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Sapna Rajderkar
Department of Agricultural Botany, Post Graduate Institute, Dr. PDKV, Akola, Maharashtra, India

Dr. SB Sakhare
Department of Agricultural Botany, Post Graduate Institute, Dr. PDKV, Akola, Maharashtra, India

Corresponding Author:
Sapna Rajderkar
Department of Agricultural Botany, Post Graduate Institute, Dr. PDKV, Akola, Maharashtra, India

Studies on effective mutagen using physical and chemical mutagens in soybean (*Glycine max*)

Sapna Rajderkar and Dr. SB Sakhare

Abstract
In the present investigation, the mutagenic effectiveness and efficiency of a physical mutagen i.e. Gamma rays and chemical mutagen i.e. Ethyl Methane Sulphonate was studied in the Soybean (*Glycine max* (L) Merill.) varieties JS-335 and JS-9560. The seeds of soybean varieties JS-335 and JS-9560 were mutagenised with the increasing doses of gamma rays 10kR, 20kR, 30kR and 40kR and Ethyl Methane Sulphonate 0.1%, 0.2%, 0.3% and 0.4%. The effectiveness and efficiency was determined by accounting lethality and seedling injury in M_1 generation of mutagenised seeds and frequency and spectrum of chlorophyll mutations in M_2 generation. The increasing doses of mutagens decreased plant survival and seedling height. Five types of chlorophyll mutants albina, xantha, chlorine, striata and xanths-viridis were screened in M_2 progeny. It was observed that the frequency of mutations increased with increasing doses of mutagen. The higher mutation frequency was noticed in chemical mutagen than physical mutagen. The highest mutagenic effectiveness was recorded 2.5% in 0.2% EMS and lowest 0.02% in 30kR dose of gamma rays. Whereas maximum mutagenic efficiency was recorded 0.23% in 10kR and minimum 0.13% in 40kR dose of gamma rays. Thus the lower doses of mutagen were effective and efficient than the higher doses in both varieties of JS-335 and JS-9560 and Chemical mutagen EMS found more effective and efficient mutagen in soybean than physical mutagen gamma rays which may create genetic variability.

Keywords: Soybean, mutagen, mutagenic effectiveness, mutagenic efficiency, mutation frequency

Introduction

The genus *Glycine* is divided into two subgenera, *Glycine* (30 perennial species) and *Soja* (annuals) which includes *Glycine max* having chromosome number 2n = 2x = 40. Soybean is one of the most economic and valuable agricultural commodities because of its unique chemical composition and multiple uses as food, feed and industrial material. Furthermore, soybean also contain many biologically active components including iso-flavins, lecithin, saponine, oligosaccharides, phyto sterols, trypsin inhibitor, lectins etc. (Watson *et al.*, (2000). It is an excellent soil improving crop with respect to available nitrogen. Mutation breeding has been found very useful tool in crop improvement as it inducing genetic variability necessary in plant breeding programmes. Many desirable varieties of crops have been developed through mutation breeding (Gabriyal *et al.*, 2009) [6]. In recent years, induction of mutations employing various mutagens has widely been accepted as an excellent tool for creating genetic variability and as supplementary approach in the crop improvement programmes in several crops (Singh and Pal, 1998) [29]. Induced mutations have been utilized to achieve success in improving plant yield. A number of chemical and physical mutagens are widely used to induce genetic variability in plants. Mutagens are specific to their action but varietals variations are present with respect to formation of mutations. The mutagenic effectiveness and efficiency of mutagens and their doses are prerequisites for induction and utilization of mutations (Sharma *et al.*, 2005) [28]. Mutagenic effectiveness means the rate of mutations as related to dose while efficiency refers to the mutation rate in relation to biological damage such as seedling injury and lethality in M_1 generation (Nilan *et al.*, 1965) [14]. Mutagenic effectiveness and efficiency gives an idea to evaluate a mutagen. Hence these two parameters were used in the present investigation in soybean variety JS-335 and JS-9560. The parameters of M_1 generation are the best indicator in measuring efficiency of mutagens and helped in comparing the effectiveness and efficiency of mutagen.

In the present investigation, attempt was made to analyze the mutagenic effectiveness and efficiency of a physical and chemical mutagen in soybean variety JS-335 and JS-9560 by using biological damages like seedling injury and lethality observed in M_1 generation in relation with the frequency and spectrum of chlorophyll mutations in M_2 generation.

