The inheritance of resistance of barley (Hordeum vulgare L.) to Puccinia hordei Otth.
by Elias Elias

A thesis submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Plant Pathology
Montana State University

Abstract:
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The susceptible cultivar Austral was crossed to the resistant cultivars CI 4974, CI 11577, CCI-M-13, Ford 1203, Modjo, Menelik, and 386-16-2 to determine the number of genes for resistance to P. hordei in each resistant cultivar. In addition, crosses were made between resistant cultivars to determine whether they carried the same gene or genes for resistance. The F2 plants resulting from the different crosses were screened for seedling reaction to three isolates of Puccinia hordei.

Most cultivars possessed either one or two genes for resistance. In addition, the cultivar 386-16-2 was shown to possess the same gene (Pa3) as the cultivar Estate.

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Signature Elias Elias

Date June 15, 1982

In dedication to:

my wife Christy Lee, my daughter Tamar Lee,
my father and mother.

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in

Plant Pathology

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ABSTRACT

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The susceptible cultivar Austral was crossed to the resistant cultivars CI 4974, CI 11577, CCI-M-13, Ford 1203, Modjo, Menelik, and 386-16-2 to determine the number of genes for resistance to P. hordei in each resistant cultivar. In addition, crosses were made between resistant cultivars to determine whether they carried the same gene or genes for resistance. The F_2 plants resulting from the different crosses were screened for seedling reaction to three isolates of Puccinia hordei.

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Chapter 1

INTRODUCTION

Leaf rust of barley, Hordeum vulgare L., caused by Puccinia hordei otth is a common disease of barley. It is distributed generally in most countries where barley is grown. The disease is considered of minor importance in the northcentral North American spring barley area, but causes considerable damage in the Eastern and Southern United States. The disease seems to be more prevalent in warm and dry summers and in some seasons develops in epiphytotic form, especially in the Southern winter barley area. In severe epidemic years the yield losses range from 50 to 80 percent.

Puccinia hordei is known to vary with respect to pathogenicity due to the sexual cycle which occurs on the alternate host Ornithogalum umbellatum L. (the common Star-of-Bethlehem). In completing the sexual cycle, variability may occur as numerous virulence types. Such development of new virulence types on the alternate host may cause difficulty in controlling the disease. However, the rust appears to survive adequately independent of host alternation. The fungicide triadimefon (Bayleton 1-(4-chlorophenoxy)-3, 3-dimethyl-1 (1,2,4-triazol-1-yl) butan-2-one) has been used to control the pathogen. However, the most economical control of this disease can be achieved by genetic resistance. It is very important that changes

in the pathogenicity be detected so that plant breeders can develop and maintain resistant cultivars. At present, nine major genes conditioning resistance have been identified and are designated as Pa through Pa9 genes.

The present study revealed that the number of genes governing resistance to leaf rust may be small. If more genes conditioning resistance can be identified then recurrent selection techniques represent a valuable tool in building quantitative resistance.

The objectives of the research described in this thesis were to:

i. Study the relationship of different isolates of *P. hordei* to different sources of resistance.

ii. Study the genetic inheritance of resistance of *P. hordei*.

iii. Find and evaluate sources of resistance to *P. hordei*.

Chapter 2

LITERATURE REVIEW

Puccinia hordei Otth, is the causal organism of leaf rust of barley (Hordeum vulgare L.). It is placed in the class BASICIOMYCETES in the order UREDINALES. P. hordei is heteroceious and the aecial host of this fungus is Ornithogalum umbelatum L. (common Star-of-Bethlehem) and some other species, Mains and Jackson (1924), Tranzschel (1914) and Dickson (1947). It is a macrocyclic rust because it has the gametophytic and the sporophytic phases. The two forms of sori that are chiefly concerned in the continued life of macrocyclic species are the aecia and telia (Arthur, 1929). The aecia and pycnia in P. hordei occur as elevated light orange yellow areas on the leaves of Ornithogalum. The aeciospores are globoid, hyaline, and minutely verrucose (Sickson, 1947). The aecia in their maturation have to do with the pairing of nuclei together with their chromosomes (Arthur, 1929). The uredia are small, round and light yellowish-brown in color. This rust is relatively inconspicuous until uredial development is quite abundant (Dickson 1947). The uredia serve to multiple the reproductive capacity of the rust, and their spores act in a similar manner to aeciospores (Arthur 1929). The telia are round to oblong brown, covered by the epidermis and

produce mainly one celled spores. The teliospores germinate to form the characteristic four-celled basidium and sporidia. Telia formation is less abundant in the more northern sections of the barley area as compared to southern sections of the United States (Dickson 1947). Both uredia and telia develop on the leaf blades, leaf sheaths and stems but rarely occur on the floral structure.

EPIDIOMOLOGY

Puccinia hordei is distributed generally in most of the areas where barley is grown: North Africa, Middle East, Europe, and the United States. It occurs extensively in both the winter and spring barley areas of the Eastern and Central United States, is found in almost every season, and in some seasons it develops in epiphytotic form, especially in the southern spring barley area (Dickson 1947). The disease seems to be more prevalent in warm and dry summers but the reason for extensive epidemic attacks in some seasons as compared with others with superficially similar weather patterns is not fully understood (Martin and Morris 1979).

Melville and Griffin (1976) showed a statistical relationship between the level of disease and grain yield. Plants exhibiting a disease severity of 10 percent on the penultimate leaf were reduced in yield by 7.7 percent.

