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Search for physiological and anatomical parameters of salt tolerance in beans (*Phaseolus vulgaris* L.)

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Key words: Salinity, Hydroponics, *Phaseolus vulgaris*, Morphological parameters, Anatomical

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Abstract

The mechanisms of tolerance or sensitivity are experimentally investigated on a local variety of *Phaseolus vulgaris* L. grown under a growing regime of NaCl and $CaCl_2$ salts (control, 100 and 200 meq.gl-1 NaCl + $CaCl_2$), under semi-controlled conditions. Bean (*Phaseolus vulgaris* L.) appears as a plant more or less sensitive to salt during its growth. The action of salt results in decreased stem and root growth in *Phaseolus vulgaris* L. The plants cultured in salty medium have morphological characteristics different from those of the controls, because the results obtained show that the growth of the stem and the root are not affected by the nutrient solution (control) by cons to high concentrations of salt (200meq.L^{-1}, NaCl, $CaCl_2$, mixture), the stem shows a marked regression, while the plants treated at the 100meq.L^{-1} concentrations exhibit stress sensitivity characteristics. The action of salinity is illustrated by a reduction in the length of the stem in the lens (benaceur, 2001) and can result in a stunting of the plant until complete dwarfism (Belkhodjaand Soltani, 1992). When the plants receive saline from NaCl, $CaCl_2$ and the mixture (NaCl, $CaCl_2$) 100meq.L^{-1}, the diameter of xylem vessels is reduced as compared to the diameter of the xylem of the plants sprayed with the nutrient solution. After 40 days of growth, the diameter of xylem vessels decreased sharply after just one day of stress, this diameter slowly decreased in plants treated with 100 and 200meq.L^{-1}NaCl, $CaCl_2$ and (NaCl + $CaCl_2$). At the level of the stems, the results clearly show the action of the salt on the conductive tissue compared to the control, results in an increase in the number of the xylem vessel and the writing of its diameter. The diameter of the vessels of the root xylem and compared with that of the stems and much more affected by the action of the salt since it shows a strong reduction compared to the vessels of plants watered to the nutrient solution and whatever saline treatment to bring or Adure of exposure. Most of the plants are able to adapt to saline environments. This adaptation is accompanied by morphological, anatomical and biochemical changes (Kylin, 1975;Paljakouf, 1988). The biomass of the aerial part hydroponically grown is more developed and greater compared to culture on substrate. It is advisable in the last 20 years to use the technique of hydroponic cultivation for several economic advantages. Understanding these phenomena will be very useful for better conduct of natural plant communities, as well as for defining the ideal characteristics for plants of agricultural importance

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Introduction

Plants respond to environmental constraints by numerous changes, revealing the multifactorial character of mechanisms of tolerance and adaptation to abiotic stresses. The response to the salt of plant species depends on the species itself, its variety, the salt concentration and the stage of development of the plant (Bennaceur *et al*., 2001). Under stressful conditions, plants can react with physiological mechanisms (Kylin and Quatrano, 1975; Parida and Das, 2005) and biochemical's (Brugnoli and Björkman, 1992) involving an enzymatic activity (Erdei *et al*, 1980). The most common criteria for salt tolerance identification include yield, vigor, foliar damage and plant size. Other tolerance indices have been proposed, based on specific physiological characteristics, including ion accumulation or the production of specific metabolites. Nevertheless, salt tolerance is usually determined in terms of growth or yield (Aceve *et al*., 1975).

Plant responses to saline stress have been studied by the use of anatomical, ecological, physiological and molecular approaches (Ashraf, 2002). Morphological and anatomical modifications at the plant level are able to minimize the undesirable effects of salt stress (Poljakoff, 1988). The bean *Phaseolus vulgaris* L. is a saline sensitive plant is a source of dietary proteins in many developing countries. As for the agricultural sector, beans represent the third largest crop of legumes in the world (La Haye and Epstein, 1971,). Among bean legumes, beans (*Phaseolus vulgaris* L.) are the subject of numerous studies dealing exclusively with agronomic aspects in relation to salinity, while physiological and anatomical aspects remain to be done.

Materials and methods

Plant material

Experiments were carried out on bean seeds (*Phaseolus vulgaris* L.) (variety provided by the Es-senia plant ecophysiology laboratory, Oran-Algéria). Medium-sized seeds were kept in a refrigerator at 7°C for a long time, in anticipation of dormancy. This work was carried out in the botanical laboratory at the University of Mostaganem-Algéria, the conditions of cultivation were kept semi-controlled.

For use, these seeds underwent a first bath in 8% sodium hypochlorite solution for three minutes to disinfect and remove impurities. They are then rinsed several times with distilled water to remove all traces of chlorine. The seeds, once dried under ambient conditions, are placed in a petri dish for germination. From the first germinations, the seedlings are carefully transplanted into pots while waiting for the application of the saline solution.

Preparation of culture

The culture is carried out in plastic jars with a capacity of 2 kg, a diameter of 15 cm and a height of 20 cm, the bottom of which is lined with gravel in order to ensure good drainage. The sand used as a substrate was recovered at the beach of the Sidi Madjdoub station (wilaya of Mostaganem-Algéria), then it was sieved, treated with the spirit of salt, and finally, a silver nitrate test was carried out in order to check the purity of the substrate which led to the clarity of the solution, several successive washing operations with running water and then with distilled water before potting. The pots are then filled with a mixture of sand and compost respectively 1v / 4v.

Culture substrate

Composition potting compost based on bark compost of deciduous and coniferous trees horticultural peat litter of peat mix of vegetable materials extruded fiber wood fertilizer based on calcium and magnesium, fertilizers (Table 1).

Determination of retention capacity

To determine the irrigation dose, we took a sample of 100g (P1) of sand previously dried in the open air that we put in a small pot that we soaked to the saturation point; 24 hours after we weighed (P2).

P1 = Wet weight

P2 = Dry Weight

Hydroponics Techniques

Cultivation on BAC the cultivation boards with a wire-mesh bottom are placed above a watertight tank filled with nutrient solution in which the roots that draw water and mineral elements (Franzpennings feld) plunge.

