International Journal of Agriculture Innovations and Research
Volume 4, Issue 2, ISSN (Online) 2319-1473

Fighting against Desertification: Studying Physiological Markers of Halophytes Response to Salinity

AMROUNE Mohamed Lamine*, BELKHODJA Moulay
Laboratory of Plant Physiology, University of Oran 1 Ahmed Benbella
*Email: amrounemohamed2003@yahoo.fr

Abstract **– This work is a comparative study of photosynthetic gaseous exchange and respiratory activity of isolated leaf chloroplasts and leaf discs on a halophytic plant (*Atriplex halimus* L.). Plants were stressed with a saline solution consisting of 400 meq.l^{-1} NaCl+$CaCl_2$ and 600 meq.l^{-1} Haogland nutrient solution added to diluted (50%) and non-diluted seawater. Gaseous exchange was measured during 30 min using Warburg device. Oxygen released throughout photosynthetic reaction in daylight conditions and oxygen absorbed throughout respiratory activity in darkness conditions was recorded.**

Results revealed that seawater (or NaCl+$CaCl_2$) salinity affects respiratory and photosynthetic activities. They however vary according to medium concentration and stress type.

***Atriplex halimus* L. leaf chloroplasts gaseous exchange shows indeed an oxygen absorption decrease with a 400 meq.l^{-1} NaCl+$CaCl_2$ concentration. Watering with non-diluted seawater causes respiratory activity slow down on *Atriplex halimus* L. leaf chloroplasts.**

***Atriplex halimus* L. leaf chloroplasts photosynthetic activity is more intense under 400 meq.l^{-1} NaCl+$CaCl_2$ stress.**

Keywords **– *Atriplex halimus* L., Gaseous Exchange, Leaf Chloroplasts, Leaf Discs, Nacl+$Cacl_2$, Oxygen Breathing, Oxygen Photosynthesis, Salinity, Seawater, Warburg Device.**

I. Introduction

After a long lasting drought, Algerian soil has become halomorphic. This characteristic is specific to arid and semi arid countries [Osman, 1982; Munns, 2002]. On Earth, salinity covers about 954.8 millions hectares and 27% of them are farmland [Hamdy *et al*., 1999]. The Mediterranean Basin is affected by 16 millions hectares of salted land: 3.2 millions are in Algeria [Hamdy, 1999]. In these ecosystems, salinity has become a limiting factor for crop development [Tal, 1984; Hamdy *et al.*, 2002]. This is due to abnormal high soluble salt rate [Poljakof-Mayber & Gale, 1975; Kolahchi & Jalali**,** 2007]. Actually, salt excess induces nutritional poisoning phenomena due to absorption disorders [Zid, 1986; Meloni, 2001]. It is also due to accumulated compounds becoming toxic and disrupting plant metabolism [Sanchez Gonzalez & Agoyo, 1992; Liu & Zhu, 1998] and due to high osmotic potential [Immamulhaq *et al*., 1984; Yokoi *et al*., 2002; Moinuddin *et al*., 2005]. This changing biotope has led to species extinction [Chamard, 1993; Pitman & Lauchli, 2002] while others have acquired resistance mechanisms [Christian, 1992; Edward *et al*., 1999]. In addition, soils structural fertility and stability have been modified [Valentin, 1994; Benkhelifa, 1997; Regueig, 2007].

In practice, restoring salt damaged soils requires many actions. One of them is introducing legumes and halophytes. In weak rainfall regions, this eco-physiological approach allows recovering soil fertility [Sing *et al*., 1982; Belkhodja, 2004] through symbiotic nitrogen fixation by legumes [Osman *et al.*, 1986; Jensen, 1986; Belkhodja *et al.*, 2002], and through desalinization and soil fixing by halophytes [Ashraf *et al*., 1987; Le Houerou, 1992; Gorham, 1996; Batanouny, 1996]. Choosing legumes as broad beans and halophytes as *Atriplex halimus* L. is based on every species several advantages. In addition to advantages already mentioned, they also are a source of animal protein [Polignano *et al.*, 1991] and are tolerant to salinity, especially *Atriplex* [Le Houerou, 1991]. Moreover, *Atriplex* species seem nowadays to be the most adapted to steady and increase forage production in arid and semi-arid climate [Froment, 1972; Kinet *et al*., 1998].

