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DIPLOIDIZATION OF INDUCED TETRAPLOID HYBRIDS OF *DACTYLIS GLOMERATA* BY MEANS OF X-RAY IRRADIATION

by

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INTRODUCTION

The agronomic value of F_1 hybrids has been amply demonstrated in several crops, including corn and Bermudagrass. In the case of corn, the maintenance of a standard genetic type is assured by producing the hybrid seed from homozygous inbred lines or single crosses. In the case of Bermudagrass, the hybrid variety Coastal has been propagated vegetatively at considerable expense. In many forage species such as orchardgrass, Dactylis glomerata, where neither inbred lines, vegetative propagation, nor apomixis are available, a cytological system for maintaining hybridity in progeny produced by seed could be of significant importance. An amphidiploid hybrid characterized by autosyndetic pairing would constitute the cytological basis for such a true breeding hybrid. Usually, however, partial homology exists between the chromosomes of the species involved and the amphidiploid behaves as a segmental allotetraploid displaying both autosyndesis and allosyndesis. Allosyndetic pairing results in genetic segregation while irregular disjunction from quadrivalents (IV), trivalents (III), and univalents (I) results in the production of gametes with unbalanced chromosome complements and consequent sterility. By intensifying the structural differentiation of the chromosomes by means of irradiation induced chromosome interchanges, it may be possible to reinforce the allotetraploid character of the amphidiploid and thus obtain greater autosyndetic bivalent pairing with lowered genetic segregation and higher fertility. This study was undertaken for the purpose of evaluating the effectiveness of x-ray irradiation as an agent for the diploidization of induced polyploids.

LITERATURE REVIEW

Dactylis Taxonomy and Chromosome Differentiation

Early reports by Levan (1930), Kattermann (1931) and Muntzing (1933, 1937a) on the northern European forms of *Dactylis glomerata* L. reported that the common cultivated and wild forms were tetraploid, 2n = 28. They also determined that a form native to the beech forest of Southern Scandinavia, Germany, and Poland, later referred to as *Dactylis glomerata* subspecies *Aschersoniana* by Stebbins and Zohary (1959), was diploid (2n = 14). Muntzing (1937) reported that twelve natural triploid hybrids between the 4n and 2n forms exhibited a high frequency of trivalents, averaging 4.58 per cell. It was concluded that the genomes of *Aschersoniana* and *glomerata* were homologous. Muntzing (1936, 1937) postulated that the tetraploid forms had originated directly from this diploid form and that morphological differences between the two forms were a result of polyploidy *per se*. Myers (1940) findings of tetrasomic inheritance of seedling characters supported this hypothesis and for several years *D. glomerata* was considered a classical example of an autotetraploid. In the backcross of eight 3n plants to 4n glomerata, 41 of 238 progeny were tetraploid.

Clausen, Keck, and Hiesey (1945) questioned Muntzing's proposal and suggested that additional forms, possibly *D. abbreviata* Bernh. (*D. glomerata* var *abbreviata* Drejer.) or *D. hispanica* Roth, might be diploid and have played a role in the evolution of the tetraploid form. Stebbins (1947) suggested on the basis of examination of pollen grain size that *D. glomerata* subspecies *juncinella* was 2n and may have contributed to

the gene pool of the tetraploid population.

Myers (1948b) reported that the induced tetraploid of Aschersoniana morphologically resembled the diploid rather than the tetraploid D. glomerata, indicating that tetraploidy per se was not responsible for differences between the two populations. He also reported that a new diploid form from Iran, later identified as D. glomerata ssp. Woronowii, crossed freely with D. glomerata ssp. Aschersoniana and produced fertile F_1 hybrids which resembled tetraploid D. glomerata. Myers' cross offered an acceptable explanation for the origin of the Northern European forms of 4n Dactylis.

Stebbins and Zohary (1959) presented detailed information on nine additional diploid forms from the Mediterranean region and South Central Asia. They identified the following as subspecies: himalayensis from south central Asia, Reichenbachii from northern Italy, judaica from Israel, ibizensis from the Balearic Islands, juncinella from southern Spain, lusitanica from Portugal, Santai from western Algeria, Mairei from eastern Algeria, and Smithii from the Canary Islands.

Zohary (1956) investigated the cytology of several diploid subspecies and their hybrids; Aschersoniana, hookerii (himalayensis), Woronowii, lusitanica, lusitanica x Woronowii, Woronowii x hookerii, lusitanica x Aschersoniana, hookerii x lusitanica, and hookerii x Aschersoniana. These subspecies and their hybrids were found to have seven bivalents (7 II) at diakinesis and metaphase one (M I) with the exception of one plant in the Woronowii x judaica cross which showed evidence of asynapsis. Separation at anaphase one (A I) was normal. All F_1 hybrids showed a considerable reduction in pollen fertility

compared to the parents. Most hybrids were found to have 20 to 50% aborted pollen. In their comprehensive report on all 11 subspecies, Stebbins and Zohary (1959) stated that meiosis was regular (7 II) in all the diploids and their F_1 hybrids. They indicated that while pollen fertility in the parental diploids and some F_1 hybrids was high, in other F_1 hybrids the percentage of aborted pollen reached 50 to 80 per cent. The percentage of seed set in the F_1 hybrids, under good conditions, was not significantly lower than in their parents. Several F_1 hybrid plants grown under poor conditions had significantly lower seed set than did their sister plants grown under more favorable conditions. These differences were not observed in the parental strains. They concluded that the diploid subspecies of Dactylis were in general interfertile and able to exchange genes with each other at the diploid level. Tetraploid strains were found to intercross readily. The F_1 hybrids were vigorous and fully fertile. The F_2 progenies showed no signs of degeneration. The authors recommended recognizing a single species with two parallel series of subspecies, one at the diploid and one at the tetraploid level.

Stebbins (1956b) reported that over the great bulk of its geographical distribution Dactylis is represented only by tetraploid strains. The diploids in general occupy very limited areas. He postulated three phases in the evolution of this polyploid complex. The first occurred during the Tertiary period with the divergence of the diploids, and their establishment as relatively homogeneous populations, inhabiting different ecological niches in widely divergent climatic zones. The second phase was brought about by the climatic changes of the Pleistocene ice age and permitted types with different climatic preferences to come together and hybridize.

The products of this hybridization advanced into new habitats. These hybrid derivatives were more successful after they had doubled their chromosome number to become tetraploids. The third phase of evolution was associated with human activity. The tetraploids, which spread as a result of habitat alteration by man, came in contact with additional diploids with which they hybridized to form triploids. Triploids can give rise to tetraploid offspring and thus enrich the variability in the tetraploid population. Zohary and Nur (1959) studied the role of naturally occurring triploids in Israel. They concluded that the unreduced eggs produced by the triploid permitted the formation of 4n and 5n progeny. By producing a large proportion of tetraploid progeny, triploids serve as an efficient bridge for one way gene flow from diploid to tetraploid level. Jones and Borrill (1962) studied 8 triploid plants from crosses of northern and southern European tetraploids with the diploids subsp. Woronowii, Aschersoniana, judaica, and lusitanica. They concluded that the significant products from the triploid backcrosses were either triploids or tetraploids. The normal pairing of the tetraploid progeny makes further introgression possible and natural tetraploid populations can be enriched with genes from the diploids.

McCollum (1958) found no difference in quadrivalent frequency between induced tetraploids of hybrids between several diploid subspecies and induced tetraploids of the diploids themselves. He concluded that there was no evidence for preferential pairing and consequently no support for the hypothesis of structural differentiation of chromosomes between the diploid subspecies.

Jones (1962) studied chromosome pairing in 2n x 2n and 4n x 4n

hybrids and non-hybrids. He concluded that there was a gross similarity among the chromosome sets in Dactylis, but that a reduced frequency of multivalents in the hybrid 4n plants in comparison with the non-hybrid 4n plants indicated that minor genetic and/or chromosomal differences existed between tetraploid populations.

Borrill (1961a) reported that a considerable reduction in ability to exchange genes was observed when diploid taxa were hybridized. Fertility was 30 times less in the F_2 than in the parental populations. Loss in fertility when crossing tetraploids was less marked. The author states that, "Such considerations as ploidy, and potentiality for gene exchange, are misplaced as criteria for a taxonomic classification, which must recognize morphologically identifiable taxa." A taxonomic revision of the group was suggested.

Evidence of chromosome differentiation induced by x-ray irradiation was presented by Stebbins (1956a). He found that four amphidiploid plants from the lusitanica x judaica cross, where the judaica pollen had been treated with 1250 r, showed unusually low multivalent frequencies; 1.07, 1.19, 1.42, and 1.62 multivalents per cell. The diploid hybrids from which these amphidiploids were derived formed rings and chains at meiosis indicative of heterozygosity for chromosome interchanges. A fifth amphidiploid plant derived from the same cross but showing high fertility and normal meiosis on the diploid level had a mean of 1.52 multivalents per cell. McCollum (1958) reported an amphidiploid from a similar cross but non-irradiated background had 3.59 quadrivalents per cell.

Details of Cytological Studies in Dactylis

Muntzing (1933) reported an avg. 3.5 IV per cell on the basis of 19 plates in "polar view." The remaining chromosomes were associated as bivalents, univalents not being observed. Of 67 IV in "polar view," 40 were zig-zag, 17 were non-zig-zag, and 10 were uncertain. Twenty cells in "side view" gave 12 zig-zag, 12 non-zig-zag, and 3 uncertain. The lower frequencies of zig-zag IV in "side view," 50%, than in "polar view," 70%, was attributed to greater ease of analysis of non-zig-zag IV in the "side view."

