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Molecular Diversity in *Puccinia triticina* Isolates from Ethiopia and Germany

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Abstract

A total of 43 isolates of the wheat leaf rust fungus, *Puccinia triticina* Eriks, collected from Ethiopia and Germany were analysed for their genetic diversity using the amplified fragment length polymorphism (AFLP) technique. Out of 18 *Eco*RI/*Mse*I primer combinations screened, 15 produced 219 highly polymorphic fragments. The average AFLP difference between pairs of the leaf rust isolates (26 from Ethiopia, 17 from Germany) was calculated using Dice's genetic similarity (GS) coefficient. The overall GS for the 43 isolates was 0.67 ± 0.13. The Ethiopian leaf rust isolates had lower average GS (0.63 ± 0.13) than the German ones (0.76 ± 0.10). A cluster analysis and a two-dimensional principal coordinate analysis (PCoA) grouped the 43 isolates into two significantly different ($P \leq 0.01$) clusters. Isolates in cluster I (35 isolates) had an average GS of 0.76 ± 0.06 while the isolates in cluster II (8 isolates) had an average GS of 0.55 ± 0.12. Isolates were also grouped into three regions of collection, central Ethiopia, south and south-east Ethiopia, and Germany. The regions were significantly different at $P \leq 0.01$ indicating regional variation in terms of molecular diversity of the leaf rust isolates studied. Each isolate, however, had a unique AFLP fingerprint. The results indicated that the leaf rust population in central Ethiopia is genetically distinct and this might be related to the predominant cultivation of durum wheat cultivars in this area.

Introduction

The leaf (brown) rust fungus *Puccinia triticina* occurs almost everywhere where wheat is grown and is considered to be one of the most important pathogens in wheat production worldwide (Dehne and Oerke, 1998). Leaf rust causes significant yield losses every year; the disease can reduce yield by 1% for every 1% increase in the infection level (Khan et al., 1997). Although effective fungicides are available, the use of host resistance is the most economical and environment-friendly method to control the disease. Especially in developing countries where fungicides are not available or their use in low input systems is economically not justified, successful crop production depends on the use of disease-resistant cultivars. Effective breeding for disease resistance requires extensive information on the incidence and virulence of endemic pathogens; which is of high importance for rust fungi as they are able to rapidly evolve new virulent races. Knowledge on the diversity of the leaf rust fungus in Ethiopia is limited; however, information on the diversity of *P. triticina* isolates regularly occurring in Germany is also rare; few reports on the incidence and frequency of isolates differing in virulence genes have been published for Germany (Von Kröcher et al., 1992) and Western Europe (Park and Felsenstein, 1998; Park et al., 2000, 2001) respectively.

To study the molecular diversity of Ethiopian and German *P. triticina* isolates, amplified fragment length polymorphism (AFLP) analysis was chosen because AFLPs (i) generate more information per analysis than other molecular techniques, (ii) require no prior knowledge about the genetic make-up of the organism under study, (iii) are highly reproducible, and (iv) allow discrimination among closely related individuals within a species (Vos et al., 1995; Majer et al., 1996; Mueller and Wolfenbarger, 1999). AFLP markers have been successfully used in mycology and plant pathology for the differentiation of species within genera (Keiper et al., 2003; Menzies et al., 2003; Schmidt et al., 2004; Leisova et al., 2005b) as well as of isolates within species from straminopiles, ascomycetes and basidiomycetes (Schnieder et al., 2001; Rau et al.,

2002, 2005; Abd-Elsalam et al., 2003; Radisek et al., 2003; Singru et al., 2003; Martinez et al., 2004; Leisova et al., 2005a,b; Kauserud et al., 2006). Molecular techniques such as AFLP produce neutral markers in contrast to resistance-specific virulence, which is subject to strong host selection (Kolmer, 1993; Brown, 1996) and environmental conditions. Such drawbacks limit the value of virulence markers as tools for population genetics studies (Leung et al., 1993; McDonald and McDermott, 1993). Hence, only AFLP markers were used for this study with the objective of investigating the genetic variability of leaf rust isolates collected from Germany and Ethiopia.

Our general hypothesis was that genetic similarity within populations of the two countries would be considerably higher than between populations. This hypothesis might have been nullified by the existence of isolates of *P. triticina* specifically adapted to durum wheat in Central Ethiopia. Viennot-Bourgin (1941) and Ezzahiri et al. (1992) reported leaf rust pathogen genotypes specifically adapted to durum wheat [*Triticum turgidum* var. *durum* (Desf.) Husn.] in the Mediterranean region and such specificity was used to classify those pathogen genotypes into a different species, *P. tritici-duri* V.-Bourgin. As the importance of this pathogen is limited to the Mediterranean region, *P. triticina* has been widely accepted as the leaf rust fungal pathogen of cultivated wheat. Our objective was, however, to study the general molecular diversity of *P. triticina* isolates collected from Ethiopia and Germany. Our results, showing the genetic variability of isolates in central Ethiopia where durum wheat is predominantly cultivated, could be used as baseline information for further studies related to pathogen classification.

