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The inheritance of resistance to Rhynchosporium secalis in Ethiopian barley cultivars
by Segenet Kelemu

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Plant Pathology
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Abstract:
Barley scald is one of the most economically important diseases prevailing in cool, humid areas of the world. The disease can effectively be controlled by the use of resistant cultivars. This investigation was initiated to determine the number of gene loci conditioning resistance to barley scald in newly identified resistant Ethiopian barley cultivars and relate their genes to some of those previously reported. Fi, BC1, F2 and F3 progeny from different cross combinations were tested with seven isolates of Rhynchosporium secalis under controlled environment conditions. Adult F2 plants were also evaluated for their reaction to an isolate from Montana under field conditions.

. Five resistance genes, which were shown to be different from the previously reported genes, were identified in four Ethiopian cultivars. Three of these genes were dominant in action and two recessive. The symbols Rhl2 and Rh 13 were proposed for the dominant genes identified in PI-382282 and PI-382509, rhl4 for the recessive gene in PI-382282 and rhl5 and Rhl6 for the recessive and dominant genes found in PI-383036 and PI-382471, respectively. One Ethiopian barley, PI-383036, was shown to possess a gene at the Rh-Rh3-Rh4 complex locus.

Indication was found for the existence of cytoplasmic effects on scald resistance in some cross combinations.

THE INHERITANCE OF RESISTANCE TO *Rhynchosporium secalis*

IN ETHIOPIAN BARLEY CULTIVARS

by

Segenet Kelemu

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citation, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

Barley scald is one of the most economically important diseases prevailing in cool, humid areas of the world. The disease can effectively be controlled by the use of resistant cultivars. This investigation was initiated to determine the number of gene loci conditioning resistance to barley scald in newly identified resistant Ethiopian barley cultivars and relate their genes to some of those previously reported. F_1, BC_1, F_2 and F_3 progeny from different cross combinations were tested with seven isolates of *Rhynchosporium secalis* under controlled environment conditions. Adult F_2 plants were also evaluated for their reaction to an isolate from Montana under field conditions.

Five resistance genes, which were shown to be different from the previously reported genes, were identified in four Ethiopian cultivars. Three of these genes were dominant in action and two recessive. The symbols Rh12 and Rh13 were proposed for the dominant genes identified in PI-382282 and PI-382509, rh14 for the recessive gene in PI-382282 and rh15 and Rh16 for the recessive and dominant genes found in PI-383036 and PI-382471, respectively. One Ethiopian barley, PI-383036, was shown to possess a gene at the Rh-Rh3-Rh4 complex locus.

Indication was found for the existence of cytoplasmic effects on scald resistance in some cross combinations.

CHAPTER 1

INTRODUCTION

Barley is one of the most important cereal crops contributing immeasurably to the world's food and feed production.

Barley serves as an experimental plant for numerous studies. It is among the top half-dozen plant species in the number of gene loci plotted on linkage maps, probably because of its small number of chromosomes and the large number of clearly classifiable characters.

The many varietal types of barley have a wide ecological range and diverse adaptabilities which can be seen from the fact that cultivation is widespread from north to south and from lowland areas below sea level to high mountain ranges. Barley grows beside frozen pools in highland Ethiopia, and beneath date palms in the Sahara desert (Weaver, 1950). It is considered to be man's most dependable grain crop because of its ability to mature earlier than any other cereal and, as a result, escape drought or cold (Nuttonson, 1957).

There are two main centers of diversity of barley according to Vavilov. One, pivoting around Abyssinia (Ethiopia) and North-Eastern Africa, is particularly rich in long-awned hulled forms. The other, centering in China, Japan, and Tibet, is the source of hull-less short-awned, awnless or hooded types (Weaver, 1950; Smith, 1951).

Ethiopia, being one of the centers of genetic diversity for barley, has provided valuable sources of germplasm to the world. Large number of barley collections from Ethiopia are maintained in the United States and in other countries. These collections have been tested for their reactions to several plant pathogens (Moseman, 1971). The results from tests of collections of barley cultivars from Ethiopia, maintained by the United States Department of Agriculture, show that there are entries in the collections with genes for

resistance to many different pathogens. Schaller et al. (1963) found that, in the World Collection of 6689 entries, 113 of the 117 entries resistant to barley yellow dwarf virus were introductions from Ethiopia. In tests conducted with a field strain of barley stripe mosaic virus, Timian and Sisler (1955) found that four entries introduced from Ethiopia were outstanding for resistance to the virus. Metcalf and Johnston (1963) reported several introductions from Ethiopia to be resistant to *Ustilago nuda*. Qualset and Moseman (1966) compiled a report that many entries among 654 barley introductions from Ethiopia were found to be resistant to infection with cultures of *Erysiphe graminis, Ustilago nuda,* BYDV, BSMV, *Puccinia hordei, Pyrenophora teres, Septoria passerinii,* and *Rhynchosporium secalis.* Webster et al. (1980) identified 22 entries from Ethiopia resistant to barley scald in their evaluation of 18,000 entries from the USDA World Barley Collection.

