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# DIFFERENCE BETWEEN NATIVE AND MUTANT RHIZOBIUM ON GROWTH OF *VIGNA MUNGO* L.

Shijila Rani AS [1], P. Parthiban [*1] and V. Mahesh [2]

Department of Microbiology [1], Marudupandiyar College, Vallam, Thanjavur, Tamilnadu, India.
MR Govt. Arts and Science College [2], Mannargudi, Tamilnadu, India.

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*Mimosa pudica* L, *Rhizobium* strain, mutated agents, *Vigna mungo* L

**Correspondence to Author:**
**P. Parthiban**

Research Scholar
Marudupandiyar College,
Vallam, Thanjavur - 613403, Tamilnadu, India.

**Gmail:** jprpaul2010@gmail.com

**ABSTRACT:** The present study, carry out the use of plant growth promoting rhizobacteria, were used to improve the yield in crop. The *Rhizobium* strains isolated from legumes plant *Mimosa pudica* L and this strain was mutated by using UV radiation. The plant growth hormones were detected by native as well as mutant *Rhizobium* strains by paper chromatography techniques. The same strains use in conducted to green house; the compared to two strains of *Rhizobium*, mutated strain are high growth and yield when compared to normal stain.

**INTRODUCTION:** Rhizobia encompass a range of bacterial genera, including *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Allorhizobium* and *Azorhizobium*, which are able to establish a symbiosis with leguminous plants. They elicit the formation of specialized organs, called nodules, to reduce atmospheric nitrogen and make it available to the plant [1].

In legume-*Rhizobium* symbioses, variabilities in symbiotic effectiveness which are either due to variations in nitrogen fixing potential of *Rhizobium* strains [2, 3, 4] or due to host genotypic compatibility of DNA based detection technology that plastids and mitochondria of the eukaryotic cell were derived from a consortium of primitive microbes [5, 6, 7] are often observed.

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The common capacity of such symbiosis to reduce dinitrogen to ammonia and to incorporate this product into the nitrogen metabolic stream of the host plant gives the genus a place of outstanding importance in natural ecosystems and agricultural production.

In genetics, a mutagen is a physical or chemical agent that changes the genetic material, usually DNA, of an organism and thus increases the frequency of mutations above the natural background level. As many mutations can cause cancer, mutagens are therefore also likely to be carcinogens. For example of chemical mutagens are ethidium bromide, Acridine dye, etc and physical mutagens are X-rays, alpha rays, gamma rays, beta rays, cosmic rays, they distort or break DNA duplex and disturb the replication. Ultraviolet rays are non-ionizing radiations and produce thymine dimers. In this paper, they are report the symbiotic behaviour of these mutants in relation to the parent strain.

## MATERIALS AND METHODS:

**Sample collection:** Root nodules were collected from young and healthy seedling plants of *Mimosa*

*pudica L*, plant from Oruthanadu, Thanjavur (dist), Tamil nadu, India.

**Isolation of Rhizobium sp from root nodules:** To select, healthy and reddish-pink root nodules were tenderly washed with distilled water and surface sterilized by keeping in 0.1 percent $HgCl_2$ for 4-5 min and wash with sterile distilled water following by 95 percent ethyl alcohol, repeat washing with sterile distilled water [8]. The nodules were crushed in a small drop of sterile distilled water to obtain milky suspension. Then, serial dilutions were made and aliquots dilutions were spread on yeast extract mannitol agar (YEMA) medium plates were incubated at 28±1°C for 3-5 days [9].

**Identification and biochemical test of Rhizobium sp:** The isolated colonies are conformed by microscopic observation, biochemical test, [10] and cultural test were performed. Isolates were identified as per Bergey's manual of Systematic bacteriology.

**Identification of IAA:** Biosynthesized auxins were partially characterized by paper chromatography method [11]. In which three, grams of culture filtrate were extracted with 50 ml of peroxide-free ether for 2 hours at 5ºC. Extracts of the samples were simultaneously loaded and developed using isopropanol: ammonia:water (10:1:1 v/v/v) as a running solvent. The appearance of the strips under ultraviolet light and spraying with modified Salkowski reagent is indicated.

**Effect of native and mutant Rhizobium on growth of *Vigna mungo* L:** The pot trails were accomplished by Department of Microbiology Marudupandiyar College, Vallam, Thanjavur [12]. Each one had conducted triplicates. The treatments follow as:

T1 Control (with out *Rhizobium*), T2 *Rhizobium* (R Mutant seed inoculation), T3 *Rhizobium* (R Native seed inoculation), T4 *Rhizobium* (R Mutant seed inoculation + foliar spray), T5 *Rhizobium* (R Native seed inoculation + foliar spray).

