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RESEARCH ARTICLE :

Effect of gamma rays on seed germination, plant survival and quantitative characters on two varieties of soybean [*Glycine max*. (L.) Merrill.] in m_2 generation

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SUMMARY : A study was undertaken on induced mutagenesis with two varieties of Soybean, BSS-2 and RKS- 18. The mutagen used was Gamma rays (50,100,150,200 and 400 Gy). A difference was observed between the varieties BSS-2 and RKS-18 in the degree of tolerance to the mutagens. Germination and survival percentage in both the varieties was lower as compared to control. Reduction in germination percentage was associated with increase in dose of mutagen in both varieties BSS-2 and RKS-18. The reduction in germination was found more in the variety BSS-2 than the variety RKS-18, proving it to be more sensitive towards Gamma rays.Survival percentage in both the varieties BSS-2 and RKS-18 in M_2 decreased with higher doses of Gamma radiation, though there was slight increase in survival percentage at 100 Gy in variety RKS-18.

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KEY WORDS :
Induced mutagenesis, Gamma rays, Mutagen, Germination percentage, Survival percentage

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BACKGROUND AND OBJECTIVES

Soybean [*Glycine max* (L.) Merrill] also known as golden bean is an important oilseed crop. The productivity of soybean in India is much low in comparison with the world average. The main attributes identified for low productivity are limitedgenetic diversity, narrow genetic base of Indian soybean varieties and stagnant genetic potential for yield (Tiwari, 2003). Narrowing down of the genetic base is due to the repeated use of few parents for breeding programmes (Satyavathi *et al.*, 2006). Atthis context, widening of genetic base is a major concern and challenge put forward to the Indian soybean breeders. The classical breeding methods have got limited application in soybean as its small fragile flowers and complete self fertility impose limitation on the success of hybridization programme. So mutation breeding appears to play an important role in the improvement of this important pulse – cum

oil seed crop.

Earlier mutation breeding work in Soybean crop has yielded in identification of many mutant lines with desirable traits like high germination and survival percentage (Rahman *et al.*, 1994). Improvement in either single or few economic traits and quality characters can be achieved with the help of induced mutation within the shortest possible time (Manjaya and Nandanwar, 2007). Therefore, the present investigation was undertaken to study the effect of physical mutagen (Gamma rays) on some quantitative traits at different doses of two varieties of Soybean BSS-2 and RKS-18 in M_2 generation.

RESOURCES AND METHODS

Two varieties, BSS-2 and RKS-18 of Soybean formed the materials for the present investigation. Dry (9-12%) moisture and healthy seeds were obtained from Birsa Agricultural University, Ranchi and exposed to 50,100,150,200 and 400Gy Gamma rays. Irradiation was done using the Cobalt 60 sources in Gamma chamber at Bhabha Atomic and Research Centre, Mumbai. In *Kharif* 2014, two thousand (2000) treated seeds of two varieties of Soybean BSS-2 and RKS-18 irradiated with five (5) different doses of γ-rays (50Gy, 100Gy, 150Gy, 200Gy and 400Gy) were sown along with untreated control seedsin the single plot design at research farm of Birsa AgriculturalUniversity, Kanke, and Ranchi.The soil type of the experimental site was lateritic with the pH 5.8. In this region Soybean cultivation is mainly dependent on monsoon rains. The climate of this place is subtropical type and the average annual rainfall of this area is approximately 1400 mm and it is mostly erratic and punctured with occasional dry spells. Nearly 80% of the total rainfall comes during four monsoons months (mid June to mid October). Representative M_2 seeds from 5649 families were sown in *Kharif* 2015 in non replicated trial as progeny using single row method, each row having 1m row length and 20 seeds per row and having 45 cm row to row spacing and 10 cm plant to plant distance. Along with M_2 seeds three rows of M_1 seeds were also sown for a comparative study of variations having 3m row length 45cm row to row spacing and 10cm plant to plant distance.Data were recorded on germination%, survival % lethality%, (30 days after sowing and at flowering) and injury percentage. Likewise variations in quantitative characters were also observed in untreated control as well as treated plants throughout the life span of the plant.

