Journal of Phytology 2009, 1(4): 213–218

ISSN: 2075-6240
Available Online: www.journal-phytology.com

REGULAR ARTICLE

SCREENING OF MUTANTS IN BLACK GRAM (*VIGNA MUNGO* (L.) HEPPER) WITH EFFECT OF DES AND COH IN M_2 GENERATION

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

SUMMARY

Cultivars developed using induced mutants may carry improvements in a wide variety of characteristics. Induced mutant cultivars also have proved to be outstanding parents for further cultivar development. The efficiency of induced mutations in increasing genetic variability has been demonstrated in several crops and a number of varieties have been evolved. In the present investigation, chlorophyll mutants chlorino, albino, xantha, variegata and viridis and morphological mutants such as, dwarf, tall, onostem, tiny leaves, hairy leaves, male sterility, brown seed, early maturity, long pod, bottom branching, top branching, trailing, spreading, and bushy type in M_2 generation from both Diethyl sulphate (DES) and Colchicine (COH) treated populations. Mutants and mutant derivatives when used in cross breeding were found to be more productive in the development of improved varieties of black gram. Moreover, induced mutations have recently become the subject of molecular investigations leading to descriptions of the structure and function of related genes. 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.

Keywords: Diethyl sulphate, Colchicine, Chlorophyll mutants, Morphological mutants and Frequency of mutation.

D. Arulbalachandran et. al. Screening of Mutants in Black Gram (*Vigna mungo* (L.) Hepper) With Effect of DES and COH in M_2 Generation. J Phytol 1 (2009) 213-218
***Corresponding Author, *Email*: arulmutbred@yahoo.co.in**

1. Introduction

A wealth of new traits or combinations of traits is readily obtainable in mutagenized populations. Cultivars developed using induced mutants may carry improvements in a wide variety of characteristics. Genetic variability of mutant has been used as a basis for the development of new conceptual models for crop plants [1, 2]. A concept of plant architecture for crop and then sought induced mutants to complement the available genetic variation for constructing model prototypes [1]. Induced mutant cultivars also have proved to be

outstanding parents for further cultivar development. The efficiency of induced mutations in increasing genetic variability has been demonstrated in several crops and a number of varieties have been evolved [3]. Diethyl sulphate in tests on maize using the cotton packing and saturation method gave a rate of mutagenesis 40% [4]. Like ethylmethane sulphonate, diethyl sulphate also one of the alkylating agent which induce point mutation. While, Colchicine is an antimitotic agent, which blocks or suppresses cell division by inhibiting mitosis, the cell division of cell's nucleus. Specifically, it inhibits or hampers the development of spindles as the nuclei are dividing. In the present pragmatic investigation, various chlorophyll and morphological mutants were recorded at different concentration of Dithyl sulphate and Colchicine treatment on black gram.

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

Chemical mutagens namely diethyl sulphate (DES) and Colchicine (COH) were used for induction of mutation on seed propagules.

Diethyl sulphate (DES - $(C_2H_5)_2SO_4$)

This chemical was obtained from SISCO Research Laboratory, Mumbai, India which having the half-life period, one hour with molecular weight, 154.19 and density 1.17.

Colchicine (COH - $C_{22}H_{25}NO_6$)

It was obtained from S.D. fine chemicals, Limited, Mumbai, India, which is chemically known as acetyl trimethylcolchinic acid, and the molecular weight is 399.43.

Two sets of six hundred well matured healthy and uniform size of non-dormancy seeds were subjected to both DES and COH mutagenic treatment. The solutions of DES and COH were 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 ± 2°C) 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 DES in the following concentrations (%)Viz., 0.02, 0.04, 0.06, 0.08, 0.1, 0.12 and 0.14 % for six hours at room temperature (28 ± 2°C) with an hour intermittent shakes. For Colchicine treatment the following concentrations were used Viz., 0.01, 0.02, 0.04, 0.06, 0.08 and 0.1 % for six hours at room temperature (28 ± 2°C) with one hour, intermittent shakes. 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 M1 generation.

Experimental design

Both chemically treated (DES and COH) 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 from both DES and COH treated populations 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 mutations are considered as the most dependable indices for evaluating the efficiency of different mutagens in inducing the genetic variability for crop improvement and are also used as genetic markers in basic and applied research [5]. The occurrence of chlorophyll mutations after treatments with physical and chemical mutagens has been reported in several crops [6 - 10]. As well, morphological mutants play a vital role to modify the characteristic of cultivars and construct ideotype and develop new variety of crops. Morphological mutants-reduced plant height is an important trait in plant breeding, mainly because short genotypes are most resistant to lodging than standard types [11, 12]. The existence of dwarf genotypes is common in many plant species and they have been used in several crops for more efficient crop management and increased yield [13]. In the present study, chemical mutagen diethyl sulphate and Colchicine were used to induce chlorophyll and morphological mutants. The frequency of mutation on the basis of number of mutants recorded in both DES and COH treatments was varied with increasing concentration. In this study, chlorophyll mutants such as Chlorino, Albino, Xantha, Variegata and Viridis were recorded in DES and COH in M2 generation (Table 1 & 2).

