Ph.D THESIS ABSTRACT

Title: “Induced Mutation in Guar (Cyamopsis tetragonoloba (L.) Taub.).”

Name of Research Student: Manisha Shamrao Shinde.

Name of Research Guide: Dr. A. D. More.

Place of Research: Department of Botany, Fergusson College, Pune-04.

INTRODUCTION:

The Cluster bean (Cyamopsis tetragonoloba (L.) Taub. ) (2n=14) belongs to family Fabaceae. The pods grow in clusters, so the guar commonly known as cluster bean. It is protein rich leguminous crop mainly cultivated in semi-arid regions of India and Pakistan. The importance of legume has been highly appreciated as a source of nutrition to animals and humans. It regenerates soil nitrogen and the endosperm of cluster bean is an important hydrocolloid widely used across a broad spectrum of industries like food, pharmaceuticals, cosmetics, paper, textile, mining and oil drilling for thickening, stabilizing, texturizing, enhancing suspension and flow control properties. Chemically, it is a polysaccharide composed of the sugar galactose and mannose. It is used as dietary fibers in many food products and nutritional supplements. The Guar seed has been used in Indian medicine as a laxative and a digestion tonic. Guar gum is also believed to lower cholesterol (abc.herbalgram.org).

Initially it was used as green vegetable, fodder and green manure, as it is a rich source of protein. However, today Guar is highly commercialized for its economic importance due to World War II and modern chemistry. Guar was not known for its industrial application until in the times of World War II, when there was shortage of locust bean crop and the paper and textile industry of the world was searching for a substitute. They found as efficient alternative in the form of guar gum and since then, this derivative of guar ruled out locust bean from this scenario and it was readily accepted for application in many other industries (CRN India). It is cultivated as a kharif crop and it is clearly rain dependent crop. The world’s total production of guar figures around 7.5 to 10 lakh tons of Guar every year. India is the world’s number one country for the production and export (about 80%) of guar and its by-products followed by Pakistan. USA, South Africa, Malawi, Zaire, Italy, Morocco, Spain, France, Greece, Germany and Sudan are other major producing countries.
India produces 6,00,000 tons of guar annually i.e. the maximum level of production in the world. It contributes to around 80% share in the world’s total production. The major producing regions of this crop in India are Rajasthan, Gujarat, Haryana, Punjab, Uttar Pradesh, Madhya Pradesh, Tamil Nadu, Maharashtra, Karnataka, Andhra Pradesh. Guar is largely consumed as a vegetable in the Indian subcontinent. The 25000 tons of the total production in the country constitutes to the domestic market (CRN India).

We can find 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 Guar, induction of genetic variability through hybridization is difficult because of very delicate flower structure. Therefore, the induced mutation through physical and chemical mutagen is very effective tool to induce variations for significant characters. Induced mutagenesis may bring about changes in the overall morphology and physiology of the plant. Therefore, the present investigation was undertaken to study the response of Guar genotype to the physical mutagen gamma rays, chemical mutagen EMS and combination both.

**RIVIEW OF LITERATURE:**

1. Dube et al. (2011) were recorded inhibitory effect on germination, seedling height and survival of Guar through mutagenesis.
2. Sangle et al. (2011) based on the mutation studies with Pigeonpea found inhibitory effect on germination, seedling height, pollen sterility and survival of plants at maturity.
3. Patil and Wakode (2011) studied the germination, survival of plant at maturity, pollen sterility and mitotic index in Soybean. Mitotic index was reduced with increased dose/ concentration.
4. Jain and Khandelwal (2009) studied frequency and spectrum of chlorophyll and other macro mutations in Black gram. They found high frequency of Xantha mutant.
5. Arulbalachandra and Mullainathan (2009) based on their mutation studies on Black gram reported that the mutations in these chlorophyll genes are reflected in the M₂ generation in the form of different types of mutants.
6. Thilagavathi and Mullainathan (2009) studied the macro mutations and mutagenic effectiveness, efficiency in Black Gram. In present study researcher found EMS was more effective and efficient of chlorophyll and viable
mutants than gamma rays treatment.

7. Bhosale and Kothekar (2010) were recorded the maximum value of effectiveness, while mutagenic efficiency varied with different biological parameters in the mutagenesis studies on Guar.