Germination

The seeds are germinated in glass cubes of a diameter of 19 cm. The boxes are packed with 4-5 filter pads moistened with 20 ml of distilled water. A maximum of ten seeds are deposited on the filters, spaced so as to avoid an overlap of the roots which could lead to a break at the time of transplanting. The labelled cans are then placed in the light at ambient temperature of about 20°C. We considered a germinated seed when the emergence and the growth of the radical appear (Dussert *et al.*, 2002).

Transplanting

At the end of a week after the first leaves appear (4-5 leaf stage), the seedlings are carefully transplanted at the rate of one seedling per pot and then placed in a greenhouse. Every two days, these seedlings are watered with the Hoagland (1938) nutrient solution diluted 1/1000th and reduced to 30% of the retention capacity.

Preparation of watering solutions

The nutrient solution

During the rearing of the plants, watering is done every two days to the nutrient solution. It consists of a set of stock solutions of micro elements and macro elements, reported in Table 2.

Preparation of the saline solution

Two types of saline solutions were prepared (Table 3).

Irrigation

Before sowing, we pre-irrigated with distilled water, after germination, we watered the nutrient solution with volumes corresponding to 30% of the retention capacity (ie 36.54 ml) for stage 1-2 leaves. At the 3-4 leaf stage, we increased the irrigation dose to 30% of the retention capacity.

This contribution is renewed every two days.

Saline stress

Salt shock is applied to the pants as they have reached the 4-5 leaf stage.

Study of plant material

The study of the plant material was carried out by observations and biometric measurements on roots and stems, anatomical observations on stems and roots and finally the determination of some cations in the stems and roots.

Anatomical study

The anatomical study consists in making cross-sections of rods and roots, using a razor blade and then staining them. This method is based on the use of certain colorants: methyl green, Congo red.

It makes it possible to specifically stain the cell walls according to their chemical composition (Roger and al.,2001).

Results

Effect of salinity on stem growth

After 21 days of growth under saline stress stem lengths were determined (Table 4). The results show that the length of the stems decreases markedly each time the salinity increases.

Table 1. Compost composition.

Corposants	Concentration
Dry matter	20%
Organic matter	10%
pH	5-6.5
Electric conductivity (Ec)	750µs/cm
NPK	14-16-18 0.5kg/m3
Net mass	6Kg

The height of the plants increases in parallel for the plants treated with the salts, the maximum height is reached for the 100meq concentration, whereas at 200meq, the plants are lower. The action of the salt is predictable compared to the control (26.75 cm), the treatments show a very low growth at the level of the stems, especially for high concentrations 200mq. (15.37 - 14.5 - 12) cm.

Effect of salinity on root growth

The results show that root lengths decrease markedly whenever salinity increases.

The action of the salt is predictable compared to the control (11.37cm), the treatments exhibit very low growth in the roots, especially for high concentrations of 200mq. (7.95 -7.5-5.5 cm).

Table 2. Composition of the Hoagland nutrient solution (1938).

Composants	Nomenclature	weight g/l
Potassium nitrate	KNO_3	191.90
Calcium Nitrate	$(NO_3)_2Ca_4H_2O$	129.80
Ammonium nitrate	NO_3NH_4	210.00
Magnesium sulfate	SO_4Mg7H_2O	61.50
Monopotassium phosphate x	$SO_4Mg\ 7H_2O$	54.40
Di-potassium hydrogen phosphate	$PO_4K_2H\ 3H_2O$	34.23
Manganese Chloride	$Cl_2Mn\ _4H_2O$	01.00
Copper sulphate x	$CuSO_45H_2O$	0.17
zinc sulphate	$ZnSO_47H_2O$	0.22
Boric acid	H_3BO_3	2.86
Ammonium molybdate	$Mo_7\ O_{24}(NH_4)7H_2O$	0.28
Ferric EDTA complex	$C_{10}H_{12}FeN_2NaO_8$	0.05

Table 3. Preparation of the NaCl and $CaCl_2$ Salt Solutions.

Corposants	100meq /L	200meq/L
Na Cl g/L	5.85	11.7
$CaCl_2$ g/L	7.35	14.7

Anatomical study

Microscopic examination of the transverse strokes in the median root region and the stem basal region after staining by the double-staining technique (methyl green / Congo red) shows that the structural changes vary according to the mode treatment of *Phaseolus vulgaris* L. The results show that stress is reflected in stems and roots by a reduction in the number and size of conductive vessels (Zamane *et al.*, 2005). The roots represent slight structural changes in the xylem tissue. At high concentrations of salts 200 meq. (NaCl + $CaCl_2$), the xylem cells show a slight reduction in diameter, as well as thickening of the walls.

Table 4. Variation in stem length (cm) as a function of different salt-concentrations.

Salt	Control	Na Cl		$CaCl_2$		Na Cl + $CaCl_2$	
		100	200	100	200	100	200
Length (cm)	26.75±1.7	21.25±5.6	15.37±1.4	18.5±2.4	14.5±3.4	16.25±3.6	12±0.9

At the stem level, the results show the action of the salt on the conductive tissue compared to the controls. The basal zones reveal a reduction in the surface area occupied by xylem tissue at high concentrations of 200mg, NaCl, $CaCl_2$, and mixing both. At high magnification, the same rods clearly illustrate a relaxation in the arrangement of their conductive tissue and in particular the xylem.

This reaction results in a reduction in the number of large vessels and the thickening of their walls and this according to the treatment provided.

Table 5. Root length (cm) with function of different concentration at a given time (day).

	Control	Na Cl		$CaCl_2$		Na Cl + $CaCl_2$	
		100	200	100	200	100	200
Length (cm)	11.37±2.1	10.25±0.64	7.95±1.2	8.5±3.7	7.5±0.8	8.1±3.7	5.5±0.9

Table 6. Change in stem length (cm) as a function of different concentration at a given time (day).