OBSERVATIONS AND ANALYSIS

Detailed observation on all the M_2 plants in 50 Gy, 100 Gy, 150 Gy, 200 Gy and 400 Gy doses of gamma rays and control of both the varieties *viz.*, BSS-2 and RKS-18 were recorded for germination per cent, survival per cent, lethality per cent and injury per cent were recorded (Table 1 and Fig.1). Observations for

Table 1: Effect of gamma rays on germination%, survival% , lethality% and injury %in two variety BSS-2 and RKS-18 of soybean in M_2 generation

Variety	DOSE	Total seeds sown	Total rows planted (TR)	Total rows germinated (RG)	Plant population (PP)	Plt.pltn at maturity (pm)	Germination % (G%)	Survival% (S%)	Reduction in survival over control	Height reduction over control (Injury %) I
BSS - 2	Control	998	50	48	956	942	95.8	98.5	-	-
	50 Gy	28987	1449	1209	23867	19304	82.3	80.9	82.1	42.37
	100 Gy	27115	1355	997	19750	15600	72.8	79	80.2	23.72
	150 Gy	13123	654	447	8952	6752	68.2	75.4	76.6	62.71
	200 Gy	8750	434	338	4952	3411	56.6	68.9	69.9	57.62
	400 Gy	1992	99	52	559	263	28.1	47	47.8	55.93
RKS - 18	Control	1110	50	50	973	962	87.7	98.9	-	-
	50 Gy	12240	616	562	11009	9975	89.9	90.6	91.6	47.06
	100 Gy	9098	455	382	6958	5842	76.5	84	84.9	47.05
	150 Gy	5100	262	175	3741	3218	73.4	86	87	41.17
	200 Gy	3967	198	161	2809	2214	70.8	78.8	79.7	45.09
	400 Gy	530	19	15	119	62	22.5	52.1	52.7	64.7

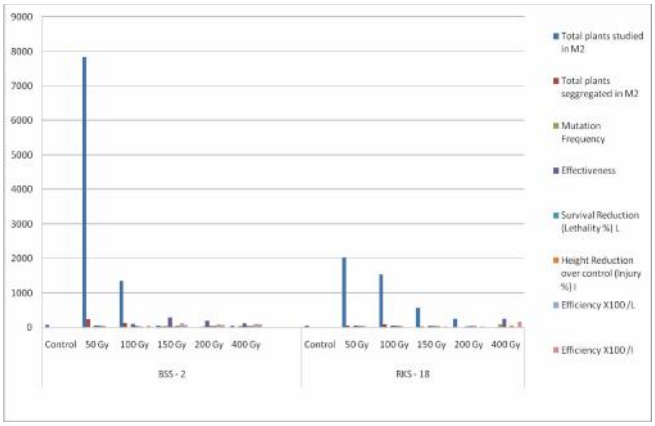

Fig 1 : Effect of gamma rays on germination%, survival%, lethality% and injury %in two variety BSS-2 and RKS-18 of soybean in M_2 generation

quantitative characters were also recorded (Table 2).

Germination per cent:

Initial germination was recorded after 10 days of sowing both in control and in irradiated seeds of the varieties BBS-2 and RKS-18 in M_2. Result observed in both the varieties BSS-2 and RKS-18 indicated that seed germination per cent decreased with an increasing dose of gamma rays clearly indicating that gamma rays as mutagen have induced an inhibitory effect on seed germination. Chaudhary and Singh (1980), Satpute and Fultambkar (2012) reported similar results in Soybean. A similar result of dose dependent germination per cent reduction in other crops was reported by different

Table 2: Mean, standard deviation, co-efficient of variability, of two varieties BSS- 2 and RKS-18 of soybean in M_2 generation

