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Induced Genetic Variability for Quantitative Traits in M_3 Generation of Cowpea by Mutagens

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ABSTRACT

Induced mutation is one of the best alternatives for the improvement of cowpea as it can help to regenerate and restore the variability, which is generally lost in the process of adaptation to various stresses. Genetic variability is the most essential prerequisite for any successful crop improvement programme as it provides spectrum of variants for the effective selection, which can be achieved through the processes of hybridization, recombination, mutation and selection. In the present investigation, variability induced by gamma rays, Ethyl methane sulphonate (EMS) and combined treatments (gamma rays with EMS) for different quantitative traits viz., plant height, days to first flower, number of branches per plant, number of leaves per plant, number of cluster per plant, number of pods per plant, number of seeds per plant and seed yield per plant in M_3 generation of cowpea. Estimate of genetic parameters (genotypic coefficient of variation, heritability and genetic advance) for the yield and its components were higher than the control in M_3 generation. The increased genetic variance in the treated material is a dependable suggestion of the effects of mutagens.

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp) is one of the most important tropical multipurpose legumes used as a protein source for human nutrition in Africa (Fery, 2002). Ionizing radiation has been used in cowpea mutation breeding programs to generate genetic variation for crop improvement by inducing heritable chromosomal changes at specific loci (Ahloowalia and Maluszynski, 2001). Mutations known to occur include altered, missed, or mismatched nucleotide bases, DNA sequence insertions and deletions, linking of pyrimidines, and double-stranded DNA breaks as well as intra strand and inter strand cross linking (Tudeja *et al*., 2001). Mutations in plants are powerful tools, not only for clarifying physiological mechanisms in plants but also for developing new plant varieties in practical breeding programs. Mutation induction is one approach for creating genetic variation in crop plants. The technology of mutation induction has become an established tool in plant breeding in order to supplement existing germplasm and to improve cultivars in specific traits. Improved varieties of many crops have been released to forms as a result of induced mutations which have been used directly as new cultivars (Gottschalk and Wolf, 1983; Micke *et al*., 1987). Plant breeding is concerned with the creation, identification, isolation, multiplication and management of genetic variability towards the development of improved cultivars. Mutation breeding has been identified as a part of plant breeding and also a method for the creation of genetic diversity for further selection and hybridization. Mutation breeding which has attained importance in recent years has yielded large number of desirable cultivars in different crops. Variability is the most important requirement for success in plant breeding programme. The estimation of heritable (genetic) and non-heritable (environmental) variations are of great value in the choice of suitable breeding programme (Amarnath *et al*., 1991). Heritability serves as a guide to the reliability of phenotypic variability in the selection programme and hence determines its success (Hamdi *et al*., 2003). The aim of the present study was to generate information on the magnitude of induced genetic variability, and magnitude of associations between yield and its components following mutagenesis of gamma rays, EMS and combined treatment of gamma rays with EMS.

Materials and Methods

The authentic seeds of cowpea (*Vigna unguiculata* (L).Walp) cultivar CO7 were obtained from Tamilnadu Agriculture University, Coimbatore, Tamilnadu. Gamma rays, ethyl methane sulphonate (EMS) and their combinations were employed in present study for the treatments of seeds of cowpea. Gamma radiation from ^{60}CO source fixed in the gamma cell 200 installed at Sugarcane Breeding Institute (ICAR), Coimbatore, Tamilnadu, India was used in the present work. Healthy, dry and uniform seeds of cowpea were treated with 20, 25 and 30KR. Ethyl methane sulphonate (Sigma chemical Co. Ltd. USA) was used for the seed treatment of cowpea. Various concentrations of EMS (20mM, 25mM and 30mM) were prepared in 0.1M phosphate buffer pH-7.0. Selected seeds were soaked in distilled water for 10 hours and the wet seeds were treated with different concentrations of EMS (such as20mM, 25mM and 30mM) for four hours. For combination treatments the gamma irradiated seeds were treated with different concentrations of EMS. The untreated seeds served as control. The seeds treated with various concentrations of EMS were washed thoroughly with tap water for two hours to terminate the reaction of chemical mutagen and to leach out the residual chemicals. The treated seeds from each treatment were used for raising M_1 generation in field. All the experiments were carried out in triplicate following RBD design. The distance between two rows and two plants was 45 X

