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FREE PROLINE RELATION TO SALINITY TOLERANCE OF THREE SUGAR BEET VARIETIES

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Abstract

Sugar beet varieties, Tribel, Maribo Marocpoly and Kawegiga Mono, differed in their osmoresponse under saline water irrigation in pot experiments during 1985/86 and 1986/87. Results revealed that Tribel is the most salt tolerant variety copmared with the other two varieties. Tribel can withstand 6000 ppm salt concentration of irrigation water without any significant reduction in fresh and dry weihts of its roots, as well as in the total soluble solids and sucrose percentage. Irrigation, generally, resulted in a very sharp decline in the amount of free proline in the leaves of the three sugar beet varieties. Increasing salt concentration of irrigation water caused a gradual and significant increase in the accumulation of proline in leaves of cv. Tribel over all salinity treatments (2000, 4000 and 6000 ppm), while insignificant differences in proline concentration were detected between the control (tap water) and 2000 ppm. For the other two varieties, the increase of proline concentration was significant under 4000 ppm treatment, while insignificant differences in proline concentration were detected between the control and 2000 ppm, as well as between 4000 ppm and 6000 ppm salinity levels. Gradual increase in the accumulation of proline in the Tribel variety provides evidence of the relationship between proline accumulation and salinity tolerance in sugar beet.

INTRODUCTION

The limited available water resources represent the main problem limiting the agricultural dexelopment in the arid and semi-arid regions. The perfect application of the different water resources is needed. Application of drainage water for irrigation depends mostly on their effect on crops. Mass and Hoffman (1977), tabulated a number of economic crops according to their tolerance to salt concentrations and their expected yield percentages. They stated that sugar beet crop is a tolerant one. Tolernace of varieties within species may differ significantly, perhaps because most commercially grown cultivars are developed under non saline conditions and not are bred to endure salt stress.

Ashour and Thalooth (1971) studied the effect of irrigation with saline water on sugar beet growth. The results revealed that saline irrigation water up to 6000 ppm greatly decreased fresh and dry weights of root. While the percentage of sucrose was not significantlt affected by using different levels of salinity (4000 and 6000 ppm) in the irrigation water.

Allam and Ali (1982) inducated that soil salinty adversely affected growth of sugar beet and that the varieties differed in their response to salinity. They added that covariance analysis showed that both the total soluble solids (T.S.S.) and sucrose content were not significantly affected by salinity. The ratio between sucrose persentage and T.S.S. was significantly affected.

Seleman (1986), in sugar beet found that all growth parameters studied were significantly affected by salt concentrations. For example fresh weight of roots decreased by 14 and 26% as NaCl concentration increased from 3500 to 7000 ppm, respectively. The corresponding values were 12 and 29.6% reduction in dry weight. He indicated that saline condition increased appreciably the quality of sugar beet roots. The increment magnitude for sucrose and T.S.S. was most pronounced than that of purity. Similar results were obtained by Yassen *et al.* (1988), and Shehata *et al.* (1994), on others varieties of sugar beet. While, Abd El-Sayed *et al.* (1993), indicated that the fresh weight sugar beet Ras Poly variety was increased with saline irrigation.

Studies regading proline accumulation in sugar beet plants are rare. While accumulation of free proline in tissues of several plant series is regarded as a general response to stresses of different organisms. Batanouny *et al.* (1985) working on different species including desert shrubs, halophytic grass and *Zea mays*, Doering and Alexander (1982) working on cotton varieties and Karamanos *et al.* (1983) and

Sairam and Dube (1984) working on wheat, they found that free proline increased under water and osmotic stresses as well as under salt stress.

In laboratory experiments with Sorghum, Pavlovskaya and Khramov (1990) found that the application of 0.1, 0.2, 0.4 or 0.8 M NaCl or Kcl decreased seedling growth and increased proline accumulation compared with seedlings grown in a non-saline medium, and the effects increased with increasing salt concentrations. Barker (1991) found also, a large increases in leaf proline with water stress occurred in the greenhouse but rarely in the field, for six variety of Sorghum.

