

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.709.421>

## Mutagenic Effects of Gamma-rays and EMS on M<sub>1</sub> Population of Rice Bean (*Vigna umbellata* Thunb, Ohwi and Ohashi)

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### ABSTRACT

Genetically pure seeds of rice bean (*Vigna umbellata* Thunb., Ohwi & Ohashi) variety viz., RBL 50 was used for induction of mutation by using three concentrations (0.2%, 0.4% and 0.6%) of Ethyl Methanesulphonate (EMS) and three doses of gamma rays (150Gy, 200Gy and 250Gy) to study the effect of mutagens on the M<sub>1</sub> population of the crop. Observations on thirteen characters were recorded and subjected to appropriate statistical analysis based on the sample mean. Numerical Taxonomy (Sneath and Sokal, 1973) method was followed to classify the mean values of all the quantitative data for all M<sub>1</sub> traits to find out the similarities among different mutagenic treatments. Invariable reduction in means of germination (%), initial plant population, plant height, length of pod and number of seeds/pod and a reverse trend for days to 50% flowering, days to maturity, branches/plant, pods/cluster, pods/plant, 100-seed weight and seed yield per plant was noticed in both the mutagenic treated populations compared to the mean of the corresponding character in control. Dose dependent reduction in means of characters for germination (%), initial plant population, plant height and seed yield per plant was observed in all the mutagenic treatments. Similar trend was also observed for clusters/plant, branches/plant, length of pod in gamma rays treatments and pods per plant and seeds per pod in EMS treated populations. Mutagenic treatments with below LD<sub>50</sub> dose of mutagens were grouped in Cluster-I (0.2% EMS, 150Gy and 200Gy), EMS (0.6%) in Cluster-II, medium dose mutagenic treatments (0.4% EMS and 250Gy) in Cluster-III and control in Cluster-IV. Grouping of mutagenic treatments of different concentrations in different clusters indicated the phenological differences among the treatments.

#### Keywords

Gamma rays, EMS,  
Mutagenic effects,  
Rice bean

#### Article Info

##### Accepted:

24 August 2018

##### Available Online:

10 September 2018

### Introduction

Rice bean (*Vigna umbellata* Thunb., Ohwi & Ohashi), a non-traditional and under-utilized legume crop of the world has drawn the attention of the researchers for its high

nutritional values, multiple uses and adaptive polymorphism for diverse and extreme environments. In Asia, the crop is primarily grown for food purposes and to a limited scale as fodder. Its distribution is sporadic and is confined to North-Eastern hill regions and

hilly tracts of Eastern and Western Ghats in Peninsular India (Arora *et al.*, 1980). Traditionally, the tender pods are used as vegetables and the whole mature seeds are primarily used for preparation of tadka, dal, snacks, soup, dessert, pastry and pickles (Wei *et al.*, 2015). Rice bean maintains high nutritional value compared to other pulses in vigna group (Katoch, 2013). The seeds contain 25% protein, 0.49% fat and 5% fiber; and are rich in amino acid and vitamins (Lohani, 1979).

Limited cultivation and poor adaptability accompanied by continuous natural selection caused narrow genetic base of the crop and resulted in eroding the genes responsible for high productivity. Creation of genetic variability to broaden the genetic base either through hybridization and induced mutagenesis is the only alternative to save the crop from further elimination. In the present investigation, an attempt was undertaken to study the effect of mutagens (Gamma-rays and EMS) on  $M_1$  populations as an indirect measure of the extent of induced genetic variability in the follow-up generations in rice bean.

## Materials and Methods

An experiment was conducted in the Experimental Block of Instructional Farm of Odisha University of Agriculture & Technology during Kharif, 2016. Five hundred numbers of genetically pure and matured dry seeds of rice bean variety *viz.*, RBL 50 were treated with different doses of gamma rays (150Gy, 200Gy and 250Gy) at Bhaba Atomic Research Centre (BARC), Trombay, India. Besides, the 500 seeds of the above variety were treated with Ethyl Methanesulphonate (EMS) at different concentrations (0.2%, 0.4% and 0.6%EMS) after pre-soaking in distilled water for a period of 14.0h. A separate lot of 500 seeds pre-

soaked simply in distilled water for 14.0h served as untreated control. The above three seed lots each treated with gamma rays and EMS along with the untreated control were sown in the field to raise  $M_1$  generation in Randomized Block Design (RBD) with three replications. Recommended agronomic practices were followed to raise the crop. Observations on thirteen characters were recorded and subjected to appropriate statistical analysis based on the sample mean. Numerical Taxonomy (Sneath and Sokal, 1973) method was followed to classify the mean values of all the quantitative data for all  $M_1$  traits. Extent of similarity among different mutagenic treatments and control were measured by using Gower's similarity coefficient (SG) and dendrogram was constructed following SHAN (Sequential Hierarchical Agglomerative and Non-overlapping) clustering strategy.

