# **The Effect of Salinity on Growth, Inorganic Ions and Dry Matter Partitioning in Sugar Beet Cultivars**

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### **ABSTRACT**

This study investigated intra-pectific variation in the growth parameters and discontinuos of sugar bect cultivars at different levels of salinify (0, 50, 150, 130, 130, 130, 130, 130, 141 and archive a trivarian cross (22 **This study investigated intra-specific variation in the growth parameters and dry matter partitioning of sugar beet cultivars at different levels of salinity (0, 50, 150, 250 and 350 mM NaCl + CaCl2 in 5:1 molar ratio) using four cvs, one British (Madison) and three Iranian cvs (7233-P<sub>12</sub>, 7233-P<sub>21</sub> and 7233-P<sub>29</sub>). The plants were grown in a controlled greenhouse environment for about 18 weeks. Although growth parameters such as leaf area and dry matter accumulation were stimulated or unaffected at a low level of salinity (50 mM), higher salt concentrations significantly decreased all growth traits. At a high**  level of salinity, cv P<sub>29</sub> showed a significantly higher leaf area and total dry matter than **others at 16 weeks of salt treatment. Pre-dawn leaf-water potential (ΨL) and shoot water**  content (SWC) were decreased by salinity. Cvs Madison and P<sub>12</sub> had significantly lower **ΨL (more negative) and shoot water content at high levels of salt treatment (250 and 350**  mM), while P<sub>29</sub> and P<sub>21</sub> showed higher Ψ<sub>L</sub> (less negative) and shoot water content at the **same salt concentrations. Analysis of ion accumulation revealed that Na<sup>+</sup> and Cl- contents**  were greatly increased in leaves under saline conditions. However, salt tolerant P<sub>29</sub> had lower Na<sup>+</sup> and Cl<sup>-</sup> concentrations than other cvs at high levels of salinity. The root dry **matter of sugar beet cvs at the lowest and the highest salt concentrations reduced by 23.3% and 89.8%, respectively compared to the non-stressed plants, while shoot dry matter decreased by 1.1% and 77% at the same salt concentrations, respectively, after 16 weeks of salt treatment. The pattern of dry matter partitioning to different parts of the plant was changed by salt stress and the dry matter allocated to the storage roots was reduced by 6% and 18% at the lowest and the highest salt concentrations, respectively, at 16 weeks after salinisation.** 

**Keywords**: Dry matter partitioning, Growth, Salinity, Sugar beet.

# **INTRODUCTION**

Salinity is one of the major constraints on crop production in numerous parts of the world, especially in arid and semi-arid regions, where soil and water-borne salts become concentrated due to inputs of irrigation water and high rates of evapotranspiration (Kingsbury *et al*., 1984). Plants vary in their ability to cope with salinity, being capable of growth in a wide diversity of habitats ranging from non-saline environments to salt marshes. Differences in salt tolerance exist not only between species but also amongst genotypes of certain species. This latter aspect attracts increasing studies on the mechanism of salt tolerance and applied research such as adaptation of crop species to saline soils (Polyakoff-Mayber and Lerner, 1999).

Sugar beet is a glycophytic member of *Chenopodiaceae* (Ghoulam and Fares, 2001). It is sensitive to elevated salinity at germination and the early seedling phase of development (Ghoulam and Fares, 2001) and tolerant at later growth stages (Katerji, 1997). The predominant influence of salinity stress on plants is growth suppression. It has

