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Response of newly collected *Acetobacter* isolates in sweet corn (*Zea mays L. saccharata*)

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Abstract

The present research was undertaken in order to study the effect of *Acetobacter* isolates on performance of sweet corn and response of nitrogen fixing ability of newly collected endophytic bacteria *Acetobacter* spp. in sweet corn. It comprised of experiments in order to select effective local isolate(s) of *Acetobacter* in especially for N accumulation, yield and yield attributing characters of sweet corn crop at maturity of crop. The Pot culture experiment was conducted in green house of the Department of Agricultural Microbiology, College of Agriculture, IGKV, Raipur in rabi, 2016-17 with *Vertisol* (medium fertility status and pH 7.6) by using same sweet corn variety (Sugar-75). Top ten isolates were selected (on the basis of growth performance) from 45 local isolates collected from Raipur and Rajnada districts of Chhattisgarh for future screening study under controlled conditions. The highest green cob yield (28.67g/plant) of sweet corn was obtained from plants raised from seeds inoculated with isolate No.18 which was followed by isolate No.16 (25.35 g/plant). Similarly, highest N uptake was associated with *Acetobacter* isolate No.18 (329.66 mg/plant) followed by *Acetobacter* isolate No.16 (301.64 mg N/plant) and minimum was observed in un-inoculated control (139.64 mg/plant). Overall findings of the experiments the performance of *Acetobacter* isolate No.18 was found superior followed by *Acetobacter* isolate No.16 with most important BNF parameters related to N and biomass accumulation. Microbial dynamic study also supported that isolate No.18 and 16 were well adapted to the rhizosphere of sweet corn and both the isolates retained their highest cell counts in soil taken from sweet corn rhizosphere during pot experiment. Hence, it was concluded that isolate No.18 and 16 were most potent N₂ fixing local *Acetobacter* isolate for sweet corn cultivation in Chhattisgarh.

Keywords: Agricultural Microbiology, performance, experiment.

1. Introduction

Chhattisgarh is regarded as the “rice bowl of India” due to more acreage under rice besides the staple food of the majority of the people. The uncertainty of rice in upland, especially in low rainfall areas lead the farmers to go for other alternative crops which give more remunerative returns. Under such circumstances, scope to grow sweet corn seems to be the better choice for upland farmers. In order to popularize its cultivation among the farming community, it is essential to standardize its biofertilizers techniques for its potential.

Sweet corn (*Zea mays L. saccharata*) is one of the highest commercialized maize type in poaceae family. The fruit of the sweet corn plant is the corn kernel. It has a sugary rather than a starchy endosperm and a creamy texture. The low starch level makes the kernel wrinkled rather than plummy. When the moisture content is higher than 74 per cent the cobs are immature and below 70 per cent they lose the sweetness and develop an unpleasant taste and texture. It has a thinner pericarp than the normal corn making it tender. The green cobs are eaten, roasted or boiled. In India, maize is grown over an area of 7.27 million ha with an annual production of 15.86 million tones and an average productivity of 2181 kg ha⁻¹ (Anonymous, 2011) [2]. In Chhattisgarh, maize is grown in an area of 102.70 thousand ha with an annual production of 185.80 thousand million tones and an average productivity of 1809 kg ha⁻¹ (Anonymous, 2010) [1].

Furthermore, Chhattisgarh soil has a demand of biological N₂-fixing and P mobilizing microbial population to reduce the use of chemical fertilizers. The low population density of endophytic, diazotrophic bacteria are mainly due to high air temperature (up-to 48 °C during summer), soil surface temperature beyond 60 °C and low humidity up to 3-4% for prolonged period of summer season resulting to loss of organic matter and population of beneficial microbes (Katre, *et al.* 1997) [13]. In addition, the soils of Chhattisgarh are low to medium in available nitrogen thus N is one of the most limiting plant nutrients. In the light of ever

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increasing prices coupled with increasing demand of chemical fertilizers and depleting soil fertility necessitates developing effective bio-inoculants like *Acetobacter* for sweet corn crop is the need of hour. So an attempt will be made to develop a suitable *Acetobacter* inoculant for Sweet corn growers of Chhattisgarh with the following objectives. Effect of *Acetobacter* isolates on performance of sweet corn and Response of nitrogen fixing ability of newly collected endophytic bacteria *Acetobacter* spp. in sweet corn.

