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Spectrum of Morphological Mutations Induced by Separate and Simultaneous Application of Gamma Rays with GA_3 in Chickpea (*Cicer arietinum* L.)

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Abstract: Three chickpea genotypes viz., Noor 91 (white), Punjab 91 (brown) and C 141 (black) were treated with 40, 50 and 60 Kr doses of gamma irradiation separately and post mutagenically with gibberellic acid (GA_3) to create genetic variability. M_2 progenies of these treatments were raised from M_1 seeds in plant to family manner. The results indicated that induced mutability is governed by the genetic architecture of the material used. Various morphological mutations induced affecting plant height, growth habit, branching and stem structure, stem and foliage colour, leaf type, flowering and maturity, pod and seed type. There were differences between the genotypes and between the two types of treatments. Frequency of these mutants increased with gamma irradiation in Noor 91 and C 141 while, with GA_3 it tended to increase in the three genotypes.

Key words: Gamma irradiation, morphological spectrum, gibberellic acid, induced mutation, *Cicer arietinum*, Chickpea

Introduction

Enhancement of the frequency and spectrum of mutations in a predictable manner and thereby achieving desired plant characteristics is an important goal of current mutation research. In the present study, various doses of gamma rays separately and in combination with were applied on the three chickpea genotypes with different seed coat colours. Induced mutations have been recognized as an important tool for crop improvement and are believed to have sufficient scope in pulses. A number of viable mutants in chickpea have earlier been described by Athwal *et al.* (1970), Nerkar and Mote, (1978) and Kalia *et al.* (1981). Haq *et al.* (1994) studied the effects of different mutagens for inducing variations in branching and stem structure, leaf and flower type, flowering and maturity, pod and seed size. Variation in morphological and yield characters have also been reported in lentil (Dixit and Dubey, 1983; 1986; Singh *et al.,* 1989; Tyagi and Gupta, 1991; Tripathi and Dubey, 1992), *Vicia faba* (Filippetti *et al.,* 1982) and in mungbean (Yadav and Singh, 1988). However, post mutagenic treatment with GA_3 for morphological mutations has not been reported. For mutation breeding, response of genotypes towards the synergistic effects also provides valuable information since it facilitates planning of experiments to achieve a high mutation frequency. During the present study of induced mutations in chickpea a number of mutations for a variety of morphological and agronomic characters were obtained and were analyzed.

Materials and Methods

Dry seeds were exposed to gamma irradiation at doses of 10, 20, 30, 40, 50, 60, 70, 90 and 110 Kr to 1000 seeds for each treatment in three genotypes at Nuclear Institute for Food and Agriculture (NIFA), Peshawar. A part of the irradiated seeds after one hour of soaking under continuous aeration seeds were subjected to 0.5 mM aqueous solution of gibberellic acid for 16 hours with constant shaking. Non irradiated seeds soaked in water were kept as control in the case. After treatment seeds were washed in running tap water and then were dried on blotting paper. On the basis of seedling performance doses of 40, 50 and 60 Kr were selected for inducing genetic variability on large scale. Treated along with control seeds were sown in split plot design at Barani Agriculture Research Institute (BARI) Chakwal to raise the M_1 generation in 1995. Seeds of 250 plants from treated populations were harvested separately, while from control population the seeds were bulked. The trial was conducted in a split plot design with three replications having 15 seeds in plot size of 1.5×0.33 meter at BARI, Chakwal in last week of September 1996.

Variations in branching and stem structure, leaf type, flower type, flowering and maturity, pod and seed size were recorded. The mutants were classified by the most contrasting character and mostly which was noticed at an early stages, e.g., many large leaf mutants had large pods and large seeds; small-leaf mutants had small pods and small seeds, but were classified into leaf type mutations. The mutation frequency was computed on M_2 family basis (percent of mutated progenies) and M_2 plant basis (percent of mutants).

