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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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
Bozeman, Montana

March 1996

D378
C997

APPROVAL

of a thesis submitted by

Jerzy Henryk Czembor

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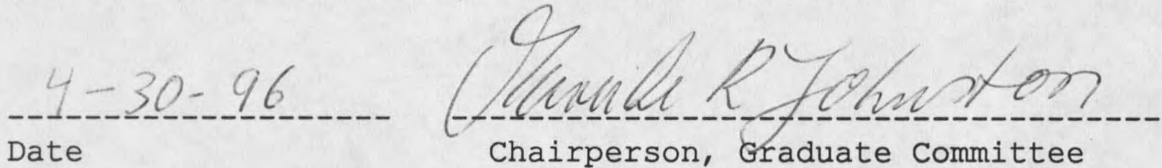

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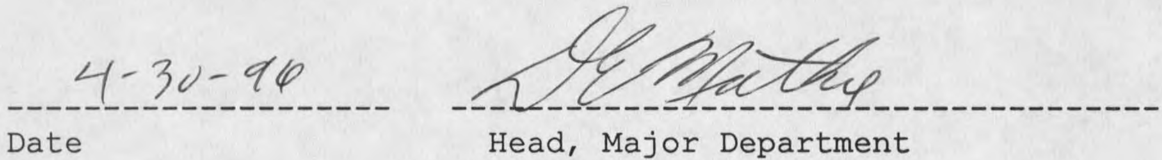

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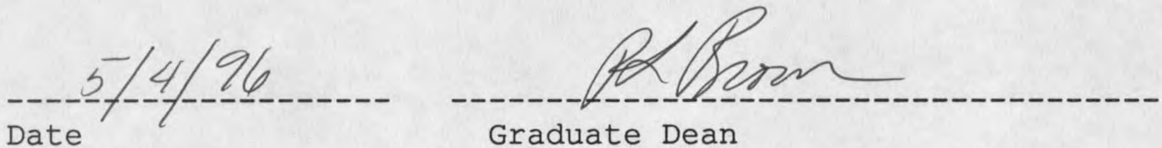

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ACKNOWLEDGMENTS

I would like to thank my wife, Elżbieta, for her love, support, encouragement and patience during this stage of our lives.

I thank my daughter, Barbara, for her patience and smile.

I would like to thank my mother, Janina Czembor, and father, Henryk J. Czembor, for emotional and moral support.

I would like to express special thanks to my major advisors, Dr. A. Scharen and Dr. M. Johnston for their advice, guidance and positive comments throughout the course of my graduate study and preparation of this thesis.

I thank Dr. J. Sherwood and Dr. J. Martin for serving on my graduate committee and giving their friendship, time and expertise.

Special thanks to Dr. L. Talbert for his support, time, technical guidance, laboratory facilities and for providing me with the environment for realization of the second part of my graduate research.

I further want to thank Dr. D. Mathre, Dr. J. Riesselman, Dr. T. Carroll and Dr. D. Sands for their friendship, interest and encouragement during my Ph.D. program.

Dr. Laura Smith deserves special thanks for her kindness and patience in teaching me most of the molecular biology techniques.

I thank the faculty, staff and fellow graduate students for their kindness and friendship.

I would like to express special thanks and acknowledgement to Dr. J. H. Jorgensen for isolates of powdery mildew and to Dr. A. Jahoor for RFLP clones which were crucial for realization of this project.

I would like to thank the USAID-ICARDA-MSU project for financial support and unconditional help.

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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; Martin 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

for powdery mildew resistance (Bothmer *et al.*, 1983; Jorgensen and Andersen, 1989; Jorgensen and Bothmer, 1988; Orton, 1979, 1980a, 1980b; Orton and Steidl, 1980; Pickering, 1988, 1989).

Mutation

In the 1960s many scientists expected that induced mutations would provide new sources for disease resistance. The results were disappointing (Jorgensen 1991a, 1991b). Dominant resistance genes have a highly specific function, whereas most mutations disrupt gene functions leading to recessive genes with non functional products (Russell, 1978). One outstanding exception is the *mlo* gene in barley. This resistance was obtained the first time by using X-rays in the German variety Haisa during the Second World War (Freisleben and Lein, 1942). This resistance now has extensive use in barley breeding and production (Andersen, 1991; Jorgensen, 1991a, 1991b, 1992b, 1994).

Types of Powdery Mildew Resistance in Barley

Race-specific Resistance

Resistance Mechanism

About fifty years ago the gene-for-gene hypothesis was developed by Flor (1942, 1955, 1956, 1971) to describe the highly specific race-variety interactions in flax rust

disease (Flor, 1942, 1946, 1947, 1951). This hypothesis states that incompatible relationships develop when a host carrying a dominant resistance allele interacts with a pathogen having a complementary dominant avirulence allele. Other combinations involving a recessive allele for susceptibility in the host or a recessive allele for virulence in the pathogen lead to the establishment of a compatible interaction and disease. (Crute and Norwood, 1986; Day, 1974; Ellingboe,1981; Flor, 1955, 1971; Sherwood *et al.*, 1991; Vanderplank, 1982). Many exceptions to this general pattern were reported (Bennett, 1984; Green, 1964; Jorgensen, 1988b; Knott, 1989; Sogaard and Jorgensen, 1984). For example, host-parasite systems were described with recessive resistance genes (Dyck and Samborski, 1968; Sawhney *et al.*, 1981; Yoshimura *et al.*, 1984) and two host genes controlling resistance to a particular pathogen genotype (Dyck and Samborski, 1982; Martens *et al.*, 1981). Modifiers which can enhance the effects of a major resistance gene (McKenzie and Martens, 1968; Samborski and Dyck, 1982) and supressors of resistance genes (Kerber and Green, 1980) have also been reported. The presence of resistance which is controlled by several genes with small additive effects is also considered as an exceptions to the gene-for-gene relation between plant and parasite (Hamid *et*

al., 1982; Lewellen and Sharp, 1968; Parlevliet, 1978). The five types of exceptions to the inheritance of resistance listed above are also found to the typical inheritance patterns of virulence in gene-for-gene systems (Green, 1964, 1965, 1966; Pedersen and Kiesling, 1979; Statler, 1977, 1982).

Race-specific resistance for powdery mildew of barley generally confirms the gene-for-gene hypothesis (Moseman, 1966). However, exceptions exist in barley-powdery mildew system to this hypothesis, i.e. recessive genes for resistance and minor gene resistance (Jorgensen, 1994; Knudsen *et al.*, 1986). In the 1930s, a barley variety, 'Pflugs Intensiv', with the first known specific gene for resistance against powdery mildew, *Mlg*, (more specifically, *Mlg* plus *Ml(CP)*, the latter being a gene of small effect that often accompanies *Mlg* in European barley varieties) was grown in Germany (Mains and Dietz, 1930; Wolfe, 1984). Since this time many powdery mildew resistance programs in barley were based on one or few race-specific resistance genes. These genes operate in the gene-for-gene system and most of them cause a resistance response resulting in a hypersensitive reaction (Jorgensen, 1993, 1994). Different responses are obtained as a result of the genes for resistance. They are sometimes initiated before the

pathogen has entered the lumen of the cell (Bushnell and Rowell, 1981). Masri and Ellingboe (1966) described some resistant reactions for the following genes: the *Mlg* gene inhibits secondary infection, the *Mla* gene causes distorion of haustoria, the *Mlk* gene causes collapse of the host tissue, and the *Mlp* gene inhibits growth and sporulation. All these genes have different effects on the fungus at different stages of fungal development.

The race-specific genes appear to have two independent functions. One of the functions is the (qualitative) recognition of the product of the pathogen's avirulence gene. The other function is the (quantitative) activation and regulation of the disease response genes involved in the biochemical and physical events leading to hypersensitive cell death (Gabriel and Rolfe, 1990; Keen, 1990; Newton and Andrivon, 1995; Sidhu, 1987). In barley plants, the response genes encode biochemical processes such as papilla formation (Inoue *et al.*, 1994a; 1994b; Koga *et al.*, 1990), accumulation of phenolic compounds (Boyd *et al.*, 1994; Carver *et al.*, 1994; Clark *et al.*, 1994; Russo and Bushnell, 1989), silicon, callose (Kita *et al.*, 1980; Kunoh *et al.*, 1986), lignin-like substances (Scott-Craig *et al.*, 1995), peroxidases (Fric and Tamas, 1993; Thordal-Christensen *et al.*,1992), chitinases (Kragh *et al.*, 1993; Sheba *et al.*,

1994), hydrolytic enzymes e.g. phosphatase and esterase (Fric and Tamas, 1993; Takahashi *et al.*, 1995), phytoalexins (Oku *et al.*, 1975), phenylalanine ammonia lyase (Shiraishi *et al.*, 1989, 1995), and pathogenesis-related proteins (Bryngelsson, 1988, 1994; Bryngelsson and Collinge, 1992; Chakravorty and Scott, 1991) in the host cells.

Durability

Before the Second World War physiological forms of powdery mildew were detected which were able to attack barley varieties with race-specific resistance. Initially, they remained relatively low in their frequency because the plants with resistance genes were never planted on a large area. But with the intensification of agriculture after 1945 the disease pressure increased, and newly introduced resistance genes were more quickly overcome by mildew populations (Wolfe and McDermott, 1994; Wolfe and Schwarzbach, 1978). Varieties with new genes for resistance became very popular and were subsequently attacked by newly emerging virulent strains of the pathogen. There are many reports that illustrate the effective life times of individual race-specific powdery mildew resistances of barley in Europe. Most genes begin to lose effectiveness within 2-4 years when distributed in widely grown cultivars (Jensen and Jorgensen, 1991; Jorgensen, 1987, 1993; Munk *et al.*, 1991; Wolfe, 1984; Wolfe and McDermott, 1994).

Race-specific Resistance Genes

Eighty-five race-specific powdery mildew resistance genes are known in barley. Ten loci (which have genes for this type of resistance) have also been mapped (Jensen, 1992; Jorgensen, 1992c, 1994).

Resistance Genes at the *Mla Locus*

The *Mla* locus is located on barley chromosome 5. This locus has 28 named alleles whose symbols range from *Mla1* to *Mla31* (Tab. 1) (Giese, 1981; Giese *et al.*, 1981; Jahoor *et al.*, 1991a, 1991b; 1993; Jensen, 1992; Jorgensen, 1992a, 1992c, 1993, 1994; Moseman and Jorgensen, 1971; Wise and Ellingboe, 1985). In spite of the deviation from general genetic nomenclature barley geneticists use symbol the *Ml* for the locus for resistance to powdery mildew. The proper symbol should be *Reg* (resistance to *E. graminis*). In some cases parentheses are used in gene symbols indicating the tentativeness, when it is not known if the gene is allelic to a named gene or segregation data are incomplete or missing. The gene with symbol *Mla15* has race-specificity identical to gene *Mla7*. Because of this the symbol *Mla15* is considered invalid (Jorgensen, 1994).

The *Mla* locus shows complex polymorphism. In many cases results indicate the presence of multigene families in

Table 1. Twenty-eight Race-specific Powdery Mildew Resistance Genes Near or at the *Mla* locus on Barley Chromosome 5 (Jorgensen, 1994).

Gene (allele)	Synonym(s)	Main donor
Mla1	*Mla*, MlA^{o}, *Mlr*, JMl^{a}_{sn}, *Regla1*, *Er*, $Pmla^{a}_{1}$	Algierian
Mla2	Mla^{2}, *Mlb*, JMl^{br}_{sn}, *Reglb2*, *Pmlb*	Black Russian
Mla3	*Ml(1036)*, *Reglc3*, *Pmlc*	Ricardo
Mla4	*Mlk*, *Ml(1063)*, *JMl*, *Reg4ae*, *Pm4*	Kwan
Mla5	*Mlgo*, *Regle5*, *Pmle*	Gopal
Mla6	MlA^{6}, Mlm^{3}, MlM^{2}, JMl_{sn}, *Reglf6*, *Pmlf*	Franger/ *H.spont.nigr*
Mla7	MlA^{10}, *MlA15*, *Reglg7*, *Pmlg*	Lyallpur
	Mla?, MlA^{1}, *Reglo*	Multan
	Mla?, *Mla15*, *Reglq*	Long Glumes/ Iso 26R
Mla8	JMl^{h4}_{sn}, *Reglh8*	Heils Hanna
Mla9	*Mlm*, MlM^{1}, JMl^{mc}_{sn}, *Regli9*	Monte Cristo
Mla10	*Reglj10*	Durani
Mla11	JMl^{al}_{sn}, *Reglk11*	A222
Mla12	*Mlas*, *Mla(Ar)*, *Reglt12*	Arabische/Emir
Mla13	*Mla?*, *Mla(Ru1)*, *Reglu13*, *Reglm*	Rupee
Mla14	*Mla(Sp2)*, *Reglv14*	Franger/ *H.spont.nigr.*
Mla15	*Mla?*, *Reglr*	Long Glumes/ Iso 26R
Mla16		*H.spont.*1B54B
Mla17		*H.spont.* RS 170-47
Mla18		*H.spont.*RS 20-1
Mla19		*H.spont.*1B-86B
Mla20		*H.spont.*RS145-39
Mla21		*H.spont.*1B-152B
Mla22	*Mlc*, MlC^{o}	HOR 1657
Mla23	*Ml(1402)*	HOR 1402
Mla24	Mlm^{1}, MlM^{o}	Engledow India
Mla25		*H.spont.*RS1707
Mla26		*H.spont.*1B-20
Mla27		*H.spont.*RS1-8
Mla28		*H.spont.*1B-151
Mla29		*H.spont.*RS110-4
Mla30	Jml^{n}_{sn}	Nigrate
Mla31	Jml^{t}_{sn}	Turkey 290

this locus (Jorgensen, 1992a; Jorgensen and Moseman, 1972; Mahadevappa *et al.*, 1994; Moseman and Jorgensen, 1973; Wise and Ellingboe, 1985). The 12 known or suspected cases of multigene families at the *Mla* locus are listed in Table 2 (Jorgensen, 1988a, 1992a, 1994; Schuller *et al.*, 1992; Wise and Ellingboe, 1985). At present there are 44 valid gene symbols for mildew resistance near or at the *Mla* locus (Tab. 1 and 2) (Giese *et al.*, 1981; Jahoor *et al.*, 1991a, 1991c; Jensen, 1992; Jorgensen, 1992c, 1994).

