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Pathotypic diversity of *Rhynchosporium secalis* (Oudem) in Tunisia

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Scald, caused by *Rhynchosporium secalis* (Oudem), is an important disease of barley in Tunisia particularly in northern, northwestern and central parts of the country where the climate is usually cold and wet during most of the barley growing season. Pathogenic variability of the barley scald pathogen in Tunisia was determined by testing the pathogenicity of 100 isolates from 5 different regions on 19 host differentials. Pathotypic diversity was high, with 93 *R. secalis* pathotypes identified on two differential sets (one comprising 9 and the other 10 barley lines) containing known resistance genes. A few pathotypes comprised 2% of the isolates; however, the majorities were represented by a single isolate. None of the differential lines was resistant to all isolates. The differential cultivar "Astrix" was the least compatible with the scald pathotypes followed by the differential cultivars "Atlas" and "Abyssinia". Compatibility of the pathotypes on "Rihane" (69%) was close to that on "Osiris" (73%) and "La Mesita" (61%). None of the pathotypes was found in all the five regions of Tunisia surveyed. Some pathotypes were specific to a single region while others were found in several regions. The incidence of pathotypes varied considerably among regions, with region 3 (northwestern Tunisia) comprising the largest number of pathotypes. Virulent pathotypes were recovered in all regions but more pathotypic variability (44%) was observed in the semi-arid region 3. Differential cultivars allowed classification of *R. secalis* in four virulence groups. Canonical discriminant analysis showed no apparent association between virulence and geographical origin of the populations. Pathogenic variability in *R. secalis* in Tunisia was found not to be associated with geographical region, hence, the necessity for deployment of different resistance sources in major barley growing areas.

Key words: Rhynchosporium secalis, barley, virulence groups, pathotypic variation.

INTRODUCTION

Scald disease of barley caused by *Rhynchosporium secalis* (Oudem) J. J. Davis, is widespread in Western and Central Asia and North Africa, particularly in areas characterized by cool winters. High infection levels were observed during the year 1998 in Eritrea, Ethiopia, Turkey, Tunisia, and Morocco. In Tunisia, this disease is currently one of the major constraints to barley production as a result of intensive production, monoculture of 1-2

varieties, and the predominant environmental conditions that are conductive to the development of this disease (Yahyaoui et al., 2002). Scald disease causes important yield losses worldwide. Current reported average yield losses vary from 1 to19% (Xi et al., 2000; Turkington et al., 1998) but could be as high as 40% (Williams et al., 2003; Yahyaoui, 2003; Salamati and Tronsmo, 1997) or greater in highly susceptible cultivars (Williams et al., 2003). The quality of malt and feed barley grain can also be drastically affected by scald (Edney et al., 1998).

Field populations of the barley scald pathogen are characterized by a high level of pathogenic variability, as has been demonstrated in different regions of the world

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Table 1. List of *Rhynchosporium secalis* isolates, and collection sites in Tunisia sampled in 2003.

Reference/site	Geographic position/rainfall[£]	No, of isolates	Origin/collection site (District)	Regions
T1	North West - HR	8	Kodia (Jendouba)	Region 1
T17	North West - HR	2	Nebeur (Kef)	Region 2
T14	North West - MR	3	Tel Ghozlene (Kef)	Region 2
T10	North West - MR	5	Touiref (Kef)	Region 2
T2	North West - LR	5	Boulifa (Kef)	Region 3
T4	North West - LR	3	Garn Halfya (Kef)	Region 3
T15	North West - MR	4	Borj Aifa (Kef)	Region 3
T16	North West - MR	4	Oued Essouani (Kef)	Region 3
T24	North West - LR	3	Borj Massoudi (Siliana)	Region 3
T8	North West - LR	7	Boulifa montagne (Kef)	Region 3
T5	North West - LR	5	Eddyr (Kef)	Region 3
T12	North West - MR	7	Choirnia (Kef)	Region 3
T13	North West - MR	5	Rihana (Kef)	Region 3
T9	North West - MR	6	Lorbous (Kef)	Region 4
T28	North West - LR	6	Massouj (Siliana)	Region 4
T23	North West - LR	7	Dehmani (Kef)	Region 4
T20	Central - LR	6	Thala (Kasserine)	Region 5
T22	Central - LR	6	Foussana (Kasserine)	Region 5
T21	Central - LR	7	Zalfana (Kasserine)	Region 5

[£]Annual Rainfall: HR=High rainfall (>400 mm); MR=Moderate (250-400 mm); LR=Low (<250 mm).

where the disease is a problem (Jørgensen and Smedegaard-Petersen, 1995). The highly variable nature of *R. secalis* may result in the selection of new pathotypes that can overcome host plant resistance genes, hence the importance of using host resistance that could provide adequate protection over a long period of time. So far, a total of 13 resistance genes have been identified (Sogaard and Von Wettstein-Knowles, 1987; Abbot et al., 1992). Harrabi et al. (1984) found that as much as 11 alleles condition resistance in barley. However, the scald pathogen has been constantly evolving, resulting in a break down of the resistance genes following deployment. Pathogenic variability in *R. secalis* has been studied by several authors worldwide (Yahyaoui et al., 2002). Tekauz (1991) used a set of 10 differential cultivars and identified 45 pathotypes in Canada. From 203 Australian scald isolates, Ali et al. (1976) identified 35 pathotypes on a set of 21 differentials. Xi et al. (2002) identified 52 pathotypes using 256 isolates from Alberta. Fukyama et al. (1998) classified 38 isolates into 36 different pathotypes according to their virulence of 14 differentials.

