ISSN : 1812-5379 (Print)
ISSN : 1812-5417 (Online)
http://ansijournals.com/ja

JOURNAL OF AGRONOMY

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Journal of Agronomy 14 (3): 102-111, 2015
ISSN 1812-5379

RESEARCH ARTICLE OPEN ACCESS

DOI: 10.3923/ja.2015.102.111

Quantitative Analysis of Induced Phenotypic Diversity in Chickpea Using Physical and Chemical Mutagenesis

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ARTICLE INFO
Article History:
Received: May 03, 2015
Accepted: July 01, 2015

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ABSTRACT
In the present scenario of variable natural environment and sky-high population, sustainable boost in the agricultural productivity is the utmost priority. Induce mutagenesis generates noble genetic combination without affecting the overall genomic makeup of crop, thus, providing essential genetic variation for any crop improvement programme. The present study has been carried out to investigate the comparative mutagenicity of gamma rays and HZ on chickpea (*Cicer arietinum* L.) genotype (avrodhi) at M_2 generation developed from seeds of treated M_1 plants population. The assessment on phenotypic expression for the studied qualitative and quantitative traits showed considerable deviations in all the treatments and significant positive shift in 0.01 and 0.02% doses compared to control while 0.04% proved to be most mutagenic with highest significant negative deviation. A broad spectrum and frequency of macro mutations were induced affecting all plant parts and different morphological variants were screened and isolated on the basis of economic importance from the treated populations. Economically important mutations like branching pattern, stem structure, plant height, dwarf and bushy growth habit, foliage type, flowering behavior and maturity were identified and the frequency of the variants were found to be more in 0.03% doses. Explicitly, HZ doses provided greater deviations in both directions in the quantitative phenotypic characters studied while frequencies of distinct morphological mutants were more in gamma rays. The induced elite phenotypes (blue flowered, double flowered, pigmented leaf, bushy and early mutants), having strong correlation with agronomic traits, will definitely be helpful in selection of improved mutants in subsequent generations.

Key words: Chickpea (*Cicer arietinum* L.), gamma rays (Gy), hydrazine hydrates (HZ), mutation breeding, quantitative phenotypic characters, morphological mutants

INTRODUCTION

Cicer arietinum L. is the only cultivated species of genus *Cicer* (Yasar *et al*., 2014) with diploid chromosomes number 16 and self pollinated due to its cleistogamic flowers (Cubero, 1987), originated from middle part of Asia Minor (Ladizinsky, 1975). Chickpea seeds contain 23% protein, 64% carbohydrates, 5% fat, 6% crude fiber, 6% soluble sugar and 3% ash (Williams and Singh, 1987), therefore, of great economic importance as one of the primary protein crop for global food security. There are two main groups of chickpea (Auckland and van der Maesen, 1980) viz., Desi (wrinkled seeded) and Kabuli (round seeded), which constituted about 85 and 15% of the total production worldwide respectively. Chickpea being the third most important pulse crop in the world, substantial increase in the global yield has been the area

of concern despite extensive breeding efforts (Gaur and Gour, 2002). An essential prerequisite for any crop improvement programme is the available genetic variation in the crop gene pool. The narrow genetic base of cultivated chickpea (*Cicer arietinum* L.), as detected from little polymorphism for isozyme, RFLP and RAPD markers (Gaur and Slinkard, 1990; Simon and Muehlbauer, 1997), is considered to be the major constraint in plant breeding for crop improvement. In chickpea, exhausted genetic variability due to adaptation to various stresses through natural selection and conventional selection methods for homozygosis resulted into limited accessible genetic variability and hence supplemented breeding strategies needs to be incorporated to serve the objective of crop improvement. Micke (1988) and Yildirim *et al*. (2013) advocated the importance of induced mutations as one of the most effective and efficient approaches to regenerate and restore the genetic variability in chickpea. Legumes generally loose different alleles for high productivity, seed quality, pest and disease resistance during the processes of adaptation to environmental stress (Dhumal and Bolbhat, 2012).

