Presence and expression of resistance genes to powdery mildew of barley in selections from Tunisian barley landraces
by Jerzy Henryk Czembor

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant Pathology
Montana State University
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Abstract:
Two hundred thirty-two accessions from barley landraces collected from Tunisia were screened for resistance to powdery mildew of barley. These accessions were tested at the seedling stage under controlled conditions with three known physiologic races of barley mildew. Sixty-three accessions showed resistance to this disease. Among these accessions twenty showed good and uniform resistant reactions to all isolates used. An attempt was made to determine the number Of genes, the type of gene action and the gene loci in these accessions. The results indicated the presence of one dominant gene in 19 accessions from landraces. One recessive gene for resistance was detected in accession from landrace T-13. This recessive gene is at a different locus than mlo. Further investigation are needed to identify genes for resistance in fourteen of these accessions. In T-40, T-14 and T-62 the genes Mla13 or Ml (Ru3), Mla6 or Mla14, and Mla1 or +?, respectively were detected. The incorporation of accessions from landraces into breeding programs and strategies for their use in the genetic control of powdery mildew were discussed.

Based on sequencing portions of the RFLP clones MWG 1H036, MWG 1H060 and MWG 1H068, sequence tagged site (STS) markers for the Mla locus were obtained. Subsequently, RFLP patterns of PCR products after DNA amplification of Pallas isolines and lines from Tunisian landraces were investigated. Potential RFLP markers for genes in Mla locus were identified and discussed.

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APPROVAL

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Jerzy Henryk Czembor

This thesis has been read by each member of the author's graduate 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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ABSTRACT

Two hundred thirty-two accessions from barley landraces collected from Tunisia were screened for resistance to powdery mildew of barley. These accessions were tested at the seedling stage under controlled conditions with three known physiologic races of barley mildew. Sixty-three accessions showed resistance to this disease. Among these accessions twenty showed good and uniform resistant reactions to all isolates used. An attempt was made to determine the number of genes, the type of gene action and the gene loci in these accessions. The results indicated the presence of one dominant gene in 19 accessions from landraces. One recessive gene for resistance was detected in accession from landrace T-13. This recessive gene is at a different locus than *mlo*. Further investigation are needed to identify genes for resistance in fourteen of these accessions. In T-40, T-14 and T-62 the genes *Mla13* or *Ml(Ru3)*, *Mla6* or *Mla14*, and *Mla1* or *+?*, respectively were detected. The incorporation of accessions from landraces into breeding programs and strategies for their use in the genetic control of powdery mildew were discussed.

Based on sequencing portions of the RFLP clones MWG 1H036, MWG 1H060 and MWG 1H068, sequence tagged site (STS) markers for the *Mla* locus were obtained. Subsequently, RFLP patterns of PCR products after DNA amplification of Pallas isolines and lines from Tunisian landraces were investigated. Potential RFLP markers for genes in *Mla* locus were identified and discussed.

CHAPTER 1

GENERAL INTRODUCTION

Powdery mildew is one of the most important diseases of barley and is of great economic importance (Corazza, 1991; Griffiths *et al.*, 1975; Munk *et al.*, 1991; Rasmusson, 1985). Breeding for resistance is considered to be the most effective and economically feasible means of powdery mildew control in barley. A number of genes for specific resistance have been used in commercial barley varieties. However, most of them are closely linked or allelic to the *Mla* locus, which limits the possible gene combinations in breeding of new varieties (Brown and Jorgensen, 1991; Jorgensen, 1994, Wolfe and McDermott, 1994). Molecular markers such as restriction fragment length polymorphisms (RFLP) and polymerase chain reaction (PCR) may be used in plant breeding programs for marker assisted selection (Murray *et al.*, 1991; Tanksley *et al.*, 1987). These markers already have broad application in breeding and genetic studies of barley (Barua *et al.*, 1993a, 1993b;

Kilian *et al.*, 1994; Laurie *et al.*, 1994, 1995; Melchinger *et al.*, 1994; Saghai Maroof *et al.*, 1994, 1995). Three RFLP markers (MWG 1H060, MWG 1H036, MWG 1H068) were identified and used for investigations of the mode of inheritance and intralocus recombination at the *Mla* locus (Jahoor *et al.*, 1993; Schuller *et al.*, 1992). Tragoonrung *et al.* (1992) described the usage of sequence tagged site polymerase chain reaction (STS-PCR) in barley. This method was developed to increase the effectiveness of genome analysis in comparison to RFLP and was successfully used in wheat and barley (Chee *et al.*, 1995; Chen *et al.*, 1994; Martin *et al.*, 1995; Talbert *et al.*, 1994; Tragoonrung *et al.*, 1992). The STS-PCR amplification product can be digested with restriction enzymes (Talbert *et al.*, 1994; Tragoonrung *et al.*, 1992) or sequenced (Wong *et al.*, 1987) to detect polymorphisms.

