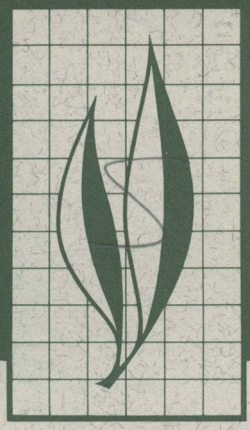
HILGARDIA

JOURNAL OF AGRICULTURAL SCIENCE PUBLISHED BY
HE CALIFORNIA AGRICULTURAL EXPERIMENT STATION

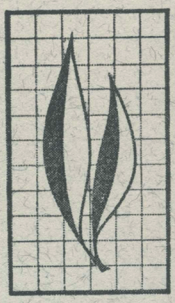


Volume 45, No. 6 · September, 1977

Genetic Male Sterility in Wheat (*Triticum aestivum* L.): Reproductive Characteristics and Possible Use in Hybrid Wheat Breeding

Chao-Chien Jan and C. O. Qualset

NIVERSITY OF CALIFORNIA DIVISION OF AGRICULTURAL SCIENCES



UC9109-9 is a genetic male-sterile wheat (*Triticum aestivum* L.) that was isolated at Davis from the cross of two fertile wheat varieties, D6301 and Ramona 50. The male-sterile plants were typical of genetic and cytoplasmic male-steriles, except that in a low proportion of the florets, the anthers were transformed to pistil-like structures. The pistilloid anthers were not fertile. UC9109-9 can be maintained by self-pollination, which occurs in about 5 percent of the florets. All progeny from selfed seeds were male-sterile. Aneuploidy was observed in populations derived from UC9109-9, but it was unrelated to male sterility. All F_1 plants were fertile when UC9109-9 was used as a female parent in crosses with eight fertile varieties. A low yield (about 30 percent of normal) of hybrid seeds was obtained in small-scale hybrid seed production trials, but improvements in seed production can be expected from manipulating growing conditions in the seed production field, or presumably by genetic improvement of the outcrossing mechanism in wheat.

THE AUTHORS:

Chao-Chien Jan is Postgraduate Research Agronomist, and C. O. Qualset is Professor of Agronomy, and Agronomist, Department of Agronomy and Range Science, Davis.

Chao-Chien Jan and C. O. Qualset

Genetic Male Sterility in Wheat (*Triticum aestivum* L.): Reproductive Characteristics and Possible Use in Hybrid Wheat Breeding[1]

INTRODUCTION

ALTHOUGH WHEAT (*Triticum aestivum* L.) is a predominantly self-pollinated crop, heterosis has been demonstrated by various workers studying F_1 populations obtained from hand emasculations and pollinations (Livers and Heyne, 1968; Grant and McKenzie, 1970; Walton, 1971; Bitzer and Fu, 1972). The nucleo-cytoplasmic type of male sterility has received the most attention as a method of pollination control because of its possibilities in the utilization of hybrid vigor. Problems encountered in hybrid wheat production, however, have not been entirely solved (Kihara and Tsunewaki, 1964; Kihara, 1967; Johnson, Schmidt, and Mattern, 1967; Ingold, 1968). Deleterious side effects appear with certain sterile cytoplasms. The restoration system seems to be genetically complex, controlled by two or more genes in most cases, and affected by modifiers and genotype-environment interactions. This has made difficult the development of lines with efficient and complete restoration. Chemical induction of male sterility in wheat was evaluated by Rowell and Miller (1971) and others; but it does not seem very promising for use in hybrid seed production, as pointed out by Stoskopf and Law (1972) for Ethrel, and by Jan, Qualset, and Vogt (1974, 1976) for RH-531, RH-532, and RH-2956. The difficulties with those methods have encouraged the investigation of genetic male sterility and its possible role in hybrid wheat development.

Genetic male sterility in wheat was reported by Pugsley and Oram (1959). Since then, male sterility controlled by nuclear genes has been discovered in progenies of varietal crosses and in induced mutation programs (Bingham, 1966–67, 1968; Fossati and Ingold, 1970; Athwal and Borlaug, 1967). Along with these discoveries, gene models were proposed; and schemes were suggested for using genetic male sterility (Hermsen, 1965; Athwal and Borlaug, 1967; Gill and Anand, 1970) in wheat breeding.

This study characterizes a male-sterile stock, derived from a varietal cross, with respect to its female and male fertility, cytological stability, outcrossing ability, and fertility in hybrid combinations. Genetic analysis and further discussion of the origin of the male sterility reported here is presented by Jan and Qualset (1977).

MATERIALS AND METHODS

The UC9109-9 male-sterile material was discovered by J. C. Williams at Davis in 1965, in a population of 140 F_3 lines from the cross of two fertile

[1] Submitted for publication June 7, 1976.

parents, D6301 and Ramona 50. All plants of one F_3 line (UC9109-9) had characteristics typical of male sterility. Individual F_3 plants in this line were designated UC9109-9-1 to 9109-9-10 (F_4 lines). D6301 is a short-statured (about 70 cm) breeding line obtained in 1961 from N. E. Borlaug in Mexico. It is a selection from the cross Mayo 54 × Norin 10-Brevor. Ramona 50 is a variety of standard height (about 120 cm), developed in California. Its parentage is [(Martin × Hard Federation^3) × Ramona^3]^2 × Ramona 44. Most anthers of the sterile plants were either shriveled, or transformed, to various degrees, into pistils. Some seeds were produced upon self-pollination, and progeny of the original F_3 plants have been maintained separately. Every season, sterile plants belonging to each of the F_4 lines were self-pollinated and randomly chosen for further planting and observation. Seeds were space-planted into either one 4.8-m row or two 2.4-m rows, with 0.3-m spacing between seeds. Pedigrees were kept so that all progenies could be traced back to their original F_3 parents.

Fertility characteristics

Plantings were made in December for the 1967–68 through 1971–72 crop seasons (normal growing season), along with a July planting in 1968 (off-season planting). Selfing bags were applied on 1 to 3 main tillers a few days before anthesis. At maturity, the selfed spikes and three open-pollinated spikes per plant, were sampled for fertility determinations in all except the July planting, when one open-pollinated spike per plant was taken. In 1969, three open-pollinated late-maturing spikes from each plant were harvested for comparison with the similar spikes from early tillers.

The low-fertility plants from the 1968 July harvest were grouped into partially sterile and sterile categories, based on the number of seeds produced on two spikes per plant. Partially sterile plants had between 12 and 50 seeds per two spikes, and the sterile plants had fewer than 12 seeds. This tentative grouping was checked by comparing progenies from these two groups in subsequent years. These comparisons were also made from the December plantings of 1968–69 through 1971–72. Four methods of fertility determination were used: the percentage of florets producing seeds under open- and self-pollination, and the number of seeds per open- and self-pollinated spike.

Progenies from selfed seeds from male-sterile plants were evaluated in 1968 and 1970–72.