Muntzing (1937a) studied meiosis in three diploid Aschersoniana plants. One with high pollen fertility (avg. 88.7%), had regular meiosis with 7 II and regular 7 - 7 disjunction at A I. Seventy-one cells had 7 II, and two cells had 6 II and 2 I. The euploid complement of a second plant with a fragment and high pollen fertility, also displayed normal meiosis. A third plant with low pollen fertility (avg. 47.0%), was heterozygous for a reciprocal translocation. The chiasma frequencies per chromosome for the first two plants above were 0.776 ± 0.011, and 0.777 ± 0.017. Analysis of two slides of the third plant yielded chiasma frequencies of 0.784 ± 0.009, and 0.824 ± 0.010. The value obtained from the first slide was not significantly different from the values of the other two plants, the value from the second slide was significantly different. This variability was attributed to environmental influences. Analysis of chiasma frequency led to the conclusion that in the plant carrying the translocation, "Four chromosomes are partially non-homologous. In some cases this results in associations of all four chromosomes at meiosis, but more frequently the chromosomes are united into two pairs. Owing to the structural dissimilarity between the members of these pairs the bivalents are

generally rod-shaped, the chiasma frequency being definitely lower than in the other five pairs." The total average chiasma frequency of this plant was not lower than that of the other two plants suggesting a unitary control of chiasma per cell. A bridge and fragment were found at A I in one sporocyte of 80.

From an analysis of several populations of northern European D. glomerata, Muntzing (1933, 1937a) concluded that D. glomerata is cytologically unstable, aneuploids being present in every population studied. The mean frequency of aneuploids was 10%. The frequency of multivalents (IV and III) at diakinesis and metaphase I varied considerably from side view to polar view, 2.95 vs. 3.81 in the variety "Skandia II." In a wild biotype from Altai, the frequency of IV at diakinesis based on 30 cells was 3.0. A determination made on 12 cells at metaphase I in the same biotype yielded a value of 2.8 IV's/cell. Of 52 IV observed in side view 25 were zig-zag rings, 12 zig-zag chains, 11 non-zig-zag rings, and 4 non-zig-zag chains. About 70% of the configurations appear as closed rings and 30% as chains. There was a marked tendency for adjacent chromosomes to go to opposite poles, about 70% of the IV having zig-zag orientation. In a "Skandia II" biotype the anaphase one separation was as follows: 44 cells 14-14, 2 cells 13-15, 4 cells 13-14 with one laggard I, and 2 cells 13-13 with 2 laggard I. A biotype from Altai had a 14-14 distribution in 13 sporocytes at A I. Dividing I were found at anaphase one in approximately 5% of the cells in "Skandia II" and 4% in Altai.

Myers and Hill (1940b) found that 59% of 116 tetraploid plants of D. glomerata examined were euploid, the remainder being aneuploid. Three plants examined had 3.8, 4.2, and 3.3 IV per cell. The percentages of A I

sporocytes showing a dicentric bridge and fragment were 3.6, 10.4, and 0.0. Inversion heterozygosity was suggested as the basis for bridge formation. A cytological study by Myers (1941b) of 20 tetraploid plants from open pollinated populations revealed a range in quadrivalent frequency of 2.4 to 4.4 IV's/cell. There were significant differences between clones. A later report by the same author (1942) on similar plant material indicated that variation in IV frequency between two collection dates was significant. The number of univalents per hundred sporocytes at M I ranged from 0 to 27.3. In 18 of 20 plants one or more sporocytes had a dicentric bridge and fragment.

A subsequent study by Myers (1943) of tetraploid D. glomerata was baised on an average of 38 diakinesis sporocytes in one year and an average of 24 such sporocytes the following year. The frequency of trivalents was low varying from 0 to 0.09 among nine clones. Utilizing data from six clones in two years, a significant year x clone interaction was found for IV frequencies at diakinesis. Position of plants in the field had little effect on IV frequency. The clones ranged from 3.0 to 4.3 in IV frequency. Differences among clones were significant. The number of half-chiasmata per chromosome ranged from 1.69 to 1.90, and the differences were significant. Again field position had no effect on this character. The per cent of sporocytes with univalents at M I for nine clones varied from 3 to 29%. Clonal differences were significant as was the clone x years interaction. Field position affected I frequency. The correlation of chiasma frequency and quadrivalent frequency was positive and significant. Analysis of the correlation coefficients of half-chiasma and quadrivalent frequency indicated that no more than 31% of the squared variation in IV frequency among

sporocytes within plants could be attributed to variation in half-chiasma.

Myers (1943) reported studies of 8 clones of tetraploid D. glomerata, known to differ significantly in meiosis, and the progeny of these clones. In six families, the average number of quadrivalents for the inbred plants was nearly identical with the value for their parents, the values for progeny and parents respectively being 4.08 - 4.09, 4.04 - 4.06, 3.99 - 3.92, 3.98 - 3.95, 3.80 - 3.79, and 3.50 - 3.50. In two families, quadrivalent frequency increased significantly with inbreeding: 3.50 - 3.04, and 3.66 - 3.00.

Myers and Hill (1943) in a more detailed report on the effects of inbreeding on meiosis, noted that a family with 20 I_1 plants had a frequency of half-chiasmata per chromosome at diakinesis of 1.78 for the parent and 1.71 for the average of the I_1. The range among the I_1 was from 1.35 to 1.90 with statistically significant differences. A second family had values of 1.77 for the parent and 1.82 as an average for the progeny with a range of 1.80 to 1.88. Chiasmata determinations were made on an average of 21 diakinesis sporocytes. Differences among the I_1 were not statistically different. While the two parents had nearly the same chiasma frequency, the means of their progenies were significantly different. It was concluded that the parents differed in factors conditioning chiasma frequency. Quadrivalent frequency per cell at diakinesis ranged from 2.62 to 4.91, and was found to be heritable. An average of 28 sporocytes per plant were used in these determinations. For the 26 inbred plants, the total chiasma frequency accounted for only 35% (r^2) of variability among plants in frequency of quadrivalents thus suggesting that other factors influence quadrivalent frequency. The results seemed consistent with the hypothesis

of segregation of two or more genes, chromosomal rearrangements, or both, with small cumulative effects upon IV formation. The average frequency of M I sporocytes with univalents was two to three times greater in the inbreds than in their respective parents. The average percentage of M I cells with univalents ranged from 3 to 24% in the 8 parents and 0.78 to 97.0% in the I_1.

Myers (1948a) reported that the I_2 progenies of two I_1 plants were unchanged in average quadrivalent and chiasma frequencies, but there was a four to six fold increase from I_1 to I_2 in frequency of M I univalents. One family had a range of 3.4 to 5.3 quadrivalents per sporocyte for 12 I_2 progeny, while chiasmata varied from 1.8 to 1.9, and the percentage of univalents at M I varied from 4.4 to 30.2.

Hanson and Hill (1953) reported a range in IV frequency at diakinesis in thirteen 4n plants of 0.88 to 2.22, with an avg. of 1.75 $\pm$ 0.10. The avg. number of half-chiasmata was 1.78 $\pm$ 0.02 with a range of 1.66 to 1.93. These plants were derived from crosses involving a tetraploid plant identified as possessing a reciprocal translocation. It was suggested that environmental conditions may have contributed to the low IV frequency. These authors also suggested that precocious disjunction of loosely held configurations at early metaphase I may contribute to the number of univalents observed at this stage. Since plants differ significantly with respect to meiotic regularity this phenomenon must be partially controlled by genotype.

Curran (1961) reported a cytological study of 27 clones of D. glomerata from the United States, Ireland, and regions of Mediterranean climate. The range in frequency of univalents at M I was from 1.3 to 28.0.

Eleven plants exhibited bridges and fragments indicative of inversion heterozygosity at A I. Some of the bridges were clearly cases of delayed separation. It was suggested, on the grounds that such bridges were found in only a small percentage of the A I and T I cells and on the assumption that inversions might involve only small segments, that inversions may have been present in more than the 11 plants. Chiasma frequencies per sporocyte were determined in four plants at diakinesis as follows: 24.3 based on 19 cells, 20.9 based on 13 cells, 20.9 based on 8 cells, and 23.7 based on 12 cells.

Zohary (1956) reported IV frequencies per cell at diakinesis of 3.64 in tetraploid maritima, 3.28 in tetraploid hispanica from Spain, and 3.18 in a tetraploid collection from Ankara, Anatolia.

Dollinger (1947) reported that temperatures up to 37 C had a significant effect on number of univalents at M I, per cent of sporocytes with univalents, chiasma frequency at diakinesis, and number of quadrivalents. Oldemeyer (1952) studied field collections from individual clones taken at different dates and concluded that higher mean or maximum temperatures 48 to 72 hours prior to collection were associated with an increase in frequency of micronuclei. Weiss, Taylor, and Johnson (1951) found significant positive correlations between frequency of quartets with micronuclei and either maximum temperatures on the day of sampling or maximum temperatures 24 or 48 hours prior to sampling.

