International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(3): 1648-1650

Received: 18-03-2018
Accepted: 20-04-2018

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

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Abstract
Present experiment was conducted 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. This experiment in order to select effective local isolate(s) of *Acetobacter*. In Sand culture experiment especially to observe N-fixing behavior of local *Acetobacter* isolates at premature stage of sweet corn crop. Out of 63 samples 45 local isolates collected from Raipur, Kawardha, and Rajnadgaon districts of Chhattisgarh. Top Ten isolates were selected (on the basis of growth performance) for future screening study under controlled conditions. Plant biomass data from N-free sand culture experiment revealed that the highest shoot dry weight was associated with isolate No.18 (1.45 g/plant) followed by isolate No.40 (1.34 g/plant). The highest N content (1.78%) was associated with *Acetobacter* isolates No. 16 followed by isolate No.18 (1.68%) as compared to control control (0.37%). In sand culture grown sweet corn resulted that, performance of *Acetobacter* isolate No.18 was found superior followed by *Acetobacter* isolate No.16 with most important BNF parameters related to N uptake and biomass accumulation.

Keywords: Response, newly collected *Acetobacter* isolates, sweet corn (*Zea mays* l. *Saccharata*), sand

Introduction
The uncertainty of rice in upland, especially in low rainfall areas lead the farmers to go for other alternative crops which give more returns. Under such conditions, scope to grow sweet corn to be the better option for upland situation. 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 crop. 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 plumy.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 tonnes 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 tonnes and an average productivity of 1809 kg ha-1 (Anonymous, 2010) [1].

Furthermore, Chhattisgarh soil has a demand of biological N_2-fixing microbial population to reduce the use of chemical fertilizers. The low population density of endophytic, diazotrophic bacteria are mainly due to high air temperature (upto 48 ^{0}C during summer), soil surface temperature beyond 60 ^{0}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 (Anonymous, 1996). 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 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 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 out of total 63 samples, for further study under controlled conditions (sand culture).

All isolates were tested for their nitrogen fixing capacity in culture media after one week of inoculation through Micro Kjedal methods (A.O.A.C.1965). The rate of nitrogen fixation was expressed in mg nitrogen fixed/ gram of sucrose consumed.

Fine grade sterilized river sand (20 lb. pressures per inch^2 for 2 hours) was filled in disposable tray of 250 g capacity. Only 225 g sterilized sand was taken in the individual cup. Seed of sweet corn was treated with the mature broth of the *Acetobacter* and seed was shown Var. sweet corn (suger-75) immediately in sand cup. N free nutrient solution and water was supplied time to time. Plant height was recorded at different intervals viz. 15, 30, and 45 days after sowing (DAS) and expressed in centimeters/plant. The plant samples are uprooted at 45 DAS and shoot fresh and dry weight were recorded (gram per plant). The plants uprooted along with roots at 45 DAS of sweet corn. Fresh biomasses of taken. The number of treatments was 11 along with control, T1 Control),T2-Isolate No. 6,T3- Isolate No. 12, T4- Isolate No. 15,T5- Isolate No. 16,T6- Isolate No. 18,T7- Isolate No. 24, T8- Isolate No. 25,T9- Isolate No. 31,T10- Isolate No 32, T11- Isolate No. 40, replicated thrice in completely randomized design.

Morphological growth parameters, plants height were recorded at 15 day's interval viz.15, 30, 45, days after sowing (DAS) and expressed in centimeters per plant. The shoots were harvested at i.e. 45 DAS and weight was expressed in grams per plant. The fresh and dry roots weight was recorded i.e. 45 DAS and weight was expressed in grams per plant. Estimation of Nitrogen in the plant samples. The nitrogen content in the plant samples was estimated by Micro-Kjeldahl method as described by Jackson (1973) [5] using auto digestion and distillation system and presented in percentage.

Result and discussion

All isolates were tested for their nitrogen fixing capacity in culture media after one week of inoculation through MicroKjedal methods (A.O.A.C.1965). The rate of nitrogen fixation maximumum 29.90 mg nitrogen fixed/ gram of sucrose consumed was recorded by Isolate no. 18.fallowed by Isolate no. 16, 24.30 mg nitrogen fixed/ gram of sucrose and minimum was observed in Isolate No. 40.

Table 1: *In vitro* nitrogen fixation capacity of selected best 10 isolates

Isolates No.	mg nitrogen/g of sugar consumed
Isolate No 6	20.00
Isolate No 12	21.30
Isolate No 15	20.80
Isolate No 16	24.30
Isolate No 18	29.90
Isolate No 24	20.80
Isolate No 25	22.50
Isolate No 31	19.8
Isolate No 32	23.3
Isolate No 40	17.4

Sand culture grown sweet corn (variety Sugar-75) experiment was conducted up to 45 DAS, in the green house with N free nutrient solution.

