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IMPROVEMENT OF MICROBES COMBINATION BY USING PROLINE IN RELATION TO SUGAR BEET YIELD UNDER NITROGEN LIMITATION IN SALINE SOIL

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ABSTRACT

Two field experiments were conducted at Sahl El-Hussinia Res. Station, El-Sharkia Governorate during the two successive seasons 2009/2010 and 2010/2011 to find out the effect of inoculation of sugar beet seeds with a mixture of N_2-fixing bacteria (*Azospirillum lipoferum*, *Azotobacter chroococcum, Bacillus polymyxa and Klebsiella pneumoniae)* compared either individually or in combination with supplementation with proline amino acid under two levels of mineral nitrogen fertilization (75 and 100 kg N/fed) on sugar beet yield, juice quality and enzymatic activities (nitrogenase and dehydrogenase of its rhizosphere soil).

Results indicated that bacterial inoculation of sugar beet seeds led to significant increases in root fresh weight, root dimensions, root and sugar yields at harvest. Increasing proline concentration from 0 to 20 and 40 ppm recorded a significant increase in root yield. Meantime, there was insignificant differences in these traits between 20 and 40 ppm which produced the highest values of root yield. N_2- fixing bacteria+ 100 kg N/fed with 20 ppm proline gave a significant increase in root yield compared with applying N_2- fixing bacteria or inorganic alone. However, the highest sugar yield was recorded by the addition of 100 kg N/fed along with biofertilization with N_2-fixing bacteria inoculation +20 or 40 ppm proline in both seasons.

Results revealed that neither the combinations among the bacterial inoculation and nitrogen inorganic-N nor the applied proline concentrations addition affected the total soluble solids (TSS), sucrose and purity percentage in both seasons. Moreover, both of nitrogenase (N_2-ase) and dehydrogenase (DHA) activities increased significantly when seeds were inoculated with N_2-fixing bacteria, especially with N_2-fixing bacteria. However, the application of N-fertilizer had insignificant influence in this respect.

The obtained results direct our attention to the importance of proline with inoculating seeds of sugar beet with *Bacillus polymyxa* which played promoting effect with inorganic-N fertilization in maximizing sugar yield/fed.

Key words: *Beta vulgaris, L., dehydrogenase activity, N_2-fixing bacteria, nitrogenase, proline, sugar beet, yield and quality.*

1. INTRODUCTION

Sugar beet *(Beta vulgaris,* L.) provides about 25% of the world sugar production ranked the second after sugarcane. However, it participates in the production of nearly 50% of sugar in Egypt in 2010 due to increasing the acreage of' sugar beet from 16.943 thousand feddans in 1982 to 320.982 thousand feddans in 2010 (Sugar Crops Council Book 2012).

One of the most important limiting factors is N-supplementation to crops by means of fertilizing plants with either inorganic or organic nitrogen source. An alternative approach for improving crop production is the biological N-fixation, as a natural resource of N-nutrition. The use of N-fixing bacteria is of economic importance to modern agriculture as they can partially replace the costly mineral N-fertilizers and minimize production costs and reduce environmental pollution as well as ensure high yields.

The beneficial effect of N_2-fixing bacteria to plant productivity was attributed to enhancing phytohormones, Auxins, Gibberellin and Cytokinin-like substance to the culture medium (Tien *et al.,* 1979).

Bacterization of sugar beet seeds with *Azotobacter chroococcum, Bacillus megatherium* and *Bacillus circulants* resulted in high significant vegetative growth characters, root length and diameter, root fresh weight and top weight than untreated plants Afify *et al.* (1994) and Sultan *et al.* (1999) found that the inoculation of sugar beet seeds with *Azotobacter* significantly increased root length

and diameter. Also, Maareg and Badr (2001) showed that inoculation of sugar beet with Cerealine (A biofertilizer consists of *Bacillus polymyxa)* caused an increase in length, diameter and weight of roots. While, Neamat-Alla (2004) found that sugar beet seed inoculation with Cerealine and Phosphorine (A biofertilizer consists of phosphate dissolving bacteria) had insignificant effect on root length and diameter and significantly affected root and sugar yields. Regarding the effect of sugar beet seed inoculation with some N- fixing bacteria on root yield, El-Badry and El-Bassal (1993) found that about 40 % of N mineral fertilizer was saved by using free living N-fixing bacteria with an increase in the average root yield of 2.8 - 6.0 ton/ fed and sugar yield. Also, Sukhovitskaya (1998) found that inoculation of sugar beet seeds with *Bacillus megatherium* increased crop yields of root and sugar by 23%. Moreover, Khalil (2002) concluded that inoculation with *Azotobacter chroococcum* and *Bacillus megatherium* saved about 25 kg N/fed of mineral fertilizer, which reduced the cost of production and the environmental pollution, in addition to the increase of sugar yield and the recoverable sugar/ fed. Furthermore, inoculation of seeds with *Azospirillum* increased sucrose content in sugar beet roots. Ali (2003) cited that inoculation of sugar beet seeds with *Azotobacter* increased significantly root and sugar yields/fed. Also, Badr (2004) mentioned that inoculation with biofertilizer significantly increased root weight, and root and sugar yields/fed.