McCollum (1958) analyzed meiosis in the following diploid subspecies, diploid hybrids, and their induced tetraploids: lusitanica, ibizensis, juncinella, Smithii, ibizensis x lusitanica, Smithii x lusitanica, lusitanica x juncinella, and judaica x lusitanica. Natural tetraploids and tetraploid hybrids were also analyzed: "typical glomerata," maritima from

Portugal, hispanica from Spain, glomerata x maritima, and ibizensis x maritima. The mean number of IV per cell in the tetraploid tissue was only slightly lower at metaphase than at diakinesis (3.44 vs. 3.50). The mean number of IV per cell ranged from 2.54 to 4.51. The effect of date of fixation was found to be sufficiently lower than the effect of plants so as to be disregarded in the main analysis of IV frequencies. Results of subjecting five tetraploid plants to low temperatures (25 - 36 F) for four or five days during flowering were such as to suggest that moderate changes in temperature played a relatively minor role in variation in IV frequency. The hybrid induced tetraploids had as high a IV frequency (3.55) as the non-hybrid induced tetraploid (3.57). The induced tetraploids had a slightly, but not statistically significant, higher IV frequency than the natural tetraploids. Natural tetraploids had a higher frequency of zig-zag IV at M I than did induced tetraploids, but all fell below the 70% zig-zags reported by Muntzing. Four plants representing collections from three different locations in Spain exhibited lower IV frequencies (less than 3.0) than are usually reported. This suggested that progress toward diploidization in local ecotypes should be considered. Analysis of the binomial distribution of IV frequency led to the conclusion that quadrivalent frequency in all the 4n material studied - - induced and natural, subspecies and hybrid - - is the same from one set of four chromosomes to the next. A highly significant correlation coefficient ($r = 0.539$) between mean number of IV and chiasmata per cell was found. The regression coefficient of quadrivalents on chiasmata ($b = 0.164$) was highly significant. It was suggested that no more than 30% (r^2) of the variation in frequencies of IV

among plants should be attributed to variation in chiasma frequency. The frequency of chiasmata per chromosome in lusitanica was .870 in the tetraploid and .897 in the diploid. In general, hybrid induced tetraploids had the same chiasma frequency as the tetraploids from pure subspecies. Natural tetraploids had a slightly higher chiasma frequency than the induced tetraploids. For the tetraploid lusitanica plant studied, the following associations were determined at diakinesis: 4.00 IV, 0.07 III, 5.60 II, and 0.67 I. Mean per cent good pollen was 88.3 for four diploid subspecies and 82.1 for three diploid subspecies hybrids involving lusitanica. Mean per cent seed set was 19.37 for the four diploid subspecies and 27.82 for the three hybrids. However, environmental conditions were sub optimal for pollination and seed development.

Jones (1962) reported on studies of the diploid subspecies: Aschersoniana, Woronowii, lusitanica, judaica, Santai, the diploid hybrids: Woronowii x Aschersoniana, Woronowii x lusitanica, Aschersoniana x lusitanica, and tetraploids from northern and Mediterranean Europe. Of 460 tetraploid plants examined only 3.2% were aneuploid. Regular pairing with 7 II at metaphase I was reported in the diploid subspecies and their hybrids. The range in mean chiasmata per cell was as follows: Aschersoniana (4 plants) 10.60 - 12.40, lusitanica (26 plants) 10.95 - 13.68, and Aschersoniana x lusitanica (5 plants) 11.68 - 12.44. On the tetraploid level a highly significant difference was found in IV frequency between natural tetraploid hispanica and glomerata plants, on the one hand, and the hybrids between them on the other hand. An analysis of chiasma frequencies failed to detect differences between the hybrids and non-hybrids. While a good positive correlation (r = 0.712) was found

between chiasma frequency and IV frequency in the non-hybrids, the correlation was much poorer in the hybrids (r = 0.198). It was suggested that something other than chiasma frequency, i.e., chromosome differentiation, reduced the frequency of IV in the hybrids. Of 25 plants derived from aged seed 12 showed evidence of translocations. It was suggested that ageing of seed may be a contributing factor in chromosomal mutations.

Tarkowski (1964) reported higher IV frequencies in wild populations of D. glomerata than in cultivated varieties. Three cultivated varieties averaged 1.86 IV per cell, while three wild populations averaged 3.43 IV per cell. Chain IV occurred more frequently in wild populations (.97) than in cultivated varieties (.13).

Stebbins and McCollum (1955) reported that a cross of lusitanica x judaica (the judaica pollen having received 1,250 r of x-rays) yielded 126 F_1 plants of which 51 had less than 20% of normal pollen. In the control non-irradiated cross, pollen fertility of the 43 F_1 plants ranged from 38 to 88% of normal. Using the same lusitanica plant as female parent in a cross with pollen of another lusitanica clone irradiated with 1,250 r, only 5 of 120 plants were found with less than 20% of normal pollen. Most of the lusitanica x judaica plants with low pollen fertility formed seven bivalents in at least some sporocytes, but some formed a ring or chain of four chromosomes, and a few had two rings or chains of four. Of the induced tetraploids of four sterile plants, two were fertile and two remained sterile. One of the fertile amphidiploids was derived from a diploid with slightly irregular meiosis but little obvious structural hybridity, the other was derived from a diploid heterozygous

for two translocations. In one of the two sterile amphidiploids, the sterility was due to disturbances of meiosis initiated at A I.

Chromosome Pairing and Diploidization

Brief synopsis of the traditional interpretation of meiosis

The traditional interpretation of meiotic events may be found in any elementary text in the fields of botany, zoology, genetics, or cytology. For purposes of comparison with recent alternative interpretations, the following salient points will be briefly noted. The chromosomes become visible at leptonema, and pair during zygonema. Genetic crossing over occurs during pachynema. Longitudinal separation of the paired chromosomes is initiated during diplonema with terminalization of chiasma at diakinesis (Swanson (1957)). This interpretation is taken as the framework for the present study. Alternative interpretations are presented as a matter of record.

Alternative interpretations of meiosis

Grell (1965) has proposed two modes of pairing at meiosis. The first in order of time is exchange pairing which takes place during interphase or very early prophase. This process is a necessary, but not sufficient cause of genetic exchange between homologues. The second mode of pairing is the distributive pairing of chromosomes which did not associate in the previous phase. Such pairing permits orientation of the chromosomes for disjunction at M I. If more than two elements are present, pairing is competitive; it may be influenced by homology but it involves nonhomologous elements as well (Grell (1962)).

Smith (1942) concluded on the basis of accumulated cytological studies that meiotic pairing consummated at pachytene is initiated at the latest by telophase of the last premeiotic division.

Maguire (1966) reported that the crossover frequency in a heterozygote for a short paracentric inversion in maize (estimated from the frequency of bridges and fragments) corresponded closely to the frequency of homologous synapsis within it at pachytene. She concluded therefore, that either crossing over is a precondition for homologous pachytene synapsis or invariably follows synapsis of the tested region even when its genetic length is substantially less than 50 units. The same author (1965) on the basis of cytological observation in maize suggested that an alignment of homologous chromosomes is achieved or initiated during the anaphase preceding meiosis. This phase is nonsaturated, more than two members may participate at any one point. This is followed by intimate two-by-two pairing of short segments spaced at considerable distance. In heterozygous aberration configurations, alignment is interrupted in immediate regions of partner exchange. There exists what may be formally interpreted as an element of resistance to the formation by a chromosome of effectively paired segments with two other chromosomes, when three chromosomes share a region of common homology. The nonhomologous pairing might be expected to follow homologous pairing in time. Pachytene chromosomes may already contain crossovers or their potential or established precursors.

Weber (1966) presented A I data on trisomic and double trisomic plants interpreted as not giving evidence of distributive pairing in maize.

Recent synaptic models

Sybenga (1966) hypothesized the existence of functional units of chromosome pairing initiation, termed zygomeres. These may be discrete units that occur in large numbers along the chromosome, or in lower frequency concentrated in certain areas of the chromosome, or a single zygomere in a large segment or entire chromosome. Three phases of chromosome pairing were postulated:

1. zygomere pairing,
2. locus - - specific protein pairing, and
3. DNA pairing.

Preferential pairing may then occur in several modes. Chromosomes may differ in type (locus) or activity of their zygomeres. Complementary activity of zygomeres may result in preferential association. When pairing is based on a series of zygomeres, disturbance of the order by a rearrangement may make pairing less efficient between normal and rearranged chromosomes. If zygomeres are activated at different periods, preferential association may result. If two populations have in common some zygomeres, but not the most active ones, preferential pairing may occur in the amphidiploid if a genetic system is introduced that either

1. decreases the overall level of zygic activity,
2. reduces the period of activation and activity, or
3. suppresses the activation or activity of common zygomeres.

Riley (1960) has established that a factor or factors on chromosome 5 B of wheat conditions bivalent pairing of homologous chromosomes and precludes pairing of homoeologues. He has suggested that chromosome

5 B appears to control pairing by reducing attraction to a low level, below which homoeologous pairing cannot be completed. This is presumably a system which could not evolve in a true autopolyploid where each chromosome is equally attracted to a number of identical homologues. He further suggested (1965) that structural differences sufficient to lead to only a moderate reduction in chiasma frequency may be great enough to preclude intergenomic synapsis in a genotype with restricted pairing specificity. It may be worthwhile to search for mutants, with altered pairing specificity, in tetraploids derived from hybrids that have slightly lower chiasma frequencies than in the parental diploids.

Diploidization

Gaul (1964) has suggested that the formation of bivalents in a polyploid as opposed to multivalents may be due to a balance of the following factors: structural differentiation of the chromosomes, asynaptic gene effect, and chromosomal interference.

Sybenga (1965) reported finding multivalent association rather than bivalents in work with translocations in polyploid rye. He suggested that inversions may offer more promise than translocations in attaining diploidization.

Grell (1961) reported that triploid Drosophila females bearing one rearranged second and two normal second chromosomes, produced progeny which indicated that the two normal chromosomes tended to pair and pass to opposite poles at the first meiotic division. Preferential segregation was related to the extent to which the sequence of the rearranged second chromosome deviated from normal chromosome sequence.

