International Letters of Natural Sciences
ISSN: 2300-9675, Vol. 64, pp 17-31
doi:10.18052/www.scipress.com/ILNS.64.17

Submitted: 2017-04-01
Revised: 2017-05-03
Accepted: 2017-05-17
Online: 2017-07-21

Mutagenic Effectiveness and Efficiency of Gamma Rays and HZ with Phenotyping of Induced Mutations in Lentil Cultivars

Rafiul Amin Laskar* and Samiullah Khan

Mutation Breeding Laboratory, Department of Botany
Aligarh Muslim University, Aligarh, 202002, U.P., India

*Email: rafihkd@gmail.com

Keywords: lentil (*Lens culinaris* Medik.), biological damage, chlorophyll mutations, phenotype category and mutant phenotyping.

Abstract. In mutation breeding, mutagenic effectiveness and efficiency are the base parameters to predict the mutagenic potency of any mutagen. Studies on mutagenic effectiveness and mutagenic efficiency of physical mutagen (gamma rays) and chemical mutagen (hydrazine hydrates; HZ) on two cultivars of lentil (*Lens culinaris* Medik.), viz. DPL 62 (macrosperma) and Pant L 406 (microsperma) have been reported. Dry and healthy seeds were treated with four doses of each gamma rays (100-400 Gy), HZ (0.1-0.4 %) and their combinations. Frequencies of the induced agro-morphological variations into different phenotypic categories were estimated in M_2 population that resulted into identification and isolation of wide range of mutants with altered phenotypes. Data on effectiveness and efficiency of various mutagenic treatments calculated on the basis of the frequency of chlorophyll mutations, which showed effectiveness and efficiency were higher at the moderate doses of gamma rays and HZ, while in case of combination treatments; lower doses were most effective and efficient with few inter-varietal exceptions. Phenotyping of the mutants revealed that growth habits was the most sensitive category to which most of the mutant belongs, followed by leaf and flower/pod/seed in both the cultivars studied. Overall, the screened and isolated mutants with economically important agronomic traits can be further propagated in the subsequent generation for development of elite lentil mutant cultivars.

Introduction

The lentil (*Lens culinaris* Medikus subsp. *culinaris*) is a self-pollinated, annual diploid (2n = 2x = 14) grain legume crop with a relatively large genome of 4063 Mbp [1]. It was among the early-domesticates in the Fertile Crescent of the Near East. Barulina [2] divided the cultigens *Lens culinaris* Medik. in to two sub-species i.e. macrosperma (seed diameter, 6–9 mm) and microsperma (seed diameter, 2–6 mm), later renewed by Cubero [3] as race *macrosperma* and race *microsperma*. Lentil is an excellent dietary staple because of their high protein content and nutrient density that complements the nutritional deficiencies of cereal based diet and its cultivation enhance the soil nutrient status by adding nitrogen, carbon and organic matter, while serving economic advantage to farmer's livelihood with high market return. It has also high level of dietary fibre, vitamin B1 and the straw is valuable animal feed [4].

The induced mutations technology for the crop improvement have successfully been used to create about 3222 mutant varieties around the world, of which India contributed 330 mutant varieties [5]. Overall, 13 mutant lentil varieties released till now with only two from India [5]. Gamma rays are the short wavelength ionizing radiation with high penetrating potential. Hydrazine can react with the pyrimidines in DNA to saturate the 5, 6 double bond, especially thymine, to form N4-aminocytosine, and to open up the pyrimidine ring with consequent loss of pyrimidines from DNA [6]. The hydrazine (HZ) induced mutations are mostly due to direct mispairing at replication rather than by error-prone repair [7] while gamma rays mostly cause chromosomal and gene mutations. Analysis on effectiveness and efficiency of mutagen is an imperative tool in mutation breeding for crop improvement. The prior information of comparative effectiveness and efficiency of various mutagens facilitate the selection, which is essential to recover high frequency of desirable mutations [8, 9]. Although, effectiveness and efficiency of a particular mutagen are

completely different properties but they together define the usefulness of any mutagen. It is not necessary that an effective mutagen shall be an efficient one also [10]. The value of effectiveness and efficiency estimation depends on various factors like biological, environmental and chemical that can modify mutation rate of different mutagens [11].

The objectives of the present study were to assess the comparative effectiveness and efficiency of the single and combination treatments of gamma rays and HZ on the induction of genetic variability for identifying the optimum concentrations of mutagens for induced mutagenesis in *Lens culinaris* Medik. and to assess the comparative sensitivity of the different phenotypic categories of lentil for understanding the spectrum of mutations induced by the different concentrations of mutagens.

Material and Methods

The genetic variability was induced in lentil microsperma cultivar Pant L 406 (Selection P-495; IIPR, Kanpur, 1996) and macrosperma cultivar DPL 62 (JLS 1 X LG 171; Govind Ballabh Pant University of Agriculture & Technology (GBPUA&T), Pantnagar, 1979) using different single and combined treatments of physical mutagen (gamma rays) and chemical mutagen (hydrazine hydrates). The accessions were obtained from National Bureau of Plant Genetic Resources (NBPGR), New Delhi and are highly recommended for the agro-climatic zone of central India. The healthy and viable seeds (moisture 11.0%) of both the cultivars were directly irradiated with 100, 200, 300 and 400 Gy of gamma rays at the dose rate of 0.695 kGy h^{-1} in Gamma chamber-900 (calibrated by Fricke dosimeter) with a radioisotope ^{60}Co (Cobalt-60), source at the National Botanical Research Institute, Lucknow, Uttar Pradesh, India. For chemical treatments, presoaked (6 hrs) seeds were treated with different doses (v/v) of hydrazine hydrates (HZ) viz, 0.1%, 0.2%, 0.3% and 0.4% at room temperature of 25±2°C for 9 hrs at pH-7. Also, a combination treatment sets of seeds viz. 100 Gy γ rays + 0.1 % HZ, 200 Gy γ rays +0.2 % HZ, 300 Gy γ rays + 0.3 % HZ and 400 Gy γ rays + 0.4 % HZ, were prepared by directly treating the gamma treated seeds with hydrazine hydrates concentrations. Control seeds were prepared simply by presoaking in distilled water for 6 hrs. Initially, the doses of chemical and physical treatments were determined based on LD_{50} values from the germination and survival test. The 300 seeds from each treatment were grown in the agricultural field of Aligarh Muslim University, Aligarh, India along with respective controls following a complete randomized block design. All the self-pollinated M_1 plants were harvested individually and 10 healthy M_2 seeds from each harvested plant were sown in plant progeny row basis for growing M_2 generation. Mutagenic effectiveness is a measure of the frequency of mutations induced by unit dose of a mutagen, while mutagenic efficiency represents the proportion of mutations in relation to biological damage. The formulas suggested by Konzak [12] were used to evaluate the mutagenic effectiveness and efficiency of the mutagens used.