		BSS – 2						RKS-18					
	DOSE	Control	50 Gy	100 Gy	150 Gy	200 Gy	400 Gy	Control	50 Gy	100 Gy	150 Gy	200 Gy	400 Gy
Days to 50 per	Mean	44	44	43	42	42	42	48	46	46	42	41	40
cent flowering	SD	1.26	1.29	1.63	1.45	1.59	1.71	3.51	2.16	2.25	1.8	2.29	1.82
	CV(%)	1.59	1.66	2.64	2.11	2.53	2.92	12.34	4.64	5.08	3.22	5.25	3.34
Days to	Mean	107	109	112	112	115	115	115	119	121	122	121	118
maturity	SD	1.71	3.68	3.73	3.6	3.32	3.22	3.12	3.67	3.37	2.74	2.66	5.22
	CV(%)	2.92	13.54	13.92	12.99	11.02	10.39	9.74	13.48	11.34	7.51	7.07	27.28
Plant height	Mean	58.68	34	45	22	25	25	51	27	27	30	28	18
	SD	2.57	9.54	8.42	7.42	10.99	10.99	2.97	8.64	7.6	7.03	5.03	5.35
	CV(%)	6.64	28.05	18.71	33.72	39.12	35.34	5.82	32	28.1	24.1	20.1	13.1
No. of	Mean	3.68	3.69	3.57	3.23	3.31	3.70	2	3.34	3.49	3.64	3.24	2
branches/ plant	SD	0.8	0.93	0.82	0.84	0.79	0.93	0.82	1.35	1.26	1.31	1.07	1.15
	CV(%)	21.73	23.25	20.5	28	26.33	23.25	41	45	42	32.7	35.6	57.5
Pod length	Mean	4	3.42	3.53	3.39	3.31	3.40	3	3.34	3.27	3.20	3.15	3.25
	SD	0.46	0.5	0.53	0.49	0.48	0.5	0.37	8.97	7.74	8.21	7.63	1.29
	CV(%)	12.92	16.67	13.25	16.34	16	16.67	12.3	14	18.6	16.6	13.3	16.6
No. of pods/	Mean	31.6	24	26	11	17	14	35	21	18	19	17	6
plant	SD	2.85	9.32	8.49	4.66	7.84	8.54	1.64	8.97	7.74	8.21	7.63	1 .29
	CV(%)	9.08	38.85	32.65	42.36	46.11	61	4.68	43	43.2	44.8	21.5	42.7
No. of seeds/	Mean	3	2.98	3.32	3.23	3.13	3.30	3	3.34	3.04	3.07	3.01	3.25
pods	SD	0.58	0.65	0.55	0.49	0.5	0.55	0.61	0.63	0.53	0.55	0.49	0.5
	CV(%)	0.33	0.42	0.30	0.24	0.25	0.31	20.3	21	17.6	18.3	16.3	16.6
100 seed	Mean	11.31	12.30	12.38	10.72	9.5	9.5	10.2	8	8.6	8.5	9.2	8.1
weight	SD	2.09	1.59	1.04	0.57	0.94	1.35	0.26	1.11	1.23	1.33	1.6	1.11
	CV(%)	4.37	2.54	1.07	0.32	9.89	14.21	2.54	14	14.3	15.6	17.3	13.7
Seed yield/	Mean	11.03	9.79	11.70	4.20	10.94	6.32	10.72	6.86	5.88	6.27	5.65	1.88
plant	SD	2.33	7.44	6.38	2.49	0.35	4.14	2.15	3.35	2.75	2.79	2.78	0.68
	CV(%)	5.43	55.37	40.76	6.20	0.12	17.13	4.61	11.25	7.59	7.80	7.71	0.46

workers *viz.,* Kumar *et al.* (2009) in Cowpea, Roy, Sagade and Apparao (2011) and Bhoslae (2013) in Urdbean. The reduction in germination may be either due to genetic cause or inhibition of physiological process in cell by mutagen.

In present investigation the variety BSS-2 was found more sensitive to gamma rays as compared to variety the RKS-18. Similar mutagenic sensitivity has been reported by Kothekar and Kothekar (1992).

Survival per cent :

In M_2 the number of plants surviving at the maturity of both the varieties, BSS-2and RKS-18 was recorded and have been presented in Table 1. A study of data recorded revealed that there was marked decrease in survival percentage in BSS-2 at higher doses of gamma rays except at 100Gy where maximum survival per cent was observed. Maximum mortality was observed ay 400 Gy. In the variety RKS-18 there was remarkable decrease in survival percentage of plants at higher doses (200 Gy and 400 Gy) of gamma radiation.Similar inverse relationship was reported by Chopde (1976) and Kharkwal (1998) in Chickpea. Satpute and Kothekar (1996) in Safflower. Potdukhe and Narkhede (2002) and Kole *et al.* (2003) in *Zinnia,* Biradar (2004) in pegionpea, Shrama *et al.* (2006) in Urdbean, Barbhat and Dhumal (2009) in Kulthi -1, Sagade and Apparoa (2011) in Urdbean, Bhosale *et al.* (2013) in Urdbean and Bashir *et al.* (2013) in Fenugreek. Gaul (1964) opinioned that chromosomal and extra chromosomal injury might had lead to disturbances at physiological and cytological level leading to decrease in survival per cent.

