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RESEARCH ARTICLE

Genetic variability and response of some sugarcane varieties to application methods of N-fixation organism (*Azospirillum brasilense*) under different inorganic nitrogen levels

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Abstract

Two field experiments were conducted at Mallawi Agric. Res. Station, El-Minia Governorate, Egypt, during 2018-19 and 2019-20 seasons to evaluate the performance of four sugarcane varieties (G.T.54-9, G.84-47, G.2003-47 and G.2004-27) in response to *Azospirillum brasilense* application methods (without, soil drench and inoculation of cane setts) and inorganic-N levels (150 and 200 kg N / fed) following split plot design. Variety G.T.54-9 surpassed the other varieties in millable cane weight, cane and sugar yields / fed. There was an insignificant difference between GT.54-9 and G.2004-27 varieties in cane yield / fed in both seasons and sugar yield / fed in the 1st one. Inoculating cane setts with Azospirillum was more effective on most of the studied traits than soil drench. Adding 200 kg N/fed significantly increased brix %, sucrose %, cane and sugar yields / fed, while purity % was reduced. The effects of the significant interactions among the studied factors on the recorded traits were discussed. Correlation between cane yield and its components was highly significant with positive directions across seasons. Cane yield and millable cane weight recorded the highest heritability %, genotypic and phenotypic coefficient of variation. Significant genotypic effects indicated existence of the genetic variability among varieties and the possibility of utilizing them in genetic improvement. Variety G.T. 54-9 attained the highest net return, followed by G. 2004-27, G. 2003-47 and G. 84-47. Under conditions of the present work, inoculating cane cutting seeds of G.T. 54-9 and / or G. 2004-27 sugarcane varieties with 2 packets / fed (one packet = 400 g) containing Azospirillum brasilense bacteria + 150 kg N/fed could be recommended to attain the economical cane and sugar yields.

Keywords: Bio-fertilizer; Correlation; Genetic variability; Nitrogen; Quality, Sugarcane; Varieties; Yield.

Introduction

Sugarcane (*Saccharum* spp.) is a robust tillering long-term crop. In Egypt, sugar manufacturers used to depend on one sugarcane variety occupying most areas and same would be replaced with another only after its damage or injury by pests and / or diseases. Sugarcane varieties viz. POJ 105, POJ 2878, Co 281 and Co 413 were subsequently replaced. Nowadays, G.T. 54-9 has almost covered all areas of sugarcane since 1983, where its productivity began to decrease, while its susceptibility with diseases such as smut and pests like soft scale and borers increases. G.T. 54-9 outperformed the two promising varieties G.2003-49 and G.2003-47 (Giza 3) in stalk dimensions and stalk weight, but the two promising varieties outperformed it with respect to purity, recoverable sugar and brix % (Makhlouf et al. 2016). El-Bakry (2018) noticed that sugarcane cultivar G.2003-47 displayed a substantial dominance in quality traits. Ali et al. (2019) opined that cultivars Cu.57-14 and G.T. 54-9 outperformed others in terms of sugar and cane yield / fed. Gadallah and Abd El-Aziz (2019) stated that variety G.T.

54-9 exceeded the other ones in terms of stalk length and cane yield per fed, meanwhile variety G. 2003-47 overpassed it in stalk thickness, brix reading, sucrose %, recoverable sugar % and sugar yield per fed. Gadallah and Mehareb (2020) observed that the maximum cane yield was scored with the commercial cultivar, while Giza 3 variety had the highest sucrose %, sugar recovery % and sugar yield. Significant and positive correlations were noticed between cane yield and millable cane weight / plant. Sugar yield was positively and appreciably correlated with purity %, recoverable sugar % and sucrose %.

Inoculation of planting materials and / or the soil plays a vital role in agriculture, as it provides significant benefits to the crop and reduces the cost of fertilizer to producers. Despite the fact that bacteria such as Bacillus, *Azospirillum brasilense* and *Pseudomonas fluorescens* have been shown to improve the production of other crops, little is known about the impacts of these micro-organisms on sugarcane (Rosa et al. 2020). Previous research using *A. brasilense* highlighted its capacity to fix N_2 and its benefits in terms of promoting plant growth through the synthesis of phyto-hormones like cytokinin, auxin, ethylene, gibberellins and salicylic acid, which significantly affect root development and may positively affect yield and increase nutrient supply (Galindo et al. 2019 a, b; Zaheer et al. 2019). Hari and Srinivasan (2005) showed that *A. brasilense* sharply increased cane and sugar yield. The response of cane yield to Azospirillum proved to be more efficient at lower N-level i.e., 200 kg / ha than 300 kg N / ha. Cock et al. (2011) cleared that using *A. brasilense* had the greatest influence on cane diameter and root systems. El-Geddawy and Makhlouf (2016) elucidated that Azotobacter-inoculated cane seeds produced considerably higher millable cane weight, sugar recovery %, purity % and cane yield. Rashed et al. (2016) mentioned that using *A. brasilense* as a foliar application and seeds inoculation insignificantly affected quality criteria of sugar beet in both growing seasons, except for sucrose % in the 2nd one, meanwhile sugar yield was markedly affected, in both seasons. Schultz et al. (2017) revealed that inoculating cane cutting seeds with diazotrophic bacterial strains and fertilizing with nitrogen improved sugar yields in RB 867515 and RB 72454 varieties. Dawood et al. (2019) found that soil application with yeast extract after 45 days from sowing, significantly improved the vegetative growth of flax, as well as, Elsayed et al. (2020) showed that soil drench with N-fixing bacteria (Rhizobium, Azospirillum and Azotobacter) markedly increased plant height of dill. Pereira et al. (2021) found that inoculating setts with a mixture of plant growth promoting rhizobacteria strains increased cane yield of 15%.

