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INDUCED MUTATIONS IN HORSEGRAM (*Macrotyloma uniflorum* (LAM.)VERDC): CHLOROPHYLL MUTATIONS, MUTAGENIC EFFICIENCY AND EFFECTIVENESS

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ABSTRACT

Induction of mutation played a vital role in the crop improvement among modern methods of plant breeding. Genetic improvement through induced mutation was found to be very effective for variability in the quantitative and qualitative characters. With this view gamma radiation (100 to 600Gy), EMS (0.2 to 0.6%) and combinations of these treatments were used to induce variability in horsegram cv. Dapoli Kulthi-1. A wide range of chlorophyll mutations affecting all plant parts were isolated in M_2 generation. The chlorophyll mutants were scored from 7 to 10 days after sowing. The different types of chlorophyll mutants such as albina, xantha, chlorina and viridis were reported due to the all mutagenic treatments. The high frequency of mutations in terms of mutants per 1000 M_2 plant population were observed in low doses of EMS (0.2% and 0.3%), gamma radiation (100Gy) as well as in their combination (100Gy +0.2% and 100Gy + 0.3% EMS). Amongst all the treatments used EMS had induced the highest chlorophyll mutation frequency, followed by gamma radiations and combination treatments. The frequency of chlorophyll mutations in horsegram increased only up to certain dose/ conc. of the mutagens and there after decreased at higher dose/conc. In the present study, effectiveness of mutagenic treatments differed considerably. The mutagenic effectiveness showed a trend which was inversely proportional to the increasing doses/ concentration of mutagens. But in combination treatments it was reverse. Effectiveness and efficiency both are the important parameters to evaluate the usefulness of the mutagens. Effectiveness of the mutagen is the number of mutations produced per unit of the mutagen dose, while efficiency is the ratio of specific desirable mutagenic changes to undesirable effects such as lethality, sterility and seedling injury. The mutation rates were calculated based on the mean values of efficiency for each treatment. It has given an idea about the average rate of mutation induction per mutagen. The mutation rates based on the lethality, pollen sterility, seedling injury and effectiveness were recorded. The treatments of EMS were very effective than GR for inducing the highest mutation with reference to pollen sterility and effectiveness. Gamma radiation gave higher rate of mutations followed by EMS and combination treatments for lethality.

Key words: Horsegram, mutagens, variability, effectiveness, efficiency, mutation rate.

INTRODUCTION

Horsegram (*Macrotyloma uniflorum* (Lam.) Verdc) is an important and very popular rainfed minor pulse crop. It occupies a prominent position in Maharashtra. In Maharashtra, during the year 2010-2011, horsegram was cultivated on 0.466 lakh ha with annual production of 0.3232 lakh tones. The average yield per hactar was 693.56 Kg. Major districts of Maharashtra such as Ahmednagar, Solapur, Sangli, Nasik, Dhule, Jalgaon, Aurangabad, Jalna, Beed, Chandrapur, Gadchiroli, Thane, Ratnagiri, Sindhudurga were cultivate this crop (Annonymous, 2011). It has high protein as well as medicinal value. It posses very high drought tolerance and nitrogen fixing ability. But it is highly susceptible to viral diseases and gives very low seed yield (Bolbhat, 2011 and Kanaka, 2012). The crop is highly neglected and limited reports are available on induced viable morphological mutations in it (Dhumal and Bolbhat, 2012). Induced mutagenesis is useful and effective method in crop improvement. Improvement in either single or few economic traits and quality characters can be achieved with the help of induced mutations within the shortest possible time (Manjaya and Nandanwar, 2007). The present investigation was undertaken to study the role of gamma rays, EMS and their combination in enlarging the genetic variability for quantitative and qualitative characters in M_2 generations in horsegram and to find out the effective and efficient mutagens.

