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Gamma irradiation sensitivity and optimal level for induction of mutation in tuberose (*Polianthes tuberosa*)

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ABSTARCT

To evaluate the sensitivity, proper bulb stage and optimal gamma irradiation dosage to induce the attractive mutant in tuberose (*Polianthes tuberose* L.), a field experiment was conducted in 2008, 2009, 2010 and 2011 at the Indian Agricultural Research Institute, New Delhi. Cultivars Prajwal and Phule Rajani with three different bulb stages, viz. freshly harvested bulb (B_0), three weeks after uprooting (B_1) and six weeks after uprooting (B_2) were irradiated by 2.5 Gy, 5.0 Gy, 7.5 Gy, 10.0 Gy and 15.0 Gy of gamma rays. Probable LD_{50} dose of gamma irradiation was between 10.0 Gy to 12.0 Gy for freshly harvested bulbs and bulbs after six weeks after uprooting in cv. Prajwal and in all the bulb stages of cv. Phule Rajani. For bulbs after three weeks after uprooting probable LD_{50} dose was 3.25 Gy in cv. Prajwal and 10.25 Gy in cv. Phule Rajani. Non sprouting of Prajwal bulbs at B1 stage beyond 2.5 Gy was supported by the histological results. The all three stages of bulbs have showed different response over gamma irradiation. In general, we have observed sprouting and all the vegetative parameters decreased over increased irradiation level. Out of three bulb stages tested, freshly harvested bulbs (B_0) responded comparably high to the gamma irradiation doses followed by bulbs after six weeks of uprooting (B_2) in both the tuberose cultivars. From this study, it was concluded that gamma irradiation dose 7.5 Gy to 11.5 Gy could yield some attractive and useful mutants in tuberose.

Key words: Bulb stages, Gamma radiation, Radiosensitivity, Tuberose

Genetic improvement of tuberose (*Polianthes tubesose* L.) is hampered by meager genetic variability, self incompatibility and seed sterility. Nuclear technology has benefited greatly in genetic improvement of seed and vegetative propagated crops worldwide (Jain 2005 and Predieri 2001). The dose to be applied for obtaining a high mutagenic efficiency generally depends on the specific properties of the radiation type, biological system and the radiation facility. The current study, to finalize the feasible gamma radiation dose which would ultimately create useful variation in tuberose, physical mutagen-gamma radiation was utilized. Gamma radiation treatment has advantages over the chemical mutagen like there is no residual effect; uniformity in penetration, less time consuming and a large number of samples can be treated in less time. Gamma radiation have provided an high number of useful mutants (Predieri 2001) and is still showing an elevated potential for improving vegetative propagated plants. Apart from the choice of the proper mutagenic agent, the dosage, the treatment conditions, plant material and the stage of plant material to be irradiated are the important criteria to enhance maximum mutation efficiency. For example: the stage in the life cycle of the plant and plant organs; the sensitivity of the plant species to the effects of the mutagenic agents; the possible genotypic differences in sensitivity in the mutagenic treatments etc. A precise method for the determination of the radiation dose absorbed has been proposed by Neville *et al*. (1998). However, the common procedure in assessing the most appropriate dosage is based on radiosensitivity, which is estimated through the response of the irradiated material.

Many vegetatively propagated plants including tuberose are with complicated physiology (e.g. dormancy) and complex genetics (e.g. high degree of heterozygocity, self-incompatible). In tuberose spontaneous mutations-'sports' have not been recorded till date. Though, an ample number of scientific literatures are available on mutation study in tuberose, resulted only in variegated new leaf mutants but the current experiment used three different bulb stage and different dose of gamma radiation in two cultivars of tuberose to obtain large number of useful mutants with respect to flower parameters. With this background, the present study was taken up with the aim to find out radiosensitivity stage and optimal gamma irradiation doses for the induction of some useful mutants in tuberose cultivars Prajwal and Phule Rajani. This experiment would yield the LD_{50} dose for tuberose. The

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LD_{50} is a dose at which 50% of the plant material survives is generally accepted as the optimum irradiation dosage for the purpose of creating new varieties because an acceptable number of cuttings survive while a large number of mutations are obtained.

