Appendices

APPENDICES

Appendix i: Research Paper Published.

Induction of a Novel, High Yielding Mutant of Pigeon Pea
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ABSTRACT
Pigeon pea (Cajanus cajan (L.) Millsp.) is one of the major pulse crops of Maharashtra grown in Khairi season. It is cultivated in 33.54 lakh hectares in Maharashtra. In spite of its demand the yield of the pulse is low. In the present investigation an effort was made to improve the yield of the crop by mutation breeding. Germplasms of authentic samples of Pigeon pea (Var. ICPL-87) were procured from Pulse Improvement Division of Maharashtra Pulse Agricultural University (M.P.K.V.), Rahuri, and District; Ahmednagar (Maharashtra). Seeds, presoaked in water for 24 hrs., were treated with different concentrations (10, 20, 30 and 40mM) of the Chemical Mutagens, Ethyl Methyl Sulphonate (EMS) for 6 hrs., at 25 - 27°C, followed by, through washing under tap water. Presoaked seeds, treated with EMS served as control. Both Control and EMS treated seeds were sown in the field to raise M₁ progeny. M₁ seeds, along with their controls were sown in next Khairi reason to raise M₂ progeny. M₂ progeny plants were screened for Yield contributing traits like, Plant Height, Number of branches per plant, number of pods per plant, Pod length, Number of Seeds per Plant, 100 seed weight and Yield per Plant. M₁ progeny were also screened for useful mutants. Results indicated that genetic variability in Yield contributing Traits, and viable mutants could be observed only at M₂ generation. The M₁ progeny raised from seeds treated with 40 mM concentration of EMS produced novel mutants showing two fold increase in number of branches per plant, number of pods per plant and Yield per plant. This mutant was named as High Yielding (Rohani) mutant. Differences observed between the High yielding (Rohani) mutant and corresponding control plants and correlation between the parameters were discussed in the text. The High yielding (Rohani) mutant seems to be very promising and can be released as variety after appropriate field trials.

KEY WORDS: Mutation Breeding, Pigeon Pea, Cajanus cajan

INTRODUCTION
Pigeon pea (Cajanus cajan (L.) Millsp.) is one of the major pulse crops of Maharashtra grown in Khairi season. It is cultivated in 33.54 lakh hectares with a production of 183.66 lakh tonnes in Maharashtra [1]. It is consumed as split Dhul, but is also consumed as green vegetable in many countries. Seed and fodder contains about 20-22% protein. Seeds are rich in iron, iodine, and essential amino-acids like lysine, cystine and arginine. In spite of its nutritional importance the yield of Pigeonpea is very low. Mutation breeding has become an alternative to conventional breeding since last three decades with the sole objective of developing better varieties of economically important crops [2]. Mutation breeding is one of the plant breeding techniques used for creating genetic variability in yield contributing traits and to improve the yield of crop plants [3]. In the present investigation, attempts were made to induce genetic variability in Yield contributing traits of Pigeonpea with an objective of isolating agronomically important, high yielding mutants, if any, at M₂ generation.

MATERIALS AND METHOD
The experimental material selected for the present study is Pigeon pea (Cajanus cajan (L.) Millsp.) var. ICPL-87. Germplasms of the variety was procured from Pulse Improvement Division of Maharashtra Pulse Agricultural University (M.P.K.V.), Rahuri, Maharashtra. The variety is a desi type, commercially and widely cultivated extensively in various parts of Maharashtra. A chemical mutagen, Ethyl Methane Sulphonate (EMS), was used in the present investigation to induce genetic variability. Test solutions of different concentrations of EMS (10, 20, 30 and 40mM) were prepared in 0.1 M Phosphate buffer (pH 7.0). Two hundred fifty seeds were used for each treatment. The seeds were immersed in distilled water for 6 hours to initiate presoaking. The presoaked seeds were dried in blotting paper and later on

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Appendices Page 180
immersed in freshly prepared solutions of mutagens for 12 hours. The volume of mutagenic solution is about 5 times as that of seed for uniform absorption. The seeds treated with chemical mutagens were thoroughly washed under running water for 1 hr. The post soaking treatments were carried out to leach out the residual chemical. The treated and control seeds were dried in blotting paper. The 200 treated and control seeds were sown on the same day in well-prepared seed beds in the field. The seeds were sown in randomized block design (R, B, D) at a spacing of 20 cm. in rows of 2M long and 45 cm between rows.

