

Research Article

GLUCONACETOBACTER DIAZOTROPHICUS AND HERBASPIRILLUM SEROPEDICAE CO-INOCULATION IMPROVES GROWTH AND YIELD OF SWEET CORN (Zea mays L. Var. Saccharata)

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Abstract: An experiment conducted to study the co-inoculation effects of *Gluconacetobacter diazotrophicus* and *Herbaspirillum seropedicae* on growth and yield of sweet com (Zea mays L.var. Saccharata) indicated profound effect of interaction between bioinoculants and different doses of nitrogen on plant growth and grain yield of sweet corn. Integrated seed treatment with *G. diazotrophicus* and *H. seropedicae* along with application of 100%, 75% and 50% of the recommended dose of nitrogen, recorded the highest plant height and dry matter production of sweet corn. Furthermore, these treatments recorded the highest number of grains per cob, number of grains per row of cob, length and girth of cob, filled to unfilled grain ratio, grain weight per plant, cob weight per plant, 100-seed weight and grain yield of sweet corn. From results of the present investigation we infer that the combined seed bacterization with *G. diazotrophicus* and *H. seropedicae* along with application of 50% of the recommended dose of nitrogen is most economical for getting higher grain yield of sweet corn.

Keywords: Gluconacetobacter diazotrophicus, Herbaspirillum seropedicae, Sweet corn

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Introduction

Among the maize types, sweet corn (Zea mays L. var. Saccharata) is one of the high value commercial used types, which is recently introduced in India. It is hybridized maize specially bred to increase sugar content and is also known as 'sugar corn'. In sweet corn, about 20 % of dry matter is sugar, compared with only 3 % in dent maize at green cob stage. These days, it is becoming popular and not only roasted green cobs but also some new maize based fast food and snack items are also getting good price for their produce. The role of biological nitrogen fixation is decisive in supplying plant with needed nitrogen, which can make agriculture more productive and sustainable without harming the environment. Hence, use of biofertilizers for crops has advanced greatly during the past few decades. Furthermore, in order to tackle food requirement of the increasing population in India, the farmers are constrained to adopt the newest technologies in agriculture like the use of high yielding and, disease and pest resistant crop varieties. However, these high yielding varieties require high amount of nutrients, making the farmers dependent on inorganic fertilizers. Nitrogen, considered as the most limiting factor in crop production, gains the highest mark of all the major nutrients from inorganic sources applied by farmers. Increased application of nitrogenous fertilizers resulted in high production for some time, but cause adverse effect on soil and other environmental conditions, if used for long run. This has been the main driving force of the search for new and safer plant growth promoting alternatives [1, 2, 3]. Several plant growth promoting rhizobacteria (PGPR), including Klebsiella, Azospirillum, Alcaligens and Enterobacter have been isolated from the rhizosphere of plants. However, a renewed interest in endophytic PGPR, such as Acetobacter and Herbaspirillum, in graminaceous plants has been arisen because of their occurrence mainly within plant tissues and evidence for significant nitrogen fixation [4]. Recent reports have indicated that Acetobacter diazotrophicus and Herbaspirillum seropedicae are the effective bioinoculants for most of the monocotyledonous crops such as sugarcane,

sorghum, wheat, maize and rice [5-8]. Maize is one of the host crops for these endosymbionts. Owing to increasing prices of nitrogenous and phosphatic chemical fertilizers, the marginal farmers cannot afford to apply recommended doses of chemical fertilizers. Therefore, biological nitrogen fixation through microorganisms has been found very economical and beneficial. *Gluconacetobacter diazotrophicus* and *H. seropedicae* are cheaper biofertilizers and will help to increase yield level of maize crop. Sevilla *et al.* [9] reported enhancement of growth and nitrogen content in sugar rich plants inoculated with *A. diazotrophicus* over uninoculated control. Oliveira *et al.* [10] demonstrated that inoculation with mixture of diazotrophs generally gave greater increases in dry matter than did individual inoculations and by far the best result in terms of N₂ fixation (29.2% N₂ derived from air) was achieved by inoculation with a mixture of diazotrophs. Keeping this in view, in the present investigation an attempt was made to evaluate effect of co-inoculation of *G. diazotrophicus* and *H. seropedicae* on growth and yield of sweet corn.