Materials and Method

Uniform 500 pure seeds with moisture content of 10-12% each of two varieties JS-335 and JS-9560 were exposed to 10kR, 20kR, 30kR and 40kR dose of gamma rays (CO^{60}) with a dose rate of 2.39kR per minute at Bhabha Automatic Research Center, Trombay in Mumbai and the same number of untreated seeds of each varieties served as control.

The EMS (Ethyl Methane Sulphonate) was used for chemical treatment at Biotechnology Center Akola. EMS is mono-functional alkylating agent. The seeds were soaked in double distilled water for 6hrs and were treated with freshly prepared 0.1%, 0.2% 0.3% and 0.4% aqueous solution of EMS at 7.0 P^H by dissolving the appropriate quantity of EMS in Phosphate buffer.

The treatment was performed for period of 8 hrs at room temperature with intermittent shaking during the treatment period. The uniform 500 seeds of both varieties were presoaked in distilled water for 6 hrs and then dipped in enough mutagenic solution of chemical mutagens of different concentrations and durations. All the treatments were given with intermittent shaking at room temperature using freshly prepared solutions.

Seeds treated with the chemical mutagen were thoroughly washed in running tap water for two hours to remove the residues of the chemical, if any and the excess moisture in seed coat was removed by using the blotting paper.

The treated and control seeds in each treatment were sown to raise the M_1 generation in the field of Department of Agriculture Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola on dated 5th July 2016 in three replications in the *kharif* season of 2016. The seeds were divided into three lots of 100 seed each and were sown in five rows of 3.0m length with 45cm spacing and 15cm between plants in three replications in the split plot experimental design with two varieties as main plot treatment and two mutagens as a sub-plot treatment.

In M_1 generation, the reduction in seedling height and plant survival were recorded and considered as injury (I) and lethality (L) expressed as percent of control (Sharma S.K. 1990, Sharma S.K. 2005, Singh A. K. and Singh R. M. 2001) [27, 28, 30, 31]. Seeds from each plant of M_1 generation were harvested separately and subsequently sown in progeny rows in the *Kharif* season of 2017 and to record observation in M_2 generation.

Various morphological mutations were scored in M_2 generation (Plants raised from seeds of selfed M_1 plants)

In M_2 generation, the treated as well as control progenies were screened for chlorophyll mutations up to first four weeks after germination, whereas, viable and morphological mutations were scored throughout the crop duration. Four types of chlorophyll mutants *viz*., albina, xantha, chlorina and striata were scored till the age of four weeks (Kharakwal, 1998) [13] in M_2 generation. Mutagenic frequency was estimated as percentage for both chlorophyll and morphological mutations in each treatment (Gaul 1964). The mutagenic effectiveness and efficiency were determined by Konzak *et al*., (1965) [14].

$$\text{Mutation frequency (\%)} = \frac{\text{Number of visible mutants}}{\text{Total population per treatment}} \times 100$$

$$\begin{array}{l}\text{Mutagenic effectiveness}\\ \text{(Physical mutagen)}\end{array} = \frac{\text{Mutation rate on the basis of } M_1 \text{ plant Progenies (MP) or } M_2 \text{ population (MS)}}{\text{Dose in kilo roentgens (kR)}}$$

$$\begin{array}{l}\text{Mutagenic effectiveness}\\ \text{(Chemical mutagen)}\end{array} = \frac{\text{Mutation rate on the basis of } M_1 \text{ plant Progenies (MP) or } M_2 \text{ population (MS)}}{\text{Concentration of mutation (\%)} \times \text{Time of Treatment (hrs)}}$$

$$\text{Mutagenic efficiency} = \frac{\text{Mutation rate on the basis of } M_1 \text{ plant Progenies (MP) or } M_2 \text{ population (MS)}}{\text{\% Lethality (L) or \% seedling injury (I) or pollen sterility (S)}}$$

Results and Discussions

Biological damages in M_1 generation

In the present study, the biological damages like seedling injury and lethality were recorded in M_1 generation. All these parameters were found increased with increasing doses of gamma rays. The seedling injury was highest 43.60% and 39.35% at 40 kR and lowest 14.58% and 10.64% at 10 kR dose of gamma rays in both JS-335 and JS-9560. The maximum higher lethality was recorded 52.69% and 57.80 at 0.4% EMS in both the varieties and lower 23.10% at 0.1% EMS in var JS-335 and 21.76% in 10kR dose in var. JS-9560. Thus these biological damages were lower at lower doses of mutagen and higher at higher doses of matagen in soybean varieties JS-335 and JS-9560 (Table 1 and Fig. 1). The increased seedling injury and lethality with increasing doses of mutagens also reported by several investigators Bhosle and Kothekar (2010) [4], S. Velu *et al*., (2007) [35], Reddy *et al*., (1991) [25] in cluster bean, The results of present investigations are in conformity with these results. Similar results were also obtained by Mathur *et al*., (2001) [17], Sonone *et al*., (2008) [32] in ground nut and Upadhyaya *et al*., (1984) [33], Khan M.F. and Tyagi S.D. in Soybean.

