Induced Mutagenesis for Realizing Temperature Sensitive Genic Male Sterility in Rice (*Oryza sativa* L.)

K. S. Anithadevi* and T. S. Raveendran
Center for Plant Breeding and Genetics, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu (641 003), India

Article History

Manuscript No. 34
Received 20th May, 2010
Received in revised form 3rd August, 2010
Accepted in final form 6th August, 2010

Correspondence to
**E-mail*: anitha_sivaram@yahoo.co.in

Keywords
TGMS, mutation, stigma exertion, pollen, spikelet fertility

Abstract

An investigation was carried out to generate genetic variability in two rice varieties viz., ADT 39 and CR 1009 by exposing them to gamma rays at 50, 100, 150, 200, 250 and 300Gy. The main focus of this study is to identify environmentally controlled genetic male sterile mutants which upon further investigation could help in hybrid breeding programme. The male sterile plants were identified in M2 generation under high temperature condition (Coimbatore) and the reverted lines (with more than 60% spikelet fertility) in the low temperature region (Gudalur) were planted again in the high temperature condition to confirm their Temperature sensitive Genic male sterilirty (TGMS) nature. Seven plants (comprising five plants from ADT 39 and two plants from CR 1009) isolated from M3 generation recorded to have 100 % pollen and spikelet sterility.

1. Introduction

Rice (*Oryza sativa* L.) is one of the agronomically and nutritionally important cereal crops and it is the principal staple food in developing countries. Approximately half of the World's population subsists wholly or partially on rice. It is cultivated in 113 countries. Hybrid rice technology has tremendously improved rice productivity as efficiently demonstrated in China and other Asian countries. Hybrid rice contributes to the increase of the grain yield of over 20% to the improved inbred varieties. Till date, the CGMS or three-line method is effective and widely used in producing hybrid rice but the exploitation of hybrid rice through CGMS is cumbersome and restricts the use of varieties or elite lines as parental materials, for want of specific maintainer (B) and restorer (R) genes. In the tropics, use of TGMS has been effective in developing hybrids and has shown prospects in increasing efficiency of hybrid rice breeding (Lu et al*.,* 1998; Lopez and Virmani, 2000). Development of TGMS lines from acceptable and agronomically superior rice varieties took place in the early 1990s through the mutation breeding programme. This has made the way for two line breeding of hybrid rice using the TGMS system, which requires a stable expressing female lines and an effective combiner.

Although several breeding methods are available, mutation is an excellent complementary tool for the breeders. In 1988, a spontaneous genic male sterile mutant named Annong 1S was isolated from the F_5 population of a cross of 40B × H285 × 6209-3. Regardless of whether the photoperiod length, Annong 1S always behaved as male sterile under 33°C, and can become fertile under 24 °C (Chen et al., 1994). It was further characterized that its male sterility is controlled by temperature, while its critical stage for fertility transformation is similar to PGMS. Since then, a series of (P/TGMS) lines were developed from various *indica* varieties, including Hengnong S, 5460 S etc. The fertility of those GMS germplasm is primarily determined by temperature. Many TGMS mutants derived from various *indica* varieties have been found and genetically characterized (Dong et al., 2000). Mutagenesis has been used for generating a spectrum of induced variability which can judiciously be manipulated for improving both oligogenic and polygenic characters in many crops (Singh, 1993). Effect of induced mutagenesis in rice for the trait of interest is governed by the genetic architecture of the material used and the nature of mutagens applied. Different mutagens have been used in inducing variability and found as useful agents in mutation breeding programmes (Bhatnagar, 1984; Kharkwal, 2000). In Japan, Maruyama et al., 1991 produced the first TGMS line exposing seeds to gamma rays. Significant progress has recently been made in hybrid rice improvement using induced mutations. The production of TGMS lines with induced mutation appears to be a practical proposition as it also avoids the genetic vulnerability due to the cytoplasmic genic male sterility.

2. Materials and Methods

2.1. Study sites

The experiments were conducted at the Department of Rice, Centre for Plant Breeding and Genetics, Coimbatore and Hybrid Rice Evaluation Centre (HREC) *Gudalur* (low temperature high altitude region) of Tamil Nadu Agricultural University (TNAU).

