SALT TOLERANCE IN FODDERBEET AND SEABEET: ANALYSIS OF BIOCHEMICAL RELATIONS

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Summary. Fodderbeet (*Beta vulgaris*) and seabeet (*Beta maritima*) were grown in a greenhouse using garden soil under increased salinity (0 mM NaCl-control and 200 mM NaCl). Growth and biochemical relations of fodderbeet and seabeet were analyzed and compared. Both plant species tolerated salinity level of 200 mM NaCl, but seabeet performed better than fodderbeet. Chlorophyll *a* content showed a progressive increase at different harvests. Chlorophyll *b* content was low at salinity level of 200 mM NaCl than the control throughout the growth period. Sugar content of fodderbeet and seabeet decreased in the presence of salt in the growth medium. The effect of salinity on the sugar content of seabeet root was not significant. However, sugar content significantly decreased in the fodderbeet under saline conditions. A significant increase ($P<0.01$) in the protein content was observed in both species. Protein content in seabeet was significantly higher compared to fodderbeet. Higher glycinebetaine content under saline conditions in both species indicate the higher degree of salt tolerance. Presence of salt ions may have helped the improvement of sucrose and protein synthesis, which in turn activates the biosynthesis of glycinebetaine. This behavior may be ascribed as one of the mechanisms adopted by the plant of salinity tolerance.

Keywords: chlorophyll, fodderbeet, glycinebetaine, salinity, seabeet, sugar, protein.

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INTRODUCTION

Soil type plays an important role in the response of crop to sodium chloride application for better sugar yield in sugar beet (Draycott and Bugg, 1978). Isla et al. (1998) reported that production of protein and nucleotide is inhibited by salinity ultimately resulting in decreased plant mass due to inhibition in cell division and cell enlargement. Niazi et al. (2002, 2004) observed comparatively higher concentration of chlorophyll in fodderbeet under saline soil conditions. Different plant species belonging to *Gramineae* have shown salt sensitiveness at various growth stages. Different cultivars of the same plant had different behavior toward salt tolerance (Flowers and Hajibagheri, 2001; Qadir et al., 2001). *Chenopodiaceae* and *Gramineae* show different behavior for biosynthesis of glycinebetaine, which is a quaternary ammonium compound accumulated in the leaves of a wide range of species (Flowers and Hajibagheri, 2001; Hajibagheri and Flowers, 1989; Wyn-Jones and Storey, 1981). Accumulation of glycinebetaine increases considerably under saline environments, especially in species belonging to *Chenopodiaceae* or *Gramineae* (Hajibagheri and Flowers, 1989; Wyn-Jones and Storey, 1981). The increase in glycinebetaine has been correlated with the salt stressed leaf expansion (Mcdonnel and Wyn-Jones, 1988). Fodderbeet and seabeet are the chenopod grown in the winter in Pakistan when there is an acute shortage of fodder for the livestock. A series of experiments are underway in our unit to explore the possibilities of improving growth and production of these plants under moderately saline-sodic soil conditions. The present study presents the results of a greenhouse experiment on the performance of fodderbeet and seabeet grown under saline soil conditions to help understand the mechanism of salt tolerance in these two plant species.

MATERIALS AND METHODS

Seeds of fodderbeet (*Beta vulgaris* cv. Majoral) and seabeet (*Beta maritima*) were germinated in sand under greenhouse conditions (temperature 30ºC/25 ºC day/night, relative humidity 70%, light 250 $\mu E\ m^{-2}\ s^{-1}$). Ten-day-old seedlings were transplanted to pots containing one seedling per pot having one kg garden soil. The physico-chemical characteristics of the soil (Potgrond mengselm, Aalsmeer) used are shown in Table 1. Two salinity levels (0 mM-control and 200 mM NaCl) were maintained in the pots. Salinity was increased stepwise one week after the establishment of the seedlings in the pots by the addition of 50 mM NaCl at two-day interval (twice added on the top of soil and third time to plate placed at the base of pot to keep salinity uniform through out the soil profile). Each treatment was repeated 20 times and randomized over the greenhouse bench. Plants were sequentially harvested five times with an interval of seven days between each harvest. Fresh plant material was ana-

lyzed for chlorophyll according to Knudson et al. (1977). The samples were also analyzed for chlorophyll fluorescence for qualitative measurement of chlorophyll 'a' and 'b'. The photochemical efficiency of photosystem II was calculated according to Oquist and Wass (1988). Plants were dried at 60°C in an oven to a constant weight and were subjected to analysis of total sugars (Bergmeyer, 1978), proteins (Peterson, 1984) and glycinebetaine (Storey and Wyn-Jones, 1977). The data were statistically analyzed using two-way ANOVA methods (Sokal and Rohlf 1981).