$$\% \text{ mutated plant progenies (Mp)} = \frac{\text{No.of mutant plant progenies segregating in M2}}{\text{Total no.of M1 plant progenies}}$$

$$\text{Mutagenic effectiveness Rate of mutation (Mp) (Gamma rays)} = \frac{\text{Rate of mutation (Mp)}}{\text{Dose in Gray (Gy)}}$$

$$\text{Mutagenic effectiveness Rate of mutation (Mp) (HZ)} = \frac{\text{Rate of mutation (Mp)}}{\text{Concentration} \times \text{duration of treatment}}$$

$$\text{Mutagenic effectiveness Rate of mutation (Mp) (Combination)} = \frac{\text{Rate of mutation (Mp)}}{\text{Dose of physical mutagen (Gy)} \times \text{concentration of chemical mutagen} \times \text{duration of treatment}}$$

$$\text{Mutagenic efficiency} = \frac{\text{Rate of mutation (Mp)}}{*\text{Biological damage in M1 generation}}$$

*Biological damage: For measuring the biological damage, three different criteria were used;

(i) Injury - i.e. percentage reduction in seedling height (Mp/I)

(ii) Sterility - i.e. percentage reduction in pollen fertility (Mp/S)

(iii) Meiotic abnormalities - i.e. percentage of meiotic abnormalities (Mp/Me)

The induced mutations in the plant morphology of the lentil cultivars were categorize into six major phenotypic categories viz. plant size, growth habit, leaf, flower, pod and seed. Each category includes various mutant phenotypes related to that particular morphology and frequencies of the mutation in each morphological category out of the total morphological mutations were calculated throughout the growing season of M_2 generations.

Results

Frequency and Spectrum of Chlorophyll Mutations

In M_2 generation, total six different types of chlorophyll mutants were recorded in the field when seedlings were 10-20 days old. The spectrum of different M_2 chlorophyll mutants included were *albina, chlorina, maculata, tigrina, viridis and xantha*. All these chlorophyll deficient mutants were lethal except *chlorina, maculata and viridis*. A brief description of the isolated different chlorophyll mutants is given in Table 1. The calculations on the chlorophyll mutations were done in plant progeny basis. The estimations showed that the induction of chlorophyll mutation did not follow a linear trend with increasing concentrations of mutagens but the higher treatments resulted more frequency of chlorophyll mutants in both the cultivars (Table 1). The obtained mutant frequencies showed that single treatments of gamma rays and HZ have produced chlorophyll mutants at a comparable frequency and the higher frequencies of chlorophyll mutants were found in cultivar Pant L 406 than the cultivar DPL 62. In both the cultivars, the frequency of *albina* mutants was the highest, followed by those of *chlorina, xantha, maculata, viridis* and *tigrina*. Among the different chlorophyll mutants, the combined gamma rays + HZ treatments and single HZ treatments produced *albina* mutations relatively in large number whereas gamma rays alone produced chlorina in maximum number. The cultivar Pant L 406 produced higher number of chlorophyll mutants than the cultivar DPL 62 (Table 2). The rate of appearance of different chlorophyll mutants could be arranged in order *Albina> Chlorina> Xantha> Maculata> Viridis> Tigrina* (Table 2) in both the cultivars. The *Albina, Chlorin, Maculata, Tigrina, Viridis* and *Xantha* were generated in number 223, 218, 68, 42, 49 and 88 in var. Pant L 406 and 210, 176, 44, 29, 32 and 62 in DPL 62, respectively (Table 2). There was no significant additive effect in producing chlorophyll mutations observed in the combination treatments in both the cultivars.

Mutagenic Effectiveness and Efficiency

Data on effectiveness and efficiency of various mutagenic treatments calculated on the basis of the frequency of chlorophyll mutations are given in Tables 3 and 4. The result showed that effectiveness and efficiency were higher at the moderate concentrations of single treatments and lower concentration of combined treatments of gamma rays and HZ. The estimates of effectiveness ranged from 3.433 to 6.667 in the cv. DPL 62 and 6.779 to 11.783 in the cv. Pant L 406 of HZ treatments, whereas the effectiveness of gamma rays treatments ranged from 0.021 to 0.040 and 0.035 to 0.060 in the cv. DPL 62 and cv. Pant L 406, respectively. The effectiveness of the combined gamma rays + HZ treatments ranged from 0.015 to 0.136 in the cv. DPL 62 and 0.017 to 0.199 in the cv. Pant L 406 (Tables 3 and 4). The decreasing order of mutagenic effectiveness was found to be HZ, gamma rays + HZ and gamma rays. Mutagenic efficiency was calculated on the basis of seedling injury (Mp/I), pollen sterility (Mp/S) and meiotic abnormalities (Mp/Me) which resulted highest efficiency to 0.2% HZ among HZ treatments and 200 Gy γ rays among gamma rays treatments in both the cultivars. Among the combination treatments, 200Gy γ rays+ 0.2% HZ in cv. DPL 62 and 100Gy γ rays+ 0.1% HZ in cv. Pant L 406 were the most efficient mutagen treatments. Overall, on the basis of seedling injury, pollen sterility and meiotic abnormalities, the efficiency of mutagens in descending order was: gamma rays + HZ > HZ > gamma rays in cv. DPL 62 (Tables 3) and HZ > gamma rays + HZ > gamma rays IN cv. Pant L 406 (Tables 4). The efficiency calculated on the basis of Mp/Me was generally higher as compared to Mp/I and Mp/S.

Frequency of M_2 Morphological Mutation and Plant Phenotyping

A wide range of mutant phenotypes were induced in the populations of two cultivars of lentil in M_2 generation, several of which are useful from the breeder's point of view. The induced variations in detectable phenotypes at different growth phases of M_2 plants from seed germination to maturation were inspected and recorded in both the cultivars. Different types of morphological variants with altered characteristic affecting plant height, growth habit, leaf, flower, pod and seed were observed at different treated population with respect to the parental populations in M_2 generation of lentil cultivars. Mutation frequency was calculated on M_2 plant basis. Overall, the frequency of morphological mutations was higher in the cv. Pant L 406 than the cv. DPL 62 (Tables 5 and 6). On combining the cultivars data, the spectrum of mutations induced was wider in HZ than that of gamma rays and the combined gamma rays + HZ treatments. Characterization of the phenotypic categories and their individual frequencies was present in Tables 5 and 6 for both the cultivars, while the frequencies of the mutation in each morphological category out of the total morphological mutations reported were presented in Figure 1. Most of the induced mutants were found to be fall under growth habit and leaf mutations category followed by pod and plant height in both the lentil cultivars DPL 62 and Pant L 406 (Fig. 1). Different mutation in growth habit like bushy, prostrate, erect etc and in plat size like tall, semi-dwarf and dwarf were observed in M_2 generation of both the cultivars. In cv. DPL 62, the highest frequency was noted in gamma rays treated population (3.48 %) and the lowest with HZ treatments (2.80%), while combination treatments of gamma rays + HZ (3.29%) were intermediate, while the frequency of mutants were highest in gamma rays + HZ (3.69%) treated population followed by HZ (3.59%) and gamma rays (2.57%) in cv. Pant L 406 (Fig. 2). In the two varieties of lentil, the combined treatments of gamma rays and HZ did not produce additive effects on morphological mutations.

