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A COMPARATIVE STUDY ON THE RESPONSES OF TWO LINES OF SUGAR BEET (B*eta vulgaris* L) TO SALINITY

YAZDANI, Mojtaba

[1] Islamic Azad University, Ashtian Branch, Department of Biology, Ashtian, Iran

** Correspondence author*
e-mail: yazdani.moj21@gmail.com

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ABSTRACT

Sugar beet is a crop able to resist high levels of soil salinity after emergence and establishment. Considering the significant difference in the effect of nitrogen forms on sugar beet performance under normal conditions, the form of nitrogen may affect the performance of sugar beet plants under abiotic stress, particularly salinity. Additionally, exploring the most appropriate type of nitrogen for sugar beet could mean optimizing sucrose content. Therefore, here using two lines, sugar beet was grown in pots (filled with 4 kg soil), salt-resistance (line 7233- p.29 x Mst), and salt-sensitive (line 3929-21939), the effect of two different forms of nitrate in the form of calcium nitrate (1 g per pot) and ammonium in the form of ammonium sulfate (1 g per pot) under normal and salt stress condition (40 Millimoles per liter sodium chloride) were evaluated. The result revealed the positive influence of nitrate over ammonium by indicating higher dry weight in both sensitive line: 19.2 and 13.6 g, and tolerance line: 20.4 and 13.6 g, respectively alone and in combination with salinity stress. Similarly, root yield levels positively influenced by nitrate treatment either alone or under salinity stress (sensitive line: 194.5 and 243.2 g and tolerance line: 207 and 249.5 g). The outcomes additionally showed the accumulation of proline aerial parts in both lines, and however, the proline accumulation of sensitive line was higher (3.9 mg/g dry weight). Moreover, induction of proline aggregation was considerably higher in nitrate nitrogen-treated sensitive line (9.3 mg/g dry weight). The absence of significant difference was obseved between nitrogen treatments in terms of extractable sucrose and root molasses sugar. Also, the root impurities increased in those treated with nitrate-nitrogen and salinity. It can be concluded that nitrate-nitrogen has improved the performance of both sugar beet lines against salinity stress, and its practical application is adviseable.

Keywords: *nitrogen, vegetative growth, root yield, extractable sucrose, salinity stress*

1. INTRODUCTION:

Sugar beet is one of the strategic industrial plants for sugar production. About 37% of the world sugar production is obtained from this plant (Bogetoft *et al.*, 2007; Rajaeifar *et al.*, 2019). In addition to producing sugar, molasses and aerial parts are also used for animal feed and fermentation products. In some countries, ethanol is also made from this plan pulp using bacteria (Zheng *et al.*, 2012; Habeeb *et al.*, 2017). Sugar beet is highly tolerant of cold, dry weather and salinity. To a large extent, this plant species has a halophytic behavior and can accumulate large amounts of sodium and chlorine ions (Zhou *et al.*, 2017; Skorupa *et al.*, 2019). The tolerance of sugar beet cultivars is varies greatly and with increasing environmental stress particularly salinity, its yield decreases (Hossain *et al.*, 2017). Sugar beets tolerate to salt is about 200 to 300 mM chlorine (Ulrich and Ohki, 1956; Gzik, 1996). In terms of salt tolerance, it is in the second place after halophytes.

Supplying good quality water in many parts of the world is fraught with limitations. In these areas, the water of the aquifers has sharply decreased due to over-exploitation for drinking, agriculture, industry, and green space, which has led to an increase in the salinity of these waters. For this reason, in the maintenance of agricultural areas, the tendency to use saline water to irrigate plants has increased, which in the long run can reduce the growth and yield of these plants (Ulrich and Ohki, 1956; Hossain *et al.*, 2017). The harmfulness of high salt concentrations to plants is due to the osmotic potential of water and the specific effects of ions on the protoplasm. Increasing the concentration of sodium and chlorine ions in the protoplasm disrupts the ionic balance and the particular impacts of these ions on membrane enzymes, resulting in photophosphorylation of the respiratory chain and

energy (Blumwald and Poole, 1987; Zhou *et al.*, 2017; Wang *et al.*, 2019).

