# EFFECT OF RESISTANCE GENE *Lr34* IN THE ENHANCEMENT OF RESISTANCE TO LEAF RUST OF WHEAT.

A Thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

By

Silvia Germán

In Partial Fulfilment of the
Requirements for the Degree

of

Master of Science

Department of Plant Science

January of 1991

ISBN 0-315-76947-5

# *EFFECT OF RESISTANCE GENE Lr34 IN THE ENHANCEMENT OF RESISTANCE TO LEAF RUST OF WHEAT*

*BY*

*SILVIA GERMAN*

A thesis submitted to the Faculty of Graduate Studies of
the University of Manitoba in partial fulfillment of the requirements
of the degree of

*MASTER OF SCIENCE*

## ACKNOWLEDGEMENTS.

I sincerely thank my advisor Dr. J. A. Kolmer for his guidance and encouragement throughout this research project and during the preparation of this manuscript.

I would like to extend my gratitude to Dr. P.L. Dyck for his valuable suggestions and for developing the wheat lines that were used in this research.

I also thank Dr. C.C. Bernier for his support and advice during my research and studies.

I extend my appreciation to Agriculture Canada Winnipeg Research Station for providing the facilities for this research and to the Canadian International Development Centre for supporting my studies.

Special thanks to students of the Department of Plant Science and technicians and summer students at Agriculture Canada for their help and friendship during my stay in Winnipeg.

I would like to dedicate this thesis to my family, especially my sons, Rafael, Martin and Alejandro and my husband Hugo.

## TABLE OF CONTENTS

# LIST OF TABLES

# ABSTRACT

Germán, S., M.Sc., The University of Manitoba. Effect of resistance gene *Lr34* in the enhancement of resistance to leaf rust of wheat. Major Professor: Dr. J.A. Kolmer.

Leaf rust resistance gene *Lr34* is present in many wheat cultivars throughout the world that have shown durable resistance to leaf rust. *Lr34* is mainly expressed in the adult plant stage but under certain environmental conditions it can also be detected in the seedling stage. Twelve pair-wise combinations of *Lr34* and seedling leaf rust resistance genes and one combination with and an adult plant resistance gene were developed by crossing the near isogenic Thatcher lines and selecting the most resistant F2 seedlings from each cross. F3 families derived from single F2 plants were tested as adults in a rust nursery to select families that were homozygous for *Lr34* and derived F4 lines were tested for segregation of the seedling gene. Homozygous F4 lines for *Lr34* and the seedling gene were tested as seedlings and adults using a wide range of leaf rust isolates, to determine if combinations of leaf rust genes with *Lr34* have enhanced resistance relative to either of the parental isogenic lines. In both seedling and adult tests pair-wise combinations with *Lr34* had enhanced resistance to different number of isolates expressing low infection type to the seedling genes. The combination Tc*Lr34,18* was an exception since it expressed enhanced resistance to specific isolates virulent to *Lr18* in seedlings and adult plant stage. In rust nursery tests, homozygous F3 families and F4 lines were more resistant than either parent if the additional leaf rust gene conditioned an effective level of resistance when present singly. Lr34 has demonstrated long lasting resistance in combinations with other genes and it has the ability to interact with effective resistance genes for superior resistance. For these reasons, the use of combinations of *Lr34* with other genes conditioning effective resistance appears to be a suitable strategy to incorporate leaf rust resistance in new wheat cultivars.

# Chapter I

## INTRODUCTION

Rust resistance in wheat (*Triticum aestivum*) has traditionally been based on the use of isolate specific genes. Resistance genes in wheat and virulence genes in *Puccinia graminis* f. sp. *tritici* and *Puccinia recondita* f. sp. *tritici* operate in what has been described as a gene-for-gene basis (Samborski and Dyck, 1968, Green, 1964 and Green, 1966). Genetic specificity in host - parasite systems was first demonstrated by Flor (1955) in the flax - flax rust system. For each resistance gene in the host there is a corresponding virulence gene in the pathogen. Incompatible infection types occur on resistant cultivars when the rust isolates have the complementary allele conditioning avirulence. Compatible infection types result when the host is susceptible or virulence genes occur in the pathogen.

The release of resistant cultivars selects isolates with the corresponding virulences in the rust population. Isolates that have matching virulence to the newly released resistance will rapidly increase in frequency to a point where the resistance is rendered ineffective. Kolmer (1989a) examined the history of virulence dynamics in populations of *P. recondita* in Canada. The use of resistant cultivars has resulted in the rapid increase of corresponding virulences in the prairie region of Canada.

In this manner the evolution of the rust populations has become a "man - guided" phenomenon (Johnson, 1961). Cultivars whose resistance has been rendered ineffective are replaced by new cultivars with effective resistance genes resulting in the continuous selection of virulent isolates. Therefore, the development of cultivars with durable resistance to the rust diseases presents a major challenge to plant pathologists and plant breeders.

Resistance to leaf rust in common wheat has been particularly short lived. One possible reason for the rapid adaptation of leaf rust populations is that resistance to leaf rust has often been based on the use of single resistance genes (Roelfs, 1988b).

In order to be virulent to lines that have combinations of resistance genes, leaf rust isolates must have the complementary virulence genes to each resistance gene present. The lower probability of rust isolates combining the needed virulences to match resistance genes in cultivars with complex resistance may prolong the duration of the resistance in these cultivars. Currently in North America durable resistance to stem rust is based on a complex of resistance genes. Wheat cultivars with similar complexes of leaf rust resistance genes may provide more durable resistance to leaf rust.

In wheat cultivars that have two or more genes for rust resistance, the genes usually act independently, exhibiting the infection type of the gene which conditions the lower infection type when present singly (epistasis) (Dyck and

Kerber, 1985; Roelfs, 1988a). Nevertheless, there are reports of enhanced levels of resistance conferred by lines with combinations of genes, indicating that interactions between resistance genes also occur.

*Lr34* is an important leaf rust resistance gene which has provided durable resistance in combinations with other resistance genes (*Lr34* + *Lr13* and *Lr34* + *Lr12*, Roelfs, 1988b). These resistance gene combinations express higher level of resistance than either gene singly.

The objective of this research was to examine paired combinations of leaf rust resistance genes with *Lr34* in a common Thatcher background, for enhanced resistance in seedling and adult plants.

# Chapter II

# LITERATURE REVIEW

## 2.1 RESISTANCE GENE INTERACTIONS

Three categories of interactions between major genes have been described in previous research: additive gene interactions, between two genes which individually condition effective resistance (Luig and Rajaram, 1972; Knott and Weller, 1988); modifying action of ineffective genes, when one ineffective gene modifies the action of an effective gene (Knott, 1957; Martens et al., 1981); and complementary gene action, when two ineffective genes are involved in the interaction (Baker, 1966; Singh and McIntosh, 1984).

Luig and Rajaram (1972) studied homozygous and heterozygous combinations of *Sr5* and *Sr9b*, *Sr5* and *Sr13*, *Sr6* and *Sr8*, and *Sr8* and *Sr9b*. Additive gene interaction was found in a number of instances, particularly when *Sr6* was present. Knott (1957) determined that resistance genes *Sr9*, *Sr10*, *Sr11* and *Sr12* are modifiers of *Sr7* which conditions resistance to race 15B of stem rust. Martens et al. (1981) provided evidence of a suppressor of *Pg12* in the cultivar Kyto or a gene for stem rust resistance from *Avena sterilis* that interacted with stem rust resistance gene *Pg12* to produce an enhanced level of resistance.

Baker (1966) demonstrated that the high level of resistance to crown rust in seedlings of the oat cultivar Bond was due to the interaction of two dominant complementary genes. Singh and McIntosh (1984a) determined that the leaf rust resistance in the Australian cultivar Gatcher was conferred by two complementary genes. The genes were separated in susceptible lines that produced resistant progenies exhibiting the Gatcher infection type when intercrossed.

Examining combinations of two and three wheat stem rust resistance genes, Knott and Weller (1988) determined that the combinations *Sr8a,9b*, *Sr8a,11*, *Sr9b,11*, *Sr7a,9b,11* and *Sr8a,9b,11* were more resistant than the most resistant single gene parent to stem rust race 56 when evaluated in field tests. The combinations *Sr7a,9b*, *Sr8a,9b*, *Sr8a,11*, *Sr7a,8a,9b* and *Sr7a,9b,11* were significantly more resistant than the most resistant single gene parent when inoculated with race 15B-1. However fewer interactions were detected in seedling tests.

Singh and McIntosh (1987) demonstrated that the adult plant resistance of the wheat cultivars Chris and W3746 to the predominant isolates of *P. graminis* in Australia appeared to be conferred by the interaction of resistance genes *Sr7a* and *Sr12* or closely linked genes.

Schafer et al. (1963) combined the leaf rust resistance of the cultivars Aniversario, Exchange, Frontana and La Prevision 25. Selections possessing a degree of resistance superior to either parent were identified in progenies of the six parental combinations. In five of these combinations the presence of resistance derived from both parents was confirmed through genetic analysis.

Samborski and Dyck (1982) examined interactions between seedling and adult plant wheat leaf rust resistance genes. When combined into a single line, the seedling genes *Lr30* and *Lr3ka* interacted to produce a higher level of resistance than either single gene parent. The adult plant resistance gene *Lr13* interacted with the seedling genes *Lr30* and *Lr11* in the seedling stage. Gene *Lr13* also interacted strongly with *Lr16* in the cultivar Columbus. In the adult plant stage, Columbus had enhanced resistance relative to *Lr13* and *Lr16* singly to three of the four isolates tested. Genes *Lr33* and *Lr34* generally conferred a 1+ and a 2+ infection types when present singly in RL6057 and RL6058 respectively, and interacted for superior resistance in RL6059.

Sawhney et al. (1989) examined the seedling and adult plant resistance of Thatcher (Tc) near isogenic lines Tc*Lr10* and Tc*Lr23*, and the substitution lines of Kenya Farmer stock with *Lr10* and *Lr23* in the Chinese Spring background. The seedling infection types of the Thatcher lines and Chinese Spring lines were similar. In the adult plant stage, the Chinese Spring substitution lines were more resistant than the equivalent Thatcher lines. This enhanced resistance was postulated to be due to the combination of the Chinese Spring adult plant resistance and the seedling resistance genes. Chinese Spring has provided a moderate level of adult plant resistance to leaf rust for a very long time, probably due to the presence of *Lr34* and *Lr12* (Dyck, in press). Chinese Spring also has *Lr31*, but it is not known whether this gene interacts with other genes for superior resistance (Dyck, in press).

Schafer (1963), Dyck (1977), Dyck and Samborski (1982) and Samborski and Dyck (1982) generically define gene interactions as when the combination of two or more genes results in higher resistance than that conferred by the individual genes. Dyck and Kerber (1985) considered the possibility that this effect may be additive.

## 2.2 PRESENCE OF INTERACTING RESISTANCE GENES IN CULTIVARS WITH DURABLE RESISTANCE TO LEAF RUST

The interactions between resistance genes may be involved in what has been termed as "durable resistance". This is defined as resistance that has been adequate for a number of years over a range of environments (Johnson, 1981).

Durable resistance to wheat leaf rust has rarely been found. According to Roelfs (1988b), the basis for most durable resistance to wheat leaf rust has been the combinations of *Lr13* and *Lr34*, and probably *Lr12* and *Lr34*. These gene combinations were most likely originally present in the cultivar Alfredo Chavez (*Lr13* and *Lr34*), a Brazilian cultivar, and Americano 44d (*Lr12* and/or *Lr13* and *Lr34*), an Uruguayan cultivar.

Dyck et al. (1966) determined the presence of *Lr12* in Exchange, and *Lr13* in Frontana, two cultivars which have been used extensively in breeding programs due to their durable resistance. Both genes required the presence of modifiers to produce the resistance of the original cultivars. One of the modifiers of *Lr13* is probably *Lr34*, which is also present in Frontana (Dyck and Samborski, 1982).

The cultivars Chris (*Lr13* + *Lr34*) (Roelfs, 1988b) and Era (*Lr13* + *Lr34* + *Lr10* + ?) (Ezzahiri and Roelfs, 1989) are grown in the northern plains of the U.S.A. and have shown durable resistance to leaf rust since their introduction in 1966 and 1971 respectively.