Chen and Wang (1991) indicated that the proline content of R 8968 *Saccharum sinensis* which was selected for its resistance to growth inhibition by hydroxyproline, was 3.8 times higher than that of the HYP- sensitive donor. In addition R8968 showed higher tolerance of NaCl. It was concluded that proline accumulation may favour the increase of tolerance to environmental stress.

Recently, Petrovic *et al.* (1991) found that the seedlings of sugar beet cv. Ns-Hy exposed to water stress had considerably higher concentrations of proline and nitrates than the control.

The present investigation was performed to compare the physiological ability of three sugar beet varieties to grown under different levels of salinity in irrigation water. The present work, also, aimed to investigate some aspects of proline accumulation in the leaves of three cultivated varieties of sugar beet in Egypt.

MATERIALS AND METHODS

Three triploid sugar beet varieties, two multigerms, Tribel and Maribo Maroc Poly, and one monogerm, Kawegiga Mono were used in the present study. Pot experiments were carried out during two seasons (1985/86 and 1986/87) to study the behavior of these varieties when grown under saline water-irrigation. Concentrations used for different salinity levels of irrigation water were 2000, 4000, 6000 ppm and the control was tap water. Water was artificially salinized by adding NaCl and $CaCl_2$ (in the ratio of 1: 1 by weight) to attain the required concentrations.

Twenty five seeds were sown at a constant depth of about 1 cm in plastic pot (35 cm in diameter and 50 cm in depth). In each pot, a 3 cm clean gravel layer was placed at the bottom to obtain good drainge conditions, then each pot was filled with 30 kg of soil. The physical and chemical analys of the soil under investigation are given in Table 1. Forty days after planting, the seedlings were thinned to five plants per pot.

Phosphorous was applied as calcium super phosphate (15.5% P_2O_2) and potassium as potassium sulfate (48% K_2O) at rates of 15 P_2O_5 kg and 24 K_2O kg/feddan, respectively, and were mixed with the top soil in each pot before planting. Nitrogen was used as ammonium sulfate (20.5% N) at the rate of 50 kg/feddan, half the amount of nitrogen from the first addition.

Irrigation was applied regularly and uniformly according to plant needs and weather conditions for attaining nearly 70% of the total water holding capacity through the course of the experiment.

All cultural practices were carried out as usual. Free proline concentration in the leaves of the three sugar beet varieties was determined according to the method of Bates *et al.* (1973). When plants were 4 months old, free proline concentration (μ molls/g fresh weight) was determined under salinity and drought stresses (2 days before irrigation). Another sample was collected and analyzed for salt stress 2 days after irrigation at 3, 4 and 5 months of age.

Fresh and dry weights of root per plant in gm were recorded at 2, 4 and 6 months during the growing season.

At harvest (6 months of age), total soluble solids (T.S.S.) were measured hand refractometer method (A.O.A.C.1955) and sucorse percentage of the root juice was determined polarimeticall according to the method of Le-Docte (1927).

A split plot design with four replications was used. The main treatment consisted of the three varieties of sugar beet, i.e., Tribel Maribo Maroc Poly and Kawegiga Mono. The sub plot treatments were salinity levels, i.e. 0000, 2000, 4000 and 6000 ppm.

All data were subjected to statistical analysis according to the procedures outlined by Sendecor and Cochran (1967). Treatment means were compared using multiple range and multiple F tests according to Duncan (1955). While the interaction effects of salinity x variety were compared using the least Significant Differences according to Waller and Duncan (1969).

Table 1 . Physical and chemical analysis of soil samples used in the present investigation.