After germination, plants were treated with 48 hrs old culture, which were foliar sprayed to the *Vigna mungo* plants every week for 4 times, starting from 10th day of germination. The plants were harvested every 15th day of intervals, after harvesting to analyse agronomical characters such as morphometric and number of root nodules analyse in standard procedure.

**RESULT AND DISCUSSION:** The isolates of rhizobia from root nodules of legume plants were characterized by opaque and milky white in appearance in YEMA medium. The rhizobial strains were isolated from the nodules of Mung bean [13], *Pisum sativum* [14] and *Cassia alata* [15]. The rhizobial strains were identified in microscopic and biochemical test. The result were compared with Bergey's manual of Determinative Bacteriology 9th edition and conformed as Rhizobium. Similarly, Singh *et al*. [16] also characterized *Rhizobium* strains on the basis of biochemical tests. The native strain of *Rhizobium* was induced by UV mutagen treated with different time intervals, (1 to 6 min). Out of six minutes treatment, four minutes were observed in maximum number of colonies (3.0±2.12) compared to other treatments recorded in **Table 1**. So, further study we have chosen in four minutes mutated isolates.

**TABLE 1: NATIVE RHIZOBIUM SPECIES ARE SPONTANEOUS MUTATION BY DIFFERENT TIME**

| S.no | Native Rhizobium treated with UV in different time (min) | No. of colonies |
|---|---|---|
| 1 | Control (untreated) | 4.09±2.04 |
| 2 | One | 1±0.7 |
| 3 | Two | 0.5±0.35 |
| 4 | Three | 2.5±1.7 |
| 5 | Four | 3.0±2.12 |
| 6 | Five | 1.5±1.06 |
| 7 | Six | 1.5±1.06 |

These Rhizobial strains were use in separation of Auxin and Gibberellin compounds by paper chromatography method by **Table 2**. Some workers reported by different legume nodulating rhizobial strains preferred different vitamins sources for IAA production reported [17, 18].

**TABLE 2: SEPARATION OF PLANT GROWTH HORMONE BY PAPER CHROMATOGRAPHY TECHNIQUES**

| S.no | Plant growth hormone | Native strain | Mutant strain |
|---|---|---|---|
| 1 | Auxin | 0.52 | 1.07 |
| 2 | Gibberellins | 0.7 | 1.3 |

The pot trial was conducted to study the response of *Vigna mungo* to inoculation with *Rhizobium* and foliar application. The result revealed that the black

gram responded well to the inoculation of *rhizobium.* The plants inoculated with mutant *Rhizobium* possessed significantly greater plant height, dry weight and nodules formation was monitor, the UV mutant *Rhizobium* species were high responsible for the plant growth (**Table 3, 4** and **5**). The current observations of the pot experiment of pea was close to Fischer *et al.* [19], who find out the ability of *Rhizobium* on wheat by development of shoot/ root fresh and dry weights. All the *Rhizobium* strains improved the root and shoot dry biomass by 100% and 70% respectively. The current conclusion goes to the results [20, 21], who obtained 70% increase in pea root/shoot dry biomass by PGPRs inoculation as contrast to control (uninoculated).

**TABLE 3: 15TH DAY MORPHOMETRIC ANALYSIS OF *V.MUNGO* PLANT**

| S.no | Parameters | T1 | T2 | T3 | T4 | T5 | T6 |
|---|---|---|---|---|---|---|---|
| 1 | Shoot length (cm) | 20.5 | 21.3 | 16.5 | 21.2 | 14.8 | 22.4 |
| 2 | Root length (cm) | 6.7 | 4.5 | 5.5 | 6.5 | 6.7 | 6.3 |
| 3 | Plant height (cm) | 27.2 | 25.8 | 22.0 | 27.7 | 20.5 | 28.7 |
| 4 | Leaf length (cm) | 4.7 | 4.7 | 4.4 | 5.5 | 4.4 | 5.6 |
| 5 | Leaf width (cm) | 1.6 | 1.7 | 1.7 | 1.7 | 1.5 | 1.8 |
| 6 | Leaf area (cm) | 3.7 | 3.8 | 3.7 | 4.3 | 3.3 | 5.0 |
| 7 | No. of nodules (Nos) | - | - | - | - | - | - |
| 8 | Plant fresh weight (mg) | 50 | 75 | 120 | 200 | 110 | 190 |
| 9 | Plant dry weight (mg) | 0.2 | 0.5 | 0.9 | 1.0 | 0.7 | 0.8 |

