Original Research Article **Allied sciences**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

INDUCED CHLOROPHYLL AND VIABLE MUTATIONS IN *Lablab purpureus* (L.) SWEET VAR. *typicus* THROUGH GAMMA RAYS AND ETHYL METHANE SULPHONATE

S. MONICA AND N. SEETHARAMAN

Department of Botany, Annamalai University, Annamalai nagar - 608 002, Tamil nadu, India

ABSTRACT

Induced mutagenesis has become an effective tool to improve a crop through creation of variability. The present study was carried out to induce mutagenesis with CO(Gb)14 variety of *Lablab purpureus* (L.) Sweet var. *typicus*. The seeds of this variety were exposed to different doses/concentrations of gamma rays and Ethyl Methane Sulphonate . The mutagenic treated seeds were sown in the M_1 field with control and harvested in bulk to raise M_2 generation to observe the characters and number of mutants in each population. A wide range of chlorophyll and morphological mutants were observed in the M_2 generations. The chlorophyll mutants identified in the treated population were albino, xantha, chlorina and viridis. The morphological mutation consisted of tall, dwarf, bushy, tendrillar, unifoliate leaf, bifoliate leaf, tetrafoliate leaf, wrinkled leaf, narrow rugose leaf, biforked leaf, triforked leaf, extra standard petal, extra wing petal, early maturity, late maturity, single seed pod and long pod. EMS induced higher proportion of chlorophyll and viable mutants than gamma rays and the highest mutation frequency was induced in 30mM of EMS followed by 25KR of gamma rays. The frequency of these mutations is agronomically desirable which may be utilized in the development of new cultivar.

KEY WORDS: ***Lablab purpureus (L.) Sweet var. typicus, Gamma rays, EMS, Mutation frequency***

S. MONICA
Department of Botany, Annamalai University,Annamalai nagar - 608 002, Tamil nadu, India

Received on: 03-11-2016
Revised and Accepted on : 18-02-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.2.b240-249

INTRODUCTION

Lablab purpureus (L.) Sweet is an important vegetable crop of Indian origin [1]. It occupies unique position for vegetable purpose among the legume vegetables and it is a good source of protein, minerals and vitamins [2]. The increase in production and productivity of this crop is very crucial to meet the protein requirement of especially under-nourished people depending on vegetable protein. Mutation breeding has become increasingly popular in recent times as an effective tool for crop improvement and more than 3222 mutant cultivars have been released worldwide[3]. Induced mutations are highly effective in enhancing natural genetic resources and have been used in developing improved cultivars of cereals, fruits and other crops[4]. Many physical and chemical mutagens have been used for induction of useful mutants in a number of crop plants[5]. Physical radiation, e.g., from gamma rays and X-rays, has been widely used for inducing mutations[6]. Gamma rays are the form of electromagnetic energy. Gamma rays have the shortest wavelength in the electromagnetic spectrum, therefore have the greatest ability to penetrate through any gap, even a subatomic one, in what might otherwise be an effective shield and has proven an adept means of encouraging the expression of recessive genes and producing new genetic variations[7]. Gamma sources are used to irradiate a wide range of plant materials, like seeds, whole plants, plant parts, flowers, anthers, pollen grains and single cell cultures or protoplasts and it interact with atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells and affect different morphology, anatomy, biochemistry and physiological characters in plants, mainly depending on the level of irradiation. These effects could changes in plant, cellular structure and metabolism, like dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative and accumulation of phenolic compounds[8]. Chemical mutagens could be successfully applied to induce mutations where no irradiation facility is available. In some cases, the efficiency of chemical mutagens has proved to be greater than those of physical mutagens[9]. Of the chemical mutagens, EMS has been quite useful in inducing point mutations in the genomes of a diverse range of plants largely because of its well established mode of action which generates G to A and C to T transitions and its effectiveness in inducing a high frequency of point mutations in a wide range of organisms without causing gross chromosomal abnormalities[10]. Chlorophyll mutations are most widely employed for assessing the potentialities of mutagens in creating genetic variabilities. Morphological mutations affecting different plant parts can be of immense practical unity and many of them have been released directly as crop varieties[11]. A study of induced variability for chlorophyll and viable morphological mutations in the M_2 generation was the most dependable tool to utilize useful mutations for efficient crop improvement[12]. The present investigation reports data on chlorophyll and viable mutants induced by different doses/concentrations of gamma rays and EMS in *Lablab purpureus* (L.) Sweet var. *typicus* cv. CO(Gb)14.