Results

The soil used for the experiment was non-saline with favorable properties for the plant growth (Table 1). Therefore, salinity levels created were representative of single salt (NaCl) salinity environments while under natural saline conditions there is always a mixture of different ions. It was observed that chlorophyll 'a' in fodderbeet increased significantly under 200 mM NaCl during 21-28 days of growth. However, chlorophyll 'b' decreased under saline conditions during the whole growth period (Table 2). Chlorophyll 'a' and 'b' content in seabeet was higher than in fodderbeet. In seabeet, higher chlorophyll content could not help the accumulation of plant biomass effectively because of the smaller leaf area compared to fodderbeet. The larger leaf area in fodderbeet successfully contributed to increased carbohydrate content by intercepting more sunlight. Consequently, it provided extra energy to overcome salt stress experienced by the plant. A decrease in chlorophyll 'a' and 'b' was recorded after 35 days of growth under saline conditions. Salinity tended to increase sugar content of root in both plants (Table 3). Sugar content in fodderbeet root increased only at salinity level of 200 mM NaCl during 28-35 days. The sugar content in seabeet increased at 200 mM NaCl since the early growth period. Seabeet showed higher sugar content than fodderbeet. Sugar content in shoot was significantly low after salt treatment in both plants (Table 3). Sugar content accumulated in the shoot during 21-35 days.

Protein content decreased in fodderbeet root at salinity level of 200 mM NaCl (Table 4). A significant increase in protein content of fodderbeet shoot was recorded at 200 mM NaCl. Protein content of shoot was higher than that in the root (Table 4). Protein content in fodderbeet (root/shoot) decreased with plant age after 14 days. On the other hand, protein content in seabeet increased significantly with plant age. Protein content in both root and shoot of seabeet increased at salinity level of 200 mM NaCl. A significantly high protein content was observed in seabeet compared to fodderbeet.

It was noted that glycinebetaine content increased at 200 mM NaCl (Table 5). Glycinebetaine content in seabeet root and shoot was higher as compared to fodderbeet. A significant increase in glycinebetaine content was observed in seven-day-old fodderbeet plants (root and shoot), while glycinebetaine content of seabeet plants (root and shoot) increased significantly after 14 days.

Table 1. Physico-chemical analysis of garden soil (Potgrond mengselm, Aalsmeer)

Physical analysis		
Moisture content (%)	Ca	64.0
Organic matter (%)	Ca	70.0
Vol. weight perlite granule	Ca	207.0
Pore vol	Ca	88.3
Vol. % water by PF 1.5	Ca	54.8
Vol. % air by PF 1.5	Ca	33.5
Chemical analysis		
Organic matter	Ca	72.0
$CaCO_3$	Ca	2.5
pH H2O	Ca	6.2
EC dS/m	Ca	1.4
Anion (mM/L extract)		
Cl	Ca	0.7
SO_4	Ca	3.2
PO_4	Ca	0.8
NO_3	Ca	2.2
Cation (mM/L extract)		
NH_4	Ca	3.1
K	Ca	2.9
Ca	Ca	1.8
Mg	Ca	0.8
Total Nitrogen	Ca	5.3

Discussion

Less sugar content in fodderbeet has been reported as compared to sugarbeet (Quin et al., 1980). Sugar content increased in both plant species with increasing salinity (Table 3). This may be due to increased Mg^{2+} concentration (Niazi et al., 1999), because of its importance as a co-factor in almost all enzyme-activated phosphorylating processes. It is also required for activation of ribulose-1,5-bisphosphate carboxylase (Bassham, 1979) and in CO_2 assimilation and related processes, such as production of sugar and starch (Greger and Linberg, 1987). Sugar yield also increased after the addition of Cl^- along with root fresh and dry weights. In addition, K^+ uptake was also increased (Niazi et al., 2004) which may have enhanced the translocation of photoassimilates from leaves to roots, thus increasing root dry matter (Hartt, 1970;

Table 2. Chlorophyll 'a' and 'b' content (mg kg^{-1} dry weight) in fodderbeet and seabeet grown under saline condition.

