Theoretical and Applied Genetics 40, **257**-**260** (1970) © by Springer-Verlag 1970

Mutagenicity of Radiations and Chemical Mutagens in Sorghum*

K. SREE RAMULU

Division of Cytogenetics & Plant Breeding, Agricultural College and Research Institute, Coimbatore (India)

Summary. A comparative study on the relative mutagenicity of radiations (X- and gamma rays) and chemical mutagens (EMS, MMS, dES and NEU) in inducing chlorophyll, viable and total mutations was made on three cultivated varieties (Co. 11, Co. 12 and Co. 18) of Eu-Sorghum. A critical comparison of the chlorophyll, viable and total mutations shows that the mutation rate increased with an increase in dose up to a certain dose level beyond which the saturation effect was observed. EMS was found to be the most potent mutagen in inducing chlorophyll, viable and total mutations. The treatments with NEU (viable mutations) and dES (viable and total mutations) also proved to be effective. In a large number of cases the relationship between chlorophyll and total mutations was more close than that of viable to total mutation rates.

Induced mutagenesis has become an important tool for the plant breeder with the expansion in knowledge of techniques for inducing mutations and of the mutation process itself. The success of an induced mutation breeding program depends on the isolation of mutants which will be useful either directly or indirectly. This can be achieved by employing a wide range of physical and chemical mutagens under diverse conditions of treatment. Chemical mutagens, in particular alkyl alkane sulphonates, have been shown to induce a higher frequency of mutations when compared with radiations and other chemical mutagens. The present study was undertaken to investigate the relative mutagenicity of different radiations and chemical mutagens in producing chlorophyll, viable and total mutations in three cultivated varieties belonging to Sorghum subglabrascens and S. durra of Eu-Sorghum.

Material and Methods

The material comprised two varieties (Co. 12 and Co. 18) of Sorghum subglabrascens and one (Co. 11) of S. durra of (series, Sativa) Eu-Sorghum. The mutagens used were two physical mutagens (X- and gamma rays) and four chemicals, ethyl methane sulphonate (EMS), methyl methane sulphonate (MMS), diethyl sulphate (dES) and N-nitrosoethyl urea (NEU).

Dry seeds with a moisture content of 11 per cent were treated with X- and gamma rays at a room temperature of 26 ± 1 °C. A Philips X-ray machine, operated at 50 KV delivering the dose rate of 500 R/sec., was used for X-irradiation. Gamma ray treatments were given from a 3500 Curie ⁶⁰Co Gamma Cell delivering the dose rate of 3200 R/min. The solutions of *EMS*, *MMS* and *dES* were prepared in phosphate buffer pH 7, while NEU was in water solution. The seeds that were pre-soaked in water for 4 h. were treated for 8 h. with the chemicals. In treatments with *dES*, the solution was changed every 30 min. The seeds soaked in buffer or water were used as control. After treatment, the seeds were washed for 30 min. and were sown in the field.

Chlorophyll mutations were scored for 8 to 15 day old seedlings. Scoring of viable mutations was done continuously over the entire life cycle of the plants. In calculating total mutation rates on M_1 plant basis, a family was counted as a segregating one if it segregated either for a chlorophyll or a viable mutation. Even if a family segregated for both chlorophyll and viable mutations it was counted as one segregating family in calculating the total mutation rate. In calculating the total mutation frequency on M_2 plant basis, the number of M_2 plants mutated for chlorophyll and viable mutations was added.

Results

Chlorophyll mutations: Chlorophyll mutations recorded in the present study include albina, viridis, xantha, chlorina, striata, maculata, tigrina and alboviridis. A dose-dependent relationship for mutation frequency was observed up to 50 KR in X-ray treatments and 20 KR in treatments with gamma rays. In treatments with chemical mutagens, as well as a linear relationship, a saturation effect was evident in all the three varieties (Figs. 1-3). The order of potency of mutagens in inducing the chlorophyll mutations as measured by the frequency at dose for 75% survival was as follows:

 M_1 plant basis: EMS > dES > Gamma rays > NEU > X-rays > MMS

 $M_{\rm 2}$ plant basis: $EMS > dES > {\rm Gamma\ rays} > X- {\rm rays} > MMS > NEU$

EMS was found to be ten times more potent than NEU and MMS. Next to EMS, dES and gamma rays proved to be equally potent.