In 1968, open-pollinated seed-set percentages were determined for three early and three late spikes on each of 51 plants. In the 5 yr from 1968 to 1972, the comparisons among lines were made by using open- or self-pollinated seed-set percentages. Yearly variations in fertility were examined with all four methods of scoring by pooling all lines in a one-way analysis of variance. Analyzed in the same way were samples from various numbers of fertile varieties (5 to 7), including D6301 and Ramona 50. Self- and open-pollinated seed-set percentages of the sterile plants were compared for all plantings except that in 1971. Samples from fertile varieties, and F_1's in 1972 were included as checks.

Cytological studies

Spikes were sampled at meiosis from 35 random sterile plants and the parents, Ramona 50 and D6301, in 1971. The samples were fixed in Carnoy's fixative (6:3:1), and stained with aceto-carmine for chromosomal observations and tetrad analyses. The male-sterile population had both monosomic ($2n = 41$) and normal ($2n = 42$) plants. Data from tetrad analyses were recorded as the percentages of abnormal

tetrads (tetrads with one or more micronuclei). More than 100 tetrads were observed for each plant. The percentages of abnormal tetrads of fertile parents, and of disomic and monosomic sterile plants, were determined.

Plants with clavate spikes were found occasionally in the sterile populations. These plants were generally shorter and weaker, and set much fewer selfed seeds, than sterile plants without clavate spikes. The fertility of clavate and normal sterile types was determined. In 1971, meiotic chromosomal analyses and progeny observations were made on seven plants which had typical clavate spikes.

Pollen fertility was examined by the technique of Alexander (1969). Pollen grains which stained deep red were scored as fertile, and those which stained green were scored as sterile.

Anther development

The pattern of structural modifications of anthers to pistil-like structures was studied in relation to pollen fertility. One early and one late spike were sampled from each of nine male-sterile plants in the 1970 spring nursery. Up to three florets per spikelet on one side of each spike were examined, and the most normal-appearing anther from each floret was examined for pollen fertility.

The degree of anther transformation was scored on a scale of 1 to 6, with 1 being normal, and 6 not distinguishable from a normal fertile pistil (Fig. 1A). Fertility of as many pollen grains as possible from anthers scored 1 to 3 was determined by Alexander's (1969) method. Anthers scored 4, 5, or 6 were considered to have zero pollen fertility since no pollen was present. Florets from each spike were further classified by position on the spike (apex, middle, and base).

Preliminary observations indicated that pollen fertility was affected by the degree of anther transformation. To study this relationship in greater detail, 24 and 5 sterile plants were sampled in 1971 and 1972, respectively. Three anthers from each plant, representing scores 1, 2, and 3, were examined for pollen fertility. A linear regression analysis of pollen fertility on the anther transformation score was done.

Fertility of normal ovary and pistilloid anthers

To test the effects of transformed anthers on the fertility of normal ovaries, and to determine the fertility of transformed anthers as female organs, sterile spikes were given various treatments before being pollinated with fertile pollen in 1970 and 1972: a) emasculation of all anthers, including transformed ones, leaving only the normal ovary in the floret; b) emasculation of normal-appearing anthers, leaving a transformed anther and normal ovary in the floret; c) emasculation of anthers and normal ovary, leaving the most advanced transformed anther in each floret; and d) emasculation of anthers and the normal ovary, leaving only the transformed anthers in the floret. Six or more spikes were prepared for each treatment in each year. After treatment, a selfing bag was placed on each spike, and 1 to 3 days later the spikes were pollinated with pollen from fertile varieties. At maturity, seed-set fertility was obtained on the basis of seeds per spike in 1970 and seed-set percentage in 1972.

Normal ovaries and the advanced type of transformed anthers were selected for histological study. They were fixed in Carnoy's solution for 24 h, dehydrated with tertiary butyl alcohol (Johansen, 1940), embedded in paraffin, sectioned serially at 10 μm, and stained with hematoxylin, safranin, and fast green.

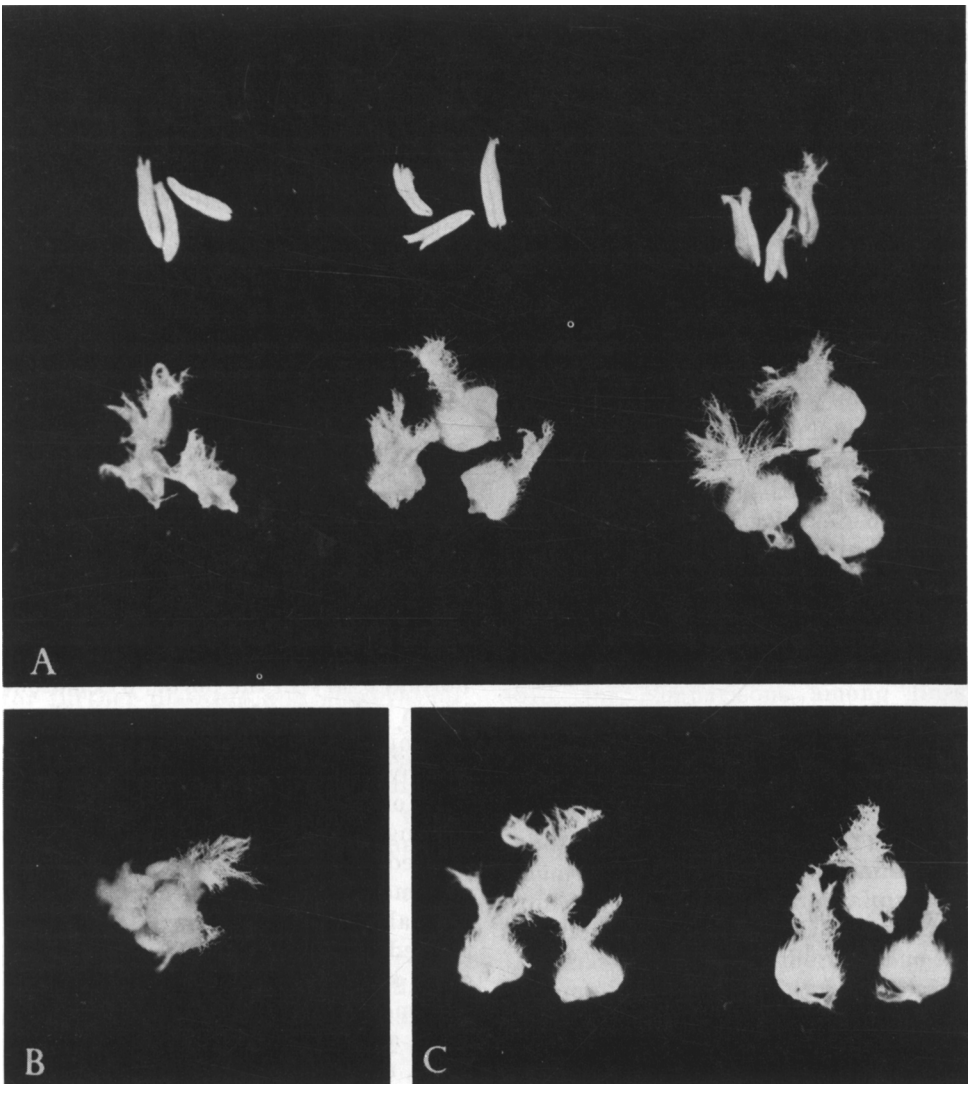


Fig. 1. Pistilloid anther development in UC9109-9. A. Different degrees of anther pistilloidy (from left to right): 1, 2, 3 (upper row) and 4, 5, 6 (lower row). B. Normal pistil in the center and three pistilloid anthers around it. C. Normal pistils on the left and complete pistilloid anthers on the right.

