

Effect of Long Term Salt Stress on Gas Exchange and Leaf Carbohydrate Contents in Two Sugar Beet (*Beta vulgaris* L.) Cultivars

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Abstract: A factorial pot experiment was conducted based on randomized complete block design in green house condition to investigate the effect of salt stress on photosynthesis, leaf carbohydrate concentration and daytime leaf carbon budget of two sugar beet cultivars (Madison and 7233-P₂₉) over photoperiod. Plants were exposed to 0, 50, 150 and 250 mM salinity (NaCl and CaCl₂ in 5:1 molar ratio) for 60 days. Measurements were done on youngest fully expanded leaves. Salinity caused a significant reduction in leaf net photosynthesis consequently total carbon fixed in both cultivars. However, salt stress significantly increased soluble carbohydrate concentration in both cultivars. Starch concentration of leaves also increased with increasing salinity. As salinity had inverse effect on photosynthesis process, the total carbon fixed decreased with increasing salinity. Daytime amount of carbon translocated from leaf was reduced by salinity.

Key words: Salt stress, sugar beet, photosynthesis, carbohydrate contents, NaCl, CaCl₂, carbon

INTRODUCTION

It has been generally recorded that salinity adversely affects plant growth and some relevant metabolic processes of glycophytic plants (Zidan and Al-Zahrani, 1994). However, the direction and magnitude of these changes varied according to the level and duration of salinization treatment as well as the plant species. 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.

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. 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 (Munns *et al.*, 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. Thus, limited CO₂ availability can alter leaf carbohydrate content and source-to-sink translocation pattern (Pelleschi *et al.*, 1997). Sharkey (1985) reported

that salinity could seriously change the photosynthetic carbon metabolize as well as photosynthetic efficiency. Carbohydrates are accumulated in plant tissues under saline condition and these substances are suspected of contributing to osmotic adjustment (Pattanagule and Thitisaksakul, 2008).

Osmotic adjustment refers to the net accumulation of solutes in cells in response to a fall in the water potential of their environment. As a consequence, the cell osmotic potential lowers and turgor pressure tends to be maintained (Blum *et al.*, 1996). It was reported that the elevated levels of the total soluble and insoluble carbohydrates to the shoot and root are considered to be playing an important role in the osmotic adjustment (Dhanapackiam and Ilyas, 2010).

Khafagi and El-Lawendy (1996) reported that the increase of NaCl level in the soil increased the concentration of total carbohydrate in leaves and roots of sugar beet plants during all stages of growth studied. The distribution of carbohydrates between plant tissues has important physiological implications since, reduction of photosynthesis may arise from feedback effects from carbohydrate accumulation due to reduced utilization.

Moreover, various tissues within a plant may respond differently to salinity (Munns and Termaat, 1986). Although, some studies have been done in the past few years, the mechanisms of salinity tolerance in plants are

not yet well understood. In fact, the understanding in terms of carbohydrate metabolism under salinity condition is still very limited. The main objectives of this study were to explore the changes in leaf carbohydrates and carbon budget of sugar beet plants in response to salt stress in the late growing period.

MATERIALS AND METHODS

A factorial pot experiment in randomized complete block design was carried out under green house condition with four replications. The experiment was conducted with two sugar beet cultivars (Madison and 7233-P₂₉) to investigate carbohydrate concentration and day time leaf carbon budget in salt stressed sugar beet leaf (youngest leaf >50% full size) over photoperiod. Plants were grown in 15 cm diameter pots filled with washed sand. Day length was 16 h during the experiment and relative humidity was between 35-55%. Four levels of salinity 0, 50, 150 and 250 mM (NaCl and CaCl₂ in 5:1 molar ratio) were added to modified Hoagland nutrient solution (Maas and Poss, 1989). Seedlings were irrigated with saline water when most were at four-leaf stage. Water lost by evapotranspiration of plants and pots was replaced by tap water.

To prevent shock to plants, irrigation started with 50 mM saline water and was increased by 50 mM every other day until reaching each salinity level. In addition, the pots were flushed out with saline water containing nutrients every week to ensure homogeneity of salinity and nutrient supply in the growth medium. This was checked by measuring the Electrical Conductivity (EC) of the drainage water.