Introducing these species in saline ecosystems requires understanding certain mechanisms such as physiological mechanisms involved in plant resistance to abiotic constraint. In accordance with [Boucaud *et al*., 1978; Girija, 2002], plant behavior varies indeed according to surrounding environment salinity: whether steady, increasing continuously or fluctuating.

II. Material and Methods

Plant material

The seeds of Atriplex, *Atriplex halimus* L., used in our experiments is taken starting from shrubs pushing in the campus of the University of Senia Oran (Algeria).

Atriplex seeds were sown in cells. Then seedlings were carefully transferred into flowerpots with sand substrate (2/3) and industrial peat (1/3). Plants were monitored during 157 days watered with 1/1000 diluted Hoagland (1938) nutrient solution before receiving saline treatment.

Experimental process

After 157 days, 400 meq.l^{-1} and 600 meq.l^{-1} NaCl+$CaCl_2$ saline solutions were poured over *Atriplex*. After one-week stress, leaves were carefully removed to extract chloroplasts and leaf discs. With 1 ml chloroplasts suspension and 10 leaf discs, gaseous exchange was monitored through Warburg device. Photosynthetic oxygen was recorded in daylight while respiratory oxygen was recorded in darkness conditions, both during 30 min.

Extraction of the chloroplasts

This operation proceeded according to the process of Walker (1970). This process normally makes it possible to obtain a suspension of chloroplasts of class I (complete), able to fix in the proportion from 50 to 80%, a CO2 high rate.

For that, preparations took place:

International Journal of Agriculture Innovations and Research
Volume 4, Issue 2, ISSN (Online) 2319-1473

Medium of crushing

Sorbitol 0.33 M

MgCl2 0,005 M

Na4P2O7, 10:2 O 0.01 M

Brought the pH of the mixture above to 6.5 with HCl, and to add, right before employment, of the sodium isoascorbate until a concentration 0,002 Mr.

Medium of handing-over in suspension

Sorbitol0.33 M

MgCl2 0,001 M

MnCl2 0,001 M

EDTA 0,002 M

HEPES (tampon acid hydroxyethylpiperazine-ethane sulfonic) 0.05 Mr.

Brought the pH to 7.6 with soda.

Extraction itself

For the extractions of the chloroplasts, the sheets are taken median part of each treated plant. 8 G of sheets are crushed in a crusher with 100 ml of medium of crushing. Then, the medium obtained is dried through 2 layers of gauze in a beaker and is filtered through 8 layers of gauze using a vacuum pump; the recovered solution is versed in a test tube then last in a blender during 3 to 5 seconds. This medium is versed in two tubes with centrifugation with equal volumes. The whole is centrifuged at low temperature (0° C) at a speed of 4000 tours/min during 90 seconds; in each tube is added 1 ml of medium of handing-over in suspension to the base recovered using a pipette (1 ml). Lastly, the suspension of chloroplasts is taken again using a syringe in two tubes of Eppendorf to then put them in a refrigerator while waiting for measurements of the gaseous exchange.

III. Results

Variations in respiratory and photosynthetic gaseous exchange

I- CHLOROPLASTS GASEOUS EXCHANGE MEASURED DURING 30 MIN.

A. Respiratory gaseous exchange

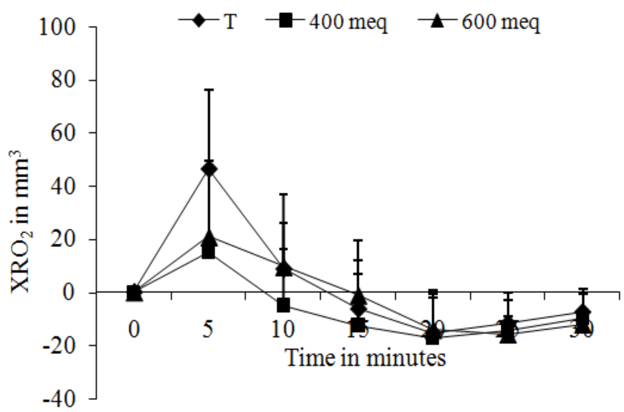

Fig.1. Variation of respiratory oxygen XRO_2 (mm^3) absorbed during 30 min by 1 ml chloroplasts suspension extracted from 165 days old *Atriplex halimus* L. leaves stressed with $NaCl+CaCl_2$.