Because mildew has the ability to overcome new resistance genes rapidly, there is a need to extend the range of resistance to this disease in barley. This is possible by using barley landraces and wild barleys (Jorgensen, 1993, 1994; Rubiales *et al.*, 1993; Russell, 1978; Wolfe, 1984; Wolfe and McDermott, 1994). Until the late nineteenth century, all barleys existed as highly heterogenous landrace populations, mixtures of inbred and hybrid segregates, the products of a low level of random crossing in earlier

generations (Brown and Munday, 1982; Brown *et al.*, 1989b; Jana and Pietrzak, 1988; Jensen, 1988). They were developed in traditional agriculture from many years of farmer-directed selection and are specifically adapted to local conditions. Mixtures of genotypes in these populations differ in reaction to certain races of pathogens. This prevented the build-up of one particular race of the pathogen to epiphytotic proportions (Brown *et al.*, 1989b; Harlan, 1975; Jensen, 1988; Plucknett *et al.*, 1987).

Barley has been grown in Tunisia since ancient times. In marginal areas of this country, barley is grown as landrace populations which give stable yields. This is due to the stabilizing effect of the genetic heterogeneity and the presence of resistance for diseases within the barley landraces (Leur *et al.*, 1989; Yahyaoui, 1986). Based on this information, these landraces may be useful sources of resistance for diseases including powdery mildew.

The major objective of this study was to characterize certain selected lines from barley landraces from Tunisia in terms of the number of genes, gene action and to identify genes for resistance to powdery mildew. An additional objective was to develop STS-PCR markers for the *Mla* locus.

CHAPTER 2

LITERATURE REVIEW

The Pathogen

The Disease Organism

Erysiphe graminis DC. f. sp. *hordei* Em. Marchal [syn. *Blumeria graminis* (DC.) Speer f. sp. *hordei* Em. Marchal], the causal agent of barley powdery mildew, belongs to the subdivision *Ascomycotina*. This is the largest subdivision of fungi and contains about 15,000 species. Sexually reproduced spores are born in a sac or ascus. Each ascus contains eight spores (ascospores) which are explosively ejected. The vegetative structure consists of single cells or septate filaments. The septum is perforated by a pore. The pore is wide enough to allow mitochondria and nuclei to pass through. Several nuclei may occur in a single cell or mycelium. Nuclei are not always genetically identical due to mutation or anastomosis of hyphae of a different genotype followed by nuclear migration (Webster, 1980).

E. graminis belongs to the class *Plectomycetes* which is characterized by an ascocarp that is rudimentary or consists

of a loose investment of hyphae or is a globose cleistocarp. This fungus is also characterized by white (colorless or hyaline) hyphae, colorless one-celled ascospores borne in asci which are enclosed in black cleistothecia on the surface of living plants. In the order *Erysiphales* two families are recognized: *Perisporiaceae*, the dark mildews, and *Erysiphaceae*, the powdery or white mildews. The *Perisporiaceae* occur in warm, humid tropical forests on adult leaves. The powdery mildews, which include *E. graminis*, are biotrophic parasites of Angiosperms, and their common name is derived from the mealy appearance of the conidia on infected foliage (Yarwood, 1978). Diseases caused by fungi from the genus *Erysiphe* include grass mildew (*E. graminis*), cucurbic mildew (*E. communis*) and pea and clover mildew (*E. polygoni*) (Smith *et al.*, 1988; Webster, 1980). Cleistothecia are dark with free-ended equatorial appendages and contain about 15-20 asci. *E. graminis* is divided into seven formae speciales: *hordei*, *tritici*, *avena*, *secalis*, *agropyri*, *bromi*, and *poae* (Hiura, 1978).

Mycelium of *E. graminis* f.sp. *hordei* penetrates the host cuticule and epidermal cell walls directly to form haustoria with fingerlike appendages in epidermal cells. From the primary mycelium arise short and simple conidiophores bearing unicelullar and ovoid conidia. This

conidial stage is named *Oidium monilioides* Desm. (Smith *et al.*, 1988; Webster, 1980; Yarwood, 1978).

Life Cycle

The life cycle of *E. graminis* f.sp. *hordei* consists of a sexual stage and an asexual stage (Butt, 1978; Smith *et al.*, 1988).