Investigations of the genetic mechanisms responsible for linkage between genes in resistance clusters were reported, but no specific process has been confirmed (Sudupak *et al.*, 1993, Robbins *et al.*, 1991). The *Hor1* and *Hor2* loci were reported to be linked to a *Mla* locus (Jensen *et al.*, 1980; Oram *et al.*, 1975). Clustering of many resistance gene families often associated with seed storage protein suggest a mechanism in which duplication, followed by multiple recombination, could be involved (Giese *et al.*, 1981; Singh *et al.*, 1990; Sudupak *at al.*, 1993, Robbins *et al.*, 1991). The same mechanism may be involved in observed complex polymorphism of the *Mla* locus (DeScenzo *et al.*, 1994; Giese *et al.*, 1981; Wise and Ellingboe, 1985)

Table 2. Sixteen Race-specific Powdery Mildew Resistance Genes Closely Linked to Named Alleles in the *Mla* Locus on Barley Chromosome 5 (Jorgensen, 1994).

Closely linked gene	Synonym(s)	Named allele	Multigene family	Donor
MlaA12		*Mla1*	i	Algierian
MlaBR2		*Mla2*	ii	Black Russian
MlaTu2		*Mla3*	iii	Turkish Sv 57/510-44
Mla14	*Mla(Sp2)* *Reg1v14*	*Mla6*	iv	Franger/ *H. spont. nigr.*
MlaNo3		*Mla7*	v	Lyallpur/Nordal
MlaNo4		*Mla7*	v	Lyallpur/Nordal
MlaLG2	*Mla?, Reg1r*	*Mla7*	vi	Long Glumes/Iso 26R
MlaLG3	*Mla?, Reg1r*	*Mla7*	vi	Long Glumes/Iso 26R
MlaMu2	*Mla?, reg1p*	*Mla7*	vii	Multan
MlaTr3		*Mla7*	viii	Triumph
MlaMC3		*Mla9*	ix	Monte Cristo
MlaMC4		*Mla9*	ix	Monte Cristo
MlaDu2	*Mla?, Reg1l*	*Mla10*	x	Durani
MlaEm2		*Mla12*	xi	Arabische/Emir
MlaRu3	*Mla?, Reg1n*	*Mla13*	xii	Rupee
MlaRu4	*Mla?, Reg1n*	*Mla13*	xii	Rupee

Resistance Genes at Loci Other than *Mla*

In addition to *Mla*, five other loci (*Mlat*, *Mlk*, *Mlra*, *MlGa*, and *Mlnn*) with race-specific powdery mildew resistance genes are known on barley chromosome five (Tab. 3) (Doll and Jensen, 1986; Giese *et al.*, 1981; Jorgensen, 1994; Wiberg, 1974a, 1974b). Other loci with race-specific powdery mildew resistance genes were mapped on barley chromosomes 4, 6, and 2. The *Mlh* locus is known to be on chromosome 6 (Hayashi and Heta, 1985), the *MlLa* locus on chromosome 2 (Hilbers *et al.*, 1992; Torp *et al.*, 1978) and the *Mlg* and *MlBo* loci on chromosome 4 (Hermansen, 1980; Jensen, 1992; Jorgensen, 1992c, 1994; Wiberg, 1974a, 1974b). Some resistance genes e.g. *Mln*, *Mlv*, have not yet been located on a chromosome (Jorgensen, 1994; Soogard and Jorgensen, 1993). In addition, 31 other race-specific genes for resistance to powdery mildew have been described (Tab. 4) (Jensen, 1992; Jorgensen, 1992c, 1994).

Mlo Resistance

Origin

Mlo resistance is a specific type of resistance which is present only in barley. It was first described in a mutagen-induced powdery mildew-resistant barley mutant, 'Mutante 66' (M66). It was induced by X-rays in the German variety 'Haisa' in 1942 (Freisleben and Lein, 1942). To

Table 3. Ten Race-specific Powdery Mildew Resistance Genes in Nine Loci other than the *Mla* Locus (Jorgensen, 1994).

Gene (allele)	Locus	Chromosome	Synonym(s)	Main donor
Mlat	*Mlat*	5	*JMl*$_{r12}$, *Pm7*	Atlas
MLGa	*MlGa*	5	*Ml(Ga)*	Galleon
Mlk1	*Mlk*	5	*Mlk, Ml(1063), JMl*$_{k}$, *Reg4ae, Pm4*	Kwan
			Mla4, Reg1d4, Pm1d	No22 Weider
Mlk2	*Mlk*	5	*JMl*$_{nz}$	Nakaizumi-zairai
Mlnn	*Mlnn*	5	*JMl*$_{nn}$	Nigrinudum
Mlra	*Mlra*	5	*Ml(41/145), Reg7a*	Ragusa
Mlg	*Mlg*	4	*JMlg, Reg2ac, Pm2, Er*$_{cp}$	Goldfoil
MlBo	*MlBo*	4	*Ml(N182)*	Mutant in Bomi
Mlh	*Mlh*	6	*Ml(37/136), JMl*$_{h}$, *Reg3ad, Pm3*	Hanna
MlLa	*MlLa*	2	*Ml(La), Mlv*	*Hordeum laevigatum*

Table 4. Thirty-one Race-specific Powdery Mildew Resistance Genes in Barley (Jorgensen, 1994).

Gene (allele)	Putative chromosome	Synonym(s)	Main donor
Mla	5		Magnif 105
Mlab			Ab 1128
Ml (Ab)			Triumph
Mlci	5		CI 6576
Ml (CP)	4		Weihenst. CP 127422
mld			Duplex
Ml (He)			Herta
Mli			H. spont. RS 42-8
Ml (1M9)			Hulda
Mlkb		JMl_{kb}	Kairyobozu-mugi
Ml (LG4)		*Ml?, Reg, s*	Long Glumes/lso 26S
Ml (Ma)			Marco
Mlmu	5	*mlmu*	Mulyan
Mlmw	5		Mian Wali
Mln	5	*Pm6*	Nepal
Mlne			
mlni		*mln*	Nigrate
Mlp		*Mlp2, Mlp3, JMlp, Reg5af, Pm5*	Psaknon
Mlr74		JMl_{r74}	Russian 74
Mlr81		JMl_{r81}	Russian 81
Ml (Ru2)			Rupee
mls			Spiti
mlw			West China
Ml (Wo)			Wong
Mlx			Arlington Awnless
Mly			Arlington Awnless
Mlz			Palmella Blue
Ml501			Gatersleben mutante 501
Ml (1192)			Stamml/Jarek
Ml (2891)			Stamml
Ml (3576)			

date, more than 150 *mlo* mutant genes have been obtained (Hentrich and Habekuss, 1991; Jorgensen, 1992, 1994). From the mid 1940s to the mid 1970s, *mlo* powdery mildew resistance genes were thought to occur only as induced mutations (Jorgensen, 1976). In 1976, Jorgensen described the *mlo* allele in the Ethiopian barley line 'Grannenlose Zweizeilige'. This line was obtained from German expeditions to Ethiopia in 1937 and 1938 (Jorgensen, 1976, 1992). Subsequently, more than twenty landraces from Ethiopia with *mlo* resistance genes have been described (Jorgensen, 1992; Negassa, 1985a).

Genes

The *mlo* locus is located on the long arm of barley chromosome 4 (Jorgensen, 1974, 1984, 1987, Negassa, 1985a). From all available data, at least 25 *mlo* powdery mildew resistance genes are described (Tab. 5) (Jorgensen, 1994). Mlo resistance is unique because: (i) it does not fit the gene-for-gene hypothesis; (ii) mlo genes originating from different mutational events map as non-complementing recessive alleles in one locus; (iii) all alleles confer the same resistance reaction, although with small quantitative differences; and (iv) it is effective against all isolates of the pathogen (Andersen, 1991; Jorgensen, 1994; Limpert *et al.*, 1991; Schwarzbach, 1979).

Table 5. Twenty-five Named *mlo* Powdery Mildew Resistance Genes at the *mlo* Locus on Barley Chromosome 4 (Jorgensen, 1994).

Gene (allele)	Synonym(s)	Barley mutant/line (mother variety)
mlo1	*reg6a*, er_m	Mutante 66 (Haisa)
mlo2	*reg6b*	H3502 (Vollkorn)
mlo3	*reg6c*	MC20 (Malteria Heda)
mlo4	*reg6d*	SR1 (=Refoma) (Foma)
mlo5	*reg6e*	Riso 5678 (Carlsberg II)
mlo6	*reg6f*	Riso 6018 (Carlsberg II)
mlo7	*reg6g*	Riso 7085 (Carlsberg II)
mlo8	*reg6h*	Riso 7372 (Carlsberg II)
mlo9	*reg6i*	SZ5139b (Diamant)
mlo10	*reg6j*	SR7 (Foma)
mlo11	*reg6k*, er_n	Grannenlose Zweizeilige (and other barleys from Ethiopia)
mlo12	*mlo/1*, *reg6-*	Mutant no. 4122 (Elgina)
mlo13	*mlo/2*, *reg6-*	Mutant no. 2018 (Plena)
mlo14	*mlo/3*, *reg6-*	Mutant no. 2029 (Plena)
mlo15	*mlo/4*, *reg6-*	Mutant no. 4123 (Elgina)
mlo16	*mlo/5*, *reg6-*	Mutant no. 2267 (Alsa)
mlo17	*mlo/6*, *reg6-*	Mutant no. 2034 (Plena)
mlo18	*reg6-*	Mutant Ml-3A (Azuma Golden)
mlo19	*reg6-*	Mutant Ml-4F (Fuji Nijou)
mlo20	*reg6-*	Mutant Ml-9F (Fuji Nijou)
mlo21	*reg6-*	Mutant Ml-13F (Fuji Nijou)
mlo22	*reg6-*	Mutant B1012 (Bomi)
mlo23		Mutant B1101 (Bomi)
mlo24		Mutant B1865 (Bomi)
mlo25		Mutant N105 (Bomi)

Phenotype

Leaves of Mlo resistant barley plants inoculated with powdery mildew spores remain resistant with no visible sign of infection except for an occasional infection type 4 (compatible) mildew colonies. These colonies originate predominantly from successful infections in the subsidiary cells next to the stomata on the barley epidermis. These cells do not have the ability to prevent infection (Andersen, 1991; Jorgensen, 1993, 1994).

The characteristic features of Mlo resistant barley plants are a tendency to necrotic or chlorotic leaf spotting and reduction of the plant height and grain yield. This is due to the pleiotropic effect of the *mlo* genes (Kjaer *et al.*, 1990; Schwarzbach, 1979). The intensity of this effect depends mainly on the gene background in the Mlo-resistant barley, environment, and apecific *mlo* allele (Bjornstad and Astreit, 1990; Hentrich and Habekuss, 1991; Jorgensen 1992; Lundqvist, 1991). However, high yielding Mlo resistant barley lines can be produced if proper adjustments are made to the genetic background. The first Mlo resistant barley variety in Europe derived its resistance from the Ethiopian accession L92 that gave rise to the variety 'Atem' released in The Netherlands in 1979. Since that time a significant number of commercial barley varieties with Mlo resistance have been released in Europe (Jorgensen, 1984, 1992).

Mechanism

When Mlo resistant barley leaf tissue is inoculated with powdery mildew, the host epidermal cells develop wall appositions (papillae) more rapidly and of a greater size than non-Mlo barley. These papillae prevent the pathogen from penetrating (Bayles *et al.*, 1990; Skou, 1985; Skou *et al.*, 1984; Wolter *et al.*, 1993). The papilla depositions consist of a variety of chemical elements and compounds such as callose, calcium, proteins and carbohydrates (Aist *et al.*, 1988; Bayles and Aist, 1987; Clark *et al.*, 1995; Zeyen *et al.*, 1993). Their presence in the papillae have to involve many structural genes. A reasonable assumption is that the *mlo* locus has a wild-type gene with a regulatory function for papillae formation. The implication of this hypothesis is that Mlo resistance will be durable, impossible to overcome by powdery mildew by a single mutation (Jorgensen, 1984, 1994).

Partial Resistance

Partial resistance is characterized by a low infection level and a susceptible infection type at all stages of plant development (Hwang and Heitefuss, 1982a, 1982b). It is often environmentally labile with many components which interact individually or pleiotropically with environmental factors (Newton, 1990, 1993; Newton and McGurk, 1991).

Infection of seedling leaves under highly controlled environmental conditions can provide detailed information on infection frequency, colony biomass and latent period. These factors influence both overall mildew damage and spore production during an epidemic, and may therefore have significant effects on final yield (Balkema-Boomstra and Mastebroek, 1995; Newton, 1990, 1993; Parlevliet and Ommeren, 1975). Quantitative or partial resistance may reduce the selection pressure for virulence in the pathogen population and thus could stabilize the host-pathogen system. Because the effects of the individual genes cannot be monitored, the understanding of the mechanisms of partial resistance are not known (Boyd *et al.*, 1994). Quantitative differences in resistance of barley to powdery mildew have been identified (Asher, 1981; Asher *et al.*, 1983; Carver, 1986; Heun, 1986; Jones and Davies, 1985; Jones *et al.*, 1981; Knudsen, 1984; Knudsen *et al.*, 1986; Newton, 1989; Newton and Thomas, 1993). The genetics of these forms of resistance have been analyzed by several authors. Asher and Thomas (1987) and Heun (1987a, 1987b) have reported a predominance of additive gene effects in this type of resistance. Partially resistant barleys for powdery mildew are known to show adult plant resistance. It is defined by an initial susceptible infection type that shifts to a more

resistant infection type at later growth stages (Hwang and Heitefuss, 1982a, 1982b; Wright and Heale, 1984). Recently, many studies focused on the evaluation of sources of partial resistance to powdery mildew (Balkema-Boomstra and Mastebroek, 1993; Mastebroek and Balkema-Boomstra, 1991b; Newton, 1990). The aim of those studies was to provide sources of resistance more durable than the major gene resistance (Jorgensen, 1983; Newton and Thomas, 1993).