MATERIALS AND METHODS

The authentic seeds of horsegram (*Macrotyloma uniflorum* (Lam.)Verdc) cv. Dapoli Kulthi-1 were obtained from Depatment of Botany, Dr. B.S.K.K.Vidyapeeth, Dapoli, Dist- Ratnagiri, (M.S., India). Dry seeds with moisture content of 10-12 % approx. were irradiated with100, 200, 300, 400,500 and 600Gy of gamma rays from ^{60}Co source at Bhabha Automic Research Center (BARC), Trombay, Mumbai. (MS). Few irradiated and few fresh seeds were soaked in water for 10 hours. Wet seeds were treated with different concentrations of EMS (0.2, 0.3, 0.4, 0.5 and 0.6%) for four hours. Treated seeds washed thoroughly with tap water for two hours and then used for raising M_1 generation. There were total 42 treatment combinations in M_1 generation including control. Untreated dry seeds were used as control. Treated and control seeds were sown in field at a spacing of 30 X 15 cm in randomized block design. 1000 seeds for each treatment were used to raise M_1 generation. Each M_1 plant was harvested individually. Seeds of individual M_1 plants were sown to raise the M_2 generation in a plot to family manner. The M_2 populations were screened for mutations at regular intervals from seedling stage until maturity of the crop.

Chlorophyll mutations

The M_2 populations were screened for frequency and spectrum of chlorophyll mutations per 1000M_2 plants at regular intervals from seedling stage until maturity of crop. The spectrum of chlorophyll mutations was studied and mutants were classified as per the Gustafson (1940) with modifications.

Seedling injury

Seedling height was recorded on 7th day. Reduction in the mean seedling length as compared to the control was regarded as seedling injury and expressed as percentage.

Pollen sterility

Pollen sterility was determined from 20 randomly selected plants per treatment, along with control. The pollen grains from freshly dehisced anthers were stained with 1% aceto-carmine. Pollen grains stained as uniform deep red colour were counted as fertile and others as sterile.

Mutagenic effectiveness and efficiency

Mutagenic effectiveness is a measure of the frequencies of mutations induced by a unit dose/ concentrations of mutagen, which gives an idea of the biological damages such as lethality, seedling injury and pollen sterility. Mutagenic effectiveness and efficiency (rate of mutations to damage or lethality/ injury/ sterility) were determined by using the formula of Konzak et al., (1965).

Mutagenic effectiveness = Mutation frequency (Mf)/ Dose or (Time x Conc.)

= Mf / Gy or Mf / TC

Mutagenic efficiency =Mutation frequency (Mf)/ Biological damage= Mf /L, Mf /I, Mf/S

Where,

L = Percentage of lethality in M_1 generation.

I = Percentage of seedling injury in M_1 generation.

S = Percentage of pollen sterility in M_1 generation.

Mutation rate

Mutation rate gives an idea of mutations induced by a particular mutagen, irrespective of dose/ conc. It was calculated by the following formulae

Mutation rate = Sum of values of effectiveness or efficiency of a particular mutagen/ Number of treatments of that particular mutagen

RESULTS

The M_2 progeny was screened thoroughly and various mutations were scored throughout the crop duration. The mutations affecting different morphological features of the treated plants are described below.

Chlorophyll mutations

The M_2 generation was raised from the seeds of M_1. The seeds of individual plant were collected separately and sown in experimental field in family manner with RBD design. The chlorophyll mutants were scored from 7 to 10 days after sowing. The different types of chlorophyll mutants such as albina, xantha, chlorina and viridis were reported due to the all mutagenic treatments. The frequency and spectrum of different types of chlorophyll mutants were recorded in (Table-1). Amongst all the treatments used EMS had induced the highest chlorophyll mutation frequency, followed by gamma radiations and combination treatments. Dalvi (1990), Prakash and Halaswamy, (2006), Manigopa-Chakraborty et al., (2005) and Dhumal and Bolbhat (2012) have also reported induction of chlorophyll mutation in horsegram with GR, EMS and their combination. The work of Kartika and Subba Lakshmi (2006) in soybean supported the above findings. Kumar et al., (2009) also reported highest range of chlorophyll mutations in mungbean with EMS as compared to GR and combination treatments. Singh et al., (2000) in mungbean and Kumar et al., (2007) in blackgram reported chlorophyll mutants with GR, EMS and their combinations. The frequency of chlorophyll mutations in horsegram increased only up to certain dose/ conc. of the mutagens and there after decreased at higher dose/conc. Barshile et al., (2005) and Auti (2005) supported the above findings.

Table 1

Effect of mutagens on the frequency and spectrum of chlorophyll mutations in M_2 generation of horsegram cv. Dapoli Kulthi-1.