Table 1. Characteristic features of chlorophyll mutants and percentage mutated plant progenies induced by gamma rays, HZ and their combinations in two varieties of lentil in M_2 generation.

Isolated mutant types and their characteristics	Treatment	No. of M_1 plant progenies	No. of plant progenies segregating in M_2	% mutated plant progenies (Mp)
	Var. DPL 62			
1. Albina Lethal mutation characterized by entirely white leaves of seedlings; seedlings survived for 10-12 days after germination.	Control	269	-.--	-.--
	0.1% HZ	243	5	2.06
	0.2% HZ	225	18	8.00
	0.3% HZ	211	21	9.95
	0.4% HZ	193	19	9.84
2.Chlorina Light green colour of leaves; most of the seedlings died within 20 days, however, few vigorous plants survived and were late in maturity.	100 Gy γ rays	236	5	2.12
	200 Gy γ rays	215	17	7.91
	300 Gy γ rays	203	16	7.88
	400 Gy γ rays	186	19	10.22
3.Maculata Seedlings showed yellow or whitish dots on leaves; mutants survived till maturity and produced few seeds.	100Gy γ rays+0.1% HZ	220	18	8.18
	100Gy γ rays+0.2% HZ	198	24	12.12
	200Gy γ rays+0.1% HZ	181	22	12.15
	200Gy γ rays+0.2% HZ	175	25	14.29

	Var. Pant L 406			
	Control	260	-.--	-.--
4.Tigrina Leaves yellow with green patches typical of the colour of the skin of Indian frog; survived for 15 days.	0.1% HZ	217	13	5.99
	0.2% HZ	198	28	14.14
	0.3% HZ	184	29	15.76
	0.4% HZ	166	27	16.27
5.Viridis Reduced plant height and viridine green colour of leaves; leaflets size reduced; plants were slow growing and had a low seed yield.	100 Gy γ rays	225	9	4.00
	200 Gy γ rays	208	25	12.02
	300 Gy γ rays	192	23	11.98
	400 Gy γ rays	172	24	13.95
6.Xantha Leaves were bright yellow in colour; seedlings survived for 10-20 days.	100Gy γ rays+0.1% HZ	201	24	11.94
	100Gy γ rays+0.2% HZ	181	25	13.81
	200Gy γ rays+0.1% HZ	163	23	14.11
	200Gy γ rays+0.2% HZ	155	25	16.13

Table 2. Frequency and spectrum of chlorophyll mutants induced by gamma rays, HZ and their combinations in two cultivars of lentil in M_2 generation.

Treatment	N*	Chlorophyll mutant types						CMS**	F (%)	k#
		Albina	Chlorina	Maculata	Tigrina	Viridis	Xantha			
Var. DPL 62										
Control	2502	-	-	-	-	-	-	-	-	-
0.1% HZ	2219	16	13	-	2	0	7	38	1.71	-
0.2% HZ	1995	15	10	-	-	5	5	35	1.75	-
0.3% HZ	1800	18	12	6	4	4	7	51	2.83	-
0.4% HZ	1608	14	11	5	-	2	4	36	2.24	-
100 Gy γ rays	2171	18	16	2	-	-	4	40	1.84	-
200 Gy γ rays	1942	19	17	4	2	3	3	48	2.47	-
300 Gy γ rays	1779	22	16	5	5	5	6	59	3.32	-
400 Gy γ rays	1569	13	24	4	4	-	5	50	3.19	-
100Gy γ rays+0.1% HZ	1965	17	-	5	-	-	-	22	1.12	0.32
200Gy γ rays+0.2% HZ	1709	19	18	4	2	-	-	43	2.52	0.60
300Gy γ rays+0.3% HZ	1533	18	20	6	6	7	12	69	4.50	0.73
400Gy γ rays+0.4% HZ	1418	21	19	3	4	6	9	62	4.37	0.80
Var. Pant L 406										
Control	2427	-	-	-	-	-	-	-	-	-
0.1% HZ	2004	-	11	2	2	-	9	24	1.20	-
0.2% HZ	1716	13	-	5	-	3	-	21	1.22	-
0.3% HZ	1515	18	11	8	-	6	8	51	3.37	-
0.4% HZ	1328	15	8	4	1	1	5	34	2.56	-
100 Gy γ rays	2025	19	25	-	4	-	4	52	2.57	-
200 Gy γ rays	1858	24	28	6	4	6	11	79	4.25	-
300 Gy γ rays	1638	25	28	10	5	8	12	88	5.37	-
400 Gy γ rays	1422	15	27	7	7	7	10	73	5.13	-
100Gy γ rays+0.1% HZ	1715	18	20	3	-	-	-	41	2.39	0.63
200Gy γ rays+0.2% HZ	1520	21	26	5	5	-	7	64	4.21	0.77
300Gy γ rays+0.3% HZ	1309	29	20	10	8	10	12	89	6.80	0.78
400Gy γ rays+0.4% HZ	1209	26	14	8	6	8	10	72	5.96	0.78

N*= Number of M_2 seedlings; CMS**= Chlorophyll mutated seedlings; F: Frequency; $k^{\#}$ = Coefficient of interaction based on % M_2 chlorophyll mutation frequency.

Table 3. Effectiveness and efficiency of gamma rays, HZ and their combinations in lentil cv. DPL 62 in M_2 generation.

