USING FREE PROLINE CONCENTRATION AS AN INDICATOR FOR IDENTIFYING SUGAR BEET VARIETIES RELATIVELY TOLERANT TO SALINITY

MONA M. SHEHATA; T.S. EL-AMMARI AND NOUR EL-HODA M. TAHA

Sugar Crops Res. Institute., Agric. Res. Cent., Giza, Egypt.

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Abstract

Two pot experiment were conducted during two successive seasons 1996/1997 and 1997/1998 to investigate the effect of different levels of saline irrigation water on nine sugar beet varieties (*Beta vulgaris* L.).

The results indicated that Ras poly and Oscar poly multigerm varieties and Eva monogerm variety could withstand a salinity level up to 6260 mgm/liter without a significant adverse effect on quality, weight and sugar yield of the root, however their proline concentration significantly increased with each increase in salt concentration of irrigation water. These varieties selected as very tolerant to salinity stress. On the other hand, all the studieded criteria except proline concentration of Gazelle stress. On the other hand, all the studieded criteria except proline concentration of Gazelle andDelitzsch poly varieties were affected by salinity treatments. Therefore they may be considered as the most sensitive varieties to salinity stress.

Observations over the Monte Bianco, Delamon, Vede and Top varieties showed that they were affected, to some extent by salinity, however no differences were detected between their proline concentration under 4260 and 6260 mgm/l salinity levels. They were moderatly tolerent to salinty stress.

Results indicated that, there were high and positive signifficant correlation detected between salinity level and proline concentration in moderate and very tolerant sugar beet varieties. In sensitive varieties no correlation between salinity level and their proline accumulation was observed.

Thus the results suggest that the concentration of amino acid proline in sugar beet leaves can be used as suitable indicator in screening varieties of sugar beet according to their tolerance to salinity and can be grown and produce a profitable yield under saline conditions.

INTRODUCTION

In Egypt sugar beet cultivated area reached about 103770 feddan in 1998, with productivity of 18.8 ton/fed. This area produced about 1.95 millons tons of sugar beet

roots.. In spite of this productivity the gap between sugar production and consumption is continually widening, making it imperative to be filled by enhancing the sugar crops production especially in new marginal lands, where this crop can withstand adverse environmental conditions. Mass and Hoffman (1977) stated that sugar beet is tolerant to salinity. Tolerance of sugar beet varieties may differ significantly, perhaps because most commercial grown cultivars are developed under non-saline conditions and are not bred to endure salt stress.

Effect of salinity on growth, yield and chemical composition of sugar beet were reported by Bhattacharyya (1986); Shehata (1989); Abd-El Sayed et al (1993) and El-Noemani (1996), they indicated that the adverse effect of salinity is dependent on the concentration of salt, the duration of salt stress as well as growth stage of the plant. They also indicated that the varieties of sugar beet differ in their response to salinitty.

The are certain mechanism to increase the ability of crops to overcome stress injury at certain lemits. Hanson and Hitz (1982) stated that proline was considered as an important amino acid, which served as an osmoprotectant in many plant families. Biochemical studies of McCue and Hanson (1990) indicated that certain metabolites, such as proline, accumulated at high level under drought and salinity stresses. Tanji (1990) postulated that the osmatic potential of the medium, when become lower than that of the plant cell. The plant cell must adjust it self osmoticaly to survive. This phenomenon could be achieved either by absorption of ions from the outer medium which localized in the vacuole or synthesis some organic compounds such as proline which was required in the cytoplasm to achieve the low osmotic potential of the whole cell. Similar conclusion was reported by Delauney and Verma (1993).

Proline accumulation in sugar beet leaves under salinity stress was reported by Shehata (1989), Petrovic et al (1991), Mostafa (1996), El-Noemani (1996) and Gzik (1996). They found that seedling of sugar beet exposed to salt stress are higher in their proline concentration than those grown and non saline condition.

