

FREQUENCY AND SPECTRUM OF MORPHOLOGICAL MUTANTS INDUCED BY GAMMA RAYS AND EMS IN M₂ GENERATION OF CHICK PEA

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Abstract	<i>The present study was undertaken in order to find out the frequency and spectrum of morphological mutants induced by gamma rays and EMS in Chick pea. The Co – 4 variety of chick pea were treated with different doses/concentration of Gamma rays and EMS to create genetic variability. The M₂ progenies of these treatments were raised from M₁ seeds. Various morphological mutations were carefully screened from M₂ generation. The result revealed a wide spectrum of morphological mutations which altered the plant height, growth habit, leaf architecture, flower characteristics, pod and seed. Of all the morphological mutation, the highest frequency is observed for flower mutation followed by seed mutation. The spectrum of morphological mutation induced by EMS was comparatively wider than that of gamma rays. It indicates, the induced variability is governed by the genotype of the material used and the mutagenic specificity for different traits.</i>
Keywords	<i>Chick pea, Mutation, Morphological mutants</i>

INTRODUCTION

Legume crops hold an important place in Indian agricultural system because of their high nutritional content. And they play an important role especially in India, where majority of the population following vegetarian diet (Wani et al., 2011). The pulses occupy an area of 10.84 million hectares with an annual production of 18.24 million tonnes with an average yield of 694 kg/ha. In India, it was grown over an area of 8.31 million hectares with the production of 7.58 million tonnes with an average yield of 912kg/ha. The current scenario of annual production of chick pea seems to vary low and has remained stagnant for the past year, it might be due to the lack of genetic variability. The rapid enhancement in chick production can be accomplished by fully exploiting the yield potential of existing varieties through crop improvement techniques.

The genetic improvement in chick pea has largely been attempted through conventional breeding methods. But the conventional breeding has its own limitations like less genetic variability due to self-pollination. Induced mutations have been recognised as an important tool for crop improvement and are believed to have sufficient scope in pulses. Mutagen – induced quantitative variation not only serves as an alternative source of Germplasm for natural variation and it is also useful in generating appropriate linked gene complexes which are responsible for improvement in yield and other characters of economic interest. The main advantage of mutation breeding is the potential to improve 1 or 2 characters without changing the rest of the genotype. 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 (Khan et al., 2011). In view of the above considerations, an attempt has been made to induce alterations in the genotype to enhance the genetic variability so as to isolate morphological mutants that could lead to improvement in yield and other economically important traits through the use of physical and chemical mutagens in chick pea.

MATERIALS AND METHODS

SEED: The CO- 4 variety of chick pea obtained from Tamil Nadu Agricultural University,

Coimbatore and Tamil Nadu were used for the study.

TREATMENT: Two types of mutagens namely; EMS (10mM to 50mM) and Gamma rays (20kR to 60kR) at different concentrations were used. A set of 50 seeds with three replication were used for each treatment and control in order to raise the further generations. The progenies of M₁ generation were used to raise the M₂ generation and successively up to M₄ generation were done. The mutations which alter the morphological traits were observed and recorded in all the generation studied. All the recommended cultural practices were carried out during the cultivation of crop

RESULT

A wide range of morphological mutants were isolated from the M₂ population. These mutants involved traits affecting plant height, growth habit, leaf, flower, pod and seed. Mutation frequency was calculated on M₂ plant basis. The various mutants observed and isolated in M₂ generation were tabulated in Table.1. The frequency and spectrum of morphological mutation was given in Table.2. The spectrum of mutation induced by EMS was comparatively wider than that of gamma rays. Mutation affecting flower and seed appeared more frequently followed by plant height, growth habit, leaf and pod. Among the different dose/conc. of mutagenic treatments, 30mM of EMS followed by 40kR gamma rays were found to be more effective for inducing morphological mutation.