Barley and its pathogens have co-existed for many centuries. *Rhynchosporium secalis* (Oud.) J. J. Davis, the causal organism of barley scald, is one of the pathogens threatening barley production in cool, humid areas of the world. Schaller (1951) reported losses in grain yields as high as 35 percent in California, and Ali et al. (1976) yield losses of 70 percent in Australia due to barley scald. Losses of up to 35 percent have been reported in Great Britain (Jenkins and Jemmett, 1967). Yield losses of 1-10 percent are not uncommon in many countries (Evans, 1969; Jenkins and Jemmett, 1967).

Resistance to *Rhynchosporium secalis* in barley has been reported on several occasions (Hansen and Magnus, 1973; Jenkins and Jemmett, 1967; Moseman, 1956; Reed, 1957; Riddle and Suneson, 1948; Roane and Starling, 1952; Rodriguez, 1948; Sarasola and Campi, 1947; Schein, 1960; Skoropad, 1960; Webster et al., 1980), but inheritance of resistance has been studied in relatively few cultivars (Baker and Larter, 1963; Bryner, 1957; Dyck and Schaller, 1961; Frecha, 1967; Habgood and Hayes, 1971; Riddle and Briggs, 1950; Starling et al., 1971; Wells and Skoropad, 1963). In these studies, 17 genes for resistance to

various isolates of *R. secalis* were identified. Starling et al. (1971) expressed doubt as to whether the number of gene loci identified was as large as reported in the literature.

Due to the appearance of new races or shifts in the pathogen populations, identification of potentially new sources of resistance genes to control barley scald disease appears to be a pertinent step. A notable example is the case of Atlas 46 in U.S.A. Atlas 46, having Rh2 and Rh3 resistance genes (Dyck and Schaller, 1961), was released in 1947 as resistant to California races of *R. secalis*, but by 1956 it was completely susceptible.

With the discovery of resistant Ethiopian barley cultivars from the USDA world barley collections, it seemed very desirable to study the inheritance of resistance in these cultivars to scald. The objectives of this research were to: (1) determine the number of gene loci conditioning resistance to barley scald in several Ethiopian barley cultivars, and (2) relate their genes to some of those previously reported.

CHAPTER 2

LITERATURE REVIEW

The Pathogen: *Rhynchosporium secalis* (Oud.) Davis

Mycological History and Taxonomy

The first preserved sample of *Rhynchosporium secalis* was collected in Norway in 1880 (Shipton et al., 1974) and identified as *Helminthosporium gramineum*. Oudemans (1897) made a collection in the Netherlands on rye and he described and named the pathogen *Marsonia secalis* (Oud.). The organism was isolated in Germany on both barley and rye in 1897 and was named *Rhynchosporium graminicola* Hein. (Frank, 1897). Later, Davis, in the United States, proposed the name *Rhynchosporium secalis* (Oud.) Davis in 1921 and the description of the genus was amended by both Davis (1921) and Caldwell (1937).

The genus *Rhynchosporium* belongs to the division Deuteromycotina, class Deuteromycetes, Order Moniliales, and family Moniliaceae.

The mycelium of the fungus is hyaline to light gray and the conidia are hyaline, one-septate, cylindrical to ovate with a short apical beak on most spores, and measure 12-20 by 2-4 microns (Dickson, 1956).

Variability of the Fungus

Heinsen (1901) found that isolates of *Rhynchosporium secalis* sporulated abundantly on some media, but were highly mycelial on others. Caldwell (1937), after culturing nine monoconidial isolates from barley on various media, concluded that the volume of mycelium and abundance of conidia varied directly with the concentration of dextrose or soluble carbohydrate present. Schein and Kerelo (1956) found that media without sugar allow

greatest sporulation and sucrose in the medium allows more sporulation than dextrose. They reported sporulation of the fungus to be practically nil on potato-dextrose agar and very high on lima-bean agar. The isolates grown on lima-bean agar had a pink color quite in contrast to the brown and black colonies on other media they used.

