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Induced mutagenic effectiveness and efficiency studies on pigeon pea (*Cajanus cajan* L. Millsp.)

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ABSTRACT

The present investigation was carried out to study mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulfonate (EMS) treatments in Pigeon pea (*Cajanus cajan* L. Millsp.) Var CO-7. The relative effectiveness and efficiency of the both mutagen used were assessed from the data on biological damage in M1 generation and frequency of chlorophyll and viable mutants in M2 generation. The spectrum of chlorophyll mutants such as xantha, albino, chlorina and viridis, viable mutants such as tall, dwarf, early flower, early maturity, late maturity, bushy, high yield and seed mutants were observed in both the mutagenic treatments. Among the chlorophyll mutants xantha was found more in number. The mutagenic effectiveness and efficiency were found to be higher at 20 kR of gamma irradiation and 25 mM of EMS. The mutation rate of gamma rays was higher in terms of effectiveness than that of EMS. More number of chlorophyll and viable mutants was induced in gamma rays treatment when compared EMS treatment.

KEY WORDS: Chlorophyll, effectiveness, efficiency, frequency, injury, lethality, mutagen, viable

INTRODUCTION

Pigeon pea (*Cajanus cajan* L. Millsp.) are also known as arhar, tur, redgram, etc., is one the most important crops cultivated in India. Its grains are highly nutritious and rich in protein (20-25%), carbohydrates, fibers, and minerals. India ranks first in pigeon pea production with 90% of the world area and 85% of production. In terms of global grain legume production, it is sixth after phaseolus beans, peas, chickpeas, broad beans, and lentils. Induced mutations are known to be useful to decipher specific problems in crop and it is also helpful to smash undesirable linkage between two characters. Among several breeding tools, mutation breeding has been proved to be one of the potent tools to increase the genetic variability and yield potentiality of crop plants (Singh *et al.,* 2000, Shah *et al.,* 2011).

Mutation breeding is comparatively a quicker method for improvement of crops. It has been observed that induced mutations can increase yield as well as other quantitative traits in plants (Dhulgande *et al.,* 2010). Most of the crop improvement programs attempted through conventional breeding methods have exploited only the natural variability available in the germplasm. Adequate variability is not available in the gene pool to change the plant ideotypes. The induced mutagenesis can be efficiently employed as an alternative to induce the variability in morphological and physiological characters. The artificial induction of mutation in a crop species is achieved through the use of physical and chemical mutagens that increase the mutation frequency when compared to the spontaneous mutation.

The physical and chemical mutagens cause three types of effects such as physical damage, gene mutation, and chromosomal aberrations (Swaminathan, 1965). Chlorophyll mutants are employed as markers for the evaluation of gene action of mutagenic factors in induced mutation studies (Gaul, 1964). Mutagenic effectiveness is a measure of the frequency of mutations induced by a unit dose of a mutagen, while mutagenic efficiency gives an idea of the proportion of mutations in relation to other associated undesirable biological effects such as chromosomal aberrations, lethality, injury, and sterility induced by the mutagen (Khan and Tyagi 2010). The usefulness of any mutagen in plant breeding depends not only on its mutagenic effectiveness but also on its mutagenic efficiency. The present investigation was

carried out with the aim to study the effects of gamma rays and ethyl methane sulfonate (EMS), on the frequency and spectrum of macro and micro mutants to evaluate the relative effectiveness and efficiency in various concentration/dose both mutagenic treatments.

MATERIALS AND METHODS

The genetically pure dry seeds of pigeon pea (Variety - CO-7) were irradiated with (physical mutagen) gamma rays with various doses such as 15, 20, and 25 kR and also treated with various concentrations of chemical mutagen (EMS), viz., 20, 25, and 30 mM. Gamma irradiation was carried out at Indra Gandhi Centre for Atomic Research, Kalpakam, Tamil Nadu, India. The various concentrations of EMS were prepared using distilled water and the seed were treated for 6 h with intermediate soaking. Control seeds were soaked in distilled water for 6 h. After the EMS treatment, the seeds were washed thoroughly with tap water and were immediately sown in the field in randomized block design with three replications along with control as the M_1 generation. The seeds were sown at a distance of 30 cm between the plants and 45 cm between the rows. All the agricultural practices, namely, irrigation, weeding, and plant protection methods were carried out during the growth period of the crop. The seed germination, lethality, seedling injury, and plant survival at maturity were recorded in M_1 generation.