With respect to sugar beet quality, Afify *et al.* (1994) reported that application of NPK and inoculation of sugar beet seeds with *Azotobacter dhroococcum, Bacillus megatherium* and *Bacillus circulans* individually or in combinations resulted in the highest sucrose percentage. Sultan *et al.* (1999) and Maareg and Badr (2001) showed that inoculation of sugar beet with N_2-fixing bacteria and phosphate dissolving bacteria caused increases in TSS, sucrose and purity percentages compared to control treatment. Ali (2003) stated that the percentages of TSS and sucrose increased significantly when sugar beet plants were inoculated with a biofertilizer. On the other hand, Favilli *et al.* (1993) found that inoculation of sugar beet with *Azospirillum lipoferum* had no significant effect on sucrose content. Hassouna and Hassanein (2000) concluded that bio and mineral N-fertilization had a slight positive effect on both sucrose and purity % and tended to decrease TSS %. Zalat *et al.* (2002) pointed out that purity and sugar content significantly decreased with the application of one or two recommended dose of Cerealin. Ali (2003) found that purity percentage of sugar beet was not affected by the inoculation with *Azospirillum brasilense, Azotobacter chroococcum* and *Bacillus megatherium.* Neamat-Alla (2004) reported that there was no evidence for significant differences in the TSS % as well as in sucrose and juice purity percentages due to inoculation of sugar beet seeds with P and N-bio fertilizers.

Many workers studied the effect of N-fertilizer on sugar beet yield and quality. El-Essawy (1996) found that juice purity percentage tended to increase with increasing the level of N fertilizer. El-Zayat (2000) showed that increasing N rate from 70 to 90 kg N/fed exhibited significant differences in the percentage of juice purity. However, Mostafa and Darwish (2001) and Badr (2004) revealed that juice purity percentage was reduced linearly as N level was increased. In respect to the effect of N- level on sugar beet quality, El-Essawy (1996) found that increasing N rate increased sucrose percentage, whereas Badr (2004) stated that increasing N level decreased sucrose percentage. Neamat-Alla (2004) showed that increasing N level from 90 to 140 kg/fed did not affect sucrose and TSS percentages. While, Neamat-Alla *et al.* (2002) indicated that increasing N-level caused a significant decrease in TSS percentage. However, Badr (2004) showed that TSS percentage was increased by increasing N level.

Regarding the effect of N-fertilizer on sugar yield El-Hawary (1999), El-Zayat (2000) and Badr (2004) showed that increasing N level significantly increased sugar yield/fed. Meanwhile, Azzazy (1998) found that increasing N rate from 40 to 80 kg/fed insignificantly increased sugar yield. Also, Zalat *et al.* (2002) indicated that N application up to 240 kg/ha was important for high yield production. With respect to sugar beet root length and diameter, Sorour *et al.* (1992), Neamat-Alla *et al.* (2002) and Badr (2004) stated that root length and diameter at harvest were significantly increased by increasing N fertilizer level up to 90 kg N/fed while, Neamat-Alla (2004) stated that there was insignificant effect on root length by applying 20, 40 and 60 kg N/fed.

The aim of this work was to study the effect of fertilizing sugar beet with different combinations of bio-and-inorganic nitrogen levels under different levels of proline on some growth, quality characteristics and yields of sugar beet as well as rhizoshere enzyme activities under saline conditions.

2. MATERIAL AND METHODS

Two field experiments were carried out at Sahl El-Hussinia Res. Station, El-Sharkia Governorate

during the two successive seasons 2009/2010 and 2010/2011 to find out the effect of N_2-fixing bacteria and proline under two levels of nitrogen fertilizer and their interaction effect on sugar beet plants. The present study included fifteen treatments represented the combinations among three proline concentrations (0, 20 and 40 ppm) and five bio and inorganic-N treatments, which were:

1- 100 kg N/fed.
2- 75 kg N/fed.
3- N_2-fixing bacteria.
4- N_2-fixing bacteria + 100 kg N/fed.
5- N_2-fixing bacteria + 75 kg N/fed.

Where: N_2-fixing bacteria: Equal portions of vinasse cultures of Log phase $\approx 10^{10}$ CFU ml^{-1} *Azotobacter chroococcum, Azospirillum lipoferum, Bacillus polymyxa* and *Klebsiella pneumoniae* were used in a mixture as bacterial inoculation. Otherwise, N_2- fixing bacteria were isolated from sugar beet rhizosphere at Sahl El-Hussinia Res. Station, El-Sharkia Governorate.

