

Induced genetic variability with EMS and studies on frequency and spectrum of chlorophyll mutations in Pigeonpea

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Abstract

Four hundred seeds in each of two long duration pigeonpea (*Cajanus cajan* (L.) Millsp.) Cultures viz., MAL 13 and MA156 were treated with three different concentrations (0.01M, 0.015M and 0.02M) of EMS. At lab condition, germination varied from 71% in MAL 13 (at 0.02M) to 88% in MA 156 (at 0.01M). Average root and shoot length decreased with increase in dose concentration in both the genotypes. In M₂, the highest in frequency of chlorophyll mutants (3.31%) at 0.015 M in MAL 13, followed by 2.73% at 0.015M in MA 156 and lowest (0.72%) was found at 0.01M in MA 156. Chlorophyll mutants viz., Albino, Xantha, Chlorina and Viridis were observed. MAL 13 is very sensitive to EMS and high efficiency was observed in 0.015M concentration.

Key words: Pigeonpea, EMS, Chlorophyll mutation.

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.], the second most important pulse crop of India belongs to the tribe Phaseoleae and the sub tribe Cajaninae. It is mostly consumed as split Dhal and also as green vegetable. The protein content is approximately 21 per cent (Saroj *et al.* 2014). Seeds are rich in iron, iodine, and essential amino-acids like lysine, cystine and arginine. Though pigeonpea has rich nutritional properties, the yield potential is very low. To create genetic variability in yield contributing traits and to improve the yield of crop plants, Mutation breeding has become an alternative conventional breeding method. Induced mutagenesis by EMS have manifested high yielding mutants in pigeonpea (Giri *et al.* 2010). Chlorophyll mutants are employed as markers for the evaluation of gene action of mutagenic factors in induced mutation studies (Gaul, 1964). The mutagenic effect is reflected in the segregation of chlorophyll mutants. The present study was undertaken to resolve the extent of genetic variability and possible spectrum of chlorophyll mutants through induced mutagenesis using EMS mutagen, an alkylating group which creates mostly point mutations.

Materials and Methods

The study was conducted at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, and Varanasi during Kharif seasons 2009-2010 and 2010-2011. The experimental area comes under sub-tropical zone.

Two pigeonpea varieties have been mutagenized for this experiment.

Variety 1 - MA 156: It has a vigorous, compact and determinate plant type, pods produced in clusters having bold seed; flower petals have red streaks with a maturity duration of 260 days. **Variety II- MAL 13 (Malviya Chamatkar):** Resistant to wilt, SMD and tolerant to *Phytophthora* blight, spreading type and bears long constricted pods containing medium bold seed, matures within 230 days.

Ethyl Methane Sulphonate (EMS) was prepared in three different concentrations viz., 0.01, 0.015 and 0.02M in phosphate buffer for inducing mutation. Four hundred pure seeds in each of the above genotypes were presoaked in distilled water for 6 hours at room temperature ($25 \pm 2^\circ\text{C}$) and dried on blotting paper for 30 minutes, then treated with different concentrations of EMS by submerging the seed in the freshly prepared solution for 6 hours and were washed thoroughly for 4 hours in running tap water to minimize the residual effect of chemical. 400 pure seeds (untreated) were presoaked in distilled water used as control. 20 seeds from each treatment have been utilized for Lab studies. At field condition, a total of eight treatments including control were immediately sown in R.B.D. with three replications during Kharif, 2009-2010. Each plot consisted of 2 meter length with spacing of 75 x 25 cm. The recommended agronomic practices were followed to raise a good crop. Germination percentage, pollen fertility, ovule fertility and survival upto maturity was recorded in M₁ generation and 50

plants with high fertility from each treatment were harvested separately.