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20 cm and the distance between two adjacent plots was one meter. The seeds of individually harvested M_1 plants were sown in the experimental field to raise M_2 generation in separate rows during kharif season. The treated as well as control plants were screened for quantitative traits to study the induced variability. From each replication and treatment including control 20 plants were randomly selected for recording data on different quantitative characters in M_2 generations. Data on eight quantitative traits such as days to first flowering, plant height, number of branches per plant, number of leaves per plant, number of cluster per plant, number of pods per plant, number of seeds per plant and yield per plant were recorded. All the surviving M_2 plants were harvested individually and seeds of single plant from each treatment were kept separately for raising M_3 generation. Observations on quantitative characters in M_3 generation were similar to that of M_2 generation. Data on following eight quantitative traits were recorded.

Days to first flower

The number of days taken from sowing to first flower was recorded and expressed as number of days to first flower.

2. Plant height (cm)

The height of the plant from the base to the top of the plant at maturity was measured and expressed in centimetres at the time of harvesting.

3. Number of branches per plant

Number of branches arising from the main stem were counted and recorded at the maturity.

4. Number of leaves per plant

Total number of leaves at maturity were counted and recorded as the number of leaves per plant.

5. Number of clusters per plant

Total number of clusters at maturity time were counted and recorded as the number of clusters per plant.

6. Number of pods per plant

Total number of pods at maturity time were counted and recorded as the number of pods per plant.

7. Number of seeds per plant

Total number of seeds from individual plant were counted and recorded as the number of seeds per plant.

8. Seed yield per plant

Seed yield was worked out by using digital electronic balance and expressed in gram per plant.

Statistical analysis

The statistical analysis of the data on the individual characters was carried out on the mean values of five random plants. Analysis of variance (Annova for RBD) was to use to analyze yield and its component traits calculated using the software NPRCSTAT, developed in National Pulse Research Center, Vamban, Pudukottai, TN, India. The variance observed among the replication was exclusively non-heritable and hence treated as environmental variance. The variance of M_3 populations was partitioned into heritable and non-heritable components (Mather and Jinks, 1971). Phenotypic and genotypic coefficient of variation (PCV and GCV) was computed using the formula adopted by Burton (1952) and categorized of the range of variation was done as proposed by Sivasubramanian and Madhavamenon (1978). Heritability (h^2) was computed using the formula according to Lush (1940) and it was classified according to Robinson (1966).Genetic advance was estimated, adopting the method suggested by Johnson *et al*. (1955).

Results

Quantitative characters in M_3 generations

Gamma radiations and EMS proved to be very effective to induce variability in quantitative traits in M_3 generations. The variance of M_3 generations, the data on mean performance, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h^2) and genetic advance (GA) were studied for the following characters like days to first flower, plant height, number of branches per plant, number of leaves per plant, number of clusters per plant, number of pods per plant, number of seeds per plant and seed yield per plant (Table-1 to 8).

Days to first flower

A slight decrease in number of days to first flower was observed in gamma rays and EMS treatment than in the control. Whereas, there was an increase in number of days to first flower was noticed in combined treatment. The treatment at 25KR+25mM of combined treatment took more number of days to first flower (42.08 days) while the minimum number of days (35.77 days) to first flower was observed at 25mM of EMS treatment. The maximum PCV and GCV values (12.78 and 8.63 per cent respectively) were recorded by 25mM of EMS. The minimum values (7.23 and 5.21 per cent) were recorded by 20KR+25mM of combined treatment. The heritability values were high in all mutagenic treatments. The maximum heritability of 70.32 per cent was recorded by 25mM of EMS while the minimum heritability of 30.28 per cent was observed at 25KR+25mM of combined treatment. All the treatments showed low GA as per cent of mean.

Plant height (cm)

There was a general increase in plant height at all mutagenic treatments than in the control. It was maximum plant height was observed at 25mM of EMS (56.38cm) while the minimum plant height was observed at 25KR+25mM combined treatments (47.28cm). The greatest PCV and GCV values were recorded at 25mM of EMS (25.26 and 9.80 per cent respectively). The lowest PCV and GCV values were recorded at 20KR of gamma rays (9.95 and 4.87 per cent respectively). The maximum heritability (66.42 per cent) was observed at 25mM of EMS and minimum heritability was observed at 25KR+25mM of combined treatment (16.41 per cent). Almost all the treatments showed a low GA as per cent of mean.