Mutation breeding is used to induce mutations at loci controlling economically important traits and/or eliminates undesirable genes from elite breeding lines (Lippert *et al*., 1964). Demand on mutation breeding to contribute to sustainable global food security and livelihood is increased tremendously in recent times. Several morphological mutants have been found and utilized in chickpea improvement as well as in linkage studies (Dahiya *et al*., 1984; Pundir and Reddy, 1998; Gaur and Gour, 2002, 2003; McNeil *et al*., 2007; Rajesh *et al*., 2007; Salimath *et al*., 2007; Srinivasan *et al*., 2006; Wani and Anis, 2008; Ali *et al*., 2010; Kharkwal *et al*., 2010; Si *et al*., 2010; Wani, 2011).

The induction of morphological macro mutations for creating phenotypic or probably genotypic diversity are of great interest as it provides additional genetic markers for genetic enhancement and linkage studies in chickpea (*Cicer arietinum* L.). In the context of selection in plant breeding, it is necessary to understand that the estimated variations in quantitative characters only explains for diversity in the observed phenotype, not for the presence/absence of particular alleles as in marker-based analyses. However, for the reason that selection generally based on phenotypes, not on genotypes, it appears decidedly pertinent to concentrate on statistical interpretation of variances in phenological parameters, which facilitate the understanding about how far the plants from mutagen treated seeds captures the induced variations up to maturity for efficient selection at the phenotypic level. From this background idea, the present investigation was undertaken 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 screened M_2 chickpea individuals from the progeny of M_1 "avrodhi" parentals grown from seeds exposed to different concentrations of gamma rays (Gy) and Hydrazine hydrates (HZ). Also, treatment doses were statistically verified comparatively to obtain the extent of genotype sensitivity and concentrations mutagenicity for future references.

MATERIALS AND METHODS

Genetic variability was induced in chickpea genotype avrodhi using physical mutagen (gamma rays) and chemical mutagen (HZ). 'Avrodhi' is a desi-type of developed disease resistant well adapted chickpea variety of central India, considered to widen genetic variability for its overall genetic improvement (yield and nutrition) into an elite variety. The healthy and viable seeds (moisture 11.0%) were treated with different doses of HZ viz, 0.01, 0.02, 0.03 and 0.04% at room temperature of 25±2°C for 9 h after 6 h of soak. For physical treatment, dry seeds were directly irradiated with 100, 200, 300 and 400 Gy of gamma rays with a radioisotope 60Co, Cobalt-60, source at the National Botanical Research Institute, Lucknow, Uttar Pradesh, India. The doses of the chemical treatments were determined for LD_{50} through an initial laboratory experiment. The individually harvested seeds of normal looking M_1 plants per treatment were advanced for raising M_2 generation in the agricultural fields of Aligarh Muslim University, Aligarh, India from mid-October 2013-April 2014. The experiment was designed in triplicate (30 seeds of 5 M_1 plant each / replication) in three rows for each treatment following a complete randomized block design.

Data on phenotypic quantitative characters were taken throughout the season and tabulated. Comparative analysis on leaflet shapes and arrangements were done according to (IBPGR, ICRISAT and ICARDA, 1985). Chlorophyll from fresh secondary emergent leaflets was extracted in 80% acetone in mg g^{-1} and estimated by the Mackinney (1941) method. Nitrate Reductase Activity (NRA) was measured following the method adopted by Jaworski (1971). Morphological variations induced in the M_2 population related to agronomic traits were recorded using descriptors for chickpea (IBPGR, ICRISAT and ICARDA, 1993) and control population was considered as standard. Observed data in different quantitative traits were tested for significance through ANOVA and the mean separation was estimated according to Duncan Multiple Range Test $p<0.01$ (Duncan, 1955).