T1 Control (with out *Rhizobium*), T2 *Rhizobium* (R Mutant seed inoculation), T3 *Rhizobium* (R Native seed inoculation), T4 *Rhizobium* (R Mutant seed inoculation + foliar spray), T5 *Rhizobium* (R Native seed inoculation + foliar spray) T6 Combined effect of Native and *Rhizobium.*

**TABLE 4: 30TH DAY MORPHOMETRIC ANALYSIS OF *V.MUNGO* PLANT**

| S.no | Parameters | T1 | T2 | T3 | T4 | T5 | T6 |
|---|---|---|---|---|---|---|---|
| 1 | Shoot length (cm) | 22.0 | 23.1 | 18.0 | 23.9 | 16.4 | 24.6 |
| 2 | Root length (cm) | 7.2 | 7.4 | 6.4 | 8.0 | 7.0 | 8.3 |
| 3 | Plant height (cm) | 29.2 | 30.5 | 24.4 | 31.9 | 23.4 | 32.9 |
| 4 | Leaf length (cm) | 3.8 | 5.3 | 5.1 | 5.2 | 5.3 | 5.9 |
| 5 | Leaf width (cm) | 2.1 | 2.3 | 2.1 | 2.5 | 2.1 | 2.3 |
| 6 | Leaf area (cm) | 4.0 | 6.1 | 5.4 | 6.5 | 5.6 | 6.8 |
| 7 | No. of nodules (Nos) | 1 | 2 | 2 | 5 | 1 | 2 |
| 8 | Plant fresh weight (mg) | 200 | 300 | 200 | 400 | 250 | 400 |
| 9 | Plant dry weight (mg) | 2.0 | 5.0 | 5.0 | 10.0 | 3.5 | 5.0 |

T1 Control (with out *Rhizobium*), T2 *Rhizobium* (R Mutant seed inoculation), T3 *Rhizobium* (R Native seed inoculation), T4 *Rhizobium* (R Mutant seed inoculation + foliar spray), T5 *Rhizobium* (R Native seed inoculation + foliar spray), T6 Combined effect of Native and *Rhizobium*.

**TABLE 5: 45TH DAY MORPHOMETRIC ANALYSIS OF *V.MUNGO* PLANT**

| S.no | Parameters | T1 | T2 | T3 | T4 | T5 | T6 |
|---|---|---|---|---|---|---|---|
| 1 | Shoot length (cm) | 21.6 | 24.2 | 21.3 | 23.5 | 22.0 | 24.3 |
| 2 | Root length (cm) | 9.4 | 9.8 | 11.4 | 11.6 | 12.5 | 13.0 |
| 3 | Plant height (cm) | 31.0 | 35.0 | 32.7 | 34.9 | 34.5 | 37.3 |
| 4 | Leaf length (cm) | 4.4 | 5.9 | 4.5 | 5.8 | 6.0 | 6.3 |
| 5 | Leaf width (cm) | 2.8 | 3.0 | 2.9 | 3.5 | 3.1 | 3.6 |
| 6 | Leaf area (cm) | 6.2 | 8.9 | 6.5 | 10.2 | 9.3 | 11.3 |
| 7 | No. of nodules (Nos) | 2 | 2 | 6 | 10 | 2 | 5 |
| 8 | Plant fresh weight (mg) | 550 | 800 | 600 | 900 | 700 | 850 |
| 9 | Plant dry weight (mg) | 5.0 | 17.5 | 10.0 | 20.0 | 10.0 | 15.0 |

T1 Control (with out *Rhizobium*), T2 *Rhizobium* (R Mutant seed inoculation), T3 *Rhizobium* (R Native seed inoculation), T4 *Rhizobium* (R Mutant seed inoculation + foliar spray), T5 *Rhizobium* (R Native seed inoculation + foliar spray) T6 Combined effect of Native and *Rhizobium*.

**CONCLUSION:** In the current investigation, the UV mutant *Rhizobium* sp were treated by *V.mungo* L., the foliar spray treatment seen highly responsible growth in plant height, leaf area and no. of root nodules, while only treated UV mutant *Rhiozobium* sp. when compared to control plant.

From this study conclude by some mutation occur in DNA basepair (AGTC), so get high results.

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