Materials and Methods

Sources of pathogen isolates

Ethiopian isolates of leaf rust (*Puccinia triticina* Eriks.) were collected from farmers' wheat (*Triticum* spp.) fields in the main cropping season (summer) of 2003. The collection was made in 10 km intervals along the roadsides. The German *P. triticina* isolates were kindly provided by Dr Kerstin Flath, Federal Biological Research Center for Agriculture and Forestry (BBA), Kleinmachnow, Germany (Table 1).

Production of monopustule isolates

Bulk collections of each sample were sparsely inoculated on detached leaf segments placed on 5% water agar in Petri dishes. The inoculated leaf segments were incubated in a chamber adjusted to 20°C with continuous white light. A single pustule was isolated with a moistened cotton swab and inoculated on leaf segments for multiplication. The spores harvested from the leaf segments were used to inoculate 7- to 9-day-old seedlings of the susceptible cv. Monopol, with no known leaf rust resistance gene described, for further spore production. For each isolate, six to eight pots ($7 \times 7 \times 8$ cm) thickly sown with the susceptible cultivar were used for inoculation. The inoculated plants were kept in a moist chamber for 24 h under a dark system at ambient temperature. Then each pot was covered with a cellophane bag (145×235 mm) and tied with a rubber band at the base of the pot to prevent cross-contamination. While preventing contaminants, the cellophane bag allows air exchange for use by the plants inside. The inoculated seedlings were transferred to a growth chamber with 16 h/8 h light/dark system and a temperature of 20–22°C. Spores of each isolate were harvested from infected plants 10–12 days after inoculation by tapping over aluminium foil. A total of 43 monopustule isolates were developed from bulk samples collected from the two countries.

Spore germination

Germination of spores (100 mg from each isolate) of the *P. triticina* isolates was basically carried out as described by Kolmer et al. (1995) except that spores were left to germinate for 12 h instead of 6 h.

DNA extraction

The matt of germinated spores of individual isolates were ground with sterilized sand and liquid nitrogen. The DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) was used to extract DNA from the germinated matt of spores and germ tubes of each isolate. The working concentration of DNA was adjusted to 50 ng/μl.

Determination of AFLP genotypes

Digestion of template DNA with Tru9I and EcoRI enzymes The template DNA of each isolate (5 μl of 50 ng/μl concentration) was digested with 0.25 μl *Tru*9I (10 U/μl) (Promega, Mannheim, Germany), 2 μl of 10X buffer F in 12.75 μl of ddH_20 for 2 h at 65°C. The *Tru*9I products (20 μl) were again digested with 0.25 μl of 12 U/μl *Eco*RI (Promega) and 2.5 μl of 10X buffer $H_{mod.}$ in a total reaction volume of 25 μl.

Adaptor ligation Ligation of adaptors was carried out for 2 h at 20°C using 12.5 μl of the digested product, 0.125 μl of each adaptor: 1 mM AD-*Eco* F [5′-CTC GTA GAC TGC GTA CC-3′], 1 mM AD-*Eco* R [5′-AAT TGG TAC GCA GTC TAC–3′], 1 mM AD-*Mse*I F [5′-GAC GAT GAG TCC TGA G-3′], and 1 mM AD-*Mse*I R [5′-TAC TCA GGA CTC AT-3′], and 0.084 μl of 3 U/μl T4 DNA-ligase (Promega) with 2.5 μl 10X ligase buffer and 9.416 μl ddH_20. The total reaction volume was 25 μl. The ligation product was diluted to 1 : 20 for use in the preamplification step.

Preamplification The preamplification polymerase chain reaction (PCR) was carried out using 5 μl of 1 : 20 diluted ligation product as template DNA, and 2.5 μl 10X PCR buffer, 2.5 μl $MgCl_2$ (25 mM), 2.5 μl dNTP-mix (2 mM), 1 μl of primer E01 (10 μM) (5′-GAC TGC GTA CCA ATT CA-3′), 1 μl primer M02 (10 μM) (5′-GAT GAG TCC TGA GTA AC-3′), 0.1 μl of 1 U *Taq*-polymerase (Promega) with 10.4 μl ddH_20