Harvested seed were sown as single plant to progeny in M_2 generation. The germination percentage, frequency and spectrum of the different chlorophyll mutants were scored in M_2 . Both mutagenic effectiveness and efficiency were scored as per the following procedure recommended by Konzak *et al.* (1965).

$$\text{Mutagenic effectiveness} = \frac{\text{Mutation frequency (Mf)}}{\text{time (t)} \times \text{concentration (C)}}$$

$$\text{Mutagenic efficiency} = \frac{\text{Mutation frequency (Mf)}}{\text{Biological damage (S)}}$$

Where, Mf = Percentage of families segregating for chlorophyll mutations

t = period of treatment with chemical mutagen

c = concentration of chemical mutagen in terms of percentage

S = percentage of sterility in M_1 generation

Pollen and Ovule fertility %:

Pollen and ovule fertility were calculated by the following formula:

$$\text{Pollen fertility (\%)} = \frac{\text{Number of fertile pollen grains}}{\text{Total number of pollen grains} \times 100}$$

$$\text{Ovule fertility (\%)} = \frac{\text{Number of underdeveloped seed}}{\text{Total number of ovule scars} \times 100}$$

Results and Discussion:

At Lab condition

The range of germination varied from 71% in MAL 13 (at 0.02M) to 88% in MA 156 (at 0.01M) as compared to control, MA 156 and MAL 13, being 94 and 92%, respectively. Average root and shoot length was highest at lower dose (0.01M) and lowest at higher doses (0.02M) in both the genotypes. However average reduction in root and shoot length was much higher in MAL 13 as compared to MA 156 (Table 1).

At Field condition

The induction in M_1 caused by physical and/or chemical mutagens towards physiological damage and chromosomal mutation in the biological material could be measured qualitatively by the degree of reduction in germination, plant survival, pollen fertility and ovule sterility (Gaul *et al.* 1972; Sagade and Apparao, 2011; Sangle and Kothekar, 2013). The data on M_1 generation for the following traits viz., Pollen fertility, ovule fertility (mention the percentage) and survival upto maturity (Table 2) showed that invariably, the maximum percentage of germination was recorded

in MA 156 at 0.01M among all the mutagenic treatments. Considering the effect of different concentrations separately, it was obvious that the reduction in germination percent (70.26 and 67.89%) was more pronounced at higher concentration of EMS (0.02M) in both the genotypes. The minimum pollen fertility was observed at 0.02M concentration (75.74 % in MA 156 and 79.89 % in MAL 13) while maximum at 0.01M in both the genotypes. The percentage of ovule sterility (give the procedure of pollen fertility and ovule sterility) was obviously higher at higher doses of mutagen in both the genotypes as it was maximum i.e. 28.57 in MA 156 followed by 26.31% in MAL 13 at 0.02M concentrations. Percent plant survival at maturity indicated that maximum survival upto 91.23% was evident in MA 156 at 0.01M concentration while minimum of 78.63% in MAL 13 at 0.02M concentration in treated population. The results of present investigation showed that there was a decrease in seed germination, plant survival, ovule and pollen fertility with the increase in different doses of EMS in M_1 generation. Venkateswarlu *et al.* (1978) reported that the treatment with mutagens reduced germination, seedling height, pollen fertility and survival of plants at maturity. The reduction in pollen and ovule fertility with increase in doses/concentrations as noted in this experiment is in accordance with the reports of Giri *et al.* (2010) in *Cajanus cajan*, Sagade and Apparao (2011) in *Vigna mungo* and Sangle and Kothekar (2013) in *Cajanus cajan*. Ignacimuthu and Babu (1988) suggested that EMS induced sterility might be caused by gene mutations and fewer chromosomal aberrations. Thus pollen fertility might have resulted due to cumulative effects of all abnormalities caused by mutagen at various stages of anther development (Gaul, 1961).

Chlorophyll mutations

Chlorophyll mutations provide one of the most dependable indices for the evaluation of genetic effects of mutagenic treatments and have been reported in various pulse crops by Gautam *et al.*, 1992. The spectrum of chlorophyll mutations was studied and the mutants were classified as per the scheme of Gustafsson (1947) with modifications:

- Albino- white and relatively smaller than normal seedlings and survived normally upto 7 days.
- Xantha- yellow to yellowish white, lethal, carotenoids present but chlorophyll absent.
- Chlorina - uniform green colour with white on tips, viable.
- Viridis - uniform light yellow green colour of leaves, viable.

- *Maculata*: Leaves with whitish dots on entire leaflet and may attain pod maturity.