Net photosynthesis was measured at three times of the day (4, 8 and 12 h into photoperiod) at a photon flux density of 500 $\mu\text{mol}/\text{m}^2/\text{sec}$ at the temperature of the growth condition by using Combined Infra Red Gas Analysis System (CIRAS-1 portable photosynthesis system). Total carbohydrate concentration of the youngest expanded leaf including ethanol soluble carbohydrate and insoluble carbohydrate (starch) concentration were measured based on Dubois *et al.* (1956). The rate of carbohydrate accumulation/depletion calculated from these data. Translocation (export) was calculated as the difference between rates of total gas exchange and carbohydrate accumulation in the leaf (Farrar and Farrar, 1985).

The data for all characters were analysed 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 AND DISCUSSION

Photosynthesis significantly ($p = 0.001$) decreased with increasing salt concentration (Fig. 1). Plants grown at low and high levels of salinity had rates of 81 and 21%, respectively compared to controls.

Analysis of variance revealed that there were no significant differences between cultivars in amount of net photosynthesis but cv Madison had higher net photosynthesis than cv 7233-P₂₉. The adverse effect on photosynthesis was associated with a significant ($p = 0.001$) decrease in the stomatal conductance (gs) (Fig. 2). Salinity treatments caused a significant increase in soluble carbohydrate concentration of leaves in both sugar beet cultivars (Fig. 3a) from around 50 mmol in the control to 150 mmol at highest salt treatment.

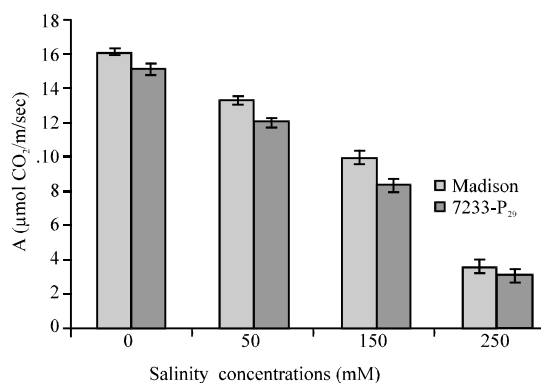


Fig. 1: Net photosynthesis (A) of youngest fully expanded leaf of two sugar beet cultivars at different levels of salinity. Each bar is the average of four measurements. Vertical lines are standard error of the means

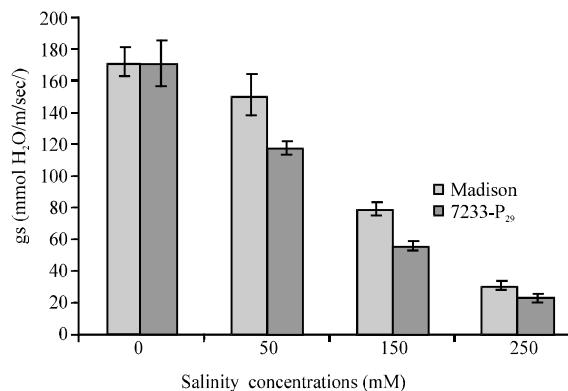


Fig. 2: Stomatal conductance (gs) of youngest fully expanded leaf of two sugar beet cultivars at different levels of salinity. Each bar is the average of four measurements. Vertical lines are standard error of the means

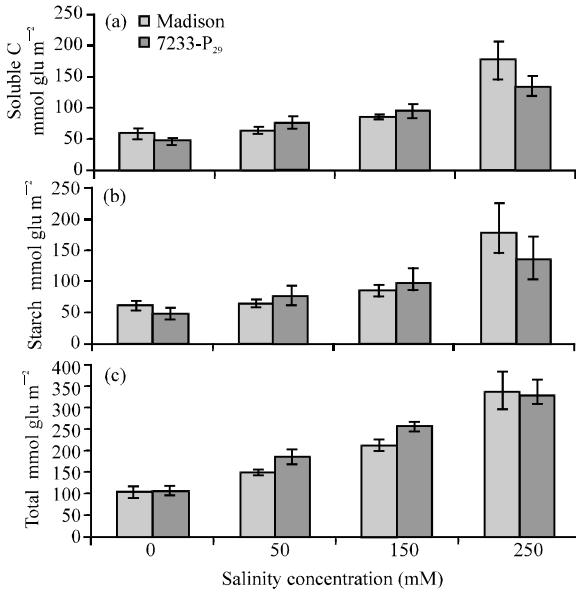


Fig. 3: Salinity concentration and soluble carbohydrate (a) Starch (b) and total carbohydrate (c) content in the youngest fully expanded leaf lamina of two sugar beet cvs, Madison and 7233-P₂₉. Each bar is the mean of three replications