Materials and Methods

A field experiment to study the effect of *G. diazotrophicus* and *H. seropedicae* on growth and yield of sweet corn was conducted during *rabi*, 2014 growing season at the Instructional Farm of Division of Plant Pathology and Agricultural Microbiology, College of Agriculture, Kolhapur (Maharashtra), India. The location has latitude 74°16' E and longitude 16°42' N and an elevation of 569 m MSL. Soil of the experimental plot had pH 7.22; EC 0.31 dS/m; available N, P and K, 260, 12.06 and 257.6 kg ha⁻¹, respectively. Lignite based inocula of the bioinoculants were prepared in laboratory using the efficient cultures of *G. diazotrophicus* and *H. seropedicae*. The population of *G. diazotrophicus* and *H. seropedicae* in both the inoculants was estimated at 1 x 10⁹ cfu/g. Seed treatment with lignite based inoculum of *G. diazotrophicus* and *H. seropedicae* was done @ 25 g/kg seed of sweet corn cv. Priya.

There were 12 treatment combinations resulting from three main plot treatments {N1-50 % of the recommended dose of nitrogenous fertilizer (RDN), N2-75 % RDN and N3-100 %RDN} and four sub-plot treatments {I1-uninoculated, I2-seed inoculation with G. diazotrophicus (Gd), I3-seed inoculation with H. seropedicae (Hs) and I4-seed inoculation with Gd +Hs) The resulting treatments, thus, were: T1: 50 % RDN, T2: 50 % RDN + Gd, T3: 50 % RDN + Hs, T4: 50 % RDN + Gd + Hs, T5: 75 % RDN, T6: 75 % RDN + Gd, T7: 75 % RDN + Hs, T8: 75 % RDN + Gd + Hs, T9: 100 % RDN, T10: 100 % RDN + Gd, T11: 100 % RDN + Hs and T12: 100 % RDN + Gd + Hs. The treatments were arranged in a split plot design with three replications, in plots having gross size of 5.25 x 4.20 m and net plot size of 2.25 x 3.40 m. Spacing between rows and plants was maintained at 75 and 20 cm, respectively, with 1 m spacing between each block. Nitrogen fertilization with inorganic fertilizer in the form of urea was done according to the treatments as 50%, 75% and 100%, based on the original recommendation of 120 kg N ha-1. Phosphorus and potassium fertilization was done as basal application to all the treatments as per the original recommendations of 60 kg P2O5 ha-1 in the form of single super phosphate and 40 kg K₂O ha⁻¹ in the form of muriate of potash, respectively. All the recommended agronomic package of practices and plant protection measures were followed for raising the crop. Field data were recorded on plant height, dry matter production, number of grains per cob, average number of grains per row of cob, length and girth of cob, ratio of filled to unfilled grain, grain weight per plant, cob weight per plant, 100-seed weight and grain yield.

Statistical Analysis

The data pertaining to growth parameters and yield and yield contributing attributes were subjected to analysis of variance prescribed for split plot design.

Results and Discussion

Plant Growth

Data on plant height and dry matter production in sweet corn, recorded at 30 and 60 days after sowing (DAS) and at harvesting of the crop, as influenced by coinoculation of G. diazotrophicus and H. seropedicae in conjunction with different levels of nitrogen are presented in [Table-1]. Perusal of the data overtly implies that inoculation of sweet corn seeds with G. diazotrophicus and H. seropedicae, either singly or in combination, and different doses of nitrogen had significantly profound effect on plant height and dry matter production of sweet corn. Interaction effect due to inoculation of the bioinoculants and different doses of nitrogen was as well statistically significant. Among the different treatments of diazotrophs, integrated seed treatment with G. diazotrophicus and H. seropedicae recorded significantly highest plant height of 56.17 cm (at 30 DAS), 144.62 cm (60 DAS) and 161.73 cm (harvesting) and highest dry matter production of 41.29 g ha-¹ (30 DAS), 81.86 g ha⁻¹ (60 DAS) and 85.95 g ha⁻¹ (harvesting). Thus, this treatment was found to be significantly superior over inoculation with either of the bioinoculants and uninoculated control. Significantly least plant height and dry matter production of the crop was observed in the uninoculated control plots. Among the three levels of nitrogen, 100% of recommended dose of nitrogen recorded significantly highest plant height of 53.99, 143.16 and 156.80 cm and highest dry matter production of 38.89, 77.10 and 77.29 g ha⁻¹ at 30 and 60 days after sowing and at harvesting of the crop, respectively. This treatment, thus, was found significantly superior over rest of the treatments in improving plant height and biomass of sweet corn. Interaction between bioinoculants and different doses of nitrogen had substantial effect on plant height and dry matter production. Integrated seed treatment with G. diazotrophicus and H. seropedicae along with application of 100%, 75% and 50% of the recommended dose of nitrogen, recorded the highest plant height and dry matter production. Plant height at 30 days after sowing with these treatments was 56.79, 56.20 and 55.53 cm, respectively; whereas at 60 days after sowing, it was 144.75, 144.67 and 144.43 cm, respectively. At harvesting of the crop, plant height with these treatments ranged between 161.50 and 162.11 cm. In regard to dry matter production, at 30 days after sowing it was 41.39, 41.26 and 41.23 q ha-1, respectively with these treatments; whereas at 60 days after sowing, it was 82.04, 81.91 and 81.63 g ha-1, respectively. At harvesting of the crop, the dry matter production with these treatments ranged between 85.71 and 86.16 g ha-1. Thus, combined seed