Fig. 1 shows that the amount of oxygen absorbed by control plants chloroplasts increases to a maximum of 46.248 mm^3 during the first five minutes. Then the amount decreases to 8.742 mm^3 during the next five minutes. Oxygen absorption fall continues for 20 minutes gaseous exchange until the end of experiment.

With chloroplasts from plants fed with 400 $meq.l^{-1}$ $NaCl+CaCl_2$, the amount of respiratory oxygen increases to a maximum of 14.865 mm^3 during the first five minutes and then decreases to -9.644 mm^3 during the last 20 minutes. With chloroplasts from plants fed with 600 $meq.l^{-1}$ $NaCl+CaCl_2$, the amount of oxygen exchange increases to a maximum of 20.899 mm^3 after five minutes and then decreases until the end of experiment.

B. Photosynthetic gaseous exchange

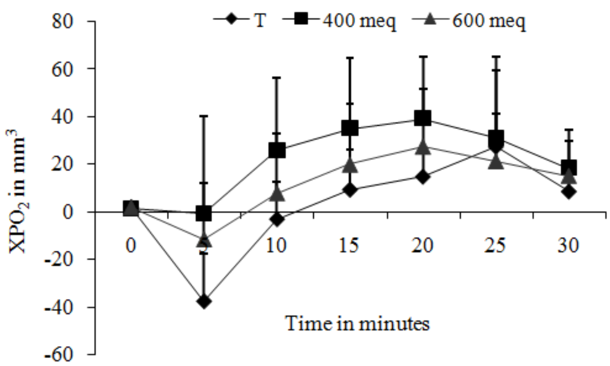

Fig.2. Variation of photosynthetic oxygen XPO_2 (mm^3) released during 30 min by 1 ml chloroplasts suspension extracted from 165 days old *Atriplex halimus* L. leaves stressed with $NaCl+CaCl_2$.

Fig. 2 shows that the amounts of photosynthetic oxygen released by control plants and stressed plants have a parallel evolution after 10 minutes of gaseous exchange. Control plants chloroplasts release goes up to 27.05 mm^3 photosynthetic oxygen during 15 minutes and then falls down to 8.32 mm^3.

In stress conditions, oxygen released is kept on a high level on chloroplasts from plants watered with 400 $meq.l^{-1}$ $NaCl+CaCl_2$ concentration and then decreases slowly after 20 minutes.

In 600 $meq.l^{-1}$ $NaCl+CaCl_2$ medium, photosynthetic activity evolves in a intermediary way between chloroplasts from control plants and from 600 $meq.l^{-1}$ $NaCl+CaCl_2$ stressed plants. It should be noted that for stressed plants, amount of oxygen released has a similar evolution than amount recorded with 400 $meq.l^{-1}$ salt treated plants.

II- LEAF DISCS GASEOUS EXCHANGE MEASURED DURING 30 MIN.

A. Photosynthetic gaseous exchange

Fig. 3 shows variations in oxygen released during photosynthetic reaction in daylight conditions. In the beginning of the experiment, oxygen released by leaf discs increases with all plants, whatever the treatment has been. After 5 minutes, a maximum of oxygen is released by leaf discs recording 34.966 mm^3 with control plants, 21.465 mm^3 with 400 $meq.l^{-1}$ $NaCl+CaCl_2$ treated plants and 30.628 mm^3 with 600 $meq.l^{-1}$ $NaCl+CaCl_2$ treated plants.

IJAIR
International Journal of Agriculture Innovations and Research
Volume 4, Issue 2, ISSN (Online) 2319-1473

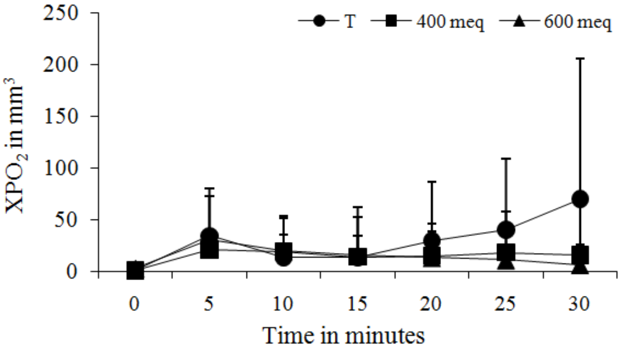

Fig.3. Variation of photosynthetic oxygen XPO_2 (mm^3) released during 30 min by 165 days old *Atriplex halimus* L. leaf discs stressed with $NaCl+CaCl_2$.