Induced Resistance

The term 'induced resistance' is used to describe resistance induced by inoculation of plants with an incompatible isolate of a pathogen, a saprophyte, or a nonpathogen (Gregersen and Smedegaard, 1989; Hwang and Heitefuss, 1982c; Jorgensen, 1994). This inoculation triggers a series of biochemical changes, which cause a reduction in the level of susceptibility to compatible pathogen isolates. This type of resistance was induced in barley by infection by avirulent isolates of powdery mildew and it was expressed as a reduction of the symptoms of infection by subsequent inoculation with a virulent isolate (Ouchi *et al.*, 1974). This type of resistance may include different defense mechanisms which inhibit the virulent pathogen in qualitative or quantitative ways. Induced resistance to powdery mildew in barley extends the latent

period of infection, reduces the rate of pathogen development and growth (Hwang and Heitefuss, 1982a, 1982b) or decreases the number of colonies and spores produced (Martinelli, 1993; Ouchi *et al.*, 1974, 1976a, 1976b;). The biochemical changes involved in induced resistance of barley to powdery mildew include production of phytoalexins (Ouchi and Oku, 1982) and phenolic compounds (Shiraishi *et al.*, 1989), the synthesis of enzymes (Sako and Stahmman, 1972), other proteins (Bryngelsson *et al.*, 1988), and papilla formation (Bushnell and Berquist, 1975). The practical importance of induced resistance is not well studied. However, it is assumed that barley plants grown in the field are exposed to many biotic factors which result in expression of induced resistance. This may have a some importance because barley plants, after being exposed to biotic factors, will become less susceptible to infection by virulent powdery mildew isolates (Jorgensen, 1994).

Passive Resistance

Passive resistance is a type of resistance in which there is no defensive reaction of the host to the pathogen. Two major ways are possible to obtain this resistance in barley for powdery mildew. The first way is often described as a disease escape. It is present when barley plants escape the distribution peak of powdery mildew. In Europe, winter

barley crops are less damaged by powdery mildew because the winter barley plants start senescence before the disease reaches its epidemic peak (Jorgensen, 1994). The physical structure of surface waxes on the leaves represent another way to obtain passive resistance for powdery mildew in barley. This wax structure affects development of the appressorial germ tubes (Carver *et al.*, 1990). The genetics of this form of resistance is assumed to be complex. However, by changing the leaf surface topography it may be possible to obtain durable passive resistance in barley for powdery mildew (Jorgensen, 1993, 1994).

Molecular Markers in Genetic Analysis of Barley

Restriction Fragment Length Polymorphisms (RFLP)

Restriction fragment length polymorphisms (RFLPs) result from specific differences in DNA sequence that alter the size of the fragments obtained after digestion of genomic DNA with restriction endonucleases (Hulbert and Michelmore, 1987). Botstein *et al.* (1980) was the first to propose the use of RFLPs as genetic markers. This type of molecular marker has genetic characteristics which are very useful in genetic improvement programs. They include lack of dominance, multiple allelic forms, and absence of pleiotropic effect on other characters (Beckman and Soller, 1983). They are generally phenotypically neutral and

independent of allelic and non-allelic interaction (Jahoor *et al.*, 1991a). Their environmental stability and nearly unlimited availability have made RFLP markers an ideal tool for plant breeding (Graner *et al.*, 1990, 1991). Conventional detection of RFLPs by Southern blot analysis (Southern, 1975) is laborious and costly (Beckman and Soller, 1983). The polymerase chain reaction (PCR) provides a rapid, safe and efficient method (Mullis and Faloona, 1987). Saiki *et al.* (1985) described cleavage of PCR products with a restriction endonuclease. Correlations between RFLP markers and qualitative or quantitative traits have been reported (Barone *et al.*, 1990; Klein-Lankhorst *et al.*, 1991; Lander and Botstein, 1989; Paterson *et al.*, 1988; Sarfatti *et al.*, 1989; Yu *et al.*, 1991). It is also possible to use RFLPs in plant breeding programs for marker assisted selection (Murray *et al.*, 1991; Tanksley *et al.*, 1987). These markers are used in evolutionary studies, in characterization of germplasm, and for surveying genetic diversity of populations both nuclear and organellar genomes (Debener *et al.*, 1990; Dudley *et al.*, 1992; Melchinger *et al.*, 1991; Miller and Tanksley, 1990; Zehr *et al.*, 1992; Zhang *et al.*, 1993). RFLP maps have been constructed for the genomes of various major crop plants (Bernatzky and Tanskley, 1986a, 1986b,; Bonierbale *et al.*, 1988; Burr *et*

al., 1988; Gebhardt *et al.*, 1989; Helentjaris *et al.*, 1986; Landry *et al.*, 1987; McCouch *et al.*, 1988; Tanksley *et al.*, 1987).

In barley, RFLP markers have broad application, including evaluation of malting quality (Saghai Maroof *et al.*, 1994), evolutionary studies (Petersen *et al.*, 1994; Saghai Maroof *et al.*, 1995; Svitashev *et al.*, 1994), genetic analysis of physiological processes (Barua *et al.*, 1993b; Laurie *et al.*, 1994, 1995), genetic diversity of population (Melchinger *et al.*, 1994; Zhang *et al.*, 1993), location of genes for resistance (Graner and Bauer, 1993; Kilian *et al.*, 1994), and mapping of quantitative resistance (Heun, 1992). Partial RFLP maps of the barley genome have been published (Hinze *et al.*, 1991, Kleinhofs *et al.*, 1988; Shin *et al.*, 1990). Recently, more extensive RFLP linkage maps of the barley genome have been constructed (Graner *et al.*, 1991; Heun *et al.*, 1991; Kleinhofs *et al.*, 1993).

Polymerase Chain Reaction Based Markers

Polymerase chain reaction (PCR) is a method which involves repeated cycles of DNA strand synthesis directed by sequence-specific synthetic oligonucleotide primers, permitting exponential amplification of that specific sequence out of a crude genomic DNA preparation (Mullis and

Faloona, 1987). A PCR based marker system was proposed by Welsh and McClelland (1990) as arbitrarily primed-PCRs (AP-PCRs), and by Williams *et al.* (1990) as random amplified polymorphic DNA (RAPDs). The RAPD technique has several advantages. It requires only nanograms of DNA, and because it exploits the use of universal primers it does not need a previously characterized DNA sequence. In contrast to RFLPs, genetic analysis with RAPDs is fast and does not involve the use of radioisotopes (Rafalski *et al.*, 1991, 1994, Tingey and Tufo, 1993). However, problems with reproducibility in using these markers may arise. This is connected with the instability of the reaction due to concentration of DNA, used polymerase, and the assay conditions (Giese *et al.*, 1994). Also, repetitive DNA sequences are often amplified (Devos and Gale, 1992; Talbert *et al.*, 1994).

The possibility of using of RAPD markers in construction of linkage maps and locating genes of agronomic importance was reported (Rafalski *et al.*, 1991; Waugh and Powell, 1992). This possibility also includes use with cereals (Devos and Gale, 1992; D'Ovidio *et al.*, 1990; Penner *et al.*, 1993; Weining and Langridge, 1991). In barley, RAPD markers have broad applications, e.g. for identification of regions in the barley genome involved in the control of

important developmental processes (Barua *et al.*, 1993b), and markers for resistance loci (Barua *et al.*, 1993a). Distribution of RAPD markers on a linkage map of barley was also described (Giese *et al.*, 1994, Kleinhofs *et al.*, 1993).

Olson *et al.* (1989) described the use of sequence tagged site polymerase chain reaction (STS-PCR) in the human genome mapping project. This method was developed to increase the effectiveness of genome analysis in comparison to RFLP and RAPDs. STS-PCR is an enzymatic amplification of a mapped DNA fragment flanked by a pair of oligonucleotide primers (Mullis and Faloona, 1987; Saiki *et al.*, 1985). PCR primer sequences are designed from well characterized (sequenced) low-copy DNA sequences (RFLP clones). The 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. Tragoonrung *et al.* (1992) applied the STS-PCR method to barley, using eight sequences previously mapped in the barley genome. Four pairs of primer sequences were obtained from published sequences, and four pairs were obtained by sequencing portions of DNA clones from genomic clones derived from a random genomic library used in the North America Barley Genome Mapping Project (NABGMP). This method was used also in wheat (Chee *et al.*, 1995; Chen *et al.*,

1994; Martin *et al.*, 1995; Talbert *et al.*, 1994). Based on the results from these investigations, it may be concluded that STS approach for genome analysis is very useful. A disadvantage of the STS-PCR method in comparison to RAPD-PCR is the need for sequence analysis before primers can be designed (Talbert *et al.*, 1994). Advantages of this technique include safety (no need to use radioactive reagents), efficiency over traditional RFLP analysis and the elimination of confusing results due to repetitive DNA sequence amplification by RAPD-PCR. Additionally, once primers are developed and tested, published sequences can be easily shared with other researchers. This is much easier to do than shipping recombinant RFLP clones (Olson *et al.*, 1989; Talbert *et al.*, 1994).

Molecular Markers for Genes for Resistance to Powdery Mildew in Barley

Genes at the *Mla* Locus

The *Mla* locus of barley is a complex locus. At least 28 alleles or closely linked loci have been indicated for this locus (Giese, 1981; Giese *et al.*, 1981; Jahoor *et al.*, 1991a, 1991b, 1993; Jorgensen, 1992, 1993, 1994; Jorgensen and Moseman, 1972; Wise and Ellingboe, 1985). Schuller *et al.* (1992) identified three RFLP markers (MWG 1H060, MWG 1H036, MWG 1H068) closely linked to the *Mla* locus. By

polymorphisms obtained with RFLP marker MWG 1H036, it was possible to separate the *Mla* alleles into 11 different groups. Jahoor *et al.* (1993) used the same three RFLP markers for further investigations of the mode of inheritance and intralocus recombination at the *Mla* locus. In this investigation F_1 plants were used to determine the mode of inheritance of several *Mla* alleles. The results obtained were generally the same as obtained by Giese *et al.* (1981). In the same study, seven *Mla* alleles were selected for the study of intralocus recombination. These *Mla* alleles represented a large range of geographic origins which should increase chances for recombination. However, the results may be interpreted in two ways: it is possible that the Mla region may include at least two very closely linked loci or all of the alleles of the Mla region belong to the same locus.

The Mla complex is flanked by two endosperm storage protein loci, *Hor1* and *Hor2* (Jensen *et al.*, 1980). Mahadevappa *et al.* (1994) developed a high-resolution mapping population for the region between *Hor1* and *Hor2*. This mapping population consisted of 270 individual lines, each representing an independent recombination event between the *Hor1* and *Hor2* loci. These recombinant lines were used by DeScenzo *et al.* (1994) to construct a high-resolution

RFLP map of the Hor1/Mla/Hor2 region. In this RFLP map were also integrated markers from three previously reported maps (Heun *et al.*, 1991; Graner *et al.*, 1991; Kleinhofs *et al.*, 1993).

Genes at Loci Other Than *Mla*

RFLP markers for the *Mlo* locus were reported (Hinze *et al.* 1991). Giese *et al.* (1993) identified an RFLP marker closely linked to the Laevigatum resistance gene for powdery mildew. In this investigation RFLP markers located on chromosome 2 provided by T. Blake (Shin *et al.*, 1990) and A. Graner (Graner *et al.*, 1991) were used.

CHAPTER 3

DETERMINATION OF NEW SOURCES OF RESISTANCE TO POWDERY MILDEW OF BARLEY IN SELECTED LINES FROM TUNISIAN BARLEY LANDRACES

Introduction

Barley powdery mildew is a severe foliar disease and it occurs wherever barley is grown (Rasmusson, 1985). This fungus show great genetic variability (McIntosh, 1978; Russell, 1978; Wolfe and McDermott, 1994). The result of this great genetic variability is very rapid loss of effectiveness of a number of race-specific resistance genes, which can lead to epidemics of disease (Brown *et al.*, 1989a, 1989b; Jensen and Jorgensen, 1991; Munk *et al.*, 1991; Wolfe and Schwarzbach, 1978). These epidemics are also caused by the fact that modern homogenous, genetically uniform barley varieties have the same gene or genes for resistance to this fungus in each plant. If the fungus possesses a gene for virulence capable of establishing infection on the particular barley variety, then all plants are liable to be infected and the disease epidemic occurs (Robinson, 1976; Vanderplank, 1968, 1982).

The importance of genetic diversity and the potential consequences associated with narrowing the genetic base of cultivated barley has long been recognized by plant pathologists and plant breeders (Jensen, 1988; Simmonds, 1987). As a result, new strategies for deploying genes for resistance to powdery mildew of barley have been explored and searches for new sources of resistance in cultivated barleys and in their wild relatives have been conducted (Gacek and Czembor, 1983; Jahoor and Fischbeck, 1987a, 1987b, 1993; Jorgensen, 1994; Moseman *et al.*, 1980, 1981, 1983; Rubiales *et al.*, 1993; Xu and Kasha, 1992).

West Asia and North Africa are the primary centers of diversity of barley. In marginal areas of Tunisia farmers still rely on landraces of barley which show a stable performance. These landraces are often collections of seeds that have been exchanged for other goods among nomad tribes or from the seeds that were handed down from generation to generation (Yahyaoui, 1986). These landraces are characteristically genetically heterogenous. This is manifested by obvious diversity in the appearance of the plants. There can be much variation in length of straw, architecture of the head and color of the grain (Brown *et al.*, 1989b; Jensen, 1988). This diversity extends to characters which cannot be readily seen, such as in genes

for resistance and susceptibility to various pathogens (Harlan, 1975). In the landrace farming system, powdery mildew rarely develops to levels that significantly damage the yield. This is due to the stabilizing effect of the genetic heterogeneity and the presence of a certain level of resistance within the barley landraces (Leur *et al.*, 1989). This indicates that Tunisian barley landraces may be useful as a source of genes for resistance for this disease (Ceccarelli *et al.*, 1987, 1992; Harlan, 1976a; Moralejo *et al.*, 1994; Wendorf *et al.*, 1979, 1984; Yahyaoui, 1986; Zohary, 1969, 1973; Zohary and Hopf, 1988).

The objective of this study was to characterize barley landraces from Tunisia in terms of the number of genes, gene action and to identify genes for resistance to powdery mildew.

Materials and Methods

The Host

Seeds of single plants from 232 landraces were collected by Dr. N. Kurauchi in central Tunisia during May and June 1990/1991 (Appendix Fig. 6). In 1992 these accessions were increased near Tucson, Arizona.

The Pathogen

Three isolates of *E. graminis* f. sp. *hordei* were used: Bzm-1, KM 18-75, R13C. The isolate Bzm-1 was collected in 1985 from the greenhouse at Montana State University, Bozeman (Reinhold *et al.*, 1990). The isolates KM 18-75 and R13C were provided by J. H. Jorgensen (Riso National Laboratory, Roskilde, Denmark). The three isolates were purified by single pustule isolation, maintained, and increased on young seedlings of the variety 'Manchuria'. The Pallas isolines differential set for powdery mildew of barley (Tab. 6) (Kolster *et al.*, 1986) was used to determine the virulence spectrum of all isolates used (Tab. 7). Frequent virulence checks assured their purity throughout the experiment.