Distribution of the rust apparently involves a northward

spread of uredial inoculum from the Southern winter-barley area (Dickson 1947). The aecial stage does not normally develop abundantly in the United States. However, Mains and Jackson (1924) reported the aecial stage in Indiana, and it has been produced at Madison, Wisconsin when barley straw containing telia was placed near Ornithogolum umbellatum (Dickson 1947). Telial development is limited in the spring barley area; the main infection occurs in this area when urediospores are blown in from the south. The infections in the northern areas frequently are not evident until relatively late in the spring or early summer. Secondary spread from urediospores is abundant during warm humid weather and urediospores do not over-winter in the spring barley area (Dickson 1947). However, the fungus does over-winter as urediospores in the southern winter barley area. In The Netherlands in normal and mild winters the winter barley can grow, replacing its leaves slowly. Under these conditions the pathogen must produce urediospores continuously in order to survive. Only in severe winters with continuous snow cover may a more static situation exist where dormant mycelia may help to bridge the winter. Therefore, the size of the rust population in early spring is determined by the rate of leaf replacement by the barley and the rate or reproduction by the pathogen (Parlevliet and Ommeren 1976).

CONTROL

Chemical means have been used to control the pathogen and a systemic fungicide triadimefon (=25% WP Bayleton) is notable in this regard (Martin and Morris 1979). Tridimefon is a member of the triazole group with the chemical name 1-(4-chlorophenoxy)-3, 3-dimethyl-1(1,2,4-triazol-1-yl) butan-2-one (Martin and Morris 1979). However, the most economical control of this rust is through the use of resistant cultivars. Many studies have been done on the inheritance of resistance and many cultivated barleys were reported as being resistant.

HOST DIFFERENTIALS FOR IDENTIFYING PHYSIOLOGIC RACES OF PUCCINIA HORDEI

In order to base breeding programs on reliable information, investigations are necessary on variation within the pathogen, virulence types as indicated by physiologic races, and on resistance sources of barley and the genetical background of these resistance sources.

In the past, Straig (1931), Hey (1931), and Ronsdor (1934), used host differentials of unknown genotypes. Some of these differentials were considered not to be critical and as a result have fallen out of favor (Levine and Cherewick, 1942). However, recently Clifford proposed an international set of host differentials to study physio-

logic specialization in barley leaf rust (Tan 1977). This set is comprised of the barley cultivars Sudan (Pa) Reka 1 (Pa2), Estate (Pa3), Gold (Pa4), Quinn (Pa2+Pa5), Bolivia (Pa2+Pa6) and Egypt 4 (Pa8), (Tan, 1977). The respective Pa genes for resistance indicated for these cultivars will be discussed later. Tan (1977) inoculated the proposed international differentials plus the old European differentials (Austral C.I. 6483, Friedrichswetter Berg, Cruzat CI. 6482, Chilean D, C.I. 1433, Kinver C.I. 2361, Club Mariout C.I. 261) and some other cultivars, Peruvian (Pa2), Juliaca C.I. 1114 (Pa2), Ricardo (Pa2), Weider (Pa2), Batna (Pa2) with 20 different single urediospore cultures from different locations of P. hordei. He used a 0-4 reaction scale; 0 and 1 were considered resistant, 2 moderately resistant, 3 moderately susceptible and 4 fully susceptible. His results showed that cultivars reported to possess the gene Pa2 each reacted differently, except Peruvian and Juliaca which were similar to each other in behavior. Peruvian and Juliaca are postulated to carry Pa2 alone whereas Reka 1, Ricardo, Weider and Batna each are believed to have one or more unknown genes in addition to Pa2. He also noted that monogenic differentials that are universally resistant or are resistant to a great majority of strains, serve perhaps more purposefully as supplemental differentials in regional sets, which are usually more relevant to resistance

breeding programs. In addition the number of strains that can be identified will be influenced by the number of differentials used. Tan (1977) concluded that among seven barley cultivars proposed as international differentials of P. hordei, only Sudan, Estate, Gold and Egype 4 were appropriate. Tan (1977) suggested that Peruvian and Austral replace Reka 1 and that cultivars monogenic for Pa5 and Pa6 be isolated in individual cultivar to replace Quinn and Bolivia, respectively. However, there is still no international differential set which is universally acceptable. In Montana, a differential set with known genotypes is comprised of: Cebada Capa (Pa7), Sudan (Pa), Peruvian (Pa2), Egypt (Pa8), Ricardo, (Pa2+?), Gold (Pa4), Quinn (Pa2+Pa5), Estate (Pa3), Bolivia (Pa2+Pa6), Batna (Pa2+?), and C.I. 1242 (Pa9) (Sharp and Reinhold, 1982).

PHYSIOLOGICAL SPECIALIZATION

Puccinia hordei is known to vary in pathogenicity due to mutation or sexual recombination. It is very important to monitor and detect changes in pathogenicity to allow plant breeders to develop and maintain resistant cultivars. These changes can be determined by a coordinated study of the host and the pathogen. New pathogenic strains can be detected by looking for susceptible type pustules on selected cultivars with known combinations of genes conditioning resistance and additional cultivars exhibiting resistance

to leaf rust in the field (Moseman and Roane 1949). Between 1956-1958 Moseman and Roane isolated 39 cultures of P. hordei from barley grown in the United States. They inoculated these 39 isolates on nine cultivars: Speciale, Sudan, Oderbrucker, Lechtaler, Gold, Reka 1, Bolivia, Quinn and Egypt. These cultivars were used by Levine and Cherewick (1942) as standard differentials, along with seven supplemental cultivares: Cebada Capa, Franger, Hispont, Ariana, Ricardo, Aim and Marocaine 079 which had been outstanding for resistance to leaf rust when grown in the uniform rust nurseries and in the international barley disease nurseries: The infection types produced on the 16 cultivars when inoculated with seven cultures of P. hordei are shown in Appendix Table 1.