Plants with altered phenotypes can serve as chlorophyll mutants which are employed as markers for the evaluation of gene action of mutagenic factors in induced mutation studies (Gaul, 1961; Kousaret *al.*, 2013). Chlorophyll mutants also serve as index of mutagenic sensitivity of various mutagenic agents and their dose effects. In this study, different doses of EMS produced different frequency of chlorophyll mutants which varied from 0.72% to 2.73% in MA 156 and 2.36% to 3.31% in MAL 13. Vig (1969) reported that the genetic basis of the chlorophyll mutations may be plastome or nuclear mutations at loci that control chlorophyll development or pigment synthesis or due to induced chromosomal aberrations causing deletion of key chlorophyll genes which are perpetuated through leaves cell lineage. Genetic differences even of a single gene induce significant changes in mutagen sensitivity, which influence not only the rate but also the spectrum of recoverable mutations (Kaul and Bhan, 1977).

In the present investigation *viridis* type chlorophyll mutant is frequently observed followed by *albino*, *xantha* and *chlorina*. Similar results were also observed by Prakash and Khanure, (2000) in ricebean. Chlorophyll development seems to be controlled by many genes located on several chromosomes (Goud, 1967) that could be adjacent to centromeres and proximal segments of the chromosome. (Swaminathan *et al.* 1964). Ramulu (1970) suggested that differences in the mutation spectrum and rate in different genotypes may be due to difference in the location of chlorophyll genes in relation to the centromere. 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 as identified by many workers (Giri and Apparao 2011 and Sangle and Kothekar, 2013) in pigeonpea. Chlorophyll mutations are recessive as revealed by segregating pattern. The segregations of *Viridis*, *Albina*, *Xantha* and *Chlorina* indicated that these are governed by independent single recessive gene (Bahl and Gupta, 1982) where as Santos (1969) observed that *Chlorina* is governed digenic recessive genes.

Efficiency and effectiveness of the mutagen

The efficient mutagenesis is the production of desirable changes free from association with undesirable ones. Mutagenic efficiency is a

measure of the proportions of mutations in relation to undesirable changes (sterility, germination and chlorophyll mutation) while the mutagenic effectiveness denotes the frequency of mutation induced by a unit dose of mutagen. Giri and Apparao (2011) reported that effectiveness and efficiency of EMS at lower concentration 10 to 20mM was the effective and preferable concentration for induction of desirable mutations in pigeonpea. Sangle and Kothekar (2013) concluded that optimum dose induced the highest mutation frequency, whereas low concentrations of chemical mutagens were the most effective in pigeonpea.

From the Table 3, it is inferred that the different concentration of EMS showed variation in mutagenic efficiency and effectiveness. Among all the treatments higher efficiency and effectiveness was observed at 0.01M in MAL 13 and 0.015M in MA 156. In general, mutagenic efficiency and effectiveness decreases with increasing doses of EMS except for 0.02M in MA 156. Medium concentration was found most effective and efficient in MA 156 while in MAL 13 lower concentrations was found more effective and efficient. Konzak *et al.* (1965) reported that greater efficiency at the lower doses of mutagens appeared to be due to the fact that lethality, injury and sterility increases with higher doses of mutagens at faster rate than the rate of occurrence of mutations. In fact at lower dose, relatively low damage caused to the biological systems may result high ability to induce mutations. Konzak *et al.* (1965) attributed these results (low damage caused to the biological systems) due to differences in experimental material as well as treatment conditions and methods used to calculate mutation frequencies. The higher efficiency of the mutagenic agents not only depends upon the biological system but also on physiological damage, chromosomal aberration and sterility in addition to mutations. The decrease in effectiveness with increasing concentrations/dose of mutagen has been reported by Khan *et al.* 1973 and Giri and Apparao, 2011 in pigeonpea.

Conclusion

Average shoot and root length have decreased with increasing dose of EMS. Similar result was observed at field condition for germination, pollen fertility, ovule sterility and plant survival up to maturity. Genotype showed differential response to induced mutation with different doses of mutagens. Among the concentrations 0.015M is more effective and potent to induce chlorophyll mutants. In this study, *Viridis* type chlorophyll mutations were most frequent and *Chlorina* type is least common. Genetic differences even of a single gene

induce significant changes in mutagen sensitivity that influences not only the rate but also the spectrum of recoverable mutations.. The genotype MAL 13 is highly sensitive for EMS than MA 156.

