Epidemiology of Puccinia hordei and new sources of resistance in barley
by Amor Hassine Yahyaoui

A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant Pathology
Montana State University

Abstract:
New virulence types of Puccinia hordei phenotypes were detected in various geographic regions in Tunisia. Highly virulent isolates able to overcome many sources of resistance were identified. The P. hordei virulence types reported in this investigation have not been previously identified. They are important not only because they are virulent on the commonly grown barley cultivars Martin' and 'Ceres', but also because many resistance (Pa) genes are ineffective against these isolates. The effectiveness of Pag to these virulence types is questionable. Pa3 and Pa7 were very effective against all P. hordei isolates tested. The naturally occurring Ornithogalum spp. , in Northern and Northwestern Tunisia, may be a breeding ground for new physiologic races of the P. hordei fungus. Isolates originating from the alternate host were as variable in virulence as those isolated from barleys in the same fields.

New genes for resistance to P. hordei appeared to be common in several collections of barley (Hordeum vulgare L.) land race cultivars originating in Central and Southern Tunisia. Response of five land race cultivars to a number of different isolates of P. hordei from the Mediterranean region differentiated them from the known genotypes. A dominant resistance gene that behaved as Pa3 was found in Tu32. Three of the land race cultivars (Tu17, Tu27, and Tu34) each have a dominant resistance gene that is different from the previously known resistance genes. The dominant resistance genes identified in this study were as effective as Pa3 and Pag, and hence, should be considered as new sources of resistance. Further testing is needed to determine the genetic relationships between these genes.

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Amor Hassine Yahyaoui

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

14 Apr 86 — Date

E. L. Sharp — Chairperson, Graduate Committee

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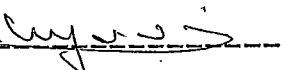
Signature 

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ABSTRACT

New virulence types of Puccinia hordei phenotypes were detected in various geographic regions in Tunisia. Highly virulent isolates able to overcome many sources of resistance were identified. The P. hordei virulence types reported in this investigation have not been previously identified. They are important not only because they are virulent on the commonly grown barley cultivars 'Martin' and 'Ceres', but also because many resistance (Pa) genes are ineffective against these isolates. The effectiveness of Pa_9 to these virulence types is questionable. Pa_3 and Pa_7 were very effective against all P. hordei isolates tested. The naturally occurring Ornithogalum spp., in Northern and Northwestern Tunisia, may be a breeding ground for new physiologic races of the P. hordei fungus. Isolates originating from the alternate host were as variable in virulence as those isolated from barleys in the same fields.

New genes for resistance to P. hordei appeared to be common in several collections of barley (Hordeum vulgare L.) land race cultivars originating in Central and Southern Tunisia. Response of five land race cultivars to a number of different isolates of P. hordei from the Mediterranean region differentiated them from the known genotypes. A dominant resistance gene that behaved as Pa_3 was found in Tu32. Three of the land race cultivars (Tu17, Tu27, and Tu34) each have a dominant resistance gene that is different from the previously known resistance genes. The dominant resistance genes identified in this study were as effective as Pa_3 and Pa_9, and hence, should be considered as new sources of resistance. Further testing is needed to determine the genetic relationships between these genes.

GENERAL INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the most important cereal crops in Tunisia. It occupies about thirty percent of the total cereal production area which is over half a million hectares. Barley is mainly a food crop, generally grown in southern and central Tunisia, where climatic conditions are less favorable for growing wheat. In the northern region, barley is grown in marginal areas or as an alternative crop in rotation. Recently, farmers in this region have become more interested in using barley for forage and feed, and in some instances for malting. Malting barley can become an important crop in this region due to its high cash value and its potential as an export crop. In recent years a major interest in increasing cereal production in the northwestern, central and southern regions with a major emphasis on barley, has become a priority in agricultural development programs in Tunisia.

As interest in barley increases, and consequently the acreage, the lack of resistance to diseases such as leaf rust, will become the most limiting factor in barley

production. Yield losses could be especially high in the northern region where the climatic conditions are more favorable for pathogen development.

Leaf rust, caused by the fungus Puccinia hordei Otth, has increased in intensity on the currently grown barley cultivars. This disease was not considered to be a significant factor in limiting barley production because there were no large scale epidemics observed by growers. Nevertheless, the severity of this disease is becoming obvious to breeders, pathologists and agronomists in Tunisia. Thus there is an urgent need to develop resistant cultivars. It is very important then, to detect the changes in pathogenicities of the fungus so that breeders will be able to develop and maintain resistant cultivars.

Presently, nine major genes conditioning resistance to leaf rust have been identified and are designated as the Pa through Pa_9. Barley leaf rust, as well as other barley diseases have received very little attention in Tunisia. To develop high yielding, adapted leaf rust resistant barley lines, the following studies were initiated:

Part I. Epidemiology of barley leaf rust (*Puccinia hordei* Otth) in Tunisia.

Part II. New sources of resistance to *Puccinia hordei* Otth in Tunisian barley land races.

PART I

EPIDEMIOLOGY OF BARLEY LEAF RUST (*PUCCINIA HORDEI* OTTH) IN TUNISIA

PART I

I. INTRODUCTION

Disease surveys are an important tool for plant breeders, and provide useful information pertinent on the distribution of pathogenic entities and on shifts in populations of the pathogens. Virulence in leaf rust of barley, caused by Puccinia hordei Otth, its importance, and the variability in host resistance are being studied in many parts of the world. Very little, however, is known about the importance of this disease in Tunisia, except for the work done in 1982 (Reinhold and Sharp) which characterized the relative effectiveness of resistance genes against one Tunisian leaf rust isolate.

Leaf rust disease of barley, as well as other barley diseases have received very little attention in Tunisia. Recently there has been marked changes in cultural practices used to grow barley which may directly effect the epidemiology of many diseases, particularly leaf rust. Therefore, the objectives of the first part of this thesis revolve around a study of the epidemiology

of barley leaf rust in Tunisia and are as follows:

1. To determine the virulence pool of leaf rust in various barley growing areas of Tunisia.
2. To determine the changes in virulence within each region and within the country over a period of two to three years.
3. To determine the effectiveness or ineffectiveness of known resistance gene(s) against Tunisian *P. hordei* isolates.

II. LITERATURE REVIEW

Epidemiology Studies

Race surveys provide valuable information on the distribution and frequency of various rust physiologic races in different geographic regions. Such information has not been effectively exploited by breeders, but renewed interest may enhance its use. Chester (1946) showed that certain races of wheat leaf rust were present in the same area year after year. According to Roelfs (1974), such a pattern still exists but the reasons are unclear.

Virulence of barley leaf rust, its importance, and host resistance variability have been studied by many authors. The appearance of a new race in a certain geographic area could result from one or more of the following: (1) an input of exogenous inoculum (Luig, 1977), (2) a mutation for virulence or avirulence in an existing race (Stakman et al., 1930), (3) asexual or parasexual recombination (Newton et al., 1930) , or (4) detection of a race previously below the detection threshold (Roelfs et al., 1982). Also, the effects of alternate host(s) and wild grasses that could be host(s) must not be ignored. D'Oliveira (1960) showed that among

the Ornithogalum species in the Mediterranean region, only O. arabicum was incompatible with the rust. Critopoulos (1956) demonstrated the significance of the alternate host in the perennation of P. hordei in Greece. Studies by Anikster (1982) showed that indigenous Ornithogalum spp. play an important role in the completion of the life cycle of P. hordei. Wahl (1984) reported that P. hordei can also attack various grasses of the Hordeum genus; H. bulbosum, H. murimum, and H. spontaneum. It is not clear, however, whether or not P. hordei from these Hordeum spp. should be classified as a different formae specialis, as suggested by Anikster (1984).

Physiologic Specialization

The occurrence of physiologic races in Puccinia was first demonstrated by Stakman in the early 1920's, a decade which he referred to as "the decade of the race" (Stakman, 1929). The earliest reports of races in cereal rusts are shown in Appendix Table 20.

The virulence of isolates of physiologically specialized pathogens, such as the rusts, is neither simple nor easy to describe. As data relating to host-parasite systems accumulate, and as new differentials are

discovered and used, the description becomes increasingly complex and difficult to interpret. This aspect of complexity has been reviewed by Browder (1971) and Browder et al. (1980).

The pathogenicity formula method proposed by Green (1965) seems to be a good way to present results of virulence studies. In this method physiologic races were described by a "virulence formula" of the form "effective/ineffective host genes". This method has since been adapted to describe pathogenicity differences in P. striiformis (Volin and Sharp, 1973), P. graminis avenae (Marten et al., 1979), and P. recondita (Loegering and Browder, 1971).

Differential Sets

Differential host sets are used in studies of physiologic specializaion of rust fungi. A set consists of a selected group of genotypes, each of which has a specific gene or gene combination for resistance to a specific rust entity. In the case of barley the genotypes are characterized by a USDA CI number and a resistance gene designated Pa, after the former scientific name, Puccinia anomala. Nine resistance Pa gene(s) or gene combinations have been identified in different barley genotypes, and are used as host

differentials of P. hordei. The resistant Pa genes in various barley cultivars have been described and reviewed by many authors (Clifford, 1974, 1977; Parlevliet, 1976; Tan, 1977).

Infection Types

Infection type, as a measurement of disease, was adapted by Stakman and co-workers at Minnesota in the early 1920's. This system is described in Appendix Table 21 and has been adapted to most cereal rusts.

Life Cycle

The pathogen P. hordei is an obligate parasite with a highly complex life cycle. Two spore forms develop on Hordeum spp. and three additional forms develop on the alternate host. Of these spore forms, urediospores are the most important because they enable repeated cycles, spread of the disease from field to field, and survive from year to year. In temperate Europe, P. hordei, overwinters in winter barley rather than on its alternate host (Tan, 1976). Thus the alternate host Ornithogalum spp. appears to be unimportant in the perennation of P. hordei in this region. Tan (1976) showed that the fungus survives the winter in the form of dormant urediomycelium on the primary host. In parts of the Mediterranean, the

fungus is known to cycle between the main host Hordeum and the alternate host Ornithogalum spp. (Anikster, 1982). D'Oliveira (1960), reported that the fungus can also cycle between Hordeum and Dipcadi erythraeum Webb. et Bert. or Hordeum and Leopoldia eburnea Eig. et Feinbr. Anikster (1982) proved that these plant species are also potential alternate hosts of P. hordei.

Wahl et al. (1984) showed that the sexual stage contributes to the diversification of the spectrum of parasitism of P. hordei. He found that the rust population from the alternate host showed new virulence patterns on barley genotypes.

Detection of New Virulences

A major goal of any race survey is to detect new virulence phenotypes. By using host differential lines, each with a "single gene" for resistance, it is possible to detect changes in virulence, and then to determine if that results in changes in a virulence combination that is capable of overcoming the combination of resistance in commercially grown cultivars or advanced breeding material. Rust cultures from Ornithogalum rendered ineffective all known genes for resistance to leaf rust, including Pa_3, Pa_7, and Pa_9 (Golan et al., 1978). Barley

leaf rust isolates virulent on Pa_7 have been reported by Parlevliet et al. (1981).

Control

Biological control through plant breeding has been the principal method used to control the cereal rusts. This method has been highly effective against barley leaf rust.

Fungicides applied as foliar sprays can be used effectively to protect the cereal crop, but are usually uneconomical.

III. MATERIALS AND METHODS

Differential Hosts

Thirteen spring barley genotypes, possessing different Pa genes, were subjected to detailed analyses of their reactions to Tunisian cultures of *P. hordei*. The Pa designation by Clifford (1974, 1977) and the USDA CI number are given in parenthesis following the common name of each genotype used. The differentials used include the following barley cultivars: 1) Estate (Pa_3, CI 3410), 2) Cebada Capa (Pa_7, CI 6193), 3) Hor 2596 (Pa_9, CI 1243), 4) Ricardo (Pa_{2+}, CI 6306), 5) Bolivia (Pa_2+Pa_6, CI 1257), 6) Quinn (Pa_2+Pa_5, CI 1024), 7) Magnif (Pa_5, CI 13806), 8) Peruvian (Pa_2, CI 935), 9) Sudan (Pa, CI 6489), 10) Egypt (Pa_8, CI 6481), 11) Batna (Pa_{2+}, CI 3391), 12) Gold (Pa_4, CI 1145), and 13) Reka I (Pa_{2+}, CI 5051). Throughout this study the differential cultivars will be listed in the same order so that the arabic number given to each can be used in the virulence formula method (Green, 1965). The differentials are also arranged, as much as possible, according to their spectrum of resistance. The most resistant genotype begins each table in the thesis.

Leaf Rust Cultures

Samples of P. hordei were collected from four different geographic regions in Tunisia. Two representative sites were chosen in each region. Collections were made over a period of three years (1982-1984). Samples were taken at the same site every year when feasible. Table 1 shows the region, site, year of collection, and the number of monouredial isolates tested from each collection site. The sites and regions are also shown in Appendix Figure 15.

Isolate Designation

The designation given for the isolates include the country, site and year of collection and isolate number within each site. A complete list of the isolates is shown in Appendix Table 22. Throughout the study the isolates are listed in an ascending order of virulence, ie. the least virulent isolate will be listed first and the most virulent last.

Table 1. Collection sites of Tunisian barley leaf rust, Puccinia hordei, isolates from 1980-1984.

Region	Leaf Rust Collection Site	Year	No. of Monouredial Isolate(s) Tested
North	Mateur (Ma)	1980	1[1]
	Mateur (Ma)	1983	16
	Mateur (Ma)	1984	6
	BouRbia (Br)	1982	2
Northwest	Beja (Bj)	1982	11
	Beja (Bj)	1984	8[2]
	Le Kef (Ke)	1982	10
	Le Kef (Ke)	1984	3
Central	Kairaouan (Kr)	1982	2
	El Jem (Ej)	1983	1
South	Oasis (Oa)	1982	8
	Oasis (Oa)	1984	2
	Mareth (Mr)	1983	10

[1] Isolate collected by Dr. E. L. Sharp (Reinhold and Sharp, 1982).

[2] Five of the eight isolates were collected from Ornithogalum.

Inoculum

Rust spores from a single uredium were isolated from green or dried leaves collected in Tunisia. In one case monouredial cultures were derived from a single aecium from the alternate host at the Beja (Bj) location. Monouredial cultures were multiplied on the universal susceptible barley cultivar, Moore (CI 7251). Inoculum that could not be used within a few days was vacuum dried and stored at 4 C, until further use, according to a technique described by Sharp (1957).

The P. hordei isolates were introduced into the United States according to U.S.D.A. quarrantine permit regulations and were tested under controlled environmental conditions.

Inoculation

Three to five seeds of each of the differential cultivars were sown in 10 cm diameter plastic pots in sterilized Bozeman silt loam soil. Initial single uredium inoculation of the universal susceptible was done by gently rubbing spores that had been suspended in a drop of distilled water, on the leaf using the thumb and index finger.

Prior to inoculation, the spores were hydrated for four hours in 100 percent relative humidity. Single-

leaf-stage barley seedling differentials were misted with distilled water, then dusted with rust spores that had been mixed with talc (1mg spores/5mg talc) using a small hand powder duster. Urediospores were collected only from the cultivar Moore which was kept in isolation following each inoculation. Inoculated seedlings were kept for 20 to 24 hours in a dew chamber maintained at 20+1 C and in 100 percent relative humidity. They were then placed in controlled environment chambers maintained at 20/15 C and a 16/8hr photoperiod (2.2-3.3 x 10^4erg/cm^{2}sec) day/night regime.

