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Effects of Gibberellic Acid and Indole Acetic Acid producing *Azotobacter spp.* on the growth of *Cicer arietinum* L. (Variety - Phule G 0517)

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ABSTRACT

The modern agriculture is exclusively based on externally applied agrochemicals that make the soil fertility vulnerable. The external application of soil-borne bacteria is the upcoming sustainable system which will maintain the soil fertility and simultaneously enhances the plant growth. The total of 15 bacterial isolates belonging to *Azotobacter spp.* were isolated from different rhizospheric soils collected from different agricultural fields of Pune district. All the isolates were screened for their plant growth promoting traits, i.e. Gibberellic Acid (GA) and Indole Acetic Acid (IAA) production and were characterized biochemically. Out of these 15 isolates 3 (Azo1, Azo2 and Azo3) isolates showed highest GA (0.10, 0.15 and 0.30 mg/ml) and IAA (0.29, 0.25 and 0.15 mg/ml) production efficiency. These three promising isolates showed positive effects on growth and health of chickpea (*Cicer arietinum L*).

Key words: *Azotobacter spp.,Cicer arietinum L., Gibberellic acid, Indole acetic acid*

Introduction

Cicer arietinum L., commonly well known as chickpeas, is one of the most significant legume crops in all over the world and represents an important source of protein for humans and food for domestic animals in moderate and semiarid climates (Siddiqui *et al*., 2001). *Azotobacter spp.*is non-symbiotic and usually found as gram negative rods with very high respiration rates. It is an obligate aerobe, although it can grow under low oxygen potential. The distribution of *Azotobacter* is very complex and variety of factors determines the presence of this bacterium in the rhizosphere region (Dobereiner *et al*., 1987). The plant growth promoters can exhibit a variety of characteristics responsible for improving growth of plant. The widespread traits comprise production of plant growth regulators (auxins, cytokinins, gibberellins, ethylene etc.), siderophore, HCN and antibiotics (Bolton *et al*., 1992).

Indole Acetic Acid (IAA) is one of the most physiologically active plant hormone. *Azotobacter* secretes IAA into culture media and considerably enhances the growth of plant (Barea *et al*., 1974). The IAA producing *Azotobacter* isolates have been isolated from mungbean rhizosphere screened for their ability to produce IAA (Kumar *et al*., 2014). Phytohormones (IAA, GA) are plant growth regulators, which have

stimulatory effects on plant growth (Megala *et al*., 2013). Gibberellic acid production was confirmed from *B. pumilus, B. licheniformis and B. siamensis* as well (Gutierrez-Manero *et al*., 2001 and Ambawade *et al*., 2015).

The rhizobia present in the soil create a symbiotic relationship with the leguminous plants, chickpea developing root nodules and thus support biological nitrogen fixation (Ahamad *et al*., 2019). Azo-biofertilizers stimulate the growth of plant and application of such biofertilizers during cultivation of plants results in superior resistance towards diseases along with higher synthesis of phytohormones and water soluble vitamins and enhance the plant growth and yield (Behl *et al*., 2003).

Materials and Methods

Isolation of *Azotobacter* strains from rhizosphere soil

The 5 g of garden soil sample was inoculated in each of the three Erlenmeyer flasks containing 100 ml of sterile Ashby's Mannitol Broth separately and flasks were kept in steady condition at room temperature for 24-96h for incubation. A loopfulof surface pellicle from enriched broth streaked (four quadrate method) on Ashby's Mannitol agar plates. The plates were incubated at room temperature for 48-72 h. Bacterial colonies with mucoid growth that turned brown upon aging were isolated and purified on the respective medium and identified as *Azotobacter spp.* by cultural, morphological and biochemical tests with reference to Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

Bioassay of GA and IAA

Quantification of GA and IAA were carried out by using potassium ferricynide and zinc acetate method (Borrow *et al*., 1955) and Salkowski method (Ehmann, 1977), respectively.

