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Research Article

Mutation frequency of chlorophyll mutants in *Vigna unguiculata* L. (Walp.) variety Lobia and *GNU* induced through mutagenesis

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Abstract

Mutation breeding is one of the cheapest and surest method of plant breeding. In mutation breeding program effectiveness and efficiency of mutagens could be helpful to realize the spectrum of desirable mutations in the treated populations. The effectiveness and efficiency of two mutagens EMS and gamma rays were studied in two varieties of cowpea [*Vigna unguiculata* (L.)Walp.] In the present investigation, the seeds of two varieties of cowpea, Lobia and GNU were treated with gamma rays and EMS to obtain the spectrum and frequency of chlorophyll mutations in M2 generation. Mutation frequency were calculated using frequency of chlorophyll mutations. The individual treatment of gamma rays was found to be more efficient than EMS to induce chlorophyll mutants. A progressive increase in mutation frequency of chlorophyll mutations was observed with increasing doses. Four different types of chlorophyll mutants, such as, *Chlorine*, *Albina*, *Xantha and Viridis* were induced with effect of mutagens. The highest frequency of chlorophyll mutations was reported in the gamma rays. There was a dose dependent increase in the spectrum and frequency of chlorophyll mutations. The lower dosages of gamma rays and EMS were proved to be most effective and efficient. Among two mutagens used EMS was much more effective than gamma rays in both the varieties viz.v ariety Lobia and GNU .

INTRODUCTION

Among the legumes, cowpea is important cultivated legume crop in the world. Cowpea grows well in poor dry conditions, thrive in soils up to 85% sand (Obatolu, 2003). The optimum temperature for cowpea growth is 30 °C (86 °F), making it the only available one as a summer crop for most of the world. Cowpeas are grown mostly for their edible beans like other legumes. Cowpea seeds consist of 25% protein as well as minerals and vitamins and have very low fat content and are usually cooked to make them edible. The seed is a rich source of folic acid, some antinutritional elements, notable phytic acid and protease inhibitors, which reduce the nutritional value of the crop. In India Cowpea is cultivated in Rajasthan, Gujarat, Punjab, Maharashtra and Tamilnadu with an average yield of cowpea 7089 kg/ha (2017) which is very low; roughly was about two-third of the world's average of 10,095 kg/ha. Thus, the efforts should be made to enhance the production and productivity of cowpea by adopting any breeding method.

Mutation breeding might be the effective alternate to induce genetic variation, particularly for traits having low level of genetic variation (Szarejko and Forster 2007). The chlorophyll deficient sectors formed in M_1 generation and chlorophyll mutants formed in M_2 generation are being used conveniently as preliminary index of the mutagenic effectiveness and efficiency. The improvement aspects like quality, quantity and morphological characters along with chlorophyll mutations have been successfully attained by several researchers through mutation breeding in different crops like mustard (Julia*et al*. 2018), chickpea (Gaur *et al.* 2007) and sesame (Savant and Kothekar 2011).The usefulness of a mutagen in mutation breeding depends on its mutagenic effectiveness and efficiency (Savant *et.al.* 2010). The selection of effective and efficient mutagen is very essential to recover a high frequency and spectrum of desirable mutations (Mahabatra 1983; Solanki and Sharma 1994). The success in mutation breeding program for any crop can be achieved by increasing the spectrum and frequency in viable mutations. Therefore the present investigation was undertaken to study the mutagenic response of EMS and gamma rays in cowpea.

MATERIALS AND METHODS

In the present investigation the seeds of two varieties of cowpea, Lobia and GNU were obtained from pulse crop research substation, Pandharpur, Maharashtra, India. The two mutagens used for mutagenic treatment are Ethyl methane sulphonate (EMS) and Gamma rays. Initially, a pilot experiment were conducted to know LD50. LD50 is the dose in which half of the individuals among the treated population dies. It is a parameter to decide the effective dose for a mutagen treatment in any crop species. Suitable concentrations and dosage of mutagens were decided on the basis of LD50.