Disease Assessment

Reaction types exhibited by barley plants after infection with *E. graminis* f. sp. *hordei* were determined using a 0 through 4 scale (Tab. 8) developed by Mains and Dietz (1930).

Table 6. The Recurrent Parent Pallas and 24 Near-isogenic Barley Lines with Specific Genes and Their Parent Cultivars (Kolster *et al.*, 1986).

Near-isogenic line	Resistance gene		
	Designation	Origin	Reference***
Pallas	*Mla8*	Mut. in Bonus	1
P1	*Mla1, +?**	Algerian	2
P2	*Mla3*	(Ricardo)	3
P3	*Mla6, Mla14*	Franger	2, 4
P4A	*Mla7, Mlk, +?*	Heine 4808	4, 5
P4B	*Ma7, +?*	Heine 4808	6
P6	*Mla7, Ml(LG2)***	Multan	2, 5
P7	*Mla9, Mlk*	Monte Cristo	4, 5
P8A	*Mla9, Mlk*	Triple Awn Lemma	4, 5, 6
P8B	*Mla9*	Triple Awn Lemma	6
P9	*Mla10, Ml(Du2)*	Durani	2, 7
P10	*Mla12*	Arabische	4, 5
P11	*Mla13, Ml(Ru3)*	Rupee	4, 5
P12	*Mlc*	(HOR 1657)	8, 9
P13	*Ml(1402)*	(HOR 1402)	8, 10
P14	*Ml(41/45)*	(Weihenstephan 41/145)	8
P15	*Ml(Ru2)*	Rupee	4, 8
P17	*Mlk*	Monte Cristo	4, 8
P18	*Mlnn*	(Nigrinudum)	11
P19	*Mlp*	Psaknon	2
P20	*Mlat*	Coast	8, 11
P21	*Mlg, Ml(CP)*	Weihenstephan MR II	5
P22	*mlo5*	Mut. in Carlsberg II	12
P23	*Ml(La)*	*H. laevigatum*	5
P24	*Mlh*	Hanna	2

* +? - indicates an unidentified resistance gene

** ( ) - the parenthesis indicates the tentative designations e.g. when it is not known whether the gene is allelic to a named gene, or when segregation data, etc. are incomplete or missing.

*** References are: (1) Jorgensen and Jensen, 1983; (2) Moseman, 1972; (3) Moseman and Schaller, 1960; (4) Giese *et al.*, 1981; (5) Torp *et al.*, 1978; (6) Kolster *et al.*, 1986; (7) Moseman and Jorgensen, 1973; (8) Wiberg, 1974b; (9) Nover *et al.*, 1968; (10) Nover, 1972; (11) Moseman, 1968; (12) Jorgensen, 1971.

Table 7. The Virulence Spectrum of Isolates Bzm-1, R13C, and KM18-75.

Differential set		Isolates		
Isolines	Gene	Bzm-1	R13C	KM18-75
Manchuria		4	4	4
Pallas	*Mla8*	4	4	4
P1	*Mla1, +?**	0	0	0
P2	*Mla3*	0	4	0
P3	*Mla6, Mla14*	0	4	0
P4A	*Mla7, Mlk, +?*	0	4	4
P4B	*Mla7, +?*	0	4	4
P6	*Mla7, Ml (LG2)***	0	4	4
P7	*Mla9, Mlk*	0	0	4
P8A	*Mla9, Mlk*	0	0	4
P8B	*Mla9*	0	0	4
P9	*Mla10, Ml (Du2)*	0	1	4
P10	*Mla12*	2	4	1
P11	*Mla13, Ml (Ru3)*	0	0	1
P12	*Mlc*	4	4	0
P13	*Ml (1402)*	1	2	3
P14	*Ml (41/145)*	3	4	3
P15	*Ml (Ru2)*	3	4	4
P17	*Mlk*	1	4	4
P18	*Mlnn*	4	4	4
P19	*Mlp*	3	3	3
P20	*Mlat*	4	2	4
P21	*Mlg, Ml (CP)*	0	4	4
P22	*mlo5*	1	0	0
P23	*Ml (La)*	4	3	4
P24	*Mlh*	2	4	4

* +? - indicates an unidentified resistance gene.

** ( ) - the parenthesis indicates the tentative designations e.g.when it is not known whether the gene is allelic to a named gene, or when segregation data, etc. are incomplete or missing.

Table 8. Infection Types Produced by *E. graminis* f. sp. *hordei* on Barley Lines (Mains and Dietz, 1930).

Infection type	Symptoms
0	No visible mycelium
1	Visible mycelium
2	Mycelium, slight sporulation and chlorosis or necrosis
3	Mycelium and moderate sporulation
4	Mycelium, strong sporulation, and sometimes green islands

Disease symptoms were assessed on the primary leaf of the seedlings. The seedlings were classified into susceptible or resistant groups, according to their reaction types. Plants scored 0, 1, and 2 were included in the resistant group and plants scored 3 and 4 were included in the susceptible group.

Preliminary Selection of Lines from Barley Landraces for the Presence of Genes for Resistance

In 1993 sixty plants per line were evaluated with the Bzm-1 isolate of *E. graminis* f. sp. *hordei*. Bzm-1 represented the most avirulent isolate available and its use allowed the expression of a maximum number of resistance genes. In this experiment six seeds of each line were sown in ten 10 cm diameter plastic pots. These pots were filled with a mixture of Bozeman loam soil, sand and peat in a

1:1:1 ratio. The variety 'Manchuria' was used as a susceptible control and was sown every six pots. Plants were kept in the greenhouse isolation room with a 14-hour photoperiod and a 21/17 C day/night temperature regime. The inoculation was carried out when plants were 10-12 days old by shaking or brushing conidia from diseased plants.

Sixty-three of these lines showed resistant reactions. Twenty-two of them showed mixed reactions i.e., some plants showed resistance and some exhibited susceptible reactions within the same landrace accession (Tab. 9).

Sixty-three lines were also tested using the KM 18-75 and R13C isolates of powdery mildew of barley. For each line, eighteen seeds were sown in three plastic cones 21 x 4 cm filled with the soil mixture described before. The cones were placed in racks measuring 60 x 30 cm, 98 cones per rack. Ten seeds of the variety 'Manchuria' were were sown in 9 x 9 plastic pots. Plants were kept in a growth chamber under mildew free conditions with a 15-hour photoperiod. The temperature regime was 12 C in darkness and 20 C during light hours. After 10-14 days 3 cm leaf segments were cut from the middle part of the primary leaf of the seedlings. These segments were laid on a 5 g/l water-agar medium containing 0.112 g/l benzimidazole and 0.472g/l $Ca(NO_3)_2$ (modified CBA) (Carver and Phillips, 1982), distributed into compartmented transparent plastic boxes. Each box had 12

Table 9. Reaction of Sixty-three Lines to Isolate Bzm-1 of Powdery Mildew of Barley.

Lines	Reaction	Lines	Reaction
T-1	R	T-33	R
T-2	R	T-34	R
T-3	R	T-35	R
T-4	R	T-36	R-S
T-5	R	T-37	R
T-6	R	T-38	R-S
T-7	R	T-39	R-S
T-8	R	T-40	R
T-9	R	T-41	R
T-10	R	T-42	R
T-11	R-S	T-43	R
T-12	R-S	T-44	R
T-13	R	T-45	R
T-14	R	T-46	R-S
T-15	R	T-47	R
T-16	R	T-48	R-S
T-17	R-S	T-49	R
T-18	R	T-50	R
T-19	R-S	T-51	R-S
T-20	R	T-52	R-S
T-21	R-S	T-53	R-S
T-22	R	T-54	R-S
T-23	R	T-55	R-S
T-24	R	T-56	R-S
T-25	R	T-57	R-S
T-26	R	T-58	R-S
T-27	R	T-59	R-S
T-28	R	T-60	R-S
T-29	R	T-61	R
T-30	R	T-62	R
T-31	R	T-63	R-S
T-32	R-S		

R - resistant reaction,
R-S - mixed reaction - presence of resistant and susceptible plants within the same line.

compartments (3.3 x 5.0 x 3.0 cm) which contained 18 ml of medium each. Two leaf segments were put into each compartment. Leaf segments of the variety 'Manchuria' were used as a control and placed in every fourth compartment. Inoculation was carried out with a spore suspension (about 10^{-6} spores per ml of the oil FC43) using sterile cotton swabs. The boxes with the inoculated leaf segments were incubated in a controlled-environment chamber with a 20 C day-night temperature regime and a 12-hour photoperiod. The light was provided by fluorescent tubes. Mildew reaction was assessed twice, 7-9 and 12-15 days after inoculation, respectively.

Following this investigation twenty lines were selected which showed consistent and uniform reaction to the three isolates of powdery mildew (Tab. 10). According to this reaction they were divided into four groups. Different genes for resistance were expected in each group of the landraces. Year and collection sites of these twenty lines are shown in Table 11 and Appendix Fig. 7.

Crosses

All crosses of barley plants used in this study were made at the Post Farm, west of Bozeman, Montana, in the summer of 1994. Standard plant breeding techniques for hand emasculating and pollinating with a pollen shower were used.

Table 10. Twenty Lines Divided into Four Groups According to Their Reaction to Three Isolates of Powdery Mildew and Expected Resistance Genes in Each of These Groups.

Group	Line	Isolate			Expected resistance genes
		Bzm-1	R13C	KM18-75	
1	T-3	R	R	R	*mlo5,*
	T-13	R	R	R	*Ml (Ru3)**,
	T-40	R	R	R	*Mla1, +?***
	T-62	R	R	R	*Mla13*
2	T-5	R	S	S	*Mla7, Mlk,*
	T-8	R	S	S	*Ml (LG2),*
	T-27	R	S	S	*Ml (CP),*
	T-31	R	S	S	*Ml (La),*
	T-33	R	S	S	*Mlg,*
	T-35	R	S	S	*Mlh, +?*
	T-47	R	S	S	
	T-49	R	S	S	
3	T-14	R	S	R	*Mla3,*
	T-18	R	S	R	*Mla14,*
	T-28	R	S	R	*Mla12,*
	T-30	R	S	R	*Mla6*
4	T-4	R	R	S	*Mla9, Mlk,*
	T-6	R	R	S	*Mla10,*
	T-37	R	R	S	*Ml (Du2),*
	T-41	R	R	S	*Ml (1402)*

R - resistant reaction for powdery mildew
S - susceptible reaction for powdery mildew

*() - the parenthesis indicates the tentative designations e.g. when it is not known whether the gene is allelic to a named gene, or when segregation data, etc. are incomplete or missing.

** +? - indicates an unidentified resistance gene.

Table 11. Year and Site of Collection of Twenty Accessions from Tunisian Landraces Selected in the Preliminary Experiment.

Accession	Origin	Year of collection
T-3	Oued Ameur	1990
T-4	Oued Ameur	1990
T-5	Oued Ameur	1990
T-6	Oued Ameur	1990
T-8	Faidh (Sidi Bonzid Center)	1990
T-13	35 km north from Gafsa	1990
T-14	35 km north from Gafsa	1990
T-18	Gabes	1990
T-27	Bir el Haffey	1991
T-28	Bir el Haffey	1991
T-30	Ben Aroun	1991
T-31	El Fedj	1991
T-33	Echabiba	1991
T-35	Oued Kbir	1991
T-37	Feriana	1991
T-40	Mlauna	1991
T-41	Thala	1991
T-47	Kalaat el Kshasba (NW of Kef)	1991
T-49	Kalaat el Kshasba (NW of Kef)	1991
T-62	selected from landrace	1990/91

All heads used for crossing were bagged after emasculation. Heads with hybrid seed were harvested and threshed individually. Three types of crosses were made:

1. The susceptible variety 'Pallas' was crossed with twenty lines selected in the preliminary experiment (Tab. 9). These crosses were made to determine the number of genes for resistance in the landraces and their mode of inheritance.
2. Lines were crossed with Pallas isolines possessing genes for resistance to powdery mildew expected to occur in these lines (Tab. 6 and 10). Progeny from these crosses should yield information regarding common loci in the Pallas isolines and tested lines.
3. Lines with identical reaction patterns were crossed among each other to identify common loci (Tab. 10).

No reciprocal crosses were conducted. The sex of the parents was chosen randomly.

<u>Testing of F_2</u>

In the winter of 1994/1995, all F_1 generations of each cross were grown in plastic pots (30 cm in diameter) in the greenhouse to obtain seeds of the F_2 generations.

The seeds of the F_2 generations were sown in cones and grown under environmental conditions as described for the preliminary experiment. The leaf segments were tested with

the Bzm-1 isolate of powdery mildew using the detached leaf technique. Scoring for powdery mildew reaction was done twice; 8-10 and 12-16 days after inoculation. Chi-square analysis was performed to determine whether the observed classes fit a hypothetical genetic ratio.

Results

Lines: Group 1

The F_2 progeny from crosses of lines T-3 and T-62 with the variety 'Pallas' gave a good fit to the 1:3 (susceptible:resistant) ratio, indicating the presence of one dominant gene for resistance to powdery mildew (Tab. 12). Segregation ratio 3:1 (susceptible : resistant) of F_2 progeny from the cross between line T-13 and variety 'Pallas' indicate the presence of one recessive gene. The gene for resistance in line T-3 and genes for resistance in Pallas isolines P1 (*Mla1, +?*), P11 [*Mla13, Ml(Ru3)*] and P22 (*mlo5*) are at different loci which indicates that this line has a gene for resistance not present in the Pallas isolines set. The reaction of the segregating population from the cross of the line T-13 and Pallas isoline P22 (*mlo*) gave a good fit to the 7:9 (susceptible : resistant) ratio suggesting the presence of two independent recessive genes for resistance. Progenies F_2 from the crosses T-13 x T-3, T-13 x T-40 and T-13 x T-62 segregated at a ratio 3:13

Table 12. The Reaction of F_2 Generations Originating from Crosses Between Lines from Group 1 and Crosses of these Lines with the Variety 'Pallas' and Three Pallas Isolines to the Bzm-1 Isolate of Powdery Mildew.