Seeds from M₁ progeny were harvested separately and carefully from each treatment as well as control and sown for M₂ progeny. In M₂ progeny plants are carefully screened for morphological mutations such as early mutant, high yielding (Robust) mutant, tall mutant, late maturing mutant etc.

**RESULTS**

Results indicated that all concentrations of EMS are effective in inducing genetic variability in yield contributing traits at M₁ generation in Pigeon pea. However, the 40 mM concentration of EMS was found to be very effective in inducing novel agronomically important, high yielding (Robust) mutant (Fig. 2).

**Fig. 3 Number of pods and branches per plant of control and high yielding mutant of Pigeon pea.**

The M₂ progeny raised from seeds treated with 40 mM concentration of EMS produced novel mutants showing two-fold increase in number of branches per plant (Table), number of Pods per plant (Fig. 3) and Yield per plant (Fig. 4). This Mutant was named as High yielding (Robust) mutant. Differences in yield contributing traits, observed between the High yielding (Robust) mutant and corresponding control plants are shown in the table. No significant difference in plant height, pod length, number of seeds per pod and hundred seed weight could be observed between the control and the mutant (Table).

**Fig. 2 Variation between the mutant and control. A- High yielding Robust Bushy Mutant, B-Control.**
### DISCUSSION

Isolation of mutants of agronomic and economic significance was a major goal of mutagen breeding [4]. In the present investigation, we were able to isolate a mutant which is high yielding and agronomically significant. Similar agronomically important mutants in Pigeon pea were also reported earlier by [5, 6]. Our results clearly indicate that mutagen breeding can be applied for inducing genetic variability in yield-contributing traits and to change specific characters in otherwise good varieties by incorporating some useful changes such as, number of branches, pods per plant and yield per plant, in a comparatively shorter time than conventional breeding methods.

### CONCLUSION

In the present investigation an effort was made to improve the yield of Pigeon pea by mutagen breeding. Seeds, presoaked in water for 12 hrs, were treated with different concentrations (10, 20, 30 and 40mM), of the chemical Mutagen, Ethyl Methyl Sulphionate (EMS) for 6 hrs., at 25 ± 2°C. Presoaked seeds, untreated with EMS served as control. Both Control and EMS treated seeds were sown in the field to raise M1 progeny. M1 seeds, along with their controls were sown in next Kharif season to raise M2 progeny. M2 progeny plants were screened for Yield contributing traits like: Plant Height, Number of Branches per plant, Number of pods per plant, Pod length, Number of Seeds per Plant, 100 seed weight and Yield per Plant. M2 Progeny were also screened for useful mutants.

The M1 progeny raised from seeds treated with 40 mM concentration of EMS produced novel mutants showing two fold increase in number of branches per plant, number of pods per plant and yield per plant. This Mutant was named as High yielding (Robust) mutant. Differences observed between the high yielding (Robust) mutant and corresponding control plants and correlation between the parameters were discussed in the text. The High yielding (Robust) mutant seems to be very promising and can be released as variety after appropriate field tests.

### ACKNOWLEDGEMENTS

Authors are grateful to Dr. S. R. Wadag, Principal and Dr. P. G. Rodey, Head Department of Botany of Pachnandi Vidya Pratishthan College of Arts, Science and Commerce, Pavana nagar, for providing laboratory facilities to carry out research work. Thanks are also due to Major R. S Shinde, Principal, Arts, Commerce and Science College, Satra, Tal-Rahuri, Dist-Ahmednagar (MS), for his keen interest and constant encouragement.

### ASIAN JEXP. BIOL. SCILSP. 2010

### REFERENCES


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SHORT COMMUNICATION

Induced Chlorophyll Mutations in Soybean Glycine max (L.) Merril

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3Jijamata College of Arts and Science, Bhendi Tal - Newasa Dist - Ahmednagar (MS), India