Data of plant height recorded at three different stages (15, 30, and 45 DAS) of crop growth and presented in Table 2. At 15 DAS, some of the microbial isolates significantly enhanced the plant height. Performance of isolate No. 18, 16, 40, 6, 12, 15 and 25 were found superior over control. At 30 DAS, almost all the isolates showed significantly better results for plant height over control. Similarly at 45 DAS, isolates gave higher values of plant height similar to that of other stages of crop growth (Fig. 4.1) It was clear from the study that plant height increased significantly from 15 to 45 DAS due to inoculation of *Acetobacter* isolates as reported by Kharbade and Sabale (2002) [9]. Similar finding also observed by Jhala *et. al.* (2016) [6] and Hari Narayan et.al. (2017) [4], they clearly reported that plant height of maize significantly increased by inoculation of *Acetobacter*. Shinde and Patil (1995) [13], Chauhana *et al*. (2010) [3], got similar type of results due to use of diazotrophs.

Data presented in Table 2, related to fresh shoot matter at 45 DAS, revealed that fresh shoot weight increased due to *Acetobacter* inoculation. The fresh shoot biomass increased significantly from 2.23 g/plant (control) to 4.95, 5.11, 5.31, 5.32, 5.78, 4.70, 5.63, 5.41, 5.24 and 5.32 g/plant due to inoculation with isolate No. 6, 12, 15, 16, 18, 24, 25, 31, 32 and 40 respectively. Among all the isolates, highest shoot fresh weight was associated with isolate No.18 *i.e.* 5.78 g/plant whereas lowest was associated with isolate No. 24 *i.e*. 4.70 g/plant among all the isolates, While, value of un-inoculated control was 2.23g./plant.

Dry matter of shoot at 45 DAS was presented in Table 4.3. The shoot dry weight of plant increased from 0.47g/plant (control) to 1.06, 1.11, 1.27, 1.33, 1.42, 1.21, 1.43, 1.40, 1.33, 1.33g /plant due to inoculation of isolate No. 6, 12, 15, 16, 18, 24, 25, 31, 32 and 40 respectively. Maximum increase in shoot dry weight was observed by isolate No.25 (1.43 g/plant), followed by isolate No.18 (1.42 g/plant), isolate No.31 (1.40 g/plant), whereas lowest shoot dry weight was observed with isolate No.12 (1.11 g/plant) among the isolates under study. Value of un-inoculated control was 0.47g. Increased dry matter production due to inoculation of *Gluconacetobacter diazotrophicus* individually and in combination with *Herbaspirillum seropedicae* had been reported by several researchers in different crops (Kharbade and Sable, 2002 [9]; Pandey S. 2004; Oliveira *et al*. 2002 [10];.

Results of root fresh matter were clearly revealed that at 45 DAS, root fresh weight increased due to inoculation of *Acetobacter* isolates. The fresh root biomass increased significantly from 0.77 g/plant (control) to 0.77, 0.169, 0.193, 0.206, 0.258, 0.238, 0.193, 0.237, 0.239, 0.222, 0.191g/plant due to inoculation with isolate No. 6, 12, 15, 16, 18, 24, 25, 31, 32 and 40 respectively. Highest root fresh weight was recorded with isolate No. 16 i.e. 0.258g /plant whereas lowest was associated with isolate No. 6 i.e. 0.169g/ plant among isolates under study. Value of un-inoculated control was 0.77g /plant. Similar type of finding was also reported by Jhala *et al.* (2016) [6].

In case of nitrogen content of plants significantly increased due to *Acetobacter* inoculation. The % N content increased significantly from 0.37of (control) to 1.50, 1.61, 1.62, 1.78, 1.66, 1.24, 1.41, 1.54, 1.68, 1.37% due to inoculation with isolate No. 6, 12, 15, 16, 18, 24, 25, 31, 32, 40 respectively. Among all the isolates, highest N content was observed with isolate No. 16 (i.e. 1.78%) whereas lowest value was associated with isolate No. 24 (i.e. 1.24%). Value of un-inoculated control was 0.37%. This observation was supported by Jhala *et.al.* (2014) [7], Hari Narayan et.al. (2017) [4].

Table 2: Sand culture inoculation efficacy of entophytic *Acetobacter* bacterial isolates on growth and N content of sweet corn c.v. Sugar -75

Name of isolates	Plant height (cm/plant)			Shoot fresh weight (g/plant) (45DAS)	Shoot Dry weight (g/plant) (45DAS)	N content (%) (45DAS)	N uptake by shoot mg/plant
	15 DAS	30 DAS	45 DAS				
Control	18.90	27.83	30.20	2.23	0.47	0.37	1.740
Isolate No 6	24.63	39.90	50.07	4.95	1.06	1.50	15.90
Isolate No 12	22.63	40.63	50.47	5.11	1.11	1.61	17.87
Isolate No 15	21.83	40.30	50.47	5.31	1.27	1.62	20.57
Isolate No 16	27.33	40.63	50.53	5.32	1.33	1.78	23.67
Isolate No 18	27.46	40.83	50.63	5.78	1.42	1.66	23.57
Isolate No 24	16.83	36.77	37.23	4.70	1.21	1.24	15.00
Isolate No 25	21.00	36.73	37.33	5.63	1.43	1.41	20.16
Isolate No 31	17.83	33.87	40.40	5.41	1.40	1.54	21.56
Isolate No 32	22.17	32.77	50.17	5.24	1.33	1.68	22.34
Isolate No 40	25.00	40.40	43.73	5.32	1.34	1.37	18.36
SEm +/-	0.54	0.36	1.82	0.31	0.08	0.13	1.42
CD (0.05)	0.62	1.10	5.52	0.94	0.24	0.40	1.96

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