

Frequency and spectrum of induced viable macromutations in chickpea (*Cicer arietinum* L.) cultivar 'Vishwas'

J. D. Barshile

Shri Anand College, Pathardi, District - Ahmednagar - 414 102 (M.S.), India

E-mail address: jagannath_barshile@rediffmail.com

ABSTRACT

Present investigation was undertaken to study the frequency and spectrum of induced viable macromutations employing SA, EMS and gamma radiation in chickpea (*Cicer arietinum* L.) cultivar Vishwas (Phule G 5). The seeds of chickpea, cultivar Vishwas were treated with three different concentrations / doses of SA (2, 3 and 4 mM), EMS (8, 12 and 16 mM) and gamma radiations (400, 500 and 600 Gy). The mutagen administered seeds were sown in experimental fields to raise M₁ progeny. Seeds of M₁ plants and control were harvested separately and sown to raise M₂ population. The M₂ progeny were screened for viable macromutations. A wide spectrum of viable macromutations was isolated in the M₂ generation. In all twenty four different types of viable morphological macromutations were observed. These included 7 types of plant type mutations and 6 types of leaf mutations, 1 types of flower mutation, 5 types of pod mutation and 5 types of seed mutations. Results indicated that all mutagenic treatments were effective in inducing viable mutations in chickpea, during M₂ generation. Differences in response to different mutagens were observed in the spectrum and frequency of viable mutations. Some mutation types occurred more frequently than others. The frequency and spectrum of viable mutations were relatively high with EMS followed by gamma radiation and SA. In the present investigation, an attempt has been made for increasing frequency and spectrum of locally important chickpea cultivar 'Vishwas' employing chemical and physical mutagens.

Keywords: Chickpea; EMS; gamma radiation and viable macromutations

1. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a widely cultivated and important food grain legume in Indian sub-continent. It is the third most important food legume in the world. The improvement of chickpea using conventional breeding approaches has been hampered due to lack of sufficient genetic variability.

It is a valuable source of dietary protein. In spite of its nutritional importance, the yield of chickpea did not witness much appreciation during the past decade (Barshile et.al, 2006). It has been argued that one of the reasons for failure to achieve breakthrough in productivity of chickpea is lack of its genetic variability. Genetic variability is the most essential prerequisite for any successful crop improvement programme as it provides a spectrum of variants for an effective selection process (Jahagirdar et.al, 2005).

Experimental mutagenesis is an important source to produce mutation in higher frequencies in cultivated crops (Pavadai, 2010). Induced mutations are playing an important role in modern plant improvement; their efficiency and application as a technology for crop improvement and plant research in foreseen to grow in the years to come (Shu, 2009).

These mutations provide beneficial variation for practical plant breeding purpose. In present investigation an attempt has been made to induce genetic variability so as to isolate viable macromutations that could be useful in chickpea improvement employing sodium azide, ethyl methane sulphonate and gamma radiation in the locally adopted chickpea cultivar, Vishwas.

2. MATERIALS AND METHODS

Seeds of Chickpea (*Cicer arietinum* L.) cv. Vishwas (Phule G-5) were procured from the Mahatma Phule Krishi Vidyapeeth, Rahuri, India. Healthy seeds containing 10-12% water were treated separately with chemical (SA and EMS) and physical (gamma radiation) mutagens. For chemical mutagen treatments, seeds were pre soaked in distilled water for 6 hours and then subjected to the concentrations of 2, 3 and 4 mM SA and 8, 12 and 16 mM EMS for 12 hours at 25 ± 2 °C.

The treated seeds were thoroughly washed under running tap water for an hour to terminate the reaction of the chemical. For physical mutagen treatment, dry seeds were irradiated with 400, 500 and 600 Gy from a ^{60}Co source available in the Department of Biophysics, Government Institute of Science, Aurangabad (M.S., India). Each treatment was carried out for 250 seeds.

