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Effect of induced chlorophyll mutation, mutagenic efficiency and effectiveness of gamma rays and EMS in paprika (*Capsicum annuum* L.) cv. Bydagi Kaddi

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ABSTRACT

The mutagenic efficiency and effectiveness of gamma-rays (10, 15 and 20 kR) and ethyl methane sulfonate (EMS) (0.05, 0.1 and 0.2%) along with control were studied in two varieties of Paprika cv. Bydagi Kaddi based on M_1 biological damages (lethality injury) and M_2 viable mutagen frequency. Further mutagenic parameters like chlorophyll and total mutation frequency were also assessed in M_2. The results indicated variable response of the variety to gamma rays and EMS. EMS has been found more effective; while, gamma irradiations were found efficient for inducing viable mutation.

Key words: Paprika, gamma-rays, EMS, mutagenic efficiency, effectiveness.

INTRODUCTION

Paprika (*Capsicum annuum* var. *longum*), the Hungarian world of plants in the genus *Capsicum* belongs to the family Solanaceae. Paprika is valued for most of its high natural colour and mildness of specific flavour. It has its origin from the Western hemisphere of the world. The fruits that are of deep red in their colour without pungency in the dried form are known as 'Paprika'. Capsicum in its fresh state is very rich in 'vitamin C' (ascorbic acid), as was shown by Szent Gyorgyi, the Hungarian scientist, who was awarded later the Nobel prize in 1937 for isolating 'vitamin C' from paprika fruits.

In recent days, the trade and use of paprika in powder form is increasing rapidly. This powder is mainly used for adding attractive natural colour to the finished products and to make the products more acceptable by the consumers. Besides colouring it is also used for flavouring and garnishing of eggs, cheese, meat dishes, sea foods and salads etc. Paprika is one of the important natural colourants next to turmeric colour extract (Anon, 1). Paprika contains remarkable quantity of the colouring material and is used as colourant in processed foods as they get the preference over synthetic products in the food colourant market. The commercial importance of paprika both as a spice and a vegetable with large scale cultivation in both tropical and sub-tropical regions are increasing at an impressive rate.

Synthetic colour and flavouring substances hitherto added in various food and cosmetic preparations are reported to be carcinogenic and therefore banned in many developed countries. This has resulted in huge demand for chilli and paprika oleoresin with high colourant and mild pungency (Tickoo and Chandra. 14). The world trade in paprika type oleoresin is showing an increasing trend in recent past years. This crop requires vast genetic diversity and variation for substantial improvement in yield and quality. However, research made so far and information on genetic improvement of this crop have been very scanty. Through induced mutagenesis, varieties resistant to pests and diseases, increased capsanthin content and high yielding potential can be obtained. It has been proved beyond doubt that the induced mutations can increase the yield as well as improve other polygenic characters in crop plants.

MATERIALS AND METHODS

The present investigation of induced mutation breeding in paprika (*Capsicum annuum* L.) was undertaken in the University Orchard of the Horticultural College and Research Institute, TNAU, Coimbatore during 2006-2009. The gamma treatments were given by using the 1000 curie ^{60}Co Gamma cell 900, located at the Centre for Plant Breeding and Genetics (CPBG), Tamil Nadu Agricultural University, Coimbatore, where cobalt- 60 serves as source of gamma rays. Ethyl methane sulphonate ($CH_3 SO_2 OC_2H_5$) with a molecular weight of 124.16 g and specific gravity of 1.20 from the Sigma Chemical Company, USA was used for treating the seeds.

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

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Table 1. Frequency of chlorophyll mutants in M_2 generation of paprika cultivar Bydagi Kaddi.

Treatment	No. of M_2 plants		Mutation frequency
	Examined	Showing chlorophyll mutants	
Gamma rays			
Control	190	-	-
10 kR	182	4	2.19
15 kR	146	8	5.47
20 kR	131	6	4.58
EMS			
Control	174	-	-
0.05%	128	5	3.90
0.1%	187	7	3.74
0.2%	143	9	6.29
Combinations (Gamma rays + EMS)			
Control	178	-	-
15 kR + 0.1%	163	4	2.45
20 kR + 0.2%	153	8	5.22

Table 2. Spectrum of chlorophyll mutants in M_2 generation of paprika cultivar Bydagi Kaddi.

