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Mutagenic effects on frequency and spectrum of chlorophyll mutations in rice bean (*Vigna umbellata* Thunb, OHWI & OHASHI)

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Abstract
An experiment was conducted to study the mutagenic effects on frequency and spectrum of chlorophyll mutations in M_2 generation of rice bean (*Vigna umbellata* Thunb., Ohwi & Ohashi) treated with three doses each of gamma rays (150Gy, 200Gy and 250Gy) and Ethyl Methanesulphonate (EMS) (0.2%, 0.4% and 0.6%). Wide spectrum of chlorophyll mutations were induced which included chlorina, xantha, albina, viridis and sectorial. Among these, chlorina was the most frequent whereas, albina type appeared to be the least in treated populations indicating high mutability of the genes encoding chlorophyll biosynthesis. Total frequency of chlorophyll mutations was higher in gamma rays treatments as compared to EMS due to higher sensitivity of the genotype to gamma rays. Variation in magnitude of total chlorophyll frequency in different doses of mutagenic treatments clearly indicates the differential response of genotype to the doses in producing the chlorophyll mutations. Higher chlorophyll mutation frequency was recovered in lower doses of mutagens and this could be due to induction of minimum lethality at lower doses. The spectrum and frequency of chlorophyll mutations could serve as an index of induced genetic variations in M_2 and follow-up generations.

Keywords: Chlorophyll mutations, EMS, Gamma rays, rice bean (*Vigna umbellata*)

Introduction

Rice bean (*Vigna umbellata* Thunb., Ohwi & Ohashi), an under-utilized legume crop is primarily grown for food purposes and to a limited extent as fodder and green manure in Asia. The distribution of the crop is confined to North-Eastern hill regions and hilly tracts of Eastern and Western Ghats in Peninsular India (Arora *et al.*,1980) [1]. This non-traditional pulse has gradually gained its attention as supplementary food crop (Gruere *et al.*, 2006) [8] for which it forms an important component of the cereal-based dietary system among the vegetarian population of the country. The nutritional values of the seeds contain 25% protein, 0.49% fat and 5% fiber and rich in amino acids (methionine and tryptophan) and vitamins (Lohani, 1979) [14].

The limited cultivation, specific adaptability coupled with natural selection have eroded important genes leading to low genetic diversity (narrow genetic base) and productivity in the crop. There is a need to increase the genetic variability and broadening the genetic base of the crop either through hybridization or induced mutagenesis. Gustafsson (1947) [10] advocated for mutation breeding approach as superior to other conventional breeding methods by using physical and chemical mutagens. However, reports on mutagenesis work in rice bean are very limited as compared to other crops including pulses.

Induction of chlorophyll mutations in an induced population indicates the most reliable indices in evaluating the efficiency of mutagens used, besides inducing genetic changes for further crop development (Usharani and Kumar, 2015) [23]. It is also used as a genetic symbol in basic and applied research (Wani and Anis, 2004) [24]. In the present investigation, an attempt was made to study the effect of both gamma rays and EMS in different doses on spectrum and frequency of chlorophyll mutations induced in M_2 generation in rice bean variety RBL 50.

Materials and Methods

An experiment was conducted in the Experimental Block of Instructional Farm of Odisha University of Agriculture &Technology during Kharif, 2016. Genetically pure and matured seeds of rice bean variety *viz*; RBL 50 was used for induction of mutation by using three doses each for Ethyl Methanesulphonate (EMS) (0.2%, 0.4% and 0.6%) and gamma rays (150Gy, 200Gy and 250Gy). The treated seeds were sown in the field to raise the M_1 generation. Seeds of all the M_1 plants from each treatment were harvested in bulk and used to grow the M_2 generation in a Randomized Block Design with three replications.

Recommended agronomic practices and appropriate plant protection measures were taken up during the crop growth period.

Chlorophyll mutants (chlorina, xantha, albina, viridis and sectorial chimaera) were recorded daily starting from 5th to 12th day after sowing. These were described and classified according to Gustafsson (1940) [9] as below:

Chlorina: Leaves with persistent yellowish green colour. The mutation is usually viable.

Xantha: A lethal mutation in which the leaf colour varies from orange yellow to yellowish white. It does not survive beyond 2-3 leaf stage.