	Control	Na Cl		$CaCl_2$		Na Cl + $CaCl_2$	
		100	200	100	200	100	200
Length (cm)	50.33±2.5	46.66±2.3	34.33±2.3	43.33±4.9	32.±5.3	31±2	26.33±1.5

The results obtained show that whatever the treatment provided, the root presents large vessels compared to the stem control (nutrient solution) treated (100meq, NaCl) (200meq, NaCl).

Cross section of the stems and roots of *Phaseolus vulgaris* L., carried out by hand and colored by double staining (methyl green / Congo red) after 21 days sampling and treatment.

Table 7. Root length variation (cm) a function of different concentration at a given time (day).

	Control	Na Cl		$CaCl_2$		Na Cl + $CaCl_2$	
		100	200	100	200	100	200
Length (cm)	22.33±2.5	12±1.0	10.66±0.7	14.66±1.5	9.33±1.5	11±1.0	7.66±1.52

Control, vessels with large diameter large area occupied by the conductive tissue. 100meq.L-1, showing large vessels 200meq.L-1 showing a reduction in the diameter of the vessels, with increasing in number (Fig. 7,9,11).

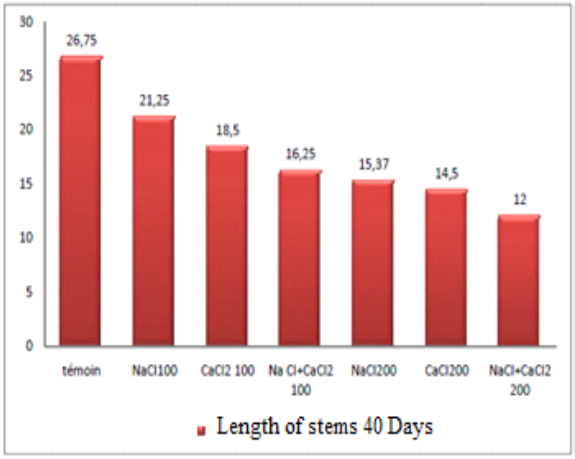

Fig. 1. The effect of salinity on the growth of *Phaseolus vulgaris* L. stems.

In addition, control, vessels with large diameter large area occupied by the conductive tissue. 100meq.L-1, presenting large vessels 200meq. L-1 with large vessels with thickening of the wall (Fig. 8, 10, 12).

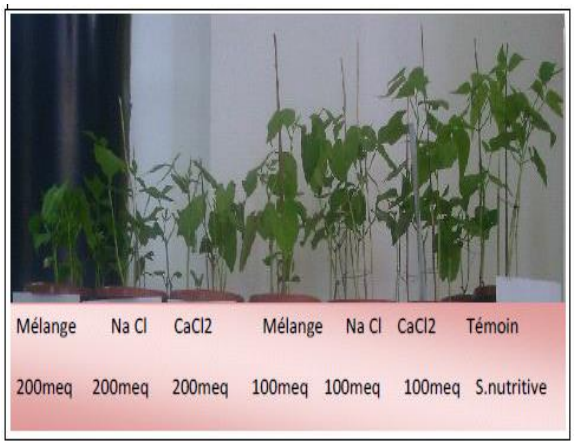

Fig. 2. Effect of salt stress on the growth of bean *Phaseolus vulgaris* L.

Effect of salinity on stem growth (hydroponics)

Effect of salinity on root growth

The results show that the lengths of the stems and the roots decrease markedly each time the salinity increases. The action of the salt is predictable compared to the control; the treatments show a very low growth in the stems and roots, especially for high concentrations 200mq.

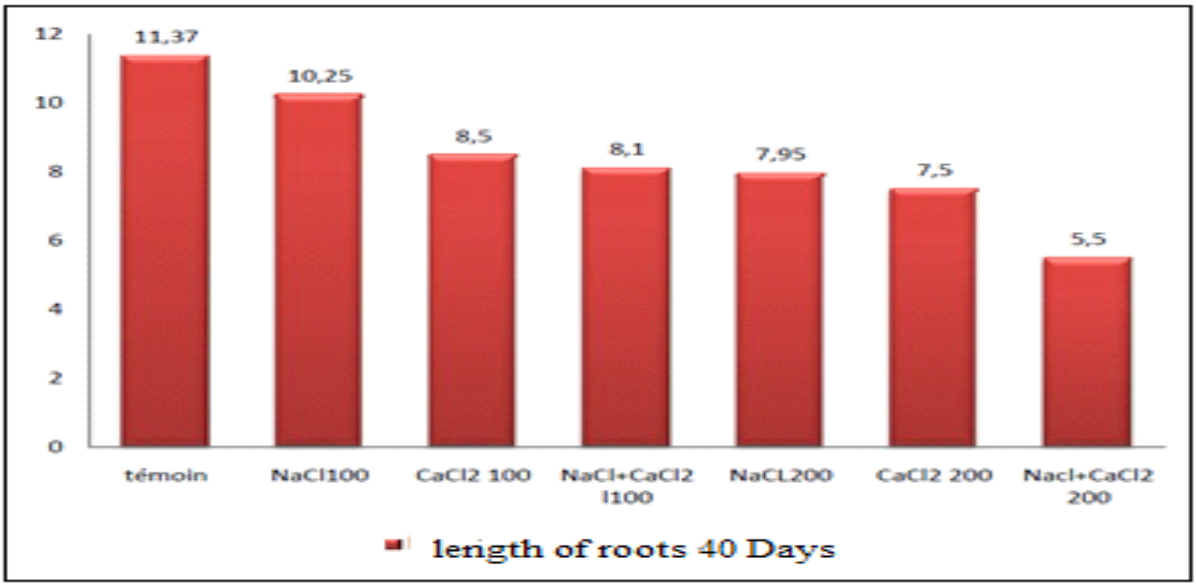

Fig. 3. The effect of salinity on the growth of *Phaseolus vulgaris* L. roots.