Table-1. Spectrum of chlorophyll and viable mutants of dES in M_2 generation

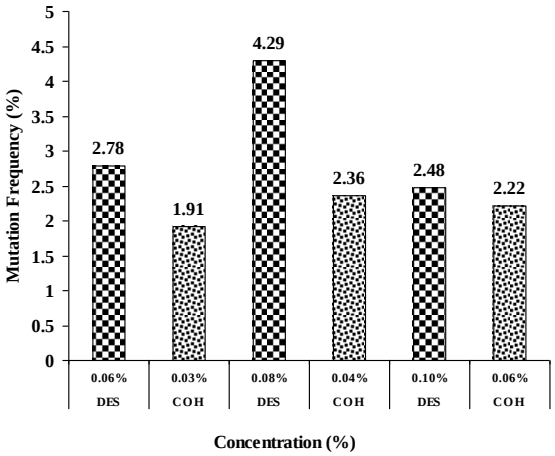
Mutants		0.06%	0.08%	0.1%
Chlorophyll Mutants	No. of plant Studied	899	815	724
	Chlorino	3	2	4
	Albino	2	4	3
	Xantha	3	3	2
	Variegata	2	4	-
	Viridis	2	3	1
Viable mutants	Dwarf	1	2	2
	Tall	1	2	-
	Monostem	2	1	-
	Tiny leaves	-	2	-
	Hairy leaves	2	2	-
	Male sterility	1	1	1
	Brown seed	-	3	-
	Early maturity	-	-	1
	Long pod	1	2	-
	Bottom branching	-	2	-
	Top branching	1	-	1
	Trailing	1	1	-
	Spreading	2	1	2
	Bushy type	1	-	1
	Total	25	35	18
	Mutation Frequency	2.78	4.29	2.48

Table-2. Spectrum of chlorophyll and viable mutants of COH in M_2 generation

Mutants		0.03%	0.04%	0.05%
Chlorophyll Mutants	No. of plant Studied	787	844	766
	Chlorino	2	2	1
	Albino	2	1	2
	Xantha	2	1	1
	Variegata	-	-	-
	Viridis	-	1	-
Viable mutants	Dwarf	-	-	2
	Tall	2	1	-
	Monostem	1	-	-
	Tiny leaves	-	-	-
	Hairy leaves	1	2	1
	Male sterility	1	1	2
	Brown seed	-	1	1
	Early maturity	-	1	1
	Long pod	-	1	1
	Bottom branching	2	2	-
	Top branching	1	1	1
	Trailing	1	2	-
	Spreading	-	1	3
	Bushy type	-	2	1
	Total	13	20	17
	Mutation Frequency	1.91	2.36	2.22

The highest mutation frequency (Fig 1) was observed in at 0.8 % DES (4.29%) and 0.04 COH (2.22%). Albino, chlorine and xantha mutants were recorded in lentil with effect of chemical

Figure 1
Spectrum of mutants induced by DES and COH in M_2 generation



mutagen [14]. 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 [5]. These types of mutations were observed in mungbean [15], in chickpea by [16] and in grass pea [17]. Chlorophyll development seems to be controlled by many genes located on several chromosomes, which could be adjacent to centromere and proximal segment of chromosomes [18]. Mutations in these chlorophyll genes are reflected in the M_2 and subsequent generations in the form of different types of mutants [5]. In general, macro-mutants play an important role in plant breeding as it may lead to the evolution of new genotypes [6]. 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 were recorded in the study at different dose of DES and COH treatment in M_2 generation (Table 1 & 2). Bushy, prostrate tendrillar, tall, dwarf, early maturity and sterile mutants etc were observed in chemical mutagenic treatments in lentil on M_2 generation [14]. These types of mutations were observed in lentil [19], in mungbean [15], in chickpea [16] and in grasspea [17]. Similar results were also recorded on different morphological mutations in lentil [19, 20]. In the present study,

dwarf, bushy type early maturity etc., were recorded in DES and COH. Reduced plant height is an important trait in plant breeding, mainly because short genotypes are most resistant to lodging than standard types [11, 12]. The existence of dwarf genotypes is common in many plant species and they have been used in several crops for more efficient crop management and increased yield [13]. In M6 seeds, 6 mutants of K-169 variety viz., dwarf, semi-dwarf, early maturity, semi-dwarf with early maturity, wax spike and chlorina types were recorded in barley [21]. The use of these mutants in breeding is expected to result in a marked change in the appearance and agronomic performance capabilities of future.

Mutants and mutant derivatives when used in cross breeding were found to be more productive in the development of improved varieties of black gram [22]. Moreover, induced mutations have recently become the subject of molecular investigations leading to descriptions of the structure and function of related genes. 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 [23]. The identification and analysis of mutants using molecular techniques of DNA fingerprinting and mapping with PCR based markers such as RAPD, AFLP and STMS and mutants tagging could bring a new dimension in gene technology [24].

