

Induction of Genetic Variability with Gamma Radiation and Its Applications in Improvement of Horsegram

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1. Introduction

Induced mutation is one of the best alternatives for the improvement of horsegram as it can help to regenerate and restore the variability, which is generally lost in the process of adaptation to various stresses. Genetic variability is the most essential prerequisite for any successful crop improvement programme as it provides spectrum of variants for the effective selection, which can be achieved through the processes of hybridization, recombination, mutation and selection.

Genetic variability has been exhausted in horsegram due to natural selection and hence conventional breeding methods are not fruitful (Wani and Anis, 2001). Legumes generally loose different alleles for high productivity, seed quality, pest and disease resistance during the processes of adaptation to environmental stress.

Gamma sources are used to irradiate a wide range of plant materials, like seeds, whole plants, plant parts, flowers, anthers, pollen grains and single cell cultures or protoplasts. Radiations have been used successfully to induce useful mutations for plant breeding. The lower doses/ concentrations of the mutagenic treatments could enhance the biochemical components, which are used for improved economic characters (Muthusamy *et al.*, 2003). Gamma radiation can induce useful as well as harmful effects on crops so there is need to predict the most beneficial dose for improvement of specific traits of crop plants (Jamil and Khan, 2002).

Improvement in yield and productivity of pulses is the need of the hour, but for this marginal land, aberrant rainfall, non availability of improved seeds, less or no input and poor crop management are the main constraints. Amongst pulses horsegram (*Macrotyloma uniflorum* (Lam.) Verdc) is highly neglected in India and hence require more emphasis on its improvement as it has nutritional, medicinal and fodder value. In Maharashtra, during the year 2008-2009, horsegram was cultivated on 0.466 lakh ha with annual production of 0.3232 lakh tones. The average yield per hactar was 693.56 Kg.

Horsegram is drought tolerant and having good nitrogen fixing ability, but receives a low priority in cropping system, soil types etc. It is grown in *kharif* and *rabbi* seasons, as main

crop, or as a mixed crop. It is cultivated in areas with annual rainfall 300-600 mm, but does not tolerate flooding or water logging. The favourable average temperature is 18 to 27°C, and adapted to a wide range of well-drained soils.

The use of dry seeds of horsegram as human food is limited due to its poor cooking quality, presence of high level of enzyme inhibitors and heamagglutinin activities (Ray 1969). The seeds are rich in tannins and polyphenols compared to the other legumes (Kadam and Salunkhe 1985). Antinutrients like phytates, tannins and oxalic acid reduce the availability of iron.

1.1 Experimental layout

The authentic seeds of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc) cultivar Dapoli Kulthi-1 were procured from Head, Department of Botany, College of Agriculture, Dr. Balasaheb Savant Konkan Agricultural University, Dapoli, Dist-Ratnagiri (M.S.) India.

The field experiments were conducted on the experimental field at Department of Botany, University of Pune, Pune 411 007 (M.S.). The crop of horsegram Dapoli Kulti-1 was grown in *Kharif* season under uniform conditions. All the experiments were carried out in triplicate, following RBD design. The size of each plot was 3.75m X 2.75m and each plot had 225 plants. The distance between two rows and two plants was 30 X 15 cm and the distance between two adjacent plots was one meter.

A total of 42 combination treatments in M_1 generation with untreated dry seeds, which was used as control. Final 675 seeds of each treatment were used to raise M_1 generation. The M_2 , M_3 , M_4 and mutants were raised in next rainy season.

2. Results

Results obtained in the present investigation are discussed in brief.

2.1 M_2 generation

2.1.1 Chlorophyll mutations

The chlorophyll mutants were scored from 7 to 10 days after sowing. The different types of chlorophyll mutants such as albina, xantha, chlorina and viridis were reported in all mutagenic treatments.

Amongst all the treatments used gamma radiations had induced the highest chlorophyll mutation frequency, followed by combination treatments. Prakash and Halaswamy, (2006) and Manigopa-Chakraborty *et al.*, (2005) and Bolbhat and Dhumal (2009) have also reported induction of chlorophyll mutation in horsegram with GR and their combination.

The frequency of total chlorophyll mutants varied for single as well as combination of gamma radiation and EMS. In gamma radiation the average percentage of chlorophyll mutation frequency was 1.47% which was slightly higher than combination treatments (1.23%). The highest chlorophyll mutation frequency was recorded in 200Gy (1.70%). The values of chlorophyll mutation frequency were ranging from 1.09% to 1.70%. Amongst all types of chlorophyll mutations, albina and chlorina types showed higher percentage

(0.64 and 0.58%). The percentage of xantha and viridis was 0.41 and 0.28 respectively. The average number of chlorophyll mutations was 24.25, while the average frequency was 1.47%.

The combination treatments showed wide range of total percentage of chlorophyll mutations. The range varied from 0.74% to 1.72%. Highest chlorophyll mutation frequency (1.72%) was noted in 100Gy + 0.3%EMS.

Reddy and Annadurai (1992) claimed that chlorophyll mutation can be used as an index for evaluating the mutagenic action of different mutagens. It is also important for assessing the potency of mutagen and also can be used as an indicator of factor mutations. Chlorophyll mutations are used as a dependable index for evaluating the genetic effects of mutagens.

Albina, xantha, chlorina and viridis were found to be the most abundant type of chlorophyll mutants induced by GR and combination treatments in horsegram Bolbhat and Dhumal (2009). Manjaya *et al.*, (2007) and Tambe *et al.*, (2010) attributed this genes concerned with the development and expression of chlorophyll pigments.

2.2 Viable mutations

The mutations affecting gross morphological changes in plant habit, leaf and pod morphology, and maturity were scored as viable mutations. These mutants were characterized and named on the basis of specific characters constantly observed in them throughout the course of investigation. Viable mutants and their characteristic features are given in Table-2. Effect of mutagens on the frequency and spectrum of different types of viable mutations in M₂ generation is illustrated in Table-1.

2.3 Frequency and spectrum of viable mutations

All the treatments used have induced the widest spectrum of viable mutations. The range of viable mutations in gamma radiation and GR + EMS (Table-1) was 0.61 to 2.16% and 0.21 to 1.81% respectively. The highest percentage of frequency of mutations noted for plant habit, leaf and pod morphology and maturity, due to various treatments of gamma radiation was 0.77%, 0.66%, 0.58% and 0.30% respectively. These results also indicated that the percentage of plant habit mutations was maximum as compared to others. In combination treatments the percentage of frequency for plant habit, leaf, pod and maturity type mutations, was 0.76%, 0.63%, 0.49% and 0.28% respectively. The highest percentage of mutations was obtained in gamma radiation (2.16%) as compared to combination treatment (1.81%).