Viable mutations: Viable mutations include those affecting the morphology of different parts of the plant such as habit, stature, leaf, stem, panicle and spikelets. A critical comparison of the viable mutations shows that the mutation rate increased with an

^{*} This work forms part of the thesis submitted for Ph. D. degree of Univ. of Madras.

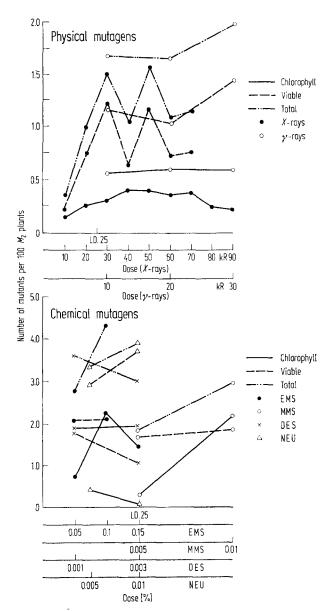


Fig. 1. Frequency of chlorophyll, viable and total mutations in M_2 of Co. 11

increase in dose up to a certain dose level, and at higher doses it levelled off in all the three varieties in treatments with radiations and chemical mutagens (Figs. 1-3). The order of potency of mutagens in inducing viable mutations as measured by the frequency at dose for 75% survival was as follows:

 M_{1} plant basis: dES > NEU > EMS > Gammarays > X-rays > MMS

 M_2 plant basis: NEU > EMS > MMS = dES > X-rays > Gamma rays

The mutagens NEU (M_1 and M_2 plant basis), EMS (M_2 plant basis) and dES (M_1 plant basis) were found to be more effective in inducing viable mutations than the rest.

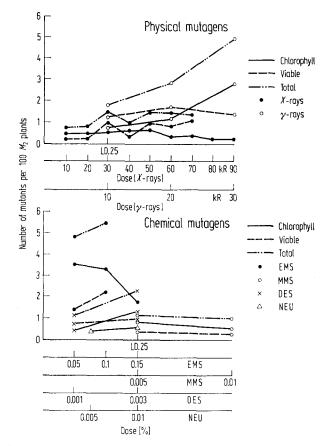


Fig. 2. Frequency of chlorophyll, viable and total mutations in M_2 of Co. 12

Total mutations: The frequency of mutations increased up to a certain dose level beyond which it decreased (Figs. 1-3). The order of potency in inducing total mutations as measured by the frequency at dose for 75% survival was as follows:

 M_1 plant basis: dES > EMS > Gamma rays > NEU> MMS > X-rays

 M_2 plant basis: EMS > NEU > Gamma rays > dES > X-rays > EMS

EMS (M_2 plant basis) and dES (M_1 plant basis) induced the maximum frequency of total mutations.

Discussion

Chlorophyll mutations: A linear relationship of mutation frequency with dose was observed in treatments with gamma rays, MMS and dES only in the variety Co. 11. A linear or exponential dose relationship was obtained in a series of experiments after irradiation and EMS treatments in barley (Gaul, 1964) and after X-irradiation in rice (Yamaguchi, 1964, Fujii, 1962). A saturation effect was observed in all the three varieties at high doses in treatments with radiations and chemical mutagens. Matsuo (1962) and Kawai (1962) in rice, Wellensiek (1965) in Vol. 40, No. 6

peas, Hildering (1963) in tomato, Ehrenberg and Nybom (1954) in barley reported a saturation effect after mutagenic treatments. 'Saturation point' was attributed to the rigour of both diplontic and haplontic selection in the biological material (Swaminathan, 1961). The low potency of NEU and MMS was due to their high toxicity. In contrast, with a high survival percentage, EMS treatments produced a high frequency of chlorophyll mutations (Sree Ramulu, 1968).

Viable mutations: The frequency of viable mutations showed not only a linear relationship with dose but also a saturation effect. Wellensiek (1965) reported that in peas the frequency of mutations increased with increasing dose of EMS and the increase was rather regular but not large in gamma ray treatments, while for X-rays, viable mutations occurred only in the highest doses. The phenomenon of saturation at high doses was also recorded by Kawai (1962) in the rate of morphological mutations by Xray treatment of florets. NEU and EMS were twice as efficient as the physical mutagens. Wellensiek (1965) working on peas reported that EMS yielded approximately five times more mutations than did radiations.

Total mutations: As in the case of chlorophyll and viable mutations, as well as a linear relationship, a saturation effect was evident at high doses. EMS and dES were found to be the most potent in inducing total mutations.