2.2. Mutation treatment and regeneration of mutant progeny

Seeds of the two selected varieties (ADT 39 and CR 1009) were exposed to irradiation in the Gamma chamber with Co^{60} was used as source. The doses of 50Gy (Gray), 100Gy, 150Gy, 200Gy, 250Gy and 300Gy of gamma rays were fixed for the treatment of rice varieties based on preliminary experiments. Well filled, uniform sized, hand picked dry seeds with 12%

moisture content were used. A total of 200 seeds packed in polythene bags were exposed to irradiation for each treatment.

M_1 generation was raised during September 2005 - January 2006 at the Department of Rice, Coimbatore. Based on the number of seeds germinated seedlings survival on 30th day and height reduction at 30th day, the LD_{50} values were fixed for all the genotypes through probit analysis.

The M_2 generation was raised during December, 2006, in progeny rows. A total of 257 families in ADT 39 and 200 families in CR 1009 were planted. In each family 60 seedlings were planted. The observations on stigma exertion, pollen and spikelet sterility were studied. A total of 131 plants with comprising 98 from ADT 39 and 33 from CR 1009 were selected in this generation and stubble planted in *Gudalur* (a low temperature region) to check their reversion to fertility.

A total of 78 plants comprising 63 from ADT 39 and 15 from CR1009 which showed fertility reversion with more than 60% of spikelet fertility at *Gudalur* were selected and their seeds were harvested and raised at Coimbatore during January 2008 in such a way that their peak flowering coincides with peak summer which is a favorable condition for expression of male sterility.

2.3. Screening mutant population for sterility reaction

2.3.1. Pollen fertility

Pollen grains from the well matured anthers of five spikelets were observed using 1% iodine potassium iodide (I-KI) solution and the pollen fertility was expressed in percent as per the formula given below.

Pollen fertility (%)=(Number of fertile pollen grains / Total number of pollen grains scored) x 100

Classification of mutant population was done as follows-

Completely sterile	100
Sterile	>70
Partial sterile	20-70
Fertile	<20

2.3.2. Spikelet fertility

One panicle in each of the tagged plants of mutant population was covered with butter paper cover at the time of panicle emergence. At maturity, the number of filled grains and total spikelets were counted and recorded to work out spikelet fertility.

Spikelet fertility (%)=Number of filled grains or Total number of grains) x 100.

Total number of grains=Number of filled grains+Number of chaffy grains.

2.4. Mean and variance studies

The mean and variance of M_2 and M_3 families were estimated for the six doses of gamma rays for three traits viz. Pollen sterility, Stigma exertion and Spikelet fertility. The mean of the families was compared with the mean of the standard check varieties ADT 39 and CR 1009.

Coefficient of variation (CV), Genotypic coefficient of variation (GCV) and Phenotypic co-efficient of variation (PCV) were calculated using standard statistical methods.

Heritability estimate (h^2) in broad sense and expected Genetic Advance (GA) at 5% selection intensity were estimated through the methods devised by Lush (1949) and Johnson and Comstock (1955) and illustrated by Allard (1960).

Genetic advance was expressed as percentage of mean.

3. Results and Discussion

3.1. Spectrum of sterile plants in M_2 generation

In ADT 39, partial sterile plants were found in all the doses of gamma irradiated population, the highest frequency of 0.80% at 100Gy was noticed. In CR 1009, these types of plants were recovered in 50, 100, 150 and 200Gy of gamma rays. The highest frequency was noticed in 150Gy (Table 1). Completely sterile plants were recovered from all the doses except 300Gy in ADT 39. The highest frequency of complete male sterile plants was identified in 100Gy treatment for ADT 39 and 50Gy for CR 1009. The values are 0.48 and 0.08% respectively (Table 1).

A total of 32,340 plants in M_2 generation was evaluated for the expression of male sterility and they were classified into three categories *i.*e., sterile (more than 70%), partially sterile (20-70%) and fertile (less than 20%) (Table 2).Among the plants of ADT 39, the highest number of male sterile plants was noticed at 50Gy and the partial sterile plants were found to be high in 150Gy with 25

