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VARIABILITY IN MEDITERRANEAN WHEAT

by

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INTRODUCTION

In 1918, Mains and Jackson (12) began the work of differentiating races of leaf rust of wheat, Puccinia recondita Rob. ex Desm. f. sp. tritici (Eriks) Cald. They originally used 11 varieties of wheat representing eight differential groups to distinguish the races but later this number was reduced, by Johnston and Mains (8), to eight varieties which included Mediterranean, CI 3332. Mediterranean was used as a resistant variety to leaf rust physiologic race 9 and related races but since has been found to have a small percentage of susceptible plants.

Numerous attempts to purify the seed stock of Mediterranean by roguing and selection of resistant plants have been made in Kansas by C. O. Johnston and others. Care was exercised with these selections to prevent natural crossing with other varieties and mechanical mixtures. However, the progeny of these purification attempts eventually had off-type plants present in approximately the same proportion as the open stock seed. Attempts to date to select stable lines of Mediterranean have not been successful.

Shulte (23) tested 562 F₃ lines from Pawnee-Mediterranean crosses and found 25 lines with off-types that varied from 1-4 in reaction to race 9. Shulte (23) also tested Mediterranean parent plants used in crosses to Pawnee and Wichita and found 3.62 percent variation in reaction to leaf rust race 9. It was thought that this variation in Mediterranean might be the cause of the off-types in the Pawnee-Mediterranean crosses. He proposed that due to the variable response of Mediterranean, it should be studied further.

The purpose of this study was to determine the extent and possible causes of this behavior of Mediterranean, CI 3332, particularly in its reaction to physiologic race 9 of leaf rust.

LITERATURE REVIEW

Natural Crossing

Wheat is classified as a "normally self-pollinated" plant. This group of plants includes species that are 96 percent or more self-pollinated (13). In 1923, Garber and Quisenberry (2) reported the results of an extensive study concerning the amount of crossing that occurred in wheat. At that time it was generally thought that wheat did not cross naturally in the field. Garber and Quisenberry determined that 4 percent of 1,461 head rows of various varieties of wheat in the breeding nursery were segregating. Further study of individual plants from these segregating rows revealed that only 0.12 percent of the total population were first generation hybrids; the rest of the off-types were attributed to crosses that had occurred in previous years. In 89 rows of Mediterranean wheat in this study 4.65 percent were segregating.

Leighty and Taylor (9) found 1.7 percent variation in Mediterranean in 1917. In that year many natural crosses were present in wheat, 34 percent being observed in a strain of Fulcaster. In five varieties of common wheat, almost six times as much natural crossing was observed in secondary tillers as in heads on primary tillers. Leighty and Taylor observed that some flowers had poorly developed anthers that failed to dehisce. These partially self-sterile flowers remained open for a longer period of time than normal flowers, which allowed a greater chance for cross-pollination to occur.

In 1927, Harrington (4) observed crosses that occurred between varieties growing eight to fourteen feet apart, with one cross occurring between plants growing 27 feet apart. From his study, Harrington concluded that there is sufficient natural crossing in wheat to be of concern to plant breeders and

farmers, particularly where varieties are planted close together as in a nursery.

Meiotic Index

In the work by Shulte (23) the behavior of Mediterranean, CI 3332, suggested cytologic instability. There is considerable literature on this phenomenon in wheat. Marquillo (2n-42), a selection from an interspecific cross between Marquis (2n-42) and Iumillo (2n-28), was studied by Powers (19, 20). He reported that 7.2 percent differed agronomically from Marquillo (19). None of these 7.2 percent off-type plants were heterozygous. A cytological study of these off-type plants showed that 30.3 percent of the tetrads had micronuclei. It was concluded that germinal instability rather than natural crossing was the cause of most of the variability in Marquillo. Non-orientation of bivalents at metaphase I and non-conjunction were correlated with the occurrence of micronuclei (20). Fragmentation and/or non-orientation of chromosomes was thought to be the major factor in micronuclei formation (19). In crosses of Marquillo with Garnett and Red Fife, the progeny were found to have more univalents present than did either parent (6). In 1936, Myers and Powers (18) reported on the progeny of 38 off-type wheat plants selected in 1933. A correlation coefficient of 0.77 with the off-type parent was determined for instability in these lines, showing that instability of germinal tissue is a heritable character. Myers and Powers reported that Sapehin found genetic factors that affect meiosis in wheat. Riley et al. (22), in 1958, reported a "gene system" on Chromosome V which restricted pairing to chromosomes that are completely homologous. This mechanism restricts intergenome pairing, causing hexaploid wheat to behave cytologically like a diploid.