Although knowledge of geographic distribution of *R. secalis* (Bouajila et al., 2004) would provide information on the distribution of the disease within the barley growing, understanding *R. secalis* pathogenic variability and population genetic structure would eventually allow better targeted resistance breeding. Hence, the purpose

of this study was to determine pathotypic diversity in *R. secalis* in the major barley growing areas of Tunisia.

MATERIALS AND METHODS

Field sampling

Barley leaves infected with *R. secalis* were sampled in March 2003 from nineteen locations in Tunisia using a hierarchical sampling method (McDonald et al., 1999; Meles et al., 2005). All leaf samples were collected in naturally infected fields. The 19 locations cover major barley growing areas in the country (Table 1) and represent five agro-ecological regions in North, North West, and Central Tunisia. The regions range from sub-humid to semi-arid. Region 1 is located in a relatively high rainfall area with mild winter temperatures and barley is used a rotational crop for hay, forage, or even silage. The two most commonly grown cultivars in this region are "Rihane" and "Martin". These two cultivars are susceptible to most foliar diseases, including scald. Region 2 is located in the semi-arid region where the average annual rainfall is around 350 mm and has extended cool temperatures during the winter. The total area is cultivated to the cultivar "Rihane". Region 3 represents the major barley growing area and is characterized as semi-arid with average annual rainfall that varies from 200-300 mm and also has extended cool winter temperatures. Barley is often grown in a wheat-barley-fallow rotation or a barley-fallow rotation. Region 4 is similar to region 3 but has lower average rainfall that varies from 200-250 mm. In the latter two regions, cultivars "Rihane, Martin, and Manel" that were obtained through selection in the breeding programs are widely cultivated in addition to the commonly grown local landraces. The varieties Rihane and Manel were bred and released by the national program, whereas Martin is an old introdu-

Table 2. Differential cultivars, CI.number, and respective resistance genes*.

Set	N	Differential/cultivar	C.I. Number	Resistance genes
I	1	Armelle		Rh, BRR1
	2	Astrix		BRR2
	3	Athene		BRR3
	4	Igri		BRR4
	5	La-Mesita		Rh4, Rh10, Rh at Rh-Rh3-Rh4, BRR5
	6	Osiris	1622	Rh4, rh6, Rh10, (Rh3?) BRR6
	7	Pirate		BRR7
	8	Digger		Partial resistance
	9	Trebi	936	Rh4, rh6=rh?, Rh at Rh-Rh3-Rh4
II	10	jet		Rh6, rh7, rh,, rh6
	11	Kitchin	1296	Rh9(incomplete)
	12	Osiris	1622	Rh4, rh6, Rh10, (Rh3) BRR6
	13	Steudel	2226	rh6, rh7
	14	Bey	5581	Rh3 (?)
	15	Atlas 46	7323	Rh, Rh2, Rh3
	16	La-Mesita	7565	Rh4, Rh10,Rh at Rh-Rh3-Rh4, BRR5
	17	Modoc	7566	(Rh4), Rh2, rh6, (Rh3?), Rh at Rh-Rh3-Rh4
	18	Forrajera	8158	unkown
	19	Abyssinia	668	(Rh1), (Rh9)
	20	Rihane-03**	local	Unkown

*Table adapted from Pinnschmidt Jakob Willas (www.crpmb.org/scald).
**Rihane-3, widely cultivated in North Africa and Syria.

ction of unknown origin; nonetheless large areas are still cultivated to indigenous landrace cultivars. Region 5 represents the central part of the country, has very low annual rainfall (200-250 mm) and is characterized by extremely cold winter temperatures that rapidly increase in spring. The larger portion of this region is grown to local landrace cultivars and "Rihane" occupies no more than 10% of the area. The scald isolates were collected from farmers' fields; except for region 5, most likely the majority of the leaf samples were collected from the cultivar "Rihane". One hundred scald isolates (Table 1) were obtained and tested on two differential sets (Table 2) that have two differential genotypes in common, "La-Mesita" (D5 & D16), and "Osiris" (D6 & D12) in differential set I (DSI) and II (DSII), respectively, as well as the test cultivar "Rihane" that was used as a standard variety.

One field population was sampled at each location and for each field population eight circular sampling spots, each 1 m in diameter, were positioned along two parallel transects, with four spots per transect. The two transects were separated by 10 m, which gave a total collection area of 10 m × 30 m including the unsampled spaces between the sampling spots. At each 1-m diameter circular spot, 10 infected leaves from different plants or tillers were sampled during a circular sweep; 80 infected leaves were thus collected in total from each field. Leaf samples were placed in paper envelopes and allowed to air-dry at room temperature for 48 h, then stored at 6°C until further analysis.