$$S_G = \frac{1}{N} S_{ijk}$$

Where,  $S_{ijk}$  is the contribution of the  $i^{\text{th}}$  character to the total distance between  $j$  and  $k$  individuals.

$$\text{Where, } S_{ijk} = 1 - \frac{|X_{ij} - X_{ik}|}{R_i}$$

$X_{ij}$  and  $X_{ik}$  are quantitative estimates of  $i^{\text{th}}$  character for Operational Taxonomic Units (OTU) on  $j$  and  $k$ , respectively,

' $R_i$ ' is the range of the character  $i$ ;

' $N$ ' is the number of characters.

Finally, the clusters were identified at appropriate (70% similarity) phenon level.

Further, the means of all the thirteen  $M_1$  characters were brought to one scale (0 to 1)

following the scaling procedure suggested below to estimate the overall effect of each mutagenic treatment in  $M_1$  generation.

$$\frac{(X_{ij} - X_{im})}{R_i}$$

Where,  $X_{ij}$  is the mean of the  $i$ th character of the  $j$ th treatment.

$X_{im}$  is the lowest value of  $i$ th character.

$R_i$  is the range of the character.

After scaling, the score for different characters were pooled to find  $M_1$  pooled mean which reflects the overall effect of a treatment. Pooled mean in respect of different clusters/classes were found out for characterizing the clusters based on the estimates of overall effect of mutagenic treatments.

## Results and Discussion

### Effect of mutagen on mean values of different characters in $M_1$

*In vogue*, embryo in each seed is the target mutagenic effect. The primary effect of mutagen in the mutation breeding programme is therefore noticed immediately in the  $M_1$  generation in the form of reduction in germination, survival per cent, plant height, growth and productive traits with some heritable changes in the plants (Kalusinh, 2015; Veni *et al.*, 2016 and Lavanya *et al.*, 2017). The present investigation revealed invariable reduction in means of germination percentage, initial plant population, plant height, length of pod and number of seeds per pod in both the mutagenic treated populations compared to the mean of the corresponding character in control (Table 1). However, a reverse trend was also noticed in all the mutagenic treatments for characters like days

to 50% flowering, days to maturity, branches per plant, pods per cluster, pods per plant, 100-seed weight and seed yield per plant. Invariably, dose dependent reduction in means of characters for germination per cent, initial plant population, plant height and seed yield per plant was observed in all the mutagenic treatments. Besides, the extent of reduction in mean values was dependent on kind of mutagens used suggesting sensitivity of plant characters to the specific mutagen. Considerable reduction in clusters per plant, branches per plant, length of pod was revealed in gamma rays treatments, while, EMS treated populations showed decrease in mean values of pods per plant and seeds per pod. The shift in mean of characters in  $M_1$  population was also reported by several workers in different pulse crops (Sevalm *et al.*, 2010; Dhasarathan *et al.*, 2014 and Lavanya *et al.*, 2017 in blackgram; Kumar *et al.*, 2010 and Olasupo *et al.*, 2016 in cowpea). The possible reason for the reduction in growth and plant characteristics could be due to magnitude of biological damage (seedling injury, seedling height, sterility etc.) caused by mutagens at cellular level (Patial *et al.*, 2015). The reduction in germination per cent in the treated population in rice bean could be attributed to the disturbance in the enzyme system involved in the repair mechanism or due to the toxic substances produced in the treated cells (Patial *et al.*, 2015). Reduction in plant height, length of pod and number of seeds per pod in  $M_1$  plants of other pulse crops have been reported earlier by many researchers (Sevalm *et al.*, 2010; Kumar *et al.*, 2010 and Olasupo *et al.*, 2016 in cowpea; Kalusinh, 2015 in green gram; Lavanya *et al.*, 2017 in black gram). In the present investigation, higher mean values were realized for characters like days to 50% flowering, days to maturity, branches per plant, pods per cluster, pods per plant, 100-seed weight and seed yield per plant in mutagenic treatment as compared to control.

