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INDUCED GENETIC VARIABILITY IN QUANTITATIVE TRAITS OF KABULI CHICKPEA (*CICER ARIETINUM* L.)

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Abstract: Dry seeds of kabuli chickpea variety Noor 91 were irradiated at 400, 500 and 600 Gy doses of gamma rays and half of the irradiated seeds were treated with 0.5mM of gibberellic acid for 16 hours. Mutagenic treatments varied highly significantly and a significant decrease in number of secondary branches, pods per plant, grain yield and harvest index was recorded in M_1 generation. Mean values of pods per plant, grain yield and harvest index shifted in positive direction either significantly or nonsignificantly in M_2 and M_3 generations. High heritability but low genetic variability and genetic advance were estimated for plant height, number of secondary branches, pods per plant and grain yield in both the generations. Correlation analysis indicated that gamma irradiation modulated the significant positive association of plant height with number of secondary branches in negative direction while reverse was true for grain yield and harvest index in M_3 generation.

Keywords: Gamma irradiation, gibberellic acid, pods, grain yield, secondary branches, harvest index

Introduction

Chickpea (*Cicer arietinum* L.), Noor 91, white seeded genotype, is a self-fertilized crop. Its somatic chromosome number is known to be 2n=16 [1] with the characteristic features of (1) a pair of very long chromosomes which are submetacentric and satellited, (2) a pair of very short chromosome, and (3) six pairs of metacentric to submetacentric chromosomes. Chickpea (*Cicer arietinum* L.), is broadly categorized into kabuli type (white flowers and whitish seeds) and desi type (pink flowers and brown to black seeds). More chromosomal lengths as compared to desi genotypes have been determined for kabuli genotypes. Noor 91 is a kabuli genotype. Studies of isozyme banding pattern and Restriction Fragment Length Polymorphism (RFLP) have revealed a limited polymorphism which may be an indication of low level of genetic variability in chickpea [1]. Low genetic variability for the major characters becomes a limiting factor for its improvement. Gamma irradiation is known to cause breakage, depolymerization of DNA, which consequently leads to transitions, transversions, or chromosomal anomalies [2,3].

In recent years, mutation breeding has been gaining ground for inducing genetic changes and creation of new genetic resources [4,5,6]. Gamma irradiation in combination with other chemicals is utilized for widening the frequency and mutation spectrum for extra genetic variability. In experimental mutagenesis mutagenic efficiency or the ratio of gene mutation to physiological damage is of both theoretical and practical importance. It has been established that the impaired growth to gamma irradiation can be restored by exogenous application of gibberellic acid. Gibberellic acid serves manifold growth related functions in plants by enhancing replication, transcription and different enzymatic systems [7,8,9,10]. Gamma rays have been used in various studies in order to induce genetic variability [11,12]. Grain yield is a complex trait and highly

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influenced by many genetic factors and environmental fluctuations. Estimates of various genetic parameters for quantitative traits can provide the mutagenic effectiveness of that very mutagen. Gamma rays are high-energy radiations, which produce peroxy radicals and ions in the cells. Radio-protective effects of gibberellic acid in seedlings and M_2 generation of chickpea have been documented by Khan *et al*., [6,11]. Improvement of any crop depends on the kind and magnitude of genetic variability existed in the population. The aim of the present study was to generate information on the magnitude of induced genetic variability, and magnitude of associations between yield and its components following mutagenesis of gamma rays separately as well as post treatment with gibberellic acid.