A white chaffed mutant found by Love (10) in the variety Golden Chaff was a monosomic; the lagging monosome formed micronuclei. Love suggested that plants with 90 percent or more normal tetrads could be considered stable. In 1951, Love (11) proposed that the term "meiotic index" be used instead of the longer term "percentage of normal quartets". The meiotic index of a plant can be determined by counting the normal quartets in 100 tetrads from a spike (11, 14). Morrison and Unrau (17) found that the most reliable stage of meiosis for index determination is when the entire tetrad is surrounded by a distinct wall. In later stages there is good evidence that micronuclei may be absorbed or lost.

Love (11) found that the most common cause of micronuclei was a failure of chromosomes to pair. The lagging chromosomes formed the micronuclei in the tetrad. Superimposed on univalents and also a cause of micronuclei were lagging bivalents. The presence of univalents caused no major disturbance in the meiosis of plants while those plants with bivalents were often accompanied by a complete disorganization of the second meiotic division. Love proposed that the disturbance may be due to genic disharmony.

Chimeras

Another cause of erratic behavior in cereals is the occurrence of somatic mutations or chimeras. Morey (16), working with Clinton oats, found 12 percent variation from the parent type. It was determined that only 0.25 percent variation in the population studied was caused by natural crossing. He observed that tillers from one plant differed from each other and from the parent clone in as many as twelve different agronomic and pathologic characters. These off-types were caused by somatic mutations early in the

development of the plant. A cytological study of the variants showed that the mutations were not caused by a loss of either a part or an entire chromosome. It was thought that the mutants possibly were caused by crossing-over between homologous chromosomes at different levels of loci or between non-homologues, possibly producing a bridge and a fragment in a later somatic anaphase. This, however, would not explain the off-type tillers which were apparent homozygous mutants. Morey did not determine the cause of these variations but found that lines differed. Lines were isolated which were stable in that these variations did not occur.

Akerman (1) reported on the occurrence of spelt-like heads in wheat. Some of the progeny from these heads were heterozygous for this character, while progeny from others were like the parent plant. In certain cases the mutation apparently had occurred late in the ontogeny of the plant and did not affect the germinal tissue.

Huskins (7), in 1946, reviewed several reports of the appearance of speltoid and fatuoid mutations in wheat and oats. Reitz (21) described a chimera in a Marquillo spike. One "row" of florets and the tip florets were awned. The mutation was apparently homozygous or was the result of the loss of some awn-suppressing character that was present in the parent.

MATERIALS AND METHODS

Pollination Study

To study the possibility that the variability in Mediterranean was caused by natural crossing, 19 plants that had been used as parents in various crosses were tested and found resistant to leaf rust race 9. In the fall of 1956, seed of these plants was planted in single rows in the field.

To study the effect of natural vs. controlled pollination on individual lines, 20 heads in each of the 19 progeny rows were covered with small glassine bags to ensure self-fertilization. Each bag was secured by folding it around another nearby culm and the flag leaf of the covered head. After drawing the fold snugly around the culms, the bag was stapled together. The bags were removed after six to eight days. Twenty naturally pollinated heads were also selected at random from each row at harvest time to be compared with the control-pollinated heads. Thus there were 717 selfed and selected heads. Seed from these was planted in three foot head rows in the fall of 1957. Seven hundred and three of the 717 head rows were harvested in 1958. The plot containing these rows was isolated from other wheat by barley and oats on all sides, with the exception of a border row of wheat that bloomed approximately two weeks earlier than Mediterranean. Of the 703 harvested head rows 358 were progeny of the first 10 original plants.

In the spring of 1958, five heads in each of 341 of the 358 progeny head rows of the first 10 original plants were bagged to ensure selfing. An attempt was made to select each head from different plants within the row. Bulk seed was also harvested from the 358 head rows of the first 10 original plants. The 1958 covered heads and samples of the bulk seed of each head row from the first 10 original plants were tested to leaf rust race 9 in the seedling stage in the greenhouse. An estimated 38,850 seedlings were tested from these 358 head rows. There were an estimated 26,490 seedlings tested from bulk seed of the 345 head rows that were harvested from the other nine original parents.

For testing with leaf rust, the seed was planted in three inch pots with approximately 25 seeds per pot in blocks of 144 pots, at two day intervals.

Four of the leaf rust differential varieties, Malakof, Webster, Carina, and Mediterranean were included in each set as race purity checks. The seedlings were inoculated with race 9 of leaf rust about 10 days after planting. The leaves were sprayed with a mist of water and then urediospores from infected seedlings were shaken and brushed onto them. The inoculated plants were then covered with a canvas moisture chamber overnight. The readings were made 10 days after inoculation and the reactions were based on the classification described by Mains and Jackson (12). In addition, a Y-type reaction was indicated for those plants that varied from a 4-type infection at the tip to a zero fleck at the base of the leaf (Plate Ic) as described by Heyne and Johnston (5). This reaction generally indicated a heterozygous plant.

