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Osmotic Adjustment in Sugar Beet Plant under Salinity Stress

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ABSTRACT

This study was carried out in the General Commission for Scientific Agricultural Research (GCSAR) at Der Ez Zour Agricultural Research Center, during 2009- 2010 growing seasons. The role of Na^+, K^+, Na^+/K^+, carbohydrates accumulation of leaves, and sugar content of roots on the osmotic adjustment was studied in 10 sugar beet genotypes (five were monogerms and five were multigerms), under salinity stress. Sugar beet plants were irrigated with saline water, with the electrical conductivity (EC) ranging from 8.6-10 $dS.m^{-1}$ in the first year and 8.4-10.4 $dS.m^{-1}$ in the second year. A randomized complete block design (RCBD) with three replicates was used. The results showed that Na^+ content in leaves and roots of all genotypes was increased in salinity stress, but the increment in leaves was higher than in roots. K^+ contents in leaves and roots were decreased in all genotypes, but this reduction was lower in roots as compared with leaves. This may be due to the substitution of Na^+ with K^+ in such condition. However, under salinity stress concentrations of inorganic solutes (Na^+, and K^+) in leaves was higher than those in roots. Kawimera (multigerm) was considered the most tolerant genotype because of high Na^+ content in its leaves and roots, whereas the most sensitive genotype was Tigris (multigerm), which had the lowest content of Na^+ in leaves and roots. Generally, the accumulation of soluble sugars in leaves was higher in tolerant genotypes as compared with non-tolerant ones. The results exhibited no correlation between sugar content in roots and salinity stress. Correlation analysis showed Na^+ content followed by soluble sugars as the main solutes for osmotic adjustment in sugar beet leaves under salinity conditions. Moreover, both sucrose and Na^+ contents in beet root could be considered the main solutes for osmotic adjustment.

Keywords: genotypes, osmotic adjustment, salinity stress, sugar beet

INTRODUCTION

Salinity is considered as a global environmental challenge, affecting crop production on over 800 million hectares, or a quarter to one-third of all agricultural land on earth (Rengasamy 2010). The 21th century is marked by global scarcity of water resources, environment pollution and increased salinization of soils and waters (Djilianov et al. 2005). The problem is particularly severe in irrigated areas (Zhu 2001), where as much as one-third of global food production takes place (Zhang et al. 2010) and where infiltration of highly saline sea water (Flowers 2004) is common. However, salinity is also increasing in dry land agriculture in many parts of the world (Rengasamy 2006). Development of crops with improved salt tolerance is proposed as part of solution to this problem (Zhu 2001).

Plants follow different behaviors to combat salinity. Detailed reviews about salinity tolerance mechanisms in different species are presented by Ashraf (2004) and Sairam and Tyagi (2004).

Osmotic adjustment under salinity stress has undoubtedly gained considerable recognition as a significant and effective mechanism of salinity tolerance in crop plants. Osmo-regulatory effects of proline, glycine betaine, and ions on water balance and salt tolerance have been shown in cotton (Rathert 1983), spinach (Di Martino et al. 2003), wheat (Abdel-Aziz and Reda 2000), bean

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Table 1. Source, germity, and salt tolerance of the studied sugar beet genotypes

No	Genotype	Source	Germity	Ploidy	Type	Salt tolerance *
1	Dita	Belgium	monogerm	Diploid	N	tolerant
2	Brigitta	Germany	monogerm	Diploid	NZ	tolerant
3	Progress	USA	monogerm	Diploid	N	Mid-tolerant
4	Rifle	Belgium	monogerm	Diploid	N	sensitive
5	Concept	USA	monogerm	Diploid	NE	sensitive
6	Tigris	Denmark	multigerm	Polyploid	N	sensitive
7	Montebaldo	Germany	multigerm	Triploid	N	tolerant
8	Prestibel	Belgium	multigerm	Polyploid	NE	Mid-sensitive
9	Waed	Germany	multigerm	Diploid	N	tolerant
10	Kawimera	Germany	multigerm	Triploid	N	tolerant

* According to Abbas *et al.* (2010)

(Shabala et al. 2000), cowpea (Freitas et al. 2001), sugar beet (Katerji et al. 1997; Ghoulam et al. 2002; Heuer et al. 1981), a halophyte sea aster (Ueda et al. 2003), and sorghum (AL-Lahham et al. 2006).

