Effect of Nitrogen Fertilization with N2- fixing Bacteria Inoculation and Boron Foliar Application on Sugar Beet Under Calcareous Soil Conditions

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ABSTRACT: The main purpose of this investigation was to evaluate the growth, yield and quality of Gazelle sugar beet cultivar under calcareous soil conditions. Two field experiments were carried out at Mariut Experimental Station, Desert Research Center, Egypt, during two successive winter growing seasons 2015/2016 and 2016/2017 to study the effect of four concentrations of boron, control (without boron), 33, 55 and 77 mg /L boron applied as foliar spraying at two times during growth seasons and assigned in the vertical plots and six treatments of nitrogen and biofertilizer; control (without adding nitrogen or biofertilizer), 40 kg N/fed., 80 kg N/fed., biofertilizer, 40 kg N/fed. + biofertilizer and 80 kg N/fed. + biofertilizer which occupied the horizontal plots. Obtained results revealed that, all studied characters i.e. leaf area/plant, leaf area index, root length, root diameter, root yield, top yield, root/top ratio, harvest index, total soluble solids %, sucrose (%), sugar yield and apparent purity percentages were significantly affected by either boron or nitrogen and biofertilization. Also, all previous studied traits were significantly affected by the interaction between nitrogen with biofertilizer and born concentrations, except root diameter in the first season, root yield, root/top ratio and harvest index in the two seasons, which were not affected significantly due to the interaction between the studied factors.

Key words: Sugar beet, Root yield, Root quality, Mineral nitrogen, Biofertilizers, Boron foliar application.

INTRODUCTION

In Egypt, there is a gap between sugar consumption and production due to steady increases in population and average consumption of sugar beside limited cultivated area of both sugar beet and sugar cane. It is well known that, sugar beet (*Beta vulgaris,* L.) is the second source of sucrose all over the world and in Egypt as well. The importance of sugar beet crop to agriculture is not confined only to sugar production, but also it is adapted to saline and alkaline soils conditions (Abdelaal and Sahar, 2015). Moreover, sugar beet is specialized as a short duration crop, where its growth period is about half that, of sugar cane. Furthermore, sugar beet requires less water, which a kilogram of sugar requires about 1.4m^3 and 4.0 m^3 water to be produced by sugar beet and sugar cane, respectively (Sohier, 2001). The main macronutrients which are nitrogen, phosphorus, and potassium influences vegetative and reproductive phase of plant growth. It is favorable to choose the optimum rate and times of application of macro or micro nutrients to produce the maximum yield and quality for sugar beet crop (Patil, 2010). Improvement of sugar beet production can be achieved through application of traditional and nontraditional methods (Hozayn *et al*., 2013). In recent years there has been an increase in the amount of sugar beet fields that, have exhibited boron deficiency symptoms. This nutrient has an essential role in promoting cell wall formation, carbohydrate metabolism, and has been associated with sugar translocation. The effects of nitrogen on the boron nutrition of plants have consistently shown that, nitrogen reduces boron toxicity, but there have been conflicting reports on the effects of nitrogen on boron deficiency. In cotton boron deficiency may have been involved in yield reductions caused by high rates of nitrogen (Gupta, 1979) but with sugar beets nitrogen fertilizers decreased boron deficiency symptoms (Hemphill, 1982). In recent years, many investigators applied biofertilizers to minimize the environmental pollution which resulted from mineral fertilizers and also to reduce its costs (Abu EL-Fotoh *et al*., 2000 and Cakmakci *et al*., 2001). Application of *Azotobacter* spp. caused solubilization of mineral nutrients and synthesis of vitamins, amino acids, auxins as well as gibberellins, which stimulate plant growth and gave the highest yields (Sprenat, 1990).

Therefore, this investigation was undertaken to study the response of sugar beet cv. Gazelle to foliar application with boron, nitrogen fertilization and nitrogen fixing bacteria inoculation to achieve maximum root productivity and quality under calcareous soil conditions at the Western Coast of Egypt.