Treatment	Total No. of mutants	Spectrum of chlorophyll mutants			
		Albina	Xantha	Chlorina	Viridis
Gamma rays					
10 kR	4	4.00	16.00	22.00	2.00
15 kR	8	-	13.00	25.00	-
20 kR	6	-	19.00	21.00	2.00
EMS					
0.05%	5	-	-	46.00	-
0.1%	7	-	11.00	23.00	3.00
0.2%	9	-	14.00	24.00	4.00
Combinations (Gamma rays + EMS)					
15 kR + 0.1%	15	-	8.00	27.00	-
20 kR + 0.2%	9	-	12.00	23.00	-

like lethality, injury and sterility. The chlorophyll and macromutations were also scored treatment wise to study the mutagenic effectiveness and efficiency of each treatment. Data on biological abnormalities such as injury and lethality in M_1 generation and chlorophyll mutation frequencies in M_2 generation were used to determine the mutagenic efficiency and effectiveness according to the formula suggested by Konzak *et al*. (6).

RESULTS AND DISCUSSION

To estimate the chlorophyll mutation, periodical scoring was carried out, starting from 5th to 15th day after sowing of the treated seed. The chlorophyll mutation frequency was estimated as percentage of plants that segregated for chlorophyll deficiency on the basis of M_2 seedling population. In the present study, the paprika cultivar Bydagi Kaddi showed lower frequency of chlorophyll mutation at 20 kR. Similarly in EMS treatment, lower frequency of chlorophyll mutation was also noticed at 0.05% EMS in Bydagi Kaddi (Table 1). The present findings are in agreement with those of Sethupathi Ramalingam (12), Pamidi Venkateswarlu (8), and Rao *et al*. (9) in chilli; and Thamaraiselvan (13) in tomato. The frequencies of occurrence of chlorophyll deficient seedlings in

Table 3. Frequency of viable mutants in M_2 generation of paprika cultivar Bydagi Kaddi.

Treatment	No. of M_2 seedlings		Mutation frequency
	Examined	Showing viable mutants	
Gamma rays			
Control	190	-	-
10 kR	182	13	7.14
15 kR	146	10	6.84
20 kR	131	6	4.58
EMS			
Control	174	-	-
0.05%	128	10	7.81
0.1%	187	8	4.27
0.2%	143	7	4.19
Combinations (Gamma rays + EMS)			
Control	178	-	-
15 kR + 0.1%	163	16	9.81
20 kR + 0.2%	153	9	5.88

Table 4. Total mutation frequency in M_2 generation of paprika cultivar Bydagi Kaddi.

Treatment	Chlorophyll mutants	Viable mutants	Non-viable mutants	Total frequency	Interaction coefficient (k)
Gamma rays					
Control	-	-	-		-
10 kR	2.19	7.14	-	9.33	-
15 kR	5.47	6.84	0.24	12.55	-
20 kR	4.58	4.58	0.31	9.47	-
EMS					
Control	-	-	-		-
0.05%	3.90	7.81	-	11.71	-
0.1%	3.74	4.27	0.21	8.22	-
0.2%	6.29	4.19	0.06	10.54	-
Combinations (Gamma rays + EMS)					
Control	-	-	-	-	-
15 kR + 0.1%	2.45	9.81	0.33	12.59	0.60
20 kR + 0.2%	5.22	5.88	0.37	11.47	0.55

the M_2 are chiefly used as a dependable measure of genetic effects of mutagens. The frequency of chlorophyll mutation in M_2 generation has been suggested as the most reliable index of mutation rate because of greater accuracy of scoring (Gustaffson, 5; Gaul, 4).

The spectrum of induced mutants obtained in the present study differed between gamma rays and ethyl methane sulphonate (Table 2). In 15 kR gamma irradiated mutant, 0.05% EMS and lower doses of combination were highly effective in producing wide spectrum of chlorophyll mutants. EMS was found to induce more of chlorina types in paprika. Swaminathan (12) explained that a high frequency of particular type of chlorophyll mutation may be due to preferential action of EMS. The chlorophyll mutant such as xantha, chlorina and viridis were also reported by Pamidi Venkateswarlu (8) in chillies, Vedamuthu (15) in coriander, and Malarkodi (7) in blackgram. The gamma rays, EMS and combined treatments

Table 5. Mutagenic effectiveness and efficiency based on chlorophyll mutants and viable mutants of paprika cultivar Bydagi Kaddi.

