Received: 15 Jul 2020 | Revised: 28 Aug 2020 | Accepted: 28 Aug 2020

Electronic Journal of Plant Breeding

Research Article

Efficiency and effectiveness of physical and chemical mutagens in cowpea (*Vigna ungiculata* (L.)Walp)

S. Priyadharshni[1*], S. Saravanan[1], K. Elanchezyan[2], A. Kavitha Pushpam[3] and M. Arumugam Pillai[1]

[1]Department of Plant Breeding and Genetics,
[2]Department of Agrl. Entomology,
[3]Department of Soil Science and Agrl. Chemistry,Agricultural College and Research Institute, Killikulam.
[1*]**E-Mail:**priyadharshnis13697@gmail.com

Abstract
A study was conducted in cowpea (*Vigna ungiculata)* varieties, VBN 3 and CO(CP) 7 to find the efficiency and effectiveness of physical (Gamma rays and Electron beam) and chemical (EMS) mutagens. A wide range of chlorophyll mutants and viable mutants were observed in the field. Overall chlorophyll mutant and viable mutant frequency was higher in EMS treatments in both the varieties. Mutagenic efficiency and effectiveness was also found to be higher in EMS treatments followed by electron beam and gamma rays. Mutagenic effectiveness and efficiency decreased with increase in doses of all mutagenic treatments.

Key words
Cowpea, Mutagenic efficiency, Effectiveness, Gamma ray, Electron beam, EMS

INTRODUCTION

Cowpea (*Vigna ungiculata*) is a dicotyledonous pulse crop which belongs to the genus *Vigna*, section *catiang*, species *Ungiculata* and comprises of four sub species *viz*., *unguiculata, dekindtiana, stenophylla* and *tenuis*. The cultivar group *Unguiculata* is the most diverse of the four and is widely grown in Africa, Asia and Latin America. Cowpea is a multipurpose legume, which is used as green fodder, pulse, green vegetable and green manure. Cowpea is now widely distributed throughout the tropics and subtropics. However, the production of cowpea is limited by both abiotic and biotic constraints. Insect pests are the major biotic limiting factors for cowpea production. The legume pod borer *Maruca vitrata* Fab. (Lepidoptera: Crambidae), one of the most devastating insect pests of cowpea, which can cause typical yield losses ranging from 20 to 80% (Atachi et al., 2007). So, Genotypes which are naturally resistance to all stress conditions proved to be an effective option in increasing the productivity of pulses without increasing the cost of cultivation of the crop. The fact that pulses are self-pollinated has led to a narrow genetic diversity in many of these crops. In order to expand the genetic diversity mutation proves to be an excellent tool in inducing variability among the cultivars thus increasing the selection efficiency and help in isolating the desired mutants for a specific trait of interest. Various mutagens may be employed to induce the desired mutants, which will be screened and evaluated at the later generations. The efficiency and effectiveness of various mutagens are estimated in M_2 generation which portrays the frequency of mutations induced by a unit dose of mutagen and the proportion of mutation in relation to biological damage induced respectively (Molosiwa et al 2016).

MATERIALS AND METHODS

The cowpea variety seeds of VBN 3 were obtained from National Pulse Research Centre, Vamban and CO(CP) 7 were obtained from Department of Pulses, Tamil Nadu Agricultural University. These seeds were free from pathogen and insect infestations. The moisture content of the seed was around 12- 14%, which is optimum for physical mutagens. For physical mutagenesis, the seeds were irradiated at six different doses of Gamma Rays (150 Gy,200 Gy,250 Gy,300 Gy,350 Gy,600 Gy) and five different doses of Electron Beam (200 Gy,300 Gy,400 Gy,500 Gy,600 Gy) at Gamma chamber facility (Co 60) and electron accelerator facility at Bhabha Atomic Research Centre, Mumbai. For chemical mutagenesis, presoaked cowpea seeds were treated with **ethyl** methanesulfonate (EMS) at five different doses 10 mM, 20 mM, 30 mM, 40 mM, 50 mM.Treated seeds were raised in M_1 generation and individually harvested and threshed. Seeds of individual plants were grown in plant to progeny rows with 30 cm between rows and 10 cm between plants during January ,2020 with suitable parent seeds as the check. Pod borer susceptible varieties Lola, Vaijyantha and Bhagyalakshmi were grown as border rows around the field plots which act as the infector rows for more pod borer attack.

