Effect of short-term salinity on photosynthesis and ion relations in two sugar beet cultivars

Alireza Dadkhah* and Ghorbanali Rassam

Higher Education Complex of Shirvan, Ferdowsi University of Mashhad, Mashhad, Iran.

_____

Abstract

A factorial pot experiment was conducted based on randomized complete blocks design in green house condition to investigate the effect of short-term salt stress on leaf photosynthesis and ion relations of two sugar beet cultivars (Madison and 7233-P_{29}). Plants were exposed to 0, 50, 150, 250, and 350 mM salinity (NaCl and $CaCl_2$ in 5:1 molar ratio) for 48 hours. Measurements were done on youngest fully expanded leaves. Salinity caused a significant reduction in stomatal conductance and consequently leaf net photosynthesis in both cultivars. Net photosynthesis of cv 7233-P_{29} at low level of salinity (50 mM) at 12 h, 24 h and 48 hours after salinity application decreased by 7.4%, 15.1% and 20%, respectively, compared to control. However, reduction in photosynthesis at highest level of salinity (350 mM) at the same times were 62.4%, 73%, and 72.3%, respectively, compared to control. For Madison the percent of reduction was significantly higher than P_{29}. Cultivar P_{29} at 12 and 24 hours after salinization had significantly higher Na^+ content than Madison up to 250 mM. Potassium concentration tended to decrease with increase in salinity. Salinity led to increased Cl^- concentration in both cultivars. However, Madison had significantly higher Cl^- content than P_{29} at the highest level of salinity at all three times of sampling. A strong correlation between photosynthesis and stomatal conductance ($R^2=0.98$) and weak correlation between photosynthesis and sodium concentration ($R^2= 0.22$), and photosynthesis and chloride concentration (R2=0.55) indicated that in short-term exposure to salinity, stomatal conductance was one of the main causes of photosynthesis.

Key words: *Beta vulgaris*; salt Stress; stomatal conductance; NaCl

_____

Introduction

Plants exposed to salinity show less CO_2 uptake by their leaves than the same plants not exposed to salinity (Hester et al., 2001; Dadkhah and Griffiths, 2004, Dadkhah, 2011). This decrease has been attributed to specific ion effects mainly from Na^+ and Cl^- which may damage photosynthesizing tissues (Dadkhah and Griffiths, 2004). However, the direction and magnitude of these changes varies according to the level and duration of salinization treatment as well as the plant species. Salinity exerts its undesirable effects through osmotic inhibition and ionic toxicity. Osmotic inhibition is the result of the salt presented in the soil solution which reduces the ability of the plant to take up water, and leads to slower growth (Dadkhah and Griffiths, 2006; Koyro, 2006). Ionic toxicity is caused by an excessive amount of salt entering the transpiration stream which eventually injures cells in the transpiring leaves and may further

reduce growth (Koyro, 2006). Reduction in growth and photosynthesis are among the most conspicuous effects of salinity stress. In addition, stomatal closure in order to reduce transpiration appears to be the main cause of the decrease in photosynthetic rate. Moradi and Esmail (2007) reported that salinity could seriously change the photosynthetic carbon metabolism as well as photosynthetic efficiency. The ability of plants to cope with salinity stress is an important determinant of crop distribution and productivity in many areas, so it is important to understand the mechanisms that confer tolerance to saline environment. The main objectives of this study were to explore the changes in photosynthesis and ion concentration of sugar beet leaves under short-term salinity.

Materials and Methods

A factorial pot experiment in randomized complete block design was carried out under greenhouse condition with four replications. The experiment was conducted with two sugar beet cultivars (Madison and 7233-P$_{29}$) to investigate short-term effect of salinity on net photosynthesis (youngest leaf over 50% full size) and ion relations of two sugar beet cultivars. Plants were grown in 15 cm diameter pots filled with washed sand. Day length was 16 hours during the experiment and relative humidity was between 35-55%. Five levels of salinity 0, 50, 150, 250 and 350 mM (NaCl and CaCl$_2$ in 5:1 molar ratio) were added to a modified Hoagland nutrient solution (Mass and Poss, 1989). Forty-day-old sugar beet plants most being at 6-leaf stage were irrigated with saline water. Net photosynthesis (A) and stomatal conductance (gs) were measured three times (12 h, 24 h, and 48 h after salinity application) at a photon flux density of 500 μmol m^{-2} s^{-1} and the temperature of the growth condition using combined infrared gas analysis system (CIRAS-1 portable photosynthesis system, LTD, Hoddesdon, UK).