Outcrossing study

Open-pollinated seeds obtained from the 1968 summer planting were planted in December 1970 and 1972 to evaluate the outcrossing ability of the sterile material. Each plot was 13.6 m long, with four rows spaced 0.3 m apart, and with a seeding rate of 101 kg/ha. The open-pollinated seeds were planted in the center two rows, and a fertile pollinator was planted in the outer two rows of each plot. Fertile varieties with different heading dates and mature heights were chosen as pollinators. In 1970, 10 plots were grown of the varieties Pitic 62, INIA 66, Siete Cerros 66, Nadadores 63, Lerma Rojo, Lerma Rojo 64, Pacific

Triple Dwarf, Nainari 60, Anza, and a composite of equal contributions from all pollinators. One plot with all four rows planted with sterile materials was grown in isolation to establish the natural selfing rate in the male-sterile stocks. In 1972, the pollinators were D6301, INIA 66, Pitic 62, Nadadores 63, and a mixture of these four varieties.

The rows were in a north-south direction. Fertile plants which appeared in the two center sterile rows were removed by hand as soon as they were recognizable. The numbers of fertile and sterile plants in the two sterile rows were recorded, and one spike from each plant was collected at maturity. In 1970, the number of seeds per spike was determined, and in 1972 the data were obtained as the percentage of florets producing seeds.

F_1 fertility

Male-sterile plants were crossed with several fertile varieties to study F_1 fertility. The male parents used were Nainari 60, Siete Cerros 66, Lerma Rojo, Ramona 50, Pitic 62, and Sonora 64. The F_1 plants were grown in 4.8-m rows with 0.3 m between rows and plants. Seed fertility percentages were obtained from two or three spikes per plant in 1968, 1969, and 1972.

RESULTS AND DISCUSSION

Male-sterile UC9109-9 plants were about the same height as their short parent, D6301. At anthesis the glumes were widely opened, and they remained open until desiccation, if pollination did not occur. Various degrees of transformation of anthers to ovaries were found (Fig. 1 B and C). The most advanced transformed anthers resembled normal ovaries to the extent that they could not be distinguished morphologically. Male-sterile plants having degree 1 anthers always produced some selfed seeds, thus allowing maintenance of the male-sterile stock. The appearance of the sterile plants was similar for all lines and plants during 5 yr of visual observations. Vegetative growth was normal, with the only abnormality noted being the occasional occurrence of clavate-spiked sterile plants in the population. These were very similar to some of the nullisomics described by Sears (1954).

Fertility characteristics of UC9109-9

In the May 1968 experiment, the sterile group averaged 2.1 seeds per spike, and the partially sterile group had 12.4 (Table 1). In the following years under self-pollination, the sterile and partially sterile groups did not differ significantly. Significant differences between the sterile and partially sterile groups in open-pollinated seed-set were found in 1969 and 1972. Uneven distribution of pollen sources in the crossing plot could have caused the different outcrossing rates in the various seasons. It is believed that the difference between the sterile and partially sterile groups in 1968 was not due to genetic differences in the expression of male sterility, and that possibility will not be considered further.

Selfed seeds from 84 male-sterile plants in 1968 and 1970 produced no fertile plants among 174 observed in the next generation. Homozygous male-sterile F_3 lines were recovered from the cross UC9109-9-10 × Ramona 50 in 1971. Selfed seeds from these lines were bulked and planted in 1972. From about 60 progenies there were no male-fertile plants. This indicated that male-sterile plants were homozygous for reasonably stable sterility

TABLE 1

PERCENTAGE SELF- OR OPEN-POLLINATED FERTILITY AND NUMBER OF SEEDS PRODUCED ON EACH SPIKE OF STERILE (S) AND PARTIALLY STERILE (PS) IN WHEAT

Year	SP fertility, %					OP fertility, %				
	S		PS			S		PS		
	%	N†	%	N	S vs. PS	%	N	%	N	S vs. PS
1968						6.9 ± 0.8	43	12.7 ± 2.1	14	**
1968‡						0.5 ± 0.2	27	1.6 ± 0.6	21	NS
1969	4.3 ± 1.4	35	4.6 ± 0.9	46	NS	8.9 ± 1.5	37	15.3 ± 1.6	46	**
1970	0.4 ± 0.1	34	0.9 ± 0.8	11	NS	1.3 ± 0.3	35	1.1 ± 0.4	11	NS
1971						6.9 ± 0.4	83	7.5 ± 1.4	24	NS
1972	1.9 ± 1.3	20	3.9 ± 2.0	3	NS	14.7 ± 2.0	20	25.6 ± 7.3	4	NS
	SP fertility, seeds/spike					OP fertility, seeds/spike				
	S		PS			S		PS		
	seeds/spike	N	seeds/spike	N	S vs. PS	seeds/spike	N	seeds/spike	N	S vs. PS
1968	2.1 ± 0.3	43	12.4 ± 1.2	14	**	4.7 ± 0.5	43	9.3 ± 1.4	14	**
1968‡						0.2 ± 0.1	27	0.6 ± 0.2	21	NS
1969	2.6 ± 0.8	35	2.7 ± 0.5	46	NS	6.7 ± 1.2	37	10.8 ± 1.2	46	**
1970	0.2 ± 0.1	34	0.3 ± 0.2	11	NS	0.9 ± 0.2	35	0.6 ± 0.3	11	NS
1971						4.5 ± 0.3	96	4.4 ± 0.9	25	NS
1972	1.0 ± 0.6	20	2.1 ± 1.1	3	NS	9.3 ± 1.4	20	18.4 ± 6.8	4	**

NS $P > 0.05$.
** $P < 0.01$.
† N = number of plants studied.
‡ Data collected in August from July planting; all others collected in May from December planting.
SP = self-pollinated (by preanthesis bagging).
OP = open-pollinated (unbagged).

factors. The sterile lines, with the low level of natural self-pollination, can be maintained by open-pollination rather than by enforced selfing. Fertile plants appearing in the next generation, arising through outcrossing in the previous generation, can be manually removed at the time of anthesis.

In 1968, open-pollinated fertility percentage data were analyzed for line differences and for differences between early and late tillers. The results indicated that fertility of early (8.0 percent) and late (7.0 percent) tillers did not differ significantly. The line differences were significant in 1968, 1969, 1970, and 1971 with respect to open-pollinated fertility percentages. However, the differences in fertility percentages under self-pollination were not significant in 1969 and 1970. The differences among lines for fertility may reflect small differences in pollen availability or stigma receptivity, rather than differences in the genetic mechanism for male sterility.

