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REGULAR ARTICLE

CHLOROPHYLL AND MORPHOLOGICAL MUTANTS OF BLACK GRAM (*VIGNA MUNGO* (L.) HEPPER) DERIVED BY GAMMA RAYS AND EMS

D. Arulbalachandran*, L. Mullainathan
Division of Plant Cytogenetics and Mutation Breeding, Department of Botany, Annamalai University
Annamalainagar – 608 002, Tamilndu, India

SUMMARY

Micro and macro-mutants play an important role to assess the dose/concentration of mutagens. In the present investigation, some of the chlorophyll mutants were observed in the different dose/concentrations of gamma rays they were chlorina albino, xantha, and viridis. Among the mutagens, Morphological mutants were observed in M_2 generation with effect of dose/concentration of mutagens and such mutants were, dwarf, tall, tiny leaves, hairy leaves, male sterility, brown seed, early, maturing, long pod, bottom branching, top branching, bushy type, trailing and spreading habit mutants were observed in M_2 generation. Mutant and its derivatives when used in cross breeding have found to be more productive in the development of improved varieties of black gram. EMS provided more number of chlorophyll and morphological mutants followed by gamma rays in this investigation.

Keywords: Chlorophyll mutants, morphological mutants, EMS and Gamma rays.
D. Arulbalachandran and L. Mullainathan. Chlorophyll and morphological mutants of black gram (*Vigna mungo* (L.) Hepper) derived by gamma rays and EMS. J Phytol 1 (2009) 236-241
***Corresponding Author, *Email*: arulmutbred@yahoo.co.in**

1. Introduction

Spontaneous variations resulting from mutations at several gene loci in different organisms are of common occurrence. However, spontaneous mutations occur at low frequency and often do not include the full range of variability possible. Hence, induced mutation using physical and chemical mutagen is one method to create genetic variation resulting in new varieties with better characteristics [1]. The induced mutations have been used to improve agronomic traits of many crops. Use of ionizing radiations, such as X-rays, gamma rays, neutrons and chemical mutagens for inducing genetic variation is well established [2]. To date worldwide, 2252 mutant varieties have been officially registered. Of which 1585 were released as direct and 667 were mutant derivatives [3]. Mutant and its derivatives when used in cross breeding have found to be more productive in the development of improved varieties of black gram [4, 5]. Gamma ray treatment has been employed for the development of 64% of the mutant varieties [3]. Of the 311 legume mutants four were in black gram achieved by gamma rays [6]. In India still today there are 7 mutant varieties of black gram released by both physical and chemical mutagens [7]. Hence, mutation breeding programme has proved to be a successful tool in bringing amelioration in self-pollinated crops [8]. Mutated genes have therefore; become valuable material to plant breeders and molecular biologists for understanding not only the function but also in isolating and shuffling the genes between varieties [3]. In the present pragmatic investigation number of chlorophyll and morphological mutants were isolated in M_2 generation by gamma rays and EMS.

2. Materials and Methods

Selection of genotype

Black gram variety vamban-1was selected for deriving chlorophyll and morphological mutants in M_2 generation. For this experiment certified seeds were collected from Vamban Pulse Research Centre (Pudukottai), Tamilnadu, India.

Mutagen Treatment

One of the physical mutagen namely gamma rays and chemical mutagen EMS were used to deriving mutants from seed treatment.

Physical Gamma rays treatment

The seeds were irradiated at Sugarcane Breeding Institute (ICAR), Coimbatore, India and the source of gamma rays was labeled Cobalt (60Co). Ten sets of three hundred well matured, non-dormancy seeds were taken for irradiation. The sets of seeds were packed in paper cover for irradiation and treated with 20, 40, 60, 80,100 and 120 kR of gamma rays. Healthy, well-matured, non-dormant, untreated seeds were used as control.

Chemical EMS Treatment

One of the chemical mutagens namely Ethylmethane sulphonate (EMS) was used for induction of mutation on seed propagules. Ethylmethan sulphonate was obtained from Himedia Laboratory Limited, Mumbai, India which having a dosimetry/half-life period is 30 hours with a molecular weight is 124.16 and density is 1.20.