Chlorophyll mutants in the field were observed at the two leaf stage to assess the effect of mutagen on seed given by Gustafsson (1940). Viable mutants are screened at further stages of crop growth. Biometric observations for micro-mutants were also recorded in each treatment. Mutagenic effectiveness is a measure of frequency of mutation induced by unit dose of mutagen whereas mutagenic efficiency gives an indication of proportion of mutation in relation to undesirable changes like lethality, injury and sterility .The effectiveness and efficiency was worked out by using the formula suggested by Konzak et al (1965).

$$\text{Mutagenic effectiveness} = \frac{M \times 100}{\text{Krad (or) c} \times t}$$

Where, M = Mutation frequency for 100 M_2 plants, c = Concentration of mutagen is mM or per cent for chemical mutagen, K rad = Dose of mutagenic radiation in Kilorad for physical mutagen, t = Time duration of chemical treatment in hours.

Mutagenic efficiency = M x 100 / L (or) I

Where, M = Mutation frequency for 100 M_2 plants, L= Percentage of lethality or survival reduction, I = Percentage of injury or reduction in seedling size.

RESULTS AND DISCUSSION

The frequency of chlorophyll mutations was estimated in M_2 generation and no chlorophyll mutants were observed in the control. Four types of chlorophyll mutants namely, chlorina, xantha, albina, maculata and other chlorophyll mutants in very few numbers were observed. In VBN 3, the overall chlorophyll mutant frequency was higher in EMS (19.39 %) followed by gamma rays (19.29%) and electron beam (17.78%) (**Fig 1**). In CO (CP) 7, highest frequency was exhibited by gamma rays treated population (18.12 %) mentioned in **Fig 2**.The experimental results were found concordant to the results obtained by Dhanavel et al(2008) in cowpea ,Sharma et al (2005) in blackgram and Vinithashri et al (2020) in rice.