Doyle (1963b) reported genetic evidence of preferential pairing, in chromosome 3 of maize trisomics, in 8 of 12 cases involving two standard chromosomes and one chromosome of exotic background. Similarly the same author (1963a) reported genetic evidence of preferential pairing in 30 of 41 plants trisomic for chromosome 3, where one chromosome 3 carried an inversion and had been subjected to 1,000 r of x-rays. It was suggested that large and small inversions, translocations, and deletions might be the basis for preferential pairing. A later report by the same author (1966) indicated the operation of positive and negative preferential pairing of chromosome 3. In this case, of 26 plants trisomic for chromosome 3 and carrying a chromosome 3 subjected to 1,000 r of x-rays, six plants showed a significantly lower transmission of the genetic marker, while six other plants displayed a significantly higher transmission of the marker. In order to explain negative preferential pairing it was suggested that at leptotene a small inversion would facilitate pairing if the chromosomes slipped past one another in reverse order. In a similar test in the same study, utilizing a different marker, six of seven plants gave indications of preferential pairing.

Shaver (1962c) reported that perennial teosinte was a modified autotetraploid, with a reduced chiasma frequency and an extension of chiasma interference across the centromere, resulting in a reduced IV frequency. These IV were restricted to rings and chains. Meiosis was generally regular and 93% of the seedlings were euploid. While pachytene observations revealed frequent exchange of pairing partners within chromosome arms, complex IV rarely occurred at M I.

Shaver (1963) reported on a study of two types of allotetraploids

of perennial teosinte x maize, one carrying an inversion, the other without an inversion. Similarly, structurally heterozygous and non-heterozygous autotetraploids of pure maize were produced. Calculations based on bridge frequency in the autotetraploids indicated that the macro rearrangement would not be a stabilizing influence in spite of its effect in increasing preferential segregation. In the allotetraploid, inversion bridge formation appeared to be absent, presumably because of a threshold effect of the inversion. It was concluded that this macro structural hybridity would be a positive influence in diploidizing this allotetraploid by greatly increasing preferential pairing, and by preserving large blocks of chromatin inviolate against interspecific crossing over. It was postulated that ordinary divergence in chromosome structure between related species may proceed through mutation or cryptic structural changes up to a threshold level, which appears surprisingly low. Beyond this level allotetraploids may retain the specific integrity of large blocks of chromatin because of the abolition of interspecific crossing over due to the presence of large inversions in heterozygous condition.

Menzel (1964) reported that in an allotetraploid of the intergeneric hybrid Lycopersicon esculentum x Solanum lycopersicoides, the chromosomes exhibited a very high degree of preferential pairing at pachytene, despite the fact that homoeologues synapse almost perfectly at pachytene in the corresponding F_1 hybrid. Preferential pairing was shown to be due to highly non-random synapsis rather than to preferential chiasma formation in the allotetraploid. The ability to discriminate exact homologues from homoeologues seemed to be uniformly distributed

along chromosomes and chromosome segments, and not attributed to differential heterochromatinization or to linear rearrangements long enough to be visible at pachytene.

Gilles and Randolph (1951) reported a decrease in IV frequency, and an increase in II frequency in maize after ten generations of selection for vigor, fertility, and other traits. It was suggested that a gene or genes influencing chromosome association had been selected for during the ten year period.

Dudley and Alexander (1966) reported an increase from 60% to 83% in seed set in an autotetraploid maize synthetic selected for seed set and agronomic desirability over a period of eight years.

Busbice and Wilsie (1966a,b) suggested, on the basis of the segregation pattern of the genetic characters Dw_1 and Dw_2 that preferential pairing may be operative in alfalfa. Buzzell and Wilsie (1963) suggested preferential pairing in duplex plants of Lotus corniculatus as a possible explanation for the inheritance of the brown keel tip character.

Doggett (1957) reported that the F_1 and F_2 from the cross of induced tetraploids of different races of Sorghum vulgare gave decidedly better seed set than that of the parents. It was suggested that geographical isolation had led to the development of local races. Sufficient differentiation of the genomes may have taken place for preferential association to occur suggesting a degree of alloploidy.

Snope (1966) reported an excess, 81.9%, of homomorphic bivalents over the expected, 33.3%, in 4n maize duplex for a large heterochromatic knob on chromosome 10.

Heinz and Elliott (1964) irradiated orchardgrass with neutron radiation

to produce translocation for use in a breeding program.

Buzzell (1965) calculated the effects of varying degrees of homogenic and heterogenic pairing on genetic ratios.

Irradiation

Gaul (1964) reported that the variability of radiation sensitivity is such that, under certain circumstances, the same dose on the same material will sometimes cause 100% killing and other times result in complete survival and no mutations.

MATERIALS AND METHODS

Plant Materials

Seeds of the following diploid subspecies of Dactylis were obtained from Dr. G. L. Stebbins: lusitanica collected at Algeuviao, Portugal, smithii collected at Iaganona, Canary Islands, Aschersoniana collected in Sweden, himalayensis collected at Gongi Tehai, India, and Woronowii from Iran. The seed was produced as follows: Aschersoniana and lusitanica - - open pollinated seed harvested in 1956 in California, himalayensis - - open pollinated seed harvested in 1958 in California, smithii - - open pollinated seed harvested in 1955 in California, and Woronowii seed harvested in 1952 at the U. S. Pasture Research Laboratory in Pennsylvania. The seed was maintained in cold storage and planted in March, 1964.

X-ray Irradiation

Two cycles of irradiation were used. The first cycle delivered to unexcised panicles prior to meiosis ranged from 450 r to 750 r. The rate was approximately 23 r/min. A General Electric Maxitron with 250 pkv, 30 ma, and filtration of .25 mm Cu and 1 mm Al was used for the irradiation. For the second cycle excised panicles with dehiscing anthers were irradiated with 750 r at 30 r/min using a source with 250 kvp, 15 ma, .5 mm Cu, and 1 mm Al. Irradiated panicles were used only as pollen parent.

Colchicine Treatments

Eight major colchicine treatments were used. These are listed below:

1. Inverted capillary tubes containing aqueous solutions of .05%, .10% and .20% colchicine were affixed over the cut surface of repotted tillers. Three fillings of .3 ml capillary tubes were applied.
2. Same as above, with the exception that the tubes were placed on tillers six inches to one foot above the base of well established plants in pots.
3. Capillary tubes with .2%, .4%, and .8% solutions of colchicine were applied to the cut surface of recently rooted tillers about two inches above the base. Treatment lasted 24 hours.
4. Immersion of tillers, freshly removed from potted material, in aqueous solutions of .1%, .2%, and .4% colchicine for nine hours.
5. Immersion of recently separated tillers for seven hours in .4% colchicine.
6. Immersion of rooted tillers in .4% colchicine for eight hours.
7. Immersion of recently separated tillers pretreated with a fertilizer solution one hour before treatment with .2% aqueous solution of colchicine.
8. Same as above, but with a 24 hour pretreatment with fertilizer.

Cytological Techniques

Root tip chromosome counts were made on material pretreated with a saturated aqueous solution of paradichlorobenzene for six hours at 12° C, fixed in Farmer's fluid, and transferred to 70% ethyl alcohol 24 hours after fixing. The following treatment was found satisfactory for 4n meiotic material: collection in Carnoy's fluid and immediate vacuum treatment followed by storage at 35° F for nine hours, and a change to fresh solution for storage at 35° F until 24 hours after collection when the material was transferred to 70% ethyl alcohol for permanent cold storage. For meiotic study of diploid material, fixation in Carnoy's solution and transfer, after 24 hours to one week, to a 70% solution of ethyl alcohol was satisfactory. Aceto-carmine smears were used for mitotic and meiotic material. A binocular phase contrast microscope with oil immersion was used for cytological analysis.

Pollen fertility determinations were made on samples of approximately 150 to 200 pollen grains from material collected and stored in 70% ethyl alcohol and stained in aceto-carmine.

Chiasma frequency was determined by predicating the minimum number of chiasma necessary to maintain the chromosome associations observed.

RESULTS

Examination of Diploid Parental Materials

The parental materials employed were found to conform to the morphological description given by Stebbins (1958). All the parental plants were determined to be diploid by root tip counts. These determinations were later verified, by meiotic analysis, in the materials utilized in further studies.

Diploid Plants Generated by the 1st Cycle of X-ray Irradiation and Intrasubspecific Hybridization

The first cycle of x-ray irradiation is described in Table 1. Only two of the sixteen irradiated panicles served successfully as pollen parents. Irradiated panicles were retarded in development, produced a large amount of sterile pollen, and in some cases failed to extrude anthers. The irradiated and control crosses yielding seed are presented in Table 2. The female parents were essentially self sterile.