Materials and Methods

Seeds of kabuli chickpea (*Cicer arietinum* L.) var. Noor 91 having 11.0% moisture were irradiated with gamma rays 100, 200, 300, 400, 500, 600, 700, 900, and 1100 Gy doses of ^{60}Co gamma cell at Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan. On the basis of seedling growth [12] doses of 400, 500 and 600 Gy were selected for inducing genetic changes. Half of the irradiated seeds were presoaked for an hour in distilled water and then transferred to 0.5 mM aqueous solution of gibberellic acid for 16 hours. Non-irradiated seeds presoaked in distilled water were kept as control. Seeds of all the treatments and control were sown in the field to raise M_1 generation in split plot design with varieties in main plots and treatments in sub plots having three replicates. Seeds harvested from individual M_1 plants were sown as M_2 families in three replications in the field. For raising M_3 generation, 40 M_2 normal looking progenies were selected having better yield components, particularly pods per plant in the M_2 generation. Seeds from each selected M_2 progenies were bulked by taking equal amount of seeds from all M_2 plants from a single M_2 progeny and thoroughly mixed. A random bulk of this was sown to obtain M_3 progeny. Data were recorded for plant height (cm), secondary branches per plant, pods per plant, grain yield per plant and harvest index on ten randomly selected plants.

ANOVA was performed to test the significance of variance by using computer software "MSTAT C". Kruskal Wallas test for M_1 generation and simple correlation among various character pairs in M_2 and M_3 generation were performed by computer software "SPSS" for windows. Heritability (h^2) in broad sense and genetic advance were calculated by the formula suggested by Singh and Chaudhary [13].

Results

Radiosensitivity studies in M_1 generation

Mean values of different characters of chickpea variety Noor 91 under different treatments for M_1 generation are presented in Table 1. Various characters responded differently to gamma rays with regard to sensitivity. Kruskal Wallas test was highly significant. This suggests that the effectiveness of different doses of gamma irradiation varied highly significantly for the characters under study. Plant height was increased either significantly or nonsignificantly at various doses of gamma irradiation and subsequently treated with gibberellic acid. This could be due to the protective effects of gibberellic acid and the prolonged vegetative growth period. The mean values of plant height at 400 and 500 Gy of gamma irradiation were nearly equal to that of control. However, significant increase was recorded at other doses of gamma irradiation. Decrease in the mean values for all other traits was recorded except at 600 Gy of gamma irradiation where an increase over control in pods per plant was noted in M_1 generation. In general, more phenotypic variability was determined for pods per plant as compared to other characters in M_1 generation.

Table 1. Radiosensitivity studies in M_1 generation of chickpea variety Noor 91.

	Plant height	Secondary branches	Pods per plant	Grain yield	Harvest index
	Mean±S.E	**Mean±S.E**	**Mean±S.E**	**Mean±S.E**	**Mean±S.E**
Control	84.78±0.22^{D}	19.80±0.50^{A}	60.10±1.26^{B}	19.12±0.52^{A}	19.80±0.50^{A}
400 Gy	85.53±0.51^{CD}	10.09±0.22^{C}	34.86±0.51^{F}	08.47±0.22^{E}	10.09±0.22^{D}
500 Gy	85.08±0.56^{D}	09.89±0.62^{C}	43.72±1.93^{E}	08.84±0.64^{E}	09.89±0.62^{D}
600 Gy	90.23±0.40^{A}	16.86±0.32^{B}	66.45±2.13^{A}	17.10±0.38^{B}	16.86±0.32^{B}
400 Gy+GA	89.01±0.50^{AB}	15.47±0.38^{B}	55.27±1.42^{BC}	16.37±0.51^{BC}	15.47±0.38^{C}
500 Gy+GA	88.91±0.45^{B}	15.78±0.84^{B}	52.50±2.28^{CD}	14.79±0.79^{CD}	15.78±0.84^{C}
600 Gy+GA	86.55±0.05^{C}	16.26±0.20^{B}	48.40±1.03^{DE}	13.31±0.18^{D}	16.26±0.20^{BC}
LSD>0.05%	**1.31**	**1.56**	**5.37**	**1.66**	**1.04**
$X^{2\dagger}$	17.612**	17.558**	18.805**	18.320**	19.325**

Means not followed by similar letters are statistically significant at >0.05% of probability by LSD.
±Standard error of the mean, ** Highly significant at >0.01% level of probability
† Kruskal Wallas test

