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Response of Sweet Corn to Microbial Inoculation by *Gluconacetobacter diazotrophicus*

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A B S T R A C T

A field experiment was conducted to study the effect of seed inoculation of *Gluconacetobacter diazotrophicus* on growth parameters, nutrient uptake and yield of sweet corn. Among different inoculation treatments, seed inoculation with *G. diazotrophicus* (Phule Madhu strain) + 50% recommended dose of nitrogen was found to be the most effective as it recorded significantly highest germination (94.33 %) and plant vigour index (3249.32) at 15 days after sowing; significantly highest plant height at 30 and 60 days after sowing and at harvest stage the crop (53.56 cm, 144.12 cm and 155.74 cm, respectively); significantly highest root length (22.96 cm and 26.79 cm) and dry matter production (37.29 q ha^{-1} and 74.31 q ha^{-1}) at flowering and harvest stage of the crop; significantly highest green cob yield (287.57 q ha^{-1}) and green fodder yield (555.01 q ha^{-1}) at 80 days after sowing; significantly highest *G. diazotrophicus* population at flowering ($6.75x10^6$ g^{-1} fresh weight of shoot) and at harvest (5.68×10^6 g^{-1} fresh weight of shoot) stage of the crop; significantly highest nitrogen uptake (488.87 kg ha^{-1}) and sugar content (18.19 % brix) of sweet corn grain, however it was as statistically at par with the treatment of seed inoculation with *G. diazotrophicus* (MPKV strain) + 50% recommended dose of N and 100% recommended dose of N without *G. diazotrophicus* for growth parameters, nutrient uptake, yield and sugar content of sweet corn grains. The results indicated saving of 50% chemical nitrogen fertilizer to sweet corn.

Introduction

Sweet corn (*Zea mays* L. *Saccharata*) cv. Phule Madhu is a variety of maize with high sugar content. Sweet corn is a naturally occurring recessive mutation in the genes which control conversion of sugar to starch inside the endosperm of the corn kernel. It is harvested at milky stage and eaten as a vegetable, rather than a grain. The area under maize crop in India is reported to be 9.43 million ha. with an annual production of 24.35 million tones. It can be taken throughout three season in India but in Maharashtra particularly taken both in *kharif* and *rabi* season.

Gluconacetobacter diazotrophicus is an endophytic bacterium first isolated from the sugarcane growing regions of Brazil

(Cavalcante and Dobereiner, 1988). It is found to live freely in the intercellular spaces of roots, stems and leaves of the sugarcane plant, actively fixes atmospheric N and provides plants with the needed nitrogen (Boddey *et al.,* 1991). Moreover, the colonization of nitrogen fixing bacterium *G. diazotrophicus* in corn plants has been reported by Tian *et al.,* (2009). Furthermore, Riggs *et al.,* (2001) reported that maize productivity has been enhanced by inoculation with endophytic diazotrophic bacteria. It has also been reported that besides N fixation, all the strains of *G. diazotrophicus* produced indole acetic acid in a culture medium supplemented with tryptophan in the range of 0.14 to 2.42 µg ml^{-1} (Fuentez-Ramirez *et al.,* 1993 and Bastian *et al.,* 1998). Furthermore, it has been reported its ability to solubilize inorganic phosphates from the soil and make available P for the inoculated crops (Crespo *et al.,* 2011). Sweet corn requires 120 kg N, 60 kg P_2O_5 and 40 kg K_2O for its growth. Due to increase in cost of chemical nitrogenous fertilizer, the marginal farmer cannot afford the fertilizer to the crop with the recommended doses. Therefore, biological nitrogen fixation through microorganisms has been found very economical and advantages (Stephan *et al.,* 1991).

Keeping this in view, in the present investigation an attempt was made to evaluate the effect of *Gluconacetobacter diazotrophicus* on growth, yield and nitrogen uptake in sweet corn.

Materials and Methods

Isolation of *Gluconacetobacter diazotrophicus* from Root Sample of Sweet Corn

Since *Gluconacetobacter diazotrophicus* is an endophyte, the isolation was done using root samples of sweet corn. The plants were uprooted and the root portion was separated and washed with tap water. The roots were washed with sterile distilled water and surface sterilized for 5 min with 5% sodium hypochloride (NaOCl) and then washed five times with sterile distilled water. The surface sterilized root samples were weighed and homogenized in a sterile sucrose solution (1%) using a sterile pestle and mortar. Aliquots (500 ml) were inoculated in the tubes containing semisolid LGIP medium (Cavalcante and Dobereiner, 1988) and incubated at 30ºC for 4-6 days. Yellowish bacterial growth from the tubes was streaked onto LGIP plates (Cavalcante and Dobereiner, 1988) and incubated at 30ºC for 6-7 days.

