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Determination of proper gamma radiation dose for creating variation in Indian garlic varieties

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Garlic (*Allium sativum* L.) an important neutraceutical crop. It is used as traditional medicine to aid digestion, respiration, parasitic infestation and to treat leprosy and various other diseases. But biologically garlic is sterile in nature hence for creating variation; traditional breeding methods are not applicable. Further clonal propagation method leads garlic more prone for various seed and soil borne diseases including virus infection. And natural genetic variation in morphological as well as biochemical traits is limited in garlic. Considering this is in view, among various methods of creating variation, treating bulbs with radiation is simple and easy technique. Main activity of mutation breeding using gamma radiation is to determine optimum dose (LD-50). Hence in present experiment, two garlic varieties were subjected to gamma radiation treatments (0, 1, 5, 10, 25, 50, 75 and 100 gray). Aim of this experiment is to identify LD-50 dose and then generate variation in garlic like increase in bulb size, bulb shape, high neutraceutical compounds like allicin etc. In results, both treated varieties recorded 50 percent germination at dose 10 grays as LD-50. Further on basis of survival of plantlets and final matured plants count, optimum gamma radiation dose of 5 gray for Bhima Omkar and in between 5 to 10 gray for Bhima Purple is identified as LD-50 respectively. In case of Bhima Purple, there is further need to screen the population for exact dose value of LD-50. All other morphological traits in both varieties recorded decreasing trend with increase in radiation doses. However no significant alterations observed visually in any treatment. This optimized radiation dose will be helpful for subjecting other genotypes for getting mutants and generating diversity in garlic which may have high neutracutical compounds and would suitable for processing and export.

Keywords: *Allium sativum* L., Conservation, Gray, LD-50, Mutation breeding

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Garlic (*Allium sativum* L.) is an important bulbous crop after onion in *Allium* genus. Worldwide this genus consisting 972 plantlist accepted (www.plantlist.org) different species which are known for their various sulphur containing compounds. Among them, mainly studied compounds are S-alk (ne) yl cysteine sulphoxide, r-Glutamyle peptide, and S-substituted cysteine[1,2]. These compounds are playing major role in maintaining characteristic flavor and quality[3] and also enhances the medicinal properties of alliums. Additionally garlic is used as traditional medicine to aid digestion, respiration, parasitic infestation and to treat leprosy[4]. Recently medicinal properties of garlic are clinically studied by many researchers and reported its use against curing diseases like Cancer[5], Hyper-tension, Cardio-vascular diseases[6,7], allergies[8], etc. Thus it is clear that garlic has huge potential of improving human health through curing diseases and adding antioxidants etc. Moreover, in future neutraceuticle values can be enhanced using different breeding strategies. But as garlic is non-flowering in nature[9,10] except few flowering genotypes occurring in its place of origin[11], traditional breeding methods are not applicable in garlic for creating variation[12]. Further clonal propagation makes garlic more prone towards various seed and soil borne diseases including virus infection[13]. This ultimately degenerates the quality and yield potential of garlic. Thus practicing *in vitro* tissue culture methods i.e., development of transgenic[14], somaclones[15,16] etc. and *in vitro* and *in vivo*[17] mutation breeding using different mutagens (chemical and physical) is becoming option for creating variability. But for adopting tissue culture protocols i.e., somaclonal variation, *in vitro* chemical mutagen treatments needs facilities and skilled hands. Considering this scenario, imposing potential garlic varieties to mutagenesis for capturing favorable

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changes in interested traits is becoming easiest way for generating variability.

Mutation is sudden heritable change in gene which may causes due to alteration of base sequence of gene, chromosomal changes, etc. This alteration result alters the morphological, physiological or biochemical behavior[18]. In organism, mutation occurs either spontaneous or induced by mutagens[19]. In physical mutagen, gamma radiation is simplest, safe and easiest way of breeding. Generally gamma radiation has wide applications in medical, industrial and agriculture field. In agriculture it is commonly used to bring genetic, morphological or physiological, and biochemical changes by applying a different intensity of gamma ray doses[20]. Therefore, this nuclear technique will broaden the selection level to identify genotypes tolerant to salinity, drought, etc. as well as enhances self-life/storage life of fruits, vegetables and processed products.

