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# Intraspecific genetic diversity of *Drechslera tritici-repentis* as detected by random amplified polymorphic DNA analysis

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## Abstract

The phytopathogenic fungus *Drechslera tritici-repentis* causes tan spot, an important disease of wheat in the southern Brazilian state of Rio Grande do Sul. Twelve *D. tritici-repentis* isolates were obtained from wheat seeds from different locations in the state. Their colony morphology on potato dextrose agar and polymorphisms in genomic DNA by the random amplified polymorphic DNA (RAPD) method were investigated. For the RAPD method, 23 primers were tested of which nine were selected for use in the study of *D. tritici-repentis* polymorphisms. The degree of similarity between isolates was calculated using a simple matching coefficient and dendrograms constructed by the unweighted pair-group method with arithmetical averages (UPGMA). The morphological and RAPD analyses showed intraspecific polymorphisms within the isolates, but it was not possible to establish a relationship between these polymorphisms and the geographical regions from where the host seeds were collected.

*Key words: Drechslera tritici-repentis*, RAPD, DNA polymorphism, genetic diversity.

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## Introduction

Tan spot of wheat leaves is caused by *Drechslera tritici-repentis* (Died.) Shoem. (anamorphic phase, the teleomorphic phase being *Pyrenophora tritici-repentis* (Died.) Drechs). The nomenclature of this fungus has been discussed by several authors, with *Pyrenophora trichostoma, Helminthosporium tritici-vulgaris* and *Helminthosporium tritici-repentis* having been considered as being the same fungus (Mehta, 1975).

Tan spot is prevalent worldwide (Hosford, 1972; Mehta, 1979; Wiese, 1991), and in Brazil it has been found in the states of Rio Grande do Sul, Paraná (Luz, 1982), Santa Catarina and Mato Grosso do Sul (Linhares and Luz, 1996). In Australia in 1982, losses due to a severe epidemic of this pathogen reached about 49% wheat-grain production (Rees *et al*., 1982).

It is recognized that *D. tritici-repentis* presents enormous variability in its morphology, genome and pathogenicity. Since traditional morphological methods of identification can be slow and tedious, other methods for the detection and differentiation of strains of *D. tritici-repentis* have been investigated by various workers. Methods based on the analysis of genomic DNA have the potential to allow the direct examination of fungal samples, eliminating the need for culturing. Random amplified polymorphic DNA (RAPD) has been extensively used for the characterization of biological material and in this work this technique was used along with colony morphology to identify polymorphisms and investigate the genetic similarity between different isolates of *D. tritici-repentis.*

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## Material and Methods

### *Drechslera tritici-repentis* isolates

Twelve *D. tritici-repentis* isolates recovered from wheat seeds (supplied by CNPT-EMBRAPA, Passo Fundo-RS, Brazil) of different cultivars growing in different regions in the southern Brazilian state of Rio Grande do Sul were used (Table I). The wheat seeds were immersed in sodium hypochlorite 2% for 2 min, rinsed three times in sterile distilled water, and subsequently transferred to Petri plates containing potato dextrose agar (PDA). The samples were then incubated at 24 ± 2 °C for approximately 9 days at a 12 h photoperiod. After confirmation of the vegetative structure of the fungi, conidia were transferred to PDA slants and incubated as above described. All strains were stored as conidia and hyphae at 4 °C.

### Morphological analysis

The morphologic analysis for each isolate followed the method described by Frazzon, *et al.* (2002, this issue).

