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RESEARCH ARTICLE

Studies on induced chlorophyll mutants in green gram (*Vigna radiata* (L.) Wilczek)

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Abstract

The seeds of mung bean variety Vamban₂ were treated with different dose/conc. of physical (Gamma rays) and chemical mutagens (Ethyl methane sulphonate) to induce mutagenesis. The phenotypic responses were studied in M₁ and M₂ generations and spectrum of chlorophyll mutation were worked out. There were four types of chlorophyll mutation was observed, i.e. albina, xantha, chlorina and viridis. While analyzing the result, it was observed that the mutation frequency increased with increase in the dose/concentration of mutagen. In general, the chlorophyll mutant was higher in EMS than the gamma rays treated plants. Among the various dose/conc. of mutagens, EMS showed more frequency was observed in 50mM and gamma rays at 60kR had higher mutation frequency.

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Introduction

Mutation breeding is a proven supplement and an effective substitute of conventional breeding so as to confer specific improvement in a variety without significantly affecting its acceptable phenotype. The practical role of induced mutation in the improvement of crop plants can best be assessed on the basis of quantitatively inherited characters. Depending upon the crop plant, character to be improved and the availability of mass screening techniques, mutation breeding not only useful but also the most appropriate one in self-fertilizing species. It is a powerful and effective narrow genetic base (Micke, 1998). The prime strategy in mutation breeding has been to upgrade the well-adapted plant varieties by altering one or two major agronomic metrical traits which limit their productivity or enhance their quality.

Chlorophyll mutants are employed as markers in genetics, physiological and biochemical investigations for the evaluation of gene action of mutagenic factor in inducing mutation studies. The spectrum and frequency of chlorophyll mutants is being used as primary index of effectiveness of mutagens and mutability of the genotypes towards the mutagen which in turn would be useful to generate the wide array of desirable mutants in the treated population (Gaul, 1964). The chlorophyll mutation do not have any economic value due to their lethal nature, such a study could be useful in identifying the threshold dose of a mutagen that would increase the genetic variability.

Material and Methods

The seeds of mungbean variety Vamban₂ were obtained from Tamilnadu Agricultural University, Coimbatore. The experimental materials were divided into two sets. The first sets of seeds were treated with gamma rays (20, 30, 40, 50 and 60kR) by irradiating seeds at Sugarcane breeding institute, Tamilnadu Agricultural University, Coimbatore. Another set of seeds were treated with Ethyl methane sulphonate at different concentrations (10, 20, 30, 40 and 50 mM). For the EMS treatment, the seeds were pre-soaked in water for four hours and different concentration of EMS solution for four hours and washed through running tap water before sowing.

The treated seeds along with controls were immediately sown after treatment. Chlorophyll mutations, as described by Gustaffson (1940) were scored throughout the plant growing period in M₂ generation. The data of chlorophyll mutants were collected and recorded at the time of maturing and expressed in percentage of control.

Results

In this experiment, only four types of chlorophyll mutants were identified. They are albina, chlorina, xantha and viridis. The chlorophyll mutation frequencies were calculated as per cent of M_2 plants (Table 1).

While analyzing the results, it was observed that the mutation frequency increased with increase in the dose/concentration of mutagen. In general, the chlorophyll mutation was higher in EMS than the Gamma rays. In EMS, frequency was observed more in 50 mM EMS and 60 kR gamma rays.

Spectrum and Frequency of Chlorophyll Mutation

Albina

In EMS treated plants albino mutant were observed in all the concentrations except in 10mM. The highest frequency (1.72) was observed in 50 mM. While in gamma rays, albino mutants were found in all the concentration, except 20 kR and 40 kR. Maximum frequency of chlorophyll mutation was occurred in 60 kR gamma rays. It was observed in both treatments that the frequency of albino mutants was increase with increase in concentration.

Xantha

The EMS treated plants showed highest frequency of xantha mutants compared to gamma treatments. The intermediate concentration of all the three treatments did not show any frequency of xantha mutants in EMS.

