Induced Structural Changes and Meiotic Aberrations in *Sorghum*¹

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In mutation studies cytological analysis with respect to either mitotic or meiotic behaviour is considered to be one of the most dependable indices to estimate the potency of a mutagen on the one hand and mutagen-response of a genotype on the other. A great supply of chromosome breaks is required as a tool in the production of genetic variation in crop plants. Induction of structural rearrangements is one of the ways of achieving new recombinations, rarely obtained by more conventional methods. In the genetic and cytogenetic analysis of the induced mutants segmental interchanges have been extensively used as markers of different chromosome segments. In the present investigation a detailed study on the types of chromosome association and the frequency and type of other nuclear and nucleolar anomalies after treatments with a wide range of radiations, chemical mutagens and combination treatments has been made in three cultivated varieties of *Sorghum*.

Materials and methods

The material comprised two varieties (Co. 12 and Co. 18) of *Sorghum subglabescens* and one of (Co. 11) *S. durra* of (Series *Sativa*) *Eu-Sorghum*. The mutagens consisted of two physical (X- and gamma rays) and four chemical mutagens, ethylmethane sulphonate (EMS), methyl methane sulphonate (MMS) diethyl sulphate (DES) and nitroso ethyl urea (NEU) and their combination treatments.

For X-irradiation dry seeds with a moisture content of 11% were treated with a Philips X-ray machine operated at 50 KV delivering at an exposure rate of 500 R/sec., while the treatments with gamma rays were given with a ⁶⁰Co gamma cell of 3500 Ci delivering at an exposure rate of 3200 R/min. at room temperature of $26 \pm 1^\circ\text{C}$. The treatments with EMS, MMS and DES were given in phosphate buffer maintained at a pH of 7, while NEU treatments were given in aqueous solution. The seeds that were pre-soaked for 4 hr were treated with chemicals for 8 hr with continuous shaking, the volume of the mutagen being 10 times that of seeds. In case of DES treatments alone,

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the chemical solution was changed every 1/2 hr and freshly prepared solution was added. For combination treatments, the dry seeds treated with X- or gamma rays at required doses were soaked in chemical solutions for 8 hr as mentioned above for different chemical treatments. The treatment temperature was maintained at $26 \pm 1^\circ\text{C}$. The seeds soaked in water or buffer were kept as control. After the treatment, the seeds were washed for 1/2 hr in running water and sown in field.

The cytological observations were made from 20 randomly selected M_1 plants per treatment treated with different mutagens and from the control plants. Young panicles were fixed in 1:3 acetic alcohol between 9 and 10 a.m. The materials were kept for 24 hr in the fixative and afterwards transferred to 70% alcohol. Pollen mother cells were scored at diakinesis and MI for chromosomal interchanges and other nuclear and nucleolar anomalies like laggards, bridges multinucleoli and micronuclei.

Observations

Scoring of pollen mother cells largely at diakinesis and MI showed the occurrence of a variety of chromosomal aberrations of which reciprocal translocations were more frequent than the rest (Tables 1 to 3). The common

Table 1. Effect of physical and chemical mutagens on chromosome association in M_1 of Co. 11

Treatments, dose in KR/%	No. of PMCs scored	Chromosome association mean (Range)			Percentage PMCs with IVs
		IV	II	I	
Control	90	—	10.00	—	
X-rays 30	121	0.02 (0-1)	9.64 (8-10)	0.60 (0-4)	2.47
60	108	0.10 (0-1)	9.14 (6-10)	0.60 (0-6)	14.81
EMS 0.05	95	..	9.98 (9-10)	0.02 (0-2)	..
0.15	202	..	9.98 (9-10)	0.03 (0-2)	..
MMS 0.005	108	0.01 (0-1)	9.82 (8-10)	0.20 (0-4)	1.85
DES 0.003	160	0.05 (0-1)	9.71 (8-10)	0.06 (0-2)	6.20
NEU 0.01	160	0.03 (0-1)	9.85 (8-10)	0.10 (0-4)	3.12

types of translocations observed were those having ring and rod configurations (Figs. 3 and 4). A positive correlation between the percentage of MI plants carrying chromosomes with interchanges and the dose of mutagens was evident from the data (Tables 1 to 3). The order of potency in inducing plants with chromosomal interchanges at biologically comparable LD. 25 survival was as follows :

