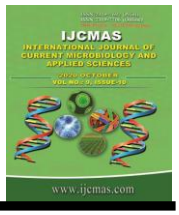
Int.J.Curr.Microbiol.App.Sci (2020) *9*(10): 693-703

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 10 (2020)**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2020.910.083

Effects of Induced Physical and Chemical Mutagen in Cowpea (*Vigna unguiculata* L. walp)

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Keywords

Mutation, EMS, Gamma rays, Cow pea

Article Info

Accepted:
07 September 2020
Available Online:
10 October 2020

A B S T R A C T

The biological effects of physical mutagen gamma rays and chemical mutagen Ethyl Methyl Sulphonate (EMS) were analyzed in two varieties of cowpea viz., VBN-1, and C-152. Five different doses of gamma rays (100 Gy, 200 Gy, 300 Gy, 400 Gy and 500 Gy) and five different concentrations of EMS (5 mM, 10 mM, 15 mM, 20 mM and 25 mM) were used for inducing mutations in these two varieties. In M1 generation, the Greater reduction was observed at higher doses of mutagens and the variety C-152 were found to be the more sensitive than VBN-1. The percentage of seed germination was reduced progressively with the increasing doses of mutagens. The observation of Shoot length not follow the linear relationship between increase in dose or concentration of mutagens but the root length follow linear relationship with increase in dose or concentration of mutagens. In case of seedling vigor, it was decreased with the proportion of increase in dose or concentration of mutagens in both the varieties. Finally, concluded to chemical mutagen EMS were most effective than the gamma rays in both the varieties of cowpea.

Introduction

Pulses were act important constitute role in human diet. They contain the essential sources of amino acids and protein and it also act as adjuncts or alternative to cereal based diet. Pulses were also called "poor man's meat" which has 20-25 per cent protein (Kumar *et al.,* 2010). Cowpea [*Vigna unguiculata* (L.) Walp.] an annual legume belonging to family Leguminosae with chromosome number 2n=22, 24 (Pandey 2010). Cowpea contains 22 to 32% of protein on a dry weight basis and 50-67% carbohydrate and starch. Because of its high protein content, cowpea has been referred to as "Vegetable meat". Cowpea's young leaves, pods and peas contain vitamins and minerals which used for human consumption and animal feeding. The 12.3 million hectares of land utilized for cow pea production in 2016 in globally (Owade *et al.,* 2020). In India around 19.3 million tons of cowpea produced annually with average productivity of 764 kg/ha. The low yield is due to nutrient deficient soils, problematic weather

conditions and poor quality of seeds (Yadav *et al.,* 2017).

Crop improvement in cowpea through hybridization and recombination is difficult, because of its self-pollination nature. Due to autogamous nature, they lack genetic variability also. In cow pea, the natural variability's were exploited in conventional breeding and it does not have favourable and adequate variability for further cow pea improvement.

Mutation breeding is one of the favorable methods to create the variability. The mutation breeding is an effective tool for creating genetic variations in self-pollinated crops and it is relatively quick method for improvement of crops. Mutants obtained from mutation breeding can also be incorporated into crossing programme as conventional alleles to obtain the desired genotype.

Through induced mutagenesis, varieties resistant to pests and diseases, increased protein content and high yielding potential can be obtained. It has been proved that the induced mutations can increase yield as well as improve other polygenic characters in crop. A number of economically important varieties are released through induced mutagenesis. Horn *et al.,* (2016) used gamma irradiation in three traditional cowpea varieties Nakare, Shindimba and Bira. They were gamma irradiated with varied doses. Gnanamurthy and Dhanavel, (2014) studied effect of EMS on induced morphological mutants and chromosomal variation in cowpea. They revealed that the improved variety of cowpea CO-7 responded more and more number of viable and economic mutants for higher productivity in 25 mM of EMS than the other mutagenic treatments in all generation.