Despite the apparent absence of a sexual state, the fungus is capable of pathogenic variation. Habgood (1973) examined variation in aggressiveness and in conidial production in vitro in a number of single-spore isolates collected from within a single naturally infected plot of barley. Aggressiveness varied significantly both between lesions in the plot, and between isolates. He concluded that conidial production is not under nuclear control. He related *Rhynchosporium secalis* to *Phytophthora infestans* in which an extra-nuclear basis for some of the variability has also been suggested.

Sarasola and Campi (1947) reported that several barley varieties resistant to barley scald in the U.S. were susceptible in Argentina. Reed (1957) observed many mutants in cultures of most collections he made. He noted extreme variation in cultural characteristics between the mutant cultures, and between the mutants, and the original isolates he used. He compared fifteen different mutants and the parent monoconidial cultures for relative pathogenicity on four different barley varieties. A majority of the mutants were non-pathogenic. Williams and Owen (1975) observed significant differences in aggressiveness among United Kingdom isolates of the fungus.

The existence of physiologic races of *Rhynchosporium secalis* has been claimed by several workers (Ayesu-Offei, 1971; Dodoff, 1963; Dyck and Schaller, 1961a; Houston and Ashworth, 1957; Jackson and Webster, 1975; Reed, 1957; Sarasola and Campi, 1947; Schein, 1957). Riddle and Suneson (1948) found no good evidence of physiologic races in field trials conducted in California. Skoropad (1960) did not find any clear evidence of pathogenic races among the fifteen isolates tested on twenty-two differential barley varieties in Canada. Jackson and Webster (1975) studied pathogenic variability of *R. secalis* in

California and differentiated one hundred seventy-five single-spore isolates into seventy-five pathogenic races on 14 barley cultivars having many of the known genes for resistance to barley scald.

Evans and Griffiths (1971) observed a substantial decline in virulence of some isolates after frequent subculturing. They maintained virulence by inoculating isolates onto barley at intervals of three months and reisolating.

Host Range

Rhynchosporium secalis has been isolated from a number of grass genera and symptoms have been induced on an extensive range. The organism has been isolated from *Agropyron ciliare, A. semicostatum, A. repens, Bromus inermis, Elymus canadensis, Holcus lanatus, Hordeum jubatum, H. leporinum, H. murinum, H. vulgare, Lolium multiflorum, L. perenne, Phalaris arundinaceae, Secale cereale*, and several other grasses (Ali, 1972; Bartels, 1928; Caldwell, 1937; Dodoff, 1963; Kajiwara and Iwata, Owen, 1958; Ozoe, 1956; Sarasola and Campi, 1947; Schein, 1958, 1960; Smith, 1937). Results of cross inoculation tests carried out with isolates of *R. secalis* from different hosts indicate that not all isolates infect barley nor are all possible grass hosts affected by all isolates.

Survival and Dissemination

Survival of the fungus has been studied both in the laboratory and under field conditions (Caldwell, 1937; Evans, 1969; Ozoe, 1956; Skoropad, 1966). It has been reported that *R. secalis* persists from season to season as mycelium in barley debris. If the first leaf of a seedling emerges close to infected debris, disease symptoms appear in a fortnight under favorable conditions. Evans (1969) found that spring barley could become infected with *R. secalis* from the previous season's barley debris, even though attempts were made to bury all sources of inoculum by ploughing. He also has shown that the extent and

severity of the disease depends on the amount of stubble debris on the soil surface. When

cool, moist conditions prevail, conidia are produced on the superficial stromata present on

infected plant debris. Either free moisture or a high relative humidity (95-98 percent) is

necessary for conidial production (Ayesu-Offei and Carter, 1971; Caldwell, 1937; Ozoe,

1956; Skoropad, 1962b). Sporulation can take place over a wide range of temperature, but

abundant conidia are produced in the range 10-20 C within 24 hours (Caldwell, 1937;

Owen, 1958; Skoropad, 1962b). The fungus loses its sporulating ability within 32 and 16

days at 10 and 18 C, respectively, when infected plant debris is kept continuously wet on a

soil surface in the laboratory. Skoropad (1966) was able to demonstrate sporulation of the

organism after 340 days following storage of infected debris in bags in the field. He also

reported that production of successive batches of conidia in the presence of free moisture,

and invasion by microbial saprophytes, destroyed the sporulating ability of the scald

lesions, and that the most rapid deterioration of stromata occurred at 18 C in continually

moist conditions on leaves in contact with the soil. Several (5-8) wetting and drying cycles

deplete the reserves of the fungus so that sporulation ceases even under optimum conditions

(Skoropad, 1962b). Bartels (1928) in his investigation on survival of the fungus under field

conditions found viability to be maintained for 6-9 months. Ozoe (1956) found that the

fungus survived for about one year on infected straw kept in the laboratory. He noted that

it usually failed to oversummer if the straw was left in the open field or buried in the soil.