**Table I** - Isolates of *Drechslera tritici-repentis* obtained from wheat seeds.

| Wheat cultivar from the seeds of which the fungi were isolated [a] | Geographical Region of Rio Grande do Sul of the host seeds[b] | *D. tritici-repentis* isolate code |
|---|---|---|
| R23 (Santa Rosa) | VII ( Highlands of Uruguai Region) | BR23-S.Rosa |
| CEP 19 (Cruz Alta) | V (Middle plateau Region) | C19-C.Alta |
| CEP 24 (Selbach) | V (Middle plateau Region) | C24-Selbach |
| EMBRAPA 15 (Lagoa Vermelha) | III ( Higher Plateau Region) | E15-L.Verm. |
| EMBRAPA 16 (Coxilha) | V (Middle plateau Region) | E16-Coxilha |
| EMBRAPA 16 (Não-Me -Toque) | V (Middle plateau Region) | E16-NMTI |
| EMBRAPA 16 (Não-Me-Toque) | V (Middle plateau Region) | E16-NMTII |
| EMBRAPA 16 (Ronda Alta) | V (Middle plateau Region) | E16-R.Alta |
| EMBRAPA 16 (Selbach) | V (Middle plateau Region) | E16-Selbach |
| EMBRAPA 16 (Vacaria) | III ( Higher Plateau Region) | E16-Vacaria |
| EMBRAPA 24 (Butiá Grande/Sertão) | III (Middle plateau Region) | E24-Butiá |
| PF90120 (Passo Fundo) | III (Middle plateau Region) | PF901210-PF |

[a]Nearest town to where the seeds were collected.
[b]According to Rio Grande do Sul. Secretaria da Agricultura e Abastecimento. Agricultural, Environmental and Economical Macroregions of the state of Rio Grande do Sul / Secretaria da Agricultura e Abastecimento; Centro Nacional de Pesquisa do Trigo – Porto Alegre, 1994. v. 2.

## Total DNA extraction

Genomic DNA was extracted from each isolate by a modification of the method of Ashktorab and Cohen (1992). The isolates were incubated in potato dextrose broth [20% potato, 2% dextrose (w/v)] for 7 days at 24 ± 2 °C with a 12 h photoperiod. The DNA extraction procedures were those described by Frazzon, *et al.* (2002, this issue).

## Analysis of amplified DNA

The genomic DNA of the *D. tritici-repentis* isolates was amplified by the RAPD technique (Williams *et al*., 1990). The primers tested were the series A (A1-10) and B (B1-10) from BIODYNAMICS SRL (Buenos Aires, Argentina) and OPB3, OPB17 and OPC13 from OPERON Technologies-CA ( Alameda, CA. US). Each 25 µL of reaction mixture contained 40 ng of DNA, 2.5 mM of each dNTP (Pharmacia, Sweden), 45 ng of primer, 3 mM of $MgCl_2$, 0.01 mg of BSA, 2.5 µL reaction buffer (10x) and 1.5U Taq polymerase (CENBIOT, Porto Alegre, Brasil), the surface of the reaction mixture being overlaid with two drops of mineral oil. RAPD was performed in a Minicycler MJ Research thermocycler for 46 cycles, one cycle of 1 min at 94 °C, 5 min at 30 °C and 2 min at 72 °C;. 44 cycles of 1 min at 94 °C, 1 min at 30 °C, 2 min at 72 °C, and a final extension of 10 min at 72 °C. Amplification products were resolved on 1.4% agarose gel and visualized after staining with ethidium bromide.

## Data analysis

The RAPD data and morphologic characteristics of the colonies were analyzed using the Statistical Package for the Social Sciences (SPSS) software, 2nd edition, thus calculating similarity coefficients and constructing the dendrogram for genetic distances. The similarity was evaluated through simple association and the genetic distance as the Euclidean Distance. The binary matrix was built pairwise, and the presence or absence of a determined RAPD band scored 1 and 0, respectively. The hierarchical groupings were based on the Unweighted Pair Group using Arithmetical Averages - UPGMA (Sneath and Sokal, 1973).

## Results

Inspection of the colony morphology of the isolates showed that there was variation in the color, borders, texture and sectors of the colonies (Figure 1).

