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PRODUCTION OF PHYTOHORMONES BY THE NITROGEN FIXING BACTERIUM, *AZOSPIRILLUM**

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The association of the nitrogen fixing associative symbiotic bacterium, *Azospirillum* with the root systems of many cereals and grasses has been amply demonstrated {Dobereiner and Day 1976; Neyra and Dobereiner 1977; Vlassak and Reynders, 1981). Substantial amount of nitrogen is being fixed by this organism in tropical environments. One of the striking responses of crop plants upon inoculation with *Azospirillum* is the increased root and shoot growth and biornass accumulation (Smith *et al*, 1978). There are convincing evidences to show that the bacterium synthesises phytohormones of various kinds in the rhizosphere which might be implicated in the response of crops to *Azospirillum* inoculation. The benefit of nitrogen fixation may add to the phytohormone effect. In this study we have examined the production of phytohormone by *Azospirillum* and by a few of its drug resistant mutants.

Materials and Methods

Cultures of *Azospirillum* were isolated from the surface sterilized root bits of pearl millet *(Pennisetum americanum)* grown under field conditions and isolates were characterised (Tarrand *et al*, 1978). For increasing the efficiency of the cultures, certain drug resistant mutants were developed making use of the mutagen ethyi methane sulphonate (Clowes and Hayes, I968). The mutants resistant to streptomycin (Str^r) and chloramphenicol (CAM) were obtained. For studying the production of phytohormone, Baldani and Dobereiner's (1980) malate broth supplemented with or without L-tryptophan (0.05 per cent) was used. The extraction of indole acetic acid and gibberellic acid fike substances present in the cell free culture filterate was done as per the methods detailed by Tien *et al*. (1979), The presence of indole acetic acid (IAA) in the sample was detected chromatographically (Stahl. 1969). For quantification of IAA the colorimetric method (Gorden and Paleg, 1957) was followed. Gibberellic acid like substances (GA) present in the sample were identified chromatographically (Mac Milan and Suter, 1963). For the quantification of GA the rice seedling bioassay technique (Murakami, 1970} was followed.

The response of pearl millet to inoculation with *Azospirillum* was studied using the Fahraeus slide technique (Fahraeus, 1957) and also by growing the plants

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in tubes containing nitrogen free nutrient solution. Vigour index was determined as per the methods of Abdul-baki and Anderson (1973).

Vigour index = Germination percentage x total length of seedling (Root length + shoot length expressed in mm).

Results and Discussion

Apart from the direct role in nitrogen fixation, production of plant growth promoting substances (phytohormones) by *AzospiriHum* has been thought to be associated with the response of plants to inoculation, AM the mutants and wild types of *Azospirillum* tested in this experiment produced indole acetic acid and gibberellic acid like substances in culture broth supplemented and unsupplemented with tryptophan. In general, shake culture condition produced maximum amount of inhole acetic acid (Table 1). Production of IAA from tryptophan by *Azospirillum* was confirmed by Tien *et at.* (1979). The mutant Str^{r} 5 produced maximum quantity of IAA (1,358.6 μg 100 ml ^{1}) in shake culture condition when tryptophan was supplemented whereas Pt 9 produced the lowest amounts under shake culture condition even in the presence of tryptophan (628.75 μg 100 ml^{-1}). The reason is quiet obvious since *Azospirillum* is aerobic in nature (Dobereiner and Boddey, 1981), the shake culture condition might have stimulated their growth in presence of combined source of nitrogen.

Under static condition when tryptophan was added, Str 5 was found to produce maximum quantity and Pt 9 produced minimum. Under static condition without tryptophan Str^{r}5 produced maximum quantity while CAM^{r}1 produced minimum. The differences in IAA synthesis may be due to the strain variation. Paper chromatographic analyses confirmed the presence of IAA compounds.

Table 1

Production of indole acetic acid by *Azospirillum* sp.

Azospirillum strains		IAA produced, μg/100 ml			
		Static condition		Shake culture condition	
		(–) Tryptophan	(+) Tryptophan	(–) Tryptophan	(+) Tryptophan
CAM^{r}	(mutant)	19.00	141.50	92.00	690.00
Str^{r} 3	(mutant)	28,25	196.87	95.38	1223.50
Str 5	(mutant)	47.00	231.13	200,50	1358.60
Pt 85	(wild)	27.00	186.15	99.00	986.70
Pt 9	(wild)	34.63	170.80	96.00	628.75

(Data represent mean of three replications)

Table 2

Production of gibberetlic acid like substances by *Azospirillum* sp.