Pollen fertility of the progeny of the irradiated and control crosses was assessed both in the field and in the greenhouse. Maximum per cent fertility of the progeny is listed in Table 3. Plants with low pollen fertility were selected in both irradiated populations for cytological analysis. No evidence of translocation was found. A few plants in the Aschersoniana population exhibited a low degree of asynapsis or desynapsis (Fig. 1A). Anaphase I bridges were observed in several plants. These results are reported in Table 4. Some plants showing low pollen fertility in the field exhibited high pollen fertility under the non-

Table 1. Clones subjected to x-irradiation prior to meiosis

Subspecies	Clone	Dosage
Smithii	F-5	750 r
	F-6	750 r
lusitanica	C-1	450 r
	C-2	750 r
	C-3	750 r
	C-5	450 r
Woronowii	G-2	450 r
	G-5	650 r
	G-6	650 r
	G-13	450 r
Aschersoniana	B-1	450 r
	B-4	650 r
	B-6	650 r
	B-5	450 r
Himalayensis	I-2	650 r
	I-4	650 r

Table 2. Seed set in irradiated and control crosses of the 1st cycle, and self fertility of the parental clones

Cross	# panicles	# seedlings
Aschersoniana		
B-5 x B-4	Portion of one panicle	86
B-5 x B-4-650r	2	40
B-5 selfed	3	1
B-4 selfed	1	0
lusitanica		
C-1 x C-3	Portion of one panicle	100+
C-1 x C-3-750r	2	100+
C-1 selfed	2	2
C-3 selfed	1	0

stress conditions in the greenhouse. Plants showing relatively low pollen fertility were selected as parents for the 2nd cycle of irradiation and concomitant intersubspecific hybridization, the rational being that the plants with lower fertility would be more likely to carry cryptic structural differentiation in their chromosomes. Plants with good pollen fertility and normal bivalent pairing (Fig. 1B) were selected as parents in the control population.

Table 3. Pollen fertility of the progeny of irradiated and control crosses of 1st cycle of irradiation

Aschersoniana				lusitanica			
Control population clone	% pollen fertility	Irradiated population clone	% pollen fertility	Control population clone	% pollen fertility	Irradiated population clone	% pollen fertility
2-1	62.9	3-2	93.5	24-1	82.0	25-1	90.4
2-2	89.1	3-3	80.1	24-7	84.4	25-2	85.5
2-3	90.6	3-4	85.5	24-11	87.6	25-3	89.2
2-4	72.2	3-5	93.3	24-12	90.2	25-6	91.4
2-5	61.9	3-6	69.4	24-13	91.1	25-7	90.8
2-6	67.6	3-7	79.9	24-16	86.9	25-11	91.0
2-7	96.2	3-9	85.7	24-17	98.3	25-12	93.8
2-8	94.6	3-10	83.7	24-18	91.6	25-13	92.5
2-9	85.4	3-11	85.6	24-20	95.0	25-14	93.9
2-10	89.4	3-12	82.9	24-21	87.6	25-16	89.1
2-11	2.7	3-13	46.0	24-22	90.8	25-17	94.9
2-12	76.3	3-14	70.2	24-24	81.4	25-18	97.9
2-13	97.7	3-15	81.0	24-27	84.9	25-21	96.7
2-14	88.7	3-16	69.8	24-28	86.1	25-23	97.3
2-15	80.0	3-17	90.3	24-29	95.1	25-24	94.2
2-16	96.9	3-18	90.5	24-30	85.4	25-25	95.6
2-18	10.9	3-19	58.6	24-32	77.1	25-26	95.4
2-19	58.3	3-20	91.8	24-33	88.8	25-29	91.6
2-20	85.4	3-21	64.1	24-38	99.3	25-30	80.6
2-21	48.3	3-22	76.4	24-39	95.6	25-31	94.0
2-22	77.1	3-23	56.8	24-40	92.8	25-32	93.7
2-23	84.1	3-24	58.0	24-42	91.8	25-33	87.8
2-24	90.0	3-25	88.2	24-43	94.6	25-34	88.6
2-25	31.7	3-26	98.5	24-46	86.1	25-36	90.4
2-26	82.5	3-27	94.3	24-47	95.9	25-39	94.5
2-27	81.3	3-28	43.1	24-49	91.8	25-41	69.3
2-29	84.8	3-30	64.0	24-51	92.9	25-42	80.7

Table 3. Continued

Aschersoniana				lusitanica			
Control population clone	% pollen fertility	Irradiated population clone	% pollen fertility	Control population clone	% pollen fertility	Irradiated population clone	% pollen fertility
2-30	83.2	3-31	65.7	24-53	89.9	25-45	94.8
2-33	52.7	3-32	73.9	24-55	86.0	25-46	86.2
2-34	0.0	3-33	66.9	24-62	85.4	25-49	92.6
2-35	82.0	3-34	37.5	24-65	93.6	25-51	93.0
2-36	94.0	3-37	91.0	24-66	91.6	25-53	83.7
2-37	82.6	3-38	87.5	24-67	87.9	25-57	95.9
2-38	84.7	3-39	62.8	24-77	94.2	25-60	74.0
2-39	26.0			24-78	85.3	25-62	93.4
2-40	78.6			24-79	85.6	25-63	89.4
2-49	90.8			24-80	96.6	25-65	13.4
2-53	81.4			24-82	95.7	25-67	81.0
2-61	92.0			24-83	76.0	25-68	94.5
				24-85	93.8	25-70	94.5
				24-86	86.0	25-71	89.9
				24-87	81.9	25-72	87.7
				24-90	92.2	25-73	88.3
				24-91	93.2	25-75	92.0
				24-92	86.4	25-76	70.3
				24-94	95.8	25-83	87.4
				24-97	89.5	25-84	79.4
						25-88	93.3
						25-89	92.5
						25-92	93.0
						25-93	74.3
						25-95	93.5
	Avg. 72.7		Avg. 75.5		Avg. 89.6		Avg. 88.1

Table 4. Chromosome associations and bridges in selected clones of 1st cycle irradiated and control populations

Clone	Diak. or M I	# cells 7II	# cells 6II+2I	# cells 5II+4I	# cells 4II+6I	% A I with bridges	# cells
Aschersoniana control population							
2-9	D	71					
2-11	D	36					
2-13	D	43				0.0	41
2-18	M I	29					
2-19	D	20					
2-25	M I	27					
2-33	D	33					
2-34	M I	23					
2-36	D	52					
2-39	M I	28					
Aschersoniana irradiated population							
3-6	M I	109	1				
3-12	M I	34					
3-13	D	41				5.3	38
3-13	M I	24	1				
3-19	D	52					
3-23	D	117				2.4	123
3-24	M I	63	6	1	1		
3-28	D	111					
3-30	D+M I	28					
3-33	D	26					
3-34	M I	27					
lusitanica control population							
24-17	D	100				24.2	66
24-42	D	187				5.6	36
24-80	D	118					
lusitanica irradiated population							
25-21	M I	27					
24	M I	70					
41	M I	61					
53	M I	41					
60	D	92					
65	D&M I	40					
76	D&M I	40					
89	M I	34				12.5	385
93	D	71	1				

Fig. 1. Meiosis in diploid tissue (Magnification 727 X)

A. Metaphase I in Aschersoniana clone 3-13 showing 6 II and 2 I

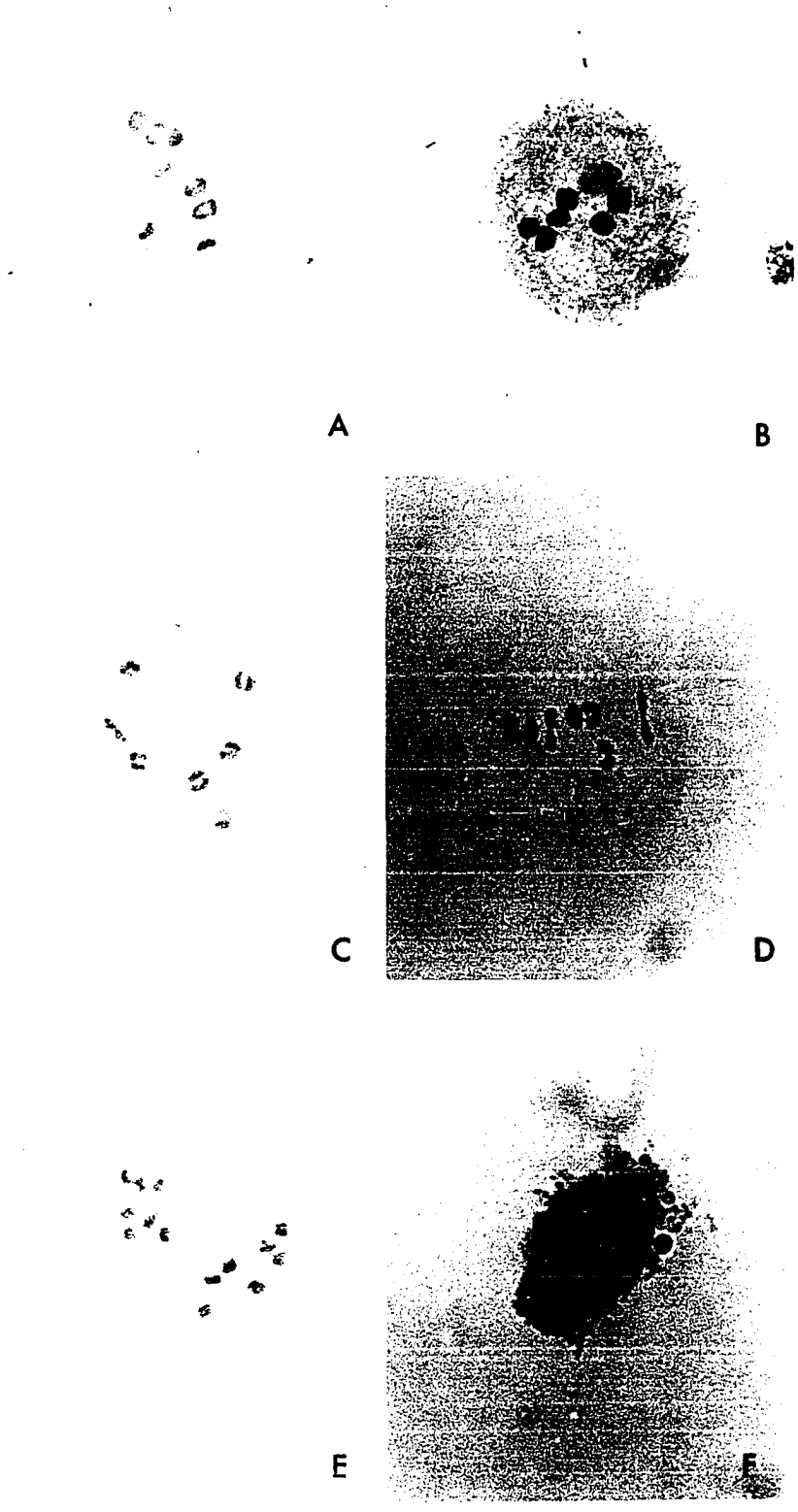
B. Diakinesis in lusitanica clone 24-42 showing 7 ring II