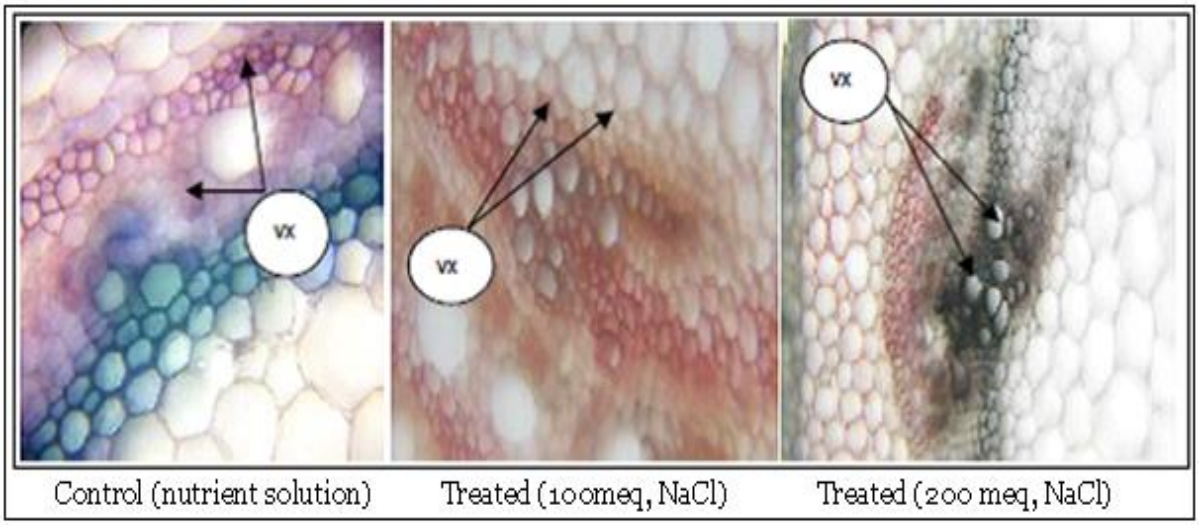

Fig. 4. Anatomy of 40-day-old *Phaseolus vulga*ris L stems grown on the substrate Vx: vessels.

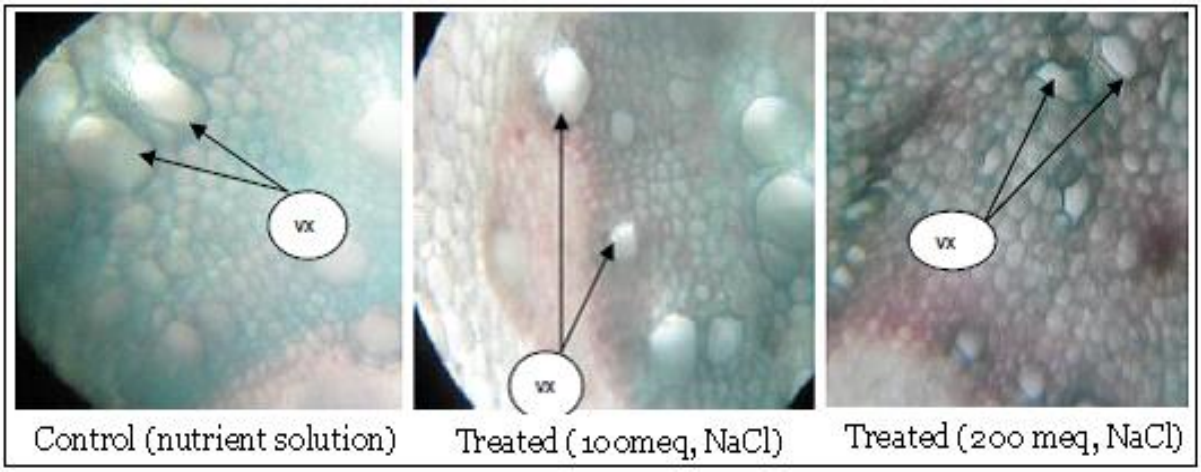

Fig. 5. Anatomy of 40-day-old *Phaseolus vulgaris* L. roots grown on the substrate Vx: vessels.

Cross-section of the stems and roots of *Phaseolus vulgaris* L, carried out freehand and colored by double staining (methyl green/Congo red) after sampling of 21 days and treatment.

Control, vessels with large diameter large area occupied by the conductive tissue. 100meq.L^{-1}, showing large vessels, 200meq. L^{-1} showing a reduction in the diameter of the vessels, with increasing in number (Fig. 13,15,17). In addition, control, large diameter vessels large area occupied by conductive tissue.100meq.L^{-1}, with large vessels, 200meq.L^{-1} with large vessels with thickened wall (Fig. 14,16,18).

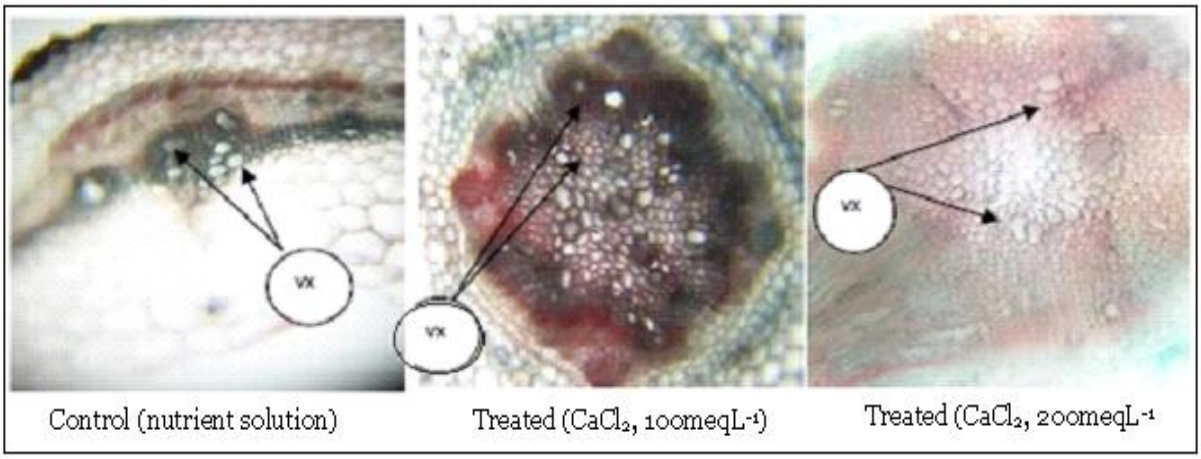

Fig. 6. Anatomy of 40-day-old *Phaseolus vulgaris* L. stems grown on the substrate. Vx: vessels.

