Asian J. Exp. Sci., Vol. 23, No. 1, 2009; 293-297 **Short Communication**

Antifungal and Phytohormone Production Potential of *Azotobacter chroococcum* Isolates from Groundnut (*Arachis hypogea* L.) Rhizosphere

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Abstract : A total number of 25 isolates of *Azotobacter chroococcum* from the rhizosphere soil of groundnut of different varieties from different localities of Sangli District were tested for their ability to produce antifungal metabolites and phytohormones- IAA & gibberellins. Out of these, isolates KG2, KG3 and KG5 were found to be more significant as compared with others. KG 2 exhibited activity against *Aspergillus flavus, Aspergillus terrus and Fusarium oxysporum* and produced IAA and Gibberellins in 55 µg/ml and 50 µg/ml amount respectively; KG3 exhibited activity against *Aspergillus flavus* and *Aspergillus terrus* and produced IAA and Gibberellins in 56 µg/ml and 58 µg/ml amount respectively whereas KG5 exhibited activity against *Aspergillus terrus, Alternaria alternata* and *Fusarium oxysporum* and produced IAA and Gibberellins in 53 µg/ml and 62 µg/ml amount respectively. Therefore, these three isolates could be successfully exploited to control fungal phytopathogens in the root region and to enhance seed germination of groundnut in order to increase the yield.

Key words : *Azotobacter chroococcum* , Antifungal metabolites, Indole acetic acid, gibberellins, groundnut.

Introduction

Azotobacter is gram negative, free living aerobic nitrogen fixing organism belonging to family Azotobacteriaceae. Among the several species, *Azotobacter chroococcum* happens to be the dominant inhabitant of the rhizosphere. There have been many reports on the beneficial effects of *Azotobacter chroococcum* on growth and yield of various agriculturally important crops. It benefits plants in multiple ways, which includes- a. ability to produce ammonia, vitamins and growth substances that enhance seed germination; b. production of indole acetic acid and other auxins such as gibberllins and cytokinins (Mratinez- Toledo *et. al.*, 1988; Verma *et. al.*, 2001) which enhance root growth and aid in nutrient absorption; c. inhibition of phytopathogenic fungi through antifungal substances (Sharma and Chahal, 1987; Verma *et. al.* 2001); d. Production of Siderophores which solubilize Fe^{3+} and suppress plant pathogens through iron deprivation.

Groundnut (*Arachis hypogea* L.), also known as peanut is an important leguminous oil seed crop grown in India. Maharashtra has the potential for this crop where it is taken in both rainy and post rainy seasons as a cash crop. Improved varieties such as JL 24, JL 286, TPG 41, TAG 24 and TG 26 are common under cultivation in Western Maharashtra. Many workers have reported low productivity of groundnut because of its cultivation under rainfed conditions which favors fungal attack

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and thereby reduces seed germination. Therefore, in the present study, native strains of *Azotobacter chroococcum* were obtained from the rhizosphere of groundnut of different varieties in Sangli District and tested for their antifungal and phytohormone production potential with a view of finding out the possible use of these isolates to control soil borne fungal phytopathogens in root region of the groundnut and thereby prevent seedling mortality to certain extent, and to enhance seed germination.

Materials and Methods

The isolates of *Azotobacter chroococcum* were obtained from the rhizosphere soil of groundnut plants of different varieties from different locations of Sangli District of Western Maharashtra, identified by studying their morphological, cultural and biochemical characteristics as described in Bergey's Manual of Systematic Bacteriology and labeled on the basis of groundnut variety and location.

To study the antifungal activity of these isolates, soil borne fungal phytopathogens viz. *Aspergillus flavus, Aspergillus terrus, Alternaria alternata and Fusarium oxysporum* were also isolated from the same soil samples and identified on the basis of morphological properties. Antifungal activity of the isolates was tested by agar diffusion method.

