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Physiological races and virulence diversity of *Puccinia graminis* f. sp. *tritici* on wheat in Ethiopia

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Summary. The physiologic races of the rust fungus *Puccinia graminis* f. sp. *tritici* in the main wheat-growing regions of Ethiopia were determined on seedlings of the standard wheat stem rust differentials following the international system of nomenclature. Forty-four races were identified among the 75 isolates studied over a period of four years. The 16 isolates collected in 2001 all belonged to different race groups. The 33 isolates collected in 2002 belonged to 23 race groups, and, the 17 and nine rust isolates collected in 2003 and 2004 respectively belonged to eight and three race groups. Most of the rust samples collected from individual wheat fields belonged to different groups with only a few belonging to the same race group. The physiologic race breakdown differed greatly from year to year. TTR was the only race identified in all cropping seasons. Races such as TTT, TTR, PTT, PTR and TTQ showed relatively wider virulence spectra. Races such as TTR and TTT showed a relatively wider spatial distribution. Generally, *P. graminis* populations in Ethiopia appear to be highly variable, and this should be an important consideration when devising a breeding programme for this country.

Key words: wheat, stem rust, physiologic race.

Introduction

Wheat (*Triticum aestivum* L.) is the fourth largest food crop cultivated in Ethiopia, being grown on more than one million hectares, with an average yield of 1.4 tons per hectare, and making up about 13% of total crop production (Central Statistical Authority, 2002). The internal demand for wheat has increased steadily in recent decades. Although over 30 fungal wheat diseases have been identified in Ethiopia, stem rust caused by *Puccinia graminis* Pers: Pers. f. sp. *tritici* Eriks. & E. Henn. is one of the major production constraints in most wheat growing areas of the country, causing yield losses of up to 100% at times of epidemic outbreaks. Such outbreaks were reported in 1993/94 in south western Ethiopia (Arsi and Bale regions), which are the major wheat producing areas of the country. Those outbreaks attacked the previously resistant bread wheat cv. Enkoy (Ayele, 2002). Another epidemic outbreak of stem rust was reported in 2003 in the same areas. Durum wheat and stem rust have co-evolved for thousands of years in the central highlands of Ethiopia, and this close association has resulted in a wide virulence spectrum for the stem rust fungus (Getinet *et al.*, 1990). Van Ginkel *et al.* (1989) also reported that stem rust races prevalent in the central highlands of Ethiopia are among the most virulent in the

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Table 1. Races of *Puccinia graminis* f. sp. *tritici* in the Arsi, Bale and Shewa regions of Ethiopia in 2001.

No.	Race	Location	Region
1	CPR	Negele	Arsi
2	DRR	Kulumsa	Arsi
3	DTR	Kulumsa	Arsi
4	GDB	Ankober	Shewa
5	HGR	Kofele	Arsi
6	HRR	Kulumsa	Arsi
7	JGH	Assasa	Arsi
8	KKR	Sinana	Bale
9	KTR	Adaba	Bale
10	KJR	Ankober	Shewa
11	LPR	Bekoji	Arsi
12	RRG	DebreZeit	Shewa
13	RTK	Wabe	Arsi
14	RTR	Negele	Arsi
15	TTR	Negele	Arsi
16	TTT	Kulumsa	Arsi

world. It is further suggested that among wheat-growing areas of eastern and north East African countries the Ethiopian plateau has favourable environmental conditions for rust disease development and epidemiology (Mahir, 2000).

The cultivation of rust-resistant wheat varieties is the most economical method of controlling the disease; however, due to sudden changes in rust race-patterns, commercial varieties tend to become vulnerable. Breeding for vertical resistance, i.e. resistance to specific races of a given pathogen, has been one of the main objectives of cereal breeding programmes for a very long time (Hoerner, 1919; Stakman *et al.,* 1962). The breeding of varieties with such specific resistance has required systematic studies on the race composition of certain pathogens. As a result, the study of the prevailing races and the dynamics of race composition of pathogens has had a significant role in developing durable resistance. The present study was conducted to investigate the prevalence, temporal variations and spatial distribution of physiologic races of *P. graminis* f. sp. *tritici* in the major wheat growing areas of Ethiopia.

Materials and methods

Field surveys were conducted in the southeast (Arsi and Bale regions), central highlands (Shewa region) and northwest (Gojjam and Gondar regions) of Ethiopia, during the 2001, 2002, 2003 and 2004 cropping seasons to collect samples of wheat stem rust. In all years the surveys were conducted in mid-October, when wheat plants were at the flag leaf stage. The surveys followed main and secondary roads on pre-selected routes where wheat was important and stem rust was known to occur. Samples were randomly collected from commercial fields every 10 km, or from the first following field. Additional biased samples were collected from breeding lines and differential cultivars in various

Table 2. Races of *Puccinia graminis* f. sp. *tritici* in the Arsi and Bale regions of Ethiopia in 2002.