I- Mechanical analysis :	Sand	Silt	Clay	
%	21.3	43.2	35.5	
II- Chemical analysis :				
pH				7.5
E.C., m mhos/cm				2.2
Total N, ppm (Jackson, 1958)				75.6
Available P, ppm (Jackson, 1958)				24.21
Cations and anions meq/L (Chapman and Pratt, 1961)				
Calcium				1.92
Magnesium				3.19
Sodium				2.6
Potassium				1.9
Carbonate				--
Chloride				2.2

RESULTS AND DISCUSSION

I. Effect of salinity treatment on growth and yield of sugar beet roots :

Growth of sugar beet varieties in terms of fresh and dry weights of root per plant at 2 &4 months of age and the yield of sugar beet in terms of fresh and dry weights of roots at 6 months (harvesting time) as affected by different salinity treatments are shown in Table 2.

The statistical analysis of data revealed that neither variety nor salinity level had significant effect on the fresh or dry weight of roots during early growth stages (60 days). But with advancing age, varieties significantly differed in their fresh and dry weights of roots. Also, a reduction was noticed in both fresh and dry weights of roots of the three studied varieties, under the highest level od salinity. However, insignificant difference was detected between the fresh weight of plants irrigated with either 4000 or 6000 ppm saline water.

As for the interaction effect of salinity levels and variety, it was noticed that the fresh and dry root weight of Tribel variety were not significantly affected by all salinity treatment. While, Maribo Maroc Poly was significantly reduced by 4000 and 6000 ppm, and Kawegiga Mono was significantly reduced by 2000, 4000 and 6000 ppm salinity level or irrigation water.

Reduction magnitude in fresh and dry weights under saline sonditions varied with the different varieties. Data of 6 months age showed that under the highest salt concentration of irrigation water (6000 ppm) fresh weight of roots were depressed by 5%, 15% and 26%, compared with those of the control, for Tribel, Maribo Maroc Poly and Kawegiga Mono, respectively. The corresponding vlues were 5%, 9% and 19% for dry weight.

The present results are in accordance with those of Allam and Ali (1982), Seleman (1986), Bhattacharyya (1986), Yassen et al. (1988) and Shehata et al. (1994). They found that the fresh and dry weights of roots and leaves of sugar beet varieties were reduced to different degrees, with increasing salt concentration of irrigation water. In addition, that the salinity and variety interaction effect was statistically significant. That means that each factor acts independently.

Table 2. Effect of salinity levels on fresh and dry weights of root per plant (g) of three varieties of sugar beet.

Variety(V)	Salinity(S)	Fresh weight 2	4	6 months	Dry weight 2	4	6 months
Tribel	0000	15.4	172.3	302.2	2.9	38.6	70.5
	2000	16.	181.7	315.9	3.0	39.6	69.6
	4000	14.7	168.2	298.3	2.7	41.0	67.3
	6000	13.9	168.6	286.8	2.7	39.7	67.1
	Mean	15.1a	172.7a	300.8a	2.8a	39.7a	68.6a
Maribo Maroc Poly	0000	16.4	144.5	301.7	3.1	36.8	68.9
	2000	14.5	145.2	295.4	2.7	35.8	69.9
	4000	14.2	131.4	262.4	2.8	31.9	64.4
	6000	14.1	126.8	256.3	2.8	33.3	63.0
	Mean	14.8a	137.0b	279.0b	2.8a	34.4b	67.6a
Kawegiga Mono	0000	16.7	126.2	271.7	3.0	31.3	65.3
	2000	14.5	132.7	248.7	2.7	33.4	61.6
	4000	13.9	113.5	218.0	2.7	29.8	59.6
	6000	13.8	112.9	200.6	2.7	29.5	52.6
	Mean	14.7a	121.3c	234.8c	2.8b	31.0c	59.8b
Overall Mean	0000	16.2a	147.7a	291.9a	3.0a	35.6a	68.3a
	2000	15.1a	153.2a	286.7a	2.8a	36.2a	67.0a
	4000	14.2a	137.7b	259.6b	2.7a	34.2a	64.8a
	6000	13.9a	136.1b	247.9b	2.8a	34.2a	60.9b
L.S.D 0.05	for VxS	N.S	N.S	18.3	N.S	N.S	3.8

II. Effect of salinity on root quality :

Table 3 presents the values of total soluble solids percentages (TSS%), sucorse % and Ourity % of three varieties of sugar beet roots at harvest time (6 months) under different salinity levels.