Table 1: Frequency (Per cent M₂ Plants) and Spectrum of morphological mutants induced by gammarays and EMS in M₂ generation of Chick pea

Mutant Type	Gamma Rays				Ems				Total Frequency (%)
	30kR	40kR	50kR	Frequency (%)	20mM	30mM	40mM	Frequency (%)	
PLANT HEIGHT									
	4	8	1	0.31	3	10	-	0.30	0.30
Dwarf mutant	1	-	2	0.07	-	2	9	0.25	0.16
Spreading mutant	-	4	1	0.11	1	5	-	0.14	0.13
GROWTH HABIT									
Bushy	-	2	1	0.07	-	4	2	0.14	0.10
Axillary branched	7	6	-	0.51	5	3	-	0.30	0.40
One side branching	-	-	3	0.11	2	3	2	0.16	0.11
Secondary branched	-	-	3	0.11	-	5	-	0.11	0.09
Dichasial branching	1	-	-	0.03	-	1	-	0.03	0.03
LEAF									
	1	6	1	0.19	1	3	2	0.23	0.16
Narrow leaf	1	4	1	0.19	2	5	1	0.18	0.16
Altered Leaf architecture mutant	1	3	2	0.19	1	2	4	0.26	0.15
Bipinnate compound leaf	2	2	2	0.19	-	2	4	0.15	0.14
FLOWER									
Double flower colour	2	6	3	0.26	3	8	1	0.28	0.27
White flower	-	-	-	-	-	14	-	0.33	0.16
Violet flower	-	11	1	0.28	-	-	-	-	0.14
Open flower	-	2	3	0.11	1	4	3	0.18	0.15
Cluster of flower	1	1	-	0.04	-	2	-	0.04	0.04
Flower with two standard petal	2	4	-	0.14	-	1	-	0.03	0.08
8 petalled flower	1	2	-	0.07	-	2	-	0.07	0.05
Presence of staminode	-	-	-	-	-	-	1	0.03	0.01
Sterile mutant	-	-	1	0.03	-	-	3	0.11	0.07
Vegetative mutant	-	-	2	0.07	-	2	4	0.14	0.09
Early flowering	2	6	2	0.23	4	8	2	0.33	0.28
Late flowering	2	-	4	0.14	2	-	5	0.16	0.15
POD									
Bold pod mutants	1	3	1	0.19	2	5	2	0.21	0.16
Small pods	1	3	3	0.15	1	2	1	0.19	0.17

SEED									
Small sized seeds	1	1	1	0.12	2	5	2	0.34	0.23
Bold sized seeds	2	6	1	0.21	3	8	1	0.28	0.24
Shrunken seeds	2	1	-	0.11	1	2	4	0.26	0.19
Seed colour mutant	3	9	4	0.38	2	10	3	0.35	0.36
Total frequency	4.50	10.41	5.39	4.09	4.03	12.11	6.78	4.71	4.40

in M₂ generation of Chick pea at different stages of growth

Mutagens	Treatments	N	Morphological mutant type						TMP	% of mutated plants
			Plant height	Growth habit	Leaf	Flower	Pod	Seed		
Control	-	854	-	-	-	-	-	-	-	-
Gamma rays	30kR	864	5	8	5	10	2	8	38	04.50
	40kR	885	8	12	15	32	6	10	90	10.41
	50kR	797	3	8	6	16	4	18	43	05.39
EMS	20mM	868	3	8	4	10	3	8	35	04.03
	30mM	900	12	21	12	41	7	25	109	12.11
	40mM	825	9	4	11	19	3	10	56	06.78

MUTANTS WITH ALTERED PLANT HEIGHT AND GROWTH HABIT

A brief description of the mutants with altered plant height and growth habit was described (Plate I – III).

Tall mutants: These mutants were observed at lower dose/conc. of gamma rays and EMS. These mutants were considerably taller than the control plant, their height being 42 – 45.7 cm in gamma rays and 44 – 47cm in EMS, where it was 40 – 42cm for the control plants. The number of branches was very less and the pods and pod setting was comparatively high. They occupied about 0.30% frequencies of the total morphological mutants.