Keeping the above points in view, the present investigation was concentrated on two varieties *viz.,* VBN-1 and C-152 for developing early mutant cowpea genotypes with yield characters, to create variability, to estimate the extent of variability generated in qualitative and quantitative traits and to assess the relative effectiveness and efficiency of different doses of the mutagens used in cowpea through induced mutagenesis.

Materials and Methods

Experimental materials

The experiment was carried out at School of Agriculture and Animal Husbandry, The Gandhigram Rural Institute- Deemed University, Gandhigram, Dindigul, Tamil Nadu during 2016-2017. The details of varieties chosen are mentioned in Table 1.

Physical mutation

The physical mutagen, gamma rays was used as to irradiate the seeds of two cowpea varieties *viz.,* VBN-1 and C-152. The treatment was done in the gamma chamber (GC 1200) installed by Board of Radiation and Isotope Technology (BRIT), Govt. of India, Mumbai at the Centre for Plant Breeding and Genetics, TNAU, Coimbatore where, Cobalt-60(^{60}Co) served as source of gamma rays. The well filled, uniform sized, dry seeds were packed in butter paper covers and placed in the Gamma cell and exposed to gamma irradiation for appropriate time as shown in the (Table 2) below, for each dose based on the half-life of the source. Non-irradiated dry seeds of each genotype were used as control.

Chemical mutagen

The chemical mutagen, Ethyl Methane Sulphonate ($CH_3SO_2OC_2H_5$, Molecular weight 124.16, Boiling point 80/100 mm Hg and density D4 25 =1.203 g / ml) were used

for induce mutation. The treatment was done at botany laboratory, School of Agriculture and Animal Husbandry, The Gandhigram Rural Institute- Deemed University, Gandhigram, Dindigul, Tamil Nadu. It was stored in dry air at 0^0C to maintain its purity. Prior to use it was taken from refrigerator and placed in a desiccator over calcium chloride to reach the room temperature. Well filled 500 seeds of both the varieties were presoaked for four hours in distilled water. Seeds were treated with different concentrations of EMS in double distilled water and pH of the mutagenic solution was adjusted to seven by using phosphate buffer. The presoaked seeds after removal from the water were packed between the folds of blotting paper to remove excess water adhering to the surface.

The seeds were immersed for six hours in the required concentrations (Table 3) of the mutagen with intermittent shaking. To ensure uniform absorption of the mutagen, the volume of the mutagen solution was maintained at a proportion of ten times to that of seed volume. The whole treatment was carried out at room temperature. After the treatment, the seeds were thoroughly washed with tap water for ten times.

Laboratory studies

The both mutagenized or treated seeds were placed in the roll towel replicated twice for the purpose of laboratory analysis. Non-irradiated dry seeds and presoaked seeds in distilled water for six hours were used as control.

Observations under laboratory condition

The morphological observation on Seed germination (%), Shoot length (cm), Root length (cm) and Seedling Vigor.

Field studies

A total of 100 seeds in each treatment were sown in the field by adopting a spacing of 30 cm between rows and 15 cm between plants under randomized block design with two replications (Fig. 1).

The recommended agronomic practices and plant protection measures were followed uniformly for all the treatments at appropriate stages during the entire crop growth period.

Data collection under field condition

The morphological observation on Pod length (cm), seed colour, seed size, Growth Pattern, Terminal Leaf Shape and Plant Twining Tendency were recorded in various plant growth stages.

Statistical analysis

Seedling vigor: The seedling vigor calculated by following formula

Seedling vigor = [mean root length + mean shoot length] x percentage of seed germination.