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References:

- Bahl, J. R. and Gupta, P. K. 1982. Chlorophyll mutations in mungbean (*Vigna radiate* L. Wilczek). *Theor. Appl. Genet.* 63: 23-26.
- Gaul, H. 1961. Use of induced mutations in seed-propagated species, *Mutation and Plant Breeding*, Nat. Acad. Sci., Nat. Res. Council, USA. 206-252.
- Gaul, H. 1964. Mutation in plant breeding. *Radiat. Bot.* 4 (3): 155-232.
- Gaul, H., Bender, K., Ulonska, E. and Sato, M. 1960. EMS induced genetic variability in barley; the problem of EMS-induced sterility and the method to increase the efficiency of EMS treatment. *Mutation in Plant Breeding*(Proc. Panel, Vienna, 1966) IAEA, Vienna. 249-252.
- Gaul, H., Frimmel, G., Gichner, T. and Uionska, E. 1972. Efficiency of mutagenesis. *Proceedings of Latin America Study Group on Induced Mutations and Plant Improvement*. IAEA, 121-139.
- Gautam, A. S., Sood, K. C. and Riccarria, A. K. 1992. Mutagenic effectiveness and efficiency of gamma rays, ethylmethane sulphonate and their synergistic effects in blackgram (*Vigna mungo* L.). *Cytologia* 57: 85-89.
- Giri, S. P. and Apparao, B. J. 2011. Studies on Effectiveness and Efficiency of EMS in Pigeonpea (*Cajanus cajan* (L.)) *Bioscience Disc.* 2(1): 29-31.
- Giri, S. P., Tambe, A. B. and Apparao, B. J. (2010). Induction of a Novel, high yielding Mutant of Pigeon pea. *Asian J. Exp. Biol. Sci. Spl.* 152-155.
- Goud, J. V. 1967. Induced polygenic mutations in hexaploid wheat. *Radiat Bot.* 7: 231-241.
- Gustafsson, A. 1947. Mutation in Agricultural plants. *Heredities* 33:1-100.
- Ignacimuthu, S. and Babu, C. R. 1988. Radio sensitivity of the wild and cultivated urd and mungbeans. *Indian J. Genet. Plant Breed.* 48(3): 331-342.
- Kaul, M. L. H. and Bhan, A. K. 1977. Mutagenic effectiveness and efficiency of EMS, DES, and Gamma-rays in rice. *Theor. Appl. Genet.* 50: 241-246.
- Khan, W. M. A., Sivaswamy, N., and Ramaswamy, K. R. 1973. Sensitivity of the red gram (*Cajanus cajan* (L.) Millsp.) strains to different mutagens. *Madras Agri. J.* 60(6): 406-407.
- Konzak, C. F., Nilan R. A., Wagner, J. and Foster, R. J. 1965. Efficient chemical mutagenesis. *Rad. Bot., (Suppl.)* 5: 49-70.
- Kousar, M., Suresh B. G., Lavanya G. R. and Kumari A. 2013. Study of chlorophyll and macromutations induced by gamma rays and sodium azide in urd bean (*Vigna mungo* L. Hepper). *African J. Agri. Res.* 8(47): 5958-5961.
- Pandey, N., Ojha, C. B., Jha, V. B. and Singh, N. B. 1996. Effect of chemical mutagens on the rate of germination, seedling mortality, and induced sterility in pigeonpea. *Intel Chickpea and Pigeonpea Newsl* 3: 65-67.
- Prakash, B. G. and Khanure, S. K. 2000. Isolation of mutants and their frequencies under M2 generation in rice bean (*Vigna umbellata* L. Thumb). *Madras Agri. J.* 86(10/12): 568-572.
- Ramulu, S. K. 1970. Sensitivity and induction of mutation in Sorghum. *Mutation Res.* 10:197-206.
- Sagade, A. B. and Apparao, B. J. 2011. M₁ Generation Studies in Urdbean (*Vigna mungo* (L.) Hepper). *Asian J. Exp. Biol. Sci.* 2(2): 372-375.
- Sangle, S. M. and Kothekar V. S. 2013. Mutagenic effectiveness and efficiency in pigeonpea. *Intl J. Adv. Sci. and Tech. Res.* (3): 4.
- Santos, I. S. 1969. Induction of mutation in mungbean (*Phaseolusaureus Roxb.*) and genetic study of some of the mutants. In *Proc. Induced Mutations in Plant Breeding Symp.* Pullman. IAEA, Vienna, Pp 169-178.
- Saroj, S. K., Dhurai, S. Y., Bhati, P. K. and Singh, M. N. 2014. D² analysis for parents, F₁'s, and F₂'s population in pigeonpea. *Progressive Res.* 9(1): 299-302.
- Swaminathan, M. S. 1964. A comparison of mutation induction in diploids and polyploids. In: *The use of induced mutation in plants breeding*. Radiation Mutation Organ. FAO/IAEA Vienna: IAEA. pp. 619-641.
- Venkateswarlu, S., Singh, R. M., Singh, R. B., and Singh, B. D. 1978. Radio sensitivity and frequency of chlorophyll mutations in pigeonpea. *Indian Journal of Genetics* 38:90-95.
- Vig, B. K. 1969. Relationship between mitotic events and leaf spotting in Glycine max. *Canadian J. Genet. Cytol.* 11(8): 142-147.
- Waghmare, V. N. and Mehra, R. B. 2001. Induced chlorophyll mutations, mutagenic effectiveness and efficiency in *Lathyrus sativus* L. *Indian Journal Genet.* 61(1): 53-56



