



## STUDIES ON CHROMIUM TOLERANCE IN *AZOSPIRILLUM*

K. GOVINDARAJAN

Department of Agricultural Microbiology  
Tamil Nadu Agricultural University  
Coimbatore - 641 003.

### ABSTRACT

*Azospirillum* cultures were examined for their tolerance to the heavy metal chromium. Different methods of assays were tested to assess the tolerance. The cultures were able to tolerate chromium upto a concentration of 1000-1400  $\mu\text{g ml}^{-1}$ . The tolerance level was found to be comparatively high in all the cultures examined.

**KEY WORDS:** *Azospirillum*, Chromium toxicity, Paper disc assay, Tube dilution assay

Tanneries discharge waste water with high concentration of chromium into nearby rivers and land. This heavy metal addition over a considerable time results in pollution and is of concern because of possible toxicity to soil microorganisms which play a significant role in crop production. The problem of chromium toxicity on the activity of beneficial microorganisms like *Azospirillum*, the nitrogen fixing diazotroph has not been examined. The present work was taken up to determine the natural level of tolerance present in promising *Azospirillum* cultures against chromium.

### MATERIALS AND METHODS

Five *Azospirillum* cultures viz., SP 7, IPI, AZ 204, AZ 208 and Y2 received from the Department of Agricultural Microbiology, Agricultural College and Research Institute Coimbatore were used in the study during 1996. Chromium was used as chromium sulphate. The amount of heavy metal in the media represented the total amount of the metal salt viz., chromium sulphate.

#### Determination of Chromium toxicity

##### Streak plate method

Petri plates containing media and different concentrations of chromium ranging from 200-2000  $\mu\text{g ml}^{-1}$  were prepared and the surfaces streaked with the *Azospirillum* cultures. The plates were incubated for a period of 3 days at 30°C before recording the observations.

##### Paper disc assay

Standard filter paper discs were soaked in the heavy metal solution of different concentrations

mentioned above and dried. Nutrient agar medium seeded with a standard cell suspension of *Azospirillum* was poured into sterile petri plates and allowed to solidify. Filter paper discs treated with specified concentrations of chromium sulphate were placed on the agar medium. Sterile filter paper discs without heavy metal served as control. Observations were recorded after 3 days incubation at 30°C. Diameter of the inhibition zones seen around the filter paper discs was measured and sensitivity to chromium was calculated.

##### Tube dilution assay

Test tubes with 10 ml of nutrient broth containing specified concentrations of chromium was prepared and 0.1 ml of standard inoculum was added to each tube and O.D. values were recorded at 520  $\mu\text{m}$  after 48 and 96 h of incubation at 30°C.

### RESULTS AND DISCUSSION

In the streak plate assay, all the 5 *Azospirillum* cultures tolerated chromium sulphate up to a concentration of 1400  $\mu\text{g ml}^{-1}$ . But the filter paper disc assay revealed that three *Azospirillum* cultures were found to be sensitive to chromium at a concentration of 1400  $\mu\text{g ml}^{-1}$  and 2 cultures were inhibited at 1600  $\mu\text{g ml}^{-1}$  (Table 1).

When tested further by the tube dilution assay up to 1000  $\mu\text{g ml}^{-1}$  no significant differences were observed in the growth of *Azospirillum* as measured by OD values. From 1200  $\mu\text{g}$  onwards growth was slow but not inhibited. Above 1600  $\mu\text{g}$  there was no growth of *Azospirillum* cultures till 96 h after inoculation (Table 2).

Table 1. Sensitivity of *Azospirillum* cultures towards chromium assessed by paper disc assay

<i>Azospirillum</i> culture	Inhibition annules (cm <sup>2</sup> )					
	1000 µg ml <sup>-1</sup>	1200 µg ml <sup>-1</sup>	1400 µg ml <sup>-1</sup>	1600 µg ml <sup>-1</sup>	1800 µg ml <sup>-1</sup>	2000 µg ml <sup>-1</sup>
SP 7	NS	NS	1.32	1.85	2.40	2.94
IP1	NS	NS	NS	1.30	2.10	2.90
AZ 204	NS	NS	1.12	1.80	2.45	2.85
AZ 208	NS	NS	NS	1.20	1.95	2.80
Y 2	NS	NS	0.90	1.62	2.40	2.95

NS : Not sensitive

Data represent mean of 3 determinations

The results indicated that the chromium tolerance level was high in all the *Azospirillum* cultures tested. The cultures were able to tolerate chromium concentration of 1000-1400 µg ml<sup>-1</sup> in the different methods of assay.

