

Original Research Article

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Mutagenic Effectiveness and Efficiency of Gamma Rays and EMS on Cape Gooseberry (*Physalis peruviana* L.)

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ABSTRACT

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The present investigation was carried out to find the efficiency and effectiveness of various mutagenic treatments of gamma rays and EMS on cape gooseberry. Genetically pure, uniform and dry seeds of cape gooseberry were subjected to 2, 5, 10, 15 and 20 kR doses of gamma rays and 0.02, 0.03, 0.04 and 0.05 M concentration of ethyl methane sulfonate along with control. Chlorophyll mutation, biological damages and viable mutant frequency was recorded in M1 and M2 generation. Highest mutation frequency 3.17% was observed in 0.02 M EMS followed by 0.03 M EMS 2.42% and the effectiveness of EMS treatments ranged from 1.09 to 4.55 and 2 kR and 0.02 M EMS were the most efficient treatments. The higher doses of both mutagen lead to lethal injury and sterility.

Introduction

The cape gooseberry (*Physalis peruviana* L.) is a member of Solanaceae family. It belongs to the genus *Physalis* with approximately 80 species cultivated in Mexico and Guatemala and originating from Mesoamerica (Menzel, 1951 and Bukasov, 1963). It is known as rasbhari in India, goldenberry in European countries and uchuva in Colombia (Puente *et al.*, 2011). The fruit is a juicy berry with rounded shape and a diameter between 1.25 to 2.65 cm, 4 to 12 g weight along with 100 to 200 small seed. Calyx protect the fruit from insects, disease, birds and adverse conditions. Cape gooseberry is an economically useful crop, especially in Colombia, where it is one

of the most important exotic fruit. It is also important in South Africa and Kenya where there have been active breeding programmes. It is used as a fresh fruit or, more commonly, as the basis for jams preparation. It is high in ascorbic acid (Barcia *et al.*, 2010).

Cape gooseberry has a very narrow genetic base so it is difficult to produce superior variety. So Mutational breeding is one of the effective tools for improvement of cape gooseberry. Mutation breeding is an effective tools for improvement of crop which having narrow genetic base such as cape gooseberry. Among different mutagenic agent gamma irradiation and EMS had been used successfully with several solanaceous crop for

creating variability. The usefulness of a mutagen in mutation breeding depends not only on its mutagenic effectiveness (mutations per unit dose of mutagens), but also its mutagenic efficiency (mutation in relation to undesirable changes like sterility, lethality, injury etc.). The selection of effective and efficient mutagens is very essential to recover a high frequency and spectrum of desirable mutations. Gamma rays and EMS (Ethyl Methane Sulphonate) are widely used mutagen for induction of viable mutant and mutational studies. Gamma irradiation as mutagen can induce useful as well as harmful mutation in plants (Gupta, 1996). Gamma rays are known to influence plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in cells and tissues. The selection of effective and efficient mutagens is very essential to recover a high frequency and spectrum of desirable mutations (Solanki & Sharma, 1994) and (Mahabatra, 1983). Therefore, present experiment was carried out to apply various gamma ray doses and different concentration of ethyl methanesulfonate (EMS) were undertaken in *Physalis peruviana* L.

Materials and Methods

Study site

The present investigation was carried out at the Horticulture Research Farm, Department of Horticulture, Banaras Hindu University, Varanasi (25° 10' North latitude and 83° 04' East longitudes with an altitude of 123.23 meter above the mean sea level), Uttar Pradesh.

Study materials

Genetically pure, uniform and dry seeds (10% moisture content) of cape gooseberry were taken for induction of mutation by using gamma irradiation and EMS. The gamma

treatments were given by using the ⁶⁰Co Gamma cell. Ethyl methane sulphonate (CH₃SO₂OC₂H₅) manufactured from the Sigma Chemical Company, USA was used for treating the seeds.

Healthy seeds packed in moist germination paper were selected for each treatment in the gamma chamber at 2, 5, 10, 15 and 20 kR doses of gamma rays in ⁶⁰Co gamma source (irradiation source capacity to release 3000 Ci delivery 7200 r/min). For EMS treatment, the seeds were pre-soaked in double distilled water for 12 hours. The pre-soaked seeds were treated with 0.02, 0.03, 0.04 and 0.05 M EMS freshly prepared solution for 6 hours.