Sugar beet (*Beta vulgaris* L., family; *Chenopodiaceae*), has halophytes ancestors. Its tolerance threshold to salinity is high (7 $dS.m^{-1}$) (Katerji et al. 1997). It is salt sensitive during seed germination and seedling emergence, but in the next stages it is salt tolerant and there are variations in sugar beet genotypes (Sadeghian et al. 2000; Ghoulam et al. 2002, Abbas et al. 2009).

Members of *Chenopodiaceae* family including sugar beet can combat salinity by having osmotic regulating mechanisms due to accumulation of Na^+ and Cl^- in their vacuoles and cytoplasm (Subbarao et al. 2001; Ghoulam et al. 2002). Sugar beet genotypes absorb Na^+ and accumulate it in their leaf tissue for regulation and adaptation of its osmotic potential with soil (Flowers 1988). This may be the reason for considering sugar beet as a tolerant crop.

Choluj et al. (2008) explained the mechanism of osmotic regulation under water shortage in sugar beet by reduced univalent ($K^+$and Na^+) cations concentrations in the petioles and divalent ($Ca^{2+}$and Mg^{2+}) ions levels in the mature and old leaves. Cation concentrations in the tap-roots are not affected by water shortage. The ratio of univalent to divalent cations was significantly increased in young leaves and petioles as a consequence of drought stress.

Table 2. Soil properties of the investigated location

Soil sample	Particle size distribution			Chemical analysis of soil paste extraction		
Season	Sand %	Silt %	Clay %	$CaCO_3$ %	E.C mmhos.cm^{-1} 25 °C	pH
2008	33.3	36.4	30.3	19.4	1.8	8.1
2009	29.3	40.7	29.6	20.7	1.9	8.2

The purpose of this experiment is to study the effect of salinity stress on Na^+, K^+, Na^+/K^+, carbohydrates accumulation of leaves and root sugar content of 10 sugar beet genotypes, and to determinate the role of these osmolytes on osmotic adjustment.

MATERIALS AND METHODS

Two field trials were sown between the beginning of August and mid-August during 2009- 2010 growing seasons. The experiments were carried out in the General Commission for Scientific Agricultural Research (GCSAR) at Der Ez Zour Agricultural Research Center, located in the east region of Syria, and considered as a dry area with irrigation being a standard agronomic measure in the sugar beet production system, to evaluate the response of ten sugar beet genotypes (five monogerm and five multigerm) as shown in Table 1 under salinity stress and control conditions. The investigated genotypes were supplied by different breeding companies. Nitrogen fertilization was added at the rate of 446 kg ha^{-1}. Phosphorous at a rate of 180 kg P_2O_5 and potassium at a rate of 185 kg K_2O were added at sowing and after thinning. Mechanical and chemical analysis of the soil at the experimental location was carried and presented in Table 2. All other agronomic practices were carried out as usually done to insure optimum production. Harvest took place after 210 days from sowing ±2 days between seasons.

Plants were irrigated with saline water in the saline stress experiment with an electrical conductivity (ECw) ranging between 8.6 to 10 dSm^{-1} in the first and between 8.4 to 10.4 $dS.m^{-1}$ in the second year. It is important to mention that the first three emergence irrigations were done with pure water, while during the season saline water was applied. A randomized complete block design (RCBD) with three replicates was used. The size of each plot was 24 m^2, which consisted of 8 m length, 50 cm

Table 3. Content of Na^+ in leaves and root for 10 sugar beet genotypes under salinity stress conditions

	Na+ content ($mg.g^{-1}$)			
	Salt conditions		±% compared with control	
Genotype*	Leaves	Root	Leaves	Root
Dita	52.17^{bc}	3.47^{b}	968.53^{a}	426.18^{cde}
Brigitta	47.72^{de}	3.43^{b}	768.46^{c}	552.87^{a}
Progress	46.07^{e}	3.26^{b}	958.06^{a}	524.26^{ab}
Rifle	38.02^{f}	2.74^{c}	737.96^{cd}	470.97^{abc}
Concept	38.72^{f}	2.64^{c}	655.39^{ef}	356.77^{e}
Tigris	37.07^{f}	1.96^{d}	599.05^{f}	216.81^{f}
Montebaldo	48.52^{de}	3.29^{b}	854.37^{b}	467.17^{abcd}
Prestibel	46.40^{de}	3.25^{b}	636.58^{ef}	386.12^{de}
Waed	49.47^{cd}	3.55^{b}	684.06d^{e}	437.80^{bcde}
Kawimera	55.42^{a}	4.31^{a}	925.03^{a}	484.87^{abc}
Mean	45.95	3.19	778.75	432.38

* Columns with the same letters do not show significant differences.

between- and 20 cm within- row spacing.