The widest spectrum and frequency of viable mutations may be due to differential mode of action of the mutagens on different base sequences in various genes. The results indicated that the variety used for study was sensitive to mutagenic treatments.

2.4 Plant habit mutations

Tall and gigas mutants obtained in the present investigation showed vigorous growth (Plate-2). According to Weber and Gottschalk (1973) and Blonstein and Gale (1984) the

Mutants	Gamma rays (Gy)							
	100		200		300		400	
Total plants studied	1560		1710		1675		1632	
	No.	%	No.	%	No.	%	No.	%
Plant habit								
Gigas	2.00	0.13±0.01	5.00	0.29±0.02	2.00	0.12±0.01	0.00	0.00±0.00
Tall	2.00	0.13±0.01	0.00	0.00±0.00	3.00	0.18±0.01	0.00	0.00±0.00
Dwarf	2.00	0.13±0.01	2.00	0.12±0.01	1.00	0.06±0.00	1.00	0.06±0.00
Compact	2.00	0.13±0.01	1.00	0.06±0.00	2.00	0.12±0.01	1.00	0.06±0.00
Bouquet	0.00	0.00±0.00	0.00	0.00±0.00	0.00	0.00±0.00	0.00	0.00±0.00
Erect	2.00	0.13±0.00	0.00	0.00±0.00	1.00	0.06±0.00	0.00	0.00±0.00
Tendrilar	0.00	0.00±0.00	2.00	0.12±0.01	0.00	0.00±0.00	0.00	0.00±0.00
Spreading	2.00	0.13±0.01	2.00	0.12±0.00	1.00	0.06±0.00	2.00	0.12±0.00
Total	12.00	0.77±0.04	12.00	0.70±0.04	10.00	0.60±0.03	4.00	0.25±0.01
Leaf								
Gigas	2.00	0.13±0.01	4.00	0.23±0.01	2.00	0.12±0.01	0.00	0.00±0.00
Broad	1.00	0.06±0.00	0.00	0.00±0.00	1.00	0.06±0.00	0.00	0.00±0.00
Narrow	0.00	0.00±0.00	2.00	0.12±0.00	1.00	0.06±0.00	0.00	0.00±0.00
Small	2.00	0.13±0.01	1.00	0.06±0.00	2.00	0.12±0.01	0.00	0.00±0.00
Stalked	0.00	0.00±0.00	0.00	0.00±0.00	2.00	0.12±0.00	1.00	0.06±0.00
Close pinnae	2.00	0.13±0.01	0.00	0.00±0.00	1.00	0.06±0.00	0.00	0.00±0.00
Curly	1.00	0.06±0.00	1.00	0.06±0.00	2.00	0.12±0.01	3.00	0.18±0.01
Long	0.00	0.00±0.00	0.00	0.00±0.00	0.00	0.00±0.00	0.00	0.00±0.00
Total	8.00	0.51±0.02	8.00	0.47±0.01	11.00	0.66±0.02	4.00	0.25±0.01
Maturity								
Early	0.00	0.00±0.00	0.00	0.00±0.00	1.00	0.06±0.00	0.00	0.00±0.00
Late	4.68	0.30±0.02	3.42	0.20±0.01	1.68	0.10±0.01	0.00	0.00±0.00
Total	4.68	0.30±0.02	3.42	0.20±0.01	2.68	0.16±0.01	0.00	0.00±0.00
Pod								
Gigas	2.00	0.13±0.01	4.00	0.23±0.01	2.00	0.12±0.00	0.00	0.00±0.00
Long	1.00	0.06±0.00	0.00	0.00±0.00	2.00	0.12±0.01	0.00	0.00±0.00
Broad	1.00	0.06±0.00	0.00	0.00±0.00	0.00	0.00±0.00	1.00	0.06±0.00
Narrow	3.00	0.19±0.01	0.00	0.00±0.00	2.00	0.12±0.00	0.00	0.00±0.00
Small	2.00	0.13±0.01	2.00	0.12±0.01	2.00	0.12±0.01	1.00	0.06±0.00
Total	9.00	0.58±0.02	6.00	0.35±0.01	8.00	0.48±0.01	2.00	0.12±0.00
High yielding								
Total freq.		2.16±0.10		1.72±0.09		1.99±0.09		0.61±0.03

Table 1. Effect of gamma radiation on frequency and spectrum of viable mutations in M₂ generation of horsegram cv. Dapoli Kulthi-1.

Mutants	Characters	Treatments
Plant habit mutants		
Gigas	These mutants were vigorous, upright and tall with larger, thick, close pinnae and thick profuse branching at the base. Pods were large containing bold seeds.	100, 200 & 300Gy, and various combination treatments.
Tall	These mutants were vigorous, tall, with medium and thick leaves, normal flowers and pods.	100 and 300Gy and various combination treatments.
Dwarf	Plant height of these mutants ranged from 15 to 20cm and had profuse branching at the base which formed a dense umbrella like canopy.	All GR treatments and combinations.
Compact	These mutants were characterized with dwarf, profuse and compact branches. The branching was more at the base, giving rise to dense, interwoven secondary branches, which ultimately made the mutant compact.	All GR treatments and combination treatments.
Bouquet	These mutants had profuse branching at the base, which remained very close to each other forming a bunch. The canopy of secondary branches and leaves together gave an appearance of bouquet.	Combination treatments
Erect	The mutant was erect and tall with shy branching and light green pinnae.	100 and 300Gy and combination treatments.
Tendrilar	These types of mutants were very weak, slender, branched or unbranched with very few leaflets. The distal portion of stem was tendrilar and had twining tendency.	200Gy and combination treatments.
Spreading	These mutants were creeping on the ground with terminal branches (30 to 60 cm length).	GR and combination treatments.
Leaf mutants		
Super gigas	These mutants had extremely large, thick, dark green leaflets with very prominent midrib, thick and semi erect stem and sparse branching.	100Gy + 0.2%EMS, 300Gy + 0.5%EMS.
Gigas	These mutants were vigorous, upright tall with large thick pinnae, profuse branching. Pods were large with bold seeds.	200Gy (0.23%) and combination treatments
Broad	These mutants were vigorous, upright, dwarf with large, thick, close pinnae.	Combination treatments
Small	These mutants were associated with dwarfness, small leaflets and light green colour with profuse branching at the base.	GR and 400Gy + 0.4%EMS.
Curly	This mutant had curly leaflets with elongated petiole. The leaflets were wedge shaped with shorter leaf lamina, curling towards centre. The plants were erect with pale green foliage	GR and various combination treatments.
Long	These mutants were dwarf having narrow and very long leaflets.	Combination treatments.