8. Shirsat et al. (2010) studied the mutagenic effectiveness, efficiency in two varieties of Horse gram. In variety SINA, SA was more effective than EMS, while in variety KS-2 EMS was more effective than NEU.

9. Girija and Dhanavel (2009) found EMS was more effective and efficient than gamma rays and combination treatment in Cowpea. The improved variety of CO7 responded more number of viable and economic mutants for higher productivity.

10. Velu et al. (2008) found the broad spectrum of morphological mutants in M2 generation of Guar.

11. Wani and Anis (2008) found the bold seeded and high yielding mutant in Chickpea. These mutants showed considerable improvement in yield, as well as increased pod size.

12. Amoatey et al. (2000) studied seed protein, carbohydrates and ash content in some legumes in Ghana.

**STATEMENT OF AIMS AND OBJECTIVES:**

The proposed research work has been undertaken with the following aims and objectives:

- Induction of morphological mutants.
- Study of various morphological changes in the mutants.
- Study of biochemical changes in mutants.

**MATERIAL AND METHODS:**

The cultivar of cluster bean ‘P.N.B.’ were treated with chemical mutagen Ethyl Methane sulphonate (EMS) at the concentration of 4mM, 7mM, 9mM, 10mM, and physical mutagen gamma rays irradiated at the dosage of 100Gy, 200Gy, 300Gy, 400Gy, and also with combination of both (Gamma rays and EMS) like 100Gy + 10mM, 200Gy + 9mM, 300Gy + 7mM, 400Gy + 4mM. The seeds of each treatment along with control (untreated seeds) were 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.

RESULTS AND DISCUSSION:

*M1 GENERATION*

Study of M1 generation comprises the collection of data on the different effects of mutagens on the various parameters like seed germination, seedling height, seedling injury, pollen sterility, survival of plant at maturity, leaf abnormalities, chlorophyll chimeras in leaves and cytological studies. These parameters help to understand the biological damage caused by mutagens, which further used to assess the effectiveness and efficiency of the mutagenic treatment.

Results indicate that the EMS and Gamma rays individually had an inhibitory effect on seed germination. Germination was high in combination treatment of 300Gy +7mM. Dube et al. (2011) suggested that in case of Cluster bean it seems probable that the cell cycle arrested by higher doses of physical and chemical mutagens might have resulted the decrease in germination.

The survival of plant at maturity was relatively equal to control in case of gamma rays treatment, while in EMS it decreases as increase in concentration of mutagen. 100Gy+10mM show the highest survival rate as compare to another treatment. According to Dube et al. (2011) in Guar, it seems probable that, the genetic damages like chromosomal aberrations and the physiological imbalance in the cells caused by the mutagens might have decreased the survival percentage.

Seedling height decreases as increase in dose of gamma rays. It increases with the increase in concentration of EMS, while the combination treatments show the increased seedling height as compare to individual treatment. According to Dube et al. (2011), it seems probable that possible interference of irradiation causing damage in the synthesis of new DNA may have lead to the inhibition of seedling height in Guar.

In individual treatment of gamma rays and EMS the pollen sterility increases as the dose /concentration of mutagen increased. However, in combination treatment the pollen sterility was less as compare to individual treatment. The pollen sterility in Guar after the gamma radiation, EMS and combination treatments showed quite lower values in a particular concentration/ dose within individual treatment. It observed that the induced mutagenic sterility in the present investigation could be due to chromosomal aberrations.

Mitotic index increases as the combination and gamma rays doses increase,
while in EMS mitotic index reduces as increase in concentration of mutagen. The similar results of reduction in mitotic index in Soybean with EMS treatment were also reported by Patil and Wakode (2011).

The different chlorophyll deficient sectors like xantha, albina, chlorina, and viridis were detected in the leaflets, totally, partially, and at the margins. All treatments were effectively induced chlorophyll deficient sectors in Guar. The frequency of such chlorophyll chimeras carrying plants was maximum in high doses of gamma rays and combination treatment. It has been observed that all mutagenic treatments induced the morphological changes in leaves like unifoliate, bifoliolate, tetrafoliate, pentafoliate, large sized leaves, small sized leaves and variegated plants. The frequency of such chlorophyll mutants was used to calculate the effectiveness and efficiency. Many researchers found that the chlorophyll mutants are not heritable.

