The inheritance of resistance to Xanthomonas campestris pv. translucens (J.J.R.) Dowson in barley
by Gurbuz Mizrak

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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Barley cultivars, Herta, Summit, Oderbrucker, Betzes, Alpine, Luther, CI 12558, CI 12569, CI 12595, CI 12777, CI 12787, CI 12866, CI 13095, PI 382511, PI 382650, PI 382720, PI 382732, and PI 383077 were found to be promising sources for resistance to bacterial leaf streak. Resistance in these cultivars varied with regard to gene action and number. Some of them may have identical genes or differrent genes in the same chromosomes. Further study is needed to determine identical, linked, and independent genes. Also, a close relation, wich suggests a linkage, was found between earliness and susceptibility.

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Gürbüz Mızrak

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

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Signature Gürbüz Mızrak

Date March 5, 1985

In dedication to:

My daughters Tuğba and Gaye,
my wife, my father and mother

VITA

Gürbüz Mızrak, son of Hüseyin and Memduha, was born on February 2, 1949 in İnziloglu, Ankara, Turkey. He grew up in İnziloglu where his father farmed.

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ABSTRACT

Inheritance of resistance to bacterial leaf streak was investigated in 23 barley cultivars. Parents, F_1s and F_2s of one complete diallel set with six varieties and those of 23 other crosses were tested with one Montana isolate (X-67) of Xanthomonas campestris pv. translucens in the field. An inoculation method is described which result in a uniform level of infection.

Barley cultivars, Herta, Summit, Oderbrucker, Betzes, Alpine, Luther, CI 12558, CI 12569, CI 12595, CI 12777, CI 12787, CI 12866, CI 13095, PI 382511, PI 382650, PI 382720, PI 382732, and PI 383077 were found to be promising sources for resistance to bacterial leaf streak. Resistance in these cultivars varied with regard to gene action and number. Some of them may have identical genes or differrent genes in the same chromosomes. Further study is needed to determine identical, linked, and independent genes. Also, a close relation, wich suggests a linkage, was found between earliness and susceptibility.

CHAPTER 1

INTRODUCTION

Bacterial leaf streak of cereals is a world wide disease caused by the bacterium, Xanthomonas campestris pv. translucens (J.J.R.) Dowson. In Montana, the disease has increased in irrigated cereals fields in the past few years, and reportedly decreases yields in barley (Hall and Sands, 1983). All commercial barley varieties grown in Montana are susceptible to this disease. Organic mercuric seed treatments apparently controlled the disease for a number of years. After the curtailment of these seed treatments, no effective method has been available to control seed transmission of the disease.

Breeding for resistance is another mechanism to control diseases. In order to implement a breeding program, breeders need resistant germplasm, and a reliable screening test. Bacteria, as with other pathogens, produce new strains which can overcome host resistance, especially if the resistance is determined by single genes in susceptible backgrounds (See Literature Review). Therefore, combining resistant genes from

different sources should be a breeding strategy.

Expression of resistance to bacterial leaf streak is influenced to a great extent by environment (See Literature Review). In many cases, the results of field and greenhouse tests are not the same(Kim, 1982). Plant cultivars resistant in the field may be susceptible in the greenhouse. It has been observed that the variation in reaction of a particular cultivar can be very large even within a small area. The difficulty in creating uniform infection has made the results of screening tests unreliable. For the above reasons, developing an appropriate screening method in the field is necessary.

The inheritance of resistance to bacterial leaf streak in barley is unknown. Previously some barley varieties and lines were screened for disease resistance and some of them were found to be promising as sources of resistance (Stewart, 1952; and Kim, 1982). The goals of this study were to determine the inheritance of resistance to bacterial leaf streak in barley by using reliable screening techniques, to find different sources of resistance, and to devise a breeding plan for developing resistant barley varieties to bacterial leaf streak. It is hoped that a long-lasting resistance to

bacterial leaf streak in barley might be developed by combining resistance from various sources.

