Effects of mutagen on chlorophyll mutation in horse gram [*macrotyloma uniflorum* (Lam) Verdcourt]

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ABSTRACT

The spectrum and frequency of chlorophyll mutations was assessed in M_2 generation in local cultivar of Horse gram [*Macrotyloma uniflorum* (Lam) Verdcourt] with a range of EMS (0.1, 0.2 and 0.3%), SA (0.05, 0.1 and 0.15%) and NMU (0.4, 0.6 and 0.8%) doses. All the mutagen induced a wider spectrum of chlorophyll mutations. In present investigation Chlorina, Xantha and Viridis were found more frequently which indicates preferential induction of certain type of mutations. Fairly high frequency of chlorophyll mutants was obtained with EMS than in SA, NMU. Dose dependent increase in chlorophyll mutation rate was observed based on plant population and segregating progenies in M_2 generation. All the three mutagenic effectiveness and efficiency were higher at higher concentrations of the mutagen. Mutagenic effectiveness, a measure of the frequency of mutations induced per unit dose of mutagen and mutagenic efficiency, proportion of mutations in relation to undesirable effects, were higher with EMS treatments indicates more effective and efficient mutagen than other two.

Key words: EMS, SA, NMU, Horse gram, chlorophyll, mutation, effectiveness, efficiency.

INTRODUCTION

Horse gram [*Macrotyloma uniflorum* (Lam) Verdcourt, family Fabaceae] a multipurpose, rich protein crop, cultivated in India, Pakistan, Bangladesh, Srilanka, Nepal and West Africa. Horse gram is wide spread pulse in India. Average crop establishment is often poor and yields are low. One of the most neglected but important in rich protein content crop of this subcontinent. In tribal localities and drought prone areas of Marathwada (region of Maharashtra), it is sown late in Kharif only.

Mutation breeding is relatively a quicker method for improvement of crops. Many chemicals mutagens have been used for induction of useful mutants in a number of crops (Sangle *et al.,* 2011; Bashir *et al.*, 2013. Induction of chlorophyll mutations in general is considered as a measure to assess the effectiveness of various mutagens (Marki and Bianu, 1970). So, it is the mutagenic effect is reflected in the segregation of chlorophyll mutants and it is also a good indicator to forecast the spectrum of genetic variability that can arise from the mutated sectors (Sengupta and Datta, 2005).

The usefulness of any mutagen in plant breeding depends not only on its effectiveness but also upon its efficiency. In the present investigation, the effect of different doses of EMS, SA and NMU mutagen on the frequency and spectrum of chlorophyll mutation were evaluated in order to determine the effectiveness and efficiency on local cultivar of Horse gram. The results of this study are reported in this paper.

MATERIALS AND METHODS

Dry (9-12 % moisture) and healthy seeds of Horse gram [*Macrotyloma uniflorum* (Lam) Verdcourt] obtained from Local Market was used in the present investigation. The experiment was categorized into three chemical Mutagens treatments. For the experiment, doses of Mutagens were prepared in Phosphate buffer of pH 7. EMS (Ethyl Methane Sulfonate), SA (Sodium Azide) and NMU (N-Nitroso-N-Methyl Urea) were used as chemical mutagens at the concentrations of 0.1, 0.2 and 0.3% for EMS, 0.05, 0.1 and 0.15% for SA and 0.4, 0.6 and 0.8% for NMU.

The treated seed were presoaked in distilled water for 4 hours at room temperature followed by six hours immersed in mutagenic solutions. Then these seeds were thoroughly washing under running water for four hours. The 300 seeds from each treatment were sown in Randomized Blocked Design (RBD) with three replications along with control for rising M_1 generation during Kharif 2009. The seeds were sown at the distance of 15cm in plant and 30 cm between rows. The field experiment was conducted at Research field of Genetics and Plant Breeding Center, JES College, Jalna (MS) India. Recommended agronomic practices were employed for preparation of field, sowing and subsequent management of the population.

Seeds of 25 randomly selected plants of M_1 generation were collected from all the treatment and control. They were used for rising the M_2 generation and chlorophyll mutations were recorded. The chlorophyll mutations were scored in the field, when the seedlings were 10-15 days old. The types chlorophyll mutation scored were *Xantha, Chlorina, Chloroxantha and Viridis*. The frequency of chlorophyll mutant was calculated.