MATERIALS AND METHODS

The present study was carried out at the Research Farm of Division of Floriculture and Landscaping, IARI, New Delhi, during 2009 to 2011. The field experiments were carried out on two single type tuberose cultivars Prajwal and Phule Rajani with three different bulb stages, i.e. B_0 - Freshly harvested bulbs, (Bulbs used for irradiation on the same day of uprooting), B_1 - three weeks after uprooting, (Bulbs stored at room temperature up to three weeks after uprooting and irradiated on next day of third week), B_2 - six weeks after uprooting (Bulbs stored at room temperature up to six weeks after uprooting and irradiated on next day of sixth week). Bulbs of each bulb stage of both the cultivars were irradiated with five different gamma radiation dose at Gamma Chamber, Nuclear Research Laboratory, IARI, New Delhi, i.e. G_1 - 2.5 Gy, G_2 - 5.0 Gy, G_3 - 7.5 Gy, G_4 – 10.0 Gy and G_5 – 15 Gy along with control (G_0 - 0 Gy) without irradiation. In total, there were 18 treatments for one tuberose cultivar, i.e. Prajwal, which includes (T_1); B_0 - Freshly harvested bulbs + G_0 - 0 Gy (T_2); B_0 - Freshly harvested bulbs + G_1- 2.5 Gy (T_3); B_0- Freshly harvested bulbs + G_2 - 5.0 Gy (T_4); B_0 - Freshly harvested bulbs + G_3 - 7.5 Gy (T_5); B_0- Freshly harvested bulbs + G_4 – 10.0 Gy (T_6); B_0 - Freshly harvested bulbs + G_5 – 15 Gy (T_7); B_1 - three weeks after uprooting + G_0 - 0 Gy (T8); B_1 - three weeks after uprooting + G_1 - 2.5Gy (T_9); B_1 - three weeks after uprooting + G_2 - 5.0 Gy (T_{10}); B_1 - three weeks after uprooting + G_3 - 7.5 Gy (T_{11}); B_1 - three weeks after uprooting + G_4 – 10.0 Gy (T_{12}); B_1 - three weeks after uprooting + G_5 – 15 Gy (T_{13}); B_2 - six weeks after uprooting + G_0 - 0 Gy (T_{14}); B_2- six weeks after uprooting + G_1 - 2.5Gy (T_{15}); B_2 - six weeks after uprooting + G_2 - 5.0 Gy (T_{16}); B_2 - six weeks after uprooting + G_3 - 7.5 Gy (T_{17}); B_2 - six weeks after uprooting + G_4 – 10.0 Gy (T_{18}); B_2- six weeks after uprooting + G_5 – 15 Gy. The same treatments were also followed in another tuberose cultivar i.e. Phule Rajani. The layout was prepared in consideration of randomized block design. There were six RBD layout replicated thrice for each of the three bulb stages in both the tuberose cultivars. Gamma irradiated bulbs were planted in 2×2 m plots. The B_0 and B_1 stages bulbs of both tuberose cultivars were planted on 12 March 2009. The B_2 stage bulbs of both tuberose cultivars were planted on 2 April 2009. Each treatment replication contained 36 bulbs. In total 3888 bulbs were irradiated from all three bulb stages of cultivars Prajwal and Phule Rajani.

The total number of plants survived among the sprouted bulbs in each treatment with respect to three bulb stages was recorded after thirty days of bulb sowing. LD_{50} (Lethal dose-50) was estimated on the basis of survival per cent. The observations of five plants from each of three replications were recorded on 6 important vegetative parameters to determine the effects of gamma irradiation on vegetative parameters of tuberose cultivars Prajwal and Phule Rajani, i.e. days to sprouting, plant height (cm), number of tillers, number of leaves at the time of heading, length and width of longest (cm) leaf. Any abnormality or variations with respect to leaf and other vegetative parameters in different treatments were also recorded.