Material Method

Isolation of *Acetobacter* and preparation of inoculums *Acetobacter* isolates were isolated from fresh root of sweet corn, sugarcane, barley, sweet potatoes, maize crop and soil sample using LGIP media. The isolated *Acetobacter* was multiplied in the departmental laboratory. After preliminary study on the basis of growth performance, 10 effective isolates selected from total 45 isolates, for further study under controlled conditions (Pot experiments).

Pot culture experiment was conducted up to maturity of crop in green house of the Department of Agricultural Microbiology, College of Agriculture, IGKV, Raipur in *rabi*, 2016-17 with *Vertisol* (medium fertility status and pH 7.6) by using same sweet corn variety (Sugar-75) with same local *Acetobacter* isolates for evaluation of different *Acetobacter* isolates especially to record crop yield parameters including biochemical parameter etc. The number of treatments was 11 along with control replicated thrice in completely randomized design. (Table 1). The medium used for growing Sweet corn crop was soil (*Vertisols*) which was well air dried and processed to good physical condition ideal for Sweet corn growth. This soil was filled in cemented pot @ 9.5 kg per pot. Soils were randomly collected from a depth of 6 inches (15cm) from soil surface and thoroughly mixed and filled in each pot 9.5 kg capacity

Details of the treatments

The experiment comprising the following 11 treatments. The treatment details are as follows.

Table 1: Detail of treatments for pot experiment

S. No.	Soil sample no.	Isolates no.
1	Control	Control 100% GRD (NPK 120:60:60)
2	6	Isolate No. 6+75%GRD
3	12	Isolate No. 12+75%GRD
4	15	Isolate No. 15+75%GRD
5	16	Isolate No. 16+75%GRD
6	18	Isolate No. 18+75%GRD
7	24	Isolate No. 24+75%GRD
8	25	Isolate No. 25+75%GRD
9	31	Isolate No. 31+75%GRD
10	32	Isolate No 32+75%GRD
11	40	Isolate No. 40+75%GRD

Morphological growth parameters, plants height were recorded at 15 day's interval viz. 15, 30, 45, and 60 days after sowing (DAS) and expressed in centimeters per plant. Fresh and dry weight of cob was taken after 75 days of sowing (DAS) and it was expressed in gram per plant. The shoots were harvested at maturity i.e. 95 DAS and weight was expressed in grams per plant. The fresh and dry roots weight was recorded i.e. 95 DAS and weight was expressed in grams per plant. Nitrogen content in the plant samples was estimated by Micro-Kjeldahl method as described by Jackson (1973) [11] using auto digestion and distillation system and presented in percentage. Available soil N was determined by alkaline

KMnO₄ method of Subbiah and Asija (1965) [23] with slight modification. 5 gram of soil sample was taken in 1000 ml digestion tube and distilled with the help of Kel plus to collect about 150 ml distillate in 10 ml boric acid solution containing mixed indicator In distillate ammonium boret was determined by titrating against 0.005 N H₂SO₄ (Bremmer, 1965) [5]. Microbial estimation with respect *Acetobacter* and total bacterial count of the soil were done by serial dilution plate method (Subba Rao, 1988) [22] and (Tuladhar, 1983) [26]

Result and discussion

In pot culture experiment was also conducted up to maturity stage of crop in green house of the Department of Agril. Microbiology, College of Agriculture, IGKV, Raipur in *rabi*, 2016-17 with *Vertisol* (medium fertility status and pH 7.6) by using same sweet corn variety (Sugar-75) with same local *Acetobacter* isolates for evaluation of different *Acetobacter* isolates especially to record crop yield parameters and N accumulation.

Data of plant height recorded at different growth stages of crop viz. (15, 30, 45 and 60 DAS) presented in Table 2. At 15 DAS some of the microbial isolates significantly enhanced the plant height. Performance of *Acetobacter* isolates No.18, 32, 31, 16, 15, 40, and 12 were found superior over control. At 30 DAS, plant height increased significantly due to local *Acetobacter* isolates No.18, 15, 16, 32, 12, 40, 31 and 25. At 45 DAS, plant height increased significantly due to local *Acetobacter* isolates No.18, 15, 40, 16, 12, 32, 6, and 24 over control. At 60 DAS, plant height also increased significantly due to *Acetobacter* isolates No.16, 18, 15, 32, 40, 12, and 6 over control.