The chemical mutagenic treatments were carried out at room temperature of 25 ± 20C. Healthy seeds of uniform size were selected and treated with 0.20%, 0.25% and 0.30% concentrations of EMS. In case of physical mutagen the dry seeds with seed moisture content of 08 per cent were exposed to gamma rays at three different doses viz., at 250 Gy (gray), 350Gy.and 450Gy. at department of Biophysics, Govt. Inst. Of Science, Aurangabad, India. Untreated seeds stock of the respective genotypes were used as control. 300 seeds of each treatment were sown in the field following randomized block design (RBD) with six replications each consisting of 50 seeds along with control for raising the M1generation. The seeds were sown keeping distance of 25cm between the plants and 50cm between the rows.

The germination percentage was counted after 10 days. The biological damage (lethality and seedling injury) was computed as the percentage reduction in plant survival, seedling height and fertility of the pollen respectively. At maturity, seeds of M1 plants from each treatment were harvested separately and used these seeds to raise M2 generation in the next season. The M2 generation were raised in Randomized Block Design (RBD) on plant to row basis. Chlorophyll mutants were recorded during 06 to 15 days after sowing of M2 generation. Chlorophyll mutants viz. Albina, Chlorina, Xantha and Viridis, were classified according to the classification of Gustafsson (1940) and Blixit (1961). . Different kinds of chlorophyll mutations were identified on the basis of amount of chlorophyll synthesized and distributed in the plants in accordance with the classification of Gustafsson (1940) and Blixit (1961). The mutant Albina was found to be Lethal mutant and characterized by entirely white leaves of seedlings. It survived for 08-10 days after emergence. The mutant Chlorina was characterized yellowish green (pale green) seedlings in colour and they survived for reasonably longer period some of theme produced flowers and seeds as well. The leaves of mutant Xantha were bright yellow in colour and their seedlings were survived for 22-28 days. Viridis were viable mutants characterized by mixed pattern of light green leaves which become normal green colour at later stages. However, this mutant died before maturity in the present study. The spectrum of chlorophyll mutations was determined as the relative proportion of different types of chlorophyll mutants to the total number of chlorophyll mutations. Mutation frequency was calculated as percentage of mutated M2 progenies for both chlorophyll and morphological mutations in each treatment as per the following formula.

$$\text{Mutation Frequency} = \frac{\text{Number of mutant plants}}{\text{Total number of } M_2 \text{ plants}} \times 100$$

RESULTS AND DISCUSSION

The tolerance level of the biological material to a particular mutagen are manifested in M1 generation itself in terms of germination, seedling injury, pollen sterility etc. (Gaul 1970). In present investigation the estimated LD50 doses for seed germination are 500Gy (gray) for gamma ray and 0.40 % concentration for EMS. The dose below 200Gy for gamma ray and 0.15 % concentration for EMS will definitely increase the availability of the population of M2 generation but the mutation spectrum induced will not be satisfying. However if the dose is more than 600Gy and 0.50 % concentration of EMS , then, enough population may not be available to grow M2 generation. Thus, doses just below 500Gy and 0.40 % concentration for EMS are selected for further mutation breeding program for inducing good mutations while insuring .

Mutation frequency and relative percentage of chlorophyll mutants were presented in Table – 1.Mutation frequency has been used as the indicator of mutagenic effect. The analysis of the mutation frequency revealed that in general, the mutation frequency increased with the increase in dose and was irrespective of the variety. The maximum amount of mutation frequencies were recorded at 450Gy gamma ray treatment which was followed by 0.3% EMS treatment in both of verities viz. variety Lobia and variety GNU .

Xantha

Albina

Chlorina

Normal

Table – 1: Effect of different mutagens on mutation frequency and Spectrum of Chlorophyll mutants in M2 generation of cowpea variety *Lobia* and GNU

Treatment	Dose	Mutation frequency **(Lobia)**	Relative % of chlorophyll mutants				Mutation frequency **(GNU)**	Relative %of chlorophyll mutants			
			Albina	Xantha	Chlorina	Viridis		Albina	Xantha	Chlorina	Viridis
Control		---	---	---	----	----	---	---	---	---	---
Gamma Ray (*Gy*)	*150*	1.84	---	33.33	40.66	20.00	1.65	12.5	37.5	25.00	25.00
	250	2.16	11.11	11.11	55.55	11.11	2.48	33.33	11.11	44.44	11.11
	350	2.72	18.18	27.27	36.63	18.18	2.56	13.33	26.66	20.20	40.00
EMS	0.15%	0.87	--	---	40.00	60.00	1.04	---	20.00	20.00	60.00
	0.20%	1.02	---	28.57	44.28	27.14	2.34	16.6	25.0	33.33	25.0
	0.25%	1.84	15.38	23.07	7.69	3.84	2.42	11.76	23.52	35.29	29.41

