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Response of Root Yield and Quality of Sugar Beet (*Beta Vulgaris*) to Salt Stress

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ABSTRACT- Pot experiment was carried out in greenhouse conditions to investigate the response of root yield and quality of sugar beet cultivars irrigated with saline water (tap water as control, 50 mM, 150 mM, 250 mM and 350 mM NaCl + $CaCl_2$ in 5 to 1 molar ratio). Root yield decreased with increasing salinity. The low level of salinity decreased root yield of Madison and P_{29} cultivars by 25% and 36.1%, respectively as compared to the control, while high level of salinity decreased root yield by 95.7% and 89.1%. Root sucrose content of the two cultivars increased with increasing salt concentration up to 150 mM but further salt concentration, tended to decrease root sucrose content of cv Madison. Salinity significantly increased the concentration of α-amino-N and Na^+ in the storage root. In contrast, potassium content tended to decrease at high levels of salt treatment. Salt stress increased unwanted sugars such as raffinose, glucose and fructose in the storage root. Although root impurity increased with increasing salinity, white sugar content (WSC) increased up to 150 mM salt treatment due to increasing sucrose content. However, higher salt concentration decreased the white sugar content of cv Madison because of the inverse effect of high levels of salinity on the sucrose content as well as greater molasses sugar. Cultivar P_{29} had greater white sugar content than Madison at high levels of salinity. The greater white sugar content in P_{29} at 150 mM and 250 mM was due to a greater root sugar content while at 50 mM salinity, it was because of lower molasses sugar. These results indicated that white sugar content increased at moderate salinity but white sugar content per plant drastically decreased because salinity had a greater effect on root yield than on white sugar content. Therefore, for saline lands, plant density per unit area should be considered.

Keywords: Sugar beet, Root quality, Sucrose, Salt stress, Root yield

INTRODUCTION

The salinity problem in agriculture is particularly important in arid and semi-arid regions where soil and irrigation water often contain high levels of salts and rain fall is insufficient to leach salts from plant root zone (14).

It is well known that in higher plants adaptation to saline conditions is associated with metabolic adjustments leading to the accumulation of specific organic solutes such as sugars, amino acids and also accumulation of ions like Na^+ which retard plant growth (4, 11, 13, 19, 23). Sugar beet is a glycophytic member of the chenopodiaceae (7). It is one of just two crops (the other one being sugar cane) which constitute the only important source of sucrose. Therefore, the main aim of sugar beet processors

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worldwide is to produce pure sugar, at least expences from the roots. However, the crystallization rate of sucrose highly depends on root quality (18).

Beet quality is not a single character which can be presented in a quantitative form by using a single numerical value. From all the factors that determine the technological value of beets, the relative proportions of crystalizable sugar and sugar in molasses are the most important. Amino acids, K^+ and Na^+ can inhibit the crystalization of sucrose from molasses (10, 22). It has been demonstrated that not only K^+ , Na^+ and amino acids, but also invert sugar degradation products (glucose + fructose), raffinose, nitrate etc. inhibit the crystalization of sucrose (8). Raffinose is an important impurity in the sugar beet. It is molassigenic, but more importantly it strongly polarizes light in the same direction as sucrose (21). Therefore, its presence in abnormally large concentrations can cause a serious over-estimation of the quantity of present sucrose and an understimation of the amount of impurity per unit weight of sugar.

The effects of salinity on various processes in plants have been studied intensively over a long period of time. However, little research has been done on the effect of salinity on the quality of crops. Since the main sugar beet growing areas in Iran are affected by salinity. The aim of this study was to assess two sugar beet cultivars for their salt tolerance and to explore the changes in the biochemical compounds of the sugar beet root in response to salt stress.