All treated seeds along with control were sown in the field at spacing of 15 cm in rows and 45 cm between rows to raise M_1 generation. For raising M_2 generation, the M_1 plants were harvested separately and seeds sown in a randomized block design (RBD) with 3 replications at experiment field of Shri Anand College, Pathardi. The M_2 progeny was screened for morphological viable macromutations. Frequency of viable mutations was scored throughout the life span of the M_2 progeny. Viable macromutations were identified and tagged for separate harvesting.

The frequency of viable mutations was calculated on the basis of total number of M_2 plants in the respective mutagen. The frequency of each mutant type was also calculated on the basis of the total number of M_2 plants screened in all the treatments. Finally, the overall frequency of viable macromutations isolated in each mutagen was calculated on the basis of the total number of M_2 plants screened.

3. RESULTS AND DISCUSSION

3.1. Frequency of viable macromutations

All treatments of mutagens were effective in inducing genetic variability and viable morphological mutations at M_2 generation, which were otherwise completely absent in the control. The frequency of viable mutations varied with dose and nature of the mutagen.

High frequency of viable mutations was observed with EMS followed by gamma radiation and SA treatments. The frequency of viable mutations ranged from 0.74 to 8.70 (Table 1). 16 mM of EMS treatment produced maximum and 2 mM SA the least frequency of viable mutations. Some mutation types occurred more frequently than others.

On the basis of total percentage of frequency of mutations (Table 1), it was observed that the chemical mutagen (EMS) was most potent in inducing viable mutations in M₂ generation than the gamma radiation and SA. Treatments of EMS induced wider spectrum of viable mutations. At 12 mM of EMS treatment mutation frequency was highest as seen in mutagenised population for plant type mutations. On the other hand SA treatments showed least spectrum and frequency for viable mutations. This could be due to differential mode of action of the mutagens on different base sequences in various genes.

The differential frequency and spectrum of viable mutations exhibited by the chickpea cultivar Vishwas in the present investigation may be due to their individual differences to the mode of action of mutagen. Kharkwal [7] have attributed the differences in frequency and spectrum of viable mutations induced by various mutagens, to genetic difference in the cultivar. Konzak et al., [9] have reported that genetic difference as small as single gene difference even could bring about significant changes not only in the spectrum but also of recoverable mutations.

Table 1. Spectrum and frequency of induced mutations in M₂ progeny of chickpea cv. Vishwas.

Treatment Conc./Dose	Spectrum of mutations (%)					Frequency of viable mutations
	M ₂ population	Leaf mutations	Plant type mutations	Pod & seed Mutations	Flower mutations	
Control	452	00	00	00	00	00
SA 2 mM	532	0.56	0.18	00	00	0.74
SA 3 mM	548	0.36	0.73	00	00	1.09
SA 4 mM	495	0.20	1.01	0.40	00	2.15
Mean		0.37	0.64	0.29	0.00	1.32
EMS 8 mM	518	1.74	2.12	0.19	0.38	4.43
EMS12 mM	573	2.44	4.53	0.69	0.17	7.83
EMS16 mM	712	3.37	4.07	0.70	0.56	8.70
Mean		2.51	3.57	0.52	0.37	6.98
GR 400 Gy	513	0.39	1.17	1.36	0.39	3.31
GR 500 Gy	535	1.86	2.05	1.12	0.19	5.23
GR 600 Gy	564	1.95	3.72	0.88	0.53	7.09
Mean		1.4	2.31	1.12	0.37	5.21

3. 2. Spectrum of viable macromutations

A wide range of viable macromutations isolated were mainly altered leaf structure, plant shape, seed size, seed colour, seed structure and days of the maturity (Figures 1-3). The

spectrum of viable mutations observed in the M₂ progeny of mutagen treated chickpea population classified into 5 major categories.

