AGROCHEMICAL RESISTANT MUTANTS OF NITROGEN FIXING CYANOBACTERIUM
TOLYPOTHRIX TENUIS AS NITROGEN FERTILIZER FOR RICE

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SUMMARY: Agrochemical resistant mutants of nitrogen fixing cyanobacterium Tolypothrix tenuis were isolated after MNNG mutagenesis. The mutants exhibited higher nitrogenase activity and released more quantities of extracellular nitrogenous substances such as ammonia, indole acetic acid like substances and amino acids when compared to the parent. They also increased the available nitrogen status of the soil in rice culture. Significant increase in the growth and yield upon inoculation of these mutants into rice culture was observed in comparison with chemical nitrogen fertilizer urea, as well as the parent strain treatment.

INTRODUCTION

Inoculation of nitrogen fixing cyanobacteria to rice culture has been reported to enhance plant performance due to a slow release of fixed nitrogen during growth and mineralization after death (Stewart et al., 1979; Tirol et al., 1982). The cyanobacterial contribution to nitrogen economy in rice culture showed that cyanobacterial supplementation results in a saving of about 30% of chemical nitrogen fertilizers (Singh and Singh, 1986). Several cyanobacterial strains are also reported to release phytohormones such as gibberellins (Gupta and Shukla, 1969) and auxins (de Caire et al., 1979; Mikhailova et al., 1984) and their culture filtrates are shown to stimulate the seed germination and to enhance the growth of rice seedlings (Tambiev et al., 1981; Antarikanonda, 1982). The application of pesticides has become a routine practice in controlling pests and diseases of rice. The heavy application of several toxic agrochemicals especially insecticides, influences the growth and nitrogen fixation of rice field cyanobacteria (Venkataraman and Rajyalakshmi, 1972). The most acceptable method to overcome this constraint is to select suitable agrochemical resistant strains that could tolerate the field dose concentrations of insecticides and liberate nitrogenous substances during growth to increase the available nitrogen status in rice culture. In the present work we report the isolation of agrochemical resistant mutants of cyanobacterium, Tolypothrix tenuis and the effect of the mutants on the growth and yield of rice plants.

MATERIALS AND METHODS

Cyanobacterium. Tolypothrix tenuis was obtained from the Division of Microbiology, IARI, New Delhi, India.

Growth Conditions. Axenic cultures of T. tenuis were maintained in Bothe's medium (1968). Cultures were grown at 25±2°C by providing light intensity of 1500 lux for 12h daily. Growth was determined by 10 days end point growth using chlorophyll values. Specific growth rate
constant values were calculated from the equation \( K = \log_{10} \left( \frac{N_t}{N_0} \right) \) where \( K \) = growth rate constant, \( t \) = growth period (10 days), \( N_t \) = absorbance at time 't' and \( N_0 \) = absorbance at time '0'.

**Agrochemicals.** BHC (Hexachlorocyclohexane, Organochlorine - 10% a. i); Demeton (Oxymethyl demeton, Organophosphorous-25% a. i) and Ekalux (Quinolphos, Organophosphorous-25% a. i) were used.

**Isolation of Agrochemical Resistant Mutants of Cyanobacterium T. tenuis.** Short filaments (5-10 cells/filament) of *T. tenuis* were treated with MNNG, 250\( \mu \)g ml\(^{-1} \) in citrate buffer pH 5.5 for 30 min. After the mutagenic treatment they were washed with phosphate buffer pH 7.0 and suspended in Bothe's medium with 100\( \mu \)g ml\(^{-1} \) of BHC, Ekalux and Demeton individually and allowed to grow for 24h in a rotary shaker (100 rpm). The mutated filaments were overlayed in 0.7% agar in the three agrochemicals (500\( \mu \)g ml\(^{-1} \)) containing 1.5% agar plates. Resistant clones were selected after 10 days and were used for further analysis.

**Nitrogen Fixation.** Nitrogenase activities were estimated by acetylene reduction assay as described previously (Thomas et al., 1991). Ethylene was resolved in a Hewlett Packard 5830A gas chromatograph using a stainless steel porapack N column at 110°C as FID temperature.

**Analytical Procedures.** Chlorophyll \( a \) was measured in 80% acetone (Thomas and Shanmugasundaram, 1986). Protein was determined using Bradfords method (1976). Ammonium was determined using phenol-hypochlorite reaction (Weatherburn, 1967). IAA like substances were determined using the method of Salkovskii (Tanner and Anderson, 1964). Total amino acids were quantified using ninhydrin reagent (Rosen, 1957). Available nitrogen content in soil samples were quantified using the method of Subbaiah and Aziza (1956) as described previously (Thomas and Shanmugasundaram, 1991). Shimadzu Graphicard UV-Visible Spectrophotometer was used for optical density measurements.

**Statistical Analysis.** This was done by using Student's 't' test.

**Rice Culture.** *Oryza sativa* IR-20 rice variety was used to evaluate the biofertilizer value of the agrochemical resistant mutants of *T. tenuis*, BHC\(^R\), DEM\(^R\) and EKA\(^R\) on the growth and yield of rice plants were conducted in pot cultures. The biofertilizer value of the added agrochemical resistant mutants was assessed against the effects of the wild type *T. tenuis* and those of urea on rice plants. The following nitrogen fertilizer treatments were employed: (1) with urea (equivalent to 120kg/hectare), (2) with *T. tenuis*, wild type (equivalent to 6kg/acre), (3) with *T. tenuis* BHC\(^R\), (4) with *T. tenuis* DEM\(^R\), (5) with *T. tenuis* EKA\(^R\). Triplicates were maintained and the pots were randomised. The pots were filled with soil and five healthy seedlings (25 days old) were planted in each pot. Urea and cyanobacteria were applied in the following stages of the plant growth along with superphosphate and potash. (a) seeding stage (basal dressing) (b) beginning of active vegetative phase (first application) (c) beginning of reproductive phase (second application). After 20 days of first and second application, plant growth parameters and finally the grain and total yield were measured. Pests and diseases were controlled by the application of insecticides, BHC, demeton and ekalux and fungicides, dithane and difolaton.

**Chemicals.** MNNG (N-methyl-N'-nitro-N-nitrosoguanidine) was purchased from Sigma Chemical Co., USA and other chemicals were from E. Merck AG, Germany or BDH, India.

**RESULTS**

*T. tenuis* developed tolerance against agrochemicals and there was considerable difference in the sensitivity of this organism to the agrochemicals tested. The organophosphorous chemicals demeton and ekalux are more deleterious than the organochlorine derivative, BHC. All the three agrochemicals at 500\( \mu \)g ml\(^{-1} \) inhibited the total growth of *T. tenuis*. Agrochemical resistant mutants of *T. tenuis* were isolated at that concentration after MNNG mutagenesis. While their growth rates in the presence and absence of the agrochemicals were checked, several mutants