Applied Biological Research 23(3): 283-292; (2021)
DOI: 10.5958/0974-4517.2021.00038.0

EFFECT OF GAMMA IRRADIATION ON SOME SELECTED VARIETIES OF INDIAN MUSTARD (*Brassica juncea* L.)

Chamim Sultana Ahmed[1], Ranjan Das[1*], Lopamudra Homchaudhuri[2], Jyotsna Baruah[1], Sangita Das[1], Meren Toshi[1] and S.J. Jumbhulker[3]

[1]*Department of Crop Physiology, Assam Agricultural University, Jorhat - 785013, Assam (India)*
[2]*Departments of Chemistry, Assam Don Bosco University, Tepesia, Guwahati - 782 402, Assam (India)*
[3]*Board of Research in Nuclear Sciences (BRNS), Bhabha Atomic Research Centre (BARC), Bombay – 400 085 Maharashtra (India)*
*e-mail:*rdassam@yahoo.com*

(Received 27 April, 2021; accepted 31 July, 2021)

ABSTRACT

An experiment was conducted to study the impact of gamma (γ) rays on some quantitative traits such as germination and survival of seeds; morpho-physiological and yield attributes of Indian mustard (*Brassica juncea* L.). Two varieties of *B.juncea viz*., 'Pusa Bold' and 'Kranti', were used in the present study. The seeds of *Brassica* were irradiated with various doses of γ-ray (900, 1000, 1100 and 1200 Gy) and a combination treatment with ethyl methane sulfonate (1000 Gy + 2% EMS). The control seeds were not exposed to either radiation or EMS. The results revealed that doses of γ-radiation altered all the studied traits for both the tested varieties. Increasing doses of γ-radiation were responsible for decreasing the seed germination, plant survival, plant height, number of siliqua plant^{-1}, seed siliqua^{-1}, dry weight of whole plant, SPAD value, weight of dry husk plant^{-1}, leaf area and photosynthetic rate. Plants in M1 generation indicated dose-dependent physiological changes of genotype as compared to the control. This alternation of each traits indicated that γ-radiation has some effect on *Brassica* seeds which might be utilized as mutant for future variety development programme.

Key words: Ethyl methane sulfonate, gamma radiation, Indian mustard, mutagen, physiological traits

INTRODUCTION

Indian mustard (*Brassica juncea* L.) belongs to the family Brassicaceae and is an important oilseed crop of India. From the nutritional point of view, it is a rich source of vitamins and minerals. Due to the less variability, it is not suitable to grow under various agroecosystems of North-Eastern India (Salem *et al.*, 2017). Therefore, induced mutation breeding may play important role in creating genetic variability for earliness, yield contributing characters and adaptability to various abiotic stresses.

Mutation breeding is vastly used for developing improved crop varieties and producing genes for controlling important traits and understanding the function and mode of action of these genes. It might be an effective alternative to augment genetic variation, particularly for the traits having low level of genetic variation (Szarejko and Forster, 2007). Induction of mutation through γ-radiation can induce cytological, genetic (Harisand Jusoff, 2013), morphogenetic (El Sherif *et al*., 2011), biochemical (Chandrashekar *et al*, 2013) and some physiological variation in cell and tissues of plants (Rahimi and Bahrani, 2011; Jan *et al*., 2012). Induced mutations have been used mainly to generate variation in a comparatively short time that could rarely be found in germplasm. Only through a careful screening and selection programme, the magnitude of genetic variability induced by mutagens could be exploited for obtaining the desirable lines (Julia *et al.*, 2018).

Applying mutation techniques, there are several opportunities for development of new lines with different genetic variants with some adaptable traits under different abiotic as well as biotic stresses. It has been reported that various doses of γ-radiation affect the morphology, anatomy, biochemistry and physiology of plants, thereby inducing mutations (Ashraf *et al.*, 2003). Rahimi and Bahrain (2011) reported that different levels of irradiation affect plant height, harvest index, 1000-seed weight, seed yield, seed oil, linolenic, linoleic and oleic fatty acids. In okra, induced variability can be accessed at M_2 generation with 400 Gy treated plants in terms of growth and yield, but growth inhibition was observed at higher doses (Asare *et. al*., 2017).