Using of free proline accumulation as screening technique to identify plants relatively to water or salt stresses tolerance was proposed by Singh et al. (1973) on barley, Chiranjivi Rao and Askan (1978) on sugar cane, Sairam and Dube (1984) on wheat and Pandey and Srivastava (1989) on rice. Therefore, this study aimed to compare the physiological response of nine sugar beet varieties to different salinity levels of irrigation water, and accumulation level of free proline in leaves of sugar beet varieties in relation to salinity, which could be used as technique in selecting sugar beet varieties in

relation to salinity, which could be used as technique in selecting sugar beet varieties adapted to salinity strees.

MATERIALS AND METHODS

This investigation was conducted in the Agricultural Research Center, Giza during two successive seasons, 1996/97 and 1997/98 to study the behavior of nine sugar beet varieties, seven of them are multgarms viz Ras poly, Oscar poly, Monte Bianco, Vede poly, Top, Gazelle and Delitzsch poly, and two are monogerm viz Eva and Delamon when grown under four treatments of saline water irrigation beign tap water (260), 2260, 4260, 6260 mgm/lite. Therefore this experiment included 36 treatments which were the combination of nine sugar beet varieties and four salinity level of irrigation water. Each treatment was replicated three times.

Eight seeds from each varietty were sown in tin pot (35-cm diameter and 50 cm hight), on 25 Septemper and 5 October in the first and second growing seasons, respectivily. The inner surface of pot was coated with three layers of bitumen as isolator to prevent the effect of the metal on the soil temperature. In each pot, two Kg of gravel were placed at the bottom to obtain good drainage condition then, each pot was filled with 30 Kg of air dried clay loam soil. The soil properties were as followes: Ec 0.6 millimhos/cm in saturated soil past extract, pH 7.5, organic matter 1.2%, total nitrogen 55.6pmm, available phosphorus 18 ppm and available potassium 565 ppm. Forty days after planting, the seedlings were thinned to three plants per pot. Normal culture practices were carried out as usual.

Artificial saline water for irrigation was prepared by adding sodium chloride and calcium chloride by adding NaCl and CaCl2 (in the ratio of 1:1 by weight) to the tap water (260 mgm/l) to obtain the required concentration. Irrigation with saline water treatmentts were commenced four weeks after planting when sugar beet was in the two - leaf stage, 20% of the pots were weighed every 3 days and the needed amount of water was added to maintain nearly 75% of the total water holding capacity capacity through out the course of the growing season.

Free proline concentration in the leaves of sugar beet, when plants were 4 months old, was determined according to the method of Bates et al (1973). At harvest time (6.5 months age) the following data were recorded:

1. Total soluble solids (T.S.S) were measured by hand refractometer method (A.O.A.C. 1986).

2. Sucrose percentage was determined according to the method of Le-Docte (1927).

3. Juice root purity percentage (sucrose/T.S.S X 100) as described by Carruthers and Oldfield (1961).

4. Fresh weight of root (g/plant).

5. Sugar yield in g/plant (root weight gmxjuice purity % X adjusted sucrose %).

All data were subjected to statistical analysis on the bases of two Factors randomized Complete Design and combined analysis over the two seasons was carried out according to the procedure outlined by Snedecor and Cochran (1981). Treatment means were compared using the Least Significant Difference test. Simple correlation coefficients were calculated between leaf-proline concentration and some characteristics of sugar beet varieties grown under different salinity levels.

RESULTS AND DISCUSSION

Varietal differences:

Data of (Table 1) indicated a clear and marked differences among the nine tested varieties. The difference in root quality of the varieties was signifificant, where Eva variety recorded the highest values of sucrose and purity. While, Gazelle variety got the lowest values of the same two critaria.

Both root weight and sugar yield per root, significantly differed among varieties of sugar beet. Data illustrated in table (1) indicated that Ras poly variety was superior in fresh weight and sugar yield per root, meanwhile, Deltizsch variety was the lowest root weight.

