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GENETIC VARIABILITY INDUCED BY GAMMA IRRADIATION AND ITS MODULATION WITH GIBBERELLIC ACID IN M_2 GENERATION OF CHICKPEA (*CICER ARIETINUM* L.)

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Abstract

Seeds of three chickpea genotypes viz., Noor 91 (white), Punjab 91 (brown) and C 141 (black) were treated at 40, 50 and 60 Kr separately and post mutagenically with gibberellic acid (GA_3). Plant height, number of primary and secondary branches, pods per plant, seeds per pod and grain yield in M_2 generation were significantly effected due to genotypes, treatments and also by their interaction. Plant height decreased nonsignificantly with both treatments except at 50 Kr of gamma irradiation. Primary branches increased significantly with gamma irradiation as compared to control. Post mutagenic application of GA_3 significantly decreased the number of primary branches at 50 and 60 Kr to that of gamma irradiation. Gamma irradiation significantly decreased number of secondary branches as compared to control. Post mutagenic application of GA_3 stimulated the secondary branches at 40 and 50 Kr as compared to irradiated population. Pods per plant significantly increased with gamma irradiation as compared to control. GA_3 application significantly decreased the pods per plant at 40 and 60 Kr which increased at 50 Kr to that of while gamma irradiation. Seeds per pod remained unchanged with gamma irradiation as compared to control while it increased significantly with GA_3. Gamma irradiation which increased the grain yield significantly as compared to control. Combined treatment stimulated the grain yield at 50 and 60 Kr to that of irradiated population.

Introduction

Mutation breeding has been used in recent years as a valuable supplement to the method of plant breeding in the development of better crop cultivars (Awan, 1991). For the improvement of a crop, the extent of genetic variability is more important than the total variability. The inheritance of important economic traits such as yield, quality, adaptation, pest and stress resistance, upon which much of the future of plant improvement depends can be understood through the analysis of a wide range of induced mutations. Several workers have attempted for induction of mutation using either physical or chemical mutagens for evolving new genotypes (Bravo, 1988; Hassan & Khan, 1991a, b; Shamsuzzaman & Shaikh, 1991; Kharkwal, 1983; Kharkwal *et al*., 1988; Haq *et al*., 1988; 1989).

It has been established that the impaired growth due to gamma irradiation can be restored by exogenous application of gibberellic acid by enhancing replication, transcription and different enzymatic systems (Callebaut *et al*., 1980; Uppal & Maherchandani, 1988; Zhebrak, 1989; Ali & Ansari, 1989; Arora *et al*., 1989). Gibberellic acid has been used as a radio protective agent mostly at the seedling stage and has not been utilized as a part of combined treatment in mutation breeding. An experiment was, therefore, conducted to determine the induced genetic variability and its modulation with the post mutagenic application of gibberellic acid in chickpea.

Materials and Methods

Fresh seeds (moisture 11.0 %) of three chickpea genotypes viz., Noor 91 (white seed coat colour). Punjab 91 (brown seed coat colour) and C141 (black seed coat colour) were irradiated @ 198.926 Kr hr^{-1} with 10, 20, 30, 40, 50, 60 70, 90 and 110 Kr doses of ^{60}Co gamma cell at Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan. On the basis of seedling growth (Khan *et al*., 2000) doses of 40, 50 and 60 Kr were selected for inducing genetical changes. To enhance the genetic variability, a part of the irradiated were presoaked for an hour in distilled water and then were transferred to 0.5 mM aqueous solution of gibberellic acid for 16 hours. Non-irradiated seeds presoaked in distilled water were kept as control. The experiment was planted in a randomized complete block design (varieties in main plots and treatments in sub-plots) with three replicates (150 seeds/replication) at the experimental fields of Barani Agriculture Research Institute (BARI), Chakwal, Pakistan during September 1995 to raise the M_1 generation. Seeds of each treatment of M_1 generation were harvested individually to raise the M_2 generation in the following season. Three rows of each treatment were sown keeping 35 and 10 cm spacing between and within rows for the M_2 generation in a randomized complete block design with varieties in main plots and treatments in sub-plots. Quantitative data i.e., plant height (cm), number of primary and secondary branches per plant, number of pods and seed yield (g) were recorded on 40 randomly selected plants. Seeds/pod were calculated by counting the total number of pods and total seeds per plant.