Table 1: Spectrum of sterile in M_2 generation for ADT 39 and CR 1009 population

Sl. No.	Types of mutants	Doses (Gy)											
		50		100		150		200		250		300	
		No	%	No	%	No	%	No	%	No	%	No	%
1.	ADT 39												
	Completely sterile (>70%)	8	0.11	6	0.48	3	0.09	2	0.05	1	0.05	-	-
	Partially sterile (20-70%)	10	0.14	10	0.79	25	0.79	20	0.53	8	0.43	5	0.36
	Total number of plants studied	7380		1260		3180		3780		1860		1380	
2.	CR 1009												
	Completely sterile (>70%)	4	0.08	1	0.04	1	0.05	2	0.07	-	-	-	-
	Partially sterile (20-70%)	10	0.21	6	0.27	5	0.28	4	0.14	-	-	-	-
	Total number of plants studied	4800		2220		1800		2820		1260		600	

plants in M_2 generation.

In M_2 generation of CR 1009 population, 50 Gy gamma treatment induced the highest number of male sterile plants (four plants with complete sterility and 10 plants with partial sterility). The sterile and partially sterile plants were found to be high in ADT 39 than CR 1009. In M_3 generation, five plants from ADT 39 and two plants from CR 1009 showed 100% pollen sterility and they were used for molecular studies to confirm the presence of the gene.

3.2. Pollen sterility

The mean value, variability parameters of both the varieties obtained at Coimbatore are presented in the Table 3. Pollen sterility ranged from 0.00 to 87.00%. In the case of ADT 39, the highest mean value was noticed in 150Gy (39.45%). A good estimate of variability parameters was recorded in all the treatments. Heritability was found to be high in ADT 39 at 50Gy dose (99.40%). The genetic advance as the % of mean were low in all treatments, while in CR 1009, highest heritability of 98.10% was observed in 200Gy .The mean value of the pollen sterility was lower when compared to ADT 39.

At *Gudalur* (low temperature region), the values for pollen sterility ranged from 0.00 to 67.00% for ADT 39 and there was a higher value of pollen fertility as compared to mean fertility values obtained at Coimbatore (Table 4). However, a good amount of variability with better heritability and genetic advance as % of mean was recorded in all the treatments. For CR 1009, high heritability with moderate genetic advance was recorded in almost all the varieties but in 150Gy, a reduction in the values for variability parameters, heritability and genetic advance was noticed.

The development of a suitable and stable two line hybrid in rice can obviate the difficulties of low productivity through conventional varieties and CMS based hybrids. In the present study, frequency of completely sterile plants was found to be in positive correlation with the dose in ADT 39. More over, overall mutant recovery was also found to be exhibiting the same type of relationship except 100Gy in ADT 39. CR 1009 also expressed good amount of sterile plants in 50Gy dose. Mean pollen sterility expression in ADT 39 was lower in the lower doses at *Gudalur* compared to Coimbatore conditions as expected. This indicates the possibility of creation of a stable male sterility genotype which is temperature dependent. However in the higher doses (250 and 300Gy), there had been an inverse relationship, i.e. higher sterility at *Gudalur* and lower at Coimbatore. The mean values, variability parameters of both the varieties are presented in the Table 5. Pollen sterility ranged from 0.00 to 100.00% in both the varieties. The highest mean value of 46.95% was recorded in 50Gy treatment. Heritability was found to be high in all doses. However, the genetic advance as % of mean was low in all the treatments, whereas in CR 1009, the values for variability parameters and heritability were found to be higher in all treatments. However, there had been moderate values of genetic advance as % of mean in 50Gy and 150Gy.

Table 3: Mean and variance for pollen sterility in M_2 generation at Coimbatore

Treatment	Range	Mean	PV	GV	PCV	GCV	h^2	GA%
ADT 39								
Control	0.00-5.00	3.05	2.20	-	-	-	-	-
50Gy	0.00-87.00	38.52	349.09	346.89	48.51	48.35	99.40	6.47
100Gy	0.00-82.00	29.31	219.17	216.97	50.51	50.26	99.00	8.47
150Gy	0.00-80.00	39.45	192.56	190.36	35.17	34.97	98.90	6.29
200Gy	0.00-71.00	36.91	199.46	197.26	38.27	38.06	98.90	6.72
250Gy	0.00-74.00	26.64	256.09	253.90	60.07	59.81	99.10	9.34
300Gy	0.00-67.00	25.77	157.18	154.98	48.65	48.31	98.60	9.60
CR 1009								
Control	0.00-7.00	3.37	3.76	-	-	-	-	-
50Gy	0.00-82.00	25.77	157.04	153.28	48.64	48.05	97.60	10.87
100Gy	0.00-75.00	25.08	147.22	143.46	48.38	47.76	97.40	11.15
150Gy	0.00-72.00	27.51	157.79	154.03	45.67	45.12	97.60	10.18
200Gy	0.00-87.00	28.22	195.68	191.92	49.57	49.09	98.10	9.97
250Gy	0.00-20.00	19.17	56.49	52.73	39.20	37.87	93.30	13.96
300Gy	0.00-15.00	21.21	21.19	17.43	21.71	19.69	82.30	11.13