Preparation of Biofertilizers

The oven dried 100 mesh black cotton soil was used as a carrier material for the preparation of biofertilizer. Three different promising isolates of *Azotobacter* spp. were selected. A loopful of suspension from each selected species inoculated in 100 ml Ashby's Mannitol Broth. The flasks were kept on rotary shaker at 130 rpm for 24 to 96 h at 28 ^{0}C. After incubation the cell density was adjusted to 10^{10} CFU/ml with the help of haemocytometer. The broths were centrifuged at 10000 rpm for 10 min. Supernatants were discarded and cell mass were washed with sterile distilled water three times.

Shelf-life study on Azo-biofertilizers

Bacterial density of each of cell mass was adjusted to 10^{12} CFU/ml as above and bacterial suspensions (5 mL each) were separately inoculated in 150 g each, oven dried 100 mesh black cotton soil samples and stored at 4 ^{0}C and 28 ^{0}C in small containers. Monthly celldensity of biofertilizers was checked as a part of shelf life study for a year. The optimal shelf lives for these three biofertilizers were 7 months for 4 ^{0}C storage to 3 months for 28 ^{0}C storage with maintenance of 1.0 X 10^{10} CFU/g of biofertilizer.

Study the effects of biofertilizers on the plant growth

To study the effects of biofertilizers on the plant growth, the pot assay was performed. The four pots viz. control, *Azo1, Azo2, Azo3* were labelled and in each of the pot about 1 Kg of black cotton soil (peat soil) with 25 seeds of Chickpea (*Cicer arietinum L)* and 50 g of prepared biofertilizer were added except in control and daily 200 ml water was added in each pot. After 15 days, the plant growth was measured in terms of average shoot length, root length, plant height dry weight and dry weight per sapling.

Statistical analysis

All the experiments were replicated in triplicates and Mean ± SD was considered for further calculations and inferences. Statistical analysis was done by using Statistical Package for Social Sciences (IBM SPSS statistics Version 25) and Microsoft Excel.

Results and Discussion

Isolation *Azotobacter spp*. from rhizospheric soil

Based on cultural, morphological and biochemical characteristics, a total of 15 bacterial isolates were characterized and identified as *Azotobacter* spp. The *Azotobacter* spp. from rhizosphere of various crops were isolated and extensively studied previously (Joseph *et al*., 2012).

Bio-assay of GA and IAA

A total of 15 *Azotobacter* spp. were selected and tested for quantitative estimation of GA and IAA.

The production of GA and IAA recorded in all isolates of *Azotobacter* strains were in the range of 0.10 – 0.29 mg/ml and 0.10 – 0.30 mg/ml, respectively. Out of these 15 isolates three (Azo1, Azo2 and Azo3) isolates showed highest GA (0.10, 0.15 and 0.30 mg/ml, respectively) as well as IAA (0.29, 0.25 and 0.15 mg/mL, respectively) production efficiency (Fig. 1). The IAA synthesis in isolates was stimulated in the presence of precursor like L-tryptophan. After the addition of 0,1% amount of L-tryptophan into bacterial culture medium, there was an increase in the biosynthesis of IAA up to 2.7 times. The L-tryptophan is an amino acid with an indole group that can work as a physiological precursor of IAA in plants as well as microorganisms, because it contains active substances that can initiate the microbial growth. In soil microorganisms, IAA biosynthesis can be triggered by the presence of L-tryptophan derived from plant root exudates (Dewi *et al*., 2016). The findings of present investigation are better to earlier reports (Dewi *et al*., 2016 and Wahyudi *et al*., 2011). The highest GA and IAA producing *Azotobacter* isolates viz. *Azo1, Azo2 and Azo3* were selected for the preparation of biofertilizers and further experimentation.