Plant Height (cm) in M_2 and M_3 generation

A variation in mean values of plant height was observed for different levels of gamma rays (Table 2 and 3) in M_2 and M_3 generations. The mean values showed shift both in positive and negative directions in M_2 and M_3 generation. Maximum genotypic coefficient of variability and genetic advance was noted at 600 Gy treated population of M_2 generation followed by 500 Gy+GA dose of gamma irradiation. However, high heritability was recorded at all treatments in M_2 generation. Maximum genotypic coefficient of variability, heritability and genetic advance was noted at 500 Gy of gamma irradiation followed by 400 Gy population, while, minimum values for these parameters were determined at 600 Gy with gibberellic acid treatment in M_3 generation. Plant height was negatively correlated (Table 4) with grain yield in control population as well as in most of the gamma rays treatments in M_2 and M_3 generation. Variation in the magnitude of means in control populations of chickpea for different years could be attributed due to the cropping in different seasons and the plastic nature of this crop.

Secondary Branches per Plant in M_2 and M_3 generation

The mean values of number of secondary branches per plant shifted in negative directions nonsignificantly in M_2 generation at 500 Gy+GA and 600 Gy+GA while decreased significantly at 500 and 600 Gy of gamma irradiation treatment. Significant increase in number of secondary branches over control was recorded at 400 Gy and 400 Gy+GA treatment. The number of secondary branches decreased significantly at 400, 600 Gy and 600 Gy+GA treatment in M_3 generation. Maximum genotypic coefficient of variation, heritability, and genetic advance were recorded at 600 Gy of gamma irradiation followed by 500 Gy+GA dose in M_2 generation. However, maximum genetic gain in number of secondary branches was recorded at 600 Gy+GA treatment in M_3 generation. Correlation studies indicated that at certain doses of gamma irradiation the association of secondary branches with other character pairs was changed in M_2 and M_3 generation.

Table 2. Estimates of mean values, shift in mean, coefficient of variation, heritability (h^2) and genetic advance (GA) for different characters in M_2 generation of chickpea variety Noor 91.

Treatment	Mean±S.E.	Shift in mean	PCV (%)	GCV (%)	H^2 (%)	GA (% of mean)
		Plant height				
Control	83.48±0.63^{B}	0.00	1.42	0.82	57.74	1.42
400 Gy	84.40±0.45^{AB}	+0.92	0.72	0.69	94.94	2.31
500 Gy	85.60±0.48^{A}	+2.12	0.82	0.78	95.50	2.29
600 Gy	84.80±0.62^{AB}	+1.32	1.38	1.35	97.35	2.36
400 Gy+GA	83.30±0.44^{B}	-0.18	0.57	0.53	93.65	2.31
500 Gy+GA	84.34±0.49^{B}	+0.86	0.86	0.82	95.68	2.33
600 Gy+GA	83.40±0.18^{A}	-0.08	0.12	0.08	69.81	1.72
LSD>0.05%	**1.63**					
		Secondary branches per plant				
Control	19.10±0.46^{C}	0.00	3.35	2.12	63.28	06.82
400 Gy	20.95±0.53^{B}	+1.85	4.17	3.95	94.72	09.31
500 Gy	17.36±0.41^{DE}	-1.74	3.12	2.85	91.49	10.85
600 Gy	16.67±0.58^{E}	-2.43	6.22	5.94	96.90	11.97
400 Gy+GA	26.20±0.48^{A}	+7.10	3.36	3.14	93.70	07.36
500 Gy+GA	18.77±0.60^{CD}	-0.33	5.92	5.68	95.91	10.52
600 Gy+GA	18.12±0.30^{CDE}	-0.98	1.44	1.20	83.48	09.48
LSD>0.05%	**1.63**					
		Pods per plant				
Control	60.52±0.68^{E}	0.00	2.24	1.65	73.66	2.50
400 Gy	79.25±0.52^{B}	+18.73	1.01	0.98	97.04	2.52
500 Gy	76.95±0.51^{C}	+16.43	1.24	0.93	96.85	2.59
600 Gy	81.27±0.67^{A}	+20.75	1.64	1.61	98.22	2.48
400 Gy+GA	82.07±0.44^{A}	+21.55	0.72	0.69	95.90	2.40
500 Gy+GA	76.62±0.46^{C}	+16.10	0.82	0.70	96.09	2.58
600 Gy+GA	71.22±0.36^{D}	+10.70	0.52	0.40	93.67	2.70
LSD>0.05%	**1.77**					
		Grain yield per plant				
Control	19.80±0.30^{D}	0.00	1.32	0.92	69.69	7.25
400 Gy	25.96±0.48^{BC}	+6.16	2.67	2.62	98.21	7.79
500 Gy	24.93±0.33^{C}	+5.13	1.27	1.22	96.07	7.93
600 Gy	26.45±0.34^{B}	+6.65	1.33	1.28	96.29	7.49
400 Gy+GA	29.27±0.31^{A}	+9.47	1.02	0.97	95.84	6.74
500 Gy+GA	26.76±0.32^{B}	+6.96	1.17	1.12	95.98	7.38
600 Gy+GA	24.88±0.26^{C}	+5.08	0.79	0.74	94.06	7.78
LSD>0.05%	**1.10**					
		Harvest index %				
Control	19.61±0.33^{D}	0.00	1.75	0.34	19.64	2.16
400 Gy	25.08±0.49^{C}	+5.47	2.91	1.81	62.24	5.36
500 Gy	25.73±0.33^{C}	+6.12	1.26	0.19	15.35	1.28
600 Gy	24.84±0.40^{C}	+5.23	2.00	0.88	35.66	3.10
400 Gy+GA	29.85±0.45^{A}	+10.24	2.03	1.11	54.67	3.95
500 Gy+GA	27.65±0.34^{B}	+8.04	1.30	0.30	23.33	1.82
600 Gy+GA	25.27±0.32^{C}	+5.66	1.26	0.17	13.86	1.18
LSD>0.05%	**1.20**					