Table II shows the nine primers selected for the analyses on the basis of the patterns obtained, after being tested

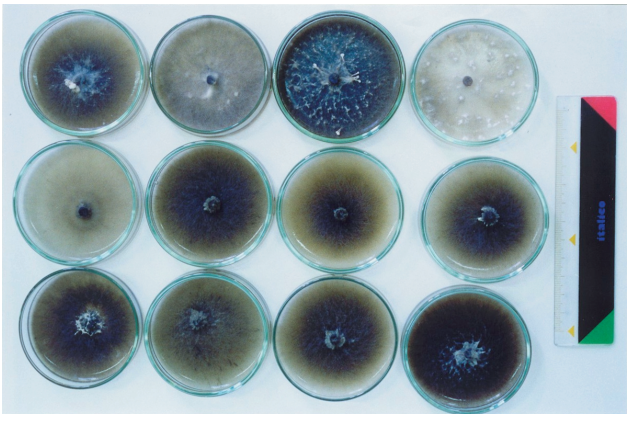

**Figure 1** - Isolates of *Drechslera tritici-repentis* showing morphological variation. (1) E16-Coxilha; (2) CEP24-Selbach; (3) E16-Ronda Alta; (4) BR23-Santa Rosa; (5) E16-Selbach; (6) E15-Lagoa Vermelha; (7) C19-Cruz Alta; (8) E16-Não-Me-Toque; (9) E24-Butiá Gr./Sertão; (10) E16-Não-Me-ToqueII; (11) E16-Vacaria; (12) PF90120- Passo Fundo.

twice in different experiments with each *D. tritici-repentis* isolate. For cluster analysis 45 fragments were used, of which 73% were polymorphic. Each primer generated a different amplification pattern with a variable number of fragments (Table II).

Primer B06 amplified four fragments, one of which was monomorphic (Figure 2), while primer B07 generated more fragments than the others, seven of which were polymorphic (Figure 3). Primer OPC13 generated six fragments, and a 0.75 kb fragment amplified only from isolates BR23-S.Rosa and E15-L.Ver (Figure 4).

Figure 5 shows the dendrogram of morphological and RAPD data combined and contains five groups. One group is formed by isolates PF90120-PF, E16-Vacaria, E16-NMTI, E16-NMTII and E24-Butiá, with isolates PF90120-PF and E16-Vacaria showing the highest similarity coefficient (0.8704). Another group is made up of isolates BR23-S.Rosa, E15-L.Ver, E16-R.Alta and C19-C.Alta. The remaining three groups contain only one isolate each, E16-Coxilha, E16-Selbach and C24-Selbach respectively. The lowest similarity coefficient (0.5556) was observed between isolates E24-Butiá and C24–Selbach (Table III).

**Table II** - Sequence of the primers tested and the size in kilobases (kb) of the *Drechslera tritici-repentis* DNA fragments amplified.

