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The Effect of Salinity on Gas Exchange on Different Developmental Stages of Mung Bean (*Vigna radiata* L. Wilczek)

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Abstract: In this study, we have put in a experimental pot, a plant of Mung bean (*Vigna radiata* L. Wilczek), this latest is subjected to salinity stress (NaCl + $CaCl_2$) during four stages of its growth (juvenile stage (5 leaves), flowering stage, after flowering stage and pod setting). Three concentrations of salt solution 100, 200 and 300 meq L^{-1} and the control (Hoagland solution) were used in irrigation. Exchangeable oxygen was determined in leaf discs and isolated chloroplasts of the stressed plants during respiration and photosynthesis processes. Results indicated that the quantity of the exchangeable oxygen during respiration increases with the increase of salinity concentration. Contrarily, the exchangeable oxygen during photosynthesis decreased with increasing salinity concentration.

Key words: Leaf discs, isolated chloroplasts, saline stress, gas exchange, *Vigna radiata* L.

INTRODUCTION

The soils salinization is one of the main reasons especially limiting the agricultural production in arid and semi arid regions (Le Houerou, 1992; Munns, 2002). Worldwide, more than 60 million hectares of irrigated land (representing some 25% of the total irrigated land in the world) have been damaged by salt (Cuartero and Fernández-Muñoz, 1998; Zhang and Blumwald, 2001). The detrimental effects of salts on plants are the consequence of both a water deficit that results from the relatively high solute concentrations in the soil as well as a stress specific to Cl^- and Na^+, resulting in a wide variety of physiological and biochemical changes that inhibit plant growth and development and disturb photosynthesis, respiration, protein synthesis and nucleic-acid metabolism (Levine *et al*., 1990; Sairam *et al*., 2002).

This situation entails a strong physical agricultural earth degradation that is often du to the choice of an intensive agriculture system for the development of these regions or in some case in an inappropriate conduct of the irrigation (Munns, 2002). This last case is essentially linked to the water quality of irrigation. Indeed, the availabilities in water become increasingly limited in the world for the quantity as for the quality, what it obliges to resort at the reuse of the waters and therefore its incontestable repercussion on the irrigation water quality (Shalhevet and Hsiao, 1986; Flowers, 2004; Wiebe *et al*., 2007). The first consequence of soils salinization is the reduction of the yield growth of cultures that can evolve toward situations where the plant is threatened with disappearance (Rathinasabapathi *et al*., 2000; Munns, 2002).

This use requires, nevertheless, more of knowledge on the engineering's of the soil science specific to this type of situation while taking into account the state of soil salinity or sodicity, of the water quality of irrigation and the cultivar tolerance of species to the salinity (Chartzoulakis and Loupassaki, 1997; Benkhelifa *et al*., 2008). Otherwise, it is important to make call to the ecophysiological approach that it can constitute an alternative for the attenuation of the effect of the soils salinity on the cultivated plant performances. This way should drive in search of tolerant species of plant that would impose a mastery of the knowledge on the mechanisms of their adaptation to the salinity.

Many of works on the adaptation of the plants cultivated to the salinity constraint evokes the related to questions the influence of the osmotic pressure in the solution of soil, the nutritional deficit of the plant that can result of it and the specific effect of some toxic ions on its physiological behavior (Kylin and Quatrano, 1975; Grattan and Grieve, 1998). The effects of these different forms of constraints linked to the salinity can be under isolated form, or under combined form whose analysis could be complex (Yeo, 1998; Hasegawa *et al*., 2000; Munns, 2002).

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Globally, a culture subject to the salt stress conditions reacts by the physiological mechanism (Kylin and Quatrano, 1975; Parida and Das, 2005) or by biochemical mechanism (Brugnoli and Lauteri, 1991; Zhu, 2002; Ashraf and Foolad, 2007).

Mung bean (*Vigna radiata* L.) is a leguminous pulse crop for its use as a vegetable protein source, animal fodder and green manure, it contains isoflavonoids having estrogen and antioxidant activities that used in prevention of much diseases such as cancer, it also exhibits antimicrobial and insecticidal activities (Brouns, 2002; Kaprelynts *et al*., 2003).