Means not followed by similar letters are statistically significant at >0.05% of probability by LSD, ± Standard error of the mean

Table 3. Estimates of mean values, shift in mean, coefficient of variation, heritability (h^2) and genetic advance (GA) for different characters in M_3 generation of chickpea variety Noor 91.

Treatment	Mean±S.E.	Shift in mean	PCV (%)	GCV (%)	H^2 (%)	GA (% of mean)
Plant height						
Control	86.14±0.61^{A}	0.00	1.33	0.74	55.63	1.33
400 Gy	86.36±0.58^{A}	+0.22	1.18	0.72	67.83	1.69
500 Gy	83.97±0.82^{BC}	-2.17	2.40	2.00	93.63	2.15
600 Gy	82.57±0.53^{C}	-3.57	1.02	0.62	61.00	1.59
400 Gy+GA	83.57±0.45^{C}	-2.57	1.17	0.77	66.32	1.71
500 Gy+GA	85.51±0.50^{AB}	-0.63	0.88	0.51	56.97	1.43
600 Gy+GA	86.33±0.42^{A}	+0.19	0.63	0.25	39.67	0.99
LSD>0.05%	**1.84**					
Secondary branches per plant						
Control	21.51±0.69^{AB}	0.00	6.65	3.65	58.40	5.59
400 Gy	19.26±0.26^{CD}	-2.25	1.05	1.05	58.12	6.51
500 Gy	22.00±0.20^{A}	+0.49	0.56	0.17	31.45	3.08
600 Gy	19.72±0.20^{CD}	-1.79	0.65	0.22	34.10	3.73
400 Gy+GA	20.38±0.38^{BC}	-1.13	2.13	1.71	80.45	8.52
500 Gy+GA	19.27±0.24^{CD}	-2.24	0.91	0.47	51.97	5.82
600 Gy+GA	18.73±0.61^{D}	-2.78	5.98	5.53	92.41	10.65
LSD>0.05%	**1.33**					
Pods per plant						
Control	75.51±0.58^{E}	0.00	1.90	1.04	54.73	1.49
400 Gy	84.16±0.53^{C}	+8.65	1.02	0.65	63.27	1.62
500 Gy	89.59±0.47^{A}	+14.08	0.76	0.40	53.37	1.28
600 Gy	78.47±0.44^{D}	+02.96	0.74	0.34	45.91	1.26
400 Gy+GA	86.24±0.53^{B}	+10.73	0.98	0.61	62.58	1.56
500 Gy+GA	82.87±0.55^{C}	+07.36	1.12	0.74	65.91	1.71
600 Gy+GA	87.39±0.61^{B}	+11.88	1.30	0.94	72.15	1.78
LSD>0.05%	**1.72**					
Grain yield						
Control	23.33±0.35^{D}	0.00	1.60	0.95	59.37	5.24
400 Gy	31.07±0.51^{BC}	+7.74	2.52	2.18	56.60	6.02
500 Gy	32.29±0.50^{AB}	+8.96	2.38	2.05	86.36	5.77
600 Gy	30.43±0.47^{C}	+7.10	2.18	1.84	84.21	5.97
400 Gy+GA	31.56±0.50^{BC}	+8.23	2.39	2.06	86.11	5.89
500 Gy+GA	32.64±0.59^{AB}	+9.31	3.24	2.91	90.07	5.96
600 Gy+GA	33.16±0.38^{A}	+9.83	1.30	0.98	75.69	4.93
LSD>0.05%	**1.57**					
Harvest index						
Control	25.20±0.64^{D}	0.00	4.89	2.26	46.21	3.77
400 Gy	36.02±0.37^{C}	+10.82	0.62	0.41	66.12	3.78
500 Gy	35.88±0.67^{C}	+10.68	3.84	3.32	86.52	5.20
600 Gy	36.02±0.91^{C}	+10.82	6.97	6.45	92.59	5.55
400 Gy+GA	37.36±0.58^{BC}	+12.16	2.72	2.22	81.72	4.72
500 Gy+GA	39.45±0.59^{A}	+14.25	2.69	2.22	82.48	4.56
600 Gy+GA	39.07±0.57^{AB}	+13.87	2.00	1.53	76.27	4.21
LSD>0.05%	**2.84**					