Mutation breeding using gamma radiation is adopted by many breeders in many crops like mung bean, groundnut, soyabean, rice, etc. and achieved potential mutants/varieties in rice[21], cotton[6] and brassica[23,23]. In case of garlic, effect of gamma radiation has been studied for sprouting inhibition, enhancing storage[24,4], morphological changes[2,13], studying biochemical behavior[25,9,4], etc. But in India, even though ICAR-DOGR is premium research Institute working on onion and garlic, no mutation study using gamma radiation has been reported in their developed potential varieties. Additionally, ICAR-DOGR also acts as National Gene Bank for Garlic and maintaining and conserving total 700 garlic accessions. Hence application of gamma radiation for creating variability as well as determining LD-50 of these varieties can be applicable for other potential genotypes. Here adopting mutation breeding strategy and determining effective dose of gamma radiation have become prerequisite. Thus present experiment planned to finalize the LD-50 dose of gamma radiation for two popular garlic varieties Bhima Purple and Bhima Omkar. Further this estimated dose will be helpful to create variability, enhancing neutraceutical values, etc.

Materials and Methods

Experimental site: Present experiment was conducted at ICAR-Directorate of Onion and Garlic Research, Rajgurunagar which is located 40 km away from Pune with 18.8550°N, 73.8875°E.

Plant material: Uniform size bulbs of two popular garlic varieties namely Bhima Omkar and Bhima Purple harvested during *rabi* 2017-18 were used as base material for this experiment. In both garlic varieties, total 250 g bulbs per dose were exposed to gamma irradiation in gamma chamber. This chamber contains CO_{60} as a source of radiation at Bhabha Atomic Research Centre (BARC), Mumbai. While exposing to radiation treatment, bulbs were packed in paper bag and then covered with aluminum foil.

Treatments: Radiation doses used in experiment are 1 gray, 5 gray, 10 gray, 25 gray, 50 gray, 75 gray and 100 gray. The dose rate of irradiation was 3.06 K kR/h. Treated bulbs were planted on 10 x 15 cm distance with plot size 1 x 6 m along with untreated control.

Methods: In observations, germination percentage and total survival (%) along with final matured plants were recorded in first seven days after planting, 30 days of planting (DAP) and at time of maturity respectively. In survived plants of all treatments, five random plants were selected for recording observations on morphological traits *viz*., plant height (cm), number of leaves per plant, 4th leaf length (cm), 4th leaf width (cm), pseudo-stem length (cm), pseudo-stem width (mm), stem pigment (green/yellow/red), foliage attitude (erect/semi erect/drooping), leaf intensity of green colour (light/medium/dark), leaf waxiness (absent/present), weight with leaves (kg), weight without leaves (kg), polar diameter (mm), equatorial diameter (mm), single bulb weight (g), average number of clove per bulb (g), weight of 50 clove (g), shape of bulb (circular/ovate/heart shape), bulb skin colour (white/purple/purple strips), clove skin colour (white/purple), total soluble solids (degree brix). Here, LD-50 was determined using Probit analysis[26,27] in Microsoft excel. Mean variance analysis for all recorded traits was carried out using software SAS 9.3.