Table 1: Effect of mutagens on biological damages in M_1 generation of soybean var. JS-335 and JS-9560

S. N.	Mutagen	Doses	Lethality (%)		Seedling injury (%)	
			JS-335	JS-9560	JS-335	JS-9560
1.	Gamma rays	10 kR	23.88	21.76	14.58	10.64
2.		20 kR	27.22	30.70	23.67	21.29
3.		30 kR	37.50	41.76	31.56	34.25
4.		40 kR	41.28	47.70	43.60	39.35
5.		Mean	32.47	35.48	28.35	26.38
1.	Ethyl Methane Sulphonate	0.1%	23.10	29.71	12.04	11.57
2.		0.2%	31.48	33.76	20.76	19.44
3.		0.3%	46.7	49.28	29.48	29.16
4.		0.4%	52.69	57.80	34.05	35.18
5.		Mean	30.79	34.11	19.26	19.07

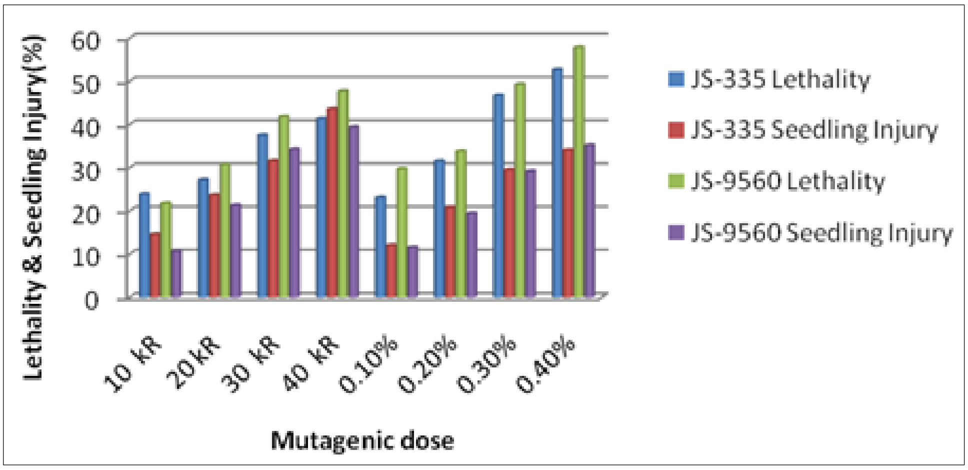

Fig 1: Effect of mutagens on biological damages in M_1 generation of soyabean var. JS-335 and JS-9560

Mutation frequency in M_2 generation

Mutation frequency is frequency of chlorophyll mutations calculated on M_2 plant basis. Chlorophyll mutants were found in almost all the mutagenic treatments. The highest chlorophyll mutation frequency 4.00% and 2.56% was reported in 0.2% EMS in variety JS-335 and JS-9560 respectively, while the lowest 0.64% and 0.59% found in 10KR treatments in variety JS-335 and JS-9560 respectively. In the segregating M_2 generation, spectrum of chlorophyll mutations indicated the presence of four types of chlorophyll mutants *viz*, albina, xantha, chlorina and striata. The variety JS-335 showed comparatively higher mutation frequency than JS-9560. Out of both the mutagens studied, ethyl methane sulphonate was found to be more effective in inducing chlorophyll mutations in both varieties of soybean. When the different doses/concentrations of mutagens were compared, all the doses were found to be effective in inducing chlorophyll mutations in both varieties.

The spectrum of chlorophyll mutation induced by mutagenic treatments was found to vary according to the mutagenic dose and variety. The Albina, Xantha, Chlorina, Viridis and Xantha viridis mutations were of common occurrence in both the varieties. Ahire and Auti (2015) [1] also reported that EMS treatment induced broad spectrum of mutations. Similar spectrum of chlorophyll mutations was also reported by Pavadai *et al.* (2013) [24], Khan and Tyagi (2013) [12], Nilhayati *et al.* (2016) [20], Basavraj *et al.* (2005), Geeta and Vaidyanathan (2000) [9] in soybean.