MATERIALS AND METHODS

Experimental material

Certified seeds of *Lablab purpureus* (L.) Sweet var. *typicus* cv. CO(Gb) 14 were procured from the Tamil nadu Agricultural University Coimbatore, Tamil nadu, India.

Mode of treatment with mutagenic agents

The seed material were treated with physical (Gamma rays) and chemical mutagen Ethyl Methane Sulphonate

1. Treatment with physical mutagen: Gamma Irradiation was performed by using a Gamma cell installed at the Sugarcane Breeding Institute, Coimbatore, India and the seeds were irradiated with ^{60}CO source at the doses of 5KR, 10KR, 15KR, 20KR, 25KR, 30KR, 35KR, 40KR, 45KR and 50KR.
2. Treatment with Chemical mutagen: EMS treatment was carried out at the Department of Botany, Annamalai University, Tamil nadu. For EMS treatment, the seeds were pre- soaked in distilled water for 6h and were treated with different concentrations of EMS (5mM, 10mM, 15mM, 20mM, 25mM, 30mM, 35mM, 40mM 45mM and 50mM) for 4 h. After the treatment, the seeds were washed in running water before sowing.

In the laboratory experiment the treated seeds were sown in absorbent cotton -wet petridishes for recording the germination test. Based on the reduction of 50% seed germination, the LD50value was fixed. Three treatments of gamma rays (20KR, 25KR and 30KR) and EMS (25mM, 30mM and 35mM) around LD50value were fixed for further studies.

Recording of data

The treated materials along with control (untreated) were immediately sown in Randomized Block Design (RBD) with three replications at Botanical Garden, Department of Botany, Annamalai University. The entire surviving M_1 crops were harvested individually to raise the M_2 generation population along with controls. Necessary cultural practices were adopted to raise a healthy crop. Observations for recording chlorophyll mutations were noted critically right from emergence to till the age of three weeks after germination. The detection of chlorophyll mutations were made as per the classification of Gustaffson[13]. The Mutations affecting gross morphological changes in branching, stem structure, growth, habit, leaf, flower, pod, seed size and maturity etc., were scored as viable mutants. Viable mutants were scored throughout the growing period as described by Blixt[14] and the mutation frequency was estimated on M_2 plant basis.

RESULTS AND DISCUSSION

Table -1
Frequency of Chlorophyll and Viable mutants in M_2 generation

Mutagens (Dose/Conc.)		Gamma rays			EMS		
		20KR	25KR	30KR	25mM	30mM	35mM
No. of plant studied		621	587	524	585	551	494
Chlorophyll Mutants	Albino	1	-	2	1	-	1
	Xantha	-	1	1	1	2	2
	Chlorina	1	1	-	-	2	2
	Viridis	1	2	2	2	2	3
Viable mutants	Tall	4	7	2	5	8	2
	Dwarf	2	4	1	3	5	2
	Bushy	4	7	2	6	9	2
	Tendrillar	1	2	1	1	3	1
	Unifoliate leaf	-	-	-	3	2	-
	Bifoliate leaf	2	2	1	2	3	1
	Tetrafoliate leaf	2	3	-	-	3	-
	Wrinkled leaf	2	2	2	3	2	1
	Narrow rugose leaf	-	-	1	-	1	-
	Biforked leaf	1	2	-	2	2	1
	Triforked leaf	-	2	-	-	3	1
	Extra standard petal	2	3	1	2	4	1
	Extra wing petal	2	2	1	2	4	-
	Early maturity	3	6	1	3	7	2
	Late maturity	2	2	1	2	2	2
	Single seed pod	-	1	2	-	1	1
	Long pod	2	2	1	3	4	1
Total		**32**	**51**	**22**	**41**	**69**	**25**
Mutation frequency		**5.15**	**8.68**	**4.19**	**7.00**	**12.52**	**5.06**