Leaf mutations - A broad spectrum consisting of six different types of leaf mutations with remarkable variation in shape, size, number and arrangement of leaflets were observed with various mutagenic treatments. The spectrum of leaf mutations observed included, curly leaf, compact leaf, gigas leaf, round leaf, small leaf and narrow leaf mutations (Fig. 1C).

i. Curly leaf mutant: These mutants had typically curly leaflets. The leaflets were wedge shaped with shorter leaf lamina and curling towards centre. This mutation was isolated with a frequency of 0.39 and 0.52 % at 4 mM SA and 8 mM EMS treatments respectively. It was also observed with a frequency of 0.37 and 0.35 % in 400 and 500 Gy gamma radiation treatments (Table 2) respectively.

ii. Compact leaf mutant: Leaflets in this type of mutant were arranged closely on the rachis. The compact mutants appeared with a frequency of 0.39 %, 0.17 and 0.28 % in 8, 12 and 16 mM concentrations of EMS respectively and with a frequency of 0.18% in 3mM SA and 3.56 % in 500 Gy gamma radiation treated M₂ populations.

iii. Gigas leaf mutant: This mutant was characterized by the largest size of leaflets arranged closely (Fig. 1C-f). Mutants having this type of mutation were vigorous in growth. This type of mutation occurred with a frequency of 0.14% in 16 mM EMS and 0.18 % in 600Gy gamma radiation treated M₂ progeny.

iv. Round leaf mutant: This mutant was characterized by the presence of round entire leaflet margins (Fig. 1 A). The leaves were dark green. This type of mutation was observed with 0.18 % frequency only in the 600Gy gamma radiation treatment. This is a late flowering mutant.

v. Small leaf mutant: This type of mutants had very small leaves and leaflets. This type of mutant was observed in all treatments with varying frequency.

vi. Narrow leaf mutant: The mutants had narrow leaflets tapering towards the tips. This type of mutation was produced only by 8 mM EMS with a frequency of 0.19 %.

Table 2. Spectrum and frequency of leaf type mutations in M₂ generation in cultivar Vishwas.

Treatment Conc./Dose	Spectrum and frequency of leaf mutations (%)						
	M ₂ population	Curly leaf	Compact Leaf	Gigas leaf	Round leaf	Small leaf	Narrow Leaf
Control	452	00	00	00	00	00	00
SA 2 mM	532	--	--	--	--	0.56	--
SA 3 mM	548	--	0.18	--	--	0.18	--
SA 4 mM	495	0.39	--	--	--	0.20	--
EMS 8 mM	518	0.52	0.39	--	--	0.58	0.19
EMS12 mM	573	--	0.17	--	--	1.05	--
EMS16 mM	712	--	0.28	0.14	--	2.10	--

GR 400 Gy	513	0.37	--	--	--	0.39	--
GR 500 Gy	535	0.35	3.56	--	--	0.93	--
GR 600 Gy	564	--	--	0.18	0.18	1.24	--

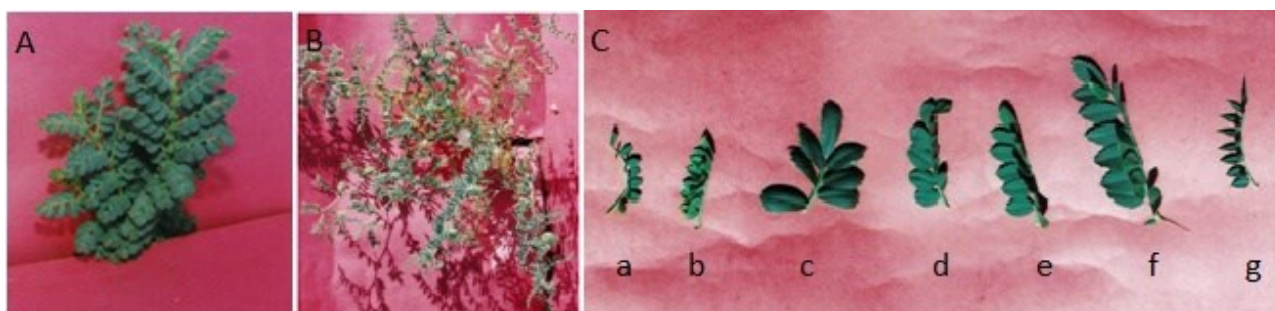


Fig. 1. A. Round leaf mutant, B. Curly leaf mutant and C. Leaf morphological mutations (a- Round leaf, b- curly leaf, c-Gigas leaf, d-Control, e- Compact leaf, f-Gigas leaf, g- Narrow leaf).

