SindhUniv. Res. Jour. (Sci. Ser.) Vol.46 (4):473-478(2014)

SINDH UNIVERSITYRESEARCH JOURNAL (SCIENCESERIES)

Gamma Rays Induced Phenotypic Mutations In Chickpea (*Cicerarietinum* L.)

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Received 11[th]May, 2014 and Revised 24[th] September, 2014

Abstract: Seven different doses of Gamma rays (0Gy, 50Gy, 75Gy, 100Gy, 150Gy, 250Gy, 300Gy) were used phenotypic mutations in chickpea (*Cicerarietinum* L.) in genotype (NCS0350). Following effects of Gamma rays were observed: (a) Variations were noticed in phenotypic expression for all the quantitative and qualitative characters. (b) Increase in germination rate, germination percentage, shoot length, root length and leaf area occurred by the application of 50Gy to 100Gy. (c) Negative effect on inter-node space was found in all the doses. (d) 100Gy induced reduction in fresh weight. (d) No change in leaf type or phylotaxis in M_0 generation observed due to Gamma rays. (e) Leaflet arrangement changed from alternate (0Gy) to apposite in 75 and 100Gy. (f) All seven new leaflet shapes were found to be acute lobed (two lobed, three lobed, four lobed), round lobed (three lobed), ovate, lanceolate, rhomboid and obvate. (g) Level of mosiacness increases with increasing the dose of Gamma rays. (h) Chlorophyll a, b and total chlorophyll content was enhanced in 100GY to 250Gy. (i) 300Gy showed inhibitory effect for most of the character studied. 100Gy to 250Gy found to be the best dose for maximum positive phenotypic mutations required for chickpea improvement.

Keywords: Gamma rays, phenotypic mutations, chickpea, germination, chlorophyll, quantitative characters

1. INTRODUCTION

Chickpea is self-pollinated diploid (2n=16) crop (Arum *et al*., 1991). There are two types of chickpeas; large seeded chickpeas are called Kabuli and small seeded are known as desi (Sexena *et al.*, 1984). Pakistan is the second major producer of chickpea (9.5%) after India (65%) followed by the Turkey (6.7%) in the world. The chickpea (*Cicerarietinum* L.) is the third important food legume of the world grown in 40 countries over an area of about 11.2 million hectors with production of 9.2 tons and average yield of 818 kg/ hector. Different physical and chemical mutagens are applied to induce useful phenotypic variations in cereals, oilseeds, vegetables and pulses (Maluszynski *et al*., 2002). A few studies focused chickpea for mutation breading. Gamma rays are one of the physical mutagen which damage DNA molecules by strand breaks and sugar bases destruction (de-Winter *et al.*; 2000). Gamma rays induced delay of germination and cause variation in growth at cytological, genetical, biochemical, physiological and morphogenetic levels (Koing *et al.*, 2008; Borzouei *et al*., 2010). Gamma rays also induce reduction in plant height, chlorophyll a, b, and total chlorophyll, increase in antioxidant enzyme activities and grain yield (Singh *et al*., 2009). The rays are also active in producing chlorophyll mutations such as, albino, coppery leaf variegated and xantha (Sangsiri *et al*., 2005 and Shah *et al*., 2006). Chickpea consist three leaf types: normal compound leaf (uni-imparipinnate), simple leaf and multi-pinnate. Simple leaf types are less commonly cultivated worldwide (Danchloueipour *et al*., 2007). The shape of leaf and leaflets arrangement is alternate and elliptical or obtuse (Pundir *et al*., 1990). Gamma rays also induce genetic alterations in leaflet shape and arrangements (Fawole, 2001; Sangsiri *et al*., 2005). Mutation breeding is highly beneficial in crop improvement by incorporating useful mutations in target loci. Several positive mutations are developed by mutagenic inductions such as new oil seed, *Brassica napus* L. cv. ABASIN-95 (Shah *et al*., 2001) and *Brassica junicea* L. cv. S-9 (Khatri *et al.*, 2005). The available chickpea germ-plasma does not possess genetic variability for economic traits. Mutation breeding is one of the most valuable method to generate elite chickpea genotypes (Shah *et al.*, 2006). Therefore present work is conducted to optimize best Gamma rays dose that induces positive phenotypic mutations before developing mutation breeding straightly for chickpea, that will prove to be milestone for future researchers to get genetically improve chickpeas with maximum grain yield protein content and leaf area.