Fungal isolates and inoculum

Leaf tissue from each field location or collection spot was placed in a paper envelope, air-dried at room temperature for at least 1 week, and then stored at 5°C. Dried leaf pieces cut from typical scald lesions were surface-sterilized by dipping them in 90% ethanol for

10 s, then in 0.5% sodium hypochlorite solution for 60 s. The pieces of leaf were then dried by pressing them between two layers of sterile filter paper; after this, they were placed on a plastic mesh that rested on rubber bands on top of damp sterile filter paper in a Petri dish. The leaf-pieces were incubated in the dark at 16°C for at least 72 h, to induce fungal growth and sporulation. Using a sterile needle, mycelial tufts were picked out of the resulting fungal colonies and grown for 14 days on lima bean agar (LBA) (100 g of lima bean soaked overnight, boiled in 1 L of water, shaken and filtered through muslin, 17 g of pure agar added, the volume adjusted to 1 L and autoclaved) amended with gentamycin sulphate at 10 mg L^{-1}. After around two weeks of growth, single colonies were picked out and macerated in a test tube containing sterile distilled water using a sterile glass rod. For each isolate, the resulting spore and mycelial suspension was transferred to a 50 ml flask containing potato dextrose broth amended with kanamycin to 50 µg/ml. The conidial/mycelial suspensions were also transferred into 2-mL microcentrifuge tubes containing sterile distilled water and several pieces of filter paper discs (6 mm diameter); the tubes were then stored at 20°C. Inoculated flasks were incubated in a shaker at 17°C for 3 to 4 weeks.

To produce inoculum, some filter paper discs were removed from storage and transferred onto petri plates containing LBA. The petri plates were incubated for 10-12 days for mycelial growth and sporulation. Developing fungal colonies were removed from the agar surface with a sterile scalpel and macerated in sterile test tubes containing sterile distilled water using a sterile glass rod. The resulting suspension was spread on the surface LBA plates and cultured for 12 days in the dark at 17°C. Mycelia/conidia were scraped off the surface of 12-day-old colonies using a sterile microscope slide and homogenized in sterile distilled water using a household blender. The resulting suspension was filtered through a fine plastic mesh. The spore concentration was determined using a
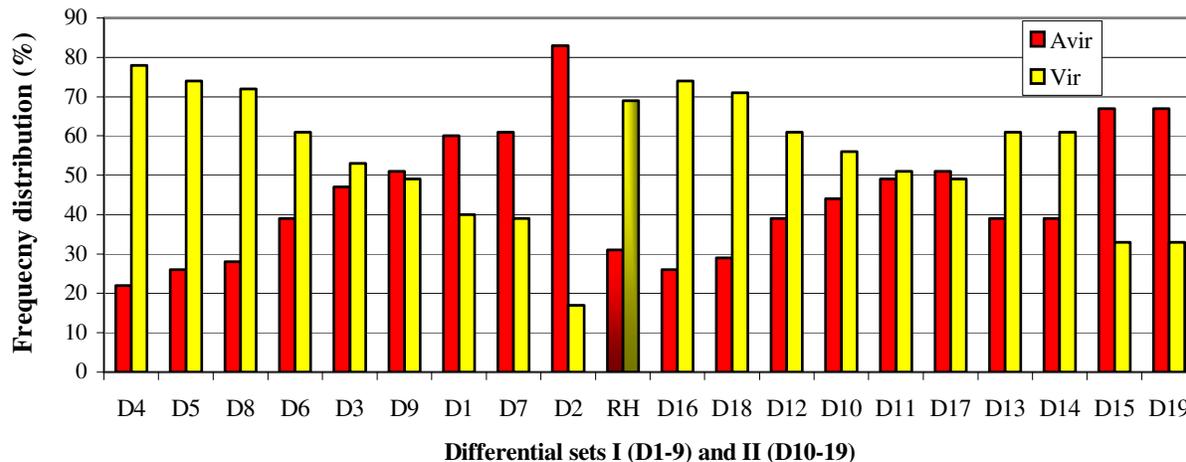
Figure 1. Pathotypic variation (virulence/avirulence) in *R. secalis* on two differential sets.

haemacytometer and adjusted to 10^6 spores/ml.

Host cultivars and inoculation

R. secalis isolates were assayed on two sets of differential cultivars of 9 and 10 cultivars, respectively (Table 2). The barley cultivar "Rihane" which covers over 70% of the barely area in Tunisia was used as a standard. The differentials were seeded in 13 cm diameter plastic pots containing a soil potting mix. Plants were grown in a greenhouse supplemented with 14 h light,10 h dark photoperiod and were inoculated at Zadoks growth stage 13 (Zadoks et al., 1974), 14-16 days after planting. The seedlings were sprayed with a spore suspension (10^6 spores/ml) amended with Tween 20 at the rate of one drop/100 ml, using a Dosage Spray Gun. Inoculated plants were then kept in a mist chamber at 12°C in darkness to maintain relative humidity at 100% for 48 h. After the mist period, the inoculated plants were moved back to the greenhouse bench with alternating temperature of 17°C day and 10°C night. The plants were watered two or three times a week from the base to prevent cross contamination. Plants inoculated with sterile distilled water served as a control.