Dwarf mutants: These mutants were observed at higher dose/conc. of gamma rays and EMS. The height of this mutant was ranged from 20 to 22cm. The mutant was characterized by short internodes. Almost all the yield components were reduced when compared to control. Frequency of this mutant was 0.16 % of the total morphological mutants.

Bushy mutants: This mutant was isolated from highest dose/conc. of gamma rays and EMS treatments. These mutants showed a considerable decrease in plant height with large number of primary and secondary branches possess' short internodes. The number of secondary branches was found to be higher when compared to primary branches. These mutants were characterized by increased number of pods per plants but most of them possess' empty pods. Frequency of these mutants was 0.10% of the total morphological mutation.

Spreading mutants: These mutants were isolated from intermediate dose/conc. of both gamma rays and EMS. These mutants had long internodes and large canopy. The leaves were mostly broad type and the yield was reduced. Their frequency was 0.13% of the total morphological mutations.

MUTANTS WITH ALTERED GROWTH HABIT

A brief description of the mutants with altered growth habit were described below.

Axillary branched mutants: These mutants were characterized by profuse number of axillary branches, short internodes small pods with small seeds and low seed setting. They appeared at a frequency of 0.40 % of the total morphological mutations.

One Sided branching mutants: In this mutant, all the branches were produced on one side of the stem. The height of the plant was 29.5cm. These mutants were characterized by late flowering, partially sterile and low pod setting. The seeds were shrinkled, small in size and most of them are viable. Frequency of these mutants was almost 0.11 % of the total morphological mutations.

Dichasial branched mutants: These mutants were characterized by dichasial primary

branches, reduced plant height, long internodes, late flowering with poor pod setting. They appeared at a frequency 0.03 % of the total morphological mutations.

Secondary branched mutants: These mutants were characterized by a profuse number of secondary branches. The mutant possesses short internodes and small sized leaves. Due to the enormous number of secondary branches, the further growth and development were disturbed. The plants remain as vegetative/non- flowering until maturity and become stunted. They appeared at a frequency of 0.09% of the total morphological mutations.

MORPHOLOGICAL MUTANTS (MUTANTS WITH ALTERED LEAF STRUCTURE)

A brief description of the mutants with altered leaf architecture was described below.

Small sized leaf mutants: Both length and width of the leaflets and length of rachis were markedly reduced in small sized leaf mutants. This small leaf mutant was observed in most of all the dose/conc. mutagenic treatments. The flower size was not reduced where as in small leaf mutants generally reduction in plant height and seed size has been observed. They appeared at a frequency of 0.16 % of the total morphological mutations.

Narrow leaf Mutants: The length of the leaflets in these mutants was normal or in some cases slightly increased, but the width of leaflet was very much reduced. The mutants were dwarfed and bearing large number of branches. The frequency of such mutant was 0.16% of the total morphological mutations.

Bipinnate compound leaf Mutants: In this mutant, each leaflet of a normal pinnate compound leaf was again modified in to a compound leaf bearing tiny leaflets. These mutants were observed at a frequency of 0.14 % of the total morphological mutations.

Altered leaf architecture Mutants: These mutants were characterized by vast variation in the leaf margins and apices. The mutants were never bearing any flower throughout the life cycle. These mutants were isolated in all most all the EMS treatments. Their frequency was 0.15% of the total morphological mutations.

Plate I: MUTANTS WITH ALTERED LEAF MORPHOLOGY



Control Small sized leaf (40kR) Narrow leaf (30mM) Bipinnate compound leaf (50kR)



Altered leaf architecture (40mM)

MORPHOLOGICAL MUTANTS (MUTANTS WITH ALTERED FLOWER CHARACTERS)

A brief description of the mutants with altered flower characters was described below. About eleven mutants affecting color, structure and flowering time were observed. They are,

Flower color mutants: White coloured flower mutants were noticed in comparison to pink coloured flower at 30mM of EMS treatments. The frequency of the white coloured flower mutant was 0.16% of the total morphological mutants.