Number of branches per plant

In general, there was an increase in number of branches in all the treatments when compared to control. The most number of branches (8.48) was observed at 25mM of EMS. The least number of branches (6.22) was observed at 25KR+25mM of combined treatment. The maximum PCV and GCV values were recorded at 25KR+25mM and 15KR+25mM of combined treatment (22.75 and 19.74 per cent respectively). The minimum PCV and GCV values were recorded at 20KR of gamma rays and 30mM of EMS (18.86 and 11.38 per cent respectively). The heritability was high in all the treatments. The maximum heritability was observed at 25mM of EMS (58.41). The minimum heritability was observed at 25KR+25mM of combined treatment (24.87 per cent). Almost all the treatments showed a low GA as per cent of mean. The maximum GA as per cent of mean was observed at 25KR of gamma rays treatment (33.66).

Number of leaves per plant

The number of leaves increased in all mutagenic treatments when compared to control. The highest number of leaves per plant was observed at 25mM of EMS (80.32) while the lowest number of leaves was observed at 25KR+25mM of combined treatment (68.56). The highest PCV and GCV values were observedat 25mM of EMS (16.75 and 8.24 per cent respectively) while the lowest amount PCV and GCV values were observed 15KR+25mM of combined treatment and 20KR of gamma rays (8.28 and 4.81 per cent respectively). The heritability was high at 25mM of EMS (69.25 per cent) while

low at 20KR of gamma rays (10.31 per cent) and moderate to high in all mutagenic treatments. The maximum GA as per cent of mean was observed at 25mM of EMS (12.17), all the remaining treatments in low GA as per cent of mean.

Number of clusters per plant

The number of clusters was increased at all dose/ concentration of treatment when compared to control. The greatest number of clusters per plant was observed at 25mM of EMS (14.14) while the lowest number of clusters per plant was observed at 25KR+25mM of combined treatment (8.52). The maximum PCV and GCV values were recorded at 25mM of EMS (24.62 and 19.35 per cent respectively). The minimum PCV and GCV values were recorded at 20KR+25mM of combined treatment and 25KR of gamma rays (15.56 and 10.18 per cent respectively). The maximum heritability was observed at 25mM of EMS (66.70 per cent) while the minimum heritability was observed at 30KR of gamma rays (35.85 per cent). The GA as per cent of mean was high in all the mutagenic treatments. The highest GA as per cent of mean was observed at 25mM of EMS (39.25).

Number of pods per plant

` The number of pods per plant was increased in all treatments, but decreased in high doses of gamma rays (30KR), EMS (30mM) and combined treatment (25KR+25mM) treatment. The maximum number of pods per plant was recorded at 25mM of EMS (42.45) while the minimum number of pods per plant was recorded at 25KR+25mM of combined treatment (28.20). The maximum values of PCV and GCV were recorded at 25mM of EMS (12.88 and 7.38 per cent respectively) while the minimum PCV and GCV value were recorded at 20KR of gamma rays (3.68 and 2.45 per cent respectively). The maximum heritability was observed at 25mM of EMS (46.36 per cent) while the minimum heritability was observed 15KR+25mM of combined treatment (14.92 per cent). All the mutagenic treatments were low in GA as per cent of mean.

Number of seeds per plant

A considerable increase in number of seeds per plant was observed among the all mutagenic treatments when compared to control. The maximum number of seeds per plant (577.42) was observed at 25mM of EMS while the minimum number of seeds per plant (342.87) was observed at 25KR+25mM of combined treatment. The highest PCV and GCV values were recorded at 25mM of EMS (33.88 and 27.33 per cent respectively). The lowest PCV and GCV values were recorded at 30KR of gamma rays and 20KR+25mM of combined treatment (20.72 and 5.24 per cent respectively).The maximum heritability was observed at 25mM of EMS (60.96 per cent) while the minimum heritability was observed at 20KR+25mM of combined treatment (4.56 per cent). The GA as per cent of mean was low in all mutagenic treatments.