The Canadian cultivar Columbus (*Lr13* and *Lr16*) has had a high level of resistance to leaf rust since its release in 1980. Isolates of leaf rust virulent on adults with *Lr13* and *Lr16* singly, are avirulent on Columbus in the adult plant stage (Samborski and Dyck, 1982). This would appear to be an exception to the gene for gene theory, which predicts that isolates virulent on both genes singly would also be virulent on lines with both genes combined. Another possibility is that Columbus may also have additional undetected resistance gene(s), or modifier(s).

The durable resistance in the previous examples has been conferred by combinations of interacting genes. However, not all interacting pairs of genes confer durable resistance. The leaf rust resistance in the Australian wheat cultivar Gatcher, which has *Lr27* and *Lr31*, two complementary seedling genes conditioning resistance (Singh and McIntosh, 1984b), did not prove to be durable. Isolates of *P. recondita* virulent on Gatcher were detected soon after its release (Singh and

McIntosh, 1984a). The identity and effectiveness of the resistance genes involved in the interacting combinations is most likely important in determining durability of the resistance. Sawhney et al. (1989) suggested that durable resistance may be achieved by combining adult plant resistance with seedling resistance genes.

### 2.3 LEAF RUST RESISTANCE GENE *Lr34*

Leaf rust resistance gene *Lr34* was located on chromosome 7D (Dyck, 1987). It had previously been designated *LrT2* (Dyck and Samborski, 1982).

Resistance gene *Lr34* was initially found in PI58548, where it interacted with *Lr33* to condition enhanced resistance (Dyck, 1977). Dyck and Samborski (1982) determined that the seedling resistance of the cultivars Terenzio (which also has *Lr3* and *Lr30*), Lageadinho, Frontana and five other lines of very diverse origin was conferred by two interacting genes: *Lr34* and *LrT3*. *Lr34* is also present in wheat accessions of very different origen (Shang et al., 1986) and in many CIMMYT cultivars (Dyck, 1987). It has been selected in many breeding programs since it enhances the expression of other resistance genes.

In the field, resistance gene *Lr34* expresses variable pustule size (M reaction) and low percentage of infection (Dyck, 1987). *Lr34* can also be detected in the seedling stage under certain temperature and light conditions as a 2 or 2+ infection type (Dyck and Samborski, 1982). The reduced pustule size in the seedling stage is accompanied by little or no chlorosis (Dyck, 1977).

Drijepondt and Pretorius (1989) studied the expression of adult plant resistance in Thatcher isogenic line *Lr34* at different temperature regimes. Tc*Lr34* had a longer latent period at 13-17 C and fewer uredinia per unit area of flag leaves than Thatcher. Tc*Lr34* had smaller uredinia size than Thatcher at cool temperature (13-17 C) and at an alternating temperature regime of 25-29/13-17 C. No chlorosis associated to the pustules was observed on Tc*Lr34*.

Perez and Roelfs (1987) examined the development of leaf rust epidemics on Tc*Lr34* and Thatcher. Tc*Lr34* exhibited "slow rusting" resistance and had a terminal severity 50% lower than Thatcher.

# Chapter III

# MATERIALS AND METHODS

## 3.1 GENERAL PROCEDURES

F2 families derived from Tc*Lr34* crossed with 14 other near isogenic Thatcher lines were evaluated for seedling resistance in greenhouse tests with two isolates of *P. recondita*. F3 families derived from seedling resistant F2 plants from each cross were evaluated as adult plants in the rust nursery for segregation of *Lr34*. F3 families homozygous for *Lr34* were harvested, and F4 lines were tested for segregation of the seedling resistance. F4 lines from each cross that were homozygous for *Lr34* and the seedling resistance gene were evaluated for enhanced resistance as seedlings in greenhouse tests, using a wide range of isolates virulent and avirulent to the seedling gene. Adult F4 plants were evaluated in greenhouse tests to selected isolates and in the rust nursery to a mixture of leaf rust isolates.

## 3.2 PLANT MATERIAL

Thatcher near isogenic line *Lr34* (RL6058), as the maternal parent, was crossed with the Thatcher isolines with the seedling resistance genes *Lr1* (RL6003), *Lr2a* (RL6016), *Lr3* (RL6002), *Lr3ka* (RL6007), *Lr10* (RL6004), *Lr11* (RL6053), *Lr16* (RL6005), *Lr17* (RL6008), *Lr18* (RL6009), *Lr21* (RL6043), *Lr26* (RL6078), and *LrB* (RL6047), and the Thatcher isoline with the adult plant resistance gene *Lr13* (RL4031) (Table 1) (McIntosh, 1983). Tc*Lr34* was also crossed with the cultivar Columbus

(*Lr16* and *Lr13*).

TABLE 1: Designation, source and chromosome location of 14 leaf rust resistance genes.

| LR GENE | RL# | SOURCE[a] | CHARACTERISTIC RESISTANT INFECTION TYPE | CHROMOSOME LOCATION[a] |
|---|---|---|---|---|
| *Lr1* | 6003 | *T. aestivum* | 0; - ; | 5DL |
| *Lr2a* | 6016 | *T. aestivum* | 0; - 2 | 2Da |
| *Lr3* | 6002 | *T. aestivum* | ; - ;1 | 6BL |
| *Lr3ka* | 6007 | *T. aestivum* | ;1- - 1; | 6BL |
| *Lr10* | 6004 | *T. aestivum* | ;1 | 1AS |
| *Lr11* | 6053 | *T. aestivum* | 1-; - 2+ | 2A |
| *Lr13* | 4031 | *T. aestivum* | | 2BS |
| *Lr16* | 6005 | *T. aestivum* | 1; - 1+N | 4A |
| *Lr17* | 6008 | *T. aestivum* | ;1- -1+ | 2AS |
| *Lr18* | 6009 | *T. aestivum* | ;1- - 2 | 5BL |
| *Lr21* | 6043 | *Ae. squarrosa* | ;1- - 2 | 1DL |
| *Lr26* | 6078 | *S. cereale* | 0; - ; | 1BL |
| *Lr34* | 6058 | *T. aestivum* | 2 - 2+3 | 7D |
| *LrB* | 6047 | *T. aestivum* | 2C - 2+C | unknown |

[a] McIntosh, 1983.

Seedlings were grown in flats and adults in 12.7 or 15.2 cm pots with a mixture of 40% soil, 30% peat and 30% sand, and fertilized weekly with a solution of a 20:20:20 NPK formulation.

### 3.3 LEAF RUST ISOLATES

Virulence formulae of the leaf rust isolates used in the study are shown in Table 2. The isolates were characterized with the Thatcher isogenic lines Tc*Lr1*, Tc*Lr2a*, Tc*Lr2c*, Tc*Lr3*, Tc*Lr9*, Tc*Lr16*, Tc*Lr24*, Tc*Lr26*, Tc*Lr3ka*, Tc*Lr11*, Tc*Lr17*, Tc*Lr30*, Tc*LrB*, and Tc*Lr18*.

TABLE 2: Virulence formulae of 26 selected *P. recondita* isolates.

| ISOLATE | VIRULENCE FORMULA |
|---|---|
| Race 1 | ---- |
| Race 15 | 3,16 |
| 35-88 | 1,3,24 |
| 44-88 | 1,3,16 |
| 63-88 | 1,3,24,26,B |
| 103-88 | 1,2c,B,18 |
| 107-88 | 3,3ka,30,B |
| 119-88 | 1,2c,3,16,17 |
| 122-88 | 1,2c,B,18 |
| 125-88 | 1,2c,3,17 |
| 127-88 | 1,2c,3,17 |
| 133-88 | 1,2a,2c,3 |
| 167-88 | 1,2a,2c,3,17,18 |
| 215-88 | 1,2a,2c,3,11,17,18 |
| 269-88 | 1,3,11 |
| 313-88 | 1,2a,2c,3,11,18 |
| 315-88 | 2a,2c,3,11 |
| 323-88 | 1,2a,2c,3,11 |
| 358-88 | 1,2c,3,3ka,17 |
| 363-88 | 1,2c,3,3ka,17,30,B |
| 364-88 | 1,2c,3,3ka,11,30,B,18 |
| 366-88 | 1,2c,3,9,3ka,B,18 |
| 369-88 | 2c,3,3ka,B,18 |
| 378-88 | 1,2c,3,3ka,B,18 |
| 384-88 | 1,2c,11,30,B,18 |
| 394-87 | 1,2c,3,3ka,11,B,18 |

The adult plant infection type of the isolates on Tc*Lr34* was also determined. No isolate was virulent on adults with *Lr34*, although variation in avirulent infection types between isolates was observed.

Leaf rust urediniospores were increased on seedlings of Little Club wheat kept in isolation.

## 3.4 INOCULATION PROCEDURE

Seedlings were inoculated with a mixture of urediniospores and talc, placed in a dew chamber overnight (sixteen hours minimum) at approximately 18 C. After incubation, flats were placed on a greenhouse bench. Temperature in the greenhouse varied from 18 C to 25 C, with eight to 12 hours of supplemental light from fluorescent bulbs.

Seedlings of F2 families were inoculated with the first isolate of leaf rust when the primary leaves were completely expanded (seven to eight days old) . The second isolate was inoculated onto the second leaf six days later, before pustules from the first isolate had erupted. Seedlings of F4 lines were inoculated as described for the first isolate on F2 seedlings.

Adult plants were inoculated at anthesis with a mixture of urediniospores and talc, or oil as carriers, and placed in a dew chamber overnight (minimum 16 hr). After incubation, plants were placed on a greenhouse bench or in a growth cabinet at a 20-25 C/15-20 C temperature setting during 16 hr/8 hr light/dark cycle. Light was supplied by a combination of fluorescent and incandescent bulbs. Adult plants of F3 families from the cross Tc*Lr34*/Tc*Lr13* were inoculated and incubated in a growth cabinet.

A mixture of leaf rust isolates from the 1988 and 1989 surveys of *P. recondita* in the prairie region of Canada (Table 3) (Kolmer, 1989b; Kolmer, in press) was used as inoculum for rust nursery evaluations of F3 families and homozygous F4 lines in 1989 and 1990 respectively.

TABLE 3: Percentage of isolates from Manitoba and Saskatchewan virulent to 13 leaf rust differential lines in 1988 and 1989.

| RESISTANCE GENE | PERCENT ISOLATES VIRULENT<br>1988[a] | <br>1989[b] |
|---|---|---|
| *Lr1* | 94.4 | 88.5 |
| *Lr2a* | 49.0 | 63.9 |
| *Lr3* | 100.0 | 100.0 |
| *Lr16* | 1.2 | 0.0 |
| *Lr26* | 23.5 | 25.8 |
| *Lr3ka* | 0.0 | 0.4 |
| *Lr11* | 11.6 | 44.3 |
| *Lr17* | 1.6 | 0.0 |
| *Lr30* | 0.0 | 0.4 |
| *LrB* | 0.8 | 0.0 |
| *Lr18* | 2.4 | 0.4 |
| *Lr21* | 0.0 | 0.0 |

[a] Kolmer, 1989b.
[b] Kolmer, in press.

A mixture of susceptible cultivars was arranged as spreader rows perpendicular and at the back of the single row plots. The spreader rows were inoculated at boot stage with a mixture of urediniospores and talc.

## 3.5 CROSSES OF Tc*Lr34* AND THATCHER ISOGENIC LINES WITH SEEDLING RESISTANCE GENES

### 3.5.1 F2 FAMILIES

From each cross, approximately 200 to 300 seedlings of two or more F2 families derived from two different crossed heads were sequentially inoculated on the first and second leaves with Race 1, which was avirulent to all the seedling genes (Table 2), and a second isolate that was virulent on the seedling gene. Only one F2 family of Tc*Lr34*/Tc*Lr10* was available. Race 1 detected the presence of the seedling gene in the segregating populations. The second isolates were used to detect the possible expression of interaction between *Lr34* and the seedling resistance genes.