RESULTS AND DISCUSSION

The findings of the inhibitory effect of chemical mutagens in the present study on reduction in seed germination were notified for further studies. It was noted that the 50% reduction in seed germination observed at 0.4% of EMS, 0.2% of SA and 1.0% of NMU. On the basis of lethality, the highest mutagenic efficiency was recorded at 0.3% of EMS (2.23%), 0.15% of SA (1.59%) and at 0.8% of NMU (1.28%). Seed germination percentage were 68.44, 55.17, 42.38 for the conc. of 0.1%, 0.2% and 0.3% of EMS, 61.27, 54.55, 48.71 for the concentration of 0.05, 0.1 and 0.15 of SA and 78.74, 62.66 and 56.03 for concentration of 0.4, 0.6 and 0.8 % of NMU as against 84.45% in control. Similar results recorded by by Gautam *et al.,* (1992) in mungbean, EMS, SA, NMU, Horse gram, chlorophyll, mutation, effectiveness, efficiency. In *Vicia faba,* Deepalakshmi and Anandakumar (2003) and Sharma *et al.*(2005) in urdbean, Jayakumar and Selvaraj (2003) in sunflower, and Jabee and Ansari (2005) in chickpea, Dhanavel *et al.*(2008) in cowpea, Kavithamani *et al.* (2008) in Soyabeans, Srivastava (1979) Bhat *et al.* (2007), Siddiqui *et al.* (2007) in *Vicia faba,* Sangle *et al.* (2011) in pigeon pea, Bashir *et al.* (2013) in fenugreek and Kulthe *et al*. (2013) in winged bean considered that reduction in germination percentage was due to weakening and disturbance of growth processes resulting in early elimination of seedlings. The survivals of seedling percentage also gradually decreased with gradual increase in concentration of mutagens were observed in present investigations.

Table 1: Effect of Chemical Mutagens on M_2 Generation of Horse gram [*Macrotyloma uniflorum* (Lam) Verdcourt]

Mutagen	Concen-tration	Seed germination (%)	Relative % frequency				Total Mutation Frequency %
			Xantha	Chlorina	Chloro-xantha	Viridis	
Control	--	84.45	--	--	--	--	--
EMS	0.1 %	68.44	0.38	0.33	0.33	0.30	1.34
	0.2 %	55.17	0.49	0.45	0.46	0.41	1.81
	0.3 %	42.38	0.65	0.58	0.49	0.51	2.23
SA	0.05 %	61.27	0.38	0.00	0.35	0.31	1.04
	0.10 %	54.55	0.51	0.42	0.47	0.00	1.40
	0.15 %	48.71	0.59	0.00	0.51	0.49	1.59
NMU	0.4 %	78.74	0.17	0.16	0.15	0.14	0.62
	0.6 %	62.66	0.27	0.21	0.23	0.00	0.71
	0.8 %	56.03	0.49	0.40	0.00	0.39	1.28

Table 2: Frequency of Chlorophyll and Viable Mutants in M_2 Generation of Horse gram [*Macrotyloma uniflorum* (Lam) Verdcourt]

Treatment	Dose	Total plants studied	Total plant segregated	Mutation frequency %
Control	000	286	0	0.00
EMS	0.1%	273	3	1.10
	0.2%	223	4	1.79
	0.3%	189	5	2.65
SA	0.05%	277	2	0.72
	0.10%	233	3	1.29
	0.15%	201	4	1.99
NMU	0.4 %	287	1	0.35
	0.6 %	251	2	0.80
	0.8 %	207	3	1.45

Table 3: Spectrum and frequency of chlorophyll and morphological mutation in M_2 generation induced by different Mutagens in Horse gram [*Macrotyloma uniflorum* (Lam) Verdcourt].

Morphological Mutation	**EMS (%)**	**SA (%)**	**NMU (%)**
Plant Growth			
Compact	0.17	0.10	0.04
Bushy	0.20	0.07	0.07
Erect	0.27	0.09	0.06
Spreading	0.02	0.03	0.00
Tall	0.02	0.03	0.00
Dwarf	0.11	0.07	0.05
Foliage			
Pentafoliate	0.02	0.00	0.01
Tetrafoliate	0.03	0.01	0.00
Lanceolate	0.05	0.02	0.01
Round Cuneate	0.03	0.01	0.00
Multiple leaf	0.04	0.00	0.00
Unifoliate	0.00	0.02	0.01
Wrinkled leaf	0.07	0.03	0.05
Total	**1.10**	**0.47**	**0.39**

In Horsegram seedling showed either Xantha, Chloroxantha or viridis leaf and died later on prematurely. A number of mutant plants were identified in M_2 generation and mutation percentage in concentration 0.1, 0.2 and 0.3 % of EMS were 1.10, 1.79 and 2.65 %; in 0.05, 0.1 and 0.15% of SA were 0.72, 1.29 and 1.99 % where as in 0.4, 0.6 and 0.8 % of NMU were 0.35, 0.80 and 1.45 % respectively (Table 2). In this experiment, no distinct mutant's plants were found regarding yield components which were died permanently. Chemicals mutagens are base specific and localized in their action (Sri Ramalu, 1971). During present experiment study, variable response of in chlorophyll mutations could have been observed due to differential action of EMS, SA and NMU on Genetic material. A mild relative mutagenic specificity was observed in relation to morphological mutation. Some of the morphological mutations viz. foliage and growth habit appeared more frequently than other types. Similar results have also been observed by Yadav (1987) and Khan (1989), Singh *et al.* (2000) in Mungbean, Sangle *et al.* (2011) in pigeon pea, Bashir *et al.* (2013) in fenugreek and Kulthe *et al*. (2013) in winged bean.

