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ORIGINAL RESEARCH

An integrated multivariate approach to net blotch of barley: Virulence quantification, pathotyping and a breeding strategy for disease resistance

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Abstract Knowledge of pathotype diversity and virulence in local populations of *Pyrenophora teres* is a prerequisite to screening for durable resistance to net blotch. The current study aimed to quantify the virulence level of Moroccan isolates, identify and designate existing pathotypes, and select resistant genotypes. We developed a method for virulence quantification of *P. teres* isolates based on a conversion of infection responses into frequencies for use in correspondence analysis. Coordinates of the first axis of this analysis had a virulence spectrum and ranked isolates from virulent to avirulent. Mixed model analysis was also devised for virulence quantification. Coordinates of the first dimension of correspondence analysis were linearly correlated to BLUPs (Best Linear Unbiased Predictors) of the mixed model. A genotype by genotype by environment model (GGE) coupled with cluster analysis differentiated *P. teres* isolates into ten and nine pathotypes for net- and spot-forms respectively. Populations of these two forms were dissimilar in terms of classes of virulence. For *P. teres* f. *maculata*, avirulent, moderately virulent and highly virulent isolates represented one-third of the population, whereas 90% of *P. teres* f. *teres* population was composed of avirulent to moderately avirulent isolates. Barley differential sets were subsequently reduced to two new sets that simplified pathotyping through a key code based on resistant or susceptible reactions. Dendrograms of cluster analysis based on GGE analysis depicted the stability of a genotype's reactions across all isolates, and using only resistant cultivars as sources of resistance to control net blotch disease would, based on this analysis, fail to control all pathotypes. Therefore, we propose an alternative breeding strategy to control net blotch effectively.

Keywords Barley (*Hordeum vulgare* L.) · Correspondence analysis · GGE model · Mixed model · *Pyrenophora teres* · Pathotypes · Virulence quantification

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Introduction

Barley (*Hordeum vulgare* L.) is one of the major cereal crops in Morocco. It represents more than one third of the total area sown to cereals. Annually, barley covers more than 2 million hectares of which more than 70% are in the arid and semi-arid regions that are known for their harsh environment with annual rainfall of 80–350 mm (Saidi et al. 2005).

Net blotch is a major foliar disease that hampers barley production and is widely distributed in most barley-growing regions of the world (Steffenson and Webster 1992; Douiyssi et al. 1998; El Yousfi and Ezzahiri 2001). In Morocco, average yield losses range from 14 to 29%, with resistant varieties outyielding susceptible ones by 39% under no disease control and by 56% under fungicide treatment (El Yousfi and Ezzahiri 2002).

Pyrenophora teres (anamorph *Drechslera teres* (Sacc.) Shoem. syn. *Helminthosporium teres*) is the causal agent of barley net blotch disease. It prevails in two forms, one producing net-type, and the other spot-type symptoms (Tekauz 1990). Infection by *P. teres* f. *teres* causes lesions that initially appear as spots and short streaks on leaves. These infection sites expand into longer longitudinal and transverse necrotic streaks that produce a net-type pattern on susceptible genotypes. McDonald (1967) described a second form of *P. teres* that was designated later as *Pyrenophora teres* Drechs. f. *maculata* Smedeg. (Smedegard-Petersen 1971). This form causes elliptical lesions that are different from the typical reticulate type. The two forms are morphologically indistinguishable (Crous et al. 1995), and sometime, it is difficult to distinguish them under field conditions because their symptoms depend on both environmental conditions and/or on genotype response (Williams et al. 2001). Currently, the two forms can be easily discriminated using molecular markers (Williams et al. 2001).

The variability in pathogenicity of *P. teres,* and the differential responses of barley lines to various isolates has been investigated for breeding purposes in many parts of the world. In western Canada, *Pyrenophora teres* f. *teres* (Ptt) was reported to be the more prevalent causal agent compared to *Pyrenophora teres* f. *maculata* (Ptm) (Tekauz 1990). Tekauz (1990) found that 82% of 224 isolates from western Canada were *P. teres* f. *teres* and only 18% were *P. teres* f. *maculata*. Other research with isolates from Morocco, Tunisia, Turkey and Montana (USA), demonstrated that some cultivars resistant to Ptt were generally not resistant to Ptm isolates causing spot-type symptoms (Bockelman et al. 1983).

The virulence of *P. teres* isolates varies on differential barley genotypes and distinct pathotypes have been identified (Tekauz 1990; Steffenson and Webster 1992; Robinson and Jalli 1996; Cromey and Parkes 2003). However, the success of screening for disease resistance depends on determination of diversity in the pathogen population based on the host/pathogen interaction (H×P) (Tekauz 1990; Cromey and Parkes 2003). This H × P interaction forms the basis of any pathotype differentiation. Its structure is an important trait for both virulence studies and studies of disease resistance. A significant H × P interaction for quantitative traits such as disease severity and infection type can significantly limit gains in selecting superior genotypes. The larger the H × P interaction the lesser is the likelihood of advances being made in selections for resistant lines, especially when significant crossover interactions are present (Crossa et al. 2002). In this regard, understanding the variation and distribution of virulent isolates of *P. teres* is important to achieving success in a resistance-breeding program.

The purpose of this study was three fold. Firstly, to quantify the virulence levels of Moroccan isolates of *Pyrenophora teres* f. *teres* and *P. teres* f. *maculata* from the semi-arid region of Morocco. Secondly, to identify and characterize the isolates into pathotypes. Thirdly, to select resistant genotypes and propose a breeding strategy to control net blotch disease.

Materials and methods

Barley differential set

A differential set of 22 and 20 genotypes (cultivars and lines) was used to characterize the virulence of Ptt and Ptm isolates, respectively (Table 1). This included two lines NDB112 and Heartland from North America, because of their known resistance to net blotch isolates (Wilcoxon et al. 1992).

Barley seed was sown into 32×46 cm² plastic containers with 24 cavities of 6×6 cm², 6 cm depth and spaced 1.5 cm apart filled with a natural soil brought from the experimental station at Sidi El Aidi (Settat, Morocco). Ten kernels of each genotype were planted per cavity. One plastic container seeded with 22 or 20 genotypes was used to test the virulence of each isolate of *P. teres* f. *teres* or *P. teres* f. *maculata,* respectively. Plants were grown in a greenhouse maintained at 20 to 25°C with a natural photoperiod (mean of 11-h of daylight) until three-leaf stage when they were subjected to inoculation.

Table 1 The 22 barley genotypes used as differentials to characterize isolates *of Pyrenophora teres* f. *teres* and *P. teres* f. *maculata*

Differentials	Codes for *P. teres* f. *teres*[a]	Codes for *P. teres* f. *maculata*[b]	Type	Origin	Row type	Data of release
Igrane	1	20	Cv	INRA-Morocco	2	1996
Amira	2	14	Cv	INRA-Morocco	6	1996
Tissa	3	6	Cv	INRA-Morocco	2	1984
Orge 628	4	3	Cv	INRA-Morocco	6	1956
Aglou	5	17	Cv	INRA-Morocco	2	1988
Amalou	6	–	Cv	INRA-Morocco	6	1997
Azilal	7	11	Cv	INRA-Morocco	2	1989
Acsad 176	8	16	Cv	ACSAD-Syria	6	1984
Acsad 68	9	–	Cv	ACSAD-Syria	6	1985
Asni	10	18	Cv	INRA-Morocco	2	1984
Rabat 071	11	1	Cv	INRA-Morocco	6	1956
Massine	12	15	Cv	INRA-Morocco	6	1994
Arig 8	13	4	Cv	RA-Morocco	6	1973
Aanacer	14	5	Cv	INRA-Morocco	6	1991
Acsad 60	15	12	Cv	ACSAD-Syria	2	1984
Taffa	16	21	Cv	INRA-Morocco	6	1994
Tamellalt	17	10	Cv	INRA-Morocco	2	1984
Rabat 077	18	9	Cv	INRA-Morocco	6	1956
Tiddas	19	13	Cv	INRA-Morocco	2	1988
Oussama	20	19	Cv	INRA-Morocco	6	1995
NDB 112 (CI 11531)	21	24	Acs	USA	6	N.A
Heartland (CR 2434)	22	2	Acs	Canada	6	N.A

[a] Barley differential codes for *P. teres* f. *teres*

[b] Barley differential codes for *P. teres* f. *maculata*

Cv Cultivar, *Acs* Accession, - Cultivar not used, *NA* Not available

Field sampling

A population of 61 isolates of Ptt and 21 of Ptm was tested. All isolates were recovered from infected barley leaves that showed typical symptoms of the two forms of net blotch disease. Samples of infected leaves were collected at random from barley plots at three experimental stations (Jemaat Shaim, Khmiss Zmamra and Sidi El Aidi) of INRA-Morocco (National Institute for Agronomic Research) and, also, from farmer's fields localized in the semi-arid regions of Morocco represented by the district of Doukkala, Abda and Chaouia. Most of the leaf samples showing spot-type symptoms were collected in the 2001-2002 winter growing season, and isolates from them were used in an earlier study, whereas leaves with characteristic net-type symptoms were collected in the 2004-05 to 2006-07 winter growing seasons (Table 2). Leaves sampled from each location were kept in labelled paper bags and dried at laboratory ambient temperature (18 to 25°C). Once dried, leaves were either used immediately or were stored in a refrigerator at 5°C.