It has been accepted that stress-induced ROSs are responsible for multiple damages to macromolecules and ultimately to cellular structure (Noreen and Ashraf, 2009) and need to be quenched to maintain normal growth. Ascorbate peroxidase (APX), catalase (CAT), and peroxidase (POD) along with low molecular weight scavengers such as ascorbate, glutathione, and proline act as the main defense against ROS produced in different parts of the plant cell. Catalase, which is located in peroxisomes, glyoxysomes, and mitochondria and is apparently absent in chloroplasts, mainly converts H_2O_2 from light or photorespiration to water and O^2 (El-Hendawy *et al.*, 2005).

Mechanisms that can be involved in plant salinity resistance are a) Lack of absorption or low uptake of salt into the plant; b) tissue resistance or tolerance; c) Accumulation of salt in vacuoles without interfering with physiological processes; d) Isolation of ions such as K+, Cl^-, Na^+ and SO_4 during root uptake and transfer to shoots; e) Various biochemical processes such as the production of some enzymes, hormones, antioxidants, etc. (Ahmad *et al*., 2019).

Increased nutrients may be associated with more or less plant tolerance to salinity stress, depending on the salinity level and abundance of nutrients in the culture medium (Mansour, 2000). Inadequate nitrogen content is considered a growth-limiting factor in plants. The addition of nitrogen fertilizer has been reported to reduce the harmful effects of salinity on some plants (El-Hendawy *et al.*, 2005). Salinity can also prevent the accumulation of nitrogen in plants. Jamil and Rha (2004); (Jafarzadeh and Aliasgharzad, 2007) reported increasing soil salinity reduces root yield in sugar beet. This decrease is quite evident when the salt content does not exceed half a percent of the soil. They also observed that when the percentage of exchangeable sodium (EPS) exceeds 10, the root yield decreases, and when it reaches 18, the root yield decreases by 50%.

In sugar beet, sucrose is transported from the cytosol to the vacuole during the proton-antiport-sucrose process, where it accumulates (Ghoulam *et al.*, 2002). This action is probably a function of the proton gradient, and it probably follows the feeding of the nitrogen form. Since the plant response to nitrate and ammonium nitrogen is different, this response may differ in saline and non-saline conditions. Because sugar beetroot should contain a high percentage of sucrose, study the effect of two nitrogen forms. Nitrate and ammonium prioritize plant growth, sucrose content, and proline content in two salinity-sensitive and sugar-tolerant sugar beet lines in saline and non-saline conditions (Ghoulam *et al.*, 2002; Abbas *et al.*, 2010; Dadkhah, 2011).

Having a critical role as a food plant, sugar beet salt stress tolerance should be evaluated specifically under different nitrogen forms. Thus, two lines of sugar beet receiving two forms of nitrogen, nitrate, and ammonium were investigated to understand the response of these cultivars and find whether nitrogen forms can influence the salt tolerance of sugar beet plant.

2. MATERIALS AND METHODS:

2.1. Plant material and pot experiment

A total of 48 pottery pots with a capacity of 5 kg of soil and a diameter of 25 cm were prepared and filled with 4 kg of soil, which was analyzed using conventional methods of the Water and Soil Research Institute, and its specifications are shown in Table 1. These pots were then placed outdoors and based on a completely randomized statistical design in 6 replications with salinity-sensitive diploid polygram seed (line 1962-2939) and salinity tolerant hybrid (line 7233-p.29 xMst) at depth 1 planted up to 2 cm and irrigated to saturation (Figure 1a). After complete germination of seeds and their growth, additional seedlings were kept in each pot, and only two seedlings were held in each pot (Figure 1b). Then nitrogen treatment was applied as follows. One gram of nitrogen from calcium nitrate was added to the soil of pots for nitrate-nitrogen treatment. One gram of nitrogen from ammonium sulfate was added to the soil of the pots for the treatment of ammonium nitrogen. Salinity stress was applied by adding salt to irrigation water. For this purpose, all seedlings treated with salinity were subjected to the same saline water stress (32 bar every 5 days with 250 ml of water containing 40 mM sodium chloride). The rest of the seedlings were irrigated with the same amount of unsalted water. The plants were harvested 167 days after sowing the seeds. During the experiment, pots were kept outdoor to simulate the field condition where average, maximum and minimum temperatures were 24ºC, 33ºC, and 16ºC. Thus, the first 0.5 g of the leaf was taken to measure the amount of proline and immediately transferred to the laboratory and frozen in liquid nitrogen at -196°C. The samples were then stored in aluminum foil at -20°C in the freezer. Also, the aerial part of the plant was washed entirely after harvest, and its dry weight was determined at 70°C