Assessment of Reaction Types

Readings of developed pustules were made 10 to 12 days after inoculation. Six infection classes and three reaction types were recorded (Table 2).

Table 2. Assessment of reaction types of Puccinia hordei on host differentials.

Infection Class	Reaction Type	Symptom Description
0	R	no visible rust pustules
0;	R	no visible rust pustules, but necrosis is present
1	R	some pustules, small, chlorosis and/or necrosis present
2	I	moderate size pustules with chlorosis
3	S	large pustules with some chlorosis
4	S	large pustules and no chlorosis

All readings of symptoms and signs were made on the first leaf of each genotype. When the reaction proved distinct, one test was considered sufficient. In less distinct cases, the test was repeated. The interaction between the 13 barley differentials and 78 monouredial Tunisian rust isolates was determined.

Classification and Analyses of the Isolates

In the interest of simplicity the rust reaction of each genotype is summarized by a single letter "R", "I", or "S" instead of the conventional system. In this study a computer program was used for ranking, sorting and comparing the different isolates. In the computerized analysis only the resistant (R) and susceptible (S) reactions were used for ranking the isolates and checking for duplicates among isolates. In the virulence formula the intermediate (I) and resistant (R) reactions were both considered as resistant (R). Differential genotypes with an R or I reaction type were considered effective, whereas only those with an S reaction type were considered ineffective. In each table, and for each leaf rust isolate, the total number of genotypes for each reaction type (R, I, or S) is listed. By listing separately the number of genotypes in the R or I

category, the reader is more able to visualize the relative effectiveness or ineffectiveness of the respective Pa resistance gene(s) or gene combinations.

The large array of data and the complexity of the analysis prompted the need for computer application. Computer manipulation of the data involved a sorting scheme based on Flor's gene-for-gene theory (Flor, 1946, 1971). This program is capable of sorting and classifying a large number of isolates in an increasing order of virulence. It also sorts out and lists any duplicate isolates separately. Computations allowing the evaluation of the relative "effectiveness/ ineffectiveness" of resistance genes and gene combinations are also performed.

IV. RESULTS

A total of 78 single uredium isolates of P. hordei were analyzed (Appendix Table 22). The virulence patterns of the P. hordei isolates at each site of collection (Table 1) are shown in Tables 3 through 9. Duplicate isolates were omitted, and the ranking of the isolates was based on the R/S reaction types.

Virulence Patterns of Puccinia hordei in Northern Tunisia

Site 1: Mateur (Ma)

Leaf rust isolate TuMa80 (Table 3) was collected at this site in 1980 and was previously studied (Reinhold and Sharp, 1982). The virulence formula for this isolate is:

1. TuMa80-1: 1,2,3,4,5,6,8,9/7,10,11,12,13

In 1983, thirteen additional P. hordei isolates were identified. Their virulence patterns are shown in Table 3. The 1983 Mateur isolates were characterized by the following virulence formulae:

1. TuMa83-15: 1,2,3,4,6,7,8,11/5,9,10,12,13
2. TuMa83-2: 1,2,3,4,6,8,9,11/5,7,10,12,13
3. TuMa83-14: 1,2,3,4,5,6,7,9/8,10,11,12,13
4. TuMa83-16: 1,2,3,4,6,7,9/5,8,10,11,12,13

5. TuMa83-3: 1,2,3,4,5,9,11/6,7,8,10,12,13

6. TuMa83-5: 1,2,3,4,5,9/6,7,8,10,11,12,13

7. TuMa83-12: 1,2,6,7,9/3,4,5,8,10,11,12,13

8. TuMa83-11: 1,2,3,4,6/5,7,8,9,10,11,12,13

9. TuMa83-6: 1,2,12/3,4,5,6,7,8,9,10,11,13

10. TuMa83-1: 1,2,4/3,5,6,7,8,9,10,11,12,13

11. TuMa83-7: 1,2,3/4,5,6,7,8,9,10,11,12,13

12. TuMa83-4: 1,2,9/3,4,5,6,7,8,10,11,12,13

13. TuMa83-8: 1,2/3,4,5,6,7,8,9,10,11,12,13

Table 3. Virulence patterns of sixteen *Puccinia hordei* isolates sampled at Mateur, Northern Tunisia, in 1980, 1983 and 1984.

	Differential Host Genotypes													Resis[1] Genes		
Isolate	1 Esta Pa_3	2 C.Ca Pa_7	3 Hor Pa_9	4 Rica Pa_{2+}	5 Bolivia Pa_2+Pa_6	6 Quinn Pa_2+Pa_5	7 Magn Pa_5	8 Peru Pa_2	9 Suda Pa	10 Egyp Pa_8	11 Batn Pa_{2+}	12 Gold Pa_4	13 Reka Pa_{2+}	R	I	S
TuMa 80-1	R	R	R	I	I	I	S	I	R	S	S	S	S	4	4	5
TuMa 83-15	R	R	R	R	S	R	R	I	S	S	I	S	S	6	2	5
TuMa 83-14	R	R	I	I	R	R	R	S	R	S	S	S	S	6	2	5
TuMa 83-2	R	R	R	R	S	I	S	I	R	S	I	S	S	5	3	5
TuMa 83-16	R	R	I	I	S	R	R	S	R	S	S	S	S	5	2	6
TuMa 83-12	R	R	S	S	S	R	R	S	R	S	S	S	S	5	0	8
TuMa 83-11	R	R	R	R	S	I	S	S	S	S	S	S	S	4	1	8
TuMa 83-5	R	R	I	I	I	S	S	S	I	S	S	S	R	3	4	6
TuMa 83-3	R	R	I	I	R	S	S	S	I	S	I	S	S	3	4	6
TuMa 83-6	R	R	S	S	S	S	S	S	S	S	S	R	I	3	1	9
TuMa 83-1	R	R	S	R	S	S	S	S	S	S	S	S	S	3	0	10
TuMa 83-7	R	R	I	S	S	S	S	S	S	S	S	S	S	2	1	10
TuMa 83-4	R	R	S	S	S	S	S	S	I	S	S	S	S	2	1	10
TuMa 83-8	R	R	S	S	S	S	S	S	S	S	S	S	S	2	0	11
TuMa 84-5	R	R	R	R	S	S	S	S	S	S	S	S	S	4	0	9
TuMa 84-3	R	R	R	S	S	S	S	S	S	S	S	S	S	3	0	10
TuMa 84-1	R	R	S	R	S	S	S	S	S	S	S	S	S	3	0	10

[1] Resistance genes: R = resistant (effective), I = intermediate (effective), S = susceptible (ineffective).

Table 3 also shows the virulence pattern of three P. hordei isolates identified from collections made at Mateur in 1984. Only one isolate differed from the isolates identified in 1983 at this same site. Its virulence formula is as follows:

1. TuMa84-5: 1,2,3,4/5,6,7,8,9,10,11,12,13

The other two isolates, TuMa84-8 and TuMa84-1, have virulence formulae that matched those of TuMa83-7 and TuMa83-1 respectively.

Site 2: BouRbia (Br)

Table 4 shows the virulence patterns of two different P. hordei isolates collected at BouRbia in 1982. The virulence formulae showing the "effectiveness/ineffectiveness" of host sources for these two isolates are as follows:

1. TuBr82-1: 1,2,3,4,5,6/7,8,9,10,11,12,13
2. TuBr82-2: 1,2,3,4,5,7/6,8,9,10,11,12,13

Virulence Patterns of Puccinia hordei in Northwestern Tunisia

Site 1: Beja (Bj)

The virulence patterns of the sixteen leaf rust isolates identified at this site in 1982 and in 1984 are shown in Table 5. The nine isolates identified in 1982

Table 4. Virulence patterns of two *Puccinia hordei* isolates sampled at BouRbia, Northern Tunisia, in 1982.

Isolate	Differential Host Genotypes													Resis[1] Genes		
	1 Esta Pa_3	2 C.Ca Pa_7	3 Hor Pa_9	4 Rica Pa_{2+}	5 Bolivia Pa_2+Pa_6	6 Quinn Pa_2+Pa_5	7 Magn Pa_5	8 Peru Pa_2	9 Suda Pa	10 Egyp Pa_8	11 Batn Pa_{2+}	12 Gold Pa_4	13 Reka Pa_{2+}	R	I	S
TuBr 82-1	R	R	R	R	R	I	S	S	S	S	S	S	S	5	1	7
TuBr 82-2	R	R	R	R	I	S	I	S	S	S	S	S	S	4	2	7

[1] Resistance genes: R = resistant (effective), I = intermediate (effective), S = susceptible (ineffective).

Table 5. Virulence patterns of sixteen *Puccinia hordei* isolates sampled at Beja, Northern Tunisia, in 1982 and 1984.

Isolate	Differential Host Genotypes													Resis[1] Genes		
	1 Esta Pa_3	2 C.Ca Pa_7	3 Hor Pa_9	4 Rica Pa_{2+}	5 Bolivia Pa_2+Pa_6	6 Quinn Pa_2+Pa_5	7 Magn Pa_5	8 Peru Pa_2	9 Suda Pa	10 Egyp Pa_8	11 Batn Pa_{2+}	12 Gold Pa_4	13 Reka Pa_{2+}	R	I	S
TuBj 82-9	R	R	R	R	R	S	S	I	S	R	S	S	S	6	1	6
TuBj 82-10	R	R	R	R	R	I	R	S	S	S	S	S	S	6	1	6
TuBj 82-2	R	R	R	R	S	S	S	I	S	R	S	S	S	5	1	7
TuBj 82-1	R	R	R	R	R	S	S	I	S	S	S	S	S	5	1	7
TuBj 82-7	R	R	I	I	S	R	I	S	R	S	S	S	S	4	3	6
TuBj 82-4	R	R	R	R	S	S	S	S	S	S	S	S	S	4	0	9
TuBj 82-11	R	R	S	R	S	S	S	S	S	S	S	S	S	3	0	10
TuBj 82-8	R	R	I	S	S	I	S	S	S	S	S	S	S	2	2	9
TuBj 82-6	R	R	S	S	S	S	S	S	S	S	S	S	S	2	0	11
TuBj 84-1	R	R	S	R	R	S	S	R	R	S	S	S	S	6	0	7
TuBj 84-2	R	R	R	R	S	I	S	S	S	S	R	S	S	5	1	7
TuBj 84-8	R	R	I	R	S	S	S	R	S	S	R	S	S	5	1	7
TuBj 84-4	R	R	R	I	S	S	S	S	S	S	I	S	S	3	2	8
TuBj 84-7	R	R	S	R	S	S	S	S	S	I	I	S	S	3	2	8
TuBj 84-3	R	R	I	R	S	I	S	S	S	S	S	S	S	3	2	8
TuBj 84-5	R	R	S	S	S	S	S	S	S	S	S	S	S	2	0	11

[1] Resistance genes: R = resistant (effective), I = intermediate (effective), S = susceptible (ineffective).

were catagorized based on the relative "effectiveness/ineffectiveness" of the barley genotype resistance genes and gene combinations and were differentiated as follows:

1. TuBj82-10: 1,2,3,4,5,6,7/8,9,10,11,12,13
2. TuBj82-9: 1,2,3,4,5,8,10/6,7,9,11,12,13
3. TuBj82-7: 1,2,3,4,6,7,9/5,8,10,11,12,13
4. TuBj82-2: 1,2,3,4,8,10/5,6,7,9,11,12,13
5. TuBj82-1: 1,2,3,4,5,8/6,7,9,10,11,12,13
6. TuBj82-4: 1,2,3,4/5,6,7,8,9,10,11,12,13
7. TuBj82-8: 1,2,3,6/4,5,7,8,9,10,11,12,13
8. TuBj82-11: 1,2,4/3,5,6,7,8,9,10,11,12,13
9. TuBj82-6: 1,2/3,4,5,6,7,8,9,10,11,12,13

In 1984, seven P. hordei isolates were identified from collections made at this site. The virulence patterns of these isolates are shown in Table 5. Four of the monouredial cultures were isolated from single aecia from the alternate host (Ornithogalum spp.). The virulence formulae of these isolates are:

1. TuBj84-8: 1,2,3,4,8,11/5,6,7,9,10,12,13
2. TuBj84-4: 1,2,3,4,11/5,6,7,8,9,10,12,13
3. TuBj84-7: 1,2,4,10,11/3,5,6,7,8,9,12,13
4. TuBj84-5: 1,2/3,4,5,6,7,8,9,10,11,12,13

The three isolates that were collected from the barley

cultivar grown in the same field as the alternate host have the following virulence formulae:

1. TuBj84-2: 1,2,3,4,6,11/5,7,8,9,10,12,13
2. TuBj84-1: 1,2,4,5,8,9/3,6,7,10,11,12,13
3. TuBj84-3: 1,2,3,4,6/5,7,8,9,10,11,12,13

Site 2: Le Kef (Ke)

At this site, nine *P. hordei* isolates were identified in 1982 and two isolates in 1984 (Table 6).

Table 6. Virulence patterns of nine *Puccinia hordei* isolates sampled at Le Kef, Northwestern, Tunisia, in 1982 and 1984.

	Differential Host Genotypes													Resis[1] Genes		
	1	2	3	4	5	6	7	8	9	10	11	12	13			
Isolate	Esta Pa_3	C.Ca Pa_7	Hor Pa_9	Rica Pa_{2+}	Bolivia Pa_2+Pa_6	Quinn Pa_2+Pa_5	Magn Pa_5	Peru Pa_2	Suda Pa	Egyp Pa_8	Batn Pa_{2+}	Gold Pa_4	Reka Pa_{2+}	R	I	S
TuKe 82-5	R	R	R	R	R	R	R	S	R	S	R	S	S	9	0	4
TuKe 82-4	R	R	R	R	R	R	I	I	R	S	R	S	S	8	2	3
TuKe 82-6	R	R	R	R	R	S	S	I	S	S	S	S	S	5	1	7
TuKe 82-3	R	R	R	R	S	S	S	S	S	S	S	S	S	4	0	9
TuKe 82-8	R	R	R	S	S	S	S	S	S	S	S	S	S	3	0	10
TuKe 82-10	R	R	I	I	S	S	S	S	S	S	S	S	S	2	2	9
TuKe 82-9	R	R	S	S	S	S	S	S	S	S	S	S	S	2	0	11
TuKe 84-2	R	R	R	I	S	S	S	S	S	S	S	S	S	3	1	9
TuKe 84-3	R	R	S	S	S	S	S	S	S	S	S	S	S	2	0	11

[1] Resistance genes: R = resistant (effective), I = intermediate (effective), S = susceptible (ineffective).