The three differential cultivars Speciale, Sudan, and Oderbrucker and the cultivar Cebada Capa were resistant to all the cultures and had similar reaction to infection. The differential cultivar Lechtaler and the two cultivars Franger and Hispont were susceptible only to culture 8 of race 40, as was the cultivar Gold, but they had a reaction differing from that of Gold with some of the other cultures. The differential cultivar Reka 1 and the cultivars Ariana, Ricardo, and Marocaine 079 were susceptible only to cultures 10 and 33 of race 44 but reacted like the cultivars Bolivia and Quinn when inoculated with the other cultures. The cultivar Aim

had the highest type resistance of all the cultivars to all cultures. Moseman and Roane (1959) concluded that physiologic race 4 made up about 70 percent of all cultures; this was in agreement with earlier studies by Levine and Cherewick (1952). Moseman and Roane (1959) suggested crossing the cultivars having similar infection type to determine if they had identicial genes for resistance to the cultures they used. By crossing the cultivars having similar infection type and inoculating the segregating progenies with a culture of race 4, the relationship of each other of the genes in these cultivars could be determined. Apparently the cultivar Aim has a gene conditioning resistance to these cultures not present in the other cultivars because it was more resistant to all cultures than the other cultivars. However, during the period 1959-1964, the cultivar Aim became susceptible to the new race 53 which was identified by Moseman and Greeley (1965). During this period (1959-1964), Moseman and Greeley made a survey to detect new pathogenic strains, and physiologic races of P. hordei. They used uniform spring barley rust nurseries grown at many locations where barley leaf rust has been an important disease. Four types of cultivars were included in the nurseries. First, were cultivars susceptible to most pathogenic strains or races of the pathogen. Second, were cultivars which had been

resistant to the pathogen throughout the United States. Third, were cultivars which had been resistant to the pathogen in some, but not all regions of the United States, and fourth, were cultivars being used as sources of resistance to the pathogen or in genetic studies. Collections of rust were made from one susceptible cultivar in the uniform spring barley rust nurseries to identify the rust species. Other collections were made from those cultivars which had been previously resistant to the pathogen to identify pathogenic strains or physiologic races that might have been new to the location. Other collections were made from plant-breeding nurseries in the winter barley region and only from cultivars being used as sources of resistance, or from cultivars which had been resistant to the pathogen at those locations. Appendix Table 2 shows the infection type when these collections were inoculated to to seedling plants of the North American standard differential set and other cultivars. The authors concluded that 64 of the 69 cultures studied were physiologic race 4 which had virulence to Pa8. Culture 63.3 was designated as phsyiologic race 53 since it differed in pathogenicity on the 9 standard differential cultivars from the other 52 races previously reported by Levine and Cherewick. They showed that culture 64.2 (race 8) was virulent on Speciale (Pa), Sudan (Pa), Oderbrucker (Pa), Gold (Pa4), and Lechtaler (Pa4).

Culture 63.3 (race 53) was virulent on Bolivia (Pa2+Pa6) and Reka 1 (Pa2). Culture 59.8 (race 40) was virulent on Gold (Pa4) and Lechtaler (Pa4). Quinn (Pa2+Pa5) was resistant to all cultures mentioned above. The similarity of reaction of Franger to Gold and Lechtaler suggested these three cultivars may have the same gene conditioning resistance. However, the cultivars Cebada Capa, Ricardo, Aim, Estate, and C.I. 4974, which were being used by plant breeders in that region, were resistant to those cultures of race 8.

Studies on physiological specialization were also done in West Germany by Rintelen (1975). He used a differential set composed Sudan, Reka 1, Quinn, Boliva, Ricardo, Weider, Estate, Gold and Egypt. This set differed from the North American differential set but had the same cultivars which were used by Moseman et al (1965). Rintelen (1975) concluded that there is essentially no resistance to P. hordei in West German cultivars. He tested 112 cultivars of the expanded 1974 European Barley disease nursery and some other cultivars classified as resistant. Of the 112 tested, 19 were found to be resistant to all isolates.

Recently Reinhold and Sharp (1981) evaluated virulence types of P. hordei from several widely separated barley growing areas. Isolates were collected from local barley cultivars in Morocco-(Merchouch, Khemis Zemara, Marrakech and Rabat), Tunisia-(Fretissa),

Egypt-(Sakha), Syria-(Homs and Tel Hadia) and Israel-(Tel Aviv). In one case a collection was from the alternate host. Collections from the United States originated from widely separated locations in Montana-(Creston, Sidney), Texas-(San Antonio), and Minnesota-(Minneapolis). All of these isolates were evaluated on the differential cultivars which represent the resistance genes Pa through Pa9. The isolates from San Antonio, Texas and Minneapolis, Minnesota appeared identical. The isolates from Creston and Sidney, Montana differed significantly from each other and showed evidence of accumulation of several virulence genes not present in collections from San Antonio, Texas or Minneapolis, Minnesota. The North African isolates from Morocco, Tunisia, and Egypt also represented significantly different virulence types. Collections from Merchouch, Khemis Zemara, and Marrakech, Morocco showed similarities but the Rabat, Morocco isolate had more virulence genes than the three previously described. A highly virulent isolate was obtained at Sakha, Egype. Isolates from the Middle East were collected in Israel, Syria and Turkey. The collection from Tel Hadia, Syria and Izmir, Turkey were similar and differed significantly only in reaction on Quinn (Pa2+Pa5). A broad based virulent isolate was found to occur in the dry land area near Homs, Syria. The virulence patterns of the Sakha isolate and the Sidney, Montana

isolate evaluated on the differential cultivars appeared to be similar.

The race number is now not used extensively because it usually doesn't give any information on genes for virulence or resistance which are of prime importance.