Results

Effect of radiation on germination and survival: Among all treatments, in medium range doses i.e., 5 gy and 10 gy, germination was commenced on 5th to 7th DOP and it was almost completed within 12 days. In doses 25 and 50 gy, germination was initiated by 7th to 10th DAP and extended up to 15 days. However nil germination was recorded in doses 75 and 100 gy. In control (without radiation), germination was initiated earliest on 3rd DOP and extended and completed within 10 days. Further in both treated varieties, decreased germination percent was observed with increase in radiation doses. Here, doses 75 and

100 gy recorded no germination. However in 1 and 5 gy treatment doses, 90 and 52 percent germination noted in Bhima Omkar and Bhima Purple respectively (Fig. 1). Both varieties showed almost 50% germination at 10 gy dose. In both varieties decreased but irregular germination recorded in doses 25 and 50 gy. Based on germination data and using Probit analysis method (Probit), gamma radiation dose 10 gy identified as a LD - 50 for germination studies in both garlic varieties (Fig. 2).

In case of final survival (recorded after 30 DOP), compare to control less survival observed at doses 1 and 5 gy, and very few plantlets survived at 10 gy. But at 25 and 50 gy doses no plants survived even after recording 5 to 30 percent initial germination (Table 1). In Egypt, Kebeish et al. (2015)[25] exposed garlic bulb to the range of doses from 10 to 150 gy and observed treated population for morphological traits along with oxidative stress and biochemical compounds. The trend in germination with respect to radiation doses is similar to our results but they observed some germination even in doses 70 gy to 150 gy. This may be purely varietal genetic difference and biochemical status of used garlic along with varietal ability to tackle the free radio-active radicals emitted through irradiation[28,29].

Fig. 1 — Effect of gamma radiation on germination of Garlic varieties

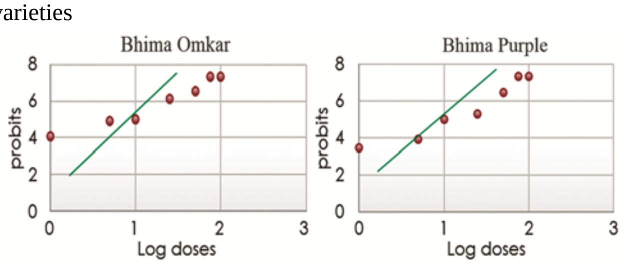

Fig. 2 — Plots of Log doses versus Probits for calculation of LD-50 of gamma radiation in Bhima Purple and Bhima Omkar at germination stage; Probit analysis (Finney,1971, 1978)

Effect of radiation on quantitative growth parameters: The result signifies the mean value of all recorded traits. In lower doses (1 gy and 5 gy) values of plant height and number of leaves per plant are statistically *at par* but in decreased proportion as compare to control (Table 2). However inhibitory effect noted in 10 grays, as significant reduction in both traits observed compare to control. Here, significantly less values and decreased trend for traits 4th leaf length (cm), 4th leaf width (cm), pseudostem length (cm) and pseudo-stem-width (mm) were recorded in all treatments as compare to control. But leaf length, pseudo stem length, weight (g) with leaves and weight without leaves (g) of 1 gy treated plantlets of Bhima purple was significantly higher than control. However all other treatments recorded decreasing trend. In both varieties, 1 gray treatment achieved positive effect for polar diameter, equatorial diameter, average bulb weight and number of cloves per bulb (Table 2). In case of weight of 50 cloves, no radiation treatments depicted positive favorable effect in any of treated variety. However highest and *at par* results were recorded for TSS in dose 1 gy and at control.

Effect of radiation on qualitative traits: In qualitative traits, significant variation was observed among mutagen treatments. Besides white and red stem pigmentation in Bhima Omkar and Bhima Purple respectively, additional yellow pigmentation also observed in treated plants**.** Spreading foliage attitude and waxiness on leaves was noted in 10 gray plants compare to erect posture and absence of wax on leaf in control plants of both varieties (Table 3). However no significant changes were observed in

Table 1 — Effect of gamma radiation on plant survival out of 240 planted cloves.