Table 1
Wheat leaf rust isolates, origin, host cultivar, and year of collection

Isolate	Origin	Host cultivar	Year of collection
Ak9-3	Akaki, CE	Pavon-76, BW	2003
Alemgena	Alemgena, CE	Gerardo, DW	2003
Bekoji	Bekoji, SSE	Galama, BW	2003
CD	Chefe Donsaia, CE	Israel, BW	2003
Dodolla	Dodolla, SSE	N/a, BW	2003
DZ7-21	Debre Zeit, CE	Arendeto, DW	2003
DZ7-23	Debre Zeit, CE	N/a, DW	2003
DZ7-24	Denkaka-Debre Zeit, CE	N/a, BW	2003
E-4	Goffer Estate Farm, SSE	N/a, BW	2003
E-8	Sama Senbet, CE	Pavon-76, BW	2003
E-16	Tefki, CE	Kubsa, BW	2003
E-19	Keta-Tulubollo, CE	N/a, BW	2003
E-22-2	Wolliso-Goro, CE	Kubsa, BW	2003
E-23	Wolliso, CE	Israel, BW	2003
E-30	Shenshicho, SSE	N/a, BW	2003
E-33	Durame, SSE	Dashen, BW	2003
E-35	Butajira, SSE	N/a, BW	2003
E-43	Womber Godeti, SSE	Gerardo, DW	2003
E-52	Gogecha-Akaki, CE	Pavon-76, BW	2003
KK	Kersana Kondaltiti, SSE	Pavon-76, BW	2003
Minjar1	Minjar, CE	Pavon-76, BW	2003
Minjar2	Minjar-Arerti, CE	Pavon-76, BW	2003
PPRC2-1	Lemo, SSE	Katar, BW	2003
PPRC2-3	Lemo, SSE	Katar, BW	2003
PPRC3-1	Adaali, SSE	Abola, BW	2003
PPRC3-3	Adaali, SSE	Abola, BW	2003
Bonn1	Bonn, Germany	Dekan, BW	2004
Bonn2	Bonn, Germany	Munk, BW	2004
K-B1	Lower Saxony, Germany	Denver, BW	2003
K-B2	Lower Saxony, Germany	Dekan, BW	2003
Mon1-p10	Mainz, Germany	Monopol, BW	2003
167-176	Germany	N/a	Before 1990
77W × R	Germany	N/a	Before 1990
WBR1	Germany	N/a	Before 1990
WBR2	Germany	N/a	Before 1990
WBR3	Aschersleben, Germany	Borenos, BW	2001
WBR4	Bonn, Germany	Dekan, BW	2003
WBR5	Bonn, Germany	Dekan, BW	2003
WBR6	Bonn, Germany	Drifter, BW	2003
WBR7	Bonn, Germany	Drifter, BW	2003
WBR10	Mainz, Germany	Punch, BW	2003
WBR12	Mainz, Germany	Dekan, BW	2003
WBR14	Mainz, Germany	Monopol, BW	

CE, central Ethiopia; SSE, south and south-east Ethiopia; BW, bread wheat *Triticum aestivum* L.; DW, durum wheat *Triticum turgidum* var *durum* (Desf.) Husn.; N/a, name of a specific variety not available.

in a total reaction volume of 25 μl. The PCR comprised 20 cycles of 94°C for 30 s, 56°C for 60 s and 72°C for 60 s. The preamplification product was then diluted 1 : 300 for use in the selective amplification.

Selective amplification In this final step, 5 μl of the diluted preamplification product, 1.5 μl 10X PCR buffer, 1.5 μl $MgCl_2$ (25 mM), 1.5 μl dNTP-mix (2 mM), 0.6 μl *Eco*RI primer with two selective nucleotides (1 μM), 0.6 μl *Mse*I primer with three selective nucleotides (10 μM) and 0.1 μl of 1 U *Taq* polymerase (Promega) were used with 4.2 μl ddH_20 for the selective amplification. The total reaction volume was 15 μl. The *Eco*RI primers were labelled with near-infrared IRD700 fluorescent dye for later detection on a Li-COR 4200 System (Li-COR Biosciences, Bad Homburg, Germany). The 'touch-down' PCR (decreasing the annealing temperature by 0.7°C per cycle) comprising 13 cycles of 94°C for 30 s to denature the sample, 65–56°C for 60 s for annealing, 72°C for 60 s for extension, and 23 cycles of 94°C for 30 s for denaturation, 65°C for 60 s for annealing and 72°C for 60 s for extension was used. A total of 18 *Eco*RI/*Mse*I primer combinations were screened.

Electrophoresis of AFLPs The PCR-amplified products were denatured at 95°C for 3 min before loading onto a 6% polyacrylamide gel. The polyacrylamide gel was prepared from 24 ml reagent (SequaGel XR; Biozym Diagnostik, Hessisch-Oldendorf, Germany), 6 ml buffer (SequaGel Complete; Biozym Diagnostik), and 240 μl 10% ammonium peroxide sulphate. The gel was poured between two 41 cm glass plates (0.2 mm spacers fixed between the plates in opposite sides) and 0.4 μl per sample was loaded onto the gel. A standard size ladder, 0.3 μl of 700 bp (Li-COR Biosciences) was loaded twice, one in the beginning and another in the centre of the gel as a sizer. The 43 isolates were divided into two sets and each set was run on a separate

gel. In every case, an internal control isolate (E-52) was loaded twice in double adjacent lanes each, one in the beginning and the other in the middle of the gel. Each isolate was amplified twice and loaded in double adjacent lanes to ensure the repeatability of the data. AFLP fragments were separated electrophoretically for 3 h in the Li-COR 4200 system (Li-COR Biosciences). Subsequently, the bands were scored manually as present (1) or absent (0).