Seed Yield per plant (g)

In general, a slight increase in seed yield per plant was observed among the mutagenic treatments than the control. The maximum seed yield per plant (72.54g) was observed at 25mM of EMS while the minimum seed yield (40.58g) was observed at 25KR+25mM of combined treatment. The PCV and GCV were generally high in all mutagenic treatments. The utmost PCV and GCV were observed at 25mM of EMS (11.88 and 9.66 per cent respectively). The least PCV and GCV were observed at 20KR of gamma rays (4.65 and 2.12 per cent respectively). The heritability was found to be high in all the treatments. The high heritability value was observed at 25mM of EMS (65.75 per cent) while the low heritability value was observed at 20KR of gamma rays (29.32 per cent). The GA as per cent of mean was low in all mutagenic treatments.

Discussion

Mean performance

In M_3 generations of the data were recorded highly significant differences for all the traits of quantitative traits in cowpea. It is clear from the tables that the mean values increased significantly for all the yield contributing traits under study. Higher values of mean for plant height, number of branches per plant, number of leaves per plant, number of cluster per plant, number of pods per plant, number of seeds per plant and seed yield per plant in M_3 generation were recorded with 25mM of EMS compared to other mutagenic treatments. It has been observed that during mutagenesis, if mutations occur at random for the quantitative traits, no significant change in expected in the mean values.

The significant decrease in the days taken for first flowering was observed in most of the treatments was suggestive of the desired direction for the isolation of useful mutants. The inducement of early flowering by physical, chemical and combined mutagens were previously reported by many workers Lamseejan *et al*. (2000) in *chrysanthemum;* Rekha and langer (2007) in *Artemisia pallens*; Diouf *et al*. (2010) in sesame; Atta *et al*. (2003) in chickpea; Chowdhury *et al*. (2009) in sesame.

The maximum number of leaves was observed at 25mM of EMS in M_3 generations. Stimulated vegetative growth induced by a narrow range of low dose/ concentration of mutagen was observed by several researchers. Similar observation was made in other plants like black gram (Deepalakshmi and Anandakumar, 2004); soybean (Cheng and Chandlee, 1999) and okra (Singh *et al*., 1998). Kumar and Dubey (1998) recorded positive shift of mean performance in primary branches per plant, pods per plant, seeds per plant, seeds per pod and 100 seed weight with effect of EMS in M_3 generation of grass pea.

The mean value recorded the positive shift for the character such as plant height, number of branches per plant and number of leaves per plant in both the physical, chemical and combined mutagenic treatments of M_3 generations. Similar observations were also made in other crops in lentil (Dixit and Dubey, 1985), rice (Lokaprakash *et al*., 1992), soybean (Dhole *et al*., 2003) and sesame (Sengupta and Datta, 2004).

A positive shift in the mean values for the characters yield per plant was observed in the M_3 generations. The maximum mean yield per plant was observed at optimum dose/ concentration of all mutagenic treatments. It was reduced the higher dose/concentration of the mutagens. Such observations were reported by previous worker in cowpea (Odeigah *et al*., 1998). Similar observations were also made in other plant like pigeon pea (Sheriff and Veeraswamy, 1977) and black gram (Juliet Hepziba and Subramanian, 2002), chickpea (Kharkwal, 2003).

Variability, heritability and genetic advance as percent of mean

Genetic improvement in the crops depends on the magnitude of genetic variation and heritability of characters of economic importance. The desired variability can be successfully utilized by various breeding methods.

Hence, knowledge about the variability using parameters like genetic coefficient of variation, heritability and genetic advance is of paramount importance for initiating an efficient breeding programme in crops like cowpea. The present study was aimed to understand the extent to which the observed variation was due to genetic factors. An evaluation of heritability, genetic advance and the extent of genetic variation available for yield attributes would be of immense help to the breeds as the success of selection in any crop improvement programme is determined by these specific genetic parameters. Similar results were observed in Senthamizhselvi *et al*. (2007).

Hence, the present study on genetic variabilities was undertaken.

The present study shows a low phenotypic co-efficient of variation (PCV) and genotypic co-efficient of variation (GCV) such as days to first flower, plant height, number of branches per plant, number of leaves per plant, number of clusters per plant number of pods per plant, number of seeds per plant and seed yield per plant was observed at moderate phenotypic co-efficient of variation (PCV) and genotypic co-efficient of variation (GCV) in M_3 generation. The maximum phenotypic co-efficient of variation and genotypic co-efficient of variation was observed at 25mM of EMS.