For Na$^+$ and K$^+$ analysis, samples of dried leaves were heated to ash in a furnace for 12 h at 550° C. The ash was dissolved in 20% sulfuric acid, diluted in distilled water, and filtered through a 90 mm Whatman No. 541 filter paper. Chloride (Cl$^-$) determinations were made on a distilled water extract of the dried sample ($\cong$ 200 mg), shaken for 24h, and then filtered through a 90 mm Whatman No. 2 filter paper. Na$^+$ and K$^+$ contents were determined by flame emission photometry. The amounts of Cl$^-$ were measured by ion-exchange chromatography using a DIONEX Model DX 500, fitted with a CD 20 conductivity detector, IP 25 Pump and AS 14 Ion Exchange Column.

The data for all characters were analyzed using the analysis of variance procedure of Statistical Analysis System (SAS) software, version 6.12. Means were compared by Duncan's multiple range tests at the 0.05 probability level for all comparisons.

Results

Photosynthesis significantly (P≤0.001) decreased with increasing salt concentration (Fig. I). Leaf photosynthesis of cv P$_{29}$ treated with low and high levels of salinity had rates of 92.6% and 37.6% (12 h after salinity application), 84.9% and 27% (24 h after salinity application) and 80% and
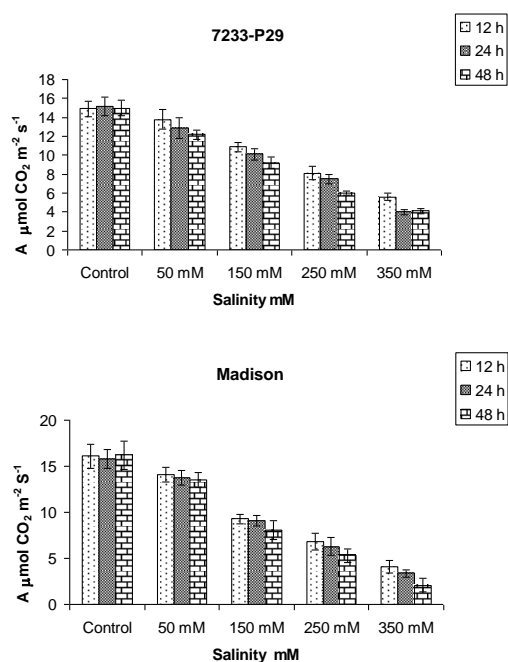

Fig. I. Net leaf photosynthesis (A) after short-term exposure of different cultivars to different salt concentrations; each bar is the average of four replications. Vertical lines are standard error of the means.

26.3% (48 h after salinity application) respectively, compared to control. However, leaf

photosynthesis of cv Madison treated with low and high levels of salinity had rates of 87.6% and 25.5% (at 12 h after salinity application), 87.3% and 22.1% (at 24 h after salinity application), and 83.3% and 13% (at 48 h after salinity application), respectively, compared to control. Analysis of variance revealed that there were significant differences between cultivars in the amount of net photosynthesis. Cultivar 7233-P_{29} had higher net photosynthesis than cv. Madison especially at high levels of salinity.

The adverse effect on photosynthesis was associated with a significant (P≤0.001) decrease in the stomatal conductance (gs) (Fig. II). Stomatal conductance of cv P_{29} treated with low and high levels of salinity had rates of 88.2% and 39.2% (12 h after salinity application), 87.5% and 32.4% (24 h after salinity application), and 80% and 30% (48 h after salinity application), respectively, compared to control. However, gs of cv Madison treated with low and high levels of salinity had rates of 90% and 26.5% (12 h after salinity application), 81.3% and 19.1% (24 h after salinity application), and 82.3% and 15.2% (48 h
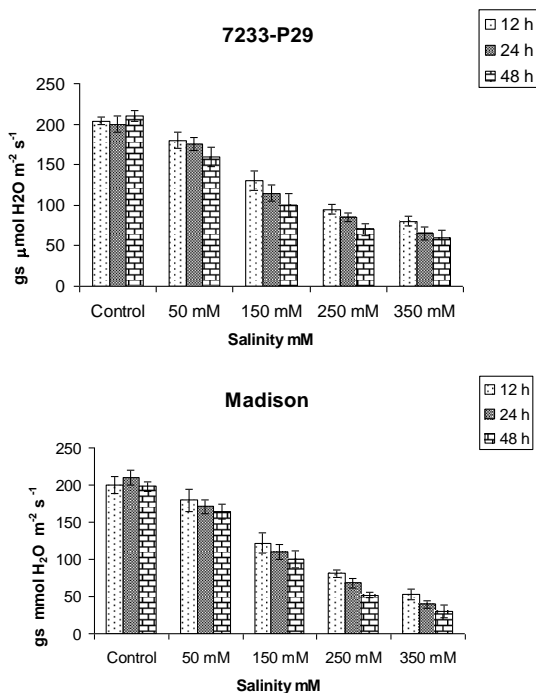
after salinity application) respectively, compared to control (Fig. II).