CHAPTER 2

LITERATURE REVIEW

Bacterial leaf streak of grasses caused by the bacterium, Xanthomonas campestris pv. translucens (J.J.R.) Dawson, has been observed since the late 1800s (Mathre, 1982). The earliest report of this disease on barley came from Madison, Wisconsin in 1912 where it appeared as an unusual leaf blight in a plot of Montana two-row barley and subsequently spread to other varieties.

This disease was first described by Jones et al. (1917). They reported bacterial leaf streak of barley in Colorado, Iowa, Minnesota, Montana, North Dakota, South Dakota, Oregon, and Wisconsin. It was found in Nebraska (Pady, 1944), in Northern Europe and Asia (Jaczewski,1935). Bacterial leaf streak is now known to occur world wide on barley, wheat, rye, triticale, and numerous grasses (Wallin, 1946; Koleva, 1981; Leyns et al., 1981; Egli and Schmidt, 1982; Mathre, 1982; and Miyajima, 1982). The disease has become prevalent in sprinkler irrigated barley and wheat fields of Montana within the past few years (Hall and Sands, 1983).

Early reports showed that local and general epiphytotics on susceptible cultivars reduced yield (Dickson, 1956). Commonly, bacterial leaf streak in barley reduces yield by 10-15% (Mathre, 1982; Hall and Sands, 1983). Yield reduction of severely infected wheat fields has been estimated to be as high as 50% (Hagborg, 1968).

Symptoms

The first symptoms on barley appear on the young green leaf blade and sheath as small, water-soaked lesions that later become light-brown and dark-brown regions. These lesions tend to develop as longitudinal streaks between the veins, then coalesce into narrow glossy-surfaced stripes extending the entire length of the leaf (Zilinsky,1983). In the later stage of development, the lesions show a characteristic translucency (Dickson, 1956). A bacterial exudate is usually present on the lesions. Under humid conditions, this exudate may appear as numerous tiny cream-like to yellow droplets giving the leaf surface a shellacked appearance. The appearance of exudate is a key characteristic in distinguishing this disease from net blotch, barley stripe, and barley stripe mosaic (Mathre, 1982). Under dry conditions the exudate quickly hardens

into yellow granular beads or glistening scales easily detached and readily softened or dissolved in water (Jones et al., 1917).

Similar lesions develop on the floral bracts and are termed "black chaff". The entire head can turn light to very dark brown. Severe late infection usually results in retarded spike elongation and blighting of the spike and adjacent tissues. An infected head may be bent and distorted (Dickson, 1956). Bacterial growth can often be seen as shellac on the surface or the seeds and a portion of the grain may be blighted (Hall and Sands, 1983).

El-Banoby and Rudolph (1979) reported that persistent water-soaked leaf spots were induced in susceptible cultivars of barley and wheat by treatment with preparations of extracellular polysaccharides (ESP) from Xanthomonas campestris pv. translucens f.sp. cerealis.

The Bacterium

Xanthomonas campestris pv. translucens (J.J.R.) Dowson, the causal organism or bacterial leaf streak of barley, belongs to the genus Xanthomonas. According to Dye (1980), the genus Xanthomonas is defined as follows: Gram negative rods 0.4 x 1.0 um, single polar flagellum,

aerobic, catalase positive, H_2S positive, oxidase negative, Tween 80 hydrolysis, nitrate not produced, and indole not produced. All Xanthomonas species recognized at present are plant pathogens and, so far as known, are found only in association with plants or plant material. Most xanthomonads produce the extracellular polysaccharide slime "gum-xanthan" on media containing glucose. Colonies of these species on nutrient agar or YDC agar are mucoid, convex, and shining.

The five nomenspecies of the genus Xanthomonas are X. campestris, X. fragariae, X. albilineans, X. axanopodis, and X. ampelina. X. campestris can be differentiated from the others by its positive test for protein digestion. The bacteria in this nomenspecies can be identified by growth characteristics on SX agar and by pathogenicity tests (Dye, 1980). X. campestris pv. translucens can not grow on SX agar and its strains are pathogenic to barley, wheat, rye, triticale, oats, rice and some other grasses (Smith et al., 1919; Reddy et al., 1924; Hagborg, 1942; Fang et al., 1950; Patel and Shekhawat, 1971; Dye, 1980; Egli and Schmidt, 1982; and Leyns et al. 1984). Previously, eight form species of X.c. pv. translucens have been described according to their host specifity (Table 1).