The Sexual Stage

The fungus is heterothallic, with two mating types occurring in about equal frequencies in natural populations (Day, 1974; Moseman, 1966). When two mildew colonies grow closely together, a sexual generation may be formed. Cleistothecia are formed by the fusion between hyphae (one ascogonium and one antheridium) and the nuclei fuse after a short dicaryotic phase. Cleistothecia develop as temperatures rise or the fungus and host mature. Cleistothecia start to crack open late in the season. The asci are then released and discharge their ascospores. Usually eight ascospores are produced in each ascus. Ascospores are able to infect living plant tissue. The ascospores within the cleistothecia can survive in a dry state for a long time and may be released under humid conditions (Butt, 1978; Jenkyn and Bainbridge, 1978; Koltin and Kenneth, 1970; Smith *et al.*, 1988; Webster, 1980; Wolfe and McDermott, 1994).

The Asexual Stage

This stage is characterized by the formation of conidia. The conidia are much more sensitive to environmental conditions than the ascospores. Secondary infection is caused by conidia which are also responsible for the spread of the disease (Butt, 1978; Smith *et al.*, 1988). Conidia germinate over a wide temperature range (5-30 C) and without free moisture. Germination is optimal at 100% relative humidity, but can occur at 85% relative humidity. Free water inhibits germination (Manners and Hossain, 1963; Sivapalan, 1993a, 1993b, 1994; Smith *et al.*, 1988; Webster, 1980). Sporulation and spore dispersal occur most readily under dry conditions. Germination, infection, and secondary sporulation are completed within 7-10 days in favorable field environments (Smith *et al.*, 1988; Wolfe and McDermott, 1994). Powdery mildew development is optimal between 15-22 C. Most stages of infection occur in darkness, except for host penetration, which requires light (Edwards, 1993; Ellingboe, 1968, 1972; Masri and Ellingboe, 1966; Webster, 1980).

Role of Sexual and Asexual Stage in Epidemic Development

Plant pathogen populations in agricultural systems in different parts of world are challenged by different

environmental conditions, especially moisture and temperature. In the Mediterranean and in the Near East, the sexual stage of drought-resistant cleistothecia is essential so that meiotically produced genotypes of the pathogen initiate new disease cycles. The asexual phase is restricted to a relatively short period of optimal multiplication conditions. In European agricultural systems, the intensity of crop rotation guarantees an almost year-round availability of green tissue, leaving only a few weeks between summer harvest and germination of the new crop in autumn, during which volunteer plants provide a green bridge. The sexual stage is thus no longer indispensable for the fungus, and yearly populations are largely composed of clones because of large-scale asexual reproduction. Generally, the asexual cycle of the pathogen is unbroken and can continue year round. Cleistothecia do not act as an overwintering stage because they produce ascospores with the autumn rains. Their impact is more important when weather conditions are unfavorable for asexual development. (Hovmoller and Ostegard, 1991; Smith *et al.*, 1988; Wolfe, 1984; Wolfe and McDermott, 1994).

Infection Process

The stages of development of *E. graminis* f.sp. *hordei* are well defined, and simultaneously inoculated spores show

highly synchronized development (Aist and Bushnell, 1991; Carver, 1988). Germinating spores produce a primary germ tube (PGT) 1-2 hours after inoculation (HAI) (Edwards, 1993). The PGT may enter the epidermal cell wall of the host plant, although this does not always appear to be essential for continued development of the spore (Carver, 1988; Carver and Bushnell, 1983; Kunoh *et al.*, 1978). An appressorial germ tube (AGT) is produced shortly after the PGT. The end of the AGT swells to form a mature appressorium in 10-12 HAI. The fungus attempts to breach the epidermal cell wall by producing an appressorial infection peg (AIP) from the appressorium. If this fails, secondary, tertiary, and even quaternary lobes may be produced from the appressorium. Each of them in turn is capable of producing an infection peg in an attempt to penetrate the cell wall (Edwards, 1993). In response, the plant deposits secondary metabolites, including callose, silicon, calcium, and phenolic-based compounds, in papillae beneath the PGT and appressorial lobes (Aist and Bushnell, 1991; Carver, 1988). If an AIP successfully breaches the cell wall, it swells to form a haustorium, through which the fungus derives nutrients from the plant. The membrane of the epidermal cell is displaced as the haustorium develops, forming digitate structures which increase the surface area

between the fungal wall and the plant cell membrane (Bracker, 1968; Bushnell and Berquist, 1975; Bushnell and Gay, 1978; Ehrlich and Ehrlich, 1963). Elongating secondary hyphae (ESH) are produced from the appressorial arm, forming a sporulating colony in a compatible interaction, and completing the asexual cycle (Aist and Bushnell, 1991).

Genetic Variation

The number of chromosomes in the haploid genome of *E. graminis* is unknown: it has been reported to be at least seven (Borbye *et al.*, 1992; Kimber and Wolfe, 1966; McKeen, 1972).