Polley (1971) thinks that, sclerotia, which give rise to hyphae and conidia on the return of

favorable conditions, are means of overwintering in Great Britain. In Australia, it has been

demonstrated that the fungus survived in the field in infected debris and could give rise to

infection for up to eight months (Shipton et al., 1974).

It has been claimed earlier that the fungus is soil-borne (Bartels, 1928; Heinsen, 1901;

Mackie, 1929). However, there is no substantial piece of evidence that the fungus actually

survives saprophytically in the soil (Brooks, 1928; Caldwell, 1937; Ozoe, 1956; Skoropad, 1962b). Seeds have also been shown to carry the fungus and are particularly important in long-range dissemination of the organism (Habgood, 1971; Ozoe, 1956; Skoropad, 1959; Smith, 1937). Coleoptiles are attacked when germination of infected seeds takes place under conditions favorable to the pathogen (Skoropad, 1959). Volunteer barley plants growing from grain shed before and during harvesting become infected with R. secalis when spores are dispersed from the stubble (Evans, 1969; Skoropad, 1960).

Ozoe (1956) and Skoropad (1960) have suggested that after development of the first lesions, secondary inoculum is dispersed by wind-borne rain splash. Ozoe (1956) claimed that the number of conidia in the air appeared to be greater in the day than at night. However, Ayesu-Offei and Carter (1971) reported that conidia may be released at any time of the day or night. Skoropad (1959) reported that conidia were most abundant during rainstorms and that they were usually trapped in clusters of three to ten, which indicated that they were transported in droplets of water.

Field observations have shown that a low level of primary infection can give rise to a severe epidemic if subsequent weather conditions are favorable for secondary infection (Jenkins and Jemmett, 1967). Skoropad (1960) has emphasized that cool, moist conditions are necessary for the establishment of the disease in Canada, whereas Ozoe (1956) reported that a warm winter with heavy rains caused more serious infection than occurred in normal years in Japan.

Ayesu-Offei and Carter (1971) found that sporulation occurred most abundantly when free water was available and that conidia were released simultaneously with rainfall or irrigation. Their experiments have shown that the conidia are not readily dislodged by wind alone, supporting the view that release and dispersal of the conidia are mainly the result of water splash. The maximum number of airborne conidia they trapped in a day was 272 which is very small compared with numbers of spores of other pathogens trapped

within cereal crops by other researchers. Hirst (1961) reported catches of up to 11,000 uredinospores of *Puccinia graminis* per cu m air in infected spring wheat, and Sreeramulu (1962) mentioned concentrations of as high as 14,300 chlamydospores of *Ustilago nuda* per cu m air over barley infected with the fungus. Stedman (1980) showed that the number of spores trapped was not related to the quantity of or duration of rainfall but was related to the mean rate of fall during brief showers only. He reported catches of up to 436 spores per cu m, most spores being trapped at ground level.

Conidia Germination and Infection Process

Conidia can germinate readily in water from one or both cells within 12 hours at 15 C. More than one germ tube may arise from either cell which may branch (Ayesu-Offei and Clare, 1970; Caldwell, 1937; Smith, 1937; Kelemu and Sharp, unpublished). Ayesu-Offei (1971) found germination continued on leaf surfaces for a number of days. A portion of germ tubes develop appressoria, which may develop either at the tips of germ tubes or be sessile on the conidia. Ayesu-Offei and Clare (1970) observed penetration occurred within 24 hours even in the absence of appressorial formation. These authors confirmed Caldwell's (1937) observation that penetration occurs directly and not through the stomata as reported by Bartels (1928) and Mackie (1929). They demonstrated that hyphae below the appressoria penetrated the cuticle and extensive mycelial mats were formed between the cuticle and the outer epidermal cell walls. Epidermal cell walls beneath the subcuticular hyphae became swollen and collapsed. Mesophyll cells, beneath the subcuticular hyphae, also collapsed and died before being entered by hyphae which penetrated between epidermal cells. These workers indicated that penetration of the cuticle and collapse of the epidermal cell walls and mesophyll cells was due to the activity of materials excreted by the fungus.