Crops with improved characteristics may have successfully been developed by induction of physical and chemical mutagens; and positive mutations created in agricultural crops by using γ-irradiation as an important tool for improving production, quality under various conditions for sustainable agriculture. Mutation breeding techniques are based on physical and chemical mutagens which lead to breaking of DNA double strand (Yadav, 2016; Lamo *et al.*, 2017). The chemical agents such as nitrous acid, a determining agent and intercalating agent (causes deletion, reading frame shifts or random based insertion) and alkylating agent (adding and ethyl group which caused the modified base formation) are used (Girija *et al.*, 2013; Yadav *et al.*, 2016; Lamo *et al.*, 2017). The most used chemicals among these is ethyl methane sulfonate (EMS) which is an alkylating chemical that cause gene mutation in high frequency and chromosome aberration in low frequency (Ramakrishna *et al*., 2018; Channaoui *et al.*, 2019). The present research was carried out to study the effects of induced mutations by physical (γ-radiation) and chemical mutagen (EMS) on the morpho-physiological and biochemical changes in M1 generation. We studied the frequency and spectrum of macro-mutations along with the mutagenic effectiveness and physiological efficiency of different doses of γ-rays in *Brassica juncea* and characterized various mutants by development of a mutant line for stress tolerant.

MATERIALS AND METHODS

The seeds of two mustard genotypes *viz.*, 'Pusa Bold' and 'Kranti' were irradiated using 0, 900, 1000, 1100 and 1200 Gy doses of γ-rays and a combination of 1000 Gy γ-ray + 2% EMS to study the physiological responses of mustard. The seed were irradiated at BRNS, BARC, Trombay, India.

Well dried, fully matured disease and insect free seeds with uniform shape, size and colour were chosen for γ-irradiation. After γ-irradiation, the experiment was conducted in Stress Physiology Laboratory, AAU, Jorhat (India). The γ-ray treated seeds as well as untreated control were replicated thrice with 1000-seeds per replication. The seeds were then allowed to germinate in petri-dishes using moist germination paper. The percentage of germination and survival were recorded. In field study, the seeds were sown as per treatment comprising three replications from each group. The experimental field was prepared by ploughing and harrowing to bring the field into a good tilt and the seeds were sown in a factorial randomized block design as per treatment. Moisture stress condition was created by withholding irrigation during the whole period of crop growth. The moisture content of soil ranged from 7 to 8% and other metrological data were also recorded during the period of experimentation. Plant parameters were recorded from the plants that survived. Various agronomic and morphological characters were measured during crop cycle *viz*., seed germination percentage, plant survival percentage, plant height, number of siliqua plant^{-1}, seed siliqua^{-1}, dry weight of whole plant, leaf area and photosynthetic rate.

The seed germination was calculated as per the method of Anjum and Bajwa (2005).

$$\text{Germination percentage (\%)} = \frac{\text{No. of seeds germinated}}{\text{Total No. of seeds}} \text{X } 100$$

$$\text{Survival percentage (\%)} = \frac{\text{No. of plants survived}}{\text{Total No. of seeds sown}} \text{X } 100$$

The plant height was measured with the help of a meter scale from the base of plant to the apex of main axis. The area of fresh green leaves in each treatment was measured using portable leaf area meter **(**LICOR 3000, USA**)** and the values expressed in cm^2 $plant^{-1}$. For measurement of total plant dry weight, the plants in each treatment were uprooted at flowering stage and thoroughly washed to remove the adhered soil. Plants were blotted to remove any free surface moisture and kept in oven for drying at 70°C till they attained a constant weight and the dry weight was recorded.