Disease severity was assessed 17 days after inoculation, using the rating scale described by Ceoloni (1980) with a slight modification: (0) no visible symptoms; (1) small lesions confined to leaf tips and brown to gray necrotic spots; (2) some what larger lesions; (3) larger and coalescing with distinct margins, and (4) total collapse of the leaf with no discrete lesions within the wilted area. For the purpose of determining pathogenic variability, lesion types 0, 1 and 2 were considered incompatible reactions (resistant) and lesion types 3 and 4 were considered compatible reactions (susceptible). Pathotypes of *R. secalis* were characterized on the two sets of differential barley cultivars. The term "pathotype" is utilized in this study to denote *R. secalis* isolates that differed in virulence on the barley differentials (Tekauz, 1991). Canonical discriminant analysis was used to assess the divergence of pathogenic variation of the isolates among regions (Zhang et al., 1992; Xi et al., 2002). The analysis was performed using PROC CANDISC (SAS Institute Inc. 1989).

RESULTS AND DISCUSSION

Traditionally, virulence in *R. secalis* has been measured in terms of compatible reaction on the host cultivar (Brown, 1990) and the assessment of the disease is based on the leaf area affected (James and Teng, 1979). Williams and Owen (1973) defined a compatible isolate as one which caused disease symptoms affecting more than 10% of the leaf area. In this study, isolates resulting in disease scores "0-2" were classified as avirulent while those that gave a severity rating of "3-4" were considered as virulent. Based on the compatible/incompatible reactions of the barley scald differential genotypes, the 100 isolates tested yielded 93 pathotypes indicating a broad pathogenicity spectrum in North West and Central Tunisia (Figure 1); such high variability in the pathogenicity of *R. secalis* was also reported earlier from Australia, California, New Zealand, Italy, Japan, Denmark and Canada. (Ali et al., 1976; Jackson and Webster, 1976; Cromey, 1987; Ceoloni, 1980; Fukuyama et al., 1998; Jorgensen et al., 1995; Xi et al., 2002).

In this study, marked and consistent differences were observed among the barley differential cultivars in reaction to infection by individual *R. secalis* isolates (Table 3), thus revealing the complex virulence capability of the isolates. Pathotype complexity referred to the ability of a pathotype to cause compatible reactions on several barley genotypes. The differentials developed typical scald symptoms in the green house tests. Ninety-three pathotypes were identified among 100 isolates obtained from the five regions surveyed in Tunisia (Tables 3, 4, 5 and 6). *R. secalis* isolates that differed in virulence on the barley differentials were designated as different pathotypes and arbitrarily assigned a numeric number for the purpose of ease of the analysis. The 100

Table 3. Virulence patterns of 93 pathotypes on 19 differential genotypes grouped on differential set I (D1-D9) and differential set II (D10-D19) and on the cultivar Rihane.

Pathotype Ref. No*	Differential set I									Differential Set II										RH
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19	RH
TP1	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
TP2	S	S	S	S	S	S	R	S	S	S	S	S	S	R	S	S	S	S	S	S
TP3	S	S	S	S	D1	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
TP4	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	S
TP5	S	R	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S
TP6	S	S	S	S	S	S	R	S	S	R	S	S	S	S	S	S	R	S	S	S
TP7	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	R	S
TP8	S	S	S	S	S	S	2	S	S	R	S	S	R	S	S	S	R	S	R	S
TP9	R	R	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S
TP10	R	R	S	S	S	S	S	S	R	S	S	S	S	S	R	S	S	S	S	S
TP11	S	R	R	S	S	S	S	S	S	S	R	S	R	S	R	S	S	S	S	S
TP12	S	R	R	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	R	S
TP13	R	R	S	S	S	S	S	S	S	R	S	S	R	S	R	S	S	S	S	S
TP14	R	R	S	S	S	S	S	S	S	R	S	S	S	R	R	S	S	S	S	S
TP15	S	R	R	S	S	S	R	S	S	S	S	S	R	S	S	S	S	S	R	S
TP16	R	R	S	S	S	S	R	S	S	S	S	S	S	R	S	S	S	S	R	S
TP17	S	R	S	S	S	S	R	S	R	R	S	S	S	S	S	S	S	S	S	S
TP18	S	R	S	S	S	S	S	R	R	R	S	S	S	S	S	S	R	S	R	S
TP19	S	S	S	S	R	S	R	S	S	S	S	S	S	S	S	R	R	S	R	S
TP20	S	R	S	S	S	S	S	S	S	S	S	S	R	S	R	S	R	R	R	S
TP21	S	R	S	S	S	R	S	S	S	R	R	S	S	S	R	S	S	S	S	S
TP22	S	S	S	S	S	S	R	S	S	S	R	S	S	S	R	S	R	R	R	S
TP23	S	S	S	S	S	S	R	S	S	S	R	S	R	R	R	S	R	S	R	S
TP24	S	S	S	S	S	R	R	S	S	S	S	R	R	R	S	S	S	S	R	S
TP25	S	R	S	S	S	S	R	S	S	R	S	S	R	R	R	S	S	S	R	S
TP26	R	R	S	S	S	S	R	S	R	S	S	S	R	R	R	S	S	S	S	R
TP27	R	R	S	S	S	S	S	S	S	R	S	S	R	S	R	S	S	S	R	S
TP28	R	R	S	S	S	S	S	S	R	S	S	S	S	R	R	S	S	S	S	S
TP29	R	R	S	S	S	S	S	S	R	R	S	S	S	S	R	S	R	S	S	S
TP30	S	S	R	S	S	S	R	S	S	S	S	S	R	S	S	S	S	S	R	S
TP31	R	S	R	S	S	S	S	R	R	S	R	S	S	S	R	S	S	S	R	S
TP32	R	R	S	S	S	R	R	S	R	S	S	R	R	S	S	S	S	R	S	R
TP33	S	R	R	S	S	S	S	R	S	S	S	S	R	R	R	S	S	S	S	R
TP34	R	R	S	S	S	S	S	S	R	S	S	S	S	R	R	S	S	S	R	S
TP35	S	R	S	S	S	S	R	S	R	S	R	S	R	S	R	S	S	R	S	R
TP36	R	R	R	S	S	S	S	S	R	S	R	S	S	R	S	S	S	S	R	R
TP37	R	R	R	S	S	S	R	S	S	S	R	S	R	S	R	S	S	S	R	S
TP38	R	R	R	S	S	S	S	R	S	R	R	S	R	S	R	S	S	S	S	S
TP39	R	R	S	S	S	S	R	S	R	S	S	S	S	R	R	S	S	S	R	R
TP40	S	R	S	S	S	S	R	S	R	R	S	S	R	R	R	S	S	S	R	S
TP41	R	R	S	S	S	S	R	S	S	R	S	S	R	R	R	S	S	S	S	S
TP42	S	R	R	R	S	S	S	S	S	R	R	S	S	R	R	S	S	S	R	R
TP43	R	R	S	R	S	S	S	S	R	S	S	S	R	S	R	S	R	S	R	S