Six hundred well matured healthy and uniform size of non-dormancy seeds were subjected to the mutagenic treatment. The solution of EMS was prepared with corresponding to the required concentration in distilled water. The volume of solution was about three times than that of volume of seeds. The seeds were pre-soaked in double distilled water for five hours at room temperature (28 ± 2ºC) prior to treatment. After the pre-soaking the excess of moisture in the seeds were removed by filter paper. Then seeds were soaked in the freshly prepared aqueous solution of EMS in the following concentrations (%) Viz 0.02, 0.04, 0.06, 0.08, 0.1, 0.12, 0.14, 0.16 and 0.18 % for six hours at room temperature (28 ± 2ºC) with an hour intermittent shaking. The pH of aqueous solution was adjusted at 8.5 by using 0.2 M solution of sodium tetra borate (Borax). After the treatment, the seeds were washed thoroughly with distilled water for eight to ten times and sown in the field as randomized block design with three replication to rise M_1 generation.

Experimental design

Both physical gamma rays irradiated and chemically treated (EMS) seeds were grown along with control (Untreated seeds) by randomized block design (RBD) with three replications at the Breeding field, Department of Botany, Annamalai University, Annamalainagar, TN, India. The plots consisted of seven rows including control at 20 cm spacing, 4 m long and 1.5 m wide. The field was fertilized with organic fertilizer. Along with all the cultural practices such as irrigation, weeding and protection measures were taken throughout the growth period.

Growth Condition

After rising M_1 generation, seeds were collected from respective dose/concentration of mutagens. Form M_1 generation, M_2 generation was raised and the chlorophyll (15th day) morphological mutants were isolated.

Isolation of mutants

In M_2 generation form both gamma rays and EMS at 15th day the following chlorophyll mutants such as Chlorino, Albino, Xantha, Variegata and Viridis and up to growth period morphological mutants such as, Dwarf, Tall, Monostem, Tiny leaves, Hairy leaves, Male sterility, Brown seed, Early maturity, Long pod, Bottom branching, Top branching, Trailing, Spreading, and Bushy type.

3. Results and Discussion

Chlorophyll mutants

Micro and macro-mutants play an important role to assess the dose/concentration of mutagens. Almost all the mutagenic treatments showed different degree of mutants with respective dose. In the present investigation, some of the chlorophyll mutants were observed in the different dose/concentrations of gamma rays they were chlorina albino, xantha, and viridis (Table-1, 2 & Fig 1). Among the mutagens, EMS provided more number of chlorophyll and morphological mutants followed by gamma rays. Albino was white and relatively smaller than the normal seedlings age of surviving was 10-20 days. Viridis was seedlings with whitish tips of leaves, leads to lethal were observed. Xantha was straw yellow seedling with normal growth in the beginning but it was started withering after 20 days. The highest frequency of these mutants was observed in EMS (Table 1 & 2).

Table 1.

Spectrum of chlorophyll and viable mutants of gamma rays in M_2 generation

Mutants		60 kR	80 kR	100 kR
	No. of plant Studied	866	783	721
Chlorophyll Mutants	Chlorino	3	3	1
	Albino	2	1	3
	Xantha	2	3	2
	Variegata	1	4	2
	Viridis	3	4	1
Viable mutants	Dwarf	2	2	2
	Tall	2	1	2
	Monostem	2	2	-
	Tiny leaves	-	1	-
	Hairy leaves	2	2	1
	Male sterility	2	1	-
	Brown seed	-	2	1
	Early maturity	-	2	1
	Long pod	2	3	1
	Bottom branching	-	-	-
	Top branching	-	1	-
	Trailing	2	1	-
	Spreading	-	2	-
	Bushy type	2	2	1
	Total	27	37	18
	Mutation Frequency	3.12	4.72	2.49

Table 2.

