Indian J. Genet., 68(3): 307-312 (2008)

Use of an rRNA internal transcribed spacer region to distinguish closely related isolates of the genera *Rhizoctonia*

D. Monga*, M. Kumar and P. K. Chakrabarty[1]

Central Institute for Cotton Research, Regional Station, Sirsa 125 055
[1]Central Institute for Cotton Research, Nagpur 440 010

(Received: February 2007; Revised: July 2008; Accepted: August 2008)

www.IndianJournals.com
Members Copy, Not for Commercial Sale
Downloaded From IP - 61.247.228.217 on dated 27-Jun-2017

Abstract

Inter-and intra specific variation among isolates of *Rhizoctonia bataticola* and *R. solani* the causal organism of root rot of cotton were evaluated by analysis of the internal transcribed spacer (ITS) sequences of the rRNA region. The ITS region was first amplified by polymerase chain reaction (PCR) with specific primers and amplification products, which ranged between 540 to 680 bp were obtained for all the isolates analyzed. The degree of polymorphism observed did not allow proper identification of most of the isolates. Analysis of two internal transcribed spacer sequences ITS 1 and ITS 2, revealed that within the isolates of *R. bataticola,* the size of ITS 1 region ranged from 135 to 184 bp while the size of ITS 2 varied by 4 bp. The size of ITS 1 ranged from 155 to 205 bp, while the size of ITS 2 ranged from 149 to 251 bp in isolates of *R. solani.* Based on phylogenetic analysis of the ITS 1 and ITS2 sequences different isolates of *R. bataticola* and *R. solani* were grouped in separate clusters.

Key words: Ribosomal RNA (rRNA), internal transcribed spacer (ITS), *R. bataticola, R. solani*, PCR

Introduction

Root rot caused by *Rhizoctonia solani* Kuhn and *R. bataticola* (Taub) is one of the most serious diseases of cotton in the irrigated northern cotton zone with fourteen lakh hectares of area and comprising the states of Haryana, Punjab and Rajasthan. The disease appears in June and is characterized by sudden and complete wilting of the plants. The affected plants can easily be pulled out of ground. The bark of roots is shattered and gives yellowish appearance. Intra specific groups within *R. solani* were earlier recognized based primarily upon cultural characters, pathogenic behavior and ecological criteria. Later, it was reported that isolates of *R. solani* could be divided into different groups based on anastomosis behaviors. The tremendous diversity in morphology, pathogenicity and physiology of genera *Rhizoctonia* has led to a classification system based on anastomosis grouping. Currently there are ISAGs of *R. solani* [1]. Grouping based on DNA hybridization also supports that based on anastomosis [2, 3]. Studies using restriction fragment length polymorphism (RFLP), analysis of nuclear DNA [4] have also been used to identify genetic differences among some Anastomosis Groups. Small subunit rRNA acts as a mosaic of different degrees of sequence conservation (from highly cpnserved to semiconserved and to highly variable regions), which allows the molecule to measure distant evolutionary relationship, as well as closer evolutionary relationships. In this study we analysed the sequence of internal transcribed spacer (ITS) region for differentiating closely related isolates of genera *Rhizoctonia.* The ITS region is located between 18S and 28S rRNA genes. At one time it was thought that these spacers have no function, but finding of Sande *et al.* [5] and Musters *et al.* [6] have shown that both ITS regions play a role in primary rRNA processing. Sequence analysis of the 18S, 28S and 5.8S transcriptional unit and the internal transcribed spacers of the rRNA genes have been used to infer taxonomic and phylogenetic relationships among the different AGs of *Rhizoctonia solani* and *Rhizoctonia* spp. [7].

In this paper we describe the results of a comparison of the ITS1 and ITS2 sequences of members of the genus *Rhizoctonia* which showed that these sequences are useful for differentiating closely related isolates of the genera *Rhizoctonia.*

Materials and methods

Fungal cultures

Twenty three isolates of *R. solani* and twenty five isolates

*Corresponding author's e-mail: mukeshhau@yahoo.com

www.IndianJournals.com
Members Copy, Not for Commercial Sale
Downloaded From IP - 61.247.228.217 on dated 27-Jun-2017

of *R. bataticola* used in this study were isolated from cotton root rot affected plants collected mainly from different places in North (Haryana, Punjab and Rajasthan), Central and South India. Pure cultures of isolates were maintained on Czapek-Dox agar slants and stored at 4°C till further use.