**M2 GENERATION:**

**Chlorophyll Mutation**

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 found in cluster bean namely albina, xantha, chlorina, and viridis.

1) **Albina:** Albina mutants were completely white in color, such seedling could not survive beyond 20-25 days after the seed germination.
2) **Xantha:** Xantha mutants were yellow in color; these mutants were survived for 35-40 days and became stunted growth.
3) **Chlorina:** Chlorina mutants were yellowish green in color few of them reverted to the normal green type, these mutants survived up to 40-45 days.
4) **Viridis:** Viridis mutants were light green in color, these colors later on change to the normal green color these seedlings survived up to 40-45 days.

The cultivar of cluster bean responds differently to the both mutagens and combination of mutagens. There was no specific trend according to increase or decrease in dose/concentration of mutagens, but gamma rays treatment shows the highest frequency of chlorophyll mutation than EMS. The combination treatment of both mutagens shows the low frequency of chlorophyll mutations than individual treatment.

**Effectiveness and Efficiency**

Induced mutation through physical and chemical mutagen is very effective
tool for plant 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 evidently important in obtaining the desire efficiency and mutation rate. Mutagens induce different genetic and cytogenetic variations. Thus, mutagenic effectiveness and efficiency will also depend upon the nature of mutations induced Bhosale and Kothekar (2010).

The mutagenic effectiveness and efficiency decreased with increase in the dose/concentration of mutagens. It simply means that low doses/concentrations were found to be more effective. However, this is the case for individual mutagens. The combination of gamma rays and EMS mutagens were found to be more superior to individual gamma rays treatment. EMS was found to be most effective mutagen in Guar as compared to gamma rays and combination treatment. The order of effectiveness and efficiency of the mutagens were EMS > GR + EMS > GR. The decrease in effectiveness with increasing concentrations of mutagens could be attributed to the biological damage like lethality, pollen sterility that increased with increase in dose/concentration at faster rate than the mutation. The exact mechanism was not yet known, by which these factors influence the mutation frequency.

Viable mutations

In M$_2$ generation cluster bean genotype shows differential viable mutations like leaf mutations, flower mutations, pod mutations, seed mutations and plant type mutations.

1. **Leaf Mutations**

In present investigation the broad spectrum of leaf mutations was observed in the M$_2$ progenies of cluster bean. Variation in number of leaflets like unifoliate, bifoliate, tetrafoliate and pentafoiative were found. Large number of variations in shape and size of leaf were observed. According to many researchers changes in leaves are due to chromosomal aberrations produced in plant because of the action of mutagens.

2. **Flower Mutations**

Generally in mutation breeding the flower color mutations are expected, but in the cluster bean this kind of flower color mutations were not observed. In cluster bean flower shows changes in its color on different stages of development itself. In present investigation the genotype of cluster bean shows terminal inflorescence while in
control it is axillary. Because of terminal position the height of flower terminates extremely dwarf.

3. Pod Mutation

In present study of mutation breeding in cluster bean, mutagens induce the variations in pods also. The increase in length of pod, seeds per pod was the major variations. Some curved shape pods, single and double seeded pods, bold pods were also found. Such variations of pods were supposed to be yield contributing. All treatments were successful to induce pod mutations, but combination treatments show the highest range of such mutations.

4. Seed Mutations

In $M_2$ generation along with other morphological variations the seed coat color variation was also accessed. The observation shows significant trend of seed coat color variation. Against the purple color seed coat of control the cream white, black, brown, dark purple colored seed coat colors were found in mutagenic plants. Variations were increased according to the increase in concentration / dose of mutagen.

5. Plant Type Mutations

In $M_2$ generation the wide spectrum of viable morphological mutants like tall mutant, dwarf mutant, sterile mutant, determinate and spreading mutant were studied. The spectrum of viable mutants was much wider in combination treatment than individual treatment. The highest frequency was induced by 400Gy + 4mM while least frequency was induced by 100Gy. The frequency of viable mutant indicated increase trend with the gradual increase in concentration of mutagen.

Quantitative characters

The data of quantitative characters like plant height, number of branches, number of clusters, number of pods per plant, length of pods, number of seeds per pod and 100 seeds weight was collected for further statistical analysis. This analysis gives the actual idea about character vise improvement of plant through mutagenesis. Therefore, these characters are supposed to be yield-contributing traits. The practical finding shows that the concentrations/dose of mutagens had promotary effect on genotype of Guar.

