

## Original Research Article

### Response of 1-Aminocyclopropane-1-carboxylic acid containing *Pseudomonas fluorescens* for rhizobial population and yield of Maize (*Zea mays* L.)

#### ABSTRACT

A field experiment entitled "Response of 1-Aminocyclopropane-1-carboxylic acid containing *Pseudomonas (P) fluorescens* for rhizobial population and yield of Maize (*Zea mays* L.)" was conducted at Research Farm, Department of Soil Science and Agricultural Chemistry, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh during 2018-19. The results revealed that the rhizobial population, irrespective of treatments, was maximum at 55 days after sowing followed by a reduction at harvest. The treatment T<sub>28</sub>+pH 7.2 (Temperature 28<sup>o</sup> C + pH 7.2) enhanced the maximum population of *P. fluorescens* in rhizosphere at 35, 55 and 75 DAS was with 1.69, 1.59 and 1.87 log fold increase over FUI [(3.7089 log cfu/g = 5.80 x10<sup>3</sup> cfu g<sup>-1</sup> soil), (5.8673 log cfu/g = (7.53x10<sup>5</sup> cfu g<sup>-1</sup> soil) and (4.676 log cfu/g 5.55x10<sup>4</sup> cfu g<sup>-1</sup> soil)]. Similar treatment T<sub>28</sub>+pH 7.2 recorded maximum number of cob plant<sup>-1</sup>, cob length<sup>-1</sup>, number of grains line<sup>-1</sup> and number of grains cob<sup>-1</sup> at harvest were with T<sub>28</sub>+pH 7.2 by 30.6, 32.1, 29.7 and 37.7% over the control FUI (2.1, 15.2 cm, 35.0 and 503), respectively. While the treatment T<sub>31</sub>+pH 7.2 (Temperature 31<sup>o</sup> C + pH 7.2) enhanced the cob girth and maximum grains line cob<sup>-1</sup> at harvest were with 26.0 and 31.3% response over FUI (13.3 cm cob<sup>-1</sup> and 13.9), respectively. The treatment T<sub>28</sub>+pH 7.2 significantly increased the seed and stover yields of maize by 24.9 and 31.6% over the control (2914 and 7133 kg ha<sup>-1</sup>), respectively.

**Keyword:** ACC-deaminase; Cob; rhizobial; population; yield; Maize

#### 1. Introduction

Maize (*Zea mays* L.) is the most widely distributed cereal crop of the world. India occupies fifth place in acreage and ranks 10<sup>th</sup> in production. Maize is one of the most versatile emerging crops showing wider adaptability under varied agro-climatic conditions. Globally, maize is known as queen of cereals because it has the highest genetic yield potential among the cereals. The average productivity in India is 1959 kg ha<sup>-1</sup> grown in 7.18 million hectares area contributing 14.1 MT of production, and the crop is the third most important food crops after rice and wheat (Agriculture Statistics at a glance, 2016). Maize in India, contributes nearly 9% in the national food basket and more than Rs. 100 billion to the agricultural

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GDP at current prices. The state of Madhya Pradesh occupies 13% of the total maize area and contributing equally to the total maize production in the country. Nutritionally, maize contains 60 to 68% starch, 1.2 to 5.7% edible oil and 7 to 15% protein (Singh *et al.*, 2003).

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*P. fluorescens* belongs to Pseudomonadaceae family, is a Gram-negative rod shaped bacteria with visible flagella extending from their cell wall, having a ratio of non-mucoid to mucoid cells approximately 9:1 ratio but a thin layer of material known as peptidoglycan outside of their cell membrane. These bacteria can secrete a fluorescent pigment 'pyoverdine' (iron binding siderophore) that exhibits bright color under certain conditions. The bacteria are obligate aerobes but certain strains are capable of using nitrate instead of oxygen as a final electron acceptor during cellular respiration. It is asexually reproduced by binary fission splitting apart into exact replica of the parent. Moreover, the bacteria are able to regulate their population density through quorum sensing (Suntharalingam and Cvitkovitch, 2005; Surette *et al.*, 1999). *P. fluorescens* has simple nutritional requirements and grows well in mineral salts media supplemented with any of a large number of carbon sources (Palleroni, 1984). Again, only small fractions of the cell populations at stationary-phase of growth as non-growing cells (8-11%) with a short generation time for all actively growing cells. Depending on the bacteria's reproduction rate, it has multifold reproduction potency in a day. It grows at an optimum growth temperature 25 – 30°C and pH 7 (Meyer and Abdallah, 1978; Palleroni, 1984).

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ACC deaminase was first discovered in soil microorganism *Pseudomonas* sp. and shown to convert ACC (1-aminocyclopropane-1-carboxylic acid), a precursor of hormone ethylene to ammonia and  $\alpha$ -ketobutyrate, both of which further metabolized by a microorganism (Honma and Shimomura, 1978). It is a multimeric sulfhydryl enzyme with a monomeric subunit having molecular mass of approximately 35-42 kDa, it utilizes pyridoxal 5-phosphate as an essential co-factor tightly bound to the enzyme @ 1 molecule per mol of an enzyme or 1 mol per trimeric subunit (Honma, 1985), required for enzyme activity. The native size of the enzyme in trimeric form with molecular mass of 110-112 kDa has been reported from *Pseudomonas* sp. The enzyme is localized within the cytoplasm of the microorganism that produces it (Jacobson *et al.*, 1994). In this case, the substrate ACC is exuded by plant tissues (Penrose *et al.*, 2001; Grichko and Glick, 2001) and is then taken up by the ACC-deaminase containing microbe (Glick *et al.*, 1998). Shaharoon *et al.*, (2006) investigated the performance of *Pseudomonas* spp. containing ACC-deaminase for improving growth and yield of maize (*Zea mays* L.) in the presence of nitrogenous fertilizer. All the three strains had significant positive effect on cob length and caused up to 18.1% increase in cob length under N-unfertilized conditions. Under N-fertilized conditions, two strains, Q14 and N3, significantly increased (up to 14%) cob length over uninoculated control (N fertilized).