NEU was found to be highly potent in inducing the frequency of viable and total mutations and also the most effective as estimated by the scheme suggested by Konzak *et al.* (1965), though the rate of chlorophyll mutations and mutagenic efficiency were lower when compared with other mutagens (Sree Ramulu, 1968).

Relationship between the chlorophyll, viable and total mutations: The rate of chlorophyll mutations increased with an increase in dose of gamma rays, MMS and NEU, when computed on both M_1 and M_2 plant basis, while the frequency of viable mutations showed a decrease at high doses. The spectrum of chlorophyll mutations was the widest in C0.18, while C 0.11 showed the widest spectrum of viable mutations. These findings emphasize that the occurrence of chlorophyll and viable mutations are different events and are independent of each other. Gaul (1964) and Rao and Natarajan (1965) working on barley have expressed similar views. It is also evident that in a large number of cases, the relationship between chlorophyll and total mutations was more close than that of viable to total mutation rates (Figs. 1 - 3).

Acknowledgement

I am grateful to Dr. B. W. X. Ponnaiya for his guidance and to Dr. M. S. Swaminathan for suggestions. My thanks

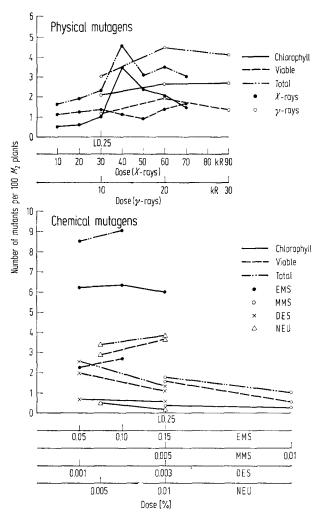


Fig. 3. Frequency of chlorophyll, viable and total mutations in M_2 of Co. 18

are due to Dr. P. Madhava Menon, Dr. S. R. Sree Rangasamy, Dr. E. A. Siddiq and Mr. V. G. Nair for their help and to Council of Scientific and Industrial Research and Indian Council of Agricultural Research for the award of research fellowships.

Literature cited

1. Ehrenberg, L., Nybom, N.: Ion density and biological effectiveness of radiation. Acta Agric. Scand. 4, 396 to 418 (1954). - 2. Fujii, T.: Radiosensitivity in plants. V. Experiments with several cultivated and wild rices. Jap. J. Breed. 12, 131-136 (1962). - 3. Gaul, H.: Mutations in plant breeding. Rad. Bot. 4, 155-232 (1964). -4. Hildering, G. J.: The mutagenic effect of ethylene imine on the tomato. Euphytica 12, 113-119 (1963). -5. Kawai, T.: The present status of mutation research and breeding in rice in Japan. IRC Newsletter 11, 10-22 (1962). -6. Konzak, C. F., Nilan, R. A., Wagner, J., Foster, R. J.: Efficient chemical mutagenesis. Symp. on use of induced mutations in Plant Breeding (FAO/IAEA, Rome) 49-70 (1965). - 7. Matsuo, T., Yamaguchi, H.: Review of research on use of radiation induced mutations in crop breeding in Japan. Euhytica 11, 245-255 (1962). - 8. Rao, R. N., Natarajan, A. T.: Mutagenicity of some alkyl 260

.

alkane sulfonates in barley. Mutation Res. 2, 133-148 (1965). - 9. Sree Ramulu, K.: Studies on induced mutations in the genus *Sorghum* (Moench.). Thesis submitted for Ph. D. degree of Univ. of Madras (1968). - 10. Swaminathan, M. S.: Effect of diplontic selection on the frequency and spectrum of mutations induced in polyploids following seed irradiations. Symp. Effects of ionizing radiation on seeds and significance for crop impro-

vement. (IAEA, Vienna), 279-288 (1961). - 11. Wellensiek, S. J.: Comparison of the effects of EMS, neutrons, gamma and X-rays on peas. Symp. Use of induced mutations in Plant Breeding (FAO/IAEA, Rome) 227-235 (1965). - 12. Yamaguchi, H.: Genetic effects of pile radiations in rice. Symp. Biological effects of neutron and proton irradiations I. (IAEA, Vienna) 371 to 382 (1964).

Received October 22, 1969

Communicated by Å. Gustafsson

Dr. K. Sree Ramulu Division of Cytogenetics and Plant Breeding Agricultural College and Research Institute Coimbatore (India)