Sugarcane production necessitates an adequate supply of water and fertilizers, especially nitrogen. The exaggeration of the applied water using flood irrigation system, as well as excessive addition of nitrogen leads to loosing nitrate-nitrogen beyond the root zone, bad soil aeration, unbalance of nutrients and difficulty in their absorption by roots resulting in yield reduction. Moreover, it causes succulence of plant tissues, leading to cane lodging and reduction in its quality, while raising costs of production and decreasing the net return. In this respect, Saleem et al. (2012) manifested that the dosage and timing of N application had a marked effect on most of the studied parameters. The greatest yields of cane and sugar / ha were obtained when nitrogen was added at 252 kg / ha in two equal splits, although the higher N dose i.e. 336 kg/ha was not statistically inferior. Abd El-Azez et al. (2018) confirmed that raising N levels to 220 kg / fed sharply increased millable height and diameter, sugar and cane yields, and TSS %, while adding 190 kg N / fed gave the maximum averages of purity % and sugar recovery %. They added that the interaction between varieties and N fertilizer was significant in its effect on stalk height, percentages of purity, sucrose and TSS. Bekheet et al. (2018) stated that raising nitrogen level up to 210 kg / fed sharply increased stalk dimensions and its weight, yields of cane and sugar, sucrose %, recoverable sugar % and brix reading. Abdel-Kader and Abdel-Aal (2019) illustrated that millable cane dimensions, cane and sugar yields/fed markedly increased by raising N rates from 180 to 220 kg/fed. Zeng et al. (2020) cleared that raising nitrogen rates from 150 to 300 kg/ha caused an increment in stalk dimensions and its weight, yields of cane and sugar / ha, while sucrose and brix % decreased. Sasy and Abu-Ellail (2021) revealed that increasing N-level up to 210 kg / fed produced a substantial improvement in dimensions and weight of millable cane, yields of sugar and cane / fed, brix reading, sucrose %, purity % and recoverable sugar %.

Agronomists and breeders have adopted yield component studies through phenotypic correlation, as a crop improvement strategy. Correlation is a term that is used to explore and show the relationship between yield and its components (Yahaya et al. 2009). Masri et al. (2014) observed positive and considerable correlations between cane yield and millable cane weight/plant. Sugar yield was positively and appreciably correlated with purity %, recoverable sugar % and sucrose %. Estimates of heritability, as well as genotypic coefficients of variation, are needed for improving any sugarcane trait because they aid in determining whether the target goal can be met with the available content (Tyagi and Singh 1998; Abu-Ellail et al. 2017). Estimating genetic variances under a limited set of environmental conditions can lead to inaccurate genetic variance estimates (Bissessur et al. 1999). Furthermore, Kang et al. (1984) stated that sugarcane genetic variation estimates based on a single year and / or position would be potentially biassed or inestimable. They suggested that the evaluation of sugarcane varieties in many crop years would be satisfactory.

Therefore, the present work aimed at decreasing inorganic nitrogen dose by the complementary use of bio-N, searching its appropriate application method, in addition to evaluate the performance of some other registered sugarcane varieties compared with the prevailed commercial one under bio and inorganic N-fertilizer.

Materials and Methods

Two plant cane field trials were carried out at Mallawi Agricultural Research Station (lat. of 28°10’ N, long. of 30°75’ E and altitude of 55 MSL), El-Minia Governorate, Egypt, during 2018/2019 and 2019/2020 seasons to evaluate the performance of four sugarcane varieties [G.T 54-9 (C 9) “control”, G.84-47, G.2003-47 (Giza 3) and G.2004-27 (Giza 4)] to find out their response to three application methods of an effective strain of *A. brasilense* N-fixing bacteria (without, soil drench, and inoculation of cane cutting seeds) and two inorganic nitrogen levels (150 and 200 kg N/ fed “fed = 0.42 ha”). Split plot design with three replications was used. Sugarcane varieties were allocated in the main plots, whereas the combinations between the application methods of bio fertilizer and inorganic nitrogen levels were randomly distributed in the sub-plots. Sub plot area was 35 m^2 including five rows of seven m in length and one m in width. At planting cane cuttings were inoculated with 2 bags / fed (one bag = 400 g) containing 10^7-10^9 cells / g approximately of *A. brasilense,* which were spread on seeds and after covering the seed cutting was irrigated immediately. The soil drench method was done with the bacterial suspension which contains 10^9 cells/ml approximately of *A. brasilense,* at a rate of 5 l / 300 liters of water / fed after 45 days from planting. Microorganism inoculant was provided from the Bio-fertilizers Prod. Unit of Agric. Microbiol. Res. Dept., Soil, Water, and Environ. Res.

Inst., ARC, Giza, Egypt. *A. brasilense* was grown on Dobereiner medium (Dobereiner and Baldini 1979).

Sugarcane varieties were planted during the 2nd week of March and harvested at twelve-month age in both seasons. The preceding crop was maize followed by fallow. Phosphorus fertilizer was added once during land preparation at 30 kg P_2O_5/ fed as mono-super phosphate (15% P_2O_5). Nitrogen fertilizer was applied as urea (46% N) in two equal doses; after two months from planting and one month later. Potassium fertilizer was added once at 48 kg K_2O/fed as potassium sulphate (48% K_2O) with the 2nd dose of N fertilizer. The other agricultural practices of growing sugarcane were carried out as usual according to Sugar Crops Res. Inst. recommendations.

Some physical properties of the experimental soil were analyzed as described by Black et al. (1981). Soil chemical analysis was determined according to Jackson (1973). Physical and chemical analyses of the experimental soil (at 40-cm depth) are presented in Table 1.

The recorded data: At harvest, a sample of 20 millable canes from each treatment was collected at random, cleaned and topped to determine the following growth and quality traits and cane and sugar yield / fed (0.42 ha).

1. Millable cane height (cm) was measured from soil surface to the top visible dewlap.
2. Millable cane diameter (cm) was measured at the middle part with no reference to the bud groove.
3. Net millable cane weight (kg)
4. Brix % was determined using Brix Hydrometer standardized at 20° C and sucrose % was determined using "Saccharimeter" according to A.O.A.C. (2005).
5. Sugar recovery % (rendment) was calculated according to Yadav and Sharma (1980); Sugar recovery % = [sucrose % - 0.4 (brix % - sucrose %) x 0.73], where 0.4 = 0.4 pound of sucrose retained (not extracted) by each pound of non-sucrose solids in cane juice, as outlined by Herbert (1973) and 0.73 is a correction factor for actual milling conditions in factories that depends on the overall mean of cane fiber % during processing, as shown by Mathur (1997).