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In view to the above, the present investigation was carried out to study the "Response of 1-Aminocyclopropane-1-carboxylic acid containing *Pseudomonas fluorescens* for yield of maize (*Zea mays* L.) and microbial population in soil".

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## 2. MATERIALS AND METHODS

The present lab or field investigation was carried out in 2 phases as Component- I (during rainy season of 2018): Kinetics of population growth of *P. fluorescens* and ACCD activity of the bacteria under different conditions of fermentation; and Component- II (during winter season of 2019): performance of selected isolates of *P. fluorescens* on maize. Studies of component- I and II were performed at the Laboratory of Microbes Research & Production Centre and the Research Farm, Department of Soil Science & Agricultural Chemistry, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur India, (latitude of 23° 13' N and longitude of 79° 57' E with an altitude of 393 m above mean sea levels) in the period of Feb-June, 2019. The field soil was deep black soil in texture, having pH, 7.48; organic carbon; 0.53%, available N, 233 kg ha<sup>-1</sup> and 14.9 kg ha<sup>-1</sup> of available P and 209 kg ha<sup>-1</sup> of available K, respectively and *P. fluorescens* population (1.35×10<sup>4</sup> cfu g<sup>-1</sup> soil) was recorded) respectively. Under fermentation experiment a local but effective strain of *P. fluorescens* was batch cultured for 13 intervals of time (at 0, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132 and 144 hr) with 16 treatments including four temperature regimes (i.e., 25, 28, 31, 34°C), four pH (i.e., 6.7, 7.2, 7.7, 8.2) and their interaction under Randomized block designs (RBD) factorial design with 2 replications. Broth samples were drained out from the fermentator at the scheduled intervals for study on the bacterial population growth and enzyme ACCD activity. Under field study, the 16 isolates derived from the treatments of different temperature and pH with additional 2 controls [Total treatments = 18 (16 isolates + 1 FUI + 1 UFUI)] were tried on maize following RBD design with 3 replications. Data were statistically treated by ANOVA, RBD to test the statistical significance of variance among different treatment means as influenced with the application of the treatments on various attributes of maize.

### 2.1. Climate

The tropic of cancer passes through the middle of the district. It has sub-tropical climate characterized by hot dry summers and cool dry winter. Jabalpur lies in the "Kymore Plateau and Satpura hills" agro- climatic zone of Madhya Pradesh. Seasonal variations prevailing during the growing period plays an important role in the developmental process, which may have great influence on the final yield of the crop. The weekly meteorological data were recorded during crop season of Meteorological Observatory College of Agricultural Engineering, JNKVV, Jabalpur and are depicted through Figure 1. It is obvious from the meteorological data that the weather conditions which prevailed during the crop season were favorable for the growth and development of maize. The mean weekly maximum temperature ranges from (26.2 to 44.4°C) whereas; mean weekly minimum temperature ranged between 3.1 to 27.3°C. Whereas, relative humidity varied from 49.0 to 85.0 % in morning and 17.0 to 47.0% in evening. The mean sunshine hours varied between 7.3 to 10.3 hours per day. Total rainfall of 77.3 mm was received.

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## 2.2. Seed inoculation, treatment and sowing

The isolates of *P. fluorescens* obtained from the laboratory experiment (Component I) performing best for population growth and ACCD enzyme activity were earmarked and specially selected for the field trial on maize to observe sustainability of the attributes. However, remaining other isolates along with the selected isolates was also included in the field trial. The isolates in broth were used for seed treatment and foliar spray on maize at three growth stages (at knee stage, tasselling and silking stage) to ascertain the population growth attributes and ACCD activity in rhizosphere and the plant growth and yield at maturity. Maize seeds in polythene bags were slightly moistened and then treated with carbendazim fungicide @ 2 g kg<sup>-1</sup> seed and air dried under shade. Then the seeds were inoculated individually with the isolated of *P. fluorescens* the recommended dose 10 ml kg<sup>-1</sup> of seed and using sterilized gum acacia (2%) as adhesive. The field experiment was carried out at research farm JNKVV Jabalpur during winter season of 2019. The seeds of maize (JM-216) were sown in the respective plot. Recommended package of practices (Depth of Sowing: 4-6 cm, where maize is generally sown on flat beds. Spacing: 70cm x 25cm for row to row and plant to plant. Plant density of 66,666/ha and intercultural operation) was followed to maintain plant population, protection and growth. Three plants from each plot were taken to measure cob plant<sup>-1</sup>, cob girth, grain line cob<sup>-1</sup>, grains line<sup>-1</sup>, grains cob and cob length<sup>-1</sup> at harvest. To evaluate the effect of different treatments on microbial population in soil, the study was done adopting serial dilution method. Soil samples collected periodically for microbial study were processed for serial dilution by suspending 10 g of soil sample in 90 ml sterilized water in flasks and were shaken thoroughly which resulted 10<sup>-1</sup> dilution. Subsequent serial dilutions were made to up 10<sup>-9</sup> dilution for plating purpose.

Plating was done by taking 1 ml of 10<sup>-7</sup> to 10<sup>-9</sup> dilutions individually in sterilized and solidified King's B medium in Petri plates for *P. fluorescens* counts in broth. Plating was performed in triplicate for each dilution. The plates were incubated upside down at 28±2°C for 3-7 days (24-72 hr). The colonies with specific growth characteristics (smooth, entire circular, convex, opaque, glistening, yellowish green colonies) of *P. fluorescens* were counted. The KB plates were examined for colony morphology and pigmentation daily up to 3 days for bacterial colonies.