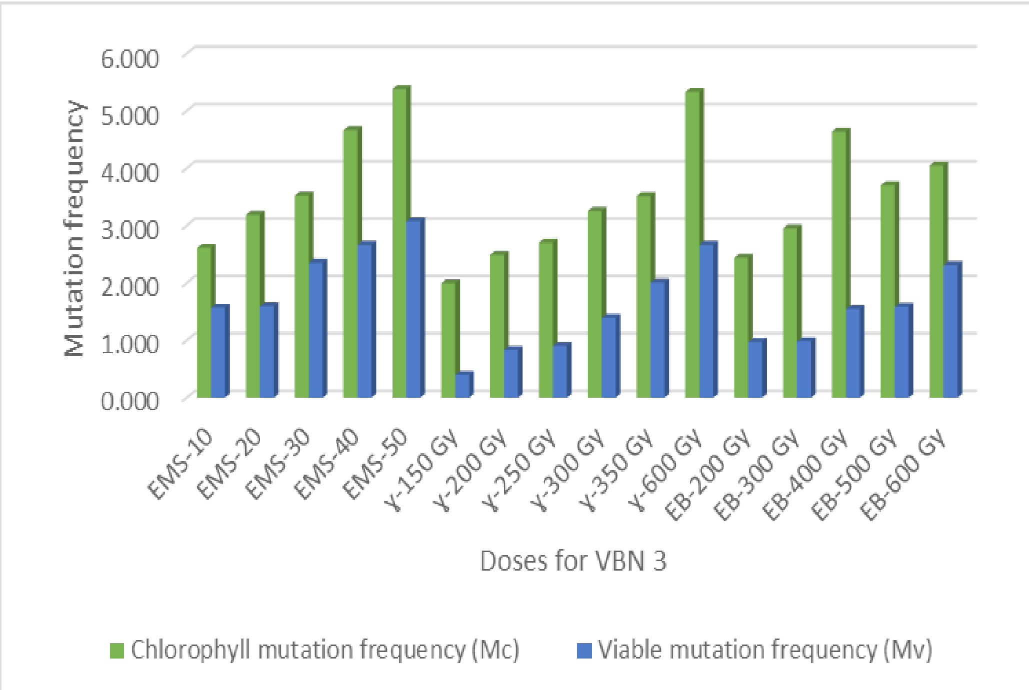

Fig. 1.Frequency of Chlorophyll mutants and viable mutants in VBN 3

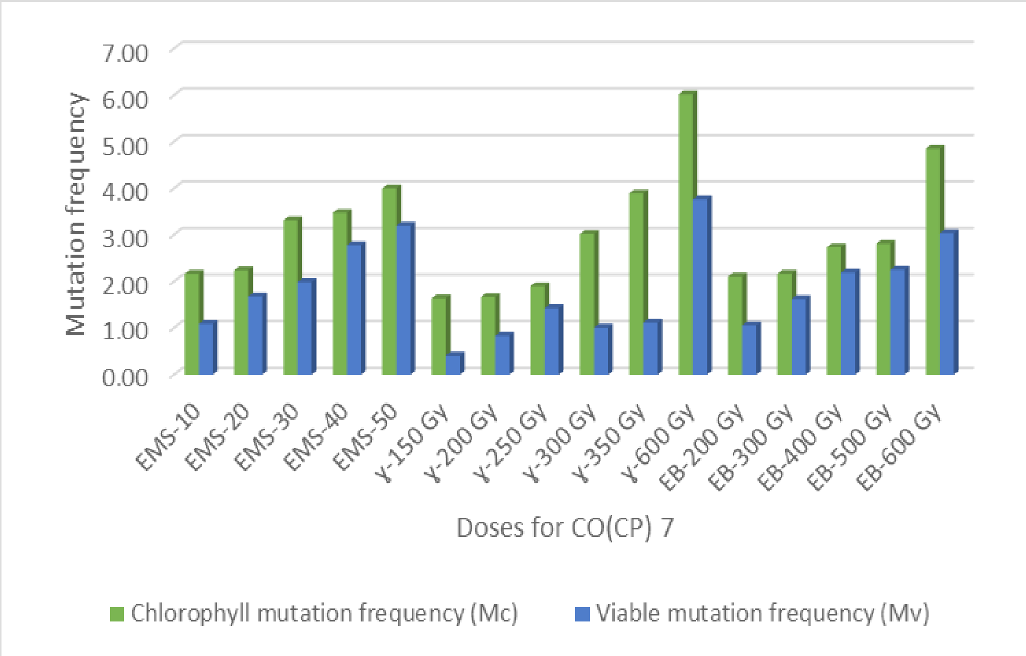

Fig 2.Frequency of Chlorophyll mutants and viable mutants in CO (CP) 7

Table 1. Effectiveness of EMS, Gamma rays and Electron beam mutagens on VBN 3

Concentration/Dose VBN 3	M2 plants studied	No of Chlorophyll mutants	No of viable mutants	Chlorophyll mutation frequency (Mc)	Viable mutation frequency (Mv)	Mc/Gy	Mv/Gy	Average
EMS-10	191	5	3	2.618	1.571	0.052	0.031	0.042
EMS-20	188	6	3	3.191	1.596	0.032	0.016	0.024
EMS-30	170	6	4	3.529	2.353	0.024	0.016	0.020
EMS-40	150	7	4	4.667	2.667	0.023	0.013	0.018
EMS-50	130	7	4	5.385	3.077	0.022	0.012	0.017
Overall EMS	829	31	18	19.390	11.263	0.153	0.089	0.121
ɣ-150 Gy	250	5	1	2.000	0.400	0.013	0.003	0.008
ɣ-200 Gy	241	6	2	2.490	0.830	0.012	0.004	0.008
ɣ-250 Gy	222	6	2	2.703	0.901	0.011	0.004	0.007
ɣ-300 Gy	215	7	3	3.256	1.395	0.011	0.005	0.008
ɣ-350 Gy	199	7	4	3.518	2.010	0.010	0.006	0.008
ɣ-600 Gy	150	8	4	5.333	2.667	0.009	0.004	0.007
Overall ɣ	1277	39	16	19.299	8.203	0.066	0.025	0.046
EB-200 Gy	205	5	2	2.439	0.976	0.012	0.005	0.009
EB-300 Gy	203	6	2	2.956	0.985	0.010	0.003	0.007
EB-400 Gy	194	9	3	4.639	1.546	0.012	0.004	0.008
EB-500 Gy	189	7	3	3.704	1.587	0.007	0.003	0.005
EB-600 Gy	173	7	4	4.046	2.312	0.007	0.004	0.005
Overall EB	964	34	14	17.784	7.407	0.048	0.019	0.033

Viable mutants were observed in the population during various stages of crop growth. In VBN 3, viable mutant frequency varied between 1.57 to 3.08 % in EMS population, 0.4 to 2.67 % in gamma rays, whereas in electron beam treated population the frequency ranged between 0.98 to 2.31 %. The overall viable mutant frequency was highest in EMS (10.72%) treated population whereas the frequency was lowest in (8.54%) gamma ray treated population in CO (CP) 7. Viable mutants based on plant habitat, stem modification, leaf modification, pod modification, seed characters and other chimeric mutants were observed in the mutant population which were found to be in argument with the findings by Horn et al (2016) and Olasupo et al (2018) in cowpea.