Albina: A lethal mutation which is characterized by white leaves of the seedlings which persist only for a few days after germination and then it withers and dies.

Viridis: It is characterized by yellowish green colour leaves in the beginning and subsequently the colour changes to green. In contrast to albina and xantha, viridis is viable mutation.

Sectorial: A viable mutation in which the green leaves has either longitudinal yellow or white strips in the green background. The number of chlorophyll mutants over three replications were divided by the total number of plants and were expressed in percentage to estimate the mutation frequencies as per the formula described below.

$$\text{Chlorophyll mutation frequency} = \frac{\text{Total number of chlorophyll mutants}}{\text{Total number of plants observed}} \times 100$$

Results and Discussion

Induction of mutations by gamma rays and EMS have been well established as the tools for creation of genetic variability both for qualitative and quantitative traits in different pulse crops (Pandey and Pandey, 2010; Ramaya, 2014 and Kalusinh, 2015) [18, 11]. Chlorophyll mutations in M_2 populations have been reported by several workers in of different crops (Devi *et al*., 2002 [3] in rice bean; Gandhi *et al*. (2014) [4], Mishra *et al*. (2013) [15], Mishra and Singh (2014) [14] in green gram; Lal *et al*.(2009) [13], Bhosale and Hallale (2011) [2], Ramchander (2017) [20] in black gram; Nair and Mehta (2014) [17] in cowpea and Patial *et al*. (2015) [19] in rice bean)**.** As many as 300 genes are *in vogue* involved in biosynthesis chlorophyll. Thus, chlorophyll mutations induced in mutagenic treated populations seem to be the initial genetic effects of mutagen used (Goyal and Khan, 2010) [7] which serve as an index of induced genetic variations in M_2 and follow-up generations.

In the present study, a wide spectrum of chlorophyll mutations such as chlorina, xantha, albina, viridis and sectorial were observed in M_2 generation of all the mutagenic treatments (Table 1 and Figure 1). Among different types of chlorophyll mutations, chlorina was the most frequent (1.2%) whereas albina types appeared in the least frequency (0.22%) in treated populations. Similar trend was reported by earlier workers in different pulse crops (Girija and Dhanavel, 2009 and Gnanamurthy and Dhanavel, 2014 in cowpea; Mishra and Singh, 2014 and Rukesh *et al*,. 2017 in green gram) [5, 6, 15, 22]**.** Highest frequency of chlorina types among all the chlorophyll mutants reveals the high mutability of the gene controlling the character. Other chlorophyll mutations were in order of viridis (0.65%), xantha (0.43%) and Sectorial (0.27%). Total frequency of chlorophyll mutations was higher in gamma rays treatments as compared to EMS treatments due to higher sensitivity of the genotype to gamma rays (Kumar *et al*., 2010 in cowpea; Gandhi *et al*., 2014 in green gram) [4]. Variation in magnitude of total chlorophyll frequency observed in different doses of mutagenic treatments clearly indicates the differential response of genotype to the doses of mutagenic treatments in producing the chlorophyll mutations (Mishra and Singh, 2014) [15]. In both the mutagenic treatment populations, no chlorophyll mutation was observed in control. Higher chlorophyll mutation frequency was recorded in lowest doses of mutagens (0.2% EMS and 150Gy) in both the cases of EMS and gamma rays treatments and decrease in number of chlorophyll mutation was observed with increase in dose in EMS mutagenic treatments (Figure 2). This finding could be due to induction of minimum lethality at lower doses. Devi *et al.* (2002) [3] reported similar findings in rice bean crop whereas other scientists have reported similar results in other pulse crops (Gandhi *et al.*, 2014 in mung bean; Kumar *et al.*, 2010 in cowpea and Ramchander *et al*.,2017 in black gram**)** [20, 4].

The frequency of induced chlorophyll mutations in M_2 generation has been considered as an important tool to know the potency of mutagen used (Usharani and Kumar, 2015) [23]. Thus, the score of spectrum and frequency acts as a scale for evaluating the efficiency and effectiveness of mutagenic treatments and also predicts the size of vital factors in mutations. The results on spectrum and frequency indicate the importance of induced mutagenesis in rice bean crop.

