STUDIES ON CROSS-POLLINATION AND MEIOTIC STABILITY IN CERTAIN VARIETIES OF COMMON WHEAT (*TRITICUM AESTIVUM* L., EM. THELL.)

By

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STUDIES ON CROSS-POLLINATION AND MEIOTIC STABILITY IN CERTAIN VARIETIES OF COMMON WHEAT (TRITICUM AESTIVUM L., EM. THELL.)

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INTRODUCTION

Pure seed stocks of common wheat varieties are needed for seed production and plant breeding work. Although in the strict sense there has probably never been a truly homozygous wheat variety released to farmers, a certain degree of uniformity is desired. The variety should be free of divergent types. The tolerance limits established by the Oklahoma Crop Improvement Association (32)[1/] allow no varietal mixture in foundation seed, five plants per acre in registered seed and 25 plants per acre in certified seed. To maintain such purity, it is essential that precautions be taken to avoid admixtures in seed produced for the Crop Improvement Association for distribution to farmers.

It has been reported that difficulty has been encountered in maintaining pure seed stocks of Kaw (C.I. 12871)[2/], a selection from a complex cross, being tested by the Oklahoma Agricultural Experiment Station[3/]. Mechanical mixtures are frequent sources of contamination while natural cross-pollination between divergent types and meiotic irregularities are also possible sources of contamination of pure seed stocks.

1/ Numbers in parentheses refer to "Literature Cited" page 45.

2/ C.I. numbers refer to the accession number of the Field Crops Research Branch, A.R.S., U.S.D.A. (Formerly Cereal Investigations).

3/ Personal communication with Dr. A. M. Schlehuber, Small Grains Section, Agronomy Department, Oklahoma State University.

It was the purpose of this study to determine if cross-pollination or meiotic irregularities are primary factors responsible for the difficulty in maintaining pure seed stocks of Kaw.

REVIEW OF LITERATURE

Natural Cross-pollination

Triticum aestivum L., em. Thell. is usually considered as a self-pollinated species. Some authors have reported the occurrence of natural cross-pollination (11, 15, 33, 37, 43, 46) while others have reported the frequency of natural cross-pollination to range from negligible to over 50 per cent (4, 5, 6, 8, 9, 10, 12, 17, 19). Difficulties encountered in purifying new lines and maintaining purity of established varieties have been attributed to cross-pollination (5, 6, 10).

According to Watkins (46), it is not logical, without further proof, to dismiss every spontaneous variation as a natural cross. The degree of certainty will be much greater if the introduction of two independently segregating characters can be observed. Carleton (1) attributed the success in making selections from older varieties to natural crossing.

In a 10-year study, Leighty and Taylor (19) found that crossing varied from zero to 34 per cent. The amount of crossing varied with year and variety. Goulden and Neatby (5) found 0.58 per cent crossing in 1927 and 16.51 per cent in 1928. One line that had poor seed set had approximately 50 per cent cross-pollination. Harrington (9) observed 0.00 per cent to 2.16 per cent crossing in a 5-year study. Others have found less than one per cent crossing (4, 17).

Leighty and Taylor (19) observed 1.5 per cent natural hybrids in progeny of primary spikes, while 8.6 per cent of the progeny from

secondary spikes were hybrids, or about six times more than from the primary tillers. Abundant pollen production by the primary tillers and scanty pollen production by secondary tillers was thought to be the primary factor involved.

Several investigators (4, 9, 10, 17) doubled the amount of crossing actually observed on the assumption that the same number of crosses would occur in either direction. However, Leighty and Taylor (19) failed to find reciprocal natural hybrids in some cases.

Goulden and Neatby (5) used the reaction of seedlings to stem rust, physiologic form 21 of Puccinia graminis tritici Eriks. and Henn., to identify hybrids between a highly resistant variety and two susceptible varieties. Most other investigators used differences in spike morphology, primarily awn development, outer glume color and covering (pubescent or glabrous), to detect hybrids resulting from natural crossing. Varieties with dominant spike characters were used as pollinators and varieties with recessive characters were used as female parents so that crosses could be detected in the F_1. According to Leighty and Taylor (19) more natural crossing will be recognized when varieties with many contrasting characters are grown in close proximity to one another. Natural crossing may appear to occur most frequently in varieties possessing several recessive characters as the hybrid can be detected in the F_1.

Several investigators (5, 9, 19) have attempted to detect the maximum amount of cross-pollination by interplanting rows of varieties with recessive characters with varieties possessing dominant characters. Others (4, 10, 17) have found natural hybrids incidentially in supposedly pure lines. Segregation among the progeny of aberrant plants was used to distinguish between hybrid plants and admixtures.

Variable dates of sowing were used to cause various varieties to bloom at the same time (5, 9). Harrington (9) used only the plots that bloomed simultaneously.

According to Smith (43), Howard et al. found many more natural crosses in an arid climate where the wheat plants were under moisture stress. Wilting caused the glumes to open and expose the stigmas. Harrington (9) found more natural crossing in humid seasons than in a dry season in Saskatchewan. Leighty and Taylor (19) observed natural crossing during both dry and wet blooming periods in a humid climate. Garber and Quisenberry (4) detected more crosses in lines that bloomed during a sunny period than in a cloudy period. Leighty and Taylor (19) found more natural crosses in varieties that were poorly adapted to an area and attributed the varietal differences to environmental conditions. Outcrossing has been found to be quite frequent in partially sterile hybrids and lines (5, 46).

According to Leighty and Taylor (19) the duration of the flowering period of a variety is influenced by the weather and by the number of tillers produced by the plants. In bright, hot weather, a variety may be expected to complete its blooming in four days. However, rain and cool weather can cause a longer blooming period. Unpollinated stigmas may remain receptive to pollen for several days after the time of dehiscence of the anthers. Thus varieties in which dehiscence differs by several days may cross-pollinate.

Investigations by Hilgendorf, according to Jenkin (17), showed that at certain low temperatures the anthers may be killed while the stigmas and ovaries remain unaffected. These florets often became fertilized through cross-pollination. According to Harrington (9), a

hot wind or a light frost at flowering time may kill the pollen in some of the florets and leave the stigmas susceptible to fertilization from outside pollen.

Several authors (5, 6, 7, 8, 9, 19) found that most crosses detected, for which the male parent could be determined, occurred between plants grown close together. Leighty and Taylor (19) found that most crosses were between plants that could have come in actual contact with each other at the time of blooming. Only five of 40 natural crosses were between plants over seven feet apart in experiments conducted by Harrington (9). Aberrant and admixture plants in a variety had nearly ideal spatial position for maximum crossing according to Harrington (6).

Cytological Studies

Natural cross-pollination and self-sterility have been found to be relatively high in some plants and lines with cytological irregularities (25, 38). Variation in phenotype has been found to be associated with cytological aberrations by other workers (15, 22, 26, 27, 34, 38).

Various types of speltoids and compactoids have reportedly been caused by the loss or addition of part or all of a chromosome (14, 15, 16, 24, 36, 38, 44, 45). According to Smith et al. (45) the compactoid mutants are, both phenotypically and cytogenetically, the reciprocals of the speltoid mutations. A deficiency in or of the C chromosome results in speltoid mutants, while a duplication produces compactoid mutants. Love (24) found that all speltoid progeny from three speltoid plants tested were characterized by a heteromorphic bivalent, one member of which was deficient for the longer arm. According to Sanchez-

Monge and Mac Key (36) the C chromosome carries the inhibitor of the genes for bearded and speltoid, situated in the B chromosome. Sears (38) has designated the C chromosome as IX. Smith et al. (44) found that the deletion of a small segment of the C chromosome could cause speltoids. The segment was about 30 cross-over units in length and could not be detected cytologically but could be determined genetically.

Love (22, 26) found a mutant in Dawson's Golden Chaff that was shorter and had long white glumes. The mutant plant had a telocentric pair of chromosomes while all chromosomes in normal Dawson's Golden Chaff had median or submedian centromeres. Sebesta (40) observed fragmentation of a chromosome in a bronze-chaffed variety in which white-chaffed plants were also found.