treatment with both the bioinoculants in conjunction with all the three nitrogen levels tried was found conspicuously superior over rest of the treatments, in augmenting plant height and dry matter production. Significantly least plant height and dry matter production was recorded in uninoculated control plots. Fuentes Ramirez et al. [11] reported that strains of Acetobacter diazotrophicus produced IAA. Likewise, Bastian et al. [12] had reported the presence of IAA and gibberellins in cultures of A. diazotrophicus and their positive influence on plant growth. Patil et al. [13] found that A. diazotrophicus L1 increased IAA level of the fresh root tissue of maize plant. Furthermore, they reported that the strain produced high amount of IAA than reported earlier. This biosynthesis of plant growth promoting substances like IAA and gibberellins suggests that the bacterium could promote height of the crop. Increased plant height and biomass observed in the present investigation can be explained on these bases. Furthermore, results of the present investigation are in conformity with those of Song and Zheng [14], Sharma and Vasudeva [15], Chauhan et al. [16] and Patil et al. [13] who obtained increased plant height and or increased dry matter production in different crops due to use of these diazotrophs.

Grain Yield and Yield Contributing Traits

Data pertaining to number of grains per cob, average number of grains per row of cob, length and girth of cob, filled and unfilled grain ratio, grain weight per plant, cob weight per plant, 100-seed weight and grain yield of sweet corn as influenced by co-inoculation with G. diazotrophicus and H. seropedicae and different levels of nitrogen unveiled that inoculation of seeds with G. diazotrophicus and H. seropedicae, either singly or in combination and the different levels of nitrogen had profound effect on yield and yield contributing attributes of sweet corn. Interaction effect due to inoculation with bioinoculants and different levels of nitrogen was as well statistically significant [Table-2] and [Table-3]. Among the different treatments of diazotrophs, integrated seed treatment with G. diazotrophicus and H. seropedicae recorded significantly highest number of grains per cob (464.73), highest number of grains per row of cob (39.62), longest cobs (18.94 cm), maximum cob girth (17.10 cm), least filled to unfilled grain ratio (8.62), highest grain weight (64.29 g plant⁻¹), highest cob weight (261.95 g plant⁻¹), highest 100-seed weight (15.39 g) and highest grain yield (27.69 q ha-1) of sweet corn. Thus, integrated seed bacterization with G. diazotrophicus and H. seropedicae was found to be significantly superior over single inoculation with either of the bioinoculants and uninoculated control. Inoculation of seeds either with G. diazotrophicus or H. seropedicae alone was the next best treatments. However, these two treatments did not differ significantly from each other and thus both the inoculants had similitude effect on yield and yield contributing traits of sweet corn. The least number of grains per cob, average number of grains per row of a cob, length and girth of cob, filled to unfilled grain ratio, grain weight per plant, cob weight per plant, 100-seed weight and grain yield of sweet corn were obtained in uninoculated control plants. Among the three levels of nitrogen, tried in the investigation, application of 100% of recommended dose of nitrogen had significantly highest number of grains per cob (463.13), highest number of grains per row of cob (38.91), longest cobs (17.45 cm), maximum cob girth (16.24 cm), least filled to unfilled grain ratio (11.64), highest grain weight (62.50 g plant⁻¹), highest cob weight (254.46 g plant-1), highest 100-seed weight (14.66 g) and highest grain yield (25.62 q ha-1) of sweet corn. Thus, this treatment was found to be significantly superior over rest of the treatments in ameliorating grain yield and other traits contributing to augmentation in yield. Interaction between bioinoculants and different doses of nitrogen had profound effect on grain yield and yield contributing traits of sweet corn. Integrated seed treatment with G. diazotrophicus and H. seropedicae along with application of 100%, 75% and 50% of the recommended dose of nitrogen, recorded the highest number of grains per cob (464.83, 464.73 and 464.63), number of grains per row of cob (40.11, 39.64 and 39.11), length (19.02, 18.93 and 18.87 cm) and girth of cob (17.20,17.10 and 17.00 cm), filled to unfilled grain ratio (8.55, 8.60 and 8.70), grain weight per plant (64.37, 64.30 and 64.20 g), cob weight per plant (262.04, 261.93 and 261.87 g), 100-seed weight (15.47, 15.40 and 15.30 g) and grain yield of sweet corn(27.78, 27.67 and 27.62 g ha-1). Thus, combined seed treatment with both the bioinoculants in conjunction with all the three nitrogen levels tried was found