Results and Discussion

Mutation frequency: Many morphological mutations were observed in all the treatments (Table 1). Data show that these mutations occurred more frequently in the simultaneous application of gamma radiation and GA_3 than in gamma irradiation separately except in Punjab 91, where more morphological mutations occurred with gamma irradiation. The stimulation in mutation frequency with GA_3 may be due to the elimination of gross chromosomal changes, while creating point mutations, which were being expressed. Results of a similar nature have been reported in lentil (Dixit and Dubey, 1986; Tripathi and Dubey, 1992) where the simultaneous application of gamma rays, NMU and EMS increased the frequency of segregating families. A dose dependent increase in the frequency of morphological mutations was observed with gamma irradiation and with simultaneous application of GA_3, both on the percentage of segregation of M_2 families and population basis. Viable mutation frequency ranged from 4.80 to 17.60% on progeny basis and 0.36 to 1.02% on population basis. Results of a similar nature have been reported in french bean (Yankulav *et al*., 1980) and in soybean (Boreiko, 1976; Nikolov, 1980).

Single and multiple mutations: Relative frequencies of families segregating for single and multiple morphological mutation types are presented in Table 2. Most of the families

Table 1: Frequency of morphological mutants in M_2 generation of three chickpea genotypes

Variables		Frequency on M_2 family basis			Frequency on M_2 population basis		
Genotype	Treatment	Total	Segregating	Percent	Total	Mutants	percent
Noor 91	Control	10	00	0.00	265	00	0.00
	40 Kr	250	17	6.80	5438	23	0.42
	50 Kr	250	20	8.00	5613	27	0.48
	60 Kr	250	29	11.60	5140	40	0.77
	40 Kr+GA_3	250	21	8.40	5529	28	0.50
	50 Kr+GA_3	250	25	10.00	5430	30	0.55
	60 Kr+GA_3	250	27	10.80	5260	29	0.55
Punjab 91	Control	10	00	0.00	274	00	0.00
	40 Kr	250	41	16.40	5381	55	1.02
	50 Kr	250	37	14.80	5470	56	1.02
	60 Kr	250	44	17.60	5290	55	1.03
	40 Kr+GA_3	250	35	14.00	5476	47	0.85
	50 Kr+GA_3	250	39	15.60	5310	54	1.01
	60 Kr+GA_3	250	42	16.80	5332	54	1.01
C 141	Control	10	00	0.00	268	00	0.00
	40 Kr	250	12	4.80	5511	20	0.36
	50 Kr	250	17	6.80	5476	28	0.51
	60 Kr	250	22	8.80	5380	37	0.68
	40 Kr+GA_3	250	16	6.40	5490	25	0.45
	50 Kr+GA_3	250	21	8.40	5481	34	0.62
	60 Kr+GA_3	250	22	8.80	5415	33	0.60

Table 2: Relative frequency of M_2 families segregating for varying number of morphological mutation types

	Genotypes								
Treatments	Noor 91			Punjab 91			C 141		
	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
40 Kr	94.33	3.77	1.88	97.77	2.23	0.00	100.00	0.00	0.00
50 Kr	95.74	4.26	0.00	90.69	6.97	2.32	95.23	4.77	0.00
60 Kr	94.44	2.77	2.77	88.23	8.82	2.94	88.23	11.77	0.00
40 Kr+GA_3	97.91	2.09	0.00	100.00	0.00	0.00	86.95	8.69	4.34
50 Kr+GA_3	94.11	3.92	1.96	95.23	4.77	0.00	100.00	0.00	0.00
60 Kr+GA_3	93.33	6.67	0.00	100.00	0.00	0.00	100.00	0.00	0.00

segregated for one type of mutation. The proportion of segregating families ranged from 86.95 to 100% for one type, 2.09 to 11.77% for two types of morphological mutants, respectively. Noor 91 segregated for two and three types with both mutagenic treatments. A relationship was observed between frequency of families segregating for three types. Punjab 91 segregated for three types with gamma irradiation while, two types with GA_3. In C141 three types were only observed with GA_3. These segregating families increased with an increase in gamma irradiation in Punjab 91 and C141 as reported by Nerkar and Mote (1978). The present results are contrary to the findings of Haq *et al.* (1994) where no such relationship was recorded in three Kabuli chickpea genotypes.

Spectrum of morphological mutants: The mutagenic treatments induced mutations affecting plant height, growth habit, branching and stem structure, stem and foliage colour, leaf size, flowering and maturity, seed and pod size (Table 3). There were differences in mutation spectrum between the genotypes.

Plant height mutants: These could be further categorized into stunted, dwarf, semi-dwarf, compact and tall.