Treatment	% Seedling injury (I)	% Pollen sterility (S)	% Meiotic abnormalities (Me)	% Mutated plant progenies (Mp)	Mutagenic effectiveness	Mutagenic efficiency		
						Mp/I	Mp/S	Mp/Me
0.1% HZ	8.74	9.42	2.88	2.06	3.433	0.24	0.22	0.71
0.2% HZ	17.67	10.41	7.07	8.00	6.667	0.45	0.77	1.13
0.3% HZ	23.31	19.74	8.36	9.95	5.528	0.43	0.50	1.19
0.4% HZ	31.31	26.67	10.03	9.84	4.100	0.31	0.37	0.98
100 Gy γ rays	16.55	7.52	5.54	2.12	0.021	0.13	0.28	0.38
200 Gy γ rays	16.55	11.86	6.80	7.91	0.040	0.48	0.67	1.16
300 Gy γ rays	17.79	21.09	9.52	7.88	0.026	0.44	0.37	0.83
400 Gy γ rays	31.68	27.87	10.40	10.22	0.026	0.32	0.37	0.98
100Gy γ rays+0.1% HZ	17.98	10.58	6.09	8.18	0.136	0.45	0.77	1.34
200Gy γ rays+0.2% HZ	21.88	12.50	8.67	12.12	0.051	0.55	0.97	1.40
300Gy γ rays+0.3% HZ	27.53	21.63	8.77	12.15	0.023	0.44	0.56	1.39
400Gy γ rays+0.4% HZ	33.42	30.03	12.41	14.29	0.015	0.43	0.48	1.15

Table 4. Effectiveness and efficiency of gamma rays, HZ and their combinations in lentil cv. Pant L 406 in M_2 generation.

Treatment	% Seedling injury (I)	% Pollen sterility (S)	% Meiotic abnormalities (Me)	% Mutated plant progenies (Mp)	Mutagenic effectiveness	Mutagenic efficiency		
						Mp/I	Mp/S	Mp/Me
0.1% HZ	12.24	7.71	5.07	5.99	9.983	0.49	0.78	1.18
0.2% HZ	24.79	9.00	8.51	14.14	11.783	0.57	1.57	1.66
0.3% HZ	36.73	11.60	9.88	15.76	8.756	0.43	1.36	1.60
0.4% HZ	59.05	18.63	11.04	16.27	6.779	0.28	0.87	1.47
100 Gy γ rays	19.70	6.58	6.46	4.00	0.040	0.20	0.61	0.62
200 Gy γ rays	31.43	12.56	8.16	12.02	0.060	0.38	0.96	1.47
300 Gy γ rays	50.31	16.39	10.75	11.98	0.040	0.24	0.73	1.11
400 Gy γ rays	60.75	22.92	11.25	13.95	0.035	0.23	0.61	1.24
100Gy γ rays+0.1% HZ	28.09	7.10	8.33	11.94	0.199	0.43	1.68	1.43
200Gy γ rays+0.2% HZ	44.70	14.01	11.22	13.81	0.058	0.31	0.99	1.23
300Gy γ rays+0.3% HZ	50.72	16.10	10.96	14.11	0.026	0.28	0.88	1.29
400Gy γ rays+0.4% HZ	65.38	23.42	12.76	16.13	0.017	0.25	0.69	1.26

Table 5. Frequency and spectrum of morphological mutants induced by various mutagens in M_2 generation of lentil cv. DPL 62.

MUTANT TYPE		HZ		Gamma rays		HZ+ Gamma rays		TOTAL		GRAND TOTAL	
		N*	F%	N*	F%	N*	F%	N*	F%	N*	F%
Plant height	Tall	19	0.26	20	0.29	21	0.35	60	0.30	**90**	**0.45**
	Dwarf	14	0.19	15	0.22	1	0.02	30	0.15		
Growth habit	Compact/Bushy	7	0.10	22	0.32	18	0.30	47	0.23	**179**	**0.89**
	Prostrate	0	0.00	7	0.10	29	0.49	36	0.18		
	Spreading	4	0.06	9	0.13	4	0.07	17	0.08		
	One sided branching	23	0.32	17	0.25	3	0.05	43	0.21		
	Axillarybranches	10	0.14	7	0.10	19	0.32	36	0.18		
Leaf	Broad /Giant leaf	9	0.13	12	0.17	10	0.17	31	0.15	**128**	**0.64**
	Narrow leaf	4	0.06	9	0.13	8	0.14	21	0.10		
	Altered leaf architecture	18	0.25	19	0.27	16	0.27	53	0.26		
	Elongatedrachis	8	0.11	15	0.22	0	0.00	23	0.11		
Flower	Multiple flower	0	0.00	2	0.03	0	0.00	2	0.01	**63**	**0.31**
	Colour	6	0.08	0	0.00	0	0.00	6	0.03		
	Open flower	0	0.00	3	0.04	0	0.00	3	0.01		
	Non-flowering/Vegetative	0	0.00	5	0.07	0	0.00	5	0.02		
	Late flowering	10	0.14	8	0.12	6	0.10	24	0.12		
	Early maturity	11	0.15	4	0.06	8	0.14	23	0.11		
Pod	Small/Narrow pods	11	0.15	14	0.20	15	0.25	40	0.20	**94**	**0.47**
	Bold pods	17	0.24	20	0.29	17	0.29	54	0.27		
Seed	Pigmentation	13	0.18	13	0.19	11	0.19	37	0.18	**83**	**0.41**
	Colouration	7	0.10	8	0.12	3	0.05	18	0.09		
	Shape and Smoothness	10	0.14	12	0.17	6	0.10	28	0.14		
GRAND TOTAL		**201**	**2.80**	**241**	**3.48**	**195**	**3.29**	**637**	**3.18**		

N*=Number of mutants; F%= Mutant frequency % of M_2 plants.

Table 6. Frequency and spectrum of morphological mutants induced by various mutagens in M_2 generation of lentil cv. Pant L 406.

MUTANT TYPE		HZ		Gamma rays		HZ+ Gamma rays		TOTAL		GRAND TOTAL	
		N*	F%	N*	F%	N*	F%	N*	F%	N*	F%
Plant height	Tall	10	0.17	21	0.34	11	0.22	42	0.25	**91**	**0.54**
	Dwarf	11	0.19	18	0.29	20	0.41	49	0.29		
Growth habit	Compact/Bushy	9	0.16	14	0.23	9	0.18	32	0.19	**117**	**0.69**
	Prostrate	6	0.10	0	0.00	23	0.47	29	0.17		
	Spreading	0	0.00	12	0.19	0	0.00	12	0.07		
	One sided branching	12	0.21	0	0.00	11	0.22	23	0.14		
	Axillarybranches	4	0.07	9	0.14	8	0.16	21	0.12		
Leaf	Broad /Giant leaf	21	0.36	12	0.19	12	0.24	45	0.27	**138**	**0.82**
	Narrow leaf	15	0.26	12	0.19	7	0.14	34	0.20		
	Altered leaf architecture	22	0.38	1	0.02	10	0.20	33	0.20		
	Elongatedrachis	10	0.17	9	0.14	7	0.14	26	0.15		
Flower	Multiple flower	0	0.00	0	0.00	0	0.00	0	0.00	**30**	**0.18**
	Colour	0	0.00	0	0.00	2	0.04	2	0.01		
	Open flower	0	0.00	1	0.02	0	0.00	1	0.01		
	Non-flowering/Vegetative	0	0.00	4	0.06	1	0.02	5	0.03		
	Late flowering	6	0.10	0	0.00	2	0.04	8	0.05		
	Early maturity	4	0.07	6	0.10	4	0.08	14	0.08		
Pod	Small/Narrow pods	29	0.50	8	0.13	16	0.32	53	0.31	**100**	**0.59**
	Bold pods	19	0.33	16	0.26	12	0.24	47	0.28		
Seed	Pigmentation	12	0.21	10	0.16	12	0.24	34	0.20	**73**	**0.43**
	Colour	7	0.12	2	0.03	6	0.12	15	0.09		
	Shape and Smoothness	10	0.17	5	0.08	9	0.18	24	0.14		
GRAND TOTAL		**207**	**3.59**	**160**	**2.57**	**182**	**3.69**	**549**	**3.25**		