Single-spore isolation and inoculum production

Infected leaves were cut into 1 to 2 cm pieces, rinsed three times in sterile distilled water, dried between sheets of filter paper and placed on moistened filter paper in Petri dishes. Incubation took place at 20± 1°C with a 12-h photoperiod (fluorescent and near-UV lamps) to promote pathogen sporulation. After one to 2 days, single conidia of *P. teres* were

Table 2 Characteristics of the isolates of *Pyrenophora teres* f. *teres* and *Pyrenophora teres* f. *maculata* tested

Isolate code	Form	Geographic origin	Sampling date	Isolate code	Type	Geographic origin	Sampling date
31	Spot	Sidi El Aidi	09/01/2001	CH8	Net	Abda (-2 km from Jemaat Shaim)	14/01/2004
32 and 32b	Spot	Sidi El Aidi	09/01/2001	CH9	Net	Abda (to Jemaat Shaim)	14/01/2004
33	Spot	5 Km from Boulaaouane to Settat	10/01/2001	CH10	Net	Station de Jemaat Shaim	14/01/2004
34	Spot	15 Km from Boulaaouane to Settat	10/01/2001	CH12	Net	Boulaaouane Field 1	14/01/2004
36	Spot	20 Km from Sidi Bennour to Settat	10/01/2001	CH14	Net	Boulaaouane Field 3	14/01/2004
39	Spot	10 Km from KH.Z to Sidi Bennour	10/01/2001	J1	Net	Jemaat Shaim	14/01/2004
40	Spot	25 Km de Sidi Bennour to Settat	10/01/2001	J2	Net	Jemaat Shaim	14/01/2004
41	Spot	Boulaaouane	10/01/2001	2Jb04	Net	Jemaat Shaim	14/01/2004
42 and 42b	Spot	CRRA-Settat	18/01/2001	3J	Net	Jemaat Shaim	14/01/2004
43 and 43c	Spot	CRRA-Settat	18/01/2001	5J	Net	Jemaat Shaim	14/01/2004
44a and 44b	Spot	Sidi El Aidi	23/01/2001	6J	Net	Jemaat Shaim	14/01/2004
46	Spot	Settat	05/02/2001	7J	Net	Jemaat Shaim	14/01/2004
47	Spot	Jemaat Shaim	13/02/2001	10J	Net	Jemaat Shaim	14/01/2004
18Sspot	Spot	Sidi El Aidi	13/01/2004	13J	Net	Jemaat Shaim	14/01/2004
4JSspot	Spot	Jemaat Shaim	14/01/2004	18J	Net	Jemaat Shaim	14/01/2004
8JSspot	Spot	Jemaat Shaim	14/01/2004	19Ja	Net	Jemaat Shaim	14/01/2004
1S	Net	Sidi El Aidi	13/01/2004	19Jb	Net	Jemaat Shaim	14/01/2004
2S	Net	Sidi El Aidi	13/01/2004	20J	Net	Jemaat Shaim	14/01/2004
3S	Net	Sidi El Aidi	13/01/2004	21J	Net	Jemaat Shaim	14/01/2004
4S	Net	Sidi El Aidi	13/01/2004	CH18	Net	Ain Ali Moumen (Settat)	15/01/2004
5Sa	Net	Sidi El Aidi	13/01/2004	CH19	Net	1 km from Settat to Marrakech	15/01/2004
5Sb	Net	Sidi El Aidi	13/01/2004	CH22	Net	Dar ElKayd Ettounsi (Settat)	15/01/2004
6S	Net	Sidi El Aidi	13/01/2004	Kh_sidi_M_Benrehal	Net	Kh. Sidi Mohammed ben Rehal	20/04/2004
7S	Net	Sidi El Aidi	13/01/2004	1J05	Net	Jemaat Shaim	23/01/2005
9S	Net	Sidi El Aidi	13/01/2004	3J05	Net	Jemaat Shaim	23/01/2005
10S	Net	Sidi El Aidi	13/01/2004	5J05	Net	Jemaat Shaim	23/01/2005
11S	Net	Sidi El Aidi	13/01/2004	10J05	Net	Jemaat Shaim	23/01/2005
13Sa	Net	Sidi El Aidi	13/01/2004	11J05	Net	Jemaat Shaim	23/01/2005
13Sb	Net	Sidi El Aidi	13/01/2004	tamJ05	Net	Jemaat Shaim	23/01/2005
14S	Net	Sidi El Aidi	13/01/2004	Boulaouane_05	Net	Boulaaouane	23/01/2005
16S	Net	Sidi El Aidi	13/01/2004	tamjs_06	Net	Jemaat Shaim	02/02/2006
17S	Net	Sidi El Aidi	13/01/2004	3S06	Net	Sidi El Aidi	02/02/2006

19S	Net	Sidi El Aidi	13/01/2004	Aanacer_KH_07	Net	Khmiss Zmamra	27/02/2007
20S	Net	Sidi El Aidi	13/01/2004	Ouled Amar	Net	Ouled Amar	27/02/2007
21S	Net	Sidi El Aidi	13/01/2004	Aounate_07	Net	Aounate	15/02/2007
CH1	Net	Khmiss Zmamra	14/01/2004	Zair_07	Net	Sidi Yehia Zair	19/04/2007
CH2	Net	From KH.Z to Jemaat Shaim	14/01/2004	Merchouch_07	Net	Merchouch	08/05/2007
CH3	Net	Dar ElKayd	14/01/2004	Tassaout_07	Net	Tassaout	07/05/2007
CH4	Net	18 km from KH.Z to Jemaat Shaim	14/01/2004				

Sidi El Aidi, Khmiss Zmamra (Kh. Z), Jemaat shaim: Experimental stations of INRA. CRRA: Regional Center for Agricultural Research (Settat)

transferred to a Petri dish containing home-made V8 juice agar, using a sterilized glass needle and a binocular microscope. Dishes with monoconidial cultures were incubated under the same conditions as above.

Following incubation, conidia were harvested by adding 20 ml of sterile distilled water to Petri dishes of 10-day-old cultures and scraping the surface of the colony of *P. teres* with a small brush. The suspension was filtered through two layers of cheesecloth and the inoculum concentration was adjusted to 15.000 conidia per ml using distilled water with one drop of Tween 20 added per 100 ml. At the three-leaf stage, whole plants were sprayed with 50 ml of the inoculum suspension until run off, using a manual sprayer. Inoculated plants were then incubated in darkness for 24 h, at 20±1°C and at 100% relative humidity to promote infection. Afterwards, inoculated plants in the 24 cavity plates were returned to the greenhouse until symptom development. The inoculation experiment was repeated once in space.

Scoring of infection types

Infection types produced by *P. teres* isolates were assessed 10 days after inoculation using the two numerical scales of Tekauz (1985) for *P. teres* f. *teres* and *P. teres* f. *maculata*. Evaluation of infection types on barley differentials was undertaken on the central portion of the second seedling leaf. For each differential, the infection type was based on the mean of values from several seedlings from the two repetitions. Since these values were on an ordinal scale, their analysis was undertaken in accordance to the measurement scale.

Virulence quantification

For each isolate, infection types presented in Tables 3 and 4 were converted to frequencies within the 10 classes of the Tekauz scale (Tekauz 1985) (Tables 5 and 6). To reduce scale variability, classes of infection types from 8 to 10 for each isolate of Ptt were merged into a single class, 8 (Table 5). The ordinal scale used for Ptm isolates ranged from 1 to 9 (Tables 4 and 6).

To quantify the virulence of each isolate, correspondence analysis (Sanogo and Yang 2004) was

Table 3 Infection phenotypes types scored according to the Tekauz (1985) scale exhibited by 61 isolates of *Pyrenophora teres* f. *teres* on a set of 22 barley differentials

Differentials	Isolates																						
	1S	2S	3S	4S	5Sa	5Sb	6S	7S	9S	10S	11S	13Sa	13Sb	14S	16S	17S	19S	20S	21S	1J	2J	3J	5J
Igrane	6	2	4	4	3	1	3	4	2	5	5	1	2	2	4	2	3	7	5	5	3	5	4
Amira	4	5	4	4	5	3	2	5	2	5	4	2	3	2	5	2	1	5	2	4	7	4	6
Tissa	6	1	1	3	1	1	1	4	2	7	5	3	2	1	2	1	3	7	6	5	5	5	5
Orge628	4	2	2	3	1	1	2	2	1	4	4	2	2	1	3	1	1	5	3	5	3	5	7
Aglou	3	2	1	4	4	5	3	3	1	1	4	3	3	2	5	1	1	6	4	5	6	6	6
Amalou	5	1	3	1	3	2	3	4	3	6	4	3	2	2	3	1	2	5	2	5	4	6	2
Azilal	5	1	3	1	3	3	3	4	1	6	4	5	2	.	5	4	3	4	5	6	5	7	7
Acsad176	3	3	4	4	2	2	2	3	3	3	3	4	1	5	4	1	1	3	3	4	4	5	4
Acsad68	1	2	4	1	1	2	3	2	2	5	4	3	2	5	3	2	2	5	4	4	5	5	3
Asni	4	1	2	3	1	1	3	5	5	8	3	4	1	5	4	1	3	7	4	6	5	5	5
Rabat071	1	1	2	1	1	2	3	1	1	2	4	1	2	3	3	1	1	4	3	3	3	5	6
Massine	3	3	4	3	4	3	2	4	2	4	5	4	3	3	4	2	1	5	3	4	4	4	5
Arig8	3	3	4	3	1	2	4	1	5	4	5	2	3	5	3	2	2	5	4	3	5	2	6
Aanacer	3	4	3	1	4	3	3	1	3	6	6	3	3	4	3	2	3	5	1	3	5	5	5
Acsad60	5	1	4	3	2	3	3	2	3	4	4	3	2	3	4	1	.	5	2	4	4	6	4
Taffa	3	1	4	1	1	3	2	1	2	4	2	2	1	1	1	1	3	6	3	5	3	4	4
Tamellalt	5	2	2	4	1	4	4	3	2	6	6	4	2	2	4	1	1	7	2	4	3	5	5
Rabat077	5	1	4	4	5	4	1	2	3	5	4	3	3	2	2	1	2	4	4	3	4	3	6
Tiddas	6	1	2	2	3	2	1	1	2	6	3	3	2	2	4	1	3	7	5	6	5	4	7
Oussama	4	1	2	3	1	2	3	1	3	2	3	3	3	4	3	1	2	4	5	3	5	7	5
NDB112	3	1	2	1	1	3	3	1	1	2	2	2	2	4	3	1	1	4	1	3	3	4	3
Heartland	1	1	1	1	1	3	1	1	1	1	3	2	1	1	3	1	1	5	5	5	5	5	4