The fungus is characterized by a number of virulence genes, which are matched by resistance genes in the host varieties, mostly in a 'gene-for-gene' relationship. Generally, only spores possessing the virulence gene corresponding to the resistance gene in the host variety are able to reproduce on such a variety. When different host varieties possessing different powdery mildew resistance genes are grown, the selection caused by these varieties can lead to the creation of new subpopulations, called physiological races, and are named after their virulence genes. The potential number of races that can be recognized depends on the number of resistance factors present in a set of differentials (Wit *et al.*, 1993).

Sexual Recombination

Sexual recombination increases genetic diversity in *E. graminis* populations by promoting the reassortment of existing combinations of alleles. This is the most important process by which genetic variation is generated. The extent to which this variation is manifested in the fungal population is affected by cultural practices and climatic conditions (McDonald *et al.*, 1989; Wolfe and McDermott, 1994).

Somatic Recombination

This process has been described to occur between different isolates of *E. graminis* f.sp. *hordei*. The overall importance of somatic hybridization as a mechanism for generating variation in natural pathogen populations is unclear. At its simplest, this process involves the exchange of whole nuclei and/or cytoplasm (heterokaryosis) (McDonald *et al.*, 1989; Webster, 1980).

Mutation

Spontaneous mutation from avirulence to virulence is widely recognized as an important means whereby natural populations of a wide range of fungi respond to changes in the resistance of host populations (Torp and Jensen, 1985). Mutations from virulence to avirulence are not described. A

possible explanation is that avirulent mutants are difficult to detect. It is likely that avirulent mutants will be rare assuming that the avirulence allele produces a 'functional' gene-product, whereas the virulence allele does not (Jorgensen, 1994; Russell, 1978).

Migration

Most spores are deposited close to where they are produced. However, there is clear evidence that they can also be transported for considerable distances (Butt, 1978). The entire populations of *E. graminis* f. sp. *hordei* can move by wind hundreds of kilometers across the European continent. For example, powdery mildew populations are regularly exported from Denmark to the UK, from Germany to Denmark, and from Czech Republic and Slovakia to Switzerland and Austria (Hermansen *et al.*, 1978, Jorgensen, 1994; Limpert and Schwarzbach 1981, McDermott and McDonald, 1993; Wolfe and McDermott, 1994). Due to this fact, migration is an important factor influencing the genetic structure of European populations of *E. graminis* f. sp. *hordei*. The area of impact of emigrant spores will depend on the size of the spore population released into the atmosphere, which can be large in the case of a large area of monoculture of a susceptible cultivar, and the distance from the source. It will depend also on the availability of an appropriate niche

for the spores that land and the size of the niche (Limpert, 1987; Limpert and Schwarzbach, 1981; Limpert *et al.*, 1990, 1991; Wolfe and McDermott, 1994).

Economic Importance

Barley powdery mildew is a severe foliar disease and it occurs wherever barley is grown. Generally the disease seems to cause the most damage in temperate latitudes. In those parts of Europe where mildew is a problem, yield losses in experiments can exceed 20% although average losses are much smaller, e.g. 6-14% in England. In the USA average yield losses in barley due to powdery mildew are less than 1% (Griffiths *et al.*, 1975; Jenkyn, 1974; Jenkyn and Bainbridge, 1978; Munk *et al.*, 1991; Wolfe, 1984). However in hot, dry regions it can be important where altitude or maritime influences have a moderating effect on the climate. For example, it is a quite important disease in the Mediterranean regions (Caddel and Wilcoxon, 1975; Corazza, 1991; Velasco, 1981).

The infection by powdery mildew results in the gradual decline in vigor and growth due to a reduction of photosynthesis and an increased rate of transpiration and respiration (McAinsh *et al.*, 1991; Scholes *et al.*, 1994). Although barley plants are most susceptible when they are young, they may be attacked at all stages of development

with resultant reduction of yield up to 25% (King, 1972, 1977; Large and Doling, 1962). Yield reduction is due to loss of functional green leaf area, reduced root growth, reduced kernel weight, smoller numbers of kernels per spike and tillers per plant (Carver and Griffiths, 1981; Griffiths *et al.*, 1975; Last, 1962; Scott and Griffiths, 1980; Smedegaard-Petersen and Stolen, 1981; Walters *et al.*, 1984).

