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EFFECT OF PHYSICAL AND CHEMICAL MUTAGENS INDIVIDUAL AND IN COMBINATION IN M1 GENERATION OF COWPEA (*VIGNA UNGUICULATA* L.)

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Abstract

Effect of Gamma rays, Ethyl Methane Sulphonate (EMS) and their combination treatments on seed germination, reduction in seedling height, pollen sterility, ovule sterility and plant survival till maturity were studied in the M_1 generation of two cowpea (*Vigna unguiculata L.*) cultivars. *viz.*, Kashi Kanchan and Kashi Unnati. Different doses / concentrations of Gamma rays (10 kR, 20 kR, 30 kR, 40 kR and 50 kR), EMS (0.01 M, 0.02 M, 0.03 M, 0.04 M and 0.05 M) and their combination (10 kR +0.02 M, 20 kR+0.02 M, 30 kR + 0.02 M, 40 kR + 0.02 M and 50 kR + 0.02 M) were used to analyze their effect on above mentioned traits in M_1 generation of both the genotypes. Data collected from M_1 generation showed minimum germination percentage and maximum reduction in seedling height were observed at higher doses of combined treatments in both the cultivars. Similarly, highest pollen and ovule sterility as well as lowest plant survival were also recorded at maximum combined dose of mutagens as compared to the other treatments in both the varieties. Deleterious effects were more pronounced in higher doses, indicating almost a linear relationship.

Key words: Mutagens, Gamma rays, Ethyl methane sulphonate (EMS), Cowpea, Kashi Kanchan and Kashi Unnati.

Introduction

Cowpea (*Vigna unguiculata* L.) is an essential food legume and an important component of cropping systems in the drier regions of the tropics covering parts of Asia and Oceania, the Middle East, Southern Europe, Africa, southern USA, and Central and South America (Singh *et al*., 2002). The seeds are a major source of dietary protein in most developing countries. Cowpea seeds are a major source of plant proteins and vitamins for man, feed for animals, and also a source of cash income. The young leaves and immature pods are eaten as vegetables. In India, cowpea is developed in a territory of 3.9 million hectares with a generation of 2.21 million tons. It is developed in the semi-parched areas of Rajasthan, Gujarat, Karnataka, Tamil Nadu and Maharashtra for the most part as grain legume. The mature cowpea seed contains 24.8 percent protein, 63.6 percent carbohydrate, 1.9 percent fat, 6.3 percent fibre, 0.00074 percent thiamine, 0.00042 percent Riboflavin and 0.00281 percent Niacin. In order to improve yield and other polygenic characters, mutation breeding should be effectively utilized (Deepa lakshmi and Ananda kumar, 2004). Mutation induction has become an established tool in plant breeding to supplement existing germplasm and improve cultivars in certain specific traits (Kurobane *et al*., 1979). Induced mutations represent the same kind of changes that occur from natural causes Govindan, (2000). Mutagenesis has been widely used as a potent method of enhancing variability for crop improvement (Singh and Singh, 2001). Induced mutation, using physical and chemical mutagen, is a way to generate genetic variation, resulting in the creation of new varieties with better characteristic (Wongpiyasatid, 2000).

Physical mutagens incorporate different sorts of radiation, *viz*., "X-rays, gamma rays, alpha particles, beta particles, quick and warm (slow) neutrons and bright rays. Cobalt-60 is commonly utilized for the generation of Gamma rays. Gamma rays cause chromosomal and quality mutations like X-rays." Gamma rays are the most energetic form of electromagnetic radiation; their energy level is from ten to several hundred kilo electron volts and they are considered as the most penetrating compared to other radiations (Kovacs *et al.*, 2002). The chemical mutagens can be divided into four groups: Alkylating Agents like Ethyl Methane Sulphonate (EMS), Base Analogues, Acridine Dyes, and Nitrous Acid and Hydroxylamine. They induce chemical modification of nucleotides by mispairing, base pair substitutions, small deletion and insertion in genomes. EMS induce a high rate of mutations in both micro and higher organisms (Freese, 1963) and sometimes the mutation frequencies exceed those obtained by radiation (Goud, 1967).

