COMBINING GENES FOR WHEAT LEAF RUST RESISTANCE: EFFECT OF TWO GENES ON INFECTION TYPE

By

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RESISTANCE: EFFECT OF TWO GENES

ON INFECTION TYPE

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CHAPTER I

INTRODUCTION

Wheat has been the world's leading crop for many years. In many areas of the world, it is the principal food of man. This crop is grown primarily for grain with pasturage as a secondary consideration in some areas.

Wheat is grown in all temperate countries and in most of the subtropical countries of the world, as well as at high elevations in some tropical countries (14). Most of the crop is produced in the temperate zone, and almost 90 percent of the area covered by this crop lies in the northern hemisphere.

In the United States, wheat is second only to corn in total production. It occupies the major portion of the central plains of the United States, and in that area hard red winter wheat is the major type grown.

One of the destructive diseases of wheat, especially in the plains area is "Leaf Rust" caused by *Puccinia recondita* Rob. ex. Desm. f. sp. *tritici* (Erriks) C.O. Johnston. This disease is very destructive in the eastern half of the plains area because the weather conditions are favorable for its development. It occurs more frequently and usually affects larger acreages than does stem rust (14).

Specific resistance has long been used in wheat cultivar development to control the disease (9). This kind of resistance is effective against only certain rust races. Wheat cultivars which have utilized specified resistance usually possess only one effective gene conditioning resistance to prevalent rust races. When a new race of rust occurs which posseses pathogenicity for that gene such a cultivar readily becomes attacked by that race. More recently, efforts have been made to combine more than one gene in the same wheat cultivar, there by provide wider resistance to the pathogenicity of the current population (27) and decrease the probability that new races arising by mutations will be virulent on the cultivar.

The objective of this study was to determine if the combinations of two specific genes for resistance in the same cultivars interact to alter the expected infection type.

CHAPTER II

REVIEW OF LITERATURE

Wheat leaf rust, incited by *Puccinia recondita* f. sp. *tritici* is one of the most important foliar diseases of wheat, and can be found wherever wheat is grown in the world. It is the most common and widely distributed of all cereal rusts, particularly in humid and sub-humid regions. This disease causes significant losses in grain production all over the world. For example in 1936, Waterhouse (30) estimated the total monetary loss for the main cereal growing areas of the world at $500,000,000 and in 1938, Chester (9) estimated the loss in Oklahoma alone at about $12,000,000 due to a leaf rust epidemic that year.

Leaf rust is usually confined to the leaves and leaf sheathes and appears as orange to orange-brown pustules (31). An early infection of leaf rust can reduce the yield of a susceptible wheat variety 42 to 94 percent (13, 14). Reduction in grain yield is due primarily to a reduced number of kernels per spike and sometimes to a reduced number of spikes. The kernels also are reduced in weight but are not noticeably shriveled.

Leaf rust can be chemically controlled with several dustings with sulfur (17) or with other fungicides. However, on the basis of safety, cost and effectiveness, chemical control never has been completely successful. It has been declared by some that pesticides constitute a serious health hazard and that disease resistance should be used for control. However, others declare that the use of specific resistance has led us only into a cycle of hope and disappointment and that we should concentrate our efforts on a more generalized or non-specific resistance (32). A balance of these two types of resistance has been proposed by Young (32) and by Kayes (11) who states that the incorporation of the best available genes for race-specific resistance into genotypes having highest possible level of non-specific resistance . . . provides the best long term solution to the problem, . . .

The use of specific resistance in wheat cultivars has been a highly sucessful method of controlling of leaf rust and the reduction of yield losses (20, 21). This type of resistance is most often inherited in a simple Mendalian fashion, usually by a simple dominant gene (23). This fact has been reported by many workers and literature on the subject has been compiled by Ausemus et al. (1). However, if resistant parents with unknown genotypes are used in a breeding program, a narrow base of resistance may result (2).

Statler (27) stated that a knowledge of virulence frequencies on single gene differential lines should be useful in the production of wheat varieties resistant to the prevalent leaf rust population. Statler and Nolte (29) in 1979 suggested that host genes LR 9, LR 19, LR 24 and Transec conditioned resistant to the greatest number of _Puccinia recondita tritici_ isolates in 1978, and probably provides the best protection against the natural population.