Mutants	Characters	Treatments
Plant habit mutants		
Maturity mutants		
Early	These mutants were associated with dwarfness and showing early flowering within a short span of 30-33 days in comparison with the flowering duration of 53-57 days in control. The total duration of the crop in such mutant was 65-70 days against 100-110 days in control.	300Gy, 400Gy + 0.5%EMS and various combination treatments
Late	These mutants were with broad and thick dark green coloured foliage, tall and bearing late flowers as compare to control. Flowering in these mutants was achieved in 60 to 65 DAS against 53 to 57 DAS in control. The total duration of the crop in such mutant was 115-120 DAS against 100-110 DAS in control.	GR and combination treatments
Pod mutants		
Gigas	These mutants were associated with gigas plant type, with very large pods containing 5-6 bold seeds per pod.	GR and combination treatments.
Long	The plants were normal in appearance with comparatively much longer pods, containing 7 to 8 seeds per pod.	100 and 300Gy and combination treatments
Broad	The mutants had broad pods containing 5-6 bold seeds per pod.	100 and 400Gy and combination treatments
Small	It was associated with dwarf plant type mutation having very small pods containing 2-3 seeds per pod.	GR and combinations treatments.
High yielding	These mutants possess high yield contributing characters viz. number of pods, thousand seed weight and total seed yield per plant.	GR and combinations treatments.

Table 2. Viable mutants and their characteristic features

increase in plant height was due to the changes in the internodal length and increase in cell number and cell length or both.

2.5 Leaf mutations

Different types of leaf mutations e.g. super gigas, gigas, broad, narrow, small, tiny, stalked, close pinnae, curly and long were observed in M_2 generation of horsegram with GR and GR + EMS treatments.

Tara and Dnyansagar (1979) stated that the changes in shapes of the leaves were due to chromosomal aberrations, induced by chemical mutagens and ionizing radiation. The leaf mutations obtained in horsegram may be ascribed to above cited reasons, which may be useful as gene markers in conventional breeding. These may be useful for understanding the genetic control of leaf formation and regulation of their size, shape and form.



(a) Field preparation



(b) Seedling stage



(c) 30 days crop



(d) 45 days crop



(e) 60 days crop



(f) Mutant screening



(g) Maturity stage



(h) Harvesting stage

Plate 1. M₂ generation in field

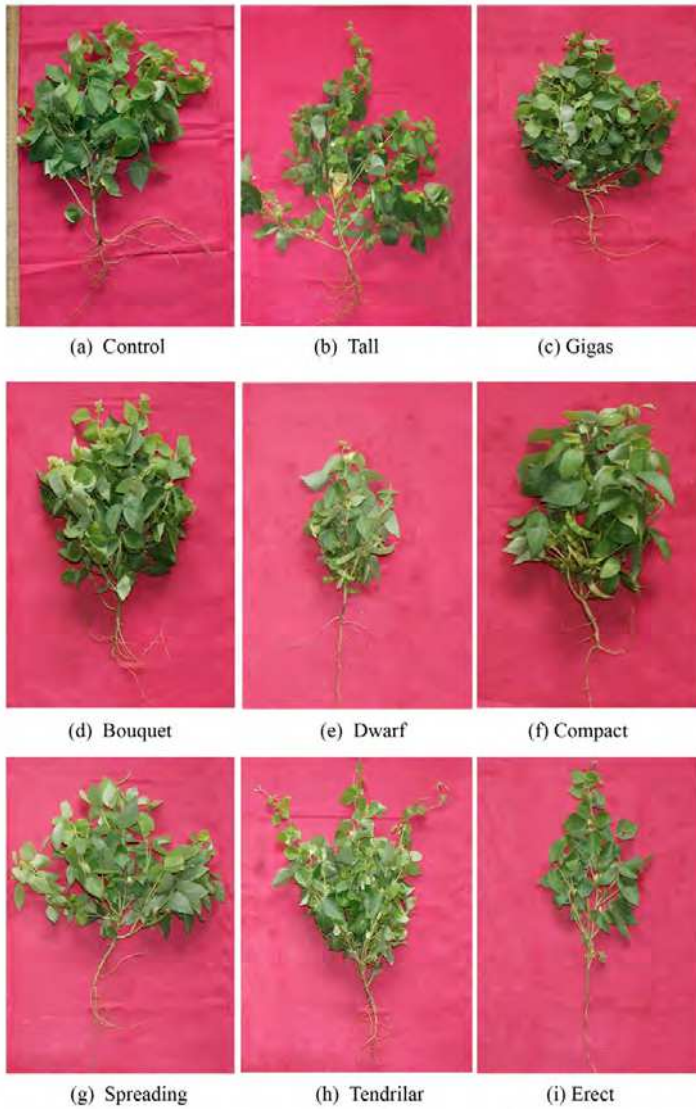


Plate 2. Plant habit mutants.

2.6 Maturity mutations

2.6.1 Early and late

The early mutants were recorded in M_2 with GR and GR + EMS. These mutants showed rapid growth and early maturity. Several workers like Dalvi (1990), Rudraswamy *et al.*, (2006) and Bolbhat and Dhupal (2010) reported early and late mutants in horsegram.

In present investigation the early mutants of horsegram show pod maturity within 50-55 DAS in the gamma radiation and combination treatments. The agronomic traits like early flowering and pod maturity have been always given paramount importance, while planning the breeding strategies. Gottschalk and Wolff (1983) explained the early mutants could be very much useful for genecological studies. The earliness was mainly achieved through rapid growth, during early stages of ontogeny and initiation of first inflorescence. Early maturity in the mutants may be due to the physiological, biochemical, enzymological and hormonal changes induced by the mutagens.