Table 3. contd.

TP44	R	R	S	S	S	S	R	R	R	S	R	S	R	S	R	S	R	R	S	S
TP45	R	R	R	S	S	S	S	R	R	R	R	S	R	R	R	S	S	S	S	S
TP46	S	R	S	S	R	R	S	R	R	R	S	R	R	R	S	R	S	S	S	R
TP47	R	R	S	S	S	R	R	S	S	S	S	R	S	R	R	S	R	S	R	S
TP48	R	R	S	S	S	R	R	S	R	S	S	R	S	R	R	S	R	S	S	S
TP49	S	S	R	R	S	R	R	S	S	S	R	R	R	R	S	S	S	R	R	S
TP50	S	R	S	R	S	R	S	S	S	R	S	R	R	R	R	S	S	R	R	R
TP51	S	R	R	S	R	S	S	S	S	S	R	S	R	R	S	R	R	S	R	R
TP52	R	R	S	S	S	S	R	S	R	S	R	S	S	R	R	S	R	S	R	S
TP53	R	R	R	S	S	R	R	S	R	R	R	R	S	S	R	S	S	S	R	S
TP54	S	R	R	S	R	S	S	R	S	R	S	S	R	R	R	R	R	S	S	S
TP55	S	S	S	S	S	R	R	R	S	R	S	R	R	R	S	S	R	S	R	R
TP56	S	R	R	S	S	R	R	R	S	R	S	R	R	R	S	S	S	S	R	S
TP57	S	R	R	R	R	S	R	S	S	S	R	S	R	R	R	R	S	R	S	S
TP58	R	R	S	R	S	S	R	S	R	S	R	S	R	R	R	S	R	R	R	S
TP59	R	R	R	R	R	R	S	S	S	S	R	R	R	R	S	R	S	S	S	S
TP60	R	R	R	R	S	R	R	S	R	R	S	R	S	S	S	S	R	S	R	S
TP61	R	R	S	R	S	S	S	R	S	R	S	S	R	R	R	S	R	S	R	S
TP62	S	R	S	S	S	S	R	S	S	R	R	S	R	R	S	S	R	R	R	R
TP63	R	R	R	S	S	S	R	S	S	S	R	S	S	R	R	S	R	S	R	R
TP64	S	R	R	S	S	S	R	S	R	R	R	S	R	R	R	S	S	R	R	S
TP65	R	R	S	S	S	R	R	S	R	S	R	R	R	R	R	S	R	S	R	S
TP66	R	R	R	S	S	R	R	S	R	S	R	R	S	R	R	S	R	S	S	S
TP67	R	R	R	S	S	R	R	S	S	R	S	R	R	S	R	S	R	S	R	R
TP68	R	R	R	S	R	R	R	S	S	S	R	R	R	R	R	R	S	S	S	R
TP69	R	R	R	S	S	S	R	S	S	R	R	S	R	R	R	S	R	S	R	S
TP70	R	R	R	S	S	R	R	S	R	S	R	R	R	R	R	S	R	S	S	S
TP71	R	R	S	S	S	R	S	S	R	R	R	R	R	R	R	S	R	S	R	S
TP72	S	R	S	S	R	R	S	R	S	R	R	R	R	R	R	R	R	S	R	S
TP73	R	R	S	S	R	S	S	R	S	R	R	S	R	R	R	R	S	R	R	S
TP74	R	R	R	S	R	R	S	S	R	S	R	R	S	R	R	R	R	S	R	S
TP75	R	R	S	S	R	R	S	S	R	R	R	R	R	S	R	R	R	R	S	S
TP76	R	R	R	S	R	S	R	R	R	R	S	S	R	R	R	R	R	R	R	R
TP77	R	R	R	S	R	R	R	S	R	S	S	R	S	R	R	R	R	S	R	S
TP78	R	R	R	R	S	S	R	R	R	S	S	S	R	R	S	S	R	R	R	S
TP79	R	R	R	S	R	R	R	S	R	R	S	R	S	R	R	R	R	S	R	S
TP80	R	R	R	R	R	R	R	R	S	S	S	R	S	S	R	R	R	R	R	R
TP81	R	R	S	S	S	R	R	S	R	R	R	R	R	R	R	S	R	R	R	S
TP82	R	R	R	S	R	R	S	R	R	S	R	R	R	R	R	R	R	S	R	R
TP83	R	R	R	R	R	R	R	R	R	S	R	R	S	S	R	R	S	R	R	R
TP84	R	R	R	S	S	S	R	R	R	R	R	S	S	R	R	S	R	R	R	R
TP85	R	R	R	R	R	R	R	S	R	R	R	R	R	S	S	R	R	S	R	R
TP86	S	R	R	R	S	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R
TP87	R	R	R	R	R	R	R	R	R	S	R	R	S	R	R	R	R	R	R	R
TP88	R	R	R	R	S	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R
TP89	R	R	R	R	R	S	R	R	R	S	R	R	R	S	R	R	R	R	R	R

