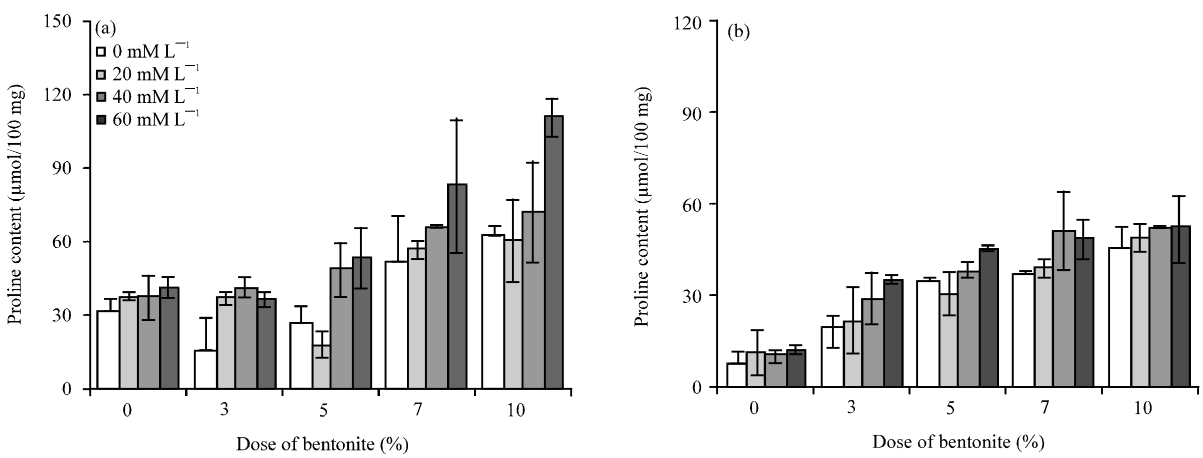

Fig. 4(a-b): Combined effect of salinity and bentonite on the proline content in (a) Aerial and (b) Root part of *Vicia faba* var. Reine mora

pressure in the soil solution. This explained the high concentration of proline in response to salt stress. The results were consistent with those of El Midaoui *et al.* (2007). The confirmed of the osmotic adjustment were a major physiological traits of tolerance to environmental stresses. It was achieved through an accumulation of osmoregulatory compounds leading to a reduction of osmotic potential, allowing the maintenance of turgor potential (Sen, 2010; Ingweye *et 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 *et al.* (2012), Choi *et al.* (2011), Latigui *et al.* (2011) and Latigui and Dellal (2009), this threshold is not harmful for vegetable crops.

CONCLUSION

The doses of 5 to 10% bentonite gave relatively the best rates for germination 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 shoot part and those of roots for two varieties. However, the amount found on Reine mora was greater than that found on Semilla violeta. This variety had higher resistance to saline caused by the bigger amount proline production.

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