A total of 63 off-type plants were selected and transplanted. Sixteen of these died, eight never matured, and 39 were grown to maturity in the greenhouse. Twelve of these 39 plants were sterile and eleven plants matured too late to be included in the summer 1959 planting. Sixty-six seed of the remaining 16 off-type plants were germinated in the greenhouse in the summer of 1959. The seeds were germinated on blotter paper until the plumules burst the pericarp. The germinated seeds were then placed in moist vermiculite in petri dishes and held at 32-36°F in a refrigerator for 60 days (15). After 60 days, the seedlings were planted in five inch clay pots and placed outside, in the fall of 1959, to submit the plants to a low growing temperature to induce tillering. A sharp freeze in early November killed 54 of the original 66 plants started. A cytological study was made of 11 of the remaining 12 plants representing eight parents. The cytological study was made on microsporocytes using the acetocarmine smear method as described by Smith (24). One plant did not mature early enough to be included in this report.

Plant Study

Powers (19) and Morey (16) found lines in cereal varieties which were more stable than other lines within the same variety. To study this possibility in Mediterranean, a sample of seed was obtained in 1957 from the leaf rust differential seed stock maintained by C. O. Johnston. The seed in this stock was maintained as pure as possible by careful handling and continuous selection and testing. This random sample of seed was space planted and the plants were harvested individually. All plants with three or more heads were saved. Three heads were selected from each of 137 plants. In the fall of 1958, seed from three heads from all but one of the original 137 plants were planted in 410 head rows. Only seed from two heads were planted from the one excepted plant. Fifteen seeds from each head were space planted in eight foot rows. This material was isolated from other wheat by about 20 feet of winter oats on the east and west sides and by over 100 feet of winter barley plots on the north and south. Throughout the spring, observations were made to determine whether any rows differed morphologically from the Mediterranean type.

One head on each of approximately 3,200 plants within the head rows was covered in the spring of 1959 to ensure a pure seed source. The plants were harvested individually and only those plants with three heads were selected to be studied for reaction to leaf rust. Seed from one head of each plant was tested with race 9 of leaf rust in the greenhouse. The seedlings were tested for reaction to leaf rust as described in the pollination study. Only 397 of the original 410 rows planted were included in the leaf rust test. The remaining 13 rows were threshed, by error, in bulk before head selections were made. In one original plant all three heads were bulked, thus eliminating

this plant from the study. Some of the off-type seedlings for reaction to leaf rust were grown to maturity.

In the fall of 1959, one head from each of 2835 plants was planted in head hills (15 seeds per hill) in the field as a continuation of the study of plant lines. The selfed head or a normal appearing head was held as a reserve seed supply of each plant tested to leaf rust in 1959.

Natural Crossing

To study the amount of natural crossing RedChief and Mediterranean were planted in a 36 square inch checkerboard pattern. The plants of each variety were surrounded by plants of the other variety in the test. These plants were harvested individually and the progeny was tested to race 9 of leaf rust in the greenhouse. RedChief was susceptible to race 9. The F_1 plants between Mediterranean and RedChief should give a Y-type reaction to race 9 of leaf rust.

Statistical Methods

All of the statistical analyses were performed according to methods described by Snedecor (25). The effects of bagging and not bagging on rust reaction were compared by the ranking test of Wilcoxin as described by Snedecor. The progeny of the naturally pollinated heads were compared with the progeny of the bagged heads within the same line.

The line study was analyzed by the nesting classification, with the original plants termed "plants", the heads selected from the parent plant termed "heads within plants" and the plants from the heads termed "progeny within heads". The mean square of "progeny within heads" was used to test

the "heads within plants" and the mean square of "heads within plants" was used to test the "plants".

EXPERIMENTAL RESULTS

Pollination Study

All of the lines grown in 1958 had some variants. Of an estimated 65,340 seedlings classified from the 703 head rows of the 19 lines 3.68 percent were off-type for reaction to race 9. When only one or two variants were found in the progeny of a single head or bulk population, the Y-type reaction was the most common.

The data were analyzed to determine whether the percent of off-type rust reaction plants was greater for either the natural or controlled type of pollination within the 19 lines. Results, using Wilcoxin's ranking test, indicated no significant difference (Table 1). The percent variants to leaf rust in the progeny of the naturally pollinated heads ranged from 0.40 percent to 11.50 percent while progeny of the bagged heads ranged from 0.20 percent to 14.90 percent. The bagged progeny of 12 of the lines had less than 2 percent variants while the naturally pollinated progeny of only six of the lines had less than 2 percent variants. Line number 6 had the largest difference; the higher percent was in the bagged progeny. Many of the heads bagged the second year were resistant but had single intermediate type plants among their progeny. Forty of the 703 head rows were segregating for reaction to leaf rust. Six of these 40 rows were observed to be morphologically different from the other head rows. However, two head rows appeared to be morphologically different and were resistant in reaction to leaf rust race 9. None of the head progeny were completely susceptible.