Plant type mutations - Seven different types of viable plant type mutations were observed in M_2 progeny of mutagen administered chickpea cultivar, Vishwas. These were miniature, tall, gigas, spreading, early, sterile and compact mutations. The gigas and tall mutants showed vigorous growth and possessed bold seeds.

i. Sterile mutant: This type of mutant did not bear any flowers and pods throughout its life cycle. This mutant was observed in all mutagenic treatments. EMS at 12 mM concentration produced high frequency of sterile mutants (1.57 %).

ii. Miniature mutant: The mutant was isolated from all mutagenic treatments except 2 and 3 mM of SA treated population in M_2 generation. Height of this mutant was much lesser than the control. It exhibited normal leaves, flowers and fruits (Figure 2 C).

iii. Tall mutant: Tall mutants were isolated from 12 and 16 mM EMS administered populations in M_2 progeny. This mutant was at least 20% taller than control.

iv. Gigas mutant: This mutant was tall with large thick leaves and profuse branches. The pods were large containing few bold and wrinkled seeds. This mutant was isolated from 3 mM of SA and 12 mM of EMS administered populations in M_2 progeny.

v. Spreading mutant: These mutants were isolated in all treatments except 2, 3 & 4 mM of SA and 8 mM of EMS treatments. These mutants were semi-erect and had creeping habit (Figure 2 B). The plant spread was about 2 feet. Flowering and fruiting was normal.

vi. Early mutant: This mutant was observed with a frequency of 0.19 % in 500Gy gamma radiation treatment. This flowered and matured at least 10 days earlier than the control.

vii. Compact mutant: This mutant was dwarf but branching was profuse at the base giving rise to dense appearance. These mutants were isolated from M₂ progeny raised from all treatment except 2 and 3 mM of SA.

Table 3. Spectrum and frequency of plant type mutations in M₂ generation in cultivar Vishwas.

Treatment Conc./Dose	Spectrum and frequency of plant type mutation (%)							
	M ₂ population	Sterile mutant	Miniature mutant	Tall mutant	Gigas mutant	Spreading mutant	Early mutant	Compact mutant
Control	452	00	00	00	00	00	00	00
SA 2 mM	532	0.18	--	--	--	--	--	--
SA 3 mM	548	0.18	--	--	0.54	--	--	--
SA 4 mM	495	0.61	0.20	--	--	--	--	0.20
EMS 8 mM	518	1.35	0.38	--	--	--	--	0.38
EMS12 mM	573	1.57	0.70	0.17	0.34	0.52	--	0.34
EMS16 mM	712	1.40	0.70	0.28	--	0.84	--	0.14
GR 400 Gy	513	0.39	0.19	--	-	0.19	--	0.39
GR 500 Gy	535	0.75	0.56		--	0.37	0.19	0.19
GR 600 Gy	564	0.89	1.24	--	--	0.89	--	0.71



Fig. 2. A. Early mutant, B. Spreading mutant and C. Miniature mutant (a- mutants and b-control).

Flower mutation - Open flower mutant: This mutation was isolated from the M₂ progenies of Vishwas treated with EMS (12 and 16 mM conc.) and gamma radiation (400 and 600 Gy) treatments. This mutation had bell shaped flower with open keel. Stamens and androecium of these flowers were exposed. The mutants were early in flowering.