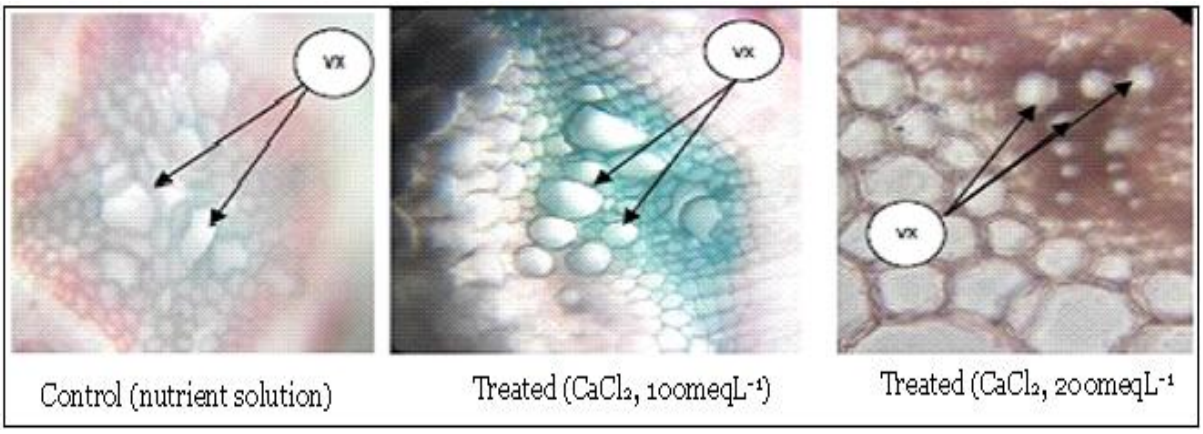

Fig. 7. Anatomy of 40-day-old *Phaseolus vulgaris* L. root grown on the substrate. Vx: vessels.

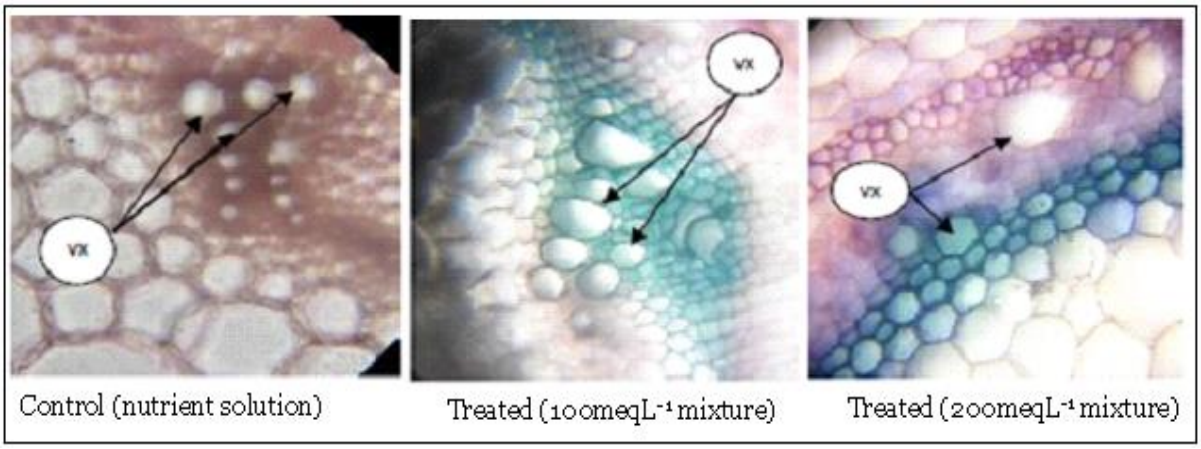

Fig. 8. Anatomy of 40-day-old *Phaseolus vulgaris* L. stems grown on the substrate. Vx: vessels.

Discussion

The plants cultured in salty medium have morphological characteristics different from those of the controls, because the results obtained show that the growth of the stem and the root are not affected by the nutrient solution (control) by cons to high concentrations of salt (200 meq. L^{-1}, NaCl, $CaCl_2$, mixture), the stem shows a marked regression, while the plants treated at the 100meq.L^{-1} concentrations exhibit stress sensitivity characteristics.

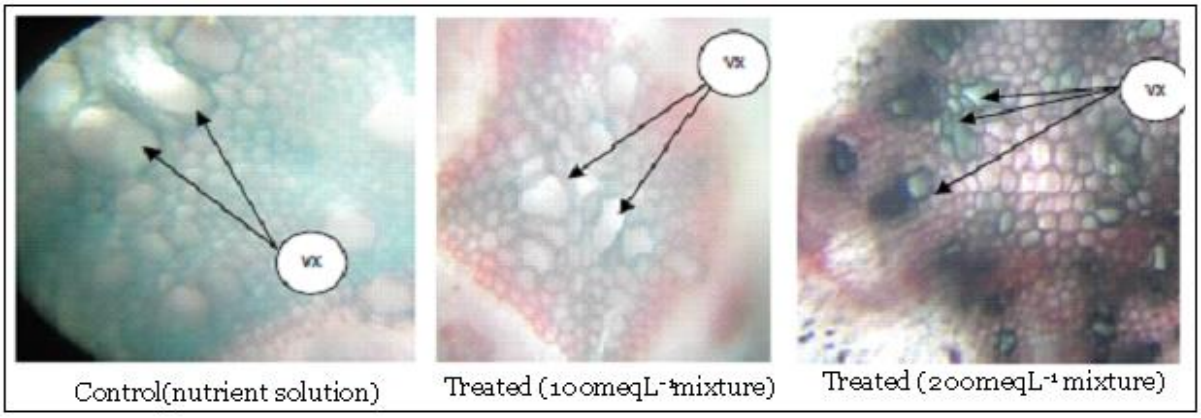

Fig. 9. Anatomy of 40-day-old *Phaseolus vulgaris* L. root grown on the substrate. Vx: vessels.