Chlorophyll and morphological mutants observed in M_2 generation followed by the method of Stadler (1930). The plant survival (L) percentage was calculated on the 30th day after sowing. The biological damage (lethality) was calculated as the reduction in plant survival. At maturity all the surviving M_1 fertile plants were harvested separately and the seeds were sown in next season to raise M_2 generation. Different kinds of chlorophyll mutants (xantha, albino, viridis and chlorina) were scored in M_2 generation and were classified method of Kharkwal (1998). Mutation frequency was in M_2 progenies for both chlorophyll and morphological mutation in each treatment. The mutagenic effectiveness and efficiency were calculated on the basis of formulae suggested by Konzak *et al*. (1965).

$$\text{Mutagenic effectiveness}\left(\text{Physical mutagen}\right)=\frac{M\times 100}{kR\,(\text{Kilo rad})}$$

$$\text{Mutagenic effectiveness}\left(\text{Chemical mutagen}\right)=\frac{M\times 100}{C\times t}$$

Mutagenic efficiency (Lethal) = M/L

Mutagenic efficiency (Injury) = M/I

Where,

M - Mutation frequency for 100 M_2 plants
t - Period of treatment with chemical mutagen in hours
C - Concentration of mutagen in mM or in per cent
kR - Dose of mutagenic radiation in kilo rad
L - Percentage of lethality or survival reduction
I - Percentage of injury or reduction in seedling size.

RESULT AND DISCUSSION

Chlorophyll and Viable Mutation Frequency

In present investigation chlorophyll and viable mutations were observed in M_2 generation. Various chlorophyll mutants such as albino, xantha, chlorina and viridis and viable mutants such as days to first flower, tall, dwarf, bushy, early maturity, late maturity, seed mutant and high yield mutant were observed in all the mutagenic treatments. In intermediate dose/concentration was found to be more effective and producing high mutation frequency of chlorophyll and viable mutations in both mutagens (Tables 1 and 3). Such type of chlorophyll and viable mutants was observed earlier workers in different plants Solanki and Sharma (2001) in lentil, Ambarkar (1997) in chickpea, Suryawanshi (2000) in urdbean. The frequency of chlorophyll and viable mutants observed in M_2 generation is mainly used as a dependable measure of genetic effects of mutagens (Gautam *et al*., 1998 and Gustafsson and Von-Wettstein, 1956). The xantha chlorophyll mutant was found to be most abundant in both the mutagenic treatments (Table 1). A similar finding was by Singh *et al*. (2000). In chlorophyll and viable mutation frequency increased with intermediate dose/concentration of mutagens. Similar results were observed by Gautam *et al*. (1992) and Asra (1995) in different plants. The maximum frequency of chlorophyll mutations was noted at 25 mM of EMS (1.68%) and the highest viable mutation frequency was observed at 20 kR (4.88) of gamma ray treatment. The least frequency of chlorophyll and viable mutations was observed at 25 kR (0.72%) of gamma rays and 20 mM (1.35%) of EMS treatments (Table 1 and 3). Chlorophyll development seems to be controlled by many genes located on several chromosomes which could be adjacent to centromere and proximal segments of chromosomes (Swaminathan, 1964; 1965). The origin of chlorophyll deficiencies is mainly due to mutations in genes, which are responsible for synthesis of photosynthetic pigments.

Effectiveness and Efficiency

Mutation plant breeding program is necessary to determine the effectiveness and efficiency of mutagen. The frequency

Table 1: Frequency and spectrum of chlorophyll mutants in M_2 generation of pigeon pea

Mutagens	Different types of chlorophyll mutants				Number of plants studied	Number of chlorophyll mutants	Percentage of chlorophyll mutants				Mutation frequency
	Albino	Xantha	Viridis	Chlorina			Albino	Xantha	Viridis	Chlorina	
Gamma rays (kR)											
Control	-	-	-	-	-	-	-	-	-	-	-
15	-	2	2	1	577	5	-	0.34	0.34	0.17	0.86
20	2	4	1	1	553	6	0.36	0.72	0.18	0.18	1.08
25	1	1	1	1	549	4	0.18	0.18	0.18	0.18	0.72
EMS (mM)											
20	2	1	1	1	589	5	0.33	0.16	0.16	0.16	0.84
25	2	3	3	1	535	9	0.37	0.56	0.56	0.18	1.68
30	1	1	2	2	521	6	0.19	0.19	0.38	0.38	1.15

EMS: Ethyl methane sulfonate

Table 2: Mutagenic effectiveness and efficiency based on the chlorophyll mutants in M_2 generation of pigeon pea