All the four types of mutants were observed in both the mutagenic treatments, whereas 10 and 20 kR treatments induced only three types of mutants. The highest frequency of albina mutant was recorded 0.35 in 40 kR in variety JS-335 and 0.47 in 0.4% EMS in variety JS-9560 whereas lowest 0.06 and 0.09 in 10 KR treatment in both the varieties. The maximum frequency (0.59) of xantha mutants was found in 40 kR in variety JS-335 and 0.71 in 0.4% EMS in JS-9560, while minimum 0.07 and 0.11 in 30 kR treatment in both the varieties. The higher frequency of chlorina mutant was reported 1.50% and 1.33 in 0.2% EMS in variety JS-335 and variety JS-9560 respectively and lower 0.16% and 0.19 in 10 kR treatment in variety JS-335 and variety JS-9560 respectively, whereas viridis mutants were found maximum 1.07% in 0.2% EMS and minimum 0.10% in 20 kR treatments in variety JS-335 and 0.47 in 0.4% EMS and 0.12 in 20 kR in JS-9560. (Table 2 and Fig. 2). The mutagen Ethyl Methane Sulphonate was found to be more effective in inducing chlorophyll mutations in both varieties of soybean than gamma radiation. As per the different doses are concerned, all the doses were found to be effective in inducing chlorophyll mutations. These findings were in conformity with Patil (2006) [22], Venkatswaralu *et al.* (1978) [36] in soybean.

Table 2: Effect of mutagens on the frequency of the chlorophyll mutations in M_2 generation of soybean var. JS-335 and JS-9560

S. N.	Mutagen	Doses	Mutagenic Efficiency		JS-335					JS-9560				
			JS-335	JS-9560	Albina	Xantha	Virdis	Xantha -Viridis	Chlorina	Albina	Xantha	Virdis	Xantha -Viridis	Chlorina
1.	Gamma rays	10kR	0.64	0.59	0.06	0.25	0.12	0.03	0.16	0.09	0.14	…	0.09	0.24
2.		20kR	1.70	0.83	0.16	0.39	0.10	…	1.11	0.19	0.32	0.12	…	0.19

3.		30kR	0.78	0.48	…	0.07	0.28	…	0.42	…	0.11	…	…	0.32
4.		40kR	2.38	1.15	0.35	0.59	0.95	…	0.47	0.31	0.20	0.20	…	0.41
5.		Mean	1.38	0.76	0.19	0.31	0.36	0.03	0.54	0.20	0.20	0.16	0.09	0.29
1.	Ethyl Methane Sulphonate	0.1%	0.77	0.75	0.11	0.19	0.26	…	0.19	0.16	0.21	…	…	0.37
2.		0.2%	4.00	2.56	0.35	0.50	1.07	0.57	1.50	…	…	0.33	…	1.33
3.		0.3%	2.83	2.06	0.21	0.32	0.97	0.43	0.87	0.15	0.31	…	0.31	1.27
4.		0.4%	2.94	2.38	0.29	0.44	1.02	0.29	0.88	0.47	0.71	0.47	…	0.71
5.		Mean	2.63	1.94	0.24	0.36	0.83	0.43	0.86	0.26	0.41	0.40	0.31	0.92

Table 3: The effectiveness of mutagens in M_2 generation of soybean var. JS-335 and JS-9560

S. N.	Mutagen	Doses	Total mutation frequency (MF)		Mutagenic effectiveness	
			JS-335	JS-9560	JS-335	JS-9560
1.	Gamma rays	10 kR	0.64	0.59	0.06	0.05
2.		20 kR	1.70	0.83	0.08	0.04
3.		30 kR	0.78	0.48	0.02	0.01
4.		40 kR	2.38	1.15	0.06	0.02
5.		Mean	1.38	0.76	0.05	0.03
1.	Ethyl Methane Sulphonate	0.1%	0.77	0.75	0.96	0.94
2.		0.2%	4.00	2.56	2.50	1.59
3.		0.3%	2.83	2.06	1.17	0.86
4.		0.4%	2.94	2.38	0.91	0.74
5.		Mean	2.63	1.94	1.39	1.03