until reaching a constant weight. The roots were immediately harvested and immediately transferred to the Sugar Technology Laboratory of the Karaj Sugar Beet Research Institute for qualitative chemical analysis.

2.2. Proline measurement

Leaf tissue proline was measured based on the method developed by Bates *et al.* (1973). For this purpose, 0.5 g of fresh leaves first weighed and homogenized in 10 ml of 3% sulfosalicylic acid solution with quartz and filtered with Whatman 42 paper. Then, in 2 ml of the filtered extract using ninhydrin reagent and 2 ml of glacial acetic acid, proline measured as a solution in toluene appeared red. The amount of proline in the tested samples was calculated in micrograms per gram of leaf fresh weight.

2.3.Nitrate test

According to Wollring (1983) method, a one-millimeter thick segment was prepared from the sugar beet plant petiole and placed on a glass plate to perform the nitrate test. Then, a drop of 1% diphenylamine reagent was added to each segment, and another glass plate was placed on them so that the petiole cuts were placed between two layers of glass. In this case, two glass plates were pressed together to extract the nitrate-containing extract in the petiole pieces. After reaction with the mentioned reagent, this extract produced a blue color, which varied depending on the quantity of nitrate in the petiole tissue. The amount of dye produced was evaluated between zero and 6 (nitrate test index). Then, by measuring the amount of nitrate in the leaf tissue, the relationship between the nitrate test index and the amount of nitrate in the tissue was determined based on the prodecure developed by Cataldo *et al.* (1975) and the amount of nitrate in leaf tissue was calculated in micrograms per gram of dry weight.

2.4.Chemical analysis of root

From the harvested roots, after complete washing, root pulp was prepared by a sampling device. The root was analyzed, and its quality factors were measured by a model 3016 -D beta analyzer and a flame photometer. A beta-analyzer and a flame photometer measured the sugar content (SC, Riyahi and Sajadi, 1984) and impurities in the root (amounts of potassium, sodium, and residue nitrogen) (Allen *et al*., 1985). Other factors such as molasses sugar (MS) and sugar content (WSC) indirectly measured using the available experimental equations and information obtained from the factors mentioned above (Clarke *et al.*, 1991). Through equations 1-3 is possible to obtain the percentage of molasses sugar.

$$\% MS = 0/343 (k + na) + 0/094 (a - amion - n) - 0/29 \quad \text{(Eq. 1)}$$

$$\% MS = 0/175 K + 0/13 Na + 0/215 (a - a min o - n) \quad \text{(Eq. 2)}$$

In these equations, potassium and sodium, and residue nitrogen impurities are mill equivalents per hundred grams of sugar beet root. WSC or percentage of extractable sugar can be obtained from reducing sugar content and molasses sugar as shown in equation 3.

$$\% WSC = \% SC - \% MS \quad \text{(Eq. 3)}$$

2.4.1 Measurement of sugar content

The percentage of sugar content or grade of sugar beet includes the percentage of extractable sugar and the percentage of sugar present in the molasses. To measure the quality parameters in the root, root paste, and lead (II) acetate in the ratio of 26 g of paste and 177.77 cubic centimeters, respectively, were thoroughly mixed using automatic mixers, then filtered with paper number 42, and its extract was separated. Its sugar percentage was determined by the polarimetry method (Clarke *et al.*, 1991), which is based on the deviation percent of polarized light using a polarimeter (Rudolph Autopol V Polarimeter APV-6W, NJ, USA).