Table 1: Physical and chemical properties of the upper 40-cm of the experimental soil

Seasons	Particle size distribution			Soil texture	EC (dS/m)	pH (1:2.5)
	Sand %	Silt %	Clay %			
2018/19	9.35	54.45	36.20	Silty clay loam	1.76	8.0
2019/20	8.65	53.52	37.83	Silty clay loam	1.97	8.1

Seasons	Soluble cations (mq l^{-1})				Soluble anions (mq l^{-1})			Available nutrients (mg/1kg soil))		
	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^{-}	SO_4^{--}	Cl^{-}	N	P	K
2018/19	8.45	2.75	4.45	0.23	3.25	7.73	4.9	37.03	7.85	175
2019/20	9.78	2.72	4.95	0.24	3.68	8.21	5.8	39.35	8.50	181

6. Juice purity % was calculated as: [(sucrose / brix) x 100].
7. Reducing sugars % was determined using the Fehling method according to A.O.A.C. (2005).
8. Cane yield/fed (ton), which was determined from the fresh weight (kg) of millable canes of each sub-plot, which was converted into tons/fed.
9. Sugar yield (ton/fed) was estimated by multiplying net cane yield/fed (ton) by sugar recovery %.
10. Economical evaluation.

Statistical analysis:The collected data were statistically analyzed according to the technique of analysis of variance (ANOVA) for the split-plot design published by Gomez and Gomez (1984) using the MSTAT-C computer software package. The least significant of difference (LSD) method was used to check differences between means at 5% level of probability as described by Snedecor and Cochran (1980).

According to Steel and Torrie (1980), a simple correlation coefficient was computed among some studied traits. On a variety mean basis, broad-sense heritability (h^2 percent) was calculated using variance components using Johnson et al. (1955) equation:

$$h^2 = \sigma^2 g / (\sigma^2 g + \sigma^2 e / r + \sigma^2 gc / rc)$$

Where, ($\sigma^2 g$) and ($\sigma^2 e$) refer to genotypic and error variance, respectively. The divisor (r) refers to the number of replications; $\sigma^2 gc$ refers to variety by crop interaction variance. The divisor (rc) refers to the number of crops. According to Burton and Devance (1953), genetic coefficients of variance (GCV) are a measure of trait genetic variation relative to its mean. The GCV percent = ($\sigma^2 g$/general mean) x 100 allows comparisons between traits of different units and scales and puts variance into perspective.

Results and Discussion

Growth characteristics

The statistical analysis showed that the tested sugarcane varieties significantly varied in millable cane diameter and its fresh weight, in both seasons, in addition to millable cane height, in the 2nd season (Table 2). The collected data clearly indicated that the commercial variety i.e. G.T.54-9 continues to outperform the others with respect to the above mentioned traits, as it had the highest average of millable cane weight/plant, surpassing G.84-47, G.2003-47 and G.2004-27 by 0.165, 0.091 and 0.091 kg respectively and 0.166, 0.054 and 0.039 kg, as well as 0.29, 0.19 and 0.15 cm and 0.14, 0.08 and 0.07 cm for millable cane diameter, in the first and second season consecutively. Variation in these characteristics among varieties of sugarcane may be due to variation in gene make-up as well as their response to the environmental conditions. The differences among varieties of sugarcane had been reported by Makhlouf et al. (2016), Fahmy et al. (2017) and Ali et al. (2019).

In terms of the impact of bio-fertilizer, Table 2 pointed out that using N-fixing bacteria with sugarcane plants produced a statistical and higher values of millable cane height, thickest and its fresh weight in both growing seasons than that left without addition of bio-fertilizer. Inoculating cane cutting seeds and soil drench methods with *A. brasilense* bacteria resulted in the highest values and substantial increments in millable cane weight reached 0.191 and 0.089 kg in the first season, corresponding to 0.086 and 0.047 kg in the second one, successively, as compared to the control treatment. The positive effect of the bio-N fertilizer is probably due to the production of bacterial phytohormones resulted from microbial activity in the root zone, as explained by Hernandez et al. (2001), which may enhance the growth of cane plants. Similar findings were obtained by Cock et al. (2011). Furthermore, the maximum growth of sugarcane plants by the influence bacterium occurred when cane cutting seeds were inoculated following the soil drench method. This method may have resulted in a more consistent distribution of bacterial cells throughout the root zone during the growth period. However, little is known about the influences of micro-organisms on sugarcane (Rosa et al. 2020), it is possible that this is due to the solubilization of some vital nutrients for the plant.

In the same table, the findings showed that feeding sugarcane plants with the higher N level i.e. 200 kg/fed significantly increased all the above-mentioned traits compared with 150 kg N/fed in both growing seasons. Raising levels of N from 150 to 200 kg/fed resulted in significant increments by 0.085 and 0.069 kg in millable cane weight, in the 1st and 2nd season, respectively. The increase in millable dimensions and its fresh weight may be due to nitrogen's role as an important nutrient in building-up plant organs and their development. These findings are consistent with those mentioned by Bekheet et al. (2018) and Zeng et al. (2020).

Quality parameters

Data in Table 3 disclosed that the examined sugarcane varieties differed markedly in sucrose % in

Table 2. Effect of application methods of N-fixing bacteria and levels of inorganic-N fertilizer on millable cane height, diameter and weight/plant of four sugarcane varieties in 2018/2019 and 2019/2020 seasons.

Treatments	**Millable cane height (cm)**		**Millable cane diameter (cm)**		**Millable cane weight (kg)**	
	1st season	**2nd season**	**1st season**	**2nd season**	**1st season**	**2nd season**
Sugar cane varieties						
G.T.54-9	298.11	310.11	2.61	2.67	1.174	1.235
G.84-47	296.94	301.78	2.32	2.53	1.009	1.069
G.2003-47	270.56	289.17	2.42	2.59	1.083	1.181
G.2004-27	274.94	294.44	2.46	2.60	1.083	1.196
LSD at 0.05	NS	13.75	0.10	0.06	0.056	0.045
Bio-nitrogen application methods						
Without	274.38	293.75	2.39	2.51	0.994	1.126
Soil drench	284.75	296.46	2.45	2.60	1.083	1.173
Seeds inoculation	296.29	306.42	2.52	2.68	1.185	1.212
LSD at 0.05	6.01	5.69	0.05	0.04	0.030	0.019
Inorganic nitrogen levels "kg/fed"						
150	276.83	292.69	2.41	2.58	1.045	1.136
200	293.44	305.06	2.49	2.62	1.130	1.205
LSD at 0.05	*	*	*	*	*	*