In this article, we propose the results of the physiological behavior of a leguminous (*Vigna radiata* L. Wilczek) subjected to the salinity constraints according to its stage of development. It is an interesting ecotype for its promising bill of particulars concerning its tolerance to the abiotic constraints of aridity and salinity of which it characterized the arid and semi arid areas in Algeria, the arid and semi arid regions cover more than 95% of the territory (Halitim, 1985). In this study, we made a follow-up of the breathing and photosynthesis at various development stages of this species to value its response to the salinity like an abiotic constraint of the arid and semi arid regions of Algeria.

MATERIALS AND METHODS

Material: Pot trial was conducted on mung bean (*Vigna radiata* L.) during summer season 2006 in sandy loam soil (2V:1V) at the experimental garden of Biology Department, Sciences Faculty, Mostaganem University. Mung bean seeds were obtained from Agriculture Research Centre of Cairo. Mung bean seeds were sterilized with 1.5% chlorox then washed with water. Seeds were planted in pots (25 cm upper diameter, 17 cm lower diameter and 25 cm in height with a bottom drainage hole), each pot contained 3 kg soil).

In this experiment a Hoagland's nutrient solution was used as a main culture solution, this solution is consisting of 1.91 mM KNO_3, 1.29 mM $Ca(NO_3)_2 \cdot 4H_2O$, 2.10 mM NH_4NO_3, 0.61 mM $Mg\,SO_4 \cdot 7H_2O$, 0.54 mM $K\,H_2PO_4$, 0.34 mM $K_2H\,PO_4 \cdot 3H_2O$, 0.02 mM $MnCl_2 \cdot 4H_2O$, 0.002 mM $CuSO_4 \cdot 5H_2O$, 0.03 mM H_3BO_3, 0.002 mM $MO_7O_{24}(NH_4) \cdot 7H_2O$, 0.001 mM EDTA and added to distilled water.

Saline solution was prepared by adding a mixture of NaCl and $CaCl_2$ in a 1:1 molar ratio. The irrigation is for one time each three days. Application of saline concentrations was practiced one week before the collecting the samples for each treatment. In this case the Hoagland's solution was stopped.

Four plants were let to grow in each pot. Three replicate pots were kept for each treatment. Plants were harvested at 21 day (juvenile stage), 45 day (vegetative growth stage), 60 day (flowering stage) and 75 day (pod setting stage). Three replicates were used for gas exchange measurement.

Methods of research: Intact chloroplasts were isolated by a modification in methods (Walker, 1980; Robinson, 1982). All procedures were carried out at O°C. Leaves (50 g) were ground for 3 s in a Polytron blender with 200 mL of 330 mM sorbitol, 30 mM Mes, 2 mM ascorbate and 0.1% BSA adjusted to pH 6.5 with Tris. The brei was squeezed through a double layer of Mira cloth containing a layer of cotton wool and the filtrate was centrifuged at 1200 g for 1 min. The pellets were resuspended in 6 mL of 330 mM sorbitol, 30 mM Hepes and 0.2% BSA adjusted to pH 7.6 with Tris. The chloroplasts were placed in two centrifuge tubes and each was under layered with 4 mL of resuspension medium plus 40% (v/v) Percoll. The tubes were centrifuged at 1200 g for 1 min and then the supernatant was aspirated and the pellets resuspended in the above medium.

The quantity of the exchangeable oxygen (XO_2) was measured as well as respiration (XO_2R) and photosynthesis (XO_2P) using Wärburg apparatus according to the method reported by Laval-Martin and Mazliak (1979) in the base of the Mariut Low (at the same volume and temperature, changes in gas quantity could be measured through changes in pressure). So exchangeable oxygen quantity of the samples which were inside Wärburg flask. Before the experiment the flasks were joined and every flask was connected to a manometer and the level of the solution was adjusted. Then samples of leaf disks and chloroplasts suspension were put in Wärburg flasks.

The exchangeable oxygen was calculated using the following equation:

$$X = h.\,K$$

Where:

X = The quantité of the exchangeable oxygen in (mm^3)
h = The change in the solution level (mm)
K = A constant for every flask

We have used the t-test ($p \leq 0.05$) statistical analysis.

RESULTS AND DISCUSSION

Quantity of absorbed oxygen (XO_2R) in function to salinity: The results indicate the variations of the quantity of absorbed oxygen at the time of the respiration

analyzed from the leaf discs and the isolated chloroplasts. The oxygen uptake measured on the leaf discs increases with the concentration in salts of the middle and in the time. At the course of the first three weeks, the oxygen absorption passes from 7.73 mm^3 at the witness at 36.61 mm^3 in the stressed plants at 300 meq L^{-1} of NaCl + $CaCl_2$. Has this same concentration, the oxygen absorption continues progressively to reach a quantity meaningfully raised at the end of 75 days of plant growth compared to the quantity of oxygen measured at the departure (Table 1).