Analysis done after applying arcsine transformation

In M_3 generation, however pollen sterility at Coimbatore in ADT 39 had revealed 100% in few families generated from 50Gy dose. Thus overall recovery of sterile mutants had been better at 50Gy for ADT 39, while for CR 1009, 200Gy had expressed 100% sterility in few plants of M_3. Thus variety dose dependency had been observed.

3.3. Stigma exertion

The stigma exertion values based on number of florets exhibiting this trait to the total ranged from 3.12 to 50.53% in the variety ADT 39, the mean values and all the variability components showed inverse dose dependent relationship. The highest value for genetic advance as % of mean was recorded in 200Gy at Coimbatore (21.45). In CR1009, the highest mean value of 12.24% was observed in 50Gy. The estimates of variance were found to be high in 50, 100, 150 and 200Gy except for the treatments 250Gy and 300Gy, where the environmental variance was high and negative values recorded. The highest value of heritability was estimated in 50Gy (99.30%). The genetic advance as % of mean was found to be moderate in all treatments (Table 6). The mean value, variability parameters of both the varieties on stubbled plants are presented

Table 4: Mean and variance for pollen sterility in M_2 generation, stubble planted at *Gudalur*

Treatment	Range	Mean	PV	GV	PCV	GCV	h^2	GA%
ADT 39								
Control	0.00-5.00	2.08	4.93	-	-	-	-	-
50Gy	0.00-32.10	10.80	44.67	39.73	61.88	58.37	89.00	25.29
100Gy	0.00-38.00	20.13	154.92	149.98	61.85	60.85	96.80	14.77
150Gy	24.00-56.00	24.85	130.28	125.35	45.93	45.05	96.20	11.89
200Gy	0.00-65.00	18.82	86.63	81.70	49.46	48.03	94.30	15.39
250Gy	12.00-50.00	31.01	137.01	132.08	37.74	37.06	96.40	9.54
300Gy	31.00-67.00	46.50	541.90	536.97	50.06	49.83	99.10	6.54
CR 1009								
Control	0.00-5.00	1.75	5.19	-	-	-	-	-
50Gy	0.00-45.67	20.79	182.98	177.79	65.06	64.13	97.20	14.53
100Gy	12.00-38.00	16.17	154.35	149.15	76.82	75.51	96.60	18.58
150Gy	24.00-38.00	29.83	24.97	19.77	16.75	14.91	79.20	8.26
200Gy	0.00-38.00	18.83	254.57	249.37	84.72	83.85	98.00	16.17
250Gy*	-	-	-	-	-	-	-	-
300Gy*	-	-	-	-	-	-	-	-

Table 5: Mean and variance for pollen sterility in M_3 generation at Coimbatore

Treatment	Range	Mean	PV	GV	PCV	GCV	h^2	GA%
ADT 39								
Control	0.00-5.00	3.01	2.38	-	-	-	-	-
50Gy	12.00-100.00	46.95	607.28	604.90	52.49	52.39	99.60	5.43
100Gy	5.00-65.00	39.55	289.09	286.71	42.99	42.82	99.20	6.41
150Gy	10.00-52.00	36.40	182.77	180.40	37.14	36.90	98.70	6.94
200Gy	0.00-33.00	23.56	69.86	67.49	35.48	34.87	96.60	10.49
250Gy	0.00-35.00	25.00	90.19	87.82	37.99	37.48	97.40	9.96
300Gy	0.00-28.00	21.79	61.51	59.14	35.99	35.29	96.10	11.28
CR 1009								
Control	0.00-6.00	3.97	3.79	-	-	-	-	-
50Gy	5.00-45.00	27.04	162.88	159.09	47.20	46.65	97.70	10.38
100Gy	8.00-67.00	34.40	325.67	321.88	52.46	52.15	98.90	8.26
150Gy	0.00-32.00	21.75	46.19	42.40	31.25	29.94	91.80	12.13
200Gy	15.00-100.00	40.30	485.18	481.39	54.66	54.45	99.20	7.08
250Gy*	-	-	-	-	-	-	-	-
300Gy*	-	-	-	-	-	-	-	-