Means not followed by similar letters are statistically significant at >0.05% of probability by LSD, ± Standard error of the mean

Pods per Plant in M_2 and M_3 generation

Considerable variation was noted for the number of pods per plant at various doses of gamma irradiation in M_2 and M_3 generations. The mean values of pods per plant shifted in positive direction at all doses of gamma irradiation as compared to the control. High degree of heritability was recorded at all doses including control population in M_2 generation. However, the genotypic coefficient of variability and genetic advance were of low value at different treatments. The heritability of pods per plant was decreased in M_3 generation as compared to M_2 generation. Association of pods per plant with plant height in the control population was positive while it became negative at 600 Gy, 400 Gy+GA, and 500 Gy+GA in M_2 and M_3 generations. The association of pods per plant with the harvest index was changed from positive to negative direction at 500 Gy dose of gamma irradiation in M_3 generation.

Grain Yield in M_2 and M_3 generation

Various doses of gamma irradiation shifted the mean values of grain yield significantly towards positive as compared to the control in both M_2 and M_3 generations. High heritability was recorded at various treatments, while low coefficient of variability and genetic advance were calculated in both generations.

Harvest Index in M_2 and M_3 generation

Mean values of harvest index shifted significantly towards positive direction at various doses of gamma irradiation in M_2 generation. However, in M_3 generation shifting of mean values was recorded towards positive as well as in negative direction. A wide range in heritability with low genotypic coefficient of variability and genetic advance was recorded in M_2 generation. However,

Table 4. Phenotypic correlation between various character pairs in M_2, and M_3 generation of chickpea variety Noor 91.