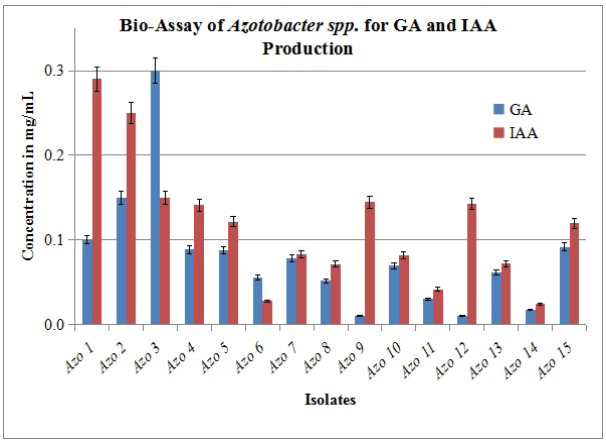

Fig. 1. Bio-Assay for GA and IAA Production

Study the effects of biofertilizer on the plant growth

The problem of chemical fertilizers is a global and researchers are working all over the world to find a solution to this problem as it is in the last century, when the chemical fertilizers were first introduced into the agriculture field and most of the problems faced by farmers to increase yield of their plantation have been solved. However, chemical fertilizers slowly started to show their side effects on human and environment (Zakaria, 2009). Biofertilizer will be the best solution to replace chemical fertilizers. Biofertilizer are the carrier-based preparations containing mainly effective strains of microorganisms in sufficient number, which are useful for nitrogen fixation also. Biofertilizers have several advantages over chemical fertilizers, they are non pollutant, inexpensive, and utilize renewable resources (Rafique *et al*., 2021).

That all the pots with added Azo-biofertilizer showed enhanced plant growth as compared to control pot. In the control pot the average plant height, average shoot length and average root length were 7.0cm, 1.8 cm and 5.5 cm, respectively while in case of Azo-1,2 and 3 pots they ranged from average plant height of 22.4-27 cm, average shoot length of 7.6-8.9 and average root length of 14.8-17.5 cm, respectively and statistically on an average about 3.49 times, 4.6 times and 2.87 times, respectively the growth promotion was found as compared to control set, while average dry weight of sapling of control set was 0.903 and that of three test pots was 2.67 g indicating 2.96 times rise in the weight in the test pots as compared to control and if all the parameters taken together then more than 4 times plant growth promotion was observed when seeds were exposed to Azo-biofertlizers prepared as compared to control (seeds without biofertilizer exposure).

It is evident from Table 1, Figure 1 and that all the pots with added Azo-biofertilizer showed enhanced plant growth as compared to control pot. In the con-

Table 1. Effects of Azo-biofertilizers on Plant Growth (chickpea)

Pots with black cotton soil	Average Dry weight / sapling(g)	Average height/ sapling (cm)	Average Shoot length/ sapling (cm)	Average root length/ sapling (cm)
Control	0.903	07.0	1.8	05.5
With Azo 1	1.841	23.9	8.9	15.0
WithAzo 2	3.886	27.0	8.5	17.5
With Azo 3	2.290	22.4	7.6	14.8

Table 2. Shelf-life studies on the Azo-biofertilizers

Sr. No.	Storage period (in months)	Azo-1		Azo-2		Azo-3		Remark
		Storage temperature						
		4 °C	28 °C	4 °C	28 °C	4 °C	28 °C	
1	0	1×10^{12}	1×10^{12}	1×10^{12}	1×10^{12}	1×10^{12}	1×10^{12}	-
2	1	0.9×10^{12}	1×10^{11}	0.7×10^{12}	0.9×10^{11}	0.8×10^{12}	0.8×10^{11}	-
3	2	0.4×10^{12}	1.6×10^{10}	0.3×10^{12}	1.5×10^{10}	0.6×10^{12}	1.8×10^{10}	-
4	3	0.1×10^{12}	1×10^{10}	0.9×10^{11}	0.9×10^{10}	0.3×10^{12}	0.85×10^{10}	Optimal shelf-life period at 4°C
5	4	0.9×10^{11}	-	0.6×10^{11}	-	0.8×10^{11}	-	-
6	5	0.7×10^{11}	-	0.4×10^{11}	-	0.5×10^{11}	-	-
7	6	0.5×10^{11}	-	0.1×10^{11}	-	0.3×10^{11}	-	-
8	7	1×10^{10}	-	0.9×10^{10}	-	0.1×10^{11}	-	Optimal shelf-life period at 28°C
9	8	1×10^{9}	-	0.6×10^{9}	-	0.3×10^{10}	-	-
10	9	0.4×10^{9}	-	0.1×10^{9}	-	0.4×10^{9}	-	-