Results of fresh shoot matter at 95 DAS, revealed that fresh shoot weight increased significantly over control due to inoculation of *Acetobacter* isolates. The fresh shoot biomass increased significantly from 22.87 g/plant (control) to 34.00, 26.33, 25.83, 29.00, 37.50, 36.47, 37.00, 26.33, 26.83 and 22.70 g/plant due to inoculation with isolate No. 6, 12, 15, 16, 18, 24, 25, 31, 32 and 40 respectively. Highest shoot fresh weight was associated with isolate No.18 i.e. 37.50 g/plant whereas lowest was associated with isolate No.40 i.e. 22.70 g/plant among all the isolates. While, value of un-inoculated control was 22.87 g/plant

The shoot dry weight of plant increased from 10.47g/plant (control) to 13.33, 11.50, 12.32, 15.67, 18.17, 12.83, 13.67, 11.17, 14.33 and 11.77g /plant due to inoculation of isolate No. 6, 12, 15, 16, 18, 24, 25, 31, 32 and 40 respectively. Maximum increase in dry weight was observed by isolate No.18 (18.17 g/plant), followed by isolate No.16 (15.67 g/plant) and isolate No.32 (14.33 g/plant), whereas lowest shoot dry weight was associated with isolate No.31 (11.17 g/plant) among the isolates under study. Value of un-inoculated control was 10.47g/plant. Similar finding was also observed by Jhala *et.al.* (2016) [12], they mentioned that due to inoculation of *Acetobacter* in sweet corn crop the dry matter was increased significantly.

The cob fresh weight increased from 12.14 g/plant to 13.45, 13.33, 22.82, 25.35, 28.67, 18.45, 19.10, 13.46, 14.88 and 19.40g /plant due to inoculation of isolate No. 6, 12, 15, 16, 18, 24, 25, 31, 32 and 40 respectively. Maximum increase in fresh weight was observed by isolate No.18 (28.67 g/plant), followed by isolate No.16 (25.35 g/plant), isolate No.15 (22.82 g/plant), whereas lowest fresh cob weight was observed with isolate No.12 (13.33 g/plant) among the isolates under study. Value of un-inoculated control was 12.14g/plant. Results of the present investigation are

conformity with Shinde and Patil (1995) [21], Thakur and Singh (1996) [25], Riggs *et al.* (2000), Kharbade and Sable (2002) [14], Jambukar (2003) [10], Pandey (2004) [17], and, Chauhana *et al.* (2010) [6], who reported increased crop yield in different crops due to inoculation of *Gluconacetobacter diazotrophicus* individually and in combination with *Herbaspirillum seropedicae*.

The cob dry weight of plant increased from 2.58g/plant (control) to 2.79, 2.79, 5.20, 4.58, 5.31, 4.03, 3.46, 3.22, 2.85 and 3.67g /plant due to inoculation of isolate No. 6, 12, 15, 16, 18, 24, 25, 31, 32 and 40 respectively. Maximum increase in dry weight was observed by isolate No.18 (5.31 g/plant), followed by isolate No.15 (5.20 g/plant), isolate No.16 (4.58 g/plant), whereas lowest cob dry weight was observed with isolate No.6, 12 (2.79 g/plant) among the isolates under study. Value of un-inoculated control was 2.58 g/plant.

Root fresh weight of plant increased from 17.06g/plant (control) to 22.84, 13.10, 25.33, 22.31, 39.34, 40.52, 27.20, 28.53, 35.73 and 20.48g /plant due to inoculation of isolate No. 6, 12, 15, 16, 18, 24, 25, 31, 32 and 40 respectively. Maximum increase in fresh root weight was observed by isolate No.24 (40.52 g/plant), followed by isolate No.18 (39.54 g/plant), isolate No.32 (35.73 g/plant), whereas lowest fresh root weight was observed with isolate No.12 (13.10 g/plant) among the isolates under study. Value of un-inoculated control was 17.06 g/plant. Similarly finding was observed in Jhala *et al.* (2016) [12].