The virulence formulae of the 1982 isolates are:

1. TuKe82-4: 1,2,3,4,5,6,7,8,9,11/10,12,13
2. TuKe82-5: 1,2,3,4,5,6,7,9,11/8,10,12,13
3. TuKe82-6: 1,2,3,4,5,8/6,7,9,10,11,12,13
4. TuKe82-3: 1,2,3,4/5,6,7,8,9,10,11,12,13
5. TuKe82-10: 1,2,3,4/5,6,7,8,9,10,11,12,13
6. TuKe82-8: 1,2,3/4,5,6,7,8,9,10,11,12,13
7. TuKe82-9: 1,2/3,4,5,6,7,8,9,10,11,12,13

The virulence patterns of the 1984 isolates were similar if not identical to some isolates that were identified the previous year. TuKe84-1 has the same virulence formula as TuKe82-9 and TuKe84-2 differed very slightly from the TuKe82-8 isolate. The main difference was on the cultivar Ricardo (Pa_{2+}, CI 6306) which showed a susceptible reaction type to the 1982 isolate TuKe82-8, but an intermediate reaction type to the 1984 isolate TuKe84-2.

Virulence Patterns of Puccinia hordei in Central Tunisia

Site 1: Kairouan (Kr)

Only one leaf rust isolate was identified from 1982 rust collections made at Kairouan. The virulence of this isolate is shown in Table 7. The virulence formula associated with this isolate is:

1. TuKr82-1: 1,2,3,4/5,6,7,8,9,10,11,12,13

Site 2: El Jem (Ej)

Table 7 shows the virulence pattern of the isolate identified from the 1983 rust collection made at El Jem. The virulence formula that fits this isolate is:

1. TuEj83-1: 1,2,3,4,5,6,7/8,9,10,11,12,13

Table 7. Virulence patterns of two Puccinia hordei isolates sampled at Kairouan in 1982, and one isolate sampled at El Jem in 1983, in Central Tunisia.

	Differential Host Genotypes													Resis[1] Genes		
	1	2	3	4	5	6	7	8	9	10	11	12	13			
Isolate	Esta	C.Ca	Hor	Rica	Bolivia	Quinn	Magn	Peru	Suda	Egyp	Batn	Gold	Reka	R	I	S
	Pa_3	Pa_7	Pa_9	Pa_{2+}	Pa_2+Pa_6	Pa_2+Pa_5	Pa_5	Pa_2	Pa	Pa_8	Pa_{2+}	Pa_4	Pa_{2+}			
Kairouan																
TuKr 82-2	R	R	R	R	S	S	S	S	S	S	S	S	S	4	0	9
TuKr 82-1	R	R	I	R	S	S	S	S	S	S	S	S	S	3	1	9
ElJem																
TuEj 83-1	R	R	R	R	R	R	I	S	S	S	S	S	S	6	1	6

[1] Resistance genes: R = resistant (effective), I = intermediate (effective), S = susceptible (ineffective).

Virulence Patterns of Puccinia hordei in Southern Tunisia

Site 1: The Oasis (Oa)

Seven highly variable leaf rust isolates were sorted out from leaf rust samples collected in the Oasis in 1982. Table 8 shows the virulence patterns of these isolates.

Table 8. Virulence patterns of seven Puccinia hordei isolates sampled in the Oasis, Southern Tunisia, in 1982.

Isolate	Differential Host Genotypes													Resis[1] Genes		
	1 Esta Pa_3	2 C.Ca Pa_7	3 Hor Pa_9	4 Rica Pa_{2+}	5 Bolivia Pa_2+Pa_6	6 Quinn Pa_2+Pa_5	7 Magn Pa_5	8 Peru Pa_2	9 Suda Pa	10 Egyp Pa_8	11 Batn Pa_{2+}	12 Gold Pa_4	13 Reka Pa_{2+}	R	I	S
TuOa 82-6	R	R	R	R	R	S	S	S	S	R	R	S	S	7	0	6
TuOa 82-4	R	R	R	S	S	I	I	R	S	S	I	I	S	4	4	5
TuOa 82-3	R	R	I	I	I	R	S	S	I	S	R	S	S	4	4	5
TuOa 82-8	R	R	I	R	I	I	S	S	S	S	I	S	S	3	4	6
TuOa 82-7	R	R	S	I	S	R	S	I	I	S	S	S	S	3	3	7
TuOa 82-5	R	R	R	S	S	S	S	S	S	S	S	S	S	3	0	10
TuOa 82-1	R	R	S	S	S	S	S	S	S	S	S	S	S	2	0	11

[1] Resistance genes: R = resistant (effective), I = intermediate (effective), S = susceptible (ineffective).

The known resistance genes in the barley differentials used when tested for their "effectiveness/ineffectiveness" against the Oasis isolates behaved as follows:

1. TuOa82-6: 1,2,3,4,5,10,11/6,7,8,9,12,13
2. TuOa82-4: 1,2,3,6,7,8,11,12/4,5,9,10,13
3. TuOa82-3: 1,2,3,4,5,6,9,11/7,8,10,12,13
4. TuOa82-8: 1,2,3,4,5,6,11/7,8,9,10,12,13
5. TuOa82-7: 1,2,4,6,8,9/3,5,7,10,11,12,13
6. TuOa82-5: 1,2,3/4,5,6,7,8,9,10,11,12,13
7. TuOa82-1: 1,2/3,4,5,6,7,8,9,10,11,12,13

In 1984, only two virulence type were detected in the Oasis. Their virulence patterns were the same as TuOa82-5 and TuOa82-1 identified in 1982.

Site 2: Mareth (Mr)

At Mareth, more duplicate isolates were detected than at any other site (Table 9).

Table 9. Virulence patterns of five Puccinia hordei isolates sampled at Mareth, Southern Tunisia, in 1983.

	Differential Host Genotypes													Resis[1] Genes		
	1	2	3	4	5	6	7	8	9	10	11	12	13			
Isolate	Esta Pa_3	C.Ca Pa_7	Hor Pa_9	Rica Pa_{2+}	Bolivia Pa_2+Pa_6	Quinn Pa_2+Pa_5	Magn Pa_5	Peru Pa_2	Suda Pa	Egyp Pa_8	Batn Pa_{2+}	Gold Pa_4	Reka Pa_{2+}	R	I	S
TuMr 83-10	R	R	R	R	R	S	S	I	S	I	S	S	S	5	2	6
TuMr 83-6	R	R	R	S	S	R	R	S	S	S	I	S	S	5	1	7
TuMr 83-2	R	R	R	I	S	S	S	S	S	S	S	S	S	3	1	9
TuMr 83-9	R	R	I	R	S	S	S	S	S	S	S	S	S	3	1	9
TuMr 83-8	R	R	S	S	S	S	S	S	S	S	S	S	S	2	0	11

[1] Resistance genes: R = resistant (effective), I = intermediate (effective), S = susceptible (ineffective).

The virulence formulae of these isolates showing the "effectiveness/ineffectiveness" of the Pa resistance genes to leaf rust isolates that are indigenous to southern Tunisia are as follows:

1. TuMr83-10: 1,2,3,4,5,8,10/6,7,9,11,12,13
2. TuMr83-6: 1,2,3,6,7,11/4,5,8,9,10,12,13
3. TuMr83-2: 1,2,3,4/5,6,7,8,9,10,11,12,13
4. TuMr83-9: 1,2,3,4/5,6,7,8,9,10,11,12,13
5. TuMr83-8: 1,2/3,4,5,6,7,8,9,10,11,12,13

Common Virulence Patterns Across Regions

The virulence patterns of Puccinia hordei common in Tunisia are shown in Appendix Table 22. Table 10 shows all the duplicate isolates of P. hordei that were found in at least two sites of collection. Based on the virulence formula, the duplicate isolates were divided into five groups.

Group 1 contains isolates that were found throughout the country. The virulence formula asociated with this group is: 1,2,3,4/5,6,7,8,9,10,11,12,13

Group 2 contains leaf rust isolates that are common in the North and Northwest regions. The virulence formula is: 1,2,4/3,5,6,7,8,9,10,11,12,13

The duplicate isolates representing group 3 are isolates that were encountered in the North, Northwest, and in the South. The virulence formula for this group is: 1,2,3/4,5,6,7,8,9,10,11,12,13

The leaf rust isolate representative of group 4 is one of the most virulent encountered in this study, and is found throughout the country in almost all barley growing areas. The virulence formula of this isolate is: 1,2/3,4,5,6,7,8,9,10,11,12,13

The fifth group contains the least virulent isolate among these duplicates. This isolate is found only in

the Northwestern region and has the following virulence formula: 1,2,3,4,5,8/6,7,9,10,11,12,13

Table 10. Common virulence patterns of *Puccinia hordei* isolates in Tunisia in 1982, 1983, and 1984.

Isolate	Differential Host Genotypes													Resis[1] Genes		
	1 Esta Pa_3	2 C.Ca Pa_7	3 Hor Pa_9	4 Rica Pa_{2+}	5 Bolivia $Pa_{2+}Pa_6$	6 Quinn $Pa_{2+}Pa_5$	7 Magn Pa_5	8 Peru Pa_2	9 Suda Pa	10 Egyp Pa_8	11 Batn Pa_{2+}	12 Gold Pa_4	13 Reka Pa_{2+}	R	I	S
Group 1[2]	R	R	R	R	S	S	S	S	S	S	S	S	S	4	0	9
Group 2[3]	R	R	S	R	S	S	S	S	S	S	S	S	S	3	0	10
Group 3[4]	R	R	R	S	S	S	S	S	S	S	S	S	S	3	0	10
Group 4[5]	R	R	S	S	S	S	S	S	S	S	S	S	S	2	0	11
Group 5[6]	R	R	R	R	R	S	S	I	S	S	S	S	S	5	1	7

1 Resistance genes: R = resistant (effective), I = intermediate (effective), S = susceptible (ineffective).

2 Group 1: Isolate identified at Le Kef (1982, 1984), Beja (1982), Mateur (1984), Mareth (1983), Kairouan (1982).

3 Group 2: Isolate identified at Beja (1982), Mateur (1982, 1984).

4 Group 3: Isolate identified at Le Kef (1982), Beja (1982), Mateur (1983, 1984), Oasis (1982).

5 Group 4: Isolate identified at Le Kef (1982, 1984), Beja (1982, 1984), Oasis (1982), Mareth (1983).

6 Group 5: Isolate identified at Le Kef (1982), Beja (1982).

V. DISCUSSION

The results obtained (Tables 3 - 10) show the presence of several virulence types of *P. hordei* in Tunisia. The leaf rust isolates identified within each site were variable and their virulence patterns differed from year to year. Some similar virulence types were identified in at least two sites. Identical isolates were also found at almost every collection site throughout the country. Nonetheless, most of the isolates studied were site specific, even though the virulence patterns changed.

Virulence Patterns of *Puccinia hordei* in Various Geographic Regions in Tunisia

In the Northern region, the two isolates identified in 1982 at BouRbia differed from all the other isolates analyzed in this study. The two isolates have similar virulence patterns (Table 4). They differed in infection types on the barley cultivars Quinn (Pa_2+Pa_5) and Magnif (Pa_5). Since the rust collections were made only once at this site, no speculations can be made as to possible changes in virulence of these two *P. hordei* isolates.

The virulence patterns of leaf rust at the Mateur site varied over years. The virulence pattern observed in 1980 was not detected in collections made the

following years. This isolate possibly evolved to a more virulent type following sexual recombinations on the alternate host, or it was just not detected in the 1983 or 1984 samples. Isolates identified in the 1983 collection were more virulent than the 1980 isolate. The virulence patterns of these isolates (Table 4) suggest that the leaf rust population at this site was highly variable. Among the isolates identified in 1984, only one differed from those of 1983. This isolate (TuMa84-5) may not have been detected the previous year, or it actually could have been a new virulence type.

The virulence pool of *P. hordei* detected in this area presents a potential danger to barley growers. Theoretically, barley cultivars that can be cultivated in this area would be those carrying either or both Pa_3 and Pa_7 resistance genes. These two genes (Table 4) are presently the only ones effective against virulent *P. hordei* isolates identified in this region. Variability in virulence of *P. hordei* isolates observed in the North is most probably due to the presence of the alternate host, *Ornithogalum spp.* which were found in many barley fields around Mateur.

In the Northwest, significant variability in virulence patterns of *P. hordei* was observed (Tables 5

and 6). In Beja, leaf rust isolates originating from collections made from Ornithogalum spp. were as variable as those isolated from commonly grown barley cultivars. None of these isolates were virulent on Pa_3 or Pa_7. Virulence patterns detected in 1982 samples were not recovered in 1984 collections. A possible change in virulence of Puccinia hordei could have taken place in this area (Table 5). At Le Kef, however, no major changes in virulence were observed (Table 6).

Pathogenicity differences in P. hordei in Northwestern Tunisia are probably enhanced by the presence of the alternate host, particularly at Beja where Ornithogalum spp. were found in barley fields. The sexual state of P. hordei on Ornithogalum spp (Table 5), contributed to the diversification of the spectrum of virulence of P. hordei in this region.

The virulence types of leaf rust encountered in Central Tunisia were similar to some virulence types identified in the Northwest. The isolate from El Jem (Table 7) was identical to one isolate from Beja. The Kairouan isolate was found in both sites in the Northwest.

In Southern Tunisia, leaf rust virulence varied almost as much as in the North and Northwest, but fewer

virulence patterns were identified in this region (Table 8, Table 9). The microclimate in the Oasis was favorable for leaf rust development. In 1984, some barley plots found in the Oasis, mosaic-type, cropping system, were completely devasted by leaf rust. Isolates identified were all virulent and identical to some virulence types identified in 1982 (Table 8). Little variability was detected in 1984, probably due to the high frequency of the virulent types. At the other Southern site, Mareth, the isolates were collected from irrigated barley plots that were grown as an intercrop in olive orchards. The irrigation created an environment favorable for rust development in 1983, and similar virulence patterns as those in the Oasis were observed (Table 8, Table 9). The intensive agriculture practiced in the Oasis probably made it possible for leaf rust to cycle on the primary host.

Common Virulence Patterns

The *P. hordei* isolate designated "Group 4" (Table 10) was the most virulent isolate identified in Tunisia. It was found in all barley growing areas in the North, Northwest and South. Only Pa_3 and Pa_7 were effective against this isolate. Another leaf rust isolate, Group 3, found in all locations but Central Tunisia, was also

virulent on all resistance genes but Pa_3, Pa_7, and Pa_9. The North and Northwest have a common P. hordei isolate, Group 2 (Table 10), that is only avirulent on the cultivars Estate (Pa_3), Cebada Capa (Pa_7), and Ricardo (Pa_{2+}). The isolate Group 1 (Table 10) that is found in all geographic areas in Tunisia was virulent on all the genotypes but Estate, Cebada Capa, Hor 2596, and Ricardo. These genotypes and Bolivia were also resistant to the isolate common to the Northwestern region, Group 5 (Table 10).

Effectiveness of Resistance Genes to Puccinia hordei Isolates Identified in Tunisia

With the exception of Pa_3 and Pa_7, the frequency of P. hordei virulence against the other Pa genes varied from moderate (Pa_9) to very severe (Pa_4, Pa, Pa_8, Pa_{2+}) Although leaf rust isolates virulent on Pa_7 have been reported (Parlevliet, 1981), all Tunisian isolates tested in this study were avirulent on Pa_7 and on Pa_3.

The observed changes in virulence patterns of P. hordei in Tunisia, should be carefully monitored. Under favorable climatic conditions, a leaf rust epidemic could be expected, especially if susceptible barley cultivars were grown over large areas.