The ratio of intercellular to ambient CO_2 concentrations (C_i/C_a) decreased at high levels of salinity compared to controls in both cultivars of sugar beet. However, cultivar 7233-P_{29} had higher C_i/C_a than cultivar Madison especially at high levels of salinity (Fig. III).

When saline treatments were imposed on sugar beet plants, there was a significant increase in sodium concentration in both cultivars used (Madison and P_{29}) in the short-term experiment (Fig. IV). Sampling for short-term cation and anion measurement was started at 12 hours and continued to 24 hours, and 48 hours after salinization. Cultivar P_{29} 12 hours and 24 hours after salinization had significantly higher Na^+ content than Madison up to 250 mM. 48 hours after salinization differences between these two cultivars was only significant at 250 mM (Fig. IV). 12 hours after salinization plants of both cultivars grown in 150 mM had markedly the highest Na^+ concentration (Fig. IV).

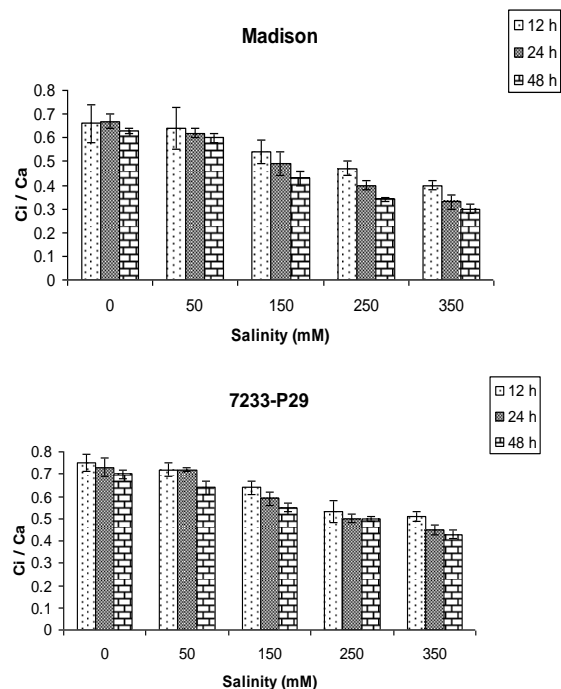Potassium concentration tended to decrease with increasing salinity (Fig. 5). At 12

Fig. II. Stomatal conductance (gs) after short-term exposure of different cultivars to different salt concentrations; each bar is the average of four replications. Vertical lines are standard error of the means.

Fig. III. The ratio of intercellular to ambient CO_2 concentrations (C_i/C_a) after short-term exposure of different cultivars to different salt concentrations; each bar is the average of four replications. Vertical lines are standard error of the means.

P$_{29}$

Madison

Fig. IV. Leaf sodium (Na$^+$) concentration (g kg^{-1} D.W) after short-term exposure of different cultivars to different salt concentrations. Each bar is the average of four replications. Vertical lines are standard error of the means.
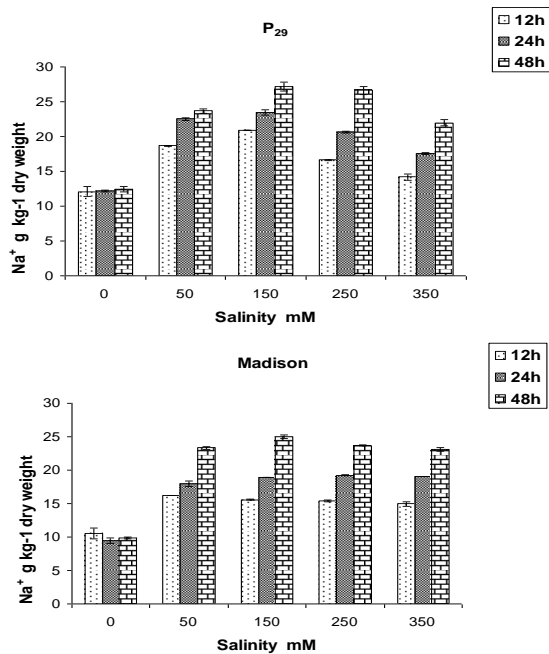
P$_{29}$