The data indicated significant differences, concerning TSS%, between Kawegiga Mono and the other two varieties. Data showed, also, that TSS% increased in the three studied varieties with increasing level of salinity. This can be due to more salt absorption by plants as salinity level increases, which in turn decreases purity and negatively affects sucorse percent, insignificant difference was detected between the TSS% of plants irrigated with 4000 ppm and 6000 ppm saline water. A significant interaction between variety and salinity level was revealed from the same data. However, the TSS% of Tribel was insignificantly affected by salinity levels, whereas, Maribo Maroc Poly and Kawegiga Mono, both showed a significant increase in TSS% under 4000 ppm as well as 6000 ppm treatments.

The differences in sucrose percentages due to varieties were significant. Tribel recorded the highest value. However, Maribo Maroc Poly and Kawegiga Mono showed insignidicant difference in their sucrose%. On the other hand, different salinity levels had no significant effect on the sucrose percentages within each variety.

Data in Table 3 clearly illustrated that the three varieties of sugar beet significantly differed in their purity (sucrose as percentage of the solid matter). Tribel recorded the highest purity followed by Kawegiga Mono and Maribo Maroc Poly.

Concerning the effect of salinity on Purity, data revealed that increasing salt concentration of irrigation water, caused a gradual decrease in the purity of sugar beet. However, insignificant differences were detected between the purity of root of plants irrigated with the control (tap water) and 2000 ppm, as well as between the purity of roots of plants irrigated with 4000 ppm and 6000 ppm salinity level.

The differences in purity percentage due to interaction effect of variety and salinity were significant. Purity percentage of the Tribel variety was significantly reduced by the highest salinity level (6000 ppm). While Maribo Maroc Poly was affected by 4000 ppm, and Kawegiga Mono was significantly reduced by 2000 ppm sa-

Table 3. Effect of salinity levels on root quality of three varieties of sugar beet at harvest time (6 months).

Variety(V)	Salinity(S) ppm	TSS%	Sucrose%	Purity%
Tribel	0000	21.6	18.5	85.6
	2000	21.6	18.4	85.2
	4000	21.8	18.7	85.7
	6000	21.8	18.4	84.4
	Mean	21.7a	18.5a	85.2a
Maribo Maroc Poly	0000	20.9	16.8	80.4
	2000	20.7	16.9	81.5
	4000	21.4	16.5	77.1
	6000	21.4	16.7	78.0
	Mean	21.1a	16.7b	79.3b
Kawegiga Mono	0000	19.8	17.3	87.4
	2000	19.8	17.0	85.9
	4000	20.4	16.8	82.4
	6000	20.7	16.8	81.2
	Mean	20.1b	17.0b	84.2a
Overall Mean	0000	20.8a	17.5a	84.9a
	2000	20.7a	17.4a	84.2a
	4000	21.2b	17.3a	81.7b
	6000	21.3b	17.3a	81.2b
L.S.D 0.05	for VxS	0.4	N.S.	1.05

linity level. These results are in full agreement with those published by Ashour and Thalooth (1971) and Allam and Ali (1982), who indicated that sucrose percentage was insignificantly affected by salinity. However, purity was significantly reduced by increasing salt concentration. Similar results were obtained by Abd El-Said et al. (1993) and Shehata et al. (1994) on others varieties of sugar beet.

Salinity effect on purity is highly important in sugar industry, since the net obtained sugar depends mainly on this character. Purity decreases as salts in juice increase. Accordingly, operational problems such as scaling will result in beet sugar processing. Also, molass production will increase at the account of sucrose recovery. Sugar, which is the end product, will be adversely affected.