. Missing value

Cv Cultivars code

	Isolates																					
	6J	7J	10J	13J	18J	19Ja	19Jb	20J	21J	1CH	2CH	Cv.	3CH	4CH	8CH	9CH	10CH	12CH	14CH	18CH	19CH	22CH	5J- 05
Igrane	7	5	5	7	10	3	3	4	6	5	4	1	4	5	4	6	3	2	3	4	2	2	6
Amira	3	4	4	5	6	5	3	2	6	7	5	2	5	4	5	5	3	1	4	4	1	3	4
Tissa	7	7	6	8	9	3	5	5	4	2	3	3	3	5	4	5	1	3	1	3	1	2	7
Orge628	3	3	3	7	7	4	1	2	5	2	3	4	2	3	3	4	1	1	2	3	2	2	3
Aglou	5	5	4	7	7	5	4	2	5	4	3	5	4	3	5	4	3	2	3	2	1	3	5
Amalou	4	4	6	7	9	2	1	2	5	4	1	6	3	2	3	6	1	3	1	2	1	2	5
Azilal	6	5	5	8	8	5	5	4	6	5	5	7	6	5	5	6	4	3	3	5	3	2	6
Acsad176	4	3	4	7	8	4	4	2	4	5	5	8	6	3	4	5	2	2	6	4	3	1	4
Acsad68	3	4	4	5	9	4	3	4	5	5	6	9	6	3	6	2	2	2	2	4	4	2	4
Asni	7	6	6	8	10	3	5	5	5	3	3	10	3	4	5	4	2	4	3	4	2	1	6
Rabat071	5	3	3	6	5	4	4	4	4	5	6	11	5	3	4	4	1	1	2	3	2	2	3
Massine	2	5	4	6	4	4	3	2	5	7	8	12	5	5	4	5	3	1	4	4	5	3	5
Arig8	4	3	6	4	7	5	3	3	4	5	8	13	5	5	2	3	3	3	4	5	5	3	5
Aanacer	2	3	4	3	6	5	2	2	6	7	6	14	3	5	5	3	4	3	4	5	4	3	6
Acsad60	6	3	3	5	8	5	2	3	4	4	8	15	4	3	6	3	1	1	3	3	4	2	5
Taffa	3	3	4	6	9	4	3	3	6	6	7	16	2	2	1	3	1	1	2	3	4	1	3
Tamellalt	2	3	4	5	9	4	5	5	3	5	7	17	4	6	6	1	4	4	3	5	5	2	7
Rabat077	4	4	4	4	6	5	4	5	7	5	6	18	4	4	4	3	3	3	6	4	6	3	5
Tiddas	4	5	6	7	9	3	5	4	7	5	4	19	3	3	1	3	3	3	3	3	1	2	6
Oussama	2	4	5	7	8	4	2	2	5	6	4	20	3	3	1	4	3	3	6	4	5	3	3
NDB112	2	3	3	5	6	2	3	1	4	3	2	21	3	3	3	1	1	3	2	2	3	2	2
Heartland	2	5	5	8	8	2	2	2	3	4	3	22	2	2	1	1	1	3	5	2	3	1	4

Table 3 (continued)

	Isolates															
	Boula-ouane_05	2Jb- 04	11 J-05	10 J-05	1 J-05	3 J-05	3 S-06	tamJ-05	Ouled Amar	Kh.S.M. Benrehal	Tamjs-06	Aounate-07	Aanacer-KhZ_07	Zair-07	Merchouch	Tassaout
Igrane	5	2	4	3	6	5	6	7	7	5	8	7	8	5	7	6
Amira	4	2	1	3	2	1	5	3	7	5	6	6	6	5	3	5
Tissa	4	1	4	5	6	6	5	4	7	6	7	7	7	3	7	5
Orge628	4	1	1	1	4	4	4	3	5	5	5	3	3	4	1	5
Aglou	5	1	5	5	5	5	6	4	7	6	6	5	4	5	5	5
Amalou	4	3	4	4	5	5	5	4	5	5	5	3	4	6	7	5
Azilal	6	4	4	6	6	4	7	5	7	6	8	7	5	4	1	6
Acsad176	5	5	4	1	2	1	6	3	5	5	3	5	5	6	3	6
Acsad68	6	4	4	1	3	1	6	3	3	5	4	5	5	5	3	5
Asni	4	5	4	7	7	6	3	6	7	6	6	8	6	6	5	5
Rabat071	5	4	6	4	3	4	4	2	8	4	5	3	5	7	3	6
Massine	5	5	6	1	3	1	6	2	5	7	6	4	7	5	7	5
Arig8	5	5	4	3	3	1	6	2	7	6	6	5	7	5	7	5
Aanacer	6	5	5	3	3	1	5	2	6	4	6	6	8	8	7	6
Acsad60	5	2	4	1	1	1	3	4	1	5	5	5	3	5	3	2
Taffa	4	4	3	3	4	3	3	4	1	3	7	2	3	1	3	1
Tamellalt	5	5	3	5	7	5	5	7	7	6	9	2	5	3	7	5
Rabat077	5	4	3	4	3	3	5	4	3	6	3	5	4	8	3	8
Tiddas	4	4	3	4	5	5	6	5	4	6	8	3	4	5	5	7
Oussama	4	3	6	2	2	3	6	4	2	7	7	4	5	5	7	5
NDB112	1	3	4	1	3	3	3	1	1	1	2	5	3	1	1	2
Heartland	3	2	4	2	2	5	5	1	3	1	4	3	2	3	5	3

Table 4 Infection phenotypes according to the Tekauz (1985) scale exhibited by 20 isolates of *Pyrenophora teres* f. *maculata* on a set of 20 barley differentials

Isolates																				
Differentials	Iso32	Iso40	Iso44a	Iso39	Iso41	Iso43	Iso42	Iso33	Iso31	Iso34	Iso36	Iso44b	Iso42b	Iso43c	Iso32b	Iso46	Iso47	18Sspot	4Jspot	8Jspot
Rabat071	1	5	7	7	8	7	5	1	7	8	2	3	3	1	1	3	5	5	7	7
Heartland	1	7	7	8	8	7	7	1	8	7	3	5	2	2	2	3	5	3	3	5
Orge628	3	5	5	5	7	5	5	1	7	7	3	3	3	5	5	2	3	3	3	5
Arig8	2	7	7	7	8	7	8	2	7	5	7	3	3	3	2	5	5	5	5	7
Aanacer	3	8	7	9	8	8	8	2	7	8	3	3	5	2	3	7	7	7	7	8
Tissa	1	5	5	7	8	7	5	2	5	5	2	2	2	1	2	2	3	5	5	8
Rabat077	7	8	7	5	7	7	5	1	5	5	2	5	3	7	3	5	3	5	7	5
Tamellalt	2	7	7	7	7	7	3	7	5	8	2	5	2	5	2	8	2	7	5	5
Azilal	2	.	5	5	7	7	3	.	.	.	.	5	3	5	3	7	2	5	5	7
Acsad60	2	5	5	3	5	5	3	1	5	3	2	5	3	5	1	3	2	5	3	3
Tiddas	1	2	5	3	5	7	7	1	5	5	1	2	2	1	2	2	8	5	3	7
Amira	2	5	7	7	7	8	8	2	5	3	2	5	3	7	5	7	8	7	5	7
Massine	1	7	7	8	7	9	8	3	7	5	1	5	2	7	3	5	8	7	7	7
Acsad176	2	5	5	5	5	7	5	3	7	5	2	5	3	3	3	5	7	5	5	5
Aglou	2	5	3	5	7	5	5	3	7	5	2	2	5	5	2	9	7	5	5	5
Asni	3	7	3	9	8	8	5	1	7	5	3	5	3	2	2	3	5	7	8	8
Oussama	3	7	7	8	8	8	7	1	8	3	3	5	5	5	2	7	7	5	5	5
Igrane	2	5	5	7	5	7	5	3	7	3	1	3	2	1	2	5	5	5	3	5
Taffa	1	3	3	5	5	5	3	1	5	3	2	1	1	1	1	3	3	3	3	5
NDB112	1	7	.	8	7	.	.	.	7	.	2	5	3	3	3	2	7	3	3	5

. Missing value

Table 5 Frequencies of infection phenotypes scored according to a modified Tekauz (1985) scale of 8 classes exhibited by *Pyrenophora teres* f. *teres* isolates

Isolates	Codes	Cls 1	Cls 2	Cls 3	Cls 4	Cls 5	Cls 6	Cls 7	Cls 8	Isolates	Codes	Cls 1	Cls 2	Cls 3	Cls 4	Cls 5	Cls 6	Cls 7	Cls 8
1	1S	3	0	7	4	5	3	0	0	32	21J	0	0	2	6	7	5	2	0
2	2S	12	5	3	1	1	0	0	0	33	1CH	0	2	2	4	9	2	3	0
3	3S	3	7	3	9	0	0	0	0	34	2CH	1	1	5	3	3	4	2	3
4	4S	8	1	7	6	0	0	0	0	35	3CH	0	3	7	5	4	3	0	0
5	5Sa	11	2	4	3	2	0	0	0	36	4CH	0	3	9	3	6	1	0	0
6	5Sb	4	7	8	2	1	0	0	0	37	8CH	4	1	3	6	5	3	0	0
7	6S	4	5	11	2	0	0	0	0	38	9CH	3	1	6	5	4	3	0	0
8	7S	8	4	3	5	2	0	0	0	39	10CH	8	3	8	3	0	0	0	0
9	9S	6	8	6	0	2	0	0	0	40	12CH	6	4	10	2	0	0	0	0
10	10S	2	3	1	5	4	5	1	1	41	14CH	2	5	7	4	1	3	0	0
11	11S	0	2	5	9	4	2	0	0	42	18CH	0	4	6	8	4	0	0	0
12	13Sa	2	6	9	4	1	0	0	0	43	19CH	5	4	4	4	4	1	0	0
13	13Sb	4	11	7	0	0	0	0	0	44	22CH	4	11	7	0	0	0	0	0
14	14S	4	7	3	3	4	0	0	0	45	tamjs_06	0	1	2	2	4	6	3	4
15	16S	1	2	9	7	3	0	0	0	46	Aounate_07	0	2	5	2	7	2	3	1
16	17S	15	6	0	1	0	0	0	0	47	Aanacer_KhZ_07	0	1	4	4	6	2	3	2
17	19S	9	5	7	0	0	0	0	0	48	Zair_07	2	0	3	2	9	3	1	2
18	20S	0	0	1	5	9	2	5	0	49	Merchouch_07	3	0	7	0	4	0	8	0
19	21S	2	4	5	5	5	1	0	0	50	Tassaout_07	1	2	1	0	11	5	1	1
20	1J	0	0	6	6	7	3	0	0	51	5J_05	0	1	4	4	6	5	2	0
21	2J	0	0	6	5	9	1	1	0	52	Boulaouane_05	1	0	1	8	9	3	0	0
22	3J	0	1	1	5	10	3	2	0	53	2Jb_04	3	4	3	6	6	0	0	0
23	5J	0	1	2	5	6	5	3	0	54	11J_05	2	0	4	11	2	3	0	0
24	6J	0	6	4	5	2	2	3	0	55	10J_05	6	2	5	4	3	1	1	0
25	7J	0	0	9	5	6	1	1	0	56	1J_05	1	4	7	2	3	3	2	0
26	10J	0	0	4	9	4	5	0	0	57	3J_05	7	0	4	3	6	2	0	0
27	13J	0	0	1	2	5	3	7	4	58	3S_06	0	0	4	2	7	8	1	0
28	18J	0	0	0	1	1	4	3	13	59	tamJ_05	2	4	4	7	2	1	2	0
29	19Ja	0	3	4	8	7	0	0	0	60	Ouled Amar	3	1	3	1	4	1	8	1
30	19Jb	2	4	7	4	5	0	0	0	61	Kh.S.M.Benrehal	2	0	1	2	7	8	2	0
31	20J	1	9	3	5	4	0	0	0										

Cls Class of frequencies

Table 6 Frequencies of infection phenotypes exhibited by *Pyrenophora teres* f. *maculata* isolates according to the Tekauz (1985) numerical scale