*Families in these doses were not selected for sterility observation; Analysis done after applying arcsine transformation

in the Table 7. The values of stigma exertion ranged from 3.67 to 7.48% in ADT 39 and there was not much difference in mean values of treatments from the control mean. The environmental variance for three treatments namely 100, 200 and 250Gy were higher and hence their values were neglected. The heritability value was recorded to be high in 50Gy with the value of 65.30%, whereas in CR 1009 the heritability estimates were observed to be low in all the treatments and the influence of environmental variance was high in 50 and 150Gy.

In the M_3 generation the values of stigma exertion in the variety ADT 39 ranged from 3.21 to 66.37% and in CR 1009, it ranged from 3.11 to 59.54% (Table 8). The highest mean value for stigma exertion in ADT 39 was recorded in 50Gy (19.15%) and the same treatment showed high values for PV, GV, PCV, GCV and heritability and the highest value for genetic advance was recorded in 150Gy doses. In CR1009, the highest mean value was recorded in 200Gy (13.22) and this treatment also exhibited the highest value of variability parameters and heritability. The genetic advance as the % of mean was high only in 100Gy (20.72).

The stigma exertion in male sterile lines should be higher for ensuring better crossability. Stigma exertion % was good in 50Gy of ADT 39 during both generations. This shows that stable mutants with good exertion % can be detected at very low doses of irradiation in ADT 39. On the contrary, 200Gy was found to be the best for CR 1009 in M_3 generation for this trait. Basic linkage, adaptability during the course of development and duration of the crop may be certain traits which greatly influence the expression of mutation in a population.

Table 6: Mean and variance for stigma exertion (%) in M_2 generation at Coimbatore

Treatment	Range	Mean	PV	GV	PCV	GCV	h^2	GA%
ADT 39								
Control	3.12-4.56	3.99	0.31	-	-	-	-	-
50Gy	5.56-50.53	14.94	130.06	129.74	76.33	76.24	99.80	10.29
100Gy	3.12-32.89	13.34	83.55	83.23	59.73	59.62	99.60	10.03
150Gy	4.68-16.72	13.15	17.12	16.81	31.48	31.19	98.20	11.51
200Gy	3.56-7.12	5.71	1.52	1.21	21.61	19.26	79.40	21.45
250Gy	4.56-6.78	5.81	0.57	0.26	13.01	8.74	45.10	11.98
300Gy	4.12-6.01	5.23	0.45	0.14	12.89	7.20	31.2	9.19
CR 1009								
Control	3.12-5.67	4.42	0.68	-	-	-	-	-
50Gy	4.56-38.28	12.24	102.96	102.28	82.92	82.64	99.30	15.18
100Gy	3.49-18.67	9.28	32.81	32.13	61.70	61.05	97.90	19.73
150Gy	4.56-12.67	9.20	10.64	9.96	35.44	34.29	93.60	19.02
200Gy	6.78-43.12	12.04	87.72	87.04	77.82	77.52	99.20	15.42
250Gy	3.67-5.12	4.54	0.25	-0.43	10.91	#	#	#
300Gy	3.05-4.89	4.54	0.25	-0.35	14.02	#	#	#

#Since environmental variance was more, negative values were ignored; Analysis done after applying arcsine transformation

Table 7: Mean and variance for stigma exertion (%) in M_2 generation in stubble planting in *Gudalur*