Madison

Fig. V. Leaf potassium (K$^+$) concentration (g kg^{-1} D.W) after short-term exposure of different cultivars to different salt concentrations; each bar is the average of four replications. Vertical lines are standard error of the means.

P$_{29}$

Madison

Fig. VI. Leaf chloride (Cl$^-$) concentration (g kg^{-1} D.W) after short-term exposure of different cultivars to different salt concentrations; each bar is the average of four replications. Vertical lines are standard error of the means.

There was not a high correlation between photosynthesis and Na$^+$ concentration (R^2= 0.22) and Cl$^-$ concentration (R^2 = 0.55) (Figs. VII and VIII). However, a strong positive linear correlation was observed between photosynthesis and stomatal conductance (R^2 = 0.98) (Fig.IX).

Discussion

The present study indicated substantially lower net photosynthesis rates with increasing salinity concentrations. Similar results were obtained with other species by Guanghni et al. (1993), Everard et al. (1994) and Kafi (2009), but not by Flanagan and Jefferies (1988) who only found slight decreases in photosynthesis rates with increasing salinity concentrations. Decreased net photosynthesis rate at high external salinities are often more pronounced in non-halophytes than halophyte species (e.g. Rowson et al., 1988; Banuls and Primo-Millo, 1992; Walker et al., 1993; Chartzoulakis et al., 1995)

In the present study increased salinity treatments often led to significantly smaller stomatal conductance. This has been found in
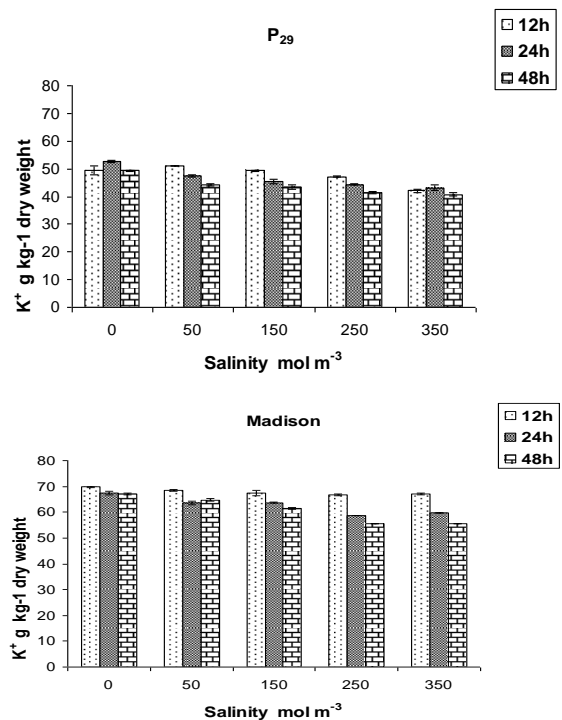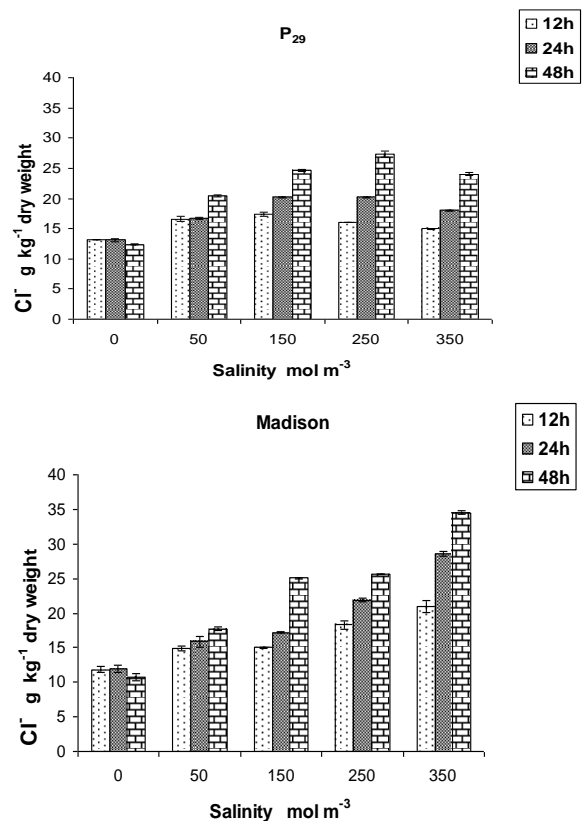
most other studies (e.g. Downton et al., 1985; Seeman and Critchley, 1985; Walker et al., 1993; Chartzoulakis et al., 1995; Kafi, 2009; Dadkhah, 2011). It was reported that a part of growth reduction due salinity is a direct consequence of stomatal closure and a reduction in the C_i which led to decrease in photosynthesis rate (Querghi et al., 2000; Netondo et al., 2007). The reduction in gs under salt stress is estimated to be substantial in spinach and *Phaselous vulgaris* with the intercellular CO_2 concentration reduced up to 30% (Seemann and Critchly, 1985).

