CHAPTER 7
SUMMARY
AND
CONCLUSION
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The Cowpea \(Vigna unguiculata\) (L.) Walp. \(2n=22\) belongs to family Fabaceae. It is protein rich leguminous crop mainly cultivated in semi-arid regions of India. The importance of legume has been highly appreciated as a source of nutrition to animals and humans. Pods are used as a vegetable for human consumption and the crop is grown for the cattle feed and as a green manure. Cowpea was used as rich protein to feed cattle’s. It was also used as green vegetable in India. The Cowpea is an annual legume. It grows best under conditions with moderate rainfall and tolerates semi-arid conditions. Cowpea is produced through Asia. It is grown on about 3, 00,000 ha. Area. Nigeria is the largest producer and consumer of Cowpea, Nigeria have about 650,000 metric ton, Cowpea production in Brazil have about 490,000mt, and the United States produced about 80,000 metric ton Cowpea annually. As Africa have long term drought many farmers turns over to production of Cowpea because of its drought tolerant property. World production of Cowpea is 1.2 million metric ton per year. In Africa due to rapid growing population per capita consumption of Cowpea is increased and trend is expected to continue. Michael et al., (2007). Production of cowpea in India state wise in not exactly known but in Madhya Pradesh it grown in both the season, in Punjab as cover crop for maize, in Uttar Pradesh it is cultivated during rainy season for its seeds and in Orissa Cowpea is grown in rotation with leguminous fodder for grazing animals and for soil reclamation, Wealth of India (1976).

We observed that the spontaneous variations occurring due to mutation in various plants. However, the frequency is very low and not induces the full range of variations. In Cowpea induction of genetic variability through hybridization is difficult because of very delicate and small size of flower. Therefore, the induced mutation through chemical and physical mutagens is effective tool to induce variations for significant, desirable and genetical improved characters.

Research and development in the area of mutation breeding is undertaken through “Induction of genetic variation in Cowpea \(Vigna unguiculata\) (L.) Walp.] through Gamma radiation and Ethyl methanesulphonate”. Induced mutagenesis may bring about changes in the overall morphology and physiology of the plants. In present study biochemical investigations such as estimation of seed protein, leaf
protein, chlorophyll, carbohydrates, minerals and characterization of morphological
mutants was studied. Research and development in this area of investigation is
necessary for quality crops improvement with increased nutritive value in Cowpea
Cowpea variety – Phule Pandhari (PCP 9708) was treated with chemical mutagen like
Ethyl Methane Sulphonate (EMS) at the concentration of 0.050%, 0.075%, 0.10% and
0.125%. and physical mutagen like Gamma rays at the doses of 20kR, 30kR, 40kR
and 50kR. and Combination of Gamma rays and EMS like 20 kR+0.050%, 30
kR+0.075%, 40 kR+0.10% and 50 kR+0.125%.

The seed material of each treatment along with control (untreated seeds) was sown in
research field by Complete Randomized Block Design (CRBD) with three
replications. The soil parameters have been analyzed before the sowing of seeds. The
statistical analysis was done with help of SPSS and Excel software.

For the present mutation-breeding programme, the entire study was spread
over four generations. Studies in the M1 generation included collection of data on the
following M1 biological parameters like seed germination percentage, seedling height
and seedling injury, survival of plants at maturity, chlorophyll deficient sectors in
leaves mitotic index, mitotic chromosomal abnormalities and pollen sterility. Results
indicated that the EMS and Gamma rays and combination treatments showed an
inhibitory effect on seed germination. Seedling height was decreases as increase in
concentration/doses of EMS, gamma rays and Combination treatment. The survival of
plant at maturity was relatively equal to control in case of gamma rays treatment,
while it decreases in EMS and Combination treatment. In Gamma radiation at
40kR, observed the highest survival rate as compare to another treatments. Mitotic
index increases as the concentration/dose of mutagen increased in EMS, Gamma rays
and Combination treatments. In individual treatment of EMS, Gamma rays and
Combination treatments the pollen sterility increases as the concentration/dose of
mutagen increased. However, in combination treatment the pollen sterility was more
as compare to other mutagenic treatments. It observed that the induced mutagenic
sterility in the present investigation could be due to chromosomal aberrations.

The different chlorophyll deficient sectors like xantha, albina, chlorina, and
viridis were recorded in the leaflets like totally, partially and at the margins. All
treatments were effectively induced the chlorophyll deficient sectors in Cowpea. The
maximum frequency of such chlorophyll chimeras carrying plants was in high doses
of gamma rays and EMS treatment. Many researchers found that the chlorophyll
mutants are not heritable. It has been observed that all mutagenic treatments induced morphological changes in leaves like bifoliate, tetrafoliate, pentafoiate, large sized leaves and small sized leaves.