The variance components of genotypes, treatments and their interaction were determined by the analysis of variance using the methodology given by Steel & Torrie (1960). The significance of variance components was determined by an F-test. Individual comparisons of variety and treatment means were accomplished by placing them in descending order and applying Duncan’s Multiple Range Test (DMRT).

Results and Discussion

Plant height: The analysis of variance for the effects of different doses of gamma irradiation separately and with the application of gibberellic acid on plant height in M_2 population of chickpea revealed high fluctuations of induced variability in genotypes across different treatments. Variability in plant height with gamma irradiation had been reported by Charumathi *et al*., (1992) in black gram. Maximum height per plant 96.09 cm was produced by C 141 as against 94.33 and 84.42 cm in Punjab 91 and Noor 91, respectively (Table 1). The data regarding the main treatments on plant height revealed non-significant ($p>0.01$) decrease at 40 and 50 Kr and significant ($p<0.01$) increase at 60 Kr with both mutagenic treatments as compared to control. Present results are compatible with Rao (1988) in chickpea, Shakoor *et al*., (1978a, b) in mungbean, Vadher *et al*., (1988) in sorghum and Ramani & Jadon (1991) in groundnut. The reports of Rao *et al*., (1988) in pigeon pea and Khan *et al*., (1989) in sorghum did not agree with these results. They observed that plant height increased with the application of gamma irradiation. This may be due to the different genetic material and environmental conditions.

With regard to genotype-treatment interaction, a differential behaviour was noted among varieties for this character. In Noor 91 gamma irradiation increased the plant height non-significantly ($p>0.01$) at various doses as compared to control. Similar results were obtained by Shakoor *et al*., (1978 a & b) in mungbean, Kasim *et al*., (1977) in M_2

generation of broad bean. However, with the application of gibberellic acid non-significant decrease was noticed at 40 and 50 Kr treatments, while at 60 Kr significant ($p<0.01$) increase as compared to control was observed. There was a progressive decrease in plant height with gamma irradiation in Punjab 91 as compared to control. The application of gibberellic acid decreased the plant height non-significantly ($p>0.01$) as compared to control. However, by comparing the two mutagenic treatments plant height increased significantly at 60 Kr with GA_3. In C141 both mutagenic treatments decreased the plant height significantly ($p<0.01$) except at 50 Kr with the treatment of gibberellic acid. GA_3 decreased the plant height significantly at 60 Kr by modulating the effects of gamma irradiation.

Primary branches: Number of primary branches per plant in M_2 population of chickpea reflects high inconsistency in the performance of genotypes for this character across the various doses. On the average, maximum number of primary branches 9.36 per plant was observed in Punjab 91, followed by 8.10 and 7.26 per plant in Noor 91 and C 141, respectively (Table 1). The data regarding the effects of main treatments on number of primary branches per plant revealed that both mutagenic treatments increased the number of primary branches. Number of primary branches per plant increased significantly ($p<0.01$) at 50 and 60 Kr with the treatment of gibberellic acid. Maximum number of primary branches per plant was observed at 40 Kr with gamma irradiation, while with the application of GA_3 at 60 Kr treatment. Charumathi *et al*., (1992) reported similar findings with gamma rays in black gram.

Differential response of the varieties was observed with respect to different type of mutagenic application. In Noor 91 number of primary branches increased significantly ($p<0.01$) with both mutagenic treatments except at 60 Kr with GA_3 the increase was non-significant as compared to control. Application of GA_3 significantly decreased the number of primary branches at 50 and 60 Kr by changing the effects of radio sensitivity. Punjab 91 exhibited significant ($p<0.01$) increase in number of primary branches with different mutagenic treatments as compared to control. Maximum number of primary branches 10.86 and 11.95 per plant were recorded at 50 Kr treatment with gamma irradiation and gibberellic acid, respectively. In C141 the number of primary branches responded similarly and significant ($p<0.01$) increase was observed at 40 Kr with both mutagenic treatments.