Indole acetic acid (IAA) production potential of these isolates was tested in Ashby's nitrogen free broth supplemented with 0.005 M concentration tryptophan at 28^0C. The concentration of IAA in the culture broth after 3 days of incubation was determined by spectrophotometric method using Salkowski reagent as described below-

3 ml of the supernatant was mixed with 2 ml of Salkowski reagent (2 ml of 0.5 M $FeCl_3$ + 98 ml 35% $HClO_4$) and the intensity of red colour developed within 30 minutes was checked at 530 nm using scanning spectrophotometer. The concentration was determined using a standard curve prepared from standard solutions of indol acetic acid. Four independent replicates of each isolates were analyzed.

Gibberellins production potential of the isolates was checked in Ashby's nitrogen free broth at 28^0C. The amount of gibberellins in the broth after 3 days of incubation was determined by spectrophotometric method using phosphomolybdic acid reagent as described below-

1 ml of the supernatant sample was taken out into 25 ml of volumetric flask, mixed with 15 ml of phosphomolybdic acid reagent and placed in a boiling water bath. After 1 hour, the temperature of the flask was reduced to room temperature and then final volume was made to 25 ml with distilled water. The absorbance of colour developed was measured at 780 nm in a spectrophotometer using distilled water as blank and the concentration was determined using a standard curve was prepared from the standard solutions of gibberellins. Four independent replicates of each isolates were analyzed.

Results and Discussion

A total number of 25 isolates of *Azotobacter chroococccum were* obtained from the rhizosphere soil of groundnut of different varieties from different localities of Sangli District and they were identified as *A. chroococcum* based on their morphological, cultural and biochemical characteristics.

Antifungal activity shown by these isolates is given the Table No.1 which reveals that there is variation in the antifungal activity among the isolates. *Aspergillus flavus* was inhibited by 13 isolates, *Aspergillus terrus* was inhibited by 10 isolates, *Alternaria alternata* was inhibited by 6 isolates and *Fusarium oxysporum* was inhibited by 8 isolates. It is also indicated that 8 isolates

Table 1 : Antifungal properties of *Azotobacter chroococcum isolates*.

Sr. No.		Activity against			
		Aspergillus flavus	***Aspergillus terrus***	***Alternaria alternata***	***Fusarium oxysporum***
1	SL1	-	-	-	+
2	KG 1	+	-	-	-
3	PL 1	-	+	-	-
4	AT 1	-	-	-	-
5	JT 1	-	-	+	-
6	SL 2	-	+	+	-
7	KG 2	+	+	-	+
8	PL2	+	-	-	+
9	AT 2	+	+	-	-
10	JT2	+	+	-	+
11	SL3	-	-	-	-
12	KG3	+	+	-	-
13	PL3	+	-	-	-
14	AT3	+	-	-	-
15	JT3	+	+	+	-
16	SL 4	-	-	-	-
17	KG4	+	-	-	-
18	PL 4	+	-	+	+
19	AT4	-	-	-	-
20	JT4	+	-	-	+
21	SL 5	-	-	-	-
22	KG 5	-	+	+	+
23	PL 5	+	-	+	-
24	AT 5	-	+	-	+
25	JT 5	-	+	-	-

Key: + : activity; - : No activity

exhibited activity only against single pathogen, 7 isolates exhibited activity against 2 pathogens, 5 isolates exhibited activity against 3 pathogens and 5 isolates do not exhibited activity against any of the pathogen. Isolate KG 2 and JT 2 exhibited activity against *Aspergillus flavus, Aspergillus terrus and Fusarium oxysporum* ; JT 3 exhibited activity against *Aspergillus flavus, Aspergillus terrus and Alternaria alternate* ; PL 4 exhibited activity against *Aspergillus flavus, Alternaria alternata and Fusarium oxysporum* whereas KG 5 exhibited activity against *Aspergillus terrus, Alternaria alternata and Fusarium oxysporum.*