The present study revealed that the cowpea varieties responded differentially to gamma ray and EMS for the production of chlorophyll mutations. The performance of chlorophyll mutations and their relative frequencies are recorded in Table- 1. Almost all the dosage of both of the mutagens are succeeded in inducing all types of the chlorophyll mutants except treatment EMS 0.15% which failed to produce Albina in variety GNU whereas in case of variety Lobia, the mutagenic treatments 150Gy, EMS 0.15% and EMS 0.20% are failed to produce Albina this could be because these lower dosage are not sufficient to induce mutation in all of the genes necessary for the synthesis of chlorophylls. In Variety Lobiya, the highest mutation frequency of chlorophyll mutants is found at dose 350 Gy. whereas the least mutation frequency is recorded at EMS treatment 0.15%. Among four chlorophyll mutants, Chlorina is recorded in highest amount than any other chlorophyll mutant in variety Lobiya and GNU. Chlorophyll mutants Albina is found to be observed in least number in both of the varieties. The highest mutation frequency (2.56) for variety GNU is recorded at dose 350 Gy.

Among two mutagens used a physical mutagen Gamma Rays is succeeded in inducing more number of chlorophyll mutants and mutation frequency than EMS in both of the varieties. Wide range of chlorophyll mutants is observed in soybean (Karthika and Subba lakshmi 2006); sesame (Savant et al. 2010) and finger millet (Ambavane et al. 2015). Variety GNU is found to be more susceptible than Lobia for both of mutagens.

All types of chlorophyll mutations were observed in both of the varieties at higher dosage of both EMS and gamma rays which are known potent mutagens in inducing point mutations and chromosomal aberrations suggesting their preferential action on genes for chlorophyll development (Goyal et.al.2019 , Bhosle and Kothekar, 2010). Higher concentrations of mutagens became more efficient in inducing chlorophyll mutations, these doses/concentrations have induced wider spectrum of chlorophyll mutations compared to lower doses of mutagens in both of the varieties (Goyal et al. 2020). Mutation frequency calculated on the basis of lethality and seedling injury in both varieties was found to be highest at lowest doses of both mutagens. Further it is reduced with increasing dose. Higher efficiency at lower concentration / dose of the mutagen is reported by Savant et.al. (2010) in sesame and Wani (2009) in chickpea. Thilagavathi and Mullainathan (2009) reported higher mutagenic effectiveness and efficiency at lower doses of EMS in black gram. Increased mutation frequency at higher doses /concentrations observed in present investigation was also reported by Dhanavel et al. (2008) and Nair et al. (2014) in cowpea. In the present study, GNU proved to be more sensitive to mutagen than Lobiya as seen elsewhere. Variations in sensitivity within crops and even within genotype may depend on their genetic architecture, and the mutagens employed (Raina et al.2020). EMS concentration induced high chromosomal aberrations than gamma ray treatments which indicate greater efficiency of EMS for inducing mitotic abnormalities in the cells of treated population.

CONCLUSION

In present investigation the optimum response of both of the mutagens were observed at lower doses. The frequency of chlorophyll mutations may be included as an indicator to determine the capacity, power, efficacy, influence, potency of plant mutagen. However, an effective mutagen may not necessarily show high efficiency and vice versa. The mutation frequency increases with increase in dose / concentration of mutagen and was found higher at higher doses. The mutagenic doses inducing mutations with less biological damage are proved to be more effective and efficient. Efficiency of a mutagenic agent depends on a number of attributes like the reactivity of the agent with the biological material, its applicability to the biological system, the level of physiological damage, chromosomal aberration, pollen sterility, lethality seedling injury and alteration in genes necessary for chlorophyll synthesis.

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REFERENCES

Ambavane AR , Sawardekar SV, Sawantdesai SA and Gokhale NB, 2015. Studies on mutagenic effectiveness and efficiency of gamma rays and its effect on quantitative traits in finger millet [*Eleusine coracana*(L.) Gaertn]. *J. Radiat. Res. and Appl. Sci.* **8**:120-125.