Proline treatment: liquid solution of amino acid L-proline ($C_5H_9NO_2$) containing 0, 20 and 40 mg l^{-1}

Sugar beet seeds cv. Ras poly were washed with tap water and soaked in the vinasse cultures of N_2-fixing bacteria mixture and in liquid solutions of L- proline (0, 20 and 40 mg l^{-1}) for 3 hrs. Soaked seeds were air-dried under shade before sowing. Moreover, the same soaking treatments were sprayed after 30, 45 and 60 days from seed sowing.

The two inorganic levels of nitrogen fertilizer were applied as ammonium nitrate (33 % N) in two equal doses, the first was added after thinning (45 days from sowing) and the second was added 21 days later. Phosphorus fertilizater was applied as calcium superphosphate 15% at 100 Kg P_2O_5 /fed during seed bed preparation.

A split plot design with three replications was used, where the three proline concentrations occupied the main plots, while the five N fertilization combinations were randomly allocated in the sub-plots. The sub - plot area was 14 m^2 consisted of 4 rows, of 7 m long and 0.5 m width. The other agronomic practices were carried out as recommended by the Ministry of Agriculture in sugar beet fields.

2.1. Soil biological and chemical analyses

Soil samples were analyzed 120 days after sowing for soil biological and chemical properties as follows:

2.1.1. Soil biological activity: Soil enzymes *i.e.* dehydrogenase activity (DHA) was estimated according to Casida *et al.* (1964) and nitrogenase activity was measured by acetylene reduction assay as described by Total bacterial counts were performed on nutrient agar medium using the spread plate technique (APHA, 1992). The most dominating N_2-fixer isolates of 8 *Azospirillum*, 12 *Azotobacter*, 8 *Bacillus* and 10 *Klebsiella* were isolated from rhizospheric soil samples of sugar beet grown at Sahl El–Hossainya Research Station experimental sites. Isolates were purified and identified as *Azospirillum lipoferum*, *Azotobacter chroococcum, Bacillus polymyxa and Klebsiella pneumoniae* (Bergy's Manual, 1984). The abilities of these isolates to fix N_2 were tested by determining activity (μg N_2 fixed/ ml culture/day) as described by Hardy *et al.* (1973) and shown in Table (2). The highest isolates in N-ase activity were used as a mixture (No.3 for *Azospirillum* sp., No. 11 for *Azotobacter* sp., No.7 for *Bacillus* sp No.9 for *Klepsiella* sp.). The highest active strains of *Azospirillum, Azotobacter, Bacillus and Klebsiella* were grown separately on semi-solid malate medium (Döbereiner, 1978), modified Ashby's medium (Abdel-Malek and Ishac, 1968), (Hino and Wilson, 1959) (Yoch and Pengra.1966) for 7 days at 28± 2 ºC, respectively.

Table (1): The abilities of isolated bacteria to fix N_2 .

Isolate No.	*Azospirillum* sp.	*Azotobacter* sp.	*Bacillus* sp.	*Klebsiella* sp.
1	31.6	91.1	24.3	23.7
2	34.2	18.4	6.00	59.11
3	**88.7**	86.76	36.68	88.4
4	78.1	77.6	29.7	61.7
5	41.3	12,8	18.30	84.40
6	6.60	50.3	19.70	55.11
7	80.4	66.00	**64.80**	40.8
8	14.0	40.5	12.3	20.6
9	_	11.9	_	**105.70**
10		52.1	_	40.0
11	_	**475.20**	_	---
12		65.6	_	_

2.1.2. Soil chemical analyses

Soil available nitrogen in the upper 30-cm of the experimental site was determined according to Black (1965). Available phosphorus was determined spectrophotometrically. Available sodium and potassium were determined using flame-photometric method (APHA, 1992). Soil pH was measured in 1:2.5 soil water extract using

Table (2): Physiochemical properties of the soil before sugar beet planting.

Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Texture		O.M (%)		$CaCO_3$ (%)
2.10	46.91	12.32	38.67	Clay		0.62		10.36
pH (1:2.5)	EC in soil paste ($dS.m^{-1}$)	Cations ($meq.l^{-1}$)				Anions ($meq.l^{-1}$)		
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO^{-}_{3}	Cl^{-}	SO^{--}_{4}
8.30	24.37	14.22	27.12	201	1.34	9.93	186	47.75
Available nutrients ($mg.kg^{-1}$ soil)								
N	P	K	Fe	Mn		Zn		Cu
31	3.77	197	1.70	2.92		0.52		0.063

glass electrode pH meter (Model 955), and electric conductivity (EC) was measured in 1:5 soil water extract using glass electrode conductivity meter Model Jenway 4310. Nutrient uptake was determined as nutrient concentration X root dry weight.