Figure 1
Albino (30KR)

Figure 2
Xantha (30mM)

Figure 3
Chlorina (25KR)

Figure 4
Viridis (20KR)

Figure 5
Tall (25KR)

Figure 6
Dwarf (35mM)

Figure 7
Bushy (30mM)

Figure 8
Tendrillar (35mM)

Figure 9
Unifoliate leaf (25mM)

Figure 10
Bifoliate leaf (35mM)

Figure 11
Narrow rugose leaf (30KR)

Figure 12
Triforked leaf (35mM)

Figure 13
Extra standard petal (30mM)

Figure 14
Extra Wing petal (20KR)

Figure 15
Single seed pod (35mM)

Figure 16
Long Pod (30mM)

CHLOROPHYLL MUTANTS

Macro- mutations are generally used to evaluate the genetic effects of various mutagens. Chlorophyll mutation is the indicator of the mutagenic effect. The occurrence of chlorophyll deficient mutant must be attributed due to change in gene and a set of genes responsible for chlorophyll mutations. In the present investigation, gamma rays and EMS induced four types of chlorophyll mutant's *viz.,* albino, xantha, chlorina and viridis (Table-1). EMS induced higher propotion of chlorophyll mutants than gamma rays. The high incidence of chlorophyll mutations induced by EMS may be due to its specificity to affect certain regions of the chromosome[15]. This is in agreement with the earlier reports of Kumar *et al.*[16]. No such mutations were observed in the controls.

Albina mutant

Albina mutant were characterized by their dull white color and were devoid of chlorophyll, carotenoid and other pigments. The seedlings of albino mutants were smaller in height and survive upto a maximum of 15-20 days after germination (Fig: 1). The albino seedling itself has no practical value; however, such seedlings may be used as genetic markers for estimation of natural selfing[17]. This type of mutation was observed in Black gram[18] and Musk Okra[19].

Xantha mutant

The xantha mutant varies from deep yellow to yellowish white colour (Fig: 2). The growth of the mutants is retarded and most of them die with in 17 to 20 days after emergence due to a block in chlorophyll synthesis[20]. This mutant was also recorded in mung bean[21] and green gram[22].

Chlorina mutant

Normally chlorina mutants do not survive. These mutant seedlings have light yellowish/ yellowish green leaves (Fig: 3).The mutants breed true for the altered characters. Similar results were recorded by Khan and Tyagi in Soyabean[23]

Viridis mutant

Viridis mutant seedlings were dark green in the early stages of development and turn normal green in the later stages (Fig: 4). The mutant produces normal looking flowers and also set pods. Similar observations were made by Kulthe *et al*. in winged bean[24] and Kulkarni and Mogle in Horse gram[25].