F2 families were planted in four to six 25.4 cm x 30.5 cm flats, 7.6 cm deep, containing approximately 50 F2 plants, Tc*Lr34*, and the Thatcher lines isogenic for the seedling resistance genes.

Infection types were assessed 10 to 12 days after inoculation, according to the scale proposed by Stakman et al. (1962) (Appendix 1). Infection types 0 to 2 were considered resistant and infection types 3 to 4 susceptible. A range of infection types was indicated with the most frequent infection type listed first (Roelfs, 1988a). Infection types to Race 1 were grouped into resistant, intermediate and susceptible. Infection types to the second isolate were grouped into lower, and equal or higher infection type than either gene singly. Chi square values were estimated for both isolates to test if observed segregation fitted expected segregation ratios.

Ten to 16 F2 plants showing the lowest infection type to isolate Race 1 and to the second isolate were selected per family (20-32/cross).

### 3.5.2 F3 FAMILIES AND F4 LINES

F3 families derived from selected single F2 plants from each cross were tested in the rust nursery as adults to determine which families were segregating for *Lr34*. Seventy to

80 seeds from each family were planted in 2 m rows separated by 0.30 m. Rust severity on the F3 and F4 lines was evaluated on basis of the modified Cobb scale (Peterson et al. 1948)(Appendix 2), and response was evaluated according to Stakman et al.(1962)(Appendix 1). Readings were taken when severity on the susceptible control Thatcher was 80% susceptible (80 S). The five most resistant F3 families homozygous for *Lr34* from each cross were selected and progeny tested in the greenhouse as seedlings (15 plants per F3 family) to determine which families were homozygous for the seedling gene. In this manner, F4 lines homozygous for both resistance genes were obtained. F4 lines (four repetitions) were evaluated in the same manner as F3 families in the 1990 rust nursery.

### 3.5.3 F4 SEEDLING TESTS

To test further the expression of enhanced levels of resistance in the resistance gene pair combinations, two homozygous F4 lines each of *Lr34,2a*, *Lr34,3ka*, *Lr34,11*, *Lr34,16*, *Lr34,17*, *Lr34,18*, *Lr34,21*, and *Lr34*,Columbus, and one F4 line of Tc*Lr34,B* were tested as seedlings to 26 isolates of leaf rust with different virulence phenotypes. The parental Thatcher lines and Thatcher were included as controls. Six to ten seeds per genotype were planted in clumps in 20.3 cm x 26.4 cm flats, 7.6 cm deep. The first and second tests included all the genotypes. The third test included all the genotypes except *Lr34,2a* and *Lr34,21* which had very consistent results in the previous tests. A fourth test was done for selected genotype-isolate combinations. Infection types were assessed as described for F2 seedlings.

### 3.5.4 F4 GREENHOUSE ADULT PLANT TEST

Homozygous F4 lines were tested in the adult plant stage, with specific isolates of leaf rust. Three to four plants of the F4 lines (one F4 line per cross), Tc*Lr34*, seedling resistance gene parents and Thatcher were grown in 15.2 cm pots, and tested with four isolates of leaf rust at anthesis. Infection types were assessed as described for seedlings.

### 3.6 Tc*Lr34* / Tc*Lr13*

Approximately 150 F2 plants from Tc*Lr34*/Tc*Lr13* were evaluated for resistance in the 1989 rust nursery. Sixteen plants with lower rust reaction than Tc*Lr34* and Tc*Lr13*, were selected and progeny tested as adults (12 plants per F3 family) in a growth cabinet. Thirty eight F3 plants were selected from five families that were homozygous resistant and progeny tested in the field in 1990.

In all tests, expression of a higher level of resistance in the gene combinations than in either Thatcher parental line was considered as an indication of the expression of interaction between the resistance genes.

# Chapter IV

# RESULTS

## 4.1 F2 FAMILIES

The infection type segregation of the F2 families to isolate Race 1 is shown in Table 4.

TABLE 4: Segregation to Race 1 of F2 families of Tc*Lr34* crossed with 13 Thatcher isogenic lines with different seedling resistance genes.

| CROSS | No. OF FAMILIES | OBSERVED NUMBERSa | EXPECTED RATIO[b] | $X^2$ | | | |
|---|---|---|---|---|---|---|---|
| | | | | TOTAL[c] | PROB. | HOMO.[*] | PROB. |
| TcLr34/TcLr1 | 2 | 298:10:91 | 3R:1IS | 0.02 | .70-.90 | 0.19 | .50-.70 |
| TcLr34/TcLr2a | 5 | 893:9:246 | 3R:1IS | 4.76 | .01-.05 | 2.03 | .70-.90 |
| TcLr34/TcLr3 | 2 | 103:167:91 | 1R:2I:1S | 2.82 | .20-.30 | 0.39 | .50-.70 |
| TcLr34/TcLr3ka | 2 | 114:134:329 | 1R:3IS | 8.46 | .001-.01 | 0.82 | .50-.70 |
| TcLr34/TcLr10 | 1 | 42:88:50 | 1R:2I:1S | 0.80 | .50-.70 | | |
| TcLr34/TcLr11 | 2 | 253:101:217 | 1R:3IS | 113.53 | < .001 | 2.43 | .10-.20 |
| TcLr34/TcLr16 | 1 | 157:44:88 | 3R:1IS | 65.88 | < .001 | | |
| | 1 | 118:135:55 | 1R:3IS | 29.11 | < .001 | | |
| TcLr34/TcLr17 | 2 | 64:249:199 | 1R:3IS | 42.67 | < .001 | 0.60 | .30-.50 |
| TcLr34/TcLr18 | 2 | 129:105:368 | 1R:3IS | 4.10 | .01-.05 | 3.12 | .05-.10 |
| TcLr34/TcLr21 | 1 | 156:13:71 | 3R:1IS | 12.80 | < .001 | | |
| TcLr34/TcLr26 | 2 | 212:0:85 | 3R:1IS | 2.08 | .10:.20 | 0.06 | .70-.90 |
| TcLr34/TcLrB | 2 | 230:95:218 | 1R:3IS | 87.25 | < .001 | 6.13 | .01-.05 |
| TcLr34/Col | 2 | 306:134:52 | 3R:1IS | 43.02 | < .001 | 0.11 | .70-.90 |

[a] number of seedlings with infection type equal or lower than the seedling gene; higher than the seedling gene and lower than *Lr34*; equal or higher than *Lr34*.

[b] R,I,S: resistant, intermediate and susceptible infection types respectively

[c] calculated according to expected ratio.

[d] homogeneity, between families.

F2 families of Tc*Lr34*/Tc*Lr1* and Tc*Lr34*/Tc*Lr26* segregated in a 3:1 ratio of resistant to intermediate, and susceptible infection types. F2 families of the crosses Tc*Lr34*/Tc*Lr2a* also fitted a 3:1 resistant to intermediate, and susceptible infection types. The significant $X^2$ value (P 0.01-0.05) of the pooled data in the *Lr2a* cross can be explained by a consistent deviation observed in all the families towards the resistant infection type. Infection type segregation fitted a 1:2:1

resistant:intermediate:susceptible ratio in F2 families of Tc*Lr34*/Tc*Lr3* and Tc*Lr34*/Tc*Lr10*.

Segregation of resistant, intermediate, and susceptible progenies in F2 families from the crosses of Tc*Lr34* / Tc*Lr3k*a, Tc*Lr11*, Tc*Lr16*, Tc*Lr17*, Tc*Lr18*, Tc*Lr21*, Tc*LrB* and Columbus did not fit a Mendelian ratio corresponding to a single gene segregation.

When tested with isolates virulent to the seedling resistance genes, no seedlings with enhanced resistance relative to the parental lines were observed in F2 progenies from the crosses of Tc*Lr34* / Tc*Lr1*, Tc*Lr2a*, Tc*Lr3*, Tc*Lr3ka*, Tc*Lr10*, Tc*Lr11* (tested with isolate 323-88), Tc*Lr17*, Tc*Lr26* and Tc*LrB* (Table 5).

Table 5: Number of F2 families, infection type of leaf rust isolates to the seedling gene in 10 crosses which had no seedlings with lower infection than the Thatcher lines.

| CROSS | NUMBER OF FAMILIES | LEAF RUST ISOLATE | INFECTION TYPE TO SEEDLING GENE |
|---|---|---|---|
| Tc*Lr34*/Tc*Lr1* | 2 | 323-88 | HIGH |
| Tc*Lr34*/Tc*Lr2a* | 2 | 323-88 | HIGH |
| Tc*Lr34*/Tc*Lr3* | 2 | 323-88 | HIGH |
| Tc*Lr34*/Tc*Lr3ka* | 2 | 394-88 | HIGH |
| Tc*Lr34*/Tc*Lr10* | 1 | 323-88 | HIGH |
| Tc*Lr34*/Tc*Lr11* | 1 | 323-88 | HIGH |
| Tc*Lr34*/Tc*Lr17* | 2 | 363-88 | HIGH |
| Tc*Lr34*/Tc*Lr26* | 2 | 63-88 | HIGH |
| Tc*Lr34*/Tc*LrB* | 2 | 394-87 | HIGH |
| Tc*Lr34*/Columbus | 2 | RACE 15 | LOW |

F2 families from the cross Tc*Lr34*/Columbus were tested with Race 15 which produced a 2c to 2+c infection type on Columbus. Seedlings with lower infection type than Columbus were not recovered.

When two F2 families of Tc*Lr34*/Tc*Lr11* were tested with isolate 366-88, seedlings with lower infection type than either parent were observed. Seedlings with lower infection type than either gene singly were also recovered in two F2 families from the crosses of Tc*Lr34* with Tc*Lr16*, Tc*Lr18* and Tc*Lr21*, tested with Race 15, 366-88, and 394-87 respectively (Table 6).

TABLE 6: Parental infection types[a] to the second leaf rust isolate and proportion of seedlings with lower infection type than the parental lines in two F2 families from the crosses Tc*Lr34*/Tc*Lr11*, Tc*Lr34*/Tc*Lr16*, Tc*Lr34*/Tc*Lr18* and Tc*Lr34*/Tc*Lr21*[b]

| | INFECTION | NUMBER OF PROGENY | | EXPECTED | | |
|---|---|---|---|---|---|---|
| GENOTYPE | TYPE | OBSERVED | EXPECTED | RATIO | $X^2$ | PROBABILITY |
| *Lr34* | 3- -3+ | | | | | |
| *Lr11* | 2+3 -3 | | | | | |
| F2-1 | ;2 -2+ | 24 | 16.4 | 1 | | |
| | 2+3 -4 | 238 | 245.6 | 15 | 3.76 | .05-.10 |
| F2-2 | ;1+ -2+ | 35 | 50.3 | 3 | | |
| | 2+3 -4 | 233 | 217.7 | 13 | 5.73 | .01-.05 |
| *Lr34* | 3 -3+ | | | | | |
| *Lr16* | 22+ -3+ | | | | | |
| F2-1 | 22+ -2+3 | 40 | 51.2 | 3 | | |
| | 3 -4 | 233 | 221.8 | 13 | 3.02 | .05-.10 |
| F2-2 | 1 -2 | 50 | 54.9 | 3 | | |
| | 2+ -4 | 243 | 238.1 | 13 | 0.54 | .30-.50 |
| Total (Tc*Lr34*/Tc*Lr16*) | | | | | 3.01 | .05-.10 |
| Homogeneity between families | | | | | 0.55 | .30-.50 |
| *Lr34* | 3 | | | | | |
| *Lr18* | 3 -4 | | | | | |
| F2-1 | ;2 -2+3 | 19 | 18.4 | 1 | | |
| | 3 -4 | 275 | 275.6 | 15 | 0.02 | .70-.90 |
| F2-2 | 2 -2+3 | 25 | 18.3 | 1 | | |
| | 3 -4 | 268 | 274.7 | 15 | 2.62 | .10-.20 |
| Total (Tc*Lr34*/Tc*Lr18*) | | | | | 1.55 | .20-.30 |
| Homogeneity between families | | | | | 0.55 | .30-.50 |
| *Lr34* | 3 -3+ | | | | | |
| *Lr21* | ;2- -;2 | | | | | |
| F2-1 | ; -;1 | 41 | 40.9 | 3 | | |
| | ;2 -4 | 177 | 177.1 | 13 | 0.00 | .95-1.00 |
| F2-2 | ;1 -;1+ | 10 | 14.0 | 1 | | |
| | ;2 -4 | 214 | 210.0 | 15 | 1.22 | .20-.30 |

---

[a] Stakman et al., 1962.