Materials and Methods

Dry, healthy and uniform sized seeds of cowpea cultivars *viz*., Kashi Kanchan and Kashi Unnati were treated with gamma rays at 10, 20, 30, 40 and 50 kR doses, ethyl methane sulphonate at 0.01, 0.02, 0.03, 0.04 and 0.05 M concentrations and combination treatment at 10kR +0.02M, 20kR+0.02M, 30kR + 0.02M, 40kR + 0.02M and 50kR + 0.02M. One ninety five (195) seeds in each set were presoaked for 6 h., in water initially. Then, the seeds were immersed for 6 h., in the requisite concentration of mutagen ethyl methane sulphonate. The treated seeds were washed thoroughly in running tap water to eliminate the residual effect of chemical. The gamma irradiated seed of both varieties with five different doses (10kR, 20kR, 30kR, 40kR and 50 kR) were pre-soaked in distilled water for 6 hrs and then treated with 0.02 M EMS for 6 hrs.

The treated seeds of gamma rays, ethyl methane sulphonate, combination treatments and control seeds were immediately sown in the field in a randomized block design (RBD) with three replications at proper spacing between row to row and plant to plant respectively. Remaining 15 seeds of each treatments including control were raised on moist blotter paper in the laboratory for observing data on seed germination, seedlings height reduction besides observation of pollen sterility, ovule sterility and plant Survival till Maturity in the field in M_1 generation.

Results and Discussion

In present investigation, combined mutagenic

Table 1: Effect of different doses / concentrations of Gamma rays and Ethyl Methane Sulfonate (EMS), individual and in combination treatments on growth characters of Kashi Kanchan and Kashi Unnati in M1 Generation.

Treatments	Germination Percentage		Seedling height reduction as percent of control		Pollen s Sterility (%)		Ovule Sterility (%)		Plant survival as percent of control	
	Kashi Kanchan	Kashi Unnati	Kashi Kanchan	Kashi Unnati	Kashi Kanchan	Kashi Unnati	Kashi Kanchan	Kashi Unnati	Kashi Kanchan	Kashi Unnati
Control	95.00	94.00	0.00	0.00	0.00	0.00	0.00	0.00	100.0	100.0
10 kR Gamma rays	91.33	90.67	6.25	2.39	8.20	10.6	3.5	4.6	93.69	90.62
20 kR Gamma rays	89.00	88.67	8.68	5.16	17.6	21.4	9.3	10.4	88.05	86.35
30 kR Gamma rays	85.33	85.00	13.19	13.63	25.6	32.6	18.6	21.3	79.64	80.57
40 kR Gamma rays	81.00	80.67	16.67	24.13	32.8	40.6	24.9	27.9	61.56	73.17
50 kR Gamma rays	77.33	75.00	23.78	27.07	39.4	49.5	28.4	35.6	48.44	66.81
0.01 M EMS	93.00	92.00	3.13	3.68	5.8	9.8	9.3	3.9	95.68	96.46
0.02 M EMS	89.67	86.67	5.90	6.26	16.6	20.6	16.8	12.3	76.39	88.24
0.03 M EMS	79.33	75.00	10.07	20.07	24.4	36.4	21.0	24.8	67.46	80.05
0.04 M EMS	76.67	72.00	13.89	26.89	28.2	43.7	26.4	33.9	62.67	70.79
0.05 M EMS	72.00	70.33	20.49	32.78	33.0	46.3	30.9	40.6	59.17	63.44
10 kR + 0.02 M (Gamma rays + EMS)	91.00	90.00	4.69	4.60	19.7	22.4	10.2	13.2	91.68	84.82
20 kR + 0.02 M (Gamma rays + EMS)	81.33	86.33	6.94	14.55	27.6	27.6	19.5	24.9	83.89	75.57
30 kR + 0.02 M (Gamma rays + EMS)	78.00	76.00	11.11	22.65	36.3	40.2	24.3	30.6	73.36	57.62
40 kR + 0.02 M (Gamma rays + EMS)	72.67	74.00	17.53	25.78	42.6	47.7	27.9	38.4	60.43	52.98
50 kR + 0.02 M (Gamma rays + EMS)	66.00	60.67	23.96	34.44	49.5	51.4	32.4	45.6	57.72	50.81

treatment (gamma rays + EMS) had more drastic effect on percent seed germination in both the varieties than gamma rays and EMS treatments as compared to control (Table 1). This clearly shows that the mutagens have exerted an inhibitory effect on physiological and biological processes necessary for seed germination; they include enzyme activity on seed germination. Progressive decrease in seed germination by the mutagenic treatments has also been reported earlier by Uma and Salimath, (2001); Gaur *et al.,* (2003); Nawale *et al.,* (2006) in cowpea. Among the all mutagenic treatments, combination treatment, 50 kR + 0.02 M EMS caused the most adverse effects. Similar reduction was also obtained by Kumar *et al.,* (2010) in cowpea, Ramya *et al.,* (2014) in black gram, Kamble and Patil, (2014) in chickpea who observed that the combination of physical and chemical mutagens was drastic, in their effect. Point mutations, enzyme inhibitions and chromosomal aberrations occur due to EMS and gamma rays.