The variations recorded in the tolerance level of different cultures to chromium might be due to the genetic make up of the organism. The differences in the uptake of the metal ions might also provide the observed resistance.

Microbiological studies are generally performed in synthetic growth media and the different organic constituents bind large amounts of heavy metals (Martensson, 1991 ; Ramamoorthy and Kushner, 1975). These substances may decrease the free concentration of the metal ions and thus influence the results. Even in solid media the presence of agar may influence the mode of action. The cell wall of *Azospirillum* being a complex three layered structure binds and immobilizes many metal ions (Beveridge and Koval, 1981) which might be the

Table 2. Chromium tolerance of *Azospirillum* cultures assessed by tube dilution assay

Chromium concentration µg ml <sup>-1</sup>	Incubation period (h)	Growth of <i>Azospirillum</i> cultures (OD values)				
		SP 7	IP 1	AZ 204	AZ 208	Y 2
Control	48	0.18	0.20	0.20	0.16	0.18
	96	0.28	0.28	0.30	0.28	0.28
1000	48	0.16	0.20	0.18	0.15	0.15
	96	0.26	0.27	0.30	0.28	0.28
1200	48	0.10	0.10	0.12	0.12	0.10
	96	0.14	0.20	0.22	0.22	0.20
1400	48	0.10	0.10	0.12	0.12	0.10
	96	0.14	0.18	0.18	0.18	0.18
1600	48	0.08	0.08	0.08	0.08	0.08
	96	0.10	0.12	0.12	0.10	0.10
1800	48	0.05	0.05	0.05	0.05	0.05
	96	0.05	0.05	0.05	0.05	0.05
2000	48	0.05	0.05	0.05	0.05	0.05
	96	0.05	0.05	0.05	0.05	0.05

reason for the higher level of tolerance observed in *Azospirillum* cultures towards chromium.

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## CORH 2 - A NEW MEDIUM DURATION RICE HYBRID

M. RANGASWAMY, K. THIYAGARAJAN, P. RANGASAMY,  
P. JAYAMANI, A.S. PONNUSAMY, R. LATHA and P. VAIDYANATHAN

Centre for Plant Breeding and Genetics  
Tamil Nadu Agricultural University  
Coimbatore 641 003.

#### ABSTRACT

CORH 2 a medium duration rice hybrid was developed by three line breeding system using cytoplasmic genic male sterile line (CMS), maintainer line and restorer line (A/B/R) and tested as TNRH 16. The parentage of this hybrid is IR 58025A/C20R. This hybrid has a duration of 125 days, grows upto a height of 95 cm, with high tillering ability of 15 productive tillers per hill. The average grain number is 230 per panicle and 1000 grain weight is 24 g. The grain size is medium and rice is white in colour. It is suitable for July-October sowing in Tamil Nadu. It also comes up well in saline and alkaline soils. On an average it yields 6071 kg/ha which is 20.5 per cent increase over the check variety ADT 39.

**KEY WORDS:** Cytoplasmic Genic Male Sterile line, Rice, CORH 2, Hybrid

Rice in India occupies an area of 44 m.ha which represents 41% of the area cropped to cereals. Since rice is the major food grain of the total cereals consumed in India, any effort to achieve food security in the future must focus on this crop. The prospects for accelerating the growth in rice production by conventional means, as in the past is less encouraging as there is little scope for any expansion of the area under rice. The high yielding varieties that sustained the growth in rice production over the last 25 years have also tended to plateau. The additional production required to keep pace with the expected demand by the year 2006 is about 20 million tonnes, which represents a 25% increase over the current level of production. Development and use of hybrid rice is one of the approaches by which the productivity can be increased. This strategy follows the highly successful example of the Chinese, where the commercialisation of hybrid has increased total production of rice by 350 m.tonnes over the last 20

years, without any expansion in the area of 33 m.ha., Tamil Nadu Agrl. University, Coimbatore initiated research on hybrid rice during 1979. The first hybrid CORH 1 was released in the year 1994. Another hybrid of medium duration group was released as CORH 2 during January 1998 for commercial cultivation.

#### MATERIALS AND METHODS

The hybrid rice culture TNRH 16 was developed through three line breeding system (A/B/R) utilizing cytoplasmic genic male sterile line (IR58025A), maintainer line (IR58025B) and restorer line (C20R). This culture was tested in six Preliminary Yield Trials (PYT) conducted at Paddy Breeding Station, Coimbatore since 1994. The performance of this culture over locations was also studied by conducting Multi Location Trials (MLT) at six locations during *Rabi*, 1995 and *Rabi* 1996. During *Kharif* 1996 it was tested at nine hybrid rice research centres of India in the National