Annual variations were highly significant for all methods of scoring fertility. Open-pollinated seed-set percentage ranged from 1.6 to 17.0, and seeds per spike ranged from 1.1 to 10.3 over the 5 yr, 1968–72. Fertility, as determined by number of seeds per spike and by percentage data, corresponded closely. The male-sterile plants were more sensitive than fertile plants to environmental modification. Environmental factors such as relative humidity, rainfall, temperature, cloudiness, wind, and soil water content can all affect cross-pollination, causing differences in seed-set from year to year. In addition, environmental effects might also have altered the development of anthers and ovaries, and therefore the expression of sterility genes. Kihara (1951) suggested that variation in photoperiod might be important. Fisher (1972) demonstrated complete pistilloidy on fertile wheat varieties by shifting plants from 16- to 10-h photoperiods at specific growth stages. This treatment resulted in phenotypes similar to those of UC9109-9 male-sterile plants grown under normal field conditions. It may be possible to enhance or reduce the UC9109-9 male sterility by manipulating photoperiods, or perhaps by application of a specific chemical that would restore normal fertility in male-sterile plants. Environmental modification of the expression of male sterility would facilitate hybrid wheat production. It would be a great advantage to define the environmental conditions that would increase selfing capacity for maintenance of the male-sterile stock. Similarly, defining other environments that reduced selfing ability and retained female fertility would be useful for producing hybrid seed.

Comparisons were made between self- and open-pollinated seed-set for the sterile plants, fertile plants, and the F_1. Seed-set on sterile plants as percentage (2.3 vs. 10.4 percent), or number of seeds per spike (1.3 vs. 7.1), was significantly lower under enforced self-pollination than under open-pollination, based on a 3-yr mean. F_1 plants obtained from the cross of male-sterile × male-fertile varieties had self-pollinated fertility (89.9 percent) and open-pollinated fertility (88.3 percent) similar to that of fertile parents (89.9 and 94.7 percent). It was concluded that the fertile plants and the F_1 did not differ significantly in seed fertility.

Progenies from open-pollinated seeds taken from sterile plants were evaluated for outcrossing percentage, which was determined as (number of fertile plants/total plants) × 100 (Table 2). Progeny analysis revealed a 27.9 percent outcrossing rate in 1968 when the self- and open-pollinated seed-sets were similar. However, in the summer planting of 1968, the number of open-pollinated seeds per spike was significantly higher than that of the self-pollinated seed-set. The outcrossing rate was 58.3 percent. The results were quite differ-

TABLE 2

OUTCROSSING PERCENTAGE OF MALE-STERILE WHEAT PLANTS AS CONFIRMED BY PROGENY TESTS

OP seed source	Progeny tests		Outcrossing %
	No. of sterile plants	No. of fertile plants	
1970	151	41	21.4
1970*	147	48	24.6
1968	921	357	27.9
1968*	1351	1886	58.3
1971	24	107	81.7

* Data collected in August from July planting; all others collected in May from December planting.

TABLE 3

CORRELATIONS BETWEEN METHODS OF DETERMINING FERTILITY FOR FERTILE PARENTS, F_1 S, AND MALE-STERILE WHEAT PLANTS

Material	Method	Year	Method correlated		
			OP seeds/spike	OP, %	Self, seeds/spike
Fertile parents	SP, %	1972	−0.11	0.32	0.28
	SP, seeds/spike	1968	0.20	0.44	
	SP, seeds/spike	1972	0.86*	−0.35	
	OP, %	1968	0.46		
	OP, %	1972	−0.38		
F_1 plants	SP, %	1972	0.32**	0.42**	0.59**
	SP, seeds/spike	1968	0.38**	0.46**	
	SP, seeds/spike	1972	0.38**	0.32**	
	OP, %	1968	0.74**		
	OP, %	1972	0.55**		
Male-sterile plants	SP, %	1969	0.27*	0.30**	0.99**
	SP, %	1970	0.13	0.17	0.96**
	SP, %	1971	−0.01	0.02	1.00**
	SP, seeds/spike	1968	0.58**	0.47**	
	SP, seeds/spike	1969	0.26*	0.28*	
	SP, seeds/spike	1970	0.17	0.20	
	SP, seeds/spike	1972	0.00	0.20	
	OP, %	1968	0.97**		
	OP, %	1969	0.98**		
	OP, %	1970	0.99**		
	OP, %	1971	0.96**		
	OP, %	1972	0.97**		

* $0.01 < P < 0.05$.
** $P < 0.01$.
SP = self-pollinated (by preanthesis bagging).
OP = open-pollinated (unbagged).

ent in 1970, when the open-pollinated percentage was about four times the self-pollinated percentage, and the outcrossing was only 21.4 percent. In the July 1968 planting, male-sterile plants had zero self-pollinated seeds per spike, whereas their open-pollinated progenies had 58 percent fertile plants as a result of outcrossing.

The results revealed adverse effects of bagging on seed-set of male-sterile plants in most years. It has been noticed that relative humidity increased in the selfing bags. Although moderately high relative humidity could be beneficial for anther dehiscence, extremely high relative humidity might prevent the dehiscence of fertile or partially fertile anthers.

A correlation analysis was done for fertility data obtained by different methods (Table 3). The fertile parents and F_1 plants had reasonably good correlations between the percentage of

TABLE 4

TETRAD ANALYSIS AND MEIOTIC CHROMOSOME NUMBER OF FERTILE PARENTS AND MALE-STERILE UC9109-9 WHEAT PLANTS IN 1971

Identification	Number of plants	Mean number of tetrads	Mean number of abnormal tetrads	Mean meiotic index, %	Chromosome number
Ramona 50	1	167	8	95.2	21II
D6301	1	152	8	94.7	21II
UC9109-9-2	5	28	36	46.8	20II+1I
UC9109-9-3	1	194	34	82.5	21II
UC9109-9-3	4	184	94	48.9	20II+1I
UC9109-9-4	1	458	233	49.1	20II+1I
UC9109-9-4	5	425	31	92.7	21II
UC9109-9-5	4	199	18	91.1	21II
UC9109-9-6	4	187	22	88.1	21II
UC9109-9-7	2	246	45	81.7	21II
UC9109-9-8	6	182	37	79.9	21II
UC9109-9-8	2	347	121	30.3	20II+1I

florets producing seeds and the number of seeds per spike under open-pollination. Spikes from the main tillers provided more reliable data when the number of seeds per spike was used as a measure of fertility.

For male-sterile plants, the results were much more clear-cut, and differed from those of the fertile parents and the F_1. A very strong correlation existed between self-pollinated percentage and seeds per spike and between open-pollinated percentage and seeds per spike. There was little or no correlation between open- and self-pollinated seed-sets. This result would be expected if outcrossing leads to greater seed-set in the open-pollinated spikes.

We found that the number of seeds per spike, either open- or self-pollinated, is a useful measurement of fertility, and the procedure takes much less time and effort than determining percentage seed-set. The correlation between self- and open-pollinated percentages was low, but either the open-pollinated percentage or open-pollinated seeds per spike was adequate for distinguishing sterile plants from fertile plants.