2.2. Growth criteria and juice quality

At harvest, a sample of ten sugar beet plants was taken at random from the two guarded rows of each sub- plot to determine the following growth and juice quality traits:

1. Root fresh weight (g/plant), root length (cm) and root diameter (cm).
2. Total soluble solids (TSS %) was determined using handle Refractometer.
3. Sucrose percentage was determined using sacchrimeter according to the procedure outlined by Le-Docte (1927).
4. Purity percentage: Sucrose percentage x 100 / TSS%
5. Yield and its components : To determine yield and its components, the four rows of each treatment were harvested, topped and weighed to determine root yield (ton fed^{-1}) and sugar yield (ton fed^{-1}) which was calculated by multiplying root yield x sucrose percentage.

The obtained data were subjected to the proper statistical analysis for the split plot design according to Snedecor and Cochran (1980). Least significant difference (L.S.D.) at 5% level of significance was used for treatment means comparison.

3. RESULTS AND DISCUSSION

3.1. Growth criteria

Results in Table (3) clear that increasing the applied proline levels from 0 to 20 and 40 ppm led to a significant increase in sugar beet root fresh weight in both seasons. The highest and significant value was produced by a concentration of 40 ppm proline. The possible roles of proline have been attributed to stabilizing the structure of macromolecules and organelles through stabilizing proteins and membranes against the denaturating effect of high concentrations of salts and other harmful solutes (Munns, 2002).

Moreover, with bacteria caused significant increase in root weight in both seasons. Inoculation of sugar beet seeds with *N_2-fixing bacteria* with 100 kg N/fed gave the highest values of beet root fresh weight 1529.2 and 1612.4 g/plant in the 1st and the 2nd seasons, respectively. Application of inorganic N-fertilization only (75 and 100 kg N/fed) led to a significant increase in root weight compared with N_2- fixing bacteria alone, where an application of 100 Kg N/fed gave the highest mean values of root weight 1135 and 1179 g in both seasons, respectively. This increase amounted to about 150.7 and 146.2% over 75 kg N/fed in both seasons, reductively. These results are in agreement with those obtained by Basha (1999).

Root fresh weight was significantly affected by the interactions between fertilization treatment sources and proline levels in both seasons. N_2-fixing bacteria +100 kg N/fed with 20 or 40 ppm proline was superior to other treatments in this trait and produced the highest values (1797.4 and 1801.6 g/plant) in the 1st season and (1899.7 and 1940.8 g/plant) in the 2nd one, respectively.

Root length of sugar beet was insignificantly affected by proline, fertilization treatments and the interaction. This result is in line with Neamat-Alla (2004).

Concerning root diameter the results revealed that root diameter was significantly increased by applying proline levels in both seasons (Table 3). The effective concentration of proline was 40 ppm. This result is in line with Mohamed Faten *et al.* (2011). Root diameter increased significantly when N level was raised from 75 up to 100 kg

N/fed in both seasons. These findings are in accordance with those reported by Neamat-Alla *et al.,* (2002) and Badr (2004) who stated that root-length and diameter at harvest were significantly increased by increasing N-fertilizer level up to 90 Kg N/ fed. Meanwhile, inoculation with N_2-fixing bacteria alone led to the lowest value in root diameter ;4.5 in the 1st and 4.73 the 2nd seasons. However, the combination between N_2-fixing bacteria and 100 kg N/fed recorded the highest values 12.43 and 12.77 cm in both seasons, respectively.

The interaction effect of the studied factor N_2-fixing bacteria + 100 kg N with 20 ppm proline caused significant increases in this trait. Also, the obtained results are in harmony with those obtained by Neamat-Alla (2004) who reported that sugar beet seed inoculation with some N-fixing bacteria had significant effect on root length and diameter at either growth or harvest stages.

Table (3): Effect of inoculation with some N_2-fixing bacteria and inorganic N- fertilizer on some growth traits of sugar beet roots at harvest in (2009/2010 and 2010/2011).