The rate of photosynthesis was measured on leaves using a portable infrared gas analyzer (IRGA, LI-COR-6400, Lincoln, Nebraska, USA). The targeted leaf was inserted in the leaf chamber facing the sun. The vapour pressure was monitored on the display board after inserting the leaf, and flow rate was adjusted till a constant rate was obtained. The incident photon flux density was monitored from the quantum sensor mounted on leaf chamber. Assimilation by the enclosed leaf area caused depletion of CO_2 concentration inside the chamber and this depletion was measured for three consecutive times of 10 sec each. Based on CO_2 depletion and other factors such as temperature, pressure and volume of enclosure, the rate of photosynthesis was computed by the inbuilt micro-computer of IRGA, and the values expressed in µmol $m^{-2}s^{-1}$.

The number of siliqua was counted from tagged plant, then the average values calculated and expressed as the number of siliqua $plant^{-1}$. The number of seed was counted from individual siliqua and then the average value was calculated and expressed as number of seed $siliqua^{-1}$.

The data collected was statistically analyzed as per the method of analysis of variance (Panse and Sukhatme, 1967). The critical difference values were calculated at 5% probability level.

RESULTS AND DISCUSSION

Seed germination

The highest seed germination in both the mustard varieties tested was found in control. Among the treatments, the highest seed germination was recorded in 'Pusa Bold' at 900 Gy, followed by 'Kranti' at 900 Gy (Table 1). The lowest seed germination was noted in 'Kranti' treated with 1200 Gy. The mustard variety, 'Pusa Bold' seemed to have some degree of tolerance for radiation within

Table 1: Effect of γ-irradiations on seed germination of *Brassica* spp.

Treatments (T)	*Brassica* varieties		Mean
	Pusa Bold	Kranti	
Control	89.09	86.57	87.83
1000 Gy + EMS	27.98	24.52	26.25
900 Gy	47.53	44.16	45.84
1000 Gy	35.78	29.28	32.53
1100 Gy	27.62	21.13	24.37
1200 Gy	22.42	20.57	21.50
Mean	41.74	37.71	-
$CD_{0.05}$			
T		0.008	
Variety (V)		0.014	
T × V		0.020	

In case of combined action of mutagen (1000 Gy + EMS) treatment, a significant decrease in seed germination was recorded as compared to the control, 900 Gy and 1000 Gy treatments. This might be due to the combined action of mutagen and their effect on cellular damage. Abdel-Hady *et al.* (2008) reported that lower doses of γ-ray may activate RNA or protein synthesis during early stages of germination. Further, the higher doses of γ-radiation might have affected the meristematic tissues of seed resulting in reduced seed germination. In maize, Marcu *et al.* (2013) reported that with increase in irradiation dose, the germination potential, root and shoot length and pigment content decreased with increasing dose of irradiation. Mashev *et al*. (1995) reported that irradiation with doses above 0.1 k Gy caused a significant decline in grain yield of wheat. Similarly, γ-ray treatments in three wheat cultivars caused a delay in germination and decrease the survival and plant height (Irfaq and Nawab, 2001). Growth inhibition induced through high-dose irradiation is attributed to the cell cycle arrest in G2/M phase during somatic cell division and/or to a variety of damages in the entire genome (Preuss and Britt, 2003). Processes like auxin destruction, changes in ascorbic acid content, physiological and biochemical disturbances could induce the inhibition of plant germination and development (Shah *et al*., 2008). γ-radiation could reduce rooting and root lengths thus affect seed vigour in some crops (Chaudhuri *et al*., 2002).

Plant survival

In the present study, a decreasing trend of survival percentage was observed with the increasing dose of radiation in both the varieties (Fig. 1). In control, higher plant survival was noticed in cv. ‘Pusa Bold’ as compared to that of cv. ‘Kranti’. Among the γ-irradiated varieties, cv. ‘Pusa Bold’ treated with 900 Gy showed highest plant survival, whereas cv. ‘Kranti’ treated with 1000 Gy + EMS depicted lowest plant survival. Higher exposures of γ-ray might have caused seed injury, thereby adversely affecting the plant survival in higher dose of γ-radiation.