MATERIALS AND METHODS

A factorial pot experiment in a randomised complete block design was carried out in greenhouse conditions with four replications. The minimum temperature in the greenhouse was 10 ºC and the maximum depended on the duration and intensity of sun light, varying between 15-35 ºC. The relative humidity was between 35-55%. Maximum photon flux density was about 300 µmol $m^{-2}s^{-1}$ on sunny days and the minimum about 80 µmol $m^{-2}s^{-1}$ on cloudy days at midday Two sugar beet cvs (Madison and 7233-P_{29}) were grown in 35cm diameter plastic pots containing one part loamy soil and two parts washed sand. Five levels of salinity including control, 50, 150, 250 and 350 mM NaCl and $CaCl_2$ in a 5:1 molar ratio were added to the modified Hogland nutrient solution (17). Three weeks after emergence, seedlings were irrigated with saline water. 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. Day length ranged from 15.50 h at the start of the experiment on 10 May to the maximum 18 h on 20 June to 12 h at the end of experiment on 25 September. The plants were harvested at the end of the growth season.

Leaf area was determined using a leaf area meter (Delta-T Devices Lt.d. Cambridge, U.K.). High-Performance Liquid Chromatography (HPLC, DIONEX, DX-100, USA) was used to measure concentration of specific neutral sugars (glucose, fructose, sucrose and raffinose). Metabolite extraction was based on Christopher and Holtum'method (3). The total concentration of free amino acids of storage roots was determined by using the ninhydrine method described by Liu (15). Sodium and potassium contents were determined by using a flame photometer (JENWAY, PEP-7).

Since root impurities (K^+, Na^+ and α-amino-N) are important in determining white sugar content (WSC) in the factory process, they were calculated using the following formula:

WSC = Sugar Content (SC) – Molasses Sugar (MS)

Marlander *et al*. (16) developed a new formula which is used for determining the amount of sugar lost to molasses in the factory process as follows:

$$MS\ (\%beet) = 0.12\ (K^+ + Na^+) + 0.24 *\ \alpha\text{-amino-N} + 0.48$$

Sugar beet root yield (RY) was the fresh weight of clean beet storage roots per plant (g $plant^{-1}$) and sucrose content (SC) was as the percentage of sucrose in the fresh weight of storage roots.

A factorial experiment based on a randomized complete block design was carried out. The data for all characters was analysed using the analysis of variance procedure of the 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

Leaf area was greatly reduced by high levels of salinity. The leaf area of sugar beet plants at high salinity (350 mM) was decreased to 72.8% (average of two cvs) as compared to the leaf area of non-stressed plants (Table 1). Salt stress also significant ly ($P\leq 0.001$) reduced the root yield of sugar beet cultivars.

Table 1. Mean leaf area of sugar beet (cm^2 $plant^{-1}$) at different levels of salinity

	Salinity (mM)				
Cultivar	**0**	**50**	**150**	**250**	**350**
Madison	**$4889^{A\dagger}a$**	**$4306^{B}{}_{a}$**	**$2366^{B}{}_{b}$**	**$1294^{B}{}_{c}$**	**$793^{B}d$**
P_{29}	**$5030^{A}a$**	**$5370^{A}a$**	**$2650^{A}b$**	**$2201^{A}c$**	**$1923^{A}c$**

†-Numbers followed by the same letter are not significantly (P 0.01) different by Duncan's multiple range test (different upper case and lower case letter show significantly differnces within columns and rows, respectively)

The root yield of cv Madison was significantly greater than cv P_{29} up to 150 mM salinity. However, at high levels of salt concentration P_{29} had a significantly higher root yield than Madison (Fig. 1).