Fertilizer treatments	2009/010				2010/011			
	Proline concentrations (ppm)				Proline concentrations (ppm)			
	0	20	40	Mean	0	20	40	Mean
Root fresh weight (g/plant)								
100 kg N/fad	683.2	1294.0	1427.7	1135.0	703.23	1344.00	1489.7	1179.0
75 kg N/fad	419.6	450.9	487.6	452.7	428.1	489.9	518.7	478.9
N_2-fixing bacteria	289.2	391.3	411.9	364.1	319.2	421.1	471.1	403.8
N_2-fixing bacteria + 100 kg N/fad	988.5	1797.4	1801.6	1529.2	996.8	1899.7	1940.8	1612.4
N_2-fixing bacteria + 75 kg N/fad	786.5	1396.5	1394.4	1192.5	789.3	1411..5	1442.4	1214.4
Mean	633.4	1066.0	1104.6		647.3	1113.2	1172.5	
LSD 0.05								
Proline (P)				44.5				64.1
Nitrogen (N)				89.5				100.6
P x N				100.3				113.9
Root length (cm)								
100 kg N/fad	23.33	28.33	29.44	27.0	21.00	24.80	28.86	24.9
75 kg N/fad	27.22	29.45	31.66	29.4	23.93	25.76	29.20	26.3
N_2-fixing bacteria	25.55	24.44	29.44	26.7	23.70	24.40	28.00	25.4
N_2-fixing bacteria + 100 kg N/fad	26.11	29.99	28.33	28.1	24..33	27.53	25.70	52.9
N2-fixing bacteria + 75 kg N/fad	25.55	28.06	29.72	27.8	23.24	25.62	27.94	25.6
Mean	25.60	28.10	29.40		23.20	25.50	27.94	
LSD 0.05								
Proline (P)				NS				NS
Nitrogen (N)				NS				NS
P x N				NS				NS
Root diameter (cm)								
100 kg N/fad	9.6	9.9	10.5	10	9.9	10.6	10.9	10.5
75 kg N/fad	7.2	7.7	7.9	7.6	7.8	8.1	8.8	8.2
N_2-fixing bacteria	3.9	4.5	5.1	4.5	4.1	4.6	5.5	4.7
N_2-fixing bacteria + 100 kg N/fad	11.5	12.8	13.0	12.43	11.8	12.9	13.6	12.77
N2-fixing bacteria + 75 kg N/fad	8.1	9.6	9.8	9.2	8.6	9.6	10.3	9.5
Mean	8.06	8.90	9.26		8.4	9.16	9.82	
LSD 0.05								
Proline (P)				0.33				0.44
Nitrogen (N)				1.31				1.57
P x N				2.11				1.98

3.2.Root and sugar yields

Results obtained in Table (4) show that increasing the application of proline concentrations from 0 to 20 and 40 ppm recorded a significant increase in sugar beet root yield. Meantime, there was a significant difference in this trait between 20 and 40 ppm which produced the highest values of root yield ton/fed (16.14, 17.34, 18.08 and 19.34 ton/fed in the 1st and the 2nd seasons,respectively.) The possible roles of proline have been attributed to stabilizing the structure of macromolecules and organelles through stabilizing proteins and membranes against the denaturating effect of high concentrations of salts and other harmful solutes (Munns, 2002). Exogenous addition of proline was very effective in counteracting the effect of salt. El-Enany *et al.* (1995) reported that addition of proline (100 mg/L) to the medium containing 100 and 150 mM NaCI counteracted the inhibitory effect of NaCl and enhanced shoot regeneration, especially at high NaCI levels in tomato cell cultures. The higher levels of proline served presumably as a compatible osmotic buffer in the cytoplasm against high vacuolar ion concentrations. In addition, the amelioration effects of proline and glycine betaine on plant growth, stability of leaf membranes, leaf relative water content, chlorophyll content and leaf osmotic potential should be taken in account.

Regarding root yield in both seasons, the results cleared that inoculation of sugar beet seeds with N_2-fixing bacteria + 100kgN/fed gave significant increases in root yield compared with applying the other treatments. This treatment was the superior one. It gave the highest mean values, 24.67 and 27.40 ton / fed. in the 1st and the 2nd seasons, respectively. These results are in line with those reported by

Table (4): Effect of inoculation with some N_2-fixing bacteria and inorganic N - fertilizer on root and sugar yields of sugar beet at harvest (2009/2010 and 2010/2011).

Fertilizer treatments	2009/010				2010/011			
	Proline concentrations (ppm)				Proline concentrations (ppm)			
	0	20	40	Mean	0	20	40	Mean
Root yield (ton/fed)								
100 kg N/fad	15.8	19.9	21.7	19.13	17.3	22.1	23.7	21.03
75 kg N/fad	10.1	11.6	14.6	12.10	12.5	13.9	16.2	14.20
N_2-fixing bacteria	5.3	6.4	6.9	6.2	6.6	7.8	8.3	7.57
N_2-fixing bacteria + 100 kg N/fad	21.7	25.9	26.4	24.67	24.2	28.4	29.6	27.40
N_2-fixing bacteria + 75 kg N/fad	15.3	16.9	17.1	16.4	14.7	18.2	18.9	17.27
Mean	13.64	16.14	17.34		15.06	18.08	19.34	
LSD 0.05								
Proline (P)				0.76				1.19
Nitrogen (N)				1.84				3.43
P x N				1.92				2.89
Sugar yield (ton/fed)								
100 kg N/fad	2.61	3.18	3.92	3.24	2.75	3.45	4.19	3.45
75 kg N/fad	1.73	1.99	2.58	2.10	2.10	2.38	2.86	2.45
N_2-fixing bacteria	0.87	1.16	1.12	1.05	1.07	1.39	1.38	1.28
N_2-fixing bacteria + 100 kg N/fad	3.57	4.23	4.14	3.98	4.05	4.61	4.87	4.51
N_2-fixing bacteria + 75 kg N/fad	2.54	2.85	2.90	2.76	2.41	3.04	3.23	2.89
Mean	2.26	2.68	2.93		2.47	3.02	3.31	
LSD 0.05								
Proline (P)				0.19				0.24
Nitrogen (N)				0.29				0.41
P x N				0.34				0.53