INHERITANCE OF REACTION TO PUCCINIA HORDEI IN BARLEY

Interest in a study of inheritance of reaction to P. hordei was stimulated by the need for more fundamental knowledge of the inheritance of disease reaction. A series of genetic studies were conducted to determine the number of gene loci conditioning rust reaction in the North American differentials and in those cultivars previously studied by Moseman and Roane (1962). In this regard Roane (1962) crossed the 9 American differential cultivars with each other and with two susceptible cultivars, Moore and Egypt. Seedlings of F_2 and F_3 generations were inoculated with culture 57-19 of P. hordei race 4. Plants with infection types 0, 1 and 2 were classified as resistant and those with type 3 and 4 were classified as susceptible. Crosses were made between resistant cultivars and the susceptible cultivars Moore and Egypt to determine the number of gene loci for resistance in each resistant differential cultivar. Gold, Lechtaler, Oderbrucker, Reka 1, Speciale, Sudan crossed to Moore and Egypt respectively

showed a 3:1 monohybrid ratio. Bolivia and Quinn crossed to Moore and Egypt, respectively, showed a dihybrid ratio 15:1. Crosses were made between resistant cultivars to determine whether genes for resistance are at common loci. Data from these crosses showed that Bolivia, Quinn and Reka 1 each had a gene for resistance at the same locus (A). Although the behavior of Quinn and Bolivia was inconsistent in crosses with both susceptible and resistant cultivars, each of these had a second gene (locus--B in Quinn and X in Bolivia). The X designation was chosen because it was not known whether the second gene in Bolivia was at the same locus as B in Quinn. Oderbrucker, Speciale and Sudan also had a resistant gene (locus C) in common. Similarly, Gold and Lechtaler had common genes for resistance at the D locus. As indicated by the dihybrid ratios, the gene loci for rust resistance in Reka 1 and Lechtaler were different as were the loci in Reka 1 and Gold. Since Gold and Lechtaler have genes at the same locus, the reason why the corss Reka 1 X Gold and Reka 1 X Lechtaler behaved as it did, was not understood. In the case of trihybrid ratios, the gene loci in Quinn and Bolivia are perhaps different from those of the Oderbruker, Speciale, Sudan and from the Gold, Lechtaler group. Linkage was believed to be the cause of some of the deviations; however, the role of linkage needs further investigation. Henderson (1945) reported that Bolivia had only the gene Pa. Roane (1962)

reported that Bolivia had genes at two loci but it was not certain which of these was Henderson's Pa gene. Henderson (1945) found a second gene, Pa1, in the cultivar Estate. Roane and Starling (1966) determined that Estate has the gene Pa3 and not the gene Pa1. Roane and Starling (1966) crossed the eight North American differential cultivars and Moore with Weider and Estate. The latter two cultivars were designated to carry the genes Pa and Pa1, respectively, by Henderson (1945). In contrast, Watson and Butler (1948) reported that the cultivar Wider had the Pa2 gene for resistance. The F_2 plants in Roane and Starling's studies (1966) were inoculated with culture 57-10, race 4, of P. hordei. The 3:1 ratios resistant/susceptible obtained from crosses Weider X Moore, Weider X Egypt 4 and Estate X Moore showed that Weider and Estate had only one gene conditioning reaction to P. hordei. Further crosses of Weider with the differential cultivars disclosed that one gene is allelic with the gene in Weider and the gene in Estate is at a different locus from any of those described for the differential cultivars. Estate crossed to Weider gave a 15:1 R/S ratio which showed that these two cultivars have different gene loci. The final results of their study indicated that the gene in Weider and the A locus of Reka 1, Quinn and Bolivia are allelic and the gene in Estate is not represented among the differential cultivars. Obviously a reassignment of gene symbols in these cultivars is justified on the basis

of work by Roane and Starling (1966). Locus C, found in Oderbrucker, Speciale, and Sudan became Pa as first reported and designated by Watson and Butler (1948); the A locus which occurs in Reka 1, Bolivia, and Quinn, and allelic with the gene in Weider, became Pa2. The Estate gene was designated as Pa3 and the D locus found in Gold and Lechtaler were designated Pa4, with the second gene in Quinn designated as Pa5. The X locus of Bolivia (PaX) was designated later by Roane and Starling (1969) as Pa6. The relationship of the proposed gene designations to those previously assigned is summarized in Appendix Table 3.

Since Pa3 was not included among the North American race differential cultivars, it was suggested by Roane that Estate be included among them in all future studies on physiologic specialization of P. hordei. Later Nover and Lehamn (1974) reported a new gene, Pa7, in the cultivar Cebada Capa. Tan (1977) reported Pa8 in the cultivar Egypt 4. He also evaluated four Ethiopian cultivar-Abyssinian Schwarz, Uadera, Ab 14 and CI 1243 to six races of P. hordei. His F_2 data indicated that Abyssinian, Schwarz and Uadera each possess apparent identical partially dominant genes. His results also showed independence between this respective gene on the one hand and Pa3, Pa4 and Pa7. He concluded that the gene conditioning resistance in Abyssinian and Uadera, as well as in Ab 14 and CI 1243, was new and had not been described previously. It was

designated Pa9. Pa9 appears to be a valuable gene for differentiating certain isolates; it was overcome by only one isolate that originated from the alternate host Ornithogalum spp. At present, 9 genes conditioning resistance to leaf rust have been found and they are designated as Pa through Pa9.

RESISTANCE SOURCES IN BARLEY TO PUCCINIA HORDEI

Compared to other cereal rusts, little emphasis has been placed to date on the determination and use of resistance sources in barley to leaf rust (Sharp and Reinhold 1981). Sharp and Reinhold evaluated 178 barley cultivars/lines to 12 isolates of P. hordei from the United States and the Mediterranean area. Among the 178 cultivars/lines tested, only seven were resistant to all isolates and included Aim, Cebada Capa, Estate, Forrajera Klein X Rika 7 CI 11801, La Estanzuela, Giza 117 X Baktim X Giza 118 X Fa086, and Giza 119 X Tanekasse 105. The first five cultivars had the genes Pa3, Pa7, Pa3, Pa7, and Pa7 respectively. The resistance genes contained in the latter two lines have not been identified. Twenty-one other cultivars/lines represented excellent sources of resistance against more than 50 percent of the isolates evaluated. The Pa9 gene in CI. 1243 showed broad based resistance and gave a 1-2 reaction type with most all isolates; however, the Pa9 gene was overcome by one isolated which originated from the

alternate host (Ornithogalum sp.).