The heritability was found moderate to high for all characters in the M_3 generation. Variability created in the M_3 generation was high compared to the control. In M_3 generation, some putative mutants with improved storability had been produced (Addai and Safo- Kantanka, 2006). The mutants isolated with high mean values high heritability and genetic advance as per cent of mean may be useful for utilizing them in crop improvement programmes. Similar results were made in lentil (Rasul *et al.,* 1994; Singh and Singh, 2004), green gram (Khan *et al.,* 2004) and chickpea (Kharkwal, 2000).

Conclusion

Both the mutagens proved to be very effective to induce variability in all quantitative traits in M_3 generations. High variance combined with moderate to high heritability for economic traits such as number of branches per plant, number of pods per plant, and number of seeds per plant suggested that induced variability can be exploited for improving characters. In general it was found that the treatment at 25mM of EMS produced high mean and high variance in the mutagenic population. This enhanced variability in the genotype cowpea var. CO7 due to mutagenic treatments provides an opportunity to utilize the generated variability for selection and further improvement in these characters.

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Table 1: Components of variance for days to first flower in M_3 generation

Treatment (Dose/Conc.)		Mean (Days)	PCV (%)	GCV (%)	h^2 (%)	GA as % of mean
Control		39.78	-	-	-	-
Gamma rays (KR)	20KR	39.16	8.36	6.65	35.38	9.26
	25KR	38.28	7.55	5.28	65.64	12.85
	30KR	41.56	11.62	7.64	41.87	10.44
EMS (Conc.mM)	20mM	38.77	8.22	6.18	68.64	12.58
	25mM	35.77	12.78	8.63	70.32	17.94
	30mM	37.88	11.32	7.84	56.48	15.25
Combined treatment (Gamma rays + EMS)	15KR+25mM	40.87	10.15	7.48	53.36	10.86
	20KR+25mM	38.65	7.23	5.21	60.84	10.29
	25KR+25mM	42.08	10.26	5.60	30.28	5.37

Table 2: Components of variance for plant height in M_3 generation

Treatment (Dose/Conc.)		Mean (cm)	PCV (%)	GCV (%)	h^2 (%)	GA as % of mean
Control		49.86	-	-	-	-
Gamma rays (KR)	20KR	50.78	9.95	4.87	51.67	12.56
	25KR	54.35	21.34	8.83	29.42	7.63
	30KR	51.27	11.49	6.72	36.27	7.48
EMS (Conc.mM)	20mM	51.44	10.33	6.64	45.86	13.25
	25mM	56.38	25.26	9.80	66.42	15.37
	30mM	50.83	12.72	8.62	42.67	15.40
Combined treatment (Gamma rays + EMS)	15KR+25mM	50.67	11.44	7.62	48.14	8.52
	20KR+25mM	52.86	14.92	8.27	52.43	11.76
	25KR+25mM	47.28	12.08	6.73	16.41	6.72

Table 3: Components of variance for Number of branches per plant in M_3 generation

Treatment	(Dose/Conc.)	Mean	PCV (%)	GCV (%)	h^2 (%)	GA as % of mean
Control		6.85	-	-	-	-
Gamma rays (KR)	20KR	7.52	18.86	12.86	38.23	20.21
	25KR	8.14	20.88	17.96	52.95	33.66
	30KR	6.90	20.13	14.32	43.38	9.56
EMS (Conc.mM)	20mM	7.64	20.56	14.86	32.56	28.53
	25mM	8.48	22.73	18.22	58.41	33.60
	30mM	6.93	21.77	11.38	41.32	9.09
Combined treatment (Gamma rays + EMS)	15KR+25mM	7.28	19.18	19.74	39.86	4.12
	20KR+25mM	7.92	20.26	14.18	50.15	28.28
	25KR+25mM	6.22	22.75	12.36	24.87	1.92

