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Meiotic stability and kernel fertility in hexaploid triticale (X *Triticosecale* Wittmack) following combined mutagen treatment *[1]/

H. K. SRIVASTAVA**

Resumen

Se observaron mejoras en la estabilidad meiótica, en la fertilidad y en el rendimiento en grano en las poblaciones M_3 de tres variedades de triticales hexaploides. Quedó discutida la eficacia del tratamiento combinado de 5 kr de irradiación gama y 0,15 por ciento (EMS) para estimular el comportamiento reproductivo de los triticales. La relación parece ser directa, es decir, que la estabilidad meiótica mejorada produjo alta fertilidad en el polen y en el grano y alto rendimiento en grano en la generación M_3. Ello sugiere que los genes mutantes en la combinación, podrían producir algún grado de dependencia entre la estabilidad meiótica y la fertilidad, posterior a las micromutaciones de la proporción del trigo hacia el genomio del centeno en el triticale hexaploide. Además, se discuten los resultados desde el punto de vista de que un tratamiento mutagénico puede promover un balance génico-citoplásmico entre los sistemas genéticos trigo-centeno, lo cual queda evidentemente reflejado en el comportamiento reproductivo superior de los triticales en la generación M_3.

Introduction

Triticale is a new plant genus produced artificially from crossing either hexaploid or tetraploid wheat (*Triticum* sp) with diploid rye species (*Secale* sp) followed by the doubling of the chromosome complement of the sterile F_1 hybrid. In the last decade a great deal of information on cytogenetics of 42 - chromosome triticale (X *Triticosecale* wittmack) has been accumulated (14, 23, 36, 44), but still the presently available hexaploid triticales have not met all the requirement necessary for general use as a commercial cultivar. The crop suffers from comparatively poor yields, inconsistant quality, winter kill, ergot disease, and sterility. Primary triticales are characterized by their cytological instability and partial fertility which have been observed in both the hexaploid and octoploid types (43). The most promising hexaploid triticales are the secondary types which have been obtained from intercrosses of hexaploid triticales with octoploid triticales (21, 28) or with hexaploid wheats (16, 22, 26, 42). High fertility is the major improvement of these types over the primary hexaploids. Zillinsky and Borlaug (42) have indicated that one of their relatively most fertile hexaploid lines ("Armadillo") having 2n = 6x = 42 chromosomes contains chromosomes of the A and B genomes, some chromosomes, or at least some genes from the D genome of hexaploid wheat (*Triticum aestivum* L em Thell) as well as chromosomes from the R genome of diploid rye (*Secale cereale* L). As might be expected in a newly synthesized species derived by hybridization of autogamous and allogamous species, there is considerable reproductive instability even in near-homozygous lines. Nakajima (27), upon viewing the morphological characteristics of F_{2-3} hexaploid derivatives of the triple cross (tetraploid wheat x diploid rye) x hexaploid wheat, rightly concluded that both R – and D – genomes chromosomes must be present in the chromosome complement of the new improved triticale lines.

The synthetic amphidiploid triticale characteristically shows a number of unpaired chromosomes at first meiotic metaphase (17). Meiotic disorders, sterility and malformed kernels (endosperm shrivelling) are common, especially in progeny from intercrosses among hexaploid triticales (25). Chromosome pairing are generally affected by chromosome homologies, genetic factors and the cellular environment during meiosis (15). These in turn can also be influenced by the external environment (1, 2, 39). Recent comprehensive studies by Bennett et al. (3, 6) on the duration of meiosis and its component stages in wheat, rye and triticale have demostrated that rye required a longer time in which to complete meiosis

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** Professor H. K. Srisvatava Professor of Plant Breeding Gujarat Agricultural University Ahemdabat - 38004 (Gujarat State) India.

than either triticale or wheat. They contended that insufficient time for normal pairing of rye chromosomes in the triticale nucleus may be the cause of the partial failure of chromosome pairing which is evidenced as univalents at metaphase I.