Morphological and Cultural Characterization of the Isolate

The isolate was examined for cell morphology and gram reaction as per the standard procedures given by Cappuccino and Sherman (1987). The colony morphology of the isolate was compared with *Gluconacetobacter diazotrophicus* MPKV strain.

Biochemical and Physiological Characterization

The isolate was further characterized using a series of biochemical tests *viz*., gelatin liquefaction, catalase test, oxidase test, growth on carbon source, growth at various concentrations of sugar and growth at various temperatures according to Dong *et al.,* (1995) and Muthukumarasamy *et al.,* (1999).

Utilization of different carbon sources by *G. diazotrophicus*

The culture medium was amended with different carbon sources *viz*., Glucose, sucrose, ethanol and mannitol and autoclaved. Sterilized petriplates were poured with 15-20

ml medium with different carbon sources and allowed to solidify. After solidification, 10 µl of 24 hrs old culture of the test organism was spotted on plates with each carbon source. The plates were incubated for 48 hrs at 28 ± 2°C and the ability of the isolate to grow on different carbon sources was noted.

Nitrogen Fixing Ability of the Isolate

The 48 hour old culture of freshly isolated *G. diazotrophicus* strain was inoculated to 5 ml of N free semisolid broth of LGIP medium. It was incubated for 48hrs and 1 ml of this broth was inoculated to 50 ml semisolid LGIP medium.

Then it was incubated for 15 days. 10 ml of this culture was used for N estimation by following the standard procedure of Microkjeldhal technique (Reis *et al.*, 1994).

The formula for N_2 estimation is:

$$N_2\ (mg/g) = \frac{\text{ml of } H_2SO_4 \text{ in the sample} \times \text{Normality of } H_2SO_4 \times 14.01}{\text{Weight of the sample (carbon used in grams)}}$$

Field Experiment

A field experiment was conducted during *kharif* 2018 at the Instructional Research Farm, Central Campus, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri to study the effect of seed inoculation of *G. diazotrophicus* on growth parameters, nutrient uptake and yield of sweet corn. The sweet corn hybrid *Phule Madhu* was used as a test crop. The seeds of sweet corn (var. Phule Madhu) were treated with *G. diazotrophicus* by following slurry method of seed inoculation and inoculated seeds were dried in shade before sowing. The experiment was laid out in randomized block design with three replications and seven treatments.

Treatment details

The sweet corn seeds were inoculated with *Gluconacetobacter diazotrophicus* before sowing as follows.

T_1: *G. diazotrophicus* (Phule Madhu strain) alone
T_2: *G. diazotrophicus* (Phule Madhu strain) + 50% recommended dose of N
T_3: *G. diazotrophicus* (Phule Madhu strain) + 75% recommended dose of N
T_4: *G. diazotrophicus* (MPKV strain) + 50% recommended dose of N
T_5: *G. diazotrophicus* (MPKV strain) + 75% recommended dose of N
T_6: 100% recommended dose of N without *G. diazotrophicus*
T_7: Uninoculated control

Note: P_2O_5 @ 60 kg $ha^{-1}$and K_2O @ 40 kg ha^{-1} (as per recommendation) was applied to all the treatments except uninoculated control.

The observations on germination (%), plant vigour index, plant height (cm) at 30, 60 days after sowing and at harvest stage (80 DAS), root length (cm) at flowering (40 DAS) and harvest stage (80 DAS), dry matter production (kg ha^{-1}) at flowering (40 DAS) and harvest stage (80 DAS), green cob yield (q ha^{-1}) and green fodder yield (q ha^{-1}) at milky stage (80 DAS) of the sweet corn were recorded.

Plant Vigour index

Plant vigour index was computed at 15 days after sowing using the procedure suggested by Abdul Baki and Anderson (1973):

Plant vigour index= Germination % x [shoot length (cm) + root length (cm)]

Estimation of Nitrogen

Nitrogen content of plant was estimated by following Modified Kjeldahl's process as

described by Jackson (1973) and accordingly N uptake (kg ha^{-1}) was estimated as N% x total dry matter yield (kg ha^{-1})/100.

Brix reading using hand refractometer

The sugar content of sweet corn grain at milky stage was estimated as Brix (%) using hand refractometer.