The late mutants were noted in M_2 generation of horsegram with gamma radiation and GR + EMS. The main reason attributed to the late maturity were inadequate production of flowering hormones, physiological disturbances, enhanced production of floral inhibitors and reduced ability to respond to the floral stimulus in the shoot apex (Beveridge and Murfet, 1996). According to Zakri and Jalani (1998) late or early maturity has agronomic significance as these mutants suit for the specific requirement of breeding strategy. The lateness in maturing is worthwhile for prolonging the vegetative phase and allowing the development of a strong sink, which may help to enhance the yield. In addition the period from flowering to maturity should also be long enough, for better seed filling. The late mutants were noted in horsegram with the treatments of gamma radiation and their combinations.

2.7 Pod mutations

Pod mutations such as long, large, narrow and small were recorded in M_2 generation (Plate-3). These mutants were also reported in horsegram Bolbhat and Dhupal (2010).

2.8 High yielding mutants

The high yielding mutants obtained in horsegram due to treatments of GR and GR + EMS showed increased number of pods and grain yield per plant over control.

2.9 Quantitative characters in M_2 and M_3 generations

Gamma radiations and GR + EMS proved to be very effective to induce variability in quantitative traits like plant height, primary branches per plant, days required for first flowering and first pod maturity, number of pods per plant, pod length, number of seeds per pod, 1000 seed weight and yield per plant in M_2 and M_3 generations (Table-3 and 4).

2.10 Quantitative traits

2.10.1 Plant height

The treatments of gamma radiations and combinations were effective for reducing the plant height (Table-3) and the maximum reduction was noted in 100Gy.

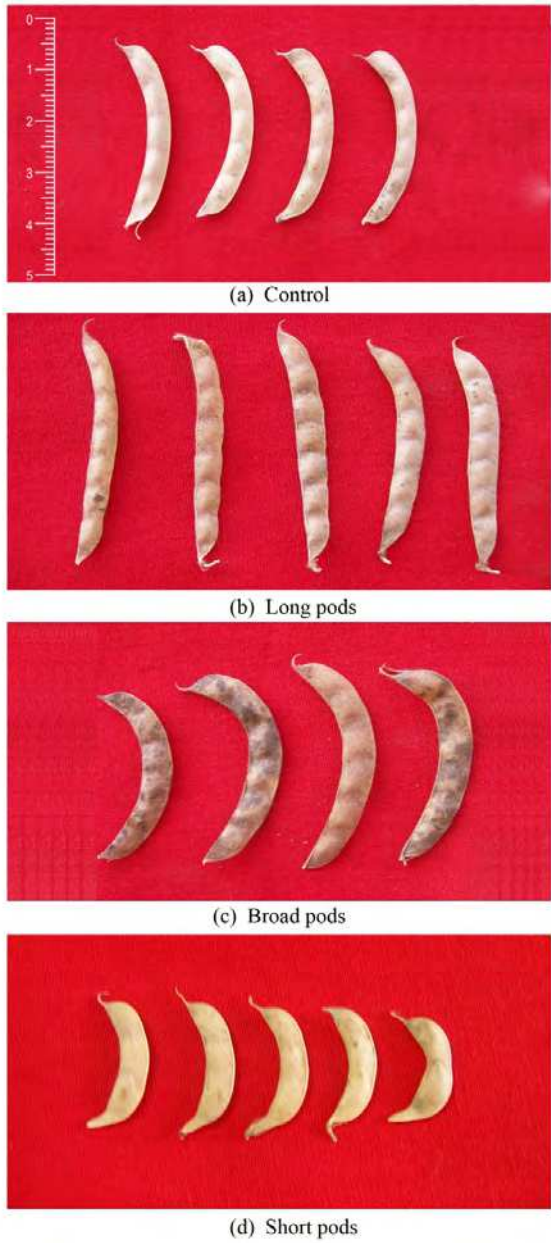


Plate 3. Pod mutants.

Treat	Plant height (cm)	Pri. br./ plant	DAS for first flowering	DAS for first pod maturity	No. of pods/ plant	No. of seeds/ pod	1000 seed Wt. (g)	Seed yield/ plant (g)
Control	46.70±1.87	6.30±0.25	53.30±2.13	85.20±3.41	71.20±2.85	7.20±0.29	26.80±1.07	14.54±0.58
100Gy	35.40±1.77	6.66±0.33	53.07±2.65	81.70±4.09	67.70±3.39	6.66±0.33	28.30±1.42	13.36±0.67
200	39.40±2.76	6.20±0.43	53.70±3.76	78.90±5.52	59.50±4.17	6.33±0.44	28.20±1.97	11.22±0.79
300	41.30±1.24	7.66±0.23	54.80±1.64	84.60±2.54	83.80±2.51	7.10±0.21	27.50±0.83	16.76±0.50
400	40.10±2.41	6.10±0.37	54.70±3.28	84.40±5.06	71.30±4.28	6.33±0.38	26.30±1.58	12.87±0.77
100Gy + 0.2%EMS	28.70±1.72	6.10±0.37	49.40±2.96	79.20±4.75	38.40±2.30	6.20±0.37	25.60±1.54	8.83±0.53
100 + 0.3	29.40±1.18	8.40±0.34	47.60±1.90	77.30±3.09	49.70±1.99	6.10±0.24	26.50±1.06	7.64±0.31
100 + 0.4	26.50±1.86	5.60±0.39	47.30±3.31	79.70±5.58	37.80±2.65	6.30±0.44	26.20±1.83	8.46±0.59
100 + 0.5	31.20±0.94	5.10±0.15	47.50±1.43	78.60±2.36	41.40±1.24	6.00±0.18	27.50±0.83	8.29±0.25
200 + 0.2	33.20±1.66	5.90±0.30	49.30±2.47	77.80±3.89	38.70±1.94	6.50±0.33	27.60±1.38	8.87±0.44
200 + 0.3	25.70±0.77	6.10±0.18	49.40±1.48	79.30±2.38	41.30±1.24	6.20±0.19	26.60±0.80	9.12±0.27
200 + 0.4	27.50±1.65	6.20±0.37	47.60±2.86	79.80±4.79	40.70±2.44	6.00±0.36	27.80±1.67	9.76±0.59
200 + 0.5	30.20±2.11	7.10±0.50	49.20±3.44	78.50±5.50	39.80±2.79	6.50±0.46	26.30±1.84	8.90±0.62
300 + 0.2	34.30±1.37	6.50±0.26	54.90±2.20	79.40±3.18	54.80±2.19	7.10±0.28	26.40±1.06	13.10±0.52
300 + 0.3	31.20±1.56	7.10±0.35	54.40±2.72	79.60±3.98	50.70±2.54	7.00±0.35	26.60±1.33	11.53±0.58
300 + 0.4	29.10±2.04	5.20±0.36	54.30±3.80	78.70±5.51	33.50±2.35	7.10±0.50	30.50±2.14	9.98±0.70
300 + 0.5	35.30±1.06	9.10±0.27	50.80±1.52	80.60±2.42	71.70±2.15	7.10±0.21	29.80±0.89	16.01±0.48
400 + 0.2	42.50±2.13	7.80±0.39	50.40±2.52	76.80±3.84	71.40±3.57	6.50±0.33	28.70±1.44	14.44±0.72
400 + 0.3	33.20±1.99	6.60±0.40	56.30±3.38	77.60±4.66	70.40±4.22	7.10±0.43	25.80±1.55	12.69±0.76
400 + 0.4	40.90±1.64	6.70±0.27	40.80±1.63	57.70±2.71	52.80±2.11	6.20±0.25	27.70±1.11	10.38±0.42
400 + 0.5	47.50±1.43	8.30±0.25	40.60±1.22	66.80±2.00	74.40±2.23	6.20±0.19	27.70±0.83	14.10±0.42
SEM±	1.42	0.27	2.14	3.30	2.25	0.27	1.41	0.47
F-value	42.03	29.99	8.26	3.64	95.80	4.82	2.45	69.09
P-value	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
LSD _{0.05}	2.78	0.53	4.19	6.47	4.41	0.53	2.76	0.92

