

Phylogenetic analysis of *Rhizoctonia solani* using rDNA-ITS sequences – a potential tool for identification of anastomosis groups

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Introduction

Rhizoctonia solani Kühn (teleomorph = *Thanatephorus cucumeris* Frank (Donk)) is a soil-borne fungus capable of causing diseases on field and horticultural crops. Isolates of multinucleate *Rhizoctonia solani* Kühn are genetically diverse in their cultural, morphological and physiological characteristics as well as in their pathogenic range of host plants (2,3,4,5,6). Using traditional methods (vegetative hyphal fusion with tester isolates), *R. solani* can be separated into at least twelve anastomosis groups (AGs). Given the laborious nature of the traditional methods, ribosomal DNA (rDNA)- internal transcribed spacer (ITS) sequence analysis may be an appropriate method for comprehensive classification of multinucleate *Rhizoctonia* spp. into 14 AGs (AG 1 to AG 13 and AG-BI) (1,5,6). The goal of this study was to develop a quick and reliable method of assigning AG groups to *R. solani* isolates based on phylogenetic analysis of their ribosomal DNA -internal transcribed spacer (rDNA-ITS) sequences.

Materials and Methods

Rhizoctonia sequences database (2,3) was downloaded from the National Center for Biotechnology Information (NCBI <http://www.ncbi.nlm.nih.gov>), for sequence information to generate a phylogram. Alignment gaps were treated as missing data. The aligned sequences were analyzed by the neighbor joining method and maximum parsimony for the phylogenetic tree construction. A distance analysis was performed using Tamura-Nei plus Gamma Model, model selected using FindModel (7, Fig 1.) (Akaike information criterion (AIC) score = 4535.54). The strength of the tree branches was tested by 1,000 bootstrap trials. *Rhizoctonia oryzae* sequences from were used as an out-group in the phylogram model.

Validation of the model used selected ITS sequences of *R. solani* isolates identified on different North Dakota crops. Pure cultures of single-spored *Rhizoctonia* isolates plated on full-strength Potato Dextrose Agar (PDA, Difco) dishes and maintained at room temperature, 24 hours dark conditions for a week. DNA was isolated from the lyophilized mycelium of the individual isolates and resuspended in 50 μ L⁻¹ of rehydration solution (1% TE buffer) using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI). A single fragment approximately 650-700 bp in length was generated and sequenced (McLab, South San Francisco, CA) following polymerase chain reaction (PCR) with primers ITS4 and ITS5 (8). ITS sequence analysis was initially performed using BLAST via GenBank nucleotide database to confirm *R. solani* before the model was used to predict the AG. Selected isolates were also used to conduct traditional anastomosis grouping using established testers for the respective AGs.

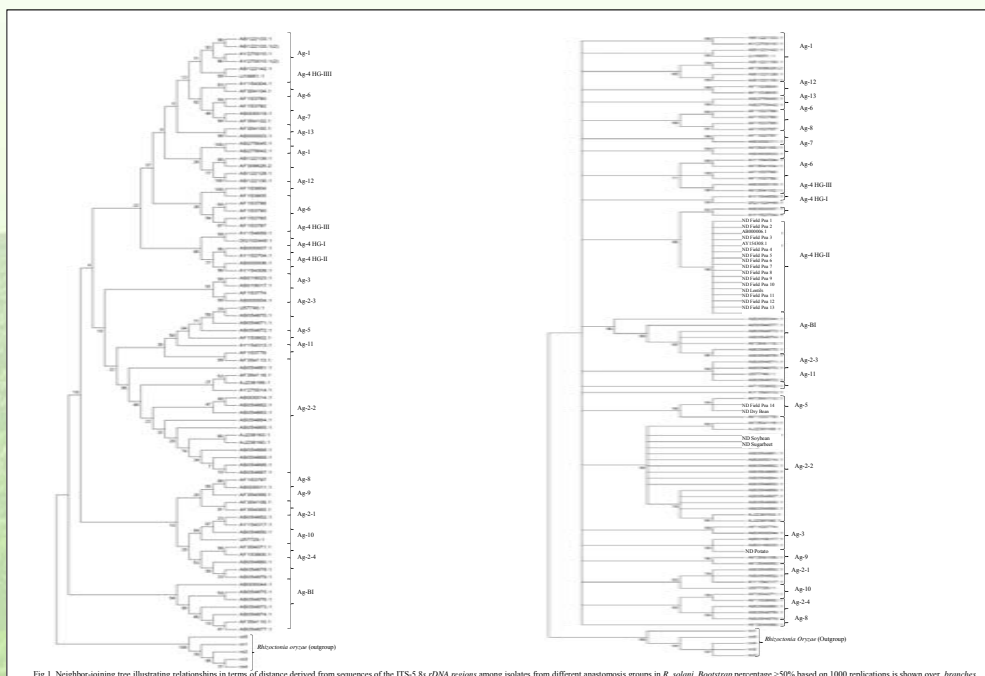


Fig 1. Neighbor-joining tree illustrating relationships in terms of distance derived from sequences of the ITS-5.8r rDNA regions among isolates from different anastomosis groups in *R. solani*. Bootstrap percentage >50% based on 1000 replications is shown over branches.

Discussion

Analysis of the ITS region of ribosomal DNA using Tamura-Nei plus Gamma Model, was supported by corresponding parsimony tree and in the scope of this project appears to be a reliable tool for determination of *R. solani* anastomosis group and subset. The sequence based anastomosis groupings were also supported by previous reports and current hyphal fusion experiments with tester isolates. A comprehensive diagnostic phylogram of multinucleate *R. solani* AGs is currently under construction. This will be deposited in TreeBASE (www.treebase.org) after further validation and can be easily used to ascertain AGs of new isolates by adding the sequences to the program and generating a modified tree.

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