Table 3. contd.

TP90	R	R	R	S	R	R	R	S	R	S	R	R	R	R	R	R	R	R	R		R
TP91	R	S	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R		R
TP92	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R		R
TP93	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R		R

*TP: Tunisian pathotype

Table 4. Distribution of pathotypes* across regions.

Regions	Pathotypes distribution /region (Individual pathotypes (TP—'s) identified within each region)	No. Path/Iso	Freq. (%)
Region 1	TP: 2, 18, 20, 31, 71,84, 86, 91	8/8	8.6
Region 2	TP: 1, 5, 14, 16, 21, 24, 26, 27, 55, 80	10/10	10.7
Region 3	TP: 3, 4, 7, 9, 12, 13, 19, 23, 25, 29, 30, 32, 34, 35, 40, 42, 43, 47, 49, 52, 52, 53, 56, 57, 58, 59, 60, 62, 64, 65, 67, 68, 69, 72, 73, 74, 75, 77, 78, 85, 86, 88, 93, 93.	41/44	(44.1)**
Region 4	TP: 1, 6, 8, 9, 11, 13, 22, 38, 39, 45, 51, 61, 63, 70, 76, 79, 81, 87, 88	15/19	(16.1)**
Region 5	TP: 10, 15, 17, 28, 33, 36, 37, 41, 44, 46, 48, 50, 54, 66, 82, 83, 89, 90, 92	19	20.4

*Identical pathotypes have the same ref. Number. Underlined pathotypes are repeats of same pathotype identified at other regions or within same region.
**Pathotypes identified in previous region were excluded from total frequency distribution

Table 5. Pathotypic distribution on virulence groups as discriminated by Differential set I (DSI).

V.Group*	Number**		Distribution of pathotypes in Virulence groups and Number of pathotypes	
	Vir.Gen	Path	Grouped Pathotypes***	Individual Pathotypes
Group I (avir)	0	1+	(83, 87, 92, 93)	
	1	4		80, 85, 89, 88
Group II (avir+)	2	8+	(77, 79, 90)	59, 60, 76, 78, 82, 86, 91,
	3	4+	(53, 66, 70)	68, 74, 84
Group III (vir-)	4	9+	(32, 48, 65, 81)	44, 45, 46, 56, 57, 58, 67, 75
	5	15+	(26,39,52); (37,69)	31, 36, 38, 43, 47, 49, 54, 61, 63, 64, 71, 72, 73
	6	10+	(34,29,10,28), (15,12), (17,35,40), (41,16)	18, 33, 42, 50, 51, 55
Group IV (vir)	7	6+	(9,13,14,27), (21,25,62)	11, 19, 24, 30
	8	4+	(2, 6, 23), (5, 20), (3,22)	7
	9	1+	(1, 4, 8)	
No. Path		62	16	46
Tot. No	Pathotypes: 93		Pathotypes discriminated by DSI: 61	

*V.Group: Virulence group; **Number of virulence genes/defeated resistance genes (Vir.Gen) and Number of pathotypes; ***Grouped pathotypes: pathotypes that showed same virulence patterns on DSI.

isolates were thus designated as Tunisia Pathotype 1 "TP1" up to pathotype 93 "TP93". Among the 93 pathotypes identified across the five regions, only seven pathotypes were recovered twice. Pathotypes TP1, TP9, TP13, TP86, and TP88 were recovered at two different regions, whereas TP52 and TP93 were recovered within the same region; none of thepathotypes from region 5 were recovered in other regions (Table 5). Often one or more of the differentials used may be incompatible with all local isolates (Tekauz, 1991). That was not encountered in this study, which may be explained at

least in part by the fact that we used a combination of two differential sets. The differential cultivar "Atlas 46", which was the most resistant in several other studies (Tekauz, 1991; Ali et al., 1976; Brown, 1985; Ceoloni, 1980; Cromey, 1987), had a compatible reaction with 28 isolates. Among thedifferential cultivars tested (Tables 2, 3) Astrix, Abyssinia, and Atlas 66 were the least compatible genotypes whereas Rihane, and the differential genotypes Athens, La Mesita, and Digger were very compatible (Table 3). All *R. secalis* isolates showed different pathotypic reaction on the 19 differ-

