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EFFECT OF EMS, DMS AND HYPOXANTHINE ON NATIVE *RHIZOBIUM* OF *CYMOPSIS TETRAGONOLOBA* L.

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In the present work the genetic effects of EMS, DMS and hypoxanthine on native rhizobial strain of *Cymopsis tetragonoloba* (GC) obtained from normal plant has been worked out. On the basis of antibiotic sensitivity, various types of rhizobial mutants (GR_5, GR_6, GR_7, GR_8, GR_9) have been detected. Five antibiotic resistant/sensitive mutant strains have been isolated after the mutagenic treatments with EMS, DMS and hypoxanthine given to the native rhizobium strain of *C. tetragonoloba*. The data on effectivity of native as well as two antibiotic resistant and one sensitive mutant strain of rhizobia indicated that all these strains differ markedly from each other with regard to influencing growth and nodulation in *C. tetragonoloba*.

Keywords : Antibiotic test; *Cymopsis tetragonoloba*; Dimethylsulphate; Ethyl methane sulphonate, Hypoxanthine; Mutant; Resistance; *Rhizobium*; Sensitive.

Introduction

Chemical mutagens including alkylating agents have been commonly used to induce mutations in different types of bacteria[1,2]. In the past induced mutational studies of *Rhizobium* have been done by many workers[3-5]. After the EMS treatment, Kalra *et al.*[6] isolated auxotrophic mutant of *Rhizobium japonicum*. Azide resistant mutant with better symbiotic effectivity and N_2 fixing capacity of *R. leguminosarum* was isolated by Ram *et al*[7]. Dogra[1] isolated non-infective pigmented mutants of *R. meliloti* by UV irradiation and observed that pigmentation in *Rhizobium* and nodulation ability are genetically linked and might have some pleiotropic effects. Dhar *et al.*[8] obtained streptomycin and erythromycin resistant mutants cowpea *Rhizobium* by nitrosogunidine treatment.These mutants differed in growth pattern, phage sensitivity and infectivity. Verma and Dedarwal[9] detected antibiotic resistant mutants of chickpea *Rhizobium* by gamma rays and found that majority of antibiotic resistant mutants were inferior to parent strain with regard to effectivity. In that context, the aim of present study was to investigate the effect of alkaline agents (EMS and DMS)and hypoxanthine on native rhizobial strain of *C. tetragonoloba*.

Materials and Methods

Native strain of *Rhizobium* and isolates of native strain were isolated and purified from the nodules of normal and induced plants of *C. tetragonoloba*, as per the method given by Subba Rao[10]. The purified rhizobial culture was maintained on yeast extract mannitol agar medium (YEMA).

For induction of mutations in native *Rhizobium* of *C. tetragonoloba*, 0.1ml over night culture of native strain was added to 5 ml of YEM broth in test tube and after this 0.1 ml mutagen was added to give a final concentrations of 0.01, 0.001% in case of DMS; 0.16 nM, 0.20 nM, in case of EMS and 0.01, 0.001% in case of hypoxanthine. Five replicates for each treatment were used. The test tubes were incubated for 24h at 28° C±1and thereafter, optical density (OD) was measured for each sample and a graph was plotted between OD and their respective concentrations for determining the growth of *Rhizobium*.The treated samples were washed with sterile distilled water,diluted and plated on YEMA medium and later on incubated for 72h at 28° C±1. From each treated sample of *Rhizobium* culture, thirty to forty randomly selected colonies were isolated and purified. Purified rhizobial colonies (both treated and untreated) were examined for antibiotic sensitivity (samples were tested according to Crabtree and Hinodill[11]) using penicillin, streptomycin, tetracyclin, neomycin, erythromycin and ampicillin disc, and later a few antibiotic

resistant/sensitive mutants of *Rhizobium* were identified. The morphological, cultural and physiological characteristics of the rhizobial isolates were tested according to the methods described by Vincent[12].

Results and Discussion

In the present work the genetic effects of EMS, DMS and hypoxanthine on native rhizobial strain of *C. tetragonoloba* (GC) obtained from normal plant has been worked out. On the basis of antibiotic sensitivity, various types of rhizobial mutants have been detected. The important cultural and physiological characteristics of the mutants are summarised (Table 1).