Root dry weight of plant increased from 5.56g/plant (control) to 7.67, 6.45, 8.83, 10.37, 11.91, 8.94, 8.10, 7.68, 9.62 and 7.83g /plant due to inoculation of isolate No. 6, 12, 15, 16, 18, 24, 25, 31, 32 and 40 respectively. Maximum increase in dry root weight was observed by isolate No.18 (11.91 g/plant), followed by isolate No.16 (10.37 g/plant), isolate No.32 (9.62 g/plant), whereas lowest fresh root weight was observed with isolate No.12 (6.45 g/plant) among the isolates under study. Value of un-inoculated control was 5.56 g/plant. Similar type of finding was also reported by Jhala *et al.* (2016) [12].

Acetobacter population density at harvest stage increased significantly over control due to inoculation of local isolates. However, highest population of *Acetobacter* in soil was observed in rhizosphere of plant raised from seed inoculated with *Acetobacter* isolate No. 18 (327.67×10^{-5}) followed by 40 (308.67×10^{-5}) and 15 (307×10^{-5}) whereas lowest population associated with isolate No. 31 (253×10^{-5}) excluding control. Several scientists have reported varying results related to population of these diazotrophs. Dobereiner *et al.* (1988) [7] observed *Acetobacter diazotrophicus* in many sugarcane varieties and numbers were increased significantly. However, Fuentes *et al.* (1999) [8] found that colonization of sugarcane by *Acetobacter diazotrophicus* is inhibited by high dose of nitrogen fertilizer.

Total bacteria population at harvest stages increased significantly over control. However, highest population density of total bacteria in soil was associated with inoculation of isolate No. 24 (341×10^{-7} /g soil) followed by isolate No. 40 (334.6×10^{-7} /g soil) whereas lowest was

observed in isolate No. 15 (274×10^{-7} /g soil) among the isolates under study. Similar type of finding was observed by Muthukumarasamy *et al.* (2006) [15] they observed that the establishment of inoculated *Herbaspirillum sp.* remained stable with the age of the crop up to 180 days, while there was reduction in population of *G. diazotrophicus* during the same period. Archana *et al.* (2008) [3] found that the population of *G. diazotrophicus* was more at N 75 compared to N0 and N150, whereas *Herbaspirillum* population increased from N0 to N150.

Data clearly indicated that N content of plants significantly increased due to *Acetobacter* inoculation treatment. The % N content increased significantly from 1.69%/ plant (control) to 1.65, 1.76, 1.77, 1.93, 1.81, 1.39, 1.56, 1.33, 1.83, 1.52% due to inoculation with isolate No. 6, 12, 15, 16, 18, 24, 25, 31, 32 and 40 respectively. Among all the isolates, highest N content was observed with isolate No.18 (*i.e.* 1.81%) whereas lowest value was associated with isolate No.24 (*i.e.* 1.39%). Value of un-inoculated control was 1.69%. The N-uptake increased significantly from 188.72 mg/plant (control) to 220.36, 202.94, 218.45, 301.64, 329.66, 178.90, 213.84, 139.94, 262.30, 178.58 mg/plant due to inoculation with isolate No. 6, 12, 15, 16, 18, 24, 25, 31, 32, and 40 respectively along with application of 75% GRD (NPK :: 120,60,60). Among all the isolates, highest N uptake was observed with isolate No.18 (*i.e.* 329.66 mg/plant) whereas lowest value was associated with isolate No.31 (*i.e.* 139.64 mg/plant). Value of un-inoculated control was estimated 188.72 mg/plant. Many scientists reported increased nitrogen uptake due to inoculation of *Gluconacetobacter diazotrophicus* individually and in combination with *Herbaspirillum seropedicae* (Boddy *et al.*, 1991; Sevilla *et al.*, 2001; Oliveira *et al.*, 2002; Hari *et al.*, 2005; Suman *et al.*, 2005 and Chauhana *et al.*, 2010) [4, 20, 16, 5, 24, 6].

Data clearly indicated that residual N of soil significantly increased due to *Acetobacter* treatment. The available soil N increased significantly from 139.60 kg/ha. control to 176.41, 197.57, 208.65, 209.07, 219.93, 213.49, 175.07, 151.38, 163.22, 210.49 kg/ha due to inoculation with isolate No. 6, 12, 15, 16, 18, 24, 25, 31, 32 and 40 respectively. Among all the isolates, highest available N was observed with isolate No.18 (*i.e.* 219.93 kg/ha) whereas lowest value was associated with isolate No.32 (*i.e.* 163.22). The value of un-inoculated control was 139.60 kg/ha observed.