The Environment

Disease symptom expression and the rate of symptom development are determined by host and pathogen genotypes and the existing environmental conditions. Phenotypic variability of the complex interaction involved complicates the genetic inferences that have to be made. Genetic factors cannot cause a character to develop unless they have the proper environment, and, conversely, no amount of manipulation of the environment will cause a character to develop unless the necessary genetic factors are present. Environment plays a role in contributing variability among individuals. Distinguishing among genotypes on phenotypic grounds is facilitated if the environmental component of variability is reduced. An appreciation is thus necessary of the non-genetic factors contributing to phenotypic variability of disease symptom expression.

Temperature and Moisture

Moisture is essential for the production of inocula. The atmosphere must be humid enough for the tissue to absorb moisture (Caldwell, 1937; Ozoe, 1956; Skoropad, 1966). Production of conidia is favored by temperatures between 10 and 20 C (Ozoe, 1956; Skoropad, 1957, 1960, 1962b). The period of time during which *R. secalis* retains its ability to sporulate in lesions of naturally infected barley leaves is strongly influenced by an interrelationship of moisture, temperature, and location of leaves in relation to the soil. Conidial production ceases during a dry period and a new crop is produced when the stroma is wet again.

Optimum infection occurs at a soil temperature of 16 C; it decreases sharply at 20 C, and is almost absent at 22 C. Lesion development proceeds normally when the post-inoculation temperature is 12-24 C, but few lesions develop and development is slow when the

post-inoculation conditions are 6-12 C or above 24 C; exposure to lower temperature initially followed by exposure to higher temperature favors lesion development (Caldwell, 1937; Skoropad, 1957).

Ali (1972) showed that the effect of temperature on symptom expression is subject to the particular combination of isolate and host genotypes examined. He observed that at high diurnal temperature regimes (18 C min./30 C max.), the ability of certain isolates to infect hosts normally susceptible to them was greatly impaired, while other isolates could infect hosts normally resistant to them. It was observed that at low temperature regimes (8 C min./20 C max.) the rate of symptom development proceeded most rapidly for certain isolates whilst higher temperatures (15 C min./24 C max.) favored the rate of symptom development of others. Evidence was also found to indicate that variability in the pathogenic characteristics of isolates may likewise be differentially influenced by environmental modification.

Nutrition

The effect of mineral nutrition on disease severity is largely unknown. Vandervalk (1942), as cited by Shipton et al. (1974), reported that the disease disappeared following cultivation and the application of nitrate. The disappearance of the disease, however, may be attributed to the destruction of much of the inoculum by cultivation. Jenkins and Jemmett (1967) occasionally found higher levels of disease in some variety trial plots receiving higher levels of nitrogen. According to the report by Ozoe (1956) inorganic nitrogen sources enhance susceptibility to disease.

Experiments conducted by Jenkyn and Griffiths (1976) showed that nitrogen fertilizer did decrease number of infections which contrasts with field observations that crops receiving most nitrogen are usually more severely diseased (Doling, 1964). The increased disease in the field may have to be attributed to enhanced lesion development or sporulation

rather than increased infection. Jenkyn and Griffiths (1978) found the susceptibility of cultivars to barley scald negatively correlated with water-soluble carbohydrate content and positively with nitrogen content.

Interaction: Host, Pathogen and Environment

Symptoms

The disease of barley caused by *Rhynchosporium secalis* is commonly referred to as *Rhynchosporium* leaf blotch, barley leaf blotch, *Rhynchosporium* scald, or simply scald. Symptoms produced on barley are usually distinctive for the pathogen on that host. Lesions on naturally or artificially infected leaves begin as grey, water soaked areas 8-14 days after inoculation. At this stage, lesions dry out but retain a grey-green color which persists even when the uninfected parts of a leaf have become straw colored. Usually the lesions become oval-shaped with dry, pale-brown or white centers surrounded by dark-brown margins. Leaves of a susceptible seedling barley cultivar completely wilt and show no discrete lesions after infection by the pathogen. Barley scald symptoms can appear on leaf sheaths, floral bracts, pericarp and awns of barley.