Control

Strategies for reducing powdery mildew of barley are faced with a highly mobile pathogen, whose gene-pool forms an almost infinite source of genetic variation. Control of powdery mildew is attempted by the introduction of genes for qualitative disease resistance into the host plant (McIntosh, 1978; Russell, 1978; Wolfe and McDermott, 1994). A number of genes for specific resistance have been used in commercial barley varieties since the first gene, *Mlg*, was introduced on a large scale in the 1930s in Germany (Jorgensen, 1994; Wolfe and Schwarzbach, 1978). For example, in this century in Europe approximately 660 cultivars of barley have been used with different combinations of 33 alleles for race-specific resistance to powdery mildew. However, 28 of these alleles are closely linked or allelic, which limits the possible number of gene combinations in breeding of new varieties (Brown and

Jorgensen, 1991; Jorgensen, 1994; Wolfe and McDermott, 1994). All these genes were successively overcome by the appearance of pathotypes with matching virulence. This is one of the classic examples of this type relationship between host and parasite (Wolfe and Schwarzbach 1978; Jorgensen 1993). An attempt for durable control of powdery mildew by using genes for resistance was very successful using the recessive alleles of the *mlo* gene. The Mlo resistance is the basis of the single gene control strategy that is now used over a vast area of barley production (Wolfe and McDermott, 1994).

In general, new varieties with race-specific resistance have been regularly available, but farmers preference for a limited number of successful varieties has tended to 'overexpose' them, with corresponding large shifts in the *E. graminis* f. sp. *hordei* population (Russell, 1978). These varieties, when their race-specific resistance breaks down, must be discarded because they are far too disease susceptible to be of any further value. This susceptibility is due to a host erosion of partial resistance during breeding for race-specific resistance. Vanderplank (1968) has named this kind of host erosion of partial resistance the 'vertifolia effect', after the potato cultivar 'Vertifolia' which had been bred for race-specific

resistance and which proved exceptionally susceptible when holding of that resistance broke down. If this partial resistance will be retained during breeding, eventual breakdown of race-specific resistance in new variety would be relatively slight.

Powdery mildew of barley is one of the main diseases for which diversification strategies are proposed. They are based on the principle of deploying many varieties with different resistance genes in space or time. Only a proportion of the *E. graminis* f. sp. *hordei* population can attack each (Limpert, 1987; Smith *et al.*, 1988; Wolfe, 1984). Thus, eliminating of a 'green bridge' may be obtained by using different resistance genes in spring and winter barleys. An example of this is that the *mlo* gene is not used in winter barley (Gacek and Czembor, 1983; Johnston, pers. comm.; Slootmaker *et al.*, 1984; Smith *et al*, 1988; Wolfe, 1984). It was also postulated that there should be an international agreement on the sequence of resistance genes to be released and a predetermined maximum permitted acreage of individual varieties with race-specific resistance. However, such an agreement would be difficult to enforce (Russel, 1978; Wolfe, 1985).

The durability of resistance genes may be increased by use of multiline varieties and by combining ('pyramiding')

different resistance genes into one variety (Huang *et al.*, 1995b; Mastebroek and Balkema-Boomstra, 1991a; Petersen and Leath, 1988). Combining different resistance genes into one variety, however, has difficulties in usage for barley in Europe. First, many of the desired genes are alleles at, or closely linked to, the *Mla* locus and so cannot be easily combined. Second, it would be impossible to prevent prior use of the component resistance genes alone or in simple combinations, allowing a degree of pre-selection within the European pathogen population before the introduction of the complex variety. Third, if such a complex variety could be produced, its commercial introduction and success could not be guaranteed (Brown and Jorgensen, 1991; Jorgensen, 1994; Wolfe and McDermott, 1994).

These problems led to the development of another strategy to enhance durability of resistance to *E. graminis* f. sp. *hordei* which is the use of variety mixtures (Czembor and Gacek, 1987; Gacek *et al.*, 1991; Wolfe, 1991; Wolfe and McDermott, 1994). The principle of using variety mixtures is simple. By growing intimate mixtures of plants with different resistances, the spread of the pathogen selected on any one plant will be restricted (Gacek and Nadziak, 1988; Wolfe and McDermott, 1994). Gacek and Nadziak (1988) have shown that the use of mixtures of varieties reduced

mildew levels up to 70 percent. Results obtained in Poland indicate yield increase from 3 to 15 percent by mixtures of varieties, in comparison to the mean of the cultivars separately (Czembor and Gacek, 1987). Because of the nature and flexibility of the system, the yield advantage can be exploited in two ways. First, by continually changing the mixture composition to take advantage of the improved yield of new varieties, the yield potential of mixtures can be maintained close to the maximum possible (Gacek and Nadziak, 1988; Wolfe, 1984). Second, variety mixtures are higher yielding and more predictable in yield performance than most single varieties (Wolfe, 1984). Their stability appears to be similar to that of the mean of their components, with the bonus of a yield increase, particularly if disease occurs (Gacek *et al.*, 1991; Wolfe, 1984, 1991; Wolfe and McDermott, 1994). Until 1990, up to 90 percent of spring barley in the former German Democratic Republic was grown as variety mixtures (Gabler and Fritsche, 1991). There is still an increasing area of mixtures in Poland (currently about 20 percent; E. Gacek, pers. comm.), in the UK, and in Denmark (Wolfe, 1991). Wolfe (1985) proposed to release newly registered varieties first in mixtures in order to extend the effectiveness of their disease resistance. Usage of partially resistant varieties in mixtures is proposed by