The alternate host has also been implicated in the development of a new virulent strain of P. hordei able to overcome resistance of the Pa7 gene (Golan, et al, 1978). These results support the hypothesis that the alternate host plays an important role in the development of new virulence types. In another study, Reinhold and Sharp (1981) tested 72 cultivars which were earlier rates as resistant or moderately resistant. These 72 barley cultivars, the international differential cultivars, and a susceptible check, Moore, were grown and evaluated in a San Antonio, Texas field nursery. Twenty-five out of 72 entries were completely resistant to P. hordei under field conditions in San Antonio. Nine cultivars/lines exhibited a low infection type in combination with low severity and were rated moderately resistant. It was concluded that among the thirteen differential cultivars, only four were susceptible to P. hordei in Southern Texas. This demonstrated clearly that the pathogen has not accumulated a large number of virulence genes as described for other regions. This agrees with van der Plank's (1968) thesis of stabilizing selection in favor of races of the pathogen with no unnecessary virulence genes. North American cultivars generally lack resistance to P. hordei, thus no selection pressure for higher virulence has been placed on

the pathogen (Reinhold and Sharp, 1981). Considerable resistance also occurs in collections of Hordeum spontaneum Koch, a wild ancestor of barley cultivars (Sharp and Reinhold 1981). Tan (1977) found that certain Ethiopian barley cultivars such as Abyssinian, Schwarz, Uadera, Ab14 and CI 1242 showed resistance to P. hordei. Clifford and Jones (1981) confirmed that the cultivar Simon carries the resistance gene Pa3 and the cultivar Triumph carries the resistance gene Pa9 with additional resistance factors.

Chapter 3

MATERIALS AND METHODS

Crosses were made first between resistance and susceptible cultivars to determine the number of gene loci for resistance in each resistant cultivar. Second, crosses were made between resistant cultivars to determine whether genes for resistance were at common loci. F_1 and F_2 populations of these crosses were tested to three different single urediospore isolates of Puccinia hordei from three different locations.

The cultivars used were Menelik, C.I. 11577, Modjo, C.I. 4974, CCI-M-13, Ford 1203, 386-16-2, Cebada Capa and Estate and were supplied by the Plant Pathology Department, Montana State University. These cultivars were chosen on the basis of earlier tests which indicated that these cultivars were resistant to some isolates of Puccinia hordei. In the summer of 1980, the susceptible cultivar Austral was corssed to Menelik, CI 11577, Modjo, CI 4974, CCI-M-13, Ford 1203, and 386-16-2. Crosses were made between the resistant cultivars (386-16-2 X Cebada Capa), (Cebada Capa X 386-16-2), (386-16-2 X Estate) and (Estate X 386-16-2) to determine if genes for resistance were at common loci. Ten F_1 seeds of each of these crosses were sent to Arizona and grown during the winter of 1980-81. F_2 seeds were harvested in most cases from the most

vigorous F_1 plants. The rest of the F_1 seeds were tested to the three different single urediospore isolates. F_2 seed was divided into two parts. Part one was evaluated in growth chambers. Two hundred F_2 seeds from each cross were planted as 25 seeds in each of eight rows across the width of metal flats 36 X 25 X 8 cm. At the same time 10 seeds from each line of the host differential set (Sudan Pa, Reka 1 Pa_2 Peruvian Pa_2, Ricardo Pa_2, Estate Pa_3, Gold Pa_4, Quinn Pa_2+Pa_5, Bolivia Pa_2+Pa_6, Cebada Pa_7, Egypt Pa_8, CI 1243 Pa_9 and Batna Pa_2) were planted in small pots to determine if there were any changes in the pathogenicity of the single urediospore isolates of the pathogen. Ten seeds of each parent were planted in small pots to use as checks for the parental reactions. The second portion of the F_2 seeds was planted as 50 seeds in three meter rows at the A. H. Post Field Research Laboratory of Montana State University to provide F_3 seed for further studies by other students.

INOCULUM PREPARATIONS

A single pustule of three different isolates (Merchouch, Morocco; Tel Hadia, Syria, Sidney, Montana) were supplied by the Plant Pathology Department, Montana State University. A susceptible cultivar Moore was used to increase each isolate. The increased inoculum was divided into two parts; the first was used immediately

for inoculations and in the second part of the urediospores were vacuumed dried and stored at 4C for later evaluation (Sharp, 1957).

INOCULATION PROCEDURE

Plant progenies from six crosses were planted in each of six different pots in Bozeman silt loan soil. The pots were then placed in a controlled environment chamber with a 16-hour daily photoperiod ($2.2\text{-}3.3 \times 10^4$ erg/cm^{2}sec) at 15/24 $\pm$ 1C (dark/light). Inoculation was performed 10-12 days after sowing. Freshly collected urediospores were suspended in distilled water and rubbed onto the leaves using the thumb and index finger. When lyophilized urediospores were used, they were hydrated 6-8 hours in 100 percent R.H. before inoculation. The inoculated seedlings were placed for 24 hours in a dew chamber at 20C to allow spore germination and then returned to the growth chamber. Disease readings were taken after 10-14 days based on the following disease index:

0 - no visible pustules	resistant
1 - small pustules, infrequently with chlorosis and necrosis	intermediate
2 - definite chlorosis surrounding moderate size pustules	intermediate
3 - large pustules, some chlorosis	susceptible

In this study, reaction type 0 was considered resistant, types 1 and 2, intermediate, and reaction type 3 considered susceptible.