There were no observed significant differences between two cultivars in soluble carbohydrate. Starch concentration of leaves significantly increased with increasing salinity again approximately 3-fold. However there were cultivars differences, cv 7233-P₂₉ having significantly greater starch content than Madison up to 50 mM salinity but not at 250 mM salinity (Fig. 3b).

Total leaf carbohydrate also increased by a factor of three with increasing salt concentration in growth medium. Cultivar 7233-P₂₉ had significantly greater total carbohydrate than Madison only at 50 and 150 mM salinity (Fig. 3c).

Figure 4a shows total carbon fixed per square meter leaf area in photosynthesis process of sugar beet plants. As salinity has inverse effect on photosynthesis process, the total carbon fixed decreased with increasing salinity, these decreases being mostly significant. At control condition the total carbon fixed by leaves of cultivars Madison and 7233-P₂₉ were 238.7 and 229.3 mmol C m⁻² leaf, respectively while at high level salt concentration (250 mM) only 67.1 (Madison) and 98.3 (7233-P₂₉) mmol C m⁻² fixed (Fig. 4a).

Madison's total was significantly lower than that of 7233-P₂₉ at 150 and 250 mM salinity. Daytime amount of carbon translocated from leaf was also significantly reduced by salinity (Fig. 4b).

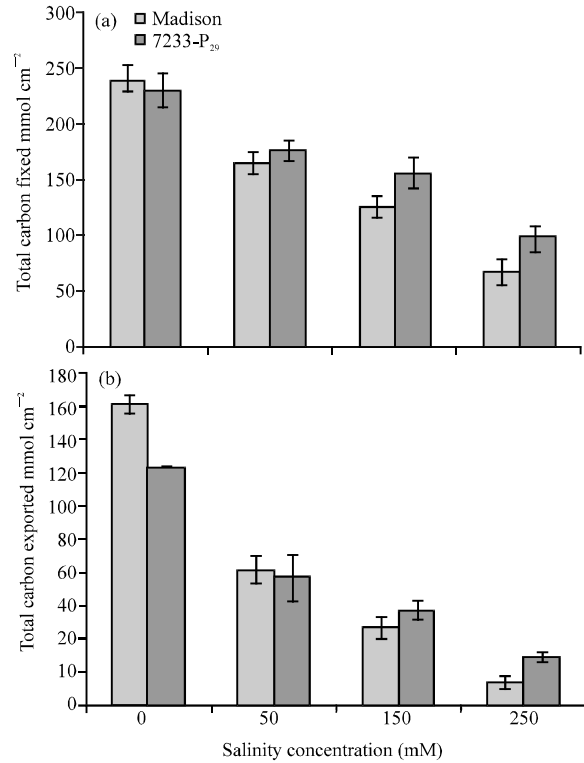


Fig. 4: The amount of total carbon fixed (a) and carbon exported from youngest fully expanded leaf (b) of two sugar beet cvs, Madison and 7233-P₂₉ under different salt concentrations. Each bar is the mean of three replications. Vertical lines indicate standard error of the means

At control condition the amount of carbon exported from leaf was 161.9 and 128.2 mmol C m⁻² for Madison and 7233-P₂₉, respectively. As shown in Fig. 4b, the amount of carbon exported in Madison was significantly >P₂₉. There was no observed significant difference between two cultivars in amount carbon exported at 50 and 150 mM salinity but at the highest salt concentration the amount of carbon exported in cultivars 7233-P₂₉ was greater than Madison.

The present study indicated substantially lower net photosynthesis rates and stomatal conductance with increasing salinity concentrations. Similar results were obtained with other studies (Guanghui and Sternberg, 1993; Everard *et al.*, 1994).