Newton and Thomas (1993). Generally, it may be assumed that the use of mixtures may be regarded as an inexpensive and simple strategy for disease control that can be added to or integrated with other strategies (Chin and Wolfe, 1984; Gacek *et al.*, 1991; Huang *et al.*, 1991, 1994, 1995a, 1995b; Wolfe, 1991). However, the acceptance of variety mixtures on a large scale is constrained by the prevailing industrial system (maltsters) of using the crop product, by existing laws concerned with variety rights (registration) and seed trade (price) with plant material (Wolfe, 1984, 1985; Wolfe and McDermott, 1994).

Since 1969, fungicide treatment against *E. graminis* f. sp. *hordei* has become routine in Europe (Russell, 1978, Smith *et al.*, 1988). Control of powdery mildew of barley using fungicides can increase the number of heads by 20% or more and the grain size by 5-10% (Smith *et al.*, 1988). The availability of ethirimol and triadimenol as fungicides and subsequently a great variety of fungicidal compounds and formulations has been aimed at overall disease control on cereals (Bent, 1978; Limpert, 1991). However, *E. graminis* f. sp. *hordei* shows differing levels of resistance to most of these chemicals (Brown, 1991; Brown and Wolfe, 1991; Brown *et al.*, 1992; Hollomon and Butters, 1991; Limpert, 1987; Wolfe, 1984). Because of this situation,

effectiveness of fungicides may be diminished and problems of loss of control of powdery mildew using fungicides may arise (Smith *et al.*, 1988). Wolfe (1984) proposes that the diversification strategies advocated for varieties with powdery mildew resistance should be integrated with diversification of fungicide use. Also, agronomic practices may help in control of powdery mildew of barley. They include reducing crop size and field size, extending rotations, delaying autumn sowing and optimizing fertilizer inputs (Wolfe and McDermott, 1994).

Any usage of chemicals (pesticides, fungicides, herbicides, and mineral fertilizers) in agriculture is increasingly criticized in societies of many developed countries. Also, generally required environmental standards are becoming higher throughout the world (Brown and Kane, 1994). Future strategies for the control of powdery mildew will have to focus increasingly on ecologically acceptable methods (Russell, 1978; Wolfe, 1984). However, the need to reduce chemical input to a minimum must be supported by the evaluation of novel cropping practices, and by breeding for durable resistance in barley (Johnson, 1981, Russell, 1978).

The Host

Importance

Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop in the world, after wheat, maize and rice. Russia is the world's largest producer, with Canada and the USA following. In the USA barley is the fourth cereal in production, after corn, wheat, and sorghum, and the fifth cereal in value, after corn, wheat, sorghum, and rice. In Africa, barley is an important crop in Ethiopia and in countries along the northern border of the continent. In Tunisia and Algeria it is grown on about one-third of the area planted to cereals. Other African countries in which production of barley is important are Egypt, Morocco and Libya. In these countries barley is often grown in marginal agricultural areas with an average precipitation less than 220 mm (Cocks and Thomson, 1988; Keatinge *et al.*, 1986; Rasmusson, 1985).

Over half of the world barley crop is used for animal feed and 10% is turned into malt. For human consumption barley is ground into a flour used to make porridge or flat bread. It is also polished to produce 'pearl barley' commonly used in soup (Rasmusson, 1985).

Origin

Centers of diversity for the genus *Hordeum*, based on areas containing the highest number of species, are found in four areas in the world:

- central and southwestern Asia,
- western North America,
- southern South America,
- the Mediterranean (Bothmer *et al.*, 1991, Hsu, 1975, Zhou and Shao, 1981).

In the most accepted theory, barley was derived from its wild ancestor *Hordeum spontaneum* C. Koch when Neolithic men selected spikes with tough rachis (Harlan and Zohary, 1966, Zohary and Hopf, 1988). The original area of cultivation and the center of origin of *H. vulgare* L. is assumed to be the area of the Fertile Crescent (from present day Israel and Jordan via Syria and southern Anatolia to the Zagros Mountain area in Western Iran) (Zohary, 1969; Zohary and Hopf, 1988). This assumption is based on archaeological evidences which indicates the earliest signs (7000-8000 B. C.) of cultivation of barley (Harlan, 1976a; Wendorf *et al.*, 1979, 1984; Zohary, 1973).