Treat	No. of M_2 plants	Total chl. mutants	Fre. of chl. mutations (%)	Spectrum of chl. mutations (%)			
				Albina	Xantha	Chlorina	Viridis
Control	1795	--	--	--	--	--	--
100Gy	1560	17	1.09	0.19	0.26	0.32	0.26
200	1710	29	1.7	0.64	0.47	0.58	--
300	1675	26	1.55	0.6	0.54	--	0.48
400	1632	25	1.53	0.49	0.37	0.55	0.12
500	1540	22	1.43	0.39	--	0.52	0.45
600	1505	14	0.93	--	0.33	0.27	0.33
Average		22.17	1.37	0.39	0.33	0.37	0.27
0.2 % EMS	1495	19	1.27	0.2	0.47	0.33	0.27
0.3	1460	24	1.64	0.27	0.62	0.21	0.55
0.4	1442	25	1.73	0.62	0.42	0.42	0.28
0.5	1411	20	1.42	0.28	0.43	0.5	0.21
0.6	1365	15	1.1	0.22	0.29	0.44	0.07
Average		20.6	1.43	0.32	0.44	0.38	0.28
100Gy + 0.2%EMS	1579	19	1.2	0.13	0.44	0.38	0.25
100 + 0.3	1553	27	1.72	0.32	0.45	0.52	0.39
100 + 0.4	1530	23	1.5	0.52	0.72	--	0.46

100 + 0.5	1513	24	1.59	0.33	--	0.79	0.46
100 + 0.6	1492	16	1.07	0.07	0.34	0.4	0.27
200 + 0.2	1472	13	0.88	0.34	0.27	0.07	0.2
200 + 0.3	1451	22	1.52	0.28	0.34	0.41	0.48
200 + 0.4	1419	21	1.48	0.14	0.42	0.42	0.49
200 + 0.5	1403	20	1.43	0.29	--	0.64	0.5
200 + 0.6	1367	18	1.32	--	0.15	0.66	0.51
300 + 0.2	1510	15	0.99	0.07	0.33	0.46	0.13
300 + 0.3	1482	19	1.28	0.07	0.2	0.47	0.54
300 + 0.4	1460	20	1.37	0.14	0.41	0.55	0.27
300 + 0.5	1443	16	1.11	0.07	0.14	0.49	0.42
300 + 0.6	1411	14	0.99	0.14	0.43	0.28	0.14
400 + 0.2	1489	11	0.74	0.07	0.13	0.34	0.2
400 + 0.3	1463	17	1.16	0.07	0.55	0.41	0.14
400 + 0.4	1439	16	1.11	0.42	0.49	0.21	--
400 + 0.5	1427	14	0.98	0.07	--	0.49	0.42
400 + 0.6	1409	12	0.85	--	0.57	0.07	0.21
500 + 0.2	1425	9	0.63	0.07	0.21	0.28	0.07
500 + 0.3	1414	14	0.99	--	0.28	0.5	0.21
500 + 0.4	1402	13	0.93	0.14	--	0.43	0.36
500 + 0.5	1378	11	0.8	0.07	0.44	0.29	--
500 + 0.6	1254	8	0.64	0.16	0.16	--	0.32
600 + 0.2	1418	7	0.49	0.07	0.28	0.14	--
600 + 0.3	1306	12	0.92	0.23	0.31	0.38	--
600 + 0.4	1285	10	0.78	0.08	--	0.47	0.23
600 + 0.5	1267	6	0.47	--	0.16	0.24	0.08
600 + 0.6	1241	4	0.32	0.08	0.16	0.08	--
Average		15	1.04	0.15	0.28	0.36	0.26

Types of chlorophyll mutants

Albina

These mutants were characterized by total absence of chlorophyll or carotenoids, emerged 1 to 2 DAS after the normal seedling, possessing very small first pair of leaves and survived for 7 to10 days (Plate-1).

Xantha

The seedlings of this mutant were uniformly yellow coloured with total absence of chlorophyll and they survived for 7 to 8 days (Plate-1).

Chlorina

The seedlings of this mutant were very light green or pale green survived up to maturity, with normal flowering and pod formation, but had arrested growth (Plate-1).