After the EMS treatment, the treated seeds were washed thoroughly in running tap water for 4 h to terminate the residual effect of the mutagenic chemicals. After the completion of the treatment all treated seeds were sown immediately in the field along with their respective controls to obtain the M₁ generation. Mutagenic effectiveness is defined as a measure of frequency of mutation induced by a unit of mutagen, while mutagenic efficiency gives an idea of the proportion of mutation in relation to deleterious effects like lethality, injury and sterility. The mutagenic efficiency and effectiveness were measure by the formula suggested by Konzak *et al.*, (1965). The chlorophyll and macromutations were also scored treatment wise to study the mutagenic effectiveness and efficiency of each treatment. Observation on biological abnormalities such as injury and lethality in M₁ generation and chlorophyll mutation frequencies in M₂ generation were recorded which was used to determine effectiveness and efficiency of both mutagen.

$$\text{Mutation effectiveness} = \frac{\text{Rate of mutation (Mp)}}{\text{Dose of gray (Gy)}} \times 100$$

$$\text{Mutation effectiveness (EMS)} = \frac{\text{Rate of mutation (Mp)}}{\text{Concentration} \times \text{Duration of treatment}} \times 100$$

$$\text{Mutation efficiency} = \frac{\text{Rate of mutation (Mp)}}{\text{Biological damage of M1 generation}} \times 100$$

Biological damage: For measuring the biological damage, three different criteria were used;

Injury - *i.e.*, percentage of reduction in seedling height (Mp/I)

Sterility - *i.e.*, percentage of reduction in pollen fertility (Mp/S)

Meiotic abnormalities - *i.e.*, percentage of meiotic abnormalities (Mp/Me)

Chlorophyll mutation

All the treated populations were screened carefully for the frequency and spectrum of chlorophyll mutations. Lethal chlorophyll mutations were scored within 10 day old seedlings, whereas viable chlorophyll mutations were scored throughout the life cycle of plants. They were identified and classified according to Gustafsson (1940). Chlorophyll mutation frequency in term of number of mutants per 1000 M₂ plants were calculated.

$$\text{Mutation frequency (\%)} = \frac{\text{Total number of mutant seedlings}}{\text{Total number of M}_2 \text{ seedlings}} \times 100$$

Results and Discussion

Chlorophyll mutant

Macromutations described the genetic effects of various mutagens. Chlorophyll mutations are employed as markers for the evaluation of

gene action of mutagenic factors in inducing mutation and the appearance of more number of viridis type mutations could be attributed to the involvement of polygenes in chlorophyll formation (Gaul, 1964). Chlorophyll mutations provide most dependable indices for the evaluation of genetic effects of mutagenic treatments in various crops (Gautam *et al.*, 1992). The frequency of chlorophyll mutants in M₂ generation mainly indicates dependable measure of genetic effects in mutagens (Nilan and Konzak, 1961).

In this study, the highest mutation frequency 3.17% was observed in 0.02 M EMS followed by 2.42% in 0.03 M EMS. However, the lower dose of EMS was found to be more effective than gamma rays treatments (Table 1). Higher frequencies of chlorophyll mutation with lower doses of mutagen were reported in different crops by Yamaguchi *et al.*, (2009) and Pawar *et al.*, (2010). The decrease in chlorophyll mutation frequency at the highest doses of mutagen may be attributed to saturation in the mutational events which result in the elimination of the mutant cells during growth. The decrease in chlorophyll mutation frequency as observed at the higher doses of mutagens may be attributed to saturation in the mutational events which may result in the elimination of the mutant cells during growth. Swaminathan (1969) explained that the high frequency of chlorophyll mutations in EMS treatment is perhaps due to preferential action of EMS on genes for chlorophyll development located near the centromeres.

Viable mutant

In the present study, the highest frequency was noted in EMS treated population (3.17%) and the lowest with gamma rays treatments (1.31%). The spectrum of mutations induced by EMS was comparatively wider than that of gamma rays (Table 2).