Treatment	Range	Mean	PV	GV	PCV	GCV	h^2	GA%
ADT 39								
Control	3.87-5.16	4.48	0.24	-	-	-	-	-
50Gy	3.92-7.48	5.43	0.70	0.46	15.41	12.45	65.30	17.40
100Gy	3.89-5.12	4.72	0.24	0.00	10.41	#	#	#
150Gy	4.27-6.34	5.28	0.28	0.04	10.06	3.74	13.90	3.80
200Gy	5.67-7.12	6.57	0.31	0.06	8.41	3.83	20.70	4.56
250Gy	3.67-5.42	4.69	0.23	-0.01	10.25	#	#	#
300Gy	4.12-5.89	4.77	0.22	-0.02	9.85	#	#	#
CR 1009								
Control	3.45-5.08	4.26	0.37	-	-	-	-	-
50Gy	3.15-5.09	4.51	0.33	-0.05	12.64	#	#	#
100Gy	2.42-4.11	3.52	0.39	0.02	17.80	4.00	5.1	2.31
150Gy	4.23-5.39	4.74	0.23	-0.15	10.05	#	#	#
200Gy	2.97-5.32	4.37	0.55	0.17	16.94	9.57	31.9	11.75
250Gy*	-	-	-	-	-	-	-	-
300Gy*	-	-	-	-	-	-	-	-

#Since environmental variance was more, negative values were ignored; *Families in these doses were not selected for sterility observation; Analysis done after applying arcsine transformation

3.4. Spikelet fertility

In the *M_2 generation* raised at Coimbatore, the values of spikelet fertility ranged from 62.83 to 92.70% (ADT 39) and 57.05 to 92.81% (CR 1009).In ADT 39, the mean values in all the treatments showed a shift towards the negative direction. The PV and GV values recorded were high in all the treatments but the PCV, GCV and genetic advance values were found to be low in all the treatments (Table 9). A good estimate of heritability was recorded in 200Gy (96.43%). In CR 1009, the mean values of spikelet fertility in all the treatments were more or less similar to the control mean, whereas the components of variability had an inverse dose dependent relationship. However, a good estimate of heritability was recorded in 50Gy (97.78%). Table 10 shows that at *Gudalur* in *M_2* generation the values of spikelet fertility ranged from 32.12 to 98.67% in the variety ADT 39 and 30.25 to 90.12% in CR 1009. The mean values in the variety ADT 39 showed a shift in mean towards negative direction and the highest mean value was recorded in 50Gy (91.23%).Values of PV and GV were high in all the treatments. The genetic advance as % of mean was observed to be low in all the treatments. CR 1009 variety also exhibited a similar trend for this trait. The highest mean value recorded was in 200Gy (73.25%), the PV and GV values were high in all the treatments. The highest value of heritability was recorded in 200Gy (94.50). The genetic advance as % of mean was low in all the treatments.

Table 8: Mean and variance for stigma exertion (%) in M_3 generation in Coimbatore

Treatment	Range	Mean	PV	GV	PCV	GCV	h^2	GA%
ADT 39								
Control	3.54-5.12	4.36	0.39	-	-	-	-	-
50Gy	6.12-66.37	19.15	197.82	197.43	73.44	73.36	99.8	8.50
100Gy	4.32-17.89	9.39	9.92	9.53	33.54	32.87	96.00	16.68
150Gy	3.45-12.89	7.97	8.15	7.76	35.81	34.94	95.20	19.47
200Gy	3.21-5.89	5.13	0.82	0.42	17.61	12.69	51.90	16.50
250Gy	5.12-7.19	6.57	0.65	0.26	12.30	14.39	39.80	9.88
300Gy	4.08-6.67	5.47	0.45	0.05	12.23	4.25	12.10	3.61
CR 1009								
Control	2.78-4.08	3.73	0.25	-	-	-	-	-
50Gy	3.12-4.86	4.05	0.25	-0.01	12.28	#	#	#
100Gy	4.56-7.12	5.19	0.96	0.70	18.87	16.18	73.50	20.72
150Gy	3.11-15.26	7.98	14.39	14.14	47.53	47.11	98.20	17.99
200Gy	5.22-59.54	13.22	163.97	163.72	96.88	96.81	99.80	11.04
250Gy*	-	-	-	-	-	-	-	-
300Gy*	-	-	-	-	-	-	-	-

#Since environmental variance was more, negative values were ignored; *Families in these doses were not selected for sterility observation; Analysis done after applying arcsine transformation