Data in the same table indicated that there are significant differences between varieties in their free proline concentration. Generally, it can be noticed that the Oscor poly, Ras poly and Eva varieties were insignificant differed and exhibted highest proline concentrations. On the other hand, Delamon, Monta Bianco, Gazellae and Delitzsch poly varieties had the lowest insignificantly deffered values of proline concentrations, while Vede poly and Top got moderate values of free proline concentrations.

The above variation among sugar beet varieties was expected. Obead (1988), Nassar (1992) and El-Sayed (1997) explained that this variation was due to their genetic background.

Salinity Effect on sugar beet

Data of Table (1) indicated that there was insignificant differences for all the studied parameters of sugar beet plants which irrigated with lower salinity level (2260 mgm/l) compared with those of control (tap water 260 mgm/l).

Table 1. Effect of varieties and salinity levels on some criteria of sugar beet (Average of 1996/97 and 1997/98 seasons).

Variety		Sucrose %	Sucrose %	Sucrose %	Sucrose %	Sucrose %	Sucrose %
Varieties	Ras poly	16.06	20.50	78.13	718	72.63	2.92
	Oscar poly	15.59	20.25	76.90	613	59.02	3.08
	Eva	16.20	20.09	80.49	609	64.21	2.83
	Monte Bianco	14.50	19.43	74.82	552	47.63	2.19
	Delamon	15.00	19.34	77.47	692	64.00	2.21
	Vede poly	14.69	19.42	75.73.	494	43.52	2.67
	Top	14.60	19.54	74.62	470	40.68	2.47
	Gazelle Poly	14.40	19.89	72.41	482	40.12	2.19
	Delitzsch Poly	14.80	19.43	76.51	293	26.69	2.10
LSD at 0.05		0.16	0.27	1.37	7.79	1.51	0.29
	260	15.21	19.55	77.74	571	54.96	1.98
	2260	15.24	19.47	78.25	567	54.77	2.14
	4260	14.96	19.83	75.57	539	49.12	2.79
	6260	14.91	20.21	73.80	501	44.93	3.27
LSD at 0.05		0.11	0.18	0.91	5.91	1.01	0.19

On the contrary, significant variation was due to the effect of moderate and highe salinity levels of moderate and highe salinity levels of irrigation water (4260 and 6260 mgm/1) in comparison with control treatment.

Sucrose percentage did not significantly differe at salinity levels of 4260 and 4260 mgm/l, although it decreased by 1.5% and 1.97%, respectively, in these two saline treatments in comparison to the control. The corresponding values were 2.8% and 5.1% for purity percentage. While, T.S.S. increased by 1.4% under moderate salinity level (4260 mgm/l) and by 3.4% under 6260 mgm/l in comparison with the control treatment.

Root and sugar yields of studied sugar beet varieties in terms of fresh root weight and sugar yield per plant, significantly decreased by increasing salt concentration in irrigation water. Comparing to the control, the decrease in treatments of 4260, and 6260 mgm/l reached 5.6% and 12.3% for fresh root weight, and 10.6% for sugar/root, respectively. It can be observed that the effect of salinity was more harmful on sugar yield than the root yield.

The influence of the high salinity level on sugar beet yield may be attributed to

the low avaiability of water to plant and to the energy spent to maintain turgo pressure on the expense of growth. This might cause changes in enzymatic activities in the plant cell. These results were supported by the research of Bhattacharyya (1986), Seleman (1986), Abd El-Sayed et al (1993) and Shehata et al (1994). They confirmed that the growth and yield of sugar beet were not markedly affected under the low salinity level, while an obvious reducton was detected under the high salinity level.

All salinity treatments increased proline concentration which increased gradually and significantly. In comparison to the control, this increase reched 8.1%, 40.9% and 65.2%, respectively. The parallel increase of both salt stress and free proline concentration may confirm that proline acted as osmolytes and played an important role in osmoprotection against salt stress in plant cells. Petrovic et al (1991) found that seedling of sugar beet exposed to salt strees had higher concentration of proline than the control. Similar results were obtained by Mostafa (1996), El-Noemani (1996) and Gzik (1996).