Cultures of *P. hordei* virulent on Pa_3 and/or Pa_7 genes may be developed by recombination on *Ornithogalum* or otherwise, but be unable to compete due to associated factors for non-aggressiveness. This aspect should be further studied.

PART II

NEW SOURCES OF RESISTANCE TO *PUCCINIA HORDEI* OTTH IN TUNISIAN BARLEY LAND RACES

Part II

VI. INTRODUCTION

Barley leaf rust has not been a significant factor affecting barley production in Tunisia. This favorable situation may not continue because the disease has now been commonly observed throughout the country. Highly virulent leaf rust isolates, virulent on many sources of resistance, have been detected (Part I). The virulence types discussed earlier are important since they have not been detected before, and they are a threat to the commonly grown cultivars.

The search for new sources of resistance among some Tunisian barley land races led to the identification of host genotypes which express seedling resistance to most, if not all, *P. hordei* isolates collected from Tunisia, Morocco, Egypt, Jordan, and Syria in 1982, 1983, and 1984. This study was carried out to investigate the expression and genetic relationships of these unknown resistance genes.

VII. LITERATURE REVIEW

Importance of Barley in Tunisia

Among the major crops of Tunisia, cereals occupy about 1.6 million hectares. Barley, durum and bread wheat are the major cereal crops cultivated. Barley is by far the best adapted cereal grain in the semi-arid to arid regions, but durum wheat is the most cultivated crop. Barley occupies 30% of the cereal production area and is the main crop in the central and southern regions. Climatic conditions in these regions are less favorable for growing wheat. In the northern region, barley is grown as an alternative crop or in marginal areas, and is seldom considered as a major crop. Recently, farmers in this region have become more interested in barley, primarily for forage and feed, and also for malting. Due to its high cash value, malting barley can become an important crop in the north, and could become a potential export commodity. If barley production is to be expanded in these areas, diseases are major risks that need to be considered.

Disease Problems

The prevalence of barley foliar diseases has been observed. Researchers involved in the "Montana-AID Barley Project" have reported the following diseases (Table 11) that they have observed during their visits to Tunisia over a period of eight consecutive years.

Table 11. Prevalent barley diseases in Tunisia, from 1978-1985, according to observers with the Montana-AID barley project.

Year	Barley Diseases[1]											Observers	Reference
	Nb	Sc	Frr	Pm	Lr	Cs	Ls	BYDV	Hf	Hs	others		
1978	-	+	+	+	-	+	-	-	+	+	-	Bockleman, Scharen	Sharp, 1979
1979	+	-	+	-	-	+	-	+	+	+	-	Sharp, Sands	Sharp, 1979
1980	+	+	-	+	-	-	+	-	-	+	Yr[2]	Scharen, Langhans	Sharp, 1980
1981	+	+	-	-	+	-	-	-	-	+	-	Carroll, Harrabi	Sharp, 1981
1982	+	+	+	+	-	+	-	+	+	-	Un[3]	Sands, Yount	Sharp, 1983
1983	-	-	-	-	+	-	-	-	-	+	Ps[4]	Sands, Ruff	Sharp, 1983
1984	+	+	+	-	+	-	-	+	+	-	-	Sharp, Yahyaoui	Sharp, 1984
1985	+	+	+	-	+	-	-	+	-	-	-	Carroll, Grey	Sharp, 1985

1 Nb: Net blotch; Sc: Scald; Frr: Fusarium root rot; Pm: Powdery mildew; Lr: Leaf rust; Cs: Covered smut; Ls: Loose smut; BYDV: Barley yellow dwarf virus; Hf: Hessian fly; Hs: Helminthosporium stripe

2 Yr: Yellow rust

3 Un: Unknown white striping disease (10-20% incidence)

4 Ps: Pseudomonas syringae

Sources of Resistance

The importance of genetic diversity and the potential consequences associated with narrowing the genetic base of cultivated species has long been recognized by plant pathologists and most plant breeders. One purpose of testing and screening germplasm is to evaluate its reaction to diseases and insect pests. A decrease in genetic variablity can then be identified and in some instances can lead to genetic vulnerability. Thus, the most obvious method of increasing genetic variability, to avoid severe epidemics, is by introducing germplasm from distantly related species into breeding populations of the cultivated crop. In this aspect the literature reveals many examples of successful transfer of disease resistance from one species to another (Feldman and Sears, 1981). It also reveals a wide range of problems that can arise in interspecific hybridization (Knot and Dvorak, 1970; Price, 1979).

Exotic germplasm may vary in its capacity to contribute positively to the improvement of cultivated barleys; in this respect, land races could be an alternative. They offer the advantage of being adapted to cultivation even though some may have undesirable agronomic traits such as excessive height, weak rachis,

and severe shattering problems.

Land race cultivars are found mostly in parts of developing countries where modern high yielding varieties have not been introduced. They are common in the high mountains of Ethiopia (Harlan, 1979), in the Near Eastern region (Weltzein and Fischbeck, 1985), in central and southern Tunisia and possibly in other North African countries.

Ethiopian barleys have been a useful source of resistance to many diseases. Harlan (1979) reported resistance in Ethiopian barleys to barley yellow dwarf virus, powdery mildew, leaf rust, scald, and net blotch. Kelemu (1984) found new resistance genes to Rhynchosporium secalis in Ethiopian barleys. It appears that the breeding populations of cultivated barleys encompass great genetic variability that can be exploited. There are 18,000 entries of barley catalogued in the world collection (Reid and Wiebe, 1979) currently available for use by barley researchers. Out of this collection, 332 barley lines were screened for scald reaction in California and Montana. A total of 131 lines showed resistance at both sites (Bockelman, personal communication). Twenty three lines were resistant to powdery mildew (Reinhold, personal communication). Ten

of these lines were resistant to both powdery mildew and scald.

Inheritance and resistance genes in barley

In the early 1900's, Biffen demonstrated that the absence of stripe rust on a host plant was an inherited character. This was the beginning of many great and successful programs of breeding for resistance to the rust fungi. Waterhouse (in Roane, 1962) was the first investigator to study the inheritance of resistance to leaf rust in barley. He found that resistance in several cultivars was conditioned by a single dominant factor. Later, in crosses between resistant barley cultivars, Waterhouse found that all genes conditioning resistance occurred at the same locus. Watson and Butler (in Roane, 1962) showed that resistance to leaf rust in several barley varieties was conditioned by independent genes. Recently several major genes for resistance to leaf rust (P. hordei) have been described (Roane and Starling, 1967; Clifford, 1974; Parlevliet, 1976 and Tan, 1977). These genes are designated Pa, Pa_2, Pa_3 etc. and are assumed to operate on a gene-for-gene basis with corresponding virulence genes in the pathogen.

Early genetic studies of host reaction to P. hordei were re-evaluated by Roane and Starling (1970). They

proposed the gene symbols Pa, Pa_2, Pa_3, Pa_4, and Pa_6. Parlevliet (1976) concluded that barley cultivars "Gondar", "La Estanzuela", "Cebada Capa", and "Dabat" all carried the Pa_7 resistance gene. This was confirmed by Clifford and Udeogalanya (1976).

Effectiveness of the Pa Genes

Virulence on barley cultivars having the resistance (Pa) genes occurs widely in Europe (Clifford, 1974). The Pa_7 gene is effective throughout Europe, while virulence against Pa_3 occurs rather infrequently (Parlevliet, 1976). Studies of virulence in British populations of P. hordei revealed widespread virulence to host resistance genes Pa, Pa_2, Pa_4, Pa_5, and Pa_6. Sharp and Reinhold (1982) showed that barley lines with Pa_3 or Pa_7 were resistant to 12 isolates from the United States and the Mediterranean area. They also identified two barley lines that did not possess Pa_3 or Pa_7 but were resistant to all the 12 isolates. Pa_9 was overcome by one isolate originating from the alternate host (Sharp and Reinhold, 1982). Clifford and Udeogalanya (1976) have observed differential response given by CI 1243, the Pa_9 carrier. Pretorius and Wilcoxson (1983) showed that Pa_3, Pa_7, and Pa_9 were effective against all races of P. hordei known

to occur in the United States. They recommended incorporating these genes into cultivars grown in the upper midwest to eliminate the potential threat of leaf rust in this region. Two cultivars with the Pa_7 gene (Monroe, CI 15691 and Henry, CI 15690) have been released and are still showing good field resistance (Roane, personal communication).

VIII. MATERIALS AND METHODS

Parent Selection

Single heads of 180 barley land race cultivars were collected from farmer's fields in central and southern Tunisia. They were screened for their reaction to isolates of *P. hordei* from the Mediterranean area. Thirty four lines showed intermediate to resistant reaction to most of the *P. hordei* isolates tested. Five of these barley cultivars were selected and seeds from single heads were multiplied in the field. At maturity, the off-types were rogued. Harvested seed from each head row was bulked. These lines were given a Tu (Tunisian) number and were further screened against various isolates of *P. hordei*.

Selected lines (Tu16, Tu17, Tu27, Tu32, Tu34; Table 12) were crossed with four differential genotypes with known resistance Pa genes. Reka I (Pa_{2+}, CI 5051) was completely susceptible (IT:4) to *P. hordei* isolates, and was used as the susceptible parent. Parents and five to ten F_1 seeds were planted in Tucson, Arizona, where backcrosses and additional single crosses were made. The backcrosses and F_3 hybrids

Table 12. Land race barley cultivars used in this study and their site of collection in Tunisia.

Cultivar	Site of Collection[1]	Year
Tu 16	Gafsa - Central Tunisia	1982
Tu 17	Oasis - Southern Tunisia	1982
Tu 27	El Jem - Southeast Tunisia	1983
Tu 32	El Jem - Southeast Tunisia	1983
Tu 34	El Jem - Southeast Tunisia	1983

[1] see Appendix Figure 15.

were later screened in environmentally controlled growth chambers.

Inoculum and Inoculation Techniques

Inoculum from the three selected isolates (TuKe82-5, TuOa82-1, MoMe84-5) was multiplied on the universal susceptible cultivar Moore (CI 725). Isolates were separated from each other and kept in different growth chambers to minimize the chances of contamination. Screening of the hybrids and parents was conducted in three separate growth chambers maintained at the same temperature and photoperiod regimes as discussed in Part I.

Inoculation procedures were the same as discussed in Part I, with the following exception; due to the large

number of plants screened, the rust spores were suspended in Soltrol 170 oil in a ratio of 1 mg spores/1 ml oil. The spore suspension was then sprayed on the plants to be screened, using a DeVilbiss atomizer attached to a compressed air hose (15-20psi).

Hybrids and parents were grown in a 2:1 soil:sand mixture. Two hundred F_2 seedlings of each cross were planted in metal flats (34cm x 25cm x 8cm). They were sown in eight rows of 25 seeds per row. Parents, the universal susceptible, back crosses, and F_3 seedlings were planted in plastic pots (10 cm diameter). Parents and hybrids that were to be transplanted into the field for seed production of the successive generations (ie. F_2, F_3, and BCF_2) were planted in peat pots (5 seeds/pot). Following seedling disease assessment, the seedlings were sprayed with Bayleton following the removal of the heavily infected leaves. Only one plant was kept in each pot and was then transplanted into the field. F_2 transplants were color-coded according to their disease reaction, and marked in the field. Thus the reaction type of the F_3 generation could be traced back to the reaction type of individual selfed F_2 plants. The advanced generation (F_3) and some F_2s that were transplanted, but matured late, and had to be harvested

before reaching physiological maturity. Hence, their germination was poor.

Since the *P. hordei* isolates used in this study were of foreign origin, all the plant material and soil were autoclaved following each experiment.

Statistical Analysis

The probability values for goodness of fit to expected ratios were calculated using Chi-square method. In both F_2 and F_3 progenies, where more than one F_1 plant was studied, a Chi-square test for homogeneity was used to determine whether different F_2 families displayed similar genetic behavior. Combined data are presented in the tables.

IX. RESULTS

Parents

The five land race barley cultivars selected for this study had outstanding resistance to almost all of the *P. hordei* isolates from the Mediterranean region and specifically those identified from Tunisia, in 1982, 1983 and 1984. Evidence presented (Table 13) suggests that these cultivars may carry new resistance genes to *P. hordei*. Their reactions to different isolates were invariably associated with some chlorosis and, as such, were normally distinguishable from those conditioned by Pa_3, Pa_7, and Pa_9 and for that matter those typical to Pa, Pa_2, Pa_4, Pa_{2+}, Pa_2+Pa_5, Pa_2+Pa_6, and Pa_8. Table 13 shows the reaction patterns of nine *P. hordei* isolates that were observed on these selected cultivars.

The three isolates TuKe82-5, MoMe84-5, and TuOa82-1 were collected from Le Kef (Tunisia), Merchouch (Morocco), and the Oasis (Tunisia). Their virulence patterns on the differential set are shown in Table 14.

Table 13. Reactions of five Tunisian barley lines and four differential barley genotypes to nine *Puccinia hordei* isolates from several locations.

Isolates[1]	Barley Genotypes								
	Reka Pa_{2+}	Hor2596 Pa_9	Est Pa_3	CCapa Pa_7	Tu16 -	Tu17 -	Tu27 -	Tu32 -	Tu34 -
1 TuMa83-16	S[2]	R	R	R	I	R	R	R	R
2 TuKe82-5	S	R	R	R	R	R	R	R	R
3 TuOa82-1	S	S	R	R	S	R	R	R	R
4 MoMe84-5	S	I	R	R	R	R	R	R	R
5 MoRb84-1	S	R	R	R	R	R	R	R	R
6 JoAm84-4	S	S	R	R	R	R	R	R	R
7 SyAl84-1	S	I	R	R	R	R	R	R	R
8 EgGg84-1	S	S	R	R	S	R	R	R	R
9 EgSk84-1	S	R	R	R	R	R	R	R	R

[1] Isolates 1, 2, 3 are from Tunisia; 4, 5 from Morocco; 6 from Jordan, 7 from Syria, and 8, 9 from Egypt.

[2] R=resistant reaction, I=intermediate, S=susceptible reaction

Table 14. Virulence patterns of three *Puccinia hordei* isolates on barley differential cultivars.