Chlorophyll 'a'

Crop	Treatment	Harvest (days)					
		7	14	21	28	35	
Fodderbeet	Control	0.850	0.717	0.890	0.810	1.113	0.876 ab
	200mM	0.735	0.658	1.070	0.980	0.660	0.821 b
Seabeet	Control	0.763	1.203	0.803	1.403	0.957	1.026 a
	200mM	0.609	0.879	0.810	0.760	1.258	0.863 b
		0.739 c	0.864 bc	0.893 ab	0.988 ab	0.997 a	

LSD ($P < 0.01$) Harvest = 0.132, Treatment = 0.150

Chlorophyll 'b'

Plant	Treatment	Harvest (days)					
		7	14	21	28	35	
Fodderbeet	Control	0.213	0.257	0.253	0.330	0.247	0.260 b
	200mM	0.309	0.220	0.203	0.302	0.227	0.252 b
Seabeet	Control	0.223	0.400	0.300	0.563	0.423	0.382 a
	200mM	0.170	0.260	0.238	0.242	0.422	0.266 b
		0.229 c	0.284 b	0.249 c	0.359 a	0.330 a	

LSD ($P < 0.01$) Harvest = 0.035, Treatment = 0.039
Values followed by the same letter in each row are not significant at $P< 0.01$. Same applies to means in the last column.

Table 3. Sugar content (mg kg^{-1} dry weight) in shoot and root of fodderbeet and seabeet grown under saline condition.

Shoot

Plant	Treatment	Harvest (days)					
		7	14	21	28	35	
Fodderbeet	Control	34.3	46.0	511.3	869.0	718.3	435.8 b
	200mM	23.9	280.9	51.4	182.0	567.5	221.1 d
Seabeet	Control	39.7	241.7	447.0	859.7	1406.3	598.9 a
	200mM	380.7	256.9	312.5	306.4	520.4	355.4 c
		119.7 e	206.4 d	330.6 c	554.3 b	803.1 a	

LSD ($P < 0.01$) Harvest = 19.7, Treatment = 31.8

Root

Plant	Treatment	Harvest (days)					
		7	14	21	28	35	
Fodderbeet	Control	34.0	76.3	1209.7	1506.7	5547.0	1674.7 a
	200mM	92.9	45.8	75.2	834.0	1821.7	573.9 b
Seabeet	Control	118.0	647.7	1817.7	1593.3	2776.0	1390.5 a
	200mM	518.4	866.9	1868.0	2292.7	2517.7	1612.7 a
		190.8 c	409.2 c	1242.7 b	1556.7 b	2915.6 a	

LSD ($P < 0.01$) Harvest = 438.7, Treatment = 472.9

Values followed by the same letter in each row are not significant at $P < 0.01$. Same applies to means in the last column.

Table 4. Protein content (mg kg^{-1} dry weight) in shoot and root of fodderbeet and seabeet grown under saline condition.

Shoot

Crop	Treatment	Harvest (days)					
		7	14	21	28	35	
Fodderbeet	Control	242.5	244.7	307.7	234.6	173.4	240.6 d
	200mM	502.6	457.4	375.2	294.2	148.1	355.5 c
Seabeet	Control	233.3	143.3	519.8	617.6	456.5	394.1 b
	200mM	524.1	716.4	500.6	868.7	660.8	654.1 a
		375.6 cd	390.5 c	425.8 b	503.8 a	359.6 d	

LSD ($P < 0.01$) Harvest = 17.2, Treatment = 29.5

Root

Crop	Treatments	Harvest (days)					
		7	14	21	28	35	
Fodderbeet	Control	136.6	126.4	152.9	76.8	95.4	117.6 c
	200mM	165.8	90.8	111.2	83.0	78.4	105.8 c
Seabeet	Control	168.4	68.8	546.8	398.7	475.2	331.6 b
	200mM	297.4	404.3	283.5	479.3	386.8	370.3 a
		192.1 b	172.6 c	273.6 a	259.4 a	259.0 a	

LSD ($P < 0.01$) Harvest = 18.4, Treatment = 18.2
Values followed by the same letter in each row are not significant at $P< 0.01$. Same applies to means in the last column.