Seconary branches: The number of secondary branches per plant in M_2 population of chickpea (Table 1) shows the differences among genotypes for this character across different doses. Main genotypic effects revealed that C141 exhibited maximum number of secondary branches 20.09 per plant followed by 19.60 and 16.17 per plant in Noor 91 and Punjab 91, respectively (Table 1). It is apparent from the results that number of secondary branches reduced significantly ($p< 0.01$) at various mutagenic treatments except at 40 Kr treatment with gibberellic acid as compared to control.

In Noor 91 the number of secondary branches increased significantly ($p<0.01$) at 40 Kr with both mutagenic treatments as compared to control (Table 1). Application of GA_3 significantly increased the number of secondary branches at all levels of irradiation. In Punjab 91 significant ($p<0.01$) decrease in the number of secondary branches was noticed at all mutagenic treatments as compared to control. However, minimum number of secondary branches and obtained at 60 Kr with gamma irradiation and at 40 Kr with the treatment of gibberellic acid. Number of secondary branches in C141 responded differentially to different mutagenic treatments. The number of secondary branches decreased significantly ($p<0.01$) with gamma radiation as compared to control. However, with gibberellic acid these were first increased significantly ($p<0.01$) at 40 Kr treatment and then decreased significantly at 50 and 60 Kr as compared to control. Charumathi *et al*., (1992) also observed a marked variability in the number of branches in black gram with gamma irradiation.

Pods per plant: The analysis of variance for the effect of different doses of gamma irradiation on number of pods per plant (Table 1) in M_2 population of chickpea revealed highly inconsistent performance of genotypes across various treatments. Punjab 91 exhibited maximum number of pods 83.87 per plant followed by 75.41 and 72.86 per plant in Noor 91 and C 141, respectively (Table 1). It is evident from the results that number of pods responded differentially to the two mutagenic treatments. Number of pods increased significantly ($p<0.01$) with two mutagenic treatments as compared to control. Comparatively, GA_3 treatment increased the number of pods significantly ($p<0.01$) at 50 Kr, which decreased at 40 and 60 Kr of gamma irradiation. In the previous research similar results have been reported by Shakoor *et al*., (1978 a & b) in mungbean, Khan *et al*., (1989) in sorghum, Alexieva & Nikolov (1991) in soybean, Bhatnagar (1991) in chickpea, Sarma *et al*., (1991) in green gram and Charumathi *et al*., (1992) in black gram.

Varietal response of pods per plant was found to be different towards the two mutagenic treatments. In Noor 91 mutagenic treatments increased the number of pods per plant significantly ($p<0.01$) as against the respective control. However, the number of pods increased significantly at 40 Kr and decreased at 60 Kr with the application of gibberellic acid (Table 1). In Punjab 91 number of pods per plant decreased significantly with gamma irradiation at 40 and 60 Kr, while with gibberellic acid significantly ($p<0.01$) decreased at 40 Kr and increased at 50 Kr as compared to control. GA_3 treatment modulated the effects of gamma irradiation and number of pods decreased significantly at 40 Kr and increased at 50 and 60 Kr. In C141 number of pods per plant increased significantly ($p<0.01$) at all irradiation treatments except at 40 Kr with GA_3 treatment. Number of pods decreased significantly with GA_3 at 40 and 60 Kr while it increased at 50 Kr treatment. Present investigations are in line to those of Shakoor *et al*., (1978 a) in mungbean, Sinha & Bharati (1990) in urdbean and Bhatnagar (1991) in chickpea where they observed that the number of pods per plant increased at various irradiation dosages irrespective of the cultivars.