Treatment	Plant height vs secondary branchess	Plant height vs pod per plant	Plant height vs. grain yield	Plant height vs. Harvest index	Secondary branches per plant vs. pods per plant	Secondary branches per plant vs. grain yield	Secondary branches per plant vs. Harvest index	Pods per plant vs. grain yield	Pods per plant vs. Harvest index	Grain yield Harvest index
M_2										
Control	0.648	0.581	-0.582	-0.832	0.696	0.996	0.962	1.00**	0.935	0.945
400 Gy	-0.958	0.914	-1.00**	-0.972	-0.992	0.995	0.999*	-0.909	-0.948	0.969
500 Gy	0.436	-0.591	-0.997*	0.851	0.469	-0.506	-0.102	0.524	-0.926	-0.806
600 Gy	0.679	-0.196	-0.721	0.990	0.588	0.019	0.566	0.821	-0.336	-0.814
400 Gy+GA	0.998*	-0.935	0.496	-0.539	-0.956	0.551	-0.484	-0.772	0.205	0.464
500 Gy+GA	1.00**	-0.995	-0.651	0.404	-0.997*	-0.674	0.432	0.725	-0.496	-0.957
600 Gy+GA	0.305	0.379	0.792	-0.511	0.997*	-0.339	0.662	-0.264	0.601	-0.929
M_3										
Control	0.698	0.384	-0.892	-0.982	0.320	0.998*	0.967	0.864	0.749	0.951
400 Gy	-0.896	0.377	-1.00**	0.963	-0.673	0.884	0.983	0.401	0.113	0.955
500 Gy	0.111	0.591	0.467	-0.939	0.867	0.930	-0.446	0.989	-0.832	-0.743
600 Gy	-0.897	-0.484	-0.591	0.784	0.821	0.887	-0.430	0.992	0.163	0.036
400 Gy+GA	-0.830	-0.980	-0.960	-0.633	0.703	0.951	0.094	0.888	0.774	0.396
500 Gy+GA	-0.607	-0.971	-0.961	-0.932	0.780	0.802	0.853	0.999*	0.992	0.996
600 Gy+GA	0.098	0.961	-0.467	0.533	-0.370	-0.834	-0.894	-0.204	0.747	0.498

in M_3 generation low values of heritability, genotypic coefficient of variability and genetic advance were determined at 400 Gy of gamma irradiation.

Discussion

In experimental mutagenesis, mutagenic treatments with low physiological damage and strong genetic effects are desirable. In the present study various doses of gamma irradiation shifted the mean of various characters under study either in positive or negative direction in M_1 generation. Radiation effects on various characters have been described in other studies [4,6]. The inheritance of the important economic traits of plants such as yield, pods per plant, number of branches and harvest index upon which rest much of the future of plant improvement. Response to selection for quantitative traits is directly proportional to the function of its heritability, genetic advance and its genotypic variance [14,15,16,17]. Heritability has enabled the plant breeder to recognize the genetic differences among the traits and the genotypic variance indicates the potential for the improvement of a particular trait. Increase in (PCV), (GCV) and heritability has also been documented for various characters in M_3 generation [19,20]. The estimates of various genetic parameters viz., genotypic coefficient of variation (GCV), heritability (h^2) and genetic advance (GA) for five quantitative characters of the chickpea variety, Noor 91, provide ample evidence that mutagenic treatments could alter mean values and create additional genetic variability for quantitative traits. Increase in mean values of grain yield and other traits in M_3 generation over the M_2 and the control could be a result of direct selection for yield exercised in M_2 generations. Khan et al. [14] had also reported similar result in Vigna radiata. Shift in mean values for various characters in positive direction have also been studied in M_3 generation (21,22). In the present study, high heritability estimates in broad sense but moderate to low genetic advance for plant height, secondary branches, grain yield, and pods per plant were calculated at various doses of gamma irradiation for M_2 and M_3 generation. These results indicate that these characters are under the control of non-additive gene action (dominance and epistasis) and they will not respond to early selection. Improvement of yield is based on simultaneous selection for the desirable yield components in crop plants. Possible association among the five characters in M_3 generation show that the mutagenic treatments succeeded in generating more favourable association between various yield components. The magnitude and direction of the induced change varied at different doses. Significant positive association of plant height with number of secondary branches was changed towards negative direction at various doses in M_3 generation. Similarly, negative association between grain yield and harvest index was changed into positive association at various treatments of gamma irradiation. This was highly desirable from the point of view of improvement of more than one trait. Such desirable changes in association with yield contributing characters have also been reported in Cicer arietinum [16] and in Vigna radiata [14]. These studies lead to the consideration of a worthwhile ideotype in kabuli chickpea that more stress should be given on progenies having medium height with greater number of secondary branches and pods per plant.

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