The present data showed that all the studied parameters of Tribel variety, were less affected by salinity treatments than those of the other two varieties. Therefore, Tribel variety may be considerd as the most tolerant variety to salinity.

III- Relation of proline to salinity tolerance :

a. Effect of water stress on proline accumulation:

Data in Table 4 clearly reveal, consistent and wide differences between the values of free proline concentration in samples both before and after irrigation. Irrigation, generally, resulted in a very sharp decline in the amount of free proline. Free proline concentration of sugar beet leaves showed reversed response when eliminating the stress condition by applying water. Free proline accumulation ratio, i.e., before and after irrigation (B/A) at the higher salinity level (6000 ppm) were 3.31, 3.89 and 4.15 for Tribel, Maribo Maroc Poly and Kawegiga Mono, respectively. It is evident from these results that the lower the B/A ratio, the more tolerant is the variety. Abo El-Khaeir (1985) and Batanouny *et al.* (198), indicated that the leaves of *Zea mays* exhibited very wide differences in free proline content before and after irrigation, similar results of Barker (1991) on Sorghum and Petrovic *et al.* (1991) on sugar beet cv. Ns-Hy 11.

b. Effect of salinity stress on free proline accumulation :

Data in Table 4 revealed that, increasing salt concentration of irrigation water above 2000 ppm caused a gradual an significant increase in the accumulation of proline in the tested varieties. This increase was 33%, 11% and 17% at the 6000 ppm as compared with the control for Tribel, Maribo Maroc Poly and Kawegiga Mono, respectively before irrigation. After irrigation their percentages were 105%, 70% and 64% for the three varieties, respectively. Under stress, Tribel accumulates more free proline to tolerate high salt concentrations than the other two varieties. This variety responds faster and accumulates a higher percentage of free proline in response to water and salinity stresses.

Table 4 . Effect of salinity levels on irrigation water on free proline accumulationin leaves of three varieties of sugar beet at four months of age.

Variety(V)	Salinity(S) ppm	μMoles /GFW Before Irrigation (B)	After Irrigation (A)	B/A ratio
Tribel	0000	19.80 (100)	3.86 (100)	5.13
	2000	20.15 (102)	3.86 (100)	5.22
	4000	23.30 (118)	6.20 (161)	3.76
	6000	26.15 (133)	7.90 (205)	3.31
	Mean	23.35a	5.46a	
Maribo Maroc Poly	0000	19.08 (100)	3.19 (100)	5.98
	2000	18.75 (98)	3.15 (99)	5.95
	4000	20.76 (109)	5.31 (166)	3.91
	6000	21.12 (111)	5.43 (170)	3.89
	Mean	19.93b	4.26b	
Kawegiga Mono	0000	19.45 (100)	3.30 (100)	5.89
	2000	18.81 (97)	3.50 (100)	5.37
	4000	22.74 (117)	5.10 (155)	4.46
	6000	22.75 (117)	5.40 (164)	4.15
	Mean	20.15b	4.33b	
Overall Mean	0000	19.44a (100)	3.45a (100)	5.63
	2000	19.24a (99)	3.50a (101)	5.50
	4000	22.27b (115)	5.54b (161)	4.02
	6000	23.11c (119)	6.24c (181)	3.70
L.S.D. 0.05 for V x S		1.5	0.47	

In support of these findings, Doering and Alexander (1982), working on cotton varieties, reported that under saline conditions, an accumulation of free amino acids, could be found in the leaves of all tested varieties; proline is the chief constituent of free amino acids in response to water and salinity stresses. Karamanos *et al.* (1983) and Sairam and Dube (1984) found that the more negative values of leaf water potenial were associated with higher amounts of free proline in the various organs of different wheat cultivars, grown in the field with or without irrigation. Similar results were obtained by Batanouny *et al.* (1985) working on different plant species including desert shrubs, halophytic grass and *Zea mays.*