N*=Number of mutants; F%= Mutant frequency % of M_2 plants.

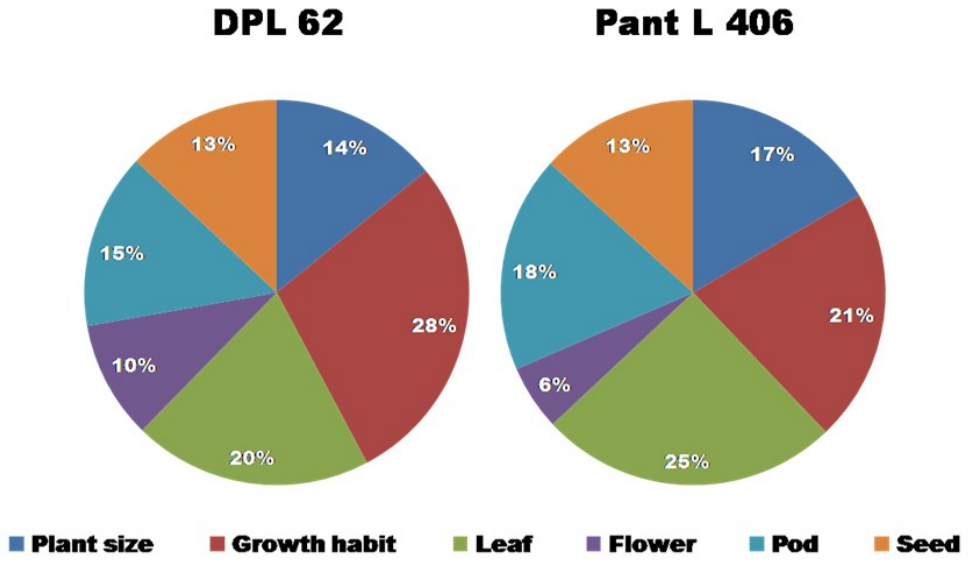

Figure 1. The comparative grouping of phenotypic mutants by six major phenotypic categories in M_2 plants of mutagenized populations of lentil cultivars DPL 62 and Pant L 406.

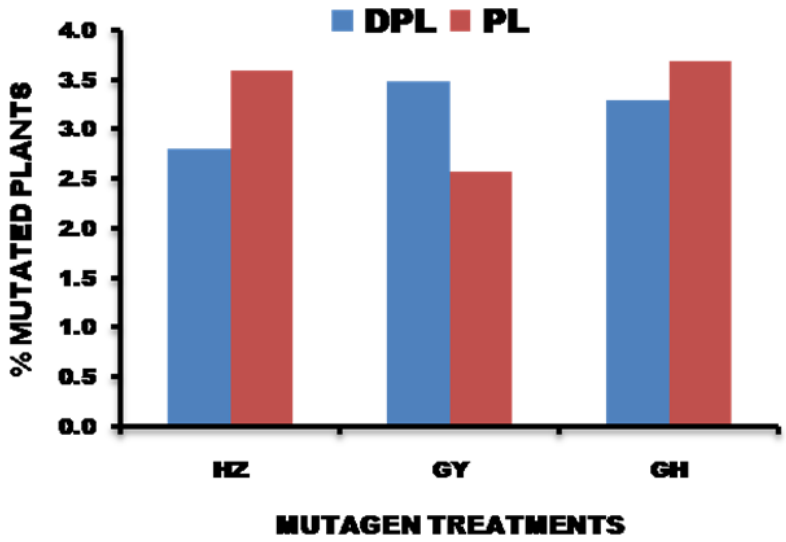

Figure 2. The comparative estimation of phenotypic mutants induced by Hydrazine hydrates-mutagenized populations (HZ), Gamma rays-mutagenized populations (GY) and Gamma rays+HZ-mutagenized populations (GH) in M_2 plants of lentil cultivars DPL 62 and Pant L 406.

Discussion

Mutagenic Effectiveness and Efficiency

Mutagenic effectiveness and efficiency is a measure of usefulness of a particular mutagen for mutation breeding in a particular crop genotype. Mutagenic effectiveness is a measure of the frequency of mutations induced by the unit dose of the mutagen, whereas the mutagenic efficiency of a treatment indicates the extent of genetic damage recorded in M_2 generation in relation to the biological damage caused in M_1 [12]. Therefore, mutagenic effectiveness is an indicator of the genotypic sensitivity towards the increasing mutagenic concentrations, while mutagenic efficiency explains the proportion of mutations in relation to the undesirable biological effects, such as, seedling injury, pollen sterility and meiotic abnormalities induced by the particular mutagen. In the present pursuit, the order of mutagenic effectiveness as determined on the basis of frequencies chlorophyll mutants was HZ > gamma rays + HZ > gamma rays. Superiority of effectiveness of chemical mutagens over gamma rays was also reported by in rice [13], lentil [14], mung bean [15], chickpea [16], urd bean [17], cowpea [18] and soybean [19]. In the cultivar DPL 62, moderate concentrations of gamma rays and HZ treatments were found to be more effective while lower concentrations of combination treatments were proved more effective. On estimation of mutagenic effectiveness in cultivar Pant L 406, it was found interestingly that the lower concentrations were more effective in all gamma rays, HZ and combination treatments. The comparative deviation in the effectiveness of identical mutagen concentration in both the cultivars indicates the presence of genetic divergence among them, and the sensitivity level of two genomes towards the employed mutagenic concentrations were independent. The decline the effectiveness at the higher treatments apparently showed that the mutations are independent events and their rate of occurrences is not proportional to the increasing strength of mutagen concentrations. The efficiency of the gamma rays, HZ and their combination treatments were determined by considering the three indices viz., seedling injury (Mp/I), pollen sterility (Mp/S) and meiotic abnormalities (Mp/Me) recorded in the M_1 generation of the crop populations. Each of these criteria resulted different efficiencies for same mutagenic concentrations, which suggests the flexibility of applying any one or all at a time depending on the objective of assessments. The results revealed that the mutagenic efficiency calculated from the meiotic abnormalities was lower as compared seedling injury and pollen sterility in both the cultivars. The observation is due to the fact that the induced meiotic abnormalities were less detrimental than the amount of seedling injury and pollen sterility in both single and combined gamma rays and HZ treatments. In the present study, the combined treatments

