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Pathogenic Variation in *Rhynchosporium secalis* on Barley in Ethiopia

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الاختلافات المرضية الموجودة في فطر رينكوسبوريم يسكالس على محصول الشعير في أثيوبيا

كيروس ميليس و مينجيستو هولاوكا و ميكل ديدمان

خلاصة: يتضمن هذا البحث أول دراسة تفصيلية لإختلاف الأمراض التي تسببها Rhynchosporium secalis في إثيوبيا. تم عزل أربعة و عشرين فصيلة من Rhynchosporium secalis من أصناف الشعير آرس، بالي، و شوا المزروعة في مناطق زراعة الشعير في إثيوبيا، وقد تم إختيارها على عشرة أصناف من R. secalis من الشعير عرفت بمقاومتها لهذا المرض. لقد كانت أكثر الفصائل ظهورا تلك التي تسبب تفاعلات فرضية على الصنفين استيدولي و كتشن، بينما كانت أقلها تعقيداً . وقد تسببت في إظهار تفاعلات مرضية على هذين الصنفين دون غيرهما. أما الفصليين ١٦ و ٧ فكانت أكثرها تعقيداً و قد تسببت في إظهار تفاعلات مرضية على صنفي الشعير ١٠ و ٩ على التوالي. جمعت هذه الفصائل الفطرية في محطات البحوث وتم عزلها في أصناف الشعير المحسنة التي تم تنبيتها بالنقع في الماء. وكان الفصيل ٦ أكبر فصائل الفطر ظهوراً و الذي تم عزله في أربع مجموعات من مناطق آرس و بيلي و شوا. ظهرت الإختلافات المرضية بين الأبواغ (spores) التي جمعت من ذات الحقل و في ذات المنطقة الجغرافية. وقد أظهرت أصناف الشعير ترك، لاميسيتا، بي، نيفرينورم جيت، و فوراجيرا فعالية كبيرة لمقاومة الأمراض.

ABSTRACT: This paper presents the first detailed study on pathogenic variability in *Rhynchosporium secalis* in Ethiopia. Twenty four isolates of *R. secalis*, collected from Arsi, Bale and Shoa, major barley growing locations in Ethiopia, were tested on ten differential host cultivars, with known genes for resistance to the disease. The most frequent pathotypes were those inducing susceptible reactions on cvs Steudelli and Kitchen and the least complex pathotype identified was able to induce a susceptible reaction on these two cultivars only. Pathotypes 16 and 7 were the most complex and were able to induce susceptible reactions on 10 and 9 of the differential host cultivars respectively. These pathotypes were collected from research stations and were isolated from improved barley cultivars belonging to the malting barley type. The most frequent pathotype was pathotype 6 which was represented by four isolates from different locations in Arsi, Bale and Shoa. Pathogenic variation was detected amongst spores collected from the same field and from the same geographical location. The most effective resistance genes were those possessed by Turk, La-Mesita, Bey, Nigrinudum, Jet and Forrajera.

Keywords: barley, pathogenic variation, *Rhynchosporium secalis*, genes, disease resistance.

In Ethiopia, barley (*Hordeum vulgare* L.) is the most important food crop after tef (*Eragrostis tef* (Zucc) Trotter) and sorghum (*Sorghum bicolor* (L) Moench). During the period 1979 to 1986 the average total area under barley was 876,000 ha, about 14 percent of the total cultivated land in Ethiopia (Central Statistical Authority, 1987).

Scald, caused by *Rhynchosporium secalis* (Oud) Davis, is common in the cool and semi-humid barley growing regions of Ethiopia where the disease has been known to occur since 1967 (Dagnatchew, 1967). Yield losses as high as 67% result from scald infection (Eshetu, 1985).