According to several investigators (27, 28, 31, 34, 35, 41) the percentage of microspore quartets showing micronuclei may be taken as a general measure of germinal stability. Frequencies were easily determined as the proper stage was relatively easy to find and counts could be made rapidly. Love (27) proposed the term "Meiotic Index" for the percentage of normal microspore quartets and considered any plant with a meiotic index of 90 or above as cytologically stable. Powers (35) found high positive correlations between non-conjunction and non-orientation and the frequency of micronuclei in the microspore quartets. Semeniuk (41) found that seed set was of little use in evaluating the chromosomal stability of lines. Estimates of pollen sterility were useful but not precise. The frequency of microspore quartets with micronuclei was more reliable. Lines with significant differences in meiotic stability have been established (31, 41). In experiments reported by Morrison and Unrau (30) consistent micronuclei

frequencies were obtained for different anthers of a floret, different florets of a plant and different plants of each monosomic line. Powers (34) found at least a small amount of aberrations in all plants of three varieties that were examined. Sebesta (40) found that the meiotic index of the Triumph plants examined ranged from 98.8 to 100.0.

Failure of homologous chromosomes to pair, lagging bivalents and genetic disturbance of the meiotic process have been considered major types of abnormalities causing meiotic instability (27, 28, 31). Love (27) found no major disturbance of the meiotic process due to lagging univalents, but the process was much disturbed in cells containing lagging bivalents. Li et al. (20) discovered desynaptic plants in which the meiotic process was greatly disturbed. Microspore configurations with many micronuclei and microcytes were observed. Desynapsis was transmitted as a simple mendelian recessive character. However, there were stable and unstable types of desynapsis which were possibly controlled by modifying genes. Highly significant correlations between the frequency of metaphase I cells with univalents and the total abnormalities observed in later stages, including microspore quartets, were reported by Semeniuk (41). Morrison and Unrau (30) found that the microspore quartet must be surrounded by a distinct wall to insure reliable counts. Older microspore quartets had lower frequencies of micronuclei than young quartets because micronuclei frequently formed microcytes and slight pressure forced them out of the quartet arrangement. Also micronuclei were observed to be diffuse and indistinct in older microspore quartets. Significant differences in micronuclei frequencies were reported by Myers and Powers (31) between dates of collection, indicating that the environment may also be a factor in determining differences in meiotic irregularities.

The number of microspore quartets analyzed from each plant is dependent upon the accuracy desired. Marshall and Schmidt (28) counted 100 microspore quartets. Powers (35) suggested counting as many as 500, especially if the frequency of micronuclei is high.

MATERIALS AND METHODS

Description of Materials

Frequency of natural cross-pollination and abnormal microspore quartets were determined for Kaw and Triumph (C.I. 12132), varieties of hard red winter wheat. This was done to ascertain if either of these factors could be responsible for the appearance of aberrant plants in Kaw. Triumph was used as a check on the amount of natural cross-pollination that may be expected in a standard variety and as a stable check in the microspore quartet analysis. Concho (C.I. 12517), a bronze-chaffed variety, was used as the paternal parent in the natural crosses with the white-chaffed varieties, Triumph and Kaw, so that the F_1's could be easily detected, by their bronze chaff color, among the normally white-chaffed plants. Mediterranean (C.I. 3332), one of the parents of Kaw, was included to serve as an unstable check in the microspore quartet analysis.

Kaw is a selection from the cross Early Blackhull-Tenmarq x Oro-Mediterranean-Hope. It is a mid-tall hard red winter wheat that matures early, has white stems, medium straw strength; awned, fusiform, mid-dense spike; white, glabrous glumes and short beaks. High test weight and long mixing time are two highly desirable agronomic characteristics of this variety.

Triumph is a short, early maturing hard red winter wheat with strong white stems; awned, fusiform, mid-dense spikes; white, glabrous glumes and short beaks.

Concho is a mid-tall, medium early hard red winter wheat with mid-strong white stems; awned, fusiform, mid-dense spikes; bronze or brown glabrous glumes with mid-long beaks.

Mediterranean is a tall, mid-season to late soft red winter wheat with mid-strong, coarse stems; awned, oblong to clavate, mid-dense to lax spikes; bronze or brown glabrous glumes with short beaks.

The seed stock of Kaw was obtained by bulking five grams of remnant seed from each of 25 head hills grown by the Small Grains Section of the Agronomy Department of Oklahoma State University. Triumph and Concho were obtained from pure seed increases grown by the Small Grains Section. Mediterranean was supplied by Dr. E. G. Heyne, Agronomy Department, Kansas State University. Greenhouse plantings were made using seeds from plants grown in the initial year of this study.

Experimental Methods

Field cross-pollination

The two white-chaffed varieties, Triumph and Kaw, were grown in alternate rows with the bronze-chaffed variety, Concho. The F_1 of a cross between the two chaff types should have bronze chaff. The frequency of bronze-chaffed plants in the progeny of the normally white-chaffed varieties was used as the per cent natural cross-pollination.

In the fall of 1958 single 10 foot rows of Concho were planted on each side of single rows of Triumph and Kaw, the spacing between rows

being one foot. At the Stillwater station, Concho normally heads a few days later than the other two varieties. Therefore, Triumph and Kaw were seeded on the same date, one and two weeks later than Concho, as suggested by Goulden and Neatby (5) and Harrington (9), so that Concho and the two white-chaffed varieties would bloom at approximately the same time in at least one date of seeding. All three varieties were seeded on the 29th of September and Triumph and Kaw were also planted one and two weeks later. Nine 10-foot rows of Triumph and of Kaw were seeded on each date.

When ripe, each row of Triumph and Kaw was carefully harvested to avoid any mixture from adjacent rows of Concho. Each row was later threshed with a Vogel thresher.

Three rows of Triumph and Kaw were selected from each date of seeding for replanting in the fall of 1959. Thus a total of nine plots of each of the two white-chaffed varieties was grown. Each plot consisted of a block of eight 10-foot rows spaced 12 inches apart. The rate of seeding was increased from the customary 60 pounds per acre to 80 pounds per acre so that more plants would be grown in each row.

When chaff color became evident in the spring of 1960, white and bronze-chaffed spikes were counted and recorded for each plot. Occasionally spikes were discolored to such an extent that chaff color could not be determined. These spikes were not counted. The per cent bronze-chaffed spikes found was used as the per cent natural crossing.

Cytological studies

Phenotypic variations may also be caused by irregularities in the meiotic process. The procedure used in making the microspore

quartet analysis was essentially the same as suggested by Love (27). Further observations were made on meiosis in certain plants.

Approximately 200 seeds of each of the varieties Triumph, Kaw and Mediterranean were spaced approximately six inches apart in rows one foot apart in the fall of 1958. In the spring of 1959, two or more whole spikes in the early boot stage were collected from about 60 vigorous, well-tillered plants of each of the three varieties. The whole spikes were fixed at room temperature for one day in a fresh 6:3:1 mixture of 95 per cent ethyl alcohol, chloroform and glacial acetic acid and stored in a refrigerator at 0 to 5° C.

The meiotic index (percentage of normal microspore quartets, i.e., free of micronuclei and microcytes) was determined for each plant. A total of 200 young microspore quartets, 100 from each of two anthers from different spikes, were counted for each plant at a magnification of 645X.

Meiosis was studied in some detail in some Mediterranean plants in an attempt to determine the reasons for the irregularities found in microspore quartets. Observations were made from slides prepared using the acetocarmine smear technique described by Smith (42) at magnifications of 645X and 1445X. Photomicrographs were made from temporary smear preparations with a Bausch and Lomb type K camera at the magnifications indicated on the legend for Plate I.