Doses	No. of plants survived		No. of plants matured	
	Bhima Omkar	Bhima Purple	Bhima Omkar	Bhima Purple
1 Gy	330	380	324	367
5 Gy	210	350	198	330
10 Gy	5	8	0	0
25 Gy	0	0	0	0
50 Gy	0	0	0	0
75 Gy	0	0	0	0
100 Gy	0	0	0	0
Control	405	408	405	408

Table 2 — Effect of gamma radiation on different growth parameters of garlic varieties

Traits/Doses	PH	NL/P	4 LL	4LW	PSL	PSW	WWL	WWOL	PD	ED	ABW	NC/B	W50C	TSS
BO-1 Gy	45.47± 0.05^{ab}	7.30± 0.12^{b}	29.99± 0.03^{ab}	1.49± 0.02^{c}	3.42± 0.04d	6.80± 0.04	1.22± 0.25	1.08± 0.47	25.41± 0.03	25.54± 0.02	10.80± 0.12	11.10± 0.06	35.75± 0.56	43.17± 0.21
BO-5 Gy	35.98± 0.03^{a}	7.20± 0.13^{b}	25.56± 0.05^{a}	1.06± 0.03^{a}	2.27± 0.04^{c}	4.57± 0.04	0.43± 0.12	0.37± 0.87	21.28± 0.04	21.62± 0.03	8.07± 0.43	10.80± 0.06	33.50± 0.43	44.58± 0.23
BO–10 Gy	14.56± 0.03^{c}	4.40± 0.13^{b}	11.74± 0.06^{e}	0.34± 0.03^{e}	1.44± 0.04^{de}	3.22± 0.05	-	-	-	-	-	-	-	-
BO(C)	52.00± 0.02^{ab}	7.90± 0.13^{b}	35.90± 0.07^{c}	1.61± 0.03^{ab}	3.46± 0.03^{d}	8.34± 0.04	1.32± 0.21	1.10± 0.47	22.72± 0.04	23.78± 0.02	10.95± 0.56	10.90± 0.05	41.25± 0.42	43.17± 0.21
BP-1 Gy	48.70± 0.05^{ab}	7.50± 0.12^{ab}	37.20± 0.06^{cd}	1.59± 0.03^{ab}	3.31± 0.03^{ab}	8.69± 0.05	1.69± 0.23	1.48± 0.32	22.81± 0.05	25.29± 0.03	10.75± 0.43	12.20± 0.07	34.60± 0.85	43.60± 0.11
BP-5 Gy	40.15± 0.05^{a}	7.00± 0.13^{b}	29.02± 0.06^{ab}	1.24± 0.03^{b}	3.17± 0.03^{b}	6.56± 0.05	0.58± 0.17	0.50± 0.79	21.71± 0.05	24.29± 0.03	8.91± 0.34	15.70± 0.03	33.80± 0.47	40.75± 0.12
BP-10 Gy	16.00± 0.06^{f}	4.00± 0.13^{ab}	10.84± 0.08^{ef}	0.26± 0.03^{e}	1.10± 0.03^{e}	2.12± 0.05	-	-	-	-	-	-	-	-
BP(C)	49.57± 0.05^{ab}	7.50± 0.12^{b}	35.21± 0.09^{c}	1.84± 0.03	3.17± 0.03^{b}	9.21± 0.04	1.35± 0.18	1.12± 0.32	23.21± 0.03	23.10± 0.03	8.50± 0.43	10.60± 0.04	38.75± 0.67	42.33± 0.14

BO- Bhima Omkar, BP- Bhima Purple, C- Control, PH- Plant Height (cm), 4LL- 4th lead length, 4LW- 4th leaf width, PSL- Pseudostem length (mm), PSW- Pseudostem width (mm), WWL- Weight with leaves (g), WWOL- Weight without leaves (g), PD- Polar Diameter (mm), ED- Equatorial Diameter (mm), ABW- Average bulb weight (g), NC/B- Number of cloves per bulb, W50C- Weight of 50 cloves, TSS- Total Soluble Solids (degree brix); Data shown is mean SD of two replicates.