Cytological examination

The meiotic chromosome number was normal for most male-sterile plants; however, monosomics were found in these UC9109-9 progeny lines (Table 4). The monosomic plants had abnormal tetrads, and the meiotic index (Love, 1949, 1951) (percent normal tetrads) was low (mean 43.8 percent). Plants with 21II had meiotic indices from 67.3 to 93.9 percent (mean 86.0), compared with about 95 percent for the two parents, D6301 and Ramona 50. Lines with greater meiotic stability were obtained by selecting disomic male-sterile plants with high meiotic indices.

The mean open-pollinated seed-set percentage was 6.9 (range 0 to 15.6) for the disomic male-sterile plants, and 6.8 (range 1.9 to 20.5) for the monosomic male-sterile plants. These two groups had the same degrees of sterility. One trisomic sterile plant was found; it had a meiotic index of 68.5 and the open-pollinated fertility was 17.9 percent. The meiotic index for a nullisomic sterile plant was about the same as for fertile plants (95.9 percent). These observations indicated clearly that there was no relationship between male sterility and chromosome number in the UC9109-9 male-sterile.

Seven plants from the 1971 December planting had clavate spikes. Two were examined cytologically and proved to be nullisomics ($2n = 40$). In the 1972 winter planting, open-pollinated progenies of these seven plants were observed.

TABLE 5

ANTHER TRANSFORMATION SCORES AND POLLEN FERTILITY PERCENTAGES (%) WITH RESPECT TO FLORET LOCATION ON THE WHEAT SPIKE, AND IN THE SPIKELET, ON EARLY AND LATE TILLERS IN 1970

Floret order in spikelet†		Position on spike for early and late tillers											
		Upper third			Middle third			Basal third			Mean		
		Early	Late	Mean	Early	Late	Mean	Early	Late	Mean	Early	Late	Mean
1	Score	2.8	2.4	2.6	4.3	3.1	3.7	2.6	2.4	2.5	3.2	2.6	2.9
	%	52.7	69.9	61.3	15.3	51.2	33.3	53.9	66.3	60.1	40.6	62.5	51.6
2	Score	3.6	3.3	3.5	4.5	3.5	4.0	2.5	2.4	2.4	3.5	3.1	3.3
	%	48.4	51.8	50.1	18.5	42.2	30.4	64.4	71.8	68.1	43.8	55.3	49.5
3	Score	3.6	2.9	3.3	4.1	3.1	3.6	2.8	2.8	2.8	3.5	2.9	3.2
	%	47.3	58.0	52.7	34.9	63.9	49.4	54.1	79.5	66.8	45.4	67.1	56.3
Mean	Score	3.3	2.9	3.1	4.3	3.2	3.8	2.6	2.5	2.6	3.1	2.9	3.1
	%	49.5	59.9	54.7	22.9	55.8	37.7	57.5	72.5	65.0	43.3	61.6	52.5

Analysis of variance

Source of variance	Score			Pollen fertility, %	
	df	MS	Standard error	MS	Standard error
Early vs. late tillers (E vs. L)	1	12.29**	0.09	13598**	2.61
Spikelet position on spike	2	18.43**	0.11	10285**	3.20
Position × E vs. L	2	3.19*	0.16	1349	4.53
Floret order in spikelet	2	1.84	0.11	652	3.20
E vs. L × floret order	2	0.06	0.16	468	4.53
Position × floret order	4	1.86*	0.19	1096	5.55
E vs. L × position × floret order	4	0.10	0.27	148	7.84
Plants	8	4.57*		3682*	
Error	136	0.68		554	
Total	161				

* $0.01 < P < 0.05$.
** $P < 0.01$.
† The floret order in each spikelet was numbered 1 to 3 from the oldest floret to the youngest one.

They were either clavate-sterile or fertile; no typical male-sterile plants appeared. Four such fertile plants had 2n = 41 chromosomes, indicating that they resulted from outcrossing of clavate nullisomics with the surrounding disomics. In general, clavate-spike plants were more sterile than typical male-sterile plants. The clavate-spike condition, and aneuploidy in general, were not related to male sterility in UC9109-9.

Floret position and pistilloidy. The degree of anther transformation to pistils, and pollen fertility in florets of early and late tillers at different positions within the spike, were examined (Table 5). Analysis of variance indicated that early tillers had greater pistilloidy (score = 3.4) than did late tillers (score = 2.9); and that the middle portion of each spike had greater pistilloidy (score = 3.8) than either the apical or the basal positions (scores = 3.1 and 2.6). The mean differences due to floret order within a spikelet were not significant, but the interaction of spikelet position with early or late tiller was significant. In the middle and apical portions of the spike, the degree of anther transformation was greater for the second floret of each spikelet; but this trend did not hold for the basal portion of the spike.

Results were similar for pollen fertility, considering that transformed anthers with score 4 or greater had zero fertility. Late tillers had greater pollen fertility (61.6 percent) than did early tillers (43.3 percent). The apical and basal portions of the spikes had greater pollen fertility (65.0 and 54.7 percent) than the middle portion (37.7 percent). No significant differences were detected in pollen fertility among floral positions within each spikelet.

Spikelet differentiation in wheat begins in the middle of the spike and proceeds toward both base and apex (Bonnett, 1966). Within the spikelet, differentiation begins at the base and proceeds upward. Srinivas and Swaminathan (1969) proposed a genetic system controlling basal sterility. They suggested that different genes are responsible for the normal development of different flower organs. In addition, a gene Q is responsible for the normal functioning of the development genes. In the absence of Q, another gene I^F inhibits the activity of the development genes. The systematic induction of sterility was associated with the direction of the differentiation. The reaction between the product of the gene I^F and the development genes might be vigorous during the early stages of the differentiation of each spike, and be gradually reduced later. This recovery with time was ascribed to a type of feedback reaction.

Similar explanations can be applied to the present study. A specific inhibitory gene I interacts with the genes governing normal anther differentiation and somehow diverts differentiation toward the ovary-like structure. Sterility genes block the very early stage of normal stamen differentiation, and subsequently effect the initiation of sporogenous tissue following stamen initiation. This was clearly shown by the close relationship between degree of anther transformation and pollen fertility in 1971 and 1972 (discussed in the next section). When the sterility genes are present, normal stamen differentiation is blocked, and the stamen primordia develop into pistils to degrees varying with the strength of the gene effect at that time. It is suggested that the effects of the sterility genes are strongly manifested at the beginning of spike differentiation, and less so at later stages. This is a reasonable explanation for the regular occurrence of more fertile anthers at the base and apex of the spike, and for the appearance of more fertile anthers on late than on early spikes. We suggest that a specific gene product is important for normal anther development.

TABLE 6

NUMBER OF SEEDS PER SPIKE AND PERCENTAGE OF SEED-SET FOLLOWING ARTIFICIAL POLLINATION OF FLORETS WITH THE SPECIFIED KINDS OF FEMALE ORGANS

Year†	Female organs pollinated	No. of spikes pollinated	Seed-set per spike	Seed-set, %
1970	Normal ovary	6	17.7 ± 3.0	
	Normal ovary and all TA's‡	8	5.8 ± 1.6	
	One TA	12	0	
	All TA's	10	0.2 ± 0.2	
1972	Normal ovary	31		48.4 ± 3.6
	Normal ovary and all TA's	20		25.3 ± 4.8
	One TA	12		0
	All TA's	12		0

† Data collected in May from December plantings; differences among pollination treatments were highly significant ($P < 0.01$) in both years.
‡ TA = transformed anther.