Codes	Isolates	Infection class						
		Class 1	Class 2	Class 3	Class 5	Class 7	Class 8	Class 9
1	Iso32	7	8	4	0	1	0	0
2	Iso40	0	1	1	8	7	2	0
3	Iso44a	0	0	3	7	9	0	0
4	Iso39	0	0	2	6	6	4	2
5	Iso41	0	0	0	5	8	7	0
6	Iso43	0	0	0	4	10	4	1
7	Iso42	0	0	4	8	3	4	0
8	Iso33	9	4	4	0	1	0	0
9	Iso31	0	0	0	7	10	2	0
10	Iso34	0	0	5	8	2	3	0
11	Iso36	3	10	5	0	1	0	0
12	Iso44b	1	3	5	11	0	0	0
13	Iso42b	1	6	10	3	0	0	0
14	Iso43c	5	3	3	6	3	0	0
15	Iso32b	3	9	6	2	0	0	0
16	Iso46	0	4	5	5	4	1	1
17	Iso47	0	3	4	5	5	3	0
18	18Sspot	0	0	4	11	5	0	0
19	4Jspot	0	0	7	8	4	1	0
20	8Jspot	0	0	1	10	6	3	0

applied to the frequency data to find optimal scores for rows (isolates) and columns (categories) on a small number of dimensions that accounted for the largest proportion of the chi-square value (Goodman 1991). Biplot representation of the first two dimensions of row and column coordinates of the correspondence analysis were depicted with the help of the Plotit macro in the SAS software package that prevented the plotting procedure from independently scaling the axes of the joint plots (Greenacre and Hastie 1987) (Figs. 1 and 6). Note that the only non-meaningful distances in the biplot are those between the row points and the column points (Carroll et al. 1989). Correspondence analysis was carried out with the Corresp procedure in SAS/STAT of SAS V 9.1 (2002) coupled with Plotit for meaningful graphical representation. From the output of the correspondence analysis, six dimensions (representing 96% of the variability) were subjected to cluster analysis using a complete linkage method to classify isolate virulence into classes of virulence (Figs. 4 and 9).

Because of missing data for the interactions of some differentials with spot-type isolates (Table 4), correspondence analysis, by definition, dropped all values for differentials Azilal and NDB112, however the mixed model analysis evaluated the individual missing values by default.

Mixed model analysis (Demidenko 2004; Littell et al. 1996) was also used for virulence quantification, where genotype was taken as a fixed effect and isolates as the random effect. The estimation of isolate effects was obtained from the Best Linear Unbiased Predictor (BLUP) option of the model (Figs. 2 and 7).

Isolate pathotyping

Infection types (Tables 3 and 4) representing the interaction of Ptt and Ptm isolates with the differentials were analyzed by the GGE model (Yan et al. 2000). In theory, GGE refers to the genotype main effect (G) plus the genotype×environment interaction (GEI) and this statistical methodology is used in

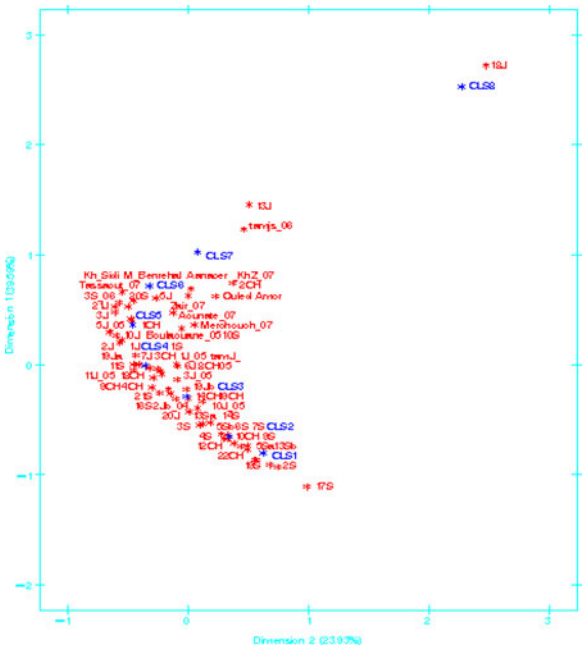

Fig. 1 Biplot of correspondence analysis based on frequencies of infection types of *Pyrenophora teres* f. *teres* isolates. Class labels represent classes of infection types according to the Tekauz (1985) scale. Star icons represent coordinates of the 61 isolates

analysis of data from multi-environment trials. By analogy, we adopted the same nomenclature, however we changed the G (genotype) to I (Isolate) and, thus, obtained an Isolates×Isolates×Environment (IIE) interaction. In this IIE interaction, the E represented genotypes (differentials) and the IIE is herein analyzed and used for pathotyping net blotch isolates. The additive part of the model was evaluated using the SAS/GLM procedure with differentials (genotypes) in the model option. The interaction part is represented by the residuals left after modelling the additive mean effects. These residuals were then transformed into a data matrix with rows as isolates and columns as differentials. The data matrices were centered by genotypes and the residual data matrix obtained was single-value-decomposed (Gabriel 1971) using SAS/IML. Row and column scores when depicted in a biplot using symmetric representation of two dimensions (Yan and Tinker 2005) explained little of the variation (data not shown). To explain most of the variation we adopted cluster analysis based on 15 and 7 axes, to represent 96% and 93% of the variability existing in the data for the net- and spot-type isolates, respectively (Figs. 3 and 8). Ward's method and squared euclidean distances were used to help in classifying these isolates into pathotypes.

Evaluation of barley differentials

The GGE analysis (Yan et al. 2000) graphically displays the genotype main effect (G) plus the genotype x environment interaction (GEI). To identify any similarity among barley differential responses to all isolates, barley differentials represented genotype

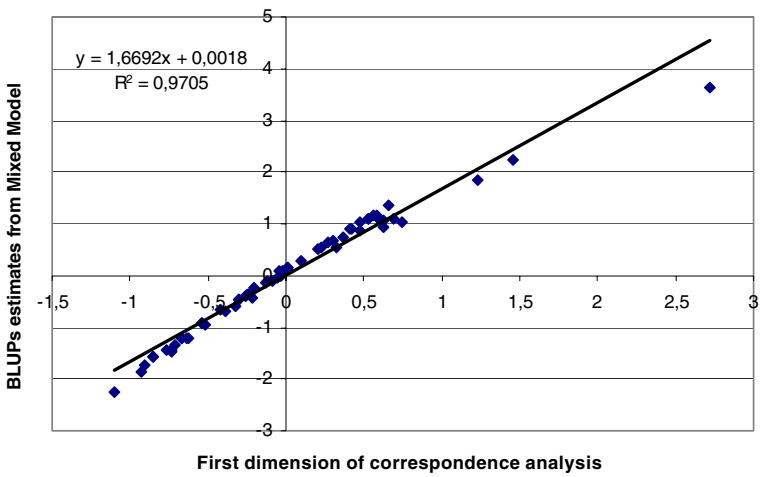

Fig. 2 Linear relationship between coordinates of the first dimension of correspondence analysis and best linear unbiased predictors (BLUPs) estimates of mixed model analysis applied to infection types of *Pyrenophora teres* f. *teres* isolates (R^2= 0.97, p<0.001). BLUPs represent estimates of the random factor of mixed model analysis

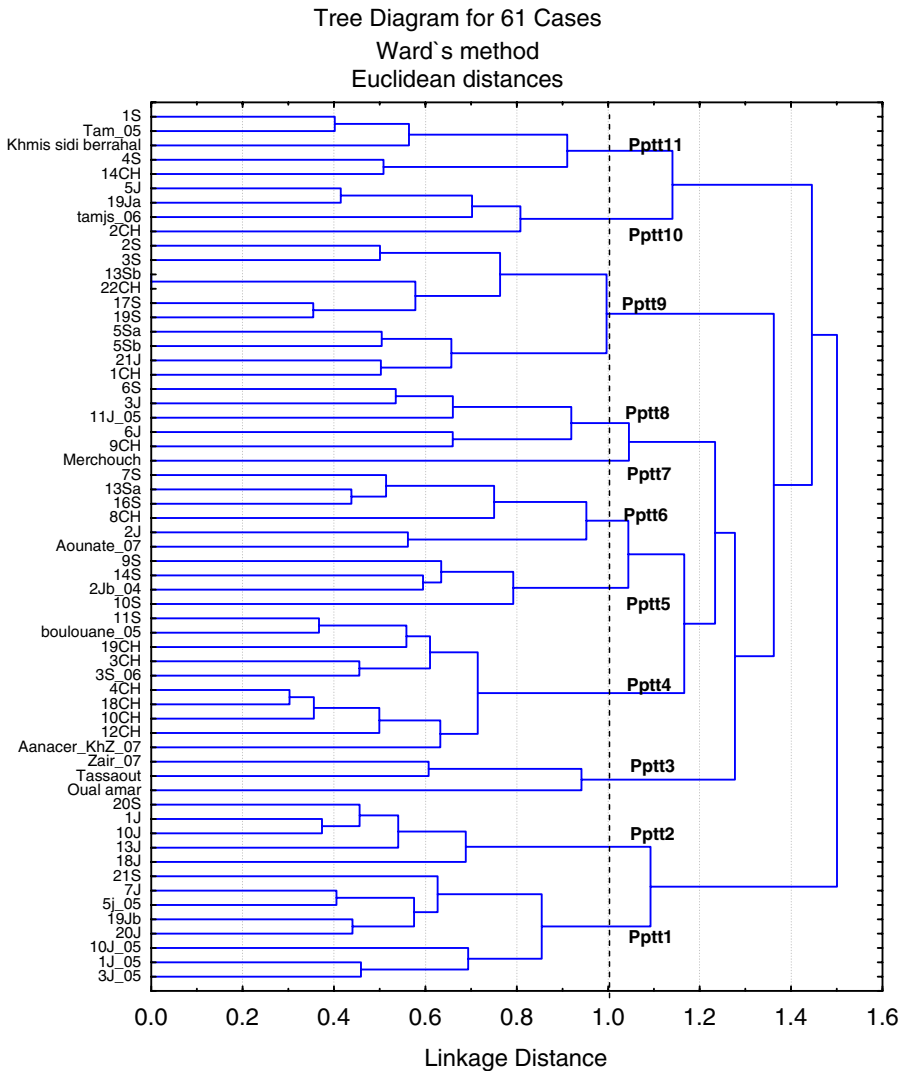

Fig. 3 Eleven pathotypes of *Pyrenophora teres* f. *teres* identified by cluster analysis of fifteen dimensions of the residuals of the IIG Model that explains 96% of the variability. Cases refer to the 61 pathogen isolates, and Pptt1 to Pptt11 to pathotype designations at the truncation level

main effect and *P. teres* isolates the environmental effect, giving a GGI interaction. As was done to analyze the IIG interaction, we analyzed the GGI data matrices with the Pattern procedure of the IRRISTAT software package using the incremental SS (Ward) option to classify the genotypes using cluster analysis (Figs. 5 and 10).