Seven progeny from each of eight selected Mediterranean plants were planted in the greenhouse in September, 1959. It was deemed desirable to obtain further information pertaining to meiosis in Mediterranean plants and to make crosses with normally stable Triumph plants so that other studies could be made on the meiotic irregularities

encountered. The approach method described by Curtis and Croy (2) was used to make the crosses.

During the early spring of 1960, one or two whole spikes were collected from each Mediterranean plant, fixed and stored as previously described. When only one spike was available for the microspore quartet survey, 100 microspore quartets from each of two anthers from one floret were analyzed.

During the spring of 1959, four spikes from each spaced plant of each of the three varieties chosen for cytological studies were bagged as the spikes emerged from the boot. This was done so that self-pollination would be insured and irregularities caused by cytological irregularities per se might be observed in the progeny. Bags were prepared from cellulose tubing. Two spikes were enclosed in one bag. The bags and spikes were supported with no. 9 wire. After pollination was completed the spikes were tagged and the bags were removed to reduce the development of saprophytes. Each plant was harvested separately, keeping bagged and unbagged spikes separate. Triumph and Kaw were harvested when ripe, however, it was necessary to harvest the Mediterranean plants before becoming completely mature to prevent further destruction by birds. These spikes were dried in an oven at 100° F. for about 24 hours.

Seed from bagged and unbagged spikes of selected Mediterranean, Triumph and Kaw plants were planted as paired plant rows in October of 1959. In addition, 14 to 50 seeds from bagged spikes of each Mediterranean plant chosen for replanting were spaced at one foot intervals in rows one foot apart so that individual plants could be observed.

RESULTS

Natural Cross-pollination

The number and per cent of bronze-chaffed spikes resulting from cross-pollination in the spring of 1959 and appearing in the plots of white-chaffed Triumph and Kaw in the spring of 1960 are shown in Table I. The figures for the grand totals show crossing to be about twice as frequent in Triumph as in Kaw. Considerable variability is evident among the progeny of the three plots grown in each date of seeding for both varieties. Essentially the same amount of crossing was observed among the progeny from each date of seeding of Triumph. About one-half as much crossing was detected in the progeny of dates I and II of Kaw as in Triumph and only one-third as much crossing was observed in the progeny of date III of Kaw as in Triumph. It is doubtful if the differences among the three dates of seeding of either variety are of significant importance.

Crossing appeared to be similar in all dates of seeding for each variety, but tillering was greatly reduced in the later dates of seeding. Although tillers were not counted, reduction in tillering was apparent from visual observations and from grain yields, which were reduced by about 10 per cent in Triumph and Kaw in the second date of seeding and by about 40 per cent in the third date of seeding. The reduction in yield and tillering was probably caused by several factors, including competition from adjacent rows of Concho and the effect of later planting. Date of seeding apparently had no effect on the date

TABLE I

Number and per cent bronze-chaffed spikes observed in Triumph and Kaw in the spring of 1960

Variety	Plot No.	1958 Date of Seeding	Total No. Spikes	No. Spikes with White Chaff	No. Spikes with Bronze Chaff	% Bronze-Chaffed Spikes
Triumph (C.I. 12132)	1	Date I	3033	3015	18	0.59
	2		3037	2960	77	2.54
	3		3119	3098	21	0.67
		Total	9189	9073	116	1.26
	1	Date II	3004	2971	33	1.10
	2		2758	2753	5	0.18
	3		2639	2566	73	2.77
		Total	8401	8290	111	1.32
	1	Date III	2626	2586	40	1.52
	2		2773	2724	49	1.77
	3		2803	2791	12	0.43
		Total	8202	8101	101	1.23
Grand Total			25792	25464	328	1.27
Kaw (C.I. 12871)	1	Date I	3074	3026	48	1.56
	2		3032	3021	11	0.36
	3		2985	2983	2	0.07
		Total	9091	9030	61	0.67
	1	Date II	2513	2506	7	0.28
	2		2866	2840	26	0.91
	3		2812	2789	23	0.82
		Total	8191	8135	56	0.68
	1	Date III	2920	2906	14	0.48
	2		2609	2598	11	0.42
	3		2980	2970	10	0.34
		Total	8509	8474	35	0.41
Grand Total			25791	25639	152	0.59

of heading. In the spring of 1959, when the crossing occurred, Triumph headed on the last day of April, while Kaw and Concho headed on the first and second day of May respectively, regardless of date of seeding.

Microspore Quartet Analysis

Meiotic indices were determined for 59 Triumph, 68 Kaw and 58 Mediterranean plants. Figure 1 of Plate I illustrates a microspore quartet without micronuclei and Figure 2 illustrates a microspore quartet with one micronucleus. Only those microspore quartets enclosed by the pellicle, as shown in Figures 1 and 2, were counted.

All Triumph plants examined (Table II) had high meiotic indices

TABLE II

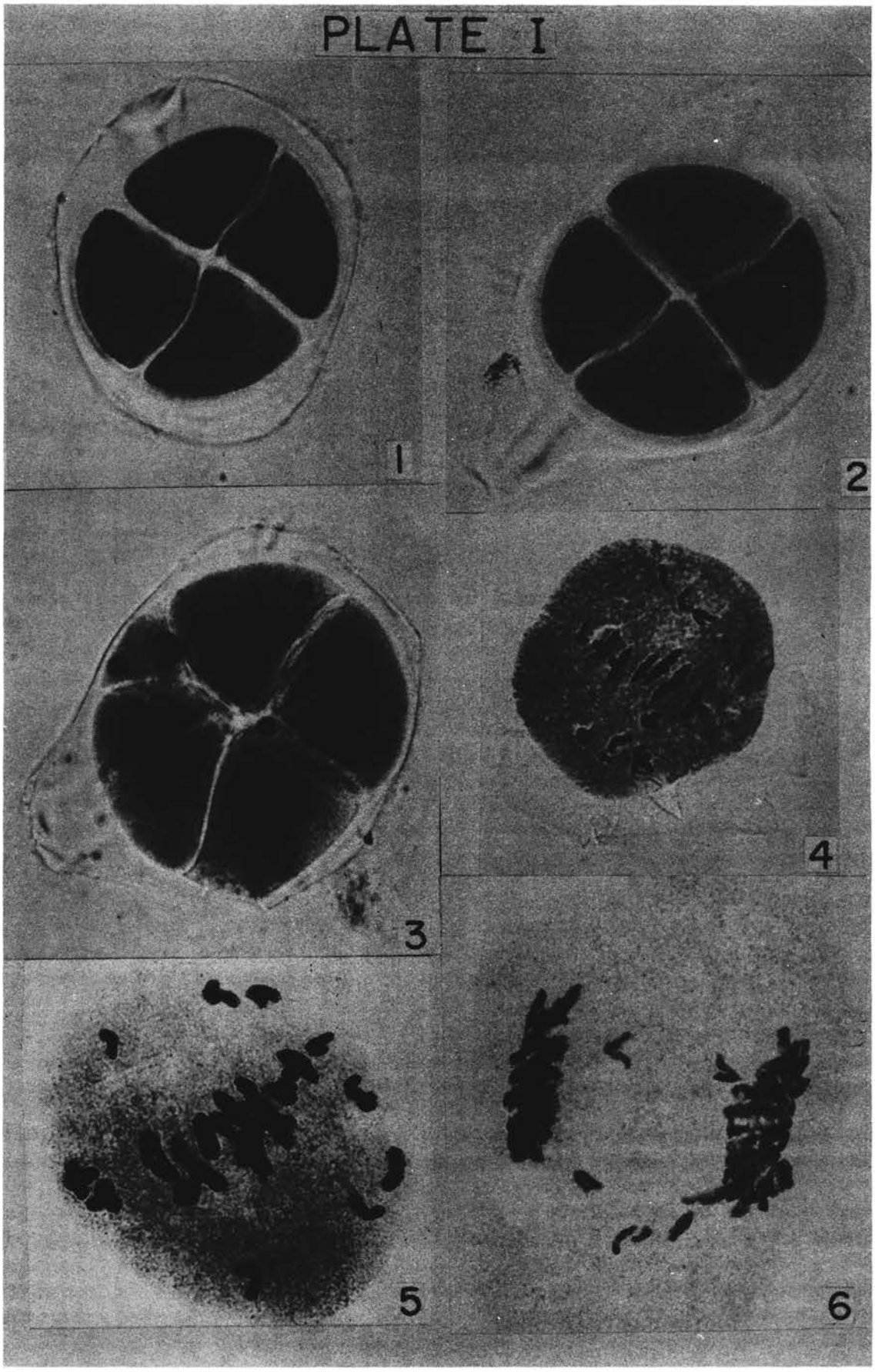
Meiotic indices for Triumph and Kaw determined from 200 microspore configurations from each plant