Anther transformation and pollen fertility. A pollen grain was considered sterile if it was extremely small and empty or only partially filled. The mean pollen fertilities for scores 1, 2, and 3 (see Fig. 1A) were, respectively, 81, 66, and 38 percent in 1971, and 90, 56, and 7 percent in 1972, with means of 83, 64, and 33 percent for the 2 yr. The regression coefficients of percent pollen fertility on score for 1971, 1972, and the 2 yr combined were, respectively, 21.6, 41.4, and 25.0 percent, all of which were highly significant ($P<0.01$). This indicated that pollen fertility and degree of anther transformation are closely related, and perhaps share a common biosynthetic pathway during anther differentiation. A block in the pathway leading to normal differentiation of anthers could cause both abnormal anther and abnormal pollen development.

Anthers which scored 3, and most anthers with score 2, did not dehisce fertile pollen grains. The bursting of anthers depends on the quantity of fertile pollen grains as well as on climatic conditions during the flowering season. Polovinkina and Lubimova (cited by Rajki and Rajki, 1966) found that at a low relative humidity (30 to 35 percent), approximately 60 to 65 percent fertile pollen grains were sufficient for anther dehiscence. However, in warm weather, at high atmosphere and soil humidity, 40 to 45 percent fertile pollen grains were sufficient for dehiscence.

Fertility of normal ovaries and transformed anthers

Pollination test. Pistilloidy is a striking feature of the UC9109-9 male-sterile. Similar male sterility with pistilloidy has been reported by Kihara (1951, 1966, 1967) in male-sterile wheat having *Aegilops caudata* cytoplasm, which indicated a dominant effect of *A. caudata* cytoplasm on pistilloidy. Connor and Purdie (1970) demonstrated another pistilloid form which was controlled by one recessive nuclear gene pair. Neither of these cases produced fertile ovaries from transformed anthers, as determined either by histological studies or artificial pollination tests. Table 6 shows the results of the pollination tests on fertility of normal ovaries and that of the advanced types of transformed anthers in this study. For florets in which all anthers and transformed anthers were removed, crossed seed-set for normal ovaries was 17.7 seeds per spike in 1970 and 48.4 percent in 1972. When only anthers up to and including score 3 were removed, and the transformed anthers with score 4 or higher and normal ovaries were left intact, seed-set was much lower, with 5.8 seeds per spike in 1970 and

TABLE 7

SEED-SET PERCENTAGES FOR CROSSES OF SEED-BEARING PLANTS AND POLLINATORS, AND THE EFFECTS OF CLIPPING ON SEED-SET OF MALE-STERILE PLANTS POLLINATED WITH FERTILE POLLEN, 1972

Female Male	Spikes	Florets	Seeds	Seed Set
		Number		*Percent*
Fertile × sterile	8	180	9	5.0
Fertile × fertile monosomics	7	206	115	55.8
Fertile monosomics × fertile	10	339	221	65.2
Sterile × fertile*	4	174	99	56.9
Sterile × fertile†	4	196	37	18.9

* No emasculation, spikelet tips clipped before pollination.
† No emasculation, no clipping before pollination.

25.3 percent in 1972. This clearly indicates the adverse effects of transformed anthers on seed-set capabilities of normal ovaries. If the male-sterile plant had a low degree of anther transformation, relatively higher percentages of outcrossed seed-sets could be expected.

When the normal ovary was removed and either one or all of the transformed anthers were left for pollination, no seeds were obtained from 424 florets pollinated on 24 spikes in 1972. In 1970, only one seed was produced from approximately 350 florets on 21 pollinated spikes. The production of a single seed out of about 770 florets could have resulted from an error in emasculation. We therefore conclude that transformed anthers are practically sterile.

In 1972, other types of crosses were evaluated (Table 7). Pollen from male-sterile plants fertilized fertile plants, although with very low success (5.0 percent). When fertile plants were pollinated with pollen from fertile monosomic plants, seed-set was 55.8 percent. When fertile monosomic plants were pollinated with fertile pollen, seed set was 65.2 percent. According to Sears (1954), monosomics should be as fertile as normal disomics, and these results essentially conform to this expectation. Seed-set on male-sterile plants pollinated without emasculation (18.9 percent) was increased (56.9 percent) when the tips of the lemmas and paleas were clipped, indicating that the lemma and palea impeded entry of pollen to the stigma. It appears that the fertility of normal ovaries on sterile plants is not significantly affected by male-sterility genes.

Histological observations. Normal ovaries and transformed anthers were examined in 10-μm serial sections. Normal ovaries always had a distinctive embryo sac containing the usual nuclei. The transformed anthers usually did not have an ovarian cavity. Transformed anthers with a small central cavity were found occasionally, but these cavities did not have the shape of the ovarian cavity in a normal ovule, and did not contain nuclei (Fig. 2). These observations indicated that transformed anthers would not be expected to be fertile, and confirmed the results of the pollination studies (Table 6).

Outcrossing study

Outcrossing tests were conducted with the same male-sterile stock in 1970 and 1972 (Table 8). In 1970, plots having different male pollinators differed significantly in seed-set per spike. Overall seed-set was poor (only about 8 seeds per spike). Sterile plants in isolation also produced 8 seeds per spike. In 1970, the weather was not favorable for selfing or outcrossing.

In 1972, to get a better estimate of fertility, the open-pollinated seed fertility was recorded both as seed-set percentage and number of seeds per spike (Table 8). The overall number of seeds