C. Diakinesis in intersubspecific hybrid from the irradiated population, clone 113-5, with 6 ring II and 1 rod II

D. Metaphase I in intersubspecific hybrid of the control population with 7 II, clone 115-6

E. Anaphase I in clone 3-13 showing normal 7-7 separation

F. Anaphase I in clone 2-9 showing a bridge


A
B
C
D
E
F

Diploid Plants Generated by the 2nd Cycle of Irradiation and Intersubspecific Hybridization

Cross and self fertility data are presented in Table 5. Lusitanica plants were chosen as the female parent because they exhibited relatively higher pollen fertility than the Aschersoniana parent, and produced a greater number of florets per panicle. Seed was obtained in these crosses with considerably greater facility than with the premeiotic irradiation of the first cycle.

Pollen fertility, chiasma frequency, and A I bridge frequency of approximately six progeny plants from each of three crosses are presented in Table 6. Parental plants are also included for comparison.

Table 5. Seed set in irradiated and control crosses of the 2nd cycle, and self fertility of the female parent

Cross	# panicles	# seedlings
25-60 x 3-23 (750r)	Portion of one panicle	40
25-60 selfed	1	0
25-93 x 3-19 (750r)	1	23
25-93 selfed	3	0
25-89 x 3-13 (750r)	2	20
25-89 selfed	1	1
24-17 x 2-36	2	24
24-17 selfed	2	2
24-42 x 2-13	1/2	100+
24-42 selfed	3	0
24-80 x 2-9	Portion of one panicle	100+
24-80 selfed	2	3

Table 6. Pollen fertility, chiasma frequency at diakinesis, and % of A I cells with bridges for parental clones, 1st cycle clones used as parents of the 2nd cycle, and intersubspecific hybrids of the 2nd cycle

Clone	Maximum % pol. fert.	Chiasma per chr.	# of cells	% of A I cells with bridges	# of cells
parental clones					
B-4	60.5	.915	37	0.0	66
B-5	69.8	.893	169	1.9	108
C-1	95.9	.977	271	0.0	106
C-3	91.7	.984	234	2.6	77
irradiated population					
25-93	82.0	.951	38	19.2	167
3-19	58.5	.918	34	2.1	242
108-1	91.5	.954	109		
108-2	96.0	.933	64	0.0	28
108-3	89.7	.969	135		
108-4	95.1	.900	38		
108-5	93.6	.922	185		
108-6	83.6	.947	35		
25-60	76.6	.977	92	0.0	38
3-23	56.8	.920	52	2.4	123
109-1	96.2	.973	24	2.4	83
109-2	87.3	.949	59		
109-3	94.7	.957	25		
109-4	94.5	.862	65	0.0	95
109-5	90.7	.972	140	3.1	162
109-6	78.8	-	-		
25-89	92.5	.943	80	12.5	385
3-13	46.0	.929	30	5.3	38
113-1	86.5	.945	26	13.8	58
113-2	79.0	.889	275	1.2	241
113-3	70.2	.934	30		
113-4	78.1	.897	34	10.7	261
113-5	87.2	.899	140	2.7	73
113-6	84.7	.923	24	12.9	170
avg. of hybrids	87.63	.931		5.2	130
control population					
24-42	91.8	.953	140	5.6	36
2-13	97.7	.929	43	0.0	41

Table 6. Continued

Clone	Maximum % pol. fert.	Chiasma per chr.	# of cells	% of A I cells with bridges	# of cells
89-1	93.7	.958	24	0.7	153
89-2	85.8	.988	24	0.0	251
89-3	96.8	.964	204	0.6	308
89-4	99.5	.934	435	4.0	100
89-5	95.5	.949	92		
89-6	97.6	.978	125		
24-80	96.6	.984	118	9.7	62
2-9	85.4	.881	71	1.7	230
102-1	73.7	.919	198		
102-2	98.9	.952	37	1.8	113
102-3	99.2	.956	90		
102-4	95.6	.984	127	1.7	121
102-5	94.7	.954	80		
102-6	96.5	.939	46	0.0	36
24-17	98.3	.974	84	24.2	66
2-36	96.5	.863	50	0.0	89
115-1	98.2	.950	41	1.0	194
115-2	85.7	.952	36	6.2	32
115-3	88.8	.918	151		
115-4	95.6	.940	88		
115-5	95.2	.984	27		
115-6	95.4	.980	28		
avg. of hybrids	93.69	.956		1.8	145.3

Pollen fertility in the intersubspecific hybrids is generally quite high and no cytological evidence of translocations was found in any of these plants (Figs. 1C and 1D). Pollen fertility data were transformed by the arc sine method. It was determined that the pollen fertility of the irradiated population was lower than that of the control population at the 1% level of probability (Table 7). Anaphase I bridges were observed with

Table 7. Analysis of variance of the % pollen fertility data, subjected to the arc sine transformation, from the 2nd cycle irradiated and control diploid populations

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F
Total	35	1,879.60		
Treatment	1	396.61	396.61	9.09**
Error	34	1,482.99	43.62	

**1% significance level.

varying frequencies in both the irradiated and control populations. The data are difficult to interpret, but it appears from Table 8 that environmental variation has considerable effect on the frequency of bridges, the highest percentages occurring during the winter months. Chiasma frequency appeared consistently higher in the lusitanica parental clones than in the Aschersoniana parental clones. The irradiated population was significantly lower in chiasma frequency than the control population (Table 9).

Table 8. Monthly distribution of anaphase I bridge frequency in diploid clones

Clone	Dec. 66	Jan. 67	Feb. 67	March 67	April 67	May 67	June 67
B-4	0.0						
B-5						1.9	
C-1						0.0	
C-3							1.9
108-2				0.0			
109-1				2.4			
109-4				0.0			
109-5				3.1			
25-89	15.5						
3-13			0.0				
3-13			5.3				
113-1				13.8			
113-2				2.9			
113-4	12.2	20.8				1.6	
113-5							
113-6	14.5		13.3				
113-6			36.0				
2-13					0.0		
89-1			0.0			1.1	
89-2		0.0					
89-3				0.7		0.0	
89-4				4.0			
24-80	9.7						
2-9			1.7				
102-2						1.8	
102-4				1.7			
102-6						0.0	
24-17	24.2						
2-36		0.0					
115-1			1.8			0.0	0.0
115-2						6.2	
avg.	12.7	6.9	8.3	3.2	0.0	1.4	1.0

Table 9. Analysis of variance of chiasma frequency at diakinesis of diploid plants of the 2nd cycle of irradiation and control populations

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F
Total	34	29,671		
Treatment	1	5,298	5,298	7.17*
Error	33	24,373	738.6	

*5% significance level.

Colchicine Treatment

The results of the colchicine treatment are given in Table 10. Panicles were assessed for the presence of tetraploid tissue by examination of at least three florets from three different spikelets on several glomerules of the panicle. Treatments one and two did not produce the usual syndrome of colchicine shock and, after examination of only a few panicles, material treated in this manner was discarded. The highest rate of doubling was obtained in the fourth treatment with a .4% solution. Pretreatment with a fertilizer solution did not appear to contribute to the efficacy of colchicine treatment.

Table 10. Results of colchicine treatments

Colch. treatment	1	2	3			4			5	6	7	8	Total
			.2%	.4%	.8%	.1%	.2%	.4%					
No. tillers treated	23	39	21	21	22	18	18	18	21	25	54	108	388
No. plants surviving	all	all	18	13	15	18	13	16	20	21	51	106	353
No. panicles examined	11	6	11	10	10	38	22	18	20	17	56	64	283
No. 4n panicles	0	0	2	1	1	5	2	5	1	2	9	8	36
Percent 4n panicles	0	0	18	10	10	13	9	28	5	12	16	12	12.7

Cytological Analysis of Induced Amphidiploids

Data on chromosome associations and chiasma frequency are presented in Table 11. The frequency of IV at diakinesis (Figs. 2A and 2B) are considerably higher than at metaphase (Figs. 2C, 2D, and 2E), while the frequency of I is appreciably less at diakinesis. It appears that terminalization of chiasma or desynapsis has greatly reduced the frequency of IV from diakinesis to metaphase I. Chiasma frequency was greater in the control populations at the 10% level of probability (Table 12). The frequency of IV at metaphase I was lower in the irradiated population; however, the difference was not statistically significant (Table 13).