Viridis

These mutants were characterized by light green to yellow leaves which latter changed to normal green and survived up to maturity. These mutants also had normal flowering and pod formation (Plate-1). Reddy and Annadurai (1992) claimed that chlorophyll mutation can be used as an index for evaluating the mutagenic action of different mutagens. Chlorophyll mutations are used as a dependable index for evaluating the genetic effects of mutagens. Chlorophyll mutations have been used for assessing the effectiveness / efficiency of mutagens and predict the size of vital factors of mutations. Mehraj-ud-din et al.,(1999) in *Vigna*, Sharma et al., (2006), Sagade (2008) in urdbean, Ahire (2008), Tambe et al.,(2010) in soybean and Bolbhat (2011) in horsegram reported chlorophyll mutants like albina, xantha, chlorina and viridis (Plate-1).

Frequency and spectrum of chlorophyll mutations

The frequency of total chlorophyll mutants varied for single as well as combination of gamma radiation and EMS. In gamma radiation the average percentage of chlorophyll mutation frequency was 1.37% which was slightly higher than combination treatments (1.04%). The highest chlorophyll mutation frequency was recorded in 200Gy (1.70%). The values of chlorophyll mutation frequency were ranging from 0.93% to

1.70%. Amongst all types of chlorophyll mutations, albina and chlorina types showed higher percentage (0.64 and 0.58%) respectively. The percentage of xantha and viridis was 0.54 and 0.48 respectively. The average number of chlorophyll mutations was 22.17, while the average frequency was 1.37% (Table-1). In the EMS treatments the percentage of total chlorophyll mutation frequency increased up to 0.4% conc. and ranged from 1.10% to1.73%. Amongst all the total chlorophyll mutations, the higher percentage was showed by albina in 0.4%EMS (0.62%), xantha in 0.3%EMS (0.62%), viridis in 0.3%EMS (0.55%) and chlorina in 0.5%EMS (0.50%). The albina and viridis showed very low average percent of total chlorophyll mutation frequency as compared to xantha and chlorina. The average number of chlorophyll mutations due to EMS was 20.6, while average frequency was 1.43%. The combination treatments showed wide range of total percentage of chlorophyll mutations. The range varied from 0.32% to 1.72%. Highest chlorophyll mutation frequency (1.72%) was noted in 100Gy + 0.3%EMS. Albina, xantha, chlorina and viridis were found to be the most abundant type of chlorophyll mutants induced by GR, EMS and combination treatments in horsegram (Table-1). Amongst the mutagens used EMS emerged as most effective mutagen to produce highest frequency of chlorophyll mutations; it was followed by gamma rays and combination treatments. Mehraj-ud-din et al., (1999) reported decrease in mutation frequency at higher dose, as attributed to chromosomal aberrations or saturation in the mutational events. Some workers like Nerkar and Mote (1978) similarly reported that the chlorophyll mutations were independent of the dose of gamma rays. Maximum chlorophyll frequency with EMS was also reported by Kharkwal (1998) in chickpea, Koli and Ramkrishna (2002) in *Trigonella*. Chlorina, xantha, albina and viridis type of chlorophyll mutants were recorded by Vanhirajan et al., (1993) in blackgram and Mehraj-ud-din et al., (1999) in mungbean during M_2 generation. Manjaya et al., (2007) and Tambe et al., (2010) also observed albina, xantha, chlorina and virescent chlorophyll mutations in gamma rays treated soybean. They attributed these genes concerned with the development and expression of chlorophyll pigments. Kumar et al., (2007) in blackgram, Singh et al., (2007) in lentil, Ignacimathu and Babu (1993) in mungbean and urdbean reported that frequency of chlorophyll mutation was very low and fluctuating under different dose/ concs. of mutagens. It is inconformity with the results of present study. Kumari (1996) also reported that spectrum and frequency of chlorophyll mutation was dependant on the dose/ concs. of mutagens.

Mutagenic effectiveness

Mutagenic effectiveness pertaining to the rate of induction of mutation was related to mutagenic dose/ conc. In the present study, effectiveness of mutagenic treatments differed considerably. The mutagenic effectiveness showed a trend which was inversely proportional to the increasing doses/ concentration of mutagens. But in combination treatments the mutagenic effectiveness showed a trend which was directly proportional to the increasing doses/ concentration of mutagens. In M_2 generation the values of effectiveness of gamma radiation were ranging from 0.002 to 0.011 (Table-2). The highest effectiveness was recorded in 100Gy, while the lowest was noted in 600Gy. The values of effectiveness of EMS were in the range of 0.46 to 1.59. The highest effectiveness was recorded in 0.2%EMS, while the lowest in 0.6%EMS. The effectiveness of the combination treatments of gamma radiation and EMS showed highest effectiveness in 100Gy + 0.5 %EMS, while the lowest in 400Gy + 0.2 %EMS and 500Gy + 0.2 %EMS. The trend of effectiveness was: EMS > combination treatments > gamma radiation

Mutagenic efficiency

Results obtained on mutagenic efficiency of various mutagens in the present investigation are presented in Table-2.