Table 5. Glycinebetaine content (mg kg^{-1} dry weight) in shoot and root of fodderbeet and seabeet grown under saline condition.

Shoot

Plant	Treatment	Harvest (days)					
		7	14	21	28	35	
Fodderbeet	Control	2.9	2.6	3.7	4.1	2.0	3.0 d
	200mM	3.5	4.8	4.5	6.3	4.0	4.6 c
Seabeet	Control	6.2	3.9	6.0	4.5	5.0	5.1 b
	200mM	6.1	5.0	16.2	15.9	11.5	10.9 a
		4.7 c	4.1 d	7.6 a	7.7 a	5.6 b	

LSD ($P < 0.01$) Harvest = 0.5, Treatment = 0.5

Root

Plant	Treatments	Harvest (days)					
		7	14	21	28	35	
Fodderbeet	Control	0.2	2.2	3.8	1.8	0.7	1.7 c
	200mM	3.5	5.0	6.5	6.5	2.9	4.9 b
Seabeet	Control	3.3	5.0	5.2	4.3	5.1	4.6 b
	200mM	3.5	3.8	16.1	19.3	10.5	10.6 a
		2.6 d	4.0 c	7.9 a	8.0 a	4.8 b	

LSD ($P < 0.01$) Harvest = 0.6, Treatment = 1.0
Values followed by the same letter in each row are not significant at $P< 0.01$. Same applies to means in the last column.

Magat and Goh, 1988). Maximization of root yield has been related to nitrogen which leads to depressed sucrose concentration in the root (Anderson and Peterson, 1988). In the present study, nitrogen was not added to pots in any form as it could favour an increase in sugar content.

A decrease in root protein content was observed in fodderbeet during the second and third weeks. This may be attributed to translocation of proteins to the shoot, which was confirmed by the subsequent increase in shoot weight till the fourth week. There was a significant increase in the protein content in seabeet shoot and even the increasing salinity could not reduce it. Frigany et al. (1981) reported an increase in sugarbeet protein due to micronutrient nutrition. The protein content in leaves was also shown to increase under salinity (Haeder and Mengel, 1972). Our results indicated that chlorophyll content remained unaffected upon salinity. However, due to the comparatively larger leaf area in fodderbeet chlorophyll content was higher than that in seabeet. The light energy interception in fodderbeet was greater than the one in seabeet. Hence, the synthesis of sugar and protein was higher. It is known that plants need more energy under saline soil environment. Extra energy could be provided by increased content of sugar and protein which are energy rich compounds. The results of the present study confirm that protein content increases under saline conditions. An important factor that controls growth and especially dry matter accumulation is the photosynthetic activity of a plant. The role of photosynthesis does not only determine the accumulation of plant structural and storage material, but the products of photosynthesis may also be important for osmoregulation. It is therefore expected that photosynthesis may show sensitivity to salinity in salt-tolerant species in which growth and yield are less inhibited by salinity.

Wyn-Jones and Storey (1981) have reported that the accumulation of glycinebetaine is a good indicator of salt tolerance in different plants. The content of glycinebetaine is very mobile (Mcdonnel and Wyn-Jones, 1988). Betaine increases with increasing K^+ concentration in storage root (Beringer et al., 1986), which may be the reason for the significantly high betaine content observed in the present study. The fodderbeet plants showed a significant salt tolerance during the five week vegetative growth period under saline soil conditions. The mechanism of salt tolerance may be achieved by plants through accumulation of K^+, Ca^{2+} and Mg^{2+} ions. These ions may help to improve the synthesis of sucrose and proteins which activate the biosynthesis of glycinebetaine, thus increasing the salt tolerance of plants. Seabeet is an ancestral plant. It grows in the salt marshy coastal areas. Its aerial part is extensively branched and has significantly higher number of leaves. However, the leaf area of seabeet is significantly smaller than that of fodderbeet. On the other hand, fodderbeet is a domesticated cultivar with a significantly larger leaf area which helps the plant in the interception of greater sunlight. This accounts for the accumulation of higher organic as well as biochemical content like sugar, protein and glycinebetaine resulting in the higher energy storage by the plants. The release of this energy during

respiration may help to overcome the salt stress conditions.

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