MATERIALS AND METHODS

Two field experiments were conducted at Mariut Experimental Station, Desert Research Center, Egypt during two successive growing seasons 2015/2016 and 2016/2017. The field experiments were laid out in strip plot design with three replications. The horizontal strips were assigned to four foliar application of boron, control (without boron), 33, 55 and 77 mg /L boron in the form of borax (11% boron) which sprayed on foliage parts of sugar beet two times (60 and 90 days after planting). The foliar solutions volume was to 100 L/fed. conducted by hand sprayer. The vertical strips were occupied by six treatments of nitrogen as ammonium nitrate (33.5% N) and nitrogen fixing bacteria as biofertlizer, control (without adding nitrogen or biofertilizer), 40 kg N/fed., 80 kg N/fed., biofertilizer, 40 kg N/fed. + biofertilizer and 80 kg N/fed. + biofertilizer. Seeds of sugar beet (*Beta vulgaris* L.) cv. Gazelle were obtained from the agricultural commercial market, Egypt and biofertilizer (*Azotobacter crococcum)* was obtained from the Agriculture Research Center, Giza, Egypt. Sugar beet seeds were inoculated with *Azotobacter crococcum* as biofertilizer at a rate of 0.8 kg/fed. then were left for a quarter hour after treating in a shaded place just before sowing. Nitrogen fertilizer treatments were added at two equal doses before third and fifth irrigation. The chemical and physical properties of soil before planting are presented in Table (1).

Each plot consist 5 ridges, each of 60 cm apart and 3.5 m long, comprising an area of 10.5 m^2 (1/400 fed.). The experimental soil was fertilized with 31 kg P_2O_5/fed. in the form of calcium superphosphate (15.5 % P_2O_5) during soil preparation and potassium at the rate of 48 kg K_2O/fed. in the form of potassium sulphate (48 % K_2O) in two equal portions added before second and fourth irrigations. Sowing took place on October 12^{th} and 17^{th} in the first and second seasons, respectively. Sugar beet balls were hand sown (3-5 balls/hill) using dry planting method on one side of the ridge and hills 20 cm apart. Experimental plots were irrigated immediately after planting, then irrigation frequently every 10 days.

Plants were thinned after 30 days from planting to one plant/hill to produce 35000 plants/fed. Plants were kept free from weeds, which were manually controlled by hoeing two times before the second and third irrigations. The agricultural practices for growing sugar beet were followed according to Ministry of Agriculture recommendations.

Studied treatments

Boron treatments:

without boron application (Control),
33 mg B/L. as borax,
55 mg B/L. as borax, and
77 mg B/L. as borax.

Nitrogen and biofertilizer:

Without nitrogen and inoculation (control),
40 kg N/fed. as ammonium nitrate,
80 kg N/fed. as ammonium nitrate,
Biofertilizer [nitrogen fixing bacteria **(***Azotobacter crococcum***)**],
40 kg N/fed. as ammonium nitrate with biofertilizer, and
80 kg N/fed. as ammonium nitrate with biofertilizer.

Studied characters

A representative samples were taken during the growth period (150 days from planting), i.e. five guarded plants were chosen at random from second and fourth ridges of each plot to determinate the following traits:
- Leaf area/plant (cm^2): It was determined using Field Portable Leaf Area Meter AM-300 (Bio-Scientific, Ltd., Great Amwell, Herforshire, England).
- Leaf area index: (LAI) = leaf area per plant (cm^2)/plant ground area (cm^2).

At maturity (180 days from planting) five guarded plants were chosen at random from the second and fourth ridges of each plot to determine yield components and quality characters as follows:
- Root length (cm).
- Root diameter (cm).
- Root yield (ton/fed.).
- Top yield (ton/ fed).
- Root/ top ratio = Root yield (ton/fed.) /Top yield (ton/fed.).

Yield of three inner ridges of each plot were harvested and cleaned. Roots and tops were separated and weighted to estimate:
- Harvest index (HI): It was calculated by using the following equation.

$$HI = \frac{\text{Root yield (ton/ha)}}{\text{Top yield (ton/ha)} + \text{Root yield (ton/ha)}}$$

- Total soluble solids (TSS %) in roots was measured in juice of fresh roots by using Hand Refractometer.
- Sucrose percentage (%): It was determined Polarimetrically on lead acetate extract of fresh macerated roots according to the method of (Carruthers and Oldfield, 1960).