Chapter 4

RESULTS

The barley cultivars used as a differential set in this study and their disease reaction to three isolates of P. hordei used are shown in Table 1. The Sidney isolate had a wider range of virulence than the Merchouch and Tel Hadia isolates. The Merchouch and Tel Hadia isolates appear to possess different genes for virulence as is indicated by their reaction with Sudan (Pa), Peruvian (Pa2), Egypt (Pa8), Bolivia (Pa2 + Pa6) and Reka 1 (Pa2). The barley cultivars which were used as parents and their disease reaction to the same three isolates are shown in Table 2. The cultivars most resistant to the three isolates are Cebada Capa (Pa7), Estate (Pa3) and 386-16-2 (Pa3). Austral was included as a susceptible cultivar to the three isolates used.

Table 3 shows the disease reaction to F_1 plants, resistant X susceptible and resistant X resistant parents. It appears that resistance to the Tel Hadia isolate in the cultivars Estate and Cebada Capa was dominant, while resistance in the cultivars Menelik, CI 4974 and Ford 1203 was recessive. The cultivars CI 11577, CCI-M-12 and 386-16-2 possessed an incomplete dominant gene or genes. In response to the Merchouch isolate, resistance appears to be incompletely dominant in the cultivars Menelik, CI 11577,

Table 1. Barley cultivars used as a differential set and their disease reaction to Tel Hadia, Syria, Merchouch, Morocco, and Sidney, Montana isolates of Puccinia hordei.

Cultivar	Genes for resistance	Disease Reaction Isolate		
		Tel Hadia	Merchouch	Sidney
Batna	Pa_2 + ?	S	-	-
Bolivia	Pa_2 + Pa_6	R	S	I
Cebada Capa	Pa_7	R	R	R
CI 1243	Pa_9	I	I	I
Estate	Pa_3	R	R	R
Egypt	Pa_8	R	I	S
Gold	Pa_4	S	R	S
Peruvian	Pa_2	S	I	S
Quinn	Pa_2 + Pa_5	S	S	S
Ricardo	Pa_2	-	S	S
Reka 1	Pa_2 + ?	I	S	S
Sudan	Pa	S	I	S

R = Resistance, reaction types 0

I = Intermediate, reaction types 1, 2

S = Susceptible, reaction type 3

Table 2. Barley cultivars used as parents and their disease reaction to Tel Hadia, Syria, Merchouch, Morocco, and Sidney, Montana isolates of Puccinia hordei.

Cultivar	Disease Reaction Isolates		
	Tel Hadia	Merchouch	Sidney
Austral	S	S	S
CI 4974	R	I	S
CI 11577	I	I	S
CCI-M-13	I	I	S
Cebada Capa	R	R	R
Estate	R	R	R
Ford 1203	I	I	S
Modjo	S	I	S
Menelik	I	I	I
386-16-2	I	R	R

R = Resistant reaction types 0

I = Intermediate reaction types 1, 2

S = Susceptible type 3

Table 3. Reaction of F_1 plants to Tel Hadia, Syria, Merchouch, Morocco and Sidney, Montana isolates of Puccinia hordei in crosses between resistant X susceptible and resistant X resistant cultivars.

Cross	Disease Reaction Isolate Tel Hadia	Merchouch	Sidney
Austral[A/] X Menelik	S	I	S
Austral X CI 11577	I	I	S
Austral X Modjo	S	I	S
Austral X CI 4974	S	S	S
Austral CCI-M-13	I	I	S
Austral X Ford 1203	S	I	S
Austral X 386-16-2	I	R	I
386-16-2 X Cebada Capa	R	R	R
386-16-2 Estate	R	R	I
Cebada Capa X 386-16-2	R	R	R
Estate X 386-16-2	R	R	I

R = Resistance reaction types 0

I = Intermediate reaction types 1, 2

S = Susceptible type 3

[A/] Austral is a susceptible cultivar to the three isolates.

Modjo, CCI-M-13 and Ford 1203, while resistance was dominant in the cultivars Cebada Capa, Estate and 386-16-2 and the resistance in the cultivar CI 4974 was recessive. In reaction to the Sidney isolate the resistance in the cultivar 386-16-2 was incompletely dominant, but was recessive in the cultivar Menelik. There were no genes for resistance in the cultivars CI 4974, CI 11577, CCI-M-13 and Modjo. However, Estate and Cebada Capa possess a dominant gene or genes for resistance to the three isolates.

The data concerning F_2 plants is divided into two groups:

i. That from crosses of resistant cultivars with the susceptible cultivars Austral to determine the number of gene loci for resistance in each resistant cultivar (Tables 4, 5, 6).

ii. That from crosses between resistant cultivars to determine whether genes for resistance are at common loci (Tables 7, 8, 9).

The barley cultivars Menelik, CI 11577, Modjo, CI 4974, CCI-M-13, Ford 1203 and 386-16-2 were crossed to the susceptible Austral and the F_2 plants were inoculated with the Tel Hadia isolate. Most of the cultivars showed monogenic or digenic inheritance except the cultivar Modjo, which possessed no genes for resistance (Table 4). Menelik gave satisfactory fits to either

Table 4. Reaction of F_2 plants to the Tel Hadia, Syria isolate of Puccinia hordei in crosses between resistant X susceptible cultivars.

Cross	Parental reaction	Number of Plants Resistant (0, 1, 2)	Number of Plants Susceptible (3)	*Probability 3:1	13:3	1:3	3:13	9:7	7:9
Austral[A/] X Menelik	I	26	115			.888	.979		
Austral X CI 11577	I	98	32	1.00	.6444				
Austral X Modjo	S	-	104						
Austral X CI 4974	R	83	30	.7878	.6375				
Austral X CCI-M-13	I	91	50	.558				.7287	
Austral X Ford 1203	I	56	133			.1658		.8374	
Austral X 386-16-2	I	116	40	.9263	.1732				

[A/] Austral is a susceptible cultivar.