Variety	No. of plants examined	Number of plants with indicated meiotic indices		
		100.0 - 98.0	97.9 - 96.0	95.9 - 95.0
Triumph	59	58	1	-
Kaw	68	57	9	2

similar to those previously reported by Sebesta (40). Only 0.28 per cent of the 11,800 microspore quartets examined contained micronuclei. The lowest meiotic index of any of the Triumph plants was 97.0 as shown in the table. As might be expected the anther with the most abnormal microspore quartets was from this plant. However, only four per cent of the microspore quartets in this anther contained micronuclei.

High meiotic indices also were obtained for all Kaw plants examined (Table II). A total of 13,600 microspore quartets were examined, micro-

PLATE I



nuclei being observed in 1.97 per cent of the quartets. The greatest variation in per cent normal microspore quartets between anthers of a Kaw plant and the lowest meiotic index was found in a plant with a meiotic index of 95.0. Fifteen per cent of the microspore quartets in one anther contained micronuclei and microcytes. Only two per cent of the microspore quartets in the second anther contained micronuclei.

Entirely different results were obtained from Mediterranean plants (Table III). Meiotic indices ranged from 98.5 to 1.0. There appeared to be an even distribution of plants ranging from very low to high meiotic indices. It is evident that in some plants the per cent aberrant microspore quartets was different in the two anthers examined, while in other plants similar or the same percentages were found in both anthers from one plant. The most extreme difference between anthers of a single plant was found in plant 33. In no instance was the meiotic index above 90 in one anther and below 90 in the other anther from any one Mediterranean plant. The difference in per cent of abnormal microspore quartets between the two anthers of each plant was 12 or less in over two-thirds of the plants.

It is evident from the data presented in Table III that meiosis was seriously disturbed in many of the Mediterranean plants examined. A microspore quartet with micronuclei and a microcyte is shown in Figure 3 of Plate I. Polyspory was frequent as only four plants (plants 14, 29, 41 and 57) were observed in which no polyspory was noted. The meiotic indices of these four plants ranged from 98.5 to 81.0. No more than four micronuclei were observed in any one microspore quartet in these four plants. Low frequencies (one per cent or

TABLE III

Frequency of polyspory and micronuclei in 200 microspore configurations in each of 58 Mediterranean plants

Plant No.	Anther No.	Quartets: No. of Micronuclei										Pentads: No. of micronuclei						Hexads: No. of Micronuclei						Septads and Octads	Meiotic Index*
		0	1	2	3	4	5	6	7	8	8+	0	1	2	3	4	4+	0	1	2	3	4	4+		
1	1	72	7	7	1	2	1	-	-	-	-	6	-	2	-	-	1	-	1	-	-	-	-	-	
	2	66	12	6	7	2	2	-	-	-	-	-	1	1	-	-	1	-	2	-	-	-	-	-	69.0
2	1	76	4	3	3	-	-	1	-	-	-	6	2	1	-	-	-	1	2	1	-	-	-	-	
	2	68	5	3	1	1	-	1	-	-	-	12	5	-	-	-	-	1	1	-	-	-	-	2	72.0
3	1	20	20	20	14	13	3	2	1	-	1	-	-	1	-	-	3	-	-	1	-	1	-	-	
	2	62	9	7	2	1	1	-	1	-	-	8	6	1	1	1	-	-	-	-	-	-	-	-	41.0
4	1	31	15	26	7	9	4	1	-	1	1	-	-	2	1	1	1	-	-	-	-	-	-	-	
	2	32	19	21	10	6	4	2	1	-	-	-	-	-	-	-	2	-	-	-	1	-	1	1	31.5
5	1	84	6	7	2	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	
	2	78	4	8	4	2	1	-	-	-	-	2	1	-	-	-	-	-	-	-	-	-	-	-	81.0
6	1	37	19	6	7	4	4	1	3	2	2	1	-	2	4	1	5	-	-	-	-	-	2	-	
	2	84	7	2	1	-	-	-	-	-	-	4	-	1	-	-	1	-	-	-	-	-	-	-	60.5
7	1	71	15	3	3	1	-	-	-	-	-	1	1	3	-	-	-	-	1	1	-	-	-	-	
	2	76	8	5	2	1	-	1	-	-	-	3	1	-	-	-	-	1	1	-	-	-	-	1	73.5
8	1	68	9	10	-	4	1	1	-	-	-	1	3	2	-	-	-	-	-	-	-	-	1	-	
	2	75	16	1	2	2	-	-	-	-	-	-	2	-	-	1	-	-	-	-	1	-	-	-	71.5
9	1	8	16	20	17	9	12	3	3	5	3	-	-	2	1	1	-	-	-	-	-	-	-	-	
	2	15	26	17	10	17	4	2	1	-	1	-	-	2	-	2	3	-	-	-	-	-	-	-	11.5

TABLE III--Continued

Plant No.	Anther No.	Quartets No. of Micronuclei										Pentads No. of Micronuclei						Hexads No. of Micronuclei						Septads and Octads	Meiotic Index*
		0	1	2	3	4	5	6	7	8	8+	0	1	2	3	4	4+	0	1	2	3	4	4+		
10	1	31	22	20	7	5	1	-	-	-	-	2	5	2	2	-	-	-	-	1	1	1	-	-	
	2	57	10	13	5	2	1	1	-	-	-	-	3	1	1	-	3	-	-	1	-	-	2	-	44.0
11	1	99	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	
	2	96	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	97.5
12	1	3	3	8	16	22	1	9	7	7	7	1	-	-	-	1	12	-	-	-	-	1	-	2	
	2	1	1	9	16	19	6	5	2	4	6	-	-	1	3	3	11	-	1	-	1	-	10	1	2.0
13	1	37	19	17	5	7	1	1	-	-	-	2	2	-	2	1	3	-	-	1	1	-	1	-	
	2	43	16	20	1	5	1	1	-	-	-	-	4	2	1	-	1	-	-	1	1	3	-	-	40.0
14	1	86	8	4	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	2	76	6	8	5	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	81.0
15	1	20	18	21	10	7	3	3	1	2	-	2	-	3	1	1	2	-	-	-	1	2	1	2	
	2	32	16	18	7	6	1	-	1	-	-	3	2	3	1	1	6	-	-	-	-	-	2	1	26.0
16	1	36	17	15	8	6	3	4	-	-	-	4	-	1	1	2	2	-	-	-	-	1	-	-	
	2	44	30	12	7	2	1	-	-	-	-	2	1	-	1	-	-	-	-	-	-	-	-	-	40.0
17	1	54	22	7	4	3	-	-	1	-	-	1	1	1	-	1	2	-	-	-	2	-	1	-	
	2	54	22	13	3	2	-	-	-	-	-	-	2	2	1	1	-	-	-	-	-	-	-	-	54.0
18	1	97	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	2	91	4	3	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	94.0

