ITS ribosomal DNA phylogeny of *Gaeumannomyces graminis*

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H. M. Fouly
Department of Natural Resources and Environmental Sciences, University of Illinois, Urbana, IL 61801, U.S.A.

ABSTRACT

Gaeumannomyces graminis causes take-all disease on cultivated cereal grasses (wheat, barley, oats, bentgrass, blugrass and other related grasses). In the present study the, 5.8S and the internal transcribed spacers (ITS) regions of the ribosomal DNA from 21 isolates belonging to G. graminis varieties [tritici (5), avenae (4), and graminis (9)], G. incrustans (1), G. leptosporous (1), and G. cylindrosporous (1) were sequenced and then compared to each other. Phylogenentic parsimnoy analysis among the isolates was carried out. Together, including gaps, ITS and 5.8S regions totaled 660 aligned sites, 220 of which were informative. Only 18 of the informative sites were located in the 5.8S rDNA. All of the isolates tested formed a major clade with high bootstrap support value (100%). G. leptosporous and G. cylindrosporous formed a distict subclade. G. graminis var. aveane grouped together as sister isolates. G.g. var. tritici isolates formed two monophyletic groups. Four G. g. var. graminis isolates formed two distict groups, while two additional isolates were associated with Ggt and Gga groups. The rest of the isolates were randomly positioned. These data support our previous results using Random amplified polymorphic DNA (RAPD) and Restriction fragment length polymorphism (RFLP) of rDNA.

Key words: *ITS rDNA, phylogeny, G. graminis.*

INTRODUCTION

Gaeumannomyces graminis (Sacc.) Arx & D. Olivier, a filamentous soil borne fungus, parasitizes the roots and crowns of susceptible members of the Gramineae family. The species *G. graminis* is subdivided into four varieties: *G. g.* var. *tritici* (Walker), *G. g.* var. *avenae*, *G.g.* var. *graminis* and *G. g.* var. *maydis*.

Gaeumannomyces genera, species and varieties have been classified using morphological characteristics of the teleomorphic, anamorphic and mycelial states; cultural characteristics; and host-parasite relationships. The most commonly used criteria for separation of *G. graminis* isolates into varietal groups are hyphopodial type, growth on media containing cystine (Turner, 1959; 1961), and morphology and size of asci and ascospores, respectively.

A complementary approach to fungal taxonomy or phylogeny is represented by DNA analysis of specific genome regions. Methods that can be used include random amplified polymorphic DNA (RAPD), restriction fragment length polymorphisms (RFLPs) and Amplified fragment length polymorphism (AFLP) that permit evaluation of phylogenetic relationships without knowing the nucleotide

sequence. However, DNA sequence-based methods are more accurate and allow more estimation of precise differences among taxa. Sequence data of rDNA subunits allow the evaluation of phylogenetic relationships among different taxa, being highly conserved within species but relatively variable at higher taxonomic levels (among species, genera, etc.). The sequence of the internal transcribed spacers (ITS) flanking the 5.8S gene is commonly used to study the phylogeny of organisms.

It has been reported (Ward and Akrofi, 1994; Fouly *et al.*, 1996) that restriction digests of the ITS region of the 5.8S ribosomal DNA of *Gaeumannomyces* species and varieties could be used to distinguish among species of *Gaeumannomyces*. The presence of length variability of the *G. cylindrosporous* ITS region and the detectable restriction polymorphisms of the ITS regions between isolates of *G. graminis* var. *tritici* and *G. graminis* var. *graminis* isolates (Fouly *et al.*, 1997) prompted us to determine the DNA sequence of this region. The DNA sequence of the IT'S region of members of the North American isolates of *Gaeumannomyces* species and varieties was determined. We attempted to construct a molecular phylogeny of the species and varieties of *Gaeumannomyces*.

MATERIALS AND METHODS

Fungal cultures and DNA extraction

Twenty-two isolates, representing four species of *Gaeumannomyces* were used in this study. Table (1) lists the cultures, their isolate number, and their geographic origin. Cultures were maintained on 1/5 strength potato dextrose agar. To obtain mycelium for DNA extraction, the isolates were grown in GYP medium (Glucose 2%; yeast extract 0.5%; and peptone 0.5%), filtered, and ground in liquid nitrogen using a mortar and pestle. DNA was extracted according to the methods used by Murray and Thompson (1980).

