TABLE I

Percentage free activity of lysosomal enzymes during growth of Yoshida ascites sarcoma*

Enzymes	% Free activity				
	Days				
	1	2	3	4	5
Acid phosphatase ..	12·00	8·20	6·00	7·80	20·00
Acid ribonuclease ..	6·00	5·28	4·32	8·91	10·00
β-Glucuronidase ..	6·50	6·41	3·78	4·52	7·83
Arylsulfatase A ..	3·00	0 80	0·93	1·26	3·29
do. B ..	2·87	0·72	0·83	1·73	4·21
Cathepsin D ..	20·09	18·28	11·00	22·61	29·20

Data are mean of three experiments.

* Free activity was calculated by the formula $F/B+F \times 100$, where 'F' is free activity in cytosol, and 'B' is the bound activity in the lysosomal fraction.

new hosts. Parry and Ghadially[13] reported an increase in lysosomes during tumour growth and their rupture during the terminal phase.

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CYTOLOGICAL STUDIES IN NORMAL AND MUTAGEN TREATED STRAINS OF TRITICALE (*TRITICALE HEXAPLOIDE*, LART)

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CYTOGENETIC investigation in many strains of 6 x triticales have demonstrated significant differences in meiotic instabilities, frequency of aneuploids and kernel fertility. Sporadic attempts have been made to increase the fertility level and quality of 6 x triticale, which is superior in seed and fertility characteristics to the 8 x forms but still not as good as wheat[1-3]. Ruebenbaver and Nalepa[4] have, however, demonstrated that exposure to ionizing radiation can lead to the isolation of mutants with higher seed fertility and increased winter hardiness in triticales. Our main objective in the present study was to develop some 6 x triticale lines which will have meiotic stability, pollen viability and kernel fertility comparable to wheat.

We treated three strains of 6 x triticales, *viz.*, Arm. 130, PC 186 and BC 245 with gamma-rays (5, 10, 15 Kr), EMS (0·15%, 0·30% and 0·45%) and gamma irradiation *plus* EMS in combination (5 Kr + 0·15% EMS, 10 Kr + 0·15% EMS, and 15 Kr + 0·15% EMS) to see whether mutagen treatment is helpful in improving the reproductive behaviour. Normal and mutagen treated seeds were grown in the field in *Rabi*, 1972. Meiosis was studied in both normal and mutagen treated M_1 populations. The young spikelets were fixed in a freshly prepared mixture of absolute alcohol and acetic acid (3 : 1) for 24 hr and finally stored in 70% alcohol. The anthers were then squashed in acetocarmine and meiosis was studied in detail. The frequency of univalents was observed at first metaphase, frequency of lagging chromosome and of chromatin bridges at first anaphase and frequency of micronuclei in tetrads. Pollen fertility was recorded as count of fertile pollen seen as completely filled, round and deeply stained with acetocarmine under microscope. Percent kernel setting was estimated for each treatment after counting the number of spikelets per spike. One hundred kernel weight in gm was also recorded.

The two strains Arm 130 and B C 245 were found sensitive to 5, 10, 15 Krs gamma irradia-

tion in terms of percent irregular cells and mean univalents per cell observed at first metaphase (Table I.) Two mutagen treatments, *viz.*, 0·15% EMS and 5 Kr gamma irradiation + 0·15% EMS were found to reduce the meiotic instability as evidenced by the low percentage of irregular cells and relatively low mean univalents per cell at first metaphase in Arm 130 and BC 245. Other treatments, *viz.*, 0·30% EMS, 0·45% EMS, 10 Kr + 0·15% EMS, 15 Kr + 0·15% EMS seemed to increase the percent irregular cells and mean univalents per cell when compared with the control. The third strain PC 186 did not show any significant effect due to mutagen treatments over control. It is interesting to note that in 0·15% EMS and 5 Kr + 0·15% EMS treated plants, the percent irregular cells and mean laggard number per cell at first anaphase decreased significantly as compared to the control and other mutagen treatments (Table II). The same two treatments further exhibited some reduction in percent irregular cells and mean laggards number per cell at second anaphase as compared with the control and other mutagen treatments (Table III). The percent pollen viability and kernel setting in 0·15% EMS and 5 Kr + 0·15% EMS treatments were greater in Arm 130 and BC 245 strains (Table IV). There did not seem to be any marked effects over control of mutagen treatment on the PC 186 strain in regard to pollen viability and kernel setting. The 100 kernel weight was found increased in case of 0·15% EMS and 5 Kr + 0·15% EMS treated plants, and the results of other treatments were in keeping with the data on pollen viability and kernel setting (Table V).