[b] *Lr34/Lr11* 366-88; *Lr34/Lr16* Race 15; *Lr34/Lr18* 366-88; *Lr34/Lr21* 394-87.

When tested with isolate 366-88, the first F2 family of Tc*Lr34*/Tc*Lr11* segregated in a 1:15 ratio for progenies with lower infection type than both parental lines, and those with equal or higher infection types, respectively. The second F2 family segregated in a 3:13 ratio although with a significant $X^2$ value (P .01-.05, Table 6). The infection type of *Lr34* to isolate 366-88 was 3-3+ but the infection type of *Lr11* was not completely compatible (2+3 to 3). F2 families from Tc*Lr34*/Tc*Lr16* segregated for progenies with enhanced resistance relative to the parents in a 3:13 ratio when inoculated with Race 15 (Table 6). The infection type of Tc*Lr34* to Race 15 was 3-3 when both families were tested. The infection type of *Lr16* was 33+ when the first family was tested (F2-1) but was 22+ to 2+3 when the second family (F2-2) was tested. F2 families of Tc*Lr34*/Tc*Lr18* segregated in a 1:15 ratio of progenies with lower infection type than both parents, and those with equal or higher infection types respectively, when tested with *Lr18* virulent isolate 366-88. For *Lr21* an avirulent isolate that had a higher infection type than Race 1 was used. In the first F2 family, a 3:13 segregation ratio of infection types lower than *Lr21*, and equal or higher infection types occurred. In the second F2 family, the segregation fitted a 1:15 ratio (Table 6).

F2 plants with infection types lower or equal to the seedling resistant parent when inoculated with Race 1 and/or the second isolate used, were selected from each cross. The F2 derived F3 families were planted in the rust nursery and evaluated for homozygosity of Tc*Lr34*. Selected F2 seedlings with enhanced resistance relative to both parents to the second isolate were not necessarily homozygous for *Lr34* and the seedling gene as predicted from the 1:15 F2 segregation ratio observed in one F2 family (F2-1) of Tc*Lr34*/Tc*Lr11*, both F2 families of Tc*Lr34*/Tc*Lr18* and one F2 family (F2-2) of Tc*Lr34*/Tc*Lr21* (Table 7).

TABLE 7: Number of F3 families from 14 crosses homozygous or segregating for *Lr34* and a seedling resistance gene.

| | Lr34 | | | | SEEDLING GENE | |
|---|---|---|---|---|---|---|
| N° OF FAMILIES<br>CROSS \ | HOMOZYGOUS RESISTANT | SEGREGATING | HOMOZYGOUS SUSCEPTIBLE | SELECTED FAMILIES | HOMOZYGOUS RESISTANT | SEGREGATING |
| 1989 | | | | | | |
| Tc*Lr34*/Tc*Lr2a* | 8 | 5 | 0 | 5 | 5 | 0 |
| Tc*Lr34*/Tc*Lr3ka* | 7 | 16 | 0 | 5 | 5 | 0 |
| Tc*Lr34*/Tc*Lr11*-1 | 2 | 9 | 1 | 2 | 2 | 0 |
| -2 | 6 | 4 | 0 | 3 | 3 | 0 |
| Tc*Lr34*/Tc*Lr16* | 15 | 16 | 1 | 5 | 5 | 0 |
| Tc*Lr34*/Tc*Lr17* | 2 | 9 | 13 | 2 | 2 | 0 |
| Tc*Lr34*/Tc*Lr18* | 13 | 8 | 1 | 5 | 5 | 0 |
| Tc*Lr34*/Tc*Lr21*-1 | 6 | 6 | 0 | 3 | 3 | 0 |
| -2 | 3 | 9 | 0 | 2 | 2 | 0 |
| Tc*Lr34*/Tc*LrB* | 3 | 12 | 9 | 3 | 1 | 2 |
| Tc*Lr34*/Col | 16 | 34 | 7 | 16 | 16 | 0 |
| 1990 | | | | | | |
| Tc*Lr34*/Tc*Lr1* | 7 | 17 | 2 | 5 | | |
| Tc*Lr34*/Tc*Lr3* | 7 | 9 | 5 | 5 | | |
| Tc*Lr34*/Tc*Lr10* | 6 | 8 | 1 | 5 | | |
| Tc*Lr34*/Tc*Lr26* | 8 | 14 | 0 | 5 | | |
| Tc*Lr34*/Tc*Lr13* | 35 | 1 | 0 | 5 | | |

In the first family from the cross Tc*Lr34*/Tc*Lr11*, nine F3 families segregated for *Lr34*, one lacked *Lr34* and only two were homozygous for *Lr34*. In the cross Tc*Lr34*/Tc*Lr18*, from 22 F3 families derived from F2 seedlings expressing enhanced resistance, 13 were homozygous for *Lr34*, eight segregated for *Lr34*, and one was homozygous susceptible. From the second family of the cross Tc*Lr34*/Tc*Lr21*, three seedlings with infection type equal to Tc*Lr21*, and nine with infection type lower than both parents were selected. Nine F2 derived F3 families were heterozygous and three homozygous for *Lr34*.

The other crosses had varying numbers of plants homozygous for *Lr34* and most field selected plants were homozygous for the seedling resistance gene. In the crosses Tc*Lr34*/Tc*Lr17* and Tc*Lr34*/Tc*LrB*, most F3 families were heterozygous or lacked *Lr34*.

### 4.2 F4 SEEDLING TEST

Infection types recorded in the third test are presented for most resistance gene combinations in Table 8. Infection types from the second test are presented for Tc*Lr2a*, Tc*Lr18*, Tc*Lr21* and Columbus and the corresponding homozygous F4 lines. Tc*Lr18* was susceptible in the third test to many isolates that had been classified previously as avirulent. The results from the first and second tests were very consistent for Tc*Lr2a* and Tc*Lr21* and Tc*Lr34,2a* and Tc*Lr34,21*; these lines were not further tested. Infection types from the fourth test are presented for selected genotype-isolate combinations. In the fourth test, Thatcher had infection types 3+, 33+, 3 and 3+, and Tc*Lr34* 3, 23+, 3 and 23, to isolates 44-88, 366-88, 378-88, and 394-87, respectively.

Infection types from one of the two tested F4 lines are given since the two F4 lines within each cross had very consistent readings (Table 8).

Table 8: Seedling infection type of homozygous Thatcher F4 lines and isogenic Thatcher lines, to 26 leaf rust isolates.

| Tc | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LINE | TEST[a] | RACE1 | RACE15 | 35-88 | 44-88 | 63-88 | 103-88 | 107-88 | 119-88 | 122-88 | 125-88 | 127-88 | 133-88 | 167-88 |
| Tc | 2 | 3[b] | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| Lr34[c] | 2 | 3 | 3 | 3+ | 4 | 3 | 4 | 3+ | 3+ | 3 | 4 | 3 | 3 | 4 |
| Tc | 3 | 4 | 33+ | 3 | 33+ | 3+ | 4 | 34 | 3+4 | 34 | 4 | 34 | 3+4 | 4 |
| Lr34 | 3 | 3 | 3 | 33+ | 33+ | 33+ | 3 | 3 | 33+ | 33+ | 33+ | 3 | 33+ | 33+ |
| Lr2a | 2 | 0; | 0; | ;1- | 0; | ; | 1 | ;1 | ;1 | ;1 | ;1 | ;1- | 4 | 3+ |
| F4[d] | 2 | 0 | 0 | 0 | 0 | 0 | 0;1= | 0 | 0;1= | 0;1= | 0;1= | 0; | 3 | 4 |
| Lr3ka | 3 | ;1- | 1-; | ;1 | 1; | 1-; | 1-; | 33+ | ;1- | 1-; | 1-; | 2- | 1- | 1-; |
| F4 | 3 | 0;1= | 0;1- | 0;1 | 0;1- | ;1- | 0;1- | 3 | 0;1- | 0;1= | 0;1- | 0;1- | 1- | 0;1- |
| Lr11 | 3 | 2 | 2 | 2 | 2+ | 2 | 2+3+ | 1-; | 2 | 23 | 1-; | 1 | 2 | 2+3 |
| F4 | 3 | ;11+ | 0;1- | 0;2 | 2+ | 12 | 13-; | 0;1= | ;1- | 23 | 0;1- | 0;1- | ;1-*[e] | ;23 |
| Lr16 | 3 | 1 | 2+3+ | 1 | 22+[f] | 1; | 1 | 1 | 1 | 1 | 1+ | 1 | 1 | 1 |
| F4 | 3 | 0;1-* | 2+3+ | 1 | 22+*[f] | ;1-* | 1 | 1 | 1 | 1 | 1+* | 1* | 1* | 1* |
| Lr17 | 3 | ;1- | 1-; | 1 | 1-; | 1; | 2 | ;1- | 3 | 1 | 3+4 | 34 | 1 | 33+ |
| F4 | 3 | 0;1-* | 0;1-* | 12* | 1*; | ;1* | 2* | ;1-* | 33+ | 1-* | 34 | 33+ | 1* | 33+ |
| Lr18 | 2 | 1 | ;1 | ;1- | ;1- | ;1- | 4 | ;1= | 2 | 4 | 1-; | ;1- | ;1- | 4 |
| F4 | 2 | 0; | 0; | 0; | 0; | 0; | 3+ | 0; | ;1- | 4 | 0;1=* | 0;1= | 0; | 3+ |
| Lr21 | 2 | ;1 | ;2 | ;2 | ;1- | ;1+ | 2 | ;1 | ;1- | ;1 | 2 | 2 | ;1 | 2 |
| F4 | 2 | 0; | 0; | 0; | 0; | 0; | 0; | 0; | 0;1= | 0;1- | 0;1= | 0;1= | 0; | ;1= |
| LrB | 3 | 2 | 2 | 2 | 2+ | 2+ | 4 | 33+ | 2 | 3+ | 2+ | 2 | 2 | 22+ |
| F4 | 3 | 2* | 2 | 2 | 2+ | 2+ | 33+ | 2+3+ | 2 | 34 | 2+ | 2 | 2* | 2+3+ |
| Col | 2 | ;1- | 1 | ;1= | 2 | ;1- | ;1 | ;1- | ;1- | 1 | 2 | 1; | ;1 | 1- |
| F4 | 2 | ;1=* | 12* | 0;1=* | 2* | 0; | 1* | ;* | ;1* | 1* | 0;1- | 1;* | ;1* | ;1-* |

Table 8.