**Chart 1: Composition of King's B medium for *P. fluorescens* (King et al. 1954)**

Ingredient	Quantity (g/litre)
Peptone (proteose)	= 20.0 g
Glycerol	= 10.0 ml
K <sub>2</sub> HPO <sub>4</sub>	= 1.5 g
MgSO <sub>4</sub> .7H <sub>2</sub> O	= 1.5 g
Agar-Agar	= 15-18 g
Distilled water	= 1000 ml

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### 3. RESULTS AND DISCUSSION

Results of field experiments revealed that inoculation with selected *P. fluorescens* isolates, containing ACCD activity, under field conditions significantly promoted the growth and yield contributing parameters of maize including cob plant<sup>-1</sup>, cob girth, grain line cob<sup>-1</sup>, grains line<sup>-1</sup>, grains cob and cob length<sup>-1</sup>, yield of maize and rhizobial population. However, the rate of enhancement varied with isolates obtained from component (I). Inoculation of rhizobacterial isolates in the presence of chemical fertilizer significantly increased yield contributing parameters of maize.

#### ***P. fluorescens* population in rhizospheric soil at 35 DAS, 55 DAS and 75 DAS**

The population of *P. fluorescens* in rhizospheric soil at 35 DAS increased from 3.5534 log cfu/g ( $4.00 \times 10^3$  cfu g<sup>-1</sup> soil) to 6.2502 log cfu/g ( $3.74 \times 10^6$  cfu g<sup>-1</sup> soil) with the average value of 5.066 log cfu/g ( $1.01 \times 10^5$  cfu g<sup>-1</sup> soil). Among all the treatment combinations T<sub>28</sub>+pH 7.2 responded maximum by 6.2502 log cfu/g ( $3.74 \times 10^6$  cfu g<sup>-1</sup> soil) 1.69 log fold increase over control (3.7089 log cfu/g =  $5.80 \times 10^3$  cfu g<sup>-1</sup> soil), followed by the response of T<sub>31</sub>+pH 7.2, T<sub>25</sub>+pH 7.2 and T<sub>34</sub>+pH 7.2 by 6.1327 log cfu/g ( $2.85 \times 10^6$  cfu g<sup>-1</sup> soil), 5.9417 log cfu/g ( $2.85 \times 10^5$  cfu g<sup>-1</sup> soil) and 5.7893 log cfu/g ( $1.47 \times 10^5$  cfu g<sup>-1</sup> soil) with the responses of 1.65, 1.60 and 1.56 log fold increase over FUI ( $3.7089$  log cfu/g  $5.80 \times 10^3$  cfu g<sup>-1</sup> soil). The data on *P. fluorescens* population in rhizospheric soil at 55 DAS ranged from 4.8060 log cfu/g ( $6.43 \times 10^4$  cfu g<sup>-1</sup> soil) to 9.3687 log cfu/g ( $3.03 \times 10^9$  cfu g<sup>-1</sup> soil) with the mean value of 7.4617 log cfu/g ( $3.02 \times 10^7$  cfu g<sup>-1</sup> soil). By the close inspection among all the treatment combinations, the response of T<sub>28</sub>+pH 7.2 was exhibited the best by 9.3468 log cfu/g ( $3.03 \times 10^9$  cfu g<sup>-1</sup> soil) 1.59 log fold over the control of FUI ( $5.8673$  log cfu/g =  $7.53 \times 10^5$  cfu g<sup>-1</sup> soil), followed by T<sub>31</sub>+pH 7.2, T<sub>25</sub>+pH 7.2 and T<sub>34</sub>+pH 7.2 by 9.1151 log cfu/g ( $2.06 \times 10^9$  cfu g<sup>-1</sup> soil), 9.0360 log cfu/g ( $2.87 \times 10^9$  cfu g<sup>-1</sup> soil) and 8.8060 log cfu/g ( $1.54 \times 10^8$  cfu g<sup>-1</sup> soil) with the responses of 1.55, 1.54 and 1.50 log fold, respectively over the control of FUI ( $5.8673$  log cfu/g =  $7.53 \times 10^5$  cfu g<sup>-1</sup> soil). The population of *P. fluorescens* in rhizospheric soil at 75 DAS ranged from 4.4270 log cfu/g ( $2.70 \times 10^4$  cfu g<sup>-1</sup> soil) to 8.7431 log cfu/g ( $3.36 \times 10^8$  cfu g<sup>-1</sup> soil) with the mean value of 6.6848 log cfu/g. Among all the treatments, the treatment combination of T<sub>28</sub>+pH 7.2 responded the best by 8.7431 log cfu/g ( $3.36 \times 10^8$  cfu g<sup>-1</sup> soil) with the responses of 1.87 log fold over FUI ( $4.676$  log cfu/g =  $5.55 \times 10^4$  cfu g<sup>-1</sup> soil), followed by T<sub>31</sub>+pH 7.2, T<sub>25</sub>+pH 7.2 and T<sub>34</sub>+pH 7.2 by 8.3962 log cfu/g ( $2.69 \times 10^8$  cfu g<sup>-1</sup> soil), 8.3902 log cfu/g ( $3.79 \times 10^8$  cfu g<sup>-1</sup> soil) 8.4559 log cfu/g ( $3.47 \times 10^8$  cfu g<sup>-1</sup> soil) with the responses of 1.81, 1.80 and 1.79 log fold over FUI ( $4.676$  log cfu/g =  $5.55 \times 10^4$  cfu g<sup>-1</sup> soil).

The results of the present investigation have shown that increase in microbial population in the rhizosphere soil was possibly due to the improvement in the more availability of nutrients (especially phosphorus) to the microbes. Shinde and Bangar 2003; Sushila and Giri (2000) reported an increase in microbial population in rhizosphere of wheat with *Azospirillum* and *Azotobacter* inoculations. The result was in harmony with that found by Chand *et al.*, (2010); Jarak *et al.*, (2012) and Singh *et al.*, (2013). PGPR have high rate of oxidation and thus its proliferation depends on the

availability of organic carbon to a greater extent and increased population could be attributed to increase of mineral stable carbon as reported by Biswas and Mukherjee (1996); Sardana (1997) and Subba Rao, 2000). Similar results were obtained by Cvijanovic *et al.*, (2007) who found an increase of microbial number and maize yield in response to *Azotobacter chroococcum*, *Azotobacter vinelandii*, *Azospirillum lipoferum*, *Bacillus megatherium*, *Bacillus subtilis* and *Klebsiella planticola* application. Some previous studies suggested that the presence of microorganisms in the maize rhizosphere varied and that total number of microorganisms and number of *Azotobacter* increased in the course of the growing season (Bjelic *et al.*, 2010).