Methods for utilizing specific resistance other than the use of single genes have been suggested. Borlaug (4) worked with stem rust and suggested that wheat cultivars could be developed which have the possibility of remaining rust-resistant indefinitely. Such a cultivar must be constituted so that its resistance can be modified to meet the changing relative prevalence of different races of the stem rust population. This cultivar would be a "composite" or mixture of a number of phenotypically similar lines, which differ genotypically for stem rust resistance (4). It has been suggested that cultivars with two or more genes for resistance be developed to obtain a longer term of protection against both the leaf rust and stem rust diseases (19, 24). Schafer et al. (22) studied combining resistant genes for controlling leaf rust. The cultivars, Aniversario (C.I. 12578), Exchange (C.I. 12635), Fontana (C.I. 12470), and La Prevision 25 (C.I. 12596) were crossed in all six possible paired combinations. All four of these cultivars have a broad coverage of resistance to races of P. recondita as reported by Levine et al. (15). Cultures of races 5, 35, 45, 76, 89, and 104 of P. recondita were used to test the progeny derived from those crosses and lines were produced which were highly resistant to all the races. Loegering and Powers (16) in similar studies with stem rust found that the combinations of resistance from two parents had a lower infection type than either of the parents singly.

CHAPTER III

MATERIALS AND METHODS

Three different wheat lines were used in this study:

1. Sage, having one known gene for resistance to leaf rust, (LR 24).
2. 5* WI/TF (5), also having one known gene for resistance to leaf rust, (LR 9).
3. Sage// 5* WI/TF. This line resulted from a cross between the above two lines and is known to have both LR 24 and LR 9.

Cultures of five races of _Puccinia recondita_ f. sp. _tritici_ used in this study:

Local race name	Pathogenicity on:	
	LR 24	LR 9
2AAG	+	-
TF	-	+
6B	-	-
Can A	-	-
Can C	-	-

The first three races were isolated from collections made in commercial wheat fields or disease observation plots in Oklahoma. The latter two races were obtained from Dr. D. J. Samborski, Rust Research Laboratory, Canada Department of Agriculture, 25 Dafoe Road, Winipeg, Canada. They were identified and classified on the basis of differential cultivars suggested by Basile (3) and the additional cultivars; Westar (CI 12110), Wesel (CI 13090), Agent (CI 13523), Transfer (CI 13296), and Anniversario (CI 12578).

Race 2AAG constitutes about one half of the leaf rust population in Oklahoma, TF and 6B are found rarely and Can A and C have been found only in Canada.

Theurediospores of these five rust races were supplied by Dr. Francis J. Gough and Dr. Harry C. Young, Jr., Department of Plant Pathology, Oklahoma State University, Stillwater, Oklahoma.

The inoculum of each race was first increased on 6 pots containing 2025 seedlings of the universally susceptible cultivar 'Danne' (CI 13876). The initial inoculation for increasing the cultures was made by using the oil technique (6) when the seedlings were seven days old. The temperature at the time of inoculation was approximately 25-27 C. Inoculated plants were kept overnight in a moist chamber.

Subsequent increase of inoculum was made using the brushing technique (6). In each case, urediospores were harvested approximately 8 to 10 days after inoculation. This method was used to increase the inoculum for all five rust races separately. Four 10 cm. pots were planted on the same day that the second increase of urediospores of each races was inoculated. In this way, fresh inoculum was ready to use when the plants to be tested had attained the desired area (8 to 10 days).

The test plants were inoculated using the brushing technique and kept overnight in separate moist chambers. Each chamber contained plants inoculated with one race to avoid contamination. The plants of all lines were inoculated on the same day. The morning after inoculation the moist chambers were opened and the plants allowed to dry slowly before being removed to a greenhouse at 25°C±5. Seven to ten days after inoculation notes were taken on infection type based on the system of Stakman et al. (26), which hereafter in this paper will be referred to as the classical system, and also according to the coding system of Browder (7) and Browder and Young (8), which hereafter will be referred to as the three-digit system.

CHAPTER IV

RESULTS

Sage, known to possess one gene conditioning resistance to leaf rust (LR 24), was resistant to three of the races used in this study, 6B, TF and Can A. The infection type according to the classical system was the same, "0", regardless of which of these three races was used. Using the three-digit system, however, there was a small difference in lesion size. Race 6B on Sage produced a "02C" infection type, but races TF and Can A produced a "03C" (Tables I, II and III).