Mutagenic effectiveness and efficiency

It is a measure of the frequency of induced mutants by a unit dose of mutagen (Gy or Concentration x Time). Data on abnormalities such as injury, lethality and viable mutants in M_1 generation were used to determine the mutagenic efficiency and effectiveness according to the formula suggested by Konzak *et al.*, (1965).

$$\text{Mutagenic effectiveness } (ME_v) = \frac{\text{Mutation frequency (MF)}}{\text{Dose or (Concentration x Time)}}$$

$$\text{Mutagenic efficiency } (ME_f) = \frac{\text{Mutation frequency (MF)}}{\text{Biological Damage}}$$

Biological damage *i.e.*, MF/L, MF/I, MF/S
L = Lethality, I= Injury, S= Sterility

Mutation Rate

Mutation Rate (MR) was calculated by the formula

$$MR = \frac{\text{Sum of values of the efficiency or effectiveness of particular mutagen}}{\text{Number of treatments of a particular mutagen}}$$

Results and Discussion

Effect of mutagens in M_1 generation under laboratory condition

The Effect of mutagens on both the varieties *viz.*, VBN-1 and C-152 in M_1 generation presented in Table 4 &5.

Effect of mutagens on germination

In cowpea variety VBN-1, germination percentage ranged from 22.00 per cent (500 Gy) to 72.00 per cent (100 Gy) in gamma ray treatments. Similarly, in case of C-152, germination percentage ranged from 42.00 per cent (500 Gy) to 86.00 per cent (100 Gy) using the gamma ray treatments.

In case of chemical mutagen EMS, germination percentage of VBN 1 ranged from 70.00 per cent (25mM) to 86.00 per cent (5 mM) whereas in C-152, the germination percentage ranged from 58.00 per cent (25 mM) to 88.00 per cent (10 mM).

On the whole, highest germination percentage following mutagenic treatments was observed at lower doses of both mutagens *viz.*, 100 Gy of gamma rays and 5 mM of EMS in both the varieties. While, maximum reduction in germination due to mutagenic treatments was observed at higher doses of both the mutagens 500 Gy of gamma rays and 25 mM of EMS.

The both the varieties displayed a dose dependent negative linear relationship between dose and germination percentage. So, these may due to the activation of RNA or protein synthesis and it may be occurred during the early stage of germination after the seeds were irradiated (Abdel-Hady *et al.*, 2008). It may also be attributed to disturbances at cellular level (caused either at physiological level or at physical level) including chromosomal damages or due to the combined effect of both (Khan and Tyagi, 2010).

Effect of mutagens on Shoot length

Under laboratory condition Mean shoot length of VBN-1 following gamma ray treatments ranged from 19.50 cm (500 Gy) to 26.00 cm (100 Gy) over the control (14.90 cm) whereas in C-152 it was ranged from 12.50 cm (500 Gy) to 27.60 cm (200 Gy) over the control (18.90). In EMS treatments, mean shoot length of VBN-1 ranged from 19.84 cm (25mM) to 24.50 cm (5 mM) over the control (23.20 cm) while in C-152 mean shoot length ranged from 20.80 cm (10 mM) to 26.62 cm (20 mM) over the control (16.18 cm).

The shoot length of VBN-1 and C-152 after treatment with gamma radiation does not follow linear relationship between increase in dose or concentration and shoot length. In VBN-1, higher reduction in shoot length was noticed in gamma rays of higher dose 100 Gy and 5 mM of EMS whereas in C-152 higher reduction was observed in 200 Gy of gamma radiation and 20 mM of EMS. Tabasum *et al.*, (2011) reported that, shoot length did not increase or decrease in definite pattern in response to different doses.

Table.1 Pedigree details of the selected varieties

Variety	Pedigree	Source
VBN-1	Selection from T 85F 2020	National Pulses Research Centre, Vamban, Pudukottai, Tamil Nadu.
C-152	Promising variety released in Karnataka for general cultivations.	State Agriculture Department, Athoor Block, Dindigul, Tamil Nadu.