Violet coloured flower mutants were found in 40kR of gamma rays treatments. The frequency of a violet-coloured flower mutant was higher compared to white coloured flower mutant. The frequency of this mutant was 0.14 % of the total morphological mutations. The flower coloured mutants don't have any significant role on yield attributes.

Double coloured flower mutants: These mutants were characterized by the presence of two different coloured flowers on the same plant as against one in control. The pods and seeds were smaller in size. Most of the pods were single seeded. The plants were normal in growth but late in flowering with a large number of flowers. The frequency of such mutant was 0.27% of the total morphological mutations.

Open flower mutants: The mutants possessed bell shaped flower with open keel and wing petals. The androecium and gynoecium were exposed. Flowers withered without fruiting. They appeared at 20mM and 30mM of EMS treatment and 40kR of gamma rays. The frequency was 0.15% of the total morphological mutants.

Cluster of flowers mutants: These mutants were characterized by the presence of two to three flowers born on the same stalk as against one in control. The flowers were withered without fruiting. Their frequency was 0.04% of the total morphological mutations.

Flower with two standard petal mutants: In this mutant, the flower was characterized by white in color, open with two standard petals. This flower is comprised of two standard petals instead of one standard petal as in control along with two wing petals and two keel petals. This mutant accounted for 0.08% of the total morphological mutations.

Eight petalled flower mutants: These mutants bear white open flower with one standard petal, five wing petals and two keel petals. The winged petals are similar in size. This mutant is characterized by large sized pod with small sized seeds. Highest percentage of empty pods was also observed in these mutants. Frequency of this mutant was 0.05% of the total morphological mutations.

Presence of Staminode mutants: Diadelphous [(9) + 1] condition of a stamen is a characteristic feature of the family leguminaceae; that is, 9 stamens with their filaments fused forming an androecial sheath and the 10th stamen completely free. In this mutant, the 10th stamen seems to in a petaloid structure. Their frequency was 0.01 per cent of the total morphological mutations.

Non – flowering/Vegetative mutants: This mutant does not bear any flowers and continued to grow vegetative until the maturity, by the time plants were setting seeds. These mutants are characterized by normal plant height, profusely branched and dark foliage. These mutants were noticed at higher doses/conc. of both EMS and gamma rays. Frequency of these mutants was 0.9% of the total morphological mutations.

Sterile mutants: This mutants bears large number of flowers but withered without fruiting. Higher percentage of pollen sterility was observed in this mutant. Frequency of these mutants was 0.07% of the total morphological mutations.

II: MUTANTS WITH ALTERED FLOWER CHARACTERS



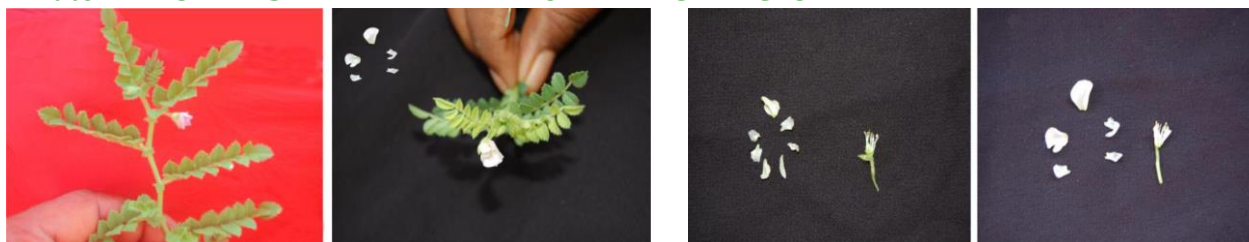
Control flower White flower (30mM)



Open Flower Mutant(30mM)

Cluster of Mutant(40mM)

Plate III: MUTANTS WITH ALTERED FLOWER ARCHITECTURE



Control flower Two standard petals (40kR)

Eight petals (30kR)

Staminode (40kR)



Sterile mutant (50kR)

Vegetative mutant (40mM)

Early flowering mutants: In these mutants, the day taken for first flowering was reduced up to 5 – 10 days ahead of their respective control. Early flowering mutant were leads to early maturity of the plant and these mutant was observed in 40kR of gamma rays and 30mM of EMS treatment. Frequency of such mutant was 0.28% of the total morphological mutations.

