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Anatomical changes of roots and steams of *Phaseolus vulgaris* L. (Fabales Fabaceae) under salinity at juvenile state

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ABSTRACT In Algeria, the region of Mostaganem is known for its agricultural soils with a sandy tendency and abnormally loaded with soluble salts affecting the yields of crops. To assess the salt tolerance threshold of the bean culture *Phaseolus vulgaris* L. (Fabales Fabaceae) variety "coco rose" was grown in plastic pots filled with two types of substrate, sand and sand amended with 7% bentonite (calcium clay of mining origin). The test was carried out in a greenhouse with controlled climatic conditions (variant temperature between 23-25°C, humidity is around 75% and a photoperiod of 12 hours). At the 5-leaf stage, irrigation with saline was provided with four saline concentrations (0, 50, 100 and 200 meq), the control is irrigated with distilled water. Two weeks later, the microscopic observations were made with an Optica type microscope, the results show a variability of the effect of saline stress depending on the organ and the concentration of the saline treatment. The anatomical structure of the treated roots and stems has shown significant anomalies; thus, the changes are marked by the decrease in the size of the parenchymal cells, that of the diameter of the xylem vessels and the increase in their number, under the action saline concentration ($NaClCaCl_2$) and according to the type of culture substrate sand (S) and sand with bentonite (SB).

KEY WORDS Anatomy; Bentonite; Salinity; *Phaseolus vulgaris*.

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INTRODUCTION

Salinity is one of the major abiotic environmental stresses affecting about 6–7% of the world's total land area and agricultural productivity specifically in arid and semi-arid regions of the world (Qadir et al., 2006). In saline soils, high concentrations of sodium (Na) and chlorine (Cl) ions within the plant root zone retards the growth of plants by either decreasing the water potential of root media or causing toxicity of Na and Cl in various plant organs (Panta et al., 2014). Salt impose both osmotic and ionic stresses on the plants which lead to several morphological and physiological changes (Jampeetong & Brix, 2009). A clear stunting of plants is noticed due to salinity stress (Takemura et al., 2002). High salt content, especially chloride and sodium sulphates, affects plant growth by modifying their morphology and anatomy (Huang & Redmann, 1995; Dolatabadian et al., 2011). The effect of salinity on root (An et al., 2003) and shoot anatomy (Ali et al., 1999) of plants had already been reported in previous works. Many researchers reported that with an increase in salinity there was a decrease in the development of the xylem. Pimmongkol et al. (2002) stated that the width of vascular bundles and

diameters of rice stems decreased in NaCl medium. The earliest response is a reduction in the rate of expansion of the leaf surface, followed by cessation of expansion as stress increases (Parida & Das, 2005). Munns & Tester (2008) reported that salt-sensitive plants have reduced survival, growth and development when exposed to even low to moderate salinities, while salt-tolerant species are able to grow and reproduce in saline environments.

The aim of this work is to study the effect of salinity not only at the scale of different soils, but also on the mechanisms of response of the plant *Phaseolus vulgaris* L. (Fabales Fabaceae) at different concentrations of $NaClCaCl_2$ on sandy soil with or without bentonite. An anatomical study of stems and roots, microscopic visualization and examination of possible correlations between anatomical changes and salinity levels were done.

MATERIAL AND METHODS

Plant materials and culture mode

Phaseolus vulgaris L. coco rose variety is used as plant material for this experience.

The crop is grown in two different types of substrate: sand and sand mixed with 7% of bentonite clay mineral of calcium origin). The substrate is placed in plastic pots of 15 cm diameter and 20 cm height with a capacity of 2 kg.

Preparation of the culture substrate

The sand is washed beforehand with dilute hydrochloric acid N, rinsed thoroughly with distilled water to remove the chlorides and dried in an oven at 105°C. Thus prepared, the sand constitutes a support of the plant (1 plant per pot), allows aeration of the roots and has the advantage of not fixing the ions. After natural drying, the bentonite is crushed then mixed with the sand with a dose of 7%. The Sand-Bentonite mixture is carefully homogenized manually, then filled into the pots. Beforehand the bottom of the pots is lined with a layer of one cm thick gravel of 0.5 cm in diameter serving as drains. On this layer is deposited a gas strip to retain the sand. *Phaseolus* seedlings were grown for 1 month, and watering with nutrient solution Hoagland & Arnon (1938). The saline solution consists of two salts NaCl and $CaCl_2$ concentrations (50, 100, and 200 meq), the control is sprayed with the nutrient solution. The stress is applied at the 5-leaf stage and then repeated at a frequency of one intake per three days for three weeks.

