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Effects of mutagen on chlorophyll mutation in horse gram (*Macrotyloma uniflorum*) Co 1 variety

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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) Co-1 with a range of EMS 10mM, 20mM, 30mM,40mM and 50mM and Gamma rays 10KR,20 KR,30 KR, 40 KR and 50 KR doses. All the mutagen induced wider spectrum of chlorophyll mutations. In present investigation Chlorina, Xantha and Viridis were found more frequently which indicated preferential induction of certain type of mutations. Fairly dependent increase in chlorophyll mutant rate was observed based on plant population and segregating progenies in M_1 generation in M_2 generation. All the three mutagenic effectiveness a measure of the frequency of mutation induced per unit dose of mutagen and mutagenic efficiency, proportion of mutation in relation to undesirable effects, were higher with EMS treatments indicates more effective and efficient mutagen than other two.

Keywords: EMS, gamma rays, horse gram, chlorophyll mutation

Introduction
Horse gram [*Macrotyloma uniflorum* (Lam) Verdcourt, family Fabaceae] a multi-use, rich protein crop, cultivated in India, Pakisthan, Bangladesh, Srilanka, Nepal and West Africa. Horse gram is large 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 [20]; Bashir *et al.*, 2013 [23]. Induction of chlorophyll mutations in general is considered as a measure to assess the effectiveness of various mutagens (Marki and Bianu, 1970) [22]. 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) [21].

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, and gamma rays 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
Selection of genotype
Horse gram variety Co-1 was selected for deriving chrolorphyll and Morphological mutants in M2 generation. For this Experiment certified seeds were collected form TNAU – Coimbatore, Tamil Nadu, India.

Mutagen Treatment
One of the Physical mutagen namely gamma rays and chemical mutagen Ems were used deriving mutants from seed treatment.

Physical Gamma rays treatment
The seeds were irradiated at Sugarcane Breeding Institute IGCAR, Kalpakkam, Chennai. Tamil Nadu, India and the source of gamma rays was labeled Cobalt (60 Co). Five sets of three hundred well matured, non-dormancy seeds were packed in paper cover for irradiation and treated with 10,20,30,40 and 50kR of gamma rays. Healthy well-matured, non-dormant, untreated seeds were used as control.

Chemical EMS Treatment
One of the chemical mutagens namely Ethyl methane sulphonate (EMS) was used for induction of mutation on seed propogules. Ethyl methane sulphonate was obtained from Hi-media Laboratory Limited, Mumbai, India Which Having a dosimetry/ half- life period is 30 hours with a molecular weight is 124.16 and density is 1.20.

Six hundred well matured healthy and uniform size of non-dormancy seeds were subjected to the mutagenic treatment. The solution of EMS was 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 ± 2ºC) prior to treatment. After the pre-soaking the excess of moisture in the seeds were removed by filter paper.

Result and discussion
The findings of the inhibitory effect of chemical and physical 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 40Mm of EMS and 30Kr Gamma Rays. On the basis of lethality the highest mutagenic efficiency was recorded at 40Mm of EMS and 40kR of gamma rays. Seed germination percentage were 93.00 in Control, 82.00% in 10KR, 67.33% in 20KR, 52.00 in 30KR, 28.22% in 40Kr and 09.33% 50Kr of Gamma Rays and 83.66 % in 10mM, 70.66% in 20mM,64.66% in 30mM,54.00% of 40mM and 20.66% of 50mM of Ems were observed. The LD 50 value observed in 52.00% in 30KR of Gamma rays and 54.00% in 40mM of EMS.

Frequency Mutants

Viable mutations have been used as an index to evaluate the mutagenic potential of various physical and chemical mutagens in number of crop plants. The viable mutation in M_2 generation is the most dependable index for evaluating the genetic effects of mutagenic treatments. In the present investigation, viable mutants *viz*., early flowering, tall, dwarf, bushy, early maturity, late maturity, high yield, leaf, flower and seed mutants were observed in both the mutagen. Among the both mutagens maximum number of viable mutants was recorded at 30KR of gamma rays and 40mM of EMS and more number of viable mutants were observed in EMS than gamma rays treated plants as shown in Table.