Gamma rays > X-rays > NEU > DES > EMS > MMS

Thus, the comparative analysis as to the efficiency of physical and chemical mutagens to produce interchanges indicated the former to be more potent as compared to the chemical mutagens. Further, the data revealed that the combined treatments did not lead to any additive effect (Table 3). The range

of translocations varied from 0-2 per PMC. No quadrivalents were noticed in treatments with EMS in Co. 11, with DES and MMS in Co. 18 and with DES in Co. 12, while NEU induced interchanges in all the three varieties

Table 2. Effect of physical and chemical mutagens on chromosome association in M_1 of Co. 12

Treatments, dose in KR/%	No. of PMCs scored	Chromosome association mean (Range)			Percentage PMCs with IVs
		IV	II	I	
Control	84	—	10.00	—	
X-rays 30	178	0.04 (0-1)	9.46 (8-10)	0.44 (0-4)	4.49
60	187	0.06 (0-1)	9.57 (9-10)	0.21 (0-2)	6.95
EMS 0.05	192	..	9.93 (9-10)	0.12 (0-2)	..
0.15	186	0.01 (0-1)	9.93 (9-10)	0.12 (0-2)	1.61
MMS 0.005	174	0.01 (0-1)	9.88 (8-10)	0.18 (0-2)	1.72
DES 0.003	171	..	10.00	..	..
NEU 0.01	173	0.08 (0-1)	9.52 (8-10)	0.27 (0-4)	8.09

Table 3. Effect of radiations, chemical mutagens and combined treatments on chromosome association in M_1 of Co. 18

Treatments, dose in KR/%	No. of PMCs scored	Chromosome association mean (Range)			Percentage PMCs with IVs
		IV	II	I	
Control	98	—	10.00	—	—
X-rays 30	142	..	9.93 (9-10)	0.12 (0-2)	..
60	112	0.08 (0-1)	9.65 (6-10)	0.39 (0-8)	8.92
Gamma rays 10	124	0.10 (0-1)	8.56 (8-10)	1.06 (0-4)	11.22
20	145	0.12 (0-1)	8.45 (8-10)	1.29 (0-4)	13.79
40	111	0.14 (0-1)	7.84 (7-10)	1.81 (0-6)	14.41
EMS 0.05	225	0.01 (0-1)	8.18 (7-10)	0.37 (0-6)	0.44
0.15	209	0.07 (0-2)	8.17 (8-10)	0.09 (0.2)	5.74
MMS 0.005	138	..	9.95 (9-10)	0.81 (0-2)	..
DES 0.003	162	..	9.92 (9-10)	0.14 (0-2)	..
NEU 0.01	152	0.07 (0-1)	9.76 (8-10)	0.15 (0-2)	7.89
Gamma rays +EMS 20+0.05	154	..	9.99 (9-10)	0.18 (0-2)	..
Gamma rays +DES 10+0.003	121	0.01 (0-1)	9.92 (9-10)	0.08 (0-2)	1.65
X-rays+NEU 40+0.01	160	..	9.95 (9-10)	0.10 (0-2)	..
60+0.01	125	..	9.92 (9-10)	0.16 (0-2)	..

revealing varietal differences with respect to the mutagens in inducing chromosome breakages.

The effect on disturbances in meiosis as judged by the association of

chromosome was measured as the mean number of univalents, bivalents and multivalents per cell. The data (Tables 1 to 3) did not show any relationship between the type of association and the dose of X- and gamma rays and EMS in varieties Co. 11 and Co. 12, while the relative frequencies of different associations was found to vary with increase in dose in Co. 18. It is, thus, evident that in Co. 18 with increase in dose of physical mutagens and EMS there was a proportionate increase in the frequency of quadrivalents and univalents (Fig. 6), while the frequency of bivalents was found to decrease with increase in dose.

Figs. 1-6. 1, diakinesis: early disjunction of a bivalent. $\times 1,120$. 2, diakinesis: two nucleoli. $\times 1,784$. 3-4, diakinesis: 1 chain quadrivalent and 8 bivalents. $\times 2,064$ and $1,544$. 5, metaphase I: 10 bivalents (Control). $\times 1,304$. 6, metaphase I: early disjunction of 2 bivalents. $\times 1,120$.

Meiotic anomalies other than translocations comprised mostly laggards, bridges, tripolar spindles, micronuclei and multinucleoli (Figs. 8-12). The percentage of PMCs with abnormalities as observed from both the reduction and equational phases of meiosis were on the increase with increasing dose in all the three varieties (Table 4). The order of potency of mutagens in inducing such meiotic disturbances at LD. 25 survival was as follows:

X-rays > NEU > MMS > EMS > DES

The PMCs from M_1 plants treated with gamma rays were, however not

scored for the other meiotic anomalies. From these results, it is evident that X-rays induced the maximum frequency of PMCs carrying meiotic anomalies. The maximum number of laggards per cell induced by MMS was to the extent of 0.21 at 0.005% followed in order by X-rays, EMS, DES and NEU. Bridges were noticed only in treatments with X-rays and MMS. Tripolar spindles were exclusively observed in X-ray treatments in all the three varieties at telophase I and telophase II, the mean number being 0.03 per cell. Micro-

Figs. 7-12. 7, anaphase I: 10/10 separation (Control). $\times 1,304$. 8, anaphase I: 9/9 separation and 2 lagging chromosomes. $\times 1,000$. 9-12, anaphase I: varying lagging chromosomes. $\times 1,808$ and $\times 1,320$.

nuclei in microspore tetrads were recorded at 60 KR of X-rays and at 0.15% of EMS treatment, the mean number being 0.02 in both the cases. While a single well stained nucleolus was observed in all the PMCs of control plants, cases were noticed in the treated plants where two nucleoli of equal size or one of them reduced in size were present.

In such cases, the nucleolar organiser was associated with only one of the nucleoli. The PMCs with two nucleoli were observed frequently in

60 KR of X-rays in Co. 12, 0.15% of EMS in Co. 11 and 0.01% of NEU treatments in Co. 18 with a mean of 0.02 in all the cases.

Table 4. Meiotic disturbances in M_1 plants treated with physical and chemical mutagens

Treatments, dose in KR/%	No. of PMC's scored	Meiotic disturbances in first and second division mean (Range)						
		Laggards	Bridges	Tripolar spindles	Extra- bodies	Micro- nuclei	Two nucleoli	Cells with distur- bances (%)
Co. 11								
Control	131	..	..	..	..	..	..	..
X-rays 30	115	0.09 (0-2)	0.03 (0-1)	..	..	..	..	9.56
60	121	0.17 (0-5)	0.08 (0-2)	0.03 (0-1)	..	..	..	18.18
EMS 0.05	109	0.02 (0-1)	..	..	..	..	..	1.83
0.15	101	0.05 (0-1)	..	..	..	..	0.02 (0-1)	4.95
MMS 0.005	119	0.09 (0-2)	0.02 (0-1)	..	..	..	..	9.24
DES 0.003	130	0.06 (0-3)	..	..	..	..	..	3.84
NEU 0.01	148	0.09 (0-3)	..	..	..	..	..	6.70
Co. 12								
Control	114	..	..	..	..	..	..	..
X-rays 30	117	0.13 (0-3)	0.02 (0-1)	0.03 (0-1)	..	..	..	12.80
60	123	0.18 (0-4)	0.05 (0-3)	0.03 (0-1)	..	..	2.02 (0-1)	17.07
EMS 0.05	108	0.06 (0-1)	..	..	..	..	..	5.55
0.15	121	0.17 (0-2)	..	..	..	0.02 (0-1)	..	10.74
MMS 0.005	114	0.13 (0-3)	..	..	..	..	..	10.53
DES 0.003	130	0.14 (0-2)	..	..	..	..	..	10.00
NEU 0.01	135	0.09 (0-2)	..	..	0.08 (0-2)	..	..	14.07
Co. 18								
Control	112	..	..	..	..	..	..	..
X-rays 30	118	0.03 (0-1)	0.03 (0-1)	0.01 (0-1)	..	..	..	6.78
60	150	0.08 (0-2)	0.03 (0-1)	0.05 (0-1)	..	0.02 (0-2)	..	16.60
EMS 0.05	117	0.14 (0-1)	..	..	..	..	..	6.83
0.15	145	0.13 (0-5)	..	..	..	..	..	10.34
MMS 0.005	121	0.21 (0-6)	..	..	..	..	..	11.57
DES 0.003	140	0.03 (0-1)	..	..	..	..	..	2.87
NEU 0.01	133	..	..	..	0.24 (0-1)	..	0.02 (0-1)	14.28

Discussion

Cytological anomalies form one of the most dependable measures of estimation of the potency of various mutagens. The cytological effects which can be studied from mitosis as well as meiosis are largely traceable to chromosome breakages, of which translocations are the readily recognizable ones in plants with small chromosomes. The nature of the reunion may be the result of a simple translocation or reciprocal translocation of which the latter is more frequent and becomes manifest through ring or chain configurations. Among

the mutagens used in the present study radiations induced not only a greater frequency of interchanges per cell but also a higher percentage of PMCs with quadrivalents than treatments with chemical mutagens. Chemical mutagens are known to produce chromosomal aberrations less frequently than radiations (Heiner *et al.* 1960, Konzak *et al.* 1961). Mitotic studies by Natarajan and Upadhyaya (1964) also revealed that alkylating agents such as EMS cause chromosome breakages. The dependence of frequency of translocations on dosage noticed in the present studies is in agreement with the findings of Caldecott *et al.* (1954). The data presented in Tables 1 to 3 reveal the differences among the three varieties in respect of response to the mutagens expressed as chromosome breakages in conformity with Hsieh's (1961) findings on varietal differences with respect to quadrivalents induced by X-rays and thermal neutrons in *Japonica* varieties of rice.

Besides translocations, other chromosomal anomalies such as laggards, bridges, fragments, tripolar spindles, cell with two nucleoli were observed. The treatment with X-rays induced the maximum meiotic disturbances and treatments with DES, the least. Chromatin bridges were noticed only in treatments with X-rays and MMS. Nilan and Konzak (1961) have reported that chromosome bridges accompanied, less frequently, with fragments occur at anaphase I in M_1 plants in the chemical treatments, indicating that these bridges may be due to inversions induced by the chemicals. Thus, the chemicals induced a higher frequency of intra-chromosomal type of aberrations, than radiations, presumably because of the characteristic delayed effect of the former on the chromosomes (Nilan and Konzak 1961). Tripolar spindles were exclusively observed in the present studies in X-ray treatments in all the three varieties at telophase I and telophase II. Nirula (1963) has also recorded spindle abnormalities in PMCs of neutron-treated *Sorghum vulgare*. In treated plants, two nucleoli of equal size or one smaller were observed, but the nucleolar organiser bivalent was associated with only one of the nucleoli. McClintock (1934) attributed the irregular organisation of nucleolus to extraneous agencies such as heat or radiation changes in the metabolic activity of cell and also to genotypic changes such as imbalance or loss of parts which by themselves do not contain any specific organisers.

Summary

Cytological observations were made in M_1 plants treated with radiations (X- and gamma rays), chemical mutagens (EMS, MMS, DES and NEU) and their combination treatments to study the effects on types of chromosome associations, the frequency and type of other nuclear and nucleolar anomalies in three cultivated varieties (Co. 11, Co. 12 and Co. 18) of *Eu-Sorghum*. Reciprocal translocations having ring or rod configuration occurred in higher frequency than the other types of structural changes. A positive correlation between the percentage of M_1 plants carrying chromosomes with interchanges

and the dose of mutagens was observed. The treatments with radiations induced not only a greater frequency of interchanges per cell but also a higher percentage of PMCs with quadrivalents than treatments with chemical mutagens. The effect on disturbances in meiosis as judged by the chromosome association was measured by the mean number of univalents, bivalents and multivalents per cell. The combined treatments did not lead to any additive effect in inducing chromosome breakages. Meiotic anomalies other than translocations comprised mostly laggards, bridges tripolar spindles, micronuclei and multi-nucleoli. The percentage of PMCs with meiotic disturbances was on the increase with increase in dose of the mutagens. Radiation treatments induced the maximum frequency of PMCs carrying meiotic anomalies as compared to treatments with chemical mutagens.

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