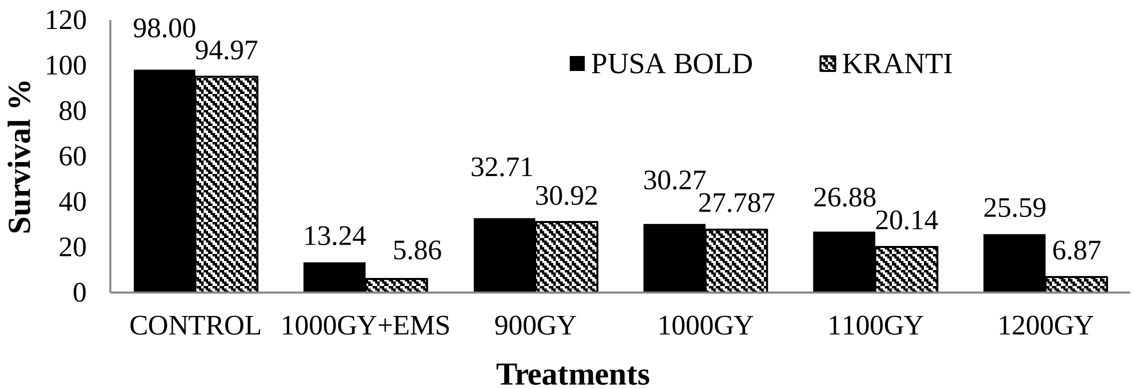

Fig. 1: Effect of γ-radiations on plant survival (%) of *Brassica* spp.

Kiong *et al.* (2008) reported that the plant survivability to maturity after mutation depends on the nature and extent of chromosomal damage. They reported that γ-irradiated seeds were unable to germinate or survive more than a few days due to metabolic disorders in the seeds. In present study, survivability of cv. ‘Pusa Bold’ was higher than cv. ‘Kranti’ under 900 Gy treatment. A significant decrease in survivability was noticed in combination treatment. Deshmukh and Malode (2018) reported that higher doses of γ-radiation affected plant survivability as compared to the lower doses of radiation in *Dianthus caryophyllus* var. ‘Chabaud’.

It was noted that mutagenic effectiveness and efficiency increased with decrease in dose or concentrations. Similar findings were reported by Anbarasan *et al.* (2015) in sesame (*Sesamum indicum*). They reported that γ-rays and EMS produced a high frequency as well as a wide spectrum of mutation and that γ-rays were more effective and efficient in causing mutations as compared to EMS treatments.

Plant height

In present study, γ-rays induced a significant variation in plant height among the varieties as well as among the treatments (Table 2). Among the varieties, effect of γ-radiation was higher in cv. ‘Kranti’ than cv. ‘Pusa Bold’. Among treatments, highest plant height was recorded in control, followed by 900 Gy treatment. A gradual decrease in plant height was noticed when radiation dose was gradually increased. Lowest plant height was found in the plants treated with 1000 Gy + EMS.

Table 2: Effect of γ-radiations on plant height and dry weight of whole plant in mustard

Treatments (T)	Plant height (cm)			Dry weight of whole plant (kg)		
	Pusa Bold	Kranti	Mean	Pusa Bold	Kranti	Mean
Control	166.01	176.16	171.085	1.67	1.56	1.62
1000 Gy + EMS	146.57	77.74	112.155	1.36	1.14	1.25
900 Gy	160.25	171.62	165.935	1.63	1.44	1.54
1000 Gy	153.84	132.56	143.2	1.46	1.15	1.31
1100 Gy	150.28	120.68	135.48	1.46	1.06	1.26
1200 Gy	148.8	77.26	113.03	1.45	1.02	1.24
Mean	154.29	1260	-	1.51	1.23	-
$CD_{0.05}$						
T		4.097			0.024	
Variety (V)		7.096			0.041	
T × V		10.035			0.059	

