Cytogenetical Effects of Chemical Mutagens in Rice

T. V. V. Seetharami Reddi and V. R. Reddi

Department of Botany, Andhra University,
Waltair 530 003, India

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The volume of work carried out with chemical mutagens is comparatively less. Progress in the effective and efficient use of chemicals is hindered by complex interplay of many physical and chemical factors that determine the ultimate yield of mutations (Konzak *et al.* 1964). The degree of cytological aberrations either in mitosis or meiosis is regarded as one of the dependable criteria for estimating the effect of a mutagen. A variety of nuclear and nucleolar anomalies in meiosis has been reported in mutagen treated rice by several workers (Parthasarathy 1938, Oka *et al.* 1952, Nishimura 1957, Hsieh 1961, Chao and Chai 1961, Katayama 1963, Siddiq 1973, Reddi and Reddi 1975, Seetharami Reddi 1977 and Ramesh 1983). The present study is undertaken to obtain information of four chemicals in inducing chromosomal aberrations in three rice cultivars.

Materials and methods

Five hundred normal seeds of each of the three rice cultivars viz: T(N)1, IR 8 and Sona with 14% moisture content, presoaked for 12h in distilled water were treated with chemical mutagens, methyl methane sulphonate (MMS), dimethyl sulphate (DMS), dichloroethyl methane sulphonate (DEMS) and diethylsulphate (dES) at $28 \pm 1^\circ\text{C}$. The concentrations used were 0.03, 0.05 and 0.1 per cent by volume except in the case of DEMS in which 0.05, 0.1 and 0.2 were used. In view of the short half-life of dES, the solution was replenished for every 30 min. The seeds were continuously shaken in the mutagen solutions throughout the 12h period of treatment. As a post-treatment care, the seeds were washed in running water for 60 min, allowed to recover in fresh water for 3h and sown directly in seed beds along with untreated controls. Material for cytological examination was collected from individual plants. Fixation of panicles of suitable stage for meiotic study was done at 14°C for 24h in acetic alcohol (1: 4) after which the material was transferred to 70% alcohol. Smear preparations of anthers were made in 1% acetocarmine.

Results

i. Frequency of aberrations: In T(N)1 cytologically abnormal plants were detected in all the mutagenic treatments. In DMS the frequency of aberrant plants decreased with increase in dose whereas in dES a linear relationship was noticed (Table 1). In IR 8 only eight aberrant plants were observed in 0.03 and 0.1 DMS and 0.05 DEMS treatments. On the other hand, Sona responded positively. In

DMS 0.1 and dES 0.03, 40% abnormality in each was observed. No abnormal plants were observed in MMS and DMS 0.05 treatments.

Table 1. Frequency of cytologically aberrant plants in M_1

Mutagen	Dose	T(N)1			IR 8			SONA		
		Total plants screened	No. abnormal	%	Total plants screened	No. abnormal	%	Total plants screened	No. abnormal	%
Control		19	—	—	21	—	—	20	—	—
MMS	0.03	44	6	13.6	29	—	—	10	—	—
	0.05	40	2	5.0	8	—	—	6	—	—
	0.1	12	4	33.3	11	—	—	—	—	—
DMS	0.03	22	10	45.4	8	2	25.0	12	2	16.6
	0.05	12	4	33.3	8	—	—	6	—	—
	0.1	18	4	22.2	12	4	33.3	10	4	40.0
DEMS	0.05	16	6	37.5	10	2	20.0	12	4	33.3
	0.1	20	6	30.0	8	—	—	16	6	37.5
	0.2	30	12	40.0	8	—	—	8	2	25.0
dES	0.03	30	2	6.6	9	—	—	10	4	40.0
	0.05	14	2	14.2	8	—	—	10	2	20.0
	0.1	18	6	33.3	7	—	—	18	6	33.3

Table 2. Variety-wise frequency of cytologically abnormal plants in M_1

Variety	Total plants	No. abnormal	% abnormality
T(N)1	276	64	23.18
IR 8	126	8	6.34
Sona	118	30	24.52

Table 3. Combined frequencies of cytologically abnormal plants in the three varieties

Mutagen	Total plants	No. abnormal	% of abnormal plants
Control	60	—	—
MMS	160	12	7.5
DMS	108	30	27.7
DEMS	128	38	29.6
dES	124	22	17.7

When comparison was made by mutagen- and variety-wise, the highest frequency of aberrations was observed in DEMS in T(N)1 and Sona and DMS in IR 8. MMS treatments were ineffective in IR 8 and Sona. The frequency of aberrations induced in Sona was relatively more (24.5%) followed by T(N)1 (23.1%) and IR 8 (6.3%) (Table 2). When comparison was made with respect to mutagens alone on the three varieties put together DEMS showed highest aberration rate (29.6%) followed by DMS (27.7%), dES (17.7%) and MMS (7.5%) (Table 3).

Table 4 shows the percentage frequency of abnormalities recorded. They were classified broadly into i) budding of nucleoli, ii) persistent nucleolus, iii) bridges

and iv) other types (laggards, more rod bivalents, more ring bivalents, multipolar spindles, univalents, delayed disjunctions, fragments and suspected translocation heterozygotes). In T(N)1 nucleolar budding of 15.3% was observed in DMS followed by others. Persistent nucleolus at metaphase I, or anaphase I or telophase I showed highest frequency of 7.6% in DMS followed by dES, DEMS and MMS. Highest frequency of 9.0% bridges was found in DEMS followed by DMS and

Table 4. Frequency of cytological abnormalities

Mutagen	Nature of abnormality	T(N)1			IR 8			SONA		
		Total no.	Abnormal	%	Total no.	Abnormal	%	Total no.	Abnormal	%
Control	Nucleolar budding	20	—	—	20	—	—	21	—	—
	Pers. nucleolus		—	—		—	—		—	—
	Bridges		—	—		—	—		—	—
	Other types		—	—		—	—		—	—
MMS	Nucleolar budding	48	2	4.1	55	—	—	45	—	—
	Pers. nucleolus		1	2.0		—	—		—	—
	Bridges		3	6.2		—	—		—	—
	Other types		—	—		—	—		—	—
DMS	Nucleolar budding	52	8	15.3	56	8	14.2	56	4	7.1
	Pers. nucleolus		4	7.6		4	7.1		—	—
	Bridges		4	7.6		—	—		4	7.1
	Other types		2	3.8		—	—		4	7.1
DEMS	Nucleolar budding	66	6	9.0	78	6	7.6	72	16	22.2
	Pers. nucleolus		2	3.0		—	—		—	—
	Bridges		6	9.0		—	—		4	5.5
	Other types		10	15.1		—	—		4	5.5
dES	Nucleolar budding	62	4	6.4	44	—	—	38	6	15.7
	Pers. nucleolus		2	3.2		—	—		—	—
	Bridges		—	—		—	—		—	—
	Other types		4	6.4		—	—		6	15.7

MMS. The frequency of other types was 15.1% in DEMS followed by dES and DMS treatments. In IR 8, nucleolar budding and persistent nucleolus were seen in DMS and nucleolar budding only in DEMS. In Sona nucleolar budding was highest in DEMS (22.2%) followed by dES and DMS. Bridges were seen in DMS and DEMS and other types in DMS, DEMS and dES treatments.

ii. Cytological observations: The most common type of abnormality noticed in the meiotic cells involved the nucleolus. The number of nucleoli varied from two to many. In the multinucleolate cells, the size of the nucleolus decreased gradually with an increase in their number. Multinucleolar condition was observed at diakinesis and persistent nucleolar bodies of varying sizes were also recorded at metaphase I (Fig. 1), anaphase I, telophase I (Fig. 5), interkinesis and even during the second division of meiosis. Other abnormalities include lagging chromosomes at anaphase II (Fig. 6), bridges with or without fragments (Figs. 2, 4 and 7) and delayed separation at anaphase I (Fig. 3). Bridges appeared even at

anaphase II in DEMS 0.2 of T(N)1. At pachytene one or more chromosomes of individual cells showed detectable structural alterations. Unpaired or loosely paired regions, buckle formations suggesting deletions or duplications were relatively more common. Although interchanges were observed in DEMS 0.05 of Sona at a lower frequency in PMCs at diakinesis they could not be established as such at pachytene stage of meiosis.

Discussion

Although it is known that physical agents are most potent in inducing chromosome breaks when compared to chemicals, increased rate of point mutations are often recorded with the latter. Kawai and Sato (1965) reported the effect of ethylene imine on rice was about five times as high as the corresponding effects of X-irradiation. Siddiq (1973) obtained higher frequency of translocations in T(N)1 and Taichung 65 irradiated with physical agents when compared to treatments with EMS and NMU. Multinucleolate condition at diakinesis and persistent nucleoli at metaphase I, anaphase I, telophase I and even during the second division of meiosis are more commonly noticed in T(N)1 and Sona than in IR 8. Bridges without fragments are more common than those with bridge and fragment which is in agreement with the findings of Shastry and Ramiah (1961) and Siddiq (1973). In the present study, plants with interchanges are relatively few. Plants with single quadrivalent per PMC were recorded earlier by Shastry and Ramiah (1961) in rice variety NP 130 treated with physical mutagenes and ^{35}S . Chromosome bridges accompanied by fragments are less frequent than multivalents in all treatments. Pachytene analysis revealed small differential segments in all treatments as observed in the present study. Nucleolar anomalies such as multinucleolate cells and persistent nucleoli are of common occurrence in the mutagen treated population. Natarajan (1958) reported a similar phenomenon in maize following radiation treatment and Siddiq (1964) in colchicine treated *Sorghum*. Multinucleolate condition observed in the present study might be due to a particular genotypic change suppressing the organising capacity of nucleolar chromosome and induce the formation of adventitious nucleoli, as suggested by Mc Clintock (1934). Genotypic changes disrupting the orderly production and dispersion of RNA are evidently more common in the present study and are also reported earlier by Siddiq (1973). Other meiotic abnormalities such as cryptic alterations noted in the pachytene chromosomes, delayed separation and bridges and fragments at anaphase I and of higher incidence of rod bivalents and univalents indicates the kind of cytogenetic effects that chemicals of the present study are capable of inducing.

Figs. 1–7. 1, T(N)1, MMS 0.05 showing persistent nucleolus at metaphase I. $\times 1500$. 2, T(N)1, MMS 0.03 showing two bridges one with fragment and one without fragment at anaphase I. $\times 1500$. 3, T(N)1, MMS 0.03 showing delayed separation of chromosomes at anaphase I. $\times 1500$. 4, T(N)1, DMS 0.1 showing bridge with fragment on the midway at anaphase I. $\times 1500$. 5, T(N)1, DEMS 0.2 showing two buds of nucleoli in between, there is one laggard chromosome at anaphase I. $\times 1500$. 6, T(N)1, DEMS 0.2 showing laggards at anaphase II. $\times 1500$. 7, T(N)1, MMS 0.03 showing bridge with fragment at anaphase II (bridge connection is in out of focus). $\times 1500$.

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Summary

Dry, well filled seeds of rice varieties T(N)1, IR 8 and Sona were treated with chemical mutagens: methyl methane sulphonate (MMS), dimethyl sulphate (DMS), dichloroethyl methane sulphonate (DEMS) and diethyl sulphate (dES) with a view to study their effects in inducing chromosomal aberrations. DEMS showed highest aberration rate (29.6%) followed by DMS (27.7%), dES (17.7%) and MMS (7.5%). The frequency of aberrations induced in Sona was relatively more (24.5%) compared to T(N)1 (23.1%) and IR 8 (6.3%).

The most common type of abnormality noticed in the meiotic cells involved the nucleolus. Other abnormalities induced include cryptic alterations in the pachytene chromosomes, delayed separation and bridges and fragments at anaphase I and higher incidence of rod bivalents and univalents.

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