RESULT AND DISCUSSION

Quantitative assessment about morphological dynamics during plant development when a wide variety of genes are perturbed individually due to mutagenesis would provide a key resource for not only to perform sensitive and objective analyses but also provide an opportunity to discover novel induced mutants. Therefore, to identify induced phenotypic

alterations in chickpea M_2 generation plant populations comprehensively, we statistically compared the information on quantitative phenotypic characters from treated population with that from untreated control. By mathematically defining quantitative phenotypic characters at different treatments, we identified diverse phenotypic alterations of morpho-physiological nature.

Effects on quantitative characters: Differential response of the genotype was observed with respect to different doses of mutagens. Quantitative analysis of the treated plants showed wide range of significant phenotypic variations (Table 1). Highest mean shoot length (52.1672 cm) was recorded at 0.02% HZ with 5.0291 cm positive shift from the control (47.1381 cm) and it was decreased by 17.9097 cm at 0.04% HZ (29.2284 cm). Lower doses of gamma rays and HZ provided positive shift while higher doses negative. Root length was higher compare to control (14.8888 cm) in 100 Gy (16.6121 cm), 0.01% HZ (15.1901 cm), 200 Gy (15.3341 cm) and 0.03% HZ (17.6450 cm) treatments, respectively while shortest (10.4877 cm) in 0.04% HZ. Treatments 100 Gy and 0.03% HZ found to induce deep rooted variants which will improve the water and mineral transportation efficiency of the crop. Kashiwagi *et al*. (2006, 2005) and Reynolds and Tuberosa (2008) in chickpeas viewed that deep and prolific root system contributes directly to productivity under water limited conditions. Number of primary branches per plant increased only in 200 Gy (15.9228) while in all other treatments it decreases compare to control (14.3166) and 0.04% HZ (7.2083) inhibited maximum. Similar mutagen induced variation in number of primary branches were also reported by Charumathi *et al*. (1992) in black gram and Khan *et al*. (2005) in chickpea. All the doses had negative effect on internodes space with 0.02% HZ (5.5898 cm) and 400 Gy (2.1778) showing least and highest inhibition respectively. Reduction in fresh weight of plant gave notable observations about organic matter accumulation in chickpea. Chickpea plants developed from 0.01 and 0.02% HZ treated seeds showed relatively increased FW (34.2496, 34.8456 g) and DW (10.0009, 10.6976 g) but highest reduction. Reduction was lowest at 400 Gy and 0.04% HZ (15.7299 and 14.7669 g, respectively) but at these doses maximum negative shift in mean compare to control was also reported in both FW and DW. The results of the present pursuit showed that lower and moderate doses of the chemical mutagens could induce useful quantitative phenotypic mutations in chickpea for screening and selection purposes. Background reasons for these phenotypic observations could be the induce growth stimulation due to enhanced hormonal signaling network or increased anti-oxidative capacity of the cells. Explicitly, the induction of growth and improved immunity against the daily biotic and abiotic stress factors in the mutagen treated chickpea plant population may possibly be the reasons for significant deviation in the expression of desirable quantitative traits.