were found to be more efficient in comparison to the individual mutagenic treatments of gamma rays and HZ. It appeared that the higher frequency of chlorophyll mutations induced was the reason behind the higher efficiency of the combined treatments. Like effectiveness, moderate concentrations of gamma rays and HZ were more efficient in cultivar DPL 62 while the lower concentrations were mostly more efficient in cultivar Pant L 406. In combination treatments of gamma rays and HZ, lower concentrations were more efficient in both the cultivars while higher concentrations of single and combine treatments were least efficient in inducing genetic variations in the lentil cultivars. Khan [20] reported higher efficiency of mutagens at the lower concentrations in black gram, however, Gautam [21] in black gram and Ganapathy et al. [22] in millet have reported that mutagenic efficiency increased with the increase in the mutagens concentrations. The higher efficiencies of the lower and moderate concentrations of mutagens were clearly due to the lower biological damage (seedling injury, pollen sterility and meiotic abnormalities) which increased with increase in the strength of the mutagen treatments. Similar observations of higher effectiveness and efficiency at the lower and moderate concentrations of mutagens were also made in *Lathyrus sativus* [23], *Pisum sativum* [24], *Glycine max* [25] and *Vigna radiata* [26].

Morphological Mutation and Plant Phenotyping

Induced mutagenesis enhances the frequency and spectrum of mutations in the desired plant characteristics for crop genetic improvement through mutation breeding. The phenotypic or probably genotypic diversity generated from the induced morphological macromutations are of immense interest to the plant breeders as it offers additional markers for genetic enhancement and linkage studies in crops. Since, the selection in plant breeding generally based on phenotypes and not on genotypes, thus, the numerical interpretation of variances in phonological parameters is significantly pertinent to assess the extent of mutations captured by the plants from mutagen treated seeds up to maturity for proficient selection of mutants at the phenotypic level. Therefore, to identify the expression level of induced novel genes or new null alleles of genes concern in the morphogenesis of plant and to obtain the feasible morphological mutants in relation to other agronomic traits, in the present study, the morphological mutations involving diverse plant characteristics were isolated on the screening of M_2 lentil populations of both the cultivars in each of the treatment conditions. The phenotyping of the isolated mutants led to the classification of these mutants into different categories like plant size, growth habit, leaf, flower, pod and seed. Although economic value of most of the mutants in different morphological categories cannot be established but some mutants can be used as a source of many beneficial genes in cross breeding programmes or for the improvement of some quantitative traits [27], may be useful in mapping studies [28] and in determining the evolution of the crops [29]. The pleiotropic effects of mutated genes or chromosomal aberrations or gene mutations were considered as the root cause for the development of such morphological mutants [26, 30]. Each category of mutants showed different frequencies of occurrence not only in the two cultivars of lentil but also within the cultivar in different mutagenic treatments, signifying the mutagen type and concentration dependency of the cultivars for induction of macromutations. The lentil cultivar DPL 62 was found to be comparatively less mutable than the lentil cultivar Pant L 406 towards the mutagens employed based on the observation on morphological mutations frequency in M_2 populations. Tyagi and Gupta [31] reported that a wide spectrum of viable mutants can be expected due to the mutability of each gene of agronomic interest in mutation experiments. It was observed that the progenies of tall, dwarf, bushy, prostrate and bold seeded mutants bred true for the altered traits in M_3 generation. The genes responsible for the altered plant size and growth habits were reported to be monogenic recessive [32-37]. While Konzak et al. [38] and Shakoor et al. [39] in wheat and triticale, respectively, reported that the polygenes are responsible for semi dwarf character in mutants; Qin et al. [40] reported a dominant dwarf mutation in the single gene in rice. Zhang et al. [41] developed s-dwarf mutant of wheat that can tolerates varying level of stresses. In the cultivar DPL 62, HZ has highest frequency of mutations followed by combination treatment and gamma rays, whereas, in cultivar Pant L 406, combination treatment followed by HZ and gamma rays was the trend. A high

frequency and broad spectrum of induced morphological mutants has been reported in *Vigna mungo* [42, 43], *Lens culinaris* [31, 44, 45], *Cicer arietinum* [46] and *Glycine max* [19]. Higher frequencies of some mutant types in some mutagenic treatments showed the relative differences in the mutability of genes for different traits. The mutant types which appeared more frequently in the treated populations of a particular mutagen indicate the higher sensitivity of the genes responsible for these traits towards the mutagen. Nilan [47] reported that different mutagens and treatment procedures may also change the relative proportion of different mutation types. Tall mutants, as observed in the present study, were also reported earlier in lentil [48] and in blackgram [32, 49] using different mutagens. Dwarf mutants were also seen earlier in barley [50], grasspea [51] and *Vigna* spp. [26]. While dwarfness may be due to reduced internode length or internode number or both [52], in the present study, reduction in internode length was mainly responsible for dwarfness. The prostrate growth habit mutants generally exhibited weak stem with long internode. Similar growth habit mutants were also reported in *Phaseolus vulgaris* [53] and *Vigna radiata* [26]. The mutants identified as bold seeded can be of useful increasing the genetic yield potential of the lentils. Bold seeded mutants in *Vigna mungo* were characterized as gene mutations by Singh [54] due to no noticeable chromosomal changes associated with them. Studies on the days to maturity resulted few maturing mutants in the treated populations of both the cultivars of lentil with normal seed yield. However, early mutants with altered agronomic characteristics like yield and growth habit were isolated in *Vigna mungo* [55, 49]. Gottschalk and Wolff [56] stated that the induce mutagenesis can effectively be applied for generating early maturity mutants. Workers studied on the induced morphological mutants in different crop plants have attributed the altered phenotypes to the chromosomal breakage, disturbed auxin synthesis, disruption of mineral metabolism and accumulation of free amino acids [33, 43, 57-59]. Flower colour mutants can be exploited as genetic markers in different breeding experiments [60]. The pleotropic effect of the genes governing most of the macromutations in the morphological traits is the reason that restricts the easier elimination of negative traits from the positive ones, but Sidorova [61] advocated the transfer of pleiotropic pattern of mutant gene into a specific genotypic background for their effective alteration. The morphological mutants identified in the present study may not be economically feasible to commercialize directly due to the presence of some undesirable traits but in context of the plant breeders, these can be further exploited as source of many elite genes or as a parents in hybridization programmes.