| Primer | Sequence 5'-3' | Molecular weight | *D. tritici-repentis* isolate |
|---|---|---|---|
| A02 | GGTGCGGGAA | 1.02 | All isolates |
| | | 1.53 | E15-L.Vermelha |
| | | 1.79 | C24-Selbach, E16-Selbach, BR23-S.Rosa, E15-L.Verm. and E16-NMTI |
| A08 | ACGCACAACC | 0.63 | All isolates, except E16-NMTI |
| | | 1.46 | All isolates, except E16-Coxilha, E16-R.Alta, E16-NMTI |
| | | 1.60 | All isolates |
| A10 | ACGGCGTATG | 0.49 | All isolates |
| | | 0.75 | All isolates except E16-Selbach |
| | | 0.84 | E24-Butiá, E16-Selbach, E16-NMTI and E16-NMTII |
| | | 1.07 | E24-Butiá, E16-Coxilha, BR23-S.Rosa |
| | | 1.32 | All isolates, except C24-Selbach, E16-Coxilha, E16-R.Alta |
| | | 1.66 | All isolates, except E16-Coxilha |
| B06 | GTGACATGCC | 0.67 | All isolates, except C24-Selbach, E16-Selbach |
| | | 0.71 | C24-Selbach, E16-Selbach |
| | | 0.79 | All isolates |
| | | 1.01 | All isolates, except C24-Selbach, BR23-S.Rosa |
| B07 | AGATGCAGCC | 0.57 | All isolates |
| | | 0.69 | E24-Butiá, E16-Selbach, E16-Vacaria |
| | | 0.77 | All isolates , except E16-Selbach, E16-Coxilha, E16-NMTI |
| | | 0.91 | All isolates except C24-Selbch, E16-Selbach, C19-C.Alta |
| | | 1.02 | All isolates |
| | | 1.33 | All isolates, except E16-NMTII |
| | | 1.79 | All isolates, except E24-Butiá, E16-Selbach |
| | | 1.94 | E16- Selbach |
| | | 2.11 | All isolates, except E16-Selbach |
| B09 | ATGGCTCAGC | 0.67 | All isolates, except C24-Selbach, PF90120-PF |
| | | 1.38 | All isolates, except C24-Selbach, E16-Selbach, E15-L.Ver. |
| | | 1.58 | C24-Selbach, E24-Butiá, E16-Selbach, E16-NMTII, E16-Coxilha, C19-C.Alta |
| OPB3 | GTCGCCGTCA | 0.95 | All isolates, except C24-Selbach, C19-C.Alta |
| | | 1.00 | E16-R.Alta, BR23-S.Rosa, E15-L.Ver., C19-C.Alta |
| | | 1.26 | All isolates |
| | | 1.54 | All isolates |
| OPC13 | AAGCCTCGTC | 0.75 | BR23-S.Rosa, E15-L.Ver. |
| | | 0.97 | All isolates |
| | | 1.22 | All isolates |
| | | 1.47 | C24-Selbach, E16-NMTII, BR23-S.Rosa |
| | | 1.69 | E16-Coxilha, PF90120-PF, E16-Vacaria |
| | | 1.81 | C24-Selbach, E16-NMTII, C19-C.Alta |
| OPB17 | AGGGAACGAG | 0.49 | All isolates, except E16-R.Alta |
| | | 0.71 | All isolates, except C19-C.Alta |
| | | 0.85 | All isolates, except E16-R.Alta |
| | | 0.97 | C24-Selbach, E16-NMTI and II, E16-Coxilha, PF90120-PF |
| | | 1.19 | All isolates, except E24-Butiá, E16-Selbach, C19-C.Alta |
| | | 1.77 | All isolates |
| | | 2.14 | All isolates |

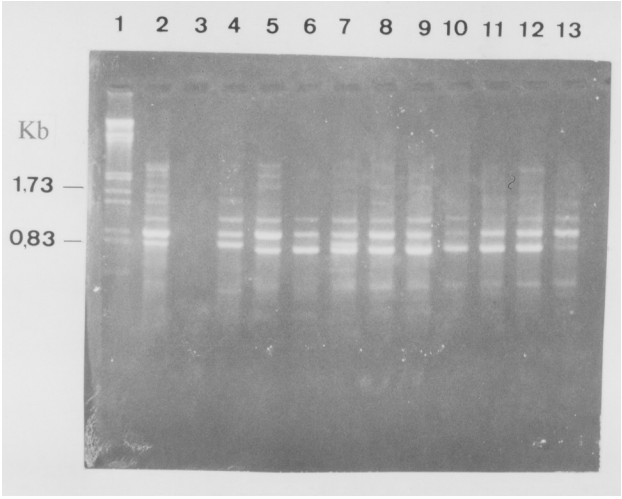


**Figure 2** - Random amplified polymorphic DNA from 12 isolates of *Drechslera tritici-repentis* using primer B06. Lanes are as follows: (1) λ; *Hind*III-*Eco*RI; (2) CEP24-Selbach; (3) E24-Butiá Gr./Sertão; (4) E16-Selbach; (5) E16-Não-Me-ToqueII; (6) E16-Coxilha; (7) C19-Cruz Alta; (8) PF90120- Passo Fundo; (9) E16-Vacaria; (10) E16-Ronda Alta; (11) BR23-Santa Rosa; (12) E15-Lagoa Vermelha; (13) E16-Não-Me-ToqueI. The numbers on the left are the lengths of the marker bands in kilobases.