Polymerase chain reaction (PCR) and sequencing

The ITS region of the rDNA was specifically amplified using the primers ITS 4 and ITS 5 (White *et al.*, 1990). The PCR primers used in this study were synthesized at the Biotechnology Center of the University of Illinois at Urbana-Champaign. PCR was run with a Perkin-Elmer Cetus Taq DNA Polymerase kit for 35 cycles (3 min denaturation step at 93°C, a primer annealing step for 1 min at 48°C, and a 1 min primer extension step at 72°C). A final extension of 10 min at 72°C was used for all PCR reactions. Negative controls, in which the DNA template solution was replaced by water, were used in all experiments to test for contamination. At least two separate PCRs were performed for each isolate.

ITS PCR products: purification, DNA sequencing and analysis

PCR products were separated in 1.4% agarose prepared with and electrophoresed in TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH 8.0) and stained with eithidium bromide. Bands were excised, crushed and purified with a QIAquick gel extraction kit (QIAgen, Chatsworth, CA). Purified PCR products were sequenced in both directions using cycle sequencing with a fluorescent dye-labeled terminator by ABI 373 automated DNA sequencer (Perkin-Elmer) at the Biotechnology Center at the University of Illinois. The ITS sequence of *Magnaporthe poae* was used as an out group. Sequences were aligned using the CLUSTAL W program (Thompson, Higgins & Gibson, 1994). Phylogenetic analysis using

parsimony (PAUP; version 4.08b, Sinnauer, Sunderland, MA) was used to determine relationship of the taxa sequenced with the following settings: heuristic search option with random addition of sequences (10 replicates), tree-bi-section-reconnection branch swapping, and steepest descent. Bootstrap values with 1000 replicates were performed to evaluate the strengths of the internal branches of the phylogenetic trees.

Table (1): Isolates of Gaeumannomyces species and varieties used in this study.

Species/Isolate	Host	Source: Collector/location
G. graminis var. *tritici*		
WF9039 (GH-90)	wheat	Bockus/KS
WF9040 (P-L)	wheat	Bockus/
WF9420 (Jo-1)	wheat	Bockus/KS
WF964(550)	wheat	Mathre/MT
WF968(698)	wheat	Mathre/MT
G. graminis var. *avenae*		
WF934	bentgrass	Wilkinson/France
WF937(93BRWI)	bentgrass	Wilkinson/WI
WF9449(FR-1)	bentgrass	Wilkinson/France
WF984	bentgrass	Wilkinson/IL
G. graminis var. *graminis*		
WF9014	zoysagrass	Wilkinson/MO
WF9124	St.Augustine	Wilkinson/CA
WF921	bermudagrass	Rhode Island
WF9236 (FL-177	St. Augustine	Elliott/FL
WF9238 (FL-199)	St. Augustine	Elliott/FL
WF9453	bermudagrass	Elliott/FL
WF9464	bermudagrass	Elliott/FL
WF9469 (92-8186-2B)	rice	Elliott/AR
WF9470 (TX-9I-1)	rice	Elliott/TX
WF9471 (PPRI-4754)	millet	South Africa
G. incrustans		
WF914	zoysiagrass	Kansas
G. cylindrosporous		
WF912	wheat	Wilkinson/KS
G. leptosporous		
WF9427		(ATCC24161)
Magnaporthe poae	bluegrass	Wilkinson/IL

RESULTS AND DISCUSSION

The primer combination ITS-4/ ITS-5 amplified single products represents the whole region of ITS (ITS1, 5.8S, and ITS2). The ITS region ranged from 547-567 bp for *G. g.* var. *tritici* isolates, and from 520-580 bp for *G.g.* var. *graminis* isolates. The ITS region contained 535 bp in *G. leptosporous*, 549 bp in *G. g.* var. *avenae*, 551 bp in *G. incrustans* and 582 bp in *G. cylidrosporous*. Table (2) gives the length of the ITS1, ITS2 and 5.8S regions for each listed species and variety of *Gaeumannomyces* isolates. DNA sequences of