Stunted: Several miniature plants having reduced height with shorter internodes were grouped under this category. All these mutants produced flowers and fruits and set seeds also. Most of these mutants were segregated in Noor 91 and only a few were noticed in Punjab 91. In C 141 no such mutants were segregated. Gamma irradiation of 60 Kr produced the most stunted seedling mutants, followed by the combined treatment of 60 Kr with GA_3. Stunted mutants have also been reported with gamma irradiation in chickpea by Haq *et al*. (1994), while in lentil by Dixit and Dubey (1983, 1986); Tripathi and Dubey (1992) and Vandana and Dubey (1994).

Dwarfs: Dwarf mutants were observed in M_2 population of various mutagenic treatments. Dwarfs were more frequently induced by combined treatments of gamma irradiation with GA_3 than by gamma irradiation alone. In some of these mutants, the leaves had small and narrow leaflets while others had normal leaves. Seed yield in the dwarfs was reduced. These dwarf mutants were found in all the three genotypes. These mutants were observed in different studies (Dixit and Dubey, 1983; Tripathi and Dubey, 1992; Vandana and Dubey, 1994; Haq *et al*., 1994).

Semi-dwarfs: The internodal length in the semi-dwarfs was greater than the dwarfs, but it was conspicuously shorter than the control. There was no apparent reduction in seed yield

Table 3: Spectrum of morphological mutation in M_2 generation of three chickpea genotypes treated with gamma irradiation separately and in combination with GA_3

	Mutational classes							
	Gamma irradiation				Gamma irradiation + GA_3			
Multinational group	Noor 91	Punjab 91	C 141	Total	Noor 91	Punjab 91	C 141	Total
Plant height								
Stunted	5	4	0	9	4	6	0	10
Dwarf	3	7	4	14	7	5	8	20
Semi-dwarf	1	3	1	5	3	2	4	9
Compact	0	1	0	1	0	0	0	0
Tall	0	13	0	13	2	19	2	23
Others	0	4	0	4	0	3	0	3
Growth habit								
Erect	11	30	19	50	13	26	15	54
Spreading	7	3	0	10	6	7	3	16
Bushy	3	2	8	13	1	3	11	15
Prostrate	1	0	0	1	0	0	0	0
Branching and stem structure								
Basal	0	5	0	5	0	3	0	3
Umbrella	0	21	1	22	0	17	4	21
Fascinated	7	0	0	7	5	0	1	6
Others	3	0	0	3	1	2	0	3
Plant and foliage colour								
Light green	11	0	0	11	8	1	0	9
Dark green	2	0	0	2	3	0	11	14
Violet green	0	3	17	20	0	1	20	21
Leaf								
Tiny	0	4	0	4	0	2	0	2
Narrow	7	20	1	28	4	25	0	29
Small	2	6	3	11	3	10	1	14
Large	0	4	0	4	0	0	0	0
Long lax	4	2	1	7	3	0	0	3
Flower								
Male sterile	0	3	0	3	0	0	0	0
Pink	0	3	0	3	0	0	0	0
Open type	0	3	0	3	0	0	0	0
Gynoecium slender	0	3	0	3	0	0	0	0
Flowering and maturity								
Early	2	7	1	10	7	10	4	21
Late	10	5	0	15	7	10	0	17
Not flowering	0	2	15	17	0	0	9	9
Pod and seed								
Large pod	0	4	0	4	0	3	0	3
Long pod	1	0	0	1	4	0	0	4
Variegated seed	0	0	15	15	0	0	0	0
Round seed	10	0	0	10	6	0	0	6
Bold seed	0	4	0	4	0	0	0	0
Total	90	166	85	321	87	155	92	324

among the semi-dwarfs. These were more frequent in the combined treatment of gamma irradiation followed by GA_3 similar to Dixit and Dubey (1983) in lentil.

Compacts: A simple bushy mutant was noticed in Punjab 91. This mutant had similar morphology to the dwarf plants but it had dense growth with increased secondary and tertiary branches, which resulted in a bushy appearance. This type of mutant was earlier reported in lentil (Sharma and Sharma, 1977b) and in genotype ILC 482 of chickpea (Haq *et al*., 1994).