Lethality per cent:

In the variety, BSS-2 lethality per cent decreased with the increase in dose of gamma rays from 50 Gy to 150 Gy doses, while 200 Gy and 400 Gy showed an increase in lethality per cent value. 50 Gy and 150 Gy recorded highest and lowest lethality per cent, respectively. In the variety, RKS-18 lethality per cent showed a random change without following any particular pattern. Highest lethality per cent was recorded in 100 Gy while lowest was recorded in 150 Gy. Zero (0) lethality per cent was recorded in 400 Gy. Similar result was reported by several workers. Ehrenberg (1955); Siddiqi and Swaminathan (1968); Kirmani (1992) and Nilan *et al.* (1964) proposed that prime cause of lethality is physiological imbalances or different types of chromosomal aberrations.

Injury per cent :

In the variety, BSS-2 lower doses (50 Gy and 100 Gy) recorded lower injury percent while higher doses (150 Gy, 200 Gy and 400 Gy) recorded higher injury per cent. 150 Gy and 100 Gy doses recorded highest and lowest injury per cent, respectively. In the variety, RKS-18 highest injury per cent was recorded in 400 Gy and lowest injury per cent was recorded in 150 Gy while 50 Gy and 100 Gy recorded a similar injury per cent value. Similar result was reported by Venkatachalam and Jayabalan (1997) in Groundnut, Khan *et al.* (2000) in Greengram. The reduction in plant height may be ascribed to different factors. Markeen *et al.* (2007) was in the opinion that reduction in plant height or seedling growth with higher dose of Sodium azide, may be due to gross injury caused at cellular level either due to gene controlled bio- chemical process or acute chromosomal aberration or both or may be due to slow rate of division of meristematic cells at shoot apex and arresting of mitotic cycle.

Observations for nine quantitative characters *viz.,* plant height, number of branches per plant, pod length, number of pods plant, number of seeds per pod, 100 seed weight, seed yield per pod, days to 50 per cent flowering and days to plant maturity were recorded. Mean, co-efficient of variance and standard deviation were calculated for all the nine quantitative characters in both the soybean varieties (Table 2).

Days to 50% flowering :

Mean values for days to 50 per cent flowering in the variety, BSS-2 showed a little change control and treatment with almost similar values. Highest mean value (44 days) was recorded in control as well as in 50 Gy while lowest (42 days) was recorded in higher doses (150 Gy, 200 Gy and 400 Gy).The estimates of variance and standard deviation values for days to 50 per cent flowering was recorded higher in radiation doses than the control. Higher co-efficient of variance and standard deviation values were recorded in 400 Gy, while lowest co-efficient of variance and standard deviation values were recorded in 50 Gy among the treatments.

In the variety, RKS-18 highest mean value for days to 50 per cent flowering was recorded in control. Treatments showed a negative deviation in mean value, as it reduced with the increase in radiation dosage.

Lowest mean value was recorded in 400 Gy. Co-efficient of variance and standard deviation values were higher in control than radiation doses. The lowest co-efficient of variance and standard deviation values were recorded in 150 Gy dose.

Days to maturity :

In the variety, BSS-2 mean values for days to plant maturity showed a positive deviation in radiation treatments and increased with increase in dose of gamma rays.The highest mean value was recorded in 200 Gy and 400 Gy (115 days). The lowest mean value was recorded in 50 Gy (109). Co-efficient of variance and standard deviation values were higher in radiation treatments than that of control. Lowest co-efficient of variance and standard deviation values were recorded in 400 Gy while highest were recorded in 100 Gy.

