ABSTRACT

*Vicia faba* is one of the oldest fodder crop known by man and used as a source of protein and carbohydrate for both human and the animals. Attempts to popularise faba bean as a pulse crop for the plains of India, have met with limited success because of lack of variability in the available germplasm. Moreover, the material is easily available, easy to grow and handle, which make it suitable for the use all over the world.

Realizing the importance of *Vicia faba*, the present work deals with the effect of gamma rays, $\gamma + SA$, $\gamma +$ caffeine, SA and aniline on seed germination, seedling and plant growth, morphology, pollen fertility, meiotic abnormalities and yield in $M_1$, $M_2$ and $M_3$ generations. Few selected mutants have been maintained in $M_3$ generation. The findings are abstracted below:

1. Seed germination and pollen fertility showed a dose dependent decrease with gamma rays, SA and aniline, while in five combination treatments ($10KR + 0.25\%$ and $20KR + 0.25\% \gamma + SA$ and $10KR + 0.25\%; \ 20KR + 0.25\%$ and $30KR + 0.25\% \gamma +$ caffeine) they showed slight increase in mean values as compared to their previous lower concentrations, giving random trend in combination treatments. Same trend was followed, in these doses in $M_2$ generation also, but the germination percentage and pollen fertility were comparatively higher than that in $M_1$ generation.
Combination treatments showed synergistic (additive) effect and induced more reduction than individual treatments. Gamma rays induced more reduction in higher doses as compared to SA and aniline.

2. Frequency of variations increased with increasing doses/concentrations in gamma rays, SA and aniline, but in above mentioned five combination treatments the frequency was slightly lesser than their lower concentrations. The mutants in $M_2$ were selected on the basis of selfing the variants of $M_1$ and maintaining them in $M_2$. The frequency of mutations was generally lower in $M_2$ and $M_3$ generations, than the variants obtained in $M_1$ generation.

3. Average height of mature plants decreased with increasing doses/concentrations in gamma rays, SA and aniline. Combination treatments showed additive effect and induced more reduction in height as compared to individual treatments. The maximum reduction in height was obtained in $\gamma + SA$ followed by $\gamma +$ caffeine, SA, gamma rays and aniline. The average height of plants decreased in $M_2$ generation also, but generally the height of plants was higher than $M_1$. Moreover, some mutants obtained as a result of segregation in $M_2$ and $M_3$ were taller than control plants, as well as general treated populations.

4. The cotyledonary and vegetative leaves of treated plants exhibited abnormalities like notching, lobe formation, rudimentary, poor development, fusion of both margins of leaflets to form cup shape structure, unequal, changes in margin and apex and increase in thickness.
in M₁ generation. These abnormalities were common in all the mutagens, with the difference in their frequencies only. In aniline, the leaflets were mostly obovate, smaller, thicker, entire and round. Almost similar abnormalities were observed in M₂ and M₃ generations also, but the frequencies were lower.

5. Older seedlings also exhibited morphological variations such as varied leaf shape and size, semi erect plants with lesser number of branches and leaves, thick, hard and woody stem, healthy seedlings with increased number of branches, delayed germination followed by retarded growth and rudimentary seedlings bearing undifferentiated branches and leaves. In M₂ generation, the variations in leaf morphology like unequal, deep notching, three lobes, deshaping, fusion and narrowing were also observed.

6. In mature plants, the morphological mutants like dwarf mutants with small narrow leaves, dwarf mutants with decreased number of branches, taller bushy plants with increased or decreased number of branches and leaflets, mutants with increased or decreased number of flowers, erect and semi erect habits, higher or lower yield than control, were obtained in various treatments of M₂ and M₃ generations.

7. Various micromutational characters such as leaf ratio, average number of leaves, branches, pods, seeds and weight of seeds on per plant basis as well as on the basis of weight of 100 seeds were isolated and studied
in M₁, M₂ and M₃ generations. Except leaf ratio, which was random and independent of doses, all other characters decreased with increasing doses/concentrations of gamma rays, SA and aniline. Combination treatments showed random effect similar to seed germination and pollen fertility. The yield was maximum in aniline followed by SA, gamma rays, γ + caffeine and γ + SA. Generally the yield was higher in M₂ and M₃ generations than M₁ due to the ceasing toxic effect of mutagens.

8. A wide spectrum of chlorophyll mutants (Albina, Xantha. Viridis. Xanthoviridis, viridoxantha and striata) were obtained in M₂ generation. The frequency of chlorophyll mutations was dose dependent and increased with the increasing doses/concentrations of mutagens. γ + SA treatments induced the highest frequency of chlorophyll mutations followed by γ + caffeine, γ rays. SA and aniline.

9. The mutagenic effectiveness was higher at lower doses of the mutagens. The order of mutagens based on effectiveness was γ rays > SA > aniline > γ + SA > γ + caffeine, γ-rays being the most effective. The mutagenic efficiency worked out on the basis of seedling injury (MP/I) and pollen sterility (MP/S), also showed a declining trend with increasing concentrations of gamma rays. SA and aniline, while in combination treatments it showed random effect. The order of mutagenic efficiency was γ + SA > γ + caffeine > SA > aniline > γ-rays.