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Iowing for adaptation to smootic through irregation rom the time<br> *Archive by* the accumulation of compati-<br> *Archive by* the concentration of compati-<br> *Archive of Ma*<sup>2</sup> (No avoid said Roagland Ir<br> *Archive of Na<sup>2</sup> infl* been reported that the fresh and dry weight of roots and leaves of sugar beet varieties were reduced with increasing salt concentration of irrigation water (Higazi *et al*., 1995; Yassen *et al*., 1998; Mekki and El-Gazzar, 1999). Growth reduction under salt stress may be because of osmotic stress due to lowering of the external water potential or the effect of ion toxicity on metabolic processes (De-Herralde *et al*., 1998). Under salt stress, plants have evolved complex mechanisms allowing for adaptation to osmotic adjustment by the accumulation of compatible solutes such as glycinebetaine and proline (Yeo, 1998; Bohnert *et al*., 1999) and lowering the toxic concentration of ions in the cytoplasm by restriction of  $Na^+$  influx or its sequestration into the vacuole and/or its extrusion (Binziel *et al*., 1988). In a saline environment, plants uptake excessive amounts of Na<sup>+</sup> at the cost of K<sup>+</sup> and Ca<sup>2+</sup> (Rengle, 1992) and can lead to nutrient deficiencies (Muhammad *et al*., 1987; Francois and Donovan, 1991). Sodium accumulation could disrupt membrane integrity and inhibit the transport of nutritional ions into the root and up to the shoot (Cramer *et al*., 1994). As the main sugar beet growing areas in Iran are affected by salinity and this problem causes a serious handicap for cultivation and production of this agricultural crop, more tolerant sugar beet varieties must be selected and recommended for the saline areas. Accurate selection requires an understanding of the mechanisms involved in salt tolerance in this species. The objective of this present work is to assess four sugar beet varieties for their salt tolerance and to gain more information on dry matter partitioning and inorganic ions accumulation under salt stress.

### **MATERIALS AND METHODS**

### **Plant Materials and Experimental Conditions**

Four varieties of sugar beet (*Beta vulgaris* L.) three of Iranian origin  $(7233-P_{12}, 7233-P_{12})$  $P_{21}$ , 7233- $P_{29}$ ) plus one of British origin (Madison) were used. The seeds of each cultivar were sown in a shallow polyethylene tray filled with vermiculite grade 0.2-1mm, and the seedlings were transplanted into 15 cm diameter plastic pots containing washed sand and with a saucer under them to prevent leaching after irrigation. Six weeks later, the plants were transplanted into 32 cm diameter plastic pots. Five salinity treatments 0, 50, 150, 250 and 350 mM (NaCl +  $CaCl<sub>2</sub>$  in 5:1 molar ratio) were imposed through irrigation from the time that most plants were at the 4-leaf stage. Salt was added to a modified Hoagland nutrient solution (Mass and Poss, 1989) in increments of 50 mM day $^{-1}$  to avoid salt shock. The plants were kept in a warm glasshouse where the temperature ranged between 15-35˚C for about 18 weeks. Relative humidity was 35% and 55% during the day and night, respectively.

### **Measurement of Growth Parameters and Leaf Water Potential**

Growth parameters were measured as leaf area, number of leaves per plant and dry matter accumulation. Plants were harvested at 16 weeks after salt treatment and were separated into leaf lamina, petioles, stem and roots. The fresh weight of the aerial parts was determined immediately after harvesting. These components were dried for 48 hours at 75˚C in a conventional oven and weighed. Dry matter partitioning in the lamina, petioles, stem and root was determined after drying the whole samples. Leaf area was determined using a leaf area meter (Delta-T Devices Lt.d., Cambridge, U.K). Pre-dawn leaf water potential  $(\Psi_l)$  of the youngest fully expanded leaves was measured by thermocouple psychrometers and a microvoltmeter Model 85 (J.R.D.Merrill Special Equipment, Logan, Utah, USA). Pre-dawn leaf water potential was determined on detached leaf discs  $(0.5 \text{ cm}^2)$ placed into a water bath at 25˚C for approximately 2 hours to equilibrate before readings were taken. To determine leaf wa-

### **Analysis of Inorganic Ions**

For Na<sup>+</sup> and K<sup>+</sup> analysis, samples of dried leaves and roots were ashed in a furnace for 24 hours at 500˚C. The ash was dissolved in 50% hydrochloric acid, diluted in distilled water and filtered through a sheet of Whatman No. 2 filter paper. The Na<sup>+</sup> and  $K^+$ contents were determined by flame photometer. Chloride (Cl<sup>-</sup>) determinations were made on a distilled water extract of the dried sample, shaken for 24 hours and then filtered through a 90mm Whatman No.2 filter paper. The amount of Cl was measured by ion-exchange chromatography using a DIONEX Model DX 500, fitted with a CD20 conductivity Detector, IP 25 pump and AS 14 Ion Exchange Column.