Data analysis

Only unambiguous polymorphic fragments giving the same results from two independent PCRs were considered for scoring in each primer combination and the statistical software, NTSYS-pc ver. 2.0 (Exeter Software, East Setauket, NY, USA), was used for analysis of the AFLP data.

Genetic similarity

The average AFLP difference between pairs of the 43 leaf rust isolates was calculated using Dice's genetic similarity (GS) coefficient (Nei and Li, 1979). The GS between two isolates i and j is equivalent to the formula, GS [DICE] $= 2a/(2a + b + c)$, where a represents bands present in both isolates i and j, b represents bands present in isolate i but absent in j, and c represents bands present in isolate j but absent in isolate i.

Grouping of pathogen isolates based on region of collection

To have more insight into the molecular diversity of the pathogen isolates, three groups were differentiated based on the region of collection. The first region consisted of 17 isolates of *P. triticina* collected from Germany: 167-176, WBR14, Bonn1, Bonn2, WBR1, K-B1, Mon1-p10, 77WxR, WBR7, WBR5, WBR4, WBR10, WBR12, K-B2, WBR2, WBR3 and WBR6. Bread wheat is the major type of wheat grown in Germany. South and south-eastern Ethiopia formed the second region with 12 isolates: E-30, E-33, E-35, E-43, KK, PPRC2-1, PPRC2-3, PPRC3-1, PPRC3-3, Bekoji, Dodolla and E-4. This region is known for its bread wheat production. According to Ensermu et al. (1998), about 75% of Ethiopia's bread wheat area is located in Arsi (south-east Ethiopia). The third region of collection, central Ethiopia, consisted of 14 isolates: Ak9-3, Alemgena, CD, DZ7-21, DZ7-23, DZ7-24, E-16, E-19, E-22-2, E-23, E-52, E-8, Minjar1 and Minjar2. This region is mainly known for its durum wheat production but bread wheat is also becoming an increasingly important crop (Badebo, 2002) due to its higher yield and wider adaptability (Geleta and Tanner, 1995).

Cluster analysis

Based on the GS values, a cluster analysis between the isolates was performed by the unweighted pair group arithmetic mean average (UPGMA) method and a dendrogram was developed for clustering isolates.

Principal coordinate analysis

A two-dimensional principal coordinate analysis (PCoA) was also developed by the NTSYS software to plot the genetic similarity of the isolates, as outlined by Pillen et al. (2000).

Allele frequency

To measure how often or common an allele was found in the respective populations, allele frequency was calculated as the percentage of isolates that the allele occupies within the population.

Results

Of the 18 *Eco*RI/*Mse*I primer combinations screened, 15 were found to produce 219 reproducible and highly polymorphic fragments that were easy to score (Table 2). Each primer combination produced 10–19 polymorphic AFLP fragments. The internal control, E-52, present in the first and second set of isolates was analysed as two separate isolates (E-52 and E-52a). The duplicates were found to be identical based on AFLP fingerprints with a GS value of 1.00 (or 100% genetic similarity) indicating the reliability of data. Overall GS for all the isolates tested was 0.67 ± 0.13. The Ethiopian leaf rust isolates had an average GS of 0.63 ± 0.13 which was lower than the average GS of the German isolates (GS 0.76 ± 0.10). The 43 wheat leaf rust isolates were clustered into two major groups (Fig. 1).

Grouping of pathogen isolates based on the region of collection

Within-region comparison Each region of collection was highly significantly different ($P \leq 0.01$) from the other in terms of the GS of isolates in the respective regions (Table 3). Isolates from Germany had the highest average GS of 0.76 ± 0.10 followed by the isolates from south and south-east Ethiopia (GS 0.70 ± 0.13). Isolates from central Ethiopia had the least average GS of 0.61 ± 0.10 of the three regions. Standard deviations within the isolates from the different regions were very similar.

Between-regions comparison The average GS between isolates collected from Germany and south and south-east Ethiopia was 0.72 ± 0.11, a value significantly lower than the average GS within the German isolates. The average GS between isolates collected from Germany and central Ethiopia was 0.65 ± 0.12, between the isolates from the two Ethiopian regions (0.64 ± 0.12).

Cluster analysis

As indicated in Table 4, the 43 isolates collected from the two countries were categorized into two significantly ($P \leq 0.01$) distinct clusters.