Table 1. The form species of *Xanthomonas campestris* pv. *translucens* and their hosts.

Form species	Hosts	References
Xct f.sp. *undulosa*	wheat, spelt, barley and rye	Smith, Jones and Reddy, 1919.
Xct f.sp. *secalis*	rye	Reddy, Godkin and Johnson, 1924.
Xct f.sp. *hordei*	barley and rice	Hagborg, 1942; Patel and Shekhawat, 1971.
Xct f.sp. *hordei-avenae*	barley and oats	Hagborg, 1942.
Xct f.sp. *cerealis*	wheat, oats, barley, rye smooth brome grass, quack grass, Italian rye grass, perennial rye grass, and meadow fescue	Hagborg, 1942; Fang et al., 1950; Egli and Schmit, 1982.
Xct f.sp. *phlei*	*Phleum* sp.	Wallin and Reddy, 1945; Egli and Schmidt, 1982.
Xct f.sp. *poae*	*Poa* sp.	Egli and Schmidt, 1982.
Xct f.sp. *arrhenatheri*	*Arrhenatherium* sp.	Egli and Schmidt, 1982

Xct= *Xanthomonas campestris* pv. *translucens*

Host Range

According to Schuster and Coyne (1974), bacterial pathogens with wide host ranges can survive between crop seasons on alternate hosts which provide a bridge during off-seasons. Such pathogens are not handicapped by discontinuous plant growth unlike the parasites that have narrow host ranges.

Boosalis (1952) investigated the overwintering of Xanthomonas campestris pv. translucens f. sp. cerealis on infected quack grass. It was pathogenic to wheat, oats, barley and rye. This example shows the importance of disease transmission from wild flora.

Previous studies show that the Xanthomonas campestris pv. translucens group has 23 different hosts in the Gramineae. These include, barley, wheat, rye, oats, triticale, rice, foxtail barley, spelt, einkorn, smooth brome, cheat grass brome, yellow bristle grass, quack grass, orchard grass, water foxtail, reed canary grass, Japanese millet, Phleum sp., Poa sp., Arrhenatherum sp., Italian rye-grass, perennial rye-grass and meadow fescue (Smith et al., 1919; Reddy et al., 1924; Hagborg, 1942; Wallin and Reddy, 1945; Fang et al., 1950; Boosalis, 1952; Patel and Shekhawat, 1971; Leyns et al., 1981; Cunfer and Scolari, 1982; Egli and Schmidt,

1982; Kim, 1982; and Leyns et al. 1984).

Barley seems to be susceptible to most of the form species of X.c. pv. translucens (Table 1). Hall et al. (1981) reported that the isolates from barley, wheat and rye in irrigated cereal fields of Montana were pathogenic on barley. Cunfer and Scolari (1982) found that strains of X. c. pv. translucens from triticale were equally virulent to triticale, wheat and rye, but much less to barley. They reported that strains from wheat and rye were usually most virulent on the host from which they were isolated, however, only one cultivar of each host species was tested. Strains from barley were restricted in host range, being pathogenic primarily to barley.

The form species hordei was originally reported to be pathogenic only to barley (Hagborg, 1942). However, Patel and Shekhawat (1971) showed that rice was susceptible to X. c. pv. t. f.sp. hordei at the 20 and 40 day old stages. According to Fang et al. (1950), X. c. pv. t. f.sp.cerealis occurs naturally on smooth brome grass, quack grass, and produces water-soaked streaking symptoms on barley following wound inoculation, but it is more virulent on grasses.

Bamberg (1936) found that strains of X. c. pv. translucens differ in pathogenicity. According to Hagborg

(1942), different isolates of the same form species of X. c. pv. translucens are different in pathogenic capablities and he suggested the need for recognizing races within this special form.