*=significant; NS=Insignificant difference.

both seasons and Brix readings in the 2nd one, meanwhile reducing sugar and purity percentages were not affected. The findings manifested that G.84-47 sugarcane variety recorded a sharp increment in sucrose % by 1.14 and 1.01 in the first season, corresponding to 1.64 and 1.52 in the second one relative to G.2003-47 and G.2004-27 respectively. It is known that sucrose % is a genetically controlled trait. In addition, variety G.84-47 had a significant increase in brix % amounted to 1.34, 2.06 and 1.97, as compared to GT.54-9, G.2003-47 and G.2004-27 varieties, successively, in the 2nd season, corresponding to insignificant increases in the 1st one. The results obtained revealed that sugarcane varieties varied with respect to their content of sucrose mainly due to their differences in maturity states which are attributed by gene-make up influence. Differences among cane varieties in these traits were found by El-Bakry (2018) and Gadallah and Abd El-Aziz (2019).

Concerning bio-fertilizer, the results pointed out that using N-fixing bacteria with sugarcane plants significantly affected Brix %, sucrose % and reducing sugar % in both seasons and purity % in the 1st one. There was a marginal variance between the two application methods with *A. brasilense* bacteria in the values of reducing sugar %. Significant effects and higher values of Brix

Table 3: Effect of application methods of N-fixing bacteria and levels of inorganic-N fertilizer on Brix, sucrose, reducing sugar and purity percentages of four sugarcane varieties in 2018/2019 and 2019/2020 seasons

Treatments	Brix %		Sucrose %		Reducing sugar %		Purity %	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Sugar cane varieties								
G.T.54-9	20.50	19.80	15.40	15.44	0.351	0.396	75.07	78.11
G.84-47	21.86	21.14	16.03	15.88	0.376	0.427	73.49	75.21
G.2003-47	20.10	19.08	14.89	14.24	0.416	0.444	74.18	74.62
G.2004-27	19.81	19.17	15.02	14.36	0.405	0.438	75.88	74.94
LSD at 0.05	NS	0.86	0.81	0.68	NS	NS	NS	NS
Bio-nitrogen application methods								
Without	19.81	19.26	14.55	14.35	0.377	0.416	73.62	74.75
Soil drench	20.60	19.91	15.34	15.16	0.390	0.430	74.58	76.17
Seeds inoculation	21.29	20.23	16.11	15.42	0.394	0.433	75.76	76.24
LSD at 0.05	0.19	0.30	0.21	0.17	0.009	0.010	1.07	NS
Inorganic nitrogen levels "kg/fed"								
150	20.17	19.42	15.13	14.82	0.376	0.417	75.11	76.41
200	20.96	20.17	15.54	15.13	0.398	0.435	74.20	75.03
LSD at 0.05	*	*	*	*	*	*	*	*

*: significant and NS: insignificant differences.

reading and sucrose % were obtained using *A. brasilense* bacteria as seeds inoculation more than soil drench. The relative benefit of bio-fertilizer in respect to sucrose % may be due to the increase in the availability of nitrogen which saves more sufficient nitrogen in soil solution which is reflected on the photosynthetic process in turn sugar production. This result was in agreement with El-Geddawy and Makhlouf (2016).

Brix, sucrose, reducing sugar and purity percentages were significantly influenced by the applied inorganic N levels, in both growing seasons. Raising N levels from 150 to 200 kg/fed attained considerable increments in brix % by 0.79 and 0.75, as well as 0.41 and 0.31 for sucrose %, corresponding to a sharp reduction in purity % reached 0.91 and 1.38, in the 1st and 2nd season, successively. A similar trend was observed by Ahmed et al. (2011), Zeng et al. (2020) and Sasy and Abu-Ellail (2021).

Sugar recovery %, millable cane and sugar yields/fed

The statistical analysis elucidated that the behaviour of sugarcane varieties appreciably varied in yields of millable cane and sugar / fed, in both growing seasons, as same as recovery sugar % in the 2nd one (Table, 4). The highest values of sugar recovery % were recorded with sugarcane variety G.84-47 followed by GT.54-9 meantime, the lowest values of this trait were obtained with G.2003-47 and G.2004-27. No statistical variances in sugar recovery % and sugar yield / fed were detected between GT.54-9 and G.84-47 varieties in the second season, as well as between G.2003-47 and

Table 4. Effect of application methods of N-fixing bacteria and levels of inorganic-N fertilizer on sugar recovery%, cane and sugar yields of four sugarcane varieties in 2018/2019 and 2019/2020 seasons.

Treatments	Sugar recovery %		Millable cane yield/fed-(ton)		Sugar yield/fed (ton)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Sugar cane varieties						
G.T.54-9	9.75	9.99	51.86	52.65	5.06	5.27
G.84-47	10.00	10.06	47.10	49.06	4.70	4.94
G.2003-47	9.34	8.98	49.89	50.42	4.67	4.53
G.2004-27	9.57	9.07	50.14	51.16	4.80	4.65
LSD at 0.05	NS	0.70	2.14	2.03	0.31	0.49
Bio-nitrogen application methods						
Without	9.08	9.04	49.06	49.94	4.45	4.52
Soil drench	9.66	9.68	49.88	50.93	4.82	4.93
Seeds inoculation	10.25	9.86	50.31	51.60	5.15	5.09
LSD at 0.05	0.21	0.21	0.67	0.88	0.12	0.15
Inorganic nitrogen levels "kg/fed"						
150	9.57	9.48	49.20	49.69	4.71	4.72
200	9.76	9.58	50.30	51.96	4.91	5.01
LSD at 0.05	*	NS	*	*	*	*

*: significant and NS: insignificant differences.