Considerable efforts in recent years towards the feasibility of further genetic improvements of triticales have been made. Rupert et al. (34) demonstrate that cytological selection for meiotic stability resulted in the improvement of hexaploid triticales especially in the third generation after colchicine treatment. Weimarck (41) observed that meiotic stability in octoploid triticale could be improved by increasing genetic variation and selecting for fertility and other yield – related characters in advanced generations. The variation of meiotic behavior and its regulation under two different genetic systems in mutagen treated (repeated X – irradiation with 15000 R) and untreated populations in the three lines of hexaploid triticales have been demonstrated (29). Recent results on the effectiveness of chemical mutagens towards improving the reproductive behavior of triticales reveal that sodium azide (35) and N –nitrosi – N – methylurea (30,31) could be used to produce useful mutations. Cytogenetical studies in the M_1 populations derived from mutagenically treated strains of hexaploid triticales revealed some relationship between meiotic disorders to that of pollen viability, kernel fertility and seed size (37). Similar studies of the M_2 populations emphasized the importance of combination treatment with 5 kilorad of gamma irradiation and 0.15 percent ethylmethanisulfonate (EMS) in promoting meiotic stability of hexaploid triticales (18). The present study evaluated this relationship in the M_3 populations obtained respectively from some promising M_2 selections of hexaploid triticales and the results observed further signify the role of combined mutagen treatment towards promoting meiotic stability, fertility, and kerneed yield.

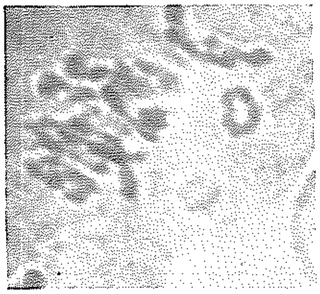
Material and Methods

The seeds harvested from the selected M_2 plants (18) of the three cultivars (Armadillo 130, Armadillo 157, and Bronco 245) of hexaploid triticale were grown under normal field conditions to obtain M_3 progeny. The field and laboratory experiments were all carried out at the research farm of the Institute of Advanced Studies, Meerut University, India, during winter of 1975-76. Fifty plants/line/mutagen treatment were randomly chosen in M_3 populations for cytological observations. The young spikes were fixed in Carnoy's solution (6 parts absolute alcohol: 3 parts chloroform: 1 part glacial acetic acid) at the appropriate stage and squashed in 2 percent acetocarmine. Meiotic irregularities were scored as number of univalents at metaphase I, laggards at anaphase I and II, and micronuclei at telophase II. Microphotographs were taken from temporary preparations.

The data on the agronomic characters like days to maturity and kernel yield/plant were recorded using similar procedures as described earlier (37, 38). A minimum of 300 plants per genotype per treatment were randomly selected for quantitative measurements. The data on the quantitative traits; number of spikelets/spike, number of kernels/spike, kernels/spikelets, pollen viability (%), kernel fertility (%) and total grain yield in gm/plant were recorded under normal field and laboratory conditions. Pollen viability was determined for ten plants randomly selected from each treatment. Several undehisced anthers were removed, and pollen grains were placed on a 'depression' slide and stained with tetrazolium bromide (40). Pollen grains were placed on the slide in the experimental field within 2 min. after the anther was excised from the plant. The number of stained (viable) and unstained (nonviable) pollen grains in a sample of at least 250 pollen grains were counted 2 to 3 hr after sampling. Each of the ten plants sampled for pollen viability was tagged, and one spike was collected from each plant at maturity. Determined for each spike were percentage fertility, kernels/spike, kernels/spikelet, and number of spikelets/spike. The data were analysed using conventional statistical procedures.