Genetics of Scald Resistance

Mackie (1929) was the first to study the inheritance of scald resistance. He found that resistance in an unnamed barley variety was governed by a single recessive gene. Twenty years later Riddle and Briggs (1950) identified a single dominant gene controlling resistance in La Mesita (CI. 7565), which was also present with a recessive gene in its derivatives, Trebi (CI. 936) and Modoc (CI. 7566) and with one or more additional dominant genes in Turk (CI. 14400). Bryner (1957) reported that resistance in Brier (CI. 7157) was conferred by a single dominant gene. Bryner (1957) was the first to use a designation for genes conferring resistance to *R. secalis*. He assigned the symbol Rha (later amended to Rh) to the dominant gene he found in Brier.

Of the several studies made on the genetics of scald resistance, that of Dyck and Schaller (1961) is the most extensive. Using four pathogenic races of *R. secalis*, they identified five dominant genes for resistance in eight varieties of barley which they designated Rh2 to Rh5. The varieties Atlas and Atlas 46 possessed the Rh2 gene which conditioned a type 1 reaction to race U.S.1 and a type 2 reaction to U.S.7 of *R. secalis*. Atlas 46 had the Rh3 gene, in addition to the Rh2 gene, which conditioned a type 0 reaction to races U.S.1, U.S.7, and U.S.8. The same gene was found in the varieties Turk and possibly Brier. Rh4 gene was reported to condition resistance of La Mesita, Trebi and Osiris (CI. 1622). Rh4 gene was closely linked with the Rh3 gene, with a recombination value of 1.0 ± 0.78 percent. An allele at the Rh4 locus, $Rh4^2$ gene was identified in Modoc. Rh5 gene, which was independent of the Rh3 and Rh4 genes, was present in Turk. These genes were demonstrated to be specific against the races of the pathogen used. In subsequent years, Baker and Larter (1963) studied the inheritance of resistance to a Saskatchewan isolate of the pathogen in five varieties of barley. They found a pair of complementary recessive genes, rh6 and rh7, in Jet (CI. 967) and Steudelli (CI. 2266), and an incompletely dominant gene, Rh9, in Kitchin (CI. 1296) and Abyssinian (CI. 668). They confirmed the presence of a single dominant gene for resistance in Turk. The effectiveness of the two complementary recessive genes for resistance was impaired by temperatures above 25 C during the infection period.

Wells and Skoropad (1963) used an Alberta isolate of *R. secalis* to determine the mode of inheritance of resistance in eight resistant barley cultivars. They found that seven of the cultivars, Turk, Bey, Rivale (CAN. 258), 36Ab1991 (CAN. 136), CI. 3515, CI. 8256 and Osiris, possessed in common a dominant gene considered to be Rh3. A recessive gene, which they designated rh8, governed resistance in Nigrinudum (CI. 2222). In 1967 Frecha reported that Osiris, Psaknon and Atlas 46 had a dominant gene for scald resistance in common, and that Atlas 46 had also an additional dominant gene.

Starling et al. (1971) recognized the predominance of the Rh-Rh3-Rh4 complex locus in the barley cultivars they studied. The results indicated that Atlas 46, Turk, Brier, La Mesita, Modoc, Gembloux 14, Alask (CI. 534 and CI. 4106), Tennessee Winter (CI. 876), Kentucky No. 36, Olympia, Tschermak, Hudson, Carstens 2-row, CI. 3515, CI. 8071, CI. 8101, CI. 8256, CI. 9042 and CI. 10176 all had a single gene or gene locus in common. CI. 8618 was found to have a dominant gene not found in any other variety they tested. Atlas 46 had an additional gene which was also present in Atlas, and CI. 3515 also had an additional dominant gene, different from that in Atlas. Evans (1969) detected a dominant gene for resistance in Atlas 46, Turk and Osiris. These genes were presumed to be Rh3 in Atlas 46 and Turk and Rh4 in Osiris since they were either allelic or closely linked.

Habgood and Hayes (1971) studied the inheritance of resistance to three isolates of *R. secalis* in 18 barley varieties. The results of their experiment suggested that the resistance genes in Turk, Atlas 46, Brier, Modoc and Cb 1084 are all situated at the same locus even though separate loci up to 1.7 cross-over units apart could give the same results. A less conclusive evidence was obtained that Hudson (CI. 8067) and Dea also contained genes at this locus. They further differentiated the gene in Modoc from the others at the same locus by its ineffectiveness against one of the isolates used and that in Cb 1084 by its incomplete dominance. The inheritance of resistance in CI. 3515, CI. 8256, Gembloux 14 and La Mesita was reported to be identical to that in Osiris. It was confirmed that resistance in Jet was controlled by two complementary recessive genes, but one of these was shown to be situated at the locus carrying the dominant genes in Osiris, Brier, Turk and Modoc. A recessive gene for resistance was found in CI. 4364 and CI. 4368 which has not been previously reported. Bockelman et al. (1977), using trisomic analysis, determined chromosomal location of the resistance genes in Kitchin and in Jet. They showed that Kitchin possessed a single gene, Rh9, on chromosome 4, whereas, Jet contained rh7 and rh6 on chromosomes 3 and 4, respectively.