Cluster I The majority of isolates from both countries – 16 from Germany and 19 from Ethiopia – were grouped together in this cluster. The 35 isolates

Table 2
Selected *Eco*RI/*Mse*I primer combinations and the corresponding polymorphic fragments produced among the 43 leaf rust isolates[a]

*Eco*RI primer	*Mse*I primer	Polymorphic fragments
5′-GAC TGC GTA CCA ATT C**AA**-3′	5′-GAT GAG TCC TGA GTA **ACA A**-3′	19
5′-GAC TGC GTA CCA ATT C**AA**-3′	5′-GAT GAG TCC TGA GTA **ACA C**-3′	14
5′-GAC TGC GTA CCA ATT C**AA**-3′	5′-GAT GAG TCC TGA GTA **ACA G**-3′	17
5′-GAC TGC GTA CCA ATT C**AA**-3′	5′-GAT GAG TCC TGA GTA **ACT A**-3′	12
5′-GAC TGC GTA CCA ATT C**AA**-3′	5′-GAT GAG TCC TGA GTA **ACT C**-3′	10
5′-GAC TGC GTA CCA ATT C**AA**-3′	5′-GAT GAG TCC TGA GTA **ACT G**-3′	19
5′-GAC TGC GTA CCA ATT C**AC**-3′	5′-GAT GAG TCC TGA GTA **ACA G**-3′	13
5′-GAC TGC GTA CCA ATT C**AC**-3′	5′-GAT GAG TCC TGA GTA **ACT A**-3′	14
5′-GAC TGC GTA CCA ATT C**AC**-3′	5′-GAT GAG TCC TGA GTA **ACT C**-3′	13
5′-GAC TGC GTA CCA ATT C**AC**-3′	5′-GAT GAG TCC TGA GTA **ACT G**-3′	10
5′-GAC TGC GTA CCA ATT C**AG**-3′	5′-GAT GAG TCC TGA GTA **ACA A**-3′	16
5′-GAC TGC GTA CCA ATT **CAG**-3′	5′-GAT GAG TCC TGA GTA **ACA C**-3′	19
5′-GAC TGC GTA CCA ATT C**AG**-3′	5′-GAT GAG TCC TGA GTA **ACA G**-3′	14
5′-GAC TGC GTA CCA ATT C**AG**-3′	5′-GAT GAG TCC TGA GTA **ACT C**-3′	14
5′-GAC TGC GTA CCA ATT C**AG**-3′	5′-GAT GAG TCC TGA GTA **ACT G**-3′	15
Total		219

[a]Selective nucleotides are indicated in **bold** faces at the end of each primer sequence.

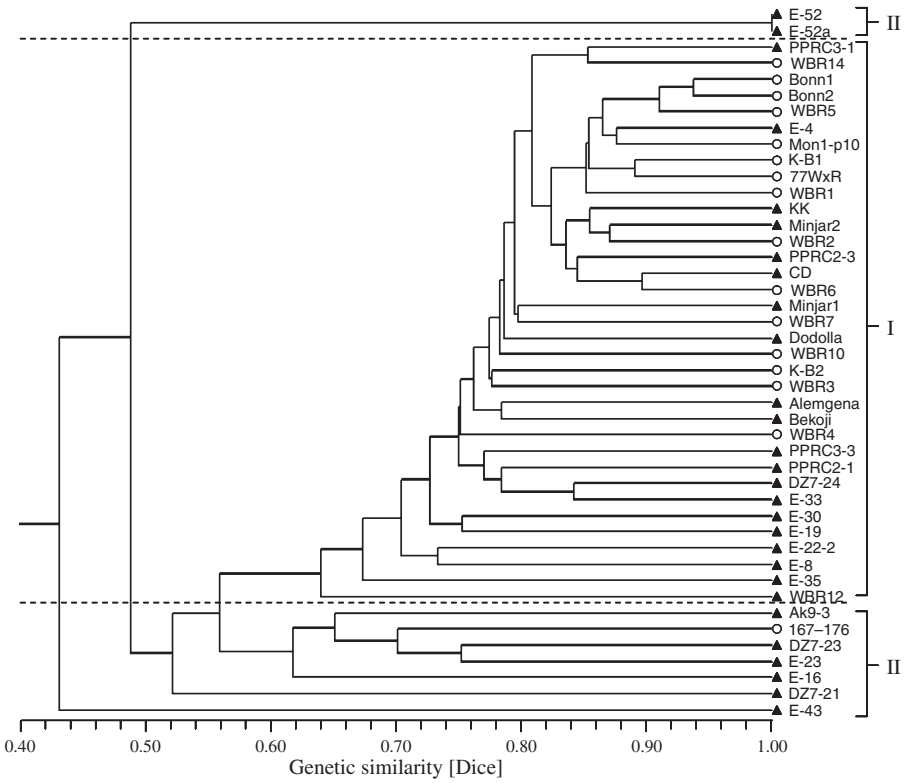

Fig. 1 Dendrogram produced using the unweighted pair-group arithmetic mean average (UPGMA) method based on GS coefficients calculated from 219 different AFLP fragments. Scale indicates Dice's GS values among the 43 isolates of *Puccinia triticina* collected from Ethiopia (▲) and Germany (○). The dendrogram is divided into two major clusters (I and II), as indicated on the right-hand side of the figure

in this cluster had an average GS value of 0.76 ± 0.06. The two genetically most similar isolates were Bonn1 and Bonn2 with a GS value of 0.94. There was also high GS (0.90) between the Ethiopian isolate CD and the German isolate WBR6. Another high similarity (GS 0.89) was observed between the two German isolates K-B1 and 77W × R. In general, there was more genetic similarity within pairs of isolates in cluster I than in cluster II.