- Sugar yield (ton/fed.): It was calculated by multiplying root yield (ton/fed.) by sucrose %.
- Apparent purity percentage (%): It was determined as a ratio between sucrose % and TSS % of roots as the method outlined by (Carruthers and Oldfield, 1960).

Statistical analyses

Data were arranged and analyzed as a strip plots design according to (Cochran and Cox, 1963) with three replicates. New L.S.D. test at a level of 5 % of significance was used for the comparison between means according to (Waller and Duncan, 1969).

Table (1). Some physical and chemical properties of the experimental soil (averages of the two growing seasons)

Particle size distribution			Texture class	Chemical Anlysis					
Sand (%)	Silt (%)	Clay (%)		pH	EC ds /m	$CaCo_3$ (%)	Available (mg /kg) N	P	K
54	21	25	Sandy clay loam	8.7	1.2	25.8	355.1	3.5	671.0

RESULTS AND DISCUSSION

Effect of Boron Concentrations:

A significant effects were detected due to boron concentrations application on leaf area/plant, leaf area index, root length, root diameter, root yield and top yield (Table 2), root/top ratio, harvest index, total soluble solids, sucrose (%), sugar yield and apparent purity percentage (Table 3) in both seasons. Increasing boron concentrations up to 55 mg/L significantly increased the previous studied traits. While application of boron at 77 mg/L, caused a slight decrease in these characters.

Foliar spraying of boron at 55mg/L as borax increased leaf area/plant, leaf area index, root length, root diameter, root yield/fed., top yield/fed., total soluble solids, sucrose (%), sugar yield and apparent purity percentage by 1.09, 1.09, 0.64, 1.52, 6.20, 6.67, 0.87, 2.77, 8.80, and 2.30 % respectively as an average of both seasons compared with control treatment. While root/top ratio and harvest index were decreased by 0.50 and 0.11 %, respectively as an average of both seasons compared to control treatment. Similar results were recorded by Kristek *et al*. (2006) who indicated that, highest root yield, yield attributes and sucrose concentration were obtained by spraying with 12% borax. Abido (2012) illustrated that, a significant effect was detected due to boron application on leaf area/plant, root length, root diameter total soluble solids, sucrose, apparent purity percentages, root yield, top yield, sugar yield and harvest index in both seasons. He added, increasing boron concentrations up to 80 mg/L significantly increased all studied traits, while application of boron at 120 mg/L came in the second rank with respect to these characters. Mirvat and Mekki (2005) revealed that, application of boron rates from zero up to 1.5 kg/acre increased root length, root diameter and root yield. Moreover, increasing boron fertilizer up to 2.0 kg/acre resulting highest sugar yield (6.611 ton / acre). Sucrose and juice purity percentages were also increased by adding higher concentration of boron might be attributed to decrease Na and K uptake in root juice. The positive effect of boron may be due to the boron role in cell elongation and turgidity where, in case of boron deficiency, plant leaves were reported to be smaller, stiff and thick (Brown and Hu, 1996). Mirvat and Mekki (2005) indicated that, root yield, sucrose and juice purity percentage increased by boron addition which may be attributed to decrease Na and K uptake in root juice. These results are in harmony with those obtained by Loomis and Durst (1992) and Kristek *et al.* (2006).