ABSTRACT

In the present work attempts were made to induce chlorophyll mutations in soybean [Glycine max (L.) Merril] by employing EMS and Gamma rays. The germplasm of soybean cultivar MACS 450 was procured from Agharkar Research Institute, Pune, Maharashtra. The uniform pressseeded seeds of soybean were treated with different concentrations of EMS (10, 20, 30 and 40 mM) for 08 hrs and dry seeds were irradiated with different doses (100, 200, 1000 and 4000 Gys) of gamma rays at Government Institute of Science, Aurangabad. Treatments as well as untreated seeds were sown in the experimental field in 8R1 during kharif 2009 in the spacing of 45 x 15 cm to raise M population. Seeds harvested from individual M plants were sown in kharif 2009 to raise M generation. Population was monitored from the first day of emergence of the seedlings up to the harvesting for chlorophyll mutations and viable mutations. Four different types of chlorophyll mutations were observed in the M, progeny of soybean. They are albino, viridis, chlorina and sataoha. The variety responded differently to the mutagens. The frequency of chlorophyll mutations increased with an increase in the concentration of the mutagens except at 20mM EMS concentrations. The frequency of chlorophyll mutations 2.12 to 8.12% in EMS, and 4.94 to 7.76% in Gamma rays. The maximum frequency of chlorophyll mutations (8.12%) was observed at 40mM EMS concentration and it was maximum (7.76%) at 400 Gy dose of gamma rays. The overall spectrum of induced chlorophyll mutations as observed in soybean was in the following order: Chlorina (17.81%) > Viridis (13.57%) > Xantina (11.07 %) > Albina (9.56 %)

KEYWORDS: Soybean, chlorophyll mutant, EMS and Gamma rays.

INTRODUCTION

Soybean [Glycine max (L.) Merril, Family, Papilionaceae (Fabaceae)], vernacularly also known as golden bean is an important oil seed crop widely cultivated in Indian subcontinent. In Maharashtra, it is widely cultivated in Ahmednagar district. Soybean forms one of the important constituents in the dietary practices of local communities. Soybean is consumed in the form of vegetable oil. It is mainly used in the preparation of idly, papad, dosa, paneer, soya flour, soya milk and other regional fermented foods. Nutritional composition of soybean indicates that it has protein content as high as 40% and 20% oil. Its beans form a nutritious item of the food, while the whole plant gives rich feed for cattle and is a good manure as well as conservation crop.

Soybean is also prescribed as a medicine to fulfill the need of malnutrition. In spite of its nutritional importance, the yield of soybean did not witness much appreciation during the past decade. It has been argued that one of the chief reasons for failure to achieve breakthrough in productivity of soybean 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. Mutation breeding techniques are the best methods to enlarge the genetically conditioned variability of a species within a short time and have played a significant role in the development of many new varieties.

The role of induced mutations in developing new and better cultivars has now been well recognized. Therefore the most popular method employed for creating genetic variability is induced mutagenesis through gamma irradiation [1] and chemical mutagen like EMS. Chlorophyll mutations are one among the few dependable parameters for evaluation of genetic effects of various mutagens and are widely used as genetic markers in basic and applied research. The present study reports the induction of different chlorophyll mutants in M, generation in soybean cultivar MACS 450.
MATERIAL AND METHODS

The experimental plant material used in the present investigation is local variety of soybean [Glycine max (L.) Merrill], MACS-453. Germplasm of this cultivar of soybean was procured from the Agarkar Research Institute (ARI), Pune, Maharashtra state, India. Seeds presoaked in water for 6 hours, were treated with different concentrations of EMS (10nm, 20nm, 30nm and 40nm) for 8 hours at room temperature. Dry seeds were irradiated with 100Gy, 200Gy, 300Gy, and 400Gy gamma radiation doses at Government Institute of Science, Aurangabad (M.S., India). Seeds not treated with the mutagens served as control. About 300 seeds of each treatment were sown in the experimental field, along with controls, following randomized block design in 3 replications for the M1 generation during kharif season of 2006. The individual seeds of M1 plant progeny were sown in the field to raise M2 progeny. Uniform cultivation methods and agricultural practices were followed for all M1 and M2 generations. Various chlorophyll mutants were identified according to Gustafsson [2]. Frequencies were calculated according to Koizumi et al. (1965). Using the data on chlorophyll mutation frequency was calculated.

RESULTS

The M2 progeny raised from the M1 seed showed the presence of four types of chlorophyll mutations. They are: viridis, chlorina, xantha and alaina.

Viridis

The seedlings were golden yellow-green in colour and survived for a reasonably long period.

Chlorina

The seedlings were light yellowish green (pale green) in colour. They survived for a reasonably long period.

Xantha

The seedlings were completely yellowish and leaves are larger than viridis. These seedlings survived for only 7-8 days.

Alaina

Leaves are white in colour and the seedlings died after few days.

