

MUTAGEN SENSITIVITY OF TWO PIGEONPEA CULTIVARS TO ETHYL METHANE SULPHONATE AND GAMMA RAYS

P. BALARAMA SWAMY YADAV and V. PADMAJA*

Department of Botany, Andhra University, Visakhapatnam - 530 003, India.

* e-mail : vpadmaja@rediffmail.com

Two cultivars of Pigeon pea (*Cajanus cajan* (L.) Millspaugh) were treated with different doses of γ -rays and EMS alone and in combination. Seedling and mature plant parameters were studied in M_1 and M_2 generations. The cultivars viz. ICPL 93115 and ICPL 93117 showed dose dependency for germination, survival, seedling, as well as for mature plant parameters in the mutagen treated population (M_1). On the other hand, combination treatments (15 Kr + 0.1% EMS) showed pronounced effects rather than in treatment with individual mutagens. Both the mutagens in general and EMS in particular appear to exert teratogenic properties manifested in the form of morphological abnormalities of the seedlings in the two varieties majority of which attain normal phenotype as the seedlings grow. Further, frequencies of deleterious effects of the two mutagens were more in ICPL 93117 variety compared to ICPL 93115.

Keywords : *Cajanus cajan* (L.) Millsp.; Induced mutagenesis; Mutagen treatments; Seedling variants.

Introduction

The role of mutagens in influencing the plant characters is well established^{1,2}. Studies on mutagen sensitivity of pigeon pea cultivars reported by earlier workers revealed amenability of the material for mutation induction^{3,4}. The present report presents a systematic evaluation of the mutagen treated population based on a number of parameters with particular reference to abnormalities at the seedling stage with the objective of arriving at an effective treatment schedule. Such information would be of use for obtaining useful mutants in the pigeon pea breeding programmes.

Materials and Methods

Two short duration, determinate type varieties of *Cajanus cajan* (L.) Millspaugh., viz., ICPL 93115 and ICPL 93117 obtained from Genetic resources unit, ICRISAT, Patancheru, A.P., India were used for mutagen treatments. Dry seeds of the two cultivars were exposed to three doses of γ -Rays (15, 25 and 35 Kr) at a dose rate of 5 Kr per minute from ⁶⁰Co source of gamma chamber '900' and three doses of EMS (0.1, 0.2 and 0.3%) besides a combination treatment of 15 Kr + 0.1% EMS. 200 seeds treated for each of the doses were sown in field plot of the experimental farm to raise M_1 generation and the untreated seeds sown to serve as controls. From each of the treated and control lines, 10-25 plants were selected at random for collecting selfed seed for raising M_2 generation.

Germination data was recorded on 5th day after sowing and seedling survival on 15th and 30th days. Data on height of the seedlings and leaf number were collected on 30th day. Height was measured from seedling base to

the tip of the last formed leaf, and leaf number was counted, excluding the cotyledonary leaves.

Results and Discussion

i. *Germination and survival* : A general delayed germination and dose dependent decrease in germination percentage was recorded in the mutagen treated material. Seedling survival values on 30th day after sowing were far less compared to the corresponding values on 15th day both in γ -ray and EMS treatments. Both germination and survival appear to be drastically affected in combination treatment compared to the corresponding treated lines with individual mutagens. Seedling survival values in the two varieties on 30th day were 65.52% and 51.22% of the control values and were much inferior compared to the corresponding values of either mutagen.

ii. *Seedling height and leaf number* : The parameters of seedling height and leaf number on 30th day, indicate a general reduction in the height of the seedlings in 35 Kr treatment compared to 15 Kr treatment (Table 1). The ICPL 93117 recorded drastic effect, compared to ICPL 93115. With respect to the number of leaves on the 30 days old seedlings, γ -ray treated ICPL 93117 variety showed only marginal changes. However, in case of EMS treatment, inferior values were recorded for both the parameters.

iii. *Morphological variants on 30th days* : At seedling stage, the treated populations showed chlorophyll deficiencies in the form of light green and yellowish green cotyledonary leaves, majority of which could be restored to normalcy after few days. Variation in the morphology

of cotyledonary leaves such as change in the color, texture, shape and size were recorded in the M_1 generation. Some of the prominent abnormalities recorded on 30 days old seedlings are as follows.