### **Data Analysis**

This experiment was carried out based on a randomized complete block design. The data for all parameters were analysied using the analysis of variance procedure of the Statistical Analysis System (SAS) soft ware, version 6.12. Means were compared using a Duncan's multiple range test at 0.05 percent probability level for all the comparisons.

# **RESULT AND DISCUSSION Leaf Dynamics**

Live leaf area was greatly reduced by high levels of salinity (Table 1). Live leaf area of plants at the highest salinity (350 mM) was decreased by 77% (average of all 4 cvs) compared to the leaf area of non-stressed plants at 16 weeks after salinisation (Table 1). The leaf area of cvs  $P_{29}$  and  $P_{21}$  were not reduced by the low level of salinity (50

*Archive of SID* mM), while the leaf area of cv Madison was decreased by about 11.9% compared with non-stressed plants at 16 weeks of salinisation. The best cv, which had the least reduction (61.8%) in leaf area and the highest area  $(1923 \text{ cm}^2)$  at the highest level of salinity was  $P_{29}$  (Table 1), while cv Madison showed the greatest reduction (83.8%) in leaf area and the lowest area  $(793 \text{ cm}^2)$  due to salinity at the same level of salt treatment (Table 1). Leaf numbers per plant decreased significantly as salinity increased in all cvs. However, a low level of salt treatment (50 mM) slightly decreased the leaf number per plant in all cvs, except cv  $P_{29}$  which had more leaves at a low level of salinity in comparison with its control. This increase was not, however, statistically significant. Cv Madison had the greatest reduction in leaf numbers due to salinity amongst cvs at 16 weeks of salt treatment (Table 2). However, the number on leaves of Madison was higher than on other cultivars at all levels of salinity but its total leaf area was less than others. Therefore, it can be concluded that Madison genetically produces more, but smaller leaves.  $P_{29}$  had the smallest reduction in leaf number due to salt at 16 weeks. The results show that leaf number was less affected than leaf area by salinity. It is suggested that most of the reduction in plant leaf area was caused by the inhibition of leaf expansion. This is consistent with the results of previous research, which showed that high levels of salinity decreased leaf area due to a combination of a decrease in cell number and in cell size (Brugnoli and Lauter, 1991; De-Herralde *et al*., 1998). Munns and Termaat (1986) demonstrated that, for a given amount of NaCl transport to the shoot, reduction in leaf expansion results in the same proportional increase in the leaf NaCl concentration. Salt stressed barley plants produced smaller leaf areas, which caused a higher  $Na<sup>+</sup>$  accumulation in a specific leaf area (Munns, 1985). Witkwski and Lamont (1991) reported that plants might reduce water loss by reducing their evaporation surface. Therefore, leaves tend to be smaller and thicker under saline conditions.



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### **Dry Matter Production and Partitioning**

Shoot dry weight was decreased by salinity  $(P \le 0.0001)$  (Table 3). The shoot dry weight of cv  $P_{29}$  was stimulated at low level of salinity (50 mM). Cultivar  $P_{29}$  had a higher dry weight than other cvs at high level of salinity, which can be attributed to greater leaf area (Table 1).