Effect on leaf type, arrangement and shape: Modifications of leaf arrangement, shape, size and colour is the most useful phenotypic marker in mutation breeding due to their wide appearance and easy detection. Chickpea leaf variants have been induced by spontaneous or induced mutations (Muehlbauer and Singh, 1987; Salimath *et al*., 2007; Toker *et al*., 2012) and there were reports of different leaf derivatives in chickpea from many workers (Rao *et al*., 1980; Muehlbauer and Singh, 1987; Toker and Cagirgan, 2004; Toker and Ceylan, 2013). The chemical mutagens employed in the study could not change the leaf type or phylotaxis in M_2 generation and no deviation from the normal leaf (Pundir *et al*., 1990) type was observed. Normal leaves in chickpea features 25-75 mm long rachis ends with a leaflet, ovate to oblique-triangular stipules, pseudo-imparipinnate with 11-15 leaflets, teeth in nearly 2/3 of the foliar blade (Toker and Ceylan, 2013). However, different derivatives of normal leaf with changed leaflet arrangement on rachis and shape have been viewed in the treated population (Fig. 1). Differences in the rachis length and shape were observed like straightness and girth was seen to be variable with more than one comparably smaller terminal leaflets. Distance between the two consecutive leaflet initials also varied and thus resulted in a wide variations in number of leaflets from the specified standard. Also, shorter rachis with overlapping leaflets were observed in some bushy and dwarf mutants at higher treatments. Smoothness of the leaflet blades showed variations and diminution of number teeth also observed in broad leaves. Length of petiole and petiolule also showed variations and 0.04% HZ induced rough tiny leaves having very short petiole with no petiolule in some sterile plants. Arrangement of leaflet changed from alternate to opposite in 300 Gy, 400 Gy and 0.04% HZ. Similarly, Fawole (2001) and Sangsiri *et al*. (2005) observed induced leaflet shape and arrangements modification in chickpea. Leaf and leaflet shape ranges from tiny, small, medium, narrow and broad with shallow to deep serration were induced in the treated population. Altered leaf colourations due to induce variations in availability of green pigment (chlorophyll) were observed. Highly expressed red pigmented leaf variants over green pigmentation were isolated at maturity from 300 Gy and 0.02% HZ treated populations while other colour variants could not survived up to maturity. Over expression or suppression of genes (one over other) due to mutagenic treatments may be the reasons. Since, normal leaf type is governed by dominant alleles of two supplementary genes (Pundir *et al*., 1990), it can be guesstimate that intra-allelic variations have been induced due to the mutagens, which resulted into variation in size and shape or due to interference of other expressed factors. Generally uniformity of leaf decreases and frequency of leaf variants increases with increasing concentrations of mutagens while gamma rays had induced more variations than HZ in number as well as type (Fig. 1).

Table 1: Statistical analysis of comparative effect of mutagens (HZ and MMS) on various quantitative phenotypic characters in M_2 generation of chickpea (*Cicer arietinum* L.) genotype "Avrodhi"

Treatment conc./doses	Quantitative phenotypic characters: Shoot length/plant (cm) Mean±SE	SD	CV (%)	Shift	Root length/plant (cm) Mean±SE	SD	CV (%)	Shift	No. of PB/plant Mean±SE	SD	CV (%)	Shift	Internode space/plant (cm) Mean±SE	SD,	CV (%)	Shift
Control	47.14±0.15^{e}	0.25	0.53	-	14.89±0.06^{cd}	0.10	0.64	-	9.32±0.02^{b}	0.04	0.43	-	5.95±0.10^{a}	0.17	2.78	-
100 Gy	47.77±0.17^{d}	0.29	0.61	0.63	16.61±0.20^{b}	0.34	2.05	1.72	8.58±0.03^{c}	0.06	0.64	-0.73	4.29±0.08^{cd}	0.13	3.11	-1.68
0.01% HZ	50.19±0.31^{b}	0.53	1.06	3.06	15.19±0.54^{c}	0.94	6.18	0.30	8.97±0.01^{bc}	0.02	0.27	-0.35	5.57±0.07^{b}	0.16	1.46	1.61
200 Gy	48.92±0.05^{c}	0.08	0.17	1.79	15.33±0.06^{c}	0.10	0.63	0.45	10.92±0.09^{a}	0.16	2.06	-0.39	4.45±0.019^{c}	0.03	0.74	-1.51
0.02% HZ	52.17±0.05^{a}	0.09	0.17	5.03	13.63±0.19^{e}	0.33	2.43	-1.26	4.60±0.06^{e}	0.10	2.08	-4.72	5.59±0.03^{b}	0.05	0.92	-0.37
300 Gy	36.26±0.08^{g}	0.13	0.37	-10.88	14.23±0.10^{de}	0.18	1.24	-0.66	7.55±0.08^{d}	0.14	1.81	-1.77	4.07±0.05^{d}	0.08	1.99	-1.89
0.03% HZ	38.62±0.05^{f}	0.08	0.21	-8.51	17.65±0.08^{a}	0.14	0.82	2.76	7.52±0.07^{d}	0.12	1.53	-1.79	3.80±0.06^{e}	0.11	2.78	-2.17
400 Gy	31.15±0.06^{h}	0.10	0.31	-15.99	12.70±0.17^{f}	0.29	2.28	-2.19	7.34±0.03^{d}	0.05	0.65	-1.96	2.1778±0.0021^{g}	0.004	0.17	-3.79
0.04% HZ	29.23±0.21^{i}	0.36	1.24	-17.91	10.49±0.06^{g}	0.11	1.00	-4.40	2.54±0.28^{f}	0.48	18.91	-6.78	3.33±0.014^{f}	0.03	0.77	-2.64
LSD	0.7145				1.0301				0.5043				0.2588			