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### Yield Attributes

The Table 2 depicts the data on number of cob, cob girth, cob length, grain lines  $\text{cob}^{-1}$ , number of grain line $^{-1}$  and number of grain  $\text{cob}^{-1}$  inoculated with *P. fluorescens* isolates screened out from different fermentation conditions of temperature and pH.

### Cob plant $^{-1}$

The number of cob plant $^{-1}$  ranged from 1.8 to 2.7 cob plant $^{-1}$  with the mean value of 2.3 cob plant $^{-1}$ . The maximum number of cob plant $^{-1}$  was 2.7 cob plant $^{-1}$  gained with the T<sub>28</sub>+pH 7.2 by 30.6% response and it was found statistically superior over the control FUI (2.1 cob plant $^{-1}$ ), followed by T<sub>31</sub>+pH 7.2, T<sub>25</sub>+pH 7.2, T<sub>34</sub>+pH 7.2, T<sub>28</sub>+pH 7.7, T<sub>31</sub>+pH 7.7 and T<sub>25</sub>+pH 7.7 with cob plant $^{-1}$  of 2.6, 2.5, 2.5, 2.4, 2.4 and 2.4 along with 25.8, 23.2, 22.6, 17.7, 16.9 and 14.8% response, respectively.

### Girth of cob

Similarly, the girth of cob varies from 12.2 to 16.8 cm  $\text{cob}^{-1}$  with the average of 14.8 cm  $\text{cob}^{-1}$ . Among all the treatments, the treatment combination of T<sub>31</sub>+pH 7.2 increased the girth of cob by 16.8 cm  $\text{cob}^{-1}$  with 26.0% response over FUI (13.3 cm  $\text{cob}^{-1}$ ), followed by the T<sub>28</sub>+pH 7.2, T<sub>25</sub>+pH 7.2, T<sub>34</sub>+pH 7.2, T<sub>28</sub>+pH 7.7, T<sub>31</sub>+pH 7.7, T<sub>25</sub>+pH 7.7, T<sub>28</sub>+pH 6.7 and T<sub>31</sub>+pH 6.7 with girth of cob 16.5, 16.4, 16.2, 15.9, 15.8, 15.4, 15.2 and 15.0 cm by 24.1, 23.2, 21.7, 19.3, 18.7, 15.5, 14.1 and 12.2% increment over FUI (13.3 cm  $\text{cob}^{-1}$ ).

### Cob length

A close inspection on the data of cob length of maize varied from 14.8 to 20.1 cm  $\text{cob}^{-1}$  having a mean value of 17.6 cm  $\text{cob}^{-1}$ . The data revealed that the cob length of maize 20.1 cm  $\text{cob}^{-1}$  recorded by the application of T<sub>28</sub>+pH 7.2 with an increment of 32.1% over the control FUI (15.2 cm  $\text{cob}^{-1}$ ), followed by T<sub>31</sub>+pH 7.2, T<sub>25</sub>+pH 7.2, T<sub>34</sub>+pH 7.2, T<sub>28</sub>+pH 7.7, T<sub>31</sub>+pH 7.7, T<sub>25</sub>+pH 7.7, T<sub>28</sub>+pH 6.7 and T<sub>31</sub>+pH 6.7 with cob length 19.9, 19.9, 19.4, 19.3, 18.7, 18.3 and 18.2 cm  $\text{cob}^{-1}$  by 30.8, 30.4, 27.1, 26.7, 22.9, 20.3 and 19.7% response, respectively. While the lowest value of cob length 14.8 cm  $\text{cob}^{-1}$  was recorded to UFUI.

### Grain line cob<sup>-1</sup>

The grain line cob<sup>-1</sup> ranged from 13.0 to 18.3 line cob<sup>-1</sup> with the mean value of 16.0 line cob<sup>-1</sup>. The maximum grain lines cob<sup>-1</sup> was 18.3 gained with the T<sub>31</sub>+pH 7.2 by 31.3% response and it was found statistically superior over the control FUI (13.9 line cob<sup>-1</sup>) followed by T<sub>28</sub>+pH 7.2, T<sub>25</sub>+pH 7.2, T<sub>34</sub>+pH 7.2, T<sub>28</sub>+pH 7.7, T<sub>31</sub>+pH 7.7, T<sub>25</sub>+pH 7.7 and T<sub>28</sub>+pH 6.7 with 18.2, 18.0, 17.7, 17.6, 17.3, 16.9 and 16.6 line cob<sup>-1</sup> of 30.6, 28.7, 27.1, 26.0, 23.8, 21.1 and 19.1% response, respectively.

### Number of grains per line

Similarly, the number of grains per line varies from 30.6 to 45.4 grains per line with the average of 39.2 grains. Among all the treatments, the treatment combination of T<sub>28</sub>+pH 7.2 increased the number of grains per lines by 45.4 grain with 29.7% response over FUI (35.0), followed by T<sub>31</sub>+pH 7.2, T<sub>25</sub>+pH 7.2, T<sub>34</sub>+pH 7.2, T<sub>28</sub>+pH 7.7, T<sub>31</sub>+pH 7.7, T<sub>25</sub>+pH 7.7, T<sub>28</sub>+pH 6.7, T<sub>31</sub>+pH 6.7 and T<sub>34</sub>+pH 6.7 with the number of grains per line 44.1, 43.9, 43.2, 43.2, 41.7, 41.1, 40.7, 39.8 and 38.5 by 26.0, 25.2, 23.4, 23.3, 19.1, 17.4, 16.2, 13.6 and 9.8% increment over FUI (35.0 grains line<sup>-1</sup>).