Table 3. Micromutations in M₂ generation of horsegram cv. Dapoli Kulthi-1.

2.11 Number of primary branches per plant

Data obtained in M₂ generation on number of primary branches per plant (Table-3) indicated that the mean values of this parameter showed positive and negative influence. Maximum number of primary branches per plant was recorded in 300Gy (7.66) over control.

All the treatments in M₃ showed increase in number of primary branches per plant. Maximum number was recorded in 100Gy (12.58) and 200Gy + 0.2 %EMS (11.25) over control (10.99). Dalvi (1990) also noted similar trend with physical as well as chemical mutagens in horsegram.

2.12 Number of days required for first flowering

GR and GR + EMS treatments have induced the variability in number of days required for first flowering in M₂ generation. However some treatments were stimulatory and others were inhibitory for inducing the flowering. The minimum number of days required for first flowering were 50 DAS in 300Gy and 42 DAS in 400Gy + 0.4%EMS.

Dalvi (1990) and Rudraswami *et al.*, (2006) also noted similar results in horsegram with different mutagens.

2.13 Number of days required for first pod maturity

The data recorded in (Table-3) indicated that all the treatments of GR and combinations had succeeded in reducing the number of days required for first pod maturity as compared to control. The combination treatments were highly significant. The average minimum number of days (57.70 DAS) required for first pod maturity was noted in 400Gy + 0.4%EMS. The data obtained for M₃ generation for this trait was on par with of M₂ generation (Table-4). All the treatments of GR + EMS, caused reduction in number of days required for first pod maturity than control. The results of Nawale (2004) supported the above findings.

Treat	Plant height (cm)	Pri. br./ plant	DAS for 1 st flowering	DAS for 1 st pod maturity	No. of pods/ plant	No. of seeds/ pod	1000 seed Wt. (g)	Seed yield/ plant (g)
Control	50.99±2.04	10.99±0.44	54.00±2.16	83.00±3.32	100.55±4.02	6.84±0.27	25.07±1.00	16.58±0.66
100Gy	49.73±2.49	12.58±0.63	53.67±2.68	82.44±4.12	106.18±5.31	6.83±0.34	25.31±1.27	18.62±0.93
200	44.57±3.12	11.49±0.80	54.72±3.83	84.45±5.91	110.04±7.70	6.84±0.48	26.05±1.82	19.14±1.34
300	49.85±1.50	11.98±0.36	50.98±1.53	79.17±2.38	110.32±3.31	6.85±0.21	25.93±0.78	19.60±0.59
400	48.75±2.93	10.03±0.60	54.77±3.29	85.78±5.15	109.62±6.58	6.88±0.41	26.70±1.60	18.90±1.13
100Gy + 0.2%EMS	45.10±2.71	6.78±0.41	54.00±3.24	83.67±5.02	86.88±5.21	6.55±0.39	24.80±1.49	14.12±0.85
100 + 0.3	48.50±1.94	6.39±0.26	52.63±2.11	81.31±3.25	100.11±4.00	6.53±0.26	24.14±0.97	16.35±0.65
100 + 0.4	49.27±3.45	7.30±0.51	54.86±3.84	84.16±5.89	90.25±6.32	6.62±0.46	24.56±1.72	14.95±1.05
100 + 0.5	46.11±1.38	8.48±0.25	51.44±1.54	79.18±2.38	93.33±2.80	6.42±0.19	22.84±0.69	15.27±0.46
200 + 0.2	50.61±2.53	11.25±0.56	57.34±2.87	88.66±4.43	85.22±4.26	7.08±0.35	25.53±1.28	13.83±0.69
200 + 0.3	45.81±1.37	11.06±0.33	52.94±1.59	81.86±2.46	93.42±2.80	6.54±0.20	23.85±0.72	15.71±0.47
200 + 0.4	50.42±3.03	10.44±0.63	58.12±3.49	89.82±5.39	97.28±5.84	7.16±0.43	27.31±1.64	16.96±1.02
200 + 0.5	47.07±3.29	9.68±0.68	53.23±3.73	82.65±5.79	86.53±6.06	6.54±0.46	25.09±1.76	14.71±1.03
300 + 0.2	50.94±2.04	9.64±0.39	55.64±2.23	85.75±3.43	83.49±3.34	6.52±0.26	25.81±1.03	13.68±0.55
300 + 0.3	50.78±2.54	9.11±0.46	54.67±2.73	84.00±4.20	87.33±4.37	6.18±0.31	24.28±1.21	13.52±0.68
300 + 0.4	47.00±3.29	8.67±0.61	45.19±3.16	76.23±5.34	87.18±6.10	6.84±0.48	24.27±1.70	13.40±0.94
300 + 0.5	46.03±1.38	9.87±0.30	46.71±1.40	78.44±2.35	92.39±2.77	6.42±0.19	24.57±0.74	14.23±0.43
400 + 0.2	44.10±2.21	9.67±0.48	43.29±2.16	72.35±3.62	102.18±5.11	5.82±0.29	23.45±1.17	13.30±
400 + 0.3	41.84±2.51	10.51±0.63	49.88±2.99	76.90±4.61	98.42±5.91	6.74±0.40	25.65±1.54	15.59±
400 + 0.4	49.12±1.96	9.22±0.37	42.34±1.69	71.14±2.85	96.08±3.84	6.18±0.25	24.40±0.98	15.20±
400 + 0.5	48.91±1.47	11.01±0.33	48.52±1.46	74.68±2.24	85.53±2.57	6.78±0.20	25.52±85.53	15.18±0.46
SEM±	1.99	0.41	2.19	3.42	3.95	0.29	1.05	0.66
F-value	3.44	32.25	7.99	4.20	9.60	2.57	2.07	18.14
P-value	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
LSD _{0.05}	3.90	0.80	4.29	6.70	7.74	0.57	2.06	1.29