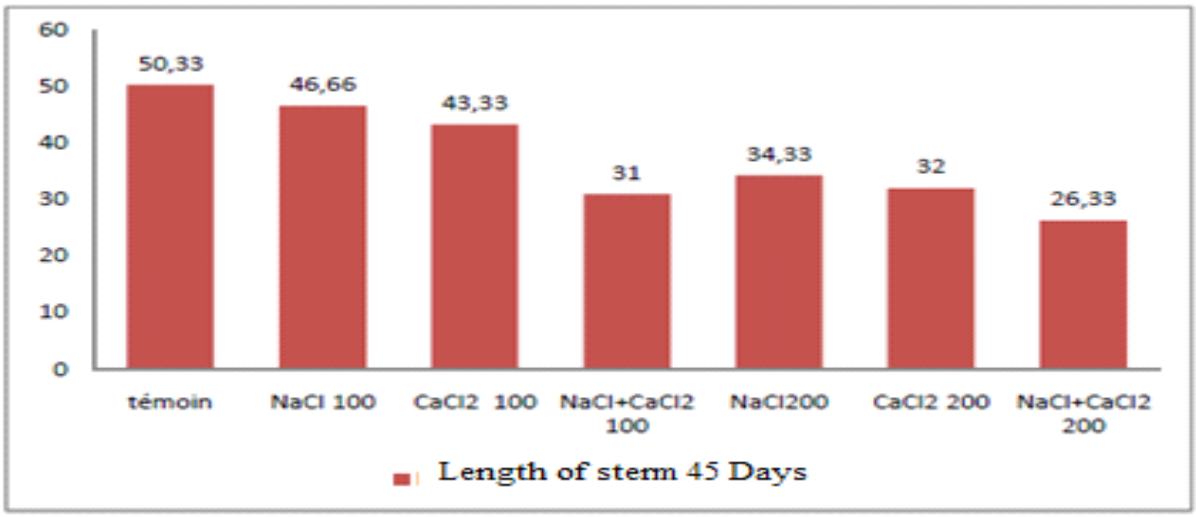

Fig. 10. Histogram shows the effect of salinity on stem growth *Phaseolus vulgaris* L.

Fig. 11. Effect of saline stress on the growth of beans *Phaseolus vulgaris* L. (hydroponics).

The effect of salinity is illustrated by a decrease in the length of the stem in the lens (Ben Taarit *et al.*, 2010) and can result in a stunting of the plant to complete dwarfism (Brun, 1980). The analysis of the observations results shows the existence of an influence of the salinity on the variation of the diameter of the xylem vessels.

The diameter of the xylem is considerably large compared to the salt-treated plants.

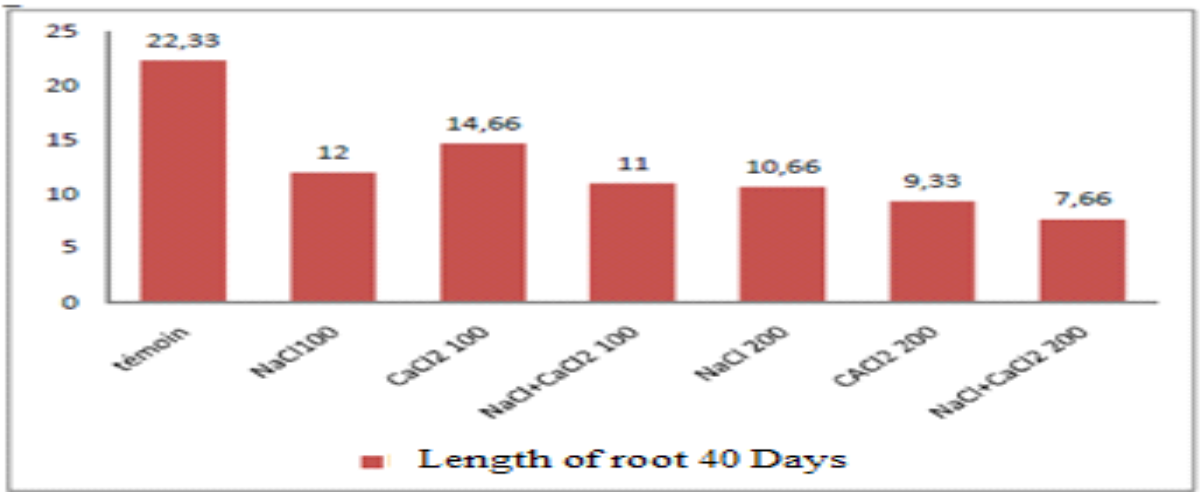

Fig. 12. Histogram shows the effect of salinity on root growth *Phaseolus vulgaris* L.

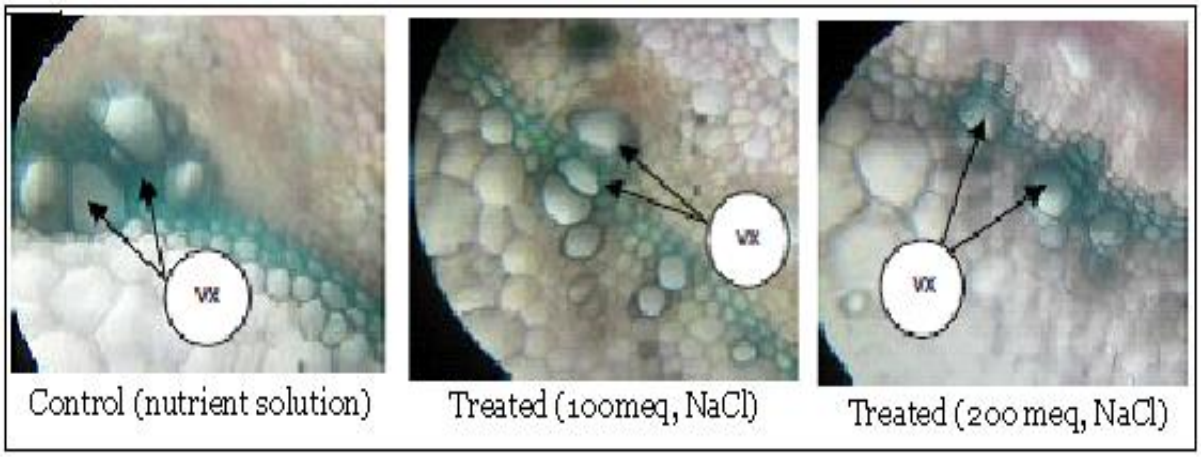

Fig. 13. Anatomy of 40-day-old *Phaseolus vulgaris* L. stems grown in hydroponics.