DNA isolation

For DNA extraction the fungi were grown on Czapak-Dox broth medium for 10 days at 25°C without shaking. Genomic DNA was extracted from 500 mg mycelium of each isolate by method of Monga *et al.* [8]. Ground samples were suspended in 500ml extraction buffer (250mM NaCl, 200mM Tris pH 8.5, 25mM Na-EDTA, 0.5% sodium dodecyl sulfate [SDS]) and extracted once with phenol: chloroform: isoamyl alcohol (25: 24:1) and once with chloroform: isoamyl alcohol (24:1). After treatment with RNase A (50 ng/ml for 30 minutes at 37°C), the DNA was re-extracted with chloroform: isoamyl alcohol, and precipitated with two volumes of cold isopropanol. The DNA pellets were washed with 80% ethanol, dried and dissolved in TE (pH 8.0) buffer.

PCR amplification of ribosomal DNA regions

The ITS1 and ITS2 and the inverting 5.8S coding rRNA gene were amplified by PCR using the primers ITS1 and ITS4 as described by White *et al.* [9]. PCR amplification was performed in 50 µl reaction volumes containing 0.5 unit *Taq* DNA polymerase, dNTP mix (0.2 µM each of dCTP, dGTP, dATP, and dTTP, 20 mM Tris-HCl (pH 8.4), 50 mM KC1, 1.5 mM $MgCl_2$, 0.5 µM primer and 25 -50 ng of genomic DNA. The reactants were carefully mixed and spun down briefly to collect the contents to the bottom of the tube. Amplification was performed in a Thermalcycler PTC-100 (M. J. Research Inc., USA) under the following conditions: Initial denaturation at 95°C for 3 min, followed by 30 cycles of denaturing at 95°C for 2min, annealing at 50°C for 30 sec, extension at 72°C for 2 min and final extension at 72°C for 10 minutes. An additional cycle at 72°C for 10 min was run at the end of these cycles. A control was kept with sterile distilled water instead of genomic DNA. The PCR products (10 µl) were resolved by gel electrophoresis using 1.5 % agarose gel in 0.5 X Tris-Borate-EDTA (TBE) buffer at a constant voltage of 50V for 3 h. Gels were stained with ethidium bromide, visualized with UV light and photographed by using Gel documentation system.

Cloning and sequencing of ITS markers

RAPD analysis of *R. bataticola* and *R. solani* isolates were performed using 23 and 15, 10-mer arbitrary primers (Operon Technologies Inc., Alameda, USA) respectively. On the basis of RAPD data the different isolates of *R. bataticola* and *R. solani* were clustered, six and four groups of *R. bataticola* and *R. solani* were found, respectively [10]. One representative isolate from each group amplified with the ITS primers was then selected for cloning purposes.

For cloning of the ribosomal RNA regions, the purified amplification product was ligated into the pGEM-T vector system (Promega) according to the manufacturer's instructions. The Alkaline Lysis with SDS: Minipreparation [11] was used to isolate plasmid DNA from the bacterial host. Inserts of six isolates of *R. bataticola* and four isolates of *R. solani* were sequenced from Banglore Genei Pvt. Ltd. Double strand sequencing was performed for each clone.

Sequence analysis

The rRNA gene sequences were aligned by using the multiple sequence alignment in Molecular Evolutionary Genetics Analysis (MEGA) version 3.1 [12]. The ITS sequences were subjected to a pairwise analysis. Level of sequence similarity was calculated and values were used to produce unrooted phylogenetic trees by unweighted pair group with mathematical average (UPGMA) cluster analysis methods. The confidence values of branches on the tree were determined by performing a bootstrap analysis in which 500 replicates were used.

Results and discussion

The internal transcribed spacer (ITS) of the rRNA regions of twenty five isolates of *R. bataticola* and twenty three isolates of *R. solani* showed length polymorphism. PCR amplification of different isolates of *R. bataticola* with specific primers for the ITS region generated bands ranging from 540 to 585 bp, while isolates of *R. solani* amplified bands ranging from 560 to 680 bp. One representative isolate from each group obtained on the basis of RAPD grouping was amplified with the ITS specific primers and used for cloning purposes. These were sequenced and submitted to GenBank (Table 1).