Accumulation of inorganic ions, predominantly Na^+ and Cl^-, has an important role in the process of osmotic adjustment. There is substantial evidence that plants of moderate to high salt tolerance may accumulate large amounts of salt under saline conditions, contributing to osmotic adjustment (Flowers, et al., 1977; Binzel et al., 1988; Ashraf, 2001; Ghoulam et al., 2002).

In short-term salinity, Na^+ concentrations were higher in tissues subjected to moderate salinity (150 mM) than high levels of salt treatments. There might be an excluding mechanism in the roots which prevents Na^+ and Cl^- loading to xylem from the growth medium at high levels of salinity. However, differences were observed between these two cultivars under low levels of salinity. Figs. (V) and (VI) show that Madison had significantly lower (or equal Na^+) and Cl^- content than P_{29} over short-term exposure up to 250 mM salinity. One explanation for low absorption of Na^+ and Cl^- in short-term in this experiment could lie in the rate of water and nutrient absorption from the rooting medium in the first hours after the changes in osmotic potential resulting from application of saline treatments. It may take time for Madison plants to lower their potential to be able to absorb water from the external solution. The higher Na^+ and Cl^- concentration of salt-tolerant cultivar P_{29}, in the short-term under moderate salinity may indicate that it started water uptake sooner than the Madison cultivar.

Potassium concentration decreased significantly with increasing salinity (Fig. V). However, this reduction was not as much as increasing sodium concentration. There were significant differences in potassium accumulation
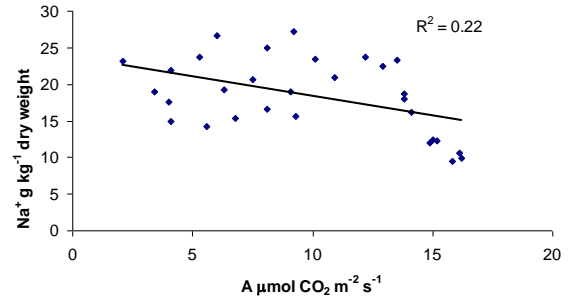

Fig. VII. Relationship between leaf sodium concentration and photosynthesis (A) in two sugar beet cultivars in the presence of short-term salinity; each point is the average of three replications.
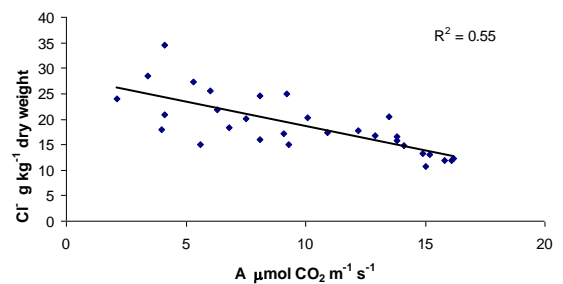