Etiology and Epidemiology

Bacterial leaf streak is a widely occuring disease affecting leaves, leaf sheaths, and glumes. The bacterium infects leaves through stomata and wounds, spreading intercellularly (Jones et al., 1917 and Bamberg, 1936). The bacterial invasion is confined to the thin-walled parenchyma possessing intercellular spaces, i.e., mesophyll of leaf, chlorenchyma and ground tissue parenchyma of the leaf sheath. In naturally infected leaves no vascular bundle elements were shown to be infected (Sheakhavet and Patel, 1978).

The seed-born nature of this disease was reported by Jones et al.(1916) and Smith et al.(1919). The bacterium penetrates the pericarp by causing infection of the plumula through wounds or stomata on the coleoptile. Spreading rapidly through these tissues, it finally reaches the enclosed foliage leaves. By elongation, the infected leaf develops the water-soaked streaks on the primary leaf (Wallen, 1946).

According to Boosalis (1952), barley plants from

infected seeds planted in sterilized soil showed a higher percentage of infection than those planted in nonsterilized soil. He found that seeds of barley, wheat and rye inoculated with X. translucens alone resulted in a few infected seedlings, whereas 25 to 40 percent of seedlings from seeds co-inoculated both with a root rot fungus (Helminthosporium sp.) and X. translucens succumbed to Xanthomonas streak. Other than seed transmission, this bacterium may persist from one season to the next on straw of barley, wheat, some other grasses, and on winter hosts.

Hedrick(1956) and Leach et al.(1957) thought that bacterial exudates behave as a hydrophilic colloid. Its high waterholding capacity may aid bacterial survival during unfavorable conditions and seasons. According to Schuster and Coyne (1974), maintenance under dry conditions commonly favors bacterial survival in plant residues. Residues on or near the soil surface are more favorable to bacterial survival than those incorporated in soil. The bacteria in dry, undecayed residues are protected from antagonistic microflora which may require moisture for movement. Probably for the above reason, X. c. pv. translucens is very tolerant to dry conditions. Jones et al.(1917) reported that the bacterium survived

for eight months on straw from blighted plants and two years on seeds. According to Boosalis (1952), X. c. pv. translucens was not able to survive long in soil kept at 27° C and in the field during the winter months unless the soil was sterile and asepsis was maintained. Bamberg (1936) reported that the bacterium survived 77 days in dry soil at St. Paul, Minnesota. He found that this organism can survive at extremely low temperatures ranging to -33° C, remaining viable in soil cultures for at least 124 days after December 15.

Bamberg (1936) found that low relative humidity retarded the growth of the bacterium in culture. The retarding effect was noticeable at 50 percent relative humidity and became greater as the R.H. decreased. Optimum temperature for the growth of this organism on agar was between 25-30° C. The maximum temperature for sustained growth was approximately 40° C with the minimum being slightly below 10° C.

Severe epidemics occur in wet seasons with the optimum temperature around 20° C (Kim,1982). According to Bamberg (1936), temperature had a marked effect on the length of the incubation period and disease development. At 20° C from two to seven days were necessary for incubation, while at 10° C from eight to 20 days were

required. Disease development was slowed even more at or below 10° C. Stewart (1952) reported that the highest infection on susceptible plants occurred at 15.5 to 18° C, moderate infection at 21° C, slight infection at 24° C, and no infection at 27° C, an optimum temperature for growth in culture. According to Kim (1982), symptom development was influenced by temperature. Symptoms did not appear under the 15.5° C day and 4.5° C night regime. Under the 21.1° C day and 12.8° C night, and 32° C day and 1.8° C night regimes, symptoms developed. Bamberg (1936) reported that for disease development, low relative humidity is more often a limiting factor than low temperature.

Effective transmission from plant to plant by aphids, wind, rain and by direct plant to plant contact, occurs only when the host is water-congested (Boosalis, 1952). Field epidemiology studies in Montana using a marked strain of X. c. pv translucens f. sp. hordei indicated that this bacterium is capable of spreading 28 square meters from a single infection locus within 39 days (Hall et al, 1981).