Fresh samples of roots and leaves were taken at harvest from each plot to determine the contents of Na^+ and K^+ in roots and leaves, carbohydrates in leaves and sugar content in roots.

Na^+ and K^+ content were measured according to AOAC (2000), by taking leaf samples that oven dried at 65 °C for 48 h and made into fine powder by mortar. A dried sample of leaves of 0.5 g was placed in crucibles in an electric furnace at 500 °C to obtain the ash. The ash was put into 50 ml volumetric flasks, then adding 5 ml of 2N HCl, mixed with boiling distilled water and filtered by Whatman paper No. 2. The Na^+ and K^+ contents were measured using flame photometer and reported as mg g^{-1} of dry weight.

Soluble sugars (carbohydrates) were determined in the previous mixture by spectrophotometer at 620 nm (Spiro, 1966).

Sugar content in roots was determined by Sacharimeter on a lead acetate of fresh macerated root according to the procedure of Le-Docte (1927).

Statistical analysis of data: Data were analyzed using GenStat program to estimate the significance of differences between the examined genotypes, in terms of the studied traits (Na^+, K^+, Na^+/K^+, carbohydrates accumulation of leaves, and sugar content of roots). Treatment means were compared by LSD method at 5 and 1% probability levels according to Waller and Duncan (1969). Simple correlation coefficients between the measured characters were also estimated.

RESULTS AND DISCUSSION

Na^+ Content

Under salinity stress, the content of Na^+ in leaves in all genotypes increased more than 7 times (778.75%) as compared with the control. Indeed, the genotypes differed significantly in this trait ($p<0.01$), and the increment in Na^+ content in leaves ranged between 599.05% in Tigris and 968.53% in Dita (Table 3).

In roots, Na^+ content was also increased in general by 432.38% in all genotypes. The increment in Na^+ content of root ranged between 216.81% in Tigris and 484.87% in Kawimera (Table 3).

The variation in Na^+ uptake could be due to some multiple adaptations to toxic ions operating concurrently within a specific plant (Tester and Davenport, 2003). This is a typical response of *Chenopodiaceae* family (halophytes), in which plant regulates osmotic potential of its tissues by Na^+ accumulation (Eisa and Ali, 2001).

These reactions is opposite to barley (a glycophyte crop) which is Na^+ excluder and in which salt tolerant genotypes accumulate less Na^+ in their shoots (Pakniyat *et al*., 2003). It seems that in tolerant sugar beet genotypes, simultaneous expression of protein vector of tonoplast membrane (H^+-ATPase port and Na^+/H^+ antiport) in cell vacuoles of leaves of tolerant genotypes are more than of that in non-tolerant ones (Parks *et al*., 2002).

K^+ Content

The content of K^+ in leaves decreased in all genotypes by an average of 31.09%, the decrement ranged between 14.80% in Rifle and 43.36% in Waed, and the differences among genotypes were highly significant ($p<0.01$) (Table 4). The same trend observed in roots but in lower rates, except in Dita, an increment in K^+ by 7.19% was observed under salinity stress. These results are in agreement with the results of Warne *et al*., 1990 and Abd-El-Motagally, 2004. This may be due to Na^+ role in regulation of sugar beet leaf osmotic potential (Lindhauer *et al.,* 1990) and substitution of Na^+ with K^+ in this regard. However, higher concentrations of inorganic solutes (Na^+ and K^+) were observed in leaves as compared to roots.

Na^+/K^+ ratio

Results indicated a significant differences ($p<0.01$) among sugar beet genotypes in Na^+/K^+ ratio (Table 5). This ratio was lower in the geno-

Table 4. Content of K^+ in leaves and root for 10 sugar beet genotypes under salinity stress conditions

	K+ content (mg.g^{-1})			
	Salt conditions		±% Compared with control	
Genotype*	Leaves	Root	Leaves	Root
Dita	37.02^{cde}	10.87^{a}	-40.94^{ab}	7.19^{a}
Brigitta	30.80^{g}	9.79^{ab}	-32.06^{cd}	-3.24^{ab}
Progress	34.88^{ef}	9.01^{bc}	-25.57^{d}	-1.01^{ab}
Rifle	45.73^{a}	10.37^{a}	-14.80^{e}	-4.38^{ab}
Concept	36.13^{def}	8.93^{bc}	-27.15^{cd}	-8.87^{b}
Tigris	38.77^{bcd}	8.52^{c}	-24.20^{d}	-1.91^{ab}
Montebaldo	33.30^{fg}	8.55^{c}	-27.21^{cd}	-6.47^{ab}
Prestibel	41.13^{b}	9.88^{ab}	-34.13^{bc}	-11.80^{b}
Waed	35.22^{def}	9.65^{abc}	-43.36^{a}	-2.70^{ab}
Kawimera	40.35^{bc}	9.62^{abc}	-41.48^{ab}	-0.88^{ab}
Mean	37.33	9.52	-31.09	-3.41

* Columns with the same letters do not show significant differences.