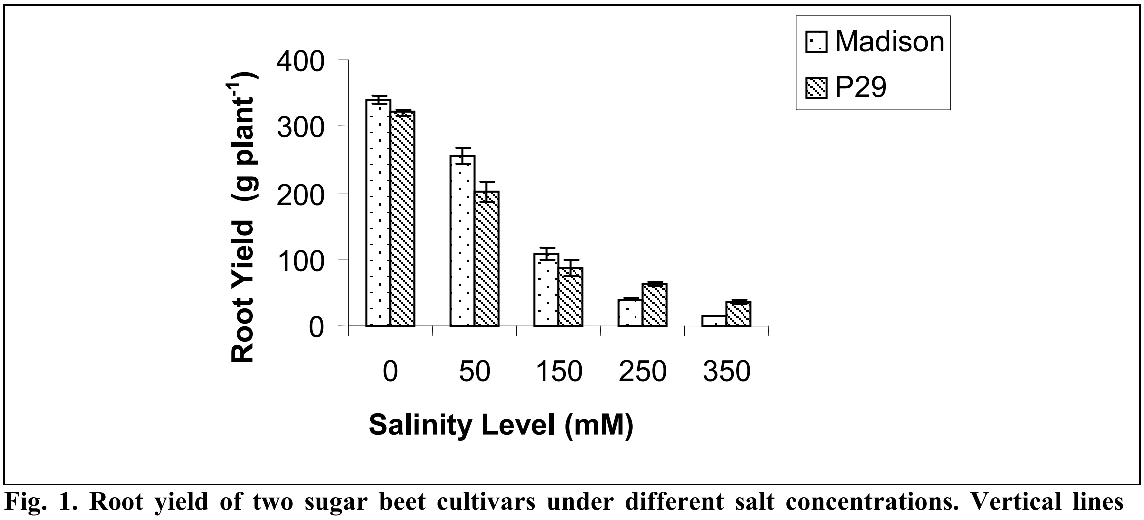

Fig. 1. Root yield of two sugar beet cultivars under different salt concentrations. Vertical lines indicate standard error of the means

Root sucrose content increased with increasing salt concentration up to 150 mM but further salt application decreased Madison root sucrose content (Fig. 2). However, there was no significant difference between 150 and 250 mM salinity levels on the root sucrose content of cv P_{29}. Although the root sucrose content of cv P_{29} decreased at high levels of salt stress, it was significantly higher than Madison at the same salt concentration.

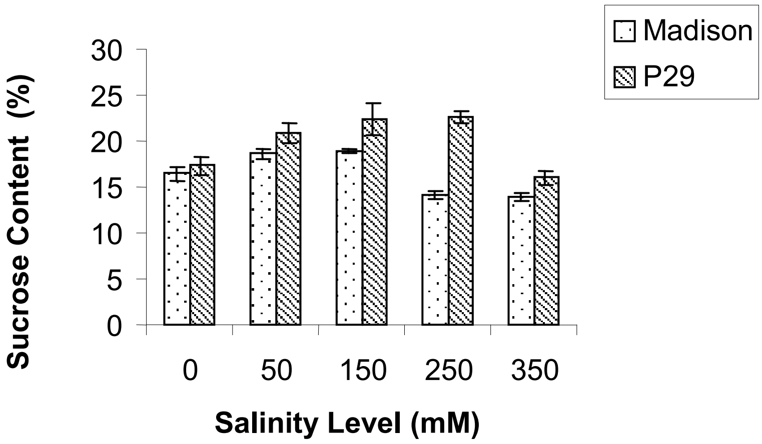

Fig. 2. Percentage root sucrose content of two sugar beet cultivars under different salt treatments. Vertical lines indicate standard error of the means

Concentration of root impurities (K^+, Na^+ and α-amino-N) play an important role in determining white sugar content (WSC) which decreases as the impurities increase. White sugar content (WSC) is represented as the percentage of extractable sugar in the factory process based on the storage root fresh weight.

The concentration of sodium (Na^+) and α-amino-N in the storage roots was significantly increased by salt stress (Tables 2 and 3). Low and high levels of salinity increased the average concentration of Na^+ in storage roots to 1.43 and 5.7 times respectively as compared to the control. Cultivar P_{29} had

Table 2. Sodium and potassium concentration (mmol per 100g root fresh weight) of different levels of salinity in two sugar beet cultivars

	Na^+ (mmol per 100g fresh weight)				
	Salinity (mM)				
Cultivar	**0**	**50**	**150**	**250**	**350**
Madison	2.09^A_e	2.86^A_d	4.99^A_c	8.79^A_b	11.61^A_a
P_{29}	1.24^B_c	1.92^B_c	4.48^B_b	7.03^B_a	7.28^B_a
	K^+ (mmol per 100g fresh weight)				
	Salinity (mM)				
	0	**50**	**150**	**250**	**350**
Madison	4.85^A_a	4.89^A_a	4.32^A_b	4.02^B_b	3.99^A_b
P_{29}	4.65^A_b	5.10^A_a	4.69^A_b	4.40^A_c	4.26^A_c

Numbers followed by the same letter are not significantly (P>0.05) different by Duncan's multiple range test (different upper case letters and lower case letters show significant differences within columns and rows)

A significantly lower Na^+ concentration than cv Madison at all levels of salinity (Table 2). The average concentration of α-amino-N in storage root increased 1.34 and 1.77 times at low and high salt concentration respectively (Table 3). In contrast, potassium concentration tended to decrease at high levels of salt treatment (Table 2). The highest salt concentration decreased potassium concentration the 13.1% as compared to the control while the lowest salinity increased potassium concentration.