the ITS region were determined for 22 isolates of *Gaeumannomyces* and for one isolate of *Magnaporthe poae*. The ratio of transition/transversion (Ti/Tv) was 1.2 for ITS1, and 1.6 for ITS2. The overall ratio for the ITS region was 1.62. Together, including gaps, the ITS and 5.8S regions totaled 660 aligned sites, 220 of which were parsimony informative. Figure (1) shows the 50% majority-rule consensus tree of the most parsimonious tree along with bootstrap values (consistency index = 0.827, retention index = 0.699, rescaled consistency index = 0.579). With exceptions of *G. incrustans* and *G. g.* var. *graminis* isolate WF9014, all the species and varieties of *Gaeumannomyces* were placed into a major clade with high bootstrap support value (100%). Within this clade, *G. leptosporous* and *G. cylindrosporous* formed a distict subclade. This arrangment is consistent with the previous conclusion that *G. cylindrosporous* and its anamorph *Phialophora graminicola* form a distict group from the other isolates of *G. graminis* and *G. incrustans* (Bryan *et al.*, 1995; Fouly *et al.*, 1996). *G. graminis* var. *avenae* isolates grouped together as sister isolates. *G.g.* var. *tritici* isolates formed two monophyletic groups. Four *G. g.* var. *graminis* isolates formed two distinct groups: WF 9236, WF 9453; and WF 9238, WF 9470. *G. g.* var. *graminis* isolates WF9471 and WF 921 showed closer relationship with *G. g.* var. *tritici* and *G. g.* var. *avenae* groups. The relationship of *G. g.* var. *graminis* isolates WF9124 and WF 9464 with other *G. g.* var. *graminis* isolates could not be resolved. Our data showed that there is a close relationship between isolates of *G. g.* var. *tritici* and *G. g.* var. *avenae*. The ITS-RFLP patterns (Ward and Akrofi, 1994; Fouly *et al.*, 1997), sequence analysis (Bryan *et al.*, 1995), and RAPD banding patterns (Fouly *et al.*, 1996) support a close relationship of *G. g.* var. *tritici* and *G. g.* var. *avenae*. *G. g.* var. *graminis* isolates are variable and could be divided into two groups. Group one is related to *G. g.* var. *tritici* and *G. g.* var. *avenae*. Group two is different and has little relation to other varieties of *G. graminis*. This is also consistent with the morphology and host range of these varieties (Walker, 1981). *G. g.* var. *graminis* produced lobed hyphopodia and short ascospores, and infect many members of *Poaceae*. However, *G. g.* var. *tritici* produces simple hyphopodia and short ascospores, and infects wheat and barley; whereas, *G. g.* var. *avenae* has longer ascospores and infect oats, wheat, barley and bentgrass. It is further known that the host range of *G. g.* var. *graminis* is broad (Elliott, 1991; Elliott *et al.*, 1993; Krauz, 1991; McCarty and Lucas, 1989; Walker, 1981; Wilkinson, 1994; Wilkinson and Kane, 1993; Wilkinson and Pedersen, 1993).

Table (2): Length (base pairs) of internal transcribed spacers of different species and varieties of Gaeumannomyces.

	Ggt1	Gga	Ggg	Gi	GI	GC
ITS	547-567	549	520-580	551	535	582
ITS1	164-168	166	138-201	172	168	209
ITS2	216-239	220	194-245	212	204	206
5.8S	163-164	163	163-164	167	163	167

1 Ggt= *Gaeumannomyces graminis* var. *tritici*; Gga= *G. g.* var. *avenae*; Ggg= *G.g.* var. *graminis* ; Gi= *G. incrustans*; Gl=*G. leptosporous*; Gc= *G. cylindrosporous*.