ITS sequence analysis

Length polymorphisms were observed between different isolates of *R. bataticola* and *R. solani* for both ITS1 and ITS2. Alignment of consensus sequence indicated that within the isolates of *R. bataticola,* the size of ITS 1 region ranged from 135 to 184 bp (Rb1 &14 had the

Table 1. Different isolates of *R. bataticolai* and *R. solani* used in the present study

Sr. No.	Isolates	State	Gene bank accession no.
	Rhizoctonia bataticola		
1.	Rb 1	Punjab	DQ2 12767
2.	Rb 2	Haryana	DQ222241
3.	Rb 14	Punjab	DQ222238
4.	Rb 16	Rajasthan	DQ222239
5.	Rb 18	New Delhi	DQ222240
6.	Rb 22	Haryana	DQ218056
	Rhizoctonia solani		
7.	Rs 11	Punjab	DQ408294
8.	Rs 12	Rajasthan	DQ223780
9.	Rs 18	Punjab	DQ223781
10.	Rs 23	New Delhi	DQ223782

smallest ITS1 and Rb 16 had the largest), while the size of ITS2 varied by 4 bp (ITS 2 of Rb 2, 16 & 22 were 156 bp long, and ITS2 of Rb 1, 14 & 18 were 160 bp long). The aligned sequences are shown in Figs. 1 & 2.

Within the isolates of *R. solani,* the size of ITS 1 ranged from 155 to 205 bp (Rs 18 had the smallest ITS 1 and Rs 11 had the largest), while the size of ITS2 ranged from 149 to 251 bp (Rs 23 had the smallest ITS 2 and Rs 12 had the largest). The aligned sequences of ITS 1 and ITS 2 of different isolates of *R. solani* are shown in Figs. 3 & 4. A pairwise analysis of sequences indicated that the nucleotide sequence similarity among isolates of *R. bataticola* was 91.0 to 100% in the ITS 1 region and 97.0 to 100% in ITS 2. The highest level of sequence similarity for ITS1 were observed with Rb1 6 and Rb2 and Rb1 and Rb18 (100%). There was no much variation in ITS 2 length among six isolates of *R. bataticola.* Nucleotide sequence similarity between the isolates of *R. solani* was maximum 92 % and 94 % for both the ITS 1 and ITS 2 regions, respectively. Phylogenetic analysis of the ITS1 sequences indicated that isolates Rbl6 and Rb 2 were identical to Rb22 and isolates Rb 1 and Rbl8 were identical to Rb 14. However, analysis of ITS2 grouped all the six isolates in two groups (Figs. 5 & 6). Phylogenetic analysis of the ITS 1 and ITS 2 sequences indicated that the different isolates of *R. bataticola* and *R. solani* did not cluster with each other.

Fig. 1. Alignment of the ITS1 sequences of different isolates of *Rhizoctonia bataticola*

www.IndianJournals.com
Members Copy, Not for Commercial Sale
Downloaded From IP - 61.247.228.217 on dated 27-Jun-2017

```
Rb14   CAACCCTCAAGCTCTGCTTGGAATTGGGCACCGTCCTCACTGCGGACGCGCCTCAAAGAC
Rb1    CAACCCTCAAGCTCTGCTTGGAATTGGGCACCGTCCTCACTGCGGACGCGCCTCAAAGAC
Rb18   CAACCCTCAAGCTCTGCTTGGAATTGGGCACCGTCCTCACTGCGGACGCGCCTCAAAGAC
Rb16   CAACCCTCAAGCTCTGCTTGGTATTGGGCACCGTCCTT--TGCGGGCGCGCCTCAAAGAC
Rb2    CAACCCTCAAGCTCTGCTTGGTATTGGGCACCGTCCTT--TGCGGGCGCGCCTCAAAGAC
Rb22   CAACCCTCAAGCTCTGCTTGGTATTGGGCACCGTCCTT--TGCGGGCGCGCCTCAAAGAC
       ********************* ***************    ***** **************

Rb14   CTCGGCGGTGGCTGTTCAGCCCTCAAGCGTAGTAGAATACACCTCGCTTTGGAGCGGTTG
Rb1    CTCGGCGGTGGCTGTTCAGCCCTCAAGCGTAGTAGAATACACCTCGCTTTGGAGCGGTTG
Rb18   CTCGGCGGTGGCTGTTCAGCCCTCAAGCGTAGTAGAATACACCTCGCTTTGGAGCGGTTG
Rb16   CTCGGCGGTGGCGTCTTG--CCTCAAGCGTAGTAGAATACACCTCGCTTCGGAGCGTAAG
Rb2    CTCGGCGGTGGCGTCTTG--CCTCAAGCGTAGTAGAATACACCTCGCTTCGGAGCGTAAG
Rb22   CTCGGCGGTGGCGTCTTG--CCTCAAGCGTAGTAGAATACACCTCGCTTCGGAGCGTAAG
       ************    *     ***************************** ******   *

Rb14   GCGTCGCCCGCCGGACGAACCTTCTGAACTTTTCTCAAGG   160
Rb1    GCGTCGCCCGCCGGACGAACCTTCTGAACTTTTCTCAAGG   160
Rb18   GCGTCGCCCGCCGGACGAACCTTCTGAACTTTTCTCAAGG   160
Rb16   GCGTCGCCCGCCGGACGAACCTTCTGAACTTTTCTCAAGG   156
Rb2    GCGTCGCCCGCCGGACGAACCTTCTGAACTTTTCTCAAGG   156
Rb22   GCGTCGCCCGCCGGACGAACCTTCTGAACTTTTCTCAAGG   156
       ****************************************
```