Table 1. Wilcoxin rank test for percent off-types for open and covered pollination in 19 lines of Mediterranean wheat.

Line number:	: Number of : : head rows : : tested :	: Polli- : : nation : : tested :	: Number of : : head rows : : tested :	: Polli- : : nation : : tested :	: Differ- : : ence : : Natural : : -Covered:	: Sign : : :	: Rank
1	20	0.86	16	0.57	0.29	/	2
2	20	6.70	17	7.10	0.40	-	3
3	20	2.50	11	0.78	1.72	/	10
4	18	1.46	16	6.90	5.44	-	16
5	18	10.30	16	6.20	4.10	/	13
6	20	1.90	14	14.90	13.90	-	19
7	22	9.20	13	4.60	4.60	/	14.5
8	19	4.90	18	6.50	1.60	-	8
9	18	2.40	21	0.90	1.50	/	7
10	20	2.40	21	0.70	1.70	/	9
11	19	5.10	22	1.20	3.90	/	12
12	14	2.30	11	1.20	1.10	/	6
13	19	9.50	17	0.90	8.60	/	17
14	20	1.30	20	1.30	0.00	/	1
15	20	4.80	18	0.20	4.60	/	14.5
16	20	4.60	19	0.90	3.70	/	11
17	18	0.40	22	0.90	0.50	-	4
18	20	1.70	24	0.70	1.00	/	5
19	20	11.50	21	0.30	11.20	/	18
Total tested	366		337			Low total - 50 n.s.*	

*Non-significant at 0.05 level.

Chimeral plants were found in this material after approximately half of the head rows had been tested. It was assumed that the chimeral plants were present in the earlier tests but were not recognized until this time. In approximately the last 38,500 plants tested there were 35 chimeral plants, giving an approximate ratio of one in 1100 plants. The type of chimera commonly found had a susceptible stripe along one edge of an otherwise resistant

leaf (Plate 2c). Others were found in which the susceptible stripe was in the middle of the leaf with a resistant stripe of tissue on either side (Plate 2a and b). The susceptible reaction was generally in the form of a Y-type while the resistant portion was usually only heavily flecked. In addition, seedlings were observed with white stripes on the primary leaf. Morphologic differences were observed among the chimeral and Y-type plants that were transplanted and grown to maturity. They differed in size, shape, and color of the leaf as well as size and shape of the head. Some of these plants produced more than one head from a single peduncle and others had club heads.

It was late in the season when the 63 off-type plants were transplanted and they were not vernalized. Sixteen of the 63 died and another eight never matured. The remaining 39 matured late in the summer and all of them showed some sterility. Eleven of the plants produced sufficient seed to test with leaf rust later.

Eight plants with a Y-type primary leaf reaction and three plants with chimeral primary leaves produced sufficient seed to test (Table 2). The progeny of these 11 plants were tested with leaf rust race 9 in 1959-1960. The progeny of five Y-type plants segregated for rust reaction, two gave resistant progeny, and progeny of one had two variants. The progeny of one chimeral plant segregated for rust reaction, the progeny of one were resistant, and the progeny of the third chimeral plant had one Y-type variant.

Scattered spikes were observed in the field, in 1958, which appeared to be male sterile because the glumes stood open longer than those in the surrounding spikes. Fifty-two head rows of the original 703, or 7.4 percent, had some sterility and male sterile spikes in 40 rows were tagged and harvested separately. Most of the tagged heads produced only a few seeds. However,

EXPLANATION OF PLATE I

Reaction types to leaf rust race 9 found among different Mediterranean plants. (a) O fleck, the resistant type characteristic of Mediterranean; (b) h, a susceptible reaction found for some plants; (c) Y-type, or intermediate type common for most Mediterranean variants.

EXPLANATION OF PLATE II

Reaction to leaf rust race 9 on chimeral leaves of variant Mediterranean plants. (a) A chimeral stripe (h-type) on one side, the other portion of the leaf being resistant; (b) a chimeral stripe in the middle of the leaf and either side of the leaf being resistant; (c) a chimeral stripe showing the Y-type reaction. Curving of the leaves was characteristic of these chimeras.