| Tc LINE | TEST | 215-88 | 269-88 | 313-88 | 315-88 | 323-88 | 358-88 | 363-88 | 364-88 | 366-88 | 369-88 | 378-88 | 384-88 | 394-87 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tc | 2 | 3 | 4 | 33+ | 4 | 4 | 4 | 4 | 4 | 4 | 3+ | 3 | 3+ | 3 |
| Lr34 | 2 | 3+ | 4 | 33+ | 4 | 4 | 3+ | 4 | 4 | 4 | 3 | 3 | 3+ | 3 |
| Tc | 3 | 4 | 3+4 | 3+ | 3+4 | 4 | 4 | 3+4 | 4 | 34 | 3+4 | 3+ | 4 | 3+4 |
| Lr34 | 3 | 33+ | 33+ | 33+ | 33+ | 33+ | 3 | 3+ | 33+ | 33+ | 33+ | 3+ | 3+ | 3+ |
| Lr2a | 2 | 3+ | 0; | 3+ | 3+ | 3 | ;1+ | 2 | 2 | 2 | ;1- | ;1- | ;1 | ;1- |
| F4 | 2 | 3 | 0 | 3+ | 4 | 4 | 0;1= | 0; | 0;1 | 0;1 | 0; | 0; | 0; | 0; |
| Lr3ka | 3 | 1; | 1; | 1- | ;1 | 1; | 4 | 34 | 34 | 4 | 4 | 3+ | 1; | 3+ |
| F4 | 3 | 0;1- | 0;1- | 0;1- | 0;1- | 0;1- | 3+ | 33+ | 3+4 | 33+ | 33+ | 3+4 | 0;1 | 33+ |
| Lr11 | 3 | 4 | 3+ | 34 | 3+4 | 4 | 2 | 2+ | 3+ | 23 | 2+ | 2+3 | 2+ | 23 |
| F4 | 3 | 34 | 33+ | 34 | 34 | 4 | 0;1 | ;12 | 3+ | ;2 | 0;2- | 23 | 0;2- | 23 |
| Lr16 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1+ | 1+ | 1 |
| F4 | 3 | 1 | 1-* | 1* | 1 | 1* | 1-; | 1-;* | 1* | 1* | 1* | 1+ | 1+* | 1* |
| Lr17 | 3 | 4 | ;1- | 1 | ;1- | 1; | 4 | 33+ | ;1- | 1-; | 1; | ;1- | 1+ | ;1- |
| F4 | 3 | 34 | ;1-* | 1-;* | 0;1- | 1;* | 33+ | 33+ | ;1-* | 1-; | 0;1-* | ;1-* | 1 | ;1-* |
| Lr18 | 2 | 3 | 1-; | 2 | 1; | 2 | ;1- | ;1= | 3 | 33+[f] | 3 | 2+3[f] | 33+ | 2+3+[f] |
| F4 | 2 | 3 | 0;1- | ;1 | 0;1-* | ;1- | ;1=* | 0; | 23 | 2-2+[f] | 22+ | 2+3[f] | 33+ | 2[f] |
| Lr21 | 2 | ;2 | 22+; | ;1 | 2; | ;1 | 2 | ;2 | ;12 | 2+ | 2 | 1 | 2 | ;2 |
| F4 | 2 | 0; | ;1- | 0; | 0;1- | 0; | 0; | 0; | 0; | 0;1- | ;1- | 0; | 0;1 | 0;1- |
| LrB | 3 | 33+ | 2 | 2 | 2+ | 2 | 2+ | 2+3+ | 4 | 34 | 33+ | 3+ | 4 | 33+ |
| F4 | 3 | 33+* | 2* | 2 | 2+ | 2* | 2+ | 2+3+ | 34 | 33+ | 3+ | 3+ | 4 | 33+ |
| Col | 2 | ; | ;1 | 1- | 1; | ; | ; | ; | ;1 | 1 | ;1- | ;1 | 1+ | 1 |
| F4 | 2 | ;1-* | 0;1= | ;1-* | 1;* | ;1-* | 0; | 0; | ;1-* | ;1* | ;1-* | ;1* | 1+* | ;1* |

[a] 2 second test; 3 third test except indicated by [f].
[b] Infection types: Stakman et al., 1962.
[c] TcLr line.
[d] F4 line from the cross Tc*Lr34* and the preceding seedling gene Thatcher line.
[e] *: reduced chlorosis or necrosis compared with the seedling gene parental line.
[f] fourth test.

All 20 isolates virulent to *Lr2a* had lower infection type on the Tc*Lr34,2a* F4 line (0 to 0;1) than on Tc*Lr2a* (0; to 2). None of the six isolates virulent to *Lr2a* had lower infection types on Tc*Lr34,2a* than on either Tc*Lr2a* or Tc*Lr34*. Tc*Lr34,3ka* expressed enhanced resistance (0;1- to ;1-) relative to *Lr3ka* (;1- to 2-) to 17 of 18 isolates avirulent to *Lr3ka*. Enhanced resistance was not observed with isolates virulent to Tc*Lr3ka*. Seventeen of 20 *Lr11* avirulent isolates had lower infection type on the Tc*Lr34,11* F4 lines (0;1= to 23) than on Tc*Lr11* (1-; to 2+3). The F4 lines did not express enhanced resistance relative to the parents to three avirulent and six virulent isolates to Tc*Lr11*. Five of 26 *Lr16* avirulent isolates had lower infection type on Tc*Lr34,16* F4 lines (0;1- to 1-) than on Tc*Lr16* (1; to 1). Sixteen isolates had reduced chlorosis on the F4 lines relative to *Lr16*. Eleven of 19 *Lr17* avirulent isolates had lower infection type on the Tc*Lr34,17* F4 lines (0;1- to 1) than on Tc*Lr17* (;1- to 1+). Sixteen *Lr17* avirulent isolates had less chlorosis on the F4 lines than on Tc*Lr17* Isolates 35-88 and 44-88 had infection type slightly higher on the Tc*Lr34,17* F4 lines than on Tc*Lr17*. Seventeen of the 18 isolates avirulent to Tc*Lr18* had a lower infection type on the Tc*Lr34,18* F4 line (0; to 2) than on Tc*Lr18* (;1= to 2+3+). Three isolates avirulent to Tc*Lr18* produced less chlorosis on Tc*Lr34,18* than on Tc*Lr18*. Isolates 364-88, 366-88 and 369-88 were virulent to Tc*Lr18* and Tc*Lr34*, and had avirulent infection types (23, 2-2+ and 22+ respectively) to Tc*Lr34,18*. Enhanced resistance was expressed in the second and fourth tests to the same isolates virulent to *Lr18*. Isolates 378-88 and 394-87 were virulent to Tc*Lr18* and avirulent to the Tc*Lr34,18* F4 line in the second test. The five isolates (364-88, 366-88, 369-88, 378-88 and 394-88), produced high infection types to Tc*Lr34,18* and to both parents in the third test. Isolates 44-88, 119-88, 313-88 and 323-88 were avirulent to Tc*Lr18* and had a lower infection type to Tc*Lr34,18* than either parent in the first, second and fourth tests. The same isolates were virulent to Tc*Lr18* and three were virulent to Tc*Lr34,18* in the third test, when higher temperatures were registered in the greenhouse. Only isolate 313-88 had high infection type on Tc*Lr18* and low infection type on Tc*Lr34,18* in the third test. All isolates tested were avirulent to Tc*Lr21* and produced lower infection types on Tc*Lr34,21* (0; to ;1-) than on Tc*Lr21* (;1- to ;22+). None of the isolates produced lower infection types on Tc*Lr34,B* compared to Tc*LrB*. One virulent and four of the *LrB* avirulent isolates had reduced chlorosis on Tc*Lr34,B* compared to Tc*LrB*. All isolates tested were avirulent to Columbus. Thirteen isolates had lower infection type on the Tc*Lr34*,Columbus F4 lines (0; to ;1) than on Columbus (;1= to 1) and four had a higher infection on the F4 lines than on Columbus. Twenty isolates had less chlorosis on the Tc*Lr34*,Columbus lines than on Columbus.

### 4.3 F4 ADULT PLANT TEST

Adult plants of Tc*Lr34* expressed variable infection types, and percentage of infection between and within tests, with different isolates of leaf rust. Infection types of Tc*Lr34* were characterized by a few moderate size pustules at the base of the flag leaf and very few smaller pustules towards the tip of the leaf. Chlorosis was not associated with the pustules. Very light hypersensitive flecks were observed throughout the leaves. All isolates tested were avirulent on adults of Tc*Lr34* (Table 9). Tc*Lr34* always had lower infection type and severity of infection than the Thatcher susceptible control.

Table 9: Adult plant infection types[a] of single gene lines, and combinations of two leaf rust resistance genes.

| Tc LINE | TEST | LEAF RUST ISOLATE<br>Race1 | Race15 | 44-88 | 125-88 | 215-88 | 323-88 | 363-88 | 364-88 | 366-88 |
|---|---|---|---|---|---|---|---|---|---|---|
| Tc | 1 | 3+4 | 3+4 | 3+4 | 3+4 | | | | | 3+4 |
| Tc*Lr34*[b] | 1 | 0;12- | 0;12- | 0;1 | 0;12- | | | | | 0;12 |
| Tc*Lr16* | 1 | 2c | 2+ | 2c | 2+ | | | | | |
| F4[c] | 1 | 0;*[d] | 0;12-* | 0;1* | 0;* | | | | | |
| Tc*Lr21* | 1 | 2 | 2 | 2 | | | | | | 22+ |
| F4 | 1 | 0;* | 0;* | 0;* | | | | | | 0;1- |
| Tc | 2 | 4 | | | | 4 | | 4 | 4 | 4 |
| Tc*Lr34* | 2 | 0;23 | | | | 0;12 | | 0;12+ | 0;23 | 0;12 |
| Tc*Lr3ka* | 2 | 0;1 | | | | | | 4 | 4 | 4 |
| F4 | 2 | 0; | | | | | | 0;12 | 0;23 | 0;12+ |
| Tc*Lr18* | 2 | 2c | | | | 4 | | | 4 | 3+ |
| F4 | 2 | 0;1-* | | | | 0;1 | | | 0;1 | 0;1 |
| Tc | 3 | 4 | | | 4 | 4 | 4 | 4 | 4 | 4 |
| Tc*Lr34* | 3 | - | | | 0;13 | 0;13 | 0;12 | 0;1 | 0;13 | ;13 |
| Tc*Lr2a* | 3 | 0; | | | | 4 | 3+ | 0;1 | | |
| F4 | 3 | 0;* | | | | 0;13 | 0;12+ | 0;* | | |
| Tc*Lr11* | 3 | 2c | | | | 4 | 4 | | 4 | |
| F4 | 3 | 0;* | | | | 0;12 | 0;1 | | 0;13 | |
| Tc*Lr17* | 3 | ;1-n | | | 12c | 4 | | ;1n | | |
| F4 | 3 | 0;* | | | 0;13* | ;23+ | | 0;12* | | |
| Tc*LrB* | 3 | 2+ | | | | | | 4 | 4 | 4 |
| F4 | 3 | 0;1* | | | | | | 0;12 | 0;13 | ;13 |

[a] Stakman et al., 1962.
[b] Tc line.
[c] F4 line from the cross Tc*Lr34* and the preceding seedling gene.
[d] * : reduced necrosis or chlorosis compared with the seedling resistance gene parental line.

Race 1 and isolate 125-88 had lower infection type, and Race 15 and isolate 44-88 had the same infection type on Tc*Lr34,16* than on Tc*Lr34*. The four isolates had less chlorosis on Tc*Lr34,16* than on Tc*Lr16*. The four isolates tested had lower infection type on Tc*Lr34,21* than on Tc*Lr34* or Tc*Lr21*.

Three of the isolates had a reduced amount of chlorosis on Tc*Lr34,21* compared to Tc*Lr21*. Race 1 had lower infection type and less chlorosis on Tc*Lr34,3ka* than on Tc*Lr34* and *Lr3ka*. *Lr3ka* virulent isolates 363-88, 364-88, and 366-88 had lower, the same and higher infection types on Tc*Lr34,3ka*, respectively, compared to Tc*Lr34*. All isolates tested (one *Lr18* avirulent and three *Lr18* virulent isolates) had a lower infection type and percentage of infection on Tc*Lr34,18* than on Tc*Lr34* and Tc*Lr18*. The two *Lr2a* avirulent isolates had lower infection type and less chlorosis on Tc*Lr34,2a* relative to Tc*Lr2a*. *Lr2a* virulent isolates 215-88 and 323-88 had the same and higher infection type on Tc*Lr34,2a* respectively, than on Tc*Lr34* . *Lr11* virulent isolates 215-88 and 323-88 had lower infection type on Tc*Lr34,11* than on *Lr34,* while 364-88 had the same infection type on Tc*Lr34,11* than on Tc*Lr34*. Isolate 125-88 had the same infection type on Tc*Lr34,17* than on either parental line. Isolates 215-88 and 363-88 had higher infection types on Tc*Lr34,17* than on Tc*Lr34* and Tc*Lr17*. Isolates avirulent on Tc*Lr17* expressed less chlorosis on Tc*Lr34,17* than on Tc*Lr17*. *LrB* virulent isolates 364-88 and 366-88 had the same infection type and 363-88 had higher infection type on Tc*Lr34,B* than on Tc*Lr34*.