Fig. 2. Alignment of the ITS 2 sequences of different isolates of *Rhizoctonia bataticola*

```
Rs11   TGAATTTAATGTAGAGTTGGTTGTAGCTGGCCTAATAAATTAATGTTGGGCATGT-GCAC
Rs12   -GAATTGAATGTAGAGTTGGTTGTCGCTGGCCCTTTCGG--------GGGCATGT-GCAC
Rs18   -------------------------------------------CCGAGCTTAT-ACAA
Rs23   ------------------------------------CAAGTGACCCCGGTCTAACCACCG
                                                      *  *      *

Rs11   A-CCTTCTCTTTCATCCACACACACCTGTGCACCTGTGAGACAGTAGGGGATTTTTAATT
Rs12   GTCCTTCTCTTTCATCCACACACACCTGTGCACTTGTGAGACGG--AGG--------AC-
Rs18   CTCATCAACCCCTGTGAACATACGCTAAAACGTTGCCTCGGCGT--CGGAACAGACGGCC
Rs23   GATGCTCATGTTCATAACCCTTTGTT-GTCCGACTCTTCGTTGCCTCCGGGGAG-CGACC
                   *   *           *        *        *

Rs11   TAATTTAATTGGACCCTCTGTCTACTTAATTCATATAAAATCAATTTAATTAAAAATGAA
Rs12   ---TTTAATTAG-TCTTCCCTCTACTTAAT-CACACAAACTCA-TTTAATTAAAT-TGAA
Rs18   C--CGTAACACGGGCCGCCCCCGCCAGAGGACCCCCTAACTCTGTTTCTATAATGTTTCT
Rs23   ---CTGCCTTCGGGCGGGGGCTCCGGGTGGACACTTCAAACTC--TTGCGTAACTTTGCA
        *         *  *                *        **       **    ***   *

Rs11   -TGTAATTGATGT-AACGCATCTAATACT   205
Rs12   -TGTAATTGATGT-AACGCATC-ATTAGA   178
Rs18   -TCTGAGTAAACA-AGCAAATAAATTA--   155
Rs23   GTCTGAGTAAACTTAATTAATAAATTA--   164
         * * * * *    *    **   * **
```

Fig. 3. Alignment of the ITS 1 sequences of different isolates of *Rhizoctonia solani*

The purpose of this study was to elucidate the genetic relationship between isolates of *R. bataticola* and *R. solani* that are isolated from root rot infected cotton plants. Monga *et al.* [10] in earlier study showed the usefulness of RAPD analysis in detecting significant polymorphism between *R. bataticola* and *R. solani* isolates. In the present study RAPD analysis and ITS sequences of isolates were used to determine their phylogenetic relationship. RAPD analysis of *R. bataticola* and *R. solani* isolates subdivided the isolates in six and four sets of genetic fingerprints, respectively.

ITS sequence data indicated a closer relationship among the different isolates of *R. bataticola* and *R. solani*