Pathogenic variation in the fungus has been reported. Kline (1960) was able to demonstrate the presence of variability in pathogenicity and the presence of distinct pathotypes in natural populations. Ayesu-offei (1971) identified a large number of races among 35 isolates examined (cited by Shipton *et al.*, 1974). Ali *et al.* (1976) were able to differentiate 203 isolates into 13

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TABLE 1

***Barley differential varieties and their designated genes conferring resistance to* R. secalis.**

Barley Differential	C.I.[1]	Resistance Genes	Reference
La-Mesita	7565	Rh4	Dyck and Schaller (1961)
		Rh[4], Rh10	Habgood and Hayes (1971)
		Rh-Rh3-Rh4	Starling *et al* (1971)
Trebi	936	Rh4[3]	Hansen and Magnus (1973)
		Rh4	Dyck and Schaller (1961)
		Rh-Rh3-Rh4	Starling *et al* (1971)
Jet	967	rh[5], rh6	Habgood and Hayes (1971)
		rh6, rh7	Baker and Larter (1963)
Kitchen	1296	Rh9	Baker and Larter (1963)
Steudelli	2266	rh6, rh7	Baker and Larter (1963)
Bey	5581	Rh	Wells and Skoropad (1963)
Modoc	7566	Rh4[2]	Dyck and Schaller (1961)
		Rh[2], rh6	Habgood and Hayes (1971)
		Rh-Rh3-Rh4	Starling *et al* (1971)
Forrajera	n/k[2]	Rh4	
Nigrinudum	2222	rh8	Habgood and Hayes (1971)
			Wells and Skoropad (1963)
Turk	5611-2	Rh3, Rh5	Dyck and Schaller (1961)
		Rh, rh6	Habgood and Hayes (1971)
		Rh-Rh3-Rh4	Starling *et al* (1971)

[1]Accession number of the Cereal Crops Research Branch, ARS, USDA, Beltsville, Maryland, USA. Cited by Ali, *et al.* (1976).
[2]Accession number not known.

pathotypes based on the reaction of 21 barley cultivars, and indicated that isolates from the same host cultivar were not necessarily of the same pathotype.

Barley scald can most effectively be controlled by growing resistant cultivars and information on the extent of variability of the pathogen population has considerable value in breeding programmes. In Ethiopia, however, no detailed studies on pathogenic variability have been undertaken. This paper presents the findings of such a study conducted under glass using isolates collected from the principal barley growing regions of the country.

Materials and Methods

Naturally infected barley leaves were collected from various locations in Bale, Arsi and Shoa regions in 1991. Single spore isolates of *R. secalis* were prepared using the method of Jackson and Webster (1976) and maintained on 2% lima bean agar (LBA) at 17°C. To prevent pathogenicity changes in culture, there was minimal subculturing of isolates between isolation and pathogenicity testing.

Inoculum for each of the 24 isolates was prepared by spreading a spore suspension, in 10 ml sterile distilled water, onto LBA. After incubation for 12 days, at 16-18°C, the mycelial mat was removed, macerated in water, and filtered through muslin to remove mycelial fragments. The resulting suspension was centrifuged at 2000 rpm for 15 minutes and the supernatant was discarded. The spore suspension was adjusted to 3×10^5 spores ml^{-1} using an haemocytometer.

Seeds of ten differential host cultivars, differing in resistant gene or gene combinations (Table 1), were immersed in hot water (51°C for 12 minutes). Seeds were pre-germinated for 36–48 hours. Five vigorous seedlings of each variety were selected and planted in pots (7 cm diameter) filled with sterile soil (containing 1:1:1 sand:clay:red soil). Seedlings were raised in a glasshouse without supplemental light.

Plants were inoculated by applying 10μl of the spore suspension to the funnel formed by the second leaf, at the two-leaf growth stage. Sterile distilled water was applied to the control plants.

Inoculated plants were kept under polythene covers for 72 hours; additional humidifiers were run for 11 hours per day throughout the experimental period. The average minimum and maximum temperature during the experimental period was 8.2-27.9°C. Cultivar-isolate combinations were replicated three times and disease ratings were made 13 and 20 days after inoculation.

Reaction types were classified on a 0-4 scale (Table 2). Where variation in symptom expression occurred among plants of a single cultivar in a single pot, the most severe reaction was taken as the score for that combination. Isolate-cultivar combinations with a reaction type (RT) of 0-2 were classified as resistant, those with RT 3 and 4 were classified as susceptible.