Isolate No.	Race	Location	Region
1	DBG	Robe	Bale
2	DBG	Robe	Bale
3	DGG	Bekoji	Arsi
4	DPR	Era	Arsi
5	FGG	Sagure	Arsi
6	FGR	Adaba	Bale
7	FGQ	Ada ali	Bale
8	JGH	Bakoji	Arsi
9	JGQ	Iteya	Arsi
10	JGR	Lemoburkitu	Bale
11	KGH	Era	Arsi
12	KGQ	Sinana	Bale
13	KGR	Sagure	Arsi
14	KKQ	Lemoburkitu	Bale
15	KQQ	Ada ali	Bale
16	RRT	Adaba	Bale
17	RTT	Agarfa	Bale
18	SGH	Iteya	Arsi
19	TPR	Agarfa	Bale
20	TPR	Agarfa	Bale
21	TPR	Sinana	Bale
22	TPT	Ada ali	Bale
23	TRK	Iteya	Arsi
24	TRR	Adaba	Bale
25	TRR	Sinana	Bale
26	TRT	Robe	Bale
27	TTR	Sagure	Arsi
28	TTR	Era	Arsi
29	TTR	Lemoburkitu	Bale
30	TTR	Bakoji	Arsi
31	TTR	Sinana	Bale
32	TTR	Sinana	Bale
33	TTR	Sinana	Bale

Table 3. Races of *Puccinia graminis* f. sp. *tritici* in the Arsi, Bale, Gojjam and Gondar regions of Ethiopia in 2003.

Isolate No.	Race	Location	Region
1	JHG	Bichena	Gojjam
2	LQG	Dejen	Gojjam
3	MRL	Dodola	Bale
4	PTR	Dodola	Bale
5	QGB	Dejen	Gojjam
6	TTR	Adaba	Bale
7	TTR	Dodota	Arsi
8	TTR	Asasa	Arsi
9	TTR	Colete	Arsi
10	TTR	Colete	Arsi
11	TTR	Washa	Bale
12	TTR	Washa	Bale
13	TTT	Adaba	Bale
14	TTT	Sinana ARC	Bale
15	TTT	Dabat	Gonder
16	TTT	Dodota	Arsi
17	PTT	Asasa	Arsi

Ethiopian Wheat Rust Trap Nursery sites. Three samples were collected from each commercial field.

A total of 75 stem rust samples (16, 33, 17 and 9 in 2001, 2002, 2003 and 2004 respectively) were collected. Seven-day-old seedlings of the bread wheat cultivar Morocco, a universal susceptible genotype to stem rust, were inoculated with a bulked spore population collected from each field. Two or three monopustules were isolated from each sample and increased in pots in a greenhouse adjusted to 25±2°C to produce enough inoculum for race breakdown to be studied. The spores of each monopustule isolate were collected in separate test tubes and stored at 4°C until they were inoculated on the standard differential sets. A suspension, prepared by mixing urediospores with lightweight mineral oil, was inoculated on seven-day-old seedlings of the standard differential hosts. Immediately after inoculation, the seedlings were placed in a humid chamber in the dark at 19–21°C for 24 h,

Table 4. Races of *Puccinia graminis* f. sp. *tritici* in the Shewa, Arsi and Bale regions of Ethiopia in 2004.

Isolate No.	Race	Location	Region
1	TTT	Debre Zeit (off-season)	Shewa
2	TTT	Debre Zeit (off-season)	Shewa
3	TTR	Debre Zeit (off-season)	Shewa
4	TTR	Debre Zeit (off-season)	Shewa
5	TTR	Debre Zeit (off-season)	Shewa
6	TTR	Debre Zeit (off-season)	Shewa
7	TTR	Dodolla	Bale
8	TTR	Bekoji	Arsi
9	TTQ	Debre Zeit (off-season)	Shewa

Table 5. Races of *Puccinia graminis* f. sp. *tritici* found in the Arsi, Bale, Shewa, Gojjam and Gondar regions of Ethiopia in 2001, 2002, 2003 and 2004.