Effect of interaction between variety and salinity level on sugar beet

A significant interaction between variety and salinity and salinity level revealed that each factor did not act independently. Data of Table (2) indicated that purity, root weight and sugar yield per root of Ras poly, Oscar poly and Eva varieties were not affected significantly by saline irrigation water. On the contrast, proline concentration in these varieties exhibited a gradual and significant increase with each increasse in salt concentration of irrigation water. Comparing to the control, the increase in free proline concentration under 2260, 4260 and 6260 mgm/l reached 1.49%, 36.33% and 56.58% for Raspoly variety. The corresponding values were 23.6%, 50.1%, and 61.9%, for Oscar poly variety and by 24.4%, 49.7% and 62.4% for Eva variety, respectively.

On the contrary, free proline concentration of the two varieties Gazelle and Delitzsch poly were not affected by irrigation with saline water, although the other parametars were significantly declined (except T.S.S which increased) by increasing salinty levels.

Concerning Monte bianco, Delamon and Vede poly vaieties data in table (2) indicated that they were affected to some extent with salinity treatments thus the lowest salinity level (2260 mgm/l) had no significant effect on all criteria (including proline concentration) as compered with control treatment, as well as there was no significant difference between the two levels of salinity of 4260 and 6260 mgm/l, while a signifi-

Table 2. Interaction effect between varieties and salinity levels on some criteria of sugar beet (Average of 1996/97 and 1997/98 seasons).

Variety	Salinity levels Mgm/l	Sucrose %	T.S.S %	Purity %	Sugar/ Root G.	Root weight G.	Relative Root weight*	Proline µ mol/ gfw	Relative proline µ mol/gfw
Ras poly	260	16.11	20.62	78.31	72.76	716.8		1.98	
	2260	15.80	20.27	78.07	71.74	722.2	1.01	2.01	1.02
	4260	15.99	20.35	77.89	71.21	715.0	1.00	3.11	1.58
	6260	16.03	20.77	77.07	71.82	718.2	1.00	4.56	2.30
Oscar poly	260	15.56	20.122	77.40	59.67	616.7		1.78	
	2260	15.79	0.2820	77.71	60.73	615.5	1.00	2.33	1.31
	4260	15.45	.5520.	75.24	58.22	602.0	0.98	3.57	2.00
	6260	15.53	57	75.13	57.47	616.2	1.00	4.67	2.62
EVa	260	16.15	20.272	79.60	63.92	613.8		1.64	
	2260	16.31	0.1319	81.21	66.03	632.3	1.04	2.17	1.32
	4260	15.95	.9520.	80.21	61.90	598.8	0.98	3.26	1.99
	6260	16.19	62	78.51	61.03	598.2	0.98	4.36	2.66
Monte Bianco	260	14.64	18.931	77.17	50.58	568.2		1.83	
	2260	14.90	8.7019	79.59	54.24	576.7	1.01	2.02	1.10
	4260	14.45	.7520.	73.13	46.08	552.8	0.97	2.51	1.37
	6260	14.09	23	69.40	37.60	491.3	0.86	2.63	1.44
Delamon	260	15.02	18.961	78.90	68.08	721.8		1.99	1.80
	2260	15.20	8.9719	80.11	67.29	713.2	0.99	2.15	1.29
	4260	14.91	.5019.	76.32	62.72	694.3	0.96	2.58	1.35
	6260	14.85	93	74.54	55.96	637.3	0.88	2.60	
Vede poly	260	14.78	19.37	76.48	46.02	514.8		2.32	
	2260	14.98	19.031	78.77	69.02	523.3	1.02	2.20	0.95
	4260	14.48	9.30	74.99	42.89	501.8	0.97	3.15	1.35
	6260	14.52	19.97	72.70	36.14	434.3	0.84	3.52	1.52
Top	260	14.75	19.171	76.84	45.62	506.8		2.28	
	2260	14.87	8.93	78.35	45.49	491.7	0.97	2.20	0.97
	4260	14.47	19.78	72.98	39.97	480.5	0.95	2.73	1.20
	6260	14.23	20.28	70.29	31.66	403.7	0.80	2.65	1.16
Gazalle	260	14.55	19.38	75.01	47.68	547.8		2.24	
	2260	14.37	19.90	72.12	41.98	509.7	0.93	2.17	0.97
	4260	14.17	20.02	70.76	38.82	456.8	0.83	2.16	0.96
	6260	14.15	20.30	69.62	31.99	413.7	0.76	2.18	0.97
Delitzsh poly	260	14.99	18.71	80.44	37.36	398.7		2.06	
	2260	14.91	19.22	77.59	30.44	331.5	0.83	2.06	1.00
	4260	14.58	19.55	75.63	22.22	253.2	0.64	2.12	1.03
	6260	14.63	20.23	72.36	16.73	198.0	0.50	2.14	1.04
L.S.D at 0.05		0.32	0.46	2.37	3.03	15.85		0.53	