Seeds per pod: The effect of different doses of gamma irradiation on the number of seeds per pod in M_2 population of chickpea reveals that sufficient variability induced in genotypes for this character across the various treatments. Similar variability in seeds/pod have also been recorded by Sarma *et al*., (1991), Charumathi *et al*., (1992) and Gupta & Sharma (1996). Maximum number of seeds 1.70 per pod were observed in Punjab 91 followed by 1.32 and 1.31 per pod in Noor 91 and C 141, respectively (Table 1). It is apparent from the results that the number of seeds responded differentially to both mutagenic treatments. There was not any significant increase or decrease in the number of seeds with gamma irradiation as compared with control. However, the number of seeds per pod increased significantly ($p<0.01$) at all treatments with gibberellic acid and maximum seeds per pod were obtained at 50 Kr treatment. The results of Khan (1984) were partially in line with these studies as that with the treatment of GA_3.

Varieties did not perform uniformly to the two mutagenic treatments. In Noor 91 number of seeds increased non-significantly ($p>0.01$) as compared to control with gamma irradiation, while the application of gibberellic acid increased the seeds per pod significantly ($p<0.01$) as compared to control at all irradiation dosages and maximum were found at 50 Kr treatment. In Punjab 91 gamma irradiation decreased the seeds per pod significantly ($P<0.01$) as compared to control at 50 and 60 Kr treatments. The application of gibberellic acid increased the seeds per pod non-significantly ($p>0.01$) as compared to control. C 141 responded differently to the two mutagenic treatments. Gamma irradiation changed the number of seeds per pod significantly ($p<0.01$) at all irradiation dosages. Our results are partially compatible with Shakoor *et al*., (1978 a) in mungbean where the number of seeds per pod decreased with gamma irradiation in M_2 population as in the genotype Punjab 91. Similarly Kumar & Sinha (1989) recorded a non-significant difference for number of seeds per pod in M_2 generation of *Cajanus cajan* and *Moghania.* These results are in agreement to the present study in genotypes C141 and Noor 91 where the number of seeds per pod obtained were statistically similar to their untreated control.

Grain yield: Highly significant variance components (Table 1) revealed that a marked variability is created at various doses in the three genotypes. In the previous research, similar observations have been reported by Sarma *et al*., (1991) and Charumathi *et al*., (1992). Maximum grain yield of 37.00 g per plant was observed in Punjab 91, followed by 25.42 and 25.21 g per plant in Noor 91 and C 141, respectively (Table 1). It is seen from the results that the grain yield increased significantly ($p<0.01$) across the different mutagenic treatments of gamma irradiation and with gibberellic acid as compared to control. Maximum increase in grain yield was observed at 50 Kr treatment with gibberellic acid. The present results are similar to those of John (1995). However, Rao (1988) found different results and reported that gamma irradiation had adverse affect on grain yield in chickpea. This may be due to the dissimilar material and environmental conditions of the experiment.

A differential response of varieties across the various mutagenic treatments was observed. In Noor 91 grain yield per plant increased significantly ($p<0.01$) with both mutagenic treatments. However, with the application of gibberellic acid significantly more grain yield was recorded at 40 and 50 Kr. Grain yield in Punjab 91 decreased significantly ($p<0.01$) with gamma irradiation at 60 Kr treatment as compared to control. Application of gibberellic acid changed the effect of gamma irradiation and significant ($p<0.01$) decrease and increase in grain yield was observed at 40 Kr and 50 Kr treatment, respectively as compared to control. In C141 gamma irradiation had non-significant ($p>0.01$) effects on grain yield, while with gibberellic acid at 50 and 60 Kr significant ($p<0.01$) increase was observed as compared to control. The results advocated in present study are similar to those of Larik *et al*., (1980) in wheat, Charumathi *et al*. (1992) in black gram and Kalia & Gupta (1989) in lentil who reported that the genotypes varied for grain yield in M_2 population. Results of Shakoor *et al*., (1978 a) are similar to genotype Punjab 91, while the results of Rao *et al*., (1988) are partially in line with the genotypes Noor 91 and C141.

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