Results and Discussion

In the present study all isolates of *R. secalis* were able to induce a susceptible reaction on ARDU 12-8C, the susceptible check cultivar. Four isolates, Lolle-9, Ticho-17, Ali-20 and Holetta-30 induced the same pattern of reactions on the differential hosts where only two of the differentials, Steudelli and Kitchen, were susceptible to them. Similarly, isolates Geredella-10 and Bekoji-23 were alike in pathogenicity. Isolates Bekoji-22 and Holetta-31 also induced similar reactions. Among the remaining isolates, no two isolates caused the same pattern of reactions and thus, based on the reaction of the set of differentials, which carry most of the known resistance genes, the 24 isolates were grouped into 19 pathotypes (Tables 2, 3).

The most frequent virulent pathotypes were those overcoming the resistant genes of Steudelli (rh6, rh7) and Kitchen (Rh9). Seventeen pathotypes incited a susceptible reaction on Steudelli, 16 were virulent on Kitchen. Varieties Modoc and Trebi had susceptible reactions to 12 and 9 of the pathotypes respectively, whilst Bey and Forrajera were susceptible to 7 pathotypes.

TABLE 2

***Reaction of barley differentials to Ethiopian pathotypes of* R. secalis.**

Cultivar	Pathotype of *R. secalis*[1]																			Number of Pathotypes on each Cultivar
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
La-Mesita			s				s								s	s	s			5
Trebi			s				s		s	s		s		s	s	s	s			9
Jet				s			s			s					s	s			s	6
Kitchen		s	s	s	s	s	s	s	s	s		s		s	s	s	s	s	s	16
Steudelli	s	s		s	s	s	s	s	s	s		s	s	s	s	s		s	s	17
Bey			s				s		s	s		s		s		s				7
Modoc	s		s	s	s		s	s		s		s		s	s	s	s			12
Forrajera							s		s	s		s			s	s	s			7
Nigrinudum		s			s		s									s	s			6
Turk					s		s								s	s	s	s		5

[1]Susceptible reaction is indicated by "s" (reaction types 3 and 4). Other reactions are resistant (reaction types 0, 1, and 2), where 0 = no visible symptoms, 1 = very small necrotic or chlorotic spots at the site of inoculation, 2 = very small lesions, 3 = large discrete lesions, 4 = total collapse of the leaf with no discrete lesions.

Differential hosts susceptible to the least number of pathotypes were Turk (with resistance genes Rh, Rh3, Rh5, rh6, Rh-Rh3-Rh4) and La-Mesita (Rh4, Rh10 and Rh-Rh3-Rh4).

The genetic basis of resistance in hosts used as differentials has not been clearly resolved (Brown, 1990). This could be due either to unrecognized differences in the genes described or to the presence of previously undetected genes for resistance (Brown, 1990). Since the use of additional differentials may or may not reveal heterogeneity in a given species, Ali *et al.* (1976) have suggested that isolates or a group of isolates, be referred to as pathotypes. Accordingly, the isolates identified in this work are labeled Pathotypes 1 to 19 (Table 3).

All differentials were susceptible to Pathotype 16 (isolate Bekoji-28). Pathotype 7 (isolates Geredella-10 and Bekoji-23) was avirulent on only one differential host, Turk. Among the others, Pathotype 15 was avirulent on only two differentials, while pathotypes 10 and 17 were avirulent on 3 differentials each. The least complex pathotype was Pathotype 13 (isolate Agarfa-21), which was virulent only on Steudelli, a variety known to carry two recessive resistance genes (Table 2).

In agreement with Zhang and Allard (1987) the results reveal high variability in pathogenicity within the *R. secalis* population in Ethiopia.

In earlier reports, pathogenic differences were detected among spores collected from the same lesion, among single spore cultures of *R. secalis* derived from the same parental isolate, between isolates collected from the same crop or between isolates collected from the same geographical location (Ali *et al.*, 1976; Brown, 1990; Hansen and Magnus, 1973). In the present study no comparisons for pathogenic variability were done among isolates derived from the same crop, the same lesion or from the same parental isolate. Nevertheless, isolates collected from the same geographical location and from the same field were noted to vary widely. Isolates collected from Bekoji were distinctly different from each other.