*Relative = Treatment/control

cat decline was detected between each one those two levels of salinity and the control (260 mgm/l). It is noticed that root weight of Monto Bianco and Vede Poly varieties significant decreased only by salinity level of 6260 mgm/l.

The present results are in accordance with those of Shehata (1989), Higazy et al (1995), Mostafa (1996) and El Noemani (1996). They stated that quality and the yield of sugar beet varieties were decreased to different degrees, with increasing salt concentration of irrigation water. The reverse trend was observed regarding proline concentration, which its accumulation was relatively more pronounced in the tolerant varieties than sensitive ones.

The previous data showed that the nine sugar beet varieties varied in their response to salt stress. Therefor it can be grouped these varieties to three groups, the first include Ras poly, Oscor poly and Eva varieties which were considered very tolerant to salinity, the second include Monte Bianco, Delamon, Vede poly and Top varieties which were moderately tolerant to salinity, the third group include Gazelle and Delitzsch poly varieties, which were the most sensitive ones.

The Correlation Between Salinity, Proline and Sugar Beet Criteria:

Data of Table (3) clearly indicate that the tolerant of sugar beet varieties to salinity exhibited significant positive correlation between salinity level and free proline accumulation levels. Other correlation's (except purity of Ras poly variety were not significant.

A highly significant positive correlation was found between salinity levels and proline concentration for moderately tolerant varieties. While purity, weight and sugar yield of root were negatively and high significant correlated with either salinity level or proline concentration.

Concerning the sensitive varieties, there was a highly significant negative correlation between salinity level on one hand and purity, weight and sugar yield of root. Whereas the correlation between proline concentration and other criteria were not significant.

The aforenamed discussion clearly indicated that there was high and positive significant correlation between salinity level and proline concentration for moderate and very tolerant of sugar beet. On contrast, varieties of sugar beet, which had no such relationship to salinity and its proline concentration were very sensitive to salinity stress.

Thus it can be concluded that the free proline accumulation under salinity stress can be used as indicator in selection salt tolerant sugar beet varieties.

Table 3. Correlation coefficients between salinity, proline and some criteria of sugar beet varieties.

Group	Variety	Proline	Purity		Root weight		Sugar	
		Slinity	Slinity	Proline	Slinity	Proline	Slinity	Proline
Very Tolerent	Ras poly	0.93**	-0.54**	-0.47	-0.05	-0.13	-0.30	-0.34
	oscar poly	0.97***	-0.23	-0.22	-0.18	-0.15	-0.30	-0.31
	Eva	0.95**	-0.16	-0.06	-0.34	-0.27	-0.34	-0.39
Moderate Tolerent	Mante binco	0.95**	0.80**	-0.80**	-0.70**	-0.67**	-0.78**	-0.78**
	Delamon	0.87**	-0.77**	-0.53**	-0.89**	-0.65**	-0.85**	-0.5**
	Vede poly	0.86**	-0.60**-	-0.64**	-0.82**	-0.67**-	-0.80**	-0.76**
	Top	0.70**	0.79**	-0.60**	-0.87**	0.43**	-0.89**	-0.56**
Very sensitive	Gazalle	0.16	-0.51**	-0.30	-0.99**	-0.15	-0.97**	-0.22
	Delitzech poly	0.03	-0.83**	-0.13	-0.83**	-0.13	-0.84**	-0.12

*Significant at 0.05 level.
**Singificant at 0.01 level.