## 4.4 RUST NURSERY TESTS

F3 and F4 lines were evaluated for leaf rust resistance in the 1989 and 1990 rust nurseries when the susceptible check

Thatcher had a severity and response ratings of 80% susceptible (80 S) and 90 S respectively (Table 10).

Table 10: Range of field severity and response of Thatcher isogenic lines and homozygous resistant F3 and F4 lines in 1989 and 1990.

| | FIELD SEVERITY[a] AND RESPONSE[b] | | | |
|---|---|---|---|---|
| R GENE | Tc ISOGENIC LINE | F3 FAMILIES[c] | Tc ISOGENIC LINE | F4 LINES[d] |
| 1989 | | | 1990 | |
| Thatcher | 80S | | 90S | |
| *Lr34* | 2-20M | | T-20M | |
| *Lr2a* | 80S | T-10M | 90S | T-20M |
| *Lr3ka* | 50MR-50MRMS | 5VR-5RMR | 20R-50MR | 5VR-5R |
| *Lr11* | 70S | T-5M | 90S | T-20M |
| *Lr16* | 50R | 5VR | 60R-70RMR | 2VR-5VR |
| *Lr17* | 50RMR-50M | 5VR-2M | 40RMR-60RMR | 5VR-5VR2R[e] |
| *Lr18* | 50VR5M | 5VR | 40VRTR-60VRTR | 5VR |
| *Lr21* | 40VR2R-40VR2M | 2VR | 20VR5R-30VR2M | 2VR-5VR |
| *LrB* | 60MRMS-70MRMS | T-10M | 70RMR-80MRMS | T-5M |
| Columbus | 10VR5R-50VR5R | 5VR | 20VR5R-40VR5R | 5VR |
| *Lr13*[f] | 40MRMS-50MRMS | 5VR | 50RMR-60RMR | 5VRTR-5VR5R |
| 1990 | | | | |
| Thatcher | 90S | | | |
| *Lr34* | T-20M | | | |
| *Lr1* | 90S | T-30M | | |
| *Lr3* | 90S | T-30M | | |
| *Lr10* | 90S | T-30M | | |
| *Lr26* | 60MS-70MS | T-20M | | |

---

[a] Modified Cobb scale (Peterson et al., 1948).

[b] Stakman et al.(1962). VR very resistant; R resistant; MR moderately resistant; MS moderately susceptible; S susceptible; M mixed.

[c] F3 families from the cross of Tc*Lr34* and the Thatcher isogenic lines.

[d] F4 line from the cross of Tc*Lr34* and the Thatcher isogenic lines.

[e] 5% severity very resistant (VR) response and 2% severity resistant (R) response on the same leaf.

[f] F2 family.

Tc*Lr34* had leaf rust ratings of 2-20 M and T-20 M in 1989 and 1990 respectively (Table 10). Rust reactions on most genotypes was very similar between the two years. Thatcher lines with *Lr1*, *Lr2a*, *Lr3*, *Lr10* and *Lr11* were almost, or as susceptible as Thatcher. Tc*Lr26* had a high percentage of infection and a moderately susceptible reaction. The Thatcher isolines with single resistance genes *Lr3ka*, *Lr13*, *Lr17* and *LrB* had varying degrees of moderate resistance. The Thatcher lines with *Lr16*, *Lr18*, *Lr21* and Coluumbus were very resistant.

The ratings of F3 families in 1989 are for families homozygous for *Lr34* and the seedling resistance gene and in 1990 for families homozygous for *Lr34*.

Tc*Lr34,3ka*, Tc*Lr34,13*, Tc*Lr34,16*, Tc*Lr34,17*, Tc*Lr34,18*, Tc*Lr34,21* and Tc*Lr34*,Columbus were more resistant than Tc*Lr34* and the other parental lines in 1989 and 1990. Tc*Lr34,1*, Tc*Lr34,2a*, Tc*Lr34,3*, Tc*Lr34,10*, Tc*Lr34,11*, Tc*Lr34,26* and Tc*Lr34,B* had the same or similar level of resistance as Tc*Lr34* in both years.

## Chapter V

## DISCUSSION

Resistance genes *Lr1*, *Lr2a*, and *Lr26* segregated in a dominant manner in F2 families when Thatcher lines with these genes were crossed with Tc*Lr34*, as indicated by the 3:1 resistant to intermediate and susceptible ratios when tested with Race 1. Progenies in these crosses segregated into two discrete classes: resistant and susceptible, with very few or no seedlings with intermediate infection type. Genes *Lr3* and *Lr10* expressed partial dominance in progenies of the crosses Tc*Lr34*/Tc*Lr3* and Tc*Lr34*/Tc*Lr10* as indicated by the 1:2:1 resistant:intermediate:susceptible segregation ratio.

The F2 segregation of infection types in crosses of Tc*Lr34* and Thatcher isogenic lines with seedling genes that express low or intermediate resistant infection types (*Lr3ka*, *Lr11*, *Lr16*, *Lr17*, *Lr18*, *Lr21* and *LrB*), and the cultivar Columbus which has *Lr16*, did not fit expected single gene ratios. There was a varying proportion of seedlings with intermediate infection type in all F2 families from these crosses. The observed non Mendelian ratios were most likely caused by modification of the expression of the seedling genes by *Lr34*, which was also segregating independently in the F2 families. The F4 seedling tests indicated that the homozygous combinations of *Lr34* and *Lr2a*, *Lr3ka*, *Lr11*, *Lr16*, *Lr17*, *Lr18* and *Lr21* were more resistant to Race 1 than were the corresponding seedling gene. In addition, Tc*Lr34,16* and

Tc*Lr34,17* had less chlorosis to Race 1 than Tc*Lr16* and Tc*Lr17* respectively. It is possible that genotypes heterozygous for *Lr11*, *Lr16*, *Lr17*, *Lr18*, *Lr21* and *LrB*, and/or *Lr34* also express lower infection type than either gene singly.

The presence of *Lr34* apparently did not affect the expected segregation ratios of the seedling genes that expressed a very low infection type (0; or ;, *Lr1*, *Lr2a* and *Lr26*), and *Lr3* and *Lr10* which had ; to ;1 and ;1 infection types, respectively.

Another factor that could have influenced the classification of infection types is the environmental sensitivity of *Lr34*. Dyck and Samborski (1982), determined that the cultivar Terenzio had four resistance genes: *Lr3*, *Lr30* and two complementary genes, *Lr34* and *LrT3*. Extreme variability of infection types was observed in segregating material from the cross Thatcher/Terenzio. Within the F3 lines that were classified as resistant, infection types ranging from 0;1 to 2++ or 3 were found. The same complementary genes *Lr34* and *LrT3* were also found in other accessions. The original accessions as well as the derived lines had variable infection types due to changes in environmental conditions, particularly temperature and light. Infection types were lower under cool temperature and low light intensity.

The expression of enhanced resistance in F2 families tested with the second isolate was consistent with the results of the F4 seedling test. The combinations *Lr34,2a*, *Lr34,3ka*, *Lr34,11* and *Lr34,17* tested with the virulent isolates 323-88, 394-87, 323-88 and 363-88 respectively; and Tc*Lr34*,Columbus tested with Race 15, avirulent to Columbus, did not express enhanced resistance in the F2 families, and also in the F4 seedling test. Enhanced resistance relative to both single gene parents was expressed in F2 families of Tc*Lr34* / Tc*Lr11*, Tc*Lr16*, Tc*Lr18*, and Tc*Lr21* and in the F4 from these crosses tested with isolates 366-88, Race 15, 366-88 and 394-88 respectively.

Tc*Lr34,18* was the only combination that expressed enhanced resistance in the seedling stage relative to both parents when inoculated with certain isolates virulent to *Lr18*. This occurred in the F2 families and F4 lines tested with isolate 366-88, and F4 lines tested with other *Lr18* virulent isolates in the F4 seedling test.

Isolates 366-88 and Race 15 had an intermediate to high infection types on Tc*Lr11* and Tc*Lr16*. It is possible that these isolates are heterozygous at the corresponding virulence loci. When heterozygous strains of *Puccinia graminis* are involved in host-pathogen interaction, an increase in the low infection type can occur, which can be altered by temperature to become a high infection type (Roelfs 1988a, 1988b). This may explain the different infection type of the isolates when tested over a period of time.

It appears that enhanced resistance in the resistance gene combinations is mainly expressed with isolates avirulent to the seedling gene, as was observed in the crosses Tc*Lr34*/Tc*Lr11*, Tc*Lr34*/Tc*Lr16* and Tc*Lr34*/Tc*Lr21*. When a third family from the cross Tc*Lr34*/Tc*Lr11* was tested with an isolate that was completely compatible with *Lr11*, no seedlings with enhanced resistance were found.

The variable expression of *Lr34* also influenced the segregation ratios observed in the F2 families of Tc*Lr34*/Tc*Lr11* and Tc*Lr34*/Tc*Lr21* when tested with isolates 366-88 and 394-87 respectively. In both cases, one F2 family segregated 1:15 for seedlings with lower infection type than either parent while in the other F2 family a 3:13 ratio was observed. Also *Lr34* and *LrT3* acted in a complementary recessive manner in some tests while in other tests the genes could be detected individually, fitting duplicate gene ratios (Dyck and Samborski, 1982). This variation might be attributed to environmental variation.

Both F2 families of the cross Tc*Lr34*/Tc*Lr16* tested with Race 15 segregated in a 3:13 ratio, indicating that one of the genes expressed in a dominant and the other in a recessive manner in the interaction between the two genes. Both F2 families of the cross Tc*Lr34*/Tc*Lr18* tested with isolate 366-88 segregated in a 1:15 ratio, as expected from the segregation of two recessive complementary genes.

Further evidence on the variability in phenotypic expression of the genotypes for rust infection type in crosses involving *Lr34*, was provided by the field progeny test of the selected F2 plants. Selected F2 plants that displayed enhanced resistance relative to both parents were not necessarily homozygous for both resistance genes. Segregating F3 families were derived from plants presumably homozygous for both resistance genes in the Tc*Lr34*/Tc*Lr11*, TcLr34/Tc*Lr18* and Tc*Lr34*/Tc*Lr21* crosses. Selected F2 plants from the crosses Tc*Lr34* / Tc*Lr1*, Tc*Lr3*, Tc*Lr3ka*, Tc*Lr10*, Tc*Lr17*, Tc*Lr26* and Tc*LrB* were almost all homozygous or heterozygous for *Lr34*. Only one homozygous F2 plant was selected in the Tc*Lr34*/Tc*LrB* cross. This is probably because in combinations with *LrB*, *Lr34* reduces the chlorosis which is characteristic of the *LrB* resistant infection type. None of the selected F2 plants that had a 2c infection type to Race 1 were homozygous for *Lr34*. The single homozygous F2 plant had a 2 infection type. This characteristic of *Lr34* in reducing chlorosis in avirulent infection types also explains the scarcity of selected homozygous F2 plants in the Tc*Lr34*/Tc*Lr17* cross.

Singh and McIntosh's results (1987) also illustrate the difficulty encountered in classifying individual F2 seedling phenotypes and demonstrated the value of progeny testing. They classified F2 seedlings from crosses of Chinese Spring and W3746 for infection type to the stem rust pathotype 34-1,2,3,4,5,6,7. Half of the seedlings classified as resistant were homozygous resistant, and half segregated as F3 lines. Non segregating resistant, segregating or nonsegregating susceptible F3 lines were derived from seedlings with intermediate or susceptible infection type.

The F4 seedling tests confirmed that *Lr34* interacted with seedling genes mostly to isolates avirulent to the seedling genes, except for the combination *Lr34,18*, which had enhanced resistance to specific isolates virulent to *Lr18*. *Lr34* enhanced the resistance of effective resistance genes. Other studies examining rust resistance gene combinations also concluded that the enhanced resistance is mostly expressed to isolates avirulent to at least one of the genes. Samborski and Dyck (1982), determined that in general isolates avirulent to both of the combined genes had lower infection types than expected, and several combinations of genes also expressed enhanced resistance to isolates avirulent to one of the genes and virulent to the other. Knott and Weller (1988), detected enhanced resistance in seedling tests only to isolates avirulent to the stem rust resistance genes they were studying in combinations.