Tall: These mutants were observed in gamma rays treatment only in Punjab 91, however, they did appeared in the other two genotypes i.e., Noor 91 and C 141 with the combined treatment of gamma irradiation and gibberellic acid. Tall mutants similar to those observed in the present study have been recorded earlier (Tripathi and Dubey, 1992; Vandana and Dubey, 1994; Haq *et al*., 1994).

Growth habit mutants: These mutants were grouped into erect, spreading, bushy and prostrate type of branching system.

Erect: These mutants were characterized by the emergence of primary branching in almost vertical direction. These mutants had more primary branches as compared to their respective controls. These mutants were recorded for all the treatments and genotypes, but they were more frequent in Punjab 91. These results are in line to those of Haq *et al*. (1994) where they also observed that the genotypes varied in their response to induce compact mutants.

Spreading: Reduced number of primary branches in combination with large number of secondary branches were the mutant characters for this group of plants. Spreading mutants were not segregated in C 141 with gamma irradiation however, they did appeared in combined treatment of gamma radiation and GA_3. These mutants were recorded with both types of treatments in Noor 91 and C 141. Similar kind of mutations were also induced with mutagenic treatments by Haq *et al*. (1994) and Vandana and Dubey (1994) in chickpea and lentil respectively.

Bushy: These mutants were characterized by having large number of primary branches with a small number of secondary branches. These branches were stiff and stout in texture. The seed yield in these mutants too was reduced. Bushy mutants were recorded earlier by Dixit and Dubey (1983), Vandana and Dubey (1994) and Haq *et al*. (1994).

Prostrate: Only a single prostrate mutant was recorded in Noor 91 with 50 Kr treatment of gamma irradiation. A number of prostrate mutants were also recorded with gamma irradiation in chickpea (Haq *et al*., 1994).

Branching and stem structure mutants: Based on branching and stem structure, the following types of mutants were observed.

Basal: Basal mutants were recorded only in Punjab 91 in treatment of gamma irradiation and also with GA_3. In these mutants all the primary branches were originated at the most basal area of the stem. Basal mutants had also been reported with gamma irradiation in one genotype of chickpea ILC 482 by Haq *et al*. (1994).

Umbrella: These mutants were characterized by the origination of primary branches at a distance in the air. Such mutants were recorded more frequently in Punjab 91 and these were not segregated in Noor 91 at all levels of treatments similar to Haq *et al*. (1994).

Fascinated: These mutants were observed in Noor 91 with treatments of gamma irradiation and a single plant was also noticed in C 141 at a dose of 60 Kr with GA_3. Such mutants were characterized by the origination of a number of peduncles from each node as against s single peduncle in case of the control. Sharma and Sharma (1977a), Dixit and Dubey (1983) in lentil and Haq *et al*. (1994) reported similar mutants chickpea.

Plant colour and other mutants: Some plants markedly different from the normal green colour and were grouped as plant colour mutants.

Light green: These otherwise normal looking plants had a light green colour and were markedly different from the normal bright green colour plants. Light green mutants were recorded in Noor 91 at all levels of gamma irradiation treatment separately and with GA_3. They were not observed in C 141 with both types of treatments. However, a single mutant for light green colour was observed at 40 Kr with GA_3 in Punjab 91. These mutants were also reported in various studies (Dixit and Dubey, 1983; Vandana and Dubey, 1994; Haq *et al*., 1994).

Dark green: These mutants were segregated in Noor 91 with gamma irradiation alone and with GA_3. In C 141, such mutants were observed only in combined treatment of gamma irradiation and GA_3. However, in Punjab 91 these mutants were not recorded. These mutants were also reported by Vandana and Dubey (1994) and Haq *et al*. (1994) in lentil and in chickpea genotype ILC 482 respectively.

Purple pigmented: A varying degree of anthocyanin pigmentation was observed in some plants. Such mutants were frequently observed in C 141 with the treatment of gamma irradiation alone and with GA_3. These were characterized by having a few numbers of primary branches and low in grain and pod setting. These mutants were not observed in Punjab 91 in both types of treatments. These mutants were earlier reported by Dixit and Dubey (1983) in lentil, but were not reported in chickpea by Haq *et al*. (1994).