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Table-1 Effect of Inoculation of G	. diazotrophicus and H. seropedicae unde	er Graded Levels of Nitrogen on Plan	It Height and Dry Matter Production of Sweet Corn

Inoculation treatment	Plant height (cm) N level				Dry matter Production (q ha-1) N level							
	50% RDN	75% F		100% RDN	Me	an	50% RDN	1 7	75% RDN	100% RDN		an
	30 DAS				30 DAS							
Control	45.62	47	.30	52.73		48.55	33.20		34.03	37.23		34.82
Gd	52.33	52	2.00	53.43		52.59	36.91		36.48	39.14		37.51
Hs	51.81	51	.77	53.00		52.19	35.33		34.46	37.80		35.86
Gd+ Hs	55.53	56	5.20	56.79		56.17	37.80		41.23	41.39		41.29
Mean	51.32	51	.82	53.99			36.67		36.56	38.89		
			60	DAS			60 DAS					
Control	131.75	13	5.33	142.56		136.55	65.30		66.98	74.09		68.79
Gd	140.78	14	0.75	142.68		141.40	73.41		72.15	77.24		74.27
Hs	138.16	13	8.13	142.66		139.65	70.16		68.30	75.01		71.16
Gd+ Hs	144.43	14	2.67	142.75	5 144.62		81.63 81.91		82.04		81.68	
Mean	138.78	13	9.72	143.16			72.63 72.34		77.10			
			Harv	/esting			Harvesting					
Control	141.59	14	6.33	152.17		146.69 70.51			71.98 78.8			
Gd	148.63	14	8.44	157.63		151.57	78.06		76.80	81.63		78.83
Hs	147.87	14	7.81	155.30 150.33		74.87 73.07		80.07		76.00		
Gd+ Hs	162.11	15	9.97	158.20		161.73	85.71		85.98	86.16		85.95
Mean	149.90	15	1.04	156.80			77.29		76.96	81.68		
		DAS 60 DAS Harvest			30 DAS 60 [
	S.E.±	CD at	S.E.±	CD at	S.E.±	CD at	S.E.±	CD at	t S.E.±	CD at	S.E.±	CD at
		5%		5%		5%		5%		5%		5%
N levels (N)	0.10	0.41	0.33	1.29	0.28	1.09	0.06	0.27	0.05	0.21	0.10	0.43
Inoculant (I)	0.18	0.54	0.32	0.95	0.27	0.82	0.11	0.33	0.11	0.32	0.11	0.34
NxI	0.31	0.94	0.55	1.64	0.47	1.43	0.19	0.58	0.19	0.57	0.20	0.59

Table-2 Effect of Inoculation of G. diazotrophicus and H. seropedicae under Graded Levels of Nitrogen on Yield Contributing Characters of Sweet Corn