* Determined by Chi-square value.

Table 5. Reaction of F_2 plants to the Merchouch, Morocco isolate of Puccinia hordei in crosses between resistant X susceptible cultivars.

Cross	Parental reaction	Number of plants: Resistant (0,1,2)	Number of plants: Susceptible (3)	*Probability: 3:1	13:3	1:3	3:13	7:9	15:1
Austral[A/] X Menelik	I	68	81			.5164		.7676	
Austral X CI 11577	I	83	29	.8159					
Austral X Modjo	I	33	64			.5305		.5781	
Austral X CI 4974	I	20	78			.3507	.6248		
Austral X CCI-M-13	I	102	12		.5057				
Austral X Ford 1203	I	122	10		.2664				.3924
Austral X 386-16-2	R	108	23		.9855				

[A/] Austral is a susceptible cultivar.

* Determined by Chi-square value.

Table 6. Reaction of F_2 plants to the Sidney, Montana isolate of *Puccinia hordei* in crosses between resistant X susceptible cultivars.

Cross	Parental reaction	Number of plants Resistant (0,1,2)	Number of plants Susceptible (3)	*Probability 3:1	*Probability 1:3
Austral[A/] X Menelik	I	55	133		.2065
Austral X CI 11577	S	-	136		
Austral X Modjo	S	-	104		
Austral X CI 4974	S	-	108		
Austral X CCI-M-13	S	-	165		
Austral X 386-16-2	R	154	50	.9356	

[A/] Austral is a susceptible cultivar to the three isolates.

* Determined by Chi-square value.

Table 7. Reaction of F_2 plants to the Tel Hadia, Syria isolate of Puccinia hordei utilizing reciprocal crosses between resistant cultivars.

Cross	Number of plants Resistant (0,1,2)	Susceptible (3)	*Probability 15:1	13:3
386-16-2 X Cebada Capa	170	13	.4298	.1837
Cebada Capa X 386-16-12	182	7	.3557	.5137
386-16-2 X Estate	187	-		
Estate X 386-16-2	169	-		

*Determined by Chi-square value.

Table 8. Reaction of F_2 plants to the Merchouch, Morocco isolate of Puccinia hordei utilizing reciprocal crosses between resistant cultivars.

Cross	Number of plants Resistant (0,1,2)	Susecptible (3)	*Probability 15:1	9:7
386-16-2 X Cebada Capa	140	5	.3673	-
Cebada Capa X 386-16-2	78	5	.9750	.2410
386-16-2 X Estate	190	-		
Estate X 386-16-2	194	-		

*Determined by Chi-square value.

Table 9. Reaction of F_2 plants to the Sidney, Montana isolate of Puccinia hordei utilizing reciprocal crosses between resistant cultivars.

Crosses	Number of plants: Resistant (0,1,2)	Number of plants: Susceptible (3)	*Probability: 15:1	*Probability: 13:3
386-16-2 X Cebada Capa	221	15	.6643	.4846
Cebada Capa X 386-16-2	110	7	.9790	.1102
386-16-2 X Estate	170	-		
Estate X 386-16-2	192	-		

*Determined by Chi-square value.

recessive monohybrid or dihybrid ratios. The cultivars CI 11577, CI 4974, CCI-M-13 and 386-16-2 gave satisfactory fits to either dominant monohybrid or dihybrid ratios, while Ford 1203, exhibiting a ratio of 7:9 resistance/susceptible, gave a satisfactory fit for two recessive genes. The segregation of the progeny from the same crosses mentioned above in response to inoculation with the Merchouch isolate are presented in Table 5. It appears that the barley cultivars Menelik, Modjo and CI 4974 have either one or two recessive genes. CCI-M-13, Ford 1203 and 386-16-2 have two dominant genes while CI 11577 gave a significant fit to the monohybrid 3:1 R/S ratio only. However, the barley cultivars CI 11577, Modjo, CI 4974 and

CCI-M-13 showed no genes for resistance to the Sidney isolate while the cultivare 386-16-2 gave a significant fit to a monohybrid 3:1 ratio resistant/susceptible (Table 6). Menelik showed segregation but no significant fit to any ratio.

To further compare the genes conditioning resistance in the cultivar 386-16-2 with other reported genes in the literature, reciprocal crosses were made between 386-16-2 and Cebada Capa (Pa7) and Estate (Pa3) known to carry specific genes for resistance. Tables 7, 8 and 9 show the results of these crosses when the progeny were inoculated respectively with the three isolates Tel Hadia, Merchouch and Sidney. None of the progeny exhibited type three pustules from the reciprocal cross 386-16-2 X Estate, which indicates that they carry the same gene. However, the reciprocal cross 386-16-2 X Cebada Capa gave type three segregates and significantly fit a dihybrid ratio which indicates the two barley cultivars each contain different single resistance genes.

Chapter 5

DISCUSSION

Since P. hordei, the causal organism of leaf rust disease of barley, is a variable pathogen, the development of resistant cultivars should be aimed at a resistance which would be effective against a wide range of virulence types. The main objective of any breeder or pathologist is to produce cultivars from their breeding program to achieve greater yield production than cultivars already in use.