Table 5. Na^+/K^+ ratio in leaves and root for 10 sugar beet genotypes under salinity stress conditions

	Na^+/K^+ Ratio			
	Salt conditions		±% Compared with control	
Genotype*	Leaves	Root	Leaves	Root
Dita	1.418^{ab}	0.355^{bcd}	1722.92^{a}	437.72^{c}
Brigitta	1.555^{a}	0.367^{bc}	1183.78^{cd}	595.47^{ab}
Progress	1.329^{b}	0.410^{a}	1333.80^{bc}	611.87^{a}
Rifle	0.833^{e}	0.277^{cd}	885.33^{e}	525.15^{abc}
Concept	1.081^{cd}	0.324^{bcd}	961.56^{e}	455.14^{bc}
Tigris	0.966^{de}	0.257^{d}	847.26^{e}	257.95^{d}
Montebaldo	1.464^{a}	0.423^{a}	1218.16^{c}	550.60^{abc}
Prestibel	1.133^{c}	0.344^{bcd}	1021.89^{de}	469.90^{abc}
Waed	1.415^{ab}	0.400^{ab}	1297.41^{bc}	505.20^{abc}
Kawimera	1.382^{b}	0.468^{a}	1658.08^{a}	510.93^{abc}
Mean	1.258	0.363	1213.02	484.79

* Columns with the same letters do not show significant differences.

types Rifle and Tigris with values of 0.833 and 0.966, respectively; whereas higher ratios were recorded for the genotypes Brigitta, Montebaldo, Dita, Kawimera and Waed figured 1.555, 1.464, 1.418, 1.415 and 1.382, respectively. The first group could be classified as salt sensitive, while the second group as salt tolerant (Abbas et al. 2010). The same tendency was also observed in roots but with a lower rate, the ratio ranged between 0.257 in Tigris and 0.468 in Kawimera. So it may be concluded that the Na^+/K^+ ratio of cytoplasmic membranes in tolerant genotypes was higher than that of non-tolerant ones. This result was confirmed by Pakniyat and Armion (2007).

Soluble sugars in leaves

Significant differences were exhibited among genotypes in terms of the accumulation of soluble sugars in leaves (Table 6) under salinity stress ($p<0.01$), with the value ranging between 60.86 mg.g^{-1} in Prestibel and 96.83 mg.g^{-1} in Dita. In spite of 52.10% average soluble sugars in leaves of all genotypes, the differences were not significant. However, the accumulation of soluble sugars in leaves was higher in tolerant genotypes (Dita, Brigitta, Montebaldo, and Kawimera) than that in non-tolerant ones (Rifle, concept, and Tigris). These results are consistent with the findings of Shannon (1977), who found variability in genotypes response regarding the content of soluble sugars under salinity stress. In other crops, Al-Lahham et al. (2006) displayed that soluble sugars play an important role in osmo-regulation process under salinity stress in *Sorghum bicolar* L. This may be explained by enhancing enzymatic activities especially amylases, or by expending more energy in cells to resist ionic imbalance (Schwarz and Gale 1981).

Table 6. Soluble sugars accumulation in leaves and sugar content in root for 10 sugar beet genotypes under salinity stress conditions

	Salt conditions		±% Compared to control	
	Soluble sugars accumulation (mg g^{-1})	Sugar content (%)	Soluble sugars accumulation (mg g^{-1})	Sugar content (%)
Dita	96.83^{a}	17.25^{a}	64.90	7.12^{ab}
Brigitta	92.33^{ab}	17.09^{a}	57.30	9.93^{ab}
Progress	79.94^{cd}	17.08^{a}	61.80	12.29^{a}
Rifle	75.11^{de}	15.64^{bc}	45.80	5.85^{b}
Concept	67.94^{ef}	16.07^{b}	40.20	10.0^{ab}
Tigris	72.56^{de}	14.56^{d}	27.60	6.57^{b}
Montebaldo	88.78^{abc}	15.37^{bc}	64.10	7.60^{ab}
Prestibel	60.86^{f}	14.82^{d}	46.60	9.64^{ab}
Waed	77.70^{cde}	15.07^{c}	54.70	4.79^{b}
Kawimera	81.21^{bcd}	15.24^{c}	57.90	6.77^{ab}
Mean	79.33	15.82	52.10	8.06

* Columns with the same letters do not show significant differences.