- a) *Thick, dark green, spotted cotyledonary leaves* : This was the most frequently encountered anomaly recorded in γ -ray, EMS and in combination treatments in both ICPL 93117 and ICPL 93115. Frequency of this anomaly was more in 0.3% EMS treatment for both the varieties compared to that of 0.2% EMS treatment.
- b) *Crumpled cotyledonary leaves* : The anomaly showing light green cotyledonary leaves with irregular margin due to shriveled condition was recorded only in 25 Kr dose of γ -ray irradiation.
- c) *Emarginate cotyledonary leaves* : The size of the cotyledonary leaves was reduced with emarginate leaf tip and the growth rate was same as that of the rest of the seedlings in the seedbeds observed at a lower frequency (5%) in 35 Kr γ -ray irradiation in both the varieties.
- d) *Linear cotyledonary leaves* : This anomalous type showing linear, pale green cotyledonary leaves was located in the combination treatments of both the varieties, the frequency of which was more (5%) in ICPL 93117 compared to that of ICPL 93115 (3%).
- e) *Partial whitening of the cotyledonary leaves* : One out of 70 seedlings of 0.3% EMS treated ICPL 93115 was found to be having one half of the cotyledonary leaf with normal green color and the second half albinic. However, the subsequent leaves were normal.
- f) *Minute cotyledonary leaves* : Two seedlings from 0.2% EMS treatment of ICPL 93115 variety and 0.1% EMS treatment of ICPL 93117 variety showed much reduced cotyledonary leaves, which however produced normal leaves subsequently.
- g) *Altered cotyledonary leaf shape* : Two seedlings of 0.3% EMS treated ICPL 93115 variety showed oval shaped cotyledonary leaves.
- h) *Shriveled trifoliate leaves* : This anomaly was recorded in EMS treatments of both the varieties and in combination treatment of ICPL 93115 variety wherein the shriveled nature of the leaves persisted for about 30 days after germination and further leaves born by these plants were apparently normal.
- i) *Deficient and dissimilar leaflets in trifoliate leaves* : Two seedlings with dissimilar leaflets were located in combination treatment in ICPL 93117. The two lateral leaflets were dissimilar in their shape and size. About 8-10 leaves present on 30th day were showing this abnormality. In very few seedlings one of the three leaflets was found to be absent.

Mature plant characters

For assessing the relative mutagen-sensitivity of the two

varieties of pigeon pea, quantitative estimates were made for a total of 10 parameters at the mature plant stage. Plant height, stem perimeter, number of primary branches, number of days to flower, size of the standard petal, pollen fertility, pod size, number of chambers per pod, number of seeds per pod and 100 seed weight were estimated by selecting 10 plants at random for each treatment.

All the mutagen treated population of both single and combination treatments showed a general reduction in each of the parameters. However, plant height in all the treated ones showed an increase over control values with one or two exceptions in EMS treated ICPL 93117 variety. The γ -ray treated ones showed delayed flowering compared to controls, while in the case of EMS treatment ICPL 93115 variety showed early flowering and ICPL 93117 variety showed delayed flowering compared to the corresponding controls. The combination treatment showed delayed flowering in both the varieties. Pollen sterility showed uniform decrease in all the treated ones. The reduction in fertility was drastic in combination treatment. The pod and seed parameters were not uniform and they showed lesser as well as greater values in the treated ones.

Morphological variants isolated from mature M_1 plants

- a) *Dwarfs* : Two plants in M_1 mature plants of 0.3% EMS treatment in ICPL 93115 were of dwarf stature. They were of about 50 cm height, late flowering with small pods and seeds.
- b) *Tall* : Five M_1 plants of 25 Kr γ -ray irradiated population in the ICPL 93115 variety were conspicuously tall compared to controls. They were about 250 cm in height while the controls were only about 160 cm.
- c) *Early flowering* : Ten plants in M_1 generation in 35 Kr γ -ray treated population of ICPL 93117 variety flowered 15 days earlier than the controls.
- d) *Late flowering* : Three plants in 0.3% EMS treated ones of ICPL 93115 variety flowered late by about 20 days compared to the controls.
- e) *Small leaflets* : Seven plants in M_1 generation in the case of 35 Kr γ -ray irradiation of ICPL 93117 variety were characterized by diminution in the breadth of leaflets. Pods and seeds were of reduced size.