The data on anaphase I bridge frequency in tetraploid microsporocytes are presented in Table 14. These data were collected in May, June, and July, with the exception of the values for 108-2 collected in February, and 109-1 and 109-3 collected in March. No pattern of seasonal variation was observed. It is evident that the frequency of bridges is higher in the control population. It may be argued that the lower level of bridge formation in the irradiated population is due to preferential pairing induced by increased chromosome differentiation resulting from the application of irradiation. The preferential pairing of homostructural chromosomes does not permit the formation of a bridge by crossing over within the inversion loop. The comparison of the diploid and tetraploid data is complicated by the environmental effect. However, it may be observed that in the control population of intersubspecific hybrids, where the environmental effect was less pronounced, the bridge frequency in the tetraploid tissue is considerably higher than that found in the diploid collections during the same interval of time. Under the

assumption of random pairing of the doubled number of homologues, in the tetraploid tissue, one would expect the bridge frequency to be 132% of the diploid. This figure is by far exceeded.

Anaphase I disjunction in the tetraploid tissue was usually characterized by 14 - 14 separation, however, unequal disjunction was not uncommon (Fig. 2G). Univalents were observed on the M I plate after the other chromosomes had disjoined (Fig. 2G). Micronuclei were observed at telophase II (Fig. 2H).

There are three prerequisites for the formation of IV associations:

1. chromosome homology
2. exchange of pairing partners
3. chiasma formation.

Estimates of the degree to which IV associations depend on chiasma frequency are in the order of 30 to 35% (Myers and Hill (1943) and McCollum (1958)). It was hypothesized that the increase in chromosome differentiation due to x-ray treatment would result in an increase in the importance of chromosome homology in IV formation, and a decrease in the importance of chiasma formation. This was tested by comparing for the irradiated and control populations the correlation coefficient of IV frequency and the number of chiasma per cell, Table 15. Had the radiation induced chromosome differentiation become a more significant factor at the expense of chiasma frequency, one would expect the correlation coefficient to be less in the x-ray population. The opposite, however, was the case, Table 16.

Fig. 2. Meiosis in tetraploid tissue of intersubspecific hybrids (Magnification 727 X)

A. Diakinesis in clone 108-5 intersubspecific hybrid from irradiated population showing 5 IV (3 pan shaped and 2 chains), and 4 rod II

B. Diakinesis in clone 115-6, intersubspecific hybrid from the control population showing 3 IV (all rings), 6 ring II, and 2 rod II

C. Metaphase I in clone 108-1 showing 2 IV, both zig-zag, 5 rod II, and 5 ring II

D. Metaphase I in clone 113-5 showing 2 IV, both rings, 2 III, both chains, 2 rod II, 2 ring II, and 6 I

E. Metaphase I in clone 89-4 showing 3 IV (2 rings and 1 chain), 1 III (v shaped), 4 rod II and 2 ring II

F. Anaphase I in clone 89-4 showing 2 bridges

G. Anaphase I in clone 89-4 showing 12 - 16 disjunction

H. Anaphase I in clone 89-4 showing 6 I on the equatorial plate

I. Telophase II in clone 89-4 showing 6 micronuclei in one quartet

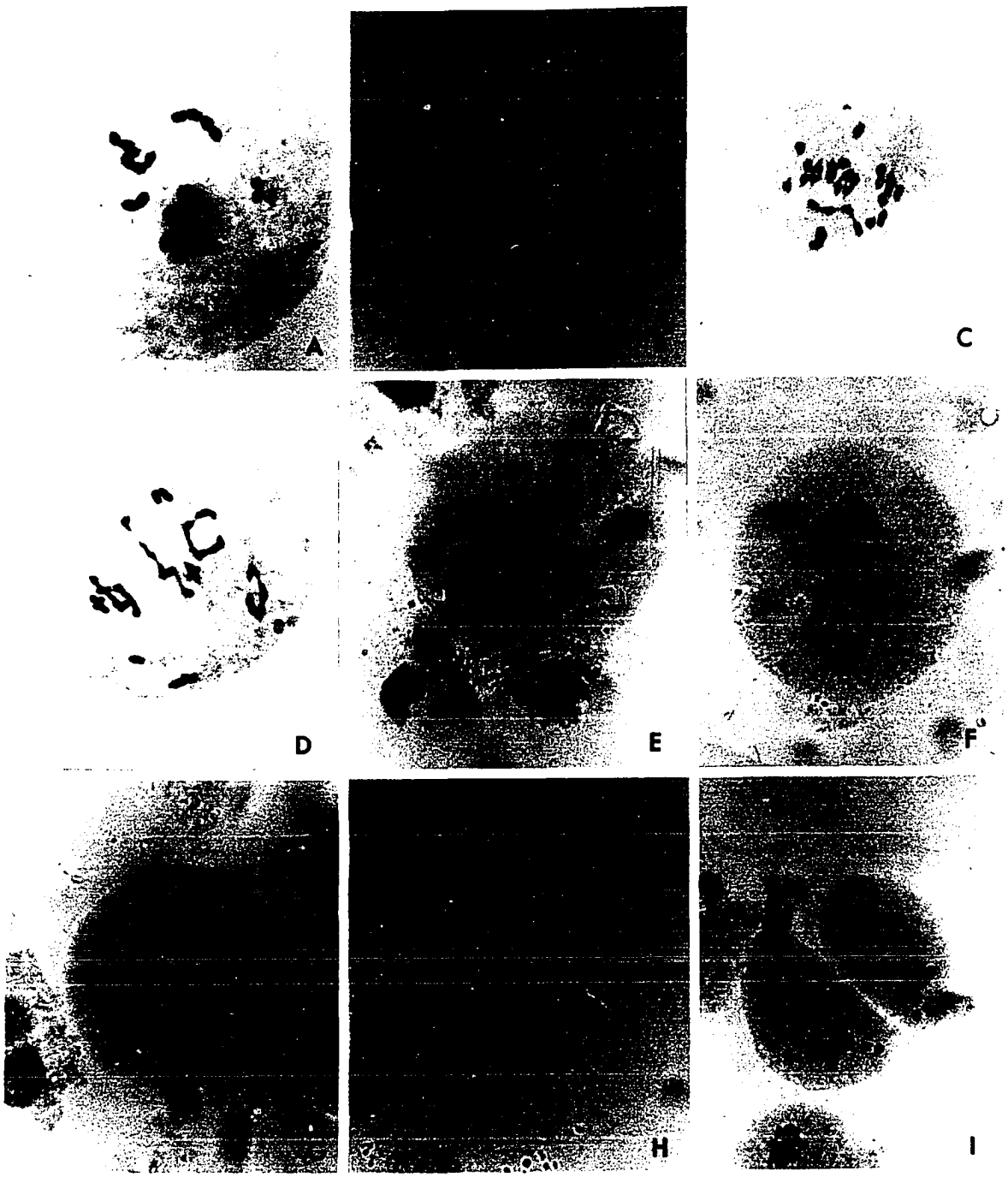

A
C
D
E
F
H
I

Table 11. Chromosome associations at M I and diakinesis, chiasma frequencies per chromosome, and numbers of cells analyzed for irradiated and control populations

Clone	Diak. or M I	IV	III	II	I	# cells	Chiasma	# cells
Control population								
89-3	M I	2.551	0.245	8.286	0.490	49	0.805	44
89-4	M I	2.026	0.421	8.658	1.316	38	0.717	38
102-3	M I	2.607	0.071	8.571	0.214	28	0.868	28
115-6	D	3.208	0.083	7.292	0.333	24	0.887	24
	M I	1.587	0.333	9.317	2.016	63	0.711	62
Avg.	M I	2.193	0.268	8.708	1.009	44.5	0.775	43
Irradiated population								
108-1	D	2.593	0.037	8.519	0.481	27	0.820	27
	M I	1.636	0.091	10.045	1.091	22	0.711	22
108-4	M I	1.643	0.310	9.667	1.166	42	0.713	39
108-5	D	3.467	0.033	6.867	0.300	30	0.814	30
109-4	M I	2.171	0.317	8.732	0.902	41	0.703	41
113-5	M I	1.774	0.806	8.032	2.419	31	0.718	31
113-6	M I	1.704	0.556	8.593	2.333	27	0.661	27
Avg.	M I	1.786	0.416	9.014	1.582	32.6	0.701	32

Table 12. Analysis of variance of chiasma frequency at metaphase one in tetraploid tissue of irradiated and control populations

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F
Total	8	31,331		
Treatment	1	12,185	12,185	4.45 †
Error	7	19,146	2,736	

† Significant at the 10% level.

Table 13. Analysis of variance of quadrivalent frequency at metaphase one in irradiated and control populations

Source of variation	Degrees of freedom	Sums of squares	Mean squares	F
Total	8	1.27		
Treatment	1	.35	.35	2.66NS[a]
Error	7	.95	.1314	

[a]NS: not significant.

Table 14. Anaphase I bridge frequency in tetraploid tissue

Clone	% A I with bridges	# cells
Control population		
89-3	6.7	134
89-4	25.0	16
102-2	5.1	59
102-3	9.8	51
102-4	10.1	79
115-1	7.1	28
115-5	9.8	51
115-6	9.2	76
Average	10.4	61.8
Irradiated population		
108-2	6.0	100
108-4	4.4	46
108-5	1.8	108
109-1	1.1	89
109-3	0.0	78
109-4	8.3	48
113-4	5.0	161
113-5	8.6	35
113-6	9.2	142
Average	4.9	89.7

Table 15. Correlation coefficients (r) between quadrivalent frequency and chiasma number per cell in irradiated and control populations

Clone	Diak. or M I	r	Chiasma per chromosome	Avg. # of quadrivalents per cell	Degrees of freedom
Control population					
89-3	M I	.1422NS[a]	.805	2.551	42
89-4	M I	.2693NS	.717	2.026	36
102-3	M I	.2797NS	.868	2.607	26
115-6	D	.4030NS	.887	3.208	22
	M I	.3464**	.711	1.587	60
Avg.	M I	.2594	.775	2.193	41
X-ray population					
108-1	D	.3159NS	.820	2.593	25
	M I	.2393NS	.711	1.636	23
108-4	M I	.4787**	.713	1.643	37
108-5	D	.5385**	.814	3.467	28
109-4	M I	.6072**	.703	2.171	39
113-5	M I	.5672**	.718	1.774	29
113-6	M I	.6196**	.661	1.704	25
Avg.	M I	.5024	.701	1.786	30

[a]NS: not significant.