In gamma irradiated plants, the xantha mutant was observed only in 50 kR and 60 kR. The lowest (0.24) and the highest (3.53) frequency of xantha mutants were exhibited by 10 mM and 50 mM EMS, respectively.

Chlorina

In EMS treatment, an increase in the frequency of chlorina mutants was noticed at 30 mM which decreased at 40 mM and 50 mM. In gamma rays, the frequencies of chlorina mutants showed an increasing trend with an increase in the dose up to 40 kR, then decreased at 50 kR and again increased at (1.80) showed in 40 mM EMS and lowest (0.24) in 10 mM EMS.

Viridis

Compared to other chlorophyll mutations, frequency of viridis mutant is less. In EMS, an increasing in concentration was associated with an increase in the frequency of mutants. In gamma rays, frequency of viridis mutants showed a decreasing trend with an increase in the concentration up to 40 kR and decreased at 50 kR. In both, the highest frequency (1.30) was noticed at 40 mM EMS and the lowest frequency (0.30) was observed in 30 kR gamma rays.

Table 1: Frequency of chlorophyll mutations recorded as per cent M_2 plants

Treatments (Dose/conc.)	Total seedlings studied	Albina	Xantha	Chlorina	Viridis	Total mutation frequency
Control	-	-	-	-	-	-
Gamma rays 20kR	378	-	-	-	0.52	0.52
30kR	328	0.30	-	0.30	0.30	0.90
40kR	254	-	-	1.18	0.39	1.57
50kR	184	1.08	1.08	0.54	0.54	3.26
60kR	147	1.36	1.36	1.36	0.68	4.76
EMS 10mM	481	-	0.24	0.24	-	0.49
20mM	401	0.40	-	0.80	0.40	1.60
30mM	245	0.80	-	0.88	0.44	2.22
40mM	150	1.30	-	1.80	1.30	4.65
50mM	113	1.72	3.53	1.7	0.88	7.07

CHLOROPHYLL MUTANTS

Chlorina (30mM)



Chlorina (50 kR gamma rays)



Xanta (40kR)



Xantha (20mM)



Albino (50kR)



Viridis (40 mM EMS)



Discussion

Chlorophyll formation in plants is the last result from a long chain of biochemical process were involved a lot of loci. According to Von Wettstein et al. (1971) nuclear genes control the biogenesis of plastids. The author found that the chlorophyll synthesis in high plants raised under the control of nuclear genes by the products of regulatory ones. Other Scientist (Van Harten, 1998) asserts that the chlorophyll synthesis is under the control of nuclear and out nuclear (cytoplasmic) genes. Chlorophyll mutations are used as a test for evaluation of genetic action of mutagenic factors (Svetleva, 2005). They are the most frequency observed and can be easily identified factorial mutations in M_2 generation, yet.

Frequencies and types of the chlorophyll mutations albino, xantha, chlorina, viridis and lutescens varied. Generally, EMS induced higher proportion of chlorophyll mutants than gamma rays. In *S. villosum*, the earliest observable mutants were chlorophyll deficiency mutants (Gustaffson, 1940) whose frequencies varied with the mutagen dose, and male sterile mutant frequency was later observed to follow a similar distribution trend (Ojiewo et al., 2006). Although, the chlorophyll mutations do not have any economic value due to their lethal nature, such a study could be useful in identifying the threshold dose of a mutagen that would increase the genetic variability. Frequency and spectrum of chlorophyll mutations increased as irradiation and chemical mutagen doses increased.

Different pattern of chlorophyll mutations were reported in mungbean mutants. Our results are contrary to the findings of Haq, (1990). He reported that the xantha and chlorina mutants were predominant. But in the present studies, same trend of predominant occurrence of xantha mutant and the maximum induction of xantha mutations suggests that genes for xanthophylls are readily available for mutagenic action. Occurrence of chlorina mutants have been attributed to different causes such as impaired chlorophyll biosynthesis, further degradation of chlorophyll and bleaching due to deficiency of carotenoids. It means that the genetic differences in genotypes under reference for inducing chlorophyll mutation type have been observed and identified by many workers in Bengal gram (Nerkar and Mote, 1978) and lentil (Sharma and Sharma, 1981).

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