Table 4. Micromutations in M₃ generation of horsegram cv. Dapoli Kulthi-1.

2.14 Number of pods per plant

The data recorded in (Table-3) revealed that the some of GR and GR + EMS treatments had stimulatory as well as inhibitory effect. In M₂ generation maximum number of pods per plant (83.80) were noted in 300Gy than control (71.20). The minimum number of pods per

plant (59.50) were recorded at 200Gy. However all the combination treatments have caused reduction in number of pods per plant. The trend in variation of pod number observed in M₃ generation was similar to that of M₂ generation (Table-4). The results of Dalvi (1990) for horsegram were in agreement with the present study.

2.15 Total number of seeds per pod

Data on total number of seeds per pod in M₂ and M₃ progeny showed non significant change as compared to control.

2.16 1000- seed weight

Results recorded on 1000-seed weight (Table-3) indicated that all the treatments of GR were non significant but the combination treatments such as 300Gy + 0.4%EMS and 300Gy + 0.5%EMS (30.50g and 29.80g) had shown considerable increase in 1000 seed weight over control. The results of M₃ generation were on par with M₂ generation (Table-4).

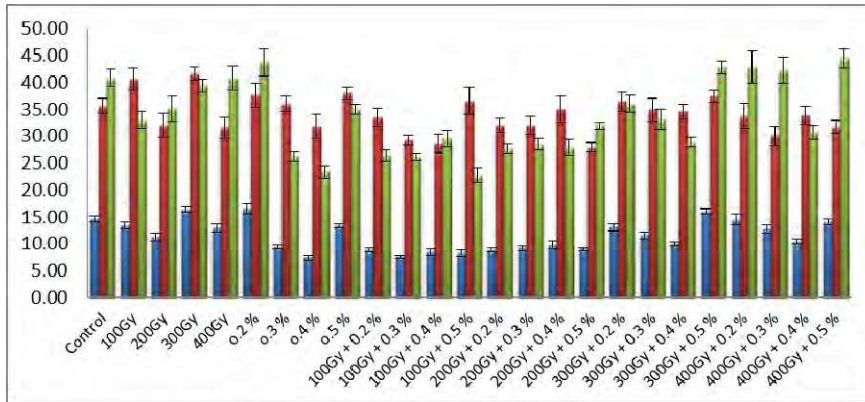
2.17 Seed yield per plant

Mean values for seed yield per plant decreased in treatments of GR and GR + EMS as compared to controls (Table-3). The maximum seed yield (16.76g) was noted in 300Gy and minimum (11.22g) in 200Gy as compared to control (14.54g). The combination treatment 300Gy + 0.5%EMS had induced maximum increase in seed yield per plant (16.01g) over control (14.54g). But all other treatments had caused reduction in seed yield per plant. In M₃ generation seed yield per plant was increased in all gamma radiation treatments, but it decreased in combinations as compared to control (Table-4). Maximum total seed yield was recorded in 300Gy (19.60g) and in 200Gy + 0.4%EMS (16.96g) as compared to control (16.58g). Hakande (1992) reported wider variability in yield due to mutagenic treatments in winged bean, which was attributed to pollen sterility and genetical as well as physiological alterations caused by mutagens. Previous studies indicated that both additive and non-additive genes contribute to yield. Luthra *et al.*, (1979), Reddy and Sree Ramulu (1982) also supported the above view.

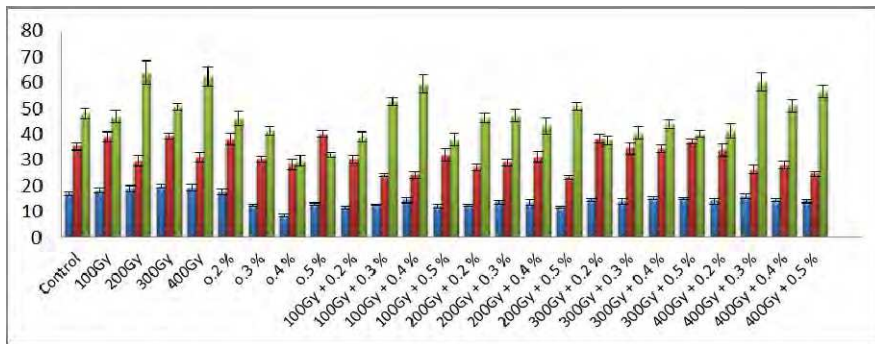
2.18 Harvest index

Mean values for seed yield per plant, biological yield and harvest index decreased with few exceptions in all mutagen treated populations as compared to their controls (Fig-1). In gamma radiation maximum seed yield (16.76g) as well as biological yield (41.55g) were recorded in 300Gy. The highest value of harvest index (40.81) was reported in 400Gy. In combination treatments seed yield 16.01g (300Gy + 0.5%EMS), biological yield 37.44g (300Gy + 0.5%EMS) and harvest index 44.77 (400Gy + 0.5%EMS) were recorded.