In this study both percent irregular cells and mean univalents per cell at first metaphase or mean laggards per cell at first anaphase were used as criteria to identify the strains for their meiotic instability. Out of nine mutagen treatments, two treatments, *viz.*, 0·15% EMS and 5 Kr + 0·15% EMS seemed to improve meiotic stability in two strains of triticale as their PMC exhibited relatively low percent irregular cells, low mean number

Table I

Chromosome analysis in the three strains of triticale at metaphase I

Metaphase I	Control	5 Kr	10 Kr	15 Kr	0·15% EMS	0·30% EMS	0·45% EMS	5 Kr +0·15% EMS	10 Kr +0·15% EMS	15 Kr +0·15% EMS
Number of cells examined										
Arm 130	120	137	140	162	132	138	141	172	162	124
PC 186	96	106	98	121	110	86	117	132	119	98
BC 245	156	167	148	150	146	132	128	156	159	137
Number of irregular cells										
Arm 130	38·8	52·3	56·1	68·2	28·1	59·6	59·7	31·8	70·7	52·8
PC 186	25·4	31·1	27·7	33·8	27·7	23·3	31·1	29·0	25·7	21·9
BC 245	67·3	75·9	72·8	72·3	28·3	65·4	61·6	25·2	75·5	64·1
Percent irregular cells										
Arm 130	32·4	38·2	40·1	42·1	21·3	43·2	42·4	18·5	43·7	42·6
PC 186	26·5	29·4	28·3	28·0	25·2	27·2	26·6	22·0	21·6	22·4
BC 245	43·2	45·5	49·2	48·2	19·4	49·6	48·2	16·2	47·5	46·8
Mean univalents per cell										
Arm 130	0·98	1·10	1·23	1·12	0·82	1·24	1·16	0·62	1·19	1·12
PC 186	0·82	0·85	0·90	0·98	0·80	0·92	0·96	0·75	0·72	0·78
BC 245	1·37	1·42	1·48	1·38	0·78	1·32	1·27	0·58	1·16	1·22

Two treatments, *viz.*, 0·15% EMS and 5 Kr + 0·15% EMS seemed to improve meiotic stability in two strains of triticale as their PMC exhibited relatively low percent irregular cells, low mean number

TABLE II

Frequency of laggards at anaphase I in both control and mutagenically treated populations in triticale

Anaphase I	Control	5 Kr	10 Kr	15 Kr	0·15% EMS	0·30% EMS	0·45% EMS	5 Kr +0·15% EMS	10 Kr +0·15% EMS	15 Kr +0·15% EMS
Number of cells examined										
Arm 130	210	185	205	187	212	189	207	185	208	195
PC 186	180	176	210	185	210	178	189	178	182	232
BC 245	235	210	230	167	200	210	176	204	168	242
Number of irregular cells										
Arm 130	84·4	76·8	93·8	86·4	56·6	89·7	96·8	35·9	101·0	97·9
PC 186	56·1	58·4	68·6	59·0	63·8	57·3	59·3	44·1	48·4	77·4
BC 245	66·7	62·2	70·4	49·7	45·8	63·2	57·3	33·2	52·4	84·7
Percent irregular cells										
Arm 130	40·2	41·5	45·8	46·2	26·7	47·5	46·8	19·4	48·6	50·2
PC 186	31·2	33·2	32·7	31·9	30·4	32·2	31·4	24·8	26·8	33·4
BC 245	28·4	29·6	30·6	29·8	22·9	30·1	32·6	16·3	31·2	34·6
Mean laggards cell										
Arm 130	0·84	0·89	0·92	0·93	0·32	0·95	0·88	0·24	0·97	0·98
PC 186	0·68	0·71	0·70	0·73	0·58	0·74	0·69	0·43	0·58	0·64
BC 245	0·49	0·54	0·53	0·51	0·24	0·35	0·29	0·38	0·47	0·48

TABLE III

Frequency of laggards at anaphase II in both control and mutagenically treated populations in triticale