Table 1: Spectrum and frequency of different chlorophyll mutations in M_2 generation of rice bean

Treatment	No. of M_2 Plants scored	Chlorophyll mutations					Total	Treatment-wise Mutation Frequency (%)
		Chlorina	Xantha	Albina	Viridis	Sectorial		
T_1 (0.2% EMS)	342.0	6	3	1	1	0	11	3.22
T_2 (0.4% EMS)	325.0	4	2	0	2	1	9	2.77
T_3 (0.6% EMS)	295.0	2	0	0	4	0	6	2.03
T_4 (150Gy)	310.0	5	2	2	0	2	11	3.55
T_5 (200Gy)	286.0	3	1	0	2	1	7	2.45
T_6 (250Gy)	282.0	2	0	1	3	1	7	2.48
Untreated Control	309.0	-	-	-	-	-	-	-
Total		22	8	4	12	5	51	16.50
Mutation frequency (%)		1.20	0.43	0.22	0.65	0.27	2.77	

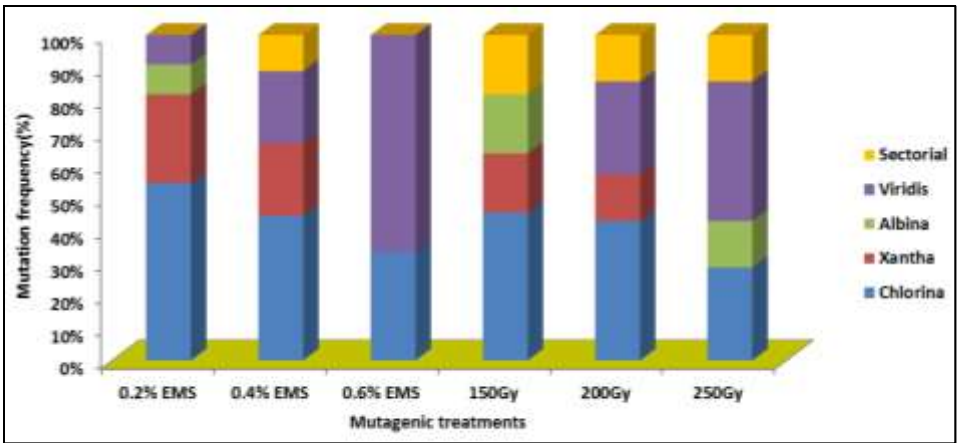

Fig 1: Spectrum of Chlorophyll mutants

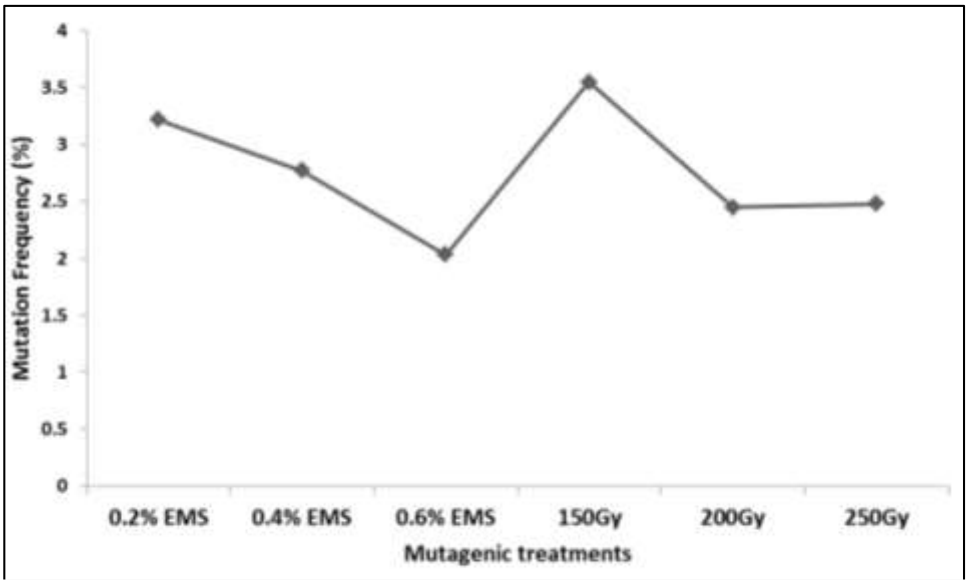

Fig 2: Frequency of Chlorophyll mutants

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