References

1. Adams, M.W. 1982. Plant architecture and yield breeding. Iowa State J. Res., 56: 225-254.
2. Blixt, S. and P.B. Vose, 1984. Breeding towards an idotype-aiming at a moving target?. P.414-426. In: P.B. Vose and Blixt (eds). Crop Breeding, a contemporary basis. Pergamon, Press, London and New York.
3. Sigurbjornsson, B and A. Micke, 1974. Philosophy and accomplishments of mutation breeding. In: polyploidy and induced mutations in plant breeding, pp. 303-343. Proc. Symp. Bari, Italy, 1972, IEAE, Vienna.
4. Ficsor, G. 1965. Chemical mutagenesis in Zea mays L. Ph.D. Thesis, Dept. of Field Crops. Univ. of Missouri, Columbia.
5. Wani, A.A. and M. Anis, 2004. Spectrum and frequency of chlorophyll mutations induced by gamma rays and EMS in *Cicer arietinum* L. J. Cytol. Genet., 5: 143-147.
6. Swaminathan, M.S., V.L. Chopra and S. Bhaskaran, 1962. Chromosome aberrations frequency and spectrum of mutations induced by EMS in barley. Indian J. Genet., 22: 192-207.
7. Prasad, A.B. and A.K. Das, 1980. Studies of induced chlorophyll mutations in *Lathyrus sativus* L. Cytologia., 45: 335-341.
8. Sharma S.K. and B. Sharma (1981). Induced chlorophyll mutations in lentil. Indian J. Genet. (41): 328-333.
9. Reddy, V.R.K. and P.K. Gupta, 1989. Induced mutations in Triticale frequency and spectrum of chlorophyll mutations. Indian. J. Genet., 49: 183-190.
10. Mitra, P.K. and G. Bhowmik, 1999. Studies on the frequency and segregation of induced chlorophyll mutations in *Nigella sativa* L. Adv. Pl. Sci., 12: 125-129.
11. Austin, R.B., J. Bingham, R.D. Blackwell, L.T. Evans, M.A. Ford, C.L. Morgan and M. Taylor, 1980. Genetic improvement in winter wheat yields since 1900 and associated physiological chances. J. Agric. Sci., 94: 675-689.
12. Fick, G.M. and J.F. Miller, 1997. Sunflower breeding. In: A.A. Schneitter (ed.,) Sunflower Technology and production. Agron. Monographs., 35: 395-439. ASA, CSSA and SSSA. Madison.

13. Abel, G.H. 1976. Inheritance of stem length and its components in safflower. Crop Sci., 16: 374-376.
14. Solanki, I.S. 2005. Isolation of Macromutations and mutagen effectiveness and efficiency in lentil (*Lens culinaris* Medik.) Indian J. Genet., 65: 264-268.
15. Singh, V.P. and P. Singh, 1989. Cytomorphological changes induced in breadwheat following seed treatment with pesticides and mutagenic chemicals. Indian J. Genet., 49: 341-349.
16. Kharkwal, M.C. 1999. Induced mutations in chickpea (*Cicer arietinum* L.) III. Frequency and spectrum of viable mutations. Indian J. Genet. Pl. Breed., 59: 451-464.
17. Waghmare, V.N. and R.B. Mehra, 2001. Induced mutations in grasspea (Lathyrus sativus L). *Lathyrus Lathyrism* Newsl., 1: 21-24.
18. Swaminathan, M.S. 1964. A comparison of mutation induction in diploids and polyploids. In: the use of induced mutations in plant breeding. Rad. Mut. Organ. FAO/IAEA, Vienna. Pp. 619-641.
19. Sarkar, A. 1985. Efficiency of early generation selection for polygenic mutations in lentil (*Lens culinaris* Mdeikus). Ph.D. Thesis, IARI, New Delhi.
20. Vandana, T.A and D.K. Dubey, 1994. Frequency and spectrum of mutations induced by ethyl methane sulphonate (EMS) and diethyl sulphate (dES) in lentil. Var. K-85. Lens Newsl., 21: 16.
21. Kumar, B and B. Ramesh, 2004. Charaterization and evolution of induced mutants in barley (*Hordeum vulgare*). Indian J. Agric. Sci., 74: 492-495.
22. Pawar, S.E., J.G. Manjaya, J. Souframanien and S.M. Bhatia, 2000. Genetic improvement of pulse crops: induced mutations and their use in cross breeding. In: Proc. FAO/IEAE workshop on the use of nuclear and molecular techniques in crop improvement. 6-8. Dec. 2000. BARC, Mumbai. 170-174.
23. Souframanien, J., S.E. Pawar and A.G. Rucha, 2002. Genetic variation in gamma ray induced mutants in black gram as revealed by RAPD and ISSR markers. Indian J. Gent., 62: 291-295.
24. Ahloowalia, B.S. and M. Maluszynki, 2001. Induced mutations. A new paradigm in plant breeding. Euphytica., 118: 167-173.