Enumeration of microbial population of *G. diazotrophicus* in shoot

The population of *G. diazotrophicus* in shoot of sweet corn plant was enumerated at 30 and 60 days after sowing and at harvesting of the crop by following serial dilution and pour plate technique (Aneja, 2003). The LGIP medium was used for enumeration of *G. diazotrophicus* population. The population was expressed as cfu g^{-1} plant sample.

Statistical Analysis

The data recorded on various parameters were subjected to statistical analysis by following standard method of analysis of variance. The level of significance used in 'F' and 't' tests was P = 0.05. Critical difference (CD) values were calculated where the 'F' test was found significant (Panse and Sukhatme, 1985).

Results and Discussion

Biochemical Characterization of Endophytic Bacterial Isolate

The isolation of endophytic nitrogen fixing bacterium *Gluconacetobacter diazotrophicus* from root sample of sweet corn (var. *Phule Madhu*) was done using semisolid LGIP medium. The endophytic bacterial isolate was tested for different biochemical characters *viz*., gram staining, motility, gelatin liquefaction, catalase test, oxidase test, growth on carbon sources, growth at various concentrations of sugar and growth at various temperatures (Table 1). The cells of endophytic bacterial isolate were motile, rod shape and gram negative in reaction. The endophytic bacterial isolate was positive for catalase and oxidase test, but was negative for gelatin hydrolysis. Glucose, sucrose, ethanol and mannitol were used as a sole carbon source for growth by the endophytic bacterial isolate. Moreover, the growth of endophytic bacterial isolate was positive at various concentrations (5, 10, 20 and 30%) of sugar. In addition, the growth of endophytic bacterial isolate was positive at 28ºC, 32ºC, and 37ºC temperature, but was negative at 4ºC. Based on biochemical and physiological characterization, the endophytic bacterial isolate was identified as *Gluconacetobacter diazotrophicus*. The results of the present investigation are in conformity with results of Hema and Savalgi (2017) that isolated nitrogen fixing endpohytic bacterium *G. diazotrophicus* from root tissue of sugarcane, maize, pineapple and carrot and further characterized these isolates biochemically for specific characters of *G. diazotrophicus* according to Burgey's Manual of Systematic Bacteriology.

Nitrogen Fixing Ability of *G. diazotrophicus* Isolate

The endophytic bacterial isolate (*Phule Madhu* strain) alongwith MPKV strain were subjected to know the nitrogen fixation by Microkjeldhal method (Table 2). The strain from sweet corn fixed highest amount of nitrogen (148.65 µg of nitrogen/mg of carbon used) than MPKV strain (121.84 µg of nitrogen/mg of carbon used). The results of the present investigation are in agreement with results of Hema and Savalgi (2017) who reported that endophytic bacterial isolate *G. diazotrophicus* from maize GdM5 fixed about 42 µg of nitrogen/mg of carbon used which was equivalent to that of reference culture of

G. diazotrophicus (MTCC1224), whereas the isolate from sugarcane GdS25 fixed highest amount of nitrogen than other strains i.e. 147µg of nitrogen/mg of carbon used.

Table.1 Selective biochemical tests of *Gluconacetobacter diazotrophicus* isolate of sweet corn (*Phule Madhu* strain)

Sr. No.	Biochemical tests	*Gluconacetobacter diazotrophicus* isolate of sweet corn (Phule Madhu strain)
1.	Cell shape	Rod shape
2.	Gram reaction	Gram negative
3.	Motility	+
4.	Gelatin liquefaction	-
5.	Catalase activity	+
6.	Oxidation of ethanol	+
7.	Growth on carbon sources	
	a) Glucose	+
	b) Sucrose	+
	c) Ethanol	+
	d) Mannitol	+
8.	Growth at various concentration of sugar	
	a) 5%	+
	b) 10%	+
	c) 20%	+
	d) 30%	+
9.	Growth at various temperatures	
	a) 4oC	-
	b) 28oC	+
	c) 32oC	+
	d) 37oC	+

Table.2 Nitrogen fixing ability of *Gluconacetobacter diazotrophicus* isolate of sweet corn by Microkjeldhal method

Sr. No.	Isolate	Nitrogen fixing ability (µg of Nitrogen/mg of Carbon)
1.	*Gluconacetobacter diazotrophicus* (Phule Madhu strain)	148.65
2.	*Gluconacetobacter diazotrophicus* (MPKV strain)	121.84