Selective Media

Selective media that have been developed for different xanthomonads are not efficient for isolating

X. campestris pv. translucens (Kim, 1982). Wilbrink's agar, not selective for the bacterium, is generally used for isolation and maintenance. Kim (1982) developed the KM-1 medium for isolation of X. campestris pv. translucens. This medium contains 10 g of lactose (Sigma Chemical Co., St. Louis, MO.), 4.0 g of D(+) trehalose (Sigma), 0.2 g of thiobarbituric acid (Sigma), 0.8 g of K_2HPO_4 and KH_2PO_4 (Sigma) respectively, 0.03 g of yeast extract (Difco Laboratories, Detroit), 1 g of NH_4Cl (Mallinckroft Chemical Works, St. Louis, MO.) and 15 g of Bacto agar per liter of double distelled water. Before adding agar, the ingredients are dissolved completely on a hot plate with stirring bar and pH is adjusted to 6.6 with 1 N NaOH solution. After autoclaving these ingredients, cycloheximide (Sigma), dissolved in ethanol, tobramycin (Sigma), dissolved in ethanol-water (1:1, v/v), and ampicillin (Sigma), dissolved in ethanol-water (1:1, v/v) with addition of 1 pellet of sodium hydroxide (Mallinckroft Inc., Paris, Kentucky) are added aseptically to final concentration of 100 µg/ml, 8 µg/ml and 1 µg/ml, respectively. Kim (1982) noted that the medium provides a sensitive and reliable detection of the bacterium. Other Xanthomonas spp. grow on the KM-1 medium, but have not been found in cereal grains. He

also added that this medium had high selectivity against soil-born plant pathogenic bacteria.

Schaad and Forster (1984) reported that many strains of X. c. pv. translucens from Idaho grew poorly on KM-1 agar when used for isolation of the bacterium from seeds. They developed a semiselective medium, XTS agar, for isolation of X. c. pv. translucens from seeds. XTS agar contains 23 g of nutrient agar, 5 g of glucose, 200 µg of cycloheximide, 100 µg gentamycin, and 8 µg of cephalexin in one liter. They reported that 91% of the saprophytic bacteria associated with wheat and barley seeds did not grow on XTS agar.

Screening for Disease Resistance

Most of the early attempts to select plants for resistance to bacterial diseases were carried out in locations where natural epidemics occur. However, it is necessary to inoculate test plants artificially to obtain a uniform disease epidemic (Russell, 1978). This is necessary so that different breeding lines and genotypes can be accurately compared for disease reaction. Historically, the following tecniques have been used to inoculate barley plants with X. c. pv. translucens:

1. Spraying bacterial suspension (Jones et al., 1917)

2. Partial vacuum techique (Boosalis, 1950).

3. Injection of bacterial suspension by hypodermic syringe (Bamberg, 1936; and Hagborg, 1936).

4. Seed inoculation by soaking seed in bacterial suspension for one hour (Wallin, 1946).

5. Seedling inoculation by poking the seedling with a sharp nichrome needle and flooding with bacterial suspension (Hagborg, 1936).

6. Mowing off the top of the barley plants in the evening, followed by immediate spraying with bacterial suspension, and sprinkler irrigation at a 3 day interval (Kim, 1982).

Kim (1982) suggested that the most effective methods were: Injecting the inoculum into the leaf of a seedling or into the leaf sheath of an older plant with a hypodermic syringe (#3), partial vacuum technique (#2), and his method (#6).

Previous studies revealed that there are resistant sources in barley to bacterial leaf streak. Jones et al. (1917) found that Chevalier, California, Summit(six-rowed and different from the cultivar recently released) and Oderbrucker (CI. 4666) were the most resistant cultivars of 53 tested barley cultivars. Stewart (1952) reported that the barley cultivars, Spartan, CI 6240, Velvon, and

some other lines showed high degrees of field resistance. Patel and Shekhawat (1971) tested 51 barley cultivars in the seedling stage for reaction to X. c. pv. translucens f. sp. hordei. They found no complete resistance, but variations in the degree of susceptiblity were observed.