PLATE I



PLATE II

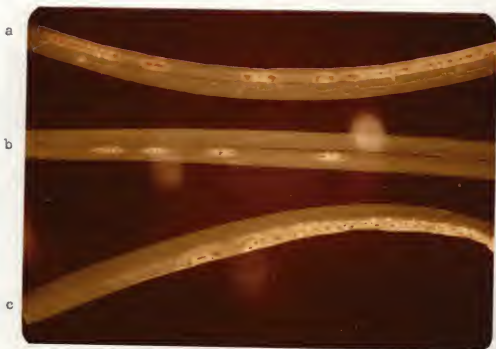


Table 2. Reaction for leaf rust race 9 on the progeny of 11 off-type Mediterranean plants.

1959 greenhouse pot number	: Leaf rust : reaction : of parent	:	Leaf rust reaction of progeny.		
			Number of seedlings per classification		
	:	:	O Fleck	: Y	: 4
778	Y		25	0	0
780	Y		7	16	6
785	Y		18	8	4
788	Y		26	0	0
789	Y		3	11	6
806	Y		8	10	4
807	Y		7	8	2
808	Y		26	2	0
812	chimeral		31	1	0
814	chimeral		10	6	5
832	chimeral		33	0	0

some tagged heads had almost a complete seed set. When tested with leaf rust race 9 the heads and the bulk seed from 38 of the rows were resistant or had single Y-type plants. Seed from two male sterile heads and the bulk seed gave segregation for reaction to leaf rust.

A cytological study of progeny of 11 plants representing eight parents that were off-type in their reaction to leaf rust race 9 (Table 3) was completed in 1960. Six of the plants studied were progeny of five plants having Y-type rust reactions. In three of the six plants from Y-type variants no abnormalities were detected. Two of these three plants had susceptible adult plant reactions to race 9 and one was resistant. One of the six plants from Y-type variants (plant 1003) was monosomic and two (plants 1002 and 1005) were meiotically abnormal. Plant 1005 had bridges in 20 of the 45 representative anaphase I cells that were counted. Of these cytologically abnormal plants 1003, 1005 and 1002 were susceptible to race 9. All five progeny plants

Table 3. Cytological data for 12 progeny plants representing eight parents off-type for reaction to leaf rust.

1960 Plant number	: 1959 Source	: Parent : leaf rust : reaction	: Plant : leaf rust : reaction	: : : Cytology
1001	785	Y	4	stable
1002	797	Y	4	unstable
1003	806	Y	4	2n-1
1004	808	Y	0;	stable
1005	809	Y	4	20 bridges in 45 cells
1006	809	Y	4	stable
1007	812	chimeral	0;	stable
1008	812	chimeral	0;	2n-1
1009	812	chimeral	0;	
1010	818	chimeral	0;	2n-2
1011	818	chimeral	0;	stable
1012	819	chimeral	0;	13 bridges in 32 cells 2n-1

of chimeral parents had adult plant resistance to race 9. These five plants came from three original chimeral plants. Plants 1008 and 1012 were monosomic and plant 1012 had 13 bridges in 32 anaphase I cells. Plant 1010 had 2n-2 chromosomes and was extremely weak. These three meiotically unstable plants represented all three chimeral parents. Two plants from the chimeral parents had no detectable abnormalities in meiosis.

Plant Study

In the plant study there were an estimated 76,000 plants tested of which 2,836 or 3.74 percent gave an off-type response to race 9. There were 81 chimeras among these 2,836 plants, giving approximately one chimeral plant

in 940 seedlings tested.

The results of the statistical analysis are given in Table 4. Both "plants" and "heads within plants" were highly significant. The LSD's for "plants" are included in Table 4. There were 14 plants in which no susceptible or chimera seedlings occurred.

Table 4. Analysis of variance for difference between plants within Mediterranean wheat by nested classification.

Source of variation	d.f.	M.S.	F	Sign.
Plants	135	12.6685	98.82	**
Heads within plants	261	0.1711	1.33	**
Progeny within heads	2438	0.1282		

**Exceeds the 0.01 level of significance

LSD's between lines

Number of heads within plants being compared.	LSD
1 and 1	1.6
1 and 2	1.4
1 and 3	1.3
2 and 2	1.1
2 and 3	1.05
3 and 3	0.95

The progeny of two susceptible plants, numbers 16 and 48, (see Appendix) headed 10 days earlier than Mediterranean. However, the progeny of plant 28 which was resistant, headed the same day. The progeny of these two susceptible plants appeared to be morphologically similar to the other Mediterranean plants in the block except for heading date. The progeny of plant 114, which was segregating, were darker green color with narrower leaves than the other progeny rows. The progeny of plant 22 was segregating and had some plants with club heads. However, the progeny of plant 97, which was resistant to leaf rust,

also had club heads. The progeny of plant 102 were segregating but were not noticeably different morphologically from Mediterranean. Progeny of plants 69 and 131 had shorter plants among the progeny but were resistant to leaf rust.