Efficiency with reference to lethality

In gamma radiation and EMS treatments the efficiency with reference to lethality showed decreased trend (0.40 to 0.02) and (0.47 to 0.03) respectively. As compared to EMS gamma radiation showed reduction in efficiency (Table-2). The results of combination treatments indicated similar trend. The trend was as follows:

gamma radiation > EMS > combination treatments.

Efficiency with reference to seedling injury

In M_2 generation, the efficiency ranged from -1.83 to 0.05 in gamma radiation. The highest efficiency was recorded at 300 and 400Gy (0.05), while lowest at 200Gy (- 1.83). In EMS treatments the highest efficiency was recorded at 0.3% (0.13) and lowest at 0.2% (- 0.35). The over all range was - 0.35 to 0.13 (Table-2). Amongst all the combination treatments 200Gy + 0.4 %EMS showed highest value (0.04). The trend was as follows: combination treatments > EMS > gamma radiation.

Efficiency with reference to pollen sterility

In M_2 generation, the efficiency ranged from 0.03 to 0.13 in gamma radiation. In EMS the highest efficiency was recorded at 0.3% (0.12) and the range was 0.04 to 0.12. Amongst all the combination treatments 100Gy + 0.3%EMS showed highest value (0.14) (Table-2). The trend was as follows: gamma radiation ≥ EMS > combination treatments Effectiveness and efficiency both are the important parameters to evaluate the usefulness of the mutagens. Effectiveness of the mutagen is the number of mutations produced per unit of the mutagen dose, while efficiency is the ratio of specific desirable mutagenic changes to undesirable effects such as lethality, sterility and seedling injury (Konzak et al., 1965 and Gaul et al., 1970). In present investigation, lower doses/conc. of gamma radiations, EMS, single and in combinations showed higher mutagenic effectiveness and efficiency(Table-2). Similar results were reported by Girija and Dhanavel (2009) in cowpea, Shah et al., (2008) in chickpea, Solanki and Sharma (1994) in lentil. Kumari (1996) had studied mutagenic effectiveness and efficiency of gamma rays, EMS and their combination in *Vicia faba*. Mutagenic effectiveness was in order of EMS > Gamma rays + EMS > Gamma rays + water > Gamma rays. Chemical mutagens were found to be more effective than gamma rays in mungbean (Singh et al., 2000, Dhanavel et al., 2008 and Auti and Apparao, 2009). Our results were also in conformity with the above findings supporting that EMS was highly effective to induce mutations in horsegram through GR and GR + EMS. In the present investigation lower concentrations of EMS showed higher mutagenic effectiveness. Sharma et al., (2005) also reported higher mutagenic effectiveness at lower dose /concentrations of gamma rays and EMS in urdbean. The decrease in effectiveness with increasing concentrations of mutagen had been reported by several authors like Koli and Ramkrishna (2002) in mungbean, Sharma et al., (2006) in urdbean, Barshile et al., (2005) and Shah et al., (2008) in chickpea, Kumar et al., (2007) in blackgram, Dhanavel et al., (2008) in cowpea, Satpute (2009) in lentil and Shirsat et al., (2010) in horsegram. The responses of physical and chemical mutagens are always influenced by number of biological, environmental and chemical factors. According to Blixt (1970), effectiveness of any mutagen depends on its dose or concentration and specificity to act on gene and genetic make-up of the cultivars. The exact mechanism, by which these factors influence mutation frequency, is not known. Mutagenic efficiency is referred to as the frequency of chlorophyll mutations in relation to damage caused in M_1 generation. Both the mutagens exhibited gradual decrease in mutagenic efficiency with the increasing concentration or doses with respect to lethality, seedling injury, and pollen sterility. Similar was the finding of Solanki and Sharma (1994), Mehraj–ud-din et al., (1999), Kavithamni et al., (2008), Koli and Ramkrishna (2002). According to Konzak et al., (1965), higher efficiency at lower concentration of a mutagenic agent is due to the biological damage (like lethality, seedling injury and sterility), which increases with increase in dose at faster rate than the mutations. Sharma et al., (2005) in urdbean reported that the lower doses of mutagens were more efficient than the higher once.