Variation in the antifungal activity of the *Azotobacter* isolates was also been observed by other workers. Chakre *et al*. (1982) reported inhibition of plant pathogenic fungi by strains of *Azotobacter*. Afrikyan (1951) stated that soils enriched with *Azotobacter* contain no plant pathogenic bacteria. Agrawal and Singh (2002) tested 22 strains of *Azotobacter* against 3 pathogenic fungi , and

observed that only 14 cultures inhibited the growth of *F. oxysporum* only. The other two were not inhibited by any of the strain. It has been reported that out of 7 isolates of *Azotobacter*, three showed broad spectrum antifungal activity against *Aspergillus* and one or more species of *Fusarium* and *Rhizoctonia*. *Azotobacter* produces an antifungal antibiotic which inhibits the growth of several pathogenic fungi in the root region thereby preventing seedling mortality to a certain extent.

Production of phytohormones- indole acetic acid and gibberellins by *Azotobacter chroococcccum* isolates as given in Table No.2 reveals that all of the isolates produce both the hormones and the production of IAA was in the range of 02 μg/ml to 56 μg/ml whereas the production of GA was in the range of 02 μg/ml to 50 μg/ml. Fifteen isolates produced IAA in less than 20 μg/ml amount, six isolates produced IAA in between 21 to 40 μg/ml and four isolates produce IAA in more than 50 μg/ml amount. Isolate PL1 exhibited minimum potential (2 μg/ml) for the production of IAA whereas isolates AT1, KG2, KG3 and KG5 produced 55, 55, 56 and 53 μg/ml of IAA respectively, which is significantly more than the other isolates.

Table 2 : Plant growth hormones production by *Azotobacter chroococcum isolates.*

Sr. No.	Isolate	Conc. of IAA in μg/ml of sample	Conc.of Gibberlin in μg/ml of sample
1	SL1	6	8
2	KG 1	3	2
3	PL 1	2	36
4	AT 1	55	25
5	JT 1	10	15
6	SL 2	15	20
7	KG 2	55	50
8	PL2	20	12
9	AT 2	8	10
10	JT2	11	14
11	SL3	21	17
12	KG3	56	58
13	PL3	7	27
14	AT3	22	29
15	JT3	30	6
16	SL 4	26	32
17	KG4	22	18
18	PL 4	7	12
19	AT4	9	32
20	JT4	15	15
21	SL 5	13	40
22	KG 5	53	62
23	PL 5	16	38
24	AT 5	18	20
25	JT 5	25	31

As regards to gibberellins production, thirteen isolates produced less than 20 μg/ml, nine isolates produced in between 21 to 40 μg/ml and three isolates produced more than 50 μ_g/ml amount of gibberellins. The minimum potential was shown by KG1 (2 μg/ml) whereas isolates KG2, KG3 and KG5 produced 50, 58 and 62 μg/ml respectively which was significantly more than the other isolates. Thus, there was variation among the isolates in the production potential of these hormones also.

Production of plant growth regulators in nitrogen free media by *Azotobacter* species has been reported by many workers. Varma *et al*. (2001) found that out of 20 isolates and mutants of *A. chroococcum* only 4 produce all the three types of hormones viz. IAA, GA and kinetin. All others produced only one of the three phytohormones. Cytokinin in the culture filtrates of *A. chroococcum*, *A.beijerinckii* and *A.vinelandii* has been also detected. *A. chroococcum* is the most prolific producer of cytokinins. Enhanced root development in crops has been found in the literature, following inoculation with *Azotobacter*. 7 *Azotobacter* isolates for their quantitative production of IAA have been found almost all were producing IAA. Small quantities of indole acetic acid were synthesized in aerated culture of *A.chroococcum*. The addition of tryptophan enhanced indole acetic acid synthesis. The accumulation of IAA in mg/ml was highest in 7 days old culture regardless of tryptophan addition.

The results obtained in the present investigation suggests that the three isolates viz. KG 2, KG 3 and KG 5 exhibit more potential for the production of antifungal metabolites as well as both the hormones as compared with the others. Therefore, these could be successfully exploited to control plant pathogenic fungi in the root region and to enhance seed germination of groundnut in order to increase the yield of the crop.

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