### Grains cob<sup>-1</sup>

A close inspection on the data of grains cob<sup>-1</sup> of maize varied from 483 to 693 grains having a mean value of 604 grains cob<sup>-1</sup>. The data revealed that the grains cob<sup>-1</sup> of maize 693 grain recorded by the application of T<sub>28</sub>+pH 7.2 with an increment of 37.7% over the control FUI (503 grains cob<sup>-1</sup>) followed by T<sub>31</sub>+pH 7.2, T<sub>25</sub>+pH 7.2, T<sub>34</sub>+pH 7.2, T<sub>28</sub>+pH 7.7, T<sub>31</sub>+pH 7.7, T<sub>25</sub>+pH 7.7, T<sub>28</sub>+pH 6.7, T<sub>31</sub>+pH 6.7, T<sub>34</sub>+pH 6.7, T<sub>25</sub>+pH 6.7 and T<sub>28</sub>+pH 8.2 with grains cob<sup>-1</sup> 681, 673, 665, 658, 645, 643, 637, 625, 618, 600 and 582 by 35.3, 33.8, 32.1, 30.7, 28.1, 27.8, 26.8, 24.1, 22.8, 19.2 and 15.7% response, respectively.

In addition to ACC-deaminase potential, these selected isolates also exhibited varied potential of multifarious properties including auxin production, siderophore production, phosphorous solubilization, chitinase activity and root colonization. A similar result was also observed by (Akhtar *et al.*, 2018) results showed that inoculation with selected strains of bacteria, exhibited percent increase in yield of fresh cob (up to 52.69%) and dry cob (40.87%), cob length (51.42%), grain yield (up to 55.34%), 1000-grain weight (up to 37.27%), K contents in grains and straw (1.756 and 0.793, respectively), % N in grains and straw (up to 2.675 and 0.997%, respectively) and %P in grains and straw (up to 1.756 and 0.793%, respectively) compared to un-inoculated control. Baral and Adhikari (2013) showed that the effect of *Azotobacter* on growth and yield of maize. The treatments were control, 120:60:40kg N P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O (RDF) ha<sup>-1</sup>, *Azotobacter* seed inoculation, *Azotobacter* soil application, *Azotobacter* + 10 t FYM ha<sup>-1</sup>, 10 t FYM ha<sup>-1</sup>, RDF + *Azotobacter*, RDF+ *Azotobacter* + 10 t FYM ha<sup>-1</sup>. Results showed that showed that grain yield, plant height, ear height, ear length, kernel per rows and 1000 grain weight were significantly affected with treatments. Only inoculation of *Azotobacter* increased 15 to 35% grain yield stover non inoculated treatments. Javed *et al.*, (1998) and Jakhar *et al.*, (2021) reported the

*Pseudomonas spp.* that significantly increased the grain yield by 18.9%, while cob weight, cob length, 1000-grain and straw weight was enhanced up to 20.8%, 11.6%, 17.2% and 27.1 %, respectively over the control.

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### Grain and Stover yield

The data related to the grain and stover yield of the maize crop is given in Table 3. The grain yield of maize ranged from 2440 to 3641 kg ha<sup>-1</sup> with the average value of 3189 kg ha<sup>-1</sup>. Among all the treatments, the treatment combination of T<sub>26</sub>+pH 7.2 yielded significantly maximum grain yield of 3641 kg ha<sup>-1</sup> that was responded 24.9% over FUI (2914 kg ha<sup>-1</sup>). The stover yield of maize increased from 5874 to 9387 kg ha<sup>-1</sup> with the mean value of 7934 kg ha<sup>-1</sup>. The highest stover yield of maize 9387 kg ha<sup>-1</sup> was recorded with the treatment combination of T<sub>26</sub>+pH 7.2 by 31.6% responses over the control FUI (7133 kg ha<sup>-1</sup>). Improvements in growth and yield following application of rhizobacterial isolates under fertilized conditions could primarily be attributed to their ACC-deaminase trait which might have reduced endogenous biosynthesis of C<sub>2</sub>H<sub>4</sub> in developing roots of seedlings, and hence resulted in formation of healthy and longer roots for efficient nutrient uptake. Indigenous C<sub>2</sub>H<sub>4</sub> biosynthesis is accelerated during seed germination which has been known for inhibitory effects on seed germination and root growth. In addition to the positive attributes of (ACC-deaminase containing PGPR) application enhanced nutrient uptake, growth and yield of maize is most likely due to promotion of root growth by the decreased ethylene levels attributed to ACC-deaminase activity (Shaharoona *et al.*, 2006). The rhizobacteria-containing ACC-deaminase are well known for improving root growth of plants as a consequence of decreased ethylene synthesis through ACC hydrolysis into NH<sub>3</sub> and  $\alpha$ -ketobutyrate in the inoculated roots (Jakhar *et al.* 2018a; Jakhar *et al.* 2018b; Jakhar *et al.* 2018; Glick, 1995; Shaharoona *et al.*, 2007). Very recently, we have reported that inoculation with PGPR containing ACC-deaminase significantly increased wheat and maize yield, under field conditions (Shaharoona *et al.*, 2006b, 2007).

**Comment [GB18]:** Don't use prehistoric references

## 5. REFERENCES

- Akhtar, Naveed N, Khalid M, Ahmad M, Rizwan, Siddique B and Saima. 2018. Effect of bacterial consortia on growth and yield of maize grown in *Fusarium* infested soil. Soil and Environment 37(1): 35-44.
- Anonymous. 2016. Agricultural statistics Division Directorate of Economics & Statistics Department of Agriculture & Cooperation, Govt of India.
- Baral BR and Adhikari P. 2013. Effect of *Azotobacter* on growth and yield of maize. SAARC Journal of Agriculture 11(2): 141-147.
- Biswas TD and Mukherjee SK. 1996. Text Book of Soil science. Publ. TataMc Graw-Hill Publishing Company Ltd. New Dehli.
- Bjelic D, Mrkovacki N, Jarak M, Josic, D and Dalovic, I. 2010. Effect of PGPR on the early growth of maize and microbial abundance in rhizosphere. *Contemporary Agriculture*, 59(3-4): 339-345.