Isolate	Differential Host Genotypes												
	1 Esta Pa_3	2 C.Ca Pa_7	3 Hor Pa_9	4 Rica Pa_{2+}	5 Bolivia Pa_2+Pa_6	6 Quinn Pa_2+Pa_5	7 Magn Pa_5	8 Peru Pa_2	9 Suda Pa	10 Egyp Pa_8	11 Batn Pa_{2+}	12 Gold Pa_4	13 Reka Pa_{2+}
TuKe82-5	R[1]	R	R	R	R	R	R	S	R	S	R	S	S
MoMe84-5	R	R	R	S	R	R	S	S	R	S	R	S	S
TuOa82-1	R	R	S	S	S	S	S	S	S	S	S	S	S

[1] R = resistant reaction, I = intermediate reaction, S = susceptible reaction

The avirulence/virulence formulae of these isolates were as follows:

TuKe82-5 1,2,3,4,5,6,7,9,11/8,10,12,13

MoMe84-5 1,2,3,5,6,9,11/4,7,8,10,12,13

TuOa82-1 1,2/3,4,5,6,7,8,9,10,11,12,13

Isolate TuOa82-1 was particularly virulent in this study since only two resistance genes were effective against it. TuKe82-5 and MoMe84-5 were largely avirulent since more resistance genes were effective against them. Resistance factors associated with land race cultivars were effective against these isolates with the exception of the resistance factor in Tu16 which was not effective against TuOa82-1. The avirulence/virulence formulae for these lines are:

TuKe82-5 -/Tu16,Tu17,Tu27,Tu32,Tu34

MoMe84-5 -/Tu16,Tu17,Tu27,Tu32,Tu34

TuOa82-1 Tu16/Tu17,Tu27,Tu32,Tu34

When land race cultivars were crossed with barley genotypes having specific resistance genes, the progeny segregated in the F_2 generation indicating the presence of different resistance genes or gene combinations. Data pertinent to these crosses were divided into two groups:

1. Those from crosses of resistant land race cultivars with the susceptible parent RekaI, to determine the number of gene loci for resistance in each cultivar (Table 15). The following crosses were made:

Tu16 x RekaI	Tu32 x RekaI
Tu17 x RekaI	Tu34 x RekaI
Tu27 x RekaI	

2. Those from crosses between the resistant land race cultivars and the three resistant genotypes (Hor 2596, Estate, and Cebada Capa) with known resistance genes, to determine if the genes for resistance are at a common locus (Tables 16, 17, 18). The following crosses were made:

Tu16 x Hor2596	Tu16 x Estate	Tu16 x Cebada Capa
Tu17 x Hor2596	Tu17 x Estate	Tu17 x Cebada Capa
Tu27 x Hor2596	Tu27 x Estate	
Tu32 x Hor2596	Tu32 x Estate	
Tu34 x Hor2596	Tu34 x Estate	

Table 15. The reaction of F_2 barley seedlings to three isolates of *Puccinia hordei* (RekaI x five land races).

Cross	Parental reaction[1]	Isolate	Observed resis.	Frequency susc.	Expected ratio	Probability
Tu16 x RekaI	R/S	TuKe82-5	154	452	1:3	.85
Tu16 x RekaI	R/S	MoMe84-5	156	459	1:3	.87
Tu16 x RekaI	S/S	TuOa82-1	116	410	1:3	.13
Tu17 x RekaI	R/S	TuKe82-5	392	142	3:1	.42
Tu17 x RekaI	R/S	TuOa82-1	371	126	3:1	.89
Tu27 x RekaI	R/S	TuKe82-5	155	52	3:1	1.00
Tu27 x RekaI	R/S	TuOa82-1	164	58	3:1	.76
Tu32 x RekaI	R/S	TuKe82-5	218	68	3:1	.68
Tu32 x RekaI	R/S	TuOa82-1	175	67	3:1	.37
Tu34 x RekaI	R/S	TuKe82-5	535	182	3:1	.84
Tu34 x RekaI	R/S	TuOa82-1	66	25	3:1	.67

[1] Reaction of first parent/second parent; R = resistant, S = susceptible.

Table 16. The reaction of F_2 barley seedlings to three isolates of Puccinia hordei (Hor 2596 x five land races).

Cross	Parental reaction[1]	Isolate	Observed Frequency resis.	susc.	Expected ratio	Probability
Tu16 x Hor2596	R/R	TuKe82-5	455	101	13:3	.76
Tu16 x Hor2596	R/R	MoMe84-5	313	242	(9:7)[2]	1.00
Tu16 x Hor2596	S/S	TuOa82-1	307	225	(9:7)	.53
Tu17 x Hor2596	R/R	TuKe82-5	720	39	15:1	.23
Tu17 x Hor2596	R/R	MoMe84-5	721	51	15:1	.73
Tu17 x Hor2596	R/S	TuOa82-1	762	243	3:1	.57
Tu27 x Hor2596	R/R	TuKe82-5	333	27	15:1	.38
Tu27 x Hor2596	R/S	TuOa82-1	72	17	3:1	.24
Tu32 x Hor2596	R/R	TuKe82-5	168	15	15:1	.35
Tu32 x Hor2596	R/S	TuOa82-1	209	43	13:3	.54
Tu34 x Hor2596	R/R	TuKe82-5	176	16	15:1	.30
Tu34 x Hor2596	R/S	TuOa82-1	140	50	3:1	.73

[1] Reaction of first parent/second parent; R = resistant, S = susceptible.

[2] Ratios in parentheses were not expected, but gave the best fit.

Table 17. The reaction of F_2 barley seedlings to three isolates of *Puccinia hordei* (Estate x five land races).

Cross	Parental reaction[1]	Isolate	Observed Frequency resis.	susc.	Expected ratio	Probability
Tu16 x Estate	R/R	TuKe82-5	561	122	13:3	.58
Tu16 x Estate	R/R	MoMe84-5	628	157	13:3	.39
Tu16 x Estate	S/R	TuOa82-1	527	163	3:1	.43
Tu17 x Estate	R/R	TuKe82-5	710	46	15:1	.91
Tu17 x Estate	R/R	MoMe84-5	642	46	15:1	.69
Tu17 x Estate	R/R	TuOa82-1	377	22	15:1	.61
Tu27 x Estate	R/R	TuKe82-5	53	6	15:1	.32
Tu27 x Estate	R/R	TuOa82-1	158	15	15:1	.25
Tu32 x Estate	R/R	TuKe82-5	77	2	(15:1)[2]	.26
Tu32 x Estate	R/R	TuOa82-1	162	0	no seg.	
Tu34 x Estate	R/R	TuKe82-5	317	14	15:1	.16

[1] Reaction of first parent/second parent; R = resistant, S = susceptible.

[2] Ratios in parentheses were not expected, but gave the best fit.

Table 18. The reaction of F_2 barley seedlings to three isolates of *Puccinia hordei* (Cebada Capa x two land races).

Cross	Parental reaction[1]	Isolate	Observed Frequency resis.	susc.	Expected ratio	Probability
Tu16 x C.Capa	R/R	TuKe82-5	477	90	13:3	.26
Tu16 x C.Capa	R/R	MoMe84-5	770	61	15:1	.22
Tu16 x C.Capa	S/R	TuOa82-1	252	71	3:1	.23
Tu17 x C.Capa	R/R	TuKe82-5	640	0	no seg	-
Tu17 x C.Capa	R/R	MoMe84-5	552	0	no seg	-
Tu17 x C.Capa	R/R	TuOa82-1	565	34	15:1	.62

[1] Reaction of first parent/second parent; R = resistant, S = susceptible.

Segregation in Resistant x Susceptible crosses

Segregation results of the crosses involving the susceptible parent, RekaI, and five land race barley cultivars are shown in Table 15. Although Tu16 was susceptible to the virulent isolate TuOAa82-1, it was found to be moderately resistant to the other two isolates (Table 13). When crossed to the susceptible cultivar RekaI, the Chi-square test for goodness of fit of the F_2 progeny gave a good fit to a 1:3 (resistant:susceptible) ratio regardless of the virulence type of the isolate tested. Resistant plants were obtained when inoculated with the virulent isolate (Figure 1).

With the crosses involving RekaI and the other four land race cultivars (Tu17, Tu27, Tu32, and Tu34) the F_2 progeny fit a 3:1 ratio (Table 15) when tested against both the avirulent and the virulent isolates. In these crosses, almost all the plants of the population reacted as resistant or susceptible to the *P. hordei* isolates. (Figs. 2, 3, 4).

Segregation in Resistant x Resistant Crosses

With crosses involving barley cultivar Hor2596, carrier of resistance gene Pa_9, different segregation ratios in the F_2 progeny were observed, some of which were not expected (Figure 5).

The F_2 progeny of (Tu16 x Hor2596) segregated in a 13:3 ratio when tested with the avirulent isolate TuKe82-5, and yet gave a good fit to a 9:7 ratio when tested with the avirulent and virulent isolates MoMe84-5 and TuOa82-1, respectively (Table 16). The observed 9:7 was not expected, especially with the virulent isolate to which both parents were susceptible, and furthermore, Tu16 probably has a resistance gene. Even though Hor2596 has the dominant resistance gene Pa_9 (Clifford and Udeogalanya, 1976), this gene was not effective

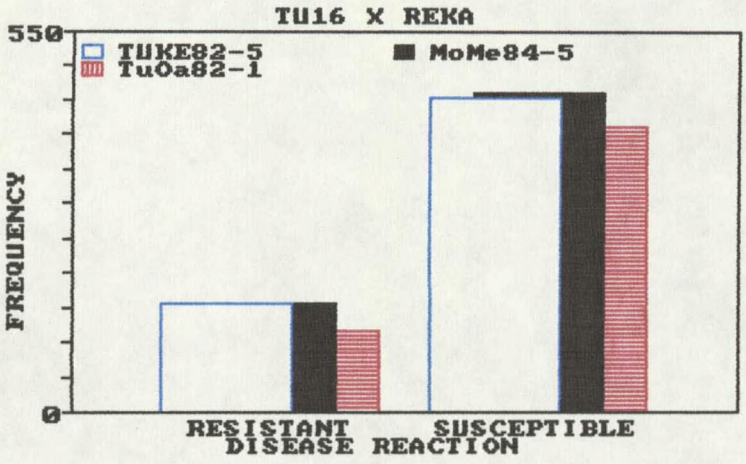

Figure 1. Frequency distribution of F_2 barley seedlings to three isolates of P. hordei (Tu16 x RekaI).

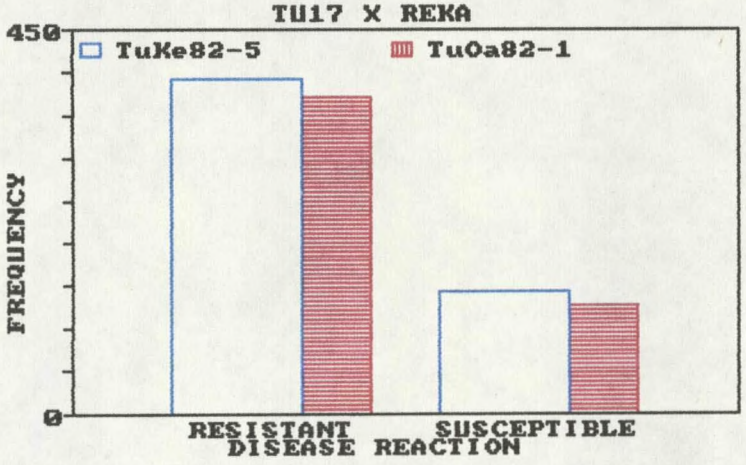

Figure 2. Frequency distribution of F_2 barley seedlings to two isolates of P. hordei (Tu17 x RekaI).

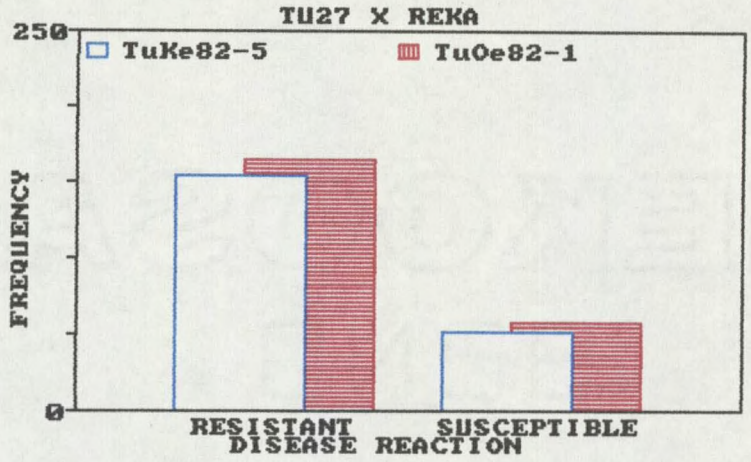

Figure 3. Frequency distribution of F_2 barley seedlings to two isolates of P. hordei (Tu32 x RekaI).

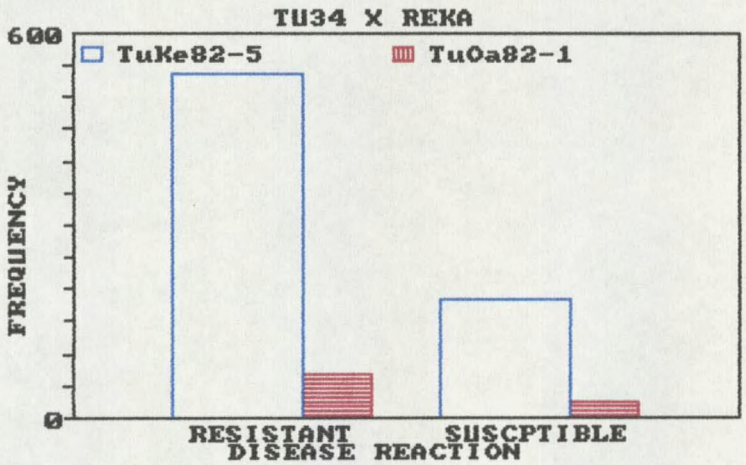

Figure 4. Frequency distribution of F_2 barley seedlings to two isolates of P. hordei (Tu34 x RekaI).

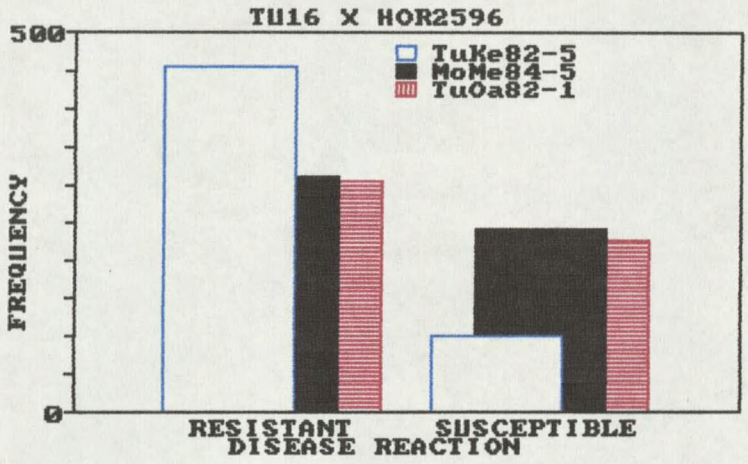

Figure 5. Frequency distribution of F_2 barley seedlings to three isolates of *P. hordei* (Tu16 x Hor2596).

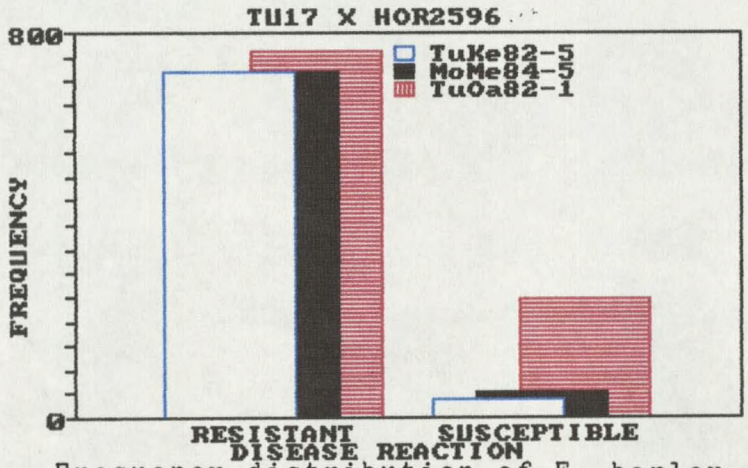

Figure 6. Frequency distribution of F_2 barley seedlings to three isolates of *P. hordei* (Tu17 x Hor2596).

against Tu0a82-1 *P. hordei* isolate, thus a 9:7 ratio would not be expected in the F_2 progeny of the Tu16 x Hor2596 cross. Since a 9:7 ratio implies complementary dominant gene action involving two genes, this would not be the case in this particular cross.