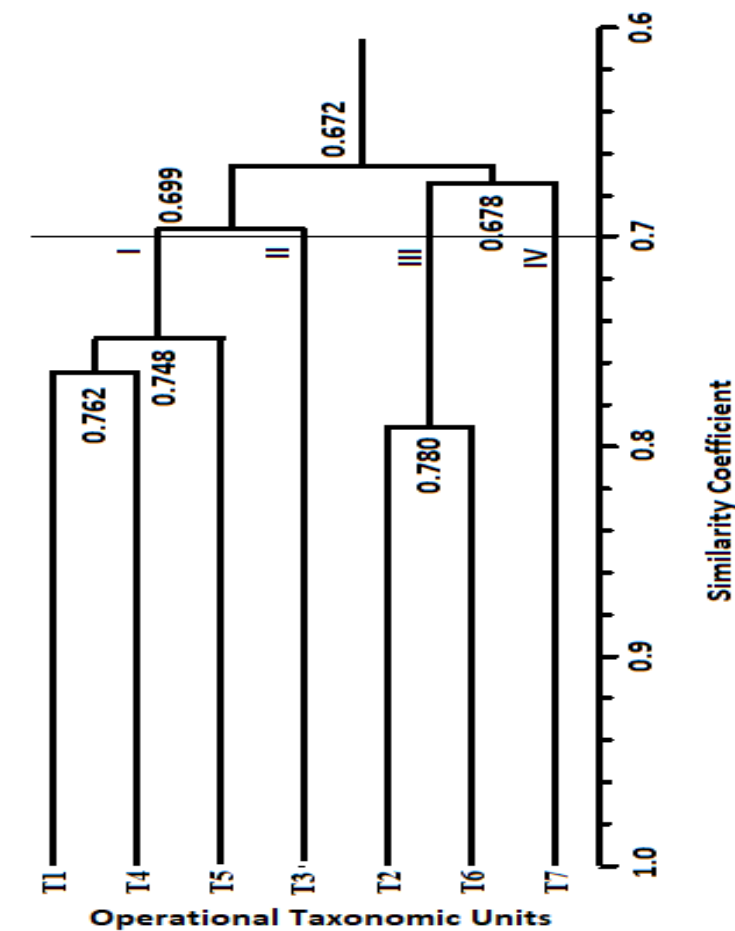
This supports the findings of Hanafiah *et al.*, (2010) in soybean and Veni *et al.*, (2016) in black gram.

**Classification of Mutagenic Treatments based on M<sub>1</sub> response**

Usually, mean along with variability parameters e.g., standard deviation, coefficient of variation, standard error is primarily taken into consideration to assess extent of induced variability in a set of mutagenic populations. In fact, the genetic variability induced in M<sub>1</sub> is said to be hidden and it becomes widely accessible in M<sub>2</sub> onwards for phenotypic

selection of desirable plants. However, the authors attempted to explore the mutagenic effect if any in M<sub>1</sub> itself by grouping all mutagenic populations using erstwhile mentioned phenotypic mean values of thirteen different characters. In this context, Numerical Taxonomy method was followed to classify the mutagenic treatments based on their overall response to mutagens used (Sneath and Sokal, 1973) and similarity between different treatments was expressed as Operational Taxonomic Units (OTUs). Dendrogram was constructed by utilizing the S<sub>G</sub> values following the SHAN clustering strategy (Fig. 1).

**Fig.1** Dendrogram showing clusters of OTUs (mutagenic treatments) in rice bean



**Dendrograms showing clusters of OTUs**

**Table.1** Means of different characters studied in M<sub>1</sub> generation of rice bean

Treatments	Germination (%)	Initial Plant Population	Days to 50% flowering	Days to maturity	Plant height	Clusters /plant	Branches / plant	No. of pods / cluster	No. of pods	Length of pod (cm)	No. of seeds /pod	100 –seed weight	seed yield /plant (g)
T <sub>1</sub> (0.2% EMS)	60.0	96.0	61.3	96.3	79.8	13.3	4.1	1.9	22.1	8.0	7.0	4.5	6.99
T <sub>2</sub> (0.4% EMS)	56.7	90.7	58.0	93.7	77.1	10.0	3.8	1.8	18.3	7.5	6.9	4.7	6.20
T <sub>3</sub> (0.6% EMS)	40.0	64.0	63.3	96.3	73.6	11.9	4.1	2.1	18.2	8.4	6.7	4.7	5.14
T <sub>4</sub> (150Gy)	79.8	127.7	62.7	96.3	82.7	13.9	4.3	1.9	19.1	7.9	6.8	4.7	7.53
T <sub>5</sub> (200Gy)	73.1	117.0	60.7	97.0	80.6	11.7	3.9	2.1	25.5	7.9	7.4	4.7	6.69
T <sub>6</sub> (250Gy)	66.7	106.7	60.7	95.3	75.9	10.5	3.8	1.9	15.3	7.7	7.1	4.8	5.81
Control	85.4	136.7	59.3	93.0	84.1	10.7	3.6	1.9	17.4	8.0	7.5	4.5	4.76
Mean	66.0	105.5	60.9	95.4	79.1	11.7	3.9	2.0	19.4	7.9	7.1	4.6	6.16
SEM (±)	3.5	5.6	0.8	1.2	3.3	0.5	0.2	0.1	1.7	0.1	0.2	0.1	0.60
CD (0.05)	10.8	17.3	2.5	3.6	10.1	1.6	0.7	0.3	5.2	0.4	0.7	0.2	1.86
CV (%)	9.2	9.2	2.3	2.1	7.1	7.6	9.6	9.0	15.1	2.9	5.4	2.9	16.99