Ali *et al.* (1976) have reported that the most effective genes for resistance were Rh5 and alleles at the Rh locus; the least effective were Rh2, rh6, rh8, Rh9 and rh11. In the present work, however, the most

TABLE 3

Isolate, pathotype groups and source cultivars.

Isolate	Source Cultivar[1]	Pathotype Group	Pathotype Complexity[2]
Huruta-1	local (fb)	1	2
Bekoji-3	HB-100 (fb)	2	3
Dinsho-5	local (fb)	3	5
Agarfa-6	local (fb)	4	4
Dinsho-7	local (fb)	5	5
Lolle-9	Holkr (mb)	6	2
Ticho-17	local (fb)	6	2
Ali-20	local (fb)	6	2
Holleta-32	local (fb)	6	2
Geredella-10	Proctor (mb)	7	9
Bekoji-11	EH-738-F2-9H (mb)	7	9
Bekoji-12	(not known) (mb)	9	3
Meraro-13	(not known) (mb and fb)	10	7
Chancho-16	local (fb)	11	2
Dinsho-19	local (fb)	12	6
Agarfa-21	local (fb)	13	1
Bekoji-22	local (fb)	14	5
Holleta-32	(not known) (fb)	14	5
Bekoji-26	Holkr (mb)	15	8
Bekoji-28	HB-120 (mb)	16	10
Bekoji-29	local (fb)	17	7
Holleta-30	HB-42 (fb)	18	3
D/Tsige-33	local (fb)	19	3

[1]fb = food barley, mb = malting barley

[2]Pathotype complexity refers to the number of host genes or gene combinations susceptible to it.

effective genes were those genes possessed by La-Mesita and Turk which were resistant to 14 of the 19 pathotypes. In addition, Bey (Rh) and Jet (rh8) were resistant to 13 pathotypes. The three differentials have at least one of the resistance genes which were reported to be effective in resisting the pathotypes mentioned by Ali *et al.* (1976), that is Rh^4 in La-Mesita, Rh and Rh5 in Turk, and Rh in Bey. The effectiveness of these genes is confirmed in the present study. The least effective genes from the results of the present study appear to be the resistance genes possessed by Kitchen (Rh9, Baker and Larter, 1963), and Steudelli (rh6 and rh7, Baker and Larter, 1963). Ali *et al.* (1976) and Jackson and Webster (1976) reported that Steudelli and Kitchen were the most effective sources of resistance. However, Nigrinudum (rh8), (Wells and Skoropad, 1963; Habgood and Hayes, 1971) which has been reported to be the least effective (Ali *et al.*, 1976) was resistant to 13 of the 19 pathotypes tested in the present study. Another differential host, Jet (rh6, Baker and Larter, 1963; Habgood and Hayes, 1971; rh7, Baker and Larter, 1963; and rh^5, Habgood and Hayes, 1971) was also resistant to 13 of the pathotypes in the current investigation. Although these two differential hosts were susceptible to the most complex pathotypes, 7 and 16, the ability of Jet to resist more than 66% of the pathotypes could be attributed to the presence of rh^5.

More than half of the isolates tested in this trial were simple, inducing a susceptible reaction in 5 or less of the barley differentials. The most frequent pathotype, Pathotype 6, which was represented by four isolates collected from different locations in Arsi, Bale and Shoa, was able to incite a susceptible reaction on only two of the most simple differentials, Steudelli and Kitchen.

The most complex isolates, Bekoji-28, Geredella-10, Bekoji-23 and Bekoji-26 were isolated from improved, malting barley types (Table 3). These four complex isolates and Bekoji-29, which was pathogenic on seven differential host cultivars and isolated from a local land race, were collected from experimental stations of the state farms and from the Institute of Agricultural Research. The presence of complex isolates (pathotypes) in experimental stations and their restriction to these sites could be related to the fact that these locations serve as testing sites for barley varieties from diverse genetic origins, introduced as seed materials from foreign sources.

Although Ethiopian barley genotypes are rich sources of resistance genes for barley yellow dwarf virus (BYDV, Schaller *et al.*, 1963; Qualset and Schaller, 1969), the resistance against scald seems to be low. If complex pathotypes, such as those observed in this study, become widespread, opportunities for developing scald resistant cultivars would be reduced.

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