Results

Virulence quantification of *Pyrenophora teres* f. *teres* isolates

The biplot, obtained through the analysis of frequencies of infection types from 61 isolates of Ptt with correspondence analysis and based on two dimensions, explained 64% of the virulence variability (Fig. 1). The biplot represents classes (Cls) of virulence ranging from 1 to 8 of the modified Tekauz scale (Tekauz 1985) (Table 4), and their orthogonal projection on the first dimension gave a biological meaning of a virulence-avirulence spectrum to the first axis of this analysis. The range of the coordinates of net type isolates on the first dimension represented the standardized scale for their virulence quantification. Therefore, isolates that are on the negative side were quantified as avirulent, those on the opposite side were virulent; isolates close to zero (Fig. 1) were moderately virulent. As such, loadings of *P. teres* isolates on only the first dimension represented a means for the quantification of their virulence level (Fig. 1).

From the biplot in Fig. 1 and Table 7, only one isolate (18 J) represented Cls 8 virulence. It had a quantified virulence level greater than 2 (coordinate on the first axis) and was considered as highly virulent. Class 7 encompassed two virulent isolates, 13J and Tamjs-06 originating from Jemaat Shaim region with a quantified virulence of between 1 and 2. Twenty-four isolates having a quantified virulence ranging from 0 and 1 were considered as moderately virulent, corresponding to Tekauz classes 4 to 6. Isolates with a quantified virulence level ranging from -1 to 0 on dimension 1 were considered moderately avirulent and numbered 33. One unique isolate 17S was classified as highly avirulent with a quantified virulence of less than -1 (Table 7).

BLUP estimates of Ptt isolates estimated by mixed model analysis were related to their corresponding coordinates on the first dimension of the previously described correspondence analysis. This relationship was linear with a coefficient of determination (R^2, $P<0.001$) of 97%. The solution of the random effect (isolates) in the mixed model based directly on infection types was similar to the quantification of the virulence by correspondence analysis based on frequencies of infection types (Fig. 2). For all Ptt isolates, the standard error (SE) for BLUPs estimates was 0.30 (not shown).

Pathotyping of *Pyrenophora teres* f. *teres* isolates

The dendrogram in Fig. 3 presents a hierarchical grouping of isolates based on fifteen factors of the IIG Model analysis that explained 96% of variability. When the dendrogram was truncated at a distance of 1.0, the isolates clustered into 11 distinct groups (Fig. 2). This truncating level gave an optimal distance at which pathotypes were found dissimilar (Fig. 3). The groups identified were labeled as pathotypes Pptt1 to Pptt11 and represented by isolates 3J-05, 18J, Ouled Amar, 19CH, 10S, Aounate-07, Merchouch-07, 9CH,

Table 7 Virulence quantification of *Pyrenophora teres* f. *teres* isolates based on frequencies of infection phenotypes subjected to correspondence analysis

Groups of virulence	*Pyrenophora teres* f. *teres* isolates			Total	Virulence range[a]
Highly virulent	18J			1 (2%)	> 2
Virulent	13Jtamjs_06			2 (3%)	]1 , 2]
Moderately virulent	2CH	5J	Boulaouane_05	24 (39%)	[0 , 1]
	Aanacer_KhZ_07	21J	10J		
	20S	3J	2J		
	Zair_07	Aounate_07	1J		
	Ouled Amar	5J_05	7J		
	Tassaout_07	1CH	6J		
	Kh_Benrehal	Merchouch_07	11S		
	3S_06	10S	11J_05		
Moderately avirulent	1J_05	16S	7S	33 (54%)	[-1 , 0]
	3CH	2Jb_04	6S		
	1S	14CH	5Sb		
	19Ja	19Jb	12CH		
	8CH	10J_05	5Sa		
	9CH	19CH	10CH		
	4CH	20J	9S		
	tamJ_05	14S	13Sb		
	18CH	13Sa	22CH		
	21S	3S	19S		
	3J_05	4S	*2S*		
Highly avirulent		17S		1 (2%)	< -1

[a] based on the loadings of the first axis of correspondence analysis

2S, 2CH and 14CH, respectively (Table 8); Table 3 shows cultivars that were resistant and susceptible to each pathotype. From this crossover interaction, differentials that best discriminated the 11 pathotypes included Igrane (1), Amira (2), Tissa (3), Orge628 (4), Azilal (7), Asni (10), Rabat071 (11), Acsad60 (15) and Rabat077 (18). Pathotype infection responses on these differentials were considered susceptible reactions when ratings were >5; those from 1 to 5 were considered resistant. On this basis, a key code for pathotype determination using these differential genotypes was developed (Table 8). Accordingly, the phenotypic characteristics of all, except one pathotype of Ptt, were different based on their reactions on the nine barley differentials. Pathotype Pptt11 (14CH) showed a similar response to pathotype Pptt4 (19CH) and was therefore dropped from subsequent analyses resulting in 10 total pathotypes.

Pathotypes Pptt4 and Pptt9 were predominant in the Ptt population with each represented by 10 isolates (Fig. 3). Pathotype Pptt4 was virulent on only one differential, whereas all the differentials were resistant to Pptt9. by contrast, most pathotypes were virulent on fewer than seven differentials except pathotype Pptt2 (18J) which was virulent on eight of nine differentials (Table 8).

The dendrogram illustrated in Fig. 4 is a hierarchical grouping of classes of pathogen virulence. These classes have no relationship to classes of pathotypes, as the former originated from correspondence analysis and the latter from the IIG model. The dendogram in Fig. 4 explains 96% of variability based on six dimensions of the correspondence analysis. Virulence of the Ptt isolates were classified into four classes when the maximum distance was truncated at 1.7 (Fig. 4). This optimal truncation level was chosen to correspond with five or less virulence classes previously identified, as shown in Table 7. The four classes of virulence were composed of avirulent isolates (31%), moderately virulent isolates (59%), virulent isolates (8%), highly virulent isolate (2%), which was virulent on eight out of the nine discriminating cultivars (Table 8).

All isolates were clustered into several distinct virulence groups but when localized, the 10 identified pathotypes were found to be not necessarily clustered into distinct virulence groups based on the correspondence analysis (Figs. 3 and 4). For example, Merchouch-07 (Pptt7) and Ouled Amar (Pptt3) have different phenotypic reactions (Table 8), but are in the same cluster in Fig. 4. Similar results were found for other pathotype combinations such as Pptt1 and Pptt8, and Pptt5 and Pptt6 (Fig. 4).

Based on the mixed model analysis, BLUP estimates of the random effect (all isolates) were all significantly different from zero ($P<0.05$) with a standard error (SE) of 0.30, except for pathotypes Pptt1 and Pptt8 (BLUP of all isolates are not shown). The zero effect in BLUP of the mixed model means that the effect is equal to the mean effect of the random factor (Isolates). We designate this zero level as a moderate level of virulence which represented a mean effect of the population. However, a significant

Table 8 A key code based on the reaction of a subset of barley differentials to infection by the pathotypes of *Pyrenophora teres* f. *teres*

Differential cultivars	Pathotypes (representative isolates)									
	Pptt1 (3j_05)	Pptt2 (18J)	Pptt3 (O.Amar)	Pptt4 (19CH)	Pptt5 (10S)	Pptt6 (Aounate)	Pptt7 (Merchouch)	Pptt8 (9CH)	Pptt9 (2S)	Pptt10 (2CH)
Igrane	R	S	S	R	R	S	S	S	R	R
Amira	R	S	S	R	R	S	R	R	R	R
Tissa	S	S	S	R	S	S	S	R	R	R
Orge628	R	S	R	R	R	R	R	R	R	R
Azilal	R	S	S	R	S	S	R	S	R	R
Asni	S	S	S	R	S	S	R	R	R	R
Rabat071	R	R	S	R	R	R	R	R	R	S
Acsad60	R	S	R	R	R	R	R	R	R	S
Rabat077	R	S	R	S	R	R	R	R	R	S

R Resistant, *S* Susceptible reactions

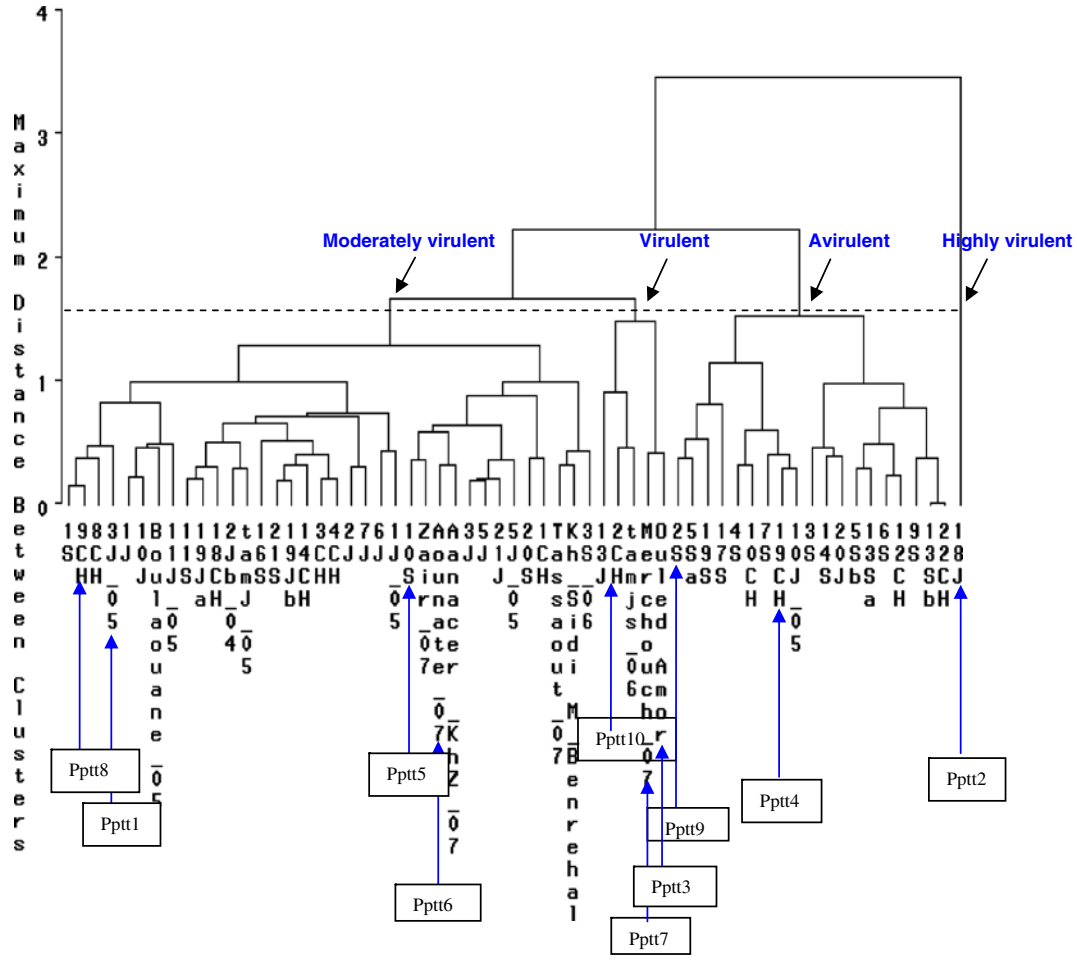

Fig. 4 *Pyrenophora teres* f. *teres* classes of virulence based on cluster analysis of six dimensions of correspondence analysis. Pptt1 to Pptt10 represent pathotype designations

BLUP estimate of any isolate means that its virulence is significantly different from the population mean effect.