2. MATERIALS AND METHODS

Seed of Kabuli chickpea (*Cicerarietinum* L.) of genotype (NCS 0503) were collected from National Agriculture Research Centre, Islamabad (NARC). Seven different doses Gamma rays in Gray (0Gy (control), 50Gy. 75Gy, 100Gy, 150Gy, 250Gy and 300Gy) were applied on dry seeds by Teletherapy machine (National Power Institute of China (NPIC) model # GWXJ 80). 0Gy dose was use as positive control. The treated seeds (18 per treatment) were

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soaked in distil water for 3 hour at room temperature in beakers. The experiment was conducted in pots with three replications. Six seeds were sown in a pot in the depth of 1 inch containing sand and mud (1:1). Equal amount of water has given to each pot when ever required till harvested.

Data for germination rate and parentage was recorded every day after the sowing till last seedling emerged. Germination rate was expressed in line graph made in MS. Excel 2003 while seedling germination percentage was calculated by following formula:

$$\textbf{S.G.P. (\%)} = \frac{\text{No: of Seedlings emerged}}{\text{Total seed sown}} \times 100$$

Shoot length (cm) measured from ground level to the tip of the seedling and root length by inch tape. Number of primary branches / plants counted. Inter node space / plant (cm) and leaf area (mm^2) was recorded with vernier caliper. Shoot fresh weight / plant (mg) and shoot dry weight / plant (mg) recorded with the help of electric balance. Decrease in shoot fresh weight (mg) was obtained by subtracting dry weight from respective fresh weight of each seedling. Comparative analysis was done of Leaflet shapes and arrangement of all the treated seedlings to control and of other treatments (Rajput *et al*., 1996).

Chlorophyll content of leaves is one of the major sign of a successful photosynthesis and dry matter accumulation. Leaf chlorophyll a,b and total chlorophyll was measured by the method developed by Torrecillas *et al*. (1984). Disk of fresh leaf samples (100 mg) were incubated in 4 ml 80% acetone at 4°C for 72 h in dark. Samples were centrifuged for 15 min at 16,000 × g. Absorbance was calculated at 649nm for chlorophyll a and 665nm for chlorophyll b against blank. The chlorophyll content is expressed in mg g^{-1} FW.

Statistic Applied:

Data of quantitative characters and chlorophyll profiles were subjected to one way analysis of variance (ANOVA) and LDS test using computer program SPSS. 13.

3. RESULTS AND DISCUSSIONS

In the Present study Chickpea shows following variations in phenotypic expression of all the quantitative and qualitative characters:

***(i)* Effects on seedling germination rate and percentage:**

The differential growth rate of chickpea seedlings is shown in **(Fig. 1).** Fastest seedling germination rate was observed in 50Gy followed by 100Gy; whereas slowest rate was exhibited by 150Gy.

Germination percentage is presented in **(Fig. 2).** Germination percentage is calculated in all the Gamma rays applied. It decreases with the increase of Gamma rays except in 250Gy, where the germination remained similar to the control. Maximum decrease was recorded in 75 and 100Gy. But in 250Gy the Germination percentage was normal as in control, therefore the correlation was not found in between doses and seedling germination percentage. Presents results are consistent with the findings of Melki *et al*. (2010), Borzouei *et al*. (2010) and Majeed *et al*. (2010).