Mutation rate

The mutation rates were calculated based on the mean values of efficiency for each treatment. It has given an idea about the average rate of mutation induction per mutagen. The mutation rates based on the lethality, pollen sterility, seedling injury and effectiveness were recorded. In M_2 generation, on the basis of lethality, seedling injury, pollen sterility and effectiveness, the mutation rate of gamma radiation was 0.15, -0.29, 0.08 and 0.005 respectively. Mutation rate of

EMS was 0.14, - 0.02, 0.08 and 1.042 respectively and in combination treatments the mutation rate was 0.08, 0.02, 0.06 and 0.08 respectively. The trends observed for mutation rate in M_2 generation were as follows,

On the basis of lethality: Gamma radiation > EMS > Combination treatments.

On the basis of seedling injury: Combination treatments > EMS > Gamma radiation.

On the basis of pollen sterility: Gamma radiation ≥ EMS > Combination treatments.

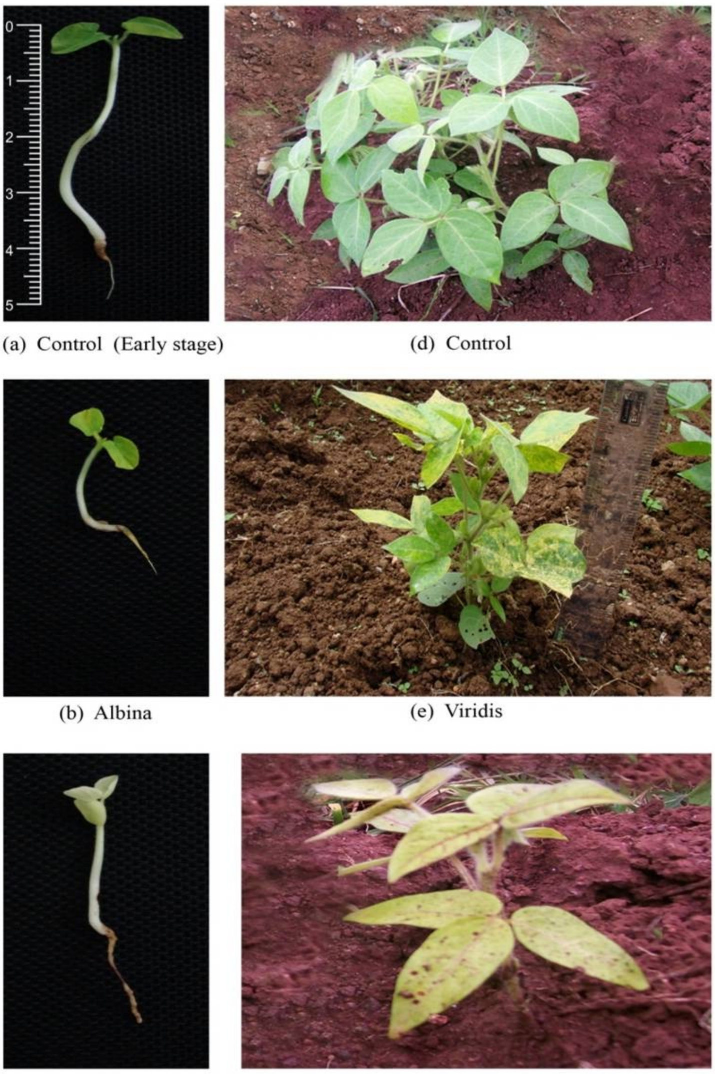
On the basis of effectiveness: EMS > Combination treatments > Gamma radiation.

The treatments of EMS were very effective than GR for inducing the highest mutation with reference to pollen sterility and effectiveness. Gamma radiation gave higher rate of mutations followed by EMS and combination treatments for lethality.

The results have clearly indicated that high mutation rates were obtained with moderate dose of mutagen. To increase the mutation frequency and mutagenic rate specific mutagen, in appropriate concentrations will be effective (Auti, 2005).