The analysis of results during respiration indicates for the leaf discs (Table 1), an important increase of absorbed oxygen with the augmentation of salinity compared to the control. In the other hand, this augmentation is more important with the duration of the stage.

Table 1 shows that the absorbed oxygen by the isolated chloroplasts lowers remarkably compared to the leaf discs. For example, the rate of fall of the absorption for the chloroplasts witnesses at the end of 21 days represents 40.62% of the absorption of the leaf discs witnesses. In the same way, under the treatment saline at 300 meq L^{-1} of NaCl+$CaCl_2$, the rate of absorption at the chloroplasts happens to à 35.11% of the one of the leaf discs. At the 75th day, the rate of absorption of the oxygen is reduced strongly, of same for the chloroplasts of the plants witnesses in relation to the leaf dicks under the same treatment (40.60%) that for the plants treated à 300 meq L^{-1} of NaCl + $CaCl_2$ for the chloroplasts (32.46%) in relation to the one registered at the leaf discs.

Isolated chloroplasts data, shows a same evolution compared to the last parameter but in more weak proportion. The horizontal comparison (Table 1), shows an important difference in amounts of XO_2 in the leaf discs between stages compared to those of isolated chloroplasts.

Table 2 shows the variations of the exchanges in XO_2R from the leaf discs and isolated chloroplasts measured at the third week and at the end of ten weeks show the differences clearly in the response of the plants to the salinity and during their development. Indeed, during the two periods, it must remark that the respiratory oxygen quantity increases quickly in the leaf discs and slowly in the chloroplasts with the concentration of the middle in NaCl+$CaCl_2$. Nevertheless, it must signal that the respiratory activity becomes more important for the leaf discs at the end of 75 days whereas it varies appreciably in the chloroplasts of the 3rd week until the end of the experimentation.

Quantity of released oxygen (XO_2P) in function to salinity: The results of exchanged oxygen during the photosynthesis in the leaf discs, show (Table 1), that is decreasing slowly with the increasing of salinity compared to the control for all stages of plant growth.

The leaf discs witnesses reject the oxygen during the photosynthesis in an important manner while those treated (Table 1), see their rate lowering quickly since 100 meq of NaCl+$CaCl_2$ whatever is the stage of plant growth. For example, at the end of 21 days, the quantity of oxygen lowers 17.09 mm^3 for the witness at 10.85 mm^3 for the leaf discs treaties at 100 meq of NaCl+$CaCl_2$. At the end of 75 days, the reduction of the released oxygen by the leaf discs is even net so that it passes 22.95 mm^3 (witness) à 5.90 mm^3 (at 100 meq L^{-1} of NaCl + $CaCl_2$). It is necessary to signal however that the oxygen rejected by the leaf discs undergoes a less brutal reduction under the

Table 1: Quantity of absorbed oxygen (XO_2R) during the respiration of the leaf discs and isolated chloroplasts of Mung bean subjected to salt stress

	Development stages (days)	Control	100 meq L^{-1}	200 meq L^{-1}	300 meq L^{-1}
Leaf disks	21	7.73±0.47	15.26±0.34	23.38±1.09	36.61±0.83
	45	8.89±0.70	19.52±1.11	26.43±1.37	40.13±0.69
	60	12.24±0.76	22.65±1.18	29.44±0.83	46.94±0.55
	75	16.43±0.50	25.80±0.62	31.72±0.41	51.78±1.24
Isolated chloroplasts	21	3.14±0.24	5.14±0.17	8.57±0.24	12.85±0.51
	45	4.16±0.22	6.20±0.22	9.64±0.30	14.49±0.18
	60	4.91±0.19	7.85±0.16	11.17±0.51	15.30±0.35
	75	6.67±0.31	9.33±0.36	12.62±0.60	16.81±0.59

Table 2: Quantity of decreased oxygen (XO_2P) during photosynthesis of the leaf discs and isolated chloroplasts of Mung bean subjected to salt stress