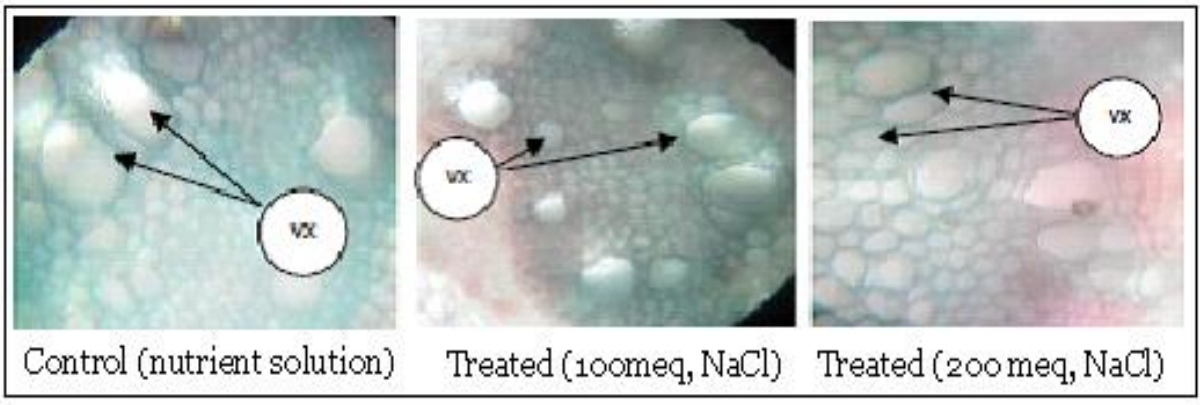

Fig. 14. Anatomy of 40-day-old *Phaseolus vulgaris* L. roots grown in hydroponics.

When plants receive saline from NaCl, $CaCl_2$ and the mixture (NaCl, $CaCl_2$) 100meq.L-1, the diameter of xylem vessels is reduced compared to the diameter of the xylem of plants watered to the nutrient solution (Fig. 7,8,9). After 40 days of growth, the diameter of xylem vessels decreased sharply after just one day of stress, this diameter slowly decreased in plants treated with 100 and 200meq.L^{-1}NaCl, $CaCl_2$ and (NaCl + $CaCl_2$).

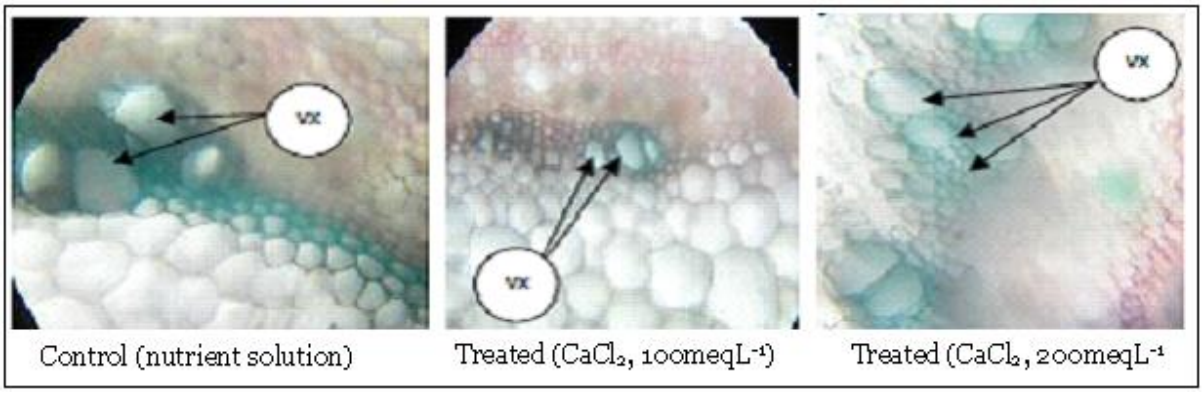

Fig. 15. Anatomy of 40-day-old *Phaseolus vulgaris* L. stems grown in hydroponics.

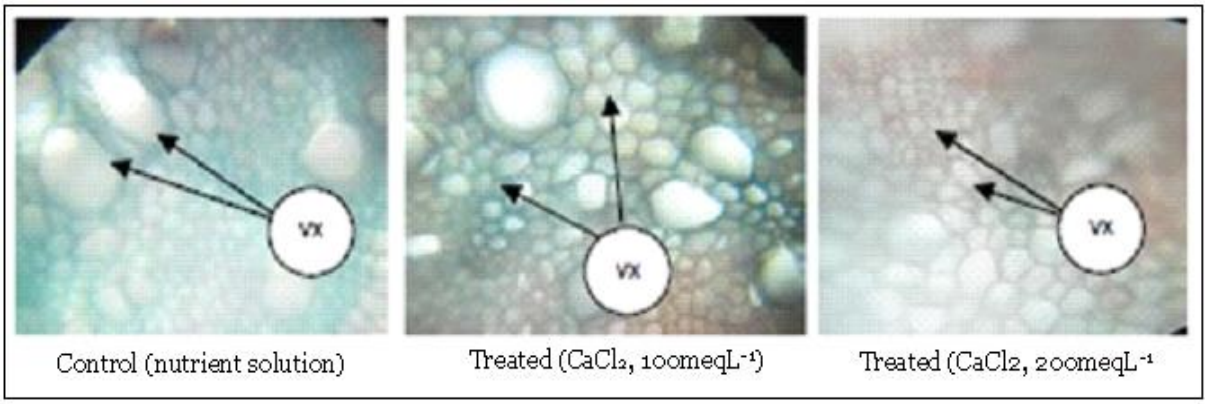

Fig. 16. Anatomy of 40-day-old *Phaseolus vulgaris* L. roots grown in hydroponics.