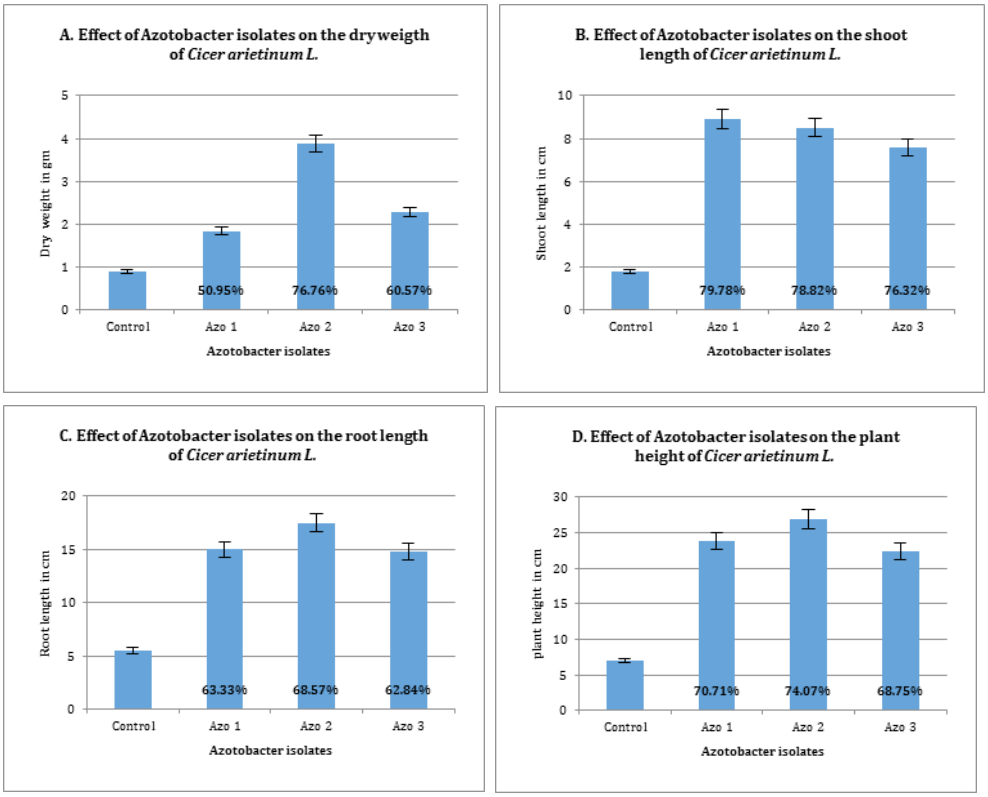

Fig. 2. Effects of Biofertilizer on Plant Growth
A – Dry Weight, B – Shoot Length, C – Root Length, D – Plant Height

trol pot the average plant height, average shoot length and average root length were 7.0 cm, 1.8cm and 5.5cm, respectively while in case of Azo-1,2 and 3 pots they ranged from average plant height of 22.4-27 cm, average shoot length of 7.6-8.9 and average root length of 14.8-17.5 cm, respectively and statistically on an average about 3.49 times, 4.6 times and 2.87 times, respectively the growth promotion was found as compared to control set, while average dry weight of sapling of control set was 0.903 and that of three test pots was 2.67 g indicating 2.96 times rise in the weight in the test pots as compared to control and if all the parameters taken together then more than 4 times plant growth promotion was

observed when seeds were exposed to Azobiofertlizers prepared as compared to control (seeds without biofertilizer exposure).

Conclusion

From the present study, it can be concluded that GA and IAA producing *Azotobacter* biofertilizers had significant growth promoting effect on various growth parameters like root length, shoot length, in the potted plant viz. *Cicer arietinum*. The results suggested that *Azotobacter* biofertilizers could be used to enhance the growth of plants as well as minimization of hazardous chemical fertilizers for the sustainable agriculture.

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