G.2004-27 varieties, in both seasons, corresponding to a significant difference was found between GT.54-9 and G.84-47 varieties in millable cane yield/fed. Sugarcane variety GT.54-9 outperformed in cane yield/fed, rising by 10.11% and 3.95% in the 1st season, corresponding to 7.32% and 4.42%, in the 2nd one, than those achieved by G.84-47 and G.2003-47 respectively. The results cleared that the commercial variety had a marked increment in sugar yield, where it exceeded G.2003-47 by 0.40 and 0.78 tons/fed in the 1st and 2nd season, successively. It could be noticed that there were insignificant differences between GT.54-9 and G.2004-27 varieties in sugar yield/fed in the 1st season, as same as cane yield/fed in the two seasons. Such varietal differences among cane varieties were mentioned by Makhlouf et al. (2016), Galal et al. (2018) and Gadallah and Abd El-Aziz (2019).

Sugar recovery % was positively affected by the use of any bio-nitrogen methods, in both seasons. Using *A. brasilense* bacteria as cane cutting seeds inoculation resulted in an appreciable increment in the values of millable cane yield/fed reached 1.25 ton (2.55%) and 1.66 ton (3.32%), corresponding to 0.82 ton (1.67%) and 0.99 ton (1.98%) using *A. brasilense* bacteria as a soil drench, in the first and second season, respectively, as compared to the check treatment. Further, inoculating cutting seeds and a soil drench with *A. brasilense* bacteria attained a substantial increment in sugar yield per fed reached 0.70 and 0.37 tons in the 1st season, as well as 0.57 and 0.41 tons, in the 2nd one, successively, as compared to the check treatment. These results are also in line with those found by El-Geddawy and Makhlouf (2016), Schultz et al. (2017) and Pereira et al. (2021). It could be observed that the bacterium enhanced growth the most when cane cutting seeds were inoculated with bio N-fixer, following soil drench method. This may have resulted in a more even and consistent distribution of bacterial cells throughout the root zone during the growth period. It's possible that the improved growth is due to the solubilization of some plant's nutrients.

The results presented in Table 4 indicated that increasing inorganic nitrogen levels from 150 to 200 kg/fed significantly raised the values of millable cane yield/fed amounted by 1.10 tons (2.24%) and 2.27 tons (4.57%), corresponding to 0.20 ton (4.25%) and 0.29 ton (6.14%) for sugar yield/fed, in the first and second season consecutively. The distinct effect of the additional level of nitrogen may be due to the low level of nitrogen in the experimental side (Table 1). Sugar recovery % was significantly affected by the applied N levels in the 1st season only similar to that of sucrose % (Table, 3) where it is known that sugar recovery % depends mainly on sucrose content. In addition, the increase may be attributed to the role of nitrogen as an important element in building-up plant organs and alteration in dry matter production and distribution, which reflected on cane yield and sugar accumulation in different sugarcane cultivars. The important role of nitrogen fertilizer in cane yield had been reported by Abdel-Kader and Abdel-Aal (2019), Zeng et al. (2020) and Sasy and Abu-Ellail (2021).

Significant interaction effect between sugarcane varieties and application methods of N-fixing bacteria

Data in Table 5 indicated that the interaction between the sugarcane varieties and application methods of N-fixing bacteria sharply affected millable cane height, purity %, yields of millable cane and sugar yield /fed in the 1st season, and millable cane diameter and its fresh weight, brix % and sugar recovery % in the 2nd one, in addition to sucrose % in both seasons. Statistical variances in the values of cane and sugar yields / fed in the 1st season were observed between GT.54-9 and each of G.84-47 and G.2003-47 varieties, as same as between G.84-47 and G.2004-27 varieties in cane yield/fed, when sugarcane plants were treated with any of bio-N application methods. Conversely, there was no substantial difference in these traits between the two cultivars GT.54-9 and G.2004-27 under the application methods of N-fixer as a soil drench. Likewise, considerable variances in the means of sucrose % in both seasons as well as brix reading and recovery sugar % in the 2nd one, were detected between cultivars G.84-47 and G.2003-47, as a result of different treatments bio-fertilizer, corresponding to marginal differences between G.2003-47 and G.2004-27 in the above-mentioned traits.

Significant differences were confirmed between the two cultivars GT.54-9 and G.2003-47 in the values of purity % in the 1st season and millable cane weight in the 2nd one, when cane cutting seeds were inoculated with *A. brasilense* bacteria. On the contrary, the differences between the two cultivars GT.54-9 and G.2004-27 in the above-mentioned traits failed to reach the significance level under the different application methods of N fixing bacterium. The commercial cultivar GT.54-9 differed with each of G.2003-47 and / or G.2004-27 appreciably in the millable cane height in the 1st season and diameter in the 2nd one in the cases of cane cutting seeds inoculation and/or soil drench with N-fixer bacteria, while the differences between

Table 5: Significant interaction effect between varieties and application methods of N-fixing bacteria on some traits of sugarcane in 2018/2019 and 2019/2020 seasons

Varieties	Bio-nitrogen application methods	Millable cane height (cm)	Millable cane diameter (cm)	Millable cane weight (kg)	Brix %	Sucrose %		Purity %	Sugar recovery %	Cane yield/fed (ton)	Sugar yield/fed (ton)
		1st season	2nd season	2nd season	2nd season	1st season	2nd season	1st season	2nd season	1st season	1st season
G.T.54-9	Without	285.50	2.57	1.186	18.81	14.25	14.98	72.20	9.82	51.45	4.53
	SD	298.33	2.68	1.238	20.14	15.36	15.45	75.11	9.91	51.68	5.03
	SI	310.50	2.77	1.281	20.44	16.60	15.87	77.89	10.25	52.46	5.36
G.84-47	Without	291.33	2.45	1.027	21.03	15.24	15.01	71.93	9.20	46.67	4.36
	SD	292.67	2.54	1.070	21.14	16.06	16.10	73.72	10.28	47.25	4.73
	SI	306.83	2.60	1.111	21.26	16.79	16.55	74.82	10.70	47.37	5.01
G.2003-47	Without	265.83	2.51	1.178	18.63	14.26	13.69	74.22	8.55	49.29	4.42
	SD	271.67	2.59	1.178	19.16	14.92	14.43	73.79	9.15	50.13	4.68
	SI	274.17	2.67	1.187	19.43	15.48	14.59	74.53	9.24	50.26	4.90
G.2004-27	Without	254.83	2.52	1.114	18.57	14.44	13.72	76.13	8.60	48.84	4.49
	SD	276.33	2.59	1.205	19.18	15.03	14.66	75.68	9.38	50.45	4.82
	SI	293.67	2.70	1.270	19.77	15.60	14.69	75.82	9.24	51.14	5.08
LSD at 0.05		12.02	0.07	0.039	0.60	0.43	0.34	2.14	0.41	1.34	0.23

SD=Soil drench; SI=seeds inoculation

the commercial variety and G.84-47 variety in the values of millable cane height were not significant under the same conditions.