2.4.2 Determination of potassium, sodium, and residue nitrogen impurities

The amounts of potassium and sodium in the extract prepared from the root paste are measured by a flame photometer that compares the sample emission spectrum obtained from lithium—subsequently, its amount in milliequivalents per gram of the resulting paste calculated from the root. A beta-analyzer was used to measure the residue nitrogen. In this device, by mixing the extract and cooper reagent, color changes are made in equal proportions compared with existing standards and expressed as milliequivalents per hundred grams of root paste

(Allen *et al*., 1985).

3. RESULT AND DISCUSSIONS:

According to the results shown in Table 2, nitrate-nitrogen treatment in comparison with ammonium nitrogen has significantly increased the dry weight of the aerial part and also the fresh weight of the root part in both lines (Figure 2), which may be due to the higher photosynthesis activity in plants treated with (DIAS and Costa, 1983; Raab and Terry, 1994; Malnou *et al.*, 2008). Ammonium nutrition also causes ammonia to accumulate within plant tissue cells, resulting in disruption of cellular pH, inhibition of phosphorylation coupling, and reduced photosynthesis (Raab and Terry, 1994). A report by Rubin *et al.* (2009) on tobacco shows that ammonium nitrogen reduces plant growth by reducing cell number, cell size, and leaf area. However, the number of leaves was equal in both nitrate and ammonium nitrogen treatments. Such a result has been reported for previously reported in sugar beet cultivars (Raab and Terry, 1994; Brentrup *et al.*, 2001; Demiral and Gündüzoğlu, 2010).

Decreased growth due to ammonium nitrogen nutrition is also associated with ammonium toxicity. It causes phosphorylation uncoupling and reduces carbohydrate compounds due to excessive consumption of soluble sugars for NH4+ reduction and detoxification (Walch-Liu *et al.*, 2000; Bittsánszky *et al.*, 2015). However, nitrate ions as a moderator in osmotic conditions increase water absorption and increase plant growth (Cordovilla *et al.*, 1995). Salinity treatment with either nitrate and ammonium nitrogen has a different effect on plant growth. Consumption of nitrate-nitrogen with salinity compared to ammonium nitrate consumption alone has significantly reduced the aerial part dry weight and the root fresh weight in the salinity sensitive line. In the line tolerating salinity, this reduction was only observed for root fresh weight. Nonetheless, the application of ammonium nitrogen combined with salinity compared with ammonium nitrogen alone in both lines did not cause a significant reduction in shoot dry weight. Still, this effect on root fresh weight was noticeable.

The data in Table 3 show the nitrate levels of leaf tissue. In nitrate-nitrogen treatment 167 days after sowing, the amount of leaf nitrate in both lines is less than the amount of ammonium nitrogen. However, this difference is not significant, probably due to the leaching phenomenon and the removal of nitrate from the soil around the roots treated with nitrate nitrogen. Ammonium ions are stored in the soil around the roots and are converted to nitrate by nitrification. Therefore, these nitrates can be gradually given to the plant (Alexander, 1965; Zaman and Blennerhassett, 2010). Salinity treatment in each of the two forms of nitrogen significantly reduced nitrate in leaf tissue than non-salinity treatment.

To achieve high-quality sugar beet, the plant must be balanced in terms of nitrogen nutrition and not suffer from insufficiency or excessiveness. Achieving a balance between sufficient nitrogen at the beginning of the growing season and its deficiency at the end of the season is critical. This matter requires the management of nitrogen fertilization according to the amount of nitrogen in the soil and the amount of nitrate in the petiole tissue of the sugar beet plant (Brentrup *et al.*, 2001; Campbell, 2002; Malnou *et al.*, 2006). Table 3 shows a direct relationship between leaf nitrate content and the index obtained from the petiole nitrate test evaluation. In other words, the higher the amount of leaf nitrate, the higher this index. By performing a nitrate test on sugar beet petiole, it is possible to speculate the plant need for nitrogen by considering its vegetative growth period.

Wollring and Koehler (1989) organized nitrogen fertilization in cereals in a balanced way and according to the plant needs by performing this nitrate test. Table 3 indicates the correlation between the nitrate concentration of sugar beet leaf tissue and plant evaluation according to the nitrate test. Completing a rapid nitrate test makes it possible to understand the need for nitrogen in sugar beet during the vegetative growth period. Thus, identifying its immediate needs for nitrogen and preventing excessive nitrogen consumption reduces the percentage of sugar in sugar beet plants.