Cluster II This group contained seven isolates (without the purposely duplicated control isolate E-52a) from Ethiopia and one isolate, 167-176, from Germany. Isolates in this cluster had an average GS value of 0.55 ± 0.12. The two genetically closest isolates in the cluster were E-23 and DZ7-23 with a GS value of 0.75. Except E-43, an isolate collected from durum wheat grown in south and south-east Ethiopia, all Ethiopian isolates in this cluster originated from the central region.

Table 3
Average genetic similarity values for the wheat leaf rust isolates grouped by region of collection

Comparison	Combinations of isolates (*n*)	Average genetic similarity[a]
Within regions		
Ger	136	0.76 ± 0.10 a
SSE	66	0.70 ± 0.13 b
CE	91	0.61 ± 0.10 c
Between regions		
Ger × SSE	204	0.72 ± 0.11 a
Ger × CE	238	0.65 ± 0.12 b
SSE × CE	168	0.64 ± 0.12 bc

Ger, Germany; SSE, south and south-east Ethiopia; CE, central Ethiopia.
[a]GS (mean ± standard deviation) with different lower-case letters are significantly different at $P \leq 0.01$ (Student–Newman–Keuls test).

Table 4
Average genetic similarity values of the wheat leaf rust isolates within and between clusters

Comparison	Combinations of isolates (*n*)	Genetic similarity[a]
Cluster 1 × cluster 1	595	0.76 a
Cluster 2 × cluster 2	28	0.55 b
Cluster 2 × cluster 1	280	0.53 b

[a]Mean GS with different lower-case letters are significantly different at $P \leq 0.01$ (Student–Newman–Keuls test).

Principal coordinate analysis

Two-dimensional principal coordinate plots based on Dice's GS values divided the 43 isolates into two major groups (Fig. 2). These are located in sections II and IV of the PCoA plot. Section II included eight isolates, seven from Ethiopia (without considering E-52a as a separate isolate) and one from Germany. These same eight isolates were also grouped together in cluster II of the dendrogram in Fig. 1. Correspondingly, the same 35 isolates (19 from Ethiopia and 16 from Germany) in section IV were grouped together in a similar manner as shown in cluster I of the dendrogram in Fig. 1.

Allele frequency

The overall frequency of AFLP alleles in the *P. triticina* isolates was 58.4 ± 10.0 (median 60.3) and varied from 30.6 (isolate E-43) to 79.0 (isolate CD). With very similar frequencies for most of the Ethiopian and German isolates, the allele frequency of isolates E-43 and DZ7-21, both isolated from durum wheat, was significantly lower (30.6% and 37.9% respectively). All alleles detected in the AFLP analysis were represented in the Ethiopian wheat leaf rust population studied, whereas eight alleles were missing in the German isolates of *P. triticina* (data not shown). Of the total 219 alleles, 20 were detected in all German isolates; none of the alleles were shared by all Ethiopian isolates tested.

The allele E-AC/M-CTG-8 was missing in both German and south and south-east Ethiopian populations (Table 5). However, this allele was shared by three isolates (allele frequency 21%) in the central Ethiopian population. Another missing allele in the German population, E-AC/M-CTC-6, was detected only in isolate E-43 from durum wheat in the south and south-east Ethiopian population while it was detected in three isolates of the central Ethiopian population (frequency 21%). Conversely, allele E-AG/M-CAG-6 was detected in all isolates of both, the south and south-east Ethiopian and the German collection. This allele, however, was detected only in eight isolates from central Ethiopia (frequency 57%). Another allele with high frequency in both German (100%) and south and south-east Ethiopian (92%) populations was E-AG/M-CAC-17. This allele was present only in eight isolates of the central Ethiopian (frequency 57%) population. Allele E-AG/M-CAG-9 was also detected in all German isolates and in 10 isolates from south and south-east Ethiopia (frequency 83%). This allele was shared only by seven isolates in the central Ethiopian population (frequency 50%).

Discussion

All isolates of *P. triticina* that were tested produced a unique pattern of AFLP alleles confirming the high genetic diversity within populations of the leaf rust fungus described by McDonald and Linde (2002). In contrast, analysis of *P. triticina* isolates from Western Europe using eight RAPD primers revealed only low variability and provided evidence of clonal migration over considerable distances in Western Europe (Park et al., 2000). The low diversity detected in these studies, however, may be also due to the lower sensitivity of RAPD-PCR to fine levels of genetic variation.