In order to find out the cause for non sprouting of cv. Prajwal bulbs (B_1 stage) beyond 2.5 Gy of gamma irradiation, histology study was done. For this, bulbs of cv. Prajwal were uprooted and stored for three weeks, under normal room temperature. After completion of three weeks the bulbs were irradiated with the gamma-rays with the same dosage level, i.e. 5.0 Gy, 7.5 Gy and 10.0 Gy, which were used during the first generation irradiation time. The central growing buds of these irradiated bulbs were fixed by putting into FA solution for 48 hr followed by 70 per cent alcohol, by this step the tissues were fixed. These fixed tissues were subjected to standard dehydration protocol using series of TBA (Tri butyl alcohol) and liquid paraffin, viz. Dehydration Series - 10% TBA (2h) - 20% TBA (Over night) - 35% TBA (1h) - 55% TBA (1h) - 75% TBA (1h) - Pure TBA 3 changes, 2 changes for 1h and 3rd change after over night - 50% Liquid Paraffin (2h) - Solid Paraffin (Over night) - Cavity Block. These blocks were cut in to equal sized cubes and microtome was done by using microtome machine (LEICA RM 2162, Leica) and slides were prepared using Houpts solution. The Staining of slides was done using Tolvidin Blue. These slides were made to follow staining steps, viz. Sections - Xylene (2h or Over night) - 1:1 Xylene : Absolute Alcohol (5 min) - 95% Alcohol (5 min) - FAST GREEN (a dip) - 95% Alcohol (5 min) - 1:1 Xylene : Absolute Alcohol (5 min) - Pure Xylene - DPX mount - Final prepared slide. After the final mounting, the slides were observed under compound microscope at 100X resolution to notice the change which was induced in the bulb cells of cv. Prajwal by gamma radiation.

RESULTS AND DISCUSSION

Effect on bulb sprouting and survival

Radiation level required, sensitivity to radiation is known to differ according to age of tissue, between varieties and even as a function of single gene differences. In our present study with respect to radiosensitivity of tuberose cultivars Prajwal and Phule Rajani (Fig 1 a,b), we have observed the sprouting per cent, survival per cent and response of all the vegetative parameters. The sprouting per cent in freshly harvested bulbs (B_0) of both the cultivars are not much affected by the lower dose of gamma rays rather the lower dose of gamma irradiation (2.5 Gy and 5 Gy) increased the sprouting per cent of freshly harvested bulbs of cv. Prajwal than the control and in higher doses 7.5 Gy and 10.0 Gy of gamma irradiation there was decreased sprouting per cent in both the cutivars. Our study is in conformity with the findings of different researchers (Uzenbaev and Nazarenko 1970, Seilluer 1975, Misra and Choudhury 1978). Their work revealed that lower doses of gamma irradiation enhance the sprouting while higher doses delayed the sprouting. B_1 bulb stage (three weeks after uprooting) of cv. Prajwal was found highly sensitive to gamma irradiation, there was no sprouting observed beyond 2.5 Gy. Even though three weeks after uprooted bulbs stage (B_1) of cv. Phule Rajani responded to all the doses of gamma irradiation, significant decrease in sprouting was observed above 2.5 Gy. This higher radiosensitivity could be due to their less developed meristems (Katagiri and Lapins 1974). In B_2 (six weeks after uprooting) bulb stage, sprouting was decreased at 7.5 Gy and 10.0 Gy in both the tuberose cultivars. This could be due to at this stage bulbs might have a large cell population, greater apical size during recovery and faster rate of growth increase the radio tolerance to irradiated pieces from the lower dose of irradiations (Langenauer *et al*. 1973). The fact for lower sprouting at higher dose of gamma irradiation could be level of auxin concentration in plants which drops after exposure to ionizing radiation (Gorden 1957). Overall we observed that there was complete lethality at 15.0 Gy gamma irradiation dose across the bulb stages and cultivars. Abraham and Desai (1976a) concluded from their study that high sensitivity of bulbs to radiation particularly to gamma rays may be due to high moisture content of the buds in the bulbs, they also said that this may be the main reason for low RBE values obtained in bulbs plant they used (tuberose , gladiolus, amaryllis). In some of previous radiation studies they stated that tuberose can tolerate gamma irradiation upto 25.0 Gy. In our study, the tolerance

