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Characterization of *Rhizobium* Isolated from Gamma Irradiated Plants of *Vigna mungo L.*

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Girish Raisinghani
Associate Professor,
Dept. of Botany,
S.P.C. Government College,
Ajmer, Rajasthan, India

Manesha Mathur
Associate Professor,
Dept. of Botany,
S.P.C. Govt. College,
Ajmer, Rajasthan, India

Renu Sharma
Associate Professor,
Dept. of Botany,
S.P.C. Govt. College,
Ajmer, Rajasthan, India

Abstract

Rhizobium isolated form different gamma irradiated mutagenized plants of *Vigna mungo L.* was subjected to morphological, cultural and biochemical tests to study the efficacy of radiation on the properties of root nodulating bacterium. As compared to native strain obtained from normal plants, 4 isolates (R1, R2, R3, R4) from mutagenized M1 plants showed characteristic variations in their behaviour. Overall recorded data revealed that the isolates R3 and R4 obtained from 40 kR and 60 kR gamma rays irradiated plants of *Vigna mungo L.* showed maximum variations in their cultural and biochemical characteristics. This could be interpreted as the consequence of direct interaction between the rhizobia and altered host physiology due to mutagenic treatments.

Keywords: *Rhizobium, Vigna mungo L.,* Gamma *Rays.*

Introduction

Vigna mungo L. occupies unique position in Indian agriculture and shows remarkable adoption under moisture stress because of its limited water requirement. As in other pulses black gram is capable of fixing atmospheric nitrogen through the symbiotic association of *Rhizobium* which form root nodules and thereby enriches the soil fertility.

Physical mutagens which are mainly radiations are widely used in inducing mutation in biological systems. They may directly break chromosomes or alter DNA bases.

In the present research seeds of *V.mungo* were treated with different doses of gamma radiation and population was raised by sowing these irradiated seeds. Nodules formed by these plants were detached and *Rhizobium* was extracted from these nodules and suspension was made separately for each dose of gamma rays. Each rhizobial suspension was treated as an isolate from irradiated plants and subjected to various tests.

Objectives of the Study

In the present investigation, an important legume *Vigna mungo* L. (common name: black gram) is used as an experimental material. As genus *Rhizobium* play an important role in biological nitrogen fixation through symbiosis in the leguminous plants, rhizobial samples were isolated from the normal and mutagenized M1 plants of *V .mungo* using gamma rays as mutagen with an aim to study the variations in morphological, cultural and biochemical characteristics of the *Rhizobium* caused due to altered physiology of the host resulted due to toxic effect of gamma radiation. Various tests were performed in aseptic conditions and the growth of rhizobial isolates and their response towards various tests were observed. Data was compared with the isolate from normal plants (native strain).

Materials and Methods

The dry seeds of *Vigna mungo L*. were irradiated with gamma radiations (cobalt 60 source) at IARI, New Delhi. Seeds were imposed to Co 60 gamma rays at 10 kR, 20 kR, 40 kR and 60 kR doses. Treated seeds as well as controls (non treated) were sown in earthen pots to raise M1 generation. Thirty five days old plants (normal and treated) were uprooted and their roots thoroughly washed in water. Nodules from the roots were surface sterilized with 0.1% HgCl2 solution and crushed in sterile water. Bacterial suspension from different irradiated and normal plants (control) was separately transferred to petriplates containing YEMA (Yeast Extract Mannitol Agar) medium to observe the growth of *Rhizobium*.

After purification isolates were subjected to various tests such as : Gram's staining, motility test,growth on YEMA medium, GPA medium and Hofer's alkaline medium, H2S production, Indole and 3 keto lactose production, getatin liquification,citrate utilization, starch hydrolysis, nitrate reduction, production of ammonia, catalase and oxidase activity, fermentation of glucose, lactose, sucrose, maltose and mannitol. Observations were recorded and tabulated (Table 1). Comparison was made with an isolate from untreated normal plants(control) to observe their behaviour against various morphological, cultural and biochemical characteristics.

Observations and Results

The native strain from *V.mungo (Rhizobium* cowpea type) was gram negative and motile rod. *Rhizobium* sp. showed luxuriant growth on YEMA medium within 24-48 hr. However, this strain was unable to grow on GPA and Hofer's alkaline medium. Few biochemical properties of the native strain noted were: production of H2S, indole and ammonia, negative for production of 3 keto lactose and liquification of gelatin, non utilization of citrate, positive hydrolysis of starch and reduction of nitrate. Further, oxidase and catalase activity was shown by native strain.Various carbon sources like glucose, lactose, maltose and mannitol were utilized by native strain with acid production.