The seeds of M₁ generation were used to raise the M₂ progenies. Plants of M₂ generation were assessed for chlorophyll mutations. These mutations were recorded at seedling stage immediately after the germination of seeds. Four types of chlorophyll mutations were observed in Cowpea like \textit{albina}, \textit{xantha}, \textit{chlorina}, and \textit{viridis}. The variety of Cowpea responds to EMS, Gamma rays mutagens and combination of mutagens. There was no specific trend according to increase or decrease in concentration /dose of mutagens, but gamma rays treatment shows the highest frequency of chlorophyll mutation than EMS. The lower concentration /dose of combination treatment show high frequency of chlorophyll mutations than individual treatment.

Data on biological damage in M₁ generation, the relative effectiveness and efficiency of the mutagenic treatments was assessed. The values for each mutagen varied according to the M₁ parameters taken for calculation. Induced mutation through chemical and physical mutagens is very effective tool for plants improvement. The usefulness of induced mutations in plant improvement depends on increasing the efficiency of mutation induction and its proper selection. The choice of proper mutagens and appropriate treatment conditions are important in obtaining the desire efficiency and mutation rate. Mutagens can induce different genetically variations.

The mutagenic effectiveness and efficiency decreased with increase in the concentration /dose of mutagens. The lower concentrations/ doses were found to be more effective. The EMS and combination of gamma rays mutagens was found to be more superior to Gamma rays treatment. EMS was found to be most effective mutagen in Cowpea as compared to Gamma rays and combination treatment. The order of effectiveness and efficiency of the mutagens were EMS > EMS+GR > GR. The decrease in effectiveness with increasing concentrations /dose of mutagens could be attributed to the biological damage like lethality and pollen sterility. Which increased with increase in concentration/dose at faster rate than the mutation?

The mutation rate was calculated by used the mean values of efficiency for each treatment. This provides an idea of the average rate of mutation induced as per mutagen. It could be noted that when the mutation rates based on efficiency was
considered the order of mutagens have varied values in relation to lethality and pollen sterility. For lethality in M₂ generation of Cowpea the order of mutagens could be framed as EMS+GR > GR > EMS. For pollen sterility the order of mutagens in the increasing direction was EMS+GR > GR > EMS.

In M₂, M₃ and M₄ generation of Cowpea plants shows differential viable mutations like leaf mutations, flower colour mutations, seed coat colour mutations and plant type mutations. A broad range of viable mutants has been observed in M₂ and M₃ generation of Cowpea. The different viable mutants obtained were of the following type:

1) Robust 2) Branched 3) Dark green 4) Early flowering 5) Late flowering 6) Tall 7) Dwarf 8) Bold and large seeded 9) Luxuriant 10) Divergently branched mutants etc.

In M₂ generation, the frequency of viable mutations increased with the increased concentration/dose in individual treatments. The EMS treatments show the increasing frequency than Gamma ray followed by Combination treatments. Some of the viable mutants can be very well exploited on a commercial basis in view of the positive attributes possess them. The tall mutant, robust mutant and divergently branched mutant, luxuriant mutant and dark green leaves mutant can be profitably incorporated in the conventional breeding programme.

The data on five quantitative characters such as plant height, number of pods per plant, length of pods, number of seeds per pod and weight of 100 seeds were collected in M₂, M₃ and M₄ generation of Cowpea. The statistical analysis of the data was carried out to understand the effect of mutagens in shifting the mean and variance in either direction. The mean, variance and coefficient of variance were computed. It was seen that the quantitative parameters were succeeded in showing a significant positive shift in mean values.

Quantitative characters are supposed to be controlled by polygene and exhibit a feature of continuous distribution. In addition, the expression of some quantitative characters is subjected to strong environmental influences. Thus, in crop plants the assessment of the practical role of induced mutations in crop improvement is based on quantitatively inherited characters which have been subjected to statistical analysis.

The improvement of cultivated plants largely depends on the genetic variability occurred within the species. The mutagenesis can help to enhance the natural mutational rate and to enlarge the genetic variability and desired selections. The induction of micro mutations in polygenic system controlling the quantitative
characters is important for crop improvement. The major objective while employing induced mutations should be to increase variability within shortest possible period and to develop useful genotypes carrying a range of beneficial attributes of high economic values.