Table 4: Components of variance for Number of leaves per plant in M_3 generation

Treatment	(Dose/Conc.)	Mean	PCV (%)	GCV (%)	h^2 (%)	GA as % of mean
Control		72.86	-	-	-	-
Gamma rays (KR)	20KR	74.97	12.39	4.81	10.31	2.96
	25KR	76.43	14.67	7.89	26.72	8.98
	30KR	73.10	13.88	5.22	27.86	6.32
EMS (Conc.mM)	20mM	76.83	14.36	7.46	26.39	8.12
	25mM	80.32	16.75	8.24	69.25	12.17
	30mM	73.82	12.22	6.96	23.88	5.78
Combined treatment (Gamma rays + EMS)	15KR+25mM	73.25	8.28	6.22	64.82	12.12
	20KR+25mM	75.14	13.82	5.62	19.67	6.18
	25KR+25mM	68.56	11.76	5.82	21.26	4.60

Table 5: Components of variance for Number of cluster per plant in M_3 generation

Treatment	(Dose/Conc.)	Mean	PCV (%)	GCV (%)	h^2 (%)	GA as % of mean
Control		10.22	-	-	-	-
Gamma rays (KR)	20KR	11.46	16.76	13.36	62.18	41.71
	25KR	12.88	17.73	10.18	38.14	18.55
	30KR	11.10	16.88	11.55	35.85	20.54
EMS (Conc.mM)	20mM	12.76	17.39	10.96	50.45	22.57
	25mM	14.14	24.62	19.35	66.70	39.25
	30mM	12.31	21.71	17.66	64.39	19.17
Combined treatment (Gamma rays + EMS)	15KR+25mM	11.22	20.14	17.55	58.26	31.55
	20KR+25mM	12.18	15.56	10.24	49.92	31.36
	25KR+25mM	8.52	21.74	16.32	50.16	30.39

Table 6: Components of variance for Number of pods per plant in M_3 generation

Treatment	(Dose/Conc.)	Mean	PCV (%)	GCV (%)	h^2 (%)	GA as % of mean
Control		30.64	-	-	-	-
Gamma rays (KR)	20KR	36.28	3.68	2.45	39.88	4.60
	25KR	39.12	8.48	5.97	45.56	12.21
	30KR	33.70	9.52	4.46	25.72	8.69
EMS (Conc.mM)	20mM	38.14	7.14	3.27	34.67	11.09
	25mM	42.45	12.88	7.38	46.36	13.78
	30mM	35.75	4.64	2.76	42.84	15.27
Combined treatment (Gamma rays + EMS)	15KR+25mM	32.88	7.74	3.43	14.92	3.71
	20KR+25mM	37.56	12.45	5.82	21.63	5.69
	25KR+25mM	28.20	7.82	3.74	21.18	6.60

Table 7: Components of variance for Number of seeds per plant in M_3 generation

Treatment	(Dose/Conc.)	Mean	PCV (%)	GCV (%)	h^2 (%)	GA as % of mean
Control		380.86	-	-	-	-
Gamma rays (KR)	20KR	470.74	26.66	6.25	5.28	1.32
	25KR	542.36	22.34	12.78	32.86	5.47
	30KR	503.82	20.72	14.22	6.86	1.56
EMS (Conc.mM)	20mM	492.18	30.66	8.28	8.37	2.46
	25mM	577.42	33.88	27.33	60.96	9.58
	30mM	462.61	27.73	21.66	48.23	17.80
Combined treatment (Gamma rays + EMS)	15KR+25mM	446.12	27.54	14.20	26.15	9.78
	20KR+25mM	488.83	25.87	5.24	4.56	3.09
	25KR+25mM	342.87	21.32	11.88	33.75	8.00

Table 8: Components of variance for Seed yield per plant in M_3 generation

Treatment	(Dose/Conc.)	Mean (g)	PCV (%)	GCV (%)	h^2 (%)	GA as % of mean
Control		49.24	-	-	-	-
Gamma rays (KR)	20KR	58.83	4.65	2.12	29.32	3.19
	25KR	62.21	11.49	7.38	65.45	11.47
	30KR	54.14	7.38	4.44	34.92	6.88
EMS (Conc.mM)	20mM	63.14	5.33	5.88	33.35	3.83
	25mM	72.54	11.88	9.66	65.75	9.96
	30mM	62.14	7.76	4.55	34.84	5.24
Combined treatment (Gamma rays + EMS)	15KR+25mM	54.40	4.74	2.26	29.39	2.25
	20KR+25mM	60.87	11.43	6.74	52.56	8.93
	25KR+25mM	40.58	6.27	3.22	29.74	5.32

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