VIABLE MUTANTS

Gaul[26] classified viable mutations as macro and micro mutations, while Swaminathan[27] grouped them as macro mutations are systematic mutations. The mutational event may be accompanied by a large or small change in phenotype. Such changes have the highest significance in plant breedingDwarf mutant was observed in the gamma rays and EMS treated plants. In this mutant, the mean plant height was reduced than the control plants (Fig: 6). Number of pods per plant was reduced. Leaf shape and size was similar as that of control. The reduction in plant height is may be due to the consequences of reduction in cell length and cell number[28] and changes in gibberellic acids[29]. This mutant showed reduced yield, thus it cannot be used as high yielding mutant but can be used in combination breeding programs. Tall mutant obtained in the present investigation showed vigorous growth and thick leaves (Fig: 5). It produced normal flowers and pods. According to Blonstein and Gale[30] the increase in plant height was due to increase in cell number and cell length or both. It has been reported by Nasare in *Ocimum sanctum*[31] and Wani *et al* in Mungbean[32]. Tendrillar and bushy mutant were observed in the physical and chemical mutagenic treatments (Fig: 7 and 8). These type of mutant have been reported earlier in Lentil[33]The leaf mutant such as unifoliate leaf, bifoliate leaf, tetrafoliate leaf, pentafoliate leaf, wrinkled leaf, narrow rugose leaf, biforked leaf and triforked leaf were observed in different doses/concentrations of mutagenic treatments. Characteristics of leaflet mutants are shown in the (Fig: 9-12). The abnormalities observed in leaves is due to various causes such as disturbances in phytochromes, chromosomal aberrations, mitotic inhibition, disrupted auxin synthesis and mineral deficiencies, disturbance in DNA synthesis, enlargement of palisade, spongy and mesophyll cells[34]. The presence of biforked and triforked leaves could be explained by the death of cells in the center of the meristematic regions which have specific influences on the development of leaves and leaf shape. Assuming that physiological activity has started in the meristematic regions toward the formation of new plants, gamma radiation may have adversely affected certain embryonal mechanisms which resulted in the non-development of leaf apices. These mutants have been reported by Bolbhat *et al.* in Horse gram[35] and Navnath *et al.* in Okra[36]. Physiological mutants such as early and late maturity were observed in all the mutagenic treatments. The maximum numbers of early maturity mutant were observed at 30mM of EMS. Early maturity mutants were reported by Sasi in bhendi[37] and Pavadai in Soyabean[38]. In the present study, flower abnormality trait is visible throughout the plant development. Observations of abnormal flowers have an extra standard petal and extra wing petal (Fig: 13 and14). All these mutant flowers were larger than normal, with increase number of petals when compared to control. Similar phenomena have been reported earlier in *Borago officinalis*[39]. Pod mutation such as single seed pod and long pod were observed in different mutagenic treatments (Fig: 15 and 16). These mutants were not found in the control populations. Therefore they were considered as the real mutants. These mutants were also reported in *Lathyrus sativus* [40]. The mutation frequency is one of the most dependable indexes for evaluating the genetic effects of mutagenic treatments. For the material grown in bulk, the mutant frequency was estimated by dividing the total number of mutants confirmed by the total number of M_2 plants in the bulk population studied. In the present results, the frequency of M_2 progenies segregating for chlorophyll and viable mutants is presented in Table-1. The highest mutation frequency was recorded in 30 mM EMS (12.52) treatment followed by gamma rays treatment at 25KR (8.68).

CONCLUSION

The chlorophyll mutations are useful in identifying the genetic effect of mutagen. Mutations in these chlorophyll genes are reflected in the M_2 and subsequent generations in the form of different mutants. The morphological mutants induced in the present study included agronomical desirable features which may possibly be utilized in future breeding programme.

ACKNOWLEDGEMENT

The authors are thankful to the Head of the Department of Botany and the authorities of Annamalai University for providing necessary facilities to carryout this work and the authors are also thankful to the UGC – BSR (SAP), New Delhi for their financial assistance.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Deka RK, Sarkar CR. Nutrient composition and anti-nutritional factors of *Dolichos lablab* L. seeds. Food Chem. 1990; 38: 239-246.
2. Basu AK, Samantha SK, Sasmala AC. Genetic analysis for some seed parameters in *Lablab* bean. Veg. Sci. 2002; 29(1): 163-164.
3. FAO/IAEA. Mutant Variety Database. 2015. http://mvd.iaea.org
4. Gnanamurthy S, Dhanavel D, Chidambaram ALA. Frequency in germination studies of chlorophyll mutants in effectiveness and efficiency using chemical mutagens. Elixir Appl. Botany. 2011; 37(A): 4083- 4086.
5. Makeen K, Suresh BG, Lavanya GR, Kumari A. Study of chlorophyll and macromutations induced by gamma rays and sodium azide in urd bean (*Vigna mungo* L. Hepper). Afr. J. Agric. Res. 2013; 8(47): 5958- 5961.
6. Park IS, Lee GJ, Kim DS, Chung SJ, Kim JB, Song HS, Goo DH, Kang SY. Mutation breeding of a spray chrysanthemum 'Argus' by gamma-ray irradiation and tissue culture. Flower Res. J. 2007; 15: 52-57.