After reaching these values, released photosynthetic oxygen decreases slowly for all plants during 10 minutes. Then, oxygen production increases back very rapidly with control plants leaf discs, reaching 70.387 mm^3 after 30 minutes. On the other hand, oxygen released becomes steady with leaf discs from 400 meq.l^{-1} $NaCl+CaCl_2$ treated plants while it slows down with leaf discs from 600 meq.l^{-1} $NaCl+CaCl_2$ watered plants.

B. Respiratory gaseous exchange

Fig. 4 shows variations in oxygen absorbed by leaf discs during respiratory activity in darkness conditions. After 5 minutes, control plants leaf discs absorbed a maximum of -39.806 mm^3, 400 meq.l^{-1} $NaCl+CaCl_2$ stressed plants leaf discs absorbed a maximum of -22.96 mm^3 and plants fed with 600 meq.l^{-1} $NaCl+CaCl_2$ leaf discs a maximum of -31.82 mm^3. Beyond this point, oxygen absorbed by control plants leaf discs slowed down during 15 minutes to reach -7.084 mm^3 at the end of the experiment.

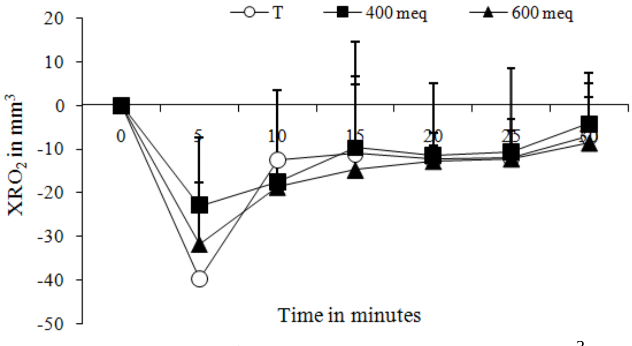

Fig.4. Variation of respiratory oxygen XRO_2 (mm^3) absorbed during 30 min by 165 days old *Atriplex halimus* L. leaf discs stressed with $NaCl+CaCl_2$.

Respiratory activity of 400 meq.l^{-1} $NaCl+CaCl_2$ stressed plants leaf discs slowed down until the end of the experiment reaching a -4.294 mm^3 value. Same phenomenon was recorded with leaf discs from plants fed with 600 meq.l^{-1} $NaCl+CaCl_2$.

IV. Conclusion and General Discussion

The purpose of this work was to monitor and compare photosynthetic and respiratory gaseous exchange of a halophytic plant (*Atriplex*) leaf discs and isolated chloroplasts. Plants were cultivated in flowerpots stored in a greenhouse to be watered with 400 meq.l^{-1} saline solution and 600 meq.l^{-1} $NaCl+CaCl_2$ gaseous exchange activity was measured through Warburg device during 30 minutes.

Results recorded assess that *Atriplex halimus* L. chloroplasts variations in oxygen absorption during respiratory activity and in oxygen release during photosynthetic activity are due to presence of salt with different concentrations in nutrient solution.

- *Atriplex chloroplasts gaseous exchange*

** concerning respiratory gaseous exchange*

While plants are stressed with $NaCl+CaCl_2$, respiratory oxygen absorbed by chloroplasts measured in darkness conditions during 30 minutes shows weak respiratory activity during the first 10 minutes compared to control plants absorbing up to 46.248 mm^3 oxygen. But during the last 20 minutes respiratory activity decreases very slowly until the end of the experiment whatever the salt concentration is.

** concerning photosynthetic gaseous exchange*

Photosynthetic activity goes up to a maximum after 20 minutes whatever the salt concentration is: 38.968 mm^3 with 400 meq.l^{-1} $NaCl+CaCl_2$ treated plants and 27.28 mm^3 with 600 meq.l^{-1} $NaCl+CaCl_2$ treated plants. Control plants chloroplasts release a maximum of oxygen after 25 minutes (27.05 mm^3).