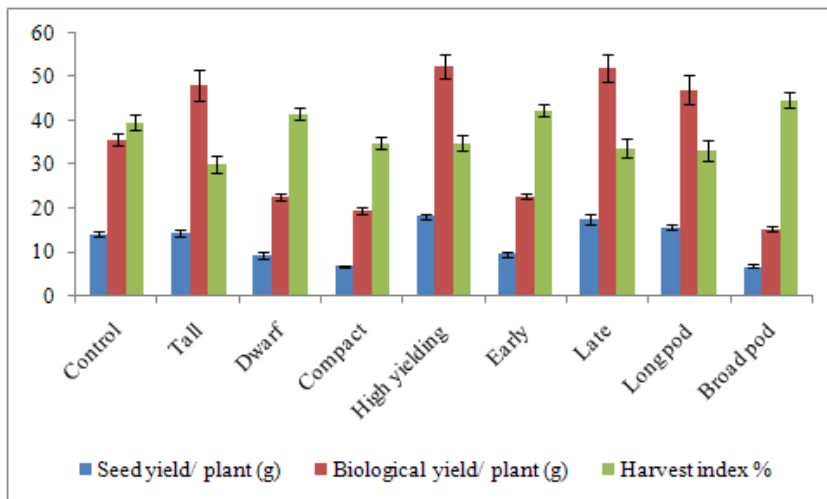
M₃ progeny showed increase in seed yield, decrease in biological yield and increase in harvest index as compare to M₂ (Fig-1). Jain (1975) claimed that high value of harvest index and dry matter production contributes to yield. The genotype with ability for converting larger part of dry matter in to economic yield is highly preferable (Donald, 1962). According to Vaghela *et al.*, (2009) the biological yield per plant and harvest index were found to be the most valuable traits for formulating the selection criteria to improve seed yield in chickpea.



a. M₂ Generation



b. M₃ generation



c. (c) M₃ Mutants

Fig. 1. Mutagenic effect on seed yield, biological yield and harvest index

The relationship of biological yield with economic yield or grain yield may help to predict the performance and yield efficiency of the genotypes.

2.19 Mutants in M₃

Mean values for seed yield per plant, biological yield and harvest index were positively or negatively influenced in all mutants as compared to controls (Fig-1). Maximum seed and biological yield was recorded in tall, high yielding, late and long pod mutants over control. The highest seed yield (18.29g) and biological yield (52.40g) in high yielding mutant and harvest index (44.72%) in broad pod mutant was recorded. In present investigation the rate of dry matter production in horsegram mutants was significantly correlated with seed yield (Fig-1). Work of Sahane *et al.*, (1995) in horsegram was inconformity with present study.

2.20 Morphological and yield attributes of M₃ mutants

Desirable mutants such as tall, dwarf, early, late, long and broad pod as well as high yielding were isolated from M₂ and M₃ generations. More than seven true breeding macromutants (Table-5) were characterized on the basis of morphological and yield attributes, which may be useful for future breeding programme in horsegram yield improvement.

Mutants	Plant height (cm)	Pri. br./ plant	DAS for 1 st flowering	DAS for 1 st pod maturity	No. of pods/ plant	No. of seeds/ pod	1000 seed Wt. (g)	Seed yield/ plant (g)
Control	56.70±2.27	6.30±0.25	61.70±2.47	102.20±4.09	71.20±2.85	6.90±0.28	26.80±1.07	14.15±0.57
Tall	71.50±3.58	7.30±0.37	63.60±3.18	105.40±5.27	82.40±4.12	6.40±0.32	27.20±1.36	14.54±0.73
Dwarf	16.30±1.14	4.20±0.29	58.30±4.08	97.70±6.84	47.00±3.29	7.00±0.49	25.80±1.81	9.49±0.66
Early	22.80±1.37	5.40±0.32	30.60±1.84	65.80±3.95	55.00±3.30	6.20±0.37	27.10±1.63	9.64±0.58
Late	60.90±3.65	8.10±0.49	65.40±3.92	110.20±6.61	85.30±5.12	7.20±0.43	28.30±1.70	17.58±1.05
Long pod	59.10±2.36	7.10±0.28	64.30±2.57	106.10±4.24	80.20±3.21	7.30±0.29	26.20±1.05	15.74±0.63
Broad pod	24.60±1.72	6.20±0.43	60.40±4.23	97.50±6.83	33.40±2.34	6.40±0.45	27.80±1.95	6.84±0.48
High yielding	59.40±1.78	8.10±0.24	62.80±1.88	109.30±3.28	87.20±2.62	7.20±0.22	28.50±0.86	18.29±0.55
SEM±	1.86	0.27	2.48	4.17	2.67	0.29	1.58	0.52
F-value	267.16	43.22	37.50	20.70	141.32	4.67	1.21	143.49
P-value	0.00	0.00	0.00	0.00	0.00	0.00	0.35	0.00
LSD _{0.05}	3.91	0.57	5.21	8.76	5.61	0.61	3.32	1.09

Data are means of three replicates ± standard deviation. Significant difference due to treatments was assessed by Fisher's LSD as a post-hoc test.

Table 5. Morphological and yield attributes of M₃ mutants of horsegram cv. Dapoli Kulthi-1.

2.21 Tall

These mutants were vigorous, tall (71.50cm), with medium and thick leaves with delayed flowers and pod formation compared to control. There was significant increase in primary branches, pods, 1000 seed weight and seed yield per plant (Plate- 4).



Plate 4. Stable mutants (M3 generation)

2.22 Dwarf

Plant height of these mutants ranged from 15 to 20cm and had profuse branching at the base, which formed a dense umbrella like canopy (Plate- 4). There was significant reduction in number of days required for first flowering and pod maturity period.

The induced dwarfness is desirable agronomic trait, because plant density per unit area, will be very high. It will help for fast translocation of metabolites from source to sink (Auti, 2005). Such mutants are valuable for investigating plant gene function and developing new crop variety with lodging resistance (Wei *et al.*, 2008).

2.23 Early

Early mutants were associated with dwarfness and showing early flowering within a short span of 30-33 days in comparison with the flowering duration of 53-57 days in control. The total duration of the crop was 65-70 days against 100-110 days in control (Plate- 4). Such type of mutants were recorded earlier in horsegram (Dalvi, 1990, Rudraswamy *et al.*, 2006 and Bolbhat and Dhupal (2010). These mutants are highly desirable to reduce the crop duration. Short duration variety of horsegram will play a key role in avoiding drought and water stress faced by rainfed agriculture.

2.24 Late

These mutants were with broad and thick dark green foliage, tall and bearing late flowers as compared to control. Flowering was achieved in 60 to 65 DAS against 53 to 57 DAS in control. Plant height, primary branches per plant, pods per plant, pod length, seeds per pod, 1000 seed weight and seed yield per plant was improved over control (Plate- 4).