Treatment conc./doses	Shoot F.W/plant (g) Mean±SE	SD	CV (%)	Shift	Shoot D.W/plant (g) Mean±SE	SD	CV (%)	Shift	Reduction in FW/plant (g) Mean±SE	SD	CV (%)	Shift
Control	31.55±0.02^{d}	0.04	0.12	-	8.36±0.006^{e}	0.01	0.12	-	23.19±0.02^{b}	0.03	0.12	-
100 Gy	30.81±0.10^{e}	0.17	0.56	-0.75	8.50±0.03^{d}	0.05	0.56	0.14	22.30±0.08^{c}	0.12	0.56	-0.89
0.01% HZ	34.25±0.09^{b}	0.15	0.45	2.70	10.001±0.03^{b}	0.05	0.45	1.64	24.25±0.06^{a}	0.11	0.45	1.06
200 Gy	32.21±0.07^{c}	0.11	0.35	0.66	8.89±0.02^{c}	0.03	0.35	0.53	23.32±0.05^{b}	0.08	0.35	0.13
0.02% HZ	34.85±0.046^{a}	0.08	0.23	3.30	10.70±0.01^{a}	0.02	0.23	2.34	24.15±0.03^{a}	0.06	0.23	0.96
300 Gy	27.19±0.17^{g}	0.29	1.06	-4.36	7.02±0.04^{f}	0.07	1.06	-1.36	20.18±0.12^{e}	0.21	1.06	-3.01
0.03% HZ	27.93±0.04^{f}	0.08	0.27	-3.62	6.90±0.01^{g}	0.02	0.27	-1.46	21.04±0.03^{d}	0.06	0.27	-2.16
400 Gy	21.06±0.05^{h}	0.09	0.44	-10.49	5.3275±0.0134^{h}	0.02	0.44	-3.034	15.73±0.04^{f}	0.07	0.44	-7.46
0.04% HZ	19.56±0.04^{i}	0.07	0.34	-11.99	4.79±0.01^{i}	0.02	0.34	-3.57	14.77±0.03^{g}	0.05	0.34	-8.42
LSD	0.3822				0.1025				0.2799			

#Means within columns followed by the same letter is not different at the 1% level of significance, based on the Duncan Multiple range test

Fig. 1(a-t): (a) Control leaf, (b) Mutant with broad leaflets showing deep serrations on the margin, (c) Opposite leaflets on long rachis with two terminal small leaflets, (d) Lowered green pigment leaf, (e) Small leaved reduced rachis with overlapping ovate leaflets, (f, g) Tiny leaved with few leaflets, (h) Few close arranged leaflets, (i) One sided close leaflet arrangement, (j) Single white flower, (k) Double flower, (l) Red pigmented leaf, (m) Early pink flowering, (n) Blue flower, (o) Bushy habitat, (p) Dwarf, (q) Increased primary branching habit with dark stem pigmentation, (r) No sec. branching with unique pri. branching pattern, (s) Simple single stem with no branches and (t) Bushy compact mutant with reduced internode length