However, the discovery of wild barley in Morocco was also reported (Molina-Cano and Conde, 1980; Molina-Cano *et al.*, 1982). This finding led Molina-Cano *et al.* (1987) to

postulate Morocco as a possible center of origin for cultivated barley. Wild barley was also discovered on the Qinghai-Xizang (Tibet) plateau in China (Hsu, 1975, Xu, 1982, Zhou and Shao, 1981). Based on these discoveries Moralejo *et al.* (1994) suggested that barley may be a multicentric crop, domesticated along the Mediterranean basin and, perhaps, also in Tibet. Ethiopia is also cited as another possible center of origin of barley (Negassa, 1985b).

Taxonomy

Barley (*H. vulgare* L.) is a diploid organism with seven large, cytologically distinct chromosomes. It belongs to the tribe *Triticeae* of the family *Poaceae* (*Gramineae*). The tribe includes a number of important cereal crops, such as wheat (*Triticum* spp.), rye (*Secale cereale*), and the artificially synthesized triticale (X*Tricosecale*). Apart from these cereals many important forage grass species are also referred to this tribe. Altogether the *Triticeae* comprises around 350 species (Bothmer *et al.*, 1991). The morphology of Hordeum is very specialized in comparison with other genera *Triticeae*. The unifying morphological characters for the *Hordeum* species are the one-flowered spikelets which are borne three together at each rachis node

(Rasmusson, 1985). All the wild species of barley are considered to be closely related to cultivated barley and to constitute a genetic resource for breeding purposes (Asfaw and Bothmer, 1990; Bothmer and Jacobsen, 1986; Bothmer *et al.*, 1983, 1991).

Ecology

H. vulgare L. occurs in a wide spectrum of habitats. It has the highest salt and highest range of temperature tolerance of all cereal crops (Ceccarelli and Grando, 1991; Papa, 1994). Cultivated barley grows well both in very moist and very dry conditions (Rasmusson, 1985).

Distribution

Cultivated barley occurs in temperate areas in both the northern and southern hemispheres. It reaches subtropical areas in central South America and arctic areas in North America and Central Asia. Because of its tolerance to cold environments it is also cultivated at very high altitudes. It has been observed to more than 4400 m in the Andes and Himalayas (Bothmer *et al.*, 1991; Harlan, 1976a).

Sources of Genes for Resistance to Powdery Mildew of Barley

Commercial Varieties

Many unique resistances for powdery mildew of barley have been introduced. In the past, these resistances have been used extensively as resistance 'sources' by practically all barley breeders. Because of this, current commercial varieties are the main 'source' of resistance in breeding of barley for powdery mildew resistance (Brown and Jorgensen, 1991). As a rule this is race-specific resistance. Knudsen *et al.* (1986) reported that a high level of partial resistance for powdery mildew of barley is still present in many modern varieties.

Landrace Varieties

As part of a movement of Europe, the United States and Australia for the improvement of agriculture in the 19th century, a few outstanding farmers and landowners (e.g. Vilmorin in France, Janasz in Poland, Rimpau in Germany), with the time and resources to experiment, began to practice selection on the variability within the landraces of crops which they were growing (Brown *et al.*, 1989b; Janasz, 1893; Jensen, 1988; Simmonds, 1987). At this point, plant breeders had interpreted variation from a mind-set conditioned by a belief in an inexhaustible supply of

variation represented in landrace varieties. It is understandable because landrace varieties were the predominant crop form and crop uniformity was not a worrisome problem (Brown *et al.*, 1989b; Jensen, 1988).

Man carried the early domesticated crops into different climatic zones, into areas of different daylengths and onto different soil types, and selected thousands of locally adapted landraces. For many temperate annual crops including barley, this process of diversification seems to have reached its peak at the middle to the end of the 19th century (Harlan, 1975, 1976b; Robinson, 1976). This proces is now in reverse. The diversity of landraces which supported agriculture for the past 9000 years is being rapidly eroded. The rapid rate of destruction of crop variability is in sad contrast to the rate of its creation - about 100 years compared to 5000 to 9000 years (Simmonds, 1987). This has happened through the growth of new, genetically uniform varieties which exist in application of increasingly sophisticated agronomic practices, including improved tillage, irrigation, artificial fertilizers and the chemical control of pests and diseases (Austin *et al.*, 1986; Baenzinger and Peterson, 1992; Vanderplank, 1968, 1982).

Pure line varieties were initially selected from landraces, but later derived from successive cycles of

crosses between established pure lines. This situation resulted in increasing genetic homogeneity of barley (Austin *et al.*, 1986; Baenzinger and Peterson, 1992; Harlan, 1975, 1976b; Nevo *et al.*, 1979; Plucknett *et al.*, 1983, 1987; Robinson, 1976; Vanderplank, 1968; Fischbeck, 1991). Domination of pure line varieties of barley and the intensification of nitrogen fertilization are causing significant increases of susceptibility to powdery mildew and other pests and diseases (Brown *et al.*, 1989a, 1989b; Mastebroek and Balkema-Boomstra, 1991a; Oerke and Schonbeck, 1990).