The expression of enhanced resistance in Tc*Lr34,18* to certain *Lr18* virulent isolates was consistent in the second and fourth tests. In warmer temperatures (third test) the higher infection type observed on Tc*Lr18* might be due to temperature sensitivity (Dyck and Johnson, 1983). In these conditions, the enhanced resistance of Tc*Lr34,18* to isolates virulent to *Lr18* was not expressed. Three isolates that were avirulent to *Lr18*, and expressed enhanced resistance to Tc*Lr34,18* in the second and fourth tests were virulent to *Lr18* and Tc*Lr34,18* in the third test. Apparently the expression of enhanced resistance in the Tc*Lr34,18* F4 lines can be affected by temperature.

In the seedling stage, certain seedling genes interacted with *Lr34* to more isolates than other genes. The combinations *Lr34,2a* and *Lr34,21* expressed enhanced resistance to all isolates avirulent to the seedling genes. Tc*Lr34,3ka*, Tc*Lr34,11* and TcLr34,18 expressed enhanced resistance to the majority of the avirulent isolates. *Lr34,17* and Tc*Lr34*,Columbus expressed enhanced resistance to some isolates and Tc*Lr34,16* to only a few isolates. The combination *Lr34,B* did not express enhanced resistance to any isolate. Dyck and Kerber (1985) stated that not all the genes expressing an intermediate infection type to avirulent isolates interact with other resistance genes for enhanced resistance.

The reduced chlorosis associated with the resistant infection types in the F4 lines was also variable. Many isolates caused reduced chlorosis on Tc*Lr34,16* and Tc*Lr34,17* and a few isolates on Tc*Lr34,11*, Tc*Lr34,18* and Tc*Lr34,B*. The reduction of chlorosis has not been previously reported in leaf rust resistance gene combinations with *Lr34*. The expression of reduced chlorosis in certain isolate-F4 line combinations is probably related to the expression of *Lr34*. The reduced pustule size typical of *Lr34* is accompanied by little or no chlorosis or necrosis in adult plants (Drijepondt and Pretorius, 1989) and in seedlings (Dyck, 1977).

The typical reaction of Tc*Lr34* in the greenhouse adult test was similar to the description of Tc*Lr34* by Drijepondt and Pretorius (1989). The infection types of the adult homozygous F4 lines were similar to the seedling tests for the majority of the F4 line-isolate combinations. Isolates avirulent to the seedling genes had lower infection types than expected while isolates virulent to the seedling genes usually had similar infection types on *Lr34* and the homozygous F4 lines. Certain F4 lines displayed enhanced resistance as adults to specific isolates avirulent and virulent to the seedling genes that was not observed in the seedling tests (Tc*Lr34,16*-125-88, Tc*Lr34,18*-215-88, Tc*Lr34,3ka*-363-88, Tc*Lr34,11*-215-88, and Tc*Lr34,11*-323-88 F4 line-isolate combinations). However in most cases involving a virulent isolate, the difference in infection type between F4 lines and Tc*Lr34* was small and may have been caused by nonuniformity in density and amount of inoculum applied, or the variability in infection types observed in genotypes with *Lr34*. The difference in infection types of Tc*Lr34* and the homozygous F4 lines in the adult plant stage was small and difficult to evaluate to many of the isolates. Pustule size and percentage of infection were variable between and within tests with different isolates. The expression of resistance of *Lr34* appears to involve quantitative aspects such as receptivity and spore production (Drijepondt and Pretorius, 1989). A quantitative comparison of the components of resistance affected by *Lr34* singly and in combinations with other resistance genes in the adult plant stage would allow a more precise characterization of the interaction between the resistance genes to specific isolates.

Confirming the results obtained in the seedling stage, in adult plant tests *Lr34* interacted with *Lr18* for enhanced resistance to isolates virulent to *Lr18*. Tc*Lr34,18* had a higher level of resistance than Tc*Lr34* to the three *Lr18* virulent isolates tested. Two of these isolates had lower infection type on the F4 lines than on the genes singly in the seedling stage. Isolate 215-88 only expressed lower infection type on the F4 line in the adult plant test.

Five F4 line-isolate combinations in the adult plant tests had higher infection types on the homozygous F4 lines than on Tc*Lr34*, although the differences were usually small. The largest differences were observed in the Tc*Lr34,17* - isolate 215-88 combination (0;13 and ;23+ on Tc*Lr34* and Tc*Lr34,17* respectively). This was also observed in the seedling stage in the Tc*Lr34,17* - 35-88, Tc*Lr34,17* - 44-88, Tc*Lr34,B* - 167-88 and Tc*Lr34*,Columbus - Race 15, 103-88, 119-88 and 323-88 F4 line - isolate combinations. Apparently certain combinations of genes express less resistance to specific leaf rust isolates than the same genes when present singly. This effect was also reported by Knott and Weller (1985). In field tests the ineffective stem rust resistance gene *Sr11* increased susceptibility in combinations with other resistance genes to stem rust race 15 B. *Sr7a* appeared to increase susceptibility to race 56 in the combination *Sr7a,8a,9b* (Knott and weller,1988).

In the greenhouse adult plant test *Lr17* was resistant to certain isolates that had been classified as virulent in the seedling tests. The adult plant test was done in July, when the higher temperatures could have affected the infection type since *Lr17* has been reported to be more resistant at higher temperatures than at lower temperatures (Dyck and Johnson, 1983).

The rust nursery resistance of the Thatcher isogenic lines was consistent with the percentage of virulence to the individual genes in the initial inoculum, and in the yearly rust surveys (Kolmer, 1989b, Kolmer, in press). Genes to which there was an intermediate to high percentage of virulence in the leaf rust population (*Lr1*, *Lr2a*, *Lr3*, *Lr10*, *Lr11*, and *Lr26*), generally had a high severity, and susceptible or moderately susceptible response, identical or close to the susceptible check Thatcher. Thatcher lines *Lr3ka*, *Lr16*, *Lr17*, *Lr18*, *Lr21* and *LrB* and Columbus expressed resistant or moderately resistant reactions in the rust nursery. The levels of virulence to these lines in the virulence surveys has been very low or nil. The only important difference in the rust populations between the two years was an increase in the percentage of virulence to *Lr11* from 11.6% in 1989 to 44.3% in 1990, which was reflected in an increase from 70 to 90% of infection on *Lr11*.

The F4 lines that expressed enhanced resistance compared to the parental Thatcher lines were the lines whose corresponding seedling resistance gene expressed some degree of effective resistance in the rust nursery when present singly. In general this agrees with the seedling tests in which the F4 lines expressed enhanced resistance mostly to isolates that were avirulent to the seedling gene.

F4 lines that expressed enhanced resistance in the field did not necessarily express enhanced resistance to most of the avirulent isolates in the seedling tests. As example the Tc*Lr34,16* line had enhanced resistance relative to either Tc*Lr34* or Tc*Lr16* in the rust nursery, but expressed enhanced resistance to only five of 26 *Lr16* avirulent isolates in the seedling tests. In the F4 adult plant test, *Lr34,16* was more resistant than either gene singly to the *Lr16* avirulent isolate 125-88. This interaction for superior resistance was not observed in the seedling test. It is possible that more interactions for enhanced resistance occurs in the adult plant stage and/or field conditions than in the seedling stage, since *Lr34* is effective mostly in the adult plant stage. Knott and Weller (1988) also detected more interactions between stem rust resistance genes in field adult tests than in seedling tests.

F4 lines that did not express enhanced resistance in the field were those whose seedling genes did not condition effective resistance when present singly. The F4 line Tc*Lr34,B* did not express enhanced resistance to isolates avirulent to *LrB* in seedling tests, and it was not more resistant than Tc*Lr34* in the rust nursery even though virulence to *LrB* is very low in the rust population.

Tc*Lr34,13* was more resistant than Tc*Lr34* or Tc*Lr13* both years in the rust nursery. The combination *Lr34,13* has been identified as one of the most durable sources of leaf rust resistance (Roelfs, 1988b). The percentage of isolates virulent to the adult plant gene *Lr13* is not regularly determined, but some tests indicate that isolates virulent to *Lr13* are present in the North American wheat leaf rust population. In spite of this, the *Lr34,13* combination expressed more resistance than either *Lr34* and *Lr13* singly, and continues to provide adequate protection to leaf rust. The combination *Lr34,13* is present in the cultivars Frontana, Era, Chris, and probably Americano 44d (Roelfs, 1988b). Schafer et al. (1963) obtained lines with enhanced seedling resistance by intercrossing the cultivars Aniversario, La Prevision 25, Frontana and Exchange. According to later studies, Frontana has *Lr34,13,T3* (Dyck et al. 1966, Dyck and Samborski, 1982) and Exchange has *Lr10*, *Lr16* (Anderson, 1961) *Lr12* (Dyck et al., 1966) and probably *Lr34* (Samborski and Dyck, 1982). Aniversario and La Prevision 25, two Argentinean cultivars, most likely derive their resistance from Americano 44d (Perez and Roelfs, 1989) which probably has *Lr34,13* or *Lr34,12*

(Roelfs, 1988b). The higher level of resistance in the lines obtained by Schafer et al. is most likely due to *Lr34* and/or *Lr13* interacting with other resistance genes, since it was demonstrated by Kolmer (unpublished) that *Lr13* also interacts for superior resistance with seedling genes that condition effective resistance.

In the cross Tc*Lr34*/Columbus three resistance genes are segregating since Columbus has *Lr13* and *Lr16*. The presence of *Lr34* was determined since the F4 lines were more resistant than Columbus in the rust nursery. It was not possible to determine the presence of *Lr13* since the field responses of Tc*Lr34*/Columbus and Tc*Lr34*/Tc*Lr16* F4 lines were equally resistant. The seedling tests detected the presence of *Lr16*, since the F4 lines were resistant to all isolates avirulent to *Lr16*. Infection types from the F4 seedling tests indicate that *Lr13* is probably present in the Tc*Lr34*/Columbus F4 lines since F4 lines of Tc*Lr34*/Columbus had lower infection types than Tc*Lr34,16* to most isolates. The isolates that were virulent to *Lr16* and Tc*Lr34,16* F4 lines in the first and second tests, were avirulent to Columbus and Tc*Lr34*,Columbus F4 lines. If Columbus does not have another resistance gene or modifier, the only possible resistance gene combination that confers seedling resistance to *Lr16* virulent isolates is *Lr13,16*, according to results obtained by Samborski and Dyck (1982). Therefore it appears that the F4 lines actually possess the *Lr34,16,13* combination. As the genetic background can modify the expression of resistance genes, the presence of *Lr13* in the F4 lines from the Tc*Lr34*/Columbus cross should be confirmed through genetic analysis.

*Lr34* appears to express its resistance in a manner similar to that of other resistance genes that confer slow rusting or partial resistance in several cereal rust diseases. Dyck (1977) stated that the phenotype of reduced pustule size expressed by *Lr34* may be one of the factors considered as a slow rusting characteristic.

Partial resistance has been characterized by a reduced rate of epidemic development resulting from some or all of the following characteristics in the resistant cultivars: lower receptivity, longer latent period, lower spore production, smaller uredinium size, and shorter infectious period (Parlevliet, 1985). Drijepondt and Pretorius (1989) reported that *Lr34* affected infection frequency, latent period and pustule size. Another similarity between partial resistance and *Lr34* is that both are best expressed in the adult plant stage (Parlevliet and Van Ommeren, 1975)(Dyck and Samborski, 1982).

Partial resistance has been reported to be polygenicaly inherited (Wilcoxon, 1981) and controlled by genes with minor effects acting in an additive manner. Even when the resistance conferred by *Lr34* in combinations with other genes is not polygenic in nature, *Lr34* appears to act similarly to the genes conferring partial resistance since the enhanced resistance expressed by the combinations of genes may be regarded as additive (Dyck and Kerber, 1985).