Statistical analysis of phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV), heritability ($h^2$) and genetic advance (GA) for plant height, number of pods per plant, length of pods, number of seeds per pod and weight of 100 seeds was carried out. Estimates of heritability and genetic advance for different traits in Cowpea population were carried out to enable selection of desired genotypes. Induced variability was calculated in EMS, Gamma rays and Combination treatments in five different yield-contributing traits of Cowpea in the M$_2$ generation.

A phenotypic coefficient of variation (PCV) was higher than its genotypic coefficients of variation (GCV) for the studied characters. This resemblance between PCV and GCV in almost all the characters suggests that the environment had effect on those characters expression. Higher heritability coupled with high genetic advance was observed for quantitative traits like Number of pods per plant, Length of pod, Number of seeds per pod, Plant height, weight of 100 seeds. In modern plant breeding one of the major trends has been supporting the traditional methods by biochemical studies so as to obtained a better value of a progeny in breeding. The economic importance of different plants is not restricted to the number and weight of seeds product. Several specific substances stored in the seeds such as proteins, oils, carbohydrates and minerals in sufficient amount of importance. In particular the seed protein and leaf protein has considerable significance for both human and animal nutrition. Since the biochemical analysis is a useful tool in understanding the basic architecture of an organism on the cellular and physiological level, a study of seed protein and leaf protein content of the control and mutants was undertaken in the present investigation.

The leaf protein seed protein content in the present studies has revealed an enhancement in majority of the M$_4$ generation. The viable mutants have developed through the EMS, gamma rays and combination treatments. Total chlorophyll content in leaves of the morphological mutants of Cowpea. It was ranged from 1.1628 mg/gm to 2.6018 mg/gm. The total chlorophyll content in leaves particular in Chl ‘a’ and Chl ‘b’ shows the fluctuations. Total carbohydrates content in morphological viable mutants of Cowpea ranges from 1.79% to 5.58 %. All viable mutants were estimated
from the seed samples of Cowpea like **Nitrogen**(N), **Phosphorus**(P), **Potassium**(K), **Calcium**(Ca), **Magnesium**(Mg), **Sulphur**(S), **Iron**(Fe), **Manganese**(Mn), **Zinc**(Zn), **Copper**(Cu) and **Sodium** (Na) were estimated. **Dwarf mutant content highest mineral percentage in Nitrogen( N ) 5.22 % ,Phosphorus(P)0.61%, Magnesium(Mg), 0.55 and Zinc(Zn) 68.66% and Robust mutant content Potassium(K.)1.70% and bold seeded mutant content Iron (Fe) 384 ppm and Manganese(Mn)19.8ppm.**

The nutritional improvement of legumes through breeding program has very immense important in world of food crisis. For this quantitative improvement of plants in yield is very important.

**Significant findings**

1. From the above observations, it can be observed that the chemical and physical mutagenic treatments employed in the present investigation have been succeeded in inducing genetic variability with significant alterations in growth and metabolism of the plant body.

2. Ten different morphological mutants were observed in present study vise, 1) robust 2) branched 3) dark green leaves 4) early flowering 5) late flowering 6) tall 7) dwarf 8) bold and large seeded 9) luxuriant 10) divergently branched mutants etc.

3. The viable mutants like robust, dark green leaves, tall, bold and large seeded and luxuriant were the high yielding mutants; therefore, these are the agronomic, biochemically and economical superior mutants.

4. Five high yielding mutants also shows the high amount of seed proteins, leaf proteins and carbohydrates content.

5. Statistical analysis of phenotypic and genotypic coefficient of variation (PCV and GCV) of all quantitative characters studied in M2, M3, and M4 generation revealed that there was less difference between two parameters indicated that the characters are controlled by genetic and environmental factors.

6. The high heritability and genetic advance of these agronomic traits suggested that they can be transferred to other plants through breeding programme for genetic improvement in Cowpea.
Conclusion

In conclusion, it can be said that various chemical and physical mutagenic treatments employed in the present research work have been succeeded in inducing superior genotypes with significant alterations in growth and metabolism of the plant body. The mutagens successfully induced genetic variability and different mutants of agronomic traits. The results obtained are quite encouraging to utilize the mutants recovered for obtaining better seed and leaf protein content besides improved yield in case of Cowpea. Five mutants like robust, dark green leaves, tall, bold and large seeded and luxuriant mutants are showing better yield contributing parameters and even more seed protein as compare to control. Such mutants could be promoted for cultivation after successful completion of seed certification.