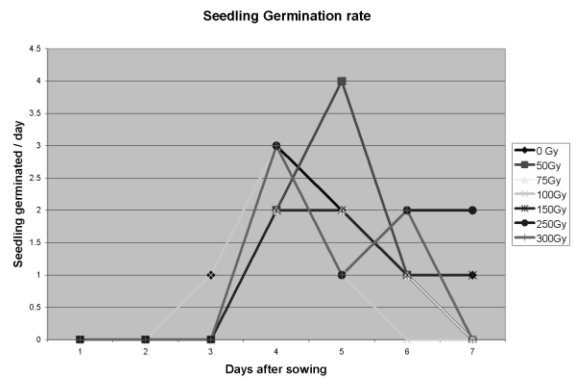

Fig. 1: Line graph showing various rats of seedling germination induced by different doses of gamma rays

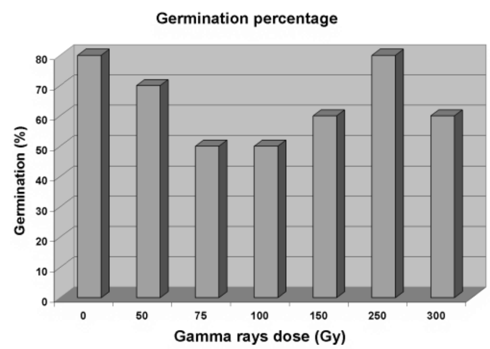

Fig. 2: Effect of gamma rays on chickpea seedling germination

***(ii)* Effects on quantitative characters:**

All the quantitative phenotypic characters exhibited mutations **(Fig. 3, 4 and Table. 1).** Shoot length was increased (45.50 cm) in seedlings treated with 50Gy and it was decreased in all the treatments. Maximum decrease (24.50 cm) occurred in 300Gy compared to control seedlings (45.17 cm). These findings are in accordance to Borzouei *et al*. (2010). Root length was higher compare to control in 100Gy (18.75 cm), 300Gy (17.75) and 50Gy (17.50) treatment respectively. In 250Gy (12.5 cm) the root length was shorter. Roots mostly occupied the pivotal position because of its role in extracting water from deeper soil profiles. In the present study 100Gy and 300Gy dose produced deeper rooted plants. Deep rooted plants have also shown to be better productive under water limited conditions (Reynolds *et al*., 2008).

Table 1 : Mean values for quantitative phenotypic characters of chickpea affected by different doses of gamma ray

(PB: primary branches; FW: fresh weight; DW: dry weight; value followed by different letters are significantly different at p<.05 using LSD test)

S..	Dose (Gy)	Shoot length/ plant (cm)	Root length/ plant (cm)	No. of PB/ plant	Internode space/ plant (cm)	Leaf area (mm²)			Shoot F.W. / plant (mg)	Shoot D.W. / plant (mg)	Reduction in FW/ plant (mg)
						Width	Length	sq			
1	0	45.17^{A}	15.84^{BC}	13.16^{A}	4.93^{A}	9.60	11.40	109.44^{C}	.026^{D}	.0065^{B}	0.0195^{C}
2	50	45.50^{A}	17.50^{A}	12.50^{A}	3.18^{AB}	10.77	13.46	144.96^{A}	.022^{D}	.0075^{B}	0.0145^{C}
3	75	44.75^{A}	15.25^{BC}	14.75^{A}	3.37^{AB}	9.80	13.66	133.86^{B}	.037^{C}	.004^{BC}	0.033^{BC}
4	100	39.25^{AB}	18.75^{A}	11.50^{AB}	3.08^{B}	7.75	12.15	94.16^{CD}	.036^{C}	.035^{A}	0.001^{DE}
5	150	32.28^{B}	15.57^{BC}	11.33^{AB}	1.73^{D}	9.28	12.28	113.95^{C}	.039^{C}	.035^{A}	0.004^{D}
6	250	25.16^{C}	12.50^{C}	12.70^{A}	3.15^{B}	7.93	11.27	89.37^{CD}	.063^{A}	.005^{BC}	0.058^{A}
7	300	24.50^{C}	17.75^{A}	5.33^{C}	3.24^{AB}	7.0	11.50	80.50^{C}	.053^{AB}	.004^{BC}	0.049^{B}