Kharakwal (2001) [1] reported that the chemical mutagens, particularly alkylating agents are more effective than ionizing radiations in inducing morphological mutations. It is possible that chemical mutagens m ay prove to be a better alternative for inducing morphological mutations, as they induce mutations at a much higher rate and cause less chromosomal disturbances than ionizing radiations (Sharma, 2001) [2].

Similarly such types of viable mutants were observed by several workers in different crops Juliethepziba and Subramanian (2002) [3] in blackgram, Jabeen and Mirza, (2004) [4] in *Capsicum annuum*, Pavadai (2006) [5] in soybean, Kumar *et al*. (2007) [6] in mungbean, Kumar *et al*. (2009) [7] in cowpea, Diouf *et al*. (2010) [8] in sesame, Wani, (2011) [9] in chickpea; Khan and Goyal (2011) [10] in chickpea, Ramya *et al*. (2014a) [13] in blackgram, Ahirwar *et al.* (2014) [19] in lentil, Mullainathan and Aruldoss (2015) [16] in chilli, Laskar *et al*. (2015) [17] and Ramchander *et al*. (2016) [18] in blackgram.

Jana (1962) [11] reported that the height of tall mutants is fundamentally due to increase in length and number of internodes. Dwarf mutants ascribed due to shortening of internodes, inhibition of auxin synthesis, genetic loss due to chromosomal aberration, interference with the synthesis of new DNA reported by Mohan *et al*. (1983) [12]. Patil (2009) [15] reported that early maturity might be due to physiological changes in production of flowering hormones caused by the mutagens. Abnormal structures of leaves have correlated the development of leaf abnormalities to the pleotropic action of mutated genes Joshua *et al*. (1972) [14].

Conclusion

From the study, it can be complete that, a dose dependent decreasing tendency was observed seed germination and seedling growth in *Macrotyloma uniflorum*. Seed germination and seedling growth decreased with increase in dose / concentration of both the mutagens. Based on the seed germination percentage, LD_{50} dose was fixed as 30KR of gamma rays and 40mM of EMS treatments. It is recommended that in any mutation breeding programme on *Macrotyloma uniflorum* 30KR of gamma rays and 40mM of EMS might be used. Among the both mutagens maximum number of viable mutants was recorded at 30KR of gamma rays and 40mM of EMS and more number of viable mutants were observed in EMS than gamma rays treated plants.

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Germination Studies of Gamma rays in *Macrotyloma uniflorum* (L)

Table 1: Frequency of chlorophyll and viable mutants in M_2 Generation

Dose/ Conc. of Mutagens		Gamma rays (KR)			EMS (mM)		
		20KR	30KR	40KR	30mM	40mM	50mM
No. of Plants Studied		567	543	542	552	565	526
Chlorophyll Mutants	Albino	1	1	1	2	1	1
	Chlorina	1	1	1	1	1	2
	Viridis	1	1	1	1	2	2
	Xantha	2	1	1	1	3	1
Viable Mutants (Flower, Capsule and Morphological Mutants)	Early flowering	1	2	-	1	2	1
	Pentafoliate	-	1	-	-	1	-
	Tetrafoliate	1	2	1	-	1	1
	lanceolatate	1	-	-	-	1	1
	Round cuneate	-	-	1	-	1	-
	Multiple leaf	1	1	-	-	2	1
	Unifoliate	-	-	2	-	-	1
	Wrinkle leaf	1	2	2	1	1	2
	Compact	-	1	1	1	2	-
	Fasicated stem	-	-	1	-	-	1
	Mono branching	1	1	1	1	2	1
	Sterile plants	1	1	2	1	1	1
	Bushy	2	2	2	1	1	2
	Tall	1	3	-	2	2	1
	Dwarf	1	1	1	1	2	2
	Early maturity	1	2	1	1	1	2
	Late maturity	1	2	2	2	2	1
	Light white colour seeds	-	1	-	-	-	1
	Light red colour seeds	-	1	1	1	2	-
	Bold size seeds	1	1	-	1	1	-
	High yield plants	1	3	1	1	2	2
	Total	20	31	23	20	34	28
	Frequency	3.52	5.50	4.24	3.62	6.01	5.32

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