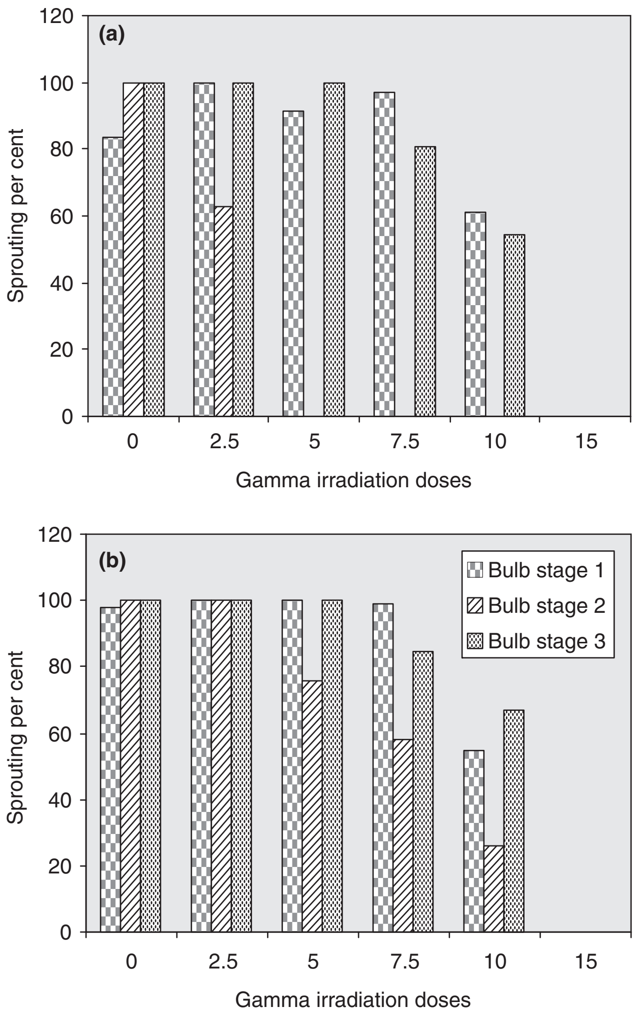

Fig 1 a,b. Effect of gamma irradiation on per cent sprouting in tuberose cv. Prajwal and Phule Rajani. Bulb stage 1- Freshly harvested bulbs; Bulb stage 2- three weeks after uprooting; Bulb stage 3- six weeks after uprooting.