In the 1st season, the interaction between the commercial variety GT.54-9 and cane cutting seeds inoculation with *A. brasilense* bacteria resulted in the highest values and sharp increments in cane yield / fed, where it exceeded variety G.2003-47 by 2.20 tons (4.38%), corresponding to 0.46 ton (9.39%) for sugar yield / fed. A similar trend was observed by El-Geddawy and Makhlouf (2016), Schultz et al. (2017) and Abd El-Azez et al. (2018). These results may be due to the varietal response to bio-fertilizers and their possible role in N fixation via free live bacteria, which reduces the pH in the rhizosphere resulting in increased availability of most essential nutrients as well as excretion of some growth substances such as IAA and GA3, which play an important role in increasing nutrient absorption.

Significant interaction effect between sugarcane varieties and inorganic nitrogen levels

Brix reading in the 1st season and millable cane height, sucrose % and cane yield/fed, in the 2nd season, and millable cane weight in both growing seasons, were significantly influenced by the interaction between the examined sugarcane varieties and N levels (Table, 6). An appreciable variance in weight of millable cane / plant in the two seasons and cane yield / fed in the second one was detected between GT.54-9 and each of G.84-47 and/or G.2003-47 as a result of fertilizing with 150 or 200 kg N/fed, corresponding to the insignificant difference between G.2003-47 and G.2004-27 in these traits in the 2nd season, when canes were fed with 150 kg N/fed. The variance between 150 and 200 kg N/fed was considerable in their influence on Brix reading in the 1st season, millable cane weight and cane yield/fed, in the 2nd one under all the tested cane varieties. Meanwhile, the difference between the two levels of N in their effect on millable cane weight in the 1st season failed to reach the level of significance with G.84-47. Substantial variances in the values of sucrose % and brix reading were found between GT.54-9 and G.2004-27 and also between G.84-47 with G.2003-47 when sugarcane plants were fed with 150 and / or 200 kg N/fed. However, the differences between GT.54-9 and G.2003-47 in brix reading failed to reach the level of significance in the case of fertilizing with 150 kg N / fed, in the 1st season.

The highest means of millable cane weight/plant in both seasons and cane yield/fed in the 2nd one, were recorded with GT.54-9 and G.2004-27, when 200 kg N/fed was added to the soil. Feeding sugarcane varieties GT.54-9 and G.2004-27 with the high level of nitrogen i.e. 200 kg N/fed resulted in a statistical increment in millable cane yield/fed which reached 7.02 % (3.54 tons) and 3.75 % (1.89 tons) respectively, as compared to that scored by G.84-47 under the same N-level, in the 2nd season. Moreover, G.84-47 recorded the highest and a marked increase in the values of sucrose and brix surpassing the commercial variety GT.54-9 by 0.68 and 1.25, consecutively, when cane plants were fertilized with 200 kg N/fed. The differences between the varieties may be attributable to differing in gene make-up and their responses to the different levels of nitrogen fertilizer under the environmental conditions. These results are in accordance with that obtained by El-Geddawy and Makhlouf (2016) and Abd El-Azez et al. (2018).

Significant interaction effect between inorganic nitrogen levels and application methods of N-fixing bacteria

Millable cane height and reducing sugar % in the 1st season were substantially affected by the interaction between nitrogen levels and application methods of N-fixing bacteria (Table 7). An appreciable variance

Table 6: Significant interaction effect between varieties and inorganic nitrogen levels on some traits of sugarcane in 2018/2019 and 2019/2020 seasons

Varieties	Inorganic N levels (kg/fed)	Millable cane height (cm)	Millable cane weight (kg)		Brix %	Sucrose %	Cane yield/fed (ton)
		2nd season	1st season	2nd season	1st season	2nd season	2nd season
G.T.54-9	150	305.89	1.116	1.197	20.04	15.32	51.35
	200	314.33	1.232	1.273	20.96	15.55	53.95
G.84-47	150	292.78	0.996	1.037	21.51	15.53	47.70
	200	310.78	1.021	1.102	22.21	16.23	50.41
G.2003-47	150	280.22	1.036	1.164	19.86	14.10	49.68
	200	298.11	1.130	1.198	20.34	14.37	51.16
G.2004-27	150	291.89	1.030	1.147	19.28	14.33	50.03
	200	297.00	1.136	1.245	20.33	14.36	52.30
LSD at 0.05		9.29	0.048	0.032	0.30	0.28	1.43

in millable cane height was detected between cane cutting seeds inoculation and soil drench with *A. brasilense* bacteria when sugarcane plants were fertilized with 150 kg N/fed. In addition, substantial differences were observed in the means of reducing sugar % with any application methods of bio-N under N-level of 150 kg/fed, as compared to the control treatment (without the addition of bio-fertilizer). However, the differences between cane cutting seeds inoculation and soil drench with *A. brasilense* bacteria in their impact on the previous traits did not reach the level of significance under fertilization with 200 kg N/fed. These findings are also in line with those found by Schultz et al. (2017).