Effect of Nitrogen and Biofertilization:

Data in Table (2) showed that, the effect of nitrogen and biofertilizer were significant on all studded characters i.e. leaf area/plant, leaf area index, root length, root diameter, root yield and top yield (Table 2), root/top ratio, harvest index, total soluble solids, sucrose (%), sugar yield and apparent purity percentages (Table 3) in both seasons. Highest value of leaf area/plant (6062 and 6098 m^2), leaf area index (5.05 and 5.08), root length (30.88 and 30.94 cm), root diameter (13.12 and 13.48 cm), root yield (31.36 and 31.68 ton/fed.), top yield (9.14 and 9.20 ton/fed.), root/top ratio (3.43 and 3.44), harvest index (77.42 and 77.50 %), sugar yield (5.08 and 5.19 ton/fed.) and apparent purity percentage (77.97 and 77.94 %) during first and second season respectively, were obtained as sugar beet plants were fertilized by 80 kg N/fed. with biofertilizer. While total soluble solids and sucrose percentage recorded highest values (21.19 and 21.34 %) and (16.34 and 16.5 %) in the first and second season respectively, by control treatment (without nitrogen and biofertilizer).

Table (2). Averages of leaf area/plant, leaf area index, root length, root diameter, root yield and top yield as affected by boron and nitrogen combined with bio fertilizer in 2015/2016 and 2016/2017 seasons

	Leaf area/plant (cm^2)		Leaf area index		Root length (cm)		Root diameter (cm)		Root yield (Ton/fed.)		Top yield (Ton/fed.)	
	15/16	**16/17**	**15/16**	**16/17**	**15/16**	**16/17**	**15/16**	**16/17**	**15/16**	**16/17**	**15/16**	**16/17**
Boron (A):												
Without boron (control)	5947	5992	4.956	4.993	30.39	30.46	12.87	13.12	29.79	29.92	8.684	8.719
33 mg/L.	5981	6030	4.984	5.025	30.51	30.53	12.98	13.29	30.86	31.07	9.052	9.055
55 mg/L.	6007	6063	5.006	5.053	30.61	30.64	13.02	13.36	31.60	32.07	9.280	9.368
77 mg/L.	5975	6034	4.979	5.028	30.54	30.58	13.00	13.33	31.55	31.97	9.229	9.289
New L.S.D. $(_{0.05})$	1.15	9.88	0.002	0.008	0.09	0.09	0.03	0.09	0.07	0.07	0.022	0.023
Nitrogen and biofertilizer (B):												
Without mineral and biofertilizer	5880	5932	4.900	4.943	29.85	29.89	12.77	13.10	30.42	30.80	8.937	8.988
40 kg N /fed.	5977	6031	4.981	5.026	30.62	30.64	12.97	13.24	30.99	31.27	9.065	9.103
80 Kg N/fed.	6029	6075	5.024	5.063	30.77	30.79	13.07	13.36	31.23	31.52	9.130	9.179
Biofertilization	5921	5994	4.934	4.995	30.35	30.39	12.86	13.18	30.58	30.86	8.993	9.030
40 Kg N/fed.+ biofertilizer	5995	6049	4.996	5.041	30.60	30.66	13.01	13.30	31.11	31.43	9.101	9.138
80 Kg N/fed.+ biofertilizer	6062	6098.	5.052	5.082	30.88	30.94	13.12	13.48	31.36	31.68	9.142	9.905
New L.S.D. $(_{0.05})$	1.41	12.10	0.002	0.010	0.11	0.10	0.04	0.11	0.09	0.09	0.027	0.028
Interaction: AXB	*	*	*	*	*	*	N.S	*	N.S	N.S	*	*