$M_3$ GENERATION

The $3^{rd}$ generation of cluster bean was assessed for the frequency of viable mutation same as $M_2$ generation. In $M_3$ generation an increase in the frequency of
viable mutations could be noted at majority of the mutagenic treatments. The relative percentage for different viable mutants was random in both the $M_2$ and $M_3$ generation. The data of quantitative characters like plant height, number of branches, number of clusters, number of pods per plant, length of pods, number of seeds per pod and 100 seeds weight was collected for further statistical analysis.

$M_4$ GENERATION

Macromutations play an important role to assess the effect of mutagenic treatments. In present investigation all the mutagenic treatments are successful in inducing macromutations in Guar, but the combination shows the highest range of such mutations. Morphological characterization of viable mutants was done in $M_4$ generation employed the EMS, Gamma rays and combination of both were described below.

1. **Tall Mutant:** The tall mutant demonstrated a height of 118 cm. to 144 cm. as against 56 cm. to 67 cm. in control. Some mutant shows 1 or 2 branches, but mostly the plants were not showing branches. Number of clusters ranges from 17 to 22 while peduncle length ranges from 16 cm. to 21 cm. Each cluster was having 13 to 17 pods. Number of pods per plant ranges from 123 to 196.

2. **Dwarf Mutant:** These mutants were characterized by an extreme reduction in plant height. It was 30 cm. to 38 cm. as against 56 cm. to 67 cm. for control. Just like control the dwarf mutants were also found no branching. Number of clusters ranges from 5 to 7 and peduncle length was also short as compare to control. It ranges from 2 cm. to 5 cm in comparison of control 5 cm. to 9 cm. Each cluster was having 4 to 10 pods. Most of the pods were single and double seeded. Number of pods per plant ranges from 18 to 29.

3. **Divergently branched Mutant:** The height of these mutants was relatively same as control which is 52 cm to 68 cm. They born quite good number of branches which were of a divergent and away going type. The branches number ranges from 4 to 13. Branches arise on all over the stem. Number of clusters, length of clusters and yield was relatively high than control.

4. **Spreading Mutant:** In spreading plants many branches were arise from single point and they were spread over the ground horizontally. The leaves were small in size and have hairy texture with increased number over the control. The peduncle length was reduced up to 1.5 cm to 4 cm. with the comparison of 5 cm to 9 cm. in control. The height of plant was greatly
reduced up to 40 cm. due to spreading nature. Number of clusters and number of pods per plant were more than control. So considered it as high yielding mutant also.

5. **Determinate Mutant**: Determinate plants show the presence of single shoot (absence of branches), broad and glabrous leaves and determinate growth habit. The peduncle length was extended up to 26 cm. with the comparison of 5 cm to 9 cm. in control. In control, plants mean height was 56 cm. to 67 cm while in determinate it grows up to 125 cm. Number of clusters per plant were more than control but in the form of yield number of pods per plant were less than control. Mostly the pods were single or double seeded.

6. **Dark green Mutant**: The main character of these mutants was leaflets carrying dark green coloration. Such mutants attained a height of 63 cm to 76 cm and number of pods per plants was 58 to 63. The number of branches was 1 or 2. The pod length was relatively same as control. In case of yield it was little more than control.

7. **Terminal inflorescence Mutant**: It was very interesting mutant found in population of cluster bean. Control plants shows axillary inflorescence, but these plants produced with terminal as well as axillary inflorescence. Because of terminal inflorescence plant height reduced from 16 cm to 48 cm. The yield was very less and no branching form. The pods were single seeded to five seeds.

8. **Luxuriant Mutant**: These mutants were found with luxuriant growth. The height of such mutants was somewhat higher than that of control. The number branches ranges from 1 to 5. Numbers of clusters were also more and number of pods per plant ranges from 87 to 104. The 100 seeds weight was also high than control.

9. **Bold seeded Mutant**: The mutant was characterized with bold seeds. The all quantitative characters were relatively same as control but 100 seeds weight was higher than control.

10. **Black seeded Mutant**: The mutant was characterized with white seed coat coloration. The all quantitative characters were relatively same as control.