Kim (1982) screened 54 commercial varieties, and 722 lines from the World Barley Collection for resistance to bacterial leaf streak. Barley varieties, Herta, Summit(MT 729), Oderbrucker, Alpine, Luther, Betzes, Wabet, and Shabet were found to be resistant. He also found 21 resistant lines, mostly from Ethiopia in the USDA World Barley Collection.

Screening tests for bacterial diseases carried out under natural conditions generally reflect the effectiveness of field resistance more accurately than greenhouse or laboratory tests (Russell,1978). Previous studies showed that there is usually no correlation between field and greenhouse tests for resistance to bacterial leaf streak on cereals. Bamberg (1936) reported that the wheat varieties, Marquis, Mindum and Kubanka appeared resistant in the field, yet were rather heavily infected when hypodermically inoculated. Stewart (1952) noted that some barley varieties were quite resistant in the field, but were susceptible in the greenhouse. Kim

(1982) did not find any significant correlation between results in the growth chamber and the field.

Mechanism of Resistance

The mechanism of resistance in barley to bacterial leaf streak is unknown. Generally, there are two types of resistance mechanisms to bacterial diseases: (i) Preformed resistance, and (ii) induced resistance. Preformed resistance involves the mechanisms which inhibit bacterial penetration into intercellular spaces of the plant (Klement,1972). Pathogenic bacteria penetrate their host plants through natural openings (stomata or lenticels) or wounds. Russell (1978) suggested that the concetration, size and morphology of stomata or lenticels as well as their distribution on the surface of the host plant, which are genetically controlled characteristics, may influence the host susceptibility to bacterial infection.

According to Klement (1972), the fluid in intercellular spaces of plants contains, in abundance, all nutrients necessary for most pathogenic and saprophytic bacteria, when cultivated *in vitro*. But inside the living plant only the host specific bacteria are able to grow. Sasser et al. (1970) reported that even though bacteria enter the host plant, the osmotic

potential in the intercellular leaf fluid is a controlling factor in the development of disease. The dry outside air normally inhibits the population increase of pathogenic bacteria. A period of high relative humidity and rainfall reduce the osmotic potential and allow bacterial multiplication within the host plant.

Garber (1961) suggested that buffering capacity of plant fluids affects resistance to bacterial diseases. As a response to bacterial infection, the pH of plant sap may change and affects the formation, penetration and effectiveness of enzymes produced by bacterial pathogens. He also found that the nutrients necessary for the pathogen may be absent from the resistant host tissues. Some antibacterial substances in plant tissue may inhibit bacterial multiplication (Garber,1961; Hildebrand and Sands,1966; and Klement,1972).

In contrast to preformed resistance, induced resistance includes defense mechanisms that start in plants as a result of infection. There are two types of induced defense reaction : (i) Preimmunity, and (ii) hypersensitivity. According to Klement and Goodman (1967), preimmunity is regarded as a nonspecific acquired immunity which manifests itself in plants pretreated (inoculated) with one bacterium that immunizes or

protects the plant from postinfection by another bacterial pathogen. Goodman (1980) noted that two different defense mechanisms are elicited for preimmunity. The first may reflect a short-lived release of antagonistic substances. The second resistance mechanism may be caused by host cell activity.

Klement (1972) defines the hypersensitive reaction as an incompatible reaction between particular host and bacterial genotypes. Following the penetration of pathogenic bacteria into the substomatal cavities, a few host cells die around the bacteria and this confines the infection so that the plant remains practically free from symptoms. Hypersenstivity seems to be associated with race specificity breakdown.

Mew et al. (1984) found that Xanthomonas campestris pv. oryzae on rice multiplied outside the water pores of the leaf and some bacteria entered the leaves through these pores. However, a strain that lost its virulence, did not multiply significantly on the leaf surface. They suggested that nonpathogenic bacteria were immobilized and inhibited from dividing by excretions from water pores.