Region	Year			
	2001	2002	2003	2004
Arsi	CPR, DRR, DTR, HGR, HRR, JGH, LPR, RTK, RTR, TTR, TTT	DGG, DPR, FGG, JGH, JGQ, KGH, KGR, SGH, TRK, TTR (3)[a]	TTR (4), TTT	TTR
Bale	KKK, KTR	DBG (2), FGR, FGQ, JGR, KGQ, KKQ, KQQ, RRT, RTT, TPR (3), TPT, TRR (2), TRT, TTR (4)	MRL, PTR, TTR (3), TTT (2)	TTR
Shewa	GDB, KJR, RRG	-	-	TTT (2), TTR (4), TTQ
Gojjam	-	-	JHG, LQG, QGB	-
Gondar	-	-	TTT	-

[a] Frequency of race detection.

Table 6. Reaction of Sr genes to different races of *P. graminis* f. sp. *tritici* from Ethiopia.

Sr gene	Virulent races
Sr5	LQG, QGB, TTT, TTR, PTT, PTR, MRL, TTQ
Sr6	LQG, QGB, JHG, TTT, TTR, PTT, PTR, MRL, TTQ
Sr7a	QGB, TTT, TTR, PTT, PTR, MRL, TTQ
Sr7b	TTT, TTR, PTT, PTR, MRL, TTQ
Sr8a	TTT, TTR, PTT, PTR, TTQ
Sr8b	-
Sr9a	TTT, TTR, PTT, PTR, TTQ
Sr9b	LQB, JHG, TTT, TTR, PTT, PTR, TTQ
Sr9d	TTT, TTR, PTT, PTR, TTQ
Sr9e	JHG, TTT, TTR, PTT, PTR, TTQ
Sr9f	TTT, TTR, PTT, PTR, MRL, TTQ
Sr9g	JHG, TTT, TTR, PTT, PTR, MRL, TTQ
Sr10	QGB, TTT,TTR, PTT, PTR, TTQ
Sr11	LQG, TTT,TTR, PTT,PTR,MRL, TTQ
Sr12	QGB, TTT,TTR, PTT, TTQ
Sr13	QGB, TTT,TTR, PTT,PTR, TTQ
Sr14	QGB, TTT,TTR, PTT,PTR, TTQ
Sr15	TTT,TTR, PTT, TTQ
Sr16	TTT,TTR, PTT, TTQ
Sr17	TTT,TTR, PTT,PTR, TTQ
Sr19	-
Sr20	TTT,TTR, PTT, TTQ
Sr21	QGB,JHG, TTT,TTR, PTT, TTQ
Sr22	JHG, TTT,TTR, PTT,PTR
Sr23	TTR,TTT,PTR
Sr24	-
Sr26+sr9g	QGB, JHG, TTT, TTR, PTT, PTR, MRL, TTQ
Sr27	LQG, TTT,TTR, PTT, PTR, MRL, TTQ
Sr28	LQG, QGB, JHG, TTT, TTR, PTT, PTR, TTQ
Sr29	PTT,TTR,TTT
Sr30	TTT, PTT
Sr31	TTT, TTR, PTT
Sr32	JHG, TTT, TTR, PTT, PTR, MRL, TTQ
Sr33	LQG, TTT, TTR, PTT, PTR, MRL, TTQ
Sr34	TTT, TTR, PTT, PTR, MRL, TTQ
Sr35	JHG, TTT, TTR, PTT, PTR, TTQ
Sr36	TTT, TTR, PTT, PTR, MRL, TTQ
Sr37	TTT,TTR, TTQ
SrTT3+sr10	QGB, JHG, TTT,TTR, PTT,PTR, TTQ
SrDP2	LQG, QGB, JHG, TTT,TTR, PTT,PTR, TTQ
Srgt	TTT, TTR, PTT, MRL, TTQ
SrpL	LQG, QGB, JHG, TTT, TTR, PTT, PTR, MRL, TTQ
SrWLD	TTT, TTR, PTT, TTQ
SrH	LQG, QGB, JHG, TTT, TTR, PTT, PTR, MRL
W3560	TTT,TTR, PTT,PTR, TTQ
SrAGI	TTT, TTR, PTT, TTQ
Sr7B,sr18,19,20	QGB, JHG, TTT,TTR, PTT, PTR, MRL, TTQ
Sr18	TTT, TTR, PTT, PTR

after which they were transferred to a greenhouse where the temperature varied between 20 and 26°C. In addition to the 12 standard differential hosts (Sr5, Sr6, Sr7b, Sr8a, Sr9b, Sr9e, Sr9g, Sr11, Sr17, Sr21, Sr30 and Sr36) used to differentiate *P. graminis* f. sp. *tritici* races, the following genotypes carrying known stem rust resistant genes were utilized (Sr7a, Sr8b, Sr9a, Sr9d, Sr9f, Sr10, Sr12, Sr13, Sr14, Sr15, Sr16, Sr18, Sr19, Sr20, Sr22, Sr23, Sr24, Sr26+Sr9g, Sr27, Sr28, Sr29, Sr31, Sr32, Sr33, Sr34, Sr35, Sr37, SrTT3+Sr10, SrDP2, Srgt, SrpL, SrWLD, SrH, W3560, SrAGI, Sr7b, 18, 19, 20).