According to Auti (2005) late maturity in these mutants was due to genetic damage caused by the mutagens. Same may be the reason for horsegram late mutants.

2.25 Long pod

The plants were normal in appearance with comparatively longer pods, containing seven to eight seeds per pod. (Plate- 4). Plant height, primary branches per plant, pods per plant, pod length, seeds per pod and seed yield per plant were significantly increased over control.

These mutants showed considerable increase in all quantitative traits, contributing to increase in yield. This type of mutants were reported earlier in mungbean (Auti and Apparao, 2009), urdbean (Sagade, 2008 and Gahlot *et al.*, 2008), cowpea (Girija and Dhanavel, 2009) and horsegram (Bolbhat and Dhupal, 2010).

2.26 Broad pod

These mutants had broad pods containing 6-7 bold seeds per pod (Plate- 4). Significant decrease was noted in days required for first flowering 60.40 DAS (control 61.70DAS) and maturity period 97.50DAS (control 102.20DAS). There was significant increase in 1000 seed weight 27.80g (control 26.80g).

2.27 High yielding

These mutants showed superior yield attributes such as length and number of pods, number of seeds, 1000 seed weight and yield per plant as compared to control (Plate- 4). Auti (2005) stated that some unknown mechanism must be inducing the expression of yield controlling genes, which are responsible to increase the yield. Same may be the reason for getting high yielding mutants in horsegram. These mutants can break the constrains of low yield of horsegram, in rainfed agriculture.

2.28 Antinutrients in seeds

2.28.1 Polyphenols

The seeds of all viable mutants showed reduced which will be of immense importance for the future development of desirable varieties of horsegram with improved nutritional quality.

2.29 Phytate

The results shown in Table- 6 revealed that all the mutants except dwarf and compact showed decrease in phytate content. Phytic acid decides the nutritional quality of cereals and seeds of legume as it is a strong antinutritional factor. It is also implicated in the "hard-to-cook" phenomenon of legumes (Stanley, 1985). Hence development of new cultivars with low phytate is the prime need of time. In present study tall, high yielding, early, late and long pod mutants showed reduced phytic acid contents (Table- 6). All such mutants can be exploited in future hybridization programme of horsegram aimed for improving nutrient quality.

Mutants	Polyphenols (mg 100g ⁻¹)	Phytate (mg 100g ⁻¹)	Trypsin inhibitors (TIU)
Control	442.25±17.69	2639.71±105.59	185.81±7.43
Tall	420.12±21.01	2565.51±128.28	110.43±5.52
Dwarf	447.14±31.30	2647.94±185.36	104.19±7.29
Compact	408.55±12.26	2643.83±79.31	120.71±3.62
Early	396.26±23.78	2425.56±145.53	98.75±5.93
Late	412.21±16.49	2544.99±101.80	126.96±
Long pod	411.37±28.80	2372.45±166.07	136.32±9.54
Broad pod	409.07±12.27	2641.59±79.25	129.98±3.90
High yielding	402.26±24.14	2289.66±137.38	138.65±8.32
SEM±	17.81	106.29	5.36
F-Value	1.88	3.24	46.18
P-Value	0.13	0.02	0.00
LSD _{0.05}	37.42	223.32	11.26

Data are means of three replicates ± standard deviation. Significant difference due to treatments was assessed by Fisher's LSD as a post-hoc test.

Table 6. Nutrient quality in seeds of M₃ mutants of horsegram cv. Dapoli Kulthi 1

2.30 Trypsin inhibitor

All the mutants showed decreased levels of trypsin inhibitor over control. Lowest values of TI were found in early mutant (98.75 TIU). The values of TI in different mutants in order as given below dwarf > tall >compact >late >long pod >high yielding.

The legume seeds like horsegram contain trypsin and chemotrypsin, which are the main anti-nutritional factors (Chavan and Hejgaard, 1981) as they decrease the digestibility of protein and cause pancreatic hypertrophy (Manjaya *et al.*, 2007). The decrease in the levels of trypsin inhibitor as a result of the treatments of mutagens was reported by Harsulkar (1994). Kim *et al.*, (2010) reported increase as well as decrease in trypsin inhibitor in mutants induced by gamma radiation. The mutants of horsegram with low TI have a great role in vegetarian diet of Indians.

3. Conclusion

In conclusion GR and GR+EMS had induced sufficient genetic variability in horsegram cv. Dapoli Kulthi-1. The agronomically and nutritionally superior mutants will be the promising material for plant breeders in future. Such cultivars will serve the purpose of protein malnutrition in vegetarian diets and will also economically benefit the resource poor farmers of rainfed area.

The high yielding mutants will play a major role to break the yield constraints in horsegram, as a result of this the farmers can be attracted towards the cultivation of this low input, nitrogen fixer legume crop and thereby it will help to improve the economic status of farmers from rainfed, drought prone areas of Maharashtra States. The early mutants will be of great help to reduce the crop duration and thereby help to avoid droughts in late stage of crop. The mutants with low TI, phytate and phenols will be of revolutionary importance, as they will be free from all antinutritional factors.

The stabilization of desirable mutants, through multilocation trials is in progress on farmers' field at Karjat (Dist-Ahmednagar), Research farms of Dr. B.S.S.K.A.U., Dapoli (Dist-Ratnagiri) and at Department of Botany, University of Pune (MS). The efforts are also being made to release the most desirable mutants of horsegram, Dapoli Kulthi-1.

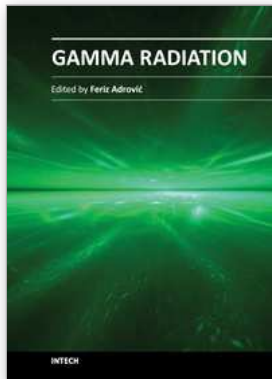
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This book brings new research insights on the properties and behavior of gamma radiation, studies from a wide range of options of gamma radiation applications in Nuclear Physics, industrial processes, Environmental Science, Radiation Biology, Radiation Chemistry, Agriculture and Forestry, sterilization, food industry, as well as the review of both advantages and problems that are present in these applications. The book is primarily intended for scientific workers who have contacts with gamma radiation, such as staff working in nuclear power plants, manufacturing industries and civil engineers, medical equipment manufacturers, oncologists, radiation therapists, dental professionals, universities and the military, as well as those who intend to enter the world of applications and problems of gamma radiation. Because of the global importance of gamma radiation, the content of this book will be interesting for the wider audience as well.

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