Many factors are involved in the complicated mechanisms of resistance to bacterial diseases in plants

and much research is still needed to understand the mechanical and molecular processes involved. Bacteria have unique advantages compared to plants, such as, the ability to multiply very rapidly, to produce new virulent strains, and thus, to overcome host resistance.

Inheritance of Resistance

Inheritance of resistance in barley to bacterial leaf streak is obscure. A lengthy literature search failed to find a single citation or reference.

The genetics of resistance to bacterial diseases follows no set pattern. Resistance to some bacterial diseases varies considerably according to location and environment. Different authors have reached conflicting conclusions about the genetic control of resistance to the same diseases. Some workers have reported that the resistance to *Xanthomonas campestris* pv. *oryzae* in rice is monogenically controlled while others indicated that it is polygenic. The resistance to the disease has also been reported by different workers to be either as a dominant or a recessive characteristic and race-specific or nonspecific (Coyne et al.,1971; Murty and Khush,1971; Ou,1973; Ezuka et al., 1975; Yamamoto et al., 1977; Panda and Chaudhary,1978; Webster et al.,1983).

These results suggest that both pathogens and hosts

have great variability, and the genetics of resistance depends not only on the genotypes of the host and pathogen involving polygenic systems, but also on the environmental conditions in which these genotypes are grown (Russell,1978).

Many cases have been found where reciprocal hybrids differ from one other and resemble their respective maternal parents in some phenotypic characteristics due to cytoplasmic inheritance. In the case of disease resistance, southern corn leaf blight disease is a good example for cytoplasmic factors. All corn hybrids containing the Texas cytoplasmic male sterility gene were heavily infected by the disease incited by Helminthosporium maydis in 1970 in the U.S.A. (Ullstrup, 1972). Therefore, reciprocal crosses might be necessary to detect cytoplasmic factors.

Vanderplank (1982) reported that the introduction of resistance to bacterial diseases into host plants results in a change toward more frequent virulence in the pathogen. According to Brinkerhoff (1970), the resistance conditioned by a few genes in cotton to bacterial blight was often unstable. In the United States, the resistance of cotton varieties with single B genes in a susceptible background was matched in a season by increased virulence

in Xanthomonas campestris pv. malvacearum. According to Vanderplank (1982), cotton breeders seem to have succeeded in synthesizing a high level of resistance that now appears to be stable.

Control

According to Jones et al. (1917), X. c. pv. translucens could be eliminated by treating barley seeds for two hours with formaldehyde solution. Nuncie (1938) reported that Ceresan at 1/2 ounce per bushel of seed, controlled the disease. The pathogen is very sensitive to mercury compounds; therefore seed treatment with organic mercury compounds has been used succesfully in controlling the disease (Dickson, 1956). However, the use of organic mercury compounds has been prohibited due to safety considerations. Goodman and Henry (1947) found that subtilin diluted 1:1000 provided adequate control of the disease. Koleva (1981) reported that hot water (at 53° C for 30 min.),0.1 phytobacteriomycin (2 liter/100 Kg seed), quinolate (200 gr/100 Kg seed) and Vitavax (200 gr/100 Kg seed) were found to be effective seed treatments.

Kim (1982) reported that the bactericide, cupric hydroxide, had been registered and recommended for seed treatment without evidence of complete control. According

to Mathre (1982), copper hydroxide could reduce seed-born inoculum.

The use of pathogen free seed produced under dryland conditions, crop rotation, the destruction of perennial weeds susceptible to the pathogen, and destroying infested plant debris are recommended as the best control measures (Jones et al., 1917; Boosalis, 1952; and Mathre, 1982).

Mathre (1982) reported that no resistant cultivars are known, although some are more susceptible than others.

CHAPTER 3

MATERIALS AND METHODS

Materials

Twenty-three barley cultivars were used as parents in the crossing program (Table 2). Of these cultivars CM 67, Kangbori, and Suweon 191 (SW 191) are very susceptible to the disease, and the remainder have some resistance (Kim, 1982).