Many of the progeny within the head rows did not develop three mature heads and the kernels in some heads were not fully developed. Consequently, plants were eliminated from among the rows. One hundred forty-nine or 36 percent of the 410 head rows had some sterile spikes on some of the plants within the rows.

Natural Crossing

Only 0.7 percent of the 4,131 progeny seedlings of the 146 Mediterranean plants grown in the block with RedChief had the expected rust reaction of an F_1 plant. There were, however, 3.64 percent off-types found in the total population. This included four segregating plants and two plants that were homozygous susceptible. In 3,601 progeny seedlings of RedChief 0.194 percent of the seedlings had the expected rust reaction of an F_1 . There were 0.98 percent variants in the RedChief plants tested which included four plants that were beyond the F_1 generation. These four parent plants were assumed to be the result of crossing in years prior to 1959.

DISCUSSION

The non-significant results obtained in the pollination study demonstrate that much of the variation in Mediterranean is due to some cause other than natural crossing. The results of the natural crossing study further substantiates this conclusion. Only 0.7 percent of the population had the Y-type

reaction to leaf rust that should be expected in a heterozygous F_1 plant. To determine the total amount of crossing, Griffee and Hayes (3) stated that it was necessary to double the number of off-types, as each plant has a 50 percent chance of pollinating itself from other spikes or florets on the same plant. Using this information, the total percent possible crosses in 1959 in Mediterranean was 1.4 and in RedChief was 0.39.

The chimeral plants found in both the pollination and plant studies indicated that cytologic instability was present. These chimeral plants were assumed to be the result of somatic mutations. Since the primary leaves were the only portion of the plant carefully studied, the number of chimerals found might have been greater if the plants had been studied to maturity.

The presence of the many single seedlings with Y-type rust reaction in the progeny of both the pollination and plant studies was taken as evidence that somatic mutations did occur. Plots for these studies were well isolated and it is doubtful that all the Y-type seedlings could have been the result of natural crosses.

The seedlings having a Y-type reaction to leaf rust were thought to be heterozygous and consequently their progeny should segregate. The progeny of five of the Y-type plants from the pollination study segregated, indicating that the plants were heterozygous. The progeny of three of the Y-type plants were resistant to race 9. It is possible that these three plants may have been improperly classified. However, considering the presence of the instability causing chimeras, these Y-type plants were probably the result of somatic mutations. The mutations may have occurred outside of the plant's reproductive tissue and did not affect the rust reaction of the progeny. This is also the proposed explanation for the resistant progeny of the

chimeral parents. The occurrence of the two Y-type seedlings from the one chimeral plant indicated that the instability was sometimes passed to the progeny. The segregating progeny of the one chimeral plant indicated that the chimeral mutants were apparently the same general type of mutant as the Y-type plants.

Various morphologic characters such as heading date and height, as well as leaf size and shape were affected when Mediterranean rows were off-type in reaction to leaf rust race 9. These factors may be located on the same chromosome as the genes controlling rust reaction. The loss of a part or entire chromosome should affect any other factors on that portion of the chromosome lost, thus giving rise to morphological differences as found in the plant and pollination studies. The mutation may be a loss of a part or the entire chromosome containing the leaf rust gene. The lack of one gene in a parent should give a segregating ratio in the progeny. The lack of two genes should cause a plant to be susceptible, assuming race 9 reaction to be controlled by a single pair of alleles. Some of the alterations caused by this deletion may be self-perpetuating while others may be especially sensitive to environmental conditions and be subject to easy elimination. If these types were self eliminated there should be a tendency to maintain the total percent off-types in a population at a fairly constant level.

There were cytological abnormalities present in six of the 11 progeny of the eight selected off-type plants studied. Although there were only 11 plants studied they gave evidence that there are cytological abnormalities in Mediterranean. Three of the six abnormal plants were monosomic and one had $2n-2$ chromosomes. The four plants that were short the normal chromosome complement added evidence to the chromosomal loss theory. Three of the

meiotically abnormal plants were resistant to race 9 and three were susceptible. Two of the cytologically abnormal plants had many anaphase I cells with chromatin bridges. The three abnormal plants that were resistant in the adult stage may have been heterozygous and may have had adult plant resistance; or chromosomes other than those concerned with leaf rust reaction also may have been unstable. The 2n-2 plant was weak and may not have been a good competitor under field conditions.