Efficient mutagenesis is the production of desirable changes with minimum undesirable effects. In mutation breeding programme, a high mutation rate accompanied by minimal deleterious effects is desirable. But, generally the mutagen dose that gives the highest mutation rate also induces a high degree of lethality, sterility and other undesirable effects (Shirsat et al., 2010).

Plate I: Chlorophyll mutants



(a) Control (Early stage) (d) Control

(b) Albina (e) Viridis

(c) Xantha (f) Chlorina

Table 2

The effectiveness and efficiency of gamma radiation, EMS and their combinations in M_2 generation of horsegram cv. Dapoli-Kulthi-1.

Treatments	Total chl. mutation fre. (%)	Mutagenic effectiveness	Lethality (%)	Efficiency	Seedling injury	Efficiency	Pollen sterility (%)	Efficiency
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	01.91±0.08	0.00±0.00
100Gy	1.09±0.05	0.01±0.00	2.71±0.14	0.40±0.02	-15.93±0.8	-0.07±0.00	10.13±0.51	0.11±0.01
200	1.70±0.12	0.008±0.00	6.48±0.47	0.26±0.02	-0.92±0.06	-1.83±0.13	13.41±0.94	0.13±0.01
300	1.55±0.05	0.005±0.00	12.59±0.38	0.12±0.00	33.64±1.01	0.05±0.00	17.24±0.52	0.09±0.00
400	1.53±0.09	0.004±0.00	19.85±1.19	0.08±0.00	29.80±1.79	0.05±0.00	20.15±1.21	0.08±0.00
500	1.43±0.09	0.003±0.00	35.72±2.14	0.04±0.00	52.99±3.18	0.03±0.00	24.37±1.46	0.06±0.00
600	0.93±0.04	0.002±0.00	43.33±1.73	0.02±0.00	55.90±2.24	0.01±0.00	29.09±1.16	0.03±0.00
Average		0.005		0.15		-0.29		0.08
0.2 % EMS	1.27±0.09	1.589±0.11	2.72±0.19	0.47±0.03	-3.63±0.25	-0.35±0.02	12.16±0.85	0.10±0.01
0.3	1.64±0.05	1.370±0.04	14.33±0.43	0.11±0.00	12.45±0.37	0.13±0.00	14.19±0.43	0.12±0.00
0.4	1.73±0.09	1.084±0.05	24.55±1.23	0.07±0.00	39.12±1.96	0.04±0.00	18.87±0.94	0.09±0.00
0.5	1.42±0.04	0.709±0.02	34.74±1.04	0.04±0.00	43.60±1.31	0.03±0.00	22.59±0.68	0.06±0.00
0.6	1.10±0.07	0.458±0.03	41.33±2.48	0.03±0.00	56.40±3.38	0.02±0.00	27.32±1.64	0.04±0.00
Average		1.042		0.14		-0.02		0.08
100Gy+ 0.2%EMS	1.20±0.08	0.010±0.00	1.28±0.09	0.94±0.07	46.37±3.25	0.03±0.00	11.19±0.78	0.11±0.01
100 + 0.3	1.67±0.07	0.020±0.00	6.67±0.27	0.25±0.01	50.50±2.02	0.03±0.00	12.36±0.49	0.14±0.01
100 + 0.4	1.50±0.08	0.024±0.00	14.85±0.74	0.10±0.01	53.56±2.68	0.03±0.00	14.80±0.74	0.10±0.01
100 + 0.5	1.59±0.11	0.032±0.00	19.84±1.39	0.08±0.01	64.08±4.49	0.02±0.00	16.36±1.15	0.10±0.01
100 + 0.6	1.07±0.03	0.026±0.00	37.33±1.12	0.03±0.00	60.60±1.82	0.02±0.00	18.98±0.57	0.06±0.00
200 + 0.2	0.88±0.04	0.004±0.00	9.33±0.47	0.09±0.00	44.59±2.23	0.02±0.00	12.66±0.63	0.07±0.00
200 + 0.3	1.52±0.09	0.009±0.00	14.67±0.88	0.10±0.01	48.36±2.90	0.03±0.00	13.98±0.84	0.11±0.01