Habgood and Hayes (1971), aided by their discovery that a single gene segregation apparent after a 14-day incubation period might be interpreted as the action of two complementary genes if re-examined after a 28-day incubation period, have detected a gene in Osiris which is complementary to the one effective 14 days after inoculation. This gene has not been previously reported since its effect is not apparent until well after the normal times of assessment. The symbol Rh10 was suggested for this gene whereas rh11 was proposed for the recessive gene in CI. 4364 and CI. 4368. These workers further proposed revised symbols for the designation of some of the resistance genes identified. Summarizing the results obtained by Habgood and Hayes (1971), there are five alleles at the Rh locus, two are dominant (Rh and Rh^2), two are incompletely dominant (Rh^3 and Rh^4) and one is recessive (rh^5).

According to studies by Ali (1972), environmental conditions of testing have considerable influence on symptom expression and thus upon interpretation of genetic results. Transgressive segregation for increased scald resistance has been indicated by Ali. Habgood (1972) detected a high level of field resistance at the adult stage which escaped detection in routine seedling inoculation studies. Quantitative differences between various barley cultivars in resistance to R. secalis at the seedling stage have been demonstrated by Jenkyn (1969), Williams (1969), Fowler and Owen (1971), Evans and Griffiths (1971) and Habgood (1972). Habgood (1974) studied the inheritance of partial resistance, which appears to be race non-specific, in European spring barley cultivars. He reported that resistance was complex in inheritance, the results being incompatible with any hypothesis involving less than four genes and that transgressive segregation occurred in all cross combinations in the F_3 material.

Fowler and Owen (1971) studied the mechanism of resistance to barley scald. Their report indicated that the earliest point at which resistance was expressed was at penetration of the cuticle. Host resistance did not affect germination or appressoria formation (Ayres and Owen, 1970; Kelemu and Sharp, unpublished).

CHAPTER 3

MATERIALS AND METHODS

Ethiopian barleys which had shown consistent and uniform resistance to isolates of *R. secalis* in both field and greenhouse tests were studied to determine the number of loci for genes conditioning resistance to several isolates of the pathogen. Each of the five Ethiopian barley cultivars chosen was crossed with Betzes, a susceptible barley variety to all of the isolates used, to determine the number of genes for resistance to barley scald in each; and all five were crossed in all possible combinations with each other to determine whether any had genes for resistance at common loci or linked. Each of these barleys was also crossed with twelve barley cultivars which had previously been studied for inheritance of reaction to *R. secalis* and assigned gene symbols. The F_1's from crosses between Ethiopian barleys and Betzes were backcrossed to Betzes. The cultivars used as parents in this investigation are listed in the Appendix, Table 19, with their origin and some agronomic characters.

Seven different isolates of the fungus from five countries were used in the experiment. Two isolates of the pathogen were from Ethiopia (Debre Zeit and Holetta areas), two from U.S.A. (California and Montana), one from Tunisia (Beja), one from Morocco (Beni-Mellal), and one from Mexico (El Batan). The isolates have been given the designation Eth-DZ-83, Eth-HT-83, US-CA-83, US-MT-83, Tun-BJ-81, Mor-BM-77, Mex-EB-83, indicating the country, the particular place and year of collection, respectively. The fungus was isolated from infected barley leaves using the method described by Schein and Kerelo (1956). The spores of the different isolates were lyophilized and kept in a refrigerator as a safeguard if attenuation of the isolates occurred in the process of subculturing.

The genetic analyses were made on the basis of F_1, BC_1, F_2 populations and some F_3 families. At least 200 seeds of F_2 populations of each cross were grown in metal flats (14" X 10" X 3") at the same time as the parents, F_1 and backcrosses to the susceptible parent in some cases. Betzes was included as a susceptible check in each flat. Sixteen F_3 lines, each line containing at least 30 seeds, were planted in one flat. The F_3 lines were separated from one another using pieces of cardboard. Parental cultivars were planted with their F_3 populations. All flats were kept in the greenhouse maintained at about 21 C prior to inoculations. In some warm days the temperature had risen above 26 C.