(Sultan *et al.,* 1999), Ali (2003) and Badr (2004) who showed that inoculation of sugar beet seed with nitrogen fixing bacteria significantly increased root yield per fed. Once more, mineral nitrogen fertilizer led to significant increases in root yields in the two seasons, where the application of 100 Kg N / fed produced the highest mean values (19.13 and 21.03 ton/fed) compared with those produced by 75 kg N/fed (12.10 and 14.20 ton/fed) in the 1st and the 2nd seasons, successively. These results are in harmony with those found by EI-Hawary (1999), Mostafa and Darwish (2001) and Zalat *et al.* (2002) who indicated that mineral N application up to 120 Kg N / fed was important for high yield production.

Interaction effects of the studied factors were significant for root yield of sugar beet in both seasons, inoculation with N_2-fixing bacteria + 100 kg N/fed in combination with 20 ppm proline attained a distinct increase in the root yield. This finding was completely true in both seasons.

With respect to Sugar yield (ton / fed), the results given in Table (4) showed that treated sugar beet seeds with 20 or 40 ppm proline attained the highest and significant sugar yield in both seasons, Results in Table (4) obviously show that sugar yield was significantly and progressively increased as inorganic nitrogen rates were increased from 75 to 100 kg N/fed. The highest sugar yield (3.24 and 3.45) was produced with the application of 100 kg N / fed. This result is in accordance with that outlined by El-Zayat (2000) and Badr (2004) who found that sugar yield was significantly increased due to the increase of N_2-levels up to 100 Kg N / fed.

Results in Table (4) also show that sugar yield was significantly affected by the applied combination of N2 fixing bacteria with 100 kg N/fed. This treatment produced 0.74 and 1.06 tons/fed higher than the un-inoculated one (100 kg N/fed) in the 1st and the 2nd season, respectively. These results clear the positive effectiveness of the used bio-N sources on sugar beet. The increase in sugar yield as affected by the used bio-N fertilizers could be attributed to the increase in root yield. These results are in agreement with those reported by Sultan *et al*., (1999) and Badr (2004) who mentioned that sugar beet seeds inoculation with biofertilizer significantly increased sugar yield/ fed. Also, applying of 80 Kg N / fed gave an increase in sugar yield (25.56% over unfertilized one).The interaction effect of the studied factors was significant on sugar yield. Increasing the applied concentration of proline under biofertilizer treatment of N_2-fixing bacteria in combination with 100 kg N/fed significantly increased the obtained value of sugar yield in both seasons. This indicates that proline with bio fertilization and N_2-fixing bacteria played a complementary role with mineral N fertilization where the highest sugar yield was recorded when sugar beet received 100 Kg N / fed.

3.3.Quality characteristics

Table (5) shows the effect of inoculation with some N_2-fixing bacteria + 20 ppm proline and mineral N fertilizer or their combination on quality characters of sugar beet juice, *i.e*. total soluble solids percentage (TSS%) sucrose and purity percentage.A general view to the presented data in Table (5) cleared that none of the studied juice quality parameters, *i.e*. the percentages of T.S.S., sucrose and purity was affected by the bio and / or the mineral nitrogen fertilizers. These results are in harmony with those obtained by Badr (2004) and Neamat-Alla (2004) who reported that there was no evidence for significant differences in T.S.S. per cent due to inoculation of sugar beet seed with phosphor and nitrogen biofertilizers. Also, Hassouna and Hassanein (2000) and Badr (2004) concluded that biological and mineral N fertilization had slightly positive effect on sugar percentage.

3.4. Microbial enzymes of sugar beet soil

3.4.a. Nitrogenase (N2-ase activity)

Table (6) shows that bacterial inoculation led to a significant increase in N2-ase activity of sugar beet rhizosphere in both seasons. N2-fixing bacteria were the most effective. It gave maximum mean values in both seasons. The corresponding values were 55.24 and 78.88 n mole g-1 rhizosphere soil day-1 for the first and the second seasons, respectively.

Active rhizosphere in N_2-ase enzyme for uninoculated plants is owing to the presence of indigenous asymbiotic bacteria in soil.