Results and Discussion

The M_3 population belonging to combined mutagen treatment (5 kr gamma irradiation plus 0.15 percent EMS) showed excellent chromosomal stability. Besides univalents, some of the commoly observed chromosome irregularities in control populations were binucleate or multinucleate PMC's at metaphase I (Fig. 2), chromatin bridges at anaphase I (Figs. 3 and 4), univalent laggards at anaphase I (Fig. 5), early dividing univalent laggards at anaphase I (Fig. 6), and late dividing univalent laggards at anaphase I (Fig. 7). Stable meiosis with regard to metaphase I, anaphase I (Figs. 8 and 9), and telophase I and II was frequently observed in plants of M_3 populations. The appearance of ring bivalents at metaphase I (Fig. 1) was considered to be irregular as none was observed in mutagen treated M_3 plants. Differences in frequencies of meiotic disorders (metaphase I univalents, anaphase I laggards, anaphase II laggards, and telophase II micronuclei) were observed between mutagen treated lines and controls (Table I). The M_3 population belonging to combined mutagen treatment (5 kr gamma irradiation plus 0.15 percent EMS exhibited excellent chromosomal stability as the mean number of univalents/PMC's at metaphase I, mean number of laggards/PMC's at anaphase I and II, mean number of micronuclei/PMC's at telophase II were markedly reduced. The other two mutagen treatments (5 kr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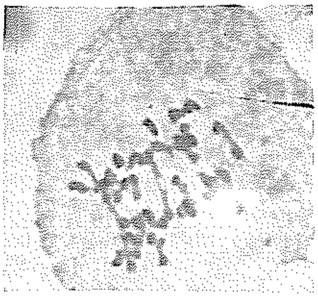
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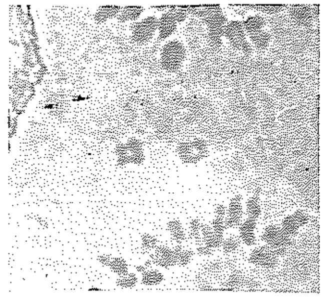
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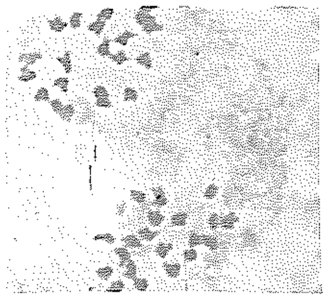
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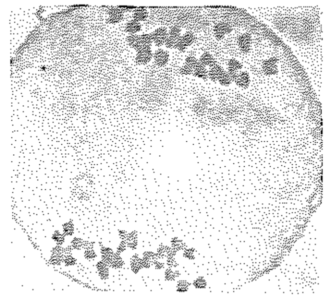
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FIGS. 1-9 MEIOTIC CHROMOSOMES BEHAVIOR IN HEXAPLOID TRITICALES (x 1500)

Fig. 1. Metaphase I showing a ring bivalent.

Fig. 2. Metaphase I showing a binucleate PMC.

Fig. 3. Anaphase I showing a chromatin bridge.

Fig. 4. Early anaphase I showing two chromatin bridges.

Fig. 5. Anaphase I showing one bivalent laggards and two early dividing univalent laggards.

Fig. 6. Anaphase I showing two bivalent laggards.

Fig. 7. Anaphase I with two dividing univalent laggards.

Fig 8. Anaphase I showing normal chromosomes in M_3 populations, treatment 5 kr gamma irradiation plus 0.15 percent EMS.

Fig. 9. Late anaphase I showing normal chromosomes in M_3 populations; treatment 5 kr gamma irradiation plus 0.15 percent EMS.

gamma irradiation or 0.15 percent EMS did not show significant reduction in the frequencies of meiotic disorders in M_3 progeny. All the three untreated lines exhibited relatively high frequencies of meiotic irregularities. The results indicated that combined mutagen treatment was effective in promoting meiotic stability.