Root dry weight was also decreased by salinity (Table 4). An increasing shoot / root ratio with increasing salt concentration (Figure 1) showed that the growth rate of the

shoot/root ratio of Madison at all levels of salinity was less than in other cvs, possibly because of production of more root dry weight and less shoot dry weight than others (Tables 3,4) and selection by its breeders for diversion of a high proportion of assimilate to the root. A possible reason for dry matter reduction could be the greater reduction in uptake and utilization of mineral nutrients by plants under salt stress. Pessarakli and Tucker (1985, 1988) reported that the total nitrogen uptake of cotton plants decreased with increasing salinity, reflecting primarily



Figure 1. The shoot: root ratio of four sugar beet cultivars at 16 weeks after salinisation under different salt treatments. The values are mean of six replications  $(\pm S.E.)$ .

shoot was not reduced as much as that of the root. It seems that root growth in sugar beet plants is more sensitive than shoot growth because, despite stimulation of shoot dry weight at low level of salinity (Table 3), root dry weight decreased by 23.3% at 16 weeks after salinisation (Table 4). This might be due to the type of sugar beet root (storage root) and also to water deficiency caused by concentration of salt in the growth medium. Abdolahian-Noghabi (1999) found that the shoot/root ratio of *Beta vulgaris* increased under drought stress conditions. The

a dry matter reduction. The uptake of nitrogen (N) in salt stressed plants might be competitively limited by Cl<sup>-</sup> (Aslam *et al.*, 1984, Ward *et al*., 1986).

The partitioning of photosynthetic materials among different organs is an important component in any description of crop growth. However, it has been given only limited attention to date, and many crop models include only simple definitions in which fixed fractions are partitioned to different organs throughout specific phases of development (Weir *et al*., 1984). Salt stress



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Figure 2. Overall effect of salinity on dry matter partitioning to leaf lamina, petiole, crown and storage root of four sugar beet cultivars at 16 weeks after salt treatment.

<sup>26</sup> 0<br>
<sup>10</sup> 0<br>
<sup>250</sup> mM <sup>560</sup> mM <sup>150</sup> mM <sup>250</sup> mM <sup>350</sup> mM<br>
<sup>260</sup> mM <sup>360</sup> mM <sup>360</sup> mM <sup>370</sup> matrix on dry matrix partitioning to leaf lamina, petical<br>
and storage root of four sugar bete cultivars at 16 weeks after salt altered the pattern of dry matter partitioning in sugar beet plants (Figure 2). Despite 6% reduction in sugar beet root dry weight due to low level of salinity (50 mM), the lamina and petioles dry weight in sugar beet were stimulated by 3% and 2%, respectively. These results demonstrate that the high level of salinity reduced the proportion of dry weight allocated to the storage root by 18%. Conversely, high level of salinity increased the proportion of dry matter allocated to sugar beet lamina by 22%. These results are in agreement with Abdollahian-Noghabi and Williams (1998) who obtained similar results for dry matter allocation in sugar beet root under drought stress conditions.

Milford *et al*. (1988) found no evidence of a discontinuity in the partitioning between shoot and root at any stage in crop development under normal conditions, and they concluded that there is normally a progressive shift in partitioning to the storage root as the crop develops. Clover (1997) found that water deficiency had a variable affect of dry matter partitioning.

### **Leaf Water Potential (ΨL) and Shoot Water Content (SWC)**

Shoot water content and leaf water potential were decreased by salinity (Tables 5 and 6). The shoot water content (SWC) of plants grown at low level of salinity (50 mM) was greater than in the control but not significantly different, except for  $P_{12}$ . The SWC of cvs Madison and  $P_{12}$  were significantly decreased by increasing the salt concentration but no significant differences were observed in SWC between the control and any of the salinity levels in  $P_{21}$  and  $P_{29}$ . Also with declining SWC, the values for leaf water potential (Ψ<sub>L</sub>) reduced. Madison and P<sub>12</sub> had significantly the lowest  $\Psi_L$  (more negative, with values of  $-2.7$  and  $-2.9$  Mpa, respectively) at high levels of salt treatment (250 and 350 mM), while  $P_{29}$  and  $P_{21}$  had higher levels (less negative) at the same salt concentrations (Table 6). The percentage reduction of  $\Psi$ <sub>L</sub> in Madison at high levels of salinity was more than twice that compared to  $P_{21}$ .