Classification of barley differentials

This study also investigated the response of the barley differentials (genotypes) to all *P. teres* isolates based on GGE analysis. At a fusion level of 2.6, five clusters having significant biological meaning were delineated (Table 5). The first cluster (node 35) comprised three resistant cultivars: Heartland, NDB112 and Taffa. The second (node 37) comprised six moderately resistant cultivars, Orge628, Rabat071, Tiddas, Acsad60, Acsad68 and Acsad176. The third (node 38) of the moderately susceptible genotypes Azilal, Amira and Aglou, while the fourth (node 39) included the susceptible cultivars Aanacer, Arig8, Massine, Rabat077 and Tamellalt. The last cluster (node 36), grouped the highly susceptible genotypes Asni, Igrane, Tissa, Amalou and Oussama (Fig. 5).

Virulence quantification of *Pyrenophora teres* f. *maculata* isolates

The infection types of the 20 isolates of *Pyrenophora teres* f. *maculata* on 20 barley differential cultivars (Table 4) were transformed into classes of frequencies (Table 6) before subjecting them to correspondence analysis. The biplot (infection classes and isolates) of the analysis explained 76% of the variability (Fig. 6). For the biological significance of the first axis, orthogonal projection of classes of *P. teres* f. *maculata* isolates on the first dimension was indicative of a

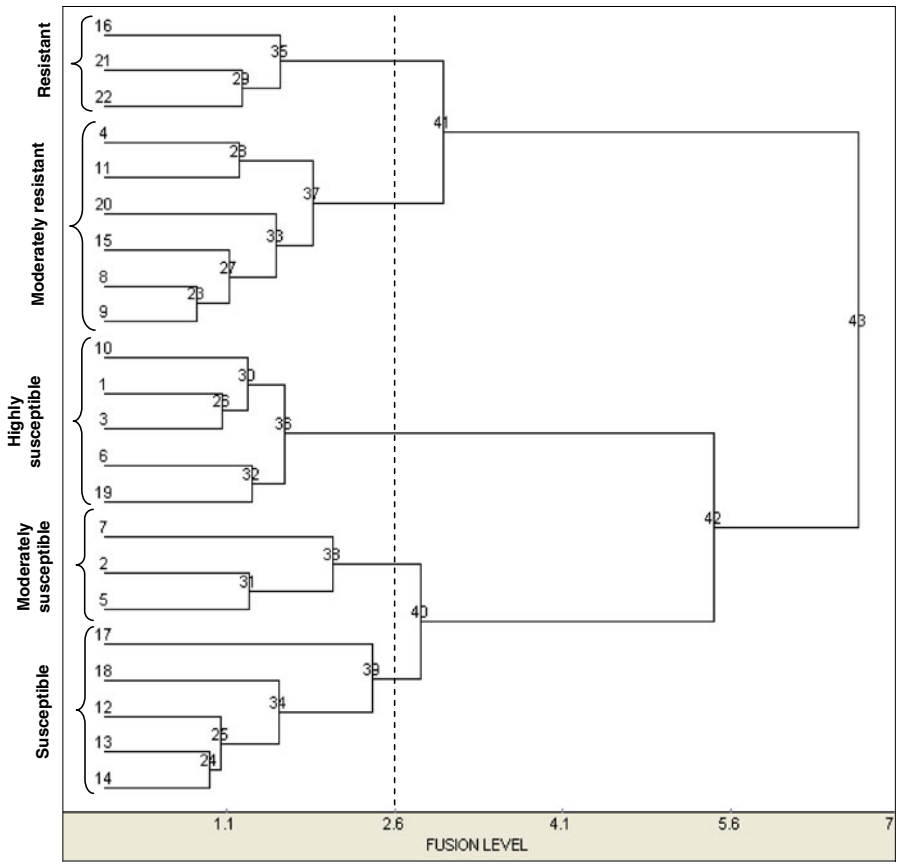

Fig. 5 Susceptibility classes of barley cultivars based on GGE analysis of their responses to isolates of *Pyrenophora teres* f. *teres*. Numbers from 1 to 22 represent the barley genotypes in the differential set listed in Table 1. Other numbers represent nodes of the dendrogram

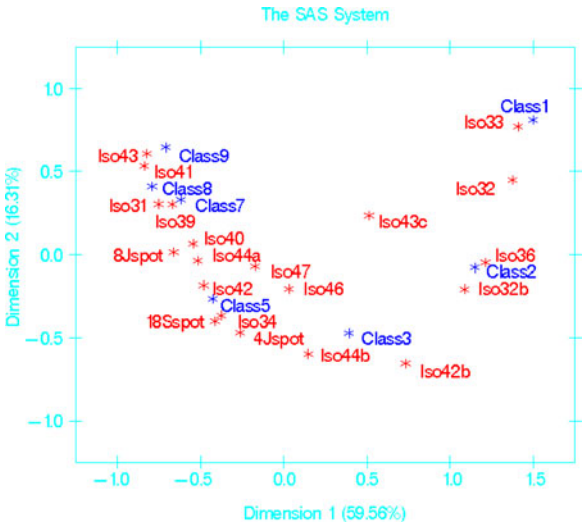

Fig. 6 Biplot of correspondence analysis based on frequencies of infection types of *Pyrenophora teres* f. *maculata*. Class 1 to 9 labels represent classes of infection types according to the Tekauz (1985) scale, and the star icons are labels for 20 isolates in Table 4

virulence-avirulence scale. Infection classes (Cls) 1, 2 and 3 are on the right side of the first dimension, whereas classes 5 through 9 are on the left (Fig. 6). Thus, isolates on the right side were described as avirulent, and those on the left virulent. Therefore, the orthogonal projection of these isolates on the first dimension is a quantification of their virulence. The avirulent isolates were Iso33, Iso32, Iso36, Iso32b, Iso42b, Iso43c, Iso44b and Iso46. Moderately virulent isolates had coordinates ranging from -0.5 to 0. This latter group includes isolates Iso47, 4Jspot, Iso34, 18Sspot and Iso42. On the Tekauz scale, these latter isolates often had infection scores of 5. Any isolate having coordinates of less than -0.5 on the first dimension is qualified as highly virulent, i.e Iso44a, Iso40, 8Jspot, Iso39, Iso31, Iso43, and Iso41 (Table 6).

Following the comparison between the first dimension of the correspondence analysis and the BLUP estimates (SE=0.46) from the mixed model analysis, a linear relationship was obtained (R^2=97%, P<0.001) with a slope factor of 1.98 (Fig. 7 and Table 9). In this instance, coordinates of the first axis of the correspondence

analysis were multiplied by a factor of -1 before relating them to the BLUP estimates (Fig. 7 and Table 9).

Pathotyping *Pyrenophora teres f. maculata* isolates

Infection responses exhibited by *P. teres* f. *maculata* also were analyzed using the adopted IIG model. Because only 20 isolates were analyzed, only seven factors were sufficient to explain 93% of the variability. IIG model analysis coupled with cluster analysis differentiated the isolates of *P. teres f. maculata* into different groups. As done for net type isolates, the dendrogram in Fig. 8 was truncated at a distance of 0.7, resulting in 10 pathotypes, labeled Pptm1 through Pptm10, and represented by isolates 4Jspot, Iso47, Iso43c, Iso32b, Iso34, Iso46, Iso33, Iso40, Iso36 and Iso42b respectively (Fig. 8). The cultivars that best discriminated the pathotypes identified, included Rabat071, Orge628, Arig8, Aanacer, Tamellalt, Acsad60, Amira and Aglou, and this relationship is shown in Table 10. Key codes related to each pathotype showed that two pathotypes Pptm4 (32b) and Pptm10 (42b) had identical infection responses on the eight differentials. Thus, pathotype Pptm10 was not included in Table 10 and from subsequent analyses.

Cultivar Acsad60 was resistant to all Ptm pathotypes. Pathotypes Pptm1 through Pptm9 were virulent on less than five cultivars and none of the eight cultivars was susceptible to pathotype Pptm4 (Iso32b) Table 10.

Cluster analysis based on six dimensions of the correspondence analysis explained 100% of the virulence variability, and the dendrogram generated grouped *P. teres f. maculata* isolates into four clusters of virulence when truncated at 1.25 maximum distance (Fig. 9).

BLUP estimates for *P. teres* f. *maculata* isolates and their levels of significance are shown in Table 9. Isolates with significant positive values were considered as virulent, those with non-significant values ($P>0.05$) moderately virulent, and isolates having values less than -1 avirulent (Fig. 9).

Classification of barley differentials

Responses of barley differentials (genotypes) to all isolates of *P. teres* f. *maculata* were analyzed by cluster analysis after applying the GGE method. At a fusion level 2.8, five classes were obtained (Fig. 10). The moderately resistant genotype class (node 31) includes three cultivars: Orge628, Heartland and NDB112. The (node 34) resistant genotype class included: Acsad60, Taffa, Aglou, Tiddas, Acsad176 and Igrane. The class of moderately susceptible (node 33) included Rabat077, Tamellalt and Azilal. The susceptible class (node 29) of susceptible genotypes was composed of cultivars Asni, Rabat071 and Tissa, and the fifth class (node 35) class of highly susceptible genotypes of Amira, Massine, Arig8, Aanacer and Oussama (Fig. 10).