The interaction of mutagenic treatment and varieties revealed lowest plant height in cv. ‘Kranti’ treated with 1200 Gy, followed by cv. ‘Kranti’ treated with 1000 Gy + EMS; whereas, the highest plant height was found in cv. ‘Kranti’ treated with 900 Gy, followed by cv. ‘Pusa Bold’ treated with 900 Gy. The reduction in plant height in higher doses of γ-irradiation and combined treatment can be attributed to the reduction in mitotic activity of meristematic tissue and reduced moisture content in seeds (Khan *et al*., 2014). The low doses of γ-irradiation could stimulate cell division/cell elongation which might have enhanced plant growth; whereas higher doses of γ-radiation could alter the metabolic processes affecting phytohormones or nucleic acids synthesis (Gunckel *et al.,* 1961; Mujeeb and Greij, 1976). The lower irradiation doses induce growth stimulation by altering the hormonal signaling network in plant cells or by increasing the antioxidative capacity of cells to easily overcome stress factors such as fluctuations of light intensity and temperature in the growth condition (Wi *et al.,* 2007). Similar results were obtained by El-Ashry *et al.* (1992) in *Lathyrus odoratus* and El-Mahrouk *et al.* (2000) in *Gomphrena globosa.*

Dry weight of whole plant

Significant variations in plant dry weight were observed in varieties and treatments (Table2). The mean plant dry weight decreased with increase in γ-radiation doses. Among the varieties, cv. ‘Pusa Bold’ yielded greater dry matter than cv. ‘Kranti’. Amongst the treatments, lowest plant dry weight was found in 1200 Gy treatment, followed by 1000 Gy + EMS treatment, and highest dry weight in control, followed by 900Gy treatment. The interaction of mutagenic treatments and varieties revealed that cv. ‘Pusa Bold’ treated with 900 Gy yield highest plant dry weight, followed by cv. ‘Pusa Bold’ treated with 1000 Gy; while cv. ‘Kranti’ at 1200 Gy showed least plant dry weight, followed by cv. ‘Kranti’ treated with 1100 Gy.

The dry weight of whole plant is directly proportional to the seed yield and plant height. The increase in plant dry weight in 900 Gy treatment could be attributed to the increase in leaf area (Ramesh *et al*., 2012). The improvement in plant height might be due to greater photosynthesis leading to greater biomass accumulation (Table 2). Our results are in line with Raghava (1989) and Baba (2015) who reported that the increase in dry matter and seed yield may be due to the increased photosynthetic rate.

Leaf area

Among the treatments, highest reduction in leaf area was observed in 1200 Gy treatment; whereas highest leaf area was found in control. Significant differences were found in interaction treatments. Lowest leaf area plant^{-1} was observed in cv. ‘Kranti’ followed by cv. ‘Pusa Bold’ treated with 1200 Gy. However, reduction in leaf area was low in cv. ‘Kranti’ with 900 Gy treatment than cv. ‘Pusa Bold’ treated with same γ-radiation dose (Table 3).

The leaf area in a plant is directly related to the leaf number, plant height and photosynthetic rate (Khalil *et al.*, 1986). In present study, high irradiation doses adversely reduced the number of

Table 3: Effect of γ-radiations on leaf area, photosynthesis rate ($\mu mol^{-1} mol^{-1} cm^{-2}$) and SPAD value in mustard

Treatments (T)	Leaf area (cm^2 $plant^{-1}$)			Photosynthesis rate (μmol^{-1} mol^{-1} cm^{-2})			SPAD value		
	Pusa Bold	Kranti	Mean	Pusa Bold	Kranti	Mean	Pusa Bold	Kranti	Mean
Control	445.0	433.0	439.0	23.31	23.90	23.60	55.48	51.52	53.50
1000 Gy + EMS	389.0	385.0	387.0	21.01	22.54	21.78	44.21	35.29	39.75
900 Gy	410.4	414.4	412.4	23.15	23.57	23.36	49.99	42.81	46.40
1000 Gy	403.8	397.8	400.1	20.89	21.03	20.96	48.87	42.05	45.46
1100 Gy	386.4	378.4	382.4	19.60	19.81	19.71	45.48	41.12	43.30
1200 Gy	373.6	371.2	372.4	19.30	19.32	19.31	47.47	40.71	44.09
Mean	401.37	396.63	-	21.21	21.70	-	48.58	42.25	-
$CD_{0.05}$									
T	1.708			0.258			0.791		
Variety (V)	2.959			0.447			1.370		
T × V	4.185			0.633			1.937		