In crosses involving Hor2596 and the land race cultivars Tu17, Tu27, Tu32 and Tu34 (Figures 6, 7, 8), the F_2 progeny fit a 15:1 and a 3:1 ratios when tested with the avirulent isolate TuKe82-5, and the virulent isolate Tu0a82-1, respectively (Table 16). The F_2 progeny of (Tu32 x Hor2596) fit a 13:3 rather than a 3:1 ratio when tested with Tu0a82-1 (Table 16, Figure 8).

The cultivar Estate has the dominant resistance gene Pa_3 (Roane and Starling, 1970) which was effective against all *P. hordei* isolates (Part I). Crosses involving this cultivar and five resistant land races are shown in Table 17. The F_2 progeny of (Tu16 x Estate) segregated in a 13:3 and a 3:1 ratio when tested with the avirulent and virulent isolates respectively (Figure 9).

The F_2 progeny resulting from Tu17, Tu27 and Tu34 being crossed to Estate, fit a 15:1 ratio (Table 17, Figure 10, 11, 12). In the F_2 progeny of (Tu32 x Estate), there were no susceptible plants detected when

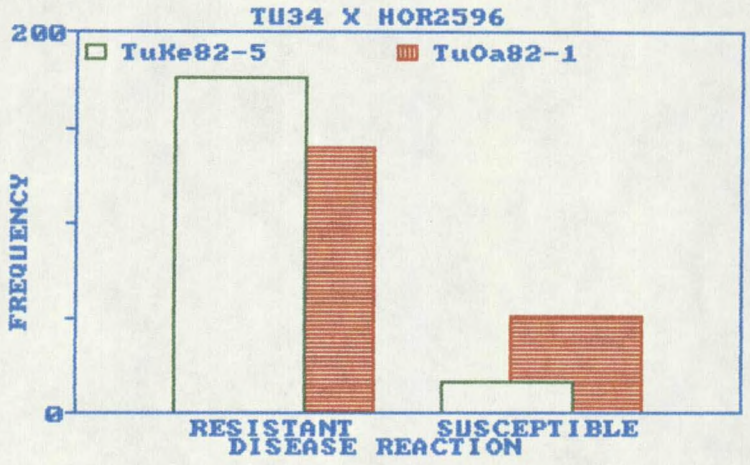

Figure 7. Frequency distribution of F_2 barley seedlings to two isolates of P. hordei (Tu34 x Hor2596).

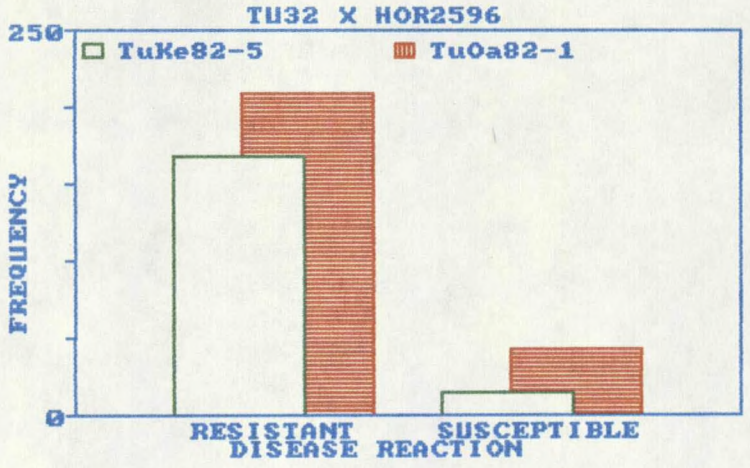

Figure 8. Frequency distribution of F_2 barley seedlings to two isolates of P. hordei (Tu32 x Hor2596).

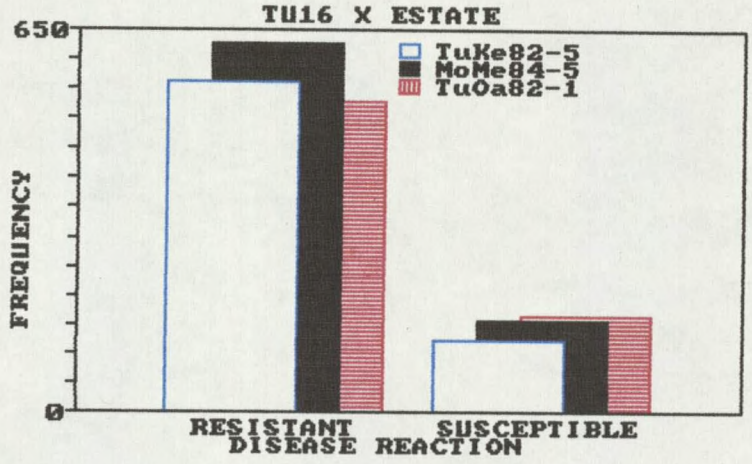

Figure 9. Frequency distribution of F_2 barley seedlings to three isolates of *P. hordei* (Tu16 x Estate).

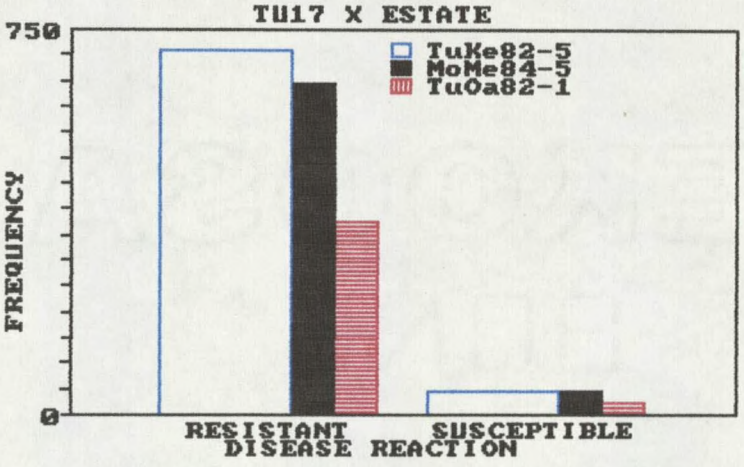

Figure 10. Frequency distribution of F_2 barley seedlings to three isolates of *P. hordei* (Tu17 x Estate).

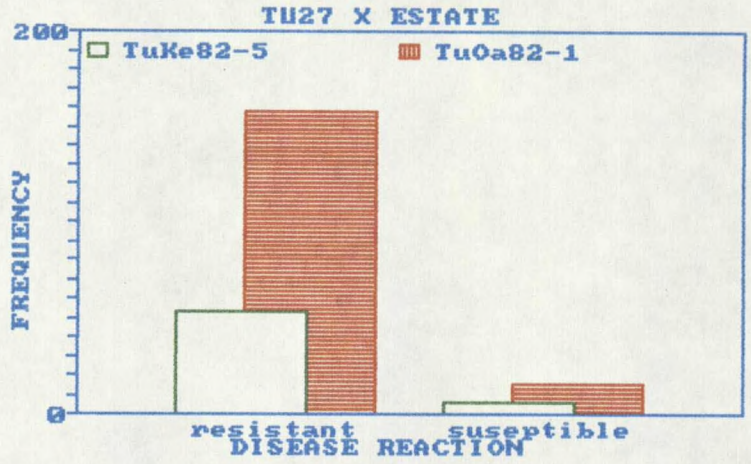

Figure 11. Frequency distribution of F_2 barley seedlings to two isolates of P. hordei (Tu27 x Estate).

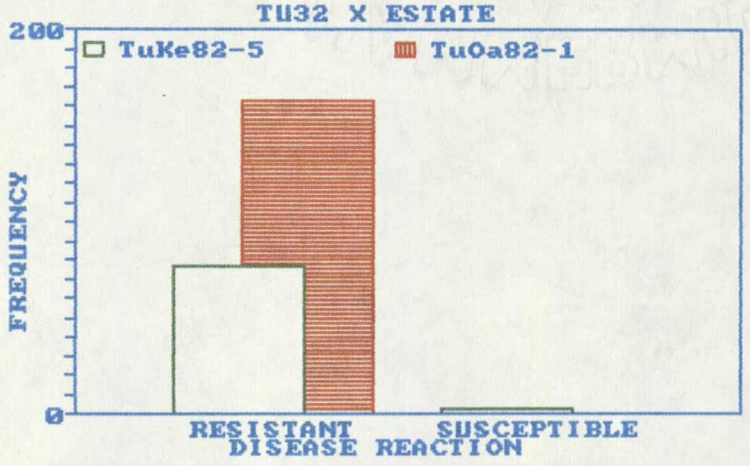

Figure 12. Frequency distribution of F_2 barley seedlings to two isolates of P. hordei (Tu32 x Estate).

tested with the virulent isolate (Figure 12). However, a good fit to a 15:1 ratio was observed when the F_2 progeny was tested with the avirulent isolate (Table 17). The latter ratio is questionable due to the low number of plants in the susceptible class which may have resulted from misclassification or mixed seed. Since no segregation was observed with the virulent isolate, this result was not expected with the avirulent isolate.

Crosses were also made with the resistant cultivar Cebada Capa which is resistant to all P. hordei isolates worldwide (Parlevliet, 1976; Reinhold and Sharp, 1982). The results pertinent to the crosses involving Cebada Capa are shown in Table 18. The F_2 progeny of (Tu16 x Cebada Capa) gave a good fit to a 13:3, 15:1 and 3:1 ratio when tested with TuKe82-5, MoMe84-5 and TuOa82-1, respectively (Figure 13). No susceptible plants were observed in the F_2 progeny of (Tu17 x Cebada Capa) when tested with the avirulent isolates, however a good fit to a 15:1 ratio was observed when the F_2 progeny was tested with the virulent isolate (Table 17, Figure 14).

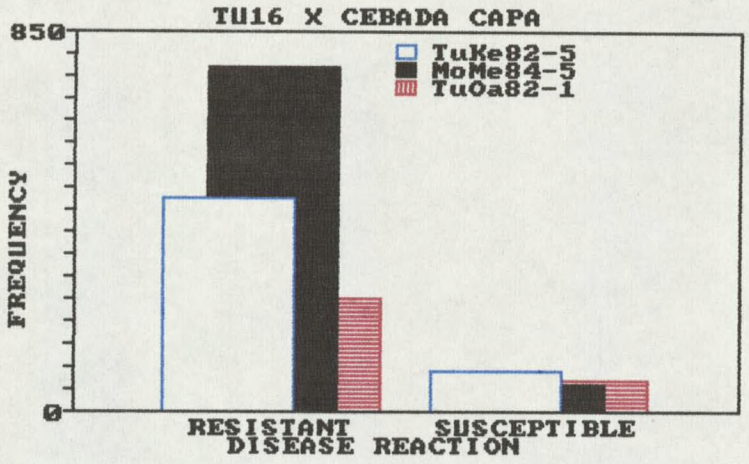

Figure 13. Frequency distribution of F_2 barley seedlings to three isolates of P. hordei (Tu16 x Cebada Capa).

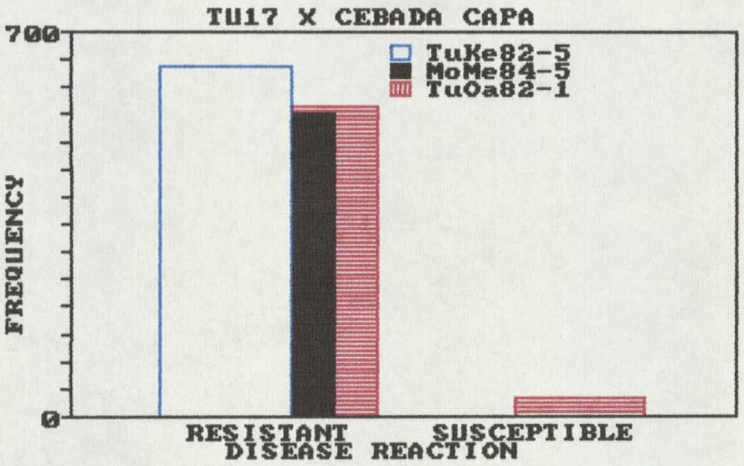

Figure 14. Frequency distribution of F_2 barley seedlings to three isolates of P. hordei (Tu17 x Cebada Capa).

Segregation in F_1, F_3, and backcross generations.

To further verify the hypotheses stated for the F_2 ratios, the F_3 and backcross hybrids in crosses with RekaI were tested (Table 19).

Table 19. The reaction of barley seedling progeny to two isolates of *Puccinia hordei* (RekaI x five land races).

Cross	Generation	Parental reaction[1]	Isolate	Observed Frequency resis.	segreg.	susc.	Expected ratio	Probability
Tu16 x RekaI	F_1	R/S	TuKe82-5	-	-	19		
Tu16 x RekaI	F_1	S/S	TuOa82-1	-	-	10		
Tu16xRekaI/RekaI	BC_1	R/S/S	TuKe82-5	-	-	19	0:1	1.00
Tu16xRekaI/Tu16	BC_2	R/S/S	TuKe82-5	12	-	15	1:1	.70
Tu16 x RekaI	F_3	R/S	TuKe82-5	12	24	17	1:2:1	.49
Tu16 x RekaI	F_3	S/S	TuOa82-1	8	24	13	1:8:7	.03
Tu17 x RekaI	F_1	R/S	TuKe82-5	13	-	-		
Tu17 x RekaI	F_1	R/S	TuOa82-1	19	-	-		
Tu17xRekaI/Tu17	BC_2	R/S/R	TuKe82-5	15	-	-	1:0	1.00
Tu17xRekaI/Tu17	BC_2	R/S/R	TuOa82-1	18	-	-	1:0	1.00
Tu17 x RekaI	F_3	R/S	TuKe82-5	8	12	2	1:2:1	.17
Tu17 x RekaI	F_3	R/S	TuOa82-1	16	26	13	1:2:1	.78
Tu27 x RekaI	F_1	R/S	TuKe82-5	10	-	-		
Tu27 x RekaI	F_1	R/S	TuOa82-1	9	-	-		
Tu27 x RekaI	F_3	R/S	TuKe82-5	11	19	13	1:2:1	.68
Tu27 x RekaI	F_3	R/S	TuOa82-1	10	13	4	1:2:1	.25
Tu32 x RekaI	F_1	R/S	TuOa82-1	10	-	-		
Tu32 x RekaI	F_3	R/S	TuOa82-1	16	28	15	1:2:1	.91
Tu34 x RekaI	F_1	R/S	TuKe82-5	9	-	-		
Tu34 x RekaI	F_1	R/S	TuOa82-1	10	-	-		
Tu34 x RekaI	F_3	R/S	TuKe82-5	12	22	14	1:2:1	.78
Tu34 x RekaI	F_3	R/S	TuOa82-1	3	24	5	1:2:1	.46

[1] Reaction of first parent/second parent; R = resistant, S = susceptible.