Regarding the effect of mineral N-fertilizer on N2-ase activity, the results show that mineral N fertilizer gave insignificant differences in N-ase.With respect to mineral N-fertilizer, the results in Table (7) show that there were no differences in DHA activity. Generally, As N-fertilizer increase up to 100 Kg N / fed, DHA activities decreased. The reaction effects of the studied factors were significant at 90 days period for the 1st season and at the 2nd season, where the best treatment was N2-fixing bacteria + 20 ppm proline inoculation without mineral N-fertilizer. From the above-mentioned results, it could be concluded that inoculation of sugar beet seeds with N_2-fixing bacteria + 20 ppm proline along with the addition of 100 Kg' N / fed was effective to maximize the root yield / fed to over 10 tons in both seasons without any adverse effect on sugar quality characteristics.

Table (5): Effect of inoculation with some N_2-fixing bacteria and inorganic N - fertilizer on some quality traits of sugar beet roots at harvest (2009/2010 and 2010/2011)

Fertilizer treatments	2009/010				2010/011			
	Proline concentrations (ppm)				Proline concentrations (ppm)			
	0	**20**	**40**	**Mean**	**0**	**20**	**40**	**Mean**
Total soluble solids (TSS %)								
100 kg N/fad	24.26	21.73	22.03	**22.67**	19.83	20.46	21.63	**20.64**
75 kg N/fad	22.53	22.90	23.60	**23.01**	20.50	19.93	19.70	**20.04**
N_2-fixing bacteria	22.60	22.73	22.26	**22.53**	20.23	20.46	20.36	**20.35**
N_2-fixing bacteria + 100 kg N/fad	22.03	23.16	23.60	**22.93**	21.56	20.03	20.03	**20.54**
N_2-fixing bacteria + 75 kg N/fad	22.86	22.63	22.88	**22.79**	20.53	20.23	20.43	**20.40**
Mean	**22.86**	**22.63**	**22.87**		**20.53**	**20.22**	**20.43**	
LSD 0.05								
Proline (P)				NS				NS
Nitrogen (N)				NS				NS
P x N				NS				NS
Sucrose %								
100 kg N/fad	16.49	15.98	18.05	**16.84**	15.90	15.63	17.66	**16.40**
75 kg N/fad	17.14	17.16	17.66	**17.32**	16.76	17.10	17.63	**17.16**
N_2-fixing bacteria	16.40	18.05	16.29	**16.91**	16.20	17.76	16.60	**16.85**
N_2-fixing bacteria + 100 kg N/fad	16..46	16.33	15.70	**16.16**	16.73	16.23	16.46	**16.47**
N_2-fixing bacteria + 75 kg N/fad	16.63	16.89	16.93	**16.81**	16.40	16.68	17.09	**16.72**
Mean	**16.6**	**16.88**	**16.93**		**16.40**	**16.68**	**17.09**	
LSD 0.05								
Proline (P)				NS				NS
Nitrogen (N)				NS				NS
P x N				NS				NS
Purity %								
100 kg N/fad	67.97	73.54	81.93	**74.48**	80.18	76.39	81.65	**79.41**
75 kg N/fad	76.08	74.93	74.83	**75.28**	81.76	0.86	89.49	**85.80**
N_2-fixing bacteria	72.57	79.41	73.18	**75.05**	80.08	86.80	81.53	**82.80**
N_2-fixing bacteria + 100 kg N/fad	74.72	70.51	66.53	**70.59**	77.60	81.03	82.18	**80.27**
N_2-fixing bacteria + 75 kg N/fad	72.75	74.64	73.99	**73.79**	79.88	82.45	83.65	**81.99**
Mean	**72.82**	**74.61**	**74.09**		**79.90**	**82.56**	**83.70**	
LSD 0.05								
Proline (P)				NS				NS
Nitrogen (N)				NS				NS
P x N				NS				NS

Table (6): Effect of inoculation with some N_2-fixing bacteria and inorganic N - fertilizer on nitrogenase enzyme activity (u mole CjHi / g / day) of sugar beet rhizosphere after 120 days from sowing (2009 and 2010/2011).