Azospirillum strains	GA 3 produced, μg/100 ml			
	Static condition		Shake culture condition	
	(—) Tryptophan	(+) Tryptophan	(—) Tryptophan	(+) Tryptophan
CAM^r 1 (mutant)	23.5	38,5	28.0	44,0
Str^r 3 (mutant)	14.0	16.5	17.0	18.0
Str 5 (mutant)	19.0	43.5	23.0	52.2
Pt 85 (wild)	13,5	43.0	15.0	45.3
Pt 9 (wild)	27,5	46.5	31.0	56.0

(Data represent mean of three replications)

Table 3

Effect of seed inoculation of *Azospirillum* on the vigour index of pearl millet on 5th day after germination

Azospirillum strains	Vigour index	Per cent increase over control
CAM^r 1 (mutant)	21,678	32.15
CAM^r 2 (mutant)	17,166	4.25
CAM^r 3 (mutant)	16,801	2.75
CAM^r 4 (mutant)	20,550	25.27
Str 2 (mutant)	19,242	17.30
Str^r 3 (mutant)	24,100	46.94
Str 4 (mutant)	19,426	18.42
Str 5 (mutant)	22,866	39.42
Pt 6 (wild)	20,860	27.16
Pt 9 (wild)	18,248	11.24
Pt 76 (wild)	18,480	12.66
Pt 80 (wild)	16,724	1.95
Pt 85 (wild)	21,646	31.95
Uninoculated control (dist. water)	16,404	—

(Data represent mean of fifty observations)

CD (0.05) -- 604.8

The production of gibberellic acid like substances (GA) was also found under *in vitro* conditions (Table 2). With trytophan, Strr5 produced maximum GA under shake culture condition while Pt 9 produced maximum GA in the absence of tryptophan. Under static condition Pt 9 produced maximum GA with and without tryptophan.

The wild strain, Pt 85 was found to produce maximum quantity of GA in shake culture condition in the absence of trytophan, while Strr3 produced minimum in the presence of tryptophan.

Inoculation with *Azospirillum* to germinated pearl millet seeds grown under Fahraeus slide technique clearly brought out the effect of inoculation. The mucigel layer of the roots was found to be very prominent and harboured abundant population of this organism. Prolific root hair elongation and abundant development of lateral roots were noticed in inoculated plants over uninoculated controls.

Inoculation of pearl millet with the mutants enhanced the vigour index. This is in congruent with the observation of Dhanapal *et al.* (1978). The mutant Str^r 3 increased the vigour index by 46.94 per cent over distilled water treatment. Among the CAM^r mutants, CAM^r 1 produced 32.15 per cent increase over distilled water treatment, while the wild type parent Pt 85 increased only 31.95 per cent. CAM^r 3 and Pt 80 were found to have no significant effect on vigour index.

The present study has re-affirmed that the inoculated plants produced higher root biomass than the control. Dewan and Subba Rao (1979) reported that root biomass of rice seedlings increased due to inoculation with *Azospirillum brasilense*. The increase in root biomass can be attributed to plant growth promoting substances, thus providing maximum surface area for the absorption of nutrients. This when coupled with the fixation of nitrogen *in vivo* may result in the increased performance of the inoculants.

Summary

Azospirillum, the nitrogen fixing bacterium associated with the root system in pear! millet *(Pennisetum americanum)* has been studied for the phytohormone production. Both the wild types of *Azospirillum* and the drug resistant mutants produced phytohormones like indole acetic acid and gibberellin like substances. Addition of the precursor tryptophan and aerated conditions of incubation helped the production of more IAA and GA. The isolates varied in their ability to produce the phytohormones. Seeds of pearl millet inoculated with *Azospirillum* resulted in profuse root hairs and lateral root development. Moreover inoculation resulted increase in the vigour index of the plant.

സംഗ്രഹം

ബജ്റ ചെടിയുടെ വേരുപടലങ്ങളിൽ കാണപ്പെടുന്ന *അസോസ്പൈറില്ലം* എന്ന നൈട്രജൻ യൗഗികീകരണ ബാക ടീരിയയുടെ ഹോർമോൺ ഉൽപാദനത്തെക്കുറിച്ച് രമിഴ്നാട് കാർഷിക സർവകലാശാലയിൽ വെച്ച് നടത്തിയ പഠനങ്ങളിൽ ഇവയുടെ സ

തവേ കാണുന്ന സ്ട്രെയിനും ഉൽപരിവർത്തി സംഭവിച്ച സ്ട്രെയിനു. ഇൻഡോം അസ റിക് അമ്ളം, ഗിബറല്ലിക് അമ്ളം മുതലായ ഹോർമോണുകളം ഉല്പാദിപ്പിക്കുന്നതായി കണ്ടു. വായുവിന്റെ സാന്നിദ്ധ്യത്തിൽ 'ട്രിപ്റോഫാൻ' എന്ന രാസവസ്തു നൽകിയ പ്പോം അവ കൂടുതൽ ഹോർമോണുകളം ഉല്പാദിപ്പിക്കുകയുണ്ടായി. എന്നാൽ ഹോർ മോൺ നിർമ്മാണത്തിൽ സ്ട്രെയിനുകളം തമ്മിൽ വലിയ അന്തരമുള്ളതായിട്ടാണ് കണ്ടത്. ബജ്റായുടെ വിത്തുവഴി അസോസ്പൈറില്ലം നിവേശനം നടത്തിയപ്പോം മൂലഭാഗങ്ങളും പാർശ്വവേരുകളും ധാരാളം വളർന്നു വരുന്നതായി കാണപ്പെട്ടു. മാത്രമല്ല, വിത്തുവഴിയുള്ള നിവേശനം ബജ്റാ ചെടിയുടെ 'ശക്തി സൂചിക' (വിഗർ ഇൻഡക്സ്) വർദ്ധിപ്പിക്കുക യും ചെയ്തു.

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