Conclusion

It has been concluded from the combined analysis of the effectiveness and efficiency of the mutagens and different morphological mutations induced and plant phenotyping in two subsequent generations of the present study, that doses of gamma rays and HZ have great potential for inducing wide range of heritable mutations in lentil cultivars DPL 62 and Pant L 406. Therefore, selected families based on desirable agro-morphological traits of phenotyped M_2 putative mutants from moderate single treatments and lower combined treatments of gamma rays and HZ, which showed stable phenotypes with complete penetrance and small variations in expressivity, could be advanced to next generations for yield, nutrition and adaptability assessment to release an extremely desirable and farmer friendly lentil mutant variety. The obtained results confirms that the high potency of the selected mutagenic concentration induced a high phenotypic diversity in the treated population and the isolated distinct mutants were of great economic as well as academic interest for future breeding programme on lentil.

References

[1] K. Arumuganathan, E.D. Earle, Nuclear DNA content of some important plant species, Plant Mol Biol. 9(3) (1991) 208-218.

[2] O.H. Barulina, Lentils of the USSR and other countries, Bulletin of Applied Botany, Genetics and Plant Breeding. 40 (1930) 1–319.

[3] J.I. Cubero, Origin, domestication and evolution, in: C. Webb, G.C. Hawtin (Eds), Lentils. Commonwealth Agricultural Bureau, Slough, UK, 1981, pp. 15-38.

[4] W. Erskine, S. Rihawe, B.S. Capper, Variation in lentil straw quality, Animal Feed Science and Technology. 28(1-2) (1990) 61–69.

[5] Joint FAO/IAEA Mutant Variety Database, 2017.

[6] R.A. Laskar et al., Induction of mutagenized tomato populations for investigation on agronomic traits and mutant phenotyping, Journal of the Saudi Society of Agricultural Sciences. (2016) In Press. Doi: 10.1016/j.jssas.2016.01.002.

[7] R.F. Kimball, The mutagenicity of hydrazine and some of its derivatives, Mutat. Res. 39(2) (1977) 111–126.

[8] H.H. Smith, Comparative genetic effects of different physical mutagens in higher plants, in: Induced Mutations and Plant Breeding Improvement, IAEA, Vienna, 1972, pp. 75-93.

[9] R. Kumar, S.C. Mani, Chemical mutagenesis in Manhar variety of rice (Oryza sativa L.), Indian J. Genet. 57(2) (1997) 120-126.

[10] N.B. Gaikwad, V.S. Kothekar, Mutagenic effectiveness and efficiency of ethylmethane sulphonate and sodium azide in lentil (Lens culinaris Medik.), Indian J. Genet. 64(1) (2004) 73-74.

[11] A. Kodym, R. Afza, Physical and chemical mutagenesis, Meth. Mol. Biol. 236 (2003) 189-203.

[12] C.F. Konzak et al., Efficient chemical mutagenesis, Rad. Bot. 5(Suppl.) (1965) 49-70.

[13] M.L.H. Kaul, A.K. Bhan, Mutagenic effectiveness and efficiency of EMS, dES and gamma rays in rice, Theor. Appl. Genet. 50(5) (1977) 241-246.

[14] I.S. Solanki, B. Sharma, Mutagenic effectiveness and efficiency of gamma rays, ethyl imine and N-nitroso-N-ethyl urea in macrosperma lentil (*Lens culinaris* Medik), Indian J. Genet. 54(1) (1994) 72-76.

[15] A.K. Singh, Mutagenic effectiveness and efficiency of gamma rays and ethylmethane sulphonate in mungbean, Madras Agric. J. 94(1-6) (2007) 7-13.

[16] T.M. Shah et al., Induced genetic variability in chickpea (*Cicer arietinum* L.) II. Comparative mutagenic effectiveness and efficiency of physical and chemical mutagens, Pak. J. Bot. 40(2) (2008) 605-613.

[17] C. Thilagavathi, L. Mullainathan, Isolation of macro mutants and mutagenic effectiveness, efficiency in blackgram (*Vigna mungo* (L.) Hepper), Global J. Mol. Sci. 4(2) (2009) 76-79.

[18] M. Girija, D. Dhanavel, Mutagenic effectiveness and efficiency of gamma rays, ethylmethane sulphonate and their combined treatments in cowpea (*Vigna unguiculata* L. Walp), Global J. Mol. Sci. 4(2) (2009) 68-75.

[19] M.H. Khan, S.D. Tyagi, Studies on effectiveness and efficiency of gamma rays, EMS and their combination in soybean (*Glycine max* (L.) Merrill), J. Plant Breed. Crop Sci. 2(3) (2010) 55-58

[20] M.N. Khan, Mutagenic effectiveness and efficiency of EMS, gamma rays and their combination in black gram (*Vigna mungo* (L.) Hepper), Ad. Plant Sci. 12(I) (1999) 203-205.

[21] A.S. Gautam, K.C. Sood, A.K. Richaria, Mutagenic effectiveness and efficiency of gamma rays, ethylmethane sulphonate and their synergistic effects in black gram (*Vigna mungo* L.), Cytologia. 57 (1992) 85-89.

[22] S. Ganapathy et al., Isolation of macromutations and mutagenic effectiveness and efficiency in little millet varieties, World J. Agric. Sci. 4(4) (2008) 483-486.

[23] Y.S. Nerker, Mutagenic effectiveness and efficiency of gamma rays, ethylmethane sulphonate and nitroso methyl urea in *Lathyrus sativus*, Indian J. Genet. 37(1) (1977) 137-141.

[24] A. Sharma et al., Induced mutagenesis for improvement of garden pea, Int. J. Veg. Sci. 16 (2010) 60-72.

[25] M.H. Khan, S.D. Tyagi, Induced morphological mutants in soybean [*Glycine max* (L.) Merrill], Front. Agric. China 4(2) (2010) 175-180.

[26] M.R. Wani, S. Khan, M.I. Kozgar, Induced chlorophyll mutations. I. Mutagenic effectiveness and efficiency of EMS, HZ and SA in mungbean, Front. Agr. China. 5(4) (2011) 514-518.

[27] S. Khan, K. Parveen, S. Goyal, Induced mutations in chickpea-morphological mutants, Front. Agric. China. 5(1) (2011) 35-39.