Effect on leaf size and physiology: Impacts of employed chemical mutagens on leaf size and physiology were tabulated in Table 2. Leaf area was increased significantly compared to control (134.8763 mm^2) in 100 Gy and 0.01% HZ (160.8371 and 142.9504 mm^2) and 200 Gy (159.5851 mm^2). Relative deviations in the mean width and length of the leaf at various treatments resulted from the morphological variations of leaf and leaflet. Estimation of physiological parameters like NRA and chlorophyll content in the treated population help to understand the mutagenic action on plants. Effects of treatments on chlorophyll content and NR activity exhibited increase in total chlorophyll content in 0.01% HZ (3.0777 mg g^{-1} FW) and 200 Gy (2.9550 mg g^{-1} FW) as compared to control (2.8428 mg g^{-1} FW) leaves, while the activity of nitrate reductase (NRA) in 0.01% HZ (503.8710 nmol h^{-1} g^{-1} FW) and 200 Gy (480.5674 nmol h^{-1} g^{-1} FW) as compared to control (2.8428 nmol h^{-1} g^{-1} FW) leaves. Maximum reduction was observed in higher doses of both the mutagens. Earlier reported results on different crop such as *Eruca sativa* (Al-Qurainy, 2009), rice (Shereen *et al*., 2009), wheat (Borzouei *et al*., 2010), *Satureja hortensis* (Rahimzadeh *et al*., 2011) showed deviation in chlorophyll content from control due to mutagenic treatments. Reddy and Vora (1986) considered the variations chlorophyll content than the control may be due to variable activity of chlorophylase enzyme. Inhibition and/or metabolic dysfunctions of the enzyme protein due to mutagenic treatments might influence the nitrate reductase activity (Hopkins, 1995).

Effect on plant morphology: Morphological mutations affecting different parts of the plants, such as branching pattern, stem structure, growth habit, foliage type, plant height, foliage color, flowering behavior and maturity were examined (Fig. 1). These mutants can be a source of many beneficial genes in cross breeding programmes or for some quantitative traits improvement (Khan *et al*., 2011), may be valuable for mapping studies (Gaur and Gour, 2003) and in evolutionary studies of the crops (Toker, 2009). Induced morphological variants considered to be either a result of pleiotropic effects of mutated genes or chromosomal aberrations or gene mutations (Gottschalk, 1987; Wani *et al*., 2011). Observed mutation frequency in the population of different treatments and also within the same treatment, suggested that the

Table 2: Statistical analysis of comparative effect of mutagens (HZ and MMS) on Leaf area and physiology in M_2 generation of chickpea (*Cicer arietinum* L.) genotype "Avrodhi"