www.IndianJournals.com
Members Copy, Not for Commercial Sale
Downloaded From IP - 61.247.228.217 on dated 27-Jun-2017

```
Rs11   GAAATCTTCAAAGTAAATCTTTTGTTAATTCAATTGGTTCTGCTTTGGTATTGGAGGTTA
Rs12   -----TCTCAAAGTAAATCTTTTGTTAATTCAACTGGTT-TGCTTTGGACTTGGAGGTCT
Rs18   ------------ACAACCCTCAGGCCCCCGGGCCTGGCGTTGGGGATCGGCGGAAGCCCC
Rs23   -------------CACCACTCAAGCCTC---GCTTGGTATTGGGCAACG-CGG----TC-
                    *   **   *          ***   **          *

Rs11   TTGCAG-CTTCACACCTGCTCCTCTTTG-TGCATTAGCTGGATCTCAGTGTTATGCTTGG
Rs12   TTGCAGATTTCACGTCTGCTCCTCTTAAATGCATTAGCTGGATCTCAGTAT-ATGCTTGG
Rs18   CTGCGG---GCACAACGCCGTCCCCCAAATACAGTGGC--GGTCCCGCCGC---A---GC
Rs23   -CGCCG------------CGTGCCTCAAATCGACCGGCTGGGTCTT-CTGT---------
         ** *             *     *     *   *   **   * **

Rs11   TTCCACTCAGCGTGATAAGTATCTATCGCTGAGGACACTGTAACAGGTGGCCAAGGTAAA
Rs12   TTCCACTCGGCGTGATAAGTATCACTCGCTGAGGACACTGTAAAAAGTGGCCAGG--AAA
Rs18   TTCCATT--GCGTAGTAGCTAACACCTCGCAACTGGAGAGCGGC--GCGGCCACG----C
Rs23   --CCCCTAAGCGTTGTGGAAACTATTCGCTAAAGGGTGCTCGG---GAGGCTACG----C
         **  *  ****  *    *          *           * *** * *

Rs11   TGCAGATGAA-CCG-CTTCTAATAGTCCATTAATTTGGACAATATTTTTATGA-------
Rs12   TGCAGATGAA-CCG-CTTCTAATAGTCTATTCAGTTAGACAATTAATTTAAGATCTGATC
Rs18   CGTAAAACAC-CCAACTTCTGAATG-----------------------------------
Rs23   CGTAAAACAAACCCATTTCTAAG-------------------------------------
        * * *  *  **   ***** *

Rs11   ---------------------   229
Rs12   TCAAATCAGGTAGGACTACCCG  251
Rs18   ---------------------   173
Rs23   ---------------------   149
```

Fig. 4. Alignment of the ITS 2 sequences of different isolates of *Rhizoctonia solani*

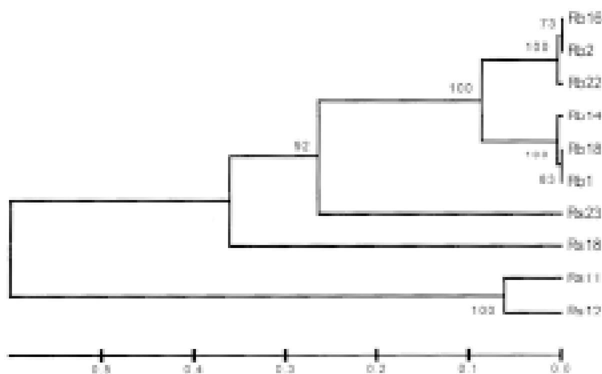

Fig. 5. Unrooted dendrogram showin the genetic relationships based on ITS 1 sequences among *R. bataticola* and *R. solani* isolates by UPGMA cluster analysis method

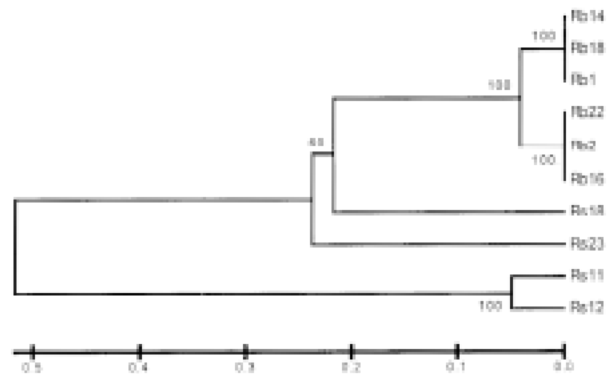

Fig. 6. Unrooted dendrogram showin the genetic relationships based on ITS 2 sequences among *R. bataticola* and *R. solani* isolates by UPGMA cluster analysis method

than RAPD data did. RAPD analysis includes multiple loci throughout the entire genome ad generates multilocus data [13, 14]. The entire genome is subject to faster evolutionary changes than the highly conserved ITS regions, which only evolve slowly [15]. Phylogenetic analysis derived from ITS1 and ITS2 sequence data grouped isolates of *R. bataticola* into one cluster; thus the two RAPD groups could not be distinguished from each other using these criteria. *R. solani* isolates on the other hand clustered separately from isolates of *R. bataticola.* This suggests that phylogenetically *R. solani* isolates are distantly related to *R. bataticola* isolates. Leavy *et al.* [16] used ITS sequence based phylogeny and PCR-RFLP for differentiation of *Tilletia walkeri* and *T. indica.* Fenille *et al.* [17] used rDNA-ITS sequences for identification of *Rhizoctonia solani* associated with soybean in Brazil.

www.IndianJournals.com
Members Copy, Not for Commercial Sale
Downloaded From IP - 61.247.228.217 on dated 27-Jun-2017

This study identified sequences differences between and among the isolates of *R. bataticola* and *R. solani* in the ITS1 and ITS2 regions. Further, ITS1 and ITS2 