Table 6. Pathotypic distribution on virulence groups as discriminated by Differential set II (DSII)

V.Group*	Number**		Distribution of pathotypes in Virulence groups and Number of pathotypes	
	Vir.Gen	Path	Grouped Pathotypes***	Individual Pathotypes
Group I (avir)	0	1+	(91,93)	
	1	5+	(90,92)	72, 81, 84, 87
	2	9		71, 73, 75, 76, 82, 86, 87, 88, 89
Group II (avir+)	3	10		50, 58, 62, 64 65, 69, 74, 79, 85
	4	14		23, 49, 51, 54, 55, 57, 61, 66, 67, 68, 70, 77, 80, 83
Group III (vir-)	5	14+	(52,63), (40,25)	20, 22, 42, 44, 45, 46, 47, 53, 56, 59, 78
	6	10		8, 24, 27, 35, 37, 38, 41, 43, 48, 60
Group IV (vir)	7	12+	(34, 39), (26, 33)	11, 13, 14, 18, 19, 21, 29, 31, 32, 36
	8	4+	(30, 15)	6, 16, 28
	9	5+	(4, 12, 7), (9, 17)	2, 5, 10
	10	1+	(1, 3)	
No. Path		83	10	72
Tot. No	**Pathotypes: 93**		**Pathotypes discriminated by DSII: 76**	

*V.Group: Virulence group; **Number of virulence genes/defeated resistance genes (Vir.Gen) and number of pathotypes; *** Grouped pathotypes: pathotypes that showed same virulence patterns on DSII; + more than one pathotype grouped together

ential cultivars, except for TP1 and TP93 that were fully compatible and incompatible, respectively, with all the barley genotypes including the check cultivar "Rihane" (Table 3, Figure 1). The virulent pathotype (TP1) was isolated from a widely grown local barley landrace cultivar "Souihli" commonly cultivated in regions 4 and 5, where as the avirulent pathotype (TP93) identified in region 3 was most likely obtained from the commonly grown commercial cultivar "Rihane".

Therefore, a classification onto virulence groups is adopted in this study to determine the magnitude of pathotypic variation in *R. secalis* that could be of use in breeding for resistance in Tunisia. Each virulence group includes pathotypes that show similar virulence patterns on the differential cultivars. Virulence group I "Avir" encompass the incompatible pathotypes that have zero virulence or virulence on only one resistance gene among the known resistance genes in the differential cultivars tested. Pathotypes in Group II "Avir+" are compatible on two to three resistance genes, those in Group III "Vir-" are compatible on 4 to 6 genes, and virulence group IV "Vir" has pathotype that are compatible on more than 7 resistance genes (Tables 5, 6; Figure 2). Virulence groups III "vir-" and IV "vir" have over 70% of the pathotypes identified that can overcome the resistance in the host cultivars.

Differential set I has nine differential genotypes (D1-D9, Tables 2 and 3) that allowed better discrimination among the scald isolates tested, 62 pathotypes were identified and assigned to respective virulence group (Table 5).

Within virulence group I, TP83, TP87, TP92, and TP93 were all incompatible with the host genes in DSI and were grouped as a single pathotype; whereas TP80,

TP85, TP89, and TP88 were virulent on a single but yet different resistance gene (Table 5). These pathotypes were classified in virulence group I. This group is considered as avirulent "Avir" and represented only 8.6% of all the pathotypes recovered across the five regions. Pathotypes in group II are considered as low virulence types (avir+) as they showed virulence on 2 to 3 different resistance genes; TP77, TP79, and TP90, and TP3, 66, and 70 had the same virulence pattern and were virulent on two and three different resistance genes, respectively. Eleven other pathotypes had different virulence patterns on 2 to 3 resistance genes and were put in this virulence group; this group covers about 17% of the pathotypes. Virulence group III has the largest number of pathotypes (50.5%) among which 8 pathotypes grouped 2 to 4 pathotypes that showed the same virulence pattern on DSI; the remaining 26 showed dissimilar virulence patterns and were virulent to 4 to 6 resistance genes. This group would be considered as virulence risk group (vir-) and could cause crop losses under high disease presssure; it would serve as source of inoculum particularly in regions 3, 4, and 5 where barley is an important cereal crop and occupies large areas. Virulence group IV has 22 virulent pathotypes, 6 of which showed the same virulence patterns with virulence to 6 to 7 resistance genes; and have unrelated virulence pattersn on 6 to 7 resistance genes in DSI (Table 5). Pathotypes TP1, 4, and 8 were compatible with all the 9 resistance in DSI (Table 3, Figure 1). This virulence group presents very high risk in barley producing areas and such pathotypes could inflict great damage on susceptible barley cultivars. Differential set II (DSII) has 10 differential genotypes (D10-D19, Tables 2, 3, 5; Fig-