	Development stages (days)	Control	100 meq L^{-1}	200 meq L^{-1}	300 meq L^{-1}
Leaf disks	21	17.09±0.52	11.95±0.65	10.23±0.50	3.95±0.28
	45	21.96±0.28	13.16±0.62	9.49±0.48	6.10±0.81
	60	22.10±0.92	10.21±0.47	9.66±0.61	3.36±0.34
	75	22.95±1.84	5.90±0.48	3.95±0.28	2.55±0.21
Isolated chloroplasts	21	10.85±0.45	8.28±0.38	5.98±0.41	4.00±0.53
	45	12.72±0.22	6.94±0.37	5.03±0.35	3.53±0.12
	60	14.19±0.60	5.29±0.34	3.78±0.25	2.82±0.18
	75	15.66±0.47	3.87±0.21	2.75±0.47	1.59±0.43

effect of the saline treatment whatever is the stage, notably at 100 and 200 meq L^{-1} on the other hand, it must notice also that under the treatment at 300 meq L^{-1} of NaCl + $CaCl_2$, the leaf discs clears a lot less photosynthetic oxygen in particular toward the last stages (60th and 75th day) with respective values of 3.36 and 2.55 mm^3 (Table 2).

These exchanges vary remarkably according to the utilized biologic material, the concentration in NaCl + $CaCl_2$ and the time. Indeed, when the salinity of the middle increases, the rejected quantity of photosynthetic oxygen decrease slowly in the leaf disks and the chloroplasts measured at the 21st day. At the 75th day, the photosynthetic exchanges undergoes a fast slowing in the rhythm of the dismissal of the oxygen in relation to the witness as soon as the plants are stressed at 100 meq L^{-1} of NaCl + $CaCl_2$; for example the leaf discs witnesses reject 23.5 mm^3 of oxygen to pass at 7.5 mm^3 under the treatment at 100 meq L^{-1} of NaCl + $CaCl_2$ whereas in the chloroplasts, this quantity of oxygen gyrates, respectively of 16.5 mm^3 at 4.5 mm^3 for the witness and under the treatment at 100 meq L^{-1} of NaCl+$CaCl_2$ beyond this concentration, the photosynthetic oxygen is increasingly attenuated.

It is important to see that the duration of stage effect, increased the XO_2 in the control, contrarily in the other treatments where it decrease this parameter. In the other hand, the values of XO_2 in the leaf discs are relatively more important that those in the isolated chloroplasts.

Oxygen during the respiration: Following our experiences, it is important to make a first remark concerning the absorbed XO_2 during the respiration that records, in general, the big proportions in the leaf discs compared to those of isolated chloroplasts. In this case, the effect of increasing doses of salinity entails a bigger increase of absorbed XO_2 compared to the witness. Witch it is in agreement with the literature (Lopez-Hoffman *et al*., 2006). This salinity stimulation of respiration is maintained, even after the osmotic adjustment. Knowing that the increase of the respiration expresses a particular adaptation form of the plant material to salinity (O'leary, 1995; Vinocur and Altman, 2005; Saqib *et al*., 2006), the plant needs some respiratory energy to maintain in activity some processes as the turnover of the proteins and the active transport. These last are indispensable for the balance of the pressure gradients of ion concentrations that doesn't necessarily have a direct liaison with the growth (De Vries, 1975; Amthor, 2000). This evolution should be assigned to the increase of the leaf internal concentration of CO_2 that permits a rate raised of assimilation of the carbon at the same in partial post-closing case of the stomata misled by the salinity (Munns, 2002).

Table 3: Part of the salinity influence and the stage of development on the global variations of respiration

Source of variation	Leaf discs		Isolated chloroplasts	
	SCE	Variation (%)	SCE	Variation (%)
Salinity	6776.09	88.54	692.23	86.98
Stage	786.17	10.27	97.98	12.31
Interaction	67.81	0.89	1.62	0.20
Mistake	23.13	0.30	4.00	0.50
CV	3.28%		3.79%	

To analyze the relative effects of the salinity and the stage of plant growth on the XO_2R, we did a statistical two way analysis of variance (Table 3), to study the relative effects of the salinity and the stage of plant growth (Dagnelie, 2006). It appears, on average, that the salinity is responsible for near 87% of the variations of XO_2R exchanged whereas the stage of development is only responsible for near 11%. It means that the salinity is the main factor that disrupts the plant respiration during the whole period of its growth. It says, the age of the plant only intervenes for 1/10 the variations on the plant faculty to tolerate the salinity. It is why, Barneix *et al*. (1988) reported that the growth rates of different varieties of some species could not be correlated with the rate of respiration.