Mutagenic efficiency and effectiveness was analysed by calculating the frequency of chlorophyll mutants and viable mutants in comparison with the control. Mutagenic effectiveness is an indicator of the genotypic sensitivity towards the increasing mutagenic concentrations. Mutagenic effectiveness was estimated based on the frequency of chlorophyll mutants and viable mutants observed during the different stages of crop growth. The overall effectiveness was highest in EMS population (0.153) followed by gamma rays (0.066) and electron beam (0.048) in VBN 3 (**Table 1**) .In CO (CP) 7, Overall Effectiveness was again highest in EMS population (0.121) (**Table 2**) . A contrasting trend was recorded by Souframanien et al (2016) in blackgram in physical mutagens. But similar results were obtained by Khursheed et al (2018) in faba bean while comparing physical and chemical mutagens.Optimizing the doses of mutants also an important factor as mentioned by Sandhya et al (2020) in sesame.

Table 2. Effectiveness of EMS, Gamma rays and Electron beam mutagens on CO(CP) 7

Concentration/Dose CO (CP) 7	M2 plants studied	No of Chlorophyll mutants	No of viable mutants	Chlorophyll mutation frequency (Mc)	Viable mutation frequency (Mv)	Mc/Gy	Mv/Gy	Average
EMS-10	185	4	2	2.16	1.08	0.043	0.022	0.032
EMS-20	179	4	3	2.23	1.68	0.022	0.017	0.020
EMS-30	151	5	3	3.31	1.99	0.022	0.013	0.018
EMS-40	144	5	4	3.47	2.78	0.017	0.014	0.016
EMS-50	125	5	4	4.00	3.20	0.016	0.013	0.014
Overall EMS	784	23	16	15.18	10.72	0.121	0.078	0.100
ɣ-150 Gy	244	4	1	1.64	0.41	0.011	0.003	0.007
ɣ-200 Gy	240	4	2	1.67	0.83	0.008	0.004	0.006
ɣ-250 Gy	211	4	3	1.90	1.42	0.008	0.006	0.007
ɣ-300 Gy	199	6	2	3.02	1.01	0.010	0.003	0.007
ɣ-350 Gy	180	7	2	3.89	1.11	0.011	0.003	0.007
ɣ-600 Gy	133	8	5	6.02	3.76	0.010	0.006	0.008
Overall ɣ	1207	33	15	18.12	8.54	0.058	0.025	0.042
EB-200 Gy	190	4	2	2.11	1.05	0.011	0.005	0.008
EB-300 Gy	185	4	3	2.16	1.62	0.007	0.005	0.006
EB-400 Gy	183	5	4	2.73	2.19	0.007	0.005	0.006
EB-500 Gy	178	5	4	2.81	2.25	0.006	0.004	0.005
EB-600 Gy	165	8	5	4.85	3.03	0.008	0.005	0.007
Overall EB	901	26	18	14.66	10.14	0.038	0.026	0.032