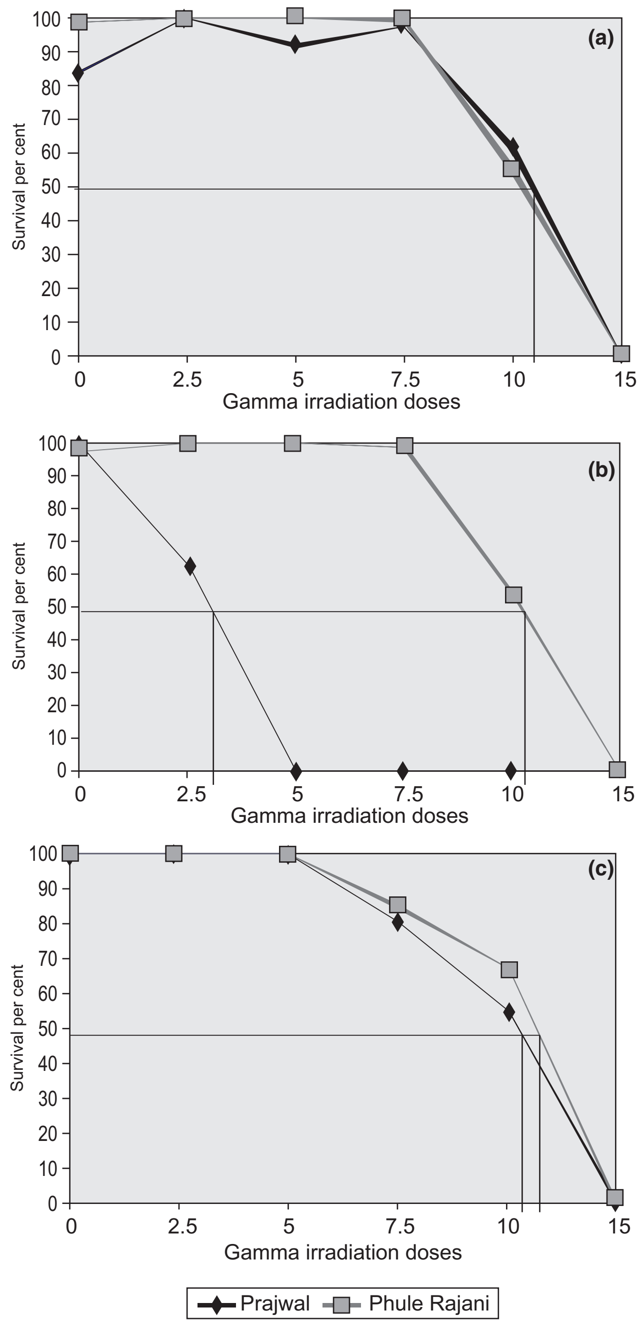

Fig 2 LD_{50} dose of gamma irradiation for Prajwal and Phule Rajani. (a) B_0 Bulb stage, (b) B_1 Bulb stage and (c) B_2 Bulb stage.

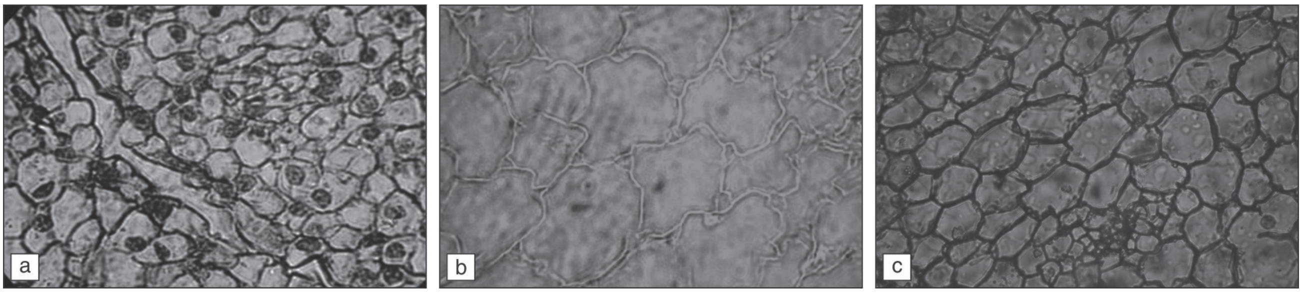

Fig 3 Effect of gamma irradiation on cells of three weeks of uprooted bulbs of tuberose cv. Prajwal. a) Cell arrangement in non-irradiated tissue, b) non nucleated deformed cells after irradiation, c) increased vacuoletion inside the cells.

limit was upto 12 Gy only. This might be due to the varied radiosensitivity of the varieties (Nilan 1956).

In our study, we observed least mortality after sprouting across the bulb stages of both the tuberose cultivars so the survival per cent was calculated based on the number of bulbs planted upon number of plants survived. By using this, we estimated the LD_{50} dose of gamma radiation for three bulbs stage of cv. Prajawl and Phule Rajani (Fig 2). According to the estimation, it was observed that LD_{50} (Lethal Dose 50) has been ascertained to be beyond 10.0 Gy for freshly harvested bulbs (B_0) and bulbs of six weeks after uprooting (B_2) in both the tuberose cultivars, which is in conformity to the finding of Raghava *et al*. (1988). But in our study B_1 bulb stage (three weeks after uprooting) was found highly sensitive to gamma irradiation in cv. Prajwal, the LD_{50} observed for this bulb stage was 3. 25Gy. This may be the first finding by using different bulb stage of tuberose cv. Prajwal and Phule Rajani to observe their response to gamma irradiation.