Delayed germination recorded in the treated seeds especially at higher concentrations of EMS for both ICPL 93115 and ICPL 93117 may be due to mitotic delay. A clear decrease in the rate of germination in all the samples treated with EMS indicates the damaging effects induced, upon exposure to the mutagen. Venkateswarlu *et al.*⁵ reported toxic effects of EMS on germination and survival of EMS on germination and survival of the pigeon pea cultivars.

Table 1. 30th day seedling height and leaf number of mutagen treated population in M₁ and M₂ generations.

Mutagen	Dose/Conc.	Generation	Seedling height (in cm) ±S.E.		Leaf Number±S.E.	
			ICPL 93115	ICPL 93117	ICPL 93115	ICPL 93117
Control	-	M ₁	26.60±0.664	24.13±0.757	10.79±0.206	9.79±0.264
γ-rays	15Kr	M ₁	28.32±0.521	24.25±0.682	10.85±0.193	9.76±0.178
	25Kr	M ₁	29.60±0.537	23.76±0.757	11.90±0.204	9.74±0.278
	35Kr	M ₁	26.67±0.567	22.17±0.643	11.01±0.206	9.17±0.278
EMS	0.1%	M ₁	25.38±0.474	18.34±0.732	10.38±0.430	8.13±0.139
	0.2%	M ₁	24.47±0.695	15.84±0.866	9.45±0.280	7.44±0.330
	0.3%	M ₁	23.32±0.884	16.67±0.848	9.30±0.310	6.98±0.276
γ-rays+EMS	15 Kr+0.1%	M ₁	24.08±0.524	16.82±0.724	10.19±0.223	7.61±0.276

S.E. = Standard Error ; ± values indicate standard errors.

Germination, survival, seedling height and number of leaves showed a significant dose dependent reduction in the treated lines. A similar trend was noted by Shrivastava⁶ while working with γ-ray irradiation (15 to 60 Kr) on diploid and tetraploid pigeon pea cultivars. Higher doses of γ-rays as well as EMS concentrations greatly affected the biological parameters studied in the present study. The reduction was greater in the cultivar ICPL 93117 than ICPL 93115. Chopde⁷ reported 50% reduction in germination in several varieties when irradiated at 20 Kr dose. The increasing doses of mutagens caused a progressive increase in the biological damage measured in terms of different parameters at mature plant stage. The differences in radiosensitivity between the two cultivars of *C. cajan* towards the same dose of irradiation could be due to the differences in their genetic make up as was also observed by earlier workers^{4,5,8}.

References

- Gustaffson A 1947, Mutation in agricultural plants. *Hereditas* **33** 1-100.
- Gaul H 1965, Mutation in plant breeding. *Radiation Botany* **5** 155-232.
- Pawar S E, Thakare R G and Joshua DC 1978, Relative biological effectiveness and Mutagenic efficiency of fast neutrons in *Cajanus cajan*. *Indian J. Expt. Biol.* **16** 656-659.
- Kumar P and Sinha S S N 1989, Effect of gamma radiation on plant attributes in two cultivars of *Cajanus cajan* and two species of *Moghania*. *Legume Research* **12** 115-122.
- Venkateswarlu S, Singh R M and Reddy L J 1980, Induced mutagenesis in Pigeonpea with gamma-rays Ethyl methane sulphonate and hydroxyl amine. *Proceedings of the International Workshop on Pigeonpeas*. 15-19 Dec., ICRISAT Center, India **2** 67-73
- Shrivastava M P 1975, Effect of gamma irradiation on diploid and tetraploid seeds of *Cajanus cajan* (L.) Millsp. *Curr. Sci.* **44** 167-168.
- Chopde P R 1969, Mutagenic effects of X-rays on *Cajanus cajan* (L.) Millsp. *Proceedings of the Symposium on Radiations and Radiomimetic substances in Mutation Breeding*. BARC, Bombay. pp. 394-403.
- Venkateswarlu S, Singh R M, Singh R B and Singh B D 1978, Radiosensitivity and frequency of chlorophyll mutations in Pigeonpea. *Indian Journal of Genetics & Plant Breeding* **38** 90-94.