- *Atriplex* leaf discs gaseous exchange

** concerning photosynthetic gaseous exchange*

Control plants leaf discs show a high photosynthetic activity during the whole experiment (70.387 mm^3). Salinity decreases leaf discs oxygen absorption on plants watered with 400 meq.l^{-1} and 600 meq.l^{-1} $NaCl+CaCl_2$ (21.465 mm^3 and 30.628 mm^3 respectively).

** concerning respiratory gaseous exchange*

Leaf discs respiratory activity of $NaCl+CaCl_2$ stressed plants is weak (-22.96 mm^3 with 400 meq.l^{-1} and -31.82 mm^3 with 600 meq.l^{-1}) compared to control plants (-39.806 mm^3).

Gaseous exchange recorded after 30 minutes allows to compare respiratory and photosynthetic activities:

- *Atriplex* chloroplasts respiratory gaseous absorption decreases as salt concentration increases.
- *Atriplex* chloroplasts photosynthetic oxygen release increases as salt concentration equals to 400 meq.l^{-1}.

Respiratory and photosynthetic gaseous exchange of *Atriplex halimus* L. chloroplasts from plants watered with saline solution has been the purpose of a certain number of laboratory works. Conclusions of works conducted on leaf discs assess that salinity has an impact on photosynthetic and respiratory gaseous exchange [Amroune, 2000]. With similar experimental conditions, it has been assessed that leaf discs absorb more oxygen during respiratory activity when plants are stressed with intermediate salinity (i.e. 400 meq.l^{-1} $NaCl+CaCl_2$). [Kabou, 1997] and [Abderrahmane, 2001] have showed that respiratory activity recorded with *Atriplex halimus* L. leaf discs increases as salt concentration increases. Moreover, these works report that photosynthetic oxygen release is very important under high salt concentration stressing conditions. Respiratory and photosynthetic exchange negative values recorded in our works likely reflect photorespiration phenomenon, a process occurring during daylight and characterized by oxygen absorption and CO_2 release.

[Bensafi & Bouchenak, 1997] have also established that salinity causes respiratory gaseous exchange increase with broad beans.

[Chahrour, 2002] has concluded that salinity has an impact on *Atriplex halimus* L. chloroplasts respiratory and photosynthetic activities. He has also concluded that amount of oxygen absorbed during respiratory activity and released during photosynthesis vary throughout duration according to salt concentration, stress type and ecotype. [Kheloufi, 2004] has demonstrated that broad beans leaf chloroplasts photosynthesis reaches higher values compared to control plants when fed with 400 $meq.l^{-1}$ $NaCl+CaCl_2$. No photosynthetic activity was recorded when salt concentration equals to 600 $meq.l^{-1}$ $NaCl+CaCl_2$.

[Björkman & Berry, 1973] have compared the impact of several factors on two *Atriplex* species photosynthesis: *Atriplex patula* (with a C3 photosynthetic metabolism type) and *Atriplex rosea* (with a C4 photosynthetic metabolism type). Both researchers have reported that C4 plants are more efficient to photosynthesis under heavy sunlight and high temperature than C3 plants. Moreover, C4 metabolism plants continue photosynthesizing while CO_2 concentration within intercellular spaces is very low, which is the case when stomata are closed.

According to results, *Atriplex halimus* leaf chloroplasts respiratory activity decreases because of salinity. This may reflect an adaptation mechanism to stress, one of halophytic plants characteristics.

Atriplex halimus L. leaf chloroplasts photosynthetic activity is very intense whatever the salt concentration is.

After comparing isolated chloroplasts and leaf discs gaseous exchange, results assess that *Atriplex halimus* leaf discs gaseous exchange is more active, probably due to the photorespiration effect taking place at the same time than photosynthesis. The phenomenon requires chloroplasts, mitochondria and peroxisomes functions. Photorespiration phenomenon takes place in case of plants being in stress conditions such as drought and salinity. This is to produce a maximum of energy and to compensate energy loss due to photosynthetic intensity decrease. [Costa *et al.*, 1978] have notified that photorespiration increases as oxygen partial concentration does.

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