Overall findings of the experiments the performance of *Acetobacter* isolate No.18 was found superior followed by *Acetobacter* isolate No.16 with most important BNF parameters related to N and biomass accumulation. Microbial dynamic study also supported that isolate No.18 and 16 were well adapted to the rhizosphere of sweet corn and both the isolates retained their highest cell counts in soil taken from sweet corn rhizosphere during pot experiment. Hence, it was concluded that isolate No.18 and 16 were most potent N₂ fixing local *Acetobacter* isolate for sweet corn cultivation in Chhattisgarh.

Table 2: Soil inoculation efficacy of entophytic *Acetobacter* bacterial isolates on growth and yield of sweet corn c.v. Sugar -75

Name of Treatments Details	Plant height (cm/plant)				Shoot weight (g/plant) at harvest		Root weight(g/plant) at harvest		Fresh cob weight (g/plant)	Dry cob weight (g/plant)
					Fresh	Dry	Fresh	Dry		
	15 DAS	30 DAS	45 DAS	60 DAS						
Control	20.27	60.73	110.53	140.10	22.87	10.47	17.06	5.56	12.14	2.58
Isolate No 6	23.20	67.83	123.67	145.63	34.00	13.33	22.84	7.67	13.45	2.79
Isolate No 12	25.17	81.83	133.50	150.47	26.33	11.50	13.10	6.45	13.33	2.79

Isolate No 15	27.33	85.00	138.73	163.57	25.83	12.32	25.33	8.38	22.82	5.20
Isolate No 16	28.67	83.33	136.67	168.57	29.00	15.67	22.31	10.37	25.35	4.58
Isolate No 18	33.60	88.83	145.27	166.97	37.50	18.17	39.34	11.91	28.67	5.31
Isolate No 24	22.53	61.00	117.00	140.27	36.47	12.83	40.52	8.94	18.45	4.03
Isolate No 25	22.30	70.67	110.60	143.60	37.00	13.67	27.20	8.10	19.10	3.46
Isolate No 31	30.00	77.67	110.53	140.60	26.33	11.17	28.53	7.68	13.46	3.22
Isolate No 32	32.20	82.00	130.30	156.70	26.83	14.33	35.73	9.62	14.88	2.85
Isolate No 40	26.27	80.67	136.90	153.53	22.70	11.77	20.48	7.83	19.40	3.76
SEm +/-	1.32	2.72	2.86	2.90	2.52	0.46	2.57	0.28	0.58	0.46
CD (0.05)	4.00	8.25	8.68	8.81	7.65	1.40	7.78	0.86	1.77	1.39

Table 3: Effect on N uptake, Available Soil Nitrogen and bacterial count due to inoculation of entophytic *Acetobacter* bacterial isolates.

Name of isolates	(60 DAS)		Available soil N kg/ha (60DAS)	Nitrogen content (%) at harvest	Nitrogen uptake (mg/plant) at harvest
	<i>Acetobacter</i> population X10 ⁻⁵ (60 DAS)	Total bacteria population X 10 ⁻⁷ (60 DAS)			
Control	199.33	245.67	139.60	1.69	188.72
Isolate No 6	280.33	277.67	176.41	1.65	220.36
Isolate No 12	261.33	276.00	197.57	1.76	202.94
Isolate No 15	307.00	274.67	208.65	1.77	218.45
Isolate No 16	306.00	256.00	209.07	1.93	301.64
Isolate No 18	327.67	323.33	219.93	1.81	329.66
Isolate No 24	297.33	341.00	213.49	1.39	178.90
Isolate No 25	266.67	286.67	175.07	1.56	213.84
Isolate No 31	255.00	309.00	151.38	1.33	139.64
Isolate No 32	290.33	326.67	163.22	1.83	262.30
Isolate No 40	308.67	334.67	210.49	1.52	178.58
SEm +/-	17.11	17.13	3.16	0.13	2.30
CD (0.05)	51.89	51.97	9.58	0.40	7.00

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