- Chand S, Somani LL and Bhandari SC. 2010. Effect of fertilizer, farmyard manure (FYM) and bio fertilizer on the population of *Azotobacter* and phosphate solubilizing bacteria (PSB) in the soil. *Journal of the Indian Society of Soil Science* 58(4): 460-463.
- Cvijanovic G, Milosevic N, Djalovic I, Cvijovic M and Paunovic A. 2007. Nitrogenization and n fertilization effects on protein contents in wheat grain. *Cereal Research Communications*, 36:251-254
- Glick BR, Karaturovic DM and Newell PC. 1995. A novel procedure for rapid isolation of plant growth-promoting pseudomonads. *Canadian Journal of Microbiology* 41:533-536.
- Glick BR, Penrose DM and Li J. 1998. A model for the lowering of plant ethylene concentrations by plant growth promoting bacteria. *Journal of the Oretical Biology* 190:63-68.
- Grichko VP and Glick BR. 2001. Amelioration of flooding stress by ACC deaminase-containing plant growth-promoting bacteria. *Plant Physiology and Biochemistry* 39:11-17.
- Honma M and Shimomura T. 1978. Metabolism of 1-aminocyclopropane-1-carboxylic acid. *Agricultural and Biological Chemistry* 42:1825-1831.
- Honma, M. 1985. Chemically reactive sulfhydryl groups of 1-aminocyclopropane-1-carboxylate deaminase. *Agricultural and Biological Chemistry* 49:567-571.
- Jacobson CB, Pasternak JJ and Glick BR. 1994. Partial purification and characterization of ACC deaminase from the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2. *Canadian Journal of Microbiology* 40:1019-1025.
- Jakhar SR, Kumar V, Mitra NG and Singh O. 2018b. Effect of Soybean (*Glycine max*) Seed inoculation with liquid and carrier based *Rhizobium* cultures and phosphorus levels on productivity and physico-chemical properties of soil. *International Journal of Current Microbiology and Applied Sciences* 7(6): 1807-1814.
- Jakhar SR, Kumar V, Mitra NG and Singh O. 2018. Effect of Soybean (*Glycine max*) seed inoculation with liquid and carrier based rhizobium cultures and phosphorus levels on productivity and physico-chemical properties of soil. *International Journal of Current Microbiology and Applied Sciences* 7(6): 1807-1814.
- Jakhar SR, Mitra NG, Chalieu R, Sahu RK, Baghel SS, Kumar V, Chaudhary B, Kumhar BL, Bangre J. 2021. Performance of 1-Aminocyclopropane-1-Carboxylic Acid Deaminase Containing *Pseudomonas fluorescens* for Enhancing Growth and Yield of Maize (*Zea mays* L.). *AMA, Agricultural Mechanization in Asia, Africa and Latin America* 51(03): 1545-1556.
- Jakhar SR, Tiwari R, Chaudhary BK and Kumhar BL. 2018a. PGPR: Heart of soil and their role in sustainable agriculture. *Rashtriya krishi* 13(1): 150-107.
- Jarak M, Mrkovacki N, Bjelic D, Josic D, Hajnal-Jafari T and Stamenov D. 2012. Effects of plant growth promoting rhizobacteria on maize in greenhouse and field trial. *African Journal of Microbiology Research* 6(27): 5683-5690.
- Javed M, Arshad M, and Ali K. 1998. Evaluation of rhizobacteria for their growth promoting activity in maize. *Pakistan Journal of Soil Science* 14:36-42.
- King EO, Ward MK and Raney DE. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. *Journal of Laboratory and Clinical and Medicine* 44: 301-307.
- Meyer JM and Abdallah MA. 1978. The fluorescent pigment of *Pseudomonas fluorescens*: Biosynthesis, purification and physicochemical properties. *Journal of General Microbiology* 107:319-328.
- Palleroni NJ. 1984. "Pseudomonadaceae". In *Bergey's manual of systematic biology*, Edited by: Kreig, NR and Holt, JG. 141-199. Baltimore: Williams and Wilkins Co.

- Penrose DM, Moffatt BA and Glick BR. 2001. Determination of 1-aminocyclopropane-1-carboxylic acid (ACC) to assess the effects of ACC-deaminase containing bacteria on roots of canola seedlings. *Canadian Journal of Microbiology* 47:77-80.
- Sardana V. 1997. Agronomic evaluation of biofertilizers to supplement inorganic fertilizers for sustained crop production-A review. *Agricultural Review* 18(2): 69-93.
- Shaharoona B, Arshad M and Zahir ZA. 2007. Effect of plant growth-promoting rhizobacteria containing ACC-deaminase on maize (*Zea mays* L.) growth under axenic conditions and on nodulation in mung bean (*Vigna radiata* L.). *Letters in Applied Microbiology* 42:155-159.
- Shaharoona B, Arshad M, Zahir ZA and Khalid A. 2006. Performance of *Pseudomonas* spp. containing ACC-deaminase for improving growth and yield of maize (*Zea mays* L.) in the presence of nitrogenous fertilizer. *Soil Biology and Biochemistry* 38: 2971-2975.
- Shaharoona B, Riffat B, Muhammad A, Zahir AZ and Hassan ZU. 2006b. 1-aminocyclopropane-1-carboxylate (ACC) deaminase rhizobacteria extenuates acc-induced classical triple response in etiolated pea seedlings. *Pakistan Journal of Botany* 38(5): 1491-1499.
- Shinde DB and Bangar ND. 2003. Studies on dual inoculation of nitrogenous and phosphatic bacterial cultures in sugarcane. *Journal of Maharashtra Agricultural University* 28:190-192.
- Singh R. 2012. Need to optimize resources use efficiency for sustainable agriculture. *Journal of Indian Society of Soil Science* 60:100-108.
- Singh NK, Chaudhary FK and Patel DB. 2013. Effectiveness of *Azotobacter* bio-inoculant for wheat grown under dryland condition. *Journal of Environmental Biology* 34:927-932.
- Singh RP, Kumar R and Singh NP. 2003. Transformation of the Indian maize economy - Different perspectives. In maize production in india — golden grain in transition TB-ICN 4:1-28.
- Subba Rao. 2000. *Soil Microbiology*. Publ. Oxford and IBH Co Pvt Ltd New Delhi. p 407.
- Suntharalingam P and Cvitkovitch DG. 2005. Quorum sensing in streptococcal biofilm formation. *Trends in Microbiology* 13:3-6.
- Surette MG, Miller MB, and Bassler BL. 1999. Quorum sensing in *Escherichia coli*, *Salmonella typhimurium*, and *Vibrio harveyi*: a new family of genes responsible for autoinducer production. *Proceedings of the National Academy of Sciences USA* 96:1639-1644.
- Sushila R and Giri G. 2000. Influence of farmyard manure, nitrogen and biofertilizers on growth, yield attributes and yield of wheat (*Triticum aestivum*) under limited water supply. *Indian Journal of Agronomy* 45(3): 590-595.