**Number of pathotypes within eah virulence group detected by
DSI, DSII, and by both DSI&II**
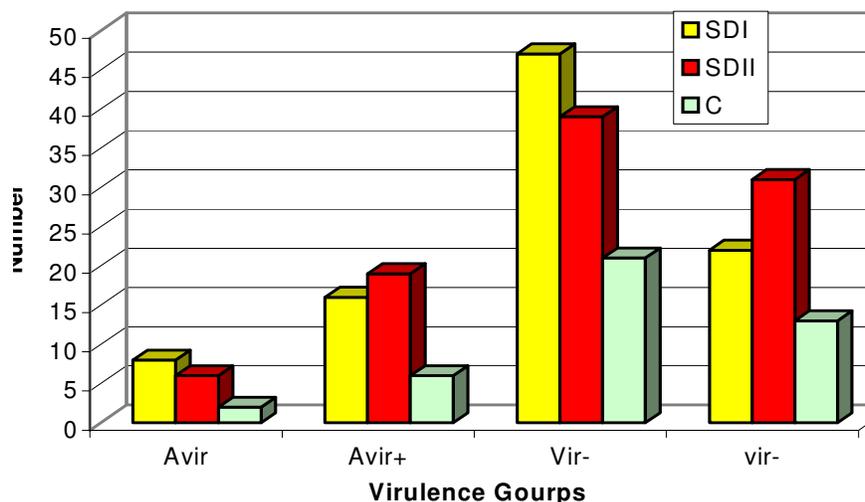
Figure 2. Virulence group distribution according to scald differential sets.

ure 1). Using the same virulence groups, the distribution of 93 pathotypes showed similar trends as with DSI. Virulence group I has two pathotypes (TP 91, and TP93) that were incompatible with all the resistance genes in DSII. TP90 and TP92 were virulent on the differential cultivar "Jet" (D10), whereas TP86 and TP72 were virulent on D15 (Atlas 46) and D18 (Forragera), respectively. Pathotypes in this virulence group are considered as avirulent and, as in the case of DSI, have the lowest frequency (8.6%) compared to the rest of the virulence groups. Pathotypes in virulence group II represent the "avir+" types on DSII (17.2%) but are very diversified, hence they would be a good source of recombination among and between pathotypes even though they have low virulence on the resistance genes. As with DSI, virulence group III has the largest number of pathotypes (73%); only four pathotypes showed same virulence patterns ("TP52 - TP63" and "TP40 - TP25") and were virulent on 5 resistance genes. As stated previously this virulence group is considered as high risk group in major barley producing areas. Virulence group IV has more pathotypes when tested on DSII compared to what was observed on DSI. Eight pathotypes were virulent to 9 resistance genes and two (TP1 and TP3) were virulent to 10 resistance genes. Thirteen pathotypes were regrouped in 5 pathotypes as they showed same virulence patterns on DSII and were compatible on 7 to 10 resistance genes (Tables 3 and 6). The pathotypes in this virulence group were detected across the five regions.

A wide virulence spectrum was found in the area surveyed and collection sites were well referenced using GIS. The canonical discriminant analysis showed some
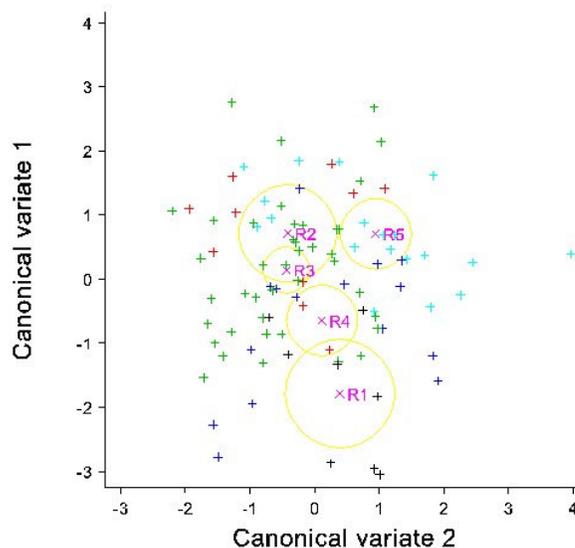
Figure 3. Coordination of the first two canonical variables based on mean disease severity using 100 isolates of R. secalis from five regions in Tunisia.

divergence in virulence among regions (Figure 3). The isolates from region 4, region 3 and region 2 were more complex in terms of their compatibility on the differential cultivars. Those in region 5 were not as diverse and were not found in other regions. We suggest that there is some association between virulence and geographical origin of the scald populations. Jørgensen and Smedgaard-Peterson (1995) found no obvious pattern between geographic distribution of races and specific virulence

patterns from various parts of Denmark. Kajiwara and Iwata (1963) classified 37 Japanese isolates of *R. secalis* into 10 groups, J1 to J10, of which J3, J4, J5, J7 and J9 were distributed in the Hokuriku district (Fukui, Ishikawa, Toyama and Yamagata). They concluded that no distinct geographical differentiation occurred in the Japanese isolates. Xi et al. (2002) observed that there was divergence in virulence among locations and Goodwin et al. (1992) found differences in complexity and diversity among scald populations from three states in the United States and observed that isolates from the same cultivars at different locations usually had different pathogenicity. These observations may reflect the extreme variability in the virulence of the pathogen. The Tunisian *R. secalis* populations are more variable than other studies. However, no satisfactory explanation has been found for the origin of variation in populations of this fungus, which could be due to spontaneous mutation, recombination, a parasexual cycle or even an unknown sexual stage (McDonald et al., 1999; Goodwin et al., 1992). We hypothesize that the extreme phenotypic variability could be an indication that sexual recombination is occurring in these regions.

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