Modification of the expression of genes conditioning hypersensitive resistance by genes conditioning partial resistance to leaf rust of barley has also been reported. Niks and Kuiper (1983) examined the histological expression in barley seedlings of two genes conferring hypersensitive reaction to barley leaf rust (*Pa3* and *Pa7*) in three genetic backgrounds that had different levels of partial resistance. Partial resistance caused a substantial early abortion of colonies before formation of haustoria; *Pa3* also caused early abortion of colonies, shortly after the formation of the first haustorium and caused host cell necrosis while *Pa7* acted several days after the establishment of the fungus and caused cell browning. The genes for partial resistance and *Pa7* acted independently and consecutively: partial resistance genes acted first and only the colonies not arrested by this resistance were affected by *Pa7*. When *Pa3* was present, the effect of partial resistance genes were greatly masked by the early abortion due to *Pa3*. The degree of necrosis caused by *Pa3* appeared to be less in the background with the highest level of partial resistance, probably caused by the action of partial resistance genes which rarely induce necrosis.

It would be interesting to investigate the effect of *Lr34* and the *Lr34* combinations at the histological level to determine if there is some similarity with the barley - barley leaf rust system. In that case, it might be possible to anticipate which resistance genes are more likely to interact with *Lr34* for increased resistance.

# CONCLUSIONS.

This study determined that combinations of *Lr34* and most leaf rust resistance genes that condition effective resistance, interact to condition higher levels of resistance than anticipated when the genes are present singly. *Lr34* also interacted with *Lr18* for superior resistance to certain isolates virulent to *Lr18*.

One of the alternatives to follow as a strategy in the incorporation of resistance genes in new cultivars is the use of combinations of *Lr34* with genes that have no or very low corresponding virulence in the leaf rust population, provided *Lr34* continues to confer adequate resistance. The resistance provided by such gene combinations could be more durable than the resistance conferred by the genes singly, since combinations of two effective resistance genes are more difficult to be overcome by the pathogen, particularly if they are not deployed individually in other cultivars. The presence of *Lr34*, which has demonstrated long lasting resistance, may also contribute to the duration of resistance in lines with two or more resistance genes. In addition, the use of *Lr34* can contribute to improved resistance to stem rust since it has been demonstrated to be associated with additional stem rust resistance on chromosome 7D (Dyck, 1987).

# LITERATURE CITED

1. Anderson, R.G. 1961. The inheritance of leaf rust in seven varieties of common wheat. Can. J. Plant Sci. 41: 342-359.

2. Baker, E.P. 1966. Isolation of complementary genes conditioning crown rust resistance in the oat variety Bond. Euphytica 15: 313-318.

3. Drijepondt, S.C. and Pretorius, Z.A. 1989. Greenhouse evaluation of adult plant resistance conferred by the gene *Lr34* to leaf rust of wheat. Plant disease 73: 669-671.

4. Dyck, P.L. 1977. Genetics of leaf rust reaction in three introductions of common wheat. Can. J. Genet. Cytol. 19: 711-716.

5. Dyck, P.L. 1987. The association of a gene for leaf rust resistance with chromosome 7D suppressor of stem rust resistance in common wheat. Genome, 29: 467-469.

6. Dyck, P.L. Genetics of adult-plant resistance in Chinese Spring and Sturdy cultivars. In press.

7. Dyck, P.L. and Johnson, R. 1983. Temperature sensitivity of genes for resistance in wheat to *Puccinia recondita*. Can. J. Plant Pathol. 5: 229-234.

8. Dyck, P.L. and Kerber, E.R. 1985. Resistance of the race - specific type, pages 469-500. In: The cereal rusts Vol II. Diseases, distribution, epidemiology and control. A.P. Roelfs and W.R. Bushnell eds., Academic Press, Orlando.

9. Dyck, P.L. and Samborski, D.J. 1982. The inheritance of resistance to *Puccinia recondita* in a group of common wheat

cultivars. Can. J. Cytol. 24: 273-283.

10. Dyck P.L., Samborski, D.J. and Anderson, R.G. 1966. Inheritance of adult - plant leaf resistance derived from the common wheat varieties Exchange and Frontana. Can. J. Genet. Cytol. 8: 665-671.

11. Ezzahiri, B. and Roelfs, A.P. 1989. Inheritance and expression of adult plant resistance to leaf rust in Era wheat. Plant Disease 73: 549-551.

12. Flor, H.H. 1955. Host - parasite interactions in flax - rust - its genetic and other implications. Phytopathology 45: 680-685.

13. Green, G.J. 1964. A color mutation, its inheritance, and the inheritance of pathogenicity in *Puccinia graminis* Pers. Can. J. Botany 42: 1653-1664.

14. Green, G.J. 1966. Selfing studies with races 10 and 11 of wheat stem rust. Can. J. Botany 44: 1255-1260.

15. Johnson, R. 1981. Durable resistance: definition of, genetic control, and attainment in plant breeding. Phytopathology 71: 567-568.

16. Johnson, T. 1961. Man guided evolution in plnt rusts. Science 133 (3450): 357:362.

17. Knott, D.R. 1957. The inheritance of rust resistance. III. The inheritance of stem rust resistance in nine Kenya varieties of common wheat. Can. J. Plant Sci. 37: 366-384.

18. Knott, D.R. and Weller, J. 1988. Genetic interactions among four genes for resistance to stem rust in bread wheat.

Genome 30: 182-185.

19. Kolmer, J.A. 1989a. Virulence and race dynamics of *Puccinia recondita* f. sp. *tritici* in Canada from 1956 - 1987. Phytopathology 79: 349-356.

20. Kolmer, J.A. 1989b. Physiologic specialization of *Puccinia recondita* f. sp. *tritici* in Canada in 1988. Can. J. Plant Pathol. 11: 431-434.

21. Kolmer, J.A. 1990. Physiologic specialization of *Puccinia recondita* f. sp. *tritici* in Canada in 1989. In Press.

22. Luig, N.H. and Rajaram, S. 1972. The effect of temperature and genetic background on host gene expression and interaction to *Puccinia graminis tritici*. Phytopathology 62: 1171-1174.

23. Martens, J.W., Rotham, P.G., Mc Kenzie, R.H.I. and Brown, P.D. 1981. Evidence for complementary gene action conferring resistance to *Puccinia graminis avenae* in *Avena sativa*. Can. J. Genet. Cytol. 23: 591-595.

24. McIntosh, R.A. 1983. A catalogue of gene symbols for wheat (1983 edition). In. Proc. 6th International Wheat Genetics Symposium, Kyoto, Japan, 1983: 1197-1254.

25. Niks, R.E. Nd Kuiper, H.J. 1983. Histology of the relation between minir and major genes for resistance of barley to leaf rust. Phytopathology 73: 55-59.

26. Parlevliet, J.E. 1985. Resistance of the non-race-specific type, pages 501-525. In: The cereal rusts Vol II. Diseases, distribution, epidemiology and control. A.P. Roelfs and W.R. Bushnell eds., Academic Prress, Orlando.

27. Parlevliet, J.E. and Van Ommeren, A. 1975. Partial resistance of barley to leaf rust, *Puccinia hoedei*. II. Relationship between field trials, micro plot tests and latent period. Euphytica 24: 293-303.

28. Perez, B.A. and Roelfs, A.P. 1987. Differences in host response of near isogenic wheat lines to leaf rust. Phytopathology 77: 1763.

29. Perez, B.A. and Roelfs, A.P. 1989. Resistance to wheat leaf rust of land cultivars and their derivatives. Phytopathology 79: 1183.

30. Peterson, R.F.; Campbell, A.B. and Hannah, A.E. 1948. A diagramatic scale for estimating rust intensity on leaves and stems of cereals. Can. J. Res. 26: sec C: 496-500.

31. Roelfs, A.P. 1988a. Genetic control of phenotypes in wheat stem rust. Ann. Rew. Phytopathology 26: 351-367.

32. Roelfs, A.P. 1988b. Resistance to leaf and stem rust in wheat. pp 10-22. In. Simmonds, N.W. and Rajaram, S. eds. Breeding strategies for resistance to the rusts of wheat. CIMMYT, Mexico D.F.

33. Samborski, D.J. and Dyck, P.L. 1968. Inheritance of virulence in wheat leaf rust on the standard differential wheat varieties. Can. J. Genet. Cytol. 10: 24-32.

34. Samborski, D.J. and Dyck, P.L. 1982. Enhancement of resistance to *Puccinia recondita* by interactions of resistance genes in wheat. Can. J. Plant Pathol. 4: 152-156.

35. Sawhney, R.N.; Nayar, S.K.; Sharma, J.B. and Bedi, R.

1989. Mechanism of durable resistance: a new approach. Theor. Appl. Genet. 78: 229-232.

36. Shang, H.S., Dyck, P.L. and Samborski, D.J. 1986. Inheritance of resistance to *Puccinia recondita* in a group of resistance accessions of common wheat. Can. J. Plant Pathol. 8: 123-131.

37. Schafer, J.F., Caldwell, R.M., Patterson, F.L. and Compton, L.E. 1963. Wheat leaf rust resistance combinations. Phytopathology 53: 569-573.

38. Singh, R.P. and McIntosh, R.A. 1984a. Complementary genes for reaction to *Puccinia recondita tritici* in *Triticum aestivum*. I. Genetic and linkage studies. Can. J. Genet. Cytol. 26: 723-735.

39. Singh, R.P. and McIntosh, R.A. 1984b. Complementary genes for reaction to *Puccinia recondita tritici* in *Triticum aestivum*. II. Cytogenetic studies. Can. J. Genet. Cytol. 26:736-742.

40. Singh, R.P. and McIntosh, R.A. 1987. Genetics of resistance to *Puccinia graminis tritici* in 'Chris' and 'W3746' wheats. Theor. Appl. Genet. 73: 846-855.

41. Stackman, E.C.; Stewart, D.M. and Loegering, W.Q. 1962. Identification of physiologic races of *Puccinia graminis* var. *tritici*. U.S. Dept. Agric. ARS - E 6/7. 53 pp.

42. Wilcoxon, R.D. 1981. Genetics of slow rusting in cereals. Phytopathology 71: 989-993.

Appendix 1. Infection types produced by physiologic races of *Puccinia graminis var tritici on standard differential varieties of Triticum spp (Stakman et al., 1962).*

| *Infection type*[a] | *Varietal reactions and reaction classes*[b] |
|---|---|
| | *Resistant* |
| 0 | IMMUNE. No uredia nor other indications of infection |
| 0; | NEARLY IMMUNE. No uredia but hypersensitive flecks present |
| 1 | VERY RESISTANT. Uredia minute; surrounded by distinct necrotic areas |
| 2 | MODERATELY RESISTANT. Uredia small to medium; usually in green islands surrounded by a decidedly chlorotic or necrotic border. |
| | *Susceptible* |
| 3 | MODERATELY SUSCEPTIBLE. Uredia medium in size; coalescence infrequent; no necrosis, but chlorotic areas may be present, especially under unfavourable growing conditions |
| 4 | VERY SUSCEPTIBLE. Uredia large, and often coalescing; no necrosis, but chlorosis may be present under unfavourable conditions |
| | *Mesothetic* |
| X | HETEROGENEOUS. Uredia variable, sometimes including all infection types and intergradations between them on the same leaf; no mechanical separation possible; on reinoculation small uredia may produce large ones, and vice versa |

[a] Plus and minus signs are used to indicate variation within a given infection type: ++ and = indicate the upper and lower limits, respectively, of each type. The symbol+- indicates a variation between + and - for the type. The symbol c indicates exceptionally pronounced chlorosis; b indicates browning with a tendency toward necrosis; n indicates a tendency toward necrosis.

[b] These classes were established primarily to facilitate the identification of rust races rather than to indicate degrees of resistance of wheat varieties. Thus, infection type 2 is considered to indicate resistance and type 3 to indicate susceptibility, although a variety with infection type 2++ may appear more susceptible for practical purposes than one with type 3=. Moreover, the mesothetic class is based solely on the presence of infection type X, and there can be a wide range of susceptibility and resistance within the class, as indicated by the plus and the minus signs after the X.

Appendix 2. Severity of leaf rust infection. Modified Cobb scale.

A refers to actual percentage of area occupied by pustules. B refers to the standard rust readings according to Peterson et al.(1948).