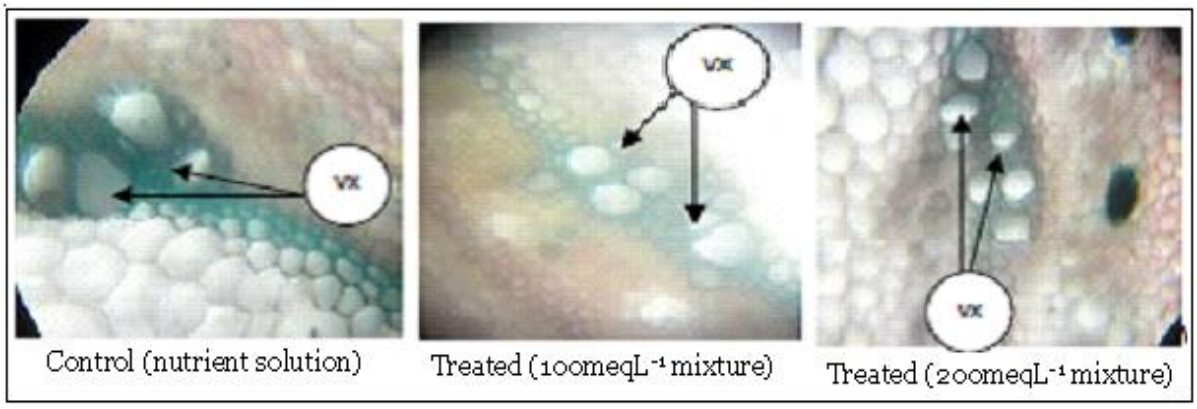

Fig. 17. Anatomy of 40-day-old *Phaseolus vulgaris* L. stems grown in hydroponics.

At the level of the stems, the results clearly show the action of the salt on the conductive tissue compared to the control, which results in an increase in the number of xylem vessels and in the writing of their diameter. At high magnification, the same rod clearly illustrates a relaxation in the arrangement of their conductive tissue and especially the xylem.

This reaction results in a reduction in the number of large vessels and in the thickening of their walls and according to the treatment treated plants treated with 200 meq. 1 of NaCl, $CaCl_2$ and mixture present after 40 days of growth a very reduced diameter compared to the plants (7,8,9) and xylem vessels in stressed plants continue to decrease but considerably.

The diameter of the vessels of the root xylem and compared with that of the stems and much more affected by the action of the salt since it shows a strong reduction compared to the vessels of plants watered to the nutrient solution and whatever saline treatment to bring or l (8,10,12).

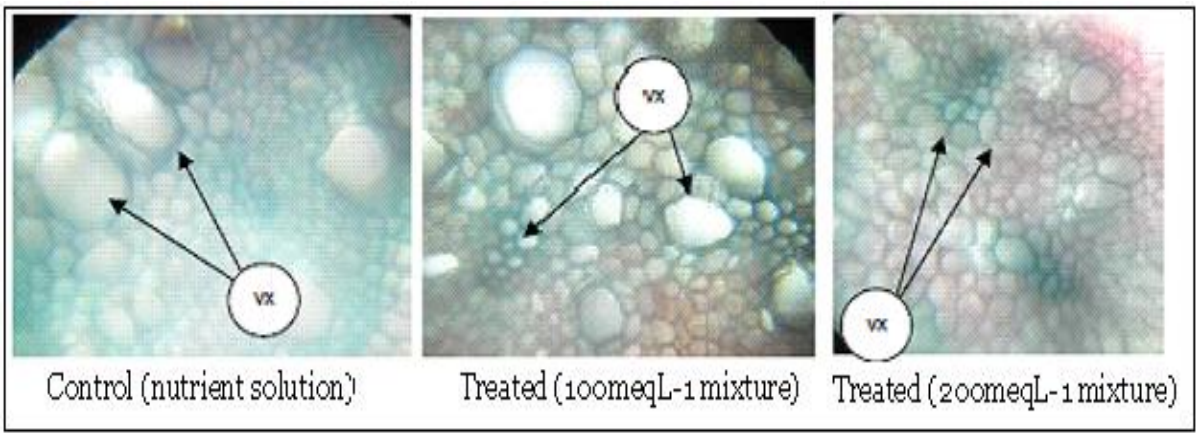

Fig. 18. Anatomy of 40-day-old *Phaseolus vulgaris* L. roots grown in hydroponics.

The results show that whatever the treatment, the root has large vessels compared to the stem. This behavior of the root can be explained as a mode of adaptation with respect to salinity. Most plants are capable of adapting to saline environments. This adaptation is accompanied by morphological, anatomical and biochemical changes (Kylin, 1975; Paljakouf, 1988). In non-halophytes, there is great variability in responses, classified from species sensitive to tolerant (Mansour *et al.*, 2003). On the other hand, salinity appears to modify the thickening of the primary xylem cell walls and percylindrical cellulosic fibers of the main stem (Belkhodja and Bidai, 2004).

The presence of salt in the plant, particularly NaCl, leads to structural modifications of the cell walls, which proves a similarity of effects and of water stress (Munns, 2002).

Conclusion

The bean (*Phaseolus vulgaris* L.) appears as a plant more or less sensitive to salt during its growth. Salt action results in decreased stem and root growth in *Phaseolus vulgaris* L. for high salt concentrations, stems and roots are more affected and their length decreases.

The salt may also cause the modification of the number and diameter of the xylem vessels in plants or sometimes create modifications in the structure of the vessels. On the other hand, most plants are capable of adapting to saline environments, this adaptation is accompanied by morphological, anatomical and biochemical changes. At the level of the stems, the results show well the action of the salt on the conductive tissue compared to the control.

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