Grouping of pathogen isolates based on the region of collection

The highly significant differences ($P \leq 0.01$) within and between regions of collection showed that there was regional variation in terms of genetic diversity of pathogen isolates. The 'within-region' comparison indicated that isolates collected from central Ethiopia had the lowest GS of the three regions. This might be attributed to the cultivation of both indigenous and commercial durum wheat cultivars as well as bread wheat cultivars in the region. Durum wheat genotypes in the region are reported to be of high genetic diversity (Tessema and Belay, 1991). In effect, isolates with high complementary genetic diversity might co-exist with a wider range of durum wheat genotypes.

South and south-east Ethiopia, on the other hand, is known for its bread wheat cultivation. The Arsi Administrative Region (south-east Ethiopia) was reported to account for about 75% of Ethiopia's total bread wheat area (Ensermu et al., 1998). As bread wheat is not indigenous to Ethiopia, these cultivars are developed through selection and crossing programmes mainly using genetic materials introduced from abroad. As a result, bread wheat cultivars in Ethiopia have a narrow genetic base (Gebre-Mariam, 1991). According to Badebo (2002),

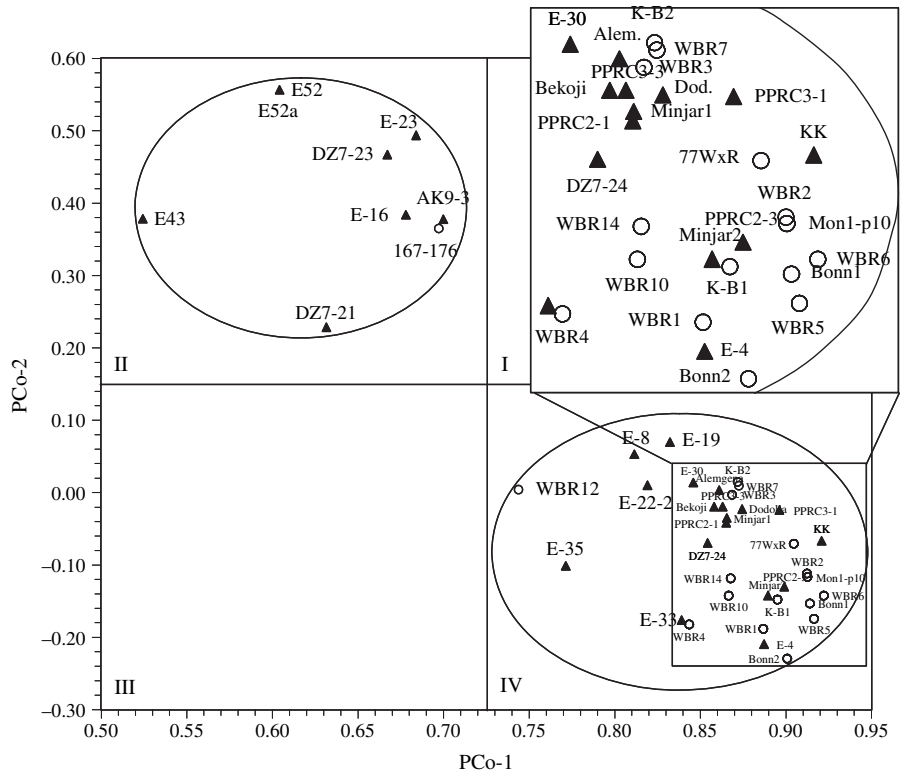

Fig. 2 Two-dimensional principal coordinate analysis (PCoA) plot of 43 *Puccinia triticina* isolates collected from Ethiopia (▲) and Germany (○) based on Dice's GS values calculated from 219 polymorphic AFLP fragments. Sections II and IV (rectangular sector magnified in insert in section I) presented the less and more genetically similar groups of isolates as shown in clusters I and II, respectively, of the dendrogram in Fig. 1

Table 5
Frequency of selected AFLP alleles in wheat leaf rust isolates from central and south and south-east Ethiopia and Germany

	Central Ethiopia ($n = 14$)		South and south-east Ethiopia ($n = 12$)		Germany ($n = 17$)	
Allele	No. isol.[a]	AF	No. isol.[a]	AF	No. isol.[a]	AF
E-AC/M-CTG-8	3	0.214	0	0.000	0	0.000
E-AC/M-CTC-6	3	0.214	1	0.083	0	0.000
E-AG/M-CAG-6	8	0.571	12	1.000	17	1.000
E-AG/M-CAC-17	8	0.571	11	0.917	17	1.000
E-AG/M-CAG-9	7	0.500	10	0.833	17	1.000

AF, allele frequency.
[a] Number of isolates in which the particular allele is observed.

the semi-dwarf bread wheat cultivars widely produced in south and south-east Ethiopia lack adequate genetic variation for resistance to rusts because they are of similar genetic background. Such cultivars are mostly released for production with a single gene for rust resistance. Depending on the selection pressure, i.e. the area grown with the cultivar, this specific disease resistance is overcome by the appearance of new virulence genes in the pathogen population which enables the new isolate(s) to attack all cultivars with a similar genetic background for leaf rust resistance. For instance, of the 26 rust resistant bread wheat cultivars released in the period 1970–1993, only three retained their resistance (Assefa, 2001). Eventually, host selection due to narrow genetic make-up reduces genetic diversity in the pathogen population. The same may be true also for the German leaf rust population. Similar to the very similar diversity of *P. triticina* isolates from Germany and south and south-east Ethiopia, the level of genetic diversity in leaf rust collections in Egypt in 1998–2000 was similar to that of collections from the Southern and Central Plains of the USA in the same period (McVey et al., 2004).