10. The effect of mutagens on meiotic chromosomes was studied in detail. The chiasma frequency (per cell and per bivalent) generally decreased
with the increasing doses/concentrations of mutagens at diakinesis and metaphase I stages, while in combination treatments (10KR + 0.25% and 20KR + 0.25% γ + SA and 10KR + 0.25%, 20KR + 0.25% and 30KR + 0.25% γ + caffeine), there was increasing trend as compared to their lower concentrations. The maximum adverse effect on chiasma frequency was observed in γ + SA, followed by γ + caffeine, γ rays. SA and aniline treatments. The same pattern was followed in M₂ generation also.

11. Multivalent associations and stickiness were observed at diakinesis and metaphase I stages. Their frequencies increased with increasing doses/concentrations of mutagens. Multivalents and stickiness were also observed in M₂ and M₃ generations but their frequencies were comparatively lower than that of M₁ generation.

12. The univalents were observed at metaphase I stage but their frequencies were very low. However, they increased with increasing doses/concentrations of mutagens. The univalents were not found in M₂ and M₃ generations.

13. Precocious separation of chromosomes was observed at metaphase I stage. Their frequency increased with increasing doses/concentrations of all the mutagens and the increase was significant at 1% level in higher doses/concentrations of all mutagens. Their frequency decreased in M₂ and M₃ generation.

14. At anaphase I, the abnormalities like laggards, bridges, unsynchronized
movement of chromosomes and unequal separation, were mainly observed. The maximum frequency of these abnormalities were observed in $\gamma$ + SA followed by $\gamma$ + caffeine, $\gamma$ rays, SA and aniline. In $M_2$ and $M_3$ generations, same abnormalities were observed, but their frequencies were lesser than $M_1$.

15. At telophase I, the main abnormalities were laggards and bridges. Their frequencies increased with increasing doses/concentrations of mutagens. The laggards were more common in $\gamma$ + SA, $\gamma$ rays and SA and the bridges in $\gamma$ + caffeine and aniline. In $M_2$ and $M_3$ generations they were lesser than $M_1$.

16. Abnormalities at second meiotic stages were lesser in treated populations in $M_1$ and $M_2$ generations. However, some of the abnormalities recorded were laggards and micronuclei at prophase II, fragments and precocious separation at metaphase II, laggards, bridges and disoriented chromosomes at anaphase II and multinucleate microspore mother cells, laggards and bridges at telophase II and these were significant in higher doses/concentrations of almost all mutagens. The frequency of these abnormalities at second meiotic stages were maximum in $\gamma$ + SA followed by $\gamma$ + caffeine, $\gamma$ rays, SA and aniline. In $M_2$ generation, the frequencies of above mentioned abnormalities were lesser than $M_1$, while in $M_3$ no such abnormalities were present in second meiotic stages. The possible cytogenetic reasons behind the variations have been discussed in detail.

17. The positive and negative mutants have been isolated in $M_3$. They showed
various morphological characters almost similar to that of $M_2$ such as
tall, dwarf, bushy, less branching, high or low yielding etc. which were
further stabilized in $M_1$ generation.

It has been concluded that the morphological and cytological variations
observed in the present investigation may be due to physiological disturbances
in metabolic activities or growth regulators (auxins and cytokinins) and the
genetic disturbances like various chromosomal associations and disturbed
spindle formation as a result of the action of different mutagens used. In the
condition of undetectable changes at chromosome level, the changes at genic
level (cryptic structural changes) have been attributed as the reasons for
phenotypic changes. Combination treatments ($\gamma + SA$ and $\gamma +$ caffeine) showed
synergistic effects and caused deleterious effects more than single mutagen
treatments (i.e. gamma rays, SA and aniline), except some random effect in
intermediary doses of $\gamma + SA$ and $\gamma +$ caffeine. Morphological variations were
higher and mostly adapted in $M_1$ generation, while in $M_2$ the mutants were either
chromosomal or genic and moreover recessive, because in aniline the frequency
of chromosomal abnormalities as compared to other mutagens was lower while
morphological mutations were significant. The mutation rates in lower doses
were higher since less damaging doses were more effective and efficient.
Chromosomal abnormalities were also dose/concentration dependent and more
or less responsible for morphological variations. The mutations were more or
less similar in all the mutagens with only differences in their frequencies and
slight specification in $\gamma + SA$ and $\gamma +$ caffeine, wherein it showed random
increasing trend in two and three concentrations of the mutagens respectively. 
It can be presumed that wherever enzymes are involved there must be the 
involvement of genes, as the genes are expressed in the forms of proteins and 
enzymes. If there is any alteration at genic or base level the mutation is found 
to occur.

Moreover, the induced cytomorphological variabilities in the present 
investigation provided greater chances of selection for different desirable 
characters in *Vicia faba* L.