Abo El-Kheir (1985) found that in *Zea mays* high soil salinity increased proline content in plants grown on moderately saline soil. Plants growing in normal soil had lower proline contents than those from other treatments. Batanouny *et al.* (1985) found that stress due to salinity caused proline accumulation in maize plants grwon on saline soil. Pavlovskaya and Khramov (1990) found increase in proline accumulation in seedling of Sorghum grown in saline condition compared with non saline medium.

c. Interaction effect of salinity and variety on proline accumulation:

Comparing plants of the three varieties grown under natural conditions (irrigated with tap water, Table 4), it can be clearly seen that they differed slightly in their proline concentration both before and after irrigation. The values of proline concentration for Tribel, Maribo Maroc Poly and Kawegiga Mono were 19.80, 19.08 and 19.45 mol/g before irrigation and 3.86, 3.19 and 3.30 mol/g after irrigation, respectively. Differences due to the interaction effect of variety and salinity levels, were significant in both samples (before and after irrigation). A significant increase was found in proline concentration of the Tribel variety with each increase in salinity level above 2000 ppm. Proline concentration of Tribel increased before irrigation by 2%, 18% and 33% compared to the control, when grown under 2000, 4000 and 6000 ppm salt concentration, respectively. No difference was dected between 4000 and 6000 ppm salt for Maribo Maroc Poly and Kawegiga Mono. Similar trends were noted for the three varieties after irrigation (Table 4).

The rate of accumulation of free proline varied widely in the different varieties of sugar beet grown under the highest level of salinity (6000 ppm). The increased amount of free proline, in Tribel compared to the other two varieties grown under similar conditions of salinity, may provide an evidence of the relationship between proline accumulation and drought tolerance mechanisms. Thus, differential accumulation of free proline by sugar beet plants under stress conditions (water or salt) may used as a useful indicator in the selection of drought resistant varieties.

In this respect, Chen and Wang (1991) in sugar cane, indicated that proline accumulation may favour the increase of tolerance to environmental stress, and the resistance variety has high concentration of proline than the sensitive one .

Based on the aforenamed discussion, further studies are needed to elucidate the biochemical aspects of water and salt stress effect on sugar beet varieties. These studies are needed to explain the relationship between growth stage stress and salt resistance of sugar beet plants and the effect on yield and quality.

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علاقة تراكم حامض البرولين الحر بتحمل أصناف بنجر السكر للملوحة

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إختلفت نباتات أصناف بنجر السكر (تريبل Tribel - ماريبو مركوبولي Maribo MarocPoly - وكاوجيجامونو Kawegiga Mono) في درجة إستجابتها لتركيزات ملوحة مياة الري وقد تفوق الصنف تريبل بالنسبة للصنفين الآخرين في مدي تحملة حيث لم يتأثر كل من الوزن الرطب والجاف لجذورة وأيضاً نسبة المواد الصلبة الذائبة والسكروز بإستخدام مياة ري تحتوي علي ٦٠٠٠ جزء في المليون من الأملاح.

وأوضحت النتائج أن زيادة تركيز الأملاح في مياة الري من ٢٠٠٠ الي ٤٠٠٠ والي ٦٠٠٠ جزء في المليون أدي الي زيادة معنوية في تراكم البرولين الحر في أوراق نباتات الصنف تريبل وكانت زيادة حامض البرولين الحر في أوراق نباتات الصنفين الآخرين معنوية عند تركيز ٤٠٠٠ جزء في المليون فقط . وكانت إستجابة الصنف تريبل لزيادة ملوحة مياة الري سريعة ومتناسبة طردياً مع تركيز الأملاح وتراكم حمض البرولين الحر مما يؤدي الي تحمل هذا الصنف للملوحة وظروف الجفاف البيئي . وقياس كمية حامض البرولين الحر مفيدة لإنتخاب أصناف مقاومة للملوحة والجفاف في أصناف بنجر السكر وتقدم وسيلة سريعة للإنتخاب لهذة الصفة في برامج تربية هذا المحصول .