TABLE III--Continued

Plant No.	Anther No.	Quartets No. of Micronuclei										Pentads No. of Micronuclei						Hexads No. of Micronuclei						Septads and Octads	Meiotic Index*
		0	1	2	3	4	5	6	7	8	8+	0	1	2	3	4	4+	0	1	2	3	4	4+		
19	1	85	2	1	-	1	-	-	-	-	-	6	-	1	-	1	1	1	1	-	-	-	-	-	
	2	56	20	7	5	3	3	-	-	-	-	1	-	-	-	1	1	-	-	1	-	-	-	2	70.5
20	1	70	13	5	2	1	-	1	-	-	-	2	1	1	1	-	-	1	-	1	-	-	1	-	
	2	60	13	3	5	2	-	-	-	-	-	5	3	2	3	1	-	-	-	2	-	-	-	1	65.0
21	1	42	20	17	11	4	2	1	-	-	-	-	-	1	-	-	-	-	-	1	1	-	-	-	
	2	56	22	10	3	1	2	-	-	-	-	2	1	1	-	-	-	1	-	1	-	-	-	-	49.0
22	1	15	8	15	10	10	5	3	4	3	4	-	-	4	2	3	8	-	-	-	-	1	4	1	
	2	7	6	15	15	16	9	6	1	1	1	-	3	1	2	4	6	1	-	-	1	2	3	-	11.0
23	1	25	25	24	7	4	2	-	-	1	-	1	3	-	2	2	-	-	1	-	1	-	1	1	
	2	35	22	17	4	7	3	-	-	2	-	-	-	1	3	1	2	-	-	2	1	-	-	-	30.0
24	1	44	18	6	4	2	-	-	-	-	-	9	8	4	2	-	-	-	3	-	-	-	-	-	
	2	40	18	16	5	2	2	1	-	1	-	1	4	2	1	1	-	2	1	1	-	1	1	-	42.0
25	1	28	19	15	15	8	3	1	3	-	-	1	-	3	3	-	-	-	-	-	-	-	1	-	
	2	64	15	4	4	1	1	-	-	-	-	3	6	-	-	-	-	-	-	1	-	-	-	1	46.0
26	1	27	20	19	14	6	4	1	1	-	1	-	1	1	1	-	-	1	1	-	1	-	1	-	
	2	15	16	18	11	14	4	1	-	1	1	2	1	1	1	1	4	1	1	-	-	1	4	2	21.0
27	1	26	15	14	20	11	2	1	1	-	-	4	1	2	1	-	2	-	-	-	-	-	-	-	
	2	50	24	10	6	6	-	-	-	-	-	-	1	1	-	-	1	-	1	-	-	-	-	-	38.0

TABLE III--Continued

Plant No.	Anther No.	Quartets: No. of Micronuclei 0	1	2	3	4	5	6	7	8	8+	Pentads: No. of Micronuclei 0	1	2	3	4	4+	Hexads: No. of Micronuclei 0	1	2	3	4	4+	Septads and Octads	Meiotic Index*
28	1	82	7	6	2	-	-	-	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-	-	
	2	65	17	7	3	1	-	-	-	-	-	1	2	1	-	1	-	-	-	-	1	-	1	-	73.5
29	1	86	3	5	4	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	2	89	9	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	87.5
30	1	2	12	14	12	15	5	4	2	2	1	-	2	3	3	7	5	1	-	1	3	1	-	5	
	2	22	15	19	13	12	3	2	-	-	-	2	-	4	3	-	-	1	-	2	-	1	1	-	12.0
31	1	51	17	10	5	8	-	-	-	-	-	2	1	-	2	1	-	-	-	1	1	-	1	-	
	2	10	12	10	12	13	1	5	1	1	2	-	1	3	2	3	14	-	2	-	-	1	5	2	30.5
32	1	45	18	12	8	9	-	1	-	-	-	1	-	3	-	-	1	-	-	-	-	-	-	2	
	2	14	9	20	16	10	10	2	1	1	1	-	-	3	3	2	4	-	1	-	1	-	2	-	29.5
33	1	68	11	11	-	-	-	3	-	-	-	4	2	1	-	-	-	-	-	-	-	-	-	-	
	2	19	13	17	11	11	2	2	-	1	-	2	4	6	1	5	1	-	2	1	1	-	-	1	43.5
34	1	39	22	12	8	3	1	1	-	1	-	1	2	5	1	-	1	1	-	-	-	1	1	-	
	2	17	16	27	15	10	5	3	1	-	-	-	-	-	1	-	2	-	-	-	-	-	2	1	28.0
35	1	15	17	23	9	11	8	1	-	1	1	-	2	1	-	3	1	-	2	-	1	-	2	2	
	2	27	26	21	8	2	1	-	1	1	-	2	1	2	2	3	-	-	2	-	1	-	-	-	21.0
36	1	85	10	2	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	2	89	6	3	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	87.0

TABLE III--Continued

Plant No.	Anther No.	Quartets: No. of Micronuclei										Pentads: No. of Micronuclei						Hexads: No. of Micronuclei						Septads and Octads	Meiotic Index*
		0	1	2	3	4	5	6	7	8	8+	0	1	2	3	4	4+	0	1	2	3	4	4+		
37	1	12	14	13	10	17	4	2	1	1	2	1	3	2	2	2	8	-	-	1	1	-	3	1	
	2	12	30	18	6	5	4	3	3	2	-	1	1	2	3	-	5	1	-	-	1	-	1	2	12.0
38	1	79	8	8	1	-	-	-	-	-	-	2	1	-	-	-	-	1	-	-	-	-	-	-	
	2	39	21	18	3	3	5	-	2	-	1	-	3	2	-	-	1	-	-	-	1	-	1	-	59.0
39	1	41	13	11	8	10	2	3	1	-	-	2	2	1	1	1	-	2	1	-	-	-	1	-	
	2	37	20	21	7	5	2	-	1	-	-	1	1	3	1	-	-	-	-	-	-	-	1	-	39.0
40	1	50	22	17	3	2	-	1	-	-	-	2	-	1	-	-	-	-	1	1	-	-	-	-	
	2	52	21	15	6	-	-	-	-	-	-	-	2	-	-	2	-	-	1	-	-	1	-	-	51.0
41	1	98	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	2	99	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	98.5
42	1	7	11	9	12	17	4	3	2	1	-	2	6	3	5	1	6	1	-	3	-	1	4	2	
	2	6	14	14	12	14	9	9	1	2	1	1	1	1	-	2	8	-	2	-	2	-	-	1	6.5
43	1	14	9	15	12	11	3	4	3	2	-	1	2	2	3	4	5	1	-	-	4	2	3	-	
	2	19	20	22	14	6	3	2	1	-	-	1	2	3	3	1	2	-	-	-	-	1	-	-	16.5
44	1	21	18	25	14	9	-	2	-	-	1	-	1	2	1	1	1	-	1	1	-	-	1	1	
	2	21	12	20	7	4	2	3	-	-	-	2	4	7	3	2	3	1	2	-	3	1	1	2	21.0
45	1	11	14	17	11	8	5	4	-	1	1	-	7	1	-	7	6	-	-	1	1	-	3	2	
	2	11	13	15	16	10	3	2	4	-	-	1	2	3	3	2	9	-	-	3	1	1	1	-	11.0