200 + 0.4	1.48±0.06	0.012±0.00	18.67±0.75	0.08±0.00	41.68±1.67	0.04±0.00	15.83±0.63	0.09±0.00
200 + 0.5	1.43±0.04	0.014±0.00	33.33±1.00	0.04±0.00	41.68±1.25	0.03±0.00	18.06±0.54	0.08±0.00
200 + 0.6	1.32±0.09	0.016±0.00	45.33±3.17	0.03±0.00	59.82±4.19	0.02±0.00	20.44±1.43	0.06±0.00
300 + 0.2	0.99±0.06	0.003±0.00	10.69±0.64	0.09±0.01	66.57±3.99	0.01±0.00	15.10±0.91	0.07±0.00
300 + 0.3	1.28±0.06	0.005±0.00	19.59±0.98	0.07±0.00	62.94±3.15	0.02±0.00	15.70±0.78	0.08±0.00
300 + 0.4	1.37±0.05	0.007±0.00	29.33±1.17	0.05±0.00	65.72±2.63	0.02±0.00	17.86±0.71	0.08±0.00
300 + 0.5	1.11±0.08	0.007±0.00	41.33±2.89	0.03±0.00	68.49±4.79	0.02±0.00	19.77±1.38	0.06±0.00
300 + 0.6	0.99±0.05	0.008±0.00	47.48±2.37	0.02±0.00	70.06±3.50	0.01±0.00	22.40±1.12	0.04±0.00
400 + 0.2	0.74±0.03	0.001±0.00	13.86±0.55	0.05±0.00	58.11±2.32	0.01±0.00	16.27±0.65	0.05±0.00
400 + 0.3	1.16±0.07	0.003±0.00	15.72±0.94	0.07±0.00	62.23±3.73	0.02±0.00	17.28±1.04	0.07±0.00
400 + 0.4	1.11±0.03	0.004±0.00	27.35±0.82	0.04±0.00	65.79±1.97	0.02±0.00	19.76±0.59	0.06±0.00
400 + 0.5	0.98±0.05	0.005±0.00	39.55±1.98	0.02±0.00	68.07±3.40	0.01±0.00	21.42±1.07	0.05±0.00
400 + 0.6	0.85±0.03	0.005±0.00	50.67±1.52	0.02±0.00	70.55±2.12	0.01±0.00	23.79±0.71	0.04±0.00
500 + 0.2	0.63±0.04	0.001±0.00	21.33±1.28	0.03±0.00	62.09±3.73	0.01±0.00	18.59±1.12	0.03±0.00
500 + 0.3	0.99±0.07	0.002±0.00	23.89±1.67	0.04±0.00	73.61±5.15	0.01±0.00	19.40±1.36	0.05±0.00
500 + 0.4	0.93±0.04	0.003±0.00	33.33±1.33	0.03±0.00	68.28±2.73	0.01±0.00	21.52±0.86	0.04±0.00
500 + 0.5	0.80±0.04	0.003±0.00	45.33±2.27	0.02±0.00	69.56±3.48	0.01±0.00	23.44±1.17	0.03±0.00
500 + 0.6	0.64±0.03	0.003±0.00	54.67±2.19	0.01±0.00	70.55±2.82	0.01±0.00	26.05±1.04	0.02±0.00
600 + 0.2	0.49±0.02	0.001±0.00	25.86±1.29	0.02±0.00	69.77±3.49	0.01±0.00	21.10±1.06	0.02±0.00
600 + 0.3	0.92±0.06	0.002±0.00	30.67±2.15	0.03±0.00	69.56±4.87	0.01±0.00	21.66±1.52	0.04±0.00
600 + 0.4	0.78±0.02	0.002±0.00	46.67±1.40	0.02±0.00	74.82±2.24	0.01±0.00	23.88±0.72	0.03±0.00
600 + 0.5	0.47±0.03	0.002±0.00	55.48±3.33	0.01±0.00	75.32±4.52	0.01±0.00	26.09±1.57	0.02±0.00
600 + 0.6	0.32±0.02	0.001±0.00	62.67±3.76	0.01±0.00	78.88±4.73	0.00±0.00	28.67±1.72	0.01±0.00
Average		0.008		0.08		0.02		0.06
SEM±	0.05	0.020	1.32	0.01	2.45	0.02	0.82	0.01
F-value	126.03	941.09	317.43	526.95	183.01	623.70	88.95	228.96
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
$LSD_{0.05}$	0.08	0.04	2.59	0.02	4.80	0.04	1.61	0.02

Data are means of three replicates ± standard deviation. Significant difference due to treatments was assessed by Fisher's LSD as a post-hoc test.

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