Table 1: Effect of different concentrations of EMS on germination in lab condition

Treatments	Concentrations (molar)	No. of seeds sown	No. of seeds germinated	Seed germination (%)	Root length(cm)			Shoot length (cm)		
					Minimum	Maximum	Average	Minimum	Maximum	Average
MA 156	0.01M	20	17	88	4.2	10.5	7.6	7	24	15.04
MA 156	0.015M	20	15	78	4	10	6.7	4	17.8	12.87
MA 156	0.02M	20	14	74	2	4.5	2.96	4.5	7	5.94
MAL 13	0.01M	20	16	83	1	6.2	3.26	2.8	15	8.72
MAL 13	0.015M	20	15	79	1	6	3.06	4	10	6.87
MAL 13	0.02M	20	14	71	1.2	3.2	1.82	3.8	8	6.43
Controls										
MA 156	-	20	19	94	4.4	11.5	8.32	4.6	18.2	17.16
MAL 13	-	20	18	92	4	11.2	7.62	13	24.5	18.06

Table 2. Germination, Pollen fertility, ovule fertility and survival upto maturity in M₁ generation in field condition

Treatments	Seed germination (%)	Pollen fertility (%)	Ovule fertility (%)	Plant Survival up to maturity (%)
MA 156	84.21	88.66	82.34	91.23
MA 156	75.00	77.42	75.02	87.45
MA 156	70.26	75.74	71.43	81.39
MAL 13	81.05	92.80	86.44	87.93
MAL 13	77.37	83.91	78.35	84.56
MAL 13	67.89	79.89	73.69	78.63
Controls				
MA 156	92.37	100	100	95.32
MAL 13	90.53	100	100	92.65

Table 3. Frequency of chlorophyll mutations in M₂ generation in pigeonpea



Treatments	Seed germination (%)	No. of seedlings analyzed	Frequency of chlorophyll mutant					Chlorophyll mutants (%)	Pollen sterility (%)	Mutagenic efficiency	Mutagenic effectiveness
			A	X	C	V	Total				
MA 156	79.5	1934	5	1	1	6	13	0.72	11.34	0.063	1.109
MA 156	62.9	965	10	3	7	7	27	2.73	22.58	0.121	2.809
MA 156	58.3	646	3	3	0	5	11	1.66	24.26	0.068	1.281
MAL 13	78.0	1486	9	9	2	18	38	2.63	7.20	0.365	4.051
MAL 13	69.0	1032	9	11	1	13	34	3.31	16.09	0.206	3.405
MAL 13	73.0	642	2	1	2	9	14	2.36	16.89	0.146	1.821
		Total	38	28	13	58	137				
Controls											
MA 156	90.0	18	-	-	-	-	-	-	100	-	-
MAL 13	95.0	19	-	-	-	-	-	-	100	-	-

A= Albina, X= Xantha, C= Chlorina and V= Viridis