Stem rust infection types were scored 14 days after inoculation using the 0–4 scale of Stakman *et al*. (1962). Races were assigned using the International code of Roelfs and Martens (1988). The results here were based on experiments conducted in two replications.

Results and discussion

Using the International system of nomenclature for *P. graminis* f. sp. *tritici* (Roelfs and Martens, 1988), 44 races were identified from the 75 isolates collected. The 16 rust isolates collected in 2001 all belonged to different race groups (Table 1). The 33 isolates collected in 2002 were from 23 race groups (Table 2), while the 17 and nine rust isolates collected in 2003 (Table 3) and 2004 (Table 4) belonged to eight and three race groups respectively. Most of the monopustules from individual fields varied in their race groups, and only some belonged to the same race group. The avirulence/virulence formulae for the 44 pathotypes are given in Table 7.

Wheat carrying genes Sr5, Sr6, Sr28, SrpL, Srgt and Sr26+Sr9g displayed consistently high rust susceptibility to most of the pathotypes identified in the four years of the survey. Wheat with the Sr30 gene had low rust susceptibility except with isolates PTT and TTT. Similarly, wheat with the Sr29 and 31 genes were susceptible only to races PTT, TTR and TTT. Genotypes carrying genes Sr8b, Sr19 and Sr24 were resistant to all races identified in Ethiopia in all years of the survey (Table 6). It is also important to note that the supplementary differential line Sr31 (Line-E /KUZ), from which most widespread wheat varieties throughout the world are bred (McIntosh *et al*., 1995) was susceptible to races PTT, TTR and TTT.

Table 7. Avirulence/virulence formula for 44 pathotypes of *Puccinia graminis* f. sp. *tritici* identified in Ethiopia on the twelve wheat stem rust standard differential varieties.