Cross	Observed frequency		Hypothesis	P-value*
	Susc.	Resis.		
Pallas x T-3	66	240	1:3	.1868
Pallas x T-13	188	72	3:1	.3519
Pallas x T-62	71	182	1:3	.2925
T-3 x P1	26	349	1:15	.6599
T-3 x P11	15	258	1:15	.6960
T-3 x P22	66	277	3:13	.8695
T-13 x P1	51	202	3:13	.6218
T-13 x P11	43	218	3:13	.3885
T-13 x P22	36	48	7:9	1.0000
T-62 x P1	0	161	0:1	
T-62 x P22	18	69	3:13	.7443
T-40 x P1	4	47	1:15	1.0000
T-40 x P11	1	291	0:1	
T-13 x T-3	5	52	3:13	.6080
T-40 x T-62	12	150	1:15	.6554
T-40 x T-13	9	115	3:13	.7808
T-62 x T-3	2	28	1:15	1.0000
T-62 x T-13	4	35	3:13	.4821

* - determined by Chi-square value

(susceptible : resistant). This ratio indicates the presence of one recessive and one dominant genes for resistance. These results confirm the presence of one recessive gene in line T-13 and one dominant gene in the lines T-3, T-40 and T-62, respectively. F_2 populations from crosses T-40 x P11 [*Mla13, Ml(Ru3)*]and T-62 x P1 (*Mla1, +?*) did not segregate, indicating the presence of a gene at a common locus in both parents. Confirmation that lines T-62 and T-40 have genes at different loci was obtained. A 1:15 (susceptible : resistant) ratio of the F_2 generation from a cross between these two lines indicated the presence of two independent dominant genes for resistance.

Lines: Group 2

The segregation ratio of 1:3 (susceptible : resistant) of F_2 generations from crosses between lines T-5, T-8, T-27, T-31, T-33, T-47 and T-49 with the variety 'Pallas' indicated the presence of one dominant gene for resistance (Tab. 13). The cross between the variety 'Pallas' and line T-35 was unsuccessful. The determination of the number of genes in this line and their mode of inheritance is based on a cross between T-35 and T-5, T-8, T-27 and T-31, respectively. Results indicate that line T-35 possesses one dominant gene for resistance. The F_2 progeny from crosses of line T-5 with Pallas isolines P21 [*Mlg, Ml(CP)*] and P23

Table 13. The Reaction of F_2 Generations Originating from Crosses Between Lines from Group 2 and Crosses of these Lines with the Variety 'Pallas' and Three Pallas Isolines to the Bzm-1 Isolate of Powdery Mildew.

Cross	Observed frequency		Hypothesis	P-value*
	Susc.	Resis.		
Pallas x T-5	76	186	1:3	.1537
Pallas x T-8	65	159	1:3	.1897
Pallas x T-27	40	139	1:3	.4632
Pallas x T-31	52	210	1:3	.0636
Pallas x T-33	24	78	1:3	.8191
Pallas x T-47	55	169	1:3	.9385
Pallas x T-49	33	98	1:3	1.0000
T-5 x P21	3	29	1:15	.7150
T-5 x P23	14	173	1:15	.5840
T-8 x P6	11	96	1:15	.1279
T-8 x P21	27	299	1:15	.1611
T-8 x P23	27	314	1:15	.2454
T-27 x P21	10	186	1:15	.6056
T-5 x T-35	9	131	1:15	1.0000
T-5 x T-27	22	241	1:15	.1972
T-5 x T-31	19	279	1:15	1.0000
T-5 x T-47	24	250	1:15	.1116
T-5 x T-33	4	58	1:15	1.0000
T-27 x T-47	2	266	0:1	
T-27 x T-49	0	307	0:1	
T-27 x T-35	2	233	0:1	
T-27 x T-33	21	215	1:15	.1220
T-31 x T-33	9	102	1:15	.5401
T-31 x T-47	11	160	1:15	1.0000
T-31 x T-35	18	221	1:15	.4935
T-33 x T-35	15	219	1:15	1.0000
T-33 x T-8	21	228	1:15	.1961
T-8 x T-53	15	181	1:15	.5067

* - determined by Chi-square value

[*Ml(La)*]; line T-27 with Pallas isoline P21 [*Mlg, Ml(CP)*]; line T-8 with Pallas isolines P6 [*Mla7, Ml(LG2)*], P21 [*Mlg, Ml(CP)*], P23 [*Ml(La)*] gave a good fit to 1:15 (susceptible : resistant) ratio suggesting the presence of two dominant genes for resistance. This indicates that these lines and Pallas isolines with which they were crossed have dominant genes at different loci. A ratio of 1:15 (susceptible : resistant) was observed for the F_2 population of the cross T-5 x T-27, supporting the conclusion that these lines have dominant genes at different loci. The F_2 generation from the crosses T-27 x T-47, T-27 x T-49 and T-27 x T-35 did not segregate, indicating the presence of genes at identical loci. A segregation ratio of 1:15 (susceptible : resistant) of F_2 generation was obtained for crosses:

- line T-5 with lines T-35, T-27, T-31, T-47 and T-33,
- line T-31 with lines T-33, T-47 and T-35,
- line T-33 with lines T-27, T-35 and T-8,
- line T-8 with line T-35.

These results indicate the presence of two dominant genes and suggest that lines T-5, T-8, T-31, T-33 each have genes at different loci which are different from genes detected in T-27, T-35, T-47 and T-49.

Lines: Group 3

A segregation ratio of 1:3 (susceptible : resistant)

obtained for F_2 progenies from crosses between lines T-14, T-18, T-28, T-30 and the variety 'Pallas' indicates the presence of one dominant gene for resistance (Tab. 14). The F_2 generation of the cross between line T-14 and Pallas isoline P3 (*Mla6, Mla14*) did not segregate indicating the presence of genes at a common locus. A 1:15 (susceptible : resistant) ratio of F_2 from crosses of lines T-18 and T-30 with Pallas isolines P3 (*Mla6, Mla14*), P10 (*Mla12*); line T-28 with Pallas isolines P10 (*Mla12*) suggest the presence of two dominant genes for resistance. The F_2 progenies from crosses of lines T-28 and T-30 with lines T-14 and T-18 gave a good fit to the 1:15 (susceptible : resistant) ratio indicating the presence of two dominant genes for resistance. These results suggest that lines T-28 and T-30 have genes for resistance at different loci than lines T-14 and T-18.

Lines: Group 4

The segregation ratio 1:3 (susceptible : resistant) of F_2 generations of crosses between the variety 'Pallas' with lines T-4, T-6, T-37, T-41 indicate presence of one dominant gene for resistance (Tab. 15). A ratio 1: 15 (susceptible : resistant) for crosses of line T-4 with Pallas isolines P7 (*Mla9, Mlk*), P8a (*Mla9, Mlk*), P8b (*Mla9*) and P13 [*Ml(1402)*]; line T-6 with Pallas isolines P8a (*Mla9, Mlk*), P8b (*Mla9*),

Table 14. The Reaction of F_2 Generations Originating from Crosses Between Lines from Group 3 and Crosses of these Lines with the Variety 'Pallas' and Three Pallas Isolines to the Bzm-1 Isolate of Powdery Mildew.

Cross	Observed frequency		Hypothesis	P-value*
	Susc.	Resis.		
Pallas x T-14	27	68	1:3	.5147
Pallas x T-18	51	191	1:3	.1815
Pallas x T-28	69	176	1:3	.2848
Pallas x T-30	62	169	1:3	.5688
T-14 x P2	9	246	1:15	.0958
T-14 x P3	0	171	0:1	
T-18 x P3	17	184	1:15	.2512
T-18 x P10	20	236	1:15	.3662
T-28 x P10	13	215	1:15	.8374
T-30 x P3	15	157	1:15	.2375
T-30 x P10	23	239	1:15	.1180
T-28 x T-14	19	188	1:15	.1102
T-28 x T-18	24	287	1:15	.3413
T-30 x T-14	23	254	1:15	.1979
T-30 x T-18	2	41	1:15	1.0000

* - determined by Chi-square value.

Table 15. The Reaction of F_2 Generations Originating from Crosses Between Lines from Group 4 and Crosses of these Lines with the Variety 'Pallas' and Five Pallas Isolines to the Bzm-1 Isolate of Powdery Mildew.

Cross	Observed frequency		Hypothesis	P-value*
	Susc.	Resis.		
Pallas x T-4	56	196	1:3	.3443
Pallas x T-6	94	254	1:3	.4210
Pallas x T-37	156	216	1:3	.3875
Pallas x T-41	83	224	1:3	.4485
T-4 x P7	5	54	1:15	.6621
T-4 x P8a	15	182	1:15	.5197
T-4 x P8b	9	119	1:15	.8551
T-4 x P13	12	187	1:15	1.0000
T-6 x P8a	11	144	1:15	.7875
T-6 x P8b	10	76	1:15	.0661
T-6 x P9	15	160	1:15	.2659
T-6 x P13	18	213	1:15	.4052
T-37 x P8b	17	203	1:15	.4437
T-37 x P13	4	37	1:15	.5453
T-41 x P8b	18	230	1:15	.5998
T-41 x P13	11	129	1:15	.5412
T-4 x T-6	0	148	0:1	
T-4 x T-37	0	248	0:1	
T-4 x T-41	14	188	1:15	.7992
T-41 x T-6	12	177	1:15	1.0000
T-41 x T-37	14	164	1:15	.4621

* - determined by Chi-square value.

P9 [*Mla10, Ml(Du2)*], P13 [*Ml(1402)*] indicate the presence of two dominant genes for resistance. The segregation data from crosses of lines T-37 and T-41 with Pallas isolines P8b (*Mla9*) and P13 [*Ml(1402)*] gave a good fit to the 1:15 (susceptible : resistant) ratio which indicates the presence of two dominant genes for resistance. These results suggest that lines T-4, T-6, T-37 and T-41 have genes in different loci than the Pallas isolines with which they were crossed. Segregation ratio 1:15 (susceptible : resistant) was obtained in F_2 progeny from the crosses of line T-4 with lines T-6, T-37 and T-41; line T-41 with lines T-6 and T-37. These results indicate that the line T-4 has a gene for resistance in a different locus than lines T-6, T-37 and T-41. The same holds true for line T-41 and lines T-6 and T-37.

Discussion

To date, no systematic study determining the powdery mildew race-specific resistance genes present in Tunisian barley landraces has been conducted. The results presented here demonstrate practical advantages of preserving the genetic diversity of barley in the form of landraces. Among 232 investigated accessions from Tunisian landraces, 20 showed good and uniform resistance to three isolates of powdery mildew. These twenty lines were investigated as

potential sources of genes for resistance. The number of genes, the type of gene action (recessive or dominant), and the gene loci in these lines were determined.

In the Mediterranean area barley powdery mildew has coevolved with many hosts over a long period of time which resulted in a diverse pathogen population. The pathogen is also forced to rely on the sexual cycle to survive the hot and dry summers (Smith *et al.*, 1988; Wolfe, 1984). This is expected to result in a high number of recombinants of existing avirulence genes (Wolfe and McDermott, 1994). It is expected that landraces should also display heterogeneity of resistance to powdery mildew (Andrivon and Vallavielle-Pope, 1992; Harlan, 1975; Leur *et al.*, 1989; Mastebroek and Balkema-Boomstra, 1991a).

Barley is a self-pollinated species. However a certain amount of outcrossing does occur, allowing the population to accumulate (pyramid) genes for resistance into one plant (Allard, 1988; Baenzinger, 1981; Giles, 1989). Among 232 tested lines, 169 were susceptible to powdery mildew, 20 were resistant and 43 segregated for resistance. It seems apparent that even susceptible plants survive in a natural population where disease epidemics are limited because the diverse nature of the host population (Leur *et al.*, 1989). Among the 20 lines with consistent reaction to powdery

mildew a number of resistance genes were detected, some known and some unknown. Somewhat surprisingly only single genes were detectable with the isolates used.

The limited number of resistance genes might be explained by the fact that genes within the *Mla* locus form very closely linked multigene families (Jorgensen, 1992a; Jorgensen and Moseman, 1972; Mahadevappa *et al.*, 1994; Moseman and Jorgensen, 1973; Wise and Ellingboe, 1985). At least 12 multigene families are suspected at this locus (Giese *et al.*, 1981; Jahoor *et al.*, 1991a, 1991c; Jorgensen, 1988a, 1992a, 1994; Schuller *et al.*, 1992; Wise and Ellingboe, 1985). This means that lines selected in the preliminary experiment with resistance expected at the *Mla* locus may possess closely linked genes. Results obtained in this investigation indicate that lines T-27, T-47, T-49 and T-35 have a gene in one common locus. This gene may be *Mla7, Mlk, Ml(LG2), Ml(La), Mlh* or *+?*. According to results from F_2 segregation data, line T-5 may carry the gene *Mla7, Mlk, Ml(LG2), Mlh* or *+?*; line T-27 *Ml(La), Mla7, Ml(LG2), Mlh* or *+?*; line T-8 *Mla7, Mlk* or *Mlh*. Two lines T-31 and T-33 may possess gene *Mla7, Mlk, Ml(LG2), Ml(CP), Mlg, Ml(La), Mlh* or *+?*. The gene *Mla3* may be present in lines T-18 and T-30. The same gene plus the genes *Mla6* and *Mla14* may be carried by line T-28. Based on F_2 segregation data line T-4

may have the *Mla10* or *Ml(Du2)* gene for resistance. The same genes plus the gene *Mlk* were expected in lines T-37 and T-41. It is also possible that each of the fourteen lines described above may have a gene that is not represented in the Pallas isolines set. Further investigations of these lines are needed to accurately identify their genes for resistance. Based on the segregation of the F_2 it was possible to determine that each of lines T-3 and T-6 has a gene for resistance which is not represented in the Pallas isolines set. Line T-40 may carry either the gene *Mla13* or *Ml(Ru3)*, line T-62 the gene *Mla1* or *+?* and line T-14 the gene *Mla6* or *Mla14*.

Based on the F_2 segregation data from a cross between the variety 'Pallas' and line T-13 it may be concluded that line T-13 carries a recessive gene. This line did not exhibit any characteristic features of Mlo resistant barley plants like necrosis or chlorotic leaf spotting when grown under field conditions. The occasional infection type 4 (compatible) pustules characteristic for Mlo resistance were also absent. These observations indicated that line T-13 has a gene for resistance not at the *mlo* locus. This was confirmed by the results of F_2 segregation from the cross between this line and Pallas isoline P22 (*mlo5*). A number of recessive resistance genes (*mlo, mld, mlni, mls,* and *mlw*)

have been described in barley (Jensen, 1992; Jorgensen, 1992c, 1994). Further tests are needed to determine if the gene for resistance carried by line T-13 is at an already known locus. This could be determined by crossing line T-13 with plants carrying only one of the known recessive genes. It might also be interesting to test this line with more isolates of powdery mildew, thus determining the effectiveness of the gene against a broader array of virulence types.