From the histology study, we have observed that cells of all three (5.0 Gy, 7.5 Gy and 10.0 Gy) gamma irradiated bulbs were affected severely after irradiation (Fig 3). In non irradiated tissue we could observe that almost all the cells were arranged properly with nucleus in them. But after irradiation it was observed that about 90% cells were without nucleus. The cells were bigger in size and shape of the cells became deformed. The other effect like outer epidermal layer was completely damaged at 10.0 Gy of gamma irradiation. Most of the bundle sheath cells were also damaged in gamma irradiated cells (5.0 Gy, 7.5 Gy and 10.0 Gy). In one of the slide we have clearly observed more vaculation in most of the cells which were devoid of nucleus.

Effect on vegetative growth parameter

In order to support our radio sensitivity study we observed effect of gamma irradiation on all the vegetative parameters such as days to sprouting, plant height and number of tillers per clump (Table 1) and on some of the leaf parameters (Table 2).

In general, there was an adverse effect of the gamma irradiation on vegetative parameters with the increased level of doses across the bulb stages on both the tuberose cultivars Prajwal and Phule Rajani. In days to sprouting more number of sprouting days was observed at 10.0 Gy in B_0 bulb stages of cv. Prajwal (32.67) and Phule Rajani (33.33). It revealed that higher doses of gamma irradiation suppressed sprouting and the time taken for sprouting was increased (Anu *et al*. 2003) in tuberose. At 10.0 Gy the plant height was harshly affected in all the bulb stages of both the cultivars. Similar kind of result has been reported earlier by Banerji *et al*. (1994) in gladiolus. This may be caused by the reduced amount of endogenous growth regulators, especially the cytokinin as a result of breakdown or lack of synthesis due to irradiation (Omar 1988). Reduction in plant height due to gamma radiation treatment

Table 1 Effect of gamma irradiation on vegetative growth parameters of tuberose cvs. Prajwal and Phule Rajani

Treatment	Days to sprouting of bulbs						Plant height (cm)						Number of tillers per clump					
	Prajwal			Phule Rajani			Prajwal			Phule Rajani			Prajwal			Phule Rajani		
	B_0	B_1	B_2	B_0	B_1	B_2	B_0	B_1	B_2	B_0	B_1	B_2	B_0	B_1	B_2	B_0	B_1	B_2
Control	28.67	20.33	16.67	28.00	19.00	15.67	74.82	81.64	91.35	71.12	69.87	72.26	8.53	8.37	9.20	10.17	10.07	12.20
2.5 Gy	26.67	25.67	19.67	26.33	20.67	18.67	70.86	18.65	79.87	66.48	63.32	73.69	7.93	0.33	8.20	10.40	9.17	10.23
5.0 Gy	27.33	0.00	17.67	26.67	23.00	20.33	58.54	0.00	74.83	59.95	59.30	62.46	7.13	0.00	6.70	8.63	8.50	6.67
7.5 Gy	29.33	0.00	21.00	30.00	28.33	22.00	48.47	0.00	59.15	52.82	53.40	53.44	4.93	0.00	6.43	7.33	3.73	10.57
10.0 Gy	32.67	0.00	23.33	31.33	29.00	23.00	12.97	0.00	8.44	11.63	9.18	5.98	3.07	0.00	3.53	2.80	0.67	3.33
Mean	28.93	9.20	19.67	28.47	24.00	19.93	53.13	20.05	62.73	52.40	51.01	53.57	6.32	1.74	6.81	7.87	6.43	8.60
CV	5.07	8.99	7.31	6.92	5.38	7.07	11.04	5.21	6.65	11.98	10.76	11.12	9.43	14.61	12.04	12.61	9.08	12.99
SE	0.85	0.20	0.83	1.14	0.75	0.81	3.39	0.49	2.41	3.62	3.17	3.44	0.23	0.15	0.47	0.57	0.34	0.64
CD (P=0.05)	2.76	0.64	2.71	NS	2.43	2.65	11.04	1.60	7.86	11.82	10.34	11.22	0.75	0.48	1.54	1.87	1.10	2.10

Table 2 Effect of gamma irradiation on leaf parameters of tuberose cv. Prajwal and Phule Rajani