Table.2 Dosage level of gamma rays

Treatments	Dosage (Gy)	Exposure Time
T_1	100	4 min 20 sec
T_2	200	8 min 40 sec
T_3	300	13 min 0 sec
T_4	400	17 min 20 sec
T_5	500	21 min 40 sec

Table.3 Dosage level of EMS

S.No	Concentration of the solution (mM)	Total volume required (phosphate buffer) (ml)	Volume to be taken(EMS)(ml)
1	5	50	0.0261
2	10	50	0.0522
3	15	50	0.0783
4	20	50	0.1045
5	25	50	0.1306

Table.4 Effect of mutagens in M_1 generation of Cowpea var. VBN-1

Treatments	No. of Seeds Evaluated	Germination (%)	Observed mortality (%)	Shoot length (cm)	Root Length (cm)	Seedling Vigor
Gamma rays						
Control	50	84.00	16.00	23.80	14.90	3250.80
100 Gy	50	72.00	28.00	26.04	13.40	2839.70
200 Gy	50	60.00	40.00	24.08	17.76	2510.40
300 Gy	50	48.00	52.00	23.96	14.78	1859.50
400 Gy	50	30.00	70.00	23.02	13.78	1104.00
500 Gy	50	22.00	78.00	19.48	12.08	694.30
EMS						
Control	50	88.00	12.00	23.20	12.82	3169.80
5 mM	50	86.00	14.00	24.50	13.90	3302.40
10 mM	50	84.00	16.00	21.56	14.44	3024.00
15 mM	50	80.00	20.00	22.22	15.40	3009.60
20 mM	50	62.00	38.00	24.30	14.50	2405.60
25 mM	50	70.00	30.00	19.84	14.08	2374.40

Table.5 Effect of mutagens in M_1 generation of cowpea var. C-152

Treatments	No. of Seeds Evaluated	Germination (%)	Observed mortality (%)	Shoot length (cm)	Root Length (cm)	Seedling Vigor
Gamma rays						
Control	50	98.00	2.00	18.92	16.40	3461.40
100 Gy	50	86.00	14.00	23.80	11.74	3056.40
200 Gy	50	84.00	16.00	27.60	14.38	3526.30
300 Gy	50	70.00	30.00	21.62	13.16	2434.60
400 Gy	50	52.00	48.00	19.44	11.64	1616.20
500 Gy	50	42.00	58.00	12.64	11.80	1026.50
EMS						
Contrl	50	82.00	18.00	16.18	13.24	2412.40
5 mM	50	82.00	18.00	21.84	13.54	2901.20
10 mM	50	74.00	26.00	20.82	12.88	2493.80
15 mM	50	70.00	30.00	21.44	12.90	2403.80
20 mM	50	66.00	34.00	26.62	11.74	2531.70
25 mM	50	58.00	42.00	22.56	10.38	1910.50

Table.6 Effectiveness and Efficiency of Mutation in M_1 generation of cowpea varieties

S. No	Dosage	Total No. of plants survived	Lethality count	Segregated	Mutants per 100 plants	Mutation effectiveness (%)	Mutation efficiency (%)
VBN-1 Gamma Rays							
1	100 Gy	73	27	8	10.96	10.96	40.59
2	200 Gy	79	21	12	15.18	07.59	75.14
3	300 Gy	64	36	1	1.56	00.52	04.33
4	400 Gy	39	61	4	10.25	02.56	16.80
5	500 Gy	24	76	2	8.33	01.66	10.96
VBN-1 EMS							
6	5 Mm	65	35	8	12.30	41.00	35.14
7	10 mM	64	36	5	7.81	13.01	21.69
8	15 mM	71	29	5	7.04	07.82	24.27
9	20 mM	66	34	8	12.12	10.10	35.64
10	25 mM	46	54	3	6.52	04.34	12.07
C-152- Gamma Rays							
11	100 Gy	67	33	11	16.41	16.41	49.72
12	200 Gy	62	38	13	20.96	10.48	55.15
13	300 Gy	49	51	6	12.24	04.08	24.00
14	400 Gy	29	71	1	3.44	00.86	04.84
15	500 Gy	15	85	1	6.66	01.33	07.83
C-152- EMS							
16	5 mM	43	57	5	11.62	38.73	20.38
17	10 mM	49	51	7	14.28	23.80	28.00
18	15 mM	46	54	6	13.04	14.48	24.14
19	20 mM	40	60	6	15.00	12.50	25.00
20	25 mM	13	87	3	15.39	10.26	17.69