Such effect of nitrogen on root yield and yield attributes may be due to its role in building up metabolites and activation of enzymes associated with accumulation of carbohydrates, which translated from leaves to roots as well as increasing division and elongation of cells, consequently increasing root size (Attia *et al*., 2011). The present results are in line with those obtained by (Awad *et al*., 2012), (Awad *et al*., 2013 a and b) and (Gehan *et al.,* 2013). The increase in root yield due to nitrogen fertilization can be explained through the fact that, nitrogen has a vital role in building up metabolites, activating enzymes and enhanced root length, diameter as well as root fresh weight and finally root yield. Similar results were recorded by (Attia *et al*., 2011), (Awad *et al*., 2012) and (Awad *et al*., 2013 a and b). Nitrogen fertilizer levels caused significant differences in yield and quality of sugar beet. These results were confirmed by El-Harriri and Mirvat (2001), Monreala *et al*. (2007), Seadh (2008) and Attia *et al*. (2011). Abou-Amouet *et al*. (1996) stated that, the highest values of purity (78.75 %) were obtained by 80 kg N/fed. El-Hawary (1999) reported that, fertilizing sugar beet with 90 kg N/fed recorded the highest values of sucrose %. El-Harriri and Mirvat (2001) pointed out that, application of 110 kg N/fed. markedly increased TSS %. The optimum means of sucrose and purity percentages were obtained from using 75 kg N/fed. in both seasons (Seadh, 2008). Monreala *et al*. (2007) stated that, the highest values of quality parameters were obtained from the lowest level of nitrogen (30 kg N/ha). The decrease in quality parameters (TSS % and sucrose %) due to excessive nitrogen application can be ascribed to its role in increasing root weight and diameter, tissue water content as well as increasing non sucrose substances such as proteins and alpha amino acid, and hence decreasing sucrose content in roots. This conclusion was confirmed by Monreala *et al.* (2007), Seadh (2008) and Awad *et al*. (2013a). The increase in yield attributes as a result of biofertilizer application may be due to its role in nitrogen fixation via free living bacteria which led to increase the availability of most essential macro and micronutrients as well as excretion some growth substances such IAA and GA_3. These compounds play important roles in formation a large and active root system and therefore increasing nutrient uptake, which stimulate vegetative growth. Favilli *et al.* (1993) found that, inoculation sugar beet seeds with *Azosperillium* accelerated the germination, seedling growth, plant growth, increased root and sugar yield and reduced nitrogen fertilizer requirement during the growth season. Many investigators confirmed this conclusion i.e. Gehan *et al*. (2013), Ibiene *et al*. (2012) and Jafarian *et al*. (2013). The increase in quality parameters due to biofertilization may be due to its role in improving growth and dry matter accumulation by increasing the uptake and availability of most nutrients, consequently enhancement sucrose content in roots. Similar results were reported by many investigators i.e. Maareg and Sohir (2001), Badr (2004) and Gehan *et al*. (2013).

Table (3). Averages of root/top ratio, harvest index, total soluble solids%, sugar percentage, sugar yield and pure percentage as affected by boron and nitrogen combined with biofertilizer in 2015/2016 and 2016/2017 seasons

Treatments	Root/top ratio		Harvest index %		Total soluble solids (TSS %)		Sucrose (%)		Sugar yield (Ton/fed.)		Apparent Purity (%)	
	15/16	**16/17**	**15/16**	**16/17**	**15/16**	**16/17**	**15/16**	**16/17**	**15/16**	**16/17**	**15/16**	**16/17**
Boron (A):												
Without boron (control)	3.43	3.43	77.429	77.435	20.87	21.01	15.97	16.06	4.76	4.81	76.49	76.46
33 mg/L.	3.41	3.43	77.320	77.433	21.02	21.16	16.36	16.50	5.05	5.13	77.85	77.98
55 mg/L.	3.40	3.42	77.299	77.393	21.08	21.17	16.39	16.55	5.18	5.31	78.39	78.17
77 mg/L.	3.42	3.44	77.368	77.486	20.95	21.13	16.38	16.50	5.17	5.27	78.19	78.07
New L.S.D. $(_{0.05})$	0.01	0.01	0.054	0.055	0.01	0.01	0.02	0.02	0.02	0.01	0.12	0.11
Nitrogen and biofertilizer (B)												
Without mineral and biofertilizer	3.369	3.428	77.292	77.410	21.19	21.34	16.34	16.50	4.97	5.08	77.26	77.35
40 kg N /fed.	3.422	3.430	77.369	77.453	20.97	21.11	16.26	16.37	5.04	5.12	77.68	77.57
80 Kg N/fed.	3.425	3.434	77.379	77.447	20.89	21.03	16.27	16.39	5.08	5.17	77.89	77.91
Biofertilization	3.398	3.419	77.275	77.363	21.07	21.18	16.32	16.42	4.99	5.07	77.60	77.51
40 Kg N/fed.+ biofertilizer	3.418	3.435	77.367	77.475	20.93	21.07	16.26	16.38	5.06	5.15	77.86	77.75
80 Kg N/fed.+ biofertilizer	3.434	3.444	77.428	77.502	20.83	20.99	16.20	16.36	5.08	5.19	77.97	77.94
New L.S.D. $(_{0.05})$	0.013	0.012	0.066	0.067	0.012	0.011	0.029	0.030	0.019	0.018	0.15	0.14
Interaction: AXB	N.S	N.S	N.S	N.S	*	*	*	*	*	*	*	*