The data on the six agronomic characters in treated and untreated M_3 populations are summarized in Table II. The mutagen treated M_3 populations appeared to be late maturing. There was, however, no effect of mutagen treatment in respect to number of spikelets/spike as the differences between the mutagen treated and untreated populations for this character were not significant. Percent pollen viability, percent fertility, kernels/spike, and kernels/spikelets were all significantly enhanced in case of the combined mutagen treatment. The kernel yield/plant was also increased in M_3 population belonging to the combined treatments as compared to the controls and other separate mutagen treatments. There was an apparent relationship between improved cytological stability and kernel fertility together with other yield related characteristics evaluated. The relationship seemed to be direct in M_3 generation, viz, improved meiotic stability resulted in high pollen and kernel fertility and yield. The results are also in keeping with the findings where mutagenic treatment was able to shift the mean values of several quantitative traits in M_2 generation including seeds per spike and 1000 seed weight (31).

The M_3 population derived from mutagen treatment in combination, 5 kr gamma irradiation plus 0.15 percent EMS, showed an improved meiotic behavior which was further reflected in increased agronomic characters. Meiotic disorders, which give rise to aneuploid plants, do not seem to have direct influence on fertility (24). As there was no significant correlation between cytological abnormalities and fertility, it has therefore been suggested that the reduced fertility in triticale is mainly of a genic nature (11, 13, 19, 32). Triticales with cytoplasm from hexaploid wheat are considered to be cytologically more stable and exhibit fewer meiotic irregularities than those having tetraploid wheat cytoplasm (33). They are also superior in pollen viability, fertility, yield and kernel quality, and they possess larger ammounts of essential amino acids (lysine, histidine, and threonine) than triticales from tetraploid cytoplasm. Higher level of total cellular RNA, total cellular protein, and nuclear histone have also been observed in triticales with hexaploid than in those with tetraploid cytoplasm (12). In view of these observations and the present results, it is likely to speculate that mutagen treatment brings about a genic-cytoplasmic balance between wheat-rye genetic systems (nuclear and cytoplasmic) which is further

Table 1. Frequencies of meiotic disorders in M_3 populations of the three lines of hexaploid triticale (*X Triticosecale* Wittmack). The data represent mean of 800 to 1000 Pollen Mother Cells studied from at least 20 M_3 plants selected at random for each treatment.

Line / Irregularity	Metaphase I Univalents	Anaphase I Laggards	Anaphase II Laggards	Telophase II Micronuclei
Armadillo 130	2.08	2.04	1.80	1.25
Armadillo + 5 kr	1.98	1.92	1.62	1.12
Armadillo + 0.15 percent EMS	1.85	1.71	1.51	1.00
Armadillo + 5 kr + 0.15 percent EMS	0.75**	0.60**	0.55**	0.40**
Armadillo 157	1.68	1.43	1.30	1.05
Armadillo + 5 kr	1.64	1.38	1.21	0.98
Armadillo + 0.15 percent EMS	1.58	1.27	1.06	0.72
Armadillo + 5 kr + 0.15 percent EMS	0.60**	0.45**	0.39**	0.30**
Bronco 245	1.72	1.37	1.23	0.98
Bronco + 5 kr	1.52	1.18	1.16	0.78
Bronco + 0.15 percent EMS	1.45	1.07	1.04	0.68
Bronco + 5 kr + 0.15 percent EMS	0.96**	0.66	0.42**	0.38**

** Population mean significant at 1% level of probability

Table 2. Agronomic characteristics in M_3 populations of the three lines of hexaploid triticale.