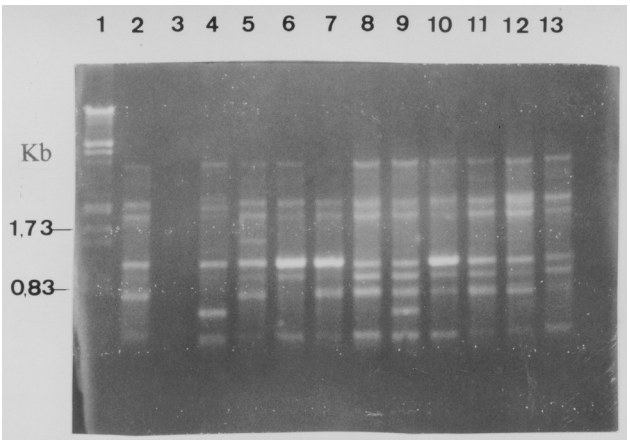


**Figure 3** - Random amplified polymorphic DNA from 12 isolates of *Drechslera tritici-repentis* using primer B07. Lanes are as follows: (1) λ; *Hind*III-*Eco*RI; (2) CEP24-Selbach; (3) E24-Butiá Gr./Sertão; (4) E16-Selbach; (5) E16-Não-Me-ToqueII; (6) E16-Coxilha; (7) C19-Cruz Alta; (8) PF90120- Passo Fundo; (9) E16-Vacaria; (10) E16-Ronda Alta; (11) BR23-Santa Rosa; (12) E15-Lagoa Vermelha; (13) E16-Não-Me-ToqueI. The numbers on the left are the lengths of the marker bands in kilobases.

The dendrogram with the morphological characters (Figure 6) consists of three groups. One group contains isolates E16-NMTI, E16-Vacaria, C19-C.Alta, E16-NMTII and E16-Coxilha. Another group is formed by isolates E16-R.Alta, PF90120-PF, E24-Butiá, E15-L.Verm, BR23-S.Rosa and E16-Selbach, all with different degrees of similarity. Isolate C24-Selbach stands alone as a separate group and has the lowest similarity coefficient (0.2222) with isolates BR23-S.Rosa, E24-Butiá, E15-l.Ver and E16-Selbach (Table IV).

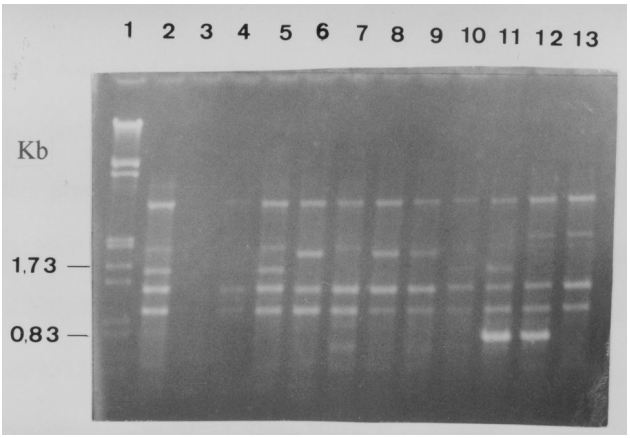


**Figure 4** - Random amplified polymorphic DNA from 12 isolates of *Drechslera tritici-repentis* using primer OPC13. Lanes are as follows: (1) λ *Hind*III-*Eco*RI; (2) CEP24-Selbach; (3) E24-Butiá Gr./Sertão; (4) E16-Selbach; (5) E16-Não-Me-ToqueII; (6) E16-Coxilha; (7) C19-Cruz Alta; (8) PF90120- Passo Fundo; (9) E16-Vacaria; (10) E16-Ronda Alta; (11) BR23-Santa Rosa; (12) E15-Lagoa Vermelha; (13) E16-Não-Me-ToqueI. The numbers on the left are the lengths of the marker bands in kilobases.

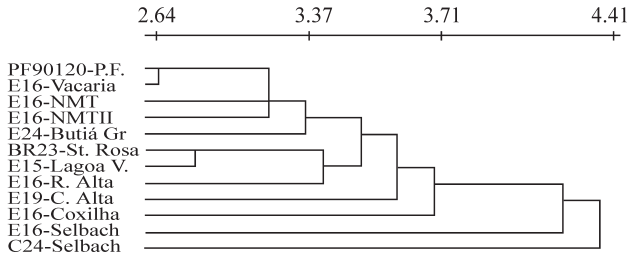


**Figure 5** - Dendrogram of the relationships between *Drechslera tritici-repentis* isolates based on both colony morphology and random amplified polymorphic DNA (RAPD) analysis.