The spectrum of induced chlorophyll mutants was broad in the local cultivar of horse gram. The mutation frequency showed gradually increased with the increasing concentration of mutagens.

The frequency of chlorophyll mutants ranged from 1.34 to 2.23 % in EMS, 1.03 to 1.89 % in SA and 0.62 to 1.28 % in NMU. Among these mutagens, 0.3 % EMS proved to be efficient in inducing mutations with highest frequency (Table1). In the present investigation, the maximum chlorophyll and viable mutation frequency observed at 0.3% of EMS (2.23%), while the minimum mutation frequency was observed at 0.6% of NMU (0.71%) (Table-1).

A detailed spectrum and analysis of frequency of chlorophyll and viable mutants observed in M_2 generation is mainly used as a dependable measure of genetic effect in mutagen (Presented in Table-3) as mentioned by Marki and Bianu (1970) in flax, Lysshenko and Ulitcheva (1971) in sunflower; Hussein *et al*. (1974) in Peas and Tsukuda *et al*. (1977) in rice, Gautam *et al.* (1998) in *Phasiolus*. Four types of chlorophyll mutations identified in the present investigation were in the order Xantha > Chroxantha> Chlorina > Viridis. Similar results noted *by* Vasu and Hasan (2011) in *Trigonella*. Recently, Bhosle and Kothekar (2010), Sangle *et al.* (2011) in pigeon pea and Bashir *et al.* (2013) in fenugreek found that SA and EMS proved to be more efficient. The frequency of chlorophyll mutation in M_2 generation can be considered as a reliable index for estimation the potency of mutagens. Though, EMS induced higher frequency of chlorophyll mutations but the order of mutagenic effectiveness as determined on M_2 plant basis was NMU>SA>EMS. The differences in molar concentrations appear to be the reason for low values of its effectiveness. In supports the earlier reports of various workers by Prasad (1972), Nerker (1977) in *Lathyrus,* Sharma and Sharma (1979) in lentil, Khan and Siddiqui (1992) in Mungbean, Bhosle and Kothekar (2010) and Kulthe *et al*. (2013) in winged bean have drawn similar conclusions. It is now well known that mutagenic efficiency and effectiveness depends not only on the type of mutagen and its dose, but also on the genetic architecture of an organism.

It was noted in M_2 generation that EMS was more pronounced in inducing chlorophyll mutations than SA and NMU. As regards the Xantha, 0.65% of EMS (0.3 %) proved to be the most effective as compared to 0.59 % in SA(0.15 %) and 0.49% in NMU (0.8%), in present study. The relative percentage of chlorophyll mutation in Chlorina 0.58% (0.3 %) of EMS, proved to be the most effective as compared to 0.30 % (0.15 %) in SA and 0.40 % (0.8%) in NMU, where as Chloroxantha was observed slightly maximum in SA 0.51% (0.15%) as compared to EMS (0.3%). The Viridis mutants were observed comparatively less than Xantha, Chlorina and Chloroxantha in all the treatments. Similar observations were made by Mehraj-ud-din *et al.* (1999) in mungbeans, Deepalakshmi and Anandakumar (2003) in urad bans, Solanki (2005) in lentil, Shah*et al.* (2006) in chickpea, Sangle *et al.* (2011) in pigeon pea and Kulthe *et al*. (2013) in winged bean.

The mutational event may be accompanied by a large or small change in phenotype. Such changes have the highest significance in plant breeding and have been stressed by several authors. In the present investigation, some of the morphological mutants were observed in M_2 generation with different concentration of EMS, SA and NMU. Treatments of 0.2 and 0.3% EMS produces the numbers of viable mutants were observed (Table-3). Similar mutants were observed by Sengupta and Datta (2005) in sesame. Tall and dwarf mutants were observed in different mutagenic treatments. Among the dose or concentration maximum number of mutants was recorded at higher doses of EMS. Similar mutants were observed by Sinha (1998) in urdbean, Kumar and Dubey (1998) in *Lathyrus sativus* and Hepziba and Subramanian (2002) in black gram. The high number of pod mutant such as single seeded pod and long pod were observed in different mutagenic treatments. Similar results were survival observed in Kumar and Dubey (1998) in *Lathyrus sativus*, Vandana *et al.* (1992) and Vandana and Dubey (1995) in lentil.

Physiological mutants such as early and late maturity were observed in all the mutagenic treatments. The maximum number of early and late maturity mutant was observed at 0.2 and 0.3% of EMS. Early maturity mutant were reported by Kumar and Dubey (1993) in *Lathyrussativus*.

The spectrum of morphological mutations includes mutants with respect to growth habit, foliage, maturity, pod size and sterility. The morphological mutants induced in the present study included agronomical desirable features which may possibly be utilized in future breeding programme.

However, the mutation rate in this experiment is rather low and thus the result needs to confirm more experiments. The mutant's plants were individually harvested or next two consecutive generations to establish pure mutant lines for further studies.

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