Mutagenic efficiency was estimated on the basis of lethality and seedling injury a using both chlorophyll and viable mutants. The chlorophyll mutant efficiency was highest in EMS (0.291) for VBN 3 population (**Table 3**) whereas for CO(CP) 7 chlorophyll mutant efficiency was highest in Gamma ray treated population (0.223) (**Table 4**) .The viable mutant efficiency was high in EMS population for both VBN 3 (0.169) and CO (CP) 7 (0.147). These results were similar to findings obtained by Shah et al. (2008) in *Cicer arietinum*, Dube et al. (20110 in cluster bean. The present study was conducted to find mutagenic efficiency and effectiveness of cowpea varieties based on chlorophyll mutants, viable mutants, lethality percentage and reduction on height with respect to the control. The results infer that the effectiveness was highest in EMS followed by gamma rays and electron beam for both CO (CP) 7 and VBN 3, while efficiency based on chlorophyll mutants was high in EMS for VBN 3 and Gamma rays for CO (CP) 7. This helps to identify the highly suitable mutagenic concentration which would induce a high variability in the mutated population and aids in selection of highly potent mutants.

Table 3. Efficiency of EMS, Gamma rays and Electron Beam mutagens on VBN 3

Concentration /Dose	Chlorophyll mutation frequency (%) (Mc)	Viable mutation frequency (%) (Mv)	Plant survival reduction (%) (L)	Plant height reduction (%)(I)	Efficiency based on chlorophyll mutant frequency			Efficiency based on viable mutant frequency		
VBN 3					**Mc/L**	**Mc/I**	**Average**	**Mv/L**	**Mv/I**	**Average**
EMS-10 mM	2.62	1.57	63.33	5.61	0.041	0.467	0.254	0.025	0.280	0.152
EMS-20 mM	3.19	1.60	49.89	5.08	0.064	0.628	0.346	0.032	0.314	0.173
EMS-30 mM	3.53	2.35	28.71	8.03	0.123	0.440	0.281	0.082	0.293	0.187
EMS-40 mM	4.67	2.67	20.13	10.13	0.232	0.461	0.346	0.132	0.263	0.198
EMS-50 mM	5.38	3.08	18.85	12.07	0.286	0.446	0.366	0.163	0.255	0.209
	19.39	**11.26**	**180.91**	**40.92**	**0.107**	**0.474**	**0.291**	**0.062**	**0.275**	**0.169**
γ-150 Gy	2.00	0.40	70.33	2.67	0.028	0.749	0.389	0.006	0.150	0.078
γ-200 Gy	2.49	0.83	61.23	2.84	0.041	0.877	0.459	0.014	0.292	0.153
γ-250 Gy	2.70	0.90	52.18	3.31	0.052	0.817	0.434	0.017	0.272	0.145
γ-300 Gy	3.26	1.40	30.14	9.9	0.108	0.329	0.218	0.046	0.141	0.094
γ-350 Gy	3.52	2.01	25.55	10.37	0.138	0.339	0.238	0.079	0.194	0.136
γ-600 Gy	5.33	2.67	15.23	15.51	0.350	0.344	0.347	0.175	0.172	0.174
γ	**19.30**	**8.20**	**254.66**	**44.6**	**0.076**	**0.433**	**0.254**	**0.032**	**0.184**	**0.108**
EB-200 Gy	2.44	0.98	59.89	5.33	0.041	0.458	0.249	0.016	0.183	0.100
EB-300 Gy	2.96	0.99	48.88	3.25	0.060	0.909	0.485	0.020	0.303	0.162
EB-400 Gy	4.64	1.55	37.56	7.03	0.124	0.660	0.392	0.041	0.220	0.131
EB-500 Gy	3.70	1.59	34.21	7.1	0.108	0.522	0.315	0.046	0.224	0.135
EB-600 Gy	4.05	2.31	25.13	19.04	0.161	0.213	0.187	0.092	0.121	0.107
	17.78	**7.41**	**205.67**	**41.75**	**0.086**	**0.426**	**0.256**	**0.036**	**0.177**	**0.107**