	Observations on leaf													
			Square (W*L)				Chlorophyll (mg g^{-1} FW)				NRA (nmolh^{-1} g^{-1} FW)			
Treatment conc./doses	Width (mm)	Length (mm)	Mean±SE	SD	CV (%)	Shift in mean	Mean±SE	SD	CV (%)	Shift in mean	Mean±SE	SD	CV (%)	Shift in mean
Control	10.6153	12.7059	134.8763±0.1216^{d}	0.2107	0.1562	-	2.8428±0.0092^{c}	0.0159	0.5585	-	447.9001±0.0987^{c}	0.1710	0.0382	-
100 Gy	11.4519	14.0448	160.8371±0.3974^{a}	0.6883	0.4279	25.9608	2.8211±0.0081^{c}	0.0141	0.4981	-0.0217	397.1343±0.0964^{d}	0.1669	0.042	-50.7658
0.01 (%) HZ	10.5275	13.5788	142.9504±0.4609^{c}	0.7982	0.5584	8.0741	3.0777±0.0012^{a}	0.0021	0.0684	0.2349	503.8710±0.5985^{a}	1.0367	0.2057	55.9709
200 Gy	10.9901	14.5210	159.5851±0.2315^{b}	0.4009	0.2512	24.7088	2.9550±0.0049^{b}	0.0084	0.285	0.1122	480.5674±0.3522^{b}	0.6100	0.1269	32.6673
0.02 (%) HZ	9.3846	14.3002	134.2011±0.0646^{d}	0.1119	0.0834	-0.6752	2.0963±0.0033^{e}	0.0057	0.2722	-0.7465	315.1712±0.5824^{e}	1.0088	0.3201	-132.7289
300 Gy	8.8344	13.1030	115.7576±0.3042	0.5269	0.4551	-19.1187	2.2683±0.0015^{d}	0.0025	0.1108	-0.5745	233.6261±0.0500^{f}	0.0866	0.037	-214.274
0.03 (%) HZ	8.1476	11.5213	93.8586±0.2174^{f}	0.3765	0.4011	-41.0177	1.7312±0.0208^{f}	0.0361	.0859	-1.1116	128.4994±0.3135^{h}	0.5430	0.4226	-319.4007
400 Gy	9.1109	13.0319	118.7315±0.1834^{e}	0.3177	0.2676	-16.1448	2.0709±0.0033^{e}	0.0057	0.2729	-0.7719	168.5224±0.3202^{g}	0.5546	0.3291	-279.3777
0.04 (%) HZ	7.2337	10.9001	78.8410±0.3432^{g}	0.5945	0.754	-56.0353	1.3183±0.0033^{g}	0.0058	0.4371	-1.5245	97.0328±0.0558^{i}	0.0967	0.0996	-350.8673
LSD			1.3552				0.0402				1.6183			

#Means within columns followed by the same letter is not different at the 1% level of significance, based on the Duncan Multiple range test

Table 3: Frequency of morphological (macro) mutants in M_2 generation of four chickpea genotype "Avrodhi"

		Mutant frequency and (%) on M_2 population basis							
		Leaf mutants		Plant growth habit mutants		Flower mutants		Frequency of viable mutants	
Treatment conc./dose	M_2 population	No.	%	No.	%	No.	%	No.	%
100 (Gy)	412	3	0.73	4	0.97	0	0.00	7	1.70
200 (Gy)	398	2	0.50	5	1.26	1	0.25	8	2.01
300 (Gy)	384	10	2.60	12	3.13	3	0.78	25	6.51
400 (Gy)	375	7	1.87	11	2.93	2	0.53	20	5.33
Total	1569	22	5.70	34	8.29	6	1.56	60	15.55
0.01(%) HZ	414	2	0.48	3	0.73	0	0.00	5	1.21
0.02(%) HZ	407	5	1.23	6	1.47	0	0.00	11	2.70
0.03 (%) HZ	394	7	1.78	9	2.28	2	0.51	18	4.57
0.04 (%) HZ	381	8	2.10	11	2.89	2	0.53	21	5.52
Total	1596	22	5.59	29	7.37	4	1.04	55	14.00