Fig. VIII. Relationship between leaf cholorid concentration and photosynthesis (A) in two sugar beet cultivars in the presence of short-term salinity; each point is the average of three replications.
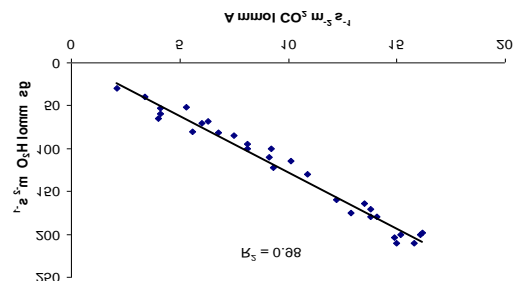

Fig. IX. Relationship between leaf stomatal conductance and photosynthesis (A) in two sugar beet cultivars in the presence of short-term salinity; each point is the average of three replications.

between cultivars in short-term exposure to salinity, cultivar P_{29} having a lower shoot potassium concentration in all salt treatments. However, the percentage reduction in potassium concentration in Madison and P_{29} under high salinity 48 h after salinization compared to their control were 20.5% and 17.9%, respectively. These results are in agreement with Heuer and Plaut (1989) and Ghoulam et al. (2002) in sugar beet, Lutts et al. (1996) in rice, and Colmer et al.

(1996) in *Sorghum bicolor*. Based on the opposing changes in leaf K^+ and Na^+ concentration with salinity, it was suggested that the K^+ mobilization was in part the result of exchange of vacuole K^+ for Na^+.

Not a high correlation was found between photosynthesis and Na^+ concentration ($R^2 = 0.22$) and Cl^- concentration ($R^2 = 0.55$) (Figs. VII and VIII). However, a strong positive linear correlation between photosynthesis and stomatal conductance ($R^2 = 0.98$) indicated that stomatal conductance was the main causes of photosynthesis reduction in short-term salinity (Fig. IX). In other words, it indicates that non stomatal parameters did not limit the gas exchanged rate. The reduction in stomatal conductance with increasing salinity can be attributed to reduction in water absorption by root due to lower water potential in soil. These observations are in agreement with those of Kafi (2009) who reported that decrease in stomatal conductance was the main factor for limited photosynthesis in wheat plants.

Acknowledgements

References

Ashraf, M. 2001. ̓Relationship between growth and gas exchange characteristics in some salt-tolerant amphidiploid *Brassica* species in relation to their diploid parentś. *Environmental and Experimental Botany*, 45, 155-163.

Banuls, J. and **E. Primo-Millo**. 1992. ̓Effects of chloride and sodium on gas exchange parameters and water relations of Citrus plantś. *Physiologia Plantarum*, 86, 115-123.

Binziel, M.L., F. D. Hess, R. A. Bressan and **P. M. Hasegawa**. 1988. ̓Intercellular compartmentation of ions in salt adapted tobacco cells̓. *Plant Physiology*, 86: 607-614.

Colmer, T.D., T.W.M. Fan, R.M. Higashi and **A. Lauchli**. 1996. ̓Interactive effects of Ca^{2+} and NaCl salinity on the ionic relations and proline accumulation in the primary root tip of *Sorghum bicolor̓*. *Plant Physiology*, 97: 421-424.

Dadkhah, A and **H. Grifiths**.2004. ̓Stomatal and non-stomatal components to inhibition of photosynthesis in leaves of sugar beet plants under salt stress̓. *Iran Agricultural Research*, 23: 35-50.

Dadkhah, A and **H. Grifiths**. 2006. ̓The effect of salinity on growth, inorganic ions and dry matter partitioning in sugar beet cultivarś. *Journal Agricultural Science and Technology (JAST)*, 8: 199-210.

Dadkhah, A. 2011. ̓Effect of salinity on growth and leaf photosynthesis of two sugar beet (*Beta vulgaris*. L.) cultivars̓. *Journal Agricultural Science and Technology,* 43: 1001-1012.

Chartzoulakis, K.S., I.N. Therios, N.D. Misopolinos and **B.I. Noitsakis**. 1995. ̓Growth, ion content and photosynthetic performance of salt-stressed kivifruit plantś. *Irrigation Science*, 16: 23-28.

Downton, W.J.S., W.J.R. Grant and **S.P. Robinson**.1985. ̓Photosynthetic and stomatal responses of spinach leave to salt stress̓. *Plant Physiology*, 78: 85-88.