Results given here are a good example of elucidating new sources of resistance to powdery mildew in landraces from Tunisia. It would be wasteful, however, to use these genes indiscriminately in breeding programs as has been done often in the past. New strategies for deploying genes for resistance to powdery mildew of barley have been suggested to increase the durability of resistance (Jorgensen, 1993; Russell, 1978; Wolfe, 1984; Wolfe and McDermott, 1994).

Diversification strategies based on deploying many cultivars with different resistance genes in space or time have been proposed (Gacek and Czembor, 1983; Priestley, 1981; Smith *et al.*, 1988). Investigations similar to this one can help supply breeders with the many different resistance sources needed to employ such strategies. The durability of resistance genes may also be increased by

combining (pyramiding) different resistance genes into one variety (Knott, 1989; Robinson, 1976; Simmonds, 1987). A combination of different resistances, even if they are each controlled by single genes, is more difficult for the pathogen to overcome than the presence of only one single gene for resistance. Lines from barley landraces with new genes for resistance may be very useful for this purpose. This strategy may be limited by the fact that many of the genes or alleles at or closely linked to the *Mla* locus cannot be easily combined within a genotype (Brown and Jorgensen, 1991; Jorgensen, 1994; Wolfe and McDermott, 1994). It may thus be useful to search for and use genes not associated with the *Mla* locus. The evaluation of landraces for powdery mildew resistance can lead to the discovery of new genes for resistance which are not linked to the *Mla* locus, such as those found in this study. However, genes occurring at or closely linked to the *Mla* locus are the most commonly reported from landraces and wild barleys (Fischbeck and Jahoor, 1991; Jahoor and Fischbeck, 1987a, 1987b; Jensen and Jorgensen, 1991; Jorgensen, 1994; Russell, 1978). This was confirmed by this investigation.

Accessions from landraces with new and different genes for resistance may play an important role in developing multilines or variety mixtures. In these strategies,

linkage relationships are of no consequence. The principle of using variety mixtures is simple. Growing a mixture of plants with different resistances will limit the spread of the pathogen selected on any one plant (Czembor and Gacek, 1987; Gacek and Nadziak, 1988; Wolfe, 1991; Wolfe and McDermott, 1994). This investigation showed that resistance represented in accessions from landraces is very similar to the resistance present in variety mixtures. In both cases many genotypes are present and each of these genotypes has different genes for resistance to powdery mildew. However, a lower number of components (genotypes) is present in variety mixtures than in landraces. It is possible to show repeatedly with many different mixtures of only three component varieties, that powdery mildew can be reduced by more than one-half in relation to the mean of the components grown alone (Gacek and Nadziak, 1988; Wolfe, 1984). Currently this strategy is gaining popularity in Western and Central Europe (Gabler and Fritsche, 1991; Gacek *et al.*, 1991; Wolfe, 1991). It is worthwhile to advocate this strategy for control of diseases in developing countries e.g. Morocco, Algeria, and Tunisia. The lines from Tunisian landraces investigated are good candidates to be used in such a strategy. In addition to the genes for resistance to powdery mildew they may also possess genes for resistance

for other diseases. These lines are also quite similar in time of maturity (field observations in 1994) and are adapted to dry land conditions. Good yielding and resistant mixtures may be composed from wisely chosen (e.g. different genes for resistance) accessions from landraces. Because of the flexibility of the system the yield advantage can be exploited by continually changing the mixture's composition according to yield and field resistance. However, further investigations of lines studied in this project are needed to determine all their agronomic characteristics.

Modern varieties must be discarded when their race-specific resistance loses effectiveness. Accessions from landraces used in a breeding program as a source of race-specific resistance will most probably also contribute some level of partial resistance. It would be desirable that partial resistance will be retained during the breeding process. However, breeding for race non-specific resistance is complicated by the polygenic inheritance of this character and the masking effect of race-specific genes (Knott, 1989; Robinson, 1976; Simmonds, 1987). Furthermore, this kind of resistance is usually only partially effective and often expressed only in adult plants (Hwang and Heitefuss, 1982a, 1982b; Martinelli, 1993; Ouchi *et al.*, 1974, 1976a, 1976b). This implies both time- and space-

consuming selection processes and limitations for agronomic use because the crop is not protected against early attacks of the pathogen. In the case of barley mildew, such early attacks decrease both the grain quality (Walters *et al.*, 1984) and the yield of the stand (Scott and Griffiths, 1980), and may therefore lead to significant economic losses.

The growing opposition among consumers regarding the use of chemicals (pesticides, fungicides, herbicides) in agricultural production demands ecologically acceptable methods for the control of plant diseases, including powdery mildew. These methods include novel agronomic practices, biological control agents and breeding for disease resistance. The use of genetic defense mechanisms of the plant is the most economic way to control plant diseases including powdery mildew of barley.

This investigation identified and characterized new sources of resistance to powdery mildew of barley in accessions from barley landraces from Tunisia. These genes may be used in many strategies of breeding for resistance and they may significantly contribute to genetic control of powdery mildew.

CHAPTER 4

DEVELOPMENT OF RESTRICTION FRAGMENT LENGTH POLYMORPHISMS (RFLPs)MARKERS FOR THE *Mla* LOCUS

Introduction

The *Mla* locus of barley, which is involved in resistance to powdery mildew, is very complex. This locus has 28 named alleles which are grouped in at least 12 multiallele families (Giese, 1981, Giese *et al.*, 1981; Jahoor *et al.*, 1991a, 1991b, 1993; Jorgensen, 1992, 1993, 1994; Jorgensen and Moseman, 1972; Wise and Ellingboe, 1985). Three RFLP clones (MWG 1H060, MWG 1H036, MWG 1H068) closely linked to the *Mla* locus were identified by Schuller *et al.* (1992). By polymorphisms obtained with RFLP clone MWG 1H036, it was possible to separate the *Mla* alleles into 11 groups (Schuller *et al.*, 1992). Jahoor *et al.* (1993) used these RFLP clones for investigations on the mode of inheritance and intralocus recombination in the *Mla* locus.

The RFLP method is based on DNA fragment size differences of defined length that are produced by cleavage of DNA with restriction endonucleases. The use of RFLPs was proposed as a new source of genetic markers for the human

genome in 1980 (Botstein *et al.*, 1980). RFLP markers have a broad application in barley genetics research (Kilian *et al.*, 1994; Laurie *et al.*, 1994, 1995; Melchinger *et al.*, 1994; Saghai Maroof *et al.*, 1994, 1995). Extensive RFLP linkage maps of the barley genome have been constructed (Graner *et al.*, 1991; Heun *et al.*, 1991; Hinze *et al.*, 1991; Kleinhofs *et al.*, 1993). Conventional detection of RFLPs by Southern blot analysis (Southern, 1975) is laborious and costly (Beckman and Soller, 1983). PCR-based marker systems were proposed by Welsh and McClelland (1990) as arbitrarily primed-PCRs (AP-PCRs) and by Williams *et al.* (1990), as random amplified polymorphic DNA (RAPDs). In contrast to RFLPs, genetic analysis with RAPDs is fast and does not involve the use of radioisotopes (Rafalski *et al.*, 1991, 1994; Tingey and Tufo, 1993). This technique has broad applications in barley genetics and breeding (Barua *et al.*, 1993a, 1993b; Giese *et al.*, 1994; Kleinhofs *et al.*, 1993). However, problems with reproducibility in using these markers may arise (Devos and Gale, 1992; Giese *et al.*, 1994; Talbert *et al.*, 1994).

An alternative to RAPDs is the sequence tagged site (STS) approach. An STS is a short, unique sequence, amplified by PCR, which identifies a known location on a chromosome (Olson *et al.*, 1989). STSs may derive from

published sequences or by sequencing portions of DNA clones (Cole *et al.*, 1991; Tragoonrung *et al.*, 1992). The STS-PCR products can be digested with restriction enzymes (Talbert *et al.*, 1994; Tragoonrung *et al.*, 1992) to detect polymorphisms. Advantages of this technique include safety and efficiency over traditional RFLP analysis (Olson *et al.* 1989; Talbert *et al.*, 1994).

The objective of this study was to develop STS-PCR markers for the *Mla* locus. To reach this goal, PCR primers were developed based on STSs which were obtained by sequencing portions of clones MWG 1H060, MWG 1H036 and MWG 1H068. Subsequently, RFLP patterns of STS-PCR products after DNA amplification of Pallas isolines (with known *Mla* alleles) and lines from Tunisian landraces were investigated.

Materials and Methods

Plant Material

Nine Pallas isolines developed by Kolster *et al.* (1986) each carried a gene for resistance in the *Mla* locus, and the variety 'Pallas' carrying only the *Mla8* allele for resistance were used (Tab. 16). Additionally, twenty lines from Tunisian landraces selected in a previous study (see chapter 3) were investigated (Tab. 17). The variety

Table 16. The Recurrent Parent Pallas and 9 Near-isogenic Barley Lines with Specific Genes and Their Parent Cultivars (Kolster *et al.*, 1986).

Near-isogenic line	Resistance gene		
	Designation	Origin	Reference***
Pallas	*Mla8*	Mut. in Bonus	1
P1	*Mla1, +?**	Algerian	2
P2	*Mla3*	(Ricardo)	3
P3	*Mla6, Mla14*	Franger	2, 4
P4A	*Mla7, Mlk, +?*	Heine 4808	4, 5
P7	*Mla9, Mlk*	Monte Cristo	4, 5
P9	*Mla10, Ml(Du2)***	Durani	2, 6
P10	*Mla12*	Arabische	4, 5
P11	*Mla13, Ml(Ru3)*	Rupee	4, 5
P22	*mlo5*	Mut. in Carlsberg II	7

* +? - indicates an unidentified resistance gene

** ( ) - the parenthesis indicates the tentative designations e.g. when it is not known whether the gene is allelic to a named gene, or when segregation data are incomplete or missing.

*** References are: (1) Jorgensen and Jensen, 1983; (2) Moseman, 1972; (3) Moseman and Schaller, 1960; (4) Giese *et al.*, 1981; (5) Torp *et al.*, 1978; (6) Moseman and Jorgensen, 1973; (7) Jorgensen, 1971.

Table 17. Twenty Lines Divided into Four Groups and Expected Resistance Genes in Each of These Groups.

Group	Tunisian lines	Expected resistance genes
1	T-3 T-13 T-40 T-62	*mlo5, Mla13, Ml(Ru3),*[*] *Mla1, +?*[**]
2	T-5 T-8 T-27 T-31 T-33 T-35 T-47 T-49	*Mla7, Mlk, Ml(LG2), Ml(CP), Mlg, Ml(La), Mlh, +?*
3	T-14 T-18 T-28 T-30	*Mla3, Mla6, Mla14, Mla12*
4	T-4 T-6 T-37 T-41	*Mla9, Mlk, Mla10, Ml(Du2), Ml(1402)*

*()- the parenthesis indicates the tentative designations e.g. when it is not known whether the gene is allelic to a named gene, or when segregation data, etc. are incomplete or missing.

** +? - indicates an unidentified resistance gene.

'Manchuria', which does not possess any genes for resistance to powdery mildew, and Pallas isoline P22 (*mlo5*) were used as controls. All plants were grown in a growth chamber with a 15-hour photoperiod. The temperature regime was 12 C in darkness and 20 C during light hours. Genomic plant DNA was isolated from young leaves as previously described (Talbert *et al.*, 1992).

DNA Sequencing

Three probes linked to the *Mla* locus were kindly provided by Dr. A. Jahoor from Technical University, Munich, Germany. Their numbers and length are as follows: MWG 1H036, 1100bp; MWG 1H060, 1300bp and MWG 1H068, 580bp. These probes were used to develop the STS-PCR primers. They were sequenced at both ends by the dideoxy chain termination method (Sanger *et al.*, 1977) using Sequenase (United States Biochemical, Cleveland, Ohio) following the manufacture's protocol.

Development of STS-PCR Primers

Twenty basepairs at both ends of sequenced clones were chosen for oligonucleotide PCR primers. These PCR primers were designed to contain 50% GC and harbor no inverted repeat sequences. They were synthesized by Bio-synthesis, Inc. (Lewisville, TX, USA). Four sets of primers were

obtained (Tab. 18). The polymerase chain reaction protocol was used as described by Talbert *et al.* (1994) for all primer sets. Additionally, for primer set 36-3A some exceptions from this protocol were made. These exceptions included: annealing temperature of 50 C, 0.5x and 2x of $MgCl_2$, twice the concentration of primers and genomic DNA.

Table 18. STS-PCR Primer Sets and their Sequences.

Primer set	Primer sequences
36-3	5' CTT GAC ATT ATA TAC TAC CA 3'
	5' CTG GTA GTT TAG TTT TAC TT 3'
36-3A	5' TAT TAC TTT TTG CAA CCG AC 3'
	5' AGC AGA GGA GCT ACT AAT TG 3'
60-3	5' CAA CGA TAC AAC AGG CTC AA 3'
	5' CTG GAT AGA GAA GCC ATG GA 3'
68-5	5' CCA TGC CAT AGT CCT GGG AA 3'
	5' GGT GAG CTG CGC CAG TTC TA 3'

Electrophoresis

Twenty-five ul of PCR products were digested with approximately 2 units of the four- or five-base-recognizing restriction enzymes *Hha*I, *Hinf*I, *Rsa*I, *Dde*I, *Hpa*II, *Hae*III, *Mbo*I, *Taq*I, *Msp*I and *BstN*I. Digestion was for 1 hour at 37 C for all enzymes with the exception of *Taq*I, for which digestion was at 65 C. Digested PCR products were separated on a 7% polyacrylamide gel with 0.5x Tris-borate buffer at 300 V for 2 hours. The gel was stained with ethidium bromide and DNA fragments were visualized under UV light. Sizes of DNA fragments were calculated by comparison to the sizes of a *Rsa*I-digested pUC18 standard.