No.	Race	Avirulence/ virulence formula
1	CPR	5, 6 , 9e, 21, 30 / 7b, 8a, 9b, 9g, 11, 17, 36
2	DBG	5, 6, 7b, 8a, 9g, 11, 17, 21, 30, 36 / 9b, 93
3	DGG	5, 6, 7b, 9b, 11, 21, 36 / 8a, 9e, 9g, 17, 30
4	DPR	5, 6, 7b, 21, 30, / 8a, 9b, 9e, 9g 11, 17, 36
5	DRR	5, 7b, 8a, 21, 30, / 6, 9b, 9e, 9g, 11, 17, 36
6	DTR	5, 7b, 21, 30, / 6, 8a, 9b, 9e, 9g, 11, 17, 36
7	FGG	5, 8a, 9g, 11, 17, 21, 30, 36, /6, 7b, 9b, 9e
8	FGR	5, 8a, 9g, 11, 21, 30, / 6, 7b, 9b, 9e, 17, 36,
9	FGQ	5, 8a, 9g, 11, 17, 21, 30 / 6, 7b ,9b, 9e, 36
10	GDB	5, 6, 7b, 9b, 9e, 9g, 11, 17, 30, 36, / 8a, 21
11	HGR	5, 8a, 9e, 9g, 11, 30,/ 6, 7b, 9b, 17, 21, 36
12	HRR	5, 8a, 9e, 30, /6, 7b, 9b, 9g, 11, 17, 21, 36
13	JGH	5, 7b, 8a, 9g, 11, 30, 36, / 6, 9b, 9e, 17, 21
14	JGQ	5, 7b, 8a, 9g, 11, 17, 30, / 6, 9b, 9e, 21, 36
15	JGR	5, 7b, 8a, 9g, 11, 30, / 6, 9b, 9e, 17, 21, 36
16	JHG	5, 7b, 8a, 11, 17, 30, 36 / 6, 9b, 9e, 9g, 21
17	KGH	5, 8a, 9g, 11, 30, 36 / 6, 7b, 9b, 9e, 17, 21
18	KGQ	5, 8a, 9g, 11, 17, 30, / 6, 7b, 9b, 9e, 21, 36
19	KGR	5, 8a, 9g, 11, 30,/ 6, 7b, 9b, 9e, 17, 21, 36
20	KJR	5, 9g, 11, 30,/ 6, 7b, 8a, 9b, 9e, 17, 21, 36
21	KKQ	5, 11, 17, 30, / 6, 7b, 8a, 9b, 9e, 9g, 21, 36
22	KKR	5, 11, 30 / 6, 7b, 8a, 9b, 9e, 9g, 17, 21, 36
23	KQQ	5, 8a, 9g, 17, 30 / 6, 7b, 9b, 9e, 11, 21, 36
24	KTR	5, 30 / 6, 7b, 8a, 9b, 9e, 9g, 11, 17, 21, 36
25	LPR	6, 7b, 9e, 21, 30 / 5, 8a, 9b, 9g, 11, 17, 36
26	LQG	7b, 8a, 9e, 9g, 17, 21, 30, 36 / 5, 6, 9b, 11
27	MRL	8a, 9b, 9e, 17, 21, 30 / 5, 6, 7b, 9g, 11, 36
28	PTR	21, 30 / 5, 6, 7b, 8a, 9b, 9e, 9g, 11, 17, 36
29	PTT	21 / 5, 6, 7b, 8a, 9b, 9e, 9g, 11, 17, 30, 36
30	QGB	7b, 8a, 9b, 9e, 9g, 11, 17, 30, 36 / 5, 6, 21
31	RRG	8a, 9e, 17, 30, 36/ 5, 6, 7b, 9b, 9g, 11, 21
32	RRT	8a, 9e / 5, 6, 7b, 9b, 9g, 11, 17, 21, 30, 36
33	RTK	9e, 36 / 5, 6, 7b, 8a, 9b, 9g, 11, 17, 21,30
34	RTR	9e, 30/ 5,6, 7b, 8a, 9b, 9g, 11, 17, 21, 36
35	RTT	9e,/ 5, 6, 7b, 8a, 9b, 9g, 11, 17, 21, 30, 36
36	SGH	7b, 8a, 9g, 11, 30, 36,/ 5, 6, 9b, 9e, 17, 21
37	TPR	6, 30,/ 5, 7b, 8a, 9b, 9e, 9g, 11, 17, 21, 36
38	TPT	6/ 5, 7b, 8a, 9b, 9e, 9g, 11, 17, 21, 30, 36
39	TRK	8a, 36 / 5, 6, 7b, 9b, 9e, 9g, 11, 17, 21, 30
40	TRR	8a, 30 / 5, 6, 7b, 9b, 9e, 9g, 11, 17, 21, 36
41	TRT	8a, / 5, 6, 7b, 9b, 9e, 9g, 11, 17, 21, 30, 36
42	TTQ	5, 6, 7b, 8a, 9b, 9e, 9g, 11, 21, 36 / 17, 30
43	TTR	30 / 5, 6, 7b, 8a, 9b, 9e, 9g, 11, 17, 21, 36
44	TTT	/5, 6, 7b, 8a, 9b, 9e, 9g, 11, 17, 21, 30, 36

Most of the pathotypes identified during the survey were virulent on most of the wheat differentials. Race TTT, for instance, was virulent to all but three (Sr8b, Sr19 and Sr24) of the stem rust standard differential lines including the additional differentials (Table 6). This race thus poses a serious threat to the country's wheat production. Race TTR, which had a wide spectrum of distribution (Table 1) was virulent to all differentials except those carrying the Sr30 gene.

Race breakdown differed greatly from year to year; only race TTR was identified in all cropping seasons. Races such as TTT, TTR, PTT, PTR and TTQ showed relatively wider virulence spectra, whereas races such as TTR and TTT showed a relatively wider spatial distribution. Some of the races (for example, TPR and TRR, TRR and TTR, TTR and TTT) differed in virulence or avirulence by only one gene. In all, in the four cropping years, 19 races were recorded from Bale, an equal number from Arsi, seven from Shewa, three from Gojjam and one from Gondar regions (Table 5). In view of the results obtained (44 races from 75 samples), it is clear that race variations in stem rusts are very wide in Ethiopia, and that with a sample size of only 75 isolates, only a minimal part of the races were actually found. It is therefore necessary to carry out more extensive and exhaustive pathotype surveys to elucidate the race make-up and distribution in the country.

In general, the absence of virulence towards the Sr8b, Sr19 and Sr24 genes in all rust races studied during the four seasons suggests that these genotypes could serve as a source of resistance to the prevailing rust races in Ethiopia. The wide virulence diversity that was found in this study across regions and over time will undoubtedly render the task of breeding durably resistant materials more difficult.

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