Anatomical study of stems and roots

After each treatment, one seedling per pot is dug up and stripped of the substrate by rinsing with distilled water. This operation is repeated three times per treatment. The organs (stems and roots) are carefully separated by means of a razor blade and then cut into pieces 1 to 2 cm long. Only the samples of the median parts are taken into consideration. Cross sections are performed "freehand" on stems and roots by means of a razor blade. Thin sections with a thickness of 20 µm are stained by the double staining technique (methyl green/Congo red). The sections are first treated with 8% sodium hypochlorite for 15 minutes. After careful rinsing with distilled water, they are etched with dilute 70% acetic acid for 2 minutes and then stained with 1% methyl green for 5 minutes; the latter colors the lignified walls in green. The pieces are then washed with distilled water and stained with 2% Congo red for 15 minutes. This dye highlights the cellulose that appears in pink or red.

Double staining technique

The sections are then washed with distilled water and mounted in a drop of water between the slide and coverslip before being observed first under ordinary microscope, then on another microscope allowing good shooting and taking pictures. The cups are kept either in pillboxes containing distilled water or in a drop of Canada balm placed between blade and coverslip. Once the sections are stained, they are observed by a microscope of the type Optica menu of apparatus of high definition in taking microscopic photo; a photo tube used for taking micrographs.

Statistical analysis

An experimental design of plots in complete random block was carried out using 3 plants per treatment. Three roots and three stems per plant were sampled for anatomical studies. Data were an-

alyzed by ANOVA using the Newman-Keuls Least Significant Difference Test (LSD) for mean comparisons using a significance level of 5%.

RESULTS

The sections obtained, used as experimental material under observation under a microscope, made it possible to measure the cells of conducting vessels specifically the root and shoot xylem. These measurements concerned measurements of the diameter of the vessels using a micrometer adapted to the microscope.

To show the effect of salinity on the evolution of the root diameter of the cylinder vessels of the bean variety tested, observations under a microscope are made on seedlings grown in substrate without bentonite (Fig. 1). The results obtained show a root cylinder diameter of (27.2 µm) in the control, this diameter decreases significantly under the effect of salinity to reach (8.77 µm) at 200 meq of $NaClCaCl_2$ (Table 1). Observations using the microscope at magnification (X40), the photos in the figure 3 (a, b, c and d) clearly show a very significant reduction in the diameter of the vessels of the root cylinder under the salinity effect.

The anatomy of the stem gives us structural changes in the cylinder elements. After 21 days of growth in the substrate without bentonite, the bean seedlings stressed with $NaClCaCl_2$ register diameters of the cylinder cells substantially identical in the control and at 50 meq around 20 µm. As soon as the salinity increases in the culture medium, the measurements made on the cells of the stem cylinder give a diameter which decreases sharply to reach (13.5 µm) at 100 meq, then (8.9 µm) at 200 meq (Table 2). These observations are confirmed with the anatomical study; thus, anatomical sections are made on the stems of bean seedlings aged 21 days grown in sand alone and stressed with $NaClCaCl_2$. Microscopic observations at magnification (X40) show variations in the number of cylinder vessel cells (Fig. 2). This explains why, when the salinity increases in the culture medium, the number of cells per vessel decreases as well as their diameter.

As shown in figure 3, results indicate the influence of salinity on the variation of the root xylem diameter of the vessels. After 21 days of growth, the roots of the bean variety have a maximum diameter of the xylem vessels with (27.2 and 28.13 µm) by the control, on the other hand, at the 200 meq of $NaClCaCl_2$, these diameters decrease to give a minimum of 8.83 and 17.03 µm grown respectively on S and SB substrate. The sand amended with bentonite made it possible to display larger xylem diameters than those of the substrate without bentonite with 23.5 and 19.37 µm against 17.67 and 13.43 µm respectively at 50 meq and 100 meq of $NaClCaCl_2$. Statistical calculations show that

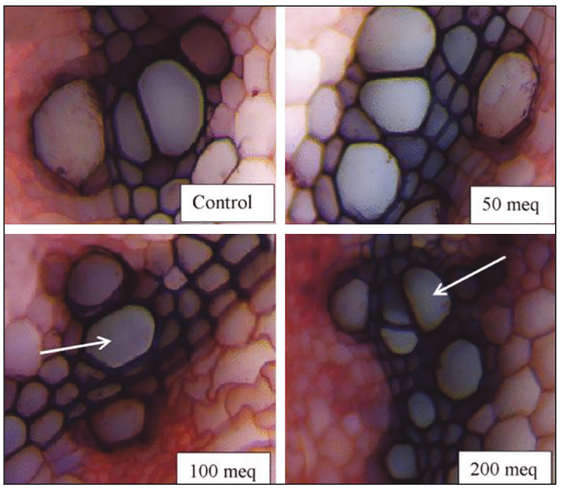

Figure 1. Root anatomy of *Phaseolus vulgaris* L. variety "coco rose", 21 days old. The arrows indicate the xylem vessels (magnificationX40).