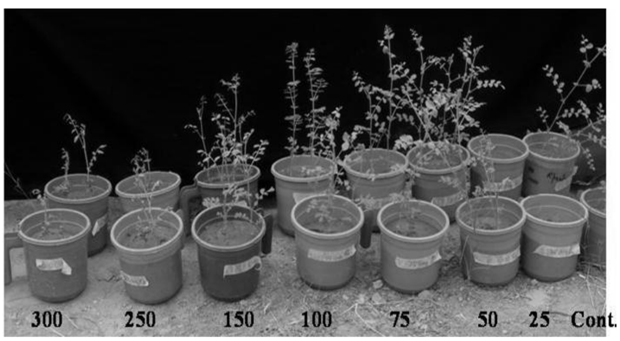

Fig. 3: Effect of gamma rays on phenotype of quantitative characters

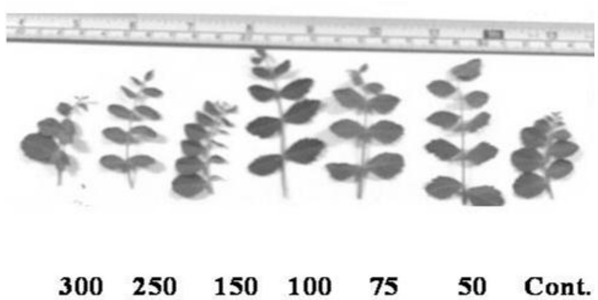

Fig.4 : Effect of gamma rays on leaflet agreement and leaf type in chickpea

Increase in number of primary branches per plant was only found in 75Gy (14.75), where as in 300Gy treatment has maximum inhibitory effect (5.33). All the doses have negative effect on internodes space. Treatment 75Gy (3.37mm) induces high level decrease in an internodes space, the minimum decrease was observed in 150Gy (1.73mm). Leaf area was increased compared to control in 50Gy, 75Gy and 150Gy treatment. In doses 100Gy, 250Gy and 300Gy. Maximum increase was observed in 50Gy (144.96mm^2), while 300Gy (80.00mm^2) exhibited maximum decrease.

In FW of seedling a notable reduction was observed in 75Gy (0.033mm), 250Gy (0.058mm) and 300Gy (0.049 mm). A low level reduction was found in FM of seedling in 50Gy (0.145mm), 100Gy (0.001mm) and 150Gy (0.004 mm). 100Gy induced minimum reduction in fresh weight which indicates that 100Gy enhanced organic matter accumulation in chickpea seedling. It could be inferred from over all quantitative phenotypic mutations in chickpea seedlings that lower doses has stimulatory effect on the quantitative characters studied. These findings are also reported by Wi *et al*. (2007) Borzouei *et al*. (2010). They give the hypothesis that the low irradiation will induce the growth stimulation by changing the hormonal signaling network in plant cells or by increasing the anti-oxidative capacity of the cells to easily overcome daily stress factors. Maximum desirable quantitative phenotypes were induced by 100Gy; after this dose most of the parameters exhibit decline in quantitative characters. These results are consistent with the observations of Chandio *et al*. (2011), worked on low dose of gamma rays in chickpea in which 100Gy is the maximum safe dose exhibited maximum gross mutations, occasional membrane damage and absence of mitosis inhibition. During the cytogenetic studies from 150GY to 300Gy inhabited maximum gross mutations, oxidative damages and inhibition of cell division in chickpea root tip cells (Arain *et al*. 2011).