Significant interaction effect among sugarcane varieties, application methods of N-fixing bacteria and inorganic nitrogen levels

The interaction among sugarcane varieties, application methods of N-fixing bacteria and nitrogen fertilizer levels had a significant influence on millable cane weight (kg), sucrose %, sugar recovery % and sugar yield / fed, in the 1st season (Table 8). The differences between G.T54-9 and G.84-47 were significant in the values of millable cane weight/plant when sugarcane varieties were fed at a rate of 150 and/or 200 kg N/fed with any application methods of bio nitrogen fixer. A statistical effect was observed between G.T54-9 with G.2003-47 varieties as a result of cane cutting seeds inoculation with *A. brasilense* under the two N levels. There was an appreciable difference between the commercial cultivar G.T54-9 and G.84-47 in the values of sucrose and sugar recovery %. The same kind of response was observed between G.T54-9 and G.2003-47 and also between G.2003-47 with G.2004-27 in sugar yield / fed using 200 kg N/fed + soil drench with AZS bacteria. However these differences did not reach the significance level using level 150 kg N/fed with the same application method of bio N-fixer. Feeding sugarcane varieties G.T54-9 and G.84-47 with 150 kg N/fed + inoculating cane seeds with *A. brasilense* resulted in a substantial increment in sugar yield / fed by 0.64 ton (12.96%) and 0.41 ton (9.03%), respectively, as compared that given using *A. brasilense* as a soil drench under the same N-level. Comparable results were reported by El-Geddawy and Makhlouf (2016). This increase in sugar yield/fed may be due to bio-fertilization treatments and the role of nitrogen element in promoting growth and dry matter accumulation

Table 7: Significant interaction effect between inorganic nitrogen levels and application methods of N-fixing bacteria on millable cane height (cm) and reducing sugar% of sugarcane in 2018/2019 season

Inorganic N levels	Bio-nitrogen application methods	Millable cane height (cm)	Reducing sugar %
150 kg/fed	Without	268.17	0.360
	Soil drench	271.75	0.381
	Seeds inoculation	290.58	0.387
200 kg/fed	Without	280.58	0.394
	Soil drench	297.75	0.400
	Seeds inoculation	302.00	0.400
LSD at 0.05		8.50	0.012

by increasing nutrient absorption and availability, which was positively reflected on the final sugar yield/fed.

Correlation among the studied traits

A term used to analyze and expose the relationship between yield and its constituents is a correlation. It's also useful for determining the relationship between quantitative attributes and yield in order to choose characters that influence yield. Except for millable cane weight, brix %, millable cane yield and sugar yield, millable cane height had a positive and no significant association with all studied traits (Table 9). Millable cane diameter, on the other hand, had a strong and highly important association with millable cane weight, cane yield, and sugar yield, but was positive but not significant with other traits. Several researchers have discovered a relationship between millable cane height and millable cane diameter and cane yield (Chaudhary et al. 2003. Sassy and Abu-Ellail 2021). Cane yield and sugar yield had a highly

Table 8: Significant interaction effect among varieties, inorganic nitrogen levels and bio-N on millable cane weight (kg), sucrose%, sugar recovery% and sugar yield/fed (ton) of sugarcane in 2018/2019 season

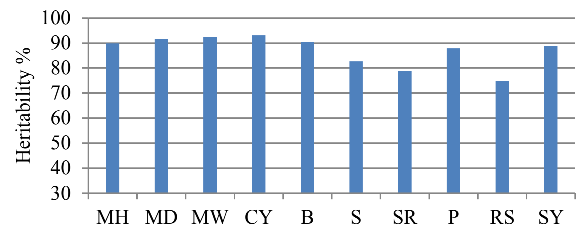
Varieties	Inorganic N levels (kg/fed)	Millable cane weight (kg)			Sucrose%			Sugar recovery %			Sugar yield/fed (ton)		
		Bio-nitrogen application methods											
		Without	SD	SI	Without	SD	SI	Without	SD	SI	Without	SD	SI
G.T54-9	150	0.989	1.130	1.230	13.83	15.26	16.47	8.51	9.74	10.76	4.32	4.94	5.58
	200	1.149	1.265	1.283	14.67	15.46	16.72	9.09	9.71	10.72	4.73	5.11	5.68
G.84-47	150	0.919	0.980	1.090	14.99	15.69	16.68	9.24	9.73	10.58	4.21	4.54	4.95
	200	0.884	0.995	1.185	15.49	16.42	16.90	9.49	10.33	10.61	4.50	4.93	5.07
G.2003-47	150	0.944	1.055	1.110	14.07	14.84	15.32	8.79	9.35	9.68	4.23	4.63	4.81
	200	1.094	1.115	1.180	14.45	15.00	15.63	9.11	9.33	9.82	4.61	4.73	4.98
G.2004-27	150	0.954	0.970	1.165	14.27	14.82	15.32	9.20	9.50	9.81	4.50	4.78	4.99
	200	1.019	1.155	1.235	14.60	15.23	15.87	9.23	9.62	10.05	4.49	4.86	5.17
LSD at 0.05		0.084			0.61			0.60			0.33		

SD: soil drench, and SI: seeds inoculation.

important and optimistic association with millable cane weight. Sucrose %, sugar recovery %, and sugar yield had positive and highly meaningful associations with brix %. It had a negative and non-significant relationship with the percentage of reducing sugar. It was discovered that sucrose and sugar yield has a clear positive and static relationship. Millable cane yield correlated with millable cane weight and sugar yield in a favorable and highly significant way. However, with purity % such associations were positive and negligible. Brix had positive associations with sucrose % sugar recovery % and sugar yield (Masri et al. 2015). Sanghera et al. (2015) found that sucrose content in the juice was positively and significantly associated with sugar recovery % and sugar yield. Abu-Ellail et al. (2019) found that sucrose content in the juice was positively and significantly associated with sugar recovery percentage and sugar yield.

Genetic variability

Estimates of heritability, as well as genotypic coefficients of variance, are critical for improving sugarcane traits and selecting the best genotypes. The data in Fig. 1 showed that broad-sense heritability estimates (h^2) differed significantly between traits and seasons. Cane yield (93.09 percent) and millable cane weight (92.41 percent) had high her-



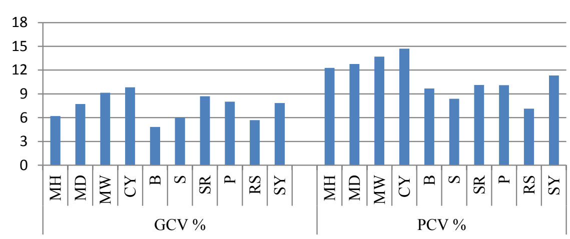
MH: Millable cane height, MD: Millable cane diameter, MW: Millable cane weight, CY: Cane yield, B: Brix%, S: Sucrose%, SR: Sugar recovery%, P: Purity%, RS: Reducing sugar% and SY: Sugar yield

Figure.1: Broad-sense heritability of studied traits under bio fertilizers treatments (average of 2018/2019 and 2019/2020 seasons)

Table 9: Correlation analysis between cane and sugar yields of sugarcane and the other studied traits (average of 2018/2019 and 2019/2020 seasons)