7. Alka, Ansari MYK. Fasciated mutant induced by the combined treatment of gamma rays and sodium azide (20 kr + 0.10% sa) in *Linum usitatissimum* L. Int. J. Pharm. Bio. Sci.. 2013; 4(2): (B) 1196- 1200.
8. Wi SG, Chung BY, Kim JH, Baek MH, Yang DH, Lee JW, Kim JS. J. Plant Biol. 2005; 48(2): 195-200.
9. Jong -Yi Fang. In Vitro Mutation induction of Saintpaulia using Ethyl Methane Sulphonate. Hort Science. 2011; 46(7): 981-984.
10. Sridevi A, Selvakumar G. Chemical mutagens induced alterations in chlorophyll mutants and flower development of Chilli (*Capsicum annuum* L.). Int. J. Modern Agric. 2013; 2(1): 39- 42.
11. Gustaffson A. Characterization and role of highly productive mutations in diploid barley. Rad. Bot. (Suppl.). 1996; 5: 323-337.
12. Kumar A, Mishra MN, Kharkwal MC. Induced mutagenesis in black gram (*Vigna mungo* (L.) Hepper). Indian J. Genet. 2007; 67(1):41- 46.
13. Gustafsson A. The Mutation System of the Chlorophyll Apparatus. Kgl. Fys. Sällsk. Handl. (Lund). Bd., 51; 1940: 3-40.
14. Blixt S. Mutation in Pisum. Agr. Hort. Genet. 1972; 30: 1-293.
15. Natarajan AT, Upadhyay MD. Localized chromosome breakage induced by EMS and HA in *Vicia faba* L. Chromosome (Berl.). 1964; 15:156-187.
16. Kumar A, Parmhansh P, Prasad R. Induced Chlorophyll and Morphological mutations in Mungbean (*Vigna radiata* L. Wilczek). Legume Res. 2009; 32 (1): 41- 45.
17. Hooda, MS, Dhillon RS, Bangarwa KS. Albinism in jojoba (*Simmondsia chinensis*). Natnl. J. Pl. Improv. 2004; 1: 69-70.
18. Arulbalachandran D, Mullainathan L. Chlorophyll and morphological mutants of blackgram (*Vigna mungo* (L.) Hepper) derived by gamma rays and EMS. J. Phytol.2009; 1(4): 236–241.
19. Warghat AR, Rampure NH, Wagh P. Effect of sodium azide and Gamma rays treatment on percentage germination, survival, morphological variation and chlorophyll mutation in Musk Okra (*Abelmoschus esculentus* L.). Int. J. Pharma Pharma Sci. 2011; 3(5): 483- 486.
20. Blixt S. Quantitative studies of induced mutations in peas. V. Chlorophyll mutations. Agr. Hort. Genet. 1961; 19:1–402.
21. Kumar A, Parmanand P, Prasad R. Induced Chlorophyll and Morphological mutations in Mung bean (*Vigna radiata* L. Wilczek). Legume Res. 2009; 32(1): 41- 45
22. Mishra D, Singh B, Sahu R. Gamma ray induced Macro- mutations in greengram (*Vigna radiata* (L.) Wilczek). Int. J. Agric. For. 2013; 3(3): 105- 109.
23. Khan MH, Tyagi SD. Studies on induction of chlorophyll mutations in Soyabean, *Glycine max* (L.) Merrill. Front. Agric. China. 2009; 3(3): 253–258
24. Kulthe M, Mogle UP, Kashid NG. Study of mutagenic efficiency of NMU in Winged beans (*Psophocarpus tetragonolobus* L.). Biosci. Discovery. 2013; 4 (1):121-123.
