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ANTIBIOTIC SENSITIVITY OF *RHIZOBIUM* ISOLATED FROM GAMMA IRRADIATED MUTANT PLANTS OF *VIGNA MUNGO* L.

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Mutagenic studies in *Vigna mungo* L. var. T9 was carried out by using different doses of gamma rays viz., 10kR, 20kR, 40kR and 60kR. Five mutants nomenclatured as tall, dwarf, round leaves, high nodulating and xanthina virescens were identified in M_3 generation and *Rhizobium* isolated from root nodules of these respective mutant plants was subjected to antibiotic sensitivity test. Erythromycin, penicillin, neomycin, tetracycline and streptomycin were few of the antibiotics against which the sensitivity of various rhizobial isolates was tested. Native strain of *Vigna mungo* was resistant to penicillin and was sensitive towards other antibiotics for 10μg / disc. Marked variations in rhizobial sensitivity was observed in different isolates from different mutant plants in contrast to native strain. Significant variations were observed in physiological characteristics of *Rhizobium* after gamma irradiation. The variations might have appeared due to rhizobial mutation inside the host plant and there could be a direct interaction between the rhizobia and altered host physiology/phenotype due to gamma irradiation.

Keywords : Gamma rays; Mutation; *Rhizobium; Vigna mungo.*

The genus *Rhizobium* play an important and vital role in biological nitrogen fixation through symbiosis in the leguminous plants. In the present study mutations were induced in *Vigna mungo* L. var T9 using different doses of gamma rays viz., 10kR, 20kR, 40kR and 60kR. In M_3 generation five prominent mutants were selected and nomenclatured as tall (VM_1), dwarf (VM_2), round leaves (VM_3), high nodulating (VM_4) and xanthina virescens (VM_5). Rhizobial samples were isolated from root nodules of normal and mutant plants and subjected to antibiotic sensitivity test with an aim to study the variations in physiological characteristics of the *Rhizobium* caused due to altered phenotype of the host resulted due to toxic effects of mutagen or genic mutation in the host plant.

Rhizobial isolates obtained from root nodules of five mutant plants of *Vigna mungo* were tested for sensitivity towards various antibiotics viz., erythromycin, penicillin, neomycin, tetracycline and streptomycin. Euqal amount of rhizobial suspension was transferred to the petriplates containing YEMA (yeast extract mannitol agar) medium in such a way that it could spread evenly on the medium. Commercially prepared different antibiotic disc (10μg antibiotic/disc) were then placed aseptically on the medium. Petriplates were incubated for bacterial growth at temprature 28±1°C. The growth of the bacterial culture around antibiotic disc was observed after 3 days of incubation. The size of inhibition zone around the antibiotic disc was measured to observe the extent of sensitivity of bacterial isolate towards a particular antibiotic. When no inhibition zone formed around the disc and completed growth of *Rhizobium* was observed, then such rhizobial isolate was regarded as a resistant isolate towards that particular antibiotic.

In the present paper, recorded observations on antibiotic sensitivity of various rhizobial isolates have been compared with the control native starin (NR) and are summarized in Table 1. Native strain of *V. mungo* was resistant to penicillin and was sensitive to streptomycin (0.5cm), erythromycin (0.5cm), tetracycline (0.5cm) and neomycin (0.2cm) for 10μg/disc of antibiotic. In contrast to native strain, isolate from tall mutant (VM_1) was found to be more sensitive towards tetracycline as large sized inhibition zone (1.2 cm) was observed around the antibiotic disc. Similarly, isolate from dwarf mutant (VM_2) also showed high sensitivity towards tetracycline (1.5cm) while 10μg/disc dose of streptomycin was not effective on this rhizobial isolate.

One of the altered antibiotic characteristic of *Rhizobium* obtained from round leaves mutant plants was its acquired resitance towards erythromycin. Isolate from high nodulating mutant (VM_4) showed marked variations in antibiotic sensitivity as compared to native strain. It was assesed to be more sensitive towards streptomycin (0.7cm) and neomycin (0.8cm) and resistant to erythromycin. More or less similar to native strain, isolate VM_5 was resistant to penicillin, sensitive to erythromycin (0.4cm) and neomycin (0.2cm). However, streptomycin and tetracycline were found to be more effective on rhizobial isolate VM_5 as 0.9 cm and 1.5cm inhibition zone was observed around these antibiotic discs respectively.

Size of the inhibition zone around individual antibiotic disc suggests that in comparison to native strain

Table 1. Antibiotic sensitivity of Rhizobial isolates obtained from induced mutant plants of *Vigna mungo* L.

Antibiotics	Mutants and their *Rhizobium* isolates (R. sp- cowpea type)					
	Native Strain NR	Tall VM_1	Dwarf VM_2	Round Leaves VM_3	High Nodulating VM_4	Xanthina Virescens VM_5
	Size of inhibition zone (cm) around disc containing 10μg of antibiotic					
Streptomycin	0.3	0.3	R	0.5	0.7	0.9
Penicillin	R	R	R	R	R	R
Erythromycin	0.5	0.2	0.7	R	R	0.4
Neomycin	0.2	0.2	0.3	0.2	0.5	1.5
Tetracycline	0.5	1.2	1.5	0.5	0.5	1.5

R = Resistance towards antibiotics

of *V. mungo,* isolates VM_4 and VM_5 were more sensitive towards streptomycin; isolate VM_4 towared neomycin and isolates VM_1, VM_2 and VM_5 towards tetracycline.

Alterations in physiological characters of *Rhizobium* obtained from irradiated mutant plants, as observed in the present work, are rarely reported in the past[1-3]. However, many reports are available in the literature regarding morphological, cultural and biochemical characteristics of various rhizobial isolates obtained from normal host plants from different sources[4-7].

The inference drawn from the present work done is that variations in cultural and physiological properties of the rhizobial isolates from mutant plants could be interpreted as the consequence of the direct interaction between the rhizobia and altered host physiology/ phenotype due to mutagenic treatments. It is hypothesized that either mutant plants have selected different rhizobial strains from the soil population or that difference have arisen by rhizobial mutation within the host plant.

Experiments related to cross inoculation studies have been initiated and preliminiary results obtained so far indicate that mutation of *Rhizobium* within the host nodule is more convincing. Tentative conclusion drawn needs further confirmation. Present interpretation corroborates the statement of Subba Rao[1], that strong irradiation with gamma rays induce mutations in *Rhizobium* present inside the nodules of irradiated plants.

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