Anaphase II	Control	5 Kr	10 Kr	15 Kr	0·15% EMS	0·30% EMS	0·45% EMS	5 Kr +0·15% EMS	10 Kr +0·15% EMS	15 Kr +0·15% EMS
Number of cells examined										
Arm 130	280	275	210	265	280	210	185	195	210	263
PC 186	210	265	189	175	180	190	192	186	148	150
BC 245	195	185	200	210	205	260	262	265	280	270
Number of irregular cells										
Arm 130	84·5	89·1	77·0	101·7	48·7	82·3	71·0	29·6	89·0	116·2
PC 186	42·0	73·6	49·5	45·5	40·1	55·8	59·1	36·8	43·2	46·8
BC 245	70·1	69·1	77·8	77·2	57·8	101·0	105·0	21·9	114 2	105·8
Percent irregular cells										
Arm 130	30·2	32·4	36·7	38·4	17·4	39·2	38·9	15·2	42·4	44·2
PC 186	24·8	27·8	26·2	26·0	22·3	29·4	30·8	19·8	29·2	31·2
BC 245	36·5	37·4	38·9	36·8	28·2	38·9	40·1	18·3	40·8	39·2
Mean laggards per cell										
Arm 130	0·72	0·78	0·81	0·86	0·40	0·90	0·85	0·34	0·72	0·78
PC 186	0·50	0·62	0·64	0·68	0·42	0·58	0·52	0·41	0·60	0·58
BC 245	0·58	0·67	0·62	0·66	0·32	0·42	0·38	0·28	0·42	0·54

TABLE IV

Pollen viability and kernel setting in both control and mutagenically treated populations in triticale. Pollen viability data represent average of 10 plants having 200 microscopic field per treatment. Data on kernel setting represent average counts of 50 primary spikes per treatment

	Control	5 Kr	10 Kr	15 Kr	0·15% EMS	0·30% EMS	0·45% EMS	5 Kr +0·15% EMS	10 Kr +0·15% EMS	15 Kr +0·15% EMS
Pollen viability (%)										
Arm 130	85·4	89·0	73·6	72·5	92·3	75·5	58·3	90·0	76·4	64·5
PC 186	98·0	86·8	72·4	67·0	95·0	83·4	53·8	74·5	81·0	59·0
BC 245	82·2	84·2	75·5	70·0	94·2	82·6	81·7	96·7	75·2	58·5
Kernel setting (%)										
Arm 130	65	68	65	60	70	54	56	75	46	47
PC 186	80	78	74	74	75	70	80	80	70	71
BC 245	72	70	65	66	80	56	52	89	62	63

TABLE V

Mean 100 kernel weight in grams
Data represent mean ± S.E. of ten samples per treatment

Treatment	Arm 130	PC 186	BC 245
Control	2·77 ±0·30	3·84 ±0·31	3·10 ± 0·12
5 Kr	2·90 ±0·23	3·08 ±0·24	3·00 ± 0·07
10 Kr	2·55 ±0·22	3·39 ±0·20	3·06 ± 0·05
15 Kr	2·76 ±0·43	3·29 ±0·20	3·04 ± 0·04
0·15% EMS	2·78 ±0·12	3·68 ±0·10	3·58 ± 0·10
0·30% EMS	2·50 ±0·25	3·10 ±0·15	3·20 ± 0·30
0·45% EMS	2·78 ±0·28	3·69 ±0·23	3·32 ± 0·21
5 Kr+0·15% EMS	2·62 ±0·33	3·74 ±0·25	3·20 ± 0·16
10 Kr+0·15% EMS	2·50 ±0·18	2·90 ±0·30	3·12 ± 0·13
15 Kr+0·15% EMS	2·47 ±0·13	2·93 ±0·11	3·00 ± 0·07

of univalents, and low mean laggards. Swaminathan[5] observed that the variability in the number of kernels per spike was much greater in 20 Kr gamma irradiation and 0·20% EMS treated population of some promising 6 x triticales as compared to the control. The results of the present study also reveal greater variability with regard to pollen viability, kernel setting and mean 100 kernel weight in gamma-rays and EMS treated population of triticale strains. Triticales characteristically exhibit some degree of sterility which has generally been attributed to meiotic abnormalities including incomplete pairing and asynchronous chromosome disjunction[6]. It is, however, suggsted that as the meiotic irregularities are polygenically controlled, the mutagen at lower doses, for example 0·15% EMS as in this study, may be producing specific alterations at chromosomal DNA level for the improvement of incomplete pairing and asynchronous chromosome disjunction. At higher doses of gamma-rays or EMS, macromutational events including nonspecific alterations at chromosomal DNA level leading to greater variability can be expected. The possibility that low doses of mutagen treatment may be responsible to bring about some dependence between meiotic stability and kernel fertility is attractive but it demands that we learn more about the operational mechanism(s) causing meiotic instabilities and low kernel setting in triticale. It is further suggested that cytological stability and high kernel fertility can be attained in 6 x triticale following vigorous progressive selection in the progenies of mutants selected for high stability and fertility.

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