Neomycin resistant mutant (GR_5) : The mutant was isolated from 0.16 nM EMS treated rhizobial populations. Mutant was resistant to neomycin in contrast to native strain which was sensitive to neomycin (0.3 Cm). Apart from this, as compared to native strain, mutant strain could grow in Hofer's alkaline medium without acid production, reduced nitrate to nitrite and produced ammonia. Mutant strain did not produce indole and did not hydrolyze starch.

Tetracycline resistant mutant (GR_6) : This rhizobial mutant strain was detected after 0.016 nM EMS treatment. The mutant strain was resistant to tetracycline as compared to native strain which was tetracycline sensitive (1.2 Cm). The other physiological characters of the mutant strain were its incapability to grow on Hofer's alkaline medium and inability to hydrolyze starch. Although mutant strain reduced nitrate to nitrite but did not produce ammonia.

Erythromycin sensitive mutant (GR_7) : This mutant was detected from 0.16 nM, 0.2 nM EMS and 0.01 DMS treated series. As compared to native strain, which was erythromycin resistant, mutant strain showed sensitivity towards erythromycin (1.0Cm). GR_7 strain did not show any growth in Hofer's alkaline medium. This strain did not hydrolyze starch and did not produce indole in trytophan broth.

Streptomycin sensitive mutant (GR_8) : Mutant was detected in 0.01% DMS and 0.001% hypoxanthine treatments. In contrast to native strain which was resistant to streptomycin, mutant strain was found to be sensitive (1.2 Cm). Mutant colonies produced ammonia. However, oxidase activity was not shown by this strain.

Streptomycin sensitive and neomycin resistant mutant (GR_9) : This strain isolated after 0.1% DMS and 0.01% hypoxanthine treatments. Mutant strain showed vigrous growth around neomycin antibiotic disc and did not show such growth around streptomycin antibiotic disc. No growth in Hofer's alkaline medium was shown by this mutant strain. However, a positive reaction towards production of ammonia was shown by this mutant. Oxidase activity and starch hydrolysis were not shown by this strain.

In the present course of investigations five antibiotic resistant/sensitive mutant strains named as neomycin resistant (GR_5), tetracycline resistant (GR_6), erythromycin sensitive (GR_7), streptomycin sensitive (GR_8) and streptomycin sensitive and neomycin resistant (GR_9) have been isolated after the mutagenic treatments of EMS, DMS and hypoxanthine given to the native rhizobial strain of *C. tetragonoloba* L. Chemical mutagens including alkylating agents have been commonly used for mutations in different types of bacteia. Various antibiotic resistant/sensitive mutant strains of *Rhizobium* species have also been screened by various investigators[3,8,12-14] using various physical and chemical mutagens.

Though hypoxanthine has been used by workers in animal and plant tissue systems[15-18], but in literature clear indication regarding its mutation induction potentiality is not there. Therefore, present results with this chemical are quite encouraging and it is worthwhile to try this group of chemical in induced mutation experiments.

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Table 1. Morphological, Cultural and Physiological Characteristics of the Various Rhizobial Isolates.

Strains/ isolates	Grams reaction test	Motility test	Growth on Hofer's alkaline medium	Nitrate reduction	NH_3 production	Oxidase activity	Catalase activity	Indole production	MR-VP test	H_2S production	Starch hydrolysis	Antibiotic Sensitivity test (Cm)					
												E	P	A	N	T	S
(GC)	-	+	AG	+	-	+	+	+	-	-	+	-	-	-	0.3	1.2	-
GR_5	-	+	G	+	+	+	+	-	-	-	-	-	-	-	-	0.8	-
GR_6	-	+	-	+	-	+	+	+	-	-	-	-	-	-	0.5	-	-
GR_7	-	+	-	+	-	+	+	-	-	-	-	1.0	-	-	0.4	1.3	-
GR_8	-	+	AG	+	+	-	+	+	-	-	+	-	-	-	0.4	1.1	1.2
GR_9	-	+	-	+	+	-	+	+	-	-	-	-	-	-	-	0.8	1.0

Key :

GC = Native Strain (Control)
GR_5 = Neomycin resistant mutant
GR_6 = Tetracycline resistant mutant
GR_7 = Erythromycin sensitive mutant
GR_8 = Streptomycin sensitive mutant
GR_9 = Streptomycin sensitive and Neomycin resistant mutant

Key :

Positive test =+
Negative test = -
Positive (Fast rate) =+ (F)
Growth with acid production = AG
Growth without acid production = G
Erythromycin = E
Penicillin = P
Ampicillin = A
Neomycin = N
Tetracycline = T
Streptomycine = S

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