[28] P.M. Gaur, V.K. Gour, Broad-few-leaflets and outwardly curved wings: two new mutants of chickpea, Plant Breed. 122(2) (2003) 192-194.

[29] C. Toker, A note on the evolution of kabuli chickpeas as shown by induced mutations in *Cicer reticulatum* Ladizinsky, Genet. Resour. Crop Evol. 56(1) (2009) 7-12.

[30] W. Gottschalk, The genetic basis of variation, in: Improving Vegetatively Propagated Crops, Academic Press Limited, London, UK, 1987, pp. 317-334.

[31] B.S. Tyagi, P.K. Gupta, Induced macromutations in lentil [Lens culinaris], Lentil Experimental News Service. 18(1) (1991) 3-7.

[32] M.K. Jana, X-ray induced tall mutants of blackgram (*Phaseolus mungo* L.), Curr. Sci. 32(10) (1963) 469-470.

[33] S.K. Sharma, B. Sharma, Leaf mutations induced with NMU and gamma rays in lentil (*Lens culinaris* Medik), Curr. Sci. 48 (1979) 916-917.

[34] V.R.K. Reddy, P.K. Gupta, Induced mutations in hexaploid triticale. Frequency and spectrum of morphological mutants, Genet. Agr. 42 (1988) 241-254.

[35] A. Satyanarayana et al., Multifoliate leaf mutants of mungbean and urdbean, Mut. Breed. Newslet. 33 (1989) 17.

[36] V.P. Singh, M. Singh, J.P. Pal, Mutagenic effects of gamma rays and EMS on frequency and spectrum of chlorophyll and macromutations in urdbean (*Vigna mungo* L. Hepper), Indian J. Genet. 59(2) (1999) 203-210.

[37] D. Talukdar, Dwarf mutations in grass pea (*Lathyrus sativus* L.): origin, morphology, inheritance and linkage studies, J. Genet. 88(2) (2009) 165-175.

[38] C.F. Konzak, S.C. Woo, J. Dickey, An induced semidwarf plant height mutation in spring wheat, Wheat Inf. Serv. 28 (1969) 10-12.

[39] A. Shakoor et al., Selection for useful semi dwarf mutants through induced mutation in bread wheat, in: Proc. 5th Int. Wheat Genet. Symp., New Delhi, Vol. I, 1978, pp. 23-28.

[40] R. Qin et al., Genetic analysis of a novel dominant rice dwarf mutant 986083D, Euphytica 160(3) (2008) 379-387.

[41] X.H. Zhang et al., A dwarf wheat mutant is associated with increased drought resistance and altered responses to gravity, African J. Biotech. 4(10) (2005) 1054-1057.

[42] D. Arulbalachandran, L. Mullainathan, Chlorophyll and morphological mutants of blackgram (*Vigna mungo* (L.) Hepper) derived by gamma rays and EMS, J. Phytology. 1(4) (2009) 236-241.

[43] S. Goyal, S. Khan, Differential response of single and combined treatment in moist seeds of urdbean, Indian J. Bot. Res. 6(1-2) (2010) 183-188.

[44] A. Tripathi, D.K. Dubey, Frequency and spectrum of mutations induced by separate and simultaneous applications of gamma rays, ethyl methane sulphonate (EMS) in two microsperma varieties of lentil, Lentil Experimental News Service. 19(1) (1992) 3-8.

[45] I.S. Solanki, B. Sharma, Induction and isolation of morphological mutations in different damage groups in lentil (Lens culinaris Medik), Indian J. Genet. 59(4) (1999) 479-485.

[46] S. Khan et al., Induction of morphological mutants in chickpea, Int. Chickpea and Pigeonpea Newslet. 11 (2004) 6-7.

[47] R.A. Nilan, Nature of induced mutations in higher plants. Induced mutations and their utilization, in: Proceed. of the Symp. Erawim Baur Gedachtnis Orle Singen IV. 1966, Acverlag Berlin, 1967, pp. 5-20.

[48] I.S. Solanki, D.S. Phogat, R.S. Waldia, Frequency and spectrum of morphological mutations and effectiveness and efficiency of chemical mutagens in Macrosperma lentil, National J. Plant Improvement. 6(1) (2004) 22-25.

[49] V. Kumar et al., Characterization of prebreeding genetic stocks of urdbean (*Vigna mungo* L. Hepper) induced through mutagenesis, in: Q. . Shu (ed.), Induced Plant Mutations in the Genomics Era, Food and Agriculture Organization of the United Nation, Rome, 2009, pp. 391-394.

[50] G.S. Sethi, Long-penduncled mutant: a new mutant type induced in barley, Euphytica. 23 (1974) 237-239.

[51] D. Talukdar, A.K. Biswas, An induced internode mutant in grass pea, in: G.K. Manna, S.C. Roy, (ed.) (Eds.), Perspectives in Cytology and Genetics. Vol. 12, AICCG Publ., Kalyani, India, 2006, pp. 267-272.

[52] J. Sjodin, Induced morphological variations in *Vicia faba* L., Hereditas. 67(2) (1971) 155-180.

[53] V. Marghitu, Mutagenic effect of X-rays and EMS in french bean in M3 and M4 generations, Striinte Agricole. 3 (1972) 105-109.

[54] R.K. Singh, Gamma ray induced bold seeded mutant in *Vigna mungo* (L.) Hepper, Indian J. Genet. 56(1) (1996) 104-108.

[55] H.L. Thakur, G.S. Sethi, Characterization and segregation pattern of some macromutations induced in blackgram (*Vigna mungo* (L.) Hepper), Indian J. Genet. 53(2) (1993) 168-173.

[56] W. Gottschalk, G. Wolff, Induced mutations in plant breeding, SpringerVerlag, Berlin, Heidelberg, New York, 1983.

[57] J.E. Gunkel, ,A.H. Sparrow, Ionizing radiations: biochemical, physiological and morphological aspects of their effect on plants, in: W. Ruhland (ed.), Encyclopedia of Plant Physiology, Springer, Berlin, 1961, pp. 555-611.

[58] S. Blixt, Mutation genetics in Pisum, Agric. Hort. Genet. 30 (1972) 1-293.

[59] C. Toker, M.I. Cagirgan, Spectrum and frequency of induced mutations in chickpea, Int. Chickpea & Pigeonpea Newslet. 11 (2004) 8-10.

[60] A.K. Datta, K. Sengupta, Induced viable macromutants in coriander (Coriandrum sativum L.), Indian J. Genet. 62(3) (2002) 273-274.

[61] K.K. Sidorova, Influence of genotypic background on the expressivity of mutant genes of pea, Pulse Crops Newslet. 1(3) (1981) 23-24.