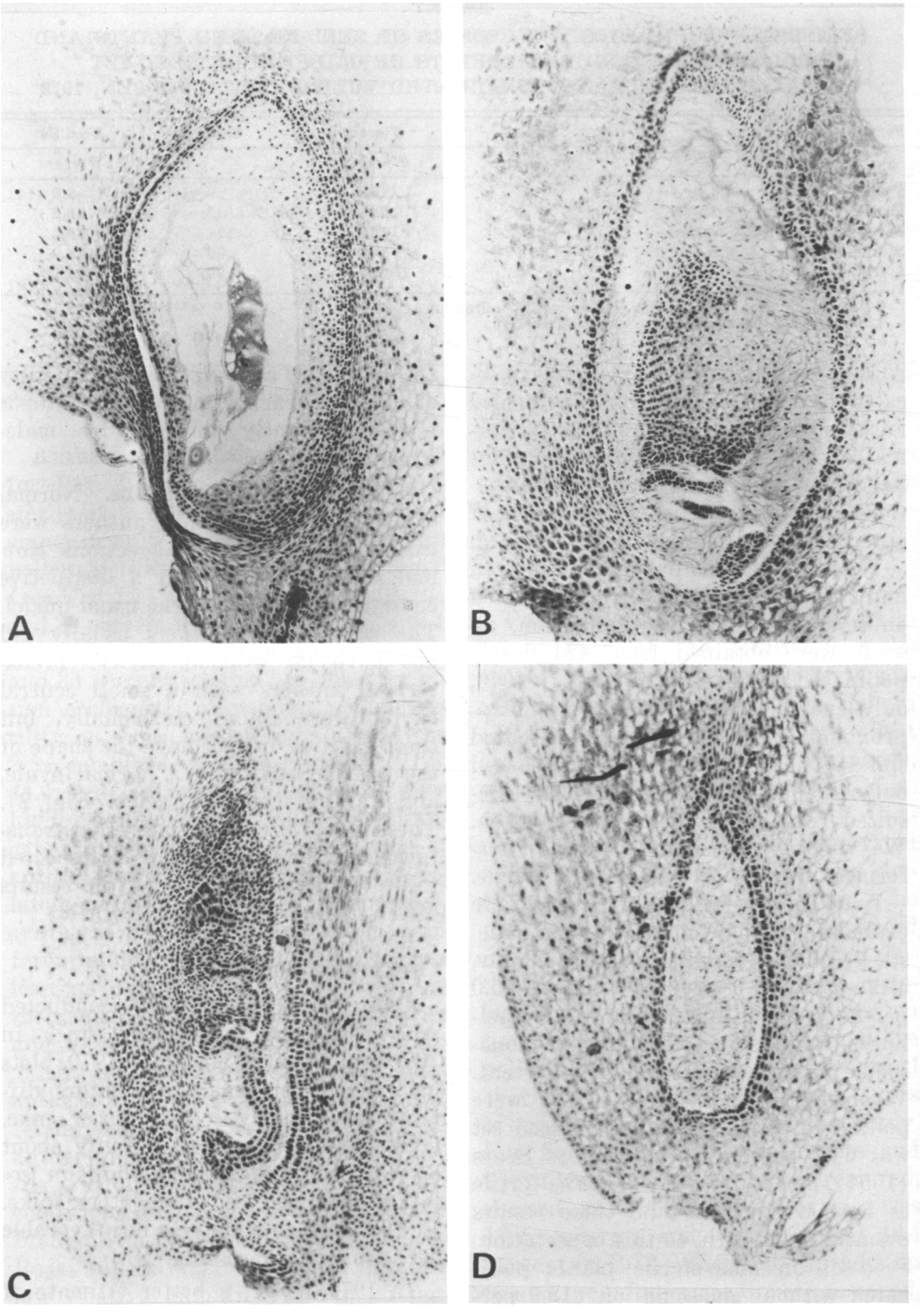


Fig. 2. Dorsiventral section of normal ovary and pistilloid anthers. (×71). **A.** Normal ovary with embryo sac, egg cell (lower left), and antipodal nuclei (center). **B, C, D.** Pistilloid anthers with no distinctive embryo sacs and no reproductive cells.

TABLE 8

OUTCROSSING TESTS FOR MALE-STERILE WHEAT PLANTS IN SPECIFICALLY DESIGNED CROSSING PLOTS

Year†	Pollen source*	Seeds/spike	Plants	Fertility	Plants
			Number	*Percent*	*Number*
1970	Composite	9.6 ± 1.1	46		
	No male pollinator	8.2 ± 0.7	102		
	Pitic 62	9.2 ± 1.0	61		
	INIA 66R	7.5 ± 0.8	59		
	Siete Cerros 66	7.8 ± 0.9	53		
	Nadadores 63	9.8 ± 1.1	57		
	Lerma Rojo	11.9 ± 1.3	47		
	Lerma Rojo 64	9.5 ± 1.4	44		
	Pacific Triple Dwarf	6.3 ± 0.9	63		
	Nainari 60	9.0 ± 1.1	56		
	Anza	9.9 ± 1.0	62		
1972	Composite	19.9 ± 0.8	68	38.2 ± 2.1	28
	Anza	25.8 ± 1.3	67	47.4 ± 4.8	27
	INIA 66	16.6 ± 1.0	60	27.4 ± 3.2	20
	Pitic 62	14.2 ± 1.0	69	27.7 ± 3.4	29
	Nadadores 63	14.8 ± 0.9	71	27.4 ± 2.6	31

* Significant differences among pollen sources were observed for seeds/spike in 1970 ($P < 0.05$) and 1972 ($P < 0.01$), and for percent fertility in 1972 ($P < 0.01$).
† Data collected in May from December plantings.

per spike was higher in 1972 than in 1970 (20 vs. 8). This increase corresponded to the greater number of seeds per spike in the regular space-planted evaluation plot (10.3 vs. 1.1), and could be attributed to the more favorable conditions, including weather, in 1972. Differences among pollinators were more clear-cut and significant in 1972 than in 1970. In the evaluation plot of 1972, seed-set from wind-borne pollen was 17.0 percent. This much higher percentage is definitely related to the contribution of pollen by pollinators. It is possible that with better pollinators, better field orientations, and proper environments, the outcrossing rate can be increased.

In the 1972 outcrossing test, the highest seed-set was 47 percent, with Anza as the pollinator. This was not satisfactory for production of hybrid seed. Although no positive indication of female sterility was noted (Table 7), pistilloidy might have contributed to the poor seed-set. However, the seed-set of this male-sterile material in the outcrossing field might have been influenced more by other factors, such as coincidence of time of anthesis (nicking) of the male and female parents, humidity, temperature, wind, and field arrangement. Male-sterile plants with less transformation of the anthers were obtained from progenies of sterile × fertile crosses. These should be included in future outcrossing studies to determine whether higher seed-set can be achieved. Direct use of the UC9109-9 male-sterile stock for production of hybrid seed is probably not feasible. It appears that better lines can be obtained by crossing and selection.

Proper nicking is very important (Bitzer and Patterson, 1967) in producing hybrid seed. The male-sterile plants should flower 1 to 2 days earlier than the pollinators. At that time, florets of female plants are opened by lodicule expansion, and are receptive to pollen by the time of anthesis of main tillers of the pollinator plants. Longevity of stigma receptivity is favored by relatively moderate temperatures (13 to 25°C) and low humidity (40 to 70 percent). The longevity of wheat pollen will be extended by relatively low temperature and high humidity. Temperature and humidity also influence the opening of the lemma and palea, and the de-

TABLE 9

PERCENTAGE SEED FERTILITY UNDER OPEN POLLINATION OF FERTILE PARENTS AND THE F_1'S OF MALE-STERILE X FERTILE PLANTS

Parent or F_1	1968†		1969†		1972†	
	%	N‡	%	N	%	N
Parent						
Nainari 60	76.8	3	94.8	3		
Sonora 64	89.4	2	99.2	3		
Ramona 50	73.1	3	97.6	3	92.9	2
Lerma Rojo	81.8	2	97.9	3		
Siete Cerros 66			98.5	3	91.3	1
Pitic 62			97.3	3		
Mean	79.2		97.6		92.4	
S.D.	6.59		2.69		2.63	
F_1						
MR9109-9-1 × Nainari 60	72.0	1	89.5	3		
MR9109-9-3 × Nainari 60	85.5	2				
MR9109-9-3 × Sonora 64	67.8	1				
MR9109-9-10 × Ramona 50	80.8	4				
MR9109-9-4 × Lerma Rojo	89.5	3	95.3	6		
MR9109-9-4 × Pitic 62			93.6	9		
MR9109-9-8 × Pitic 62			88.2	1		
MR9109-9-5 × Siete Cerros 66			93.4	3	90.6	53
MR9109-9-5 × Sonora 64			99.0	5		
MR9109-9-4 × Siete Cerros 66					92.5	15
MR9109-9-2 × Siete Cerros 66					91.5	11
Mean	78.9		94.3		91.1	
S.D.	5.25		1.79		5.31	

† Data collected in May from December plantings.
‡ N represents the number of plants sampled; 3 spikes from each plant were examined.

hiscence of anthers. These factors should be studied to establish the best environmental conditions and genotype for production of hybrid seed.