The reaction of Sage was intermediate to race Can C, exhibiting an "X" or "25X" infection type, and was susceptible to race 2AAG, with an infection type "4", or "88P".

The wheat line 5* WI/TF also possesses one gene conditioning resistance to leaf rust, LR 9; different from the gene in Sage. Races 2AAG, 6B, Can A, and Can C were avirulent on this line and had 0, 0; and 0; infection types respectively according to the classical system and 000, 02C, 03C respectively, according to the three-digit system (Tables I, II, IV and V). In this case also, the avirulent races did not have the same infection types. With race Can C the lesion size varied among replicates from 2 to 4 with an average of 3.3.

Race TF was virulent on line 5* WI/TF line and produced a "4" or "88P" infection type.

TABLE I

THE INFECTION TYPE ON PARENT LINES, SAGE, 5*WI/TF, AND ON THE CROSS SAGE//5*WI/TF INOCULATED WITH RACE 2AAG

		SAGE				5*WI/TF				SAGE//5*WI/TF			
		Replicate				Replicate				Replicate			
		1	2	3	4	1	2	3	4	1	2	3	4
Infection type of Stakman et al. (26)		4	4	4	4	0	0;	0;	0;	0	0	0	0
Infection type of Browder[a] (6)													
	PS	8	8	8	8	0	0	0	0	0	0	0	0
	LS	8	8	8	8	2	2	2	2	0	0	0	0
	TD	P	P	P	P	C	C	C	C	0	0	0	0

PS - Pustule size
LS - Lesion size
TD - Tissue damaging
[a]-3-digit classification

TABLE II

THE INFECTION TYPE ON PARENT LINES, SAGE, 5*WI/TF, AND ON THE CROSS SAGE//5*WI/TF INOCULATED WITH RACE 6B

		SAGE				5*WI/TF				SAGE//5*WI/TF			
		Replicate				Replicate				Replicate			
		1	2	3	4	1	2	3	4	1	2	3	4
Infection type of Stakman et al. (26)		0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;
Infection type of Browder[a] (6)													
	PS	0	0	0	0	0	0	0	0	0	0	0	0
	LS	2	2	2	2	2	2	2	2	2	2	2	2
	TD	C	C	C	C	C	C	C	C	C	C	C	C

PS - Pustule size
LS - Lesion size
TD - Tissue damaging
[a]-3-digit classification

TABLE III

THE INFECTION TYPE ON PARENT LINES, SAGE, 5*WI/TF, AND ON THE CROSS SAGE//5*WI/TF INOCULATED WITH RACE TF

		SAGE				5*WI/TF				SAGE//5*WI/TF			
		Replicate				Replicate				Replicate			
		1	2	3	4	1	2	3	4	1	2	3	4
Infection type of Stakman et al. (26)		0;	0;	0;	0;	4	4	4	4	0;	0;	0;	0;
Infection type of Browder[a] (6)													
	PS	0	0	0	0	8	8	8	8	0	0	0	0
	LS	3	3	3	3	8	8	8	8	3	3	3	3
	TD	C	C	C	C	P	P	P	P	C	C	C	C

PS - Pustule size
LS - Lesion size
TD - Tissue damaging
[a]-3-digit classification

TABLE IV

THE INFECTION TYPE ON PARENT LINES, SAGE, 5*WI/TF, AND ON THE CROSS SAGE//5*WI/TF INOCULATED WITH RACE CAN A

		SAGE				5*WI/TF				SAGE//5*WI/TF			
		Replicate				Replicate				Replicate			
		1	2	3	4	1	2	3	4	1	2	3	4
Infection type of Stakman et al. (26)		0;	0;	0;	0;	0	0	0	0	0	0	0	0
Infection type of Browder[a] (6)													
	PS	0	0	0	0	0	0	0	0	0	0	0	0
	LS	3	3	3	3	0	0	0	0	0	0	0	0
	TD	C	C	C	C	0	0	0	0	0	0	0	0

PS - Pustule size
LS - Lesion size
TD - Tissue damaging
[a]-3-digit classification

TABLE V

THE INFECTION TYPE ON PARENT LINES, SAGE, 5*WI/TF, AND ON THE CROSS SAGE//5*WI/TF INOCULATED WITH RACE CAN C

		SAGE				5*WI/TF				SAGE//5*WI/TF			
		Replicate				Replicate				Replicate			
		1	2	3	4	1	2	3	4	1	2	3	4
Infection type of Stakman et al. (26)		X	X	X	X	0;	0;	0;	0;	0;	0;	0;	0;
Infection type of Browder[a] (6)													
	PS	2	2	2	2	0	0	0	0	0	0	0	0
	LS	5	5	5	5	4	4	3	2	3	2	3	2
	TD	X	X	X	X	C	C	C	C	C	C	C	C