In barley, a low level of outcrossing is always present (Allard, 1988; Baenzinger *et al.*, 1981; Giles, 1989). This is probably the reason why many landraces display intermediate (Brown and Munday, 1982) or high (Jana and Pietrzak, 1988) levels of genetic diversity. In traditional farming systems which use landraces, powdery mildew rarely develops to levels that significantly damage the yield. This has been attributed both to the stabilizing effect of the genetic heterogeneity within the landraces and to the presence of resistance sufficient to control the limited disease development (Andrivon and Vallavielle-Pope, 1992; Leur *et al.*, 1989). In marginal areas of the Mediterranean region, farmers still rely on landraces that show a stable

performance (Ceccarelli *et al.*, 1987).

Currently, many genes for race-specific resistance for powdery mildew are commonly used in barley breeding programs. Unfortunately, the resistance conferred by most of these genes has not been maintained for more than a few years following commercial release of the corresponding new cultivars (Czembor, 1981; Jorgensen, 1983; Lacicowa, 1984; Wolfe and Schwarzbach, 1978). Most of the powdery mildew resistance genes used commercially are derived from the barley landrace populations (Fischbeck and Jahoor, 1991; Jorgensen, 1994). These landrace populations originated from West Asia, Ethiopia and North Africa (Fischbeck and Jahoor, 1991; Jensen and Jorgensen, 1991; Jorgensen, 1994; Russell, 1978). Based on this fact, it is assumed that many landrace accessions possess mildew resistance genes different from those genes which have already been introduced into cultivated barley varieties, and are therefore of value in the further diversification of resistance genes (Mastebroek and Balkema-Boomstra, 1991a; Russell, 1978).

Wild Barleys

Many investigations justify the conclusion that *Hordeum spontaneum* populations carry a large number of major genes for mildew resistance, which have not been used in barley

breeding (Baenziger *et al.* 1981; Fischbeck *et al.*, 1976; Harlan and Zohary, 1966; Jahoor *et al.*, 1989; Jana and Nevo, 1991; Moseman and Craddock, 1976; Moseman *et al.*, 1980, 1981, 1983; Segal *et al.*, 1982, 1987). Several new powdery mildew resistance genes are currently being incorporated into European germplasm from *H. spontaneum* (Jahoor and Fischbeck, 1987a, 1987b, 1993; Fischbeck and Jahoor, 1991; Mastebroek *et al.*, 1995). Most of these genes are at the *Mla* locus. It is estimated that about 50% of resistance genes against powdery mildew which are present in *H. spontaneum* belong to the Mla region (Jahoor and Fischbeck, 1987a, 1987b).

Hordeum bulbosum has long been known to contain several interesting agronomic characters, especially resistance to powdery mildew (Hardison, 1944; Eshed and Wahl, 1970, 1975). Variation in resistance to powdery mildew in *H. bulbosum* was evaluated (Jones and Pickering, 1978; Prasad *et al.*, 1983). The resistance of *H. bulbosum* to *E. graminis* f. sp. *hordei* was expressed in hybrids with *H. vulgare* (Xu and Kasha, 1992; Xu and Snape, 1988, 1989). *Hordeum chilense* and *Hordeum murinum* resistances to powdery mildew and their potential use in barley breeding have also been described (Giles and Barrett, 1983; Martín and Cubero, 1981; Rubiales *et al.*, 1993). Another gene for resistance to powdery

mildew was obtained from *Hordeum laevigatum* and was introduced into modern barley varieties (Jorgensen, 1994).

At present only a few genes for resistance for powdery mildew originated from wild barleys are present in commercial varieties (Fischbeck and Jahoor, 1991; Jorgensen, 1994). However, in the last decade the interest in sources of resistance from wild barley has intensified. In an extensive study of resistance of wild species of *Hordeum* to *E. graminis* f. sp. *hordei*, Gustafsson and Claesson (1988) screened 155 populations representing 28 *Hordeum* species. They used four cultures of powdery mildew which exhibited virulence to most known resistance genes. With the exception of one susceptible accession of *Hordeum marinum* all populations of the wild species showed immune or resistant reactions.

Unfortunately, the wild species of *Hordeum* have so far played only a minor role in barley improvement. This is caused by strong incompatibility barriers, usually expressed as F_1 sterility, between cultivated barley and the wild species (Bothmer *et al.*, 1983; Bothmer and Linde-Laursen, 1989; Schooler, 1974). A better understanding of the incompatibility mechanisms as well as the development of new procedures for gene transfer are necessary for the future utilization of wild *Hordeum* species in breeding of barley