Late flowering mutants: In these mutants, the days to first flowering was observed 8 – 10 days later than the control and growth period was extended by a similar number of days. These mutants were isolated at the higher concentrations of EMS. Frequency of such mutant was 0.15% of the total morphological mutation.

MUTANTS ALTERED WITH POD CHARACTERS

A brief description of the mutant with altered pod characters and seed characters were described below. The mutants affecting size, shape and colour of the pod and seeds were observed

Small pod mutants: These mutants were mostly found at the higher concentration of EMS treatments. Some of the pods having small sized two seeds and some have been moderately big sized single seeded pod. In these mutants, yield per plant was lower than the control. Frequency of these mutants was 0.17 % of the total morphological mutations.

Bold pod mutants: The mutant plants exhibited vigorous growth and large seeded pods. As a result of bold pods, a significant increase in yield was recorded. These mutants were observed in 40kR of gamma rays and 30mM of EMS treatments. Frequency of such mutants was 0.16% of the total morphological mutations.

Bold sized seed mutants: In the present study, many large – seeded mutants were isolated from lower dose/conc. of mutagenic treatments. Bold sized seed mutants fetches high price in the market. They are characterized by an enormous increase in the yield per plant and high seed weight as compared to control. The frequency of such mutant was 0.24 % of the total

morphological mutations.

Small sized seed mutants: This mutant was characterized by small pods with two seeds per pod, and seeds were smaller in size. The yield and hundred seed weight was reduced when compared to control. These mutants accounted for 0.23 % of the total morphological mutations.

Seed shape mutants: At highest dose/conc. of the gamma rays and the EMS, seeds with shrunken surface were observed. These mutants were characterized by low yield and seed weight. These mutants accounted for 0.11% of the total morphological mutations.

Seed color mutants: These mutants were observed in almost all the doses/conc. of mutagenic treatments. In these mutants, the plant exhibited different coloured seeds, which are dark brown, yellowish green, brown, ash coloured etc. Frequency of these mutants was 0.36% of the morphological mutations.

DISCUSSION

In the present study, the viable mutations affecting plant characteristics were isolated based on the screening from M_2 plants. For the isolation of induced mutants, M_1 plants are advanced as M_2 generation because about 99% of all mutants are due to recessive mutations; therefore, the mutant's genes are not discernible in M_2 plants. The selection of mutants can begin only in the M_2 generation. These mutants were differed from control plant, especially in height, growth habit, leaf architecture, pod size and their flowering habits. Some of the morphological mutants were considered as uneconomical, some mutants, nevertheless this could be used as a basis in many useful genes for the improvement of some desired quantitative traits, or it can be used in crossing programmes (Wani et al., 2011) in determining the evaluation of the crops (Khan et al., 2011). Appearance of mutants in plant is controlled by many factors; some of the most important biological factors include the mode of reproduction, number of primordial cells involved in the origin of inflorescence, genetic architecture of the organism (diploid and polyploidy) and characteristics of the locus involved in the mutation process.

Tall mutant exhibited a significant increase in the height with decreased in the number of pods and yield per plant as compared to control. Tallness is generally an undesired trait, because the stability of stem is negatively influenced, therefore, tall mutants are only in exceptional cases of agronomic interest. Tall mutants, as observed in this study were also reported earlier in lentil and black gram (Tocker, 2009; Kumar et al., 2009).