F_1 fertility

Variation in fertility under open-pollination of the male-fertile parents and F_1 hybrids was evaluated in the spring planting of 1968, 1969, and 1972 (Table 9). The fertilities of the F_1's and parents were comparable in all 3 yr. For hybrid-wheat production, the UC9109-9 genetic male-sterile does have certain advantages over cytoplasmic male sterility, and other sources of genetic male-sterility which require foreign pollen for maintenance of the female parent. Since the fertility genes are completely dominant over male sterility, specific fertility-restoration genes are not necessary for the F_1 hybrid. UC9109-9 male-sterile lines can be maintained through selfing, so hand pollination is not required for maintenance of stocks. The small fraction of selfed sterile plants in the F_1 population would probably not appreciably affect yield. The frequency of selfing of UC9109-9 in the usual environments at Davis was too low to be economically feasible for maintenance of the seed parent. The possibility of improving the rate of selfing by environmental manipulation should be investigated. The results of outcrossing experiments also were not encouraging, because the rate of hybrid seed production was low. We believe that this can be increased by improved management of the seed production field.

ACKNOWLEDGMENTS

The genetic male-sterile stock was discovered at Davis by J. C. Williams, who kindly made it available for study. This research was supported in part by grants from the California Crop Improvement Association and the Voluntary California Cereal Research Fund. C. C. J. was supported in part by a Regents Fellowship and a Henry A. Jastro Fellowship in Agriculture.

LITERATURE CITED

ALEXANDER, P.
1969. Differential staining of aborted and non-aborted pollen. Stain Technol. **44**:117–22.
ATHWAL, D.S., and N. E. BORLAUG
1967. Genetic male sterility in wheat breeding. Ind. Jour. Genet. Pl. Breed. **27**:136–42.
BINGHAM, J.
1966–67. Rep. Pl. Breed. Inst., Cambridge, England. p. 67.
1968. Rep. Pl. Breed. Inst., Cambridge, England. p. 64.
BITZER, M. J., and F. L. PATTERSON
1967. Pollen dispersal and cross-pollination of soft red winter wheat (*Triticum aestivum* L.) Crop Sci. **7**:482–84.
BITZER, M. J., and S. H. FU
1972. Heterosis and combining ability in southern soft red winter wheats. Crop Sci. **12**:35–37.
BONNETT, O. T.
1966. Inflorescence of maize, wheat, rye, barley, and oats: Their initiation and development. Univ. of Illinois, Agr. Expt. Sta. Bull. **721**.
CONNOR, H. E., and A. W. PURDIE
1970. Genetic pistilloidy in New Zealand wheat. Wheat Inf. Serv. **31**:19–21.
FISHER, J. E.
1972. The transformation of stamens to ovaries and of ovaries to inflorescences in *T. aestivum* L. under short-day treatment. Bot. Gaz. **133**:78–85.
FOSSATI, A., and M. INGOLD
1970. A male sterile mutant in *Triticum aestivum*. Wheat Inf. Service **30**:8–10.
GILL, B. S., and S. C. ANAND
1970. Genetic male sterility for hybrid seed production in wheat. Crop Sci. **10**:385–386.
GRANT, M. N., and H. MCKENZIE
1970. Heterosis of F_1 hybrids between spring and winter wheats. Can. Jour. Pl. Sci. **50**:137–40.
HERMSEN, J. G. TH.
1965. Towards a more efficient utilization of genetic male sterility in breeding hybrid barley and wheat. Euphytica **14**:221–24.
INGOLD, M.
1968. Male sterility and restorer systems in wheat. Euphytica **17** (Suppl. 1) :69–74.
JAN, C. C., and C. O. QUALSET
1977. Genetic male sterility in wheat (*Triticum aestivum* L.): Inheritance. Crop Sci. **17**: 791–94.
JAN, C.C., C. O. QUALSET, and H. E. VOGT
1974. Chemical induction of sterility in wheat. Euphytica **23**:78–85.
1976. Chemically induced sterility in wheat for hybrid seed production. Euphytica **25**: 375–86.
JOHANSEN, D. A.
1940. Plant microtechnique. McGraw-Hill, New York. pp. 130–131.
JOHNSON, V. A., J. W. SCHMIDT, and P. J. MATTERN
1967. Hybrid wheat in the United States. Qualitas Planatarum et Materiae Vegetabiles. **41**: 193–211.
KIHARA, H.
1951. Substitution of nucleus and its effects on genome manifestation. Cytologia **16**:177–93.
1966. Nucleus and chromosome substitution in wheat and *Aegilops*. I. Nucleus substitution. Proc. 2nd Int. Wheat Genet. Symp. (Hereditas, Suppl. **2**:313–27.)
1967. Cytoplasmic male sterility in relation to hybrid wheat breeding. Züchter **37**:86–93.
KIHARA, H., and K. TSUNEWAKI
1964. Some fundamental problems underlying the program for hybrid wheat breeding. Seiken Ziho **16**:1–14.
LIVERS, R. W., and E. G. HEYNE
1968. Hybrid vigor in hard red winter wheat. Proc. Third Int. Wheat Genet. Symp. Austral. Acad. Sci., Canberra. pp. 431–36.
LOVE, R. M.
1949. La citologia como ayuda practica al mejoramiento de los cereales. Rev. Argentina Agron. **16**:1–13.

1951. Varietal differences in meiotic chromosome behaviour of Brazilian wheat. Agron. Jour. 43:72–76.

PUGSLEY, A. T., and R. N. ORAM
1959. Genetic male sterility in wheat. Austral. Pl. Breed. Genet. Newsletter 14.

RAJKI, E., and S. RAJKI
1966. Research work on hybrid wheat at Martonvasar. Acta Agronomica Academiae Scientarium Hungaricae 15:199–214.

ROWELL, P. L., and D. G. MILLER
1971. Induction of male sterility in wheat with 2-chloroethylphosphonic acid (Ethrel). Crop Sci. 11:629–31.

SEARS, E. R .
1954. The aneuploids of common wheat. Missouri Agr. Expt. Sta. Res. Bull. 572.

SRINVAS, T., and M. S. SWAMINATHAN
1969. Analysis of the genetic regulation of flower morphogenesis in bread wheat. Ind. Jour. Genet. 29:62–71.

STOSKOPF, N. C., and J. LAW
1972. Some observations on Ethrel as a tool for developing hybrid wheat. Can. Jour. Pl. Sci. 52:680.

WALTON, P. D.
1971. Heterosis in spring wheat. Crop Sci. 11:422–24.

2½m-9,'77(T3403) PAD