Salt treatment induced a reduction in shoot water content and  $\Psi_L$ . This reduction was



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more important in the less tolerant variety, Madison, than in the more tolerant one,  $P_{29}$ . The decrease in SWC indicated a loss of turgor that resulted in limited water avail ability for the cell extension process (Katerji *et al*., 1997). Salinity strongly reduced leaf water potential in all cvs. However, cvs  $P_{21}$ and  $P_{29}$  had significantly higher (less negative)  $\Psi_L$  than Madison and P<sub>12</sub> at high levels of salinity at 16 weeks of salt treatment. This may be attributed to a greater water loss in Madison and  $P_{12}$  at higher levels of salinity or could be due to the transport of more  $Na<sup>+</sup>$ and Cl<sup>-</sup> to the leaves under saline conditions

(Heuer and Plaut, 1989).

#### **Inorganic Ion Concentration**

The presence of salt in the rooting medium induced a remarkable increase in the Na<sup>+</sup> concentration in leaves and roots (P≤ 0.0001) (Figure 3). There were significant differences in leaf sodium concentration among cvs at different levels of salinity. As can be seen in Figure 3, cv  $P_{29}$  had a significantly higher Na<sup>+</sup> content in its leaves at the lowest salinity (50 mM) than cvs Madison and  $P_{12}$ . However, at the highest salinity



Figure 3. Concentration of  $\mathrm{Na}^+$ ,  $\mathrm{K}^+$  and Cl in shoot and root of four sugar beet varieties submited to five salt treatments for 16 weeks. The values are means of six replications  $(\pm S.E.)$ .

archive than others at all text increases may be extrained that the teament. At high levels of saling more sensitive to salinity than  $P_{29}$  and  $P_{21}$  had significantly lower and that economic yield of states than other level cv  $P_{29}$  had significantly lower sodium content while Madison and  $P_{12}$  had the highest Na<sup>+</sup>. Leaf potassium  $(K^+)$  concentration significantly decreased with increasing salinity to around half of the control values at 350 mM (Figure 3). Cl- contents in leaves of all sugar beet varieties increased with increasing salt concentration (Figure 3). There were no significant differences in leaf Clconcentration between different cvs in control plants. However,  $P_{12}$  had a significantly higher leaf Cl content than others at all levels of salt treatment. At high levels of salinity cvs  $P_{29}$  and  $P_{21}$  had significantly lower Cl<sup>-</sup> content in leaves than other cvs. The four varieties tested accumulated more Na<sup>+</sup> and  $K^+$  and Cl ions in the leaves than in the roots under salinity (Figure 3). These varieties are qualified as includers (Yeo and Flowers, 1985). Accumulation of inorganic ions, predominantly  $Na<sup>+</sup>$  and Cl, plays 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 (Al-Kateeb, 1997; Ashraf and Ahmad, 2000; Ghoulam *et al*., 2002). The increase in Na<sup>+</sup> and Cl<sup>-</sup> concentrations in the salinised expanded leaves of all cultivars was not similar. Cultivar  $P_{29}$  (salt tolerant) accumulated less Na<sup>+</sup> and Cl<sup>-</sup> in its cells at high levels of salinity. Schachtment and Munns (1992) reported that the lower rate of Na<sup>+</sup> accumulation in expanding leaves of *Triticum* species might be due to higher leaf expansion and/or a mechanism that limits the transport of uptake of  $Na<sup>+</sup>$  by roots. The lower  $Na^+$  and Cl<sup>-</sup> contents in  $P_{29}$  at high levels of salinity can be attributed to its higher leaf expansion than other cvs at high salinity. Another possibility might be that this cv absorbs  $Na^{\dagger}$  and Cl up to a threshold level and then changes to being a salt excluder.