Everard, J.D., R. Gucci, S. C. Kann, J. A. Flore and **W.H. Leoscher**. 1994. ̓Gas exchange and carbon partitioning in the leaves of celery (*Apium graviolens* L.) at various levels of root zone salinity̓. *Plant Physiology*, 106: 281-292.

Flanagan, F.B. and **R.L. Jefferies** .1988. ̓Stomatal limitation of photosynthesis and reduced growth of halophyte, *Plantago maritima* L. at high salinity̓. *Plant, Cell and Environment.*, 11: 239-246.

Flowers, T.J., P.F. Troke and **A.R. Yeo** .1977. ̓The mechanism of salt tolerance in halophyteś. *Annual Review of Plant Physiology*, 28: 89-121.

Ghoulam, C., A. Foursy and **K. Fares**.2002. ̓Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivarś. *Environmental and Experimental Botany*, 47: 39-50.

Guanghui, L., D. S. Leonel and **L. Sternberg**. 1993. Effects of salinity fluctuation on photosynthetic gas exchange and plant growth of the red mangrove (*Rhizophora mangle* L.). *Journal of Experimental Botany*, 44: 9-16.

Kafi, M. 2009. The effect of salinity and light on photosynthesis, respiration and chlorophyll fluorescence in salt-tolerant and salt-sensitive wheat *(Triticum aestivum* L.) cultivars. *Journal Agricultural Science and Technology*, 11: 535-547.

Koyro, HW. 2006. Effect of salinity on growth, photosynthesis, water relations and solute compositions of the potential cash crop halophyte *Plantago coronopus* L. *Environ. Exp., Bot*. 56: 136-146.

Hester, M.W., I. A. Mendelssohn and **K. L. McKee** .2001. Species and population variation to salinity stress in *Panicum hemitomon*, *Spartina patens*, and *Spartina alterniflora*: morphological and physiological constraints. *Environmental and Experimental Botany*, 46: 277-297.

Heuer, B. and **Z. Plaut**. 1989. Photosynthesis and osmotic adjustment of two sugar beet cultivars grown under saline conditions. *Journal of Experimental Botany*, 40: 437-440.

Lutts, S., J.M. Kinet and **J. Bouharmont**. 1995. Changes in plant response to NaCl during development of rice (*Oryza ativa* L.) varieties differing in salinity resistance. *Journal of Experimental Botany*, 49: 1843-1852.

Maas, E.V. and **J. A. Poss**. 1989. Salt sensitivity of wheat at various growth stages. *Irrigation Science*, 10: 29-40.

Moradi, F. and **A. M. Ismail**. 2007. Responses of photosynthesis, chlorophyll fluorescence and ROS-Scavenging systems to salt stress during seedling and reproductive stages in rice. *Ann. Bot*., 99(6): 1161-1173.

Netondo, G. W., J.C. Onyango and E. Beck. 2004. Sorghum and salinity II: Gas exchange and chlorophyll fluorescence of sorghum under salt stress. *Crop Sci*., 44: 806-811.

Ouerghi, Z., G. Cornic, M. Roudani, A. Ayadi and **J. Brulfert**. 2000. Effect of NaCl on photosynthesis of two wheat species (*Triticum durum* and *Triticum aestivum*) differing in their sensitivity to salt stress. *J. Plant Physiol*., 156: 335-340.

Rawson, H.M., M. J. Long and **R. Munns**. 1988. Growth and development in NaCl-treated plants I. Leaf Na$^+$ and Cl$^-$ concentrations do not determine gas exchange of leaf blades in barley. *Australian Journal of Plant Physiology*, 15: 519-527.

Seemann, J.R. and C. **Critchley**. 1985. Effect of salt stress on the growth, ion content, stomatal behavior and photosynthesis capacity of a salt sensitive species, *Phaseolus vulgaris* L. *Planta*, 164: 151-162.

Sharkey, T. D., J. R. Seeman and **J. A. Berry**. 1989. Photosynthesis in intact leaves of C$_3$ plants: Physics, Physiology and rate limitations. *Botany Review*. 51: 53-105.

Walker, R.R., D. H. Blackmore and **S. Qing**. 1993. Carbon dioxide assimilation and foliar ion concentrations in leaves of lemon tree (*Citrus limon* L.) irrigated with NaCl and Na$_2$SO$_4$. *Australian Journal of Plant Physiology*, 20: 173-185.