leaves. Our findings are in conformity with Ochatt *et al.* (2001). Under low doses, the production of growth regulator like kinetin might have been stimulated, thereby leading to increase in leaf number (Kiong *et al.*, 2008). Dubey *et al.* (2007) noted increase in leaf number when okra seeds were irradiated with different doses of γ-rays.

Photosynthesis rate

The photosynthesis rate is affected by the exposure of seed to different doses of γ-rays (Table 3). Among the treatments control showed highest photosynthesis rate followed by 900 Gy while lowest photosynthesis rate was recorded at 1200 Gy. Among the varieties, the higher photosynthesis rate was found in cv. 'Kranti' as compared to cv. 'Pusa Bold'. Due to the treatment and variety interaction cv. 'Kranti' at 900 Gy showed highest photosynthesis rate due to the application of lower doses of γ-radiation. On the other hand, cv. 'Pusa Bold' 1200 Gy showed lowest photosynthesis rate followed by 'Kranti' 1200 Gy. Decrease in photosynthesis due to γ-irradiation has been attributed to the interaction of atoms or molecules producing free radicals in cells. These radicals have been reported to damage or modify the important component of plants e.g. thylakoid membranes, alteration in photosynthesis (Kim *et al.*, 2004; Wi *et al.*, 2007; Ashraf, 2009). Therefore, higher doses of irradiation adversely affected the rate of photosynthesis.

SPAD (Soil plant analysis development) value

Present study revealed highly significant effects of γ-ray on SPAD value varieties as well as among the treatments (Table 3). Among the varieties, the effect of γ-radiation was high in c. 'Pusa Bold' than cv. 'Kranti'. Highest SPAD value was noted in control followed by 900 Gy treatment. The application of higher dose of γ-radiation and chemical 1000 Gy + EMS showed lowest SPAD value. An increase in SPAD value is positively related to chlorophyll content in leaf.

The interaction of treatment and variety showed lowest SPAD value in cv. 'Kranti' 1000 Gy + EMS and highest in cv 'Pusa Bold' 900 Gy. According to Saha *et al.* (2010) chlorophyll content of plants decreased gradually after irradiation which indicated that photosynthetic pigments are destroyed by γ-rays with a concomitant loss of photosynthetic capacity. Similar observation was noticed in present study. Kiong *et al.* (2008) reported that γ-irradiation results in greater destruction of chlorophyll *b* than that of chlorophyll *a* due to the disturbance in biosynthesis or degradation of its precursors.

Number of siliqua per plant

A significant variation was noticed in the number of siliqua $plant^{-1}$ in various doses of γ-irradiation in the current study. Among the varieties, highest number of siliqua $plant^{-1}$ was recorded in 'Pusa

Bold' than that of cv. 'Kranti' (Table 4). The number of siliqua plant^{-1} decreased with increase in γ-radiation doses. The highest number of siliqua plant^{-1} was found in control, followed by 900 Gy; whereas lowest was noted in 1000 Gy + EMS treatment. There was a significant variation due to the interaction of treatment and variety. The increase of the number of siliqua plant^{-1} may attributed to the stimulation of growth hormones at lower γ-radiation doses. These results are in conformity with Khan *et al.* (2014) in *Brassica napus* L.