Fig.1 Field view of the experimental plot

Fig.2 Pod length variations observed in M_1 generation of cowpea

C-152 – 300Gy **C-152 – 10mM** **VBN-1 – 100Gy** **VBN-1 – 300Gy**

Fig.3 Seed colour variations observed in M_1 generation of cowpea

C-152 – 25mM **C-152 – 100 Gy** **C-152 – 500Gv** **VBN-1 – 5mM**

Fig.4 Seed size variations observed in M_1 generation of Cowpea

C-152 – 200Gy **VBN-1 – 20mM** **VBN-1 – 400Gy**

Fig.5 Terminal leaf variation observed in M_1 generation of Cowpea

C-152 – 300Gy **C-152 – 20mM** **VBN-1 – 15mM** **VBN-1 – 200Gy**

Fig.6 Twining variations observed in M_1 generation of Cowpea

C-152 – 20mM **C-152 – 20mM** **C-152 – 20mM** **C-152 – 20mM**

Effect of mutagens on root length

Under laboratory condition in gamma ray treated populations of VBN-1 mean root length of the seedlings ranged from 12.08 cm (500 Gy) to 17.75 cm (200 Gy) over the control (14.90 cm) whereas mean root length of C-152 ranged from 11.64 cm (400 Gy) to 14.38 cm (200 Gy) over the control (16.40 cm). In EMS treatments, mean root length of VBN-1 ranged from 13.90 cm (5 mM) to 15.40 cm (15 mM) over the control (12.82 cm), while in C-152 mean root length ranged from 10.38 cm (25 mM) to 13.54 cm (5 mM) over the control (13.24 cm).

The increase in root length was in positive linear fashion in both gamma irradiated and

EMS treated VBN-1 and C-152. Higher root length was observed at higher dose or concentration of gamma rays and EMS of 500 Gy and 25 mM. Kumar *et al.,* (2010) recorded the reduction in shoot and root development which related to various factors such as chromosomal abnormality with height reduction, reduction in auxin levels, inhibition of auxin synthesis, failure of assimilation mechanisms and chromosomal damage.

Effect of mutagens on seedling vigor

Under laboratory condition in gamma ray treated populations of VBN-1 mean seedling vigor of the seedlings ranged from 694.3 (500 Gy) to 2839.7 (100 Gy) over the control 3250.8 whereas mean seedling vigor ofC-152 ranged from 1026.5 (500 Gy) to 3526.3 (200 Gy) over the control 3461.4. In EMS treatments, mean seedling vigor of VBN-1 ranged from 2374.4 (500 Gy) to 3302.4 (100 Gy) over the control 3169.8, while in C-152 mean seedling vigor ranged from 1910.5 (25 mM) to 2901.5 (5mM) over the control (2412.4).

The seedling vigour was decreased with the proportion of increase in dose or concentration in both the varieties of VBN-1 and C-152. Similar results have been reported by Harding *et al.,* (2012). Cheema and Atta (2003) reported that, the seedling height was decreased with the increase of irradiation dose, but the decrease was not proportional to the increase in dosage.

Effectiveness and efficiency of mutagens

From the table 6, the viable mutant was maximum at 200 Gy (VBN-1) and 5 mM (C-152) gamma ray doses on M_1 plant basis. In EMS treatments, 5mM & 20 mM in VBN-1 and 20 mM in C-152 recorded maximum viable mutants on M_1 plant basis. The mutant frequency or mutant per 100 M_1 plant basis was calculated from segregated viable mutants. Based on the mutant frequency, in VBN-1 the dose 200 Gy Gamma rays and 5mM EMS produced maximum mutant frequency of 15.18 % and 12.30 % respectively. In variety C-152, the dose 200 Gy gamma rays and 25 mM EMS showed high mutant frequency of 20.96 % and 10.26 % respectively.