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إستخدام تركيز البرولين الحر كمؤشر لتحديد مدي تحمل أصناف بنجر السكر للملوحة

منى مكرم شحاته ، طارق سهري العماري ، نور الهدي محمد طه

مركز البحوث الزراعية ، معهد بحوث المحاصيل السكرية، جمهورية مصر العربية، الجيزة.

أجري هذا البحث بالأصص بمزرعة مركز البحوث الزراعية خلال موسمي ٩٦ / ١٩٩٧ ، ٩٧ / ١٩٩٨ لدراسة حساسية أصناف بنجر السكر للملوحة، تتضمن التجربة ٣٦ معاملة (مكررة ثلاثة مرات) عبارة عن التوافق بين تسعة أصناف من البنجر منها سبعة أصناف من بنجر السكر عديدة الأجنة هي Ras poly, Oscar poly, Monte Bianco, Vede poly, Top, Gazelle and Delitzsch poly بالإضافة إلي صنفين وحيد الأجنة هما Eva, Delamon. مع أربعة مستويات ملوحة لمياة الري هي (٢٢٦٠ و ٤٢٦٠ و ٦٦٦٠ ملليجرام/لتر بالإضافة إلي ماء الصنبور كمقارنة ٢٦٠ ملليجرام/لتر).

وقد أوضحت النتائج عدم تأثر كل من نقاوة ووزن ومحصول السكر لجذور ثلاثة أصناف من بنجر السكر وهي Ras poly, Oscar poly, Eva بزيادة تركيز الأملاح لماء الري حتي ٦٢٦٠ ملليجم/لتر، ولذلك أعتبرت هذه الأصناف متحملة للملوحة كما زاد تركيز الحمض الأميني البرولين في أوراق هذه الأصناف زيادة تدريجيا ومعنوية بتزايد تركيز الأملاح بماء الري. علي العكس من ذلك تأثرت كل الصفات المدروسة للصنفين Gazelle, De-litzsch Poly تحت كل مستويات ملوحة ماء الري وبذلك ثبت حساسية هذين الصنفين للملوحة مع عدم تزايد تركيز البرولين بأوراقها بزيادة مستوي تركيز الأملاح لماء الري. وقد تأثرت نسبة كل من السكروز والمواد الصلبة الذائبة وبالتالي نقاوة ووزن الجذور ومحصول السكر للجذر بدرجات متفارتة للأصناف Vede poly, Top Monte Bianco, Delamon, مع ملاحظة عدم وجود فروق معنوية في تركيز البرولين لأوراقها عند الري بماء الصنبور أو بماء تركيز الملاح به ٢٢٦٠ ملليجم/لتر. وكذا بين معاملتي الري بمياه تحتوي علي ٤٢٦٠ أو ٦٢٦٠ ملليجم/لتر، ويمكن إعتبار أن هذه الأصناف متوسطة التحمل للملوحة.

وقد وجد أرتباط موجب عالي المعنوية بين تركيز الأملاح بمياه الري ونسبة البرولين الحر المتراكم في أوراق أصناف بنجر السكر شديدة ومتوسطة التحمل للملوحة، بينما لم تتأكد معنوية الإرتباط بين هذين المتغيرين لنباتات الأصناف الحساسة للملوحة. وبذلك يمكن إتخاذ تركيز البرولين الحر بأوراق بنجر السكر كوسيلة لإنتخاب الأصناف المقاومة للملوحة من بين أصناف بنجر السكر. وتحديد أفضل الأصناف مقاومة للملوحة التي يمكن زراعتها في الأراضي الجديدة مع إمكانية أستخدام ماء يصل تركيز الأملاح به ٦٢٦٠ ملليجرام/لتر في ريها.