Salinity treatment in both lines (Table 4) has increased leaf tissue proline content regardless of nitrogen. The results also show that the accumulation of proline with salinity conditions in the salinity tolerant line is more than the salinity sensitive line. This result is consistent with Carillo *et al.* (2008), who reported that the accumulation of proline in plants can indicate salinity tolerance and that salt-tolerant species accumulate more proline.

The results also show (Table 4) that the salinity tolerant line under salt stress has a higher proline accumulation when nitrate nitrogen is used compared to those under ammonium nitrogen

treatment. This observation is possibly due to the lower growth rate of sugar beet in ammonium nitrogen nutrition conditions. Sumithra *et al.* (2006) found that the activity of enzymes involved in proline synthesis is higher in the vegetative stage. Therefore, any factor that affects the vegetative growth of the plant limits the synthesis of proline. Because the growth of sugar beet in ammonium nitrogen-fed conditions was less than that of nitrogen-nitrogen-fed conditions, the decrease in proline content may be related to the effect of nitrogen form.

From the information presented in Table 5, it could be inferred that there is no significant difference in the amount of sucrose, extractable sugar, and molasses sugar of root tissue between the two forms of nitrogen. Salinity treatment has significantly reduced the percentage of extractable sugar and significantly increased the percentage of molasses sugar.

Root impurities include potassium, sodium, and residue nitrogen (amino nitrogen) presented in Table 6. The amount of sodium and potassium in the roots in both lines differs from both forms of nitrogen. They were not significant, but nitrogen in nitrate-nitrogen treatment is higher than ammonium nitrogen. Salinity treatment increased root sodium regardless of the nitrogen form, while it has no significant effect on root potassium in both lines. The combined use of salinity and nitrone nitrate has led to a substantial reduction in the amount of nitrogen in the salinity-sensitive line and a significant increase in the salinity tolerant line than the nitrate-nitrogen treatment alone. On the other hand, consumption of ammonium nitrogen with salinity compared to ammonium nitrogen alone has increased the amount of residue nitrogen in both sugar beet lines.

4. CONCLUSIONS:

In nitrate-nitrogen treatment, the total amount of root impurities in both lines is significantly higher than in ammonium nitrogen treatment. Additionally, salinity treatment in both nitrogen conditions has increased the number of impurities. This increase was noticeably higher in ammonium nitrogen consumption when compared with nitrate nitrogen. Increasing the impurities of sugar beet extract may reduce the percentage of extractable sugar and increase the molasses sugar during the sucrose crystallization process. Given the significant results of this study, it's highly recommended further experiments be conducted in this area, particularly by increasing the levels of salinity stress using different lines of sugar beet.

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6. OPEN ACCESS:

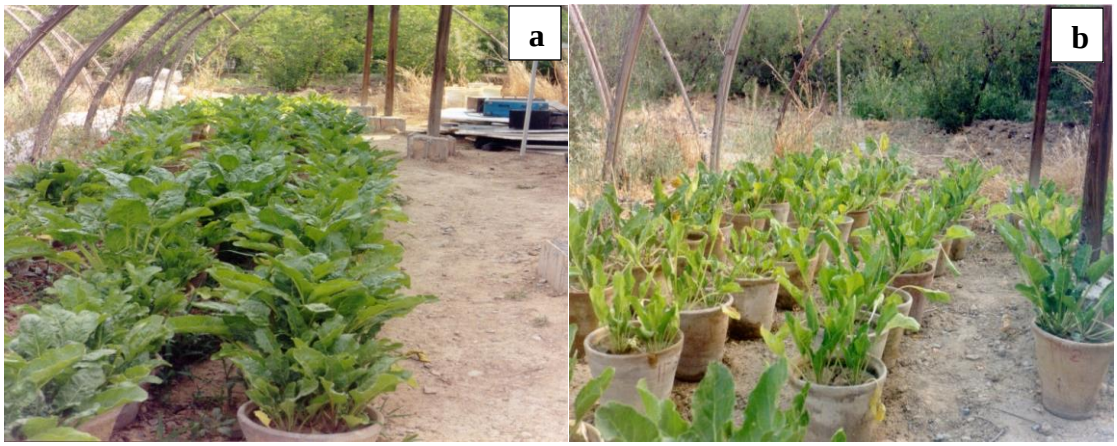

Figure 1. *Sugar beet plants in pottery pots before (a) and after (b) thinning.*

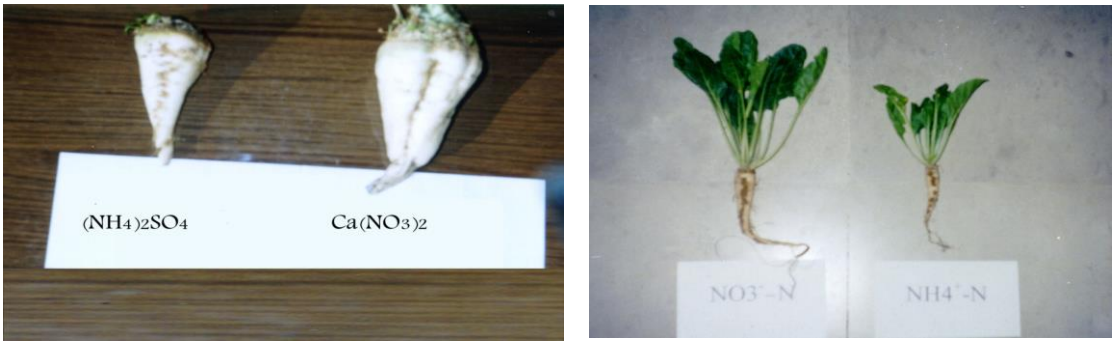

Figure 2. *The effect of nitrogen forms, nitrate, and ammonium on the growth and root yield of sugar beet.*

Table 1. *The characteristics of the soil of pots used in this study.*

Organic carbon (%)	**Neutralized compounds (%)**	**Electrical conductivity (S/m)**	**saturated soil paste acidity (pH)**	**Clay (%)**	**Silt (%)**	**Sand (%)**
1.2	16.6	3.2	7.7	28	33	39
	Micronutrient (mg/kg)					
Cu	Zn	Mn	Fe	Absorbable potassium (mg/kg)	Absorbable phosphorus (mg/kg)	NH_4-N (mg/kg)	NO_3-N (mg/kg)
1.4	7.8	13.1	12.8	440	61.4	40.3	50.9
Cations and Anions (mEq/L)of saturated soil paste)							
		Na^+	Mg^{2+}	Ca^{2+}	SO_4^{2+}	Cl^-	HCO_3^-
		7.0	8.4	32.2	29.0	7.6	3.6

Notes: Milliequivalents per litre (mEq/L); Siemens per meter (S/m);

Table 2. *Effects of N-forms and salinity on biomass production of two lines of sugar beet.*

Root Fresh Weight (g)	Shoot Dry Weight (g)	Shoot Fresh Weight (g)	Treatments
Sensitive Line			
243.2 d	19.2 d	178.4 d	NO_3-N
141.4 a	13.6 a	122.7 a	NH_4^+-N
194.5 c	17.0 c	158.3 c	NO_3-N and NaCl
163.7 b	13.7 a	123.9 a	NH_4^+-N and NaCl
Tolerant Line			
249.5 d	20.0 d	181.9 d	NO_3-N
143.1 a	13.7 a	123.9 a	NH_4^+-N
207.5 c	18.0 cd	167.3 cd	NO_3-N and NaCl
168.6 b	13.8 ab	125.5 ab	NH_4^+-N and NaCl

Table 3. *Leaf nitrate concentration and Nitrate-test index in the petiole of sugar beet lines under salinity stress and two forms of nitrogen.*