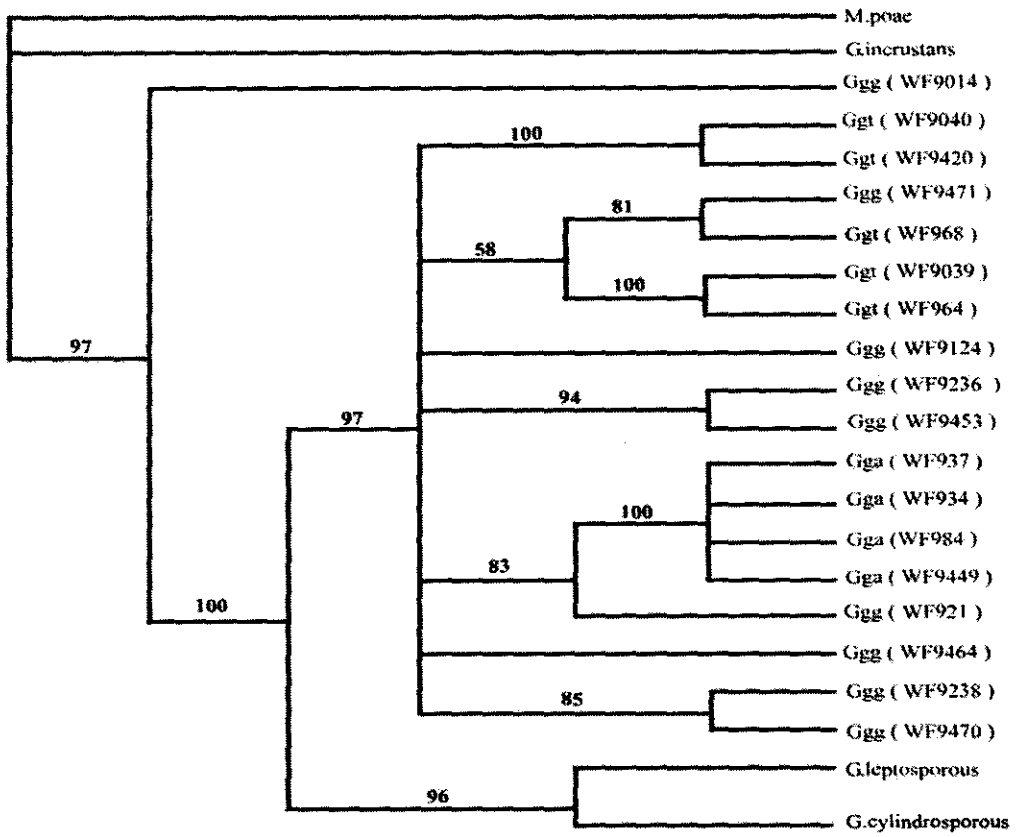

Fig. (1): The 50% Majority- rule consensus tree the three most parsimonious trees based on ITS sequence data. Bootstrap support values (100 replicates) are shown above nodes .

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الملخص العربي

استخدام التركيبات النوتيدية الواقعة بين وحدات الريبوزوم في دراسة مقارنة للعلاقة التقاربية للفطر *Gaemannomyces graminis*

حنفى محمود فولى
قسم علوم المصادر الطبيعية والبيئة – جامعة إلينوى – الولايات المتحدة الامريكية

يسبب الفطر ***G. graminis*** مرض ال Take-all لعديد من محاصيل وحشائش العائلة النجيلية (القمح و الشعير والشوفان والبنتجراس و البلوجراس).

تمت دراسة التتابع النيوتيدى (DNA Sequence) للمناطق الواقعة ما بين وحدات الريبوزوم (وهى ITS2, ITS2, 5.8S) في عدد ٢١ عزلة من الفطر منها ٥ عزلات تنتمي للصنف *tritici* و ٤ عزلات تنتمي للصنف *avenae* و ٩ عزلات تنتمي للصنف *graminis* وعزلة واحده من الأنواع الآتية , *G.leptosporous* , *G.incrustans* *G.cylindrosporous* . بعد تحديد التتابع النيوتيدى لكل عزلة عمل مقارنه بينها و تم دراسة العلاقات التقاربية بين العزلات . وجد ان العدد الكلى للتتابعات ٦٦٠ تتابعا منها ٢٢٠ تتابع له دلاله معينة محدده . واظهر التحليل *Phylogenetic parsimony* ان جميع العزلات لهامنشأ واحد وتنتمى الى فرع رئيسى major clade . عزلات النوعين , *G.leptosporous* *G.cylindrosporous* فلهما Sub-clade مميز . عزلات الصنفين *tritici* و *avenae* من الفطر *G.graminis* فهي عزلات قريبة من بعضها جدا كالاخوات . sister isolates اما عزلات الصنف *graminis* فقد صنفوا إلى ٤ مجموعات : مجموعة ذات علاقة تقاربية مع عزلات الصنفين الاخرين ومجموعتين مميزتين (اخوات) اما المجموعة الرابعة فلم تتحدد درجة قرابتها لأية مجموعة .تدعم هذه النتائج السابقة المنشورة والمتحصل عليها من دراسة هذا الفطر والتي استخدم فيها تقنيات RAPD و RFLP لتحليل التتابعات النوتيدية بين وحدات الريبوزومات .