Effect of the Interaction:

Regarding the interaction between the studied factors, boron concentrations and nitrogen with biofertilizer, data presented in Tables 2 and 3 showed a significant effect on leaf area/plant, leaf area index, root length, top yield, total soluble solids %, sucrose (%), sugar yield and apparent purity percentage. In the same time, no significant effect on root yield, root/top ratio and harvest index due to the interaction between nitrogen with biofertilizer and boron concentrations for two seasons was obtained. While root diameter were significantly affected by this interaction only in the second season.

CONCLUSIONS

Foliar application of 55 mg/L boron as a solution twice and fertilizing sugar beet plants by mineral nitrogen at a rate of 80 kg N/fed. with inoculating seeds by nitrogen fixing bacteria (*Azotobacter crococcum)* as biofertilization was satisfactory to achieve a better yield and quality of sugar beet under calcareous soil conditions.

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الملخص العربي

تأثير التسميد النيتروجينى مع التلقيح بالبكتريا المثبتة للنيتروجين والرش الورقى بالبورون على بنجر السكر تحت ظروف الأرض الجيرية

محمد قناوى محمد قناوى

قسم الإنتاج النباتى – شعبة البيئة وزراعات المناطق الجافة – مركز بحوث الصحراء – مصر

أجريت تجربتان حقليتان خلال الموسمين الشتويين ٢٠١٦/٢٠١٥ و٢٠١٧/٢٠١٦ بمحطة بحوث مريوط التابعه لمركز بحوث الصحراء لدراسة إستجابة بنجر السكر صنف جازيل لتأثير ثلاثة مستويات من البورون رشا على الأوراق (٣٣ ، ٥٥ و ٧٧ مللجم/لتر بورون) بالإضافة الى معاملة المقارنة (بدون الرش الورقى للبورون)] وستة مستويات من التسميد الأزوتى والتلقيح بالبكتريا المثبتة للنيتروجين (تسميد حيوى) [بدون تسميد (مقارنة)، ٤٠ كجم أزوت/فدان، ٨٠ كجم أزوت/فدان، التسميد الحيوى بالبكتريا المثبتة للأزوت الجوى (أزوتوباكتر) ، ٤٠ كجم أزوت/فدان مع التسميد الحيوى و ٨٠ كجم أزوت/فدان مع التسميد الحيوى]. صممت التجربة فى نظام الشرائح المتعامدة حيث شغلت معاملات الرش الورقى للبورون الشرائح الرأسية بينما وزعت معاملات التسميد بالنيتروجين والحيوي فى الشرائح الأفقية فى ثلاث مكررات. أوضحت النتائج حدوث تأثير معنوى لمعاملات الرش الورقى للبورون ومعاملات التسميد بالنيتروجين والحيوي على جميع الصفات التى تم دراستها (مساحة سطح أوراق النبات، دليل المساحة الورقية، طول وقطرالجذر، محصول الجذور/فدان، محصول العرش/فدان، نسبة الجذر للعرش ، دليل الحصاد، النسبة المئوية للمواد الصلبة الذائبة الكلية بالجذور، نسبة السكروز بالجذور، محصول السكر/فدان، نسبة النقاوة) فى كلا الموسمين، أيضا كان للتفاعل بين العوامل التى تم دراستها تأثير معنوى على كل الصفات السابقة عدا صفات قطر الجذر فى الموسم الأول ومحصول الجذور و نسبة الجذر للعرش ودليل الحصاد فى الموسمين حيث لم تتأثر هذه الصفات معنويا بالتفاعل بين العوامل التى تم تناولها فى هذه الدراسة.