Table 3 — Effect of gamma radiation on qualitative traits of garlic varieties

Traits/ Doses	SP	FA	LIGC	LW	SB	BSC	CSC
B.O. - 1 Gy	Y	E	M	A	OB	W	W
B.O. - 5 Gy	Y	E	M	A	TE	W	W
B.O. - 10 Gy	W	D	M	A	-	-	-
B.O. (C)	W	E	D	A	OB	W	W
B.P. - 1 Gy	R	E	D	A	TE	P	P
B.P. - 5 Gy	Y	SE	M	A	OB	P	P
B.P. - 10 Gy	W	D	L	P	-	-	-
B.P. (C)	R	E	D	A	TE	P	P

B.O - Bhima Omkar, B.P - Bhima Purple, C - Control, SP - Stem Pigment (Green/Yellow/Red), FA - Foliage Attitude (Erect/Semi Erect/Drooping), LIGC - Leaf Intensity of Green Colour (Light/Medium/Dark), LW - Leaf Waxiness (Absent/Present), SB - Shape of bulb, BSC-Bulb Skin Colour (White/ Purple), CSC - Clove Skin Colour (White/ Purple)

bulb skin colour and clove skin colour in mutagenic plants. In case of leaf colour although both varieties originally possess dark green colour for leaves, radiated plants of Bhima Omkar recorded medium green color and medium and light green color observed in Bhima Purple for 5 and 10 gray respectively. Some variation also reflected in bulb shape. In addition to this no other aberrant abnormality has been observed in any treatment.

Discussion

Even though garlic is non-flowering and creation of variation is main and urgent aim in breeding, for enhancing neutraceutical value and yield but crop is less exposed to physical mutagens. Irradiation is very easy and simple technique where released ions are damaging or modifying cell or its components. Such alterations led to change in cell expressions[31,32]. Therefore with rate of doses, its concentration and genetic components of crop, mutation occurred[16]. In recent reports on garlic Mahajan *et al.*[17] used chemical mutagens for creating variability in garlic and analyzed LD - 50. But they did not report effect of mutation in garlic using gamma radiation.

In present experiment, both gamma radiation treated garlic varieties showed almost similar trend in germination with change in doses. This indicates both varieties had same radio-resistance which may be due to their genetic composition, water holding in cells etc. Olasupo *et al.* in 2016[28] revealed same genotypic effect of radiation on 8 accessions of cowpea. On basis of germination percent in both varieties, 10 gy radiation dose showed almost 50% germination. This indicates that radiation damages enzyme activity and physiological actions etc. required for germination. These changes were not limited upto germination but are there till plant growth and its maturity, as out of 200 germinated plants hardly 5 to 10 plants able to grow and get survived at 10 gy dose. But final matured plants were nil in both varieties at 10 gy. Approx. 50% survival of Bhima Omkar is at 5 gy and for Bhima Purple it is between 5 to 10 gy. This indicates more radio-resistance of Bhima Purple compare to Bhima Omkar. Here reduction in 'survival up to maturity' with gamma rays may be due to inactivation of auxin and a decrease in auxin content with increased irradiation dose[15]. Main visible morphological traits in garlic like plant height, leaf length, leaf colour, bulb colour, and bulb shape including other minor traits exhibited inhibitory effect with increase in radiation doses. Same results were reported in cowpea[28], merigold[29,31] but few reports also revealed opposite reaction i.e., low doses results in positive action and higher doses gives inhibitory effect[29,30] on various interested traits of crops. Alike other crops we expect in next generation of all survived plants that we may get garlic lines with high medicinal properties i.e., containing increased level of allicin and other related compounds. Hence in consequent year experiment planned to maintain bulb-wise separate lines and further its screening for biochemical properties along with other potential traits.

Conclusion

Determining mutagen dose is key factor of mutation breeding. In present study, LD-50 on basis of germination and survival are identified for both varieties. Hence this study identified optimum range of gamma radiation dose required in garlic as higher doses are affecting severely. Further generated mutants will be helpful for generating variation in important garlic traits like allicin content, bulb size etc.

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