The 'between-regions' comparison showed that the isolates collected from Germany were more genetically similar to the south and south-east Ethiopian isolates. As both regions are used predominantly for the

production of bread wheat, the leaf rust populations in these regions may have largely adapted to *Triticum aestivum*. As there is no or little evidence that sexual reproduction plays an important role in the formation of new pathotypes (Kolmer and Liu, 2000), the genetic diversity of *P. triticina* populations observed in our study may not be attributed to recombination.

Clustering and PCoA

Distinct geographical clustering was observed among isolates of the soil-borne stramenopile *Pythium insidiosum* (Schurko et al., 2003) and species of the basidiomycete *Coprinus* (Ko et al., 2001). It was plausible to expect the Ethiopian and German leaf rust populations to cluster based on the region of collection. However, no geographical (based on country of origin) clustering was observed in the present study. The highly significant difference between the two clusters clearly justified the validity of clustering the isolates into two groups. Kolmer and Liu (2000) also found *P. triticina* isolates from Italy and Spain and from Canada and South America to be clustered together based on RAPD marker differences. A positive correlation between the genetic diversity of isolates and the geographical distance has been reported for *Gibberella zeae* in the USA (Zeller et al., 2004), *Sclerospora graminicola* in India (Singru et al., 2003) and *Alternaria brassicicola* in New South Wales (Bock et al., 2002). For isolates of *Beauveria bassiana* from Kenya (De Muro et al., 2003), *Fusarium semitectum* on cotton in Egypt (Abd-Elsalam et al., 2003) and *Peronospora sparsa* in Finland (Lindqvist-Kreuze et al., 2002), however, no correlation between physical distance of origin and genetic diversity could be established.

In cluster I of the dendrogram and section IV of PCoA, the majority of isolates from both countries were clustered together with a minimum average GS value of 0.59 ± 0.06. The fact that nearly all isolates from Germany and south and south-east Ethiopia were grouped in this cluster indicated that the isolates from the two regions were more genetically similar.

Cluster II consisted of more genetically diverse isolates than the isolates in cluster I. Seven isolates of cluster II were from Ethiopia and only one from Germany. Of the seven isolates in this cluster, six were collected from central Ethiopia. E-43, the only isolate in this group collected from south and south-east Ethiopia, was collected from the durum wheat cultivar Gerardo. In this cluster was also the German isolate, 167-176. It was collected before 1990 and no information on the region of collection or the type of the host cultivar from which the sample was collected is available. The fact that the majority of isolates in this cluster belong to central Ethiopia might show the genetic variability of central Ethiopian isolates. This high variability might be of supporting evidence for the specificity (Ezzahiri, 2000) of central Ethiopian isolates to durum wheat cultivars.

Allele frequency

The allele sharing of isolates from Germany and south and south-east Ethiopia also indicates that the isolates in both regions have a very similar genetic background. Allele E-AC/M-CTG-8 detected in isolates from central Ethiopia was missing in both regions; allele E-AG/M-CAG-6 was present in all isolates from both regions confirming a high genetic similarity between the isolates of the two regions. These two alleles were not shared in similar manners by the isolates from central Ethiopia demonstrating genetic distinctiveness of isolates from this region. This might be due to co-evolution of the isolates with the durum wheat genotypes grown in the region.

The specific adaptation of leaf rust isolates to durum wheat has been recognized since the 1940s. Isolates of the wheat leaf rust fungus collected from durum wheat cultivars were reported to be distinct from those isolates adapted to bread wheat (Ezzahiri, 2000). Huerta-Espino (1992) and Martinez et al. (2005) also found that populations of the pathogen on durum wheat cultivars were quite distinct from populations on bread wheat cultivars. Ezzahiri et al. (1994) reported on a greater diversity of virulence genes in leaf rust isolated from durum wheat than from most bread wheat cultivars. In Morocco, durum wheat is often grown in marginal areas where the alternate host *Anchusa italica* occurs. In conclusion, the results of the present study revealed that the wheat leaf rust population in central Ethiopia has a distinct genetic background. The genetic differentiation might be related to the predominant cultivation of durum wheat in the region. However, further studies, with larger number of isolates and with supporting virulence data are required to consolidate this conclusion.

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