The F_3 progeny in (Tu16 x RekaI) fit a "one resistant:two segregants:one susceptible" (1:2:1) when tested with the avirulent isolates. The F_3 progeny of (Tu16 x RekaI), when inoculated with the virulent Tu0a82-1 isolate, did not fit the expected 1:8:7 ratio. The F_1 progeny of this cross was susceptible and when crossed to the resistant parent (BC_2) it gave a good fit to a 1:1 ratio, however when crossed to the susceptible parent (BC_1) a 0:1 ratio was observed.

The F_3 progeny of (Tu17 x RekaI) fit a 1:2:1 ratio, and a 1:0 ratio was observed in BC_1, and BC_2. A segregation ratio of 1:2:1 was also observed in the F_3 progeny of (Tu27 x RekaI), (Tu32 x RekaI) and (Tu34 x RekaI).

X. DISCUSSION

Many varieties of land races of barley grown in central and southern Tunisia have an adequate level of resistance to the leaf rust pathogen. In these remote areas of Tunisia there have been no planned programs for breeding for resistance to pests or to other stress tolerances such as, drought tolerance, heat tolerance, cold tolerance, salt tolerance etc. The barley cultivars grown in these regions are often mixtures of heterogenous genotypes that have been either discarded from commercial production over the years, or are collections of seeds that have been exchanged for other goods among nomad tribes along the southern parts of North Africa. Another alternative is that the seeds were handed down from generation to generation within the farming community in these regions.

The mixed barley cultivars or land races, regardless of their origin, could become a useful source of resistance. Incorporating this valuable material into a breeding program should be a relatively easy task since there should be no problems of sterility or other abnormalities that would occur in interspecific

hybridization. Furthermore undesirable agronomic traits that are usually derived from wild relatives do not have to be bred out when using land races. It appears that little attention, if any, has been paid to the direct exploitation of land race cultivars. In this present investigation, it can be seen that from the screening of a few land race barley cultivars collected over a short period of time, an adequate source of resistance to P. hordei and maybe resistance to other plant pathogens, was present and could easily be exploited. The five land races studied in this investigation were shown to have a good level of resistance to P. hordei, which was far better than that of the present commercially grown barley cultivars in Tunisia, and probably in other regions of the world as well.

In this investigation an attempt was made to study the genetics of resistance in these cultivars with respect to their reaction to P. hordei. Crosses were made between barley genotypes with four known resistance genes, and five land races, to determine if the resistance factor(s) in these lines were dominant or recessive, and whether they were controlled by one, few, or many genes.

For the purposes of this study, the three P. hordei isolates used to test the segregating populations differed in their virulence. The avirulent isolate used allowed the detection of the largest possible number of resistance gene(s), whereas the differentially virulent isolate allowed further classification of the segregating progenies to be made. It is important, though, to emphasize that a particular isolate of the P. hordei pathogen may be comprised of different genotypes. These genotypes have only one essential factor in common, which is the possession of a virulence gene able to overcome resistance controlled by a corresponding gene in the host. Thus a single isolate may consist of individuals which have a virulence gene in common but differ in virulence to other host cultivars with other resistance genes.

It must be emphasized that the result of cultivar inoculation with a specific isolate of the pathogen is an interaction of corresponding resistance and avirulence genes giving an infection type. This in turn, gives rise to some degree of resistance. If the critical corresponding genes in the pathogen are virulent, the host is susceptible regardless of whether or not it has resistance genes (Appendix, Table 23). In cases of

quantitative inheritance, several interacting genes in both the host and pathogen may be required to give effective resistance (Appendix, Table 24). This premise is used throughout this thesis in interpretation of results.

In crosses with the susceptible cultivar RekaI, a monogenic mode of inheritance was suggested, with the avirulent isolates. F_3 and backcross results (Table 19) support this hypothesis. RekaI has a dominant gene (gene complex) Pa_{2+}, which, even though ineffective against a vast majority of the Tunisian isolates (Appendix, Table 22), was found to confer resistance to a virulent *P. hordei* isolate from Sakha, Egypt (Reinhold and Sharp, 1982). Nevertheless, the resistant reaction detected could be due to a dosage effect. Resistance genes from RekaI and Tu16 interacted in a complementary manner and conditioned resistance to the virulent Tu0a82-1 isolate. It appears that two dominant alleles at one locus and at least one dominant allele for resistance at another locus were required to counter the virulence gene(s) in the Tu0a82-1 isolate. It could be possible that the Pa_{2+} gene complex in RekaI contributed to the resistance in the segregants of (Tu16 x RekaI). This also could have been due to some other factors in the background of

either parent, such as the presence of minor genes.

In the interaction of (Tu16 x RekaI) with the virulent isolate, F_3 data (Table 19) failed to confirm the 1:3 F_2 ratio. This could have been due to either inadequate F_2 family sample sizes or experimental error in disease classification. More data are needed to either confirm the F_2 ratio proposal or another mode of inheritance.

When the remaining four land race cultivars were crossed to RekaI, the segregation ratios observed in the F_2, F_3, and BC generations implied a monogenic inheritance pattern. These cultivars could be considered as carriers of a dominant resistance gene.

The results presented for the F_2 progenies from the crosses of the five land race cultivars to four barley differentials with known resistance genes, suggest that the resistance genes in the land race cultivars were different from the Pa_3, Pa_7, and Pa_9 resistance genes.

The resistance gene in the cultivar Tu16 appears to be ineffective against the virulent isolates, but effective against the avirulent ones. Evidence of this was presented with the data from (Tu16 x Estate) and (Tu16 x Cebada Capa), especially if one contrasts the reactions of the F_2 progenies that resulted when tested

with the avirulent TuKe82-5 and virulent Tu0a82-1 isolates. A monogenic mode of inheritance was observed with the virulent isolate. The resistance gene in Tu16 was ineffective against Tu0a82-1 but interacted in a complementary manner with Pa_3 in the presence of the avirulent isolates. In (Tu16 x Hor2596), a 9:7 ratio was unexpectedly observed in the F_2 progeny. Hor2596 has the single dominant gene, Pa_9, which is temperature sensitive (Clifford and Udeogalanya, 1976). Temperature could have had an effect on the hybrid progeny, especially when Pa_9 was in a different genetic background other than that of Hor2596. Also, not enough is known about Tu16 to conclusively attribute its resistance to solely a single gene. Minor genes maybe present in both parents and could have affected reactions of progeny. Thus altering expression of major genes. There was also the possibility of accidental seed mixture or impurity of the parental seed source.

Results of testing F_2 progeny of Tu17, Tu27, and Tu34 crossed to either Hor2596 or Estate, suggested the presence of a dominant gene in each of these cultivars. These genes were also different from Pa_3 and Pa_9. Tu32, likewise possessed a dominant resistance gene as suggested by the F_2 progeny (Tu32 x Hor2596). However,

no susceptible plants were observed when Tu32 was crossed to Estate. The absence of recombinant types implies that both parents have the same resistance gene as was the case with Cebada Capa, La Estanzuela, Gondar, and Dabat barley cultivars (Parlevliet, 1976). In addition, two closely linked loci could be involved rather that a single locus. In order to distinguish between the resistance factor(s) involved in this cross, screening of the F_3 progeny would be necessary; using an isolate virulent on either parent.

The merit of using isolates of different virulence levels can be seen in the cross of Tu17 x Cebada Capa. The absence of susceptible plants in the F_2 progeny, when tested with the avirulent isolates, suggests that Tu17 and Cebada Capa have a gene in common, implying Pa_7, or that two closely linked loci could be involved. The results of the F_2 progeny when inoculated with the virulent isolate showed a 15:1 ratio implying the presence of two dominant genes. The segregation ratio obtained in the latter case could be due to either the presence of another resistance factor in Tu17 or in Cebada Capa, or it could be due to the presence of a virulence factor associated with Tu0a82-1. To clarify the results obtained with the avirulent and virulent

isolates. The virulence factor in Tu0a82-1 should be considered in this interaction (Appendix, Table 23).

The rate at which the P. hordei pathogen adapts to a resistant host can be reduced by using diversified sources of resistance. For this diversification to be efficient, it should be controlled relative to the virulence composition of the pathogen. This requires a thorough monitoring of the pathogen population. The diversification of resistance can be accomplished by various strategies as discussed below, and hence, will increase the durability of resistance.

A combination of different resistances, even if they are each controlled by a single gene, may be more difficult for a pathogen to adapt to than a single gene mechanism. A number of vertical genes (Pa_3, Pa_5, Pa_7, etc.) available in different barley cultivars (Roane and Starling, 1970; Clifford, 1974; Parlevliet, 1976) can confer adequate protection against P. hordei. However, a new race will need to change only one virulence gene to overcome a single vertical gene for resistance in the host. To circumvent this risk, the use of multiple genes in a common background could be applied, and a durable resistance in barley cultivars could be realized. In this system, two or more new and still effective

resistance genes could be placed into a new cultivar so that the pathogen population has a barrier of several resistance genes presented to it, simultaneously. This should be an effective strategy, because, for a new race to overcome the multiple resistance genes, it must go through two or three simultaneous changes toward virulence, which is quite unlikely.

Multiple sources of resistance could be developed using the adapted and resistant land race cultivars in combination with effective resistance genes, such as Pa_3 and Pa_7. For instance, in the F_2 progeny of (Estate x resistant land races) and (Cebada Capa x resistant land races), the resistant lines from each population could be intercrossed. Since the resistance background of the intercrossed lines is likely to be different, a considerable improvement could be expected.

The use of multilines is another strategy that is often overlooked in barley, and would allow the enhancement of durability of resistance to *P. hordei*. The land race cultivars analyzed are good candidates to be used in such a strategy. With the exception of Tu17, the other four land races were quite similar in maturity and were adapted to dry land conditions. Together, they could make an excellent mixture and could serve the same

purpose as a multiline. The resistance genes in these cultivars were probably different, but further testing is needed to be conclusive.

Deploying single host genes over a wide agricultural area in a monoculture can be potentially dangerous. An ideal system would be to deploy effective resistance genes over time or in restricted geographic areas. Varieties with different resistance factors can be grown. Then, whenever their resistance breaks down, they should be removed and new varieties with different resistance genes should be introduced. If varieties that have different resistance genes are available, it is possible to recycle them after they have been removed from production for a period of time. This same system could be applied in the deployment over space. In this case, different resistance sources could be planted in different restricted geographic areas. For this strategy to be effective, an adequate and intensive disease survey should be maintained.

The success of the strategies discussed above will depend on the resistance genes involved. For instance, Pa_9 was found to be temperature sensitive (Clifford and Udeogalanya, 1976) and its effectiveness is questionable in semi-arid climates that are characterized by hot

weather. Pa_7, on the other hand, gave resistance to all P. hordei isolates tested in this study and in other studies (Parlevliet, 1976; Reinhold and Sharp, 1982); as yet there is no report of a breakdown in resistance in Pa_7.

It is of major importance to test the worth of resistance genes before they are used in breeding programs. For a gene like Pa_7, a new race(s) that will attack it could be created. A virulent isolate such as the Tu0a82-1 or the Sakka isolate (Reinhold and Sharp, 1976), or any other virulent isolate could be treated with a mutagen. The mutant clones could be tested on the resistant Pa_7 gene. A mutant that would attack this gene would represent a new race. Any plant genotype that is resistant to this new race must have an unknown gene for resistance in addition to the original resistance gene. This resistant plant must have two genes effective against all races in the field. The mutant race is produced on the basis of known resistance genes, and in that sense, the mutant is a future race. The resistant genotypes used in the field will select for races with virulent genes that can overcome those resistance genes.

XI. SUMMARY AND CONCLUSIONS

Leaf rust, P. hordei, presents a real danger to barley growers in Tunisia. The variability in virulence patterns, the diversification in pathogenicity, and the distribution of virulence types in different geographic regions in Tunisia have been shown. The genetic studies indicate that the barley land race cultivars have effective sources of resistance to P. hordei. The resistance genes identified were different from those previously known (except for the Tu32 resistance gene). In summary, then, the following points can be made with respect to the epidemiology of P. hordei and the new sources of resistance.

1. Several virulence patterns of P. hordei were identified throughout the country of Tunisia.
2. Most of the P. hordei isolates were site specific, but several were found in more than one geographic region.
3. Using the virulence formula method to compare virulence patterns of the P. hordei isolates, new virulences were identified from different sites and in different years.

4. New virulent patterns resulted from recombination of *P. hordei* on the alternate host, *Ornithogalum* spp.

5. There are strong indications that in the Northern and Northwestern parts of Tunisia, *P. hordei* completes it life cycle on *Ornithogalum* spp.; whereas in the South, it oversummers on volunteer barleys in the Oasis.

6. Pa_3 and Pa_7 resistance genes were effective against all Tunisian *P. hordei* isolates.

7. The genetic analyses of crosses between land race and known resistance sources of barley cultivars suggest that, with the exception of Tu32, the resistance genes in these barley cultivars were different from those genes previously identified.

   - - Tu16 was shown to possess a dominant resistance gene that interacted in a quantitative manner when associated with Pa_{2+}.
   - - Tu17, Tu27, and Tu34 each have a dominant resistance gene.

8. Cultivation of these land races in a multiline mixture is advocated.

9. Additional genetic studies should be conducted to determine the relationships between the resistance genes in the land race cultivars.

In conclusion, to be effective, diverse gene pools should be used in breeding programs. Not only do new gene pools provide the necessary building blocks for further varietal improvement, but genetic diversity is essential, if high levels of productivity are to be sustained. New varieties with adequate resistance could be developed within a few years, if sufficient resources are provided for this vital work.

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APPENDIX

Table 20. Initial studies of physiologic specialization in the cereal rust diseases caused by *Puccinia* spp.[1]

Host	Pathogen (*Puccinia*) Species	Number of: Host differentials	Number of: Pathogen races	Date published	Author(s)
Triticum spp.	*graminis* f. sp. *tritici*	-	-	1917	Stakman and Piemcival
Triticum spp.	*graminis* f. sp. *tritici*	12	-	1922	Stakman and Levine
Avena spp.	*coronata* f. sp. *avenae*	2	4	1919	Hoerner
Avena spp.	*graminis* f. sp. *avenae*	3	5	1923	Stakman et al.
Triticum spp.	*recondita* f. sp. *tritici*	7	12	1926	Mains and Jackson
Secalis	*recondita* f. sp. *secalis*	1	2	1926	Mains
Hordeum	*hordei*	2	2	1926	Mains
Zea	*sorghi*	3	4	1926	Mains
Triticum	*striiformis*	6	4	1930	Allison and Isenbeck
Secalis	*graminis* f. sp. *secalis*	5	3	1932	Cotter and Levine

[1] from Roelfs (1984).

Table 21. Description of infection types used in physiologic specialization studies of the cereal rusts[1]

Host Response (Class)	Disease: Infection Type		Disease: Symptoms
Immune (Res)	0	low	no uredia or other macroscopic sign of infection
Nearly Immune (Res)	0;	low	no uredia, but hypersensitive necrotic or chlorotic flecks of varying size present
Very Resistant (Res)	1	low	small uredia surrounded by necrosis
Moderately Resistant (I)	2	low	small to medium uredia, often surrounded by chlorosis or necrosis, green island may be surrounded by chlorotic or necrotic border
Moderately Susceptible (S)	3		medium sized uredia that may be associated with chlorosis or rarely necrosis
Susceptible (S)	4		high amount uredia without chlorosis or necrosis

[1] Roelfs and McVey (1979); Stakman et al. (1962).