Treatments (doses/concentration)	Survival reduction (L) 30th day	Height reduction (I) 30th day	Mutation frequency	Effectiveness $\frac{M\times100}{kR(or)C\times t}$	Efficiency	
					$\frac{M}{L}$	$\frac{M}{I}$
Gamma rays (kR)						
15	37.73	7.41	0.86	1.38	0.022	0.11
20	43.35	15.01	1.08	1.08	0.024	0.07
25	54.50	27.12	0.72	0.46	0.013	0.02
EMS (mM)						
20	31.30	19.27	0.84	1.05	0.026	0.04
25	42.68	24.43	1.68	1.68	0.039	0.06
30	51.89	29.40	1.15	0.95	0.022	0.03
Total			6.33	6.60	0.087	0.33

EMS: Ethyl methane sulfonate

Table 3: Frequency and spectrum of viable mutants in M_2 generation of pigeon pea

Dose/concentration of mutagens	Gamma rays (kR)			EMS (mM)		
	15	20	25	20	25	30
Number of plants studied	577	553	549	589	535	521
Viable mutants (flower, pods, seeds, and morphological mutants)						
Early flowering	1	2	-	1	3	1
Complete sterile plants	-	-	1	-	-	1
Female sterile plants	-	1	1	-	1	-
Male sterile plants	-	-	1	-	-	1
Aborted flower	-	1	-	-	1	-
Light white colored flower	-	2	-	-	1	1
Light red colored flower	2	-	-	1		
Flower with irregular shaped petals	-	-	1	-	-	1
High pod clustered	1	2	-	-	1	1
Unusual structure of pod	-	-	2	-	-	1
Mono branching	1	1	1	1	2	-
Bushy	2	4	1	2	3	1
Tall	2	2	-	2	1	1
Dwarf	-	2	4	1	1	2
Fasicated stem	-	-	1	-	-	-
Early maturity	1	3	1	-	3	1
Late maturity	-	2	3	-	1	2
Light white color seeds	-	1	-	-	-	1
Light red color seeds	-	1	-	-	-	-
Bold size seeds	-	-	1	-	-	1
High yield plants	1	3	1	-	2	1
Total	11	27	19	8	20	17
Frequency	1.90	4.88	3.46	1.35	3.73	3.26

EMS: Ethyl methane sulfonate

of mutations induced by mutagenic treatment is an index of the effectiveness of mutagen. Mutagenic effectiveness is a measure of the frequency of mutations induced by a unit dose of mutagen. Konzak *et al.* (1965) showed that mutagenic efficiency provides the best available measure to evaluate different mutagenic treatments. The efficiency estimated on the basis of seedling injury was generally higher when compared with lethality in both the mutagens similar finding was observed by Wani (2009) and Khan and Tyagi (2010).

In both the mutagens, efficiency was found more in 20 kR of gamma rays and 25 mM of EMS treatments when compared with other doses/concentration of mutagenic treatments (25 kR and 30 mM) (Tables 2 and 4). The injury and lethality were highest in higher doses of mutagenic treatments. The efficiency of gamma rays treatments declined considerably with the increase in the dose/concentration of both mutagens (Tables 2 and 4). The decrease in efficiency at higher doses/concentration may be attributed to the failure in the recovery of chlorophyll and viable mutations proportionate to the dose of mutagens. This was consistent with the findings of Wani (2009) and Khan and Tyagi (2010). Similar differences in mutagenic response have also been reported by many

Table 4: Mutagenic effectiveness and efficiency based on the viable mutants in M_2 generation of pigeon pea

Treatments (doses/concentration)	Survival reduction (L) 30th day	Height reduction (I) 30th day	Mutation frequency	Effectiveness $\frac{M\times100}{kR(or)C\times t}$	Efficiency $\frac{M}{L}$	Efficiency $\frac{M}{I}$
Gamma rays (kR)						
15	37.73	7.41	1.90	3.05	0.050	0.25
20	43.35	15.01	4.88	4.88	0.112	0.32
25	54.50	27.12	3.46	2.21	0.063	0.12
EMS (mM)						
20	31.30	19.27	1.35	1.68	0.043	0.7
25	42.68	24.43	3.73	3.73	0.087	0.15
30	51.89	29.40	3.26	2.71	0.062	0.11
Total			15.32	18.26	0.417	1.65

EMS: Ethyl methane sulfonate

earlier workers (Bhat *et al.,* 2007; Wani, 2009 and Kulthe *et al*., 2013).

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