Figure 7 presents the dendrogram of the RAPD data and is made up of three groups. One group is formed by isolates E16-Vacaria, PF90120-PF, E24-Butiá, E16-NMTII, E16-Coxilha, E16-NMTI, BR23-S.Rosa, E15-L.Verm and E16-R.Alta, with isolates E16-Vacaria and PF90120-PF having a similarity coefficient of 0.9318 (Table V), the highest among the isolates. Another group contains isolates C19-C.Alta and C24-Selbach. Again, isolate E16-Selbach stands alone in its own group and showed the lowest genetic similarity with the other isolates. The lowest similarity coefficient (0.5682) was that between E16-Selbach and E16-R.Alta (Table V).

## Discussion

Using the RAPD technique and morphological polymorphism we detected genetic diversity among *D. tritici-repentis* isolates recovered from seeds collected in different locations in the southern Brazilian state of Rio Grande do Sul.

**Table III** - *Dreschslera tritici-repentis* similarity matrix coefficients based on both colony morphology and random amplified polymorphic DNA (RAPD) analysis.

| Isolates | C19-C. Alta | C24-Selbach | BR23-S.Rosa | E24-Butiá | E15-L. Ver. | E16-Coxilha | E16-NMTI | E16-R.Alta | E16-Selbach | E16-NMTII | PF90120-PF |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C24-Selbach | 0.7037 | | | | | | | | | | |
| BR23 s.rosa | 0.7407 | 0.6296 | | | | | | | | | |
| E24-Butiá | 0.7778 | 0.5556 | 0.7778 | | | | | | | | |
| E15-L.Ver. | 0.7407 | 0.5926 | 0.8519 | 0.7778 | | | | | | | |
| E16-Coxilha | 0.7038 | 0.6667 | 0.7037 | 0.7407 | 0.6667 | | | | | | |
| E16-NMTI | 0.7222 | 0.6481 | 0.7593 | 0.7593 | 0.7593 | 0.7963 | | | | | |
| E16-R.Alta | 0.7407 | 0.5926 | 0.7778 | 0.7778 | 0.8148 | 0.7407 | 0.7593 | | | | |
| E16-Selbach | 0.7037 | 0.5926 | 0.6296 | 0.7778 | 0.6667 | 0.5926 | 0.6852 | 0.5926 | | | |
| E16-NMTII | 0.7963 | 0.7222 | 0.7593 | 0.7963 | 0.7222 | 0.7593 | 0.8148 | 0.7593 | 0.6481 | | |
| PF90120-PF | 0.7407 | 0.6667 | 0.7778 | 0.8148 | 0.8148 | 0.7778 | 0.7963 | 0.8519 | 0.6296 | 0.8333 | |
| E16-Vacaria | 0.7963 | 0.6481 | 0.7963 | 0.7963 | 0.7963 | 0.7963 | 0.8519 | 0.7963 | 0.6852 | 0.8148 | 0.8704 |