Rhizobial isolates from gamma rays irradiated population were morphologically similar to the native strain. Four isolates when grown on YEMA medium, R1, R2 and R4 were found to be fast growers (similar to native strain) while R3 was a slow grower. All the four isolates did not grow on GPA medium. Amongst four studied isolates, R4 isolate showed growth on Hofer's alkaline medium. Similar to behaviour of native strain, 3 ketolactose was not produced by any isolate. Likewise, in general, they reduced nitrate, produced ammonia and showed positive enzymatic reaction for catalase and oxidase.

In contrast to characteristics of native strain isolates R2 and R4 did not produce H2S; isolate R3 did not produce indole, R2, R3 could liquify gelatin; R3 and R4 utilized citrate and did not hydrolyse starch and R1 could not show oxidase activity. Studied isolates utilized different carbon sugars for growth except for R1,R3,R4 isolates which did not utilize lactose and maltose.

Conclusion

When compared to the native strain, many rhizobial isolates from mutagenized M1 population showed contrasting results in various tests. In conclusion, overall data recorded for 4 rhizobial isolates revealed that relatively isolates R3 and R4 obtained from 40 kR and 60 kR gamma rays irradiated plants of *Vigna mungo L.* showed maximum variation in their cultural and biochemical characteristics as 6-9 characters were altered in these isolates. Characterization of *Rhizobium* in different legumes and alterations in culturaland physiological characters of *Rhizobium* obtained from normal and irradiated plants are also reported in the past by Jain, 1981; Subba Rao, 1989; Garg, 1991: Mahna *et al*, 1992; Roychowdhary *et. al*, 2015 and Saraf *et.al*., 2018. Major conclusion drawn from the present work done is that variations in cultural and physiological properties of the rhizobial isolates from treated plants are due to the effect of radiations caused in the host plant.

References

1. *Garg, R. (1991). Induced mutations in legumes with special reference to nitrogen metabolism. Ph.D. thesis, M.D.S. University, Ajmer, India*
2. *Jain, P.S. (1981). Studies on the causal organism, development and structure of root nodules and effect of passage in a few summer and winter leguminous weeds. Ph.D. thesis, Univ. of Raj., Jaipur, India*
3. *Mahna, S.K., Garg and M. Parvateesam (1992). Morphological, cultural and biochemical characteristics of root nodule bacteria isolated from induced mutants of Vigna mungo L. Proceedings Vistas in Seed Biology : 264-282*
4. *Roychowdhury, Debojyoti, Manibrata Paul and Sudip Kumar Banerjee (2015). Isolation, Identification and Characterization of bacteria (Rhizobium) from chick pea (Cicer arietinum) and production of biofertilizer. European Journal of Biotechnology and Bioscience. Vol 3: Issue 12; 26-29.*
5. *Saraf, Renuka, Shweta Chaudhary and Dipjyoti Chakraborty (2018). Isolation and characterization of osmotolerant Rhizobium sp. from Vigna mungo L Hepper grown in semi arid region. International Journal of Plant Research: 31.*
6. *Subba Rao, N.S. (1989). Soil micro organisms and plant growth. Oxford and IBH Publishing, New Delhi, India.*

Table 1 Morphological, Cultural and Biochemical Properties of Rhizobial isolates obtained from mutagenized population (M1) of *Vigna mungo L.*

S.N	Rhizobial Characteristics+		Isolates			
		Control	R1	R2	R3	R4
1	Gram's Reaction	-	-	-	-	-
2	Motility Test	+	+	+	+	+
3	Growth of YEMA Medium	++++	++++	++++	+++	++++
4	Growth on GPA Medium	-	-	-	-	-
5	Growth on Hofer's Alkaline Medium	-	-	-	-	+
6	H2S Production	+	+	-	+	-

7	Indole Production	+	+	+	-	+
8	Production of 3 ketolactose	-	-	-	-	-
9	Gelatin liquification	-	-	+	+	-
10	Citrate Utilization	-	-	-	+	+
11	Starch Hydrolysis	+	+	+	-	-
12	Reduction of Nitrate	+	+	+	+	+
13	Production of Ammonia	+	+	+	+	+
14	Catalase Activity	+	+	+	+	+
15	Oxidase Activity	+	-	+	+	+
16	Fermentation of Glucose	GAp	GAp	GAp	GAp	GAp
17	Fermentation of Lactose	GAp	-	GAp	-	-
18	Fermentation of Sucrose	GAp	GAp	GAp	GAp	GAp
19	Fermentation of Maltose	GAp	-	GAp	-	-
20	Fermentation of Mannitol	GAp	GAp	GAp	GAp	GAp

Key : - = Negative test G = Gas Evolution
+ = Positive Test Ap = Acid Production
++ = Growth after 72 h
+++ = Growth within 48-72 h
++++ = Growth within 24-48 h