Treatment	Number of leaves/ clump						Length of leaves (cm)						Width of leaves (cm)					
	Prajwal			Phule Rajani			Prajwal			Phule Rajani			Prajwal			Phule Rajani		
	B_0	B_1	B_2	B_0	B_1	B_2	B_0	B_1	B_2	B_0	B_1	B_2	B_0	B_1	B_2	B_0	B_1	B_2
Control	26.33	28.40	33.40	16.07	25.13	30.40	50.94	54.81	58.96	54.25	54.45	56.93	2.39	2.87	2.99	2.07	2.37	2.39
2.5 Gy	15.47	21.97	33.50	21.73	20.67	27.73	51.60	18.65	57.53	49.37	48.16	53.81	2.49	1.49	2.65	2.15	2.22	2.32
5.0 Gy	16.20	0.00	28.73	17.33	18.27	24.93	48.55	0.00	55.06	38.89	46.79	51.24	2.29	0.00	2.50	1.96	2.10	2.23
7.5 Gy	13.13	0.00	25.53	16.47	16.27	20.00	44.31	0.00	50.83	40.19	39.44	46.07	2.24	0.00	2.35	1.78	1.71	1.94
10.0 Gy	11.33	0.00	15.07	17.73	14.77	17.37	23.10	0.00	26.19	23.25	19.27	29.77	1.71	0.00	2.03	1.56	1.44	1.53
Mean	16.49	10.07	27.25	17.87	19.02	24.09	43.70	14.69	49.71	41.19	41.62	47.56	2.23	0.87	2.50	1.90	1.97	2.08
CV	14.33	19.23	10.86	12.41	7.86	8.06	7.84	7.99	7.32	7.41	7.22	5.06	5.19	8.09	2.42	5.55	5.29	3.10
SE	1.36	1.12	1.71	1.28	0.86	1.12	1.98	0.68	0.97	1.76	1.73	1.39	0.07	0.09	0.04	0.06	0.06	0.04
CD (P=0.05)	4.45	3.65	5.57	NS	2.82	3.66	6.45	2.21	16.21	5.75	5.65	4.53	0.22	0.30	0.11	0.20	0.20	0.12

NS, Non significant, B_0- Freshly harvested bulbs, B_1- Three weeks after uprooting, B_2- Six weeks after uprooting.

can be explained on the basis of harmful physiological effects which bring about a reduction in meiotic activity of the cells and retarding cell division, chromosomal aberrations and also damage to auxins (Gray 1956). Like other plant parameters which have been discussed above we found reduced number of tillers with increased gamma irradiation doses in all the bulb stages and across the cultivars of tuberose used.

Effect on leaf parameter

In general, all number of leaves/clump, length of leaf and width of leaf decreased with increase in gamma irradiation doses in all three bulb stages of cv. Prajwal and Phule Rajani. The lower dose of gamma irradiation (2.5 Gy) was not significantly affected the leaf parameters. Freshly harvested bulbs (B_0) of both the tuberose cultivars responded comparably high to gamma irradiation with their high CV obtained. There was significant reduction in the length and width of leaf in 10.0 Gy gamma irradiation dose across the bulb stages and cultivars. Similar results were reported by Banerji *et al*. (1994) in gladiolus; Anu *et al*., (2003) in tuberose. Little stimulatory effect of lower dose of gamma irradiation (2.5 Gy) on length and width of leaf in freshly harvested bulb stage (B_0) but it was non significant.

Increased gamma irradiation resulted in some of the leaf abnormality like leathery leaves, formation of white and golden yellow bands on one side of the leaf margins only in few leaves. Presumably this effect might be due to radiation damage to the cells themselves and cell division. A similar phenomenon was reported by Gunkel and Sparrow (1954). The similar results were also reported by Abraham and Desai in tuberose (1976b) and Gupta (1984). In plant, abnormalities like resetting of all the leaves of the plants and multiple sprouting shoots from single bulb was observed at 10.0 Gy. It has been demonstrated that the degree of chromosomal aberration in somatic or meiotic cells generally increase as the degree of growth inhibition become more severe (Lea 1947 and Catecheside 1948). These abnormalities are determined by the behaviour of tissues at different phases of development (Hansel 1966).