**Significant at 1% level.

DISCUSSION

Colchicine Treatment

The application of colchicine in a capillary tube appeared to result in a frequency of doubling comparable to that occurring in materials immersed in the colchicine solution, provided the capillary tube was affixed close to the tiller base.

An attempt was made to produce a concurrence of colchicine application with a high level of mitotic activity stimulated by fertilizer treatment, in order to enhance the probability of chromosome doubling. Success with these treatments was not greater than with the non-fertilizer treatments. Better control of the environmental factors light, water, nutrient availability, and temperature may permit controlling the growth-differentiation balance in a manner which would allow a measure of control of the mitotic process.

Effects of Irradiation

Irradiation prior to meiosis was unsatisfactory due to the difficulty in obtaining seed from crosses and the lack of appreciable mutagenic effect. Had small deletions and recessive lethal point mutations been caused, one would expect the plants of the 1st cycle of irradiation to be heterozygous for these defects and consequently to show a reduction in fertility in the haploid pollen. Such was not the case. As pointed out by Gaul (1964) the effects of radiation are at times erratic. It is possible that the cells which by chance escaped irradiation damage, were more efficient competitors, and these eventually gave rise to pollen, while the damaged tissue failed to

do so. Thus the pollen involved in the cross was normal.

In the second cycle of irradiation, applied after the panicles had commenced to shed pollen, the results were more satisfactory, both in terms of obtaining seed set and the induction of genetic changes. On the diploid level the reduction in chiasma frequency, and pollen fertility, may be interpreted as indicative of chromosome differentiation. The reduction in chiasma frequency may be the result of synaptic interference caused by reduced homology due to cryptic chromosomal changes. The variability in bridge frequency may be due to the inversions encompassing areas of small size with segments of high crossover frequency which is acutely sensitive to environmental changes.

Quadrivalent Frequency

It is of interest to compare the data on IV frequency with that previously reported. There is considerable variability in the reports of IV frequency: 1.86 in cultivated varieties, and 3.43 in wild populations reported by Tarkowski (1964); 1.92 calculated from Curran's (1961) diakinesis data; a range of 0.88 to 2.22 with an average of 1.75 at diakinesis reported by Hanson and Hill (1953); 2.95, 3.81, and 3.00 at diakinesis, and 2.8 at metaphase reported by Muntzing (1937); 3.8, 4.2, and 3.3 reported by Myers and Hill (1940b); a range of 2.4 to 4.4 in 20 plants reported by Myers (1941b); a range of 3.0 to 4.3 at diakinesis among six clones reported by Myers (1943); a range of 2.62 to 4.91 at diakinesis reported by Myers and Hill (1943); a range of 3.4 to 5.3 for 12 plants reported by Myers (1948a); 3.64, 3.28, and 3.18 at

diakinesis reported by Zohary (1956); an average of 3.44 at metaphase, and 3.50 at diakinesis reported by McCollum (1958); an approximate range of 2.6 to 3.0 of the averages of several populations calculated from metaphase I data by Jones (1962). In this study IV frequency ranged from 1.59 to 2.61 at M I, and 2.59 to 3.47 at diakinesis. It is unlikely that the lower frequency at M I compared to diakinesis is due to environmental variation, since the diakinesis values were obtained, in two cases, from sporocytes from the same panicle as was used for the metaphase I determinations. Cell sample size was adequate. The decrease in IV frequency was accompanied by an increase in the frequency of all the other associations; III, II, and I. "Precocious disjunction of loosely held configurations at early M I," suggested by Hanson and Hill (1953) would appear to be operative.

Diploidization

The difference in IV frequency between the irradiated and control population at M I is not as great as might be expected on the basis of Stebbins' report (1956a). The plant materials utilized here, however, differed in the use of subspecies Aschersoniana rather than judaica in the cross with lusitanica, in the degree of chromosomal rearrangement, as evidenced by pollen fertility and cytological abnormalities at meiosis.

The difference between the irradiated and control in the correlation coefficients between chiasma frequency and IV frequency may be due to a greater dependence of IV frequency on chiasma frequency when the latter factor is limiting. A minimum of three chiasma are necessary to

maintain a IV. With a maximum of seven IV per cell, IV formation is restricted when the chiasma frequency falls below 21 chiasma per cell. Thus one would expect a closer correlation between IV and chiasma in the irradiated population where the frequency of chiasma per cell averaged 19.6 as opposed to 21.7 in the control. This difference was significant at the 10% level.

The evidence presented here would indicate that x-ray induced chromosome differentiation can result in a degree of diploidization. The changes induced here were of a cryptic nature. It is possible that a more intense form of such cryptic differences produced by a higher level of exposure of the chromosomes to irradiation would result in a greater degree of preferential bivalent pairing. Such is suggested by the relatively low IV frequency of an induced tetraploid of a plant of irradiated background, but normal diploid meiosis, reported by Stebbins (1956a).

Evidence has been presented by Grell (1961), Doyle (1963a,b), and Shaver (1963), that macro chromosomal rearrangements contribute to preferential pairing. Shaver (1963) reported that the superimposition of an inversion on chromosomes already differing structurally in a cryptic fashion resulted in complete preferential pairing. In a species such as Dactylis, diploidization may require the induction of cryptic differences as well as macro rearrangements, since little differentiation appears to have taken place.

The formation of IV in Dactylis, requiring of necessity the exchange of pairing partners, indicates that pairing is initiated at a minimum of two points on the chromosomes involved. In Zybenga's (1966)

terminology at least two zygomeres are involved. Early prophase studies, which might shed light on the number of zygomeres involved, are not available. If a small number of zygomeres were involved for any one set of homologous chromosomes, it might be feasible, by shifting the zygomeres by chromosomal rearrangement to different positions in the chromosomes of different populations, to restrict the more intimate locus specific and DNA pairing to homostructural types.

It may also be possible, assuming differentiation in zygic activity, to increase preferential bivalent pairing by selection for differential timing of zygic activity. Such may have been the case in the report of a decrease in IV frequency in response to selection by Gilles and Randolph (1951). However, it would appear that selection pressure for such a system in natural populations of Dactylis has not been effective. This may be due to differences in the life cycle of the annual, maize, and the perennial, Dactylis. Presumably the selection pressure for fertility in an annual would be much greater than in a perennial with the potential for producing vast numbers of seed.

It would appear on the basis of evolutionary considerations that inversions would offer the best hope of diploidization. The compensation phenomenon of nulli-tetrasomics in wheat (Sears and Okamoto (1957)), permits us to deduce that chromosome differentiation has taken place primarily by rearrangement or genetic change within a unit chromosome, rather than between homologues. It would also appear that simple reciprocal translocations would result in higher order multivalent associations in polyploids. More complex translocation systems might result in preferential pairing, but the evolutionary

time required for establishing such a system might be accompanied by genetic differentiation, which would result in physiologically disruptive hybridity.

Riley (1960) has suggested that the genetic factor on chromosome 5 B of wheat acts by reducing attraction between chromosomes to a low level, at which the weaker homoeologous attraction is inadequate to effectuate pairing. He points out (1966) that homoeologous chromosomes may be regarded as highly heterozygous, and that among the diploid Aegilops species related to wheat, the cross pollinated heterozygous species have alleles dominant to that on 5 B, while the inbred homozygous species carry alleles recessive to that on 5 B. It is unlikely therefore that a diploidizing factor, analogous to that in wheat, is available in crosspollinated Dactylis. The alternative method of producing a true breeding hybrid, by inducing chromosome differentiation, in the form of cryptic differences and macro inversions, with consequent preferential bivalent pairing, appears more promising.

SUMMARY

This study was undertaken to assess the utility of x-irradiation in inducing chromosome differentiation of such a nature as to result in increased autosyndetic pairing in induced amphidiploids.

Two cycles of irradiation were used; the first applied to panicles of the male parent prior to meiosis, the second applied to the post meiotic panicles concomitantly with intersubspecific hybridization. Post meiotic irradiation was more satisfactory with regard to seed set.

No evidence of chromosome differentiation as measured by reduced pollen fertility and multivalent association was obtained from the first cycle diploid population. Reduced pollen fertility and lower chiasma frequency were indicative of differentiation, on the diploid level, in the irradiated second cycle population.

On the tetraploid level, the reduction in quadrivalent frequency at metaphase one in the irradiated population compared to the control population, while not statistically significant, is indicative of a minor increase in autosyndesis in the irradiated population. The higher bridge frequency in the control tetraploid tissue compared to the tetraploid tissue of the irradiated population also suggests preferential pairing of homostructural chromosomes.

The higher correlation coefficients between chiasma frequency and quadrivalent frequency in the irradiated population may be attributed to the lower chiasma values in this population.

The similarity of correlation coefficients between chiasma frequency and IV frequency from diakinesis to metaphase one suggests

that this relationship is not affected by the terminalization and desynapsis occurring at metaphase one.

Colchicine treatment by immersion of tillers in an aqueous solution, and by application of a capillary tube containing an aqueous solution in close proximity to the tiller base, gave satisfactory frequencies of tetraploid sectors. The highest frequency of colchicine doubling occurred with immersion in a .4% solution for nine hours.

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