Table 4. Efficiency of EMS, Gamma rays and Electron Beam mutagen on CO(CP) 7

Concentration /Dose	Chlorophyll mutation frequency (%) (Mc)	Viable mutation frequency (%) (Mv)	Plant survival reduction (%) (L)	Plant height reduction (%)(I)	Efficiency based on chlorophyll mutant frequency			Efficiency based on viable mutant frequency		
CO (CP) 7					**Mc/L**	**Mc/I**	**Average**	**Mv/L**	**Mv/I**	**Average**
EMS-10 mM	2.16	1.08	65.45	0.91	0.033	2.376	1.205	0.017	1.188	0.602
EMS-20mM	2.23	1.68	30.45	7.26	0.073	0.308	0.191	0.055	0.231	0.143
EMS-30mM	3.31	1.99	22.25	10.17	0.149	0.326	0.237	0.089	0.195	0.142
EMS-40 mM	3.47	2.78	18.23	14.03	0.190	0.247	0.219	0.152	0.198	0.175
EMS-50 mM	4.00	3.20	17.85	15.35	0.224	0.261	0.242	0.179	0.208	0.194
EMS	**15.18**	**10.72**	**154.23**	**47.72**	**0.098**	**0.318**	**0.208**	**0.070**	**0.225**	**0.147**
γ-150 Gy	1.64	0.41	60.23	1.82	0.027	0.901	0.464	0.007	0.225	0.116
γ-200 Gy	1.67	0.83	52.12	5.12	0.032	0.326	0.179	0.016	0.163	0.089
γ-250 Gy	1.90	1.42	44.44	9.82	0.043	0.193	0.118	0.032	0.145	0.088
γ-300 Gy	3.02	1.01	34.12	8.42	0.088	0.358	0.223	0.029	0.119	0.074
γ-350 Gy	3.89	1.11	20.33	10.27	0.191	0.379	0.285	0.055	0.108	0.081
γ-600 Gy	6.02	3.76	15.55	14.03	0.387	0.429	0.408	0.242	0.268	0.255
γ	**18.12**	**8.54**	**226.79**	**49.48**	**0.080**	**0.366**	**0.223**	**0.038**	**0.173**	**0.105**
EB-200 Gy	2.11	1.05	54.33	0.99	0.039	2.127	1.083	0.019	1.063	0.541
EB-300 Gy	2.16	1.62	42.12	6.77	0.051	0.319	0.185	0.039	0.240	0.139
EB-400 Gy	2.73	2.19	38.25	9.49	0.071	0.288	0.180	0.057	0.230	0.144
EB-500 Gy	2.81	2.25	33.33	14.19	0.084	0.198	0.141	0.067	0.158	0.113
EB-600 Gy	4.85	3.03	24.1	16.58	0.201	0.292	0.247	0.126	0.183	0.154
	14.66	**10.14**	**192.13**	**48.02**	**0.076**	**0.305**	**0.191**	**0.053**	**0.211**	**0.132**