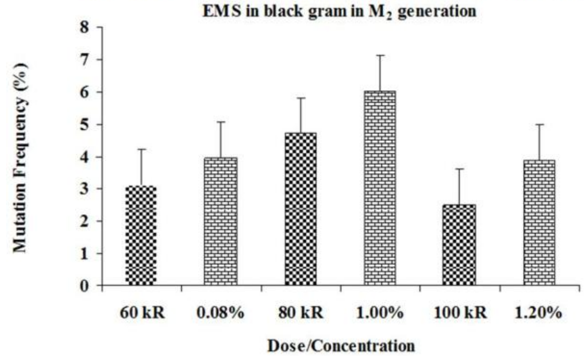
Spectrum of chlorophyll and viable mutants of EMS in M_2 generation

	Mutants	0.08%	0.1%	0.12%
Chlorophyll Mutants	No. of plant Studied	758	696	662
	Chlorino	3	2	1
	Albino	2	2	3
	Xantha	2	3	1
	Variegata	2	4	3
	Viridis	2	4	1
Viable mutants	Dwarf	2	2	2
	Tall	2	1	2
	Monostem	2	2	-
	Tiny leaves	-	1	1
	Hairy leaves	2	2	-
	Male sterility	2	1	-
	Brown seed	-	2	-
	Early maturity	-	2	-
	Long pod	2	3	-
	Bottom branching	2	2	-
	Top branching	1	3	1
	Trailing	2	2	-
	Spreading	-	2	3
	Bushy type	2	2	1
	Total	30	42	19
	Mutation Frequency	3.96	6.03	3.87

Albino, chlorine and xantha mutants in lentil with effect of EMS and SA while, EMS was found almost twice as efficient as SA [9]. This is agreement with the present investigation. Some of the chlorophyll mutants Viz., albino, chlorine, viriscence and xantha in the segregating M_2 plants based on the intensity of pigmentation at the seedling stage in the varieties in cowpea [10]. This is correlated with the present investigation. The albino seedling itself has no practical value; however, such seedlings may be used as genetic markers for estimation of natural selfing. The phenomenon of albinism is rarely exhibited by plants which characteristic deficiency of chlorophyll and subsequent whitish-yellow colour of entire seedling [11]. These types of mutations were observed in mungbean [12], in chickpea [13] and in grass pea [14]. Chlorophyll development seems to be controlled by many genes located on several chromosomes, which could be adjacent to centromere and proximal segment of chromosomes [15]. Mutations in these chlorophyll genes are reflected in the M2 and subsequent generations in the form of different types of mutants [10].

Figure-1

Spectrum of chlorophyll and morphological mutants of gamma rays and EMS in black gram in M_2 generation



Morphological mutants

Morphological mutants were observed in M_2 generation with effect of dose/concentration of mutagens and such mutants were, dwarf, tall, tiny leaves, hairy leaves, male sterility, brown seed, early, maturing, long pod, bottom branching, top branching, bushy type, trailing and spreading habit mutants were observed in M_2 generation. The highest mutation frequency was noted in EMS (1.0%) than other dose/concentration of mutagens. Bushy, prostrate tendrillar, tall, dwarf, early maturity and sterile mutants were more in EMS than SA treatments in lentil on M_2 generation [9]. Similar results were recorded on different morphological mutations in lentil [16- 18). The frequency and spectrum of morphological mutants was relatively wide with EMS followed by HZ and SA in chickpea [19]. EMS was to be higher superior to gamma rays including a higher frequency and wider spectrum of chlorophyll mutations in M_2 generation [20]. It is confirmed the present investigation, which revealed high mutation frequency and, highest number of both mutants were recorded at 0.1% EMS (42) followed by 60 kR gamma rays (37) respectively. Where as other mutagenic dose/concentration gave low number of mutants. Among the mutagens, 0.1% EMS showed highest percentage of mutation frequency (6.03%) followed by 60 kR (4.72%) gamma rays (Table-1, 2 & Fig 1).

Conclusion

Chlorophyll mutants inferred in this investigation, how chlorophyll gene response to mutagen gamma rays and EMS. Mutations in these chlorophyll genes are reflected in the M2 and subsequent generations in the form of different types of mutants. While, morphological mutants are viable and useful to breeding approach to obtain suitable ideotype in black gram. Hence, mutant and its derivatives when used in cross breeding have found to be more productive in the development of improved varieties of black gram.

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