PS - Pustule size
LS - Lesion size
TD - Tissue damaging
[a]-3-digit classification

The Sage//5* WI/TF line containing both genes LR 9 and LR 24 with race 2AAG produced no indication of infection (Table I). The infection type was classified as a classical "0", or with the three-digit system as "000". Both parents of this line had higher infection types (Sage was "4" or "88P" and 5* WI/TF was "0;" or "02C"). With race "6B", however, the cross and both parents had the same infection type "0;" or "02C".

With race Can C the infection type on the cross was lower than the Sage parent with was meothetic but was equal to the 5* WI/TF parent line as both had "0;". There were small differences, however, since the cross was classified 02C to 03C while the 5* WI/TF parent line was classified 02C to 04C according to the three-digit system. Again, the cross containing both genes had the lower infection type.

Both the Sage and 5* WI/TF parents were resistant to race Can A with "0;" and "0" infection types respectively or "03C" and "000" respectively. The cross had a "0" or "000" infection type with this race; equal to the 5* WI/TF parent but lower than the Sage parent.

The TF race produced a "0;" or "03C" infection type on Sage, and a "4" or "88P" infection type on 5* WI/TF. This race on the cross Sage// 5* WI/TF produced a "0;" or "03C" infection type. Perhaps this would be expected since only the LR 24 gene from Sage would be effective (10). However, when the cross was inoculated with race 2AAG, the infection type was lower than that of the parent which contributed the gene that was effective in that case (LR 9).

CHAPTER V

DISCUSSION

According to Flor (10) the rust fungus must possess genes for pathogenicity for each corresponding gene for resistance in the host in order for a high infection type to develop. If two genes for resistance are present in the host, they may interact cumulatively to condition a lower infection type than conditioned by either gene alone. Loegering and Powers (16) reported that the infection type of a cross with two genes was lower than that of either parent with only a single gene.

The results of this study generally support the observations of Loegering and Powers, although there were some exceptions.

The cross used here involved the resistance genes LR 9 and LR 24. One of the races used, 2AAG, was pathogenic on LR 24 but on on LR 9. Another race, TF, was pathogenic on LR 9 but not on LR 24. When race 2AAG was used, the cross produced a lower infection type than either parent, as Loegering and Powers (16) suggested. However, when race TF was used the infection type was only as low as the lowest parent.

The same situation occurred when the cross was inoculated with races that were not pathogenic on either of the host resistance genes. When race 6B was used, the corss and both parents had the same infection type. When race Can A was used, the cross and the 5* WI/TF line parent had the same infection types, which was lower than the Sage parent. When race Can C was used, however, the infection type was slightly lower than the lowest parent, although on both the cross and the 5* WI/TF parent there was a small amount of variability in infection type between replicates.

Therefore, it can be concluded that in some cases the infection type on a line or cultivar containing two genes for resistance is lower than the infection of either parent line containing only one of the two genes involved. In this study, the cross line containing both genes was always at least equal to, if not lower, than the lowest parent. This was true regardless of whether the race use was pathogenic on one or the other or neither of the genes involved or whether it was pathogenic on neither gene. In no case was the infection type of the cross higher than that of the lowest parent.

CHAPTER VI

SUMMARY

1. A cultivar of wheat, Sage, containing the leaf rust resistance gene LR 24, and another line of wheat from the cross Wichita/ Transfer backcrossed to Wichita four times and which possessed the leaf rust resistance gene LR 9 were compared for leaf rust infection types with a selection from a cross of this cultivar and a line which contained both LR 9 and LR 24.
2. Five races of leaf rust were used: one race, 2AAG was virulent on LR 24 but not on LR 9; one race, TF was virulent on LR 9 but not LR 24; and three races, 6B, Can A, and Can C, were avirulent on both LR 9 and LR 24.
3. In two cases, with races 2AAC and Can C, the infection type of the cross was lower than that of either parent.
4. In one case, race 6B, the infection type of the cross and both parents was the same.
5. In the other two cases, races TF and Can A, the infection type of the cross was equal to that of the lowest parent.

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