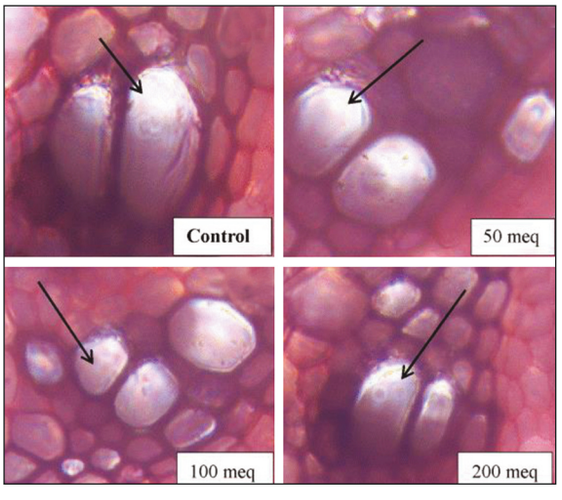

Figure 2. Stem anatomy of *Phaseolus vulgaris* L. variety "coco rose", 21 days old. The arrows indicate the xylem vessels (magnificationX40).

Substrate	Control	50 meq	100 meq	200 meq
WOB ***	27.2 $^{a\ ns}$	17.67 ^{b}	13.43 ^{d}	8.77 ^{f}
WB***	28.13 $^{a\ ns}$	23.5 ^{c}	19.37 ^{e}	17.03 ^{g}

Table 1. Effect of salinity and substrate on root cylinder diameter (µm) of *Phaseolus vulgaris* L. Means in each row followed by different letters are significantly different ($p < 0.05$). *, **, ***: Differences between substrate is significant at $P < 0.05$, 0.01, and 0.001 respectively, ns: not significant at ($P < 0.05$).

Substrate	Control	50 meq	100 meq	200 meq
WOB ***	20.9 ^{a}	18.5 ^{a}	13.5 ^{b}	8.9 ^{d}
WB***	22.9 ^{a}	21.4 ^{a}	19.3 ^{c}	16.1 ^{e}

Table 2. Effect of salinity and substrate on stem cylinder diameter (µm) of *Phaseolus vulgaris* L. Means in each row followed by different letters are significantly different ($p < 0.05$). *, **, ***: Differences between substrate is significant at $P < 0.05$, 0.01, and 0.001 respectively, ns: not significant at ($P < 0.05$). Interaction between substrate and NaClCaCl2 is not significant at $P<0.05$.

there is a very significant effect of salinity on the diameter of root xylem diameter, the sand culture substrate amended with bentonite records a very significant effect compared to the sand substrate alone ($p < 0.05$) (Table 1).

After 21 days of culture (Fig. 4), the bean plants from S and SB respectively register a maximum diameter of the stem cylinder with 20.9 and 22.9 µm by the control, on the other hand, the minimum is recorded at 200 meq of $NaClCaCl_2$ with 8.9 and 16.1 µm. Overall, the sand culture substrate improved with bentonite has larger shoot diameters with 21.4 and 19.3 µm than those of the S with 18.5 and 13.5 µm respectively under salinity with 50 and 100 meq of $NaClCaCl_2$. Statistical calculations show that there is a very significant effect of salinity on the stem cylinder diameter. The SB has a significant effect at 100 and 200 meq. However, the interaction between substrate and $NaClCaCl_2$ is not significant at $P<0.05$ (Table 2).

DISCUSSION

The salinity induced structural changes in the xylem of the stems and roots. In plants stressed with $NaClCaCl_2$, the thickness of the shoot vascular cells was much higher than in the control; the effect of salinity depended on the salt concentration and the growing medium. Al-Tardeh & Iraki (2013) studied the seedlings of two varieties of tomatoes exposed to salt stress and concluded that salinity reduces root vascular function. In addition, the profiles of the phloem and xylem parenchyma were significantly reduced in saline environments. Other studies confirm that the salinity of the environment modifies the anatomical structure of the root and leads to a decrease in the number of cells per xylem bundle and the number of layers of cortical parenchyma (Haouala et al., 2007; Farhana et al., 2014). In general, plants grown in bentonite-modified soil had a larger xylem vessel diameter than those grown in sand without bentonite, this is likely due to the role of calcium-rich bentonite in mitigating the effect of salinity, as pointed out by Hellal et al. (2015). These results were approved by Arbaoui (2016), with 10% bentonite in sandy soils, the effect of salinity is reduced on tomato plants. Saline stress is associated with a greater deposit of lignin in the vascular tissue and/or the development of xylem. $NaClCaCl_2$ causes significant lignifica-