Pod mutations - Five different pod mutants viz., gigas pod, long pod, small roundish pod, narrow elongated pod and small pod mutants (Fig. 3 A), were observed in M₂ progeny of chickpea cultivar Vishwas, treated with various concentrations / doses of the mutagens.

i. Gigas pod: It was isolated from 4 mM SA administered M₂ progeny. This mutation was associated with the gigas plant type mutation and had large pods containing 1-2 bold seeds per pod.

Table 4. Frequency of induced flower, pod and seed mutations in M₂ generation in chickpea cultivar Vishwas.

Treatment Conc./Dose	Spectrum and frequency of leaf mutations (%)			
	M ₂ population	% of flower mutation	% of pod mutation	% of seed mutation
Control	452	00	00	00
SA 2 mM	532	--	--	--
SA 3 mM	548	--	--	--
SA 4 mM	495	--	0.20	0.20
EMS 8 mM	518	--	--	0.19
EMS12 mM	573	0.17	0.17	0.34
EMS16 mM	712	0.14	0.20	0.20
GR 400 Gy	513	0.19	0.58	0.58
GR 500 Gy	535	--	0.18	0.93
GR 600 Gy	564	0.35	0.35	0.17

ii. Long pod: The plants were normal in appearance and possessed comparatively longer pods. Each pod contain 1-2 seeds. Long pod mutants were isolated from M₂ progeny raised from 400 Gy gamma radiation treatment.

iii. Small roundish pod: It was found in M₂ progeny raised form 600 Gy gamma radiation treatment. The mutant produced smaller pods that are almost round in shape.

iv. Narrow elongated pod: The mutant had comparatively elongated pods containing 1-2 small seeds per pod. It was isolated from the M₂ progeny raised from 12 mM EMS treatment.

v. Small pod: It was associated with dwarf plant type mutation. These mutants had very small pods containing 1-2 seeds per pod. This was isolated from 500 Gy gamma radiation administered M₂ progeny.

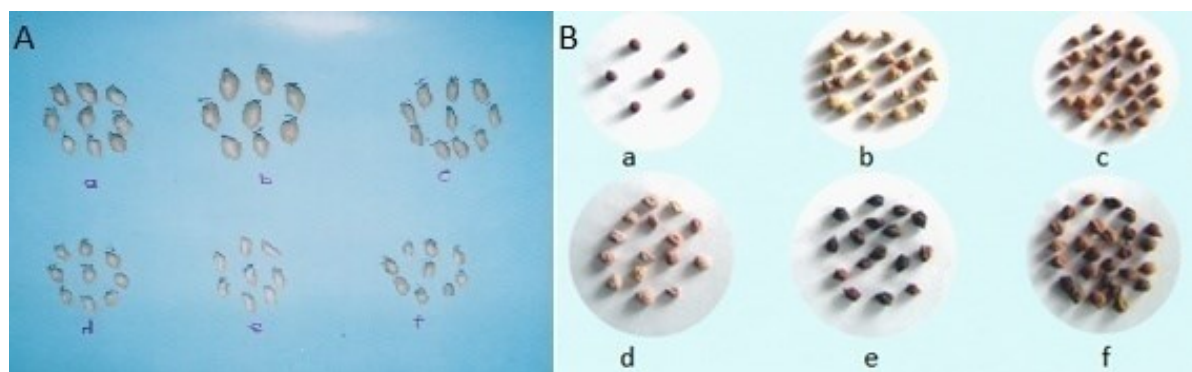


Fig. 3. A. Pod mutations (a-Control, b- Gigas pod, c- Long pod, d- Small roundish pod, e-narrow elongated pod and f- Small pod) and B. Seed mutations (a- Dark brown, b- Wrinkled bold seed, c- Control, d- Reddish wrinkled, e- black dull wrinkled and f- Bold dark brown).