Leaf mutants appeared in a large number in both gamma rays, and EMS showed changes in the length of rachis and leaflet width, number etc. In chick pea, the possibility for drastic crop improvement may be based on an increased plant density, and in these respect mutants with modified leaves can be utilized in breeding programs in order to reduce the canopy. Both the length and the width of the leaflets and length of rachis were markedly reduced in small leaf mutants. Bipinnate compound leafed mutant was due to the formation of compound leaf on a normal pinnate compound leaf. This shows that a single pair of recessive genes is responsible for this mutation. Rao and Jena (1976) reported that a single recessive gene is controlling appearance of Bipinnate leaves in a chick pea mutant. The higher concentrations of EMS induce more altered leaf architecture mutants. And the narrow leaf was characterized by reduced leaflet widths.

Many flower mutants were identified by in the majority of these cases the genetically conditioned flower anomaly resulted in sterility or a drastic reduction on fertility of the plants. Most cultivars of chick pea produced single coloured flowers but some produced distinctive coloured flower in same plant. The mutants with different flower colour can be exploited as genetic markers in breeding experiments (Rao & Jena, 1976; Atta, 2003). The mutant with different flower color was also reported earlier (Datta & Sengupta, 2002; Chowdhury et al.,

2009). The chick pea has characteristic cleistogamous flowers, which ensure the self – pollination. Though cleistogamy in chick pea is useful traits for maintaining genetic purity of cultivars, it possesses challenges in development of hybrids. In normal chick pea flowers, the reproductive organs (androecium and gynoecium) were enclosed by the keel petals which prevent the plant from cross pollination. The flowers which that exposes both male and female reproductive organs would make possible of cross pollination. Such an open flower mutant has been reported in desi chick pea (Haware et al., 1992).

The corolla in normal chick pea flower consists of one standard petal with two wing petal and two keel petals. The two keel petals enclose reproductive organs until their maturity, which ensures self – pollination. However, in the present investigation flowers with two standard petal, 8 – petalled flower and cluster of flowers were also observed after mutagenic treatments. The normal development of all the floral whorls facilitates the Sexual reproduction in an angiosperm. The abnormal development of any floral whorls disturbs the entire parts may lead to sterility (Dahiya et al., 1984). The abnormalities in floral structure were early reported by Padmavathi (2005) in *Physalis*.

A number of early maturing mutants were observed at various mutagen treatments, and such mutants were characterized by earliness in maturity with normal seed yield. The sterile plants appeared normal in every respect until flowering and produced a slightly open flower in which anthers are visible. Reddy *et al.* (1978) reported a genetic male sterile in pigeon pea identifiable by its translucent anthers, which allowed an easy and early detection of male – sterile plants. Brim and Yong (1971) reported that male sterile character in soybean was inherited as a single recessive gene pair in which pollen in viability was about 99%.

The seed mutants shows variation in size, shape and the color of seeds. Similar seed shape and sized mutants were also reported by in pigeon pea (Reddy et al., 1978), cow pea (Brim & Young, 1971) and in ground nut (Shinde, 2017). The shapes of the seed, size of the seed and the coat color were controlled by polygenes. Disruption of any one of these genes result mutations in seed. The small-sized mutants were characterized by small pods with two smaller seeds. The mutant with bold seeds is considered as a desirable variation and can be exploited a significant improvement in the number of seeds and the seed size leading to increased genetic potential of the yield. Singh (1996) characterized bold seeded mutants in *Vigna* as gene mutation because there is no visible chromosomal change was observed. Appearance of large-grained *Pisum* mutants was reported by Gottschalk(1982) and Sidorova et al, (1969). The large pod character may prove extremely useful if the size of the seed is increased.

CONCLUSION

Many morphological mutants isolated in this study, could not be able to use directly for commercial cultivation due to the presence of some unwanted traits. Though, it is not possible to eradicate the negative characters of the pleiotropic spectrum from the positive ones will alter the pattern of pleiotropic mutant gene up to a level by transferring it into a specific genotypic background.

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