Table 3. α-amino-N concentration (mmol per 100g root fresh weight) and molasses sugar (MS) of different levels of salinity in two sugar beet cvs

			α-amino-N mmol per 100g root fresh weight		
			Salinity (mM)		
Cultivar	**0**	**50**	**150**	**250**	**350**
Madison	0.169^{B}_{c}	0.227^{A}_{c}	0.359^{A}_{b}	0.361^{A}_{b}	0.409^{A}_{a}
P_{29}	0.212^{A}_{c}	0.285^{A}_{c}	0.336^{A}_{b}	0.470^{A}_{a}	0.269^{B}_{c}
			MS		
			Salinity (mM)		
	0	**50**	**150**	**250**	**350**
Madison	1.35^{A}_{d}	1.46^{A}_{c}	1.68^{A}_{b}	2.09^{A}_{b}	2.45^{A}_{a}
P_{29}	1.24^{B}_{d}	1.39^{B}_{c}	1.66^{A}_{b}	1.98^{A}_{a}	1.93^{B}_{a}

Numbers followed by the same letter are not significantly (P≥0.05) different by Duncan's multiple range test (different upper case letters and lower case letters shows significant differences within columns and rows)

Salinity also increased unwanted sugar such as raffinose, glucose and fructose in storage root (Tables 4 and 5). Low levels of salinity (50 mM) increased the average amount of glucose, fructose and raffinose to about 1.84, 2.44 and 1.78 times respectively as compared to the control. High salt concentration increased the average amount of these sugars to 5.66, 5.51 and 3.47 times, respectively compared to control.

Table 4. Glucose and fructose content (mg per g root fresh weight) of different levels of salinity in two sugar beet cvs

			Glucose mg g^{-1} F.W		
			Salinity (mM)		
Cultivar	**0**	**50**	**150**	**250**	**350**
Madison	0.37^{B}_{c}	1.12^{A}_{b}	1.30^{B}_{b}	1.77^{B}_{b}	3.35^{A}_{a}
P_{29}	0.98^{A}_{c}	1.38^{A}_{c}	2.66^{A}_{b}	4.24^{A}_{a}	4.14^{A}_{a}
			Fructose mg g^{-1} F.W		
			Salinity (mM)		
	0	**50**	**150**	**250**	**350**
Madison	0.22^{B}_{c}	1.04^{A}_{b}	1.10^{B}_{b}	2.63^{A}_{a}	2.46^{A}_{a}
P_{29}	0.68^{A}_{c}	1.16^{A}_{b}	2.44^{A}_{b}	3.24^{A}_{a}	2.50^{A}_{b}

Numbers followed by the same letter are not significantly (P≥0.05) different by Duncan's multiple range test (different upper case letters and lower case letters show significant differences within columns and rows)

Although, root impurity increased with increasing salinity, white sugar content (WSC) increased up to 150 mM with salt treatment (Table 6) but further salt concentration decreased WSC due to the inverse effect of high levels of salinity on sucrose content and also greater molasses sugar at high salt concentrations. Cultivar P_{29} had greater WSC than Madison at high levels of salinity. The greater WSC in P_{29} at 150 mM was due to greater root sucrose content (Fig. 2) while at 350 mM salinity, it was due to lower molasses sugar (Table 3).

Despite greater sugar content, the root yield of sugar beets under salt stress was less than that of unstressed plants. This could be due to the reduction in leaf area under salt stress condition (Table 1) and also inverse effect of salinity on photosynthesis. Another reason might be the reduction in uptake and utilization of mineral nutrients by plants under salt stress. El-Maghraby *et al.*(6) reported that root yield per unit area increased significantly by increasing potassium fertilizer under saline conditions.