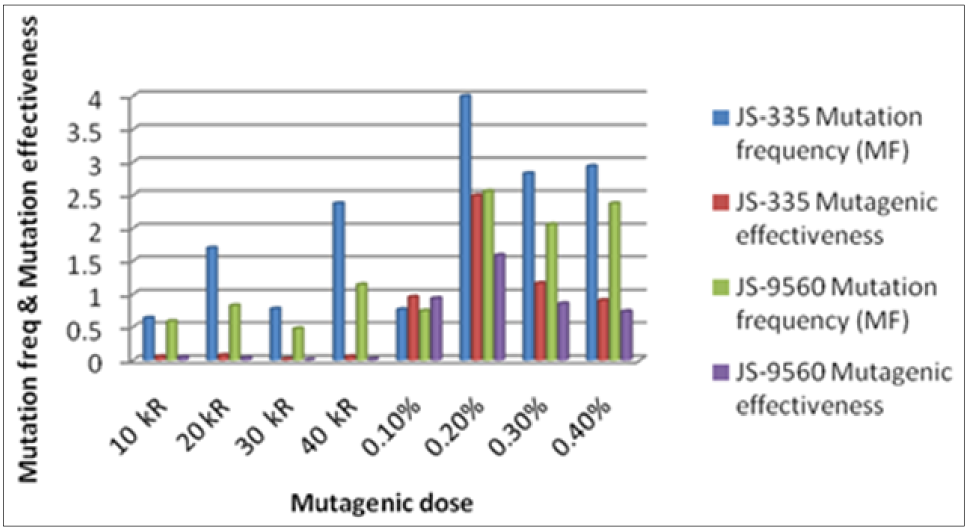

Fig 2: The effectiveness of mutagens in M_2 generation of soybean var. JS-335 and JS-9560

The spectrum of chlorophyll mutation induced by mutagenic treatments was found to vary according to the mutagenic dose and variety. The Albina, Xantha, Chlorina, Viridis and Xantha viridis mutations were of common occurrence in both the varieties. Ahire and Auti (2015) [1] also reported that EMS treatment induced broad spectrum of mutations. Similar spectrum of chlorophyll mutations was also reported by Pavadai *et al.* (2013) [24], Khan and Tyagi (2013) [12], Nilhayati *et al.* (2016) [20], Basavraj *et al.* (2005), Geeta and Vaidyanathan (2000) [9] in soybean. The variety JS-335 have shown more frequency of chlorophyll mutants as compared to variety JS-9560. The varietal difference with respect to the frequency of chlorophyll mutations may be attributed due to differences and variations in the number of genes controlling the chlorophyll development in different varieties. Such varietal difference have also been reported earlier by Ahire and Auti (2015) [1] in soybean, Usharani and AnandKumar (2015) [34] in blackgram, Paul and Singh (2002) [23] in Lentil.

However, S. Velu *et al.*, (2007) [35] calculated mutation frequency in cluster bean variety Pusa Navbahar on M_2 plant basis showed a dose dependency, where highest mutation frequency was recorded 4.00% and 2.56% at lower doses of 0.2% EMS in var. JS-335 and JS-9560 respectively. Thus they recorded decreased mutation frequency with increased doses of gamma ray and EMS. These results were in accordance to the results obtained by Gautam *et al.*, (1998) [8] in Rajmah and Kumar D. *et al.*, (2003) [8] in lima bean. The high frequency of chlorophyll mutations obtained with gamma rays is due to preferential action of this mutagen on genes controlling the chlorophyll formation in the leaves (Chopra 2005).

Mutagenic effectiveness

The mutagenic effectiveness is the ratio of mutation frequency in M_2 generation to the doses of gamma rays used. In the present investigation, it was recorded that mutagenic effectiveness decreased with increasing doses of mutagens in soybean in both the varieties under study. The highest value of mutagenic effectiveness 2.50 and 1.59 was recorded in 0.2% EMS i.e. chemical mutagenic treatment and lowest 0.02 and 0.01 in 30kR treatment in variety JS-335 and JS-9560 respectively. Thus the lower doses of EMS were most effective (and Fig. 3). These results were supported by the results obtained in cluster bean by S. Velu *et al.*, (2007) [35]. They reported higher mutagenic effect variety Pusa-Navbahar at lower doses of gamma rays and EMS. However EMS and it's doses were found to be more effective mutagen than the gamma rays. Bhosle and Kothekar (2010) [4] also reported reduction in the value of mutagenic effectiveness with the increased doses or concentration of mutagens gamma rays, EMS and SA respectively in the cluster bean. The present study revealed that mutation frequency, mutagenic efficiency measured on the basis of lethality, seedling injury and mutagenic effectiveness showed that chemical mutagen EMS was found to be better in both the varieties i.e. JS-335 and JS-9560 as compared to physical mutagenic gamma treatment.