Line Characters	Days to Maturity	Number of Spikelets	Kernels/ Spike	Kernels/ Spikelets	Pollen Viability %	Kernel Fertility %	Kernel Yield/ Plant (g)
Armadillo 130	90	23.5	30	1.65	65	55	10.5
Armadillo + 5 kr	115	22.0	34	1.72	67	58	12.0
Armadillo + 0.15 percent EMS	116	21.8	36	1.80	65	60	11.0
Armadillo + 5 kr + 0.15 percent EMS	130**	22.5	48**	2.26**	75**	71**	16.0**
Armadillo 157	95	26.2	40	1.80	67	60	14.8
Armadillo + 5 kr	118	24.8	43	1.95	68	62	15.4
Armadillo + 0.15 percent EMS	117	25.8	44	2.00	70	61	16.0
Armadillo + 5 kr + 0.15 percent EMS	135**	25.5	52**	2.34**	82**	78**	21.0**
Bronco 245	97	28.5	42	2.00	65	61	17.2
Bronco + 5 kr	110	29.7	47	1.95	69	63	18.0
Bronco + 0.15 percent EMS	116	30.2	48	2.10	67	65	18.2
Bronco + kr + 0.15 percent EMS	142**	30.5	59**	2.41**	90**	84**	24.1**

** Population mean significant at 1% level of probability

reflected in superior reproductive behavior of triticales in M_3 populations. While the precise mechanism by which the combined mutagens affect the reproductive behavior of hexaploid triticales remains unknown, the possibility that the mutagen treatment in combination brings about some dependence between meiotic stability and fertility after so-called 'micromutations' of the ratio of wheat to rye genome in hexaploid triticales is worth mentioning. The ratio of genomes is thought to be a manifestation of different amount of DNA content, which, in turn, affect meiotic duration as a whole and, in particular, those phases of meiosis related to pairing and chiasma formation (7, 17). The results, however, suggest that the meiotic stability in hexaploid triticales could be improved following combined mutagen treatment and selecting in advanced generation for high kernel fertility and other yield contributing characters.

The cause for partial fertility in triticales is not yet fully understood. A proposal has been made that cytological instability and low fertility of triticale were two unrelated phenomena and that selection for improved fertility would not automatically result in increased meiotic regularity (32). Results of some recent experiments however manifest significant correlations between improved meiotic regularity and kernel fertility. The low seed set in octaploid and hexaploid triticales was associated with the frequency of aberrations in meiosis, especially desynapsis, leading to the occurrence of aneuploid gametes and plants (20, 29). The number of normal tetrads at telophase II in this study was correlated with the seed set ($r = 0.55$) (20). An exhaustive study on meiotic stability and multivariate analyses of vegetative and reproductive characteristics in nine secondary 42–chromosome triticales and four intertriticale hybrids revealed that at least some of the variation in fertility in triticale is due to meiotic disorders (8). Differences between populations in amount of genetic variability for heading time, pollen viability and grain yield indicated that these agronomic traits might be useful as a selection criteria (10). It has also been elucidated by breeders that kernel development in triticale is more sensitive to environmental influences than in the parental species (9) and better plumper kernel development usually occurs among the most fertile plant type (37). The results of the present study suggest that a positive genetic association between meiotic stability, fertitily and kernel yield exists in the M_3 generation following combined mutagen treatment.

Improvement caused by 'micromutations' are generally detected in large groups of plants and testing for their genetic nature would require biometrical analysis in later generations. Several mutants have been isolated in M_3 populations containing strikingly improved meiotic stability, fertility, and yield related characteristics. Additional research on the genetic nature of these micromutations in hexaploid triticale is needed to determine the specific mechanism by which combined mutagen treatment produces improved meiotic stability, high fertility and superior agronomic performance.

Summary

Improved meiotic stability, fertility, and kernel yield were observed in M_3 populations of three strains of hexaploid triticales. The efficacy of combination treatment with 5 kr gamma irradiation and 0.15 percent EMS in promoting reproductive behavior of triticales has been discussed. The relationship seemed to be direct, i.e., improved meiotic stability resulted in high pollen and kernel fertility and grain yield in M_3 generation. It is suggested that the mutagens in combination may bring about some dependence between meiotic stability and fertility after micromutations of the proportion of wheat to rye genome in hexaploid triticale. The results are also discussed from the point of view that mutagenic treatment may result in bringing about a genic-cytoplasmic balance between wheat – rye genetic systems which is evidently reflected in superior reproductive behavior of triticales in M_3 generation.

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