Table 5. Raffinose and sucrose content (mg g^{-1} F.W) of different levels of salinity in two sugar beet cvs

			Raffinose mg g^{-1} F.W Salinity (mM)		
Cultivar	**0**	**50**	**150**	**250**	**350**
Madison	0.53^{A}_{b}	0.62^{A}_{b}	1.31^{A}_{a}	1.22^{A}_{a}	1.39^{A}_{a}
P_{29}	0.44^{A}_{c}	1.11^{A}_{b}	1.56^{A}_{a}	1.88^{A}_{a}	1.95^{A}_{a}
			Sucrose mg g^{-1} F.W Salinity (mM)		
	0	**50**	**150**	**250**	**350**
Madison	164.46^{A}_{b}	186.24^{B}_{a}	188.48^{B}_{a}	142.00^{B}_{c}	138.94^{B}_{c}
P_{29}	173.02^{A}_{b}	208.01^{A}_{b}	224.26^{A}_{a}	226.52^{A}_{a}	$156.88\ ^{A}_{c}$

Numbers followed by the same letter are not significantly (P≥0.05) different by Duncan's multiple range test (different upper case letters and lower case letters show significant differences within columns and rows)

Higher root yield of cv P_{29} at high levels of salinity was due to higher leaf area (Table 1) which can be attributed to a greater salt tolerance (for example higher capacity of osmotic adjusment). Moderate levels of salt stress increased the root sucrose content of the two cultivars as compared to the control. A reason for this might be due to the smaller size of cells because small cells are more efficient at accumulating sucrose per volume and weight unit than larger cells. Thus, sucrose concentration is likely to be a function of the relative proportions of large and small cells, and a storage root of many small cells would be more efficient at accumulating sucrose than composed of large cells (20).

Table 6. White sugar content (WSC %) of different levels of salinity in two sugar beet s

			WSC % Salinity (mM)		
Cultivar	**0**	**50**	**150**	**250**	**350**
Madison	15.91^{A}_{b}	18.11^{B}_{a}	18.32^{B}_{a}	13.68^{B}_{c}	13.35^{B}_{a}
P_{29}	16.79^{A}_{b}	20.29^{A}_{a}	21.90^{A}_{a}	22.12^{A}_{a}	15.30^{A}_{b}

Numbers followed by the same letter are not significantly (P≥0.05) different by Duncan's multiple range test (different upper case letters and lower case letters show significant differences within columns and rows)

Another reason might be due to the inhibition of soluble acid invertase activity under moderate salt concentration. Khafagi and El-Lawendy (12) reported that the stimulation of sucrose accumulation in sugar beet roots under salt saline conditions was probably controlled to a certain extent by the inhibition of acid invertase activity. However, severe salt stress decreased sucrose content as compared to the control. This reduction could be the result of the depressive effect of salinity on the process of photosynthesis (1, 13). It has also been reported that the activity of sucrose phosphate synthase (SPS) decreases at high levels of salt concentration due to the reduction of leaf water potential (mostly negative leaf water potential) (5, 24). Low levels of salt stress increased the average concentrations of α-amino-N and Na^+ in the storage root by 1.34 and 1.43 times while the highest salinity increased by 1.77 and 5.7 times respectively as compared to as control. Cultivar P_{29} had a significantly lower Na^+ concentration than cv Madison at all levels of salinity, especially at high levels of salt concentration. The lower Na^+ content in P_{29} at high levels of salinity can be attributed to the fact that Na^+ absorption up to the threshold level and then chnages it to a salt excluder. The results presented here are consistent with those of Hanson and Wyse (9), Gzik (8) and Ghoulam *et al*. (7) who concluded that salinity stress increased the α-amino-N and Na^+ concentration in the sugar beet. The reason may be that some of these α-amino-N compounds and presumably glycinebetaine found as impurities in the storage roots of stressed plants, result from osmotic adjustment as suggested by Brown *et al*. (2).

The actual potential of white sugar yield per unit area (WSY) is the most economically important index in sugar beet production. Although moderate salt stress increased root sucrose content, the root yield was reduced by salinity (Fig. 1). For example, low level of salt treatment increased root sucrose content of Madison and P_{29} by 2.2% and 3.5% respectively but decreased root yield by 25% and 36.1% as compared to the control. Salinity drastically decreased white sugar content per plant due to its inverse effect on root yield. Therefore, in saline lands, plant density per unit area should be considered.

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