genotype responded differently to the dose and type of mutagens employed (Table 3). Similar results of high frequency and broad spectrum of induced morphological mutants by chemical mutagens were also reported in *Vigna mungo* (Arulbalachandran and Mullainathan, 2009; Goyal and Khan, 2010), *Vicia faba* L. (Laskar and Khan, 2014; Laskar *et al*., 2015), *Lens culinaris* (Tyagi and Gupta, 1991; Tripathi and Dubey, 1992; Solanki and Sharma, 1999; Amin *et al*., 2015), *Cicer arietinum* (Khan *et al*., 2004), *Glycine max* (Khan and Tyagi, 2010) and *Cicer arietinum* (Wani, 2011). Macromutations affecting growth habit, flower color and plant type have been reported in chickpea earlier (Ahmad and Godward, 1993; Kharkwal, 1999; Gaur and Gour, 2001; Khan *et al*., 2004; Wani, 2011). Highest frequency of mutants were observed in 300 Gy (6.51%) followed by 0.04% HZ (5.52%) while total frequency was found to be more in gamma rays (15.55%) then HZ (14.00%) (Table 3).The variations in growth habits were identified and isolated in the treated populations like bushy, compact prostrate variants. The bushiness in dwarf variants was characterize with reduced internodes, condensed branches and crammed leaflets on the rachis on the other hand prostrate plants showed trailing long weak internodes. Khan *et al*. (2011) reported the wide occurrence of dwarf mutants in the treated chickpea population. Diminution in the internode length and number observed in the higher doses of mutagens may be the cause of dwarfism as also suggested by Sjodin (1971). The reduction in seedling growth might be due to genetic injury in meristemetic cells (Gray and Scholes, 1951) or due to the inhibition of auxin synthesis (Goud and Nayar, 1968). Morphological variants, bushy plant type with excessive branching and increased number of inflorescence, slightly grooved surface of primary shoots, narrow leaves, broad leaves and chlorophyll variants were investigated and compared to control. In chickpea, erect or bushy growth habit is a characteristic controlled by a single gene (Hg/hg) where erectness is dominant and bushy is recessive (Khan *et al*., 2011). Mutants were also identified with altered flower colour and number (Table 3). The available information on flower color mutations induced in chickpea is very meager (Atta *et al*., 2003). All plants in M_1 generation had pink flowers, pigmented stems and single-flowers/pods. In M_2 population, normally there were violet flowers which gradually turned into pink colour but in higher treatments light/dark pink, blue and white coloured flower were observed. Pundir *et al*. (1985) presented a survey of over 12,000 chickpea accessions that showed occurrence of three flower colour viz., pink flowers (80.67%), white flowers (18.87%) and rarely blue flowers (0.46%). Studies on inheritance of flower colour suggested that the trait is governed by two genes (Khan and Akhtar, 1934; Pal, 1934) or three genes (Ayyar and Balasubramaniam, 1936; Kumar *et al*., 2000a). However, Atanasova and Mihov (2006) confirmed the earlier suggested monogenic behavior for the trait. Phenotypically, plant with blue flower is associated with more branching with reduced seed size and white flower is associated with increased plant height with medium to large seed size. Similar phenotypic linkages were also reported by Kumar *et al*. (1982) and Atta *et al*. (2003). Observations on number of flower per peduncle resulted into double flower and vegetative non-flowering mutant isolation (Table 3). The double-flowered/double-podded trait is known to have yield advantage in chickpea (Kumar *et al*., 2000b; Gaur and Gour, 2002; Ali *et al*., 2010; Anbessa *et al*., 2007) also reported early maturity of double podded plants. It was reported that single recessive gene s or sfl governs the trait (Muehlbauer and Singh, 1987; Srinivasan *et al*., 2006). Hasan and Deb (2013) reported single flowered and pink flower color trait in chickpea is completely dominant over double- and white flower color trait respectively. Since, these traits are monogenic in nature with independent segregation; manipulation through mutation breeding has great potential for ascertaining the uniform expressivity of recessive gene which aids the selection stable high yielding mutants. The present results confirmed the mutagenic effects on expression pathways of flowering gene in M_2 generation of chickpea.

CONCLUSION

It has been concluded from the combined analysis of the different parameters considered in two subsequent generations of present study, that doses of gamma rays and HZ have great potential for inducing wide range of heritable mutations in chickpea genotype "Avrodhi". Therefore, the implication is that the isolated M_2 putative mutants, 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 chickpea mutant variety. The obtained results confirm a high phenotypic diversity has been induced in the treated population and the isolated distinct mutants were of great economic as well as academic interest, which can contribute as future breeding material in research on chickpea.

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