**Table IV** - *Drechslera tritici-repentis* similarity matrix coefficients based on colony morphology only.

| Isolate | BR23-S.Rosa | E24-Butiá | C19-C.Alta | C24-Selbach | E15-L. Verm. | E16-Coxilha | E16-NMTI | E16-R.Alta | E16-Selbach | E16-NMTII | PF90120-PF |
|---|---|---|---|---|---|---|---|---|---|---|---|
| E24-Butiá | 0.7778 | | | | | | | | | | |
| C19-C.Alta | 0.7778 | 0.7778 | | | | | | | | | |
| C24-Selbach | 0.2222 | 0.2222 | 0.4444 | | | | | | | | |
| E15-L.Verm. | 0.7778 | 1.0000 | 0.7778 | 0.2222 | | | | | | | |
| E16-Coxilha | 0.5556 | 0.5556 | 0.7778 | 0.6667 | 0.5556 | | | | | | |
| E16-NMTI | 0.6667 | 0.6667 | 0.8889 | 0.5556 | 0.6667 | 0.6667 | | | | | |
| E16-R.Alta | 0.6667 | 0.8889 | 0.6667 | 0.3333 | 0.8889 | 0.4444 | 0.5556 | | | | |
| E16-Selbach | 0.7778 | 0.7778 | 0.7778 | 0.2222 | 0.7778 | 0.5556 | 0.6667 | 0.6667 | | | |
| E16-NMTII | 0.6667 | 0.6667 | 0.8889 | 0.5556 | 0.6667 | 0.6667 | 0.7778 | 0.7778 | 0.6667 | | |
| PF90120-PF | 0.6667 | 0.8889 | 0.6667 | 0.3333 | 0.8889 | 0.4444 | 0.5556 | 1.0000 | 0.6667 | 0.7778 | |
| E16-Vacaria | 0,6667 | 0,6667 | 0,8889 | 0,5556 | 0,6667 | 0,6667 | 1,0000 | 0,5556 | 0,6667 | 0,7778 | 0,5556 |

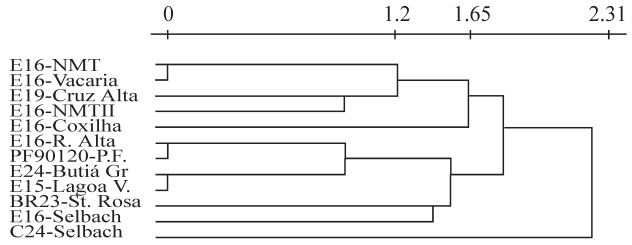


**Figure 6** - Dendrogram of the relationships between *Drechslera tritici-repentis* isolates based on colony morphology only.

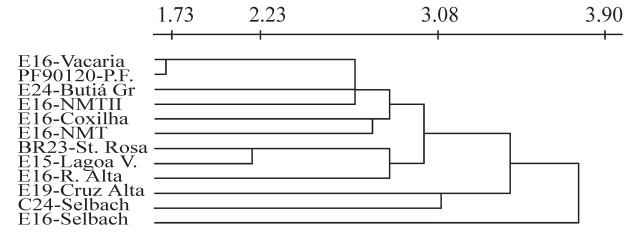


**Figure 7** - Dendrogram of the relationships between *Drechslera tritici-repentis* isolates based on random amplified polymorphic DNA (RAPD) analysis only.

The isolates of *D. tritici-repentis* used in this study were from wheat plantations located in three different regions in the State of Rio Grande do Sul, Brazil (Table I), but it was not possible to relate the polymorphisms found among the isolates with the region from where the host seeds were collected. In Figure 5 (based on morphology and RAPD data combined) isolates from the same region appeared in different groups and most of the isolates which shared the highest similarity coefficients were from different geographic regions. Analysis of morphological data (Figure 6) and RAPD data (Figure 7) separately also showed no obvious relationship with the location from which the seeds were collected so it appears that in this case there was no advantage in analyzing the data separately.

The wheat fields from which the seeds were collected are located in the middle or northern regions of Rio Grande do Sul where the soils are very variable in their chemical characteristics despite the fact that they were formed from the same geological material (Brauner, 1982). It would be interesting to know more about these variations in soil chemistry, and especially whether or not they happen in the same regions. Another aspect that might have influenced the variability of the isolates is the length of time during which the host-pathogen interaction has taken place. The *D. tritici-repentis* isolates show a great deal of polymorphism, with the similarity coefficient in the genetic analysis ranging from 59% to 93%, suggesting that the population is not in equilibrium.