REFERENCES

Atachi , P. Dannon ,E. A., and Rurema , D. G, 2007. Trap cropping and intercropping of pigeon pea (*Cajanus cajan* Millsp.) in pest management of cowpea (*Vigna unguiculata*) in southern Benin: competing risk and pest status in pod attack. *Annales des Sciences Agronomiques*, **9**: 1-20. [Cross Ref]

Gustafsson, A, 1940. The mutation system of the chlorophyll apparatus. Lunds Univ. *Arsskr. N.F.Adv* .,**36**: 1-40.

Konzak, C.P., R.A. Nilan, J. Wagner and R.J. Foster ,1965. Efficient chemical mutagenesis. The use of induced mutations in plant breeding. *Radiation Botany* , **5**: 49-70.

Sharma ,S.K., Ritu ,S., and Pandey ,D.P. 2005. Studies on mutagen sensitivity, effectiveness and efficiency in urdbean (*Vigna mungo* L. Hepper). *Indian J. Genet*., **65**(1): 20-22.

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).*African Journal of Biotechnology* ,**7** (22): 4116-4117.

Olasupo ,F.O., Ilori,C.O., Forster,B.P. and Bado,S, 2018. Selection for Novel Mutations Induced by Gamma Irradiation in Cowpea (*Vigna unguiculata* L. Walp.). *International Journal of Plant Breeding and Genetics*,**12**:1-12. [Cross Ref]

Horn,L.N., Ghebrehiwot,H.M. and Shimelis,H.A, 2016. Selection of Novel Cowpea Genotypes Derived through Gamma Irradiation. *Front. Plant Sci*., **7**:262 – 275. [Cross Ref]

Souframanien, J., Kandali S Reddy,Petwal, V.C. and Dwivedi, J., 2016. Comparative effectiveness and efficiency of electron beam and ^{60}Co γ-rays in induction of mutations in black gram (*Vigna mungo* L. Hepper). *Journal of Food Legumes,***29**(1): 1-6.

Khursheed,S., Raina,A., Laskar,R.A. and Khan, S ,2018. Effect of gamma radiation and EMS on mutation rate: their effectiveness and efficiency in faba bean (*Vicia faba* L.). *Caryologia*,**71**(4):397- 404. [Cross Ref]

Shah,T.M., Mirza,J.I., Haq,M.A., Atta,B.M , 2008. Induced genetic variability in chickpea (*Cicer arietinum* L.). Comparative mutagenic effectiveness and efficiency of physical and chemical mutagens. *Pakistan J Bot*. **40** (2):605–613.

Dube,K.G., Bajaj,A.S., Gawande,A.M, 2011. Mutagenic efficiency and effectiveness of gamma rays and EMS in *Cyamopsis tetragonoloba* L. var. Sharada. *Asiatic J Biotechnol Res.* **2**:436–440.

Molosiwa, O. O., Gwafila, C., Makore, J., and Chite, S. M ,2016. Phenotypic variation in cowpea (*Vigna unguiculata*L. Walp.) germplasm collection from Botswana. *International Journal of Biodiversity and Conservation*, **8**(7):153-163. [Cross Ref]

Vinithashri ,G.,Manonmani ,S., Anand ,G., Meena ,S., Bhuvaneswari ,K., and John Joel , A ,2020. Mutagenic efficiency and effectiveness of Sodium Azide in rice varieties. *Electronic Journal of Plant Breeding* ,**11**(1):197-203. [Cross Ref]

Sandhiya,V. ,Kumar,M. , Parameswari .C., Vanniarajan,C. , Kumaravadivel,N. , Sakthivel, N. and Anand M. Badigannavar ,2020 . Determination of optimum dose of chemical mutagen for large scale seed treatment of white seeded sesame (Sesamum indicum L.) varieties, *Electronic Journal of Plant Breeding* ,**11**(1): 238-242. [Cross Ref]