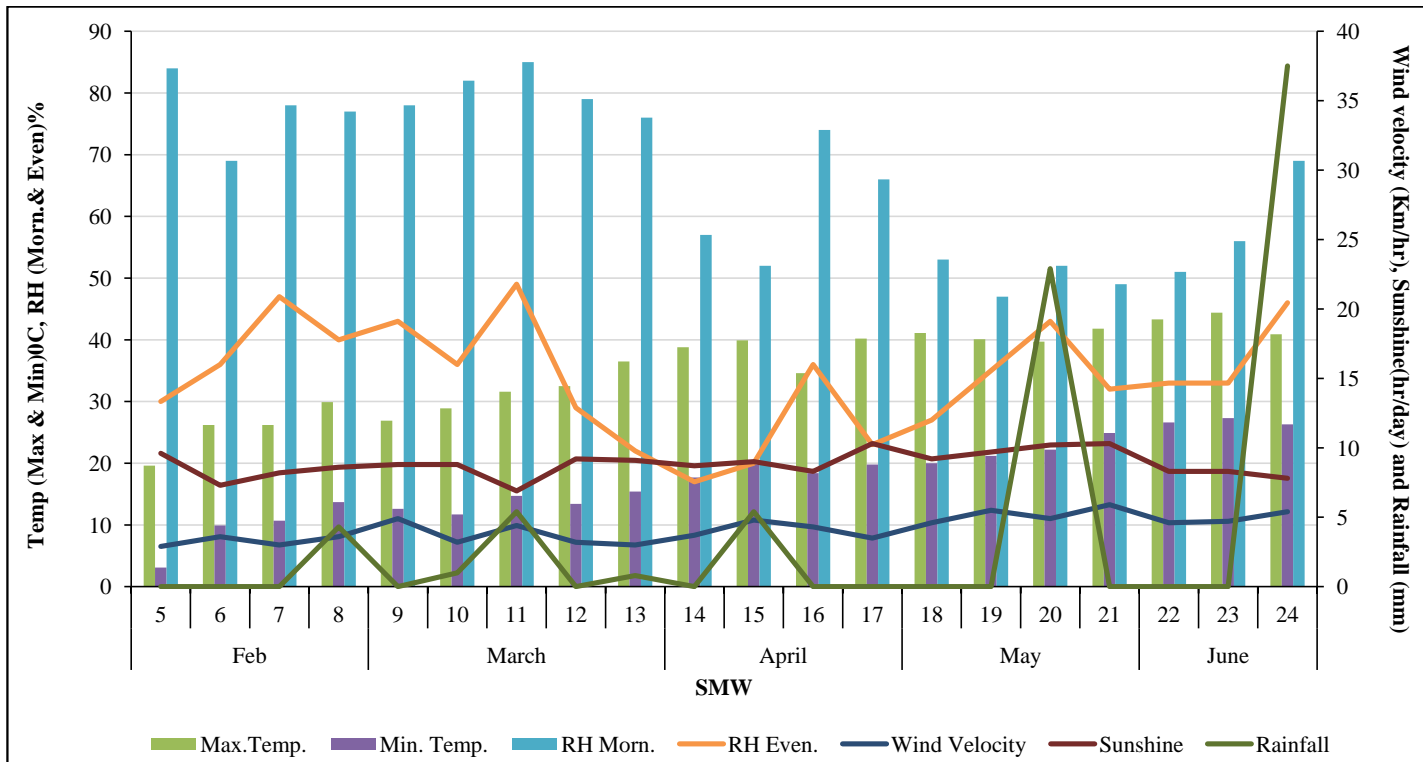


Fig. 1: Weekly meteorological data during Feb - Jun, 2018-19

**Table 1.** Effect of *P. fluorescens* isolates on rhizospheric population of maize at different growth stages

Treatment combination	<i>P. fluorescens</i> population (cfu g <sup>-1</sup> soil)		
	35 DAS	55 DAS	75 DAS
T <sub>25</sub> +pH 6.7	5.0162 (2.82x10 <sup>5</sup> )	7.1177 (3.55x10 <sup>7</sup> )	6.3292 (2.97x10 <sup>6</sup> )
T <sub>25</sub> +pH 7.2	5.9417 (2.85x10 <sup>5</sup> )	9.0360 (2.87x10 <sup>8</sup> )	8.3902 (3.79x10 <sup>8</sup> )
T <sub>25</sub> +pH 7.7	5.5157 (8.85x10 <sup>5</sup> )	8.0306 (1.26x10 <sup>8</sup> )	7.7947 (5.56x10 <sup>7</sup> )
T <sub>25</sub> +pH 8.2	4.2077 (2.19x10 <sup>4</sup> )	6.3751 (2.52x10 <sup>6</sup> )	5.7262 (5.45x10 <sup>5</sup> )
T <sub>28</sub> +pH 6.7	5.4473 (4.59x10 <sup>5</sup> )	7.9531 (3.21x10 <sup>7</sup> )	6.5486 (3.47x10 <sup>6</sup> )
T <sub>28</sub> +pH 7.2	6.2502 (3.74x10 <sup>6</sup> )	9.3468 (3.03x10 <sup>8</sup> )	8.7431 (3.36x10 <sup>8</sup> )
T <sub>28</sub> +pH 7.7	5.7103 (3.50x10 <sup>5</sup> )	8.3886 (4.61x10 <sup>8</sup> )	7.6702 (1.13x10 <sup>7</sup> )
T <sub>28</sub> +pH 8.2	4.9043 (2.16x10 <sup>4</sup> )	6.7271 (1.13x10 <sup>6</sup> )	6.1408 (2.58x10 <sup>6</sup> )
T <sub>31</sub> +pH 6.7	5.3373 (3.92x10 <sup>5</sup> )	7.8320 (2.57x10 <sup>7</sup> )	6.2086 (2.52x10 <sup>6</sup> )
T <sub>31</sub> +pH 7.2	6.1327 (2.85x10 <sup>6</sup> )	9.1151 (2.06x10 <sup>9</sup> )	8.3962 (2.69x10 <sup>8</sup> )
T <sub>31</sub> +pH 7.7	5.6207 (6.05x10 <sup>5</sup> )	8.2027 (2.26x10 <sup>8</sup> )	7.6062 (5.52x10 <sup>7</sup> )
T <sub>31</sub> +pH 8.2	4.6618 (2.42x10 <sup>4</sup> )	6.5447 (2.93x10 <sup>6</sup> )	5.7141 (1.08x10 <sup>5</sup> )
T <sub>34</sub> +pH 6.7	5.2136 (2.09x10 <sup>5</sup> )	7.5609 (5.32x10 <sup>7</sup> )	6.4896 (3.30x10 <sup>6</sup> )
T <sub>34</sub> +pH 7.2	5.7893 (1.47x10 <sup>5</sup> )	8.8057 (1.54x10 <sup>8</sup> )	8.4559 (3.47x10 <sup>8</sup> )
T <sub>34</sub> +pH 7.7	4.1156 (1.94x10 <sup>4</sup> )	6.3535 (4.09x10 <sup>6</sup> )	5.6477 (4.35x10 <sup>5</sup> )
T <sub>34</sub> +pH 8.2	4.0686 (2.25x10 <sup>4</sup> )	6.2471 (2.16x10 <sup>6</sup> )	5.3599 (2.71x10 <sup>4</sup> )