Dadkhah and Griffiths (2006), reported that photosynthesis at intermediate salinity (50 and 150 mM) in sugar beet is limited by decrease in stomatal conductance and at the highest level (350 mM), carboxylation capacity (by measuring C_i) and electron transport (fluorescence) were the apparent prevailing limitations to photosynthesis. There was an increasing

trend in the amount of all types of carbohydrates with increasing salt concentration. These results are in agreement with findings of Khafagi and El-Lawendy (1996) in sugar beet and Pattanagule and Thitisaksakul (2008) in rice.

In contrast, Delane *et al.* (1982) found no increase in soluble carbohydrate in the mature leaves of barley while there was an enhancement of soluble carbohydrate in elongating (youngest) leaves.

Although both soluble and insoluble carbohydrates were deposited in the leaf, it has been confirmed that the transportable form of carbohydrate from source (leaves) to sink (root) in sugar beet is sucrose (Giaquinta, 1979; Zamski and Azenhot, 1981; Thomas, 1986, 1999). Increasing carbohydrates in the leaves might be a response to excess accumulation of monovalent ions in the vacuole under saline stresses. Organic solute, e.g., carbohydrates, amino acids and glycinebetaine accumulated in cytoplasm act as an osmoticum which unlike the monovalent ions is not harmful to the enzyme systems and membranes and thus will balance the lowered osmotic potential of the vacuole (Jones and Storey, 1981), preventing cytoplasmic dehydration.

Another possible reason for increased carbohydrate in salt-stressed leaves could be inhibition of distribution of these sugars to storage and growing tissues (Gorham *et al.*, 1985). In this study, increasing total carbohydrate concentration in the leaves was associated with reduced carbon fixation rate in photosynthesis process (Fig. 4a).

A reduction in photosynthesis with increasing carbohydrate concentration in the leaves may arise from feedback effects from reduced carbohydrate utilisation (Hall and Milthorpe, 1978; Munns *et al.*, 1982). Amount of daytime export of carbon from leaves was also affected by salinity. Terry and Mortimer (1972) reported that plants tend to export sucrose continuously through each 24 h period but in sugar beet export is much less during the night than during the daytime.

The amount of carbon exported significantly decreased with increasing salinity (Fig. 4b). This might be due to inhibition of phloem loading under salt stress conditions. Daie and Wyse (1983) found that ABA which increases under stress conditions, inhibits the phloem loading process. Therefore, reduced loading of phloem increased the feedback inhibition of photosynthesis due to carbohydrate accumulated in leaves under saline conditions.

CONCLUSION

Higher carbohydrate content of the leaf of stressed plants could be an adaptive feature under saline conditions. Salinity significantly decreased the total

carbon fixed per leaf area in both cvs but cv. Madison showed a greater reduction than 7233-P₂₉. Cultivar 7233-P₂₉ also exported a greater amount of carbon from the leaf than Madison under high levels of salt treatments.

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REFERENCES

- Blum, A., R. Munns, J.B. Passioura, N.C. Turner and R.E. Sharp *et al.*, 1996. Genetically engineered plants resistant to soil drying and salt stress: How to interpret osmotic relations. *Plant Physiol.*, 110: 1051-1053.
- Dadkhah, A.R. and H. Griffiths, 2006. The effect of salinity on growth, inorganic ions and dry matter partitioning in sugar beet cultivars. *J. Agricult. Sci. Technol.*, 8: 199-210.
- Daie, J. and R.E. Wyse, 1983. Regulation of phloem loading in *Phaseolus vulgaris* by plant growth regulators. *Proceedings of the Meeting on Plant Growth Regulators Society of America, (PGRSA'83)*, Michigan, East Lansing, pp: 139-144.
- Delane, R., H. Greenway, R. Munns and J. Gibbs, 1982. Ion concentration and carbohydrate status of the elongating leaf tissue of *Hordeum vulgare* growing at high external NaCl. 1. Relationship between solute concentration and growth *J. Exp. Bot.*, 33: 557-573.
- Dhanapackiam, S. and M.H.M. Ilyas, 2010. Effect of salinity on chlorophyll and carbohydrate contents of *Sesbania grandiflora* seedlings. *Indian J. Sci. Technol.*, 3: 64-66.
- Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith, 1956. Colorimetric method for the determination of sugars and related substances. *Anal. Chem.*, 28: 350-356.
- 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 graveolens* L.) at various levels of root zone salinity. *Plant Physiol.*, 106: 281-292.
- Farrar, S.C. and J.F. Farrar, 1985. Carbon fluxes in leaf blades of barley. *New Phytol.*, 100: 271-283.
- Giaquinta, R.T., 1979. Phloem loading of sucrose: Involvement of membrane ATPase and proton transport. *Plant Physiol.*, 63: 744-748.