To prepare inoculum, conidia from lyophilized cultures were transferred to Petri dishes containing fresh lima bean agar and were kept in a constant temperature cabinet at 18 C. Plantings of barley and transfers of isolates were usually made the same day. A spore suspension, harvested from two week old cultures by adding distilled water and rubbing the colonies off with a glass slide, was used as inoculum. The suspension of spores and agar was filtered through four layers of cheesecloth to remove the agar fragments. Inoculations were made 14 days after planting. The spore suspension with a concentration of at least 100,000 spores per ml was applied using a spray bottle until wetness. The plants were then incubated in the dark for 24 hours at 100 percent relative humidity (21 C) and subsequently transferred to a growth chamber maintained at alternating temperatures of 21 C (light) and 16 C (dark). In 10-14 days the scald symptoms developed sufficiently for evaluation. The scale of 0 to 4, described by Dyck and Schaller (1961), was used in evaluating the reaction of individual plants (0 = no lesions, a fully resistant reaction; 1 = small lesions on the margin of the second leaf only; 2 = somewhat larger lesions on the margin of the second leaf and a large lesion at the base of the leaf or at the lower extent of the leaf area exposed when inoculated, but no lesions on the first leaf; 3 = large lesions covering almost the entire second leaf, occasionally causing the leaf to collapse and/or wilt, with a few small lesions on the first leaf; 4 = all exposed areas, both first and second leaf, wilted with no

definite lesions). Plants with 0 and 1 reactions (resistant) and those with 3 and 4 reactions (susceptible) were grouped for convenience in presenting the data. Intermediate plants (type 2 reaction) were observed only in crosses involving Kitchin, Atlas and Atlas 46. F_3 lines were classed as resistant, segregating, or susceptible. Scald reactions of the plants in each segregating F_3 line were classified using the scale of 0 to 4.

Parental cultivars and their F_2 populations were space planted at the Horticultural Farm, Bozeman, Montana on May 16 and 17 in 1984.

A spore/agar suspension was prepared by blending two-week-old cultures in distilled water (cultures from 10 Petri dishes (100 mm $\times$ 15 mm) in 1 liter of distilled water). Tillering plants were inoculated with US-MT-83 isolate of *R. secalis* twice at a weekly interval in later afternoons in June using a sprayer. All materials were sprinkler irrigated for a few minutes before inoculations. Immediately after inoculation, the plants were covered with plastic sheets for about 14 hours to create a favorable environment for infection. Scald readings were made in July using a modified 0 to 4 scale, where class 1 was modified to include plants with small lesions on the margin of the second leaves and/or lesions on leaf sheaths.

The probability values for goodness of fit to expected ratios were calculated using the Chi square method. The maximum recombination frequency which could have passed undetected was calculated using the formula outlined by Hanson (1959): $Prc = 1 - \sqrt[n]{0.05}$, where n is the number of F_2 plants or F_3 lines used, and Prc is the proportion of detectable recombinants.

CHAPTER 4

RESULTS

The seedling reactions of 18 barley cultivars to seven isolates of *R. secalis* are shown in Table 1. Tun-BJ-81 isolate was found to be the most virulent attacking 14 of the parents, whereas US-MT-83 was the least virulent infecting only one, Betzes. The more avirulent isolate will detect the largest number of resistance genes, hence, US-MT-83 was used to detect resistance genes in most instances. PI. 382488, PI. 383036 and Osiris were resistant to all of the seven isolates. PI. 382282, PI. 382471 and PI. 382509 reacted identically to all of the isolates.

There could be several pairs of cultivars whose resistances when combined would, at least theoretically, be effective against all of the isolates used in this study if the resistance of the cultivars can be combined readily. Even though PI. 382488 was resistant to all of the isolates in this study at normal temperature regime, temperatures greater than 26 C induced a susceptible reaction to isolates Tun-BJ-81, Eth-HT-83, Mex-EB-83 and Mor-BM-77. The resistance in PI. 382282 was also impaired by temperatures above 26 C to Mor-BM-77 and Eth-HT-83 isolates.

Scald lesions were observed on leaf sheaths of PI. 382282, PI. 382472, PI. 382488, PI. 382509 and PI. 383036 in the field, whereas, the leaves remained free of symptoms. Brown discoloration and subsequent wilting of leaf sheaths of these cultivars was also noted in seedling tests in growth chambers while the leaves were resistant to the isolates shown in Table 1. Both the leaf sheaths and leaves of Osiris were symptomless, whereas, those of Betzes were susceptible. Leaf sheath resistance and leaf susceptibility were noted in other