Leaf mutants: Mutants showing alterations in leaf characteristics were induced. Five types of leaf mutants were found, namely tiny, narrow, small, large and long lax. It becomes evident from the data that the genotype Punjab 91 was sensitive towards leaf mutants. Tiny and large leafed mutants were only recorded in Punjab 91 and also the narrow leafed mutants were more frequent in this genotype. The small-leaf mutants characterized by either reduction in the number and size of leaflets or reduction in both of these characters. Most of the leaf mutants recovered in the present study have also been recorded in earlier studies (Dixit and Dubey, 1983; Tripathi and Dubey, 1992; Vandana and Dubey, 1994; Haq *et al*., 1994). Bipinnate leaves recorded by Haq *et al*. (1994) were not induced in this experiment.

Flowering mutants: Three mutants with modification in flower parts were recorded in Punjab 91 at a dose of 50 Kr. These mutants were characterized by having polyandrous while sterile stamens, flower open and pink, Gynoecium was malformed with protruding beak. The ovary was also protruded out of the flower. Such mutants were not recorded in other two genotypes. Double-flowered mutants and cauliflower-head inflorescence recorded by Haq *et al*. (1994) were not induced in the present study.

Maturity mutants: The number of days from sowing to first flower in variety Noor 91 ranged from 115-122; in variety Punjab 91 from 110-117 and from 115-123 in genotype C 141. Early and late flowering plants flowering outside the range in respective controls were included in their respective groups. Late flowering had not been recorded in genotype C 141. Maturity mutants have also been reported in various studies (Dixit and Dubey, 1983; Tripathi and Dubey, 1992; Vandana and Dubey, 1994; Haq *et al*., 1994).

Sterile mutants: Sterile mutants were scored in individual treatment of gamma radiation and with combination of GA_3 in variety C 141 and two mutants were recorded at the highest dose of 60 Kr individually in genotype Punjab 91. Sterile mutants were not observed in genotype Noor 91. Such mutants were recorded earlier by Dixit and Dubey (1983) Tripathi and Dubey (1992), Vandana and Dubey (1994) and Haq *et al*. (1994).

Pod mutants: Two categories of pod mutants were recorded. Three seeded long pod (instead of normal two seeded ones) were induced by 40 Kr individually and in combination with GA_3 in genotype Noor 91, while the large pods of one seeded were recorded in 40 Kr with GA_3.dose in variety Punjab 91. The pod mutants recovered in the present study have also been recorded in earlier studies (Dixit and Dubey, 1983; Tripathi and Dubey, 1992; Vandana and Dubey, 1994; Haq *et al*., 1994).

Seed mutants: Among the different seed mutants, variation in color, shape and size of the seed were noticed. A variegated seed mutant, which was recorded in genotype C 141 of M_1 generation at 40 Kr of gamma irradiation was bred true in M_2 generation. Four mutants of bold seeds were segregated in genotype Punjab 91 at a dose of 40 Kr with GA_3. Round seeded mutants were only recorded in genotype Noor 91.

There were differences in mutation spectrum between the genotypes. The relative occurrence of various mutations in Punjab 91 with both types of treatments was higher than the other two genotypes. Compact and basal plant habit, tiny and large leaf, all the flower mutants, large pod and bold seed mutants were induced in Punjab 91. Long pod and round seed mutants were induced only in Noor 91. Variegated seed mutants appeared only in C 141 genotype. The results suggested that induced mutability has governed by the genetic architecture of the material used. There were differences in mutation spectrum between the two treatments. Prostrate mutant was isolated in Noor 91, compact, large leaf and flower mutants in Punjab 91, while variegated seed mutants in C 141 only in gamma irradiation treatments. The genotypic control of mutation process as observed in the present studies has been reported in different crops (Varghese and Swaminathan, 1968; Nilan, 1964; Tyagi and Gupta, 1991; Tripathi and Dubey, 1992; Filippetti *et al.,* 1982; Haq *et al.,* 1994; Vandana and Dubey, 1994). Some mutations, such as tall, increased branching, large pod and seed size, can be used directly in developing a variety. Other mutations like erect, sterile, early maturing, long pod and various leaf mutants increase the wealth of germplasm for breeding programme. The various beneficial mutants recovered in the present study suggest that mutation breeding can reasonably be expected to play a pivotal role in the evolution of new types suited to the new needs and niches.

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