***(iii)* Effect on leaf type and leaflet arrangement and shape**

Gamma rays could not change leaf type or phylotaxis in M_0 generation **(Fig. 5)** as leaf type differences are governed by two supplementary genes (mlsl). Simple leaf results when ml is recessive and normal leaf is expressed when dominant alleles of both genes are present and multipinnate leaf occurs when dominant allele of ml is present (Pundir *et al*. 1990). Only leaflet arrangement on rachis and shape were changed**.** Leaflet arrangement changed from alternate (0Gy) to apposite only in 75 and 100Gy. While rest of treatment showed alternate leaflet arrangement. Apart from elliptical leaflets (0Gy) overall seven new leaflet shapes found were acute lobed (two lobed, three lobed, four lobed), round lobed (three lobed), ovate, lanceolate, rhomboid and obviate. Minimum leaflet shape variants were found in 50Gy (1) and maximum were observed in 300Gy. Fawole (2001) reported that mutations affecting leaflet number and shape were most frequent in cowpeas. Sangsiri *et a*l. (2005) reported Gamma rays induced five leaf shape mutations in mung bean. Presence of many leaflet shapes suggests that gamma rays has high power of generating mosiac seedlings and number of different leaflet shapes within one seedling increase with the increase in radiation dose.

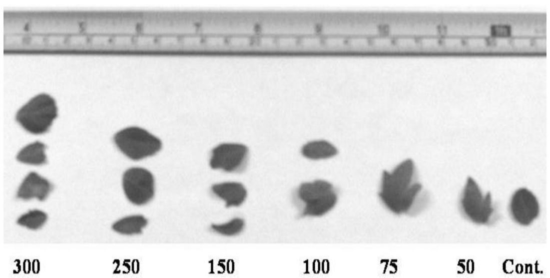

Fig. 5: Effect of gamma rays on leaflet shape in chickpea

(iv) Effect on chlorophyll profiles

Effects of Gamma rays treatment on chlorophyll profiles is shown in **(Table 2).** An increase in Chlorophyll (b) was recorded in 100Gy (0.11mg g^{-1} FW) as compared to control leaves that exhibited (0.089 mg g^{-1} FW).While reduction in chlorophyll (b) pigment was observed for rest of all the doses. Maximum reduction was observed in 150Gy (0.039 mg g^{-1} FW). In chlorophyll (a) except 50Gy (0.061 mg g^{-1} FW), all the doses caused increase in the amount of pigment as compared to control leaves that displayed (0. mg g^{-1} FW). Maximum increase in total leaf chlorophyll was observed in 250Gy (0.043 mg g^{-1} FW).

According to **(Table 2).** chlorophyll contents is irregularly distributed, similar observations are reported by Kim *et al*. (2004); Koing e*t al*. (2008); Borzouei *et al*. (2010). Once again over all chlorophyll profile suggests that 100Gy induce best increase in both the chlorophyll pigments (a, b) as well as total chlorophyll. Current research is supported by Borzouei *et al*. (2010) who reported that 100Gy induce highest amount of chlorophyll a, b and total chlorophyll in wheat genotypes. They further describe that chlorophyll content decrease with increased Gamma rays dose.

Table 2: Effect of Gamma rays on chlorophyll b, a and total chlorophyll

(C^b = Chlorophyll b; C^a = Chlorophyll a; g^{-1}= per 1 gram; FW= Fresh weight; value followed by different letters are significantly different at p<.05 using LSD test)

S.No.	Dose (Gy)	Chlorophyll (mg g^{-1}FW)		
		C^b	C^a	Total
1	0	0.08^{A}	0.018^{B}	0.098^{B}
2	50	0.07^{A}	0.016^{B}	0.086^{B}
3	75	0.02^{B}	0.025^{AB}	0.045^{C}
4	100	0.11^{A}	0.025^{AB}	0.135^{A}
5	150	0.012^{A}	0.027^{AB}	0.039^{C}
6	250	0.019^{B}	0.043^{A}	0.062^{BC}
7	300	0.051^{AB}	0.02^{B}	0.071^{BC}

4. CONCLUSION

It is concluded that different level doses of Gamma rays has different effects on the quantitative and qualitative phenotypic characters. Level of mosaic has direct proportion to the dose of Gamma rays. 50Gy to 250Gy are the best gamma rays and should be used in future researches to get desirable mutations for grain yield and associated characters.

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