25. Kulkarni GB, Mogle UP. Effects of mutagen on chlorophyll mutations in Horse gram (*Macrotyloma uniflorum* (Lam) Verdcourt. BioScience Discovery. 2013; 4(2): 214-219.
26. Gaul H. Mutation in plant breeding. Rad. Bot.1964; 4: 155- 232.
27. Swaminathan MS. A comparison of mutation induction in diploid and polyploidy. The use of induced mutation in plant breeding. Rad Bot.1964; 5:619.
28. Weber E, Gottschalk W. DieBeziehungen Zuischen Zellagrobe and internodienuange tri starhleindyzei earten *Pisum* mutant. Beitr Bio Pfl. 1973; 49: 101- 126.
29. Hedens P. The genes of the green revolution. Trends Genet. 2003; 19 (1): 5–9
30. Blonstein AD, Gale MD. Cell size and cell number in dwarf barley and semidwarf cereal mutants and their use in cross breeding II (Teidse 407) FAO/IAEA. Vienna. 1984; pp. 19-29.
31. Nasare PN.Characterisation of induced Morphological mutants in *Ocimum sanctum* Linn. Int. Interdisci. Res. J. 2011; 1(II): 14- 21.
32. Wani MR, Khan S, Kozgar MI, Goyal S. Induction of morphological mutants in Mungbean (*Vigna radiata* (L.) Wilczek) through chemical mutagens. The Nucleus. 2011; 48(3): 243-247.
33. Solanki IS. Isolation of macromutations and mutagenic effectiveness and efficiency in lentil (*Lens culinaris* Medik.). Indian J. Genet. 2005; 65 (4): 264-268.
34. Raghuvanshi SS, Singh AK. Studies on the effect of gamma rays on *Trigonella foenum-graceum*. Cytologia. 1974; 39: 473- 482.
35. Bolbhat, SN, Gawade BB, Bhoge VD, Wadavkar DS, Shendage VS, Dhumal KN. Effect of mutagens on frequency and spectrum of viable mutations in Horse gram (*Macrotyloma uniflorum* (Lam.) Verdc). Int. J. Agric. Vet. Sci.. 2012; 1(1): 45- 55.
36. Navnath, Kashid G, Slave US. Effect of induced mutation on leaf morphological changes in Okra (*Abelmoschus esculentus* L. Moench). Int. J. Rec. Adv. Multidiscip. Res. 2014; 1(1): 001-002.
37. Sasi A. Effect of chemical mutagenesis in bhendi [*Abelmoschus esculentus* (L.) Moench.]. M.Phil. Thesis, 2004, Annamalai University, Annamalainagar, Tamil Nadu
38. Pavadai P. Studies on induced mutagenesis in Soybean (*Glycine max* (L.) Merr.). Ph. D. Thesis, 2006, Annamalai University, Annamalainagar, Tamil Nadu.
39. De Haro, Del Tio A. Isolation of chemically induced mutants in Borage (*Borage officinalis* L.). JAOCS.1998; 75: 281-3.
40. Kumar S, Dubey DK. Induced morphological mutations in *Lathyrus sativus*. J. Cytol. Genet.1998; 33(2):131-137

Reviewers of this article

Dr. D. Kumarasamy M.sc., Mphil.,Ph.D

Associate Professor, Department of botany,
Annamalai University,
Annamalai Nagar, Chidambaram,
Tamil Nadu 608002, India.

Asst.Prof.Dr. Sujata Bhattacharya

Assistant Professor, School of Biological and Environmental Sciences, Shoolini University, Solan (HP)-173212, India

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript