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Induced Mutations in Sorghum Improvement*

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Recent advances in sorghum improvement are mainly due to spontaneous mutations followed by selection and hybridization. Induced mutations received relatively limited attention. A critical review of different aspects of mutagen sensitivity, considering the importance of such factors as genotypic constitution of the material, pre- and post-treatment modifications, type of mutagen and dose, techniques of handling the material and treatment procedures to maximize the induction of mutations together with the scope of induced mutations in sorghum improvement constitute the subject matter of this paper.

Hydrazine was found to be a more effective and efficient mutagen for inducing chlorophyll and viable mutations in sorghum than ethyl methanesulphonate, methyl methanesulphonate or γ-rays. Ethyl methanesulphonate among the alkylating agents and nitroso methyl urea was among nitroso compounds were the most potent mutagens. The efficient radiation dose was within the 20-35 kr range, whereas 0·015M was the effective dosage for hydrazine and ethylmethane sulphonate. The combination treatments of various physical and chemical mutagens failed to yield significant increase in the recovery of mutations, while cysteine post-treatments of γ-irradiated and hydrazine-treated material reduced seedling injury, seed sterility and increased the recovery of viable mutations compared to single treatments. There is scope for induced mutations in solving some of the current problems of sorghum improvement such as, increasing the recombination potential of tropical × temperate crosses, improving the nutritional quality of grain and forage sorghums, diversification of male sterile cytoplasmic sources, better understanding of mechanism of apomixis and augmenting the levels of resistance to sorghum insects, pests and diseases.

Key Words: Sorghum mutations, Gamma rays, Mutation, Breeding, Sorghum improvement, Hydrazine, Cysteine, Post-treatments

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Introduction

Cultivated sorghums originated about 5000–7000 years ago or earlier in North East Africa, probably in Ethiopia or Sudan. After domestication these moved to other parts of Africa, India, China and Europe. Sorghum were introduced into the Western hemisphere during the 17th and 18th centuries.

Recessive mutations at the height and maturity loci of cultivated sorghums, which took place in the tropics, resulted in the evolution of dwarf, early and photo-insensitive forms. Intensive selection and isolation of such forms, however, took place in the USA. Superimposed on the dwarf and early stature were the recessive male sterility genes (*msc*$_1$ and *msc*$_2$) which, interacting with *milo* cytoplasm resulted in cytoplasmic-genetic male sterility that laid the foundation for the development of commercial hybrid sorghums, a source of major yield advance.

Thus, while spontaneous mutations in cultivated sorghum furnished the basis for the recent advances in sorghum improvement, the work on induced mutations has not been generally rewarding. This paper presents a comprehensive review of the work on induced mutations in sorghum, identifies some of the current problems in sorghum improvement and discusses the possible role of induced mutations in providing solutions.

Mutational Studies in Sorghum—Present Status

As early as 1934, Ayyangar and Rao treated pollen grains of sorghum with X-rays. By 1942, Quinby and Karper reported several deleterious mutations from seeds exposed to X-rays and inferred that improvement of sorghum through induced favourable mutations may not be promising. Since then, there have been several studies on induced mutations in sorghum, but not with measurable success.

Several investigators have employed various physical agents such as X-rays, γ-rays, thermal neutrons and chemical mutagens such as methyl methanesulphonate (MMS), ethyl methanesulphonate (EMS), diethyl sulphate (DES), 1:2, 3:4 diepoxybutane (DEB), ethyleneimine (EI), 1-4 bisdiazo-acetylbutane (BDB), 1-methyl-3-nitrosoguanidine (NG), N-methyl-N-nitrosourea (NEU), hydrazine (HZ) and colchicine in their attempts to induce mutations in sorghum. Also various combinations of these physical and chemical mutagens and pre- and post-treatments with cysteine (CS) of γ-irradiated and hydrazine-treated material have been used to increase the mutation frequency and to attain an altered mutation spectrum in sorghum.

Mutagenic effects in M_1 generation

(a) *Plant injury and sterility:* Para-sorghum species were found to be more sensitive to γ-rays and X-rays than Eu-sorghum species. For Eu-sorghum species LD_{50} based on seed germination in the laboratory was 63.68 Kr for X-rays and 65.16 for γ-rays, while for Para-sorghum species the corresponding values were 16 Kr and 26.06 Kr respectively (Nirula 1963). Critical doses of γ-rays based on 50% sterility in M_1 generation were between 20 and 30 Kr for Redalan-60 sorghum variety and between 40 and 50 Kr for *Shallu* (Harris et al. 1965). Reddy (1977) found LD_{50} value of γ-rays in Texas-414, as 63.4 Kr in vitro and 38.6 Kr in vivo (21 days after the treatment). This could be

attributed to the fact that many seeds which meet the criteria of germination in the laboratory i.e., extrusion of radical and plumule, may be damaged too severely to sustain growth required from soil emergence.

X-rays showed greater effects on stigmas than on pollen germination (Ayyangar & Rao 1934). Nirula (1963, 1964) found X-rays to be more effective than γ-rays in causing reduction in survival, however the latter were more potent as regards seedling growth reduction, on the other hand, Sree Ramulu (1970a) reported X-rays as less effective in causing reduction not only in survival and seedling growth, but also in fertility compared to γ-ray treatments in *Sorghum durra* and *S. subglabrescens*. The relative biological efficiency of different radiations varies according to linear energy transfer of radiations.

MMS caused higher toxic effects on seed germination, survival and seedling growth than EMS, DES and NEU (Sharma 1965, Sree Ramulu 1970a, Reddy 1977). This agrees with the view that among alkylating agents, methyl groups are relatively more toxic than other chemicals, since they rapidly methylate the phosphate and purine bases and cause deploymerisation of DNA. Hydrazine showed low toxic effects (based on low seedling injury and sterility in M_1 generation) than EMS (table 1). Sree Ramulu (1971c) found DEB as more effective in reducing the percentage seed germination, survival, growth of root, coleoptile and seedlings than NMU and NG.

The combination treatments of γ-rays, EMS, MMS and DES caused less than additive effects in the reduction of survival or seedling growth or seed fertility (Sree Ramulu 1973). Reddy (1977) found that combined treatments of γ-rays, HZ and EMS reduced seed germination more than additively, while less than additive effects were observed in reducing the length of primary root, coleoptile and seedling height in M_1 generation. Fertility reduction and survival was more than additive in combination treatments of HZ and EMS, and less than additive in all combination treatments of γ-ray and hydrazine (table 1).

Cysteine by itself had no toxic effects on either seed germination or seedling injury (table 2). Post-treatments with cysteine reduced seedling injury and sterility caused by γ-irradiation of sorghum seeds (Reddy & Smith 1978a, 1978b). In HZ-treated sorghum, post-treatment with cysteine reduced seedling injury, while both pre- and post-treatments reduced seed sterility in M_1 generation (table 3). The most probable mode of action of cysteine on HZ-treated material of sorghum seed could be attributed to the scavenging of excess of mutagen when given as pre- or post-treatment. Cysteine reacts with the excess of mutagens resulting in different reaction byproducts, which by themselves are generally devoid of any biological activity (Reddy & Smith 1979). Also cystine post-treatments may reduce the effects of diplontic selection by scavenging out free radicles produced by γ-rays and HZ, which cause cellular death, thus reducing the seedling injury and sterility in HZ and γ-ray treatments.

(b) *Cytological effects:* X-rays and thermal neutrons were reported to induce chromosome breaks in root tip cells and bridges and fragments in PMC's of *S. bicolor* (Kaukis & Webster 1956). Treatments of Eu- and Para-sorghum species with thermal neutrons induced one quadrivalent per PMC.

Table 1 *Mutagenic effectiveness and effieciency of Hydrazine (HZ), Ethyl methanesulphonate (EMS) γ-rays and their combinations in grain sorghum*

Mutagenic Treatments	Injury (I) %	Sterility (S) %	No. of M_1 Panicles analysed	No. of M_2 seedlings analysed	Mutation events 100 M_1 Panicles Msp	Mutation events 100 M_2 Seedlings Msd	Mutagenic effectiveness Msp/tC(Kr)	Mutagenic efficiency Msp/I	Mutagenic efficiency Msp/S
Borate buffer	—	—	114	5,700	—	—	—	—	—
0.005M HZ	21.59	7.83	159	7,950	20.13	1.37	1.68	0.93	2.57
0.010M HZ	32.57	8.02	93	4,650	25.81	1.91	1.07	0.79	3.22
0.015M HZ	39.01	9.33	75	3,750	37.33	3.44	1.04	0.96	4.00
0.020M HZ	51.51	15.99	87	4,350	33.33	3.50	0.69	0.65	2.08
0.030M HZ	58.71	22.15	19	950	26.31	2.21	0.36	0.45	1.19
Water (Control)	—	—	449	22,450	—	—	—	—	—
0.005M EMS	17.64	14.45	52	2,600	15.38	1.73	1.28	0.87	1.06
0.010M EMS	29.94	20.37	131	6,500	18.32	2.15	0.76	0.61	0.90
0.015M EMS	44.92	32.11	83	4,150	24.10	2.77	0.67	0.54	0.75
0.020M EMS	49.73	51.03	25	1,250	20.00	2.24	0.42	0.40	0.40
0.030M EMS*	67.91	—	0	0	—	—	—	—	—
0.005M HZ+0.005 M EMS	3.79	88.26	62	3,100	20.97	1.45	0.87	5.53	0.24
0.005M EMS+0.005 M HZ	20.83	78.85	90	4,500	23.33	1.80	0.97	1.12	0.30
0.010M HZ+0.010 M EMS	34.09	93.33	4	200	25.00	2.50	0.52	0.73	0.27
0.010M EMS+0.010 M HZ	50.00	94.17	14	700	28.57	2.86	0.60	0.57	0.30
20 Kr TX—414	30.97	31.24	428	21,400	18.46	0.97	0.92	0.60	0.59
35 Kr	38.36	59.81	258	12,900	22.87	1.43	0-65	0.60	0.38
50 Kr	56.82	79.64	117	5,850	25.64	2.03	0.51	0.45	0.32
20 Kr+0.005 M HZ	28.79	23.40	122	6,100	20.49	1.14	0.08	0.71	0.88
20 Kr+0.010 M HZ	51.89	27.73	127	6,350	23.62	1.38	0 05	0.45	0.85
35 Kr+0.005 M HZ	47.35	48.54	54	2,700	23.63	1.85	0.06	0.60	0.49
35 Kr+0.010 M HZ	62.00	57.54	35	1,750	25.71	2.51	0.03	0.41	0.45
20 Kr NM—31	51.00	44.95	253	12,650	22.53	1.68	1.13	0.44	0.50
35 Kr	60.60	67.39	184	9,200	25.00	2.30	0.71	0.41	0.37
50 Kr	70.53	87.10	55	2,750	29.09	2.98	0.58	0.41	0.33

*None of the treated plants survived in the field until maturity at this concentration

I = % Seedling height reduction (% of control)

S = % Reduction in seed set (% of control)

TC = The product of treatment time × initial concentration of the mutagen

Kr = γ-radiation dose in kilorads

Table 2 *Effects of cysteine (CS) on seed germination and seedling growth in the M_1 generation of sorghum*

Treatments	Seed germination %	Length of primary root (cms) Mean ± S.E.	Length of coleoptile (cms) Mean ± S.E.	Seedling height (cms) Mean ± S.E.
Phosphate buffer (control)	100	3.14±0.09 a*	2.13±0.08 a	3.06±0.17 a
0.005 M CS	101	3.23±0.09 a	2.04±0.05 ab	3.41±0.06 a
0.010 M CS	100	3.23±0.07 a	1.91±0.07 b	3.32±0.09 a

* Means followed by the same letter are not significantly different by Duncan's Multiple Range test at the 5% level of probability

Trivalents, univalents, B-chromosomes and binucleate microsporocytes were observed in Para-sorghum species and tripolar spindles in Eu-sorghum species (Nirula 1963, 1964). In γ- and X-irradiated material of *S. durra* and *S. subglabrescens* a higher frequency of translocations and univalents was observed than in treatments with EMS and DES.

X-rays also induced a higher frequency of laggards, bridges, tripolar spindles than chemical mutagens (Sree Ramulu 1971a). Spraying of atrazine induced chromosome abnormalities in Redlan, K–33 and Martin (Liang & Liang 1972). Reddy and Bhaskar Rao (1975) isolated γ-ray induced interchange heterozygotes in sorghum varieties, IS 3941 and IS 2566. They found that translocation breakpoints were fairly well spread out in several of the chromosomes both in heterochromatin and euchromatic regions. Among the reverted diploids of the irradiated tetraploid sorghum variety, IS 3949, Bhaskar Rao and Reddy (1975b) found chimeral plants in which some spikelets showed normal meiotic behaviour while others showed rings or chains of four chromosomes at diakinesis and reduction in the chiasma frequencies.

Mutagenic effects in M_2 generation

(a) *Chlorophyll mutations:* In sorghum, chlorophyll-deficient mutants have been observed spontaneously and their spectrum was similar to that found in barley and maize (Karper & Conner 1931). Most of the chlorophyll mutants are recessives and may be lethal. These mutants are often used for the assessment of mutagenic efficiency of various agents. Chlorophyll mutants such as *albino*, *pinkish-albino viridis*, *xantha*, *Chlorina*, *albo-viridis*, *albo-xantha*, and *zebra* or *striata* types have been observed in M_2 following various mutagenic treatments (Goud et al. 1970, Harris et al. 1965, Kaukis & Reitz 1955, Nirula 1963, 1964, Reddy & Smith 1976, Reddy 1977, Sharma 1965, Sree Ramulu 1970a, 1970e). Kaukis and Reitz (1955) found a higher frequency of *albino* mutants with thermal neutrons than with X-rays. However, Haensel (1960) and Ross et al. (1961) did not obtain any chlorophyll mutants in sorghum variety Experimental-3 by EI and γ-radiation treatments. Varietal differences in the induction of chlorophyll mutations have been observed with γ-rays (Goud et al. 1970, Harris et al. 1965, Reddy & Smith 1975, Reddy 1977, Sree Ramulu 1970e). A wider spectrum of

Table 3 *Mutagenic effectiveness and efficiency of hydrazine (HZ) following cysteine (CS) pre- and post-treatments in grain sorghum*

Mutagenic treatments	Injury (I) %	Sterility (S) %	No. of M_1 panicles analysed	No. of M_2 seedlings analysed	Mutation events/ 100 M_1 panicles Msp	Mutation events/ 100 M_2 seedlings Msd	Mutagenic effectiveness Msp/tC	Mutagenic efficiency Msp/I	Mutagenic efficiency Msp/S
0.01M CS	—	—	171	8,550	—	—	—	—	—
0.01M HZ	32.57	8.02	93	4,650	25.81	1.91	1.07	0.79	3.22
0.01M HZ+0.01 M CS	7.21	4.41	125	6,250	22.40	1.95	0.93	3.20	5.08
0.01M CS+0.01 M HZ	39.67	6.17	211	10,550	28.40	2.08	1.18	0.70	4.61
0.02M HZ	51.51	15.99	87	4,350	33.33	3.50	0.69	0.65	2.08
0.02M HZ+0.01 M CS	36.07	8.41	222	11,100	24.77	2.16	0.69	0.69	2.94
0.01M HZ+0.02 M HZ	57.38	13.08	178	8,900	28.09	2.54	0.49	0.49	2.14
0.03M HZ	58.71	22.15	19	950	26.31	2.21	0.36	0.45	1.19
0.03M HZ+0.01 M CS	59.67	9.75	123	6,150	30.89	2.02	0.52	0.52	3.16
0.01M CS+0.03 M HZ	70.16	10.08	31	1,550	32.26	2.58	0.46	0.46	3.20

I = % Seedling height reduction (% of control)
S = % Reduction in seed set (% of control)
tC = The product of treatment time × initial concentration of the mutagen

chlorophyll mutations was observed in M 35–1 than in GM–2–3–1 with γ-radiation (Goud et al. 1970). Reddy (1977) found a wider spectrum of chlorophyll mutants and a higher frequency of *albino* mutants in NM–31 than in TX–414 with γ-rays.

EMS was found to be the most potent mutagen and induced the highest frequency of chlorophyll mutations followed by DES, γ-rays, MMS and NEU on M_2 plant basis when comparisons were made at LD_{25} for survival. The low potency of MMS and NEU was due to their higher toxicity (Sree Ramulu 1970a). NMU was more efficient in inducing a higher and broader spectrum of chlorophyll mutations than NG in *S. subglabrescens* (Sree Ramulu 1972).

We have observed hydrazine to be more efficient in inducing higher frequency and broader spectrum of chlorophyll mutations than γ-rays and EMS (table 1). *Albo-viridis* and *striata* types were observed only in HZ treatments, whereas *albino viridis*, *xantha* and *chlorina* types were induced by both chemicals and γ-rays (table 4.) various studies show that the *viridis* type occurs more frequently in all the mutagen treatments. The relative frequency of *albino* mutants was highest in EMS followed by HZ and γ-rays in that order, while *xantha* and *chlorina* types were more frequent in HZ than in EMS or γ-treatments (table 4).

In the combination treatments of γ+HZ, HZ+EMS or EMS+HZ, the chlorophyll mutation frequencies were less than additive (table 1). Similarly γ+MMS and γ+DES produced less than additive mutation frequencies but γ+EMS treatments induced an additive mutation frequency (Sree Ramulu 1973). Recurrent treatments with NMU induced a higher frequency and wider spectrum of chlorophyll mutations than single treatments (Sree Ramulu 1971c).

No chlorophyll mutations were observed in treatments with cysteine alone (Reddy & Smith 1978b). Broader spectrum of chlorophyll mutations was observed in CS pre- and post-treatments of HZ-treated and γ-irradiated material (table 4). *Striata* and *albo-viridis* mutants were observed in CS post-treatments of γ-irradiated material while they were not observed in γ-ray treatments alone. *Albo-xantha* was observed only in CS pre- and post-treatments of HZ-treated material. The relative proportions of *albino*, *xantha* and *chlorina* were increased in CS post-treatments of γ-irradiated material and that of *viridis* type decreased compared to γ-ray treatments alone.

(b) *Morphological mutations:* Viable mutations reported so far include those affecting plant height, maturity, panicle type, midrib colour, presence of awn, grain colour, grain size, grain type, spikelet type and tillering (Chanchal Kapoor 1967, Goud et al. 1970, Gurusiddaradhya & Goud 1977, Harris et al. 1965, Nirula 1964, Quinby & Karper 1942, Rao et al. 1969, Reddy & Smith 1976, Reddy 1977, Sree Ramulu 1975). The mutants tolerant or resistant to shootfly attack, drought-tolerant types (Goud et al. 1970, Usman & Goud 1972) and also mutants with high lysine and protein content (Mohan 1973) have been isolated in sorghum following various mutagenic treatments.

A higher frequency and broader spectrum of morphological mutants was recovered from HZ (2.14%) than EMS (0.81%) and γ (0.42%) treatments. The total viable mutation frequency was more than additive in γ+HZ (3.22%) while it was less than additive in sequential treatments of EMS+HZ (0.09%)

Table 4 *Relative proportions of different types of chlorophyll mutations recovered in M_2 generation of sorghum following various mutagenic treatments*

Mutagen treatment	Total no. of M_2 seedlings analysed	Total chlorophyll mutations %	Frequency of chlorophyll mutations (%)						
			albino	*viridis*	*xantha*	*chlorina*	*striata*	*albo-viridis*	*albo-xantha*
HZ	22,650	28.60*	10.33 (93)**	48.23 (232)	14.14 (63)	13.72 (63)	1.45 (7)	3.12 (15)	—
EMS	14,550	19.45	35.67 (117)	45.73 (180)	12.19 (40)	6.40 (21)	—	—	—
Gama rays	40,150	22.32	8.94 (44)	76.00 (377)	9.35 (46)	5.08 (25)	—	—	—
Gama + HZ	24,400	24.82	17.42 (73)	57.76 (242)	15.03 (63)	8.11 (34)	—	1.67 (7)	—
EMS + HZ	5,200	25.95	29.00 (29)	39.00 (33)	24.00 (24)	9.00 (9)	—	—	—
HZ + EMS	3,300	22.99	24.00 (12)	48.00 (24)	18.00 (9)	10.00 (5)	—	—	—
HZ + CS	23,500	26.02	15.29 (74)	59.30 (287)	25.50 (75)	5.16 (25)	—	—	4.75 (23)
CS + HZ	21,000	29.59	16.50 (79)	62.63 (300)	14.40 (69)	—	1.46 (7)	2.09 (10)	4.39 (21)
γ + CS	19,700	28.16	23.10 (76)	39.51 (130)	17.32 (57)	12.46 (41)	1.52 (5)	6.08 (20)	—

** Parenthetic numbers identify the number of independent mutations of each class which were recovered

* Chlorophyll mutations based on % M_1 segregating panicles. Mean percentage of Mutations of all treatments is given

and no mutation was observed in HZ+EMS treatments (table 5). With CS post-treatments of γ-irradiated material a higher frequency and broader spectrum of viable mutants (6.75%) were recovered compared to that of γ-rays alone (table 5). Similar observations were made with CS Pre- (7.4%) and post-treatments (3.12%) of HZ-treated material compared to HZ alone (Reddy & Smith 1976, Reddy 1977). Sree Ramulu (1970e) compared the potency of different mutagens for inducing viable mutations at LD_{25} for survival and found NEU to be the most potent, followed by EMS, MMS, DES, X-rays and γ-rays in that order.

In TX-414 a higher frequency and broader spectrum of viable mutants were recovered (0.42%) than in NM-31 (0.08%) with γ-ray treatments (Reddy 1977). Similarly Rao et al. (1969) observed that among a number of tall indigenous varieties with partly exposed internodes, only Ujjain-6 yielded mutants of brachytic dwarfs with varying maturities, while a number of medium tall Indian varieties with nodes enclosed by leaf sheaths yielded dwarf and early maturing mutants when treated with radiations and chemicals. Goud et al. (1970) found M 35-1 to be highly mutable than GM 2-3-1 both with regard to frequency as well as spectrum of viable mutations.

Reddy (1977) obtained a number of mutants simulating the characters of other species particularly those affecting the grain, spikelet and panicle types (table 5) The system proposed by Harlan and de Wet (1972) was used in classifying the spikelet type mutants. TX-414 possesses *durra* type spikelets while *caudatum*, *kafir*, *guinea* and wild types were isolated following various mutagenic treatments. Induced systematic mutants have been reported in rice (Siddiq & Swaminathan 1969) and sorghum (Chanchal Kapoor 1967, Sree Ramulu 1975, Reddy 1977). The recovery of high frequency of systematic mutants in the present study reflects the evolutionary process which has taken place in sorghum. According to Doggett (1964, 1965) cultivated sorghums seems to have developed from a single wild species through disruptive selection. The occurrence of high frequency of mutants resembling races of *S. bicolor* ssp. *bicolor*, other than that used in the present study suggest that racial differentiation proceeded through a series of independent mutations affecting grain and spikelet characteristics, either independently or stepwise in evolution of cultivated varieties of sorghum from wild to cultivated, brought together under the influence of disruptive selection.

(c) *Relationship between chlorophyll and viable mutations:* No relationship was observed between chlorophyll-deficient and morphological mutations in the present study. Though a higher frequency of chlolorophyll mutations could be recovered from all treatments, only certain agents induced a higher frequency of viable mutations (tables 4 & 5). A higher frequency of chlorophyll and low frequency of viable mutations were observed in NM-31, whereas reverse was the case in TX-414 (Reddy 1977). Similarly, Sree Ramulu (1970a) working with the cultivated varieties, C_o-11, C_o-12 and C_o-18 found that the frequency of chlorophyll mutations was highest in C_o-18, while C_o-11 showed the widest spectrum. The occurrence of chlorophyll and viable mutations are thus different events and are independent of each other (Gaul 1964).

Table 5 *Frequencies (number) of induced morphological mutations recovered in the M_2 generation of sorghum following various mutagenic treatments*

Mutagen treatments	Frequency of viable mutations (%)									Total viable mutations %	Ratio of viable/ chlorophyll mutations
	Grain mutations				Glume type	Panicle type	Awned	Tall	White Midrib		
	Color	Shape	Type	Size							
HZ	1.07 (12)*	0.67 (7)	—	0.45 (5)	—	—	—	—	—	2.14	0.07
EMS	0.36 (4)	—	—	0.27 (3)	0.18 (2)	—	—	—	—	0.81	0.04
γ rays	0.13 (8)	0.14 (8)	0.02 (1)	0.05 (3)	0.02 (1)	0.02 (1)	0.02 (1)	0.02 (1)	—	0.42	0.02
γ + HZ	0.88 (12)	0.74 (11)	0.45 (6)	0.15 (3)	0.60 (9)	0.15 (3)	0.10 (2)	0.05 (1)	0.10 (2)	3.22	0.13
EMS + HZ	0.09 (1)	—	—	—	—	—	—	—	—	0.09	0.13
HR + EMS	—	—	—	—	—	—	—	—	—	—	—
HZ + CS	1.03 (7)	0.30 (2)	0.15 (1)	0.15 (1)	0.59 (4)	0.30 (2)	0.30 (2)	0.15 (1)	0.15 (1)	3.12	0.12
CS + HZ	2.06 (14)	1.48 (10)	1.04 (7)	0.44 (3)	0.74 (5)	0.60 (4)	1.04 (7)	—	—	7.40	0.25
γ + HZ	2.25 (43)	1.45 (29)	1.40 (28)	0.25 (5)	0.60 (12)	0.35 (7)	0.30 (6)	0.10 (2)	0.05 (1)	6.75	0.24

* Parenthetic numbers identify the number of independent mutations of each class which were recovered

Factors affecting Recovery of Induced Mutations

Induction of mutations at the gene level and their subsequent recovery are two independent processes. The probability that an induced mutation will survive and give rise to a mutant at the level of organism is controlled by many factors, the important ones are listed below :

(*a*) *Genotypic constitution :* Differential genotypic response of sorghum varieties was reported following treatments with radiations and chemical mutagens (Goud et al. 1970, Harris et al. 1965, Nirula 1963, 1964, Reddy 1977, Sree Ramulu 1975). Para-sorghum species (2n=10) were more sensitive to radiations and chemicals than Eu-sorghum ones (2n=20) with respect to M_1 parameters (greater reduction in survival, seedling growth and seed fertility), but yielded low frequency of chlorophyll or morphological mutations (Nirula 1964, Sree Ramulu 1975). Para-sorghum species have relatively longer chromosomes than the Eu-sorghum ones. The plants with longer chromosomes and low diploid number are reported as more sensitive to mutagen treatments than those with higher number of smaller chromosomes (Sparrow et al. 1963). Cytogenetical investigations have pointed out that Para-sorghum section is distinctly different from Eu-sorghum with regard to speciation (Magoon et al. 1964).

Several studies indicate that differences in the quantity of the genetic material, nuclear volume, interphase chromosome volume, heterochromatin content, undefined differences in the constitution of genes or gene system and cytoplasm as well as several other factors may be responsible for inter-varietal differences to radiation sensitivity (Evans & Sparrow 1960, Sparrow & Chrinstensen 1953). The genome make-up and/or genetic constitution of the species is more important in realising the gene mutations following mutagenic treatment.

(*b*) *Type of mutagen and dose:* Effectiveness of a mutagen usually means the number of mutational events per unit of mutagen dose and the efficiency refers to the mutational rate in relation to the undesirable effects such as seedling injury and sterility.

Mutagenic effectiveness and efficiency of γ-rays decreased with increasing concentrations. (table 1). These were higher in NM-31 than in TX-414. Among the various treatments tried by us, the 20 Kr treatment was the most efficient as measured by mutation frequencies adjusted for seedling injury and seed fertility.

Mutation frequencies reached peak at 0.015M concentration and then on decreased with increasing concentrations of HZ and EMS (table 1). This phenomenon of saturation effect was also reported in comparisons of chlorophyll, viable, and total chlorophyll mutation frequencies of EMS, MMS, DES, NEU, γ-rays and X-rays (Sree Ramulu 1970e). Gradual inactivation of the repair system with increasing doses of mutagens thereby inducing high lethality and sterility could be responsible for this decreased tendency after the peak mutation rate was reached. Saturation effect was also attributed to the rigor of diplontic and haplontic selections in the biological material (Swaminathan 1961). The injurious effects at increasing concentrations increase at faster rates than gene mutations (Konzak et al. 1965).

Hydrazine was found to be more effective and efficient mutagen in inducing chlorophyll and morphological

mutations in sorghum than EMS and γ-rays (Reddy 1977). HZ was also reported to be an efficient mutagen in rice (Reddy 1972, Reddy et al. 1973, 1974) and tomato (Jain et al. 1968). The differential action of HZ and EMS on DNA molecule may be responsible for the production of higher frequency and broader spectrum of Chlorophyll and morphological mutations with HZ than EMS. Toxic effects of HZ are due to peroxides and free radicles while the mutagenic action is due to its direct action on DNA, specifically with thymine or uracil (Freese 1963). The inhibitory effect of EMS on seedling growth depression has been attributed to its hydrolytic products namely, ethyl sulphuric acid and ethyl alcohol (Konzak et al. 1965), while the mutagenic action is due to its direct action on DNA molecule, specifically by the addition of an alkyl group to the purine, guanine (Freese 1963). It also acts indirectly by making sugar phosphate backbone sensitive to breakage which can lead to further genetic damage.

(c) Combination treatments: Mutation yield and efficiency of mutagenic treatments can be considerably increased by manipulating the secondary factors such as methods of pre- and post-treatments or by combining various mutagenic treatments. In sorghum, combination treatments of γ-rays, EMS, DES, MMS and HZ, in general, produced lower efficiency values as compared to the sum of these produced by mutagens individually (Reddy 1977, Sree Ramulu 1975). A synergistic effect was observed in the recovery of viable mutations with γ-rays+HZ treatments (table 5).

The synergistic effects would be produced following post-irradiation chemical mutagen treatment, when the chemical mutagen besides inducing potential lesions, may also render the repair enzymes non-functional (Roberts & Warwick 1957) thereby promoting the fixation of already induced pre-mutational changes or when the access of alkylating agents to vulnerable sites in DNA may be increased by denaturation of DNA at sites of reaction due to pre-irradiation.

The higher dose, interaction of mutagens in increasing the toxicity, inhibition of uptake of the mutagens, sequence of treatments as well as number of other factors are presumed to be responsible causes for less than additive effects observed in combination treatments in the present study. Also it is quite probable that high seed sterility and low survival of plants in combination treatments masked the recovery of mutations. The results suggest that it is the recovery of mutations rather than their induction that determines whether less than additive or additive or synergistic effects will be obtained from the combination treatments.

(d) Pre- and post-treatments with cysteine: An increase in the mutagenic effectiveness and efficiency based on seedling injury was resulted from CS post-treatments of γ-irradiated material (Reddy & Smith 1978b) compared to γ-rays alone. The mutagenic effectiveness was increased with CS post-treatments of HZ-treated material compared to HZ alone. Similarly, the mutagenic efficiency based on sterility (Msp/S) also increased with CS pre- and post-treatments of HZ-treated material compared to HZ alone (table 3).

The increased recovery of chlorophyll and viable mutations in CS pre- and post-treatments of HZ-treated and γ-irradiated material can be explained as follows. Two types of selections are known to intervene before a mutation

induced in a seed is expressed in the M_2 generation. The first is the diplontic selection occurring in M_1 somatic tissues and the second is haplontic selection operating at gametic level (Swaminathan 1969). Only a mutation which passes through both somatic and gametic sieves will find phenotypic expression in the M_2 and subsequent generations. In γ-irradiated and HZ-treated populations, though a large number of mutations may be induced, relatively few pass through diplontic selection sieve because of intracellular death caused by free radicles. Thus, relatively few chlorophyll and viable mutations were recovered in the M_2 generation following γ-irradiation and HZ treatments. CS post-treatments reduce the effects of diplontic selection by scavenging free radicles which cause cellular death, as observed by reductions in the seedling injury and sterility. This would increase the probability of induced mutations to be transmitted and identified in the subsequent generations. If this presumed effect upon diplontic selection is correct, it suggests that the mutagenic efficiency of ionizing radiation and chemical mutagens can be greatly enhanced by post-treatment with radio-protective agents like cysteine.

Colchicine-induced Mutations

Polyploids have been induced in grain sorghum by colchicine treatments (Chin 1946, Murty et al. 1978, Schertz 1969). In general autotetraploid grain sorghums are characterised by slow growth, dark green foliage with wavy margins, short but stouter straw, larger grains, greater protein content and higher degrees of sterility. Another cytological feature of autotetraploid sorghum is the lower frequency of univalents and trivalents (Murty & Rao 1973). Low seed set in auto-tetraploids makes them of limited use in sorghum breeding. However, Doggett (1964) has shown that there is a scope for fertility improvement through crossing autotetraploids of different genotypes and also by repeated back crossing to *Sorghum almum* to incorporate its high seed setting. Differences in fertility between autotetraploids have been ascribed to variation in chromosome behaviour at meiosis and to genetic differences (Morrison & Rajathy 1960a). Rao and Murty (1972) have suggested that tetraploid sorghum from apomictic sorghum strains, may give better seed sets than sexual ones, since meiosis is circumvented in apomicts.

Murty et al. (1978) induced autotetraploids in apomictic grain sorghum R 473 by treating with colchicine, which had higher seed fertility than earlier reported ones (Chin 1946, Schertz 1962) with low percentage of univalents and quadrivalents. Absence of meiosis in the formation and development of embryo sac from diploid cells of nuclear origin, autonomous development of the endosperm and the diploid nature of the young endosperm accounts for high seed set (Murty et al. 1978). Also they explained that the genetically controlled regular segregation at A 1 and the increased chaisma frequency of the tetraploid may also be responsible for the regular disjunction of the chromosomes resulting in higher seed set.

In addition to polyploidizing effect following colchicine treatment, Franzke and Ross (1952) and Ross et al. (1954) found diploid mutants in *Sorghum bi-color*, variety Experimental-3, which bred true for many changed characters (height, stem diameter, leaf width, seed size and yield). They explained that

colchicine treatment caused the formation of haploid chromosome complement by somatic reduction followed by doubling to restore the original diploid number. Simaental and Ross (1963) have taken the formation of 10 bivalents in F_1 between the haploid and diploid mutants, as well as the presence of unlinked mutated genes, as evidence that entire chromosome complement resulted from the reduction process. Sanders and Franzke (1962) and Chen and Ross (1963a) have shown somatic reduction involving all the chromosome pairs by the induction of diploid mutants following treatments of tetraploid seedlings of Experimental-3 with colchicine. Beraho and Olembo (1971) also reported the isolation of two diploid mutants from colchicine treatments—one was tall mutant and other chlorophyll-deficient mutant.

Diploid mutants from colchicine treatment do not occur always. Their production requires infra-red light, an optimum temperature and specific genotypes. Sanders and Franzke (1976) found 20°C as the optimal temperature for colchicine treatment and concluded that conditions favourable for survival of colchicine-treated plants should be used to obtain mutants rather than conditions resulting in most rapid growth.

Future Prospects of Mutation Breeding in Sorghum

Mutation studies of immediate utility are fewer in sorghum than in other major crops (Reddy 1977, Sree Ramulu 1975). They were mostly confined to determining the mutagenic sensitivity, effectiveness and efficiency of mutagens, increasing the efficiency of mutagens by pre- and post-treatment modifications, standardizing the techniques of handling the material and treatment procedures to maximize the induction of mutations. This basic information is, of course, essential for sorghum breeders.

Compared to conventional breeding methods mutation breeding was less widely used, probably because of the enormous amounts of variability already present in the germplasm. Some areas where mutation breeding appears useful in sorghum are: overcoming problems of tight linkage between height, maturity and yield in tropical and temperate crosses; improving nutritional quality through increasing essential amino acids such as lysine or elimination of toxic products; diversification of male sterile cytoplasmic sources; increasing the frequency of apomixis and further augmenting the levels of resistance to insect pests and diseases of sorghum.

(a) Improving recombination potential in tropical and temperate crosses: Yield is a complex polygenic character and can be rarely expected to be improved by mutations in single genes, though a number of mutant cultivars with increased yield have been released in many crops (Sigurb-jornsson & Micke 1974). Tall mutants with high yielding ability have been reported in sorghum (Bhaskar Rao & Reddy 1975, Hadley et al. 1965, Landi 1974). At least four recessive loci (dw_1, dw_2, dw_3, dw_4,) inherited independently affect height (Quinby & Martin 1954). The dw_3 is unstable in the recessive state and reverts spontaneously (4.48%) to dominant condition (Landi 1974). Analysis of 52 true breeding induced mutants in sorghum has revealed that all the four height (*Dw*) genes have mutated and the genes dw_1, dw_2, appear to affect the internode length, dw_3, affects the number of nodes

and dw_4 seem to affect the panicle length (Goud & Vasudev Rao 1977).

Economically useful dwarf and early maturing mutants were reported in sorghum (Goud et al. 1970, Gurusiddaradhya & Goud 1977, Rao et al. 1969). When used as parents in hybridization programme, dwarf mutants contributed to high yield (Gurusiddaradhya & Goud 1977). Indirect improvement of yielding ability, and promotion of distinct traits by means of changes, and making the plants more adapted are the main objectives of utilising induced mutations in sorghum.

Crosses between tropical and temperate sorghums have been receiving considerable attention in recent years, but on account of the tight linkages between height, maturity and yield, the recombination potential has been very low (Rao 1971).

Localised chaisma formation results in decreased or no recombination in large chromosome segments. In such cases, genes are inherited in blocks and cannot be separated by hybridization. In most cereals, atleast crossing-over is restricted to the region distant from the centromere. Thus the available potential for recombination is not fully realised in hybridization programmes. Release of genetic variability and independent assortment of linked loci can be expected if the recombination in F_1 can be enhanced. In eukaryotic cells, radiation treatments are known to enhance crossing-over. Irradiation of F_1 plants especially during pre-meiotic stages, is expected to further enhance the variability in the F_2 population.

Increased variability in F_2 M_2 for quantitative characters was reported in rice (Jalil Miah & Yamaguchi 1968). Similarly radiation as well as chemical mutagens are reported to increase somatic recombination in *Glycine max* (Vig 1973), which can also contribute to increased variability in the F_2. This phenomenon could be explored in sorghum for obtaining desirable recombinants in tropical × temperate crosses.

From mutational studies in tropical sorghums, Rao et al. (1969) felt that a complex or unusual locus may be involved in the transformation of tropical sorghums with exposed internodes to the temperate type with enclosed internodes which is a rare event. Such a change involves the alteration of the entire genotype and the spontaneous height and maturity mutants reported by Quinby and Martin (1954) operate only after this major change has taken place. This hypothesis, however, needs verification.

(*b*) *Protein content and quality:* Protein availability is limited in sorghum due to the presence of tanins in testa layer of the grain. Protein quality is also poor due to its low lysine content. Two high lysine lines of sorghum (IS 11167 and IS 11758) were collected from Ethiopia which have shrivelled grains and high photosensitivity. High lysine and shrivelledness are tightly linked. There is need to break this linkage by incorporating high lysine gene in plump background sorghum to elevate yield level. Mohan (1973) reported the isolation of high lysine mutants of sorghum by screening for opaque kernels following DES treatments. Of the 425 mutants identified, P721 was less shrivelled and promising for protein and lysine. The inheritance of this opaque mutant is controlled by single gene that expresses partial dominance in triploid sorghum endosperm.

(*c*) *Toxic substances*: Young sorghum has pronounced ability to produce hydrocyanic acid (HCN) or prussic acid. Poisoning by this substance is a problem when livestock are grazed on young

sorghum plants, whose growth has been retarded by drought or low temperature. Mutants devoid of HCN content could be isolated by treating the sorghum seed with physical or chemical mutagens.

(*d*) *Cytoplasmic male sterility mutants:* In sorghum there is a need to diversify the male sterile cytoplasm since current hybrid sorghum programmes are based on *milo* cytoplasm. Hybrid seed production will be impaired if this cytoplasm becomes susceptible to disease as in the case of susceptibility of Texas male sterile cytoplasm of maize to southern corn leaf blight caused by *Helminthosporium maydis*, race T, and yellow leaf blight *Phyllastica zeae*. Also there is a need to diversify the nuclear genotypes to evolve new restorer lines which can restore alternate cytoplasm i.e., sources reported by Rao (1962).

The origin of cytoplasmic male sterility and fertility restoring genes are more important in higher plants. It has been postulated that some factors controlling male fertility reside in organelle DNA and their alteration causes cytoplasmic male sterility. The alteration might be a point mutation or an extensive loss of DNA and both types of events have been observed in organelle DNA of fungi and algae (Harvey et al. 1972).

There are reports of isolation of cytoplasmic male sterile mutants in barley (Favert & Ryan 1963), maize (Stoilov 1969) and Pearl Millet (Burton & Hanna 1976) following treatment with physical and chemical mutagens. In sorghum, isolation of cytoplasmic male sterile mutants by treating the seed with NMU, EI and 1, 4-bisdiazoacetyl butane is reported (Malinvosky et al. 1973).

Induction of cytoplasmic mutants through different mutagens is rather rare, but the results in various crops suggest that it may be possible.

(*e*) *Alternation of the reproductive system*: Apomixis, seed production without fertilization, occurs in sorghum (Rao & Murty 1972) and may be utilised as a system for fixing heterosis. It was first reported in a line (R 473) of sorghum derived in advanced generations from a cross between IS 2942 (a yellow endosperm *Kafir* line) and Aispuri (an Indian local variety). The frequency of apomixis could not be determined in R 473 through progeny tests because of its cross sterility. Mutagen treatments can be used to break cross sterility barriers and also to further enhance the frequency of apomixis. Attempts are being made to isolate plants with markers, either seedling marker such as chlorophyll mutant or simply inherited morphological character, from R 473, following treatments with γ-irradiation and hydrazine treatments, which can be used as pollen sources (Reddy & Rao 1979, Reddy et al. 1980).

(*f*) *Breeding for insect and disease resistance*: The primary mechanism of resistance to most sorghum pests such as shoot fly, stem borer and midge, is non-preference with low levels of antibiosis. Induced mutations could provide a means of augmenting the available sources of resistance to most sorghum pests.

Shoot fly is the most destructive insect of sorghum at the seedling stage. So far, absolute resistance is not known for shoot fly in cultivated varieties or in wild species. Attempts have been made to isolate shoot fly resistant mutants in sorghum. γ- or X-irradiation reduced the shoot fly attack in three sorghum varieties, but the resistance was only temporary and limited to initial stages of

plant growth (Subba Rao et al. 1968). Isolation of 42 mutants which were 100% resistant to shoot fly attack was reported in varieties M 35–1 and GM 2–3–1 treated with γ-rays (Nayar et al. 1969). Later, only twelve of these showed tolerance to attack (Usman & Goud 1972).

Striga, a phaneogamic root parasite in sorghum can damage the crop up to 75%. Sorghum plants produce strigol which is essential for the survival of parasite. Hence, mutants incapable of producing strigol could be isolated through induced mutations which confer a high degree of resistance to *Striga*.

In sorghum, perconia root rot, caused by the fungus *Periconia circincta* causes serious losses. Resistant mutant plants were found spontaneously in farmer's fields that bred true. Resistance was found to be due to a single gene (Schertz & Tai 1969). Maize dwarf mosaic virus (MDMV) resistant mutants have been isolated from γ-irradiated sorghum (Pai 1977).

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