Fertilizer treatments	2009/010				2010/011			
	Proline concentrations (ppm)				Proline concentrations (ppm)			
	0	20	40	Mean	0	20	40	Mean
Nitrogenase								
100 kg N/fad	15.28	18.83	17.21	**17.11**	3.02	9.59	19.28	**10.63**
75 kg N/fad	17.98	16.25	12.78	**15.67**	6.30	5.10	3.54	**4.98**
N_2-fixing bacteria	64.69	70.01	31.01	**55.24**	74.0	78.55	84.08	**78.88**
N_2-fixing bacteria + 100 kg N/fad	28.16	27.56	33.16	**29.63**	72.78	44.53	59.76	**59.02**
N_2-fixing bacteria + 75 kg N/fad	46.53	40.66	38.54	**41.91**	37.13	47.19	26.75	**37.02**
Mean	**34.53**	**34.66**	**26.54**		**38.65**	**36.99**	**38.68**	
LSD 0.05								
Proline (P)				NS				NS
Nitrogen (N)				2.11				5.8
P x N				2.29				6.9

Table (7): Effect of inoculation with some N_2-fixing bacteria and inorganic N - fertilizer on dehydrogcnasc enzyme activity (pg TPF g / day) of sugar beet rhizosphcrc after 90 days from sowing (2009/2010 and 2010/2011)

Fertilizer treatments	2009/010				2010/011			
	Proline concentrations (ppm)				Proline concentrations (ppm)			
	0	20	40	Mean	0	20	40	Mean
Dehydrogenase								
100 kg N/fad	14.85	13.77	19.12	15.91	13.3	18.14	18.45	16.63
75 kg N/fad	16.67	11.77	11.57	13.33	14.10	19.00	16.92	16.673
N_2-fixing bacteria	46.12	44.96	42.30	1566.42	35.64	29.65	38.10	34.463
N_2-fixing bacteria + 100 kg N/fad	31.02	57.80	33.80	40.87	61.42	60.45	41.79	54.553
N_2-fixing bacteria + 75 kg N/fad	42.17	45.83	41.69	29.17	41.29	39.3-1	36.32	12.107
Mean	**30.16**	**34.82**	**29.69**		**33.15**	**33.108**	**30.316**	
LSD 0.05								
Proline (P)				NS				NS
Nitrogen (N)				2.8				1.96
P x N				3.4				2.87

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تحسين المعاملات البكتيريه باستخدام البرولين وعلاقة ذلك بمحصول بنجر السكر فى الاراضى الملحية محدودة النيتروجين

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ملخص

أجريت هذه الدراسه بمحطة بحوث الحسينية بمحافظة الشرقية فى موسمى الزراعة ٢٠٠٩/٢٠١٠ و٢٠١٠/٢٠١١ لدراسة تأثير توليفات من بعض معاملات التسميد النيتروجينى الحيوى (مخلوط من أربعة أنواع من البكتريا المثبته للنيتروجين) وغير العضوى (٧٥ ، ١٠٠كجم نيتروجين للفدان) مع ثلاث تركيزات من الحمض الأمينى البرولين (٠، ٢٠ ، ٤٠ جزء فى المليون) وتفاعلاتها على حاصل وجودة محصول بنجر السكر. استخدم تصميم القطاعات المنشقة مرة واحدة فى ثلاث مكررات حيث وضعت تركيزات البرولين فى القطع الرئيسية ووزعت معاملات السماد النيتروجينى الحيوى وغير العضوى عشوائيا فى القطع الشقية.

- أظهرت النتائج ان تلقيح بذور بنجر السكر بالبكتيريا المثبته للنيتروجين واضافة ١٠٠ كجم نيتروجين للفدان أدى الى زيادة معنوية فى صفات نمو بنجر السكر تحت الدراسة (الوزن الغض للجذر ، قطر الجذر) كما أدى استخدام تركيزى البرولين ٢٠ ، ٤٠ جزء فى المليون لزيادة معنوية فى حاصل الجذور/فدان ولم تكن هناك فروقاً معنوية بين التركيزين.
- أعطت توليفة البكتريا مع ١٠٠ كجم نيتروجين / فدان مع ٢٠ أو ٤٠ جزء فى المليون من البرولين أعلى حاصل جذور مقارنة باستخدام البكتريا بمفردها ، وسجل أعلى حاصل جذور وسكر باستخدام البكتيريا مع ١٠٠ كجم نيتروجين / فدان مع البرولين ٢٠ جزء فى المليون .
- لم تتأثر صفات جودة محصول بنجر السكر تحت الدراسة (المواد الصلبة الذائبة الكلية ، النسبة المئوية لكل من السكروز والنقاوة) معنوياً بجميع المعاملات المستخدمة. بالنسبة لنشاط إنزيمى النيتروجينيز والدىهىدروجينيز فقد تأثرا معنوياً بإستخدام البكتيريا المثبته للنيتروجين فقط ولم يكن هناك تأثير معنوى للسماد النيتروجينى غير العضوى أو تركيزات البرولين.
- يمكن من النتائج التحصل عليها التوصية بإستخدام مخلوط الاربعة أنواع من البكتيريا المثبته للنيتروجين مع إضافة ١٠٠ كجم نيتروجين للفدان والرش بالبرولين بتركيز ٢٠ جزء فى المليون للحصول على أعلى حاصل جذور وسكر.

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (٦٣) العدد الرابع (أكتوبر ٢٠١٢): ٣٧٥-٣٨٥.