### **CONCLUSION**

This assessment of the effect of salinity on

the growth parameters in four sugar beet cvs allows us to conclude that all of the parameters considered were affected by salinity with a cultivar difference. Moreover, the Madison cv was the least tolerant and  $P_{29}$ was the most tolerant cv. Water loss and inorganic ion accumulation were involved in the osmotic adjustment. Salt stress altered the pattern of dry matter partitioning in sugar beet. Dry matter allocated to the shoot was increased under saline conditions, suggesting that root growth of sugar beet is more sensitive to salinity than shoot growth and that economic yield of sugar beet is likely to be adversely affected by saline conditions.

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# **تاثير تنش شوري بر رشد، ميزان يونها و توزيع ماده خشك در ارقام چغندر قند**

**ع. ر. دادخواه و ح. گريفيتز چكيده** 

عم**کر در دادخواد و ح. گویدایتز**<br>تبقی بهنظور بررسی تفاوکهای درون گویه ای واریتههای چغندر قند، در پارامترهای رشد و توزیع<br>یقی بهنظور بررسی تفاوکهای درون گویه ای واریتههای چغندر قند، در پارامترهای رشد و توزیع<br>اینم کنت تبدارها اين تحقيق بمنظور بررسي تفاوتهاي درون گونه اي واريتههاي چغندر قند، در پارامترهاي رشد و توزيع ماده خشك تحت تيمارهاي مختلف شوري (،0 ،50 ،150 250 و 350 ميلي مول مخلوط نمكهاي كلرورسديم و كلرور كلسيم به نسبت مولي 5 به يك) با استفاده از چهار واريته، يك واريته انگليسي (ماديسون) و سه واريته ايراني (12P7233- ، 21P7233- و 29P7233- (انجام شد. آزمايش در گلخانه تحت شرايط كنترل شده بمدت 18 هفته اجرا گرديد. اگر چه پارامترهاي رشد از قبيل سطح برگ گياه و تجمع ماده خشك در سطح پايين شوري (50 ميلي مول) در واريتههاي مختلف چندان تحت تأثير قرار نگرفتند، غلظتهاي بالاتر شوري بطور معني داري همه صفات رشد را كاهش دادند. در بالاترين سطح شوري (350 ميليمول) واريته 29P بطور معني داري سطح برگ و ماده خشك بيشتري نسبت به ساير واريتهها در هفته شانزدهم پس از اعمال شوري داشت. پتانسيل آب برگ (Ψl (قبل از طلوع خورشيد و محتوي آب اندامهاي هوايي (SWC) با افزايش شوري كاهش يافتند. واريتههاي ماديسون و P۱۲–۷۲۳۳ در سطوح شوري بالا (۲۵۰ و ۳۵۰ ميلي مول) بطور معني داري  $\Psi_{\text{l}}$ (منفي تر) و SWC كمتري داشتند، در حاليكه P۲۹ و P۲۱ و SWC بيشتري در همين سطوح شوري داشتند. ميزان يونهاي سديم ( Na<sup>+</sup>) و كلر Cl ) ) در شرايط شوري افزايش يافت، اما واريته 29<sup>P</sup> كمترين ميزان سديم و كلر را نسبت به ساير واريتهها - در سطوح شوري بالا داشت. در شانزده هفته پس از اعمال شوري ماده خشك ريشه واريتههاي چغندر قند در پايينترين و بالا ترين سطوح شوري بترتيب 23/3 و 89/8 درصد در مقايسه با شاهد كاهش يافت، در حاليكه ماده خشك اندامهاي هوايي در همين سطوح شوري بترتيب 1/1 و 77 درصد كاهش نشان دادند. تنش شوري الگوي توزيع ماده خشك به قسمتهاي مختلف گياه را تغيير داد بطوريكه تخصيص ماده خشك به ريشه گياه در پايين ترين (۵۰ ميليمول) و بالاترين (۳۵۰ ميليمول) سطوح شوري پس از شانزده هفته بترتيب 6 و 18 درصد در مقايسه با شاهد كاهش يافت.