FUI (Control)	3.7089 (5.80x10 <sup>3</sup> )	5.8673 (7.53x10 <sup>5</sup> )	4.6763 (5.55x10 <sup>4</sup> )
UFUI	3.5534 (4.00x10 <sup>3</sup> )	4.8060 (6.43x10 <sup>4</sup> )	4.4270 (2.70x10 <sup>4</sup> )
SE <sub>m</sub> ±	0.4	0.6	0.2
CD <sub>5%</sub>	1.3	1.7	0.7

**Table 2.** Effect of *P. fluorescens* isolates on cob filling of maize

Treatment combination	Cob plant <sup>-1</sup>	Cob girth (cm)	Cob length (cm)	Grain lines cob <sup>-1</sup>	Number of grain line <sup>-1</sup>	Number of grain cob <sup>-1</sup>
T <sub>25</sub> +pH 6.7	2.3	14.2	17.1	15.5	38.2	600
T <sub>25</sub> +pH 7.2	2.5	16.4	19.9	18.0	43.9	674
T <sub>25</sub> +pH 7.7	2.4	15.4	18.3	16.9	41.1	643
T <sub>25</sub> +pH 8.2	2.1	14.5	16.3	14.9	35.8	547
T <sub>28</sub> +pH 6.7	2.3	15.2	18.2	16.6	40.7	637
T <sub>28</sub> +pH 7.2	2.7	16.5	20.1	18.2	45.4	693
T <sub>28</sub> +pH 7.7	2.4	15.9	19.3	17.6	43.2	658
T <sub>28</sub> +pH 8.2	2.2	14.0	16.7	15.2	37.7	583
T <sub>31</sub> +pH 6.7	2.3	15.0	17.8	15.8	39.8	625
T <sub>31</sub> +pH 7.2	2.6	16.8	19.9	18.3	44.1	681
T <sub>31</sub> +pH 7.7	2.4	15.8	18.7	17.3	41.7	645
T <sub>31</sub> +pH 8.2	2.1	13.7	16.6	15.1	36.2	573
T <sub>34</sub> +pH 6.7	2.3	14.5	17.2	15.7	38.5	618
T <sub>34</sub> +pH 7.2	2.5	16.2	19.4	17.7	43.2	665
T <sub>34</sub> +pH 7.7	2.1	13.5	16.0	14.4	35.2	537
T <sub>34</sub> +pH 8.2	2.1	13.4	15.8	14.2	35.2	514
FUI (Control)	2.1	13.3	15.2	13.9	35.0	503
UFUI	1.8	12.2	14.8	13.0	30.6	483
SE <sub>m</sub> ±	0.07	0.5	1.0	0.8	1.1	24.8
CD <sub>5%</sub>	0.2	1.4	2.8	2.4	3.3	73.2

**Table 3.** Effect of *P. fluorescens* isolates on grain and stover yield of maize

Treatment combination	Yield (kg ha <sup>-1</sup> )	
	Grain	Stover
T <sub>25</sub> +pH 6.7	3033	7502
T <sub>25</sub> +pH 7.2	3552	9205
T <sub>25</sub> +pH 7.7	3353	8257
T <sub>25</sub> +pH 8.2	2950	7247
T <sub>28</sub> +pH 6.7	3319	8037
T <sub>28</sub> +pH 7.2	3641	9387
T <sub>28</sub> +pH 7.7	3452	8900
T <sub>28</sub> +pH 8.2	2999	7485
T <sub>31</sub> +pH 6.7	3235	7855
T <sub>31</sub> +pH 7.2	3590	9248
T <sub>31</sub> +pH 7.7	3427	8305
T <sub>31</sub> +pH 8.2	2977	7357
T <sub>34</sub> +pH 6.7	3182	7756
T <sub>34</sub> +pH 7.2	3478	8964
T <sub>34</sub> +pH 7.7	2918	7150
T <sub>34</sub> +pH 8.2	2935	7140
FUI (Control)	2914	7133
UFUI	2440	5874
SE <sub>m</sub> ±	142.5	233.9
CD <sub>5%</sub>	420.0	689.3