Fourteen of the original plants in the plant study had no off-types. Twelve of these 14 resistant plants had three head rows in the study. These were plants 42, 49, 68, 70, 92, 100, 116, 124, 125, 128, 133, and 138. There was a significant difference between these 12 plants and the other plants with three head rows in the study. One resistant plant, number 112, had only one head row in the study and one plant, number 115, had two head rows in the study (Appendix). The LSD's for these latter two plants were higher than for those plants that had three heads in the study. Because of the larger LSD's they did not rate as high as did the 12 plants with three head rows studied. Although these 14 plants can be selected as being statistically more stable than most of the others in this study, more data from later generations, as well as cytological examinations, will be necessary to determine if they will remain pure in reaction to leaf rust. A selected head of each of 2,835 plants from the head rows was planted in the fall of 1959 as a continuation of the plant study.

Three plants, 22, 102, and 104 from the plant study, were segregating in reaction to leaf rust and two plants, 16 and 48, were susceptible. In the remaining 344 progeny head rows, 89 rows were resistant to leaf rust. One progeny row in plant 80 segregated for reaction to leaf rust and the

plants were shorter than the other progeny row of plant 80. In the Appendix table the plants with resistant heads can be determined by comparing the "heads with off-types" column with the "number of heads studied" column. The remaining 254 progeny head rows had only a few variants in reaction to leaf rust in a row. In these 254 progeny rows the most common off-type was the Y-type.

The self-sterility present in Mediterranean in the field caused some spikes to remain open for prolonged periods which might have allowed foreign pollen to fall on the stigmas. If this pollen was from plants other than Mediterranean, F_1 plants would be expected. However, the plants in the pollination and plant studies were isolated from other wheat; so, if any crossing occurred it was probably with Mediterranean. Such a cross should be of no consequence in a homozygous variety unless the pollen parent was an off-type. There was a large difference in the percent of rows with sterile spikes in 1958 and 1959. This difference may have been due to environmental conditions.

No conclusions concerning the total percent off-types in these studies was made because the original seed source had been continuously rogued to eliminate the variants. However, there was remarkable agreement between Shulte's (23) report of 3.62 percent variants and the 3.68 percent from the pollination study, the 3.74 percent from the plant study, and the 3.64 percent from the natural crossing study. The variants for rust reaction may have reached an equilibrium, if no roguing had been practiced, at which the variants may have been eliminated at about the same rate that new ones occurred. This elimination could be caused by chromosomal alterations that are overly sensitive to environment or by the lack of hardiness or competitive

ability within a plant formed by these alterations. Thus, only the more hardy aberrations would survive and give rise to morphologic and leaf rust off-type plants like those found in the pollination and plant studies.

SUMMARY

The ranking test for the pollination study was non-significant. This indicated that natural crossing was not the main factor causing the off-type rust reactions in the 19 lines studied. The results of the natural crossing study in 1959 substantiates the conclusion drawn from the pollination study. There were only 0.7 percent F_1 type plants found, giving a total of 1.4 percent possible out-crosses in Mediterranean. RedChief, in the same block, had 0.194 percent seedlings with F_1 type rust reactions.

Chimeral plants were found in all three studies with an approximate ratio of one in 1000 plants. The presence of the chimeral and the single Y-type plants from a resistant bagged head coupled with the morphologic character of the leaf rust off-types found in the plant study gave evidence that there was chromosome instability. The findings of the cytological study further substantiated this assumption. It was assumed from the available evidence that mutations were occurring by the loss of an arm or the entire chromosome bearing the leaf rust factor.

Statistical data for one year indicated that it was possible to select plant lines that did not have variant plants for rust reaction. The 14 original plants in the line study with no off-types were significantly different from most of the other lines in the study. However, with the presence of the instability it will be necessary to further screen the material and to study those plants with no leaf rust off-types cytologically to determine

their stability. The 3.62 percent variants to leaf rust found by Shulte (23) was remarkably similar to the percent variants found in the three studies of this report; 3.68 percent for the pollination study, 3.74 percent for the plant study, and 3.64 percent for the crossing study.

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APPENDIX

Table. Plant number, number of head progeny rows and number of these head row progeny of the Mediterranean wheat variety that had variants in 1958-1959.

Plant number	: Number : of head : progeny : rows : studied	: Number : of head : progeny : rows with : variants	:	Plant number	: Number : of head : progeny : rows : studied	: Number : of head : progeny : rows with : variants
1	2	1				
2	3	2		37	3	2
3	3	2		38	3	3
4	3	2		39	3	3
5	3	3		40	3	1
6	3	1		41	3	3
7	3	3		42	3	0
8	3	2		43	3	2
9	3	1		44	3	3
10	3	1		45	3	3
11	3	3		46	3	2
12	3	2		47	3	3
13	3	2		48	3	3
14	3	2		49	3	0
15	3	3		50	3	1
16	3	3		51	3	2
17	3	2		52	3	3
18	3	1		53	3	3
19	3	3		54	3	3
20	3	3		55	3	2
21	3	1		56	3	3
22	2	2		58	3	3
23	3	3		59	3	3
24	3	2		60	3	2
25	3	3				
26	3	3		61	3	3
27	3	1		62	3	3
28	3	2		63	3	3
29	3	2		64	3	3
30	3	3		65	3	2
31	3	1		66	3	2
32	3	3		67	3	3
33	3	2		68	3	0
34	3	3		69	3	1
35	3	2		70	3	0

Table (concl.)