Results

From a total of four STS-PCR primers sets which were used for PCR amplification, readable results were only obtained using the 68-5 set of primers for amplification of the Pallas isolines (Fig. 1 and 2). From ten restriction enzymes used for digestion of PCR product of the Pallas isolines, enzymes *BstN*I, *Msp*I and *Hae*III generated polymorphic banding patterns (Tab. 19, Fig. 1 and 2). A 560bp band obtained using *BstN*I was unique to the variety 'Pallas'(*Mla8*) and P1 (*Mla1, +?*) (Tab. 19, Fig. 1). *Msp*I differentiated two groups: (i) the variety 'Pallas' (*Mla8*), P10 (*Mla12*), P11 [*Mla13, Ml(Ru3)*] and P22 (*mlo5*) (390 and

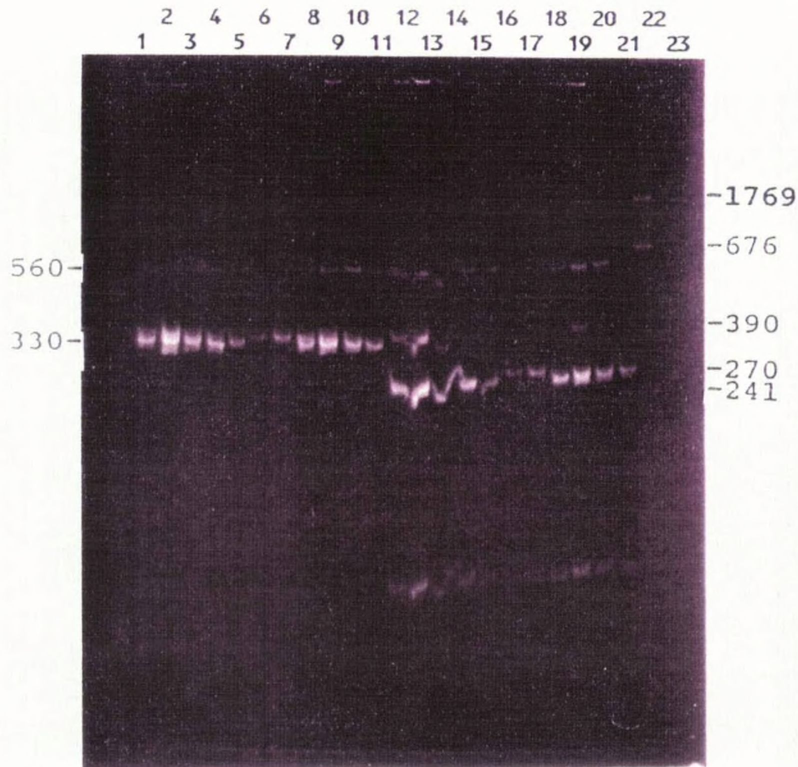

Figure 1. PCR products of primer set 68-5 separated on a 7% acrylamide gel. The samples from 1 to 11 were digested with *BstN*I and from 12 to 23 with *Msp*I. Lanes 1 to 23 represent: 1: P22, 2: P11, 3: P10, 4: P9, 5: P7, 6: P4a, 7: P3, 8: P2, 9: P1, 10: 'Pallas', 11: 'Manchuria', 12: P22, 13: P11, 14: P10, 15: P9, 16: P7, 17: P4a, 18: P3, 19: P2, 20: P1, 21: 'Pallas', 22: *Rsa*I-digested pUC18, 23: 'Manchuria'.
Sizes indicated to the left and to the right are in bp based on the sizes of a *Rsa*I-digested pUC18 standard.

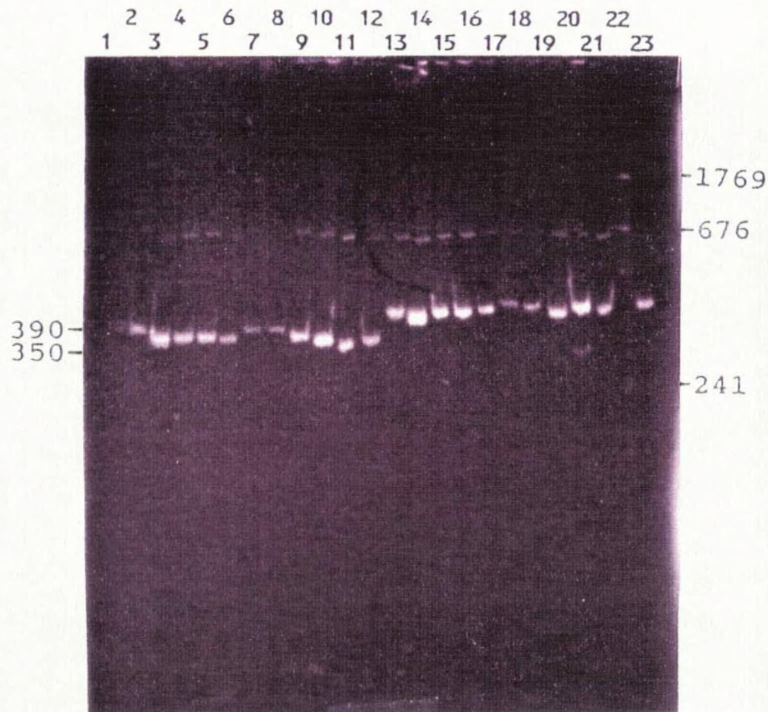

Figure 2. PCR products of primer set 68-5 separated on a 7% acrylamide gel. The samples from 1 to 11 were digested with *Hae*III and from 12 to 23 with *Hpa*II.
Lanes 1 to 23 represent: 1: P22, 2: P11, 3: P10, 4: P9, 5: P7, 6: P4a, 7: P3, 8: P2, 9: P1, 10: 'Pallas', 11: 'Manchuria', 12: P22, 13: P11, 14: P10, 15: P9, 16: P7, 17: P4a, 18: P3, 19: P2, 20: P1, 21: 'Pallas', 22: *Rsa*I-digested pUC18, 23: 'Manchuria'.
Sizes indicated to the left and to the right are in bp based on the sizes of a *Rsa*I-digested pUC18 standard.

Table 19. RFLP Patterns Obtained by Digestion of STS-PCR Products (Using 68-5 Set of Primers) of Nine Pallas Isolines, Twenty Tunisian Lines and Varieties 'Manchuria' and 'Pallas' with *BstN*I, *Msp*I and *Hae*III Restriction Enzymes.

Plant material	*BstN*I			*Msp*I		*Hae*III	
	560bp	330bp	310bp	390pb	270bp	390bp	350bp
'Manchuria'	-	+	-	-	-	-	+
'Pallas'	+	+	-	+	+	-	+
Isolines							
P-1	+	+	-	-	+	-	+
P-2	-	+	-	-	+	-	+
P-3	-	+	-	-	+	+	-
P-4a	-	+	-	-	+	+	-
P-7	-	+	-	-	+	-	+
P-9	-	+	-	-	+	-	+
P-10	-	+	-	+	+	-	+
P-11	-	+	-	+	+	-	+
P-22	-	+	-	+	+	+	-
Tunisian Lines							
T-3	-	+	-	+	+		
T-13	-	+	-	+	+		
T-40	-	+	-	+	-		
T-62	-	+	+	+	+		
T-5	-	+	-	+	-		
T-8	-	+	-	+	+		
T-27	-	+	+	+	+		
T-31	-	+	-	+	+		
T-33	-	+	-	+	+		
T-35	-	+	+	+	+		
T-47	-	+	-	+	+		
T-49	-	+	-	-	+		
T-14	-	+	-	-	+		
T-18	-	+	-	-	+		
T-28	-	+	-	+	+		
T-30	-	+	-				
T-4	-	+	-	+	+		
T-6	-	+	-	-	+		
T-37	-	+	-	-	+		
T-41	-	+	-	-	+		

\+ - presence of band
\- - absence of band

270bp) (ii) P1 (*Mla1, +?*), P2 (*Mla3*), P3 (*Mla6, Mla14*), P4a (*Mla7, Mlk, +?*), P7 (*Mla9, Mlk*) and P9 [*Mla10, Ml(Du2)*] (270bp). In the case of the variety 'Manchuria' the 390bp and 270bp bands were not present. *Hae*III divide PCR products to 2 groups: (i) P3 (*Mla6, Mla14*), P4a (*Mla7, Mlk, +?*), P22 (*mlo5*) (390bp), (ii) the varieties 'Manchuria' and 'Pallas' (*Mla8*), P1 (*Mla1, +?*), P2 (*Mla3*), P7 (*Mla9, Mlk*), P9 [*Mla10, Ml(Du2)*], P10 (*Mla12*) and P11 [*Mla13, Ml(Ru3)*] (350bp) (Tab. 19, Fig. 2).

STS-PCR primer sets 60-3 and 68-5 gave clear amplification products for the Tunisian lines (Fig. 3, 4 and 5). In the case of the 68-5 primer set digestion with restriction enzymes *BstN*I and *Msp*I of PCR products gave polymorphic banding patterns (Tab. 19, Fig. 3 and 4). Banding patterns obtained after digestion with *BstN*I of PCR products may differentiate T-62, T-27 and T-35 (310bp) (Tab. 19, Fig. 3). Patterns from digestion with *Msp*I were able to differentiate three groups: (i) T-40 and T-5 (390bp), (ii) T-3, T-13, T-62, T-8, T-27, T-31, T-33, T-35, T-47, T-28 and T-4 (390 and 270bp) and (iii) T-49, T-14, T-18, T-6, T-37 and T-41 (270bp) (Tab. 19, Fig. 4). In the case of the variety 'Manchuria' and T-30 no bands were observed, suggesting that PCR amplification was unsuccessful.

STS-PCR primer 60-3 gave good amplification of the

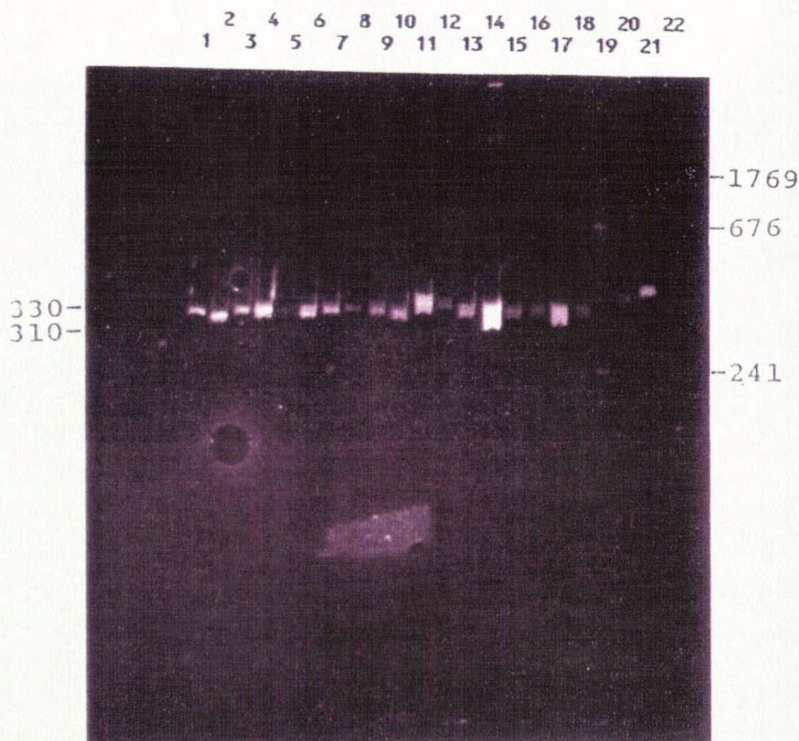

Figure 3. PCR products of primer set 68-5 separated on a 7% acrylamide gel. The samples from 1 to 22 were digested with *BstN*I.
Lanes 1 to 22 represent: 1: T-41, 2: T-37, 3: T-6, 4: T-4, 5: T-30, 6: T-28, 7: T-18, 8: T-14, 9: T-49, 10: T-47, 11: T-35, 12: T-33, 13: T-31, 14: T-27, 15: T-8, 16: T-5, 17: T-62, 18: T-40, 19: *Rsa*I-digested pUC18, 20: T-13, 21: T-3, 22: 'Manchuria'.
Sizes indicated to the left and to the right are in bp based on the sizes of *Rsa*I-digested pUC18 standard.

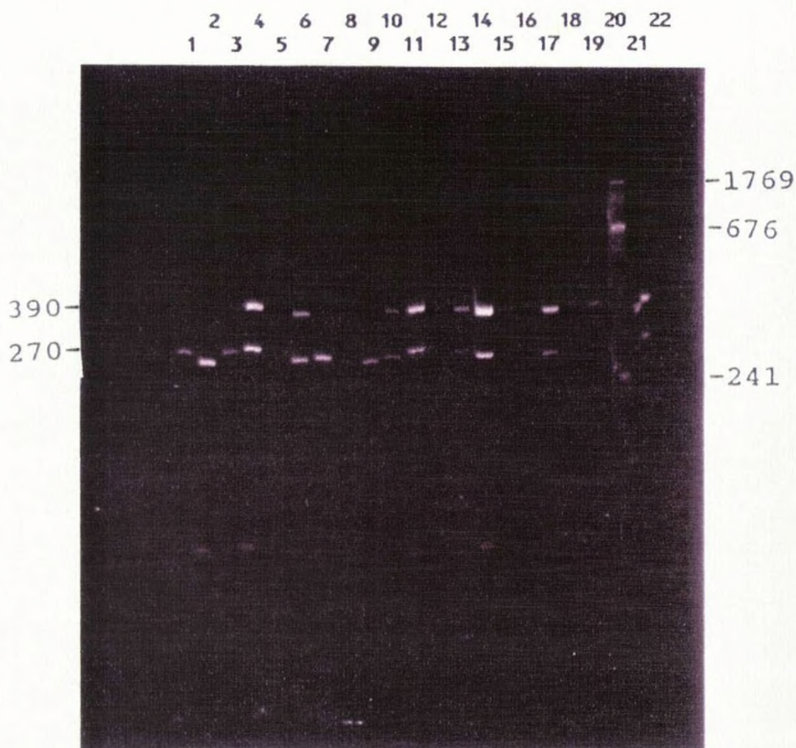

Figure 4. PCR products of primer set 68-5 separated on a 7% acrylamide gel. The samples from 1 to 22 were digested with *Msp*I.
Lanes 1 to 22 represent: 1: T-41, 2: T-37, 3: T-6, 4: T-4, 5: T-30, 6: T-28, 7: T-18, 8: T-14, 9: T-49, 10: T-47, 11: T-35, 12: T-33, 13: T-31, 14: T-27, 15: T-8, 16: T-5, 17: T-62, 18: T-40, 19: T-13, 20: *Rsa*I-digested pUC18, 21: T-3, 22: 'Manchuria'.
Sizes indicated to the left and to the right are in bp based on the sizes of *Rsa*I-digested pUC18 standard.