In the variety RKS-18 highest mean value for the character days to maturity (122 days) was recorded in 150 Gy and in higher dose of 400 Gy it was (118 days). The lowest mean value was observed in control (115 days). Values of co-efficient of variance and standard deviation for days to maturity were higher in lower doses (50 Gy and 100 Gy) than control, while in comparison to control the values of co-efficient of variance and standard deviation were found lower in higher doses (150 Gy, 200 Gy). The highest values of co-efficient of variance and standard deviation were recorded in 400 Gy.

Plant height :

The mean values of plant height shifted in negative direction in all the treatments over the control. Mean height of control was 58.68 cm. 50 Gy showed a mean value (34 cm) while the same mean value of plant height (25 cm) was recorded in 200 Gy and 400 Gy. In treatments maximum mean value (45 cm) was recorded in 100 Gy dose. The co-efficient of variance for plant height was highest in 200 Gy followed by 400 Gy, 150 Gy, 50 Gy and 100 Gy. There was increase in the value of co-efficient of variance with the increase in dose of Gamma rays except in case of 100 Gy which recorded the lowest co-efficient of variance value. For plant height, highest value of standard deviation was recorded in both 200 Gy and 400 Gy followed by 50 Gy, 100 Gy, 150 Gy and control in decreasing order.

In the variety RKS-18, maximum value of mean for plant height was observed in control. There was a negative shift in mean value for plant height in radiation treatments and was lower than control. The estimates of co-efficient of variance and standard deviation were higher than the control and showed decline in their values with the increasing dose of Gamma rays.

Number of branches per plant :

In the variety BSS-2, there was positive as well as negative shift in mean values for number of branches per plant in all treatments. Highest mean (3.70) was observed in 400 Gy, followed by 50 Gy, 100 Gy and 200 Gy while lowest mean (3.23) branches per plant was observed in 150 Gy. The co-efficient of variance for number of branches per plant was maximum in 150 Gy followed by 200 Gy. It was same in 50 Gy and 400 Gy (23.25). The lowest was recorded in 100 Gy (20.5). Standard deviation value was highest in 50 Gy and 400 Gy followed by 150 Gy, 100 Gy and 200 Gy. In control the standard deviation value was 0.8.

In the variety RKS-18, the highest value of mean for number branches per plant was shown in 150 Gy followed by 100 Gy (3.49), 50 Gy (3.34) and 200 Gy (3.24). Lowest value was recorded in both control and 400 Gy, The co-efficient of variance value was recorded highest in 400 Gy followed by 50 Gy, 100 Gy, control, 200 Gy and 150 Gy in decreasing order. Standard deviationvalue was higher radiation treatments when compared to control. Highest value was recorded in 50 Gy while lowest was in 200 Gy.

Pod length:

In the variety, BSS-2 the highest mean value for the character pod length was recorded in control followed by 100 Gy (3.53cm), 50 Gy (3.42), 400 Gy (3.40), 150 Gy (3.39) and 200 Gy (3.31) in decreasing order. For the character pod length maximum co-efficient of variance was found in 50 Gy and 400 Gy followed by 150 Gy, 200 Gy and 100 Gy. Co-efficient of variance showed a positive shift in the value over the control.Maximum value of standard deviation for pod length was found in 100 Gy followed by 50 Gy, 400 Gy, 150 Gy and 200 Gy.

In the variety, RKS-18 the mean value for the character pod length was highest in 50 Gy, followed by 100 (3.27), 400 Gy (3.25), 150 Gy (3.20) and 200 Gy (3.15). Lowest mean value was recorded in control (3). Co-efficient of variance and standard deviation values were higher in radiation treatments than that of control. Highest co-efficient of variance value was recorded in 100 Gy, while highest standard deviation value was

recorded in 50 Gy.

Number of pods per plant :

The mutagen administered progeny showed decrease in the mean number of pods per plant as compared to the control. The maximum decrease in the number of pods per plant was observed in 150 Gy. A comparison of estimates of co-efficient variance indicated that the variance value increased in radiation doses in comparison to control. There was increase in co-efficient of variance value with the increasing dose of Gamma rays. Similarly, value of standard deviation was higher in radiation doses in comparison to control for number of pods per plant.