	: Number	: Number	::	: Number	: Number	
	: of head	: of head	::	: of head	: of head	
	: progeny	: progeny	::	: progeny	: progeny	
Plant	: rows	: rows with	::	Plant	: rows	: rows with
number	: studied	: variants	::	number	: studied	: variants
71	3	3		106	3	2
72	3	1		107	3	3
73	3	3		108	3	3
74	3	2		109	3	2
75	3	1		110	1	1
76	3	3		112	1	0
77	3	2		113	1	1
78	3	2		114	2	1
79	3	2		115	2	0
80	3	3				
81	2	1		116	3	0
82	3	2		117	3	2
83	3	2		118	3	1
85	3	2		119	3	2
				120	3	1
86	3	2		121	3	1
87	3	2		122	3	3
88	3	1		123	3	2
89	3	3		124	3	0
90	3	3		125	3	0
91	3	2		126	3	2
92	3	0		127	3	1
93	3	3		128	3	0
94	3	3		129	3	1
95	3	3		130	3	3
96	3	1		131	3	1
97	3	1		132	3	2
98	3	2		133	3	0
99	3	2		134	3	3
100	3	0		135	3	2
101	3	3		136	3	2
102	3	3		137	3	2
103	3	3		138	3	0
104	3	3		139	3	2
105	3	3		140	3	2

VARIABILITY IN MEDITERRANEAN WHEAT

by

ROBERT LOVELL ELLSWORTH

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AN ABSTRACT OF A THESIS

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Abnormalities in Mediterranean wheat in reaction to leaf rust race 9 have been recognized for many years. Attempts have been made to purify this variety but none have remained stable to date. The purpose of this study was to determine the extent and possible causes of this behavior in Mediterranean.

Several causes of variation have been reported in the literature. The amount of natural crossing in wheat has been shown to vary with variety and season. Partial self sterility has been suggested as a possible cause for out-crossing in wheat. The meiotic instability in Marquillo wheat was attributed to non-orientation or the failure of chromosomes to pair. Instability of germinal tissue was shown to be a heritable character in several generations of unstable wheat plants. Chimera plants have been described which were caused by somatic mutations. In Clinton oats there was 12 percent variation caused by somatic mutations.

Approximately 65,340 seedling progeny of selfed and naturally pollinated spikes from 19 leaf rust resistant lines were tested with race 9 of leaf rust. The differences between the bagged and non-bagged heads within the 19 lines was non-significant, indicating that the abnormalities present were not caused by out-crossing. Many single Y-type seedlings were found in the progeny of both the bagged and non-bagged heads. It was proposed that some of the Y-types may have been the result of somatic mutations.

When Mediterranean and RedChief were interplanted, the progeny of Mediterranean plants had 0.7 percent variants in reaction to leaf rust typical of the expected F_1 reaction. The progeny of RedChief from this block had 0.194 percent variants that were typical of the F_1 reaction.

Another group of approximately 75,000 seedlings, progeny of 136 plants,

were tested to race 9 and the differences between plants was highly significant for one year's data. Fourteen plants had no off-types in their progeny and were significantly different from the other 122 plants in the test. Some of the leaf rust off-type rows showed morphological differences when studied in the field.

The total off-types for the three studies was 3.68 percent for the pollination study, 3.74 percent for the plant study, and 3.64 percent for the natural crossing study. There was no conclusion drawn from these percentages since the seed sources for the three studies had been rogued to remove off-types.

Chimeral plants were found in an approximate ratio of 1 in 1000 seedlings tested to leaf rust in both sets of seedlings studied. A cytological study of 11 plants from eight selected chimeral and Y-type plants showed six of the progeny to be unstable, indicating that cytological instability was present in Mediterranean. Three of the unstable plants had $2n-1$ chromosomes and one had $2n-2$.

Data from this preliminary study indicated that the occurrence of plants susceptible to leaf rust race 9 in Mediterranean was caused by cytologic abnormalities. This instability appeared to be due to a loss of a part or an entire chromosome on which the leaf rust factor was located. Further study will be necessary to determine whether the 14 plants that showed no susceptible plants are breeding true for a stable response.