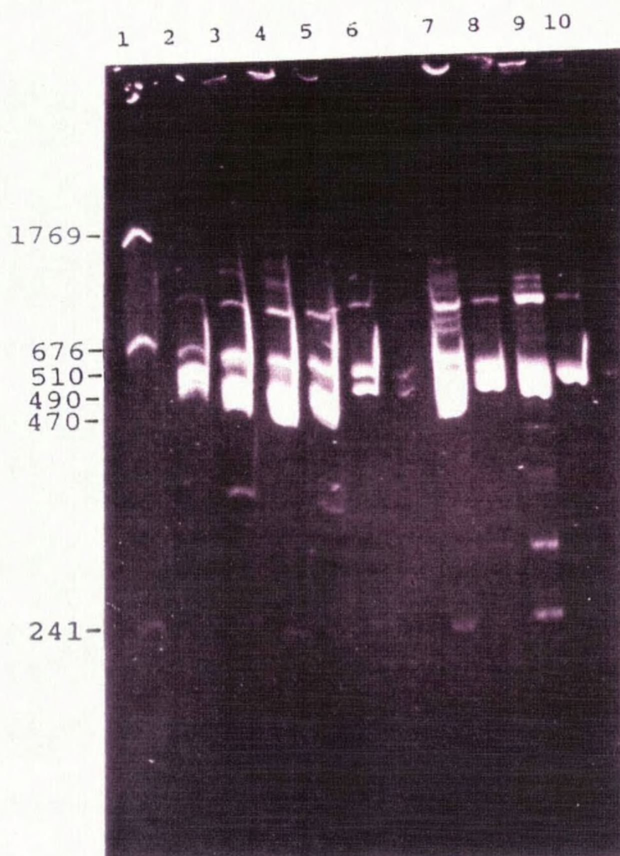

Figure 5. PCR products of primer set 60-3 separated on a 7% acrylamide gel. The samples from 1 to 10 were digested with *Hha*I.
Lanes 1 to 10 represent: 1: *Rsa*I-digested pUC18, 2: 'Manchuria', 3: T-37, 4: T-4, 5: T-28, 6: T-14, 7: T-62, 8: T-5, 9: T-27, 10: T-13.
Sizes indicated to the left are in bp based on the sizes of *Rsa*I-digested pUC18 standard.

targeted fragments of DNA from the Tunisian lines (Fig. 5). However, only one enzyme, *Hha*I, gave clear polymorphic banding patterns (Tab. 20). According to this pattern it was possible to differentiate three groups: (i) 'Manchuria', T-37, T-4, T-28 and T-62 (590, 510, 490, 470bp), (ii) T-14, T-5 and T-27 (590 and 510bp), (iii) T-13 (590bp).

Table 20. RFLP Pattern Obtained by Digestion of STS-PCR Products (Using 60-3 Set of Primers) of Eight Tunisian Lines and the Variety 'Manchuria' with *Hha*I Restriction Enzyme.

Plant material	590bp	510bp	490bp	470bp
'Manchuria'	+	+	+	+
T-37	+	+	+	+
T-4	+	+	+	+
T-28	+	+	+	+
T-14	+	+	-	-
T-62	+	+	+	+
T-5	+	+	-	-
T-27	+	+	-	-
T-13	+	-	-	-

+ - presence of band
- - absence of band

Discussion

The STS-PCR method has recently proven to be useful for analysis of the barley genome (Tragoonrung *et al.*, 1992). This was confirmed in this investigation by synthesizing PCR primers based on STSs developed by sequencing portions of RFLP clones. It was possible to associate RFLP bands using Pallas isolines with different *Mla* alleles. The RFLP differences observed in the various Pallas isolines and lines from Tunisian landraces suggests that these STS markers are linked closely to *Mla* and might be useful in assisting in segregation analysis of these alleles. An interesting fact is that band 310bp obtained with *BstNI* is present in Tunisian lines T-62, T-27 and T-35 but it is absent in Pallas isolines. This probably indicates the presence in these Tunisian lines of *Mla* alleles which are not represented in Pallas isolines or differences in the genetic background of Pallas isolines and Tunisian lines. To be able to draw genetic conclusions from the observed RFLP patterns for Tunisian lines further investigations are needed. Those investigations would include crosses between Tunisian lines and isolines with known *Mla* genes and testing of these Tunisian lines with isolates of powdery mildew differentiating alleles of the *Mla* locus.

The detected associations between RFLP bands and *Mla* alleles may be used in the future as RFLP markers to assist in breeding for resistance to powdery mildew of barley. However, further investigations are needed which will prove the linkage between the observed RFLP bands and genes at the *Mla* locus. These investigations include backcrosses of plant material used in this study with the variety 'Pallas' or 'Manchuria' and segregation analysis of progeny. This segregation analysis has to be done using isolates of powdery mildew which differentiate *Mla* genes and using potential RFLP markers.

Observed associations between RFLP bands and the *Mla* alleles present in Pallas isolines and Tunisian lines may be misleading when used as markers for the *Mla* locus without checking their linkage. Because of different genetic background, the same *Mla* alleles present in Pallas isolines and Tunisian lines may display different banding patterns with the same primer set and restriction endonuclease. This is demonstrated when the band patterns obtained for P22 (*mlo5*), the variety 'Manchuria' and Pallas isolines are compared. Future investigations are needed which include different restriction enzymes and plant materials containing *Mla* alleles in different genetic backgrounds.

During this investigation some technical problems

occurred. This was true especially with separation of digested PCR products by 7% polyacrylamide gel electrophoresis. Uneven wells often present in these gels caused difficulties with the description and interpretation of the observed polymorphic banding patterns. Also, in many cases, the observed banding patterns indicated incomplete digestion with the restriction endonucleases. However, the STS-PCR method used in this investigation was relatively efficient and safe.

Further investigations for development of RFLP markers for the *Mla* locus using identified RFLP clones are needed. In these investigations, the presented preliminary results and synthesized STS-PCR primers may be used.

This investigation showed that it is possible to identify potential RFLP markers for genes in the *Mla* locus by using the STS-PCR method.

CHAPTER 5

SUMMARY

This investigation represents the first systematic study of race-specific genes for resistance to powdery mildew in Tunisian landraces. A number of race-specific genes were detected. Among the 232 tested accessions from Tunisian landraces, 163 were susceptible to powdery mildew, 20 were resistant and 43 segregated for resistance. A number of different resistance genes were detected among the 20 accessions with resistant reactions. Surprisingly only single genes were detected with the isolates used. Some of these genes could be associated with already known loci. Nineteen genes were dominant and one recessive. The recessive gene was not located at the *mlo* locus.
Further investigations of these lines from Tunisian landraces are needed to accurately identify their genes for resistance. This may include crosses with plants carrying known genes and testing with more isolates of powdery mildew. The newly identified sources of resistance to powdery mildew in lines from Tunisian barley landraces may

be used in many strategies of breeding for disease resistance.

PCR primers were synthesized based on STSs developed by sequencing portions of MWG 1H036, MWG 1H060 and MWG 1H068 RFLP clones. Associations were detected between RFLP bands and genes in the *Mla* locus using Pallas isolines, the 68-5 set of the STS-PCR primers and *BstN*I, *Msp*I and *Hae*III restriction enzymes. Polymorphic bands were detected using lines from Tunisian landraces, 68-5 and 60-3 sets of primers and *BstN*I, *Msp*I and *Hha*I restriction enzymes. Further investigations for development of RFLP markers for the *Mla* locus using RFLP clones are needed, including proving the linkage between observed RFLP bands and genes in this locus. In these investigations, the presented preliminary results and synthesized STS-PCR primers may be used. Developed RFLP markers may be used in screening for new sources of resistance for powdery mildew of barley.

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APPENDIX

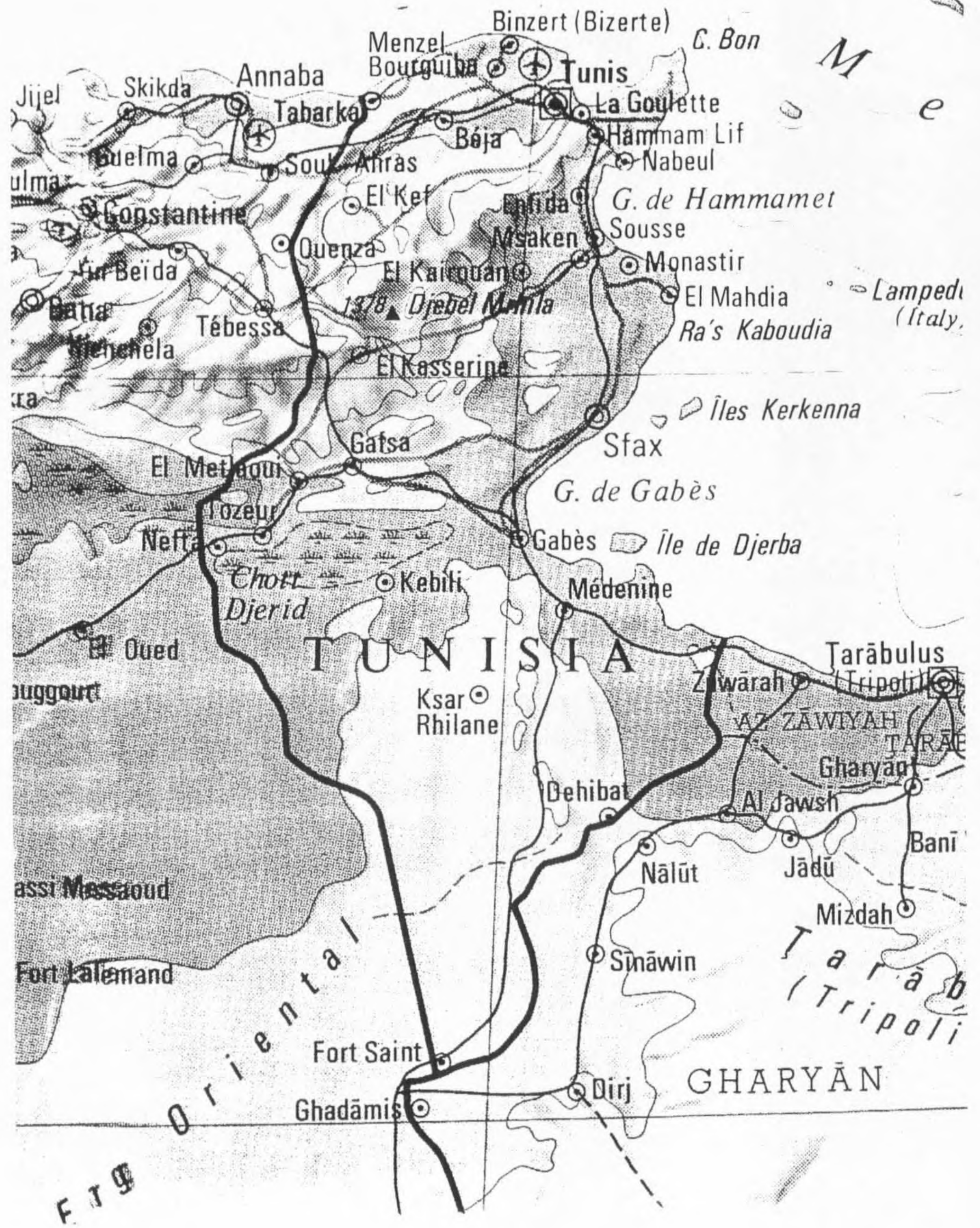

Figure 6. Map of Tunisia ('Atlas of the World', 1993).

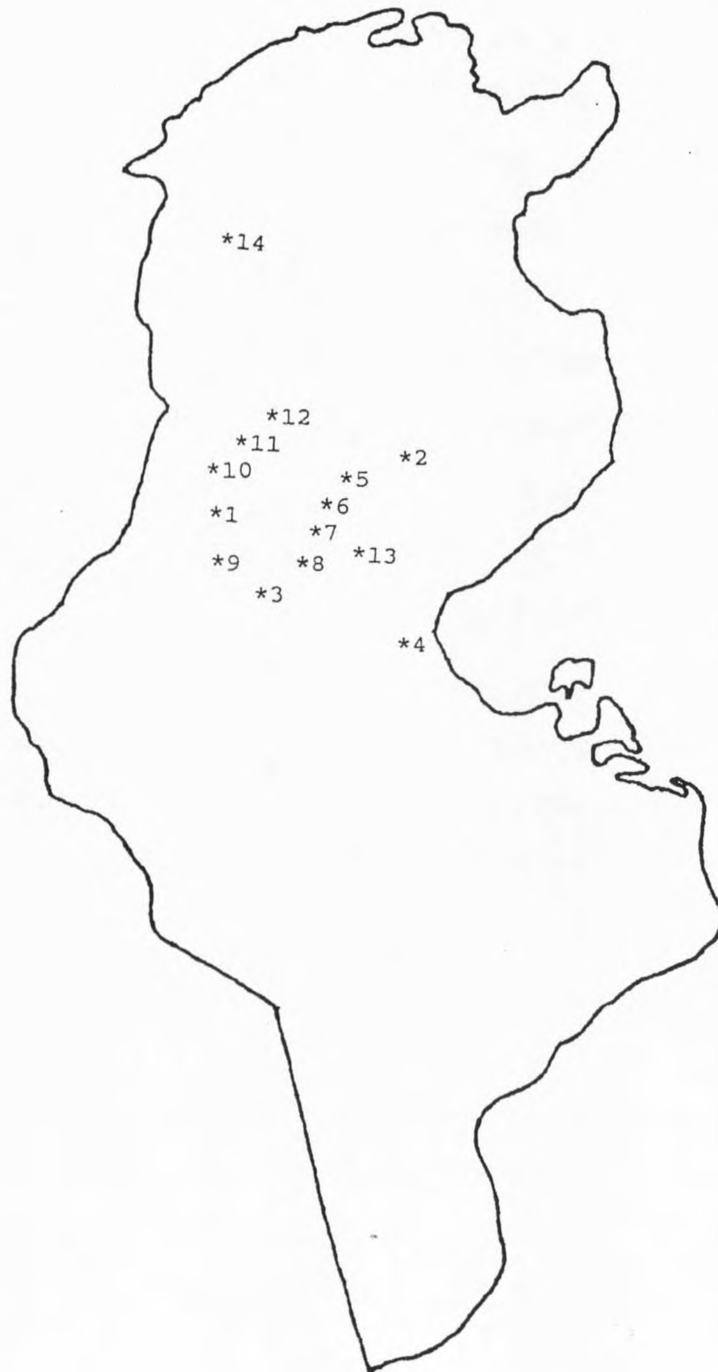

Figure 7. Collection Sites of Twenty Accessions from Tunisian Landraces Selected in the Preliminary Experiment: 1: Oued Ameur, 2: Sidi Bonzid, 3: Gafsa, 4: Gabes, 5: Bir el Haffey, 6: Ben Aroun, 7: El Fedj, 8: Echabiba, 9: Oued Kbir, 10: Feriana, 12: Mlauna, 13: Thala, 14: Kef.

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