The effectiveness of inducing viable mutations ranged from 00.52 (300 Gy) to 10.96 (100 Gy) and 0.86 (400 Gy) to 16.41 (100 Gy) on VBN-1 and C-152 respectively. Maximum effectiveness of 41.00 and 38.73 were recorded at 5 mM EMS concentrations on VBN-1 and C-152 respectively. Generally, effectiveness of viable mutants were higher in gamma ray treatment than EMS on M_1 plant bases. Gunasekaran (1992) reported that gamma rays were more effective than chemical mutagen in inducing viable mutations in cow pea. In the present study, the effectiveness decreased with increase in concentration of both gamma rays and EMS.

The efficiency of the viable mutants in gamma rays, on lethality basis maximum efficiency of 75.14 and 55.15 at 200 Gy M_1 plants of VBN-1 and C-152 respectively. In EMS concentration, based on lethality maximum efficiency 35.64 obtained at 20 mM on VBN-1 and 28.00 at 10 mM on C-152 M_1 plant basis. Efficiency of the viable mutants based on the lethality in M_1 plant the maximum efficiency of 75.14 and 55.15 was observed at 200 Gy in VBN-1 and C-152 respectively. Similarly, maximum efficiency of 35.64 was obtained at 20 mM on VBN-1 and 28.00 at 10 mM on C-152 in EMS treatments. From the present study it is inferred that 200 Gy and 20 mM on VBN-1 and 28.00 at 10 mM on C-152 were more efficient on lethality and inducing viable mutations. Konzak *et al.,* (1965) reported the mutagenic efficiency on the other hand

provides an idea of proportion of mutations in relation to other associated undesirable biological effects such as injury, lethality and sterility induced by the mutagen.

The mutagen rates were more in chemical mutagen, On the basis of effectiveness, the mutation rates in EMS were 15.25 and 19.95 in variety VBN-1 and C-152. Whereas, in case of physical mutagen gamma rays, the mutation rates were 4.66 and 6.63 in variety VBN-1 and C-152 respectively. As far as mutation rates in terms of efficiency concerned, mutation rates of lethality induced by gamma rays and EMS were more in variety VBN-1 (29.56 and 28.31) than variety C-152 (25.76 and 23.04). Nair and Mehta,(2014) reported that the effectiveness increased when the chemical concentration increase till 0.30 %, 0.35 % in pusakomal, arkagarima respectively and mutagenic efficiency decreased in higher concentration of gamma ray.

Effect of mutagens in M_1 generation under field condition

The mutagens treated seeds were sown in the field in randomized block design. The important observations were noted *viz.,* pod length (cm), seed colour, seed size, plant growth pattern, terminal leaf shape and plant twining tendency. From these observations, some of the desirable variations are recorded based on the different dosages of gamma ray and EMS treatment which is shown in the form of figures (Fig. 2, 3, 4, 5, and 6).

In conclusion the present investigation, lower concentration of EMS and gamma rays showed higher effectiveness values. In other words the effectiveness of the mutagens decreased with the increase in the concentration or dose of mutagens. Chemical mutagen (EMS) was found to be most effective than the physical mutagen (gamma rays). It also found that lower concentrations of both physical and chemical mutagens are effective. Finally concluded the most effective mutagen was EMS than the gamma rays in both the varieties of cowpea.

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How to cite this article:

Sathees, N., K. Gowthami, R. Pramila Devi, T. Gowtham, K. Mounika, M. Nivesh and Mohan, S. 2020. Effects of Induced Physical and Chemical Mutagen in Cowpea (*Vigna unguiculata* L. walp). *Int.J.Curr.Microbiol.App.Sci.* 9(10): 693-703.
doi: https://doi.org/10.20546/ijcmas.2020.910.083