In the variety, RKS-18 the mean value for number of pods per plant showed negative deviation in radiation doses. There was decrease in the mean value with increase in radiation dose. Highest mean value was recorded in 50 Gy and lowest in 400 Gy. For the character number of pods per plant, the co-efficient of variance value was higher in radiation doses than control. Highest co-efficient variance value was recorded in 150 Gy (44.8) very closely followed by 100 Gy (43.2) and 50 Gy (43),while lowest was recorded in 200 Gy (21.5). Standard deviation value for number of pods per plant was highest in 50 Gy (8.95) followed by 150 Gy, 100 Gy, 200 Gy, control and 400 Gy in decreasing order.

Number of seeds per pod:

The mean value of number of seeds per pod increased with increase in doses of Gamma rays and there was a ppositive shift in the mean value over control except in 50 Gy. Maximum increase in mean value (3.32) was found in 100 Gy.The value of co-efficient of variance and standard deviation for the character number of seeds per pod decreased in radiation treatments, except in 50 Gy which showed higher value of co-efficient of variation and standard deviation than control.

In the variety RKS-18, the mean value for number of seeds per pod was identical in both control and radiation treatments.Highest mean value was recorded in 50 Gy and lowest value was recorded in 200 Gy.For the trait seeds per pod, co-efficient of variance and standard deviation values were lower in radiation doses except 50 Gy where they were higher than those of control.

100 seed weight:

Results showed that the mean values for this parameter were shifted in both negative and positive directions. Highest was observed in 100 Gy (12.38 g) and lowest was in 200 Gy and 400 Gy (9.5 g).There was decrease in the mean value of 100 seed weight with the increasing dose of gamma rays.The value of co-efficient of variance for the character 100 seed weight was lower than the control while it was comparatively higher in higher doses.Standard deviation for 100 seed weight was lower in radiation treatments in comparision to control.

In the variety RKS-18 the mean values of 100 seed weight showed a negative deviation in treatments from control. Among treatments highest mean value in treatments was recorded in 200 Gy (9.2 g) and lowest was in 50 Gy (8 g).The co-efficient of variance and standard deviation values for 100 seed weight were higher in radiation treatments than control. Highest co-efficients of variance as well as standard deviation were recorded in 200 Gy while lowest co-efficients of variance as well as standard deviation were recorded in 400 Gy.

Yield per plant:

In the variety BSS-2, mean values for seed yield per plant showed a negative shift over the control. There was random increase in the mean value. Highest mean value was recorded in 100 Gy (11.70 g) followed by 200 Gy (10.94 g). Lowest mean value was recorded in 150 Gy (4.20 g).The values of co-efficient of variance and standard deviation for seed yield per plant were higher in radiation treatments than that of control, except in 200 Gy where both values were lower than control.

In the variety RKS-18, mean value of yield was lower in Gamma treatments than the control and showed a negative deviation. Highest mean value was recorded in 50 Gy while the lowest was recorded in 400 Gy.The values of co-efficient of variance and standard deviation for yield per plant were higher in radiation treatments than those of control except 400 Gy which has the lowest co-efficient of variance and standard deviation value. Highest value was shown in 50 Gy.

Conclusion :

Per cent seed germination and seedling growth was found inhibited due to increasing dose of mutagen. Similar trend was also observed in survival percentage also**.** All the mutagen treatment except some brought about decrease in the plant height, number of primary branches per plant, days required for flowering, number of pods per plant, pod length, number of seeds per pod and seed

yield per plant in both the varieties BSS-2 and RKS-18. Almost all report on induced mutation studies in different crop plants have revealed physical damage in M_2 generation thereby inducing change in quantitative characters. The results observed are in conformity with the findings of Bolbhat *et al.* (2012) in two varieties of Horsegram. Other similar findings were of Misra (1992) in Blackgram, Rakshit and Singh (2001) in Mungbean and Urdbean, Rybinski (2003) in Grampea, Patil *et al.* (2004) in Soybean, Sharma *et al.* (2006) in Urdbean and Senapathi *et al.* (2008) in Blackgram. The probable reason may be that genes responsible for diverse types of traits, which are distributed throughout the genome, might have been affected by mutagens which resulted in different types of micro mutations (Senapati *et al.*, 2008, Bollhat, 2012 and Dhumal and Bolbhat (2012).

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