In this study, three different groups or crosses were tested with one Montana barley isolate, X-67, in the field: (1) a complete diallel set, (2) other resistant x susceptible crosses, and (3) additional resistant x resistant crosses.

Crosses with six parents were made in a complete diallel mating design to fully investigate gene actions in these cultivars. The parents used in the diallel set were Herta, Summit, Oderbrucker, Betzes, CM 67 and SW 191 (Table 3). The first four cultivars have some resistance to bacterial leaf streak, and the last two are very susceptible to the disease (Kim, 1982). The cultivars selected as parents for the diallel set were chosen

Table 2. Barley cultivars used as parents in the crossing program

Cultivars	Number	Origin
Herta	CI 8097	Sweden
Summit	MT 729	England
Oderbrucker	CI 4666	Germany
Betzes	CI 6398	Poland
CM 67(*)	CI 13782	USA
Suweon 191 (*)		S. Korea
Kangbori (*)		"
Alpine	CI 9578	USA
Luther		"
	CI 12558	Ethiopia
	CI 12569	"
	CI 12577	"
	CI 12595	"
	CI 12776	Sweden
	CI 12777	"
	CI 12787	Ethiopia
	CI 12866	"
	CI 13095	"
	PI 382511	"
	PI 382650	"
	PI 382720	"
	PI 382732	"
	PI 383077	"

(*): Susceptible varieties

because they have good agronomic types with the exception of Oderbrucker. Oderbrucker has been reported to be resistant since 1917 (Jones et al., 1917; and Kim, 1982). All possible crosses among these cultivars were made and inheritance of the disease resistance was studied in detail.

Table 3. Crosses made for the diallel set and their parents, F_1s and F_2s which were evaluated

Cross	Parents	F_1s	F_2s
Herta x Summit	+	+	+
" x Oderbrucker	+	+	+
" x Betzes	+	+	+
" x CM 67	+	+	+
" x SW 191	+	-	+
Summit x Herta	+	+	+
" x Oderbrucker	+	+	+
" x Betzes	+	+	+
" x CM 67	+	+	+
" x SW 191	+	+	+
Oderbrucker x Herta	+	+	+
" x Summit	+	+	+
" x Betzes	+	+	+
" x CM 67	+	+	+
" x SW 191	+	+	+
Betzes x Herta	+	+	+
" x Summit	+	+	+
" x Oderbrucker	+	+	+
" x CM 67	+	+	+
" x SW 191	+	+	+
CM 67 x Herta	+	+	+
" x Summit	+	-	+
" x Oderbrucker	+	+	+
" x Betzes	+	-	+
" x SW 191	+	+	+
SW 191 x Herta	+	-	+
" x Summit	+	+	+
" x Oderbrucker	+	+	+
" x Betzes	+	+	+
" x CM 67	+	+	+

SW 191: Suweon 191
+: Evaluated
-: Not evaluated

In the second group, eighteen crosses were made between other promising resistant cultivars and one of

the susceptible varieties to detect whether the resistant cultivars had heritable resistance to the disease (Table 4).

In the third group, resistant cultivars from different origins were crossed with each other to determine if they had different genes for resistance (Table 4).

F_1s were grown in the greenhouse during the winter of 1983-84 and checked phenotypically to determine if they were true F_1s or selfed. Selfed plants were eliminated.

Parents, F_1s and F_2s were space planted at the Horticulture Farm near Bozeman, Montana on 21 May 1984. A serpentine planting design was used. Individual plants were spaced 15 cm apart in rows 3 m in length and 30 cm apart (20 seeds/row). Twenty seeds of each parent and F_1 were planted. For each F_2, 120 seeds were planted. One row of CM 67 was planted between every two rows as the spreader to enhance uniform infection (Figure 1).

Inoculation

Artificial inoculation was carried out with a strain (X-67) of *X. c.* pv. *translucens*, highly virulent to barley. This isolate was obtained from an irrigated barley field at Sidney, Montana, where the natural