Finally we can conclude that, 7.5 Gy and 10.0 Gy doses of gamma irradiation have showed more effect on vegetative paramters across the bulb stage in both the cultivars Prajwal and Phule Rajani and 2.5 Gy and 5.0 Gy have not made any significant effect. The response of bulbs stages were in the order $B_0 > B_2 > B_1$, respectively. The optimal dose for mutation induction in these two tuberose cultivars would be from 7.5 Gy to 11.5 Gy.

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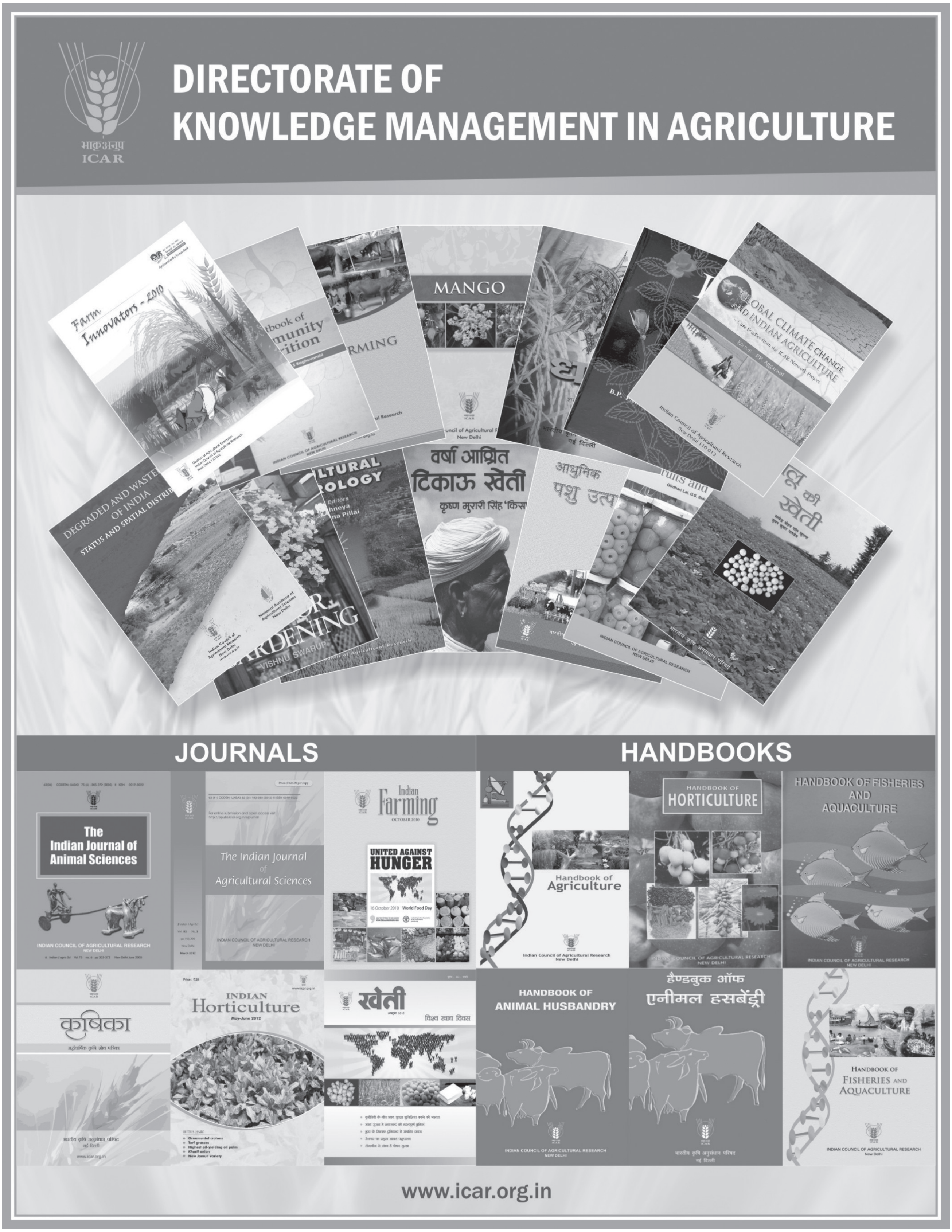
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