11. **White seeded Mutant**: The mutant was characterized with black seed coat coloration. The all other quantitative characters were same as control.
Some of the viable mutants can be very well exploited on a commercial basis in view of the positive attributes possessed by them. In this regard tall mutant, divergently branched mutant, spreading mutant and dark green mutant would be of immense application. They can be profitably incorporated in the conventional breeding program.

The data on seven quantitative characters such as plant height, number of branches, number of clusters, number of pods per plant, length of pods, number of seeds per pod and 100 seeds weight were collected in $M_2$, $M_3$ and $M_4$ generation of cluster bean. 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.

**BIOCHEMICAL STUDIES IN MUTANTS:**

In modern plant breeding one of the major trends has been supporting the traditional methods by biochemical investigation so as to obtain a better estimate of the breeding value of a strain. The economic importance of different plants is simply not restricted to the number and weight of seeds product. Several specific substances stored in the seeds such as proteins, oils, carbohydrates and minerals of a good 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.

**Seed protein content:**

In *Cyanopsis tetragonoloba* (L.) Taub. the control seed protein content was 10.42%. The dark green mutant, spreading mutant, tall mutant and luxuriant mutant shows the increased seed protein content than control. Spreading mutant has highest seed protein content that is 14.75%. Among the all mutants seed protein content ranges from 8.17% to 14.75%.

**Leaf Protein Content:**

The leaf protein content in the control of Guar was 4.52%. The dark green mutant, spreading mutant, tall mutant and luxuriant mutant shows the increased leaf protein content than control. Spreading mutant has highest leaf protein content that is 6.40%. Among the all mutants leaf protein content ranges from 3.44% to 6.40%.
**Chlorophyll Content:**

The control plant has 2.0696 mg/gm of total chlorophyll. The highest amount of total chlorophyll was found in spreading mutant, which was 2.7154 mg/gm, while the lowest amount of total chlorophyll content was found in determinate mutant which was 1.6817 mg/gm. Total chlorophyll content in morphological mutant of cluster bean ranges from 1.6817 mg/gm to 2.7154 mg/gm. Besides the total chlorophyll content, specific Chl ‘a’ and Chl ‘b’ shows the fluctuations. This fluctuation shows the direct effect on physiology.

**Total Carbohydrates Content:**

Total carbohydrates content in morphological mutant of Guar ranges from 1.02% to 7.64%. The control plant has 3.50% of total carbohydrate. The highest amount of total carbohydrates was found in spreading mutant, which was 7.64%, while the lowest amount of total carbohydrates content was found in determinate mutant which was 1.02%.

**Minerals Content:**

All macro and micro nutrients were estimated from the seed samples of cluster bean mutant. N, P, K, Ca, Mg, S, Na, Fe, Mn, Zn, Cu were estimated. Bold seeded mutant content highest nitrogen (5.10%) than control (4.41%).

The nutritional improvement of legumes through breeding program has very immense important in today’s world of food crisis. Only quantitative improvement in yield is not of much importance, while the nutritional improvement of any plant will be the solution for malnutrition.

**SIGNIFICANT FINDINGS:**

1. From the above observations, it can be seen that the mutagenic treatments tried in the present study have been succeeded in including genetic variability with significant alterations in growth and metabolism of the plant body.

2. Eleven different types of morphological mutants were found in present study vise, Tall mutant, Dwarf mutant, Divergently branched mutant, Spreading mutant, Determinate mutant, Dark green mutant, Terminal inflorescence mutant, Luxuriant mutant, Bold seeded mutant, Black seeded mutant, White seeded mutant.

3. Tall mutant, Spreading mutant, Dark green mutant and luxuriant mutant were the high yielding mutants, so they are the agronomically superior mutants.
4. The high yielding four 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 M$_2$, M$_3$, and M4 generation revealed that there is less difference between these two parameters indicating that these characters are largely controlled by genetic factors and less affected by the environmental factors.

6. The high heritability and genetic advance, of these agronomic traits strongly suggest that they can be transferred to other plants through breeding experiments aimed at genetic improvement of Cluster bean.

CONCLUSION:
The mutagens successfully induced genetic variability and different mutants of agronomic traits. Four mutants Tall mutant, Spreading mutant, Dark green mutant and luxuriant mutant 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 protocol.

[Manisha S. Shinde]  [Dr. A. D. More]
Research Student  Supervisor