Discussion

Assessment of the variation in and distribution of virulent isolates of the causal agent of barley net blotch is necessary for a successful resistance-

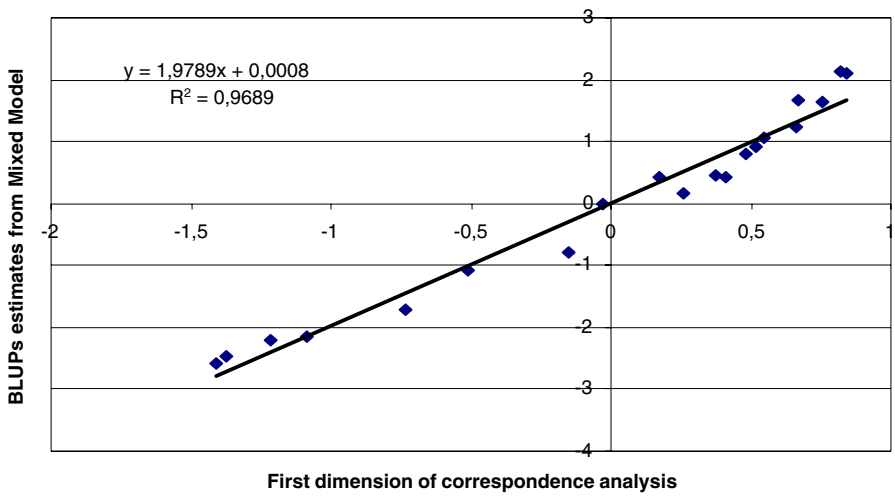

Fig. 7 Linear relationship between coordinates of the first dimension of correspondence analysis and best linear unbiased predictors (BLUPs) estimates of mixed model analysis applied to infection types of *Pyrenophora teres* f. *maculata* isolates ($R^2=0.97$, $p<0.001$). BLUPs are estimates of the random factor of mixed model analysis

Table 9 Virulence level of *Pyrenophora teres* f. *maculata* isolates according to row coordinates on the first dimension of correspondence analysis and BLUPs of Proc mixed analysis

Isolates	Correspondence analysis	Mixed model		Virulence
	Row coordinates on the first dimension[a]	BLUPs[b]	Probabilities	
Iso33	-1,408	-2,573	<.0001	Avirulent
Iso32	-1,374	-2,476	<.0001	Avirulent
Iso36	-1,214	-2,216	<.0001	Avirulent
Iso32b	-1,088	-2,139	<.0001	Avirulent
Iso42b	-0,735	-1,706	0,0003	Avirulent
Iso43c	-0,514	-1,080	0,0204	Avirulent
Iso44b	-0,148	-0,791	0,0889	Moderately virulent
Iso46	-0,031	-0,021	0,9635	Moderately virulent
4Jspot	0,261	0,171	0,7121	Moderately virulent
18Sspot	0,413	0,412	0,3751	Moderately virulent
Iso47	0,171	0,412	0,3751	Moderately virulent
Iso34	0,374	0,463	0,3287	Moderately virulent
Iso42	0,480	0,813	0,0837	Moderately virulent
Iso44a	0,515	0,914	0,0519	Moderately virulent
Iso40	0,544	1,071	0,0228	Virulent
8Jspot	0,662	1,230	0,0084	Virulent
Iso31	0,752	1,628	0,0006	Virulent
Iso39	0,669	1,664	0,0004	Virulent
Iso41	0,839	2,097	<.0001	Virulent
Iso43	0,823	2,128	<.0001	Virulent

[a] Coordinates of the first dimension were multiplied by a factor of -1

[b] Best linear unbiased predictors (BLUPs)

breeding program (Grünwald et al. 2003). Many previous studies dealing with virulence of *P. teres* revealed the existence of a large number of pathotypes (Tekauz 1990; Steffenson and Webster 1992; Wu et al. 2003). Furthermore, other studies have investigated differences in cultivar reactions to the causal agents of net blotch disease. Scott (1992) reported that *Pyrenophora teres* f. *maculata* was less damaging on susceptible cultivars than *Pyrenophora teres* f. *teres*. Wu et al. (2003) likewise showed that many isolates of *P. teres* f. *teres* exhibited high infection responses, which were greater than those of *P. teres* f. *maculata,* and concluded that the spot form isolates were less virulent than those of the net form. The net form of *Pyrenophora teres* was reported to be more prevalent across certain barley growing regions (Gupta and Loughman 2001; Tekauz 1990), which may be related to the region sampled and/or to the net-form isolates being more virulent. It is widely accepted that both forms of *P. teres* are composed of a large number of pathotypes and that the genetic resistance to them is independently inherited (Arabi et al. 2003). As such, the two forms *P. teres* isolates were treated separately in the current study.

In Morocco, little is known about the genetics of resistance of native barley cultivars to net blotch (Douiyssi et al. 1998). Furthermore, the international differential set of barley lines proposed to study the virulence frequency of the pathogen is of the type that includes cultivars, landraces, and breeding lines (Brown and Cooke 2006). Since this latter set was not available to us, we found it necessary to develop our own differential set of varieties relevant to the resistance genes used by breeders.

Conversion of infection types into frequencies for each infection category scale (Tekauz 1985) and the use of correspondence analysis allowed us to quantify

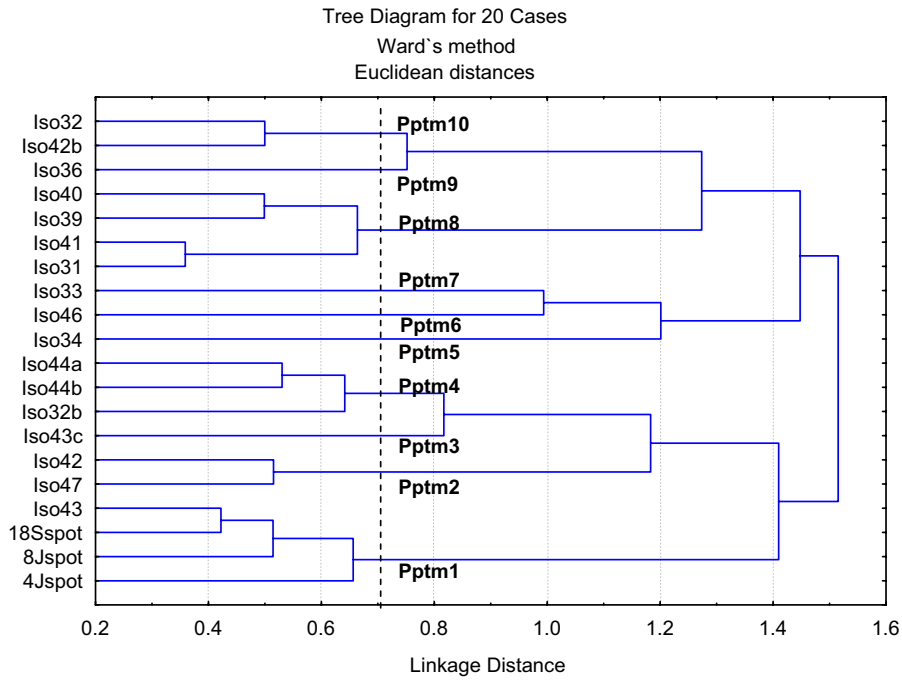

Fig. 8 Pathotyping of *P. teres* f. *maculata* isolates by cluster analysis of fifteen dimensions that explains 93% of the variability of the residuals in IIG Model. Pptm1 to Pptm10 represent pathotype designations at the truncation level. Cases refer to the 20 pathogen isolates listed in Table 4

the virulence level of all isolates of *P. teres*. Coordinates on the first dimension of correspondence analysis differentiated isolates of both forms of *P. teres* into various classes of virulence. The spectrum (virulence to avirulence) is defined as an adjective to measure pathogenicity, and avirulence of an isolate is a reduced ability to induce disease (Shaner 1992). Virulent and avirulent are related to phenotypes derived from a quantitative characterization of pathogenicity (Shaner 1992).

This study also revealed that isolates of Ptt were more prevalent than those of Ptm especially in the

Table 10 A key code based on the reaction of a subset of barley differentials to infection by the pathotypes of *Pyrenophora teres* f. *maculata*

Differential cultivars	Pathotypes (representative isolates)								
	Pptm1 (Iso4Jspot)	Pptm2 (Iso47)	Pptm3 (Iso43c)	Pptm4 (Iso32b)	Pptm5 (Iso34)	Pptm6 (Iso46)	Pptm7 (Iso33)	Pptm8 (Iso40)	Pptm9 (Iso36)
Rabat071	S	R	R	R	S	R	R	R	R
Orge628	R	R	R	R	S	R	R	R	R
Arig8	R	R	R	R	R	R	R	S	S
Aanacer	S	S	R	R	S	S	R	S	R
Tamellalt	R	R	R	R	S	S	S	S	R
Acsad60	R	R	R	R	R	R	R	R	R
Amira	R	S	S	R	R	S	R	R	R
Aglou	R	S	R	R	R	S	R	R	R

R Resistant, *S* Susceptible reactions

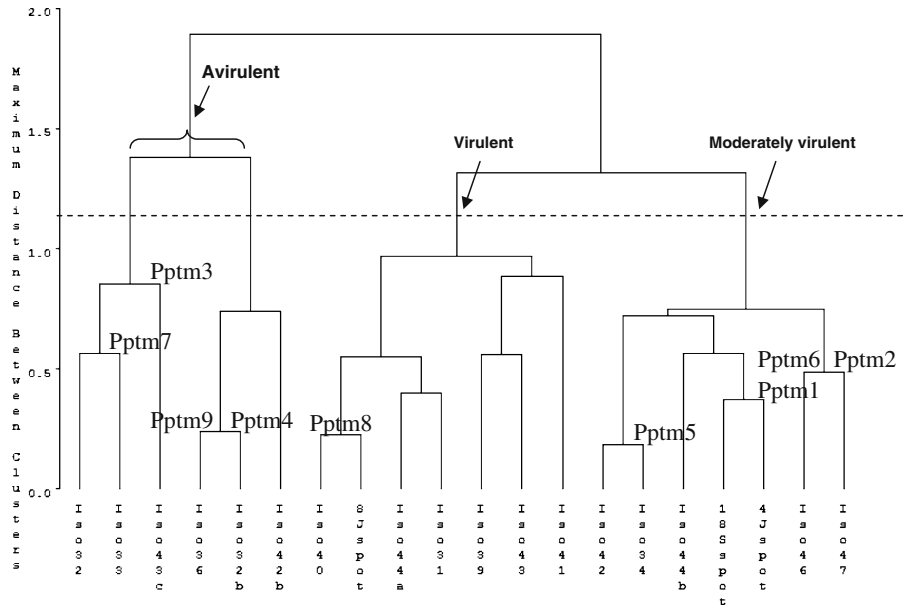

Fig. 9 Classes of virulence of *Pyrenophora teres* f. *maculata* based on cluster analysis of six dimensions of correspondence analysis. Pptm1 to Pptm10 represent pathotype designations