Frequency of these chlorophyll mutations increased with an increase in concentration/dose of the mutagens. The maximum frequency of chlorophyll mutations was observed at 40nm concentration of EMS (8.12%) and 400 Gy of gamma rays (7.78%). Chlorina chlorophyll mutation 5% was recorded at 40nm concentration of EMS and 2.79% at 400 Gy doses of gamma rays. Xantha chlorophyll mutations 1.60% at 30nm of EMS and 2.20% at 400 Gy dose of gamma rays. Viridis chlorophyll mutation was recorded 1.60% at 30nm concentration of EMS and 3.47% at 300 Gy doses of gamma rays. Alaina type chlorophyll mutation was least and only observed in 0nm of EMS (0.17%) and 300 Gy doses of gamma rays (0.34%). Both the mutations were found to be equally effective in producing high frequency of chlorophyll mutations. The overall spectrum of induced chlorophyll mutations observed in soybean was in the following order: Chlorina (17.61%) > Viridis (15.67%) > Xantha (11.07%) > Alaina (5.61%).

Increase in the frequencies of chlorophyll mutations with increase in the concentration/dose reported [3-8] see table and fig. 1 & 2.
The high frequency of chlorophyll mutations obtained with mutagens, is due to preferential action of these mutagens on genes for chlorophyll development or the preferential effect on guanine in the G-C rich chloroplast genome. Biosynthesis of photosynthetic pigments occurs in a series of biochemical reactions. EMS and gamma rays are potent mutagens well known for their action in inducing point mutations, and chromosomal aberrations. Any alteration in the nucleotide composition of the genes, that control the synthesis of enzymes involved in the biosynthesis of pigments, as result of action of the mutagens, would eventually lead to the observed chlorophyll mutations.

Table: the effect of mutagens on the spectrum of chlorophyll mutants in M_{2} generation

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<td>EMS (mM)</td>
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<td>Gamma radiation (Gy)</td>
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We are thankful to Aghariar Research Institution, Pune, for providing the germplasm. Government Institute of Science, Aurangabad for extending irradiation facility and Dr. S.R. Wali, Principal, Dr. P.G Reddy H.O.D. Botany Department, Pandharpur Vithal Patil college, Pravaranagar for providing laboratory facilities.

REFERENCES

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Appendix ii: Research Paper Accepted for Publication.

Bioscience Discovery
Journal of Life Sciences
Regd. No. MAHENG12607
ISBN 2229 - 3489

To,
Girijashanker P. S.,
Arts, Commerce and
Science College, Satara,
Tal-Rahuri, Dist-Ahmednagar (MS),
PIN 413 711.

Subject: Letter of acceptance.

Sir/Madam,

With reference to the subject cited above, your manuscript entitled “STUDIES ON EFFECTIVENESS AND EFFICIENCY OF EMS IN PIGEONPEA (Cajanus cajan (L.) Millsp.)” accepted for publication in the Bioscience Discovery tentatively in volume 2 No. 1 (Jan. 2011), subject to the conditions noted below:

1) Your manuscript need revision, hence we have returned the manuscript for revision. Your immediate attention is drawn; otherwise, further delay is inevitable.
2) You are requested to reply all the comments raised by the referee (enclosed).
3) You and your coauthors are requested to send the subscriptions, as all authors must be subscribers. Payment of subscription is a prerequisite for early processing of your paper. (already paid ignore this)
4) The papers are published in a chronological order on first come, first served basis, since there is an accumulation of reviewed, edited and processed papers.

Thanking you for your interest in the journal.

With warm regards,

Yours Sincerely,
Dr. Umesh P. Mogle,
Editor

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The supply will commence on receipt of full subscription in advance. The amount mentioned in the letter can be paid directly into our account, Dr. U. P. Mogle A/C No. 52000902227 in State Bank of Hyderabad (1015) where core banking facilities are available. Add core banking charges extra Rs. 25.00. You may avail this facility. Inform us the amount and date for account purpose. or
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Regd. No. MAHENG12607
ISSN 2229 - 3489
Appendices Page 186

“Mutation Breeding of Pigeon Pea [Cajanus cajan (L.) Millsp] for Yield Contributing Traits”, Presented in National Seminar at Baramati (MS), India.

“A Novel, High Yielding Pigeonpea Mutant”, Presented in State level Conference at Radhabai Kale Mahila Mahavidyalaya, Ahmednagar (MS), India.
“Induction of a Novel, High Yielding Mutant in Pigeonpea” Presented in National Conference at Pravaranagar (MS), India.

“Induction of a Novel, Vegetable type Indeterminate Mutant in Pigeonpea”, Presented in National Conference at Jijamata College Bhende (MS), India.

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