Treatment	% survival reduction at 30 days (lethality)	% height reduction at 30 days (injury)	Mutation (M) per 100 M_2 seedlings	Effectiveness $\frac{M \times 100}{Cxt \text{ (or) } kR}$	Mutagenic efficiency $\frac{M \times 100}{L}$	$\frac{M \times 100}{I}$
Chlorophyll mutants						
Gamma rays						
10 kR	8.98	15.74	2.19	21.9	24.39	13.91
15 kR	14.93	18.24	5.47	36.46	36.64	29.99
20 kR	15.54	23.48	4.58	22.9	29.47	19.51
EMS						
0.05%	6.77	17.32	3.90	13.00	57.61	22.52
0.1%	10.07	23.01	3.74	6.23	37.14	16.25
0.2%	17.25	30.14	6.29	5.24	36.46	20.87
Combinations (Gamma rays + EMS)						
15 kR + 0.1%	10.54	13.77	2.45	-	23.24	17.79
20 kR + 0.2%	18.80	17.91	5.22	-	27.77	29.15
Viable mutants						
Gamma rays						
10 kR	8.98	15.74	7.14	71.40	79.50	45.3
15 kR	14.93	18.24	6.84	45.60	45.80	37.5
20 kR	15.54	23.48	4.58	22.90	29.40	19.5
EMS						
0.05%	6.77	17.32	7.81	26.03	115.36	45.09
0.1%	10.07	23.01	4.27	7.11	42.40	18.55
0.2%	17.25	30.14	4.19	3.49	24.20	13.90
Combinations (Gamma rays + EMS)						
15 kR + 0.1%	10.54	13.77	9.81	-	93.00	71.24
20 kR + 0.2%	18.80	17.91	5.88	-	31.20	32.83

were found equally potent in inducing chlorophyll mutation as reported by Augustine *et al*. (2) and Pamidi Venkateswarlu (8) in chillies. Thus, it is clearly evident that number and type of chlorophyll mutations depend not only on type of mutagens but also on the cultivar used.

The highest frequency of mutants was recorded in 15 kR + 0.1% combination treatment followed by 0.05% EMS and 10 kR treatments (Table 3). The viable macro mutations with altering plant stature, leaf morphology, duration, fruit mutants and colour mutants were observed. Among these, fruit mutants was more frequently occurring viable macro mutants and mostly conspicuous for induced changes in one of the characters and also showed simultaneous alterations in other component characters as well, but to a lesser extent.

Frequency of non-viable mutants was lower when expressed on M_2 seedlings basis. In case of gamma rays, higher doses produced more non-viable mutants whereas EMS at higher concentration produced lesser number of non viable mutants. In respect of combination treatments, occurrence of non-viable mutants increased with the increase in dosage of gamma rays (Table 4). Similar observations have already been made in tomato (Thamaraiselvan, 13) and coriander (Dhanalakshmi, 3).

Among the gamma treated population of Bydagi Kaddi the most effective dose based on injury was 15 kR treatment. Among EMS treated population, the most effective and efficient dose was 0.05%. A slight decline was however observed at the highest concentration of gamma and EMS treatments. It seems that strong mutagens reach their saturation point even at a lower dose in the cultivars having highly mutable allelic sites, and any further increase in the mutagen dose add to their mutation frequency. It has also been suggested that with increase in the mutation dose beyond a certain point, the strong mutagens become more toxic in nature than higher doses of relatively weak mutagens. Similar observations have been made in fenugreek seed spice by Sahba Parveen *et al*. (11).

While considering the lethality and injury the highest efficiency was observed at 15 kR treatment. Whereas in EMS, the highest efficiency was recorded at 0.05% EMS. (Table 5). The efficiency was found greater at lower concentration of mutagens and the reasons relating to the fact that lethality increased with the mutagen level at much faster rate. So the lower concentration of mutagens causes relatively less damage enabling the organisms to manifest the induced mutations more frequently (Reddy *et al*., 10; Thamaraiselvan, 13) in tomato.

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