Seed mutations - The spectrum of induced seed mutations was observed in the cultivar Vishwas of Chickpea, as a result of treatment with different concentration of SA, EMS and GR included dark brown, wrinkled bold, reddish wrinkled, black wrinkled seed and bold dark brown seed mutations (Figure 3B). All these mutations appeared more frequently with gamma rays.

- i. **Dark brown seed mutant:** This mutant was characterized by small, round and dark brown seeds. The 100-seed weight of these mutants was less as compared to control.
- ii. **Wrinkled bold seed mutant:** It was isolated from in 4 mM SA treated progeny. The mutant seed coat had wrinkled structures while parental seed coat was smooth (Figure 3B-b).
- iii. **Reddish wrinkled seed mutant:** This type of mutant was characterized by reddish brown seed coat. The 100-seed weight did not show any variation as compared to control. It was isolated from 8 mM EMS administered M_2 progeny.
- iv. **Black dull wrinkled seed mutant:** This mutant was isolated from M_2 populations of 500 Gy gamma radiation administered progeny. Seeds of this mutant were very much similar in appearance to those of control but the seed coats were wrinkled in texture.
- v. **Bold dark brown seed mutant:** This type of mutant was characterized by the presence of dark coloured seed coats. It was isolated from 600Gy gamma rays administered M_2 progeny.

All the mutagens were effective in inducing genetic variability in size, shape and colour of leaves, flowers, pods and seeds in chickpea. Among the 9 different treatments 16 mM EMS produced wider spectra and higher frequency of mutations. The results thus indicate EMS is a highly potent mutagen in inducing high frequency and wider spectra of viable mutations in chickpea cultivar Vishwas. The differences in frequency and spectrum of viable mutations, induced by various mutagens, in chickpea, as observed in the present investigation may be due to differential mode of action of the mutagens on different base sequences in various genes. Several authors have attributed the differences in frequency and spectrum of viable mutations, induced by various mutagens, to genetic difference in the cultivars of chickpea by Kharkwal (2001) and in Sorghum by Reddy and Smith [11]. Konzak *et al.*, [9] have reported that genetic difference as small as single gene difference even could bring about significant changes not only in the spectrum but also of recoverable mutations. Differences in the mutation frequency and spectrum will depend on the interaction of three factors; mutagen,

plant genotype, and physiological state of the organism at the moment of treatment. The variation in the mutation frequency, within and between the treatments noticed in the present study may be due to the number of genes involved in the mutational process.

Several researchers have reported different morphological mutants in chickpea, similar to those observed in the present investigation. These include, Gigas and tall mutants by Kharkwal [8], Wani and Anis [14], Kharkwal [6], Khan *et. al.*, [5], Ahmad and Godward [1], simple leaf, narrow leaflets and smooth leaflet edges by Toker and Cagirgan [13], two-tier flower and open flower mutations by Kharkwal [8], gigas, long pod, large pod, narrow and elongated pod, small pod and double podded mutations by Kharkwal [7], variations in seed coat colour by Bhamburkar [3]. Viable mutations, observed in the present study, might have arisen as a result of mutations in the genes that control the ontogeny of leaves, flowers, pods or seeds. Seed shape, size and coat colour seems to be under the control of several genes (polygenes). Disruption of any one of the genes might manifest in the form of seed mutations. Various mutations obtained in the present investigation may be of immense value in understanding the genetic control of organ formation and regulation of their size, shape and form. The induced flower mutations can be exploited as genetic markers in different breeding experiments. Some of the viable mutations obtained in the present investigation can be directly used in selection while others can be used as initial material in breeding programmes.

4. CONCLUSION

The spectrum of mutations produced by a mutagen depends on the nature of the mutagen employed. The frequency and spectrum of induced mutations in the cultivar Vishwas of chickpea is quite broad by EMS as compared to gamma radiation and SA. It can be concluded that the frequency of morphological mutations is directly proportional to the conc./dose of mutagen at its LD₅₀ in M₁ corresponds to the frequency of viable mutations in M₂ generation.

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