TABLE III--Continued

Plant No.	Anther No.	Quartets: No. of Micronuclei 0	1	2	3	4	5	6	7	8	8+	Pentads: No. of Micronuclei 0	1	2	3	4	4+	Hexads: No. of Micronuclei 0	1	2	3	4	4+	Septads and Octads	Meiotic Index*
46	1	26	17	13	17	8	4	2	4	1	-	1	-	2	1	1	1	-	2	-	-	-	-	-	
	2	18	11	23	13	11	7	2	1	-	-	1	1	1	4	2	4	-	1	-	-	-	-	-	22.0
47	1	18	19	22	4	6	7	3	-	-	2	-	-	3	4	3	5	1	-	-	1	-	2	-	
	2	14	13	22	17	12	2	4	2	1	1	-	2	6	1	-	3	-	-	-	-	-	-	-	16.0
48	1	-	4	7	10	10	11	5	6	3	3	-	-	2	1	1	24	-	-	1	-	2	7	3	
	2	2	7	15	12	14	12	6	3	1	2	-	1	2	3	2	12	-	-	-	-	-	4	2	1.0
49	1	28	16	11	4	2	-	1	-	-	-	8	10	6	5	1	3	1	1	2	1	-	-	-	
	2	1	-	-	2	2	2	4	4	4	10	-	-	2	3	1	24	-	-	-	-	1	32	8	14.5
50	1	80	10	2	2	2	-	-	-	-	-	1	1	1	-	-	-	1	-	-	-	-	-	-	
	2	87	4	2	2	2	-	-	-	-	-	-	-	1	1	-	-	-	-	1	-	-	-	-	83.5
51	1	72	5	12	3	1	-	-	-	-	-	4	-	-	-	-	-	1	-	1	-	-	1	-	
	2	70	9	6	2	1	-	-	-	-	1	2	5	-	-	1	-	1	1	-	1	-	-	-	71.0
52	1	93	3	2	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	
	2	92	2	4	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	92.5
53	1	90	7	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	2	91	4	3	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-	-	-	90.5
54	1	6	22	20	13	11	7	7	3	-	-	-	2	1	2	-	2	-	1	-	2	-	-	1	
	2	13	16	16	25	11	5	6	1	-	-	-	-	1	2	2	-	-	-	-	-	-	2	-	9.5

TABLE III--Continued

Plant No.	Anther No.	Quartets No. of Micronuclei										Pentads No. of Micronuclei						Hexads No. of Micronuclei						Septads and Octads	Meiotic Index*
		0	1	2	3	4	5	6	7	8	8+	0	1	2	3	4	4+	0	1	2	3	4	4+		
55	1	87	6	4	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	2	84	7	3	2	1	-	-	-	-	-	-	-	-	-	-	-	2	1	-	-	-	-	-	85.5
56	1	72	9	8	4	1	-	-	-	-	-	-	-	-	-	-	-	-	2	2	-	2	-	-	
	2	63	15	8	5	4	-	-	-	-	-	-	-	-	-	-	3	-	-	1	1	-	-	-	67.5
57	1	89	6	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	2	79	7	10	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	84.0
58	1	42	16	13	8	6	5	4	1	-	2	1	-	1	-	-	-	-	1	-	-	-	-	-	
	2	54	21	9	4	2	1	-	-	-	-	3	1	4	1	-	-	-	-	-	-	-	-	-	48.0

*Meiotic Index denotes percentage of normal microspore quartets found in each plant.

less) of polyspory and high meiotic indices (97.5 to 90.5) were found in plants 11, 18, 52 and 53. As a group the four plants with very low frequencies of polyspory may be more stable than the four plants entirely free of polyspory. Only plant 41 in the group free of polyspory had a higher meiotic index than the four plants with low frequencies of polyspory. Pentads were found in most plants and octads were observed in 11 plants. As reported by Love (27) the number of micronuclei and microcytes per quartet increased as the frequency of microspore quartets with micronuclei and microcytes increased. Plants 12, 48 and 49 are good examples in which many micronuclei were found in a quartet.

Meiosis in Mediterranean

Meiosis was studied in some detail in 29 Mediterranean plants, including all plants with meiotic indices below 20, above 90 and selected plants with meiotic indices between 20 and 90. The types of irregularities observed in meiosis were similar in all plants. The frequency of irregularities appeared to be associated with the irregularities observed in microspore quartets. Univalents at metaphase I, laggards at anaphase I and II and at telophase I and II, non-orientated bivalents at metaphase I and non-orientated chromosomes at metaphase II were irregularities observed often in most plants. Frequently chromosomes would be seen in meiosis II that were not associated with the achromatic figure. Many times the chromatids of these chromosomes were separated but remained close together, both being included in one microspore.

Pollen mother cells with aberrant chromosome numbers were found in 15 plants. Figures 4 and 5 show cells of this type. The number of chromosomes in metaphase I cells found in different plants ranged from 19 to 37 and the cells were characterized by relatively large numbers of univalent chromosomes as shown in Figures 4 and 5. Usually metaphase I cells with aberrant chromosome numbers were found in anthers in which most of the cells were undergoing meiosis II.

One plant with a meiotic index of 2.0 (plant 12 in Table III) was found to be deficient for one chromosome in all pollen mother cells analyzed. No pollen mother cells similar to the atypical type previously described were found in this plant. Three or more univalent chromosomes were frequently seen at metaphase I. Laggards were seen in most anaphase I and II and telophase I and II cells; non-orientated chromosomes were frequent at metaphase II. Microspore quartets with large numbers of micronuclei were frequent and 24 per cent of the microspore quartets contained one or more microcytes.

Observations of Plants Grown in the Greenhouse

Seven progeny from each of two Mediterranean plants with high (plants 53 and 11 in Table IV), four with low (plants 9, 37, 42 and 45) and two with medium (plants 13 and 33) meiotic indices were planted in the greenhouse in September of 1959. These plants were grown to provide additional information on the cytological irregularities in Mediterranean. Progeny from selected Triumph plants with meiotic indices ranging from 98.5 to 100 also were grown. Collections were made during most of the month of February and in early March. In nearly all cases the first two tillers from each plant were used to make

crosses with Triumph. Thus the third tiller to reach the proper stage was collected from each plant. Meiotic indices for Triumph plants examined were 94 or higher. The meiotic indices of Mediterranean plants grown in the field and their selfed progeny grown in the greenhouse, where countable microspore quartets were found, are presented in Table IV. Only one spike was collected for cytological observations from most plants; however, in a few instances two spikes were collected.

TABLE IV

Meiotic indices determined from 200 microspore configurations from each of eight Mediterranean plants and some of their progeny

Parent plant No.[1]	Meiotic index of parent	Progeny No. 1	2	3	4	5	6	7
		Meiotic indices of progeny						
53	90.5	88.0	85.0	55.0*	31.0	21.0	-	-
11	97.5	99.0	99.0	98.5	98.5	97.5	96.5	95.5
9	11.5	93.0	90.0	79.0	66.0	39.0	34.5	-
37	12.0	90.0	84.5	73.5	49.0*	43.5	28.5	-
42	6.5	81.0	65.0	48.0*	38.0**	35.5	-	-
45	11.0	76.0	73.5	67.0*	39.0	31.5	4.0*	-
13	40.0	85.0	75.5	71.0	25.5	17.5	-	-
33	43.5	88.5	86.0	32.5*	-	-	-	-

[1] Parent plant numbers are those listed in Table III.
* Denotes monosomic plants.
** Denotes plant with 39 chromosomes.

Consistant meiotic indices were obtained from the progeny of only one plant (plant 11 in Table IV). Progeny from each of the other plants exhibited considerable variation in meiotic indices. Although

the parent plant 53 had a meiotic index of 90.5, the meiotic indices of the progeny were quite variable and one plant was monosomic. Meiotic indices of 90.0 or higher were obtained for one or more progenies of plants 9 and 37 even though these parental plants had low meiotic indices.

Considerable differences in meiotic indices were found between spikes when two spikes from one plant were examined. Countable microspore quartets were found in both spikes from only two plants. In one case the second spike was collected 28 days later than the first spike and their identity was maintained. The meiotic index determination from the earlier spike was 88.0 as shown for progeny 1 of plant 53 in Table IV. The meiotic index determination from the later spike was 12.0. It is apparent that the meiotic process was more disturbed in the later spike. The frequency of occurrence of microcytes increased only slightly while the frequency of micronuclei increased considerably in the later spike as compared with the earlier spike. Meiotic indices were determined for two spikes from only one other plant (progeny 1 of plant 45 in Table IV). However, in this case it is not known which spike was collected first, although it is known that the collections were made 20 days apart. The meiotic indices of the two spikes were 76.0 and 14.0. Only the first determined meiotic index is presented in Table IV. The frequency of microcytes was greatly increased in the spike with the lower meiotic index.