The mean values, variability parameters of both the varieties in M_3 generation are presented in Table 11. The value of spikelet fertility ranged from 5.78 to 85.55% in ADT 39 and 12.34 to 87.56% in CR 1009. In the variety ADT 39, the mean values for spikelet fertility showed a shift in mean towards the negative direction and the lowest value of mean was recorded in 50Gy (64.67%). Genetic advance was found to be low in all the treatments. In CR 1009, the highest mean value for spikelet fertility was registered in 50Gy (80.25%), while high PV, GV, PCV, GCV and heritability values were observed in 200Gy. This is the trait of commercial importance which has a direct implication on the yield plant or unit area^{-1}. Though various parameters influence the fertility of a spikelet, the fore most is the fertility level of the pollen grains. Thus, the pollen sterility % has a direct bearing on the spikelet fertility since rice is a self pollinated crop. Besides, external environment and internal nutrient balance also plays a role in determining the spikelet fertility. Least spikelet fertility % was noticed in 50Gy treatment of ADT 39 which was reflected in M_3 generation as well. However in CR 1009, though all the treatments behaved more or less uniform for this trait in M_2, M_3 generation had the least spikelet fertility % in 200Gy. This also

Table 9: Mean and variance for spikelet fertility in M_2 generation at Coimbatore

Treatment	Range	Mean	PV	GV	PCV	GCV	h^2	GA%
ADT 39								
Control	83.56-86.89	85.06	1.53	-	-	-	-	-
50Gy	66.67-91.17	78.04	40.85	39.32	8.19	8.03	96.25	2.83
100Gy	66.67-92.16	79.75	38.25	36.72	7.75	7.60	95.99	2.76
150Gy	63.57-87.58	81.48	31.11	29.57	6.85	6.67	95.07	2.67
200Gy	62.83-92.70	80.25	42.96	41.43	8.17	8.02	96.43	2.75
250Gy	68.53-90.07	80.23	36.63	35.10	7.54	7.38	95.82	2.74
300Gy	69.12-90.10	82.39	31.11	21.23	6.77	5.59	68.24	3.02
CR 1009								
Control	82.73-86.43	84.76	1.98	-	-	-	-	-
50Gy	57.05-91.67	80.09	88.77	86.80	11.76	11.63	97.78	2.98
100Gy	63.52-92.81	81.08	73.57	71.59	10.58	10.44	97.32	2.93
150Gy	62.59-91.85	83.81	55.58	53.60	8.90	8.74	96.45	2.81
200Gy	61.29-92.16	83.13	42.99	41.01	7.89	7.70	95.41	2.80
250Gy	75.46-90.23	83.18	27.68	25.70	6.32	6.10	92.86	2.73
300Gy	79.56-91.37	84.58	16.94	14.97	4.87	4.57	88.34	2.55

Analysis done after applying arcsine transformation

Table 10: Mean and variance for spikelet fertility in M_2 generation, stubble planted at *Gudalur*

Treatment	Range	Mean	PV	GV	PCV	GCV	h^2	GA%
ADT 39								
Control	88.34-98.23	93.88	15.38	-	-	-	-	-
50Gy	80.21-98.67	91.23	50.28	34.91	7.77	6.48	69.40	3.10
100Gy	67.45-90.67	76.45	89.12	73.74	12.35	11.23	82.7	4.42
150Gy	42.45-85.67	66.69	198.02	182.64	21.10	20.26	92.2	5.64
200Gy	32.12-52.31	45.20	30.78	15.40	12.28	8.68	50.00	4.52
250Gy	45.12-81.23	63.33	242.15	226.77	24.57	23.78	93.60	6.03
300Gy	42.34-82.67	65.78	192.98	177.60	21.12	20.26	92.00	5.71
CR 1009								
Control	85.45-96.42	93.16	9.60	-	-	-	-	-
50Gy	51.21-78.23	62.72	81.16	71.56	14.36	13.49	88.20	5.10
100Gy	64.23-79.21	69.70	52.00	42.40	10.35	9.34	81.50	4.24
150Gy	30.25-65.23	51.77	123.97	114.37	21.51	20.66	92. 30	6.46
200Gy	60.45-90.12	73.25	176.01	166.41	18.11	17.61	94.50	4.68
250Gy*	-	-	-	-	-	-	-	-
300Gy*	-	-	-	-	-	-	-	-

*Families in these doses were not selected for sterility observation; Analysis done after applying arcsine transformation

had sufficient genetic advance as a % of mean. An overall result of pollen fertility and spikelet fertility data indicates that 50Gy treatment for ADT 39 and 200Gy treatment for CR 1009 are influential in creating the required variability for this important trait.