**Table V** - *Drechslera tritici-repentis* similarity matrix coefficients based on random amplified polymorphic DNA (RAPD) analysis only.

| Isolates | BR23-S.Rosa | E24-Butiá | C19-C. Alta | C24-Selbach | E15-L. Ver. | E16-Coxilha | E16-NMTI | E16-R.Alta | E16-Selbach | E16-Vacaria | E16-NMTII |
|---|---|---|---|---|---|---|---|---|---|---|---|
| E24-Butiá | 0.7955 | | | | | | | | | | |
| C19-C.Alta | 0.7500 | 0.7727 | | | | | | | | | |
| C24-Selbach | 0.7045 | 0.6364 | 0.7727 | | | | | | | | |
| E15-L.Ver. | 0.8864 | 0.7273 | 0.7273 | 0.6818 | | | | | | | |
| E16-Coxilha | 0.7500 | 0.7727 | 0.6818 | 0.6818 | 0.6818 | | | | | | |
| E16-NMTI | 0.7955 | 0.7727 | 0.6818 | 0.6818 | 0.7727 | 0.8182 | | | | | |
| E16-R.Alta | 0.8182 | 0.7500 | 0.7500 | 0.6591 | 0.7955 | 0.7955 | 0.7955 | | | | |
| E16-Selbach | 0.6136 | 0.7727 | 0.6818 | 0.6818 | 0.6364 | 0.5909 | 0.6818 | 0.5682 | | | |
| E16-Vacaria | 0.8409 | 0.8636 | 0.7727 | 0.6816 | 0.8182 | 0.8182 | 0.8182 | 0.8409 | 0.6818 | | |
| E16-NMTII | 0.7955 | 0.8182 | 0.7727 | 0.7727 | 0.7273 | 0.7727 | 0.8182 | 0.7500 | 0.6364 | 0.8182 | |
| PF90120-PF | 0.8182 | 0.7955 | 0.7500 | 0.7500 | 0.7955 | 0.8409 | 0.8409 | 0.8182 | 0.6136 | 0.9318 | 0.8409 |

There are many factors affecting polymorphism analysis e.g. the number of samples selected for analysis, the organism studied, genetic flow between populations, environmental adaptation and adaptation to a new host, selective pressure and migration. Peever and Milgroom (1994) have stated that agricultural pathogens are subject to extensive extinction and re-colonization and are rarely in equilibrium. In studies with *D. teres* from different parts of the world these authors found a high degree of genetic differentiation between populations when compared with most other fungal populations studied, about 46% of the total genetic variability observed occurring in the *D. teres* populations.

Although Guthrie *et al*. (1992) and Assigbetse *et al*. (1994) were able to detect a relationship between intraspecific polymorphisms and geographic location, most work done in this field has shown no direct relationship and there is no general agreement among researchers as to whether such a relationship exists.

Fabre *et al*. (1995), in a study of *Colletotrichum lindemuthianum* from different countries, investigated grouping by DNA polymorphism in relation to the geographical origins of the isolates and found no correlation, because the isolates formed two groups with isolates from Latin America occurring in both groups. The relationship of *Trichoderma harzianum* strains from various geographical regions and presenting similar RAPD patterns is not yet understood (Zimand *et al*., 1994). Moller *et al.* (1995) detected intraspecific diversity not only between isolates of *Chaunopycnis alba* from different geographic regions or hosts, but also between isolates from a single location. In the present work with *D. tritici-repentis,* the isolates recovered from seeds collected in the towns of Selbach and Não-Me-Toque (the same geographic origin) showed both morphological and RAPD diversity (Figures 1 and 4).

Using RAPD markers it was possible for Bayman and Cotty (1993) to separate isolates based on the relationship between their genetic and morphological variability. However, this approach was not possible in the present work probably because the number of samples from each region was too small and only nine primers were used for the final analysis. The study of a larger number of primers and isolates (and re-isolating in different years) may show clearer results.

It will be very important to find a molecular marker for *D. tritici-repentis*, since *Cochliobolus sativus* and *D. tritici-repentis* produce very similar symptoms on seedlings and wheat leaves, making it very difficult to distinguish the disease based only on symptoms (Luz, 1982). It is normally necessary to isolate the fungus by plating for identification and confirmation, thus delaying diagnosis (Mehta, 1979). The RAPD technique is quick, effective and produces reliable markers which have been used for the identification of various fungal pathogens. Using this technique to identify pathogens by DNA fingerprinting is proving to be very useful and should be equally beneficial for classifying *D. tritici-repentis* isolates.

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