Lagging chromosomes in both meiotic divisions appeared to be the most frequent abnormality observed. Univalents were present in some cells in most plants. The fate of univalent chromosomes was uncertain. Many would move towards the poles intact. Others would remain on or

near the equatorial plate while some divided. Some of the chromatids would move to the poles while others remained near the equatorial plate. Three univalents have divided in the cell shown in Figure 6 of Plate I. Very small numbers of cells with aberrant chromosome numbers, as described previously, were observed in plants grown in the greenhouse.

In addition to the seven monosomic plants indicated in Table IV, a progeny of plant 33, for which countable microspore quartets were not found, was monosomic. Thus of the total of 56 plants grown, eight, or about 14.3 per cent were monosomic. None of these monosomic plants could be distinguished from sister disomic plants.

Most metaphase I cells of the plant with 39 chromosomes (progeny 4 of plant 42) appeared to have 18 bivalents and three univalents, although a cell containing 11 bivalents and eight univalents was observed. As compared to sister plants this plant was shorter in stature and the spikes were smaller. Also, this plant appeared to be sterile as no selfed or crossed seeds were obtained.

Observations of Paired Plant Rows

Progeny from bagged and unbagged spikes selected from plants grown for cytological studies in 1959 were grown in plant rows during the 1959-1960 season. The two progeny classes, i.e., from bagged and unbagged spikes, from each plant were grown side by side and considered as paired plant rows. Plants believed to be the result of natural hybridization in the spring of 1959 were observed among the plants grown from unbagged spikes. Two bronze-chaffed plants were found in one row, one or two plants in another row and one plant in a third row

of Triumph. Two rows of Kaw contained one bronze-chaffed plant while two bronze-chaffed plants were found in another row.

Cross-pollination with Concho could have occurred in each case. A row of Concho was grown two feet from the row of Triumph and three feet from Kaw. Mediterranean was grown in a row one foot from Kaw; however, Mediterranean probably flowered too late to pollinate any Triumph or Kaw florets. No other bronze-chaffed varieties or selections were grown close to these plants.

The progeny from one Mediterranean plant had white glumes and mid-long beaks except for one spike with bronze glumes and short beaks in the row seeded from unbagged spikes. The parent plant was apparently a white-chaffed segregate of a cross between a bronze-chaffed Mediterranean plant and an unknown white-chaffed, mid-long-beaked plant in some previous season. The bronze-chaffed short-beaked plant was probably the result of a natural cross involving a bronze-chaffed Mediterranean plant. There were no F_1 plants detected among the progeny from any of the bagged spikes of the three varieties.

It is possible that these aberrant types arose through cytological irregularities. However, each aberrant plant was detected by the appearance of bronze-chaffed spikes among white-chaffed plants. No other variations were detected among Kaw plant rows. Both rows grown from one Triumph plant segregated for height and maturity. It is possible that this plant resulted from a natural cross between a Triumph plant and an unknown taller and later plant.

Chaff color in Mediterranean was not distinguishable in the first year of this study as it was necessary to harvest the plants before chaff color became evident to prevent further damage by birds.

When the progeny from selected plants were grown the following season it was found that most plants were typical for the variety. However, considerable variability was noted in the progeny of several plants. Both glabrous and pubscent-glumed plants and segregation for height were noted in both rows grown from a plant with a meiotic index of 84.0. Variation in glume color and awned condition was found among the plants in the two rows grown from each of two plants. White and bronze glumes and fully awned to tip awned and awnless spikes were observed. The meiotic indices of the parent plants were 81.0 and 87.5. All the plants in the four rows grown from two plants had denser fusiform spikes than was typical of other Mediterranean plants. Both parental plants had high meiotic indices (98.5 and 97.5). All the progeny from one plant with a meiotic index of 94.0 had spikes with mid-long beaks and white chaff except for one plant with short beaks and bronze chaff. This plant was found in the row grown from unbagged spikes and may be the F_1 of a natural cross with a bronze-chaffed, short-beaked Mediterranean plant.

Progeny from the monosomic plant (plant 12 in Table III) described in a previous section were variable in height. The shorter plants were about 10 inches shorter than the taller plants.

Seeds from the bagged spikes of the parents of the paired plant rows of Mediterranean were space planted in the fall of 1959. All of the spaced plants matured late and the spikes became discolored before chaff color could be determined with any degree of certainty. Observations of this space-planted material complemented the observations made in the paired plant rows. Variations found in the paired rows, such as differences in awned condition, also were ob-

served in the spaced plants. No aberrant types were found among the spaced plants that were not observed in the paired rows. A few less vigorous plants were observed in each plot.

DISCUSSION

Natural Cross-pollination

The results of this study indicate that cross-pollination should not be any more of a factor in maintaining pure seed stocks of Kaw than of Triumph. The frequency of cross-pollination was found to be low in Kaw (0.59 per cent) and Triumph (1.27 per cent). Similar frequencies are recorded in the literature for varieties that were well adapted to the area in which the studies were being conducted (5, 9, 19). Harrington (9) found year to year differences in cross-pollination, however, low frequencies were always obtained. Also crossing may occur relatively frequently in a variety one season, but be low in another season in comparison to other varieties. Environmental factors were probably responsible for much of the variations observed. However, the way in which environment affected crossing was unknown. It is not known how frequently cross-pollination might be expected in Triumph or Kaw under the environmental effects of another season. Since no definite correlations are known between environment and the frequency of natural crossing it is impossible to make accurate predictions on the occurrence of crossing when plants are grown under different environmental conditions.

According to Leighty and Taylor (19) diseases that affect the plant prior to or during flowering, expecially those that affect the

spike, may exert some influence on male sterility and natural crossing. It is not known if diseases were responsible for the higher frequency of crossing in some plots than in others within dates of seeding or if other factors were responsible for the variations noticed. There may be actual differences, however, some of the apparent differences may be due to sampling errors.

It is evident from this study and others that some cross-pollination should be expected in common wheat. Precautions against cross-pollination may be necessary to insure purity of parental types and selfing of hybrid material for genetic investigations. If crossing is appreciably greater in the secondary tillers as reported by Leighty and Taylor (19) it may be desirable to harvest only the healthy and vigorous primary tillers for seed supplies. Whenever possible, varieties should be arranged so that divergent rather than like types are grown together and a variety with as many dominant characters as possible should be used for field borders so that crosses can be detected and removed. When dominance obscures the F_1 hybrids the evidence of natural crossing would not be apparent until the following generation when segregation occurred. Roguing will fail to remove the segregates approaching the dominant type, thereby increasing the heterogenity of the material.

Cytological Studies

All Kaw and Triumph plants examined in the microspore quartet analysis would be considered stable according to Love's (27) designation of plants with meiotic indices above 90 being considered stable. Although irregularities were slightly more frequent in Kaw

than in Triumph, the two varieties probably have essentially the same meiotic stability. It would seem that very few, if any, aberrant plants would arise in either variety as the result of cytological irregularities. Apparently the Kaw used in this study has been purified through selection prior to this study.

The level of cytological instability varied greatly among the Mediterranean plants grown in the field and in the greenhouse. In some instances considerable differences in the frequency of aberrant microspore quartets were found between two spikes of individual plants; however, in most plants similar data were obtained from both spikes. Possibly some of the variability between anthers of a single plant in this study, as shown in Table III, was due to environment, since it has been reported that environmental fluctuations may cause changes in the frequency of meiotic irregularities (20, 31). In the present study, most of the material used for cytological analysis was collected on different days and stored in one container. Therefore, no association could be made between dates of collection and the respective meiotic indices.