Table 22. Virulence patterns of *Puccinia hordei* isolates sampled in Tunisia in 1982, 1983 and 1984.

	Differential Host Genotypes												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Isolate	Esta Pa_3	C.Ca Pa_7	Hor Pa_9	Rica Pa_{2+}	Bolivia Pa_2+Pa_6	Quinn Pa_2+Pa_5	Magn Pa_5	Peru Pa_2	Suda Pa	Egyp Pa_8	Batn Pa_{2+}	Gold Pa_4	Reka Pa_{2+}
BEJA													
TuBj82-1	R	R	R	R	R	S	S	I	S	S	S	S	S
-2	R	R	R	R	S	S	S	I	S	R	S	S	S
-3	R	R	R	R	S	S	S	S	S	R	S	S	S
-4	R	R	R	R	S	S	S	S	S	S	S	S	S
-5	R	R	I	S	S	S	S	S	S	S	S	S	S
-6	R	R	S	S	S	S	S	S	S	S	S	S	S
-7	R	R	I	I	S	R	I	S	R	S	S	S	S
-8	R	R	I	S	S	I	S	S	S	S	S	S	S
-9	R	R	R	R	R	S	S	I	S	R	S	I	S
-10	R	R	R	R	R	I	R	S	S	S	S	S	S
-11	R	R	S	R	S	S	S	S	S	S	S	S	S
TuBj84-1	R	R	S	R	R	S	S	R	R	S	S	S	S
-2	R	R	R	R	S	I	S	S	S	S	R	S	S
-3	R	R	I	R	S	I	S	S	S	S	S	S	S
-4	R	R	R	I	S	S	S	S	S	S	I	S	S
-5	R	R	S	S	S	S	S	S	S	S	S	S	S
-6	R	R	S	S	S	S	S	S	S	S	S	S	S
-7	R	R	S	R	S	S	S	S	S	I	I	S	S
-8	R	R	I	R	S	S	S	R	S	S	R	S	S
KEF													
TuKe82-1	R	R	R	S	S	S	I	S	S	S	S	S	S
-2	R	R	R	R	R	R	R	I	R	S	R	S	S
-3	R	R	R	R	S	S	S	S	S	S	S	S	S
-4	R	R	R	R	R	R	I	I	R	S	R	S	S
-5	R	R	R	R	R	R	R	S	R	S	R	S	S
-6	R	R	R	R	R	S	S	I	S	S	S	S	S
-7	R	R	I	R	S	S	S	S	S	S	S	S	S
-8	R	R	R	S	S	S	S	S	S	S	S	S	S
-9	R	R	S	S	S	S	S	S	S	S	S	S	S
-10	R	R	I	I	S	S	S	S	S	S	S	S	S
TuKe84-1	R	R	S	S	S	S	S	S	S	S	S	S	S
-2	R	R	R	I	S	S	S	S	S	S	S	S	S
-3	R	R	S	S	S	S	S	S	S	S	S	S	S

† Resistance genes: R = resistant (effective), I = intermediate (effective), S = susceptible (ineffective).

Table 22. (Continued)

Isolate	Differential Host Genotypes												
	1 Esta Pa_3	2 C.Ca Pa_7	3 Hor Pa_9	4 Rica Pa_{2+}	5 Bolivia Pa_2+Pa_6	6 Quinn Pa_2+Pa_5	7 Magn Pa_5	8 Peru Pa_2	9 Suda Pa	10 Egyp Pa_8	11 Batn Pa_{2+}	12 Gold Pa_4	13 Reka Pa_{2+}
MATEUR													
TuMa82-1	R	R	R	I	I	I	S	I	R	S	S	S	S
TuMa83-1	R	R	S	R	S	S	S	S	S	S	S	S	S
-2	R	R	R	R	S	I	S	I	R	S	I	S	S
-3	R	R	I	I	R	S	S	S	I	S	I	S	S
-4	R	R	S	S	S	S	S	S	I	S	S	S	S
-5	R	R	I	I	I	S	S	S	I	S	S	S	R
-6	R	R	S	S	S	S	S	S	S	S	S	R	I
-7	R	R	I	S	S	S	S	S	S	S	S	S	S
-8	R	R	S	S	S	S	S	S	S	S	S	S	S
-9	R	R	R	R	R	R	R	R	R	R	R	R	S
-10	R	R	S	I	S	S	S	S	S	S	S	S	S
-11	R	R	R	R	S	I	S	S	S	S	S	S	S
-12	R	R	I	S	S	R	R	S	R	S	S	S	S
-13	R	R	S	S	S	R	R	S	R	S	S	S	S
-14	R	R	S	I	R	R	R	S	R	S	S	S	S
-15	R	R	I	R	S	R	R	I	S	S	I	S	S
-16	R	R	S	I	S	R	R	S	R	S	S	S	S
TuMa84-1	R	R	S	R	S	S	S	S	S	S	S	S	S
-2	R	R	R	S	S	S	S	S	S	S	S	S	S
-3	R	R	R	S	S	S	S	S	S	S	S	S	S
-4	R	R	R	R	S	S	S	S	S	S	S	S	S
-5	R	R	R	R	S	S	S	S	S	S	S	S	S
-6	R	R	I	R	S	S	S	S	S	S	S	S	S
OASIS													
TuOa82-1	R	R	S	S	S	S	S	S	S	S	S	S	S
-2	R	R	I	S	S	S	S	S	S	S	S	S	S
-3	R	R	I	I	I	R	S	S	I	S	R	S	S
-4	R	R	R	S	S	I	I	R	S	S	I	R	S
-5	R	R	R	S	S	S	S	S	S	S	S	S	S
-6	R	R	R	R	R	S	S	S	S	R	R	S	S
-7	R	R	S	I	S	R	S	I	I	S	S	S	S
-8	R	R	I	R	I	I	S	S	S	S	I	S	S

[1] Resistance genes: R = resistant (effective), I = intermediate (effective), S = susceptible (ineffective).

Table 22. (continued)

Isolate	Differential Host Genotypes												
	1 Esta Pa_3	2 C.Ca Pa_7	3 Hor Pa_9	4 Rica Pa_{2+}	5 Bolivia Pa_2+Pa_6	6 Quinn Pa_2+Pa_5	7 Magn Pa_5	8 Peru Pa_2	9 Suda Pa	10 Egyp Pa_8	11 Batn Pa_{2+}	12 Gold Pa_4	13 Reka Pa_{2+}
EL JEM													
TuEj83-1	R	R	R	R	R	R	I	S	S	S	S	S	S
MARETH													
TuMe83-1	R	R	R	R	R	S	I	I	S	I	S	S	S
-2	R	R	R	I	S	S	S	S	S	S	S	S	S
-3	R	R	R	R	S	S	S	S	S	S	S	S	S
-4	R	R	S	S	S	S	S	S	S	S	S	S	S
-5	R	R	I	R	S	S	S	S	S	S	S	S	S
-6	R	R	R	S	S	R	R	S	S	S	I	S	S
-7	R	R	S	S	S	S	S	S	S	S	S	S	S
-8	R	R	S	S	S	S	S	S	S	S	S	S	S
-9	R	R	I	R	S	S	S	S	S	S	S	S	S
-10	R	R	R	R	R	S	S	I	S	I	S	S	S
BOURBIA													
TuBr82-1	R	R	R	R	R	I	S	S	S	S	S	S	S
-2	R	R	R	R	I	S	I	S	S	S	S	S	S
KAIROUAN													
TuKa82-1	R	R	I	R	S	S	S	S	S	S	S	S	S
-2	R	R	R	R	S	S	S	S	S	S	S	S	S

[1] Resistance genes: R = resistant (effective), I = intermediate (effective), S = susceptible (ineffective).

Table 23. Possible genotypes involved in the interaction between (Tu17 x Cebada Capa) progeny and (TuKe82-5, Tu0a82-1) *P. hordei* isolates.

Host Genotypes	Genotype of *P. hordei* isolates and interactions[1] TuKe82-5	Int[2]	Tu0a82-1	Int[2]
AAbbCC (Tu17)	AABBCC	R	AABBcc	R
aaBBCC (Cebada Capa)	AABBCC	R	AABBcc	R
AaBbCC (F_1)	AABBCC	R	AABBcc	R
gene frequency F_2 progeny				
1 AABBCC	AABBCC	R	AABBcc	R
2 AABbCC	AABBCC	R	AABBcc	R
1 AAbbCC	AABBCC	R	AABBcc	R
2 AaBBCC	AABBCC	R	AABBcc	R
4 AaBbCC	AABBCC	R	AABBcc	R
2 AabbCC	AABBCC	R	AABBcc	R
1 aaBBCC	AABBCC	R	AABBcc	R
2 aaBbCC	AABBCC	R	AABBcc	R
1 aabbCC	AABBCC	R	AABBcc	S
F_2 segregation ratio		no seg.		15:1

1 Both isolates TuKe82-5 (AABBCC) and Tu0a82-1 (AABBcc) are avirulent on Cebada Capa (aaBBCC) and Tu17 (AAbbCC). The two cultivars have 'CC' in common. F_2 progeny gave some susceptible plants when inoculated with Tu0a82-1 and none with TuKe82-5 because of the 'cc'virulence gene in Tu0a82-5.

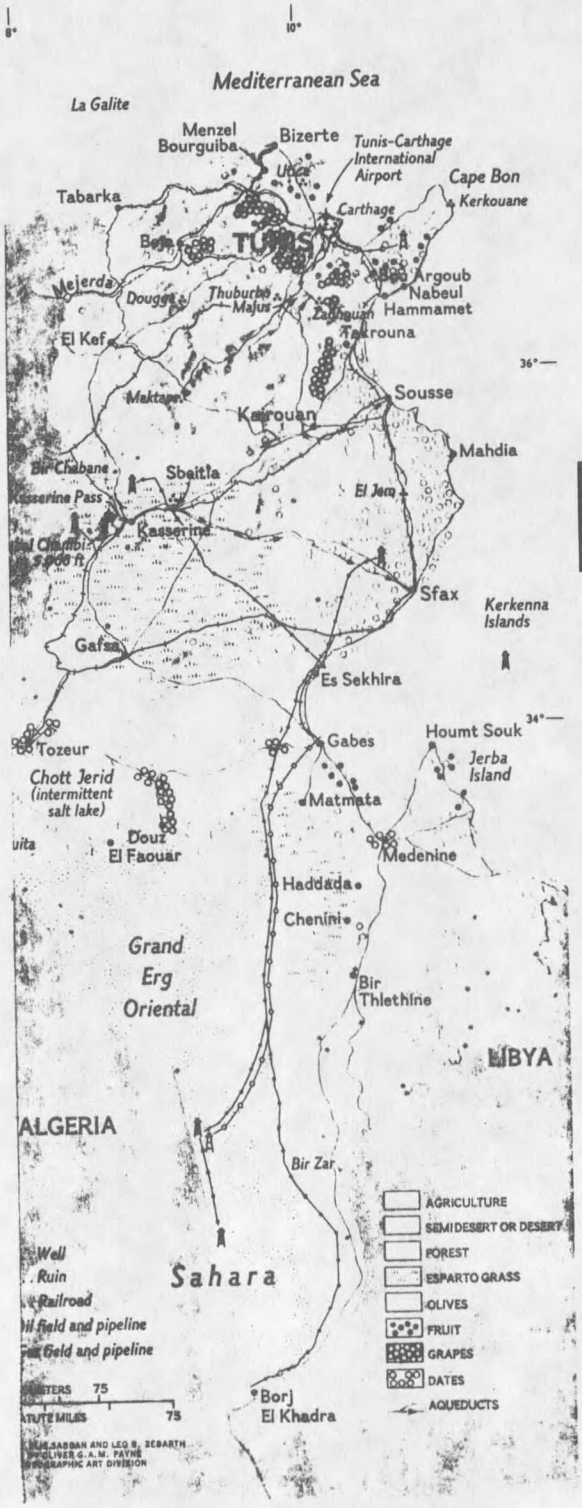
2 Int = interaction

Table 24. Possible genotypes involved in the interaction between (Tu16 x RekaI) progeny and (TuOa82-5) *P. hordei* isolate.

Host Genotypes	Genotype of *P. hordei* isolate and interaction[1] TuOa82-1	Interaction
AAbbcc (Tu16)	AABBcc	S
aaBBcc (RekaI)	AABBcc	S
AaBbcc (F_1)	AABBcc	S
gene frequency F_2 progeny		
1 AABBcc	AABBcc	R
2 AABbcc	AABBcc	R
1 AAbbcc	AABBcc	S
2 AaBBcc	AABBcc	R
4 AaBbcc	AABBcc	S
2 Aabbcc	AABBcc	S
1 aaBBcc	AABBcc	S
2 aaBbcc	AABBcc	S
1 aabbcc	AABBcc	S
F_2 segregation ratio		5:11 (1:3)

1 BB (host) in combination with BB (pathogen) = not effective, i.e. susceptible reaction. AA (host) in combination with AA (pathogen) = not effective, i.e. susceptible reaction. Two dominant alleles at one locus and at least one dominant allele at another locus are required to counter the virulence of 'cc', and the avirulence of 'AA' and 'BB' genes of the pathogen in a quantitative manner.

Resistant types can result from (susceptible x susceptible) depending on virulence genes present in the pathogen. All plants in (Tu16 x RekaI) would be susceptible if TuOa82-1 has two virulence genes, i.e. 'AAbbcc'.



TUNISIA

THIRTY CENTURIES AGO Phoenicians were the first to establish colonies on the coast of a land inhabited by a people who would later be called Berbers. Among those outposts Carthage grew to a rich and powerful city-state that traded with, then threatened, Rome.

"Delenda est Carthago—Carthage must be destroyed," was the unflinching aim of the Roman statesman Cato. And, despite the brilliant victories of Hannibal, it was destroyed in 146 B.C. Utterly.

The conquering Romans built and rebuilt and improved the water supply with aqueducts, one of which is still in use.

In time and in turn others came to control the land: Vandals, Byzantines, Arabs, Turks, and French. Not until 1957 was the Republic of Tunisia proclaimed under the leadership of Habib Bourguiba, who has since been made president for life. With traditions from both the Islamic and European worlds, Tunisia has followed a nonaligned foreign policy friendly to the West, while concentrating on social and economic development. As with other developing countries, population has grown faster than jobs; many Tunisians work abroad, primarily in France and Libya.

AREA: 164,200 sq km (63,400 sq mi). **POPULATION:** 6,000,000. **LANGUAGES:** Arabic, French. **RELIGION:** 98% Muslim. **ECONOMY:** Agriculture, tourism, textiles, phosphate, fishing, modest petroleum reserves and processing. **MAJOR CITY:** Tunis, capital, pop. 1,000,000. **CLIMATE:** Temperate in the north; hot, almost wholly arid desert in the south.

(from National Geographic, Feb.1980)

Figure 15. Map of Tunisia