Table 11: Mean and for spikelet fertility in M_3 generation at Coimbatore

Treatment	Range	Mean	PV	GV	PCV	GCV	h^2	GA%
ADT 39								
Control	85.32-88.18	86.16	3.30	-	-	-	-	-
50Gy	5.78-82.31	64.67	269.73	266.43	25.35	25.19	98.80	4.23
100Gy	56.78-85.32	68.67	89.42	86.12	13.77	13.51	96.30	3.89
150Gy	69.67-85.43	77.50	19.71	16.41	5.73	5.23	83.30	2.98
200Gy	71.82-85.55	77.65	13.63	10.33	4.75	4.14	75.80	2.71
250Gy	78.45-84.45	82.02	4.91	1.61	2.70	1.55	32.90	1.11
300Gy	79.23-82.59	81.34	4.68	1.38	2.66	1.45	29.60	1.01
CR 1009								
Control	80.45-88.72	84.06	5.38	-	-	-	-	-
50Gy	72.34-87.56	80.25	18.66	13.29	5.38	4.54	71.20	2.78
100Gy	60.78-78.12	71.82	34.34	28.96	8.16	7.49	84.30	3.68
150Gy	72.34-80.12	76.71	7.13	1.76	3.48	1.73	24.60	1.01
200Gy	12.34-78.32	64.90	327.76	322.38	27.89	27.66	98.40	4.75
250Gy*	-	-	-	-	-	-	-	-
300Gy*	-	-	-	-	-	-	-	-

*Families in these doses were not selected for sterility observation

4. Conclusion

Facing the challenge of population growth and cropland reduction, it is obvious that the only way to solve this problem is to improve the yield of cereal crops namely, rice, wheat and corn etc. (Yuan and Peng, 2005). Thus the present study was directed to create temperature sensitive genic male sterile lines using gamma rays and characterize the lines by comparing the different features in terms of morphological characters which can be useful for the development of the hybrid breeding programme.

5. Acknowledgements

The study was done with the financial support of Bhabha Atomic Research Center, Mumbai, India.

6. References

Allard, R.W., 1960. Principles of Plant Breeding. John Wiley and Sons, Inc., New York, 485.

Bhatnagar, S.M., 1984. Interaction of physical and chemical mutagens in Kabuli chickpeas. International Chickpea Newsletter, 11-17.

Chen, L.B., Zhou, G.Q., Huang, Y.X., 1994. Influence of temperature and photoperiod on the fertility and physiology of Annong S-1 and Hengnong S-1 of rice. Acta Botanica Sinica 36, 119-123.

Dong, N.V., Subudhi, P.K., Luong, P.N., Quang, V.D., Quy, H.G., Zheng, T.D., 2000. Molecular mapping of a rice gene conditioning thermosensitive genic male sterility using AFLP, RFLP and SSR techniques. Theoretical Applied Genetics 100**,** 727-734.

Johnson, H.W., Comstock, R.E., 1955. Genotypic and phenotypic correlation in soybeans and their implications in selections. Agronomy Journal 47**,** 477- 483.

Kharkwal, M.C, 2000. Induced mutations in chick pea (*Cicer arietinum* L.). Types of macro mutations induced. Indian Journal of Genetics 60**,** 305-320.

Lopez, M.T., Virmani, S.S., 2000. Development of TGMS lines for developing two line rice hybrids for the tropics. Euphytica 114**,** 211-215.

Lu, X.G., Virmani, S.S., Rencui, Y., 1998. Advances in two line hybrid rice breeding. In: Innovative Approaches in Rice Breeding. International Rice Research Institute, Manila, Philippines, 89-98.

Lush, J.L., 1949. Heritability of quantitative characters in farm animals. Hereditas (Lund) 35 (Suppl.), 356- 375.

Maruyama, K., Araki, H., Kato, H., 1991. Thermosensitive genic male sterility induced by irradiation. In: Rice Genetics II, International Rice Research Institute, Philippines, 227-235.

Singh, B.D., 1993. Plant Breeding - Principles and Methods. Kalyani Publishers (India), New Delhi, India, 598-600.

Yuan, L.P., Peng, J.M., 2005. Hybrid Rice and World Food Security. China Science and Technology Press, Beijing.