Traits	1	2	3	4	5	6	7	8	9
1. Millable cane height (cm)									
2. Millable cane diameter (cm)	0.559								
3. Millable cane weight (kg)	0.578**	0.828**							
4. Brix %	0.471**	0.121	0.023						
5. Sucrose %	0.584	0.086	0.171	0.866**					
6. Reducing sugar %	0.074	0.255	0.323	-0.179	-0.152				
7. Sugar recovery %	0.580	0.185	0.254	0.703**	0.965**	-0.121			
8. Purity %	0.238	0.361	0.344	0.195*	0.321	0.019	0.558*		
9. Millable cane yield/fed (ton)	0.423**	0.809**	0.815**	0.164	0.022	0.174	0.116	0.314	
10. Sugar yield/ fed (ton)	0.679**	0.557**	0.612*	0.480**	0.788**	0.009	0.866**	0.615**	0.596**

itability levels, Brix percent (90.33 percent) was moderate, and reducing sugar percent was low (74.86 percent). Cane yield (ton/fed) decreased broad-sense heritability (h^2), while sugar percent reduction increased it. The high heritability of cane and sugar yield, according to Chaudhary (2001) and Gulzar et al. (2014), can be used as a selection criterion. Millable cane diameter and single cane weight had high heritability estimates (Abu-Ellail et al. 2020), but millable cane diameter and length had low broad-heritability for both crops. Because of lower heritability for yield and higher heritability for yield components, heritability may be more efficient for yield components that were chosen to increase yield (Abu-Ellail, 2015 and Masri et al. 2014). For most of the traits showed a slight difference between phenotypic and genotypic coefficients of variation (PCV and GCV). For PCV % and GCV %, the high and low figures were 14.71 and 9.82 % for cane yield, 13.7 and 8.71 % for millable cane weight and 7.13 and 5.70 % for reducing sugar %. A relative indicator of genetic variation in the varieties is the genotypic coefficient of variation. Despite the fact that the PCV % and GCV % tended to be marginally higher, the crop forecasts for both seasons were similar. The probability of genetic change in these traits was suggested by small variations between GCV % and PCV %. GCV % is useful for describing the relative amounts of trait variability in varieties; they give only a partial indication of the genetic potential to improve a trait (Abu-Ellail et al. 2020). Masri et al. (2015) discovered that genotypic variation and GCV for cane yield decreased from the first to the second season. Cane yield and millable cane weight have high genotypic and phenotypic coefficients of variation (Bhatnagar 2003 and Abu-Ellail 2015).



MH: Millable cane height, MD: Millable cane diameter, MW: Millable cane weight, CY: Cane yield, B: Brix%, S: Sucrose%, SR: Sugar recovery%, P: Purity%, RS: Reducing sugar% and SY: Sugar yield.

Figure 2. Genotypic coefficient of variance (GCV%) and phenotypic (PCV%) for studied traits (average of 2018/2019 and 2019/2020 seasons)

Economical evaluation and general discussion

Results given in Table 10 pointed out that the total cost of cultivation of the four varieties differed according to the used N-fixing bacteria as well as the quantities of nitrogen applied; consequently, the total revenue of the examined varieties had differed. Based on the net return data, it can be noted that adding N fixing bacteria and raising nitrogen levels tended to increase the total revenue as a result of the continuous increase in cane yield, which corresponded to the addition of bio fertilizer and increasing nitrogen levels. However, it

Table 10: Economical evaluation for four sugarcane varieties (average of 2018/2019 and 2019/2020 seasons) under different inorganic nitrogen levels and bio-N application methods

Treatments		Cane yield/fed (ton)			Total costs/fed (L.E.)			Total revenue/fed (L.E.)			Net return/fed (L.E.)		
		Bio-nitrogen application methods											
Varieties	N-level) kg/fed)	with-out	SD	SI	with-out	SD	SI	with-out	SD	SI	with-out	SD	SI
G.T. 54-9	150	50.39	51.31	52.03	26121	26311	26257	36281	36943	37462	10160	10632	11205
	200	52.67	53.32	53.84	26569	26759	26705	37919	38387	38765	11350	11628	12060

G.84-47	150	46.23	47.34	47.67	26121	26311	26257	33282	34085	34322	7161	7774	8065
	200	48.72	49.50	49.75	26569	26759	26705	35078	35640	35816	8509	8881	9111
G. 2003-47	150	48.62	49.59	50.00	26121	26311	26257	35006	35701	36000	8885	9390	9743
	200	50.57	50.91	51.27	26569	26759	26705	36407	36655	36911	9838	9896	10206
G. 2004-27	150	48.91	50.31	50.96	26121	26311	26257	35212	36223	36688	9091	9912	10431
	200	50.13	51.49	52.14	26569	26759	26705	36094	37069	37541	9525	10310	10836

- SD: soil drench and SI: cane cutting seeds inoculation.
- Based upon the average of total cost included rent/fed (fed = 0.42 ha^{-1}) = L.E. 26569 (average of the different locations).
- Bio fertilizer's price = L.E. 18/liter of suspension, L.E. 18/one bag, and labor wage/day = L.E.100.
- Nitrogen's price = L.E. 165.5/50 kg urea (46.5 % N).
- Total revenue/fed (L.E.) = cane yield/fed (ton) x ton's price (L.E. 720).
- Net return/fed (L.E.) = total revenue/fed (L.E.) - total costs/fed (L.E.).
- L.E. One = 0.064 USD, according to the exchange rate of the Egyptian currency against the US dollar, (April 9, 2021).

could be observed that using A. brasilense bacteria as inoculation of cane cutting seeds + 150 kg N/fed resulted in an increment in net return for G.2004-27 and G.T.54-9 varieties and reached 1340 and 1045 L.E., corresponding to 1311 and 710 L.E. using the same inoculation method + 200 kg N/fed, respectively, as compared to the control treatment (without the addition of bio-fertilizer) under the same conditions of N levels. Despite the high response of sugarcane cultivar G.2004-27 to the addition of bio-fertilizers, the commercial variety GT54-9 still has the highest yield among the others under study. In spite of the superiority of the commercial variety in yield and net return, it became necessary to conduct focused studies of the promising new varieties to face any deterioration of the commercial variety.

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