Effectiveness and efficiency are two different properties of mutagen and are quite important, as far as plant breeding is considered. Mutagenic effectiveness is a measure of the frequency of mutation induced by a unit dose of mutagen while mutagenic efficiency represents the properties of mutation in relation to the associated undesirable biological effects, such as chromosomal aberrations, lethality and sterility induced by mutagen (Konzak *et al.* 1965) [14]. The usefulness of any mutagen would therefore depend on its effectiveness and efficiency. Gaul *et al.* (1972) [7] opined that the mutagenic efficiency carries lot of theoretical and practical importance in rice. Konzak *et al.* (1965) [14] have defined mutagenic efficiency as the best available measure for evaluating different mutagenic treatments. The mutagenic effectiveness and efficiency decreased with increase in the dose/concentration of mutagens. It simply means that low doses/concentrations were found to be more effective.

Several investigators have also studied mutagenic effectiveness in various crops such as Singh and Singh (2001) [30, 31] in Mung bean, Nerkar (1977) [19] in *Lathyrus sativus*, Mehta and Pandey (1998) [18] in french bean, Kumar *et al*., (2003) [15] in lima bean, Jabaraj and Marappan (1981) [10] in green gram, Sharma *et al*., (2005) [28] in urd bean, using mutagens Gamma rays, and EMS and reported higher mutagenic effectiveness at lower doses. These results are in accordance to results of present investigation. The effectiveness of any mutagen depends upon its dose or concentration and specificity to act on genes and genetic makeup of the cultivar (Blixt, 1968) [5].

Mutagenic efficiency

Mutagenic efficiency is the ratio of frequency of chlorophyll mutations induced in M_2 generation to various biological damages such as seedling injury and lethality observed in M_1 generation. In the present study, the mutagenic efficiency decreased with the increasing doses of mutagens with respect to seedling injury (I), and lethality (L). Highest value of mutagenic efficiency was recorded 1.09 (S.I) and 1.65 (L) in 0.2% EMS in Var. JS-335 as well as 0.69 (S.I) and 1.20 (L) in 0.2% EMS in Var. JS-9560. The mutagenic efficiency on lethality and seedling injury (MF/L and MF/I) was increased in lower and medium doses and decreased in higher doses in both physical and chemical mutagens. These findings are in conformity with Patil (2006) [22], Venkatswaralu *et al.* (1978) [36], Geetha and Vaidyanathan (2000) [9] in soybean.

They proved that lower doses of mutagens were more efficient than the higher doses. These results were also supported by Manrique *et al*., (1998) [16] in French bean, Khan and Tyagi (2010) [11] in Soybean, Sharma *et al*., (2005) [28] in Urd bean, Sharma and Sharma (1979) [26] in Lentil. They concluded that mutagenic efficiency was higher at lower doses of mutagens. These results are in agreement with the results of present investigation. These findings are in accordance to the observations of several workers Konzak *et al*., (1965) [14], in barley, Nerkar (1977) [19] in *Lathyrus sativus*, Gautam *et al*., (1992) [8] in black gram. Aijaz A Weni (2009) [2] studied mutagenic efficiency of EMS and gamma rays the varieties (two) of chick pea and showed that intermediate treatments were found more efficient. According to Konzak *et al*., (1965) [14] efficiency with an increase in concentration or doses of mutagenic agent is due to biological damage like seedling injury and lethality in M_1 generation which increases with increase in dose or concentration.

Conclusions

From the present investigation, it was concluded that mutagenic effectiveness and mutagenic efficiency were highest at lower dose of mutagens in the soybean varieties JS-335 and JS-9560. The chlorophyll frequency found highest at 0.2% EMS in both the varieties under study. The moderate mutagenic dose causes the highest mutagenic effectiveness compared to higher doses. The mutagenic efficiency was higher at lower doses due to the increased injury, and lethality with increasing doses of mutagens. Thus Chemical mutagen EMS found more effective and efficient mutagen in soybean than physical mutagen gamma rays which may create genetic variability useful in crop improvement.

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