Tolerant		Sensitive			
Leaf Nitrate density (mg/kg)	**Nitrate-index***	**Leaf Nitrate density (mg/kg)**	**Nitrate-index***	**Treatment**	**Growth stage (days)**
132 f	2.8 c	128 d	2.8 c	NO_3-N	**96 days**
100 d	2.0 b	94 c	1.9 b	NH_4^+-N	
120 e	2.4 b	121.0 d	2.5 bc	NO_3-N and NaCl	
95 c	1.9 b	86 c	1.7 b	NH_4^+-N and NaCl	
307 i	5.0 d	324 h	6.0 d	NO_3-N	**131 days**
252 h	4.6 d	220 f	4.3 d	NH_4^+-N	
291 f	5.0 e	307 g	5.9 d	NO_3-N and NaCl	
192 g	4.0 d	174 e	3.8 cd	NH_4^+-N and NaCl	
48 b	1.0 a	28 b	0.8 a	NO_3-N	**167 days**
51 b	1.1 a	33 b	1.0 a	NH_4^+-N	
26 a	0.6 a	6.0 a	0.3 a	NO_3-N and NaCl	
23 a	0.6 a	14 a	0.5 a	NH_4^+-N and NaCl	

*Nitrate index was measured visually according to the intensity of color produced in the reaction which is from 0 to 6. Note: mean values with the same letters indicate homogeneous subsets for $P \leq 0.05$ according to Duncan's test. Means in the same column with different letters differ at $P \leq 0.05$.

Table 4. *Effect of N-forms on leaf proline content in sugar beet lines under salinity stress.*

Proline density (mg/g fresh weight of leaves)		Treatments
Torelant	**Sensitive**	
3.6 a	3.9 a	NO_3-N
3.2 a	3.8 a	NH_4^+-N
6.5 b	9.3 c	NO_3-N and NaCl
5.9 b	7.2 b	NH_4^+-N and NaCl

Notes: mean values with the same letters indicate homogeneous subsets for P ≤ 0.05 according to Duncan's test. Mean in the same column with different letters differ at P ≤ 0.05.

Table 5*. Effects of N-form on root qualitative properties in two lines sugar beet*

Molasses sugar (%)	Extractable sugar (%)	Sucrose (%)	Treatments	Lines
3.0 bc	11.2 bc	14.2 b	NO_3-N	Sensitive Line
2.5 ab	11.8 bc	14.3 bcd	NH_4^+-N	
4.00 d	8.1 a	12.0 a	NO_3-N and NaCl	
3.00 bc	12.8 bc	15.8 cd	NH_4^+-N and NaCl	
2.5 ab	11.5 bc	14.1 b	NO_3-N	
1.8 a	13.8 c	15.6 cd	NH_4^+-N	Tolerant Line
3.1 bc	12.6 bc	15.7 cd	NO_3-N and NaCl	
2.5 ab	13.4 bc	16.0 d	NH_4^+-N and NaCl	

Notes: mean values with the same letters indicate homogeneous subsets for P ≤ 0.05 according to Duncan's test. Mean in the same column with different letters differ at P ≤ 0.05.

Table 6. *Effects of N-form and salinity on K+, Na+ and residue nitrogen in sugar beetroots*

Impurity content (milliequivalent per 100 g of sugar beetroot)					
Total residues	**Residue nitrogen**	**Na+**	**K+**	**Treatments**	**Lines**
4.4 cd	4.9 d	2.3 bc	6.00 bc	NO_3-N	**Sensitive Line**
3.3 b	1.6 a	1.8 a	6.1 bc	NH_4^+-N	
4.5 d	1.6 a	5.2 d	6.7 c	NO_3-N and NaCl	
4.1 cd	3.5 c	3.03 bc	5.6 bc	NH_4^+-N and NaCl	
3.4 bc	2.8 b	4.5 abc	4.9 a	NO_3-N	
2.4 a	1.3 cd	1.9 ab	3.8 a	NH_4^+-N	**Tolerant Line**
3.4 cd	3.9 cd	2.5 abc	6.5 bc	NO_3-N and NaCl	
3.3 b	2.4 ab	2.5 abc	5.1 bc	NH_4^+-N and NaCl	

Notes: mean values with the same letters indicate homogeneous subsets for P ≤ 0.05 according to Duncan's test. Mean in the same column with different letters differ at P ≤ 0.05.