Table.1 Frequency and spectrum of chlorophyll mutation

Treatments	Number of M2 seedling examined	Albina	Chlorina	Xantha	Viridis	Total chlorophyll mutants	Mutation frequency (%)
Gamma rays							
2 kR	775	8	3	2	3	16	2.06
5 kR	726	9	5	2	1	17	2.34
10 kR	650	5	2	2	2	11	1.69
15 kR	629	6	2	-	1	9	1.43
20 kR	591	3	3	1	0	7	1.18
EMS							
0.02 M	819	14	6	3	3	26	3.17
0.03 M	783	9	5	4	1	19	2.42
0.04 M	678	7	2	1	2	12	1.76
0.05 M	612	8	3	0	1	12	1.96
Control	893	-	-	-	-	-	-

Table.2 Frequency of viable mutants in M₂ generation

Treatments	Number of M2 Seedlings Examined	Showing Viable Mutants	Mutation Frequency (%)
Gamma rays			
2 kR	152	2	1.31
5 kR	144	4	2.78
10 kR	128	2	1.56
15 kR	102	4	3.92
20 kR	92	2	2.17
EMS			
0.02 M	146	6	4.1
0.03 M	130	4	3.07
0.04 M	110	2	1.81
0.05 M	98	2	2.04
Control	188	-	-

Table.3 Mutagenic effectiveness and efficiency based on viable mutants

Treatments	% Survival reduction at 30 DAT (lethality)	Height reduction injury % (I)	Mutation Frequency (M)	Effectiveness M × 100/ kR(or) C × T	Efficiency M/L× 100	Efficiency M/I × 100
Gamma rays						
2 kR	13.21	5.99	1.31	6.55	9.91	21.87
5 kR	18.7	18.54	2.78	5.55	14.87	14.99
10 kR	27.21	21.51	1.56	1.56	5.73	7.25
15 kR	29.56	28.87	3.92	2.61	13.26	13.58
20 kR	33.82	39.01	2.17	1.09	6.42	5.56
EMS						
0.02 M	8.29	12.5	2.73	4.55	32.94	21.84
0.03 M	12.32	30.05	3.07	3.41	24.92	10.22
0.04 M	24.08	47.83	1.81	1.51	7.52	3.78
0.05 M	31.47	48.73	2.04	4.08	19.45	12.56

Reduction in internode length was mainly responsible for dwarfness. The mutant plants which exhibited prostrate growth habit had long internodes and weak stem which are in agreement with the findings in *Vigna radiata* (Wani *et al.*, 2011).

Mutagenic effectiveness and efficiency

Mutagenic effectiveness is a measure of the frequency of mutation induced by a unit dose of mutagen while mutagenic efficiency represents the proportion of mutation in relation to the associated undesirable biological effects, such as chromosomal aberration, lethality and sterility induced by mutagen in question (Konzak *et al.*, 1965). The effectiveness decreased with increasing dose or concentration. Increasing doses of EMS or Gamma rays decreased the values obtained for all the biological criteria for M1 generation. The reduction in biological criteria may be attributed to a drop in the auxin level (Gordon & Webber, 1955) and inhibition of auxin synthesis (Skoog, 1935). EMS was found to be more effective than

gamma rays and combined treatments. Data on effectiveness and efficiency of various mutagenic treatments calculated on the basis of the frequency of chlorophyll mutations and biological damage are presented in Table 3. It was found that effectiveness and efficiency were higher at the lower doses of gamma rays and EMS. The effectiveness of EMS treatments ranged from 1.09 to 4.55. Results on mutagenic efficiency, calculated on the basis of percentage of survival reduction over control and seedling height reduction, showed that 2 kR and 0.02 M EMS were the most efficient treatments.

Similar results were reported by several authors in cowpea (Dhanavel *et al.*, 2008), in lentil (Satpute 2009), in paprika (Kumar *et al.*, 2012). In the present investigation lower concentration of EMS and gamma rays showed higher effectiveness values. In other words, the effectiveness of the mutagens decreased with increase in concentration of mutagens. Chemical mutagen (EMS) was found to be more effective than physical mutagen (gamma rays). It was also found that

the lower concentration of both chemical and physical mutagens was the most effective. Sharma *et al.*, (2005) also reported a higher mutagenic effectiveness at lower concentration or dose of EMS and gamma rays in urdbean. The decrease in effectiveness with increasing concentrations/doses of mutagen has been reported by Badere and Choudhary (2007) in linseed, Dhanavel *et al.*, (2008) and Girija and Dhanvel (2009) in cowpea, Satpute (2009) in lentil and Barshile *et al.*, (2006) in chickpea.

Results obtained during the present investigation revealed that highest mutation frequency and highest mutagenic efficiency of the mutagens were observed at lower dose of EMS (0.02 M) and gamma rays (2 kR) treatments. EMS was highly effective in enhancing the frequency of morphological mutants than gamma rays.

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