Inoculation treatment	N level N					evel			
	50% RDN	75% RDN	100% RDN	Mean	50% RDN	75% RDN	100% RDN	Mean	
	No. of grains per cob				No. of grains per row of cob				
Control	421.33	427.07	462.33	436.91	36.60	37.75	38.17	37.51	
Gd	455.73	455.47	462.80	458.00	38.11	38.09	38.29	38.16	
Hs	440.80	440.53	462.53	447.96	38.00	37.88	38.28	38.05	
Gd+ Hs	464.63	464.73	464.83	464.73	39.11	39.64	40.11	39.62	
Mean	445.63	446.95	463.13		37.95	38.34	38.71		
	S.E		C.D.			Ī.±	C.D. at 5%		
N levels (N)	0.1		0.		0.08		0.31		
Inoculants (I)	0.2	· · · · · · · · · · · · · · · · · · ·	0.61		0.13		0.38		
NxI	0.3		1.	06	0.		0.6	6	
		Length of					cob (cm)		
Control	15.03	15.72	16.62	15.79	10.93	12.09	15.90	12.97	
Gd	16.44	16.27	17.16	16.62	14.79	14.75	15.94	15.16	
Hs	16.17	16.00	17.00	16.39	13.84	13.81	15.92	14.52	
Gd+ Hs	18.87	18.93	19.02	18.94	17.00	17.10	17.20	17.10	
Mean	16.63	16.73	17.45		14.14	14.44	16.24		
	S.E		C.D.			±	C.D. at 5%		
N levels (N)	0.0		0.			24	0.94		
Inoculants (I)	0.1		0.			24	0.71		
NxI	0.3		0.	98	0.			1.22	
		Filled and unfil			Grain weight per plant (g)				
Control	19.82	18.67	13.67	17.38	53.28	55.99	61.83	57.03	
Gd	16.00	16.67	11.67	14.78	59.94	59.77	61.94	60.55	
Hs	18.00	18.33	12.67	16.33	56.96	56.90	61.86	58.57	
Gd+ Hs	8.70	8.60	8.55	8.62	64.20	64.30	64.37	64.29	
Mean	15.63	15.57	11.64		58.59	59.24	62.50		
	S.E		C.D.			.±	C.D. at 5%		
N levels (N)	0.21		0.83		0.36		1.39		
Inoculants (I)	0.20		0.60		0.42		1.26		
NxI	0.35 1.03			03	0.73 2.18				
0.1.1	Cob weight p						weight (g)		
Control	220.20	227.10	251.83	233.04	10.97	11.90	14.00	12.29	
Gd	244.70	244.53	252.00	247.08	13.90	13.87	14.60	14.12	
Hs Orthe Ha	235.67	235.47	251.97	241.03	12.77	12.63	14.57	13.32	
Gd+ Hs	261.87	261.93	262.04	261.95	15.30	15.40	15.47	15.39	
Mean	240.61	242.26	254.46		13.23	13.45	14.66		
N levels (N)	S.E		C.D. at 5%		S.E.±		C.D. at 5%		
N levels (N)	0.3		1.49		0.09		0.36		
Inoculants (I) N x I	0.2		0.61		0.13 0.23		0.39		
IN X I	0.3	00	1.	00	0.	23	0.00		

Table-3 Effect of Inoculation of G. diazotrophicus and H. seropedicae under Graded Levels of Nitrogen on Grain Yield (q ha-1) of Sweet Corn

Inoculation treatment	50% RDN	75% RDN	100% RDN	Mean		
Control	18.00	21.30	24.82	21.37		
Gd	24.71	22.00	25.00	23.90		
Hs	21.89	21.68	24.90	22.83		
Gd+ Hs	27.62	27.67	27.78	27.69		
Mean	23.05	23.16	25.62			
	S.E	.±	C.D. at 5%			
N levels (N)	0.3	32	1.27			
Inoculants (I)	0.1	17	0.50			
NxI	0.:	29	0.87			

conspicuously superior over rest of the treatments, in augmenting grain yield and yield contributing parameters in sweet corn. Results of the present investigation are in conformity with those of Patrick Riggs *et al.* [17], Pandey [18], Sharma and Vasudeva [15] and, Chauhana *et al.* [16], who reported increased yield in different crops due to inoculation of *G. diazotrophicus* individually and in combination with *H. seropedicae.* Muthukumarasamy *et al.* [19] reported 50 % reduction in N fertilizer with increase in crop productivity by 5-7 t acre⁻¹ due to use of *A. diazotrophicus*, *Azospirillum* and *Azotobacter* as a biofertilizer for sugarcane. Likewise, Thopate and Jadhav [20] reported increased sugar yield with saving of 50% of supplied nitrogen due to application of *Acetobacter* to sugarcane. Results obtained by these researchers corroborate results of our investigation that inoculation of *G. diazotrophicus* in combination with *H. seropedicae* saves 50% of the recommended dose of nitrogenous fertilizer with exuberant increase in productivity of sweet corn.

Conclusion

From results of the present investigation we infer that the combined seed bacterization with *G. diazotrophicus* and *H. seropedicae* along with application of 50% of the recommended dose of nitrogen is most economical for getting higher grain yield of sweet corn.

Application of Research: For getting higher grain yield of sweet corn with added benefit of cutback in nitrogen fertilization to the tune of 50%, farmers may follow combined seed bacterization with *G. diazotrophicus* and *H. seropedicae*.

Research Category: Biofertilizers

Abbreviations

N: nitrogen P: phosphorus K: potash N₂: nitrogen P₂O₅: Diphosphorus pentoxide K₂O: Potassium oxide ds/m: deciSiemens per metre cfu: colony forming units cv.: cultivar ha: hectare q: quintal g : gram t: tonne m: metre cm: centimetre MSL: mean sea level IAA: Indole-3-Acetic acid

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