Bhosle SS and Kothekar VS, 2010. Mutagenic efficiency and effectiveness in Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub]. J. Phytol. 2: 21 - 27.

Blixit S, 1961. Quantitative studies of induced mutations in Peas. V. Chlorophyll mutations. *Agric. Hortic.Genet.* **19**: 402-47.

Dhanavel D, Pavadai P, Mullainathan L, Mohana D, Raju G, Girija M, and Thilagavathi C,2008. Effectiveness and efficiency of chemical mutagens in cowpea [*Vigna unguiculata* (L.)Walp]. *Afr. J. Biotechnol.* **7**: 4116 - 4117.

Gaur PM, Gour VK and Srinivasan SS, 2007. An induced brachytic mutant of chickpea and its possible use in ideotype breeding. *Euphytica.* **159**(12): 35-41.

Goyal S, Wani MR, Khan S, 2019. Frequency and Spectrum of Chlorophyll Mutations Induced by Single and Combination Treatments of Gamma Rays and EMS in Urdbean. *Asian Journal of Biological Sciences,* **12**: 156-163

Goyal S, Wani MR, Laskar RA, Raina A, Khan S, 2020. Mutagenic effectiveness and efficiency of individual and combination treatments of gamma rays and Ethyl Methanesulfonate in black gram [*Vigna mungo* (L.) Hepper] *Adv. Zool. Bot.* 8:163–168.

Gustafsson A, 1940. The mutation system of the chlorophyll apparatus.Lunds.Univ. Arrsr. N.F. Avd. 30: 1-40.

Julia T, Renuka TH, Nanita H and Jambhulkar S, 2018. Mutagenic Effectiveness and Efficiency of Gamma Rays in Indian mustard. *Int. J. Curr. Microbiol. App.Sci* .7(3): 3376-3386.

Karthika R, and Subba Lakshmi B, 2006. Effect of Gamma Rays and EMS on Two Varieties of Soybean. *Asian J. Plant Sci.* **5**: 721-724.

Mahabatra BK, 1983. Studies on comparative spectrum and frequency of induced genetic variability in green gram [*Vigna radiate* (L.)Wilczek]. Ph.D. Thesis, IARI, New Delhi.

Nair R, Mehta AK, Singh KP and Sharma SK, 2014. Mutagenic effectiveness and efficiency in Cowpea. *Adv. Appl. Res.* **6**(1): 78-85

Obatolu VA, 2003. Growth pattern of infants fed with a mixture of extruded malted maize and cowpea. *Nutrition*, **19** (2): 174–178.

Raina A, Laskar RA, Tantray YR, Khursheed S, Wani MR, Khan S, 2020. Characterization of Induced High Yielding Cowpea Mutant Lines Using Physiological, Biochemical and Molecular Markers. *Sci. Rep.* 2020;1 0:3687.

Savant KD, Kulkarni GP, and Kothekar VS, 2010. Mutagenic effectiveness and efficiency in sesame (*Sesamum indicum* L.). *Geobios*, **37**(1): 49-52

Savant KD, and Kothekar VS, 2011. Induction of variability in fatty acid profile in sesame (*Sesamum indicum* L.). *Journal of Phytology*, **3**(12): 01-03.

Solanki IS and Sharma B 1994. Mutagenic effectiveness and efficiency of gamma rays, ethylene imine and N-nitroso-N-ethyl urea in macrosperma lentil (Lens culinaris Medik.). *Indian J. Genet*, **54**(1): 72-76.

Szarejko I, and Forster BP 2007. Doubled haploidy and induced mutation. *Euphytica*, **158**: 359-370.

Thilagavathi C, and Mullainathan, L 2009. Isolation of macro mutants and mutagenic effectiveness, efficiency in Black gram [*Vigna mungo* (L.)Hepper]. *Global J. Mol. Sci.* **4**: 76 - 79.

Wani A, 2009. Mutagenic effectiveness and efficiency of gamma rays, ethyl methane sulphonate and their combination treatments in Chickpea (*Cicer arietinum* L.). Asian *J. Plant Sci.*, **8**: 318 - 321.

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