Oxygen during the photosynthesis: In the case of the photosynthesis, the effect of the concentrations of 100, 200 and 300 meq L^{-1} seems to act while reducing the XO_2 free remarkably. This reduction must not be the result of the closing of the stomata (Kao *et al*., 2001). It is owed, either à lowers it of the net photosynthesis bound at an inhibition of the fixing of the CO_2 in salty environment as the case of some species as cotton, either à a reduction of the partial pressure of the CO_2 in the leaves as the case of another range of cultures as the bean (Longstreth and Nobel, 1979). This inhibition of the fixing of CO_2 in the level of the chloroplasts of the cells of guard appears by a reduction of the contents in carbohydrates in the mature leaves (Fisarakis *et al*., 2001; Prytz *et al*., 2003). The difference in values of XO_2P exchanged between the two methods of measurement is less important than in the case of the XO_2R.

We noticed, that the effect of the salinity on the XO_2P seems especially important that the plant approaches of its adult stage. On the other hand, the statistical two way analysis of variance (Table 4), reveal a part of variation on average of 88% for the salinity, 8% for the effect of interaction and less 5% for the stage of development of the culture. It shows that the salinity is

Table 4: Part of the salinity influence and the stage of development on the global variations of photosynthesis

Source of variation	Leaf discs		Isolated chloroplasts	
	SCE	Variation (%)	SCE	Variation (%)
Salinity	765.62	88.04	1886.24	87.54
Stage	12.35	1.42	91.39	4.24
Interaction	86.95	10.00	161.81	7.51
Mistake	4.71	0.54	15.32	0.71
CV	6.34%		5.72%	

the main reduction factor of the XO_2P contrary to the stage of development and the interaction. But it doesn't prevent that the stage has nevertheless a part of influence, as weak as (<5%), that added to the interaction effect (8%), show that the stage of development contributes at the reduction of the XO_2P under the effect of the salinity. It permits to deduct that the plant expresses a certain tolerance to the salinity relatively in the juvenile stages in relation to its phase of maturity. This tendency is not general since other species don't show a significant difference under the effect of the age. Indeed, Pezeshki *et al*. (1986) noticed that the salinity entailed a reduction of the stomatic conductance and the clean XO_2P as well at the young leaves as aged of cattail. What makes that this sensitivity in the salinity according to the stage of growth of the plant must vary between a species to another (Foolad and Lin, 2001; Cixin He, 2005). Although some of agronomic studies exist on this species, it has there that very few physiological studies that process its resistance to the salinity.

The reduction of XO_2P in the plant can be explained globally, by the fact that the raised salt concentrations in the substrate generally reduces the photosynthesis (Downton *et al*., 1985; Belkhodja, 1996; Fisarakis *et al*., 2001), or because of the potassic deficiency generated by the effect of the salinity that reduces the photosynthesis at a lot of plants (Morard, 1974). Indeed, since the K^+ is the major solution that maintains the turgescence of the guard cells, it is reasonable that the potassic deficiency generates the closing of the stomata (Brugnoli and Lauteri, 1991; Steduto *et al*., 2000).

CONCLUSION

This study show that during the respiration or during the photosynthesis, the values of XO_2 obtained by the leaf discs is superior to those obtained by the isolated chloroplasts submitted in similar applied condition.

In the case of the respiration, it is the salinity that controls the essential of the reduction of the XO_2R in the inverse of the age of the plant that only controls the $1/10^{ème}$ of the variations of this parameter. It doesn't appear an interaction effect between the two parameters of measures, the salinity and the stage. What makes that the salinity influences the XO_2R exchanged independently of the phase of development of the culture.

In the photosynthetic phase, it is the salinity that controls the essential of the reduction of the XO_2P via the assimilation of the carbon in the inverse of the age of the plant that only controls the $1/10^{ème}$ on behalf of controls the salinity. In this case, it appears an effect of interaction between the two parameters of measures: the salinity and the stage of development, that show that the manifest studied plant a certain shape of tolerance at the salinity during its juvenile phase in relation to its stage of maturity. This behavior deserves to be studied more deeply to prospect the capacity of tolerance of this species at the salinity during its juvenile stage.

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