Table 7. Correlation coefficients between osmolytes contents in leaves and roots of sugar beet genotypes under control and salinity stress conditions

Leaves					Root				
Parameters	Na^+	K^+	Na^+/K^+	Soluble sugars	Parameters	Na^+	K^+	Na^+/K^+	Sugar content
Under normal conditions									
Na^+	1.00				Na^+	1.00			
K^+	0.41	1.00			K^+	0.07	1.00		
Na^+/K^+	0.36	-0.68*	1.00		Na^+/K^+	0.79**	-0.55*	1.00	
Soluble sugars	-0.21	-0.09	-0.03	1.00	Sugar content	-0.02	0.15	-0.13	1.00
Under salinity stress									
Na^+	1.00				Na^+	1.00			
K^+	-0.24	1.00			K^+	-0.01	1.00		
Na^+/K^+	0.79**	-0.78**	1.00		Na^+/K^+	0.86**	-0.51*	1.00	
Soluble sugars	0.32*	-0.28	0.41	1.00	Sugar content	0.35*	-0.15	0.38*	1.00

Sugar content in roots

Data regarding sugar content in roots (Table 6) displayed significant differences among genotypes under salinity stress ($p<0.01$), with the mean values ranging between 14.56% in Tigris and 17.25% in Dita. The average of increment in sugar content of roots was 8.06% as compared with control conditions. Under control condition, the difference in sugar content between genotypes was significant ($p<0.05$). The intermediate tolerant monogerm genotype Progress achieved the highest increment (12.29%) when compared to the control, whereas the lowest values (4.79% and 6.57%) were observed in the sensitive multigerm genotypes Waed and Tigris, respectively, and the monogerm genotype Rifle (5.85%). This may be due to germity. We noticed that monogerm genotypes had higher sugar content than multigerm ones.

Simple correlation coefficients

Correlation coefficients between measured characters are shown in Table 7. Results showed a negative correlation between Na^+ and K^+ contents in leaves (r= -0.24), and also between Na^+ and K^+ content in roots (r= -0.01) under salinity condition. There was a high positive correlation between Na^+ and Na^+/K^+, and a high negative correlation between K^+ and Na^+/K^+ in both leaves and roots. This agrees with the findings of Eisa and Ali (2001), who showed a negative linear correlation between these two ions after salt stress in sugar beet leaves. They also found that the increasing of Na^+ accumulation and reduction of K^+ content played a critical role in osmotic potential adjustment of sugar beet under salt stress.

The correlation analysis exhibited a significant positive correlation between Na^+ content and soluble sugars in leaves ($r = 0.32$, $p<0.05$). This indicates that sugars and Na^+ increased under salinity stress. These two indices can be used for screening tolerant genotypes within a sugar beet population.

There was a positive correlation between Na^+ content and sugar content in roots ($r = 0.35$, $p<0.05$), indicating that sugar and Na^+ contents increased under salinity stress, and involved in osmo-regulation process under salinity stress.

CONCLUSION

Sugar beet plant has a good ability in changing its osmotic potential as a response to salt stress. This was discussed previously by Lindhauer et al. (1990) who reported that inorganic salts such as potassium, sodium and magnesium played the main role in osmotic potential adjustment in sugar beet leaves, whereas in root, sugar content dominated this process in terms of osmotic potential. Their findings are consistent with the results of the current study.

Sugar beet genotypes combat the toxicity of Na^+ by accumulating this factor in the vacuoles of leaf cells; therefore this regulates their osmotic potential under salinity stress. Besides, under the same circumstances sugar beet genotypes accumulated also more sugars in leaves and more sucrose in roots to regulate the osmotic potential. These findings are in agreement with those investigated in Atriplex, which is a halophyte and belongs to *Chenopodiaceae* family (Glenn *et al*., 1994).

In terms of genotype tolerance, the most tolerant genotype was Kawimera, while the most non-tolerant genotype was Tigris; this finding was agreed with Abbas et al. (2010). Kawimera had the highest content of Na^+ in leaves and root, whereas Tigris showed the lowest value.

Depending upon correlation analysis, Na^+ content could be considered the main solute for

osmotic potential adjustment in sugar beet leaves under salinity conditions, followed by soluble sugars. Moreover, both sucrose and Na^+ content in beet root could also be considered the main solutes for osmotic potential adjustment.

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