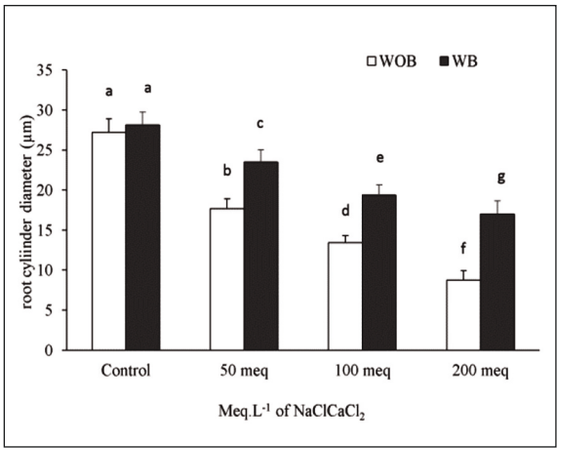

Figure 3. Root cylinder diameter (µm) after 21 days of bean variety "coco rose" cultivated on substrate (S and SB) stressed with NaClCaCl2. Data represent the mean of tree replication and error bars indicate SD. Different letters among a groups show significantly different values at $p<0.05$. The same letters show no significantly different values.

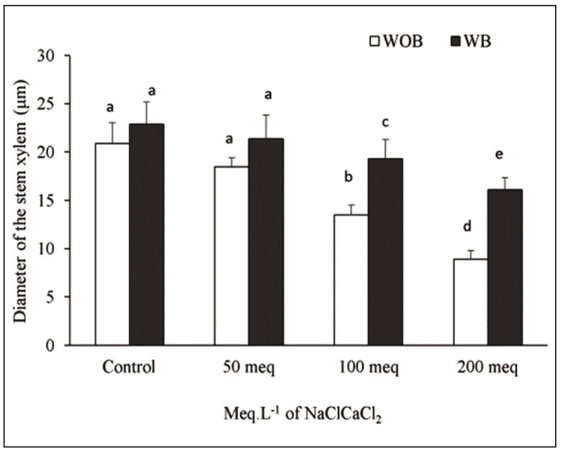

Figure. 4. Stem cylinder diameter (µm) after 21 days of bean variety "coco rose" cultivated on substrate (S and SB) stressed with NaClCaCl2. Data represent the mean of tree replication and error bars indicate SD. Different letters among a groups show significantly different values at $p<0.05$. The same letters show no significantly different values.

tion in root and caular vascular tissues, suggesting a factor that inhibits root growth and, therefore, represents an adaptation mechanism to resist the stress imposed by salinity (Cachorro et al., 1993). Vascular tissue and the size of plant cells are reduced when exposed to salinity. In addition, the cross-sectional areas of the roots of plants exposed to salinity were considerably reduced, so that the roots of salt-stressed seedlings exhibited reduced vascular function and cortical parenchyma compared to control plants. These changes in the number and diameter of xylem vessels have had a significant impact on water consumption and transport according to several authors (Choat et al., 2005; Alsafary et al., 2019). According to several studies, the adaptation of plants to salt stress is accompanied by physiological changes (Shannon, 1997) and anatomical changes (Hwang & Chen, 1995; Çavuşoğlu et al., 2007), inhibition in diameter and number of xylem. Introduced by Kiliç et al. (2007), so salinity stress induced the production of new protein bands do not occur in the control plants (see also Dawood & El-Awadi, 2015). In addition, the salt response of plant species depends on several variables, starting with the species itself, its variety, salt concentration, growing conditions and stage of plant development (Bennaceur et al., 2001; Alaoui et al., 2013). The identification of salt tolerant varieties and genotypes, capable of minimizing the depressive effects of salinity on yields, would certainly improve agricultural production in salinity-affected areas. In non-Halophytes, there is great variability in responses of sensitive or salt stress tolerant species based on the lipid composition of the roots (Greeway, 1980). The effect of salinity on the lipid composition of roots has been studied in different species, including grapes, bean and plantago (Erdel et al., 1980). However, the mechanism of adaptation of plants to salinity is not fully known. Calcium plays a crucial role in the stabilization of cell membranes. Also, it is known to have an improving effect on plant growth stress (Hyder & Greenway, 1965; Deo & Kanwer, 1969; La Haye & Epstein, 1971).

CONCLUSIONS

The presence of calcium in bentonite and in the solution of irrigation has played an important role in reducing the effect of salinity. This is due to the contribution of bentonite and the mixture of NaCl with $CaCl_2$ that made it possible to improve the number of cells of the vessels of the xylem, whether it is the root or the stem. Finally, an addition of 7% bentonite to sandy soils with a saline tendency improves the structure of sandy soils.

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