Information about different sources of resistant germplasm is a prerequisite for an effective breeding program for the development of leaf rust resistant cultivars. There are several known sources of resistance to P. hordei reported in the literature. Most resistances are conditioned by single or two dominant genes. To date, nine major genes for resistance have been reported. The most common resistance genes are Pa3, Pa9 and Pa7. The virulence against Pa7 is particularly important, as this gene is being introduced by several breeders into commercial cultivars (Parlevliet, et. al., 1978). Breeders began to use this gene since it was reported to be effective everywhere with the exception of its susceptibility to one isolate of P. hordei originating from the alternate host (Golan, et. al., 1978). For breeders planning to use or already using major genes such as Pa7 in their breeding programs,

the isolate from the alternate host may provide a warning that this gene may also be overcome by some isolated of P. hordei. However, such isolates may not be competitive with the more aggressive isolates in the rust population. This would be similar to the situation involving the use of the two stem rust resistant genes Sr6 and Sr9 in wheat. An analysis of stem rust (Puccinia graminia f. sp. tritici) races that occurred from 1954 to 1969 in North America showed that no commonly occurring race could attack the resistance gene combination Sr6, Sr7b, Sr9d, Sr17 and Sr23 because no common race combined virulence to resistance genes Sr6 and Sr9d (Green 1971). However, two rare races of stem rust did combine virulence to all of the resistance genes contained in the wheat cultivar Selkirk, including Sr6 and Sr9d, but they were not aggressive and did not survive in nature (Green, 1975). Virulence to Sr6 and Sr9d genes separately was common in the pathogen population and it is not clear why the rust was unable to combine the virulence necessary to attack Selkirk in an aggressive race (Green and Campbell, 1979). The question which arises is what will happen to barley breeding programs if they rely on the Pa7 gene when aggressive isolates to this gene occur in nature. It is clear that barley cultivars with appropriate combinations of resistance genes can be more effective in breeding programs than cultivars known to possess only one gene for resistance. There

is good evidence to show that complex resistance can be enduring and can be produced by present breeding procedures (Green and Campbell, 1979).

In this study an attempt was undertaken to gain more understanding of some barley lines and cultivars showing resistance which could be used in breeding programs. The results obtained from crosses involving Austral X Menelik to the three isolates used in this study suggested that resistance in Menelik is conditioned by one or two recessive genes. The two gene system varied in action according to the isolate used, resistance toward the Merchouch isolate showed recessive major gene action while the Tel Hadia isolate showed dominant gene action.

The cultivar CI 11577 was observed to have a single dominant gene for resistance to the Merchouch isolate while having either one or two dominant genes to the Tel Hadia isolate of P. hordei. The cultivar Modjo appeared to possess one or two genes for resistance to the Merchouch isolate. The data in Table 5 shows a satisfactory fit for either one recessive gene or two recessive major genes.

The inheritance of resistance of CI 4974 was found to be the opposite of Menelike. Barley cultivar CI 4974 expressed one recessive gene or two genes to the Merchouch isolate with higher

probability of two genes being involved. However, the same cultivar showed one or two dominant genes conditioning resistance to the Tel Hadia isolate with a lower probability of two genes being involved.

The resistance of the cultivars CCI-M-13 to the Merchouch isolate appeared to be controlled by two genes. In response to the Tel Hadia isolate, the cultivar CCI-M-13 was shown to possess either one dominant gene with a probability of 0.558 or two complimentary genes with a probability 0.7207 to the same isolate.

The cultivar Ford 1203 was observed to possess two duplicate genes for resistance to the Merchouch isolate and two recessive major genes to the Tel Hadia isolate, but it is not known whether these genes are the same or if they are four different genes. It seems that they are most likely the same two genes because the probability of finding four different genes in the same cultivar is low. None of the cultivars discussed to this point possessed any genes for resistance to the Sidney isolate. However, the cultivar 386-16-2 was resistant to the three isolates and it was crossed to the susceptible Austral to determine how many genes conditioned resistance, it was also crossed to the resistant cultivars Estate and Cebada Capa, known to have Pa3 and Pa7 respectively, to see whether it had Pa3 or Pa7. In terms of the Sidney and Tel Hadia isolates, 386-16-2 showed only one dominant

gene while showing two genes conditioning resistance to the Merchouch isolate. When 386-16-2 was crossed to the cultivar Cebada Capa and inoculated with the three isolates it showed a satisfactory fit to 15:1 R/S ratio which suggests that the two cultivars possess two different genes. However, when 386-16-2 was crossed to Estate and inoculated with the three isolates, no type 3 reaction types were observed which suggests that the cultivar 386-16-2 possesses the same gene, Pa3, as the cultivar Estate. Since no 63:1 R/S ratio was observed from the crosses involving 386-16-2 X Cebada Capa and 386-16-2 X Estate, none of the three cultivars possess more than one gene for resistance. It is not known why the cross Austral X 386-16-2 gave a satisfactory fit to 13:3 R/S ratio showing that 386-16-2 possess two genes for resistance to the Merchouch isolate.

The results of this study show that 386-16-2 and Estate have the Pa3 gene. However, there is a difference in their reaction to the Tel Hadia isolate. Table 2 shows that Estate is completely resistant and 386-16-2 is intermediate. This could be related to different backgrounds in these cultivars.

Most of the crosses used in this study were characterized at a one or two gene ratio. Further studies of F_3 plants or progenies resulting from back crossing the F_1 plants to the parents are necessary to determine whether these cultivars possess one or two

genes. Unfortunately, time and resources did not permit this to be completed. Furthermore, these cultivars should be crossed to cultivars whose genetics have previously been studies and are known to possess certain Pa genes conditioning resistance to leaf rust. These types of crosses may lead to the identification of genes for resistance in Menelik, CI 11577, Modjo, CI 4974, CCI-M-13 and Ford 1203 and may lead to identifying new resistance genes which previously have not been identified. After identifying the genes in these cultivars and if these genes for resistance are inherited independently, then recurrent selection could be an effective tool for pyramiding different resistant factors into a single cultivar. The most effective cultivar against leaf rust presently might be a cultivar which possesses the complex of the resistance genes Pa3, Pa7 and Pa9.

The number of genes governing resistance to leaf rust may be small and it is not yet known on which chromosomes they are located. Trisomic analysis may help identify the chromosome or chromosomes carrying the genes for resistance to reaf rust.

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