Table.3 Effect of inoculation of *G. diazotrophicus* on growth parameters of sweet corn

Tr. No	Treatment details	Germination (%)	Plant vigour index	Plant height (cm)			Root length (cm)		Dry matter production (q ha^{-1})	
				30 DAS	60 DAS	Harvest (80 DAS)	Flowering (40 DAS)	Harvest (80 DAS)	Flowering (40 DAS)	Harvest (80 DAS)
T_1	*G. diazotrophicus* (Phule Madhu strain) alone	87.67	2670.24	47.41	137.93	145.49	20.32	24.15	34.65	69.35
T_2	*G. diazotrophicus* (Phule Madhu strain) + 50% recommended N	94.33	3249.32	53.56	144.12	155.74	22.96	26.79	37.29	74.31
T_3	*G. diazotrophicus* (Phule Madhu strain) + 75% recommended N	90.33	2833.94	48.45	138.93	148.05	20.93	24.58	35.30	70.46
T_4	*G. diazotrophicus* (MPKV strain) + 50% recommended N	91.67	3028.68	51.38	141.37	152.27	22.02	25.85	36.35	72.55
T_5	*G. diazotrophicus* (MPKV strain) + 75% recommended N	89.33	2652.21	46.21	137.94	147.04	19.80	23.63	34.13	68.38
T_6	100% recommended N without *G. diazotrophicus*	90.67	2921.18	50.08	140.67	151.60	21.46	25.29	35.79	71.50
T_7	Uninoculated control	83.33	2202.36	41.10	131.11	140.78	17.61	21.44	31.94	64.26
	S.E.	1.32	107.04	1.53	1.35	2.03	0.67	0.73	0.67	1.25
	C.D.at 5%	4.07	329.83	4.72	4.16	6.24	2.05	2.25	2.05	3.86
	C.V.	2.55	6.64	5.50	1.68	2.36	5.57	5.15	3.29	3.10

G. diazotrophicus = *Gluconacetobacter diazotrophicus*
DAS = Days after sowing

Table.4 Effect of inoculation of *G. diazotrophicus* on yield, nutrient uptake and population of *G. diazotrophicus* in sweet corn shoot

Tr. No	Treatment details	Green cob yield (q ha^{-1})	Green fodder yield (q ha^{-1})	Nitrogen uptake (kg ha^{-1})	Sugar content of sweet corn grain (Brix %)	*Gluconacetobacter* count (x 10^6) in sweet corn shoot	
						Flowering (40 DAS)	Harvest (80 DAS)
T_1	*G. diazotrophicus* (Phule Madhu strain) alone	268.39	493.84	456.27	14.19	4.11	3.04
T_2	*G. diazotrophicus* (Phule Madhu strain) + 50% recommended N	287.57	555.01	488.87	18.19	6.75	5.68
T_3	*G. diazotrophicus* (Phule Madhu strain) + 75% recommended N	272.66	501.70	463.53	15.33	4.70	3.63
T_4	*G. diazotrophicus* (MPKV strain) + 50% recommended N	280.75	541.85	477.28	16.57	5.81	4.74
T_5	*G. diazotrophicus* (MPKV strain) + 75% recommended N	264.63	486.92	449.87	14.77	3.59	2.52
T_6	100% recommended N without *G. diazotrophicus*	276.70	534.02	470.38	12.11	1.89	1.09
T_7	Uninoculated control	218.70	427.60	371.78	11.48	1.21	1.01
	S.E.	5.55	9.11	9.44	0.78	0.58	0.56
	C.D.at 5%	17.11	28.08	29.09	2.42	1.78	1.73
	CV%	13.60	14.12	3.60	9.26	15.02	13.30

Inoculation Effect of *G. diazotrophicus* on Growth Parameters and Yield of Sweet corn