- Gorham, J., R.G.W. Jones and E. McDonnell, 1985. Some mechanisms of salt tolerance in crop plants. *Plant Soil*, 89: 15-40.
- Guanghui, L. and L.D.S.L. Sternberg, 1993. Effects of salinity fluctuation on photosynthetic gas exchange and plant growth of the red mangrove (*Rhizophora mangle* L.). *J. Exp. Bot.*, 44: 9-16.
- Hall, A.G. and F.L. Milthorpe, 1978. Assimilation source-sink relationship in *Capsicum annuum* L. effects of fruit excision on photosynthesis, leaf and stem carbohydrates. *Aust. J. Plant Physiol.*, 5: 1-13.
- Jones, R.G.W. and R. Storey, 1981. Betaine. In: *The Physiology and Biochemistry of Drought Resistance in Plants*, Paleg, L.G. and D. Aspinall (Eds.). Academic Press, Sydney, pp: 172-204.
- Khafagi, O.M.A. and W.I. El-Lawendy, 1996. Salt tolerance of sugar beet (*Beta vulgaris* L.). II. Metabolic Products and ion accumulation. *Ann. Agricult. Sci. Moshtohor prod.*, 34: 1647-1663.
- Maas, E.V. and J.A. Poss, 1989. Salt sensitivity of wheat at various growth stages. *Irrigation Sci.*, 10: 29-40.
- Munns, R. and A. Termaat, 1986. Whole-plant responses to salinity. *Aust. J. Plant Physiol.*, 13: 143-160.
- Munns, R., H. Greenway, R. Delane and J. Gibbs, 1982. Ion concentration and carbohydrate status of the elongating leaf tissue of *H. vulgare* growing at high external NaCl. II. Cause of the growth reduction. *J. Exp. Bot.*, 33: 574-583.
- Munns, R., R.A. James and A. Lauchli, 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot.*, 57: 1025-1043.
- Pattanagule, W. and M. Thitisaksakul, 2008. Effect of salinity stress on growth and carbohydrate metabolism in three rice (*Oryza sativa* L.) cultivars differing in salinity tolerance. *Indian J. Exp. Biol.*, 46: 736-742.
- Pelleschi, S., J.P. Rocker and J.L. Prioul, 1997. Effect of water restriction on carbohydrate metabolism and photosynthesis in mature maize leaves. *Plant Cell Environ.*, 20: 493-503.
- Sharkey, T.D., 1985. Photosynthesis in intact leaves of C3 plants: Physics, Physiology and rate limitations. *Botanic. Review*, 51: 53-105.
- Terry, N. and D.C. Mortimer, 1972. Estimation of the rates of mass carbon transfer by leaves of sugar beet. *Can. J. Bot.*, 50: 1049-1054.
- Thomas, T.H., 1986. Hormonal Control of Assimilate Movement and Compartmentation. In: *Plant Growth Substances*, Bopp, M. (Ed.). Springer Berlin Heidelberg, New York.
- Thomas, T.H., 1999. Sugar Beet. In: *Crop yield, Physiology and Processes*, Smith, D.L. and C. Hamel (Eds.). Springer-Verlag, Berlin Heidelberg, Germany, pp: 311-331.
- Zamski, E. and A. Azenhot, 1981. Sugar beet vasculature. I. cambial development and the three-dimensional structure of the vascular system. *Botanical Gazette*, 142: 334-343.
- Zidan, M.A. and H.S. Al-Zahrani, 1994. Effect of NaCl on the germination seedling and some metabolic changes in sweet basil (*Ocimum basilicum*). *Pak. J. Ind. Res.*, 37: 541-543.