In the present study, the seedling height reduction as percent of control was increased with an increase in dose or concentration of mutagenic treatments. In both the genotypes the maximum reduction was observed in combination treatments than individual treatments. Similar increase in seedling injury with increased concentrations of mutagens has been reported by Khan, (1990) in *Vigna mungo*, Khan *et al.*, (2006) in mungbean, Larik *et al.*, (2009) in Sorghum, in cowpea. An inverse relationship between the doses / concentrations of mutagen and plant survival was observed in both the genotypes. Similarly as germination, the plant survival rate was drastically reduced with an increase in dose/concentration of all the mutagens. Mutagens reduced plant survival has also been reported by Blixt *et al.,* 1963 and Dhanavel *et al.*, 2008. Dose dependent reduction in plant survival was observed with gamma rays, EMS and combination of both mutagens, which is in conformity with the results of Nawale *et al.*, (2006); Singh *et al.*, (2013); Ramya *et al.*, (2014); Nawale *et al.*, (2006); Dhanavel *et al.*, (2008). Reduction in plant survival after mutagenic treatments could be attributed to decrease in the assimilation mechanism (Quasteler and Baer, 1950), inhibition of mitosis and chromosomal damage (Guckel and Sparrow, 1961). This might have been due to the effect of mutagens on meristematic tissues of the seed. Morphological variations, especially leaf abnormalities are the indicators of effective mutagen treatment

Pollen sterility increased with increasing doses of gamma rays, EMS and combined treatments. These results are supported with those of the earlier researchers like Singh and Singh, (2014); Kumar and Verma, (2010) and Kumar *et al.,* (2010) who concluded that the combined treatment of gamma rays and EMS proved to be more effective in inducing abnormalities and pollen sterility as compared to individual treatment, on seeds of cowpea (*Vigna unguiculata* (L.) Walp.) in variety K 5269. Among the mutagenic treatments (50 kR gamma+ 0.02M EMS) produced maximum pollen sterility followed by gamma rays and EMS. Pollen sterility and ovule sterility increased with increasing dose / concentration of Gamma rays Ethyl methane sulphonate. The findings were in conformity to there by Lambat *et al.*, (2012) in Chilli. According to Konzak *et al.*, (1961), Sparrow and Woodweel, (1962) and Sudhakaran, (1971) induction of the pollen sterility is due to chromosomal irregularities introduced by the mutagen. Siddiq and Swaminathan, (1969) concluded that chromosomal aberrations particularly high frequency of translocations was responsible for high sterility. In M1 generation, it was expected, as the chemical compounds are known to cause high pollen sterility (Blixt *et al.,* 1963; Ramya *et al.*, 2014). The dose of mutagen and ovule sterility showed inverse relationship with increasing doses of mutagens. The maximum ovule sterility was observed at highest dose or concentration of the mutagens. The findings were in conformity to there by Vanniarajan *et al*., (1993); Ahmed john, (1996) and Sagade and Apparao, (2012) in blackgram; Singh and Singh, (2007) in green gram.

Conclusion

All the growth traits were affected by different doses / concentrations of gamma rays and ethyl methane sulphonate either singly or in combination. Minimum germination percentage and maximum reduction in seedling height were observed at higher doses of combined treatments (50 kR + 0.02 M EMS) in both the cultivars. Similarly, highest pollen and ovule sterility as well as lowest plant survival were also recorded at a maximum combined dose of 50 kR + 0.02 M EMS as compared to the other treatments in both the varieties. Gamma rays belong to ionizing radiation and interact with atoms or molecules to produce free radicals in cells. These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the irradiation level. Chemical mutagens usually cause point mutation, but the loss of a chromosome segment or deletion can also occur.

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