**Table.2** Classification of mutagenic treatments in M<sub>1</sub> generation of rice bean

Cluster	Cluster Composition	M <sub>1</sub> pooled mean	Characteristic feature of the cluster
I	T <sub>1</sub> (0.2% EMS) T <sub>4</sub> (150Gy) T <sub>5</sub> (200Gy)	0.467	Cluster comprised of two mutagenic treatments of lower dose (0.2% EMS and 150Gy) and one moderate dose of gamma rays treatment (200Gy). The M <sub>1</sub> pooled mean was the highest signifying least biological damage.
II	T <sub>3</sub> (0.6% EMS)	0.329	Monotypic cluster comprised of only one mutagenic treatment of higher dose. M <sub>1</sub> pooled mean was the lowest signifying highest biological damage.
III	T <sub>2</sub> (0.4% EMS) T <sub>6</sub> (250Gy)	0.435	Cluster containing moderate dose of EMS mutagenic treatment (0.4% EMS) and higher dose of gamma rays treatment with moderate M <sub>1</sub> pooled mean signifying moderate biological damage.
IV	T <sub>7</sub> (Control)	0.660	Monotypic cluster comprised of only control with highest M <sub>1</sub> pooled mean signifying no biological damage.

Seven treatments (three gamma ray treatments, three EMS treatments and one control) were grouped in to four distinct clusters at 70% phenon level. Mutagenic treatments with below LD<sub>50</sub> dose of mutagens were grouped in Cluster-I (0.2% EMS, 150Gy and 200Gy), while the higher dose of EMS alone (0.6%) appeared in Cluster-II. Medium dose mutagenic treatments (0.4% EMS and 250Gy) were grouped in Cluster-III. The monotypic cluster e.g., Cluster IV comprised of only the control population with highest M<sub>1</sub> pooled mean signifying no biological damage. Grouping of mutagenic treatments of different concentrations in different clusters clearly indicated the phenological differences among the treatments (Singh and Rao, 2007 and Mishra and Singh, 2013).

Further, each cluster was characterized based on the average of M<sub>1</sub> pooled mean estimates of all the thirteen characters (Table 2). Cluster-IV represented with the control exhibited the highest M<sub>1</sub> pooled mean of 0.660. Whereas, the monotypic Cluster-II represented with the highest mutagenic dose of EMS (0.6%) had the lowest M<sub>1</sub> pooled mean (0.329). Moderate M<sub>1</sub> pooled means were estimated for the cluster representing lower and moderate doses of both the mutagenic treatments. Grouping of 0.4% EMS and higher dose of gamma rays (250Gy) in Cluster-III exhibited moderate M<sub>1</sub> pooled mean (0.435). Decrease in M<sub>1</sub> pooled mean in clusters with higher mutagenic dose clearly revealed more of biological damage in higher doses of mutagenic treatment. The results was in conformity with the earlier findings of Singh and Rao (2007) and Mishra and Singh (2013) in green gram and Patial *et al.*, (2015) in rice bean.

### Acknowledgement

The authors highly acknowledge AICRN on Potential Crops, OUAT, Bhubaneswar for

providing facilities to conduct the experiment and ICAR, New Delhi for financial help.

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**How to cite this article:**

Dayanidhi Mishra, Lopamudra Bhoi, Swapan K. Tripathy, Mohima Prasad Behera and Tapas K. Mishra. 2018. Mutagenic Effects of Gamma-rays and EMS on M<sub>1</sub> Population of Rice Bean (*Vigna umbellata* Thunb, Ohwi and Ohashi). *Int.J.Curr.Microbiol.App.Sci.* 7(09): 3393-3399. doi: <https://doi.org/10.20546/ijcmas.2018.709.421>