On the basis of observations of various stages of meiosis, it is believed that the exclusion of entire chromatids from the nucleus was responsible for many of the micronuclei observed in microspore quartets. Lagging chromosomes in anaphase and telophase stages were observed much more frequently than chromosomal fragmentation. Although frequency counts were not made on the occurrence of lagging chromosomes in the various stages of meiosis, the occurrence of laggards appeared to increase as the meiotic index decreased. Therefore, it would be expected that many pollen grains from plants with low meiotic indices would be lacking one or more chromosomes. According

to Sears (38) and Morrison (29) normal pollen is much more competitive than pollen lacking a chromosome, however, no selection against n-1 female gametes was found. Pollen grains lacking more than one chromosome are usually nonviable. Increased cross-pollination could result from the competitive advantage of pollen from normal plants over abnormal pollen from plants with irregular meiotic processes. Due to competitive advantages normal pollen will function in fertilizing most female gametes and tend to decrease the frequency of aneuploid plants. At the same time the viability of female gametes with aberrant chromosome numbers would tend to increase the frequency of aneuploid plants. If it is assumed that meiotic irregularities are similar in the production of microspores and megaspores it may well be expected that chromosomal abnormalities will be found in following generations. It is doubtful if any viable gametes would develop from the pollen mother cells with aberrant (other than monosomic) chromosome numbers, as shown in Figures 4 and 5 of Plate I. Several workers (13, 21, 23, 29) have reported the infrequent occurrence of similar cells and have attributed their origin to abnormal mitotic divisions. Plants with low meiotic indices may produce very few normal pollen grains. Natural crossing may be frequent in plants with low meiotic indices and will always occur in male sterile nullisomics.

The phenotypic variability recognized in the Mediterranean plant rows was probably due to the combined effects of cytological irregularities and cross-pollination. Through cytological irregularities a plant may become partially sterile and as a consequence be more likely to outcross. If normal pollen is involved in such a cross considerable meiotic stability may be restored in some of the progeny. Meiotic

stability may be increased by restoration of the euploid condition if a monosomic or nullisomic female parent is involved or by reducing the tendency for the occurrence of univalents if either aneuploid or euploid female parents are involved. If the cross occurs between plants with divergent phenotypes segregation would be evident for several generations, although considerable meiotic stability may be obtained in some segregates. In this study the meiotic indices of the Mediterranean plants that produced segregating progeny were relatively high (81.0 to 87.5). It is possible that more careful examination would have revealed the presence of considerably more variability, expecially among the progeny of plants with low meiotic indices. In some cases, a tiller may have been considered as a weak secondary tiller when it may have been a less vigorous plant growing among stronger plants. As pointed out by Huskins (15) and Sears (38) plants lacking an entire chromosome may appear much like normal plants when grown under favorable environmental conditions. Under less favorable conditions some monosomics will develop characteristics in the direction of the nullisomic. Due to the normal appearance of many monosomic plants and the low frequencies of nullisomic plants phenotypic variation may not be outstanding in some cytologically aberrant plants. Considering the frequency of aneuploid plants found in the greenhouse, it seems probable that there were aneuploid plants in the populations grown in the field.

As shown by the progeny of plant 11 (Table IV), it should be possible to select lines with high meiotic indices. However, plants with high meiotic indices may be expected among the progeny of plants of low meiotic indices. For example, plants 9 and 37 had meiotic indices of 11.5 and 12.0 respectively and produced one or more progeny

with meiotic indices of 90.0 or higher. As shown by the progeny of plant 53, low meiotic indices may be found among the progeny of plants with high meiotic indices. From this it should be obvious that progeny testing is necessary before a reliable estimation of the meiotic stability of different lines can be made. Re-examination in later generations is necessary to remove plants possessing meiotic irregularities. Since Mediterranean is a parent of Kaw and abnormal microspores are produced in many Mediterranean plants it is possible that in early generations Kaw may have been unstable cytologically. The aberrant plants reported in Kaw may have been aneuploid plants and their segregates resulting from irregularities in meiosis. Also, meiotic irregularities in both euploid and aneuploid plants may have caused considerable sterility thus causing more cross-pollination than would be expected in normal plants. Had cytological examinations been made during early generations only the cytologically normal and agronomically desirable plants would have been retained.

Monosomic and nullisomic series developed from a reasonably vigorous variety can be used in several ways in wheat research programs. As summarized by Elliott (3) monosomics and nullisomics can be used in genetic studies, evolution studies, studies on the recessive genes present in homoeologous groups and associating genes with a particular chromosome. The high frequency of monosomic plants found among the plants grown in the greenhouse and the frequent occurrence of several univalent chromosomes at metaphase I in disomic plants grown in the field and greenhouse would indicate that a monosomic and nullisomic series should be easily obtained in Mediterranean. It is not known how many different monosomes are represented in the nine monosomic plants found in this study. However, since the monosomic plants were

derived from several different disomic plants it may be assumed that the same chromosome is not involved in each plant. Further work with Mediterranean seed stocks at hand should make it possible to recover the complete monosomic series in this variety.

SUMMARY

Triumph and Kaw, a selection from the cross Early Blackhull-Tenmarq x Oro-Mediterranean-Hope, both white-chaffed, hard red winter wheats, were planted in alternate rows with Concho, a bronze-chaffed variety of hard red winter wheat, to determine the frequency of natural cross-pollination. The per cent tillers with bronze chaff in the next generation of the white-chaffed varieties was used as the per cent natural cross-pollination. A total of 25,792 spikes were counted in Triumph plots. Of these 328 had bronze chaff. Only 152 bronze-chaffed spikes were found in 25,791 Kaw spikes. The per cent natural cross-pollination was 1.27 per cent in Triumph and 0.59 per cent in Kaw.

Meiotic indices (the percentage of normal microspore quartets) were determined for 58 to 68 selected plants of each of the varieties, Triumph, Mediterranean and Kaw. Meiotic indices were used as the basis for determining the meiotic stability of the various plants. High meiotic indices were found in all Triumph and Kaw plants. Meiotic indices in Mediterranean plants ranged from very low (1.0) to high (98.5).

Further study was made of the meiotic behavior of some Mediterranean plants to ascertain the probable causes for the occurrence of micronuclei. One monosomic (2n-1) plant was found. Univalents, non-orientation and laggards were observed in most plants.

Progeny from eight Mediterranean plants were grown in the greenhouse and examined cytologically. Progeny from one plant with a high meiotic index had uniformly high meiotic indices. The progeny from each of the other plants with high, medium and low meiotic indices were quite variable. Eight monosomic plants and a plant with 39 chromosomes were found.

Paired plant rows were grown from bagged and unbagged spikes from selected plants of each of the three varieties studied cytologically. At least eight aberrant plants were found among the progeny from unbagged spikes and were believed to be possibly due to cross-pollination during the spring of 1959. No other appreciable morphological variation was detected between the progeny from bagged and unbagged spikes from individual plants of the three varieties.

Considerable variability was evident in both rows grown from four Mediterranean plants. A combination of cytological irregularities and cross-pollination may be responsible for the variability observed. Morphological variation was detected in progeny from plants with low and high meiotic indices. Results from greenhouse studies indicated that aneuploids could be expected in Mediterranean.

CONCLUSIONS

Natural cross-pollination detected in this study was no greater than frequently reported in the literature for well adapted varieties. Although it was reported that difficulty had been encountered in maintaining pure seed stocks of Kaw, the plants examined in this study appeared to be uniform. Since cross-pollination was about twice as frequent in the established variety, Triumph, as in Kaw, it is concluded that natural crossing found to have occurred during the blooming period of 1959 would not be a major factor causing difficulty in maintaining pure seed stocks of Kaw. Under the environmental conditions of another season different results might be obtained. Cross-pollination may be an important factor in causing admixtures when different lines, strains, or varieties are grown side by side.

Results of the microspore quartet analysis indicate that the meiotic process is nearly as stable in Kaw as in Triumph and would not be a factor in causing an appreciable number of aberrant plants in Kaw. The great variability among Mediterranean plants would indicate that plants used in breeding work should be carefully examined to determine their stability. Mediterranean may be a source of aneuploids for further plant breeding work since 16 per cent of the plants grown in the greenhouse were found to be aneuploids.

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