The results in respect of growth and yield attributing characters of sweet corn are presented in (Table 3 and 4). It was revealed from the data that all the growth parameters and green cob yield and fodder yield differences were significant due to seed inoculation with *Gluconacetobacter diazotrophicus*. Among different inoculation treatments, T_2 i.e. seed inoculation with *G. diazotrophicus* (Phule Madhu strain) + 50% recommended dose of nitrogen was found to be the most effective as it recorded significantly highest germination (94.33 %) and plant vigour index (3249.32) at 15 days after sowing; significantly highest plant height at 30 and 60 days after sowing and at harvest stage the crop (53.56 cm, 144.12 cm and 155.74 cm, respectively); significantly highest root length (22.96 cm and 26.79 cm) and dry matter production (37.29 q ha^{-1} and 74.31 q ha^{-1}) at flowering and harvest stage of the crop; significantly highest nitrogen uptake (488.87 kg ha^{-1}) and sugar content (8.19% brix) of sweet corn grain and significantly highest green cob yield (287.57 q ha^{-1}) and green fodder yield (555.01 q ha^{-1}) at 80 days after sowing, however it was as statistically at par with the treatment T_4 i.e. seed inoculation with *G. diazotrophicus* (MPKV strain)+ 50% recommended dose of N and T_6 i.e. 100% recommended dose of N without *G. diazotrophicus* for growth parameters, nutrient uptake, yield and sugar content of sweet corn grains. The results indicated saving of 50% chemical nitrogen fertilizer to sweet corn.

Results of the present investigation are in conformity with Murumkar *et al.,* (2017), Partick *et al.,* (2000), Pandey (2004) and Chauhan *et al.,* (2010) who reported increased crop yield in different crops due to inoculation of *G. diazotrophicus* individually and in combination with *H. seropedicae.* Moreover, Indi *et al.,* (2017) reported the enhancement in sugarcane yield to the tune of 20-22 t/ha and CCS yield by 3 t/ha with 50 % reduction in the recommended dose of chemical nitrogen by use of *G. diazotrophicus* as set treatment before planting or spray the broth culture of *G. diazotrophicus* at 60 days after planting. Furthermore, Muthukumarasamy *et al.,* (1994) reported 50 % reduction in N fertilizer with increase in crop productivity by 5-7 t $acre^{-1}$ due to use of nitrogen fixing bacteria *Acetobacter diazotrophicus, Azospirillum* and *Azotobacter* as a biofertilizer for sugarcane. These results corroborate results of the present investigation that seed inoculation of *G. diazotrophicus* (Phule Madhu strain) saves 50% of the recommended dose of nitrogenous fertilizer with exuberant increase in crop productivity of sweet corn.

Inoculation Effect of *G. diazotrophicus* on microbial population

The results in respect of population of *G. diazotrophicus* in shoot of sweet corn plant at flowering (40 DAS) and harvest (80 DAS) stage as influenced by inoculation of *G. diazotrophicus* under graded levels of nitrogen are presented in Table 4. Among different inoculation treatments, T_2 i.e. seed inoculation with *G. diazotrophicus* (Phule Madhu strain) + 50% recommended dose of nitrogen was found to be the most effective as it recorded significantly highest *G. diazotrophicus* population at flowering (6.75 x 10^6 g^{-1} fresh weight of shoot) and at harvest (5.68 x 10^6 g^{-1} fresh weight of shoot) stage of the crop over rest of the treatments, however it was statistically at par with T_4 i.e. *G. diazotrophicus* (MPKV strain) + 50% recommended dose of N for *G. diazotrophicus* population at flowering (5.81 x 10^6 g^{-1} fresh weight of shoot) and at harvest

(4.74×10^6 g^{-1} fresh weight of shoot) stage of the crop. Different scientists have obtained varying results in regard to population of these diazotrophs. Murumkar *et al.*, (2017) reported that sugarcane set inoculation with *G. diazotrophicus* @ 10 kg in 100 lit water/ha for 30 min before planting significantly improved the *Gloconacetobacter* population in cane at 10 months. Moreover, Navadkar (2015) reported that integrated inoculation of *G. diazotrophicus* and *H. seropedicae* to sweet corn seeds resulted in highest *G. diazotrophicus* and *H. seropedicae* population in shoot of sweet corn plant at flowering and harvest stage of the crop. Furthermore, Dobereiner *et al.*, (2000) observed *Acetobacter diazotrophicus* in many sugarcane varieties and numbers were in the range of 103-107 in washed roots, 103-105 in surface sterilized roots, 103-106 in basal and apical stem; besides 104-107 in sugarcane trash. Similar results were reported by Fuentez *et al.*, (1999) and Archna *et al.*, (2008) in sugarcane and found that colonization of sugarcane by *A. diazotrophicus* was inhibited by high nitrogen fertilizer. Results of the present investigation are in conformity with the results of these scientists.

In conclusion from the present investigation it can be concluded that seed inoculation with *Gluconacetobacter diazotrophicus* + 50% recommended dose of nitrogen was found to be the most beneficial for getting higher green cob yield, green fodder yield and sugar content of sweet corn grains with 50% saving of nitrogen dose of chemical fertilizers to sweet corn.

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