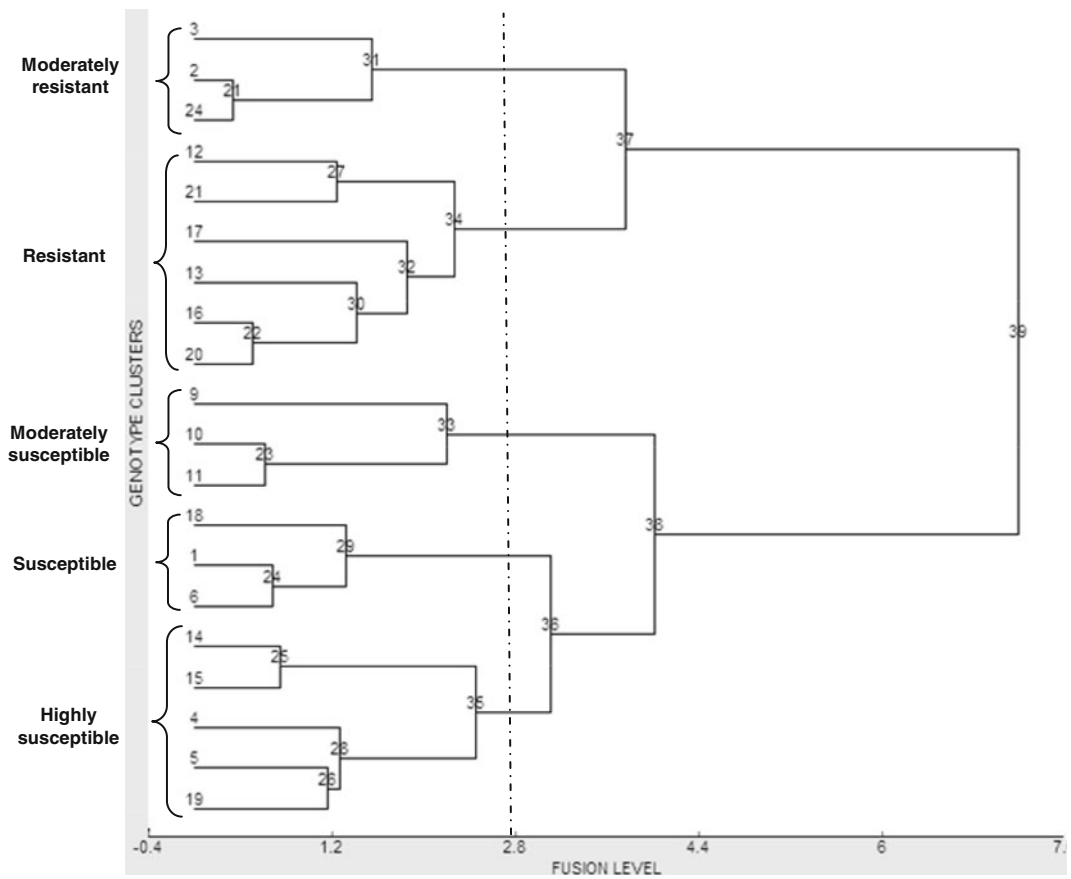

Fig. 10 Susceptibility classes of barley cultivars based on GGE analysis of their responses to isolates *Pyrenophora teres* f. *maculata*. Numbers from 1 to 24 represent the barley genotypes in the differential set listed in Table 1. Other numbers represent nodes of the dendrogram

regions of Morocco sampled. The majority of *Pyrenophora teres* f. *maculata* isolates used were sampled in 2001 and form the basis of an earlier study; this is not to infer that spotted net blotch prevailed in that growing season. In the 2003-2004 agricultural season, most isolates sampled corresponded to the net-form of *Pyrenophora teres,* and isolates of *Pyrenophora teres* f. *maculata* were less prevalent in the fields sampled. We found that 93% of the Ptt population was composed of avirulent to moderately virulent isolates. This may have arisen from the shortage in the availability of resistant cultivars to Moroccan farmers since only barley landraces or susceptible cultivars are typically planted. The prevalence of these susceptible land races has resulted in weak selection pressure on the local populations of *P. teres*. Our study indicated that the spot form population was divided into three classes of virulence, each having a similar number of the isolates. The differences found between Ptt and Ptm may arise from the co-evolution between this pathogen and his host.

Based on infection responses, Steffenson and Webster (1992) differentiated 91 Ptt isolates into 13 pathotypes, whereas in Australia, Gupta and Loughman (2001) reported 8 pathotypes for net-form isolates and only two groups for the spot-form isolates. The discrepancy between studies dealing with pathotype diversity may arise from differences in research methodologies and from the use of different sets of barley differentials. However, phenotyping barley net blotch isolates into pathotypes with the help of the IIG model and cluster analysis, as described here, is a valid alternative methodology that can be used regardless of the resistance genes involved. Discrepancies may also arise from the fact that designation of pathotypes and their numbers is dependent on the number of differentials involved. Assuming independence among genes, for a differential set containing n lines or genes, there are 2^n pathotypes or races that can be differentiated. Thus, theoretically, Steffenson and Webster (1992) should have found 2^{22} pathotypes, but in fact only differentiated 13. By contrast, the integrated approach used here generated a differential set that is fewer in number, locally relevant, easily available and less costly for phenotyping the Moroccan population of *Pyrenophora teres*.

In our study, the mixed model analysis helped in dividing the virulence classes (Correspondence analysis) into four groups for Ptt isolates, while it reduced Ptm isolates into three groups. Note that clusters represented by pathotypes Pptm3 and Pptm4 of *P. teres* f. *maculata* were joined to form one class of avirulent isolates.

The dendrogram for spot form isolates (correspondence analysis) did not match the classification made by mixed model analysis. The former gave four classes of quantified virulence while the latter gave only three. The difference between the two analyses may arise from the presence of missing values that represented 5% of the data in Table 4. In correspondence analysis, the entire isolate array that contains a missing value is deleted by default. However, in the mixed model, the missing value is evaluated. Consequently, we relied upon mixed model analysis to differentiate classes of quantified virulence into virulent, moderately virulent and avirulent groups of Ptt isolates. Furthermore, mixed model analysis is regarded as more reliable when there is missing data or the design is unbalanced (Littell et al. 1996; Demidenko 2004).

The interaction of *P. teres* f. *teres* isolates with barley differentials revealed 10 pathotypes, while nine were identified in the population of spot-form isolates. The most common pathotypes of Ptt were Pptt1 (3J-05), Pptt4 (19CH) and Pptt9 (2S) representing 16%, 16% and 13% of the population, respectively. The isolates of *P. teres* f. *maculata* were evenly distributed among nine pathotypes. In contrast, Douiyssi et al. (1998) noted in an earlier study that pathogenic variability among Moroccan net-type isolates was great and none of the fifteen tested isolates was identical.

In the current study, the presence of only four, common discriminative cultivars (Amira, Orge628, Rabat071 and Acsad60) among the barley differentials we developed for the two forms of *P. teres,* may support the genetic differences existing between the two forms outlined by Friesen et al. (2006). The original barley differential sets were reduced from 22 to 9 and from 20 to 8 discriminative genotypes for Ptt and Ptm isolates, respectively. In addition, and based on the key codes, the new differentials in Table 8 and 10 would easily differentiate *P. teres* isolates into pathotypes. Screening for sources of resistance to *P. teres* should be achievable using the pathotypes identified.

Based on the pathogen population sampled and the data generated, we can propose a protocol to breed for resistance to *P. teres* in Moroccan barley. Cultivar Acsad60 that is resistant to all pathotypes of the spot-

form isolates and all net form pathotypes except pathotypes Pptt2 (18J) and Pptt10 (2CH), should be crossed with cultivar Orge628 that is resistant to all Ptt pathotypes except the pathotype Pptt2. The cultivar Orge628 is a six-rowed cultivar well adapted to Moroccan conditions and has good agronomic characteristics, while Acsad60 is a two-rowed cultivar (Saidi et al. 2005). Since Moroccan farmers prefer agronomic characteristics such as a six-row spike, high straw and grain yield, and a leafy plant coupled with large grain size (El Yousfi and Ezzahiri 2002), screening for resistance to either form of net blotch should take these agronomic characters into account. Screening should start at the F2 generation, based on the advice of Jonsson et al. (1999) and the selected F2 six-row lines should be fixed *via* a doubled haploidization technique to facilitate the selection process. Selected six-row material resulting from the cross of Orge628 × Acsad60 should provide resistance to pathotypes of Ptt except Pptt2. The selected and fixed lines could then be crossed with cultivar Rabat071 to provide resistance to pathotype Pptt2 (18J). The resulting F2 lines when selected against the net-type forms are expected to control all pathotypes of *P. teres*. However, modifications to this breeding program, such as the use of backcrossing coupled with doubled haploidization techniques may be necessary to select and fix desirable agronomic characters in addition to selecting for resistance.

Because it is not possible to control where resistant varieties are planted and Moroccan farmers are unlikely to recognize the two forms of the disease, the Moroccan barley breeding program should develop six-rowed resistant cultivars combining resistance to both forms of net blotch. Adopting this strategy coupled with the identification and use of molecular markers would help ensure the national breeding program is successful in developing resistant cultivars whose production will minimize losses from the disease.

Host-pathogen coevolution can result in the rise of new virulent pathotypes in response to resistant cultivars being grown (Brown and Wolfe 1990). Therefore, the use of adapted cultivars as differentials is of a great value when differentiating pathogen isolates into specific pathotypes because rapid advance can be achieved in breeding programs. Furthermore, and based on our integrated method (Garret et al. 2004), scientists dealing with phenotyping of pathogen isolates can develop their own differential set and easily group their isolates into pathotypes independently of the genes that may exist in their differentials.

Conclusion

The current study demonstrated methods of quantifying virulence of *P. teres* isolates by the use of correspondence and mixed model analyses. The choice between these two statistical tools depends on the number of isolates involved. When the number of isolates is small, the use of correspondence analysis is preferable. In contrast, the use of mixed models analysis is justified when the number of isolates is greater than 30 or where missing values are involved.

To our knowledge, this is the first report that has dealt with the phenotyping of the two forms of *Pyrenophora t*eres in Morocco. It revealed that the Moroccan population of *P. teres* was comprised of 10 Ptt pathotypes and 9 Ptm pathotypes, respectively. Furthermore, IIG model analysis coupled with cluster analysis is useful for phenotyping host-pathogen interactions as per the suggestion of Yan and Falk (2002). This analysis produced two unique barley sets to differentiate *P. teres* isolates into pathotypes. These sets of barley differentials, based on key codes, are simple and will be less costly, both in time and in space, when used for phenotyping in subsequent studies. Finally, a selection scheme is proposed for the Moroccan national barley-breeding program to use the sources of resistance identified to minimize the negative impact of net blotch on barley production.

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