Table 4: Effect of γ-radiations on number of siliqua plant^{-1}, seed siliqua^{-1} and seed yield plant^{-1} in mustard

Treatments (T)	Number of siliqua plant^{-1}			Seed siliqua^{-1}			Seed yield plant^{-1} (g)		
	Pusa Bold	Kranti	Mean	Pusa Bold	Kranti	Mean	Pusa Bold	Kranti	Mean
Control	218.10	163.00	190.55	9.00	9.20	9.10	31.75	30.25	31.00
1000 Gy + EMS	191.40	93.60	142.50	5.80	4.00	4.90	14.25	12.75	13.50
900 Gy	212.60	112.00	162.30	8.00	8.60	8.30	29.75	28.25	29.00
1000 Gy	205.50	106.80	156.15	7.00	5.80	6.40	24.50	23.00	23.75
1100 Gy	207.75	99.20	153.48	6.60	5.60	6.10	20.50	20.25	20.38
1200 Gy	198.00	96.20	147.10	5.60	5.20	5.40	17.25	15.25	16.25
Mean	205.57	111.80	-	7.00	6.40	-	23.00	21.63	-
$CD_{0.05}$									
T		24.7			0.647			0.809	
Variety (V)		42.7			1.120			1.401	
T × V		60.4			1.584			1.981	

Seed siliqua^{-1}

γ-irradiation significantly affected the varieties in terms of seed siliqua^{-1} (Table 4). Rice cv. 'Kranti' was highly susceptible to the high doses of γ-irradiation than cv. 'Pusa Bold'. Among the treatment, 1000 Gy + EMS gave lowest seed siliqua^{-1} followed by 1200 Gy due to the negative effect of higher dose of γ-radiation. Control plants showed highest seed siliqua^{-1} followed by 900 Gy. Amongst treatment and variety interaction, 'Kranti' with 900 Gy treatment showed highest seed siliqua^{-1}.

Lower doses of γ-irradiation might have stimulated the enzyme and growth hormones responsible for growth and yield of seed (Wisal *et al*., 2014), while excess dose of γ-radiation reversed the whole procedure and was responsible for decrease in seed siliqua^{-1}. Increased siliqua number plant^{-1} at lower doses of γ-irradiation has been reported (Dubey *et al*., 2007, Mishra *et al.*, 2007; Sharma and Mishra, 2007). It has been observed that 400 Gy exposure stimulated increase in the number of fruits plant^{-1} in okra (Asare *et al.*, 2017). Our findings revealed increased the number of seed siliqua^{-1} under 900 Gy treatment in both the varieties indicating variation in dose response depending upon the crop species. Pushparajan *et al.* (2014) have found that 400 Gy increased fruit character. Increase in yield of crops treated with mutagens has been reported (Shamsi and Sofajy, 1980; Shamusuzzaman *et al.*, 2005).

Seed yield per plant

A significant variation was noticed in the seed yield plant^{-1} for various doses of gamma irradiation. Among the varieties, highest seed yield plant^{-1} was recorded in Pusa Bold as compared to Kranti (Table.4). In the present study, it was observed that the seed yield plant^{-1} decreases with the increasing doses of gamma radiation. The highest seed yield plant^{-1} was noted in control, followed by 900 Gy; whereas the lowest was recorded at 1000 Gy + EMS. There was a significant variation due to the interaction of treatment and varieties. Rice cv. 'Kranti' at 1000 Gy + EMS showed lowest seed yield plant^{-1} whereas cv. 'Pusa Bold' at 900 Gy showed the highest seed yield plant^{-1}.

Conclusion: From the study it may be concluded that the higher doses of γ-irradiation effects the quantitative traits including seedling survival and germination behaviour. Among the γ-irradiation treatments, 900 Gy had some stimulatory effect on various traits related to growth. Again, among the two varieties cv. 'Pusa Bold' proved superior over cv. 'Kranti' in terms of seed germination, plant survival, plant height, number of siliqua plant^{-1}, seeds siliqua^{-1}, dry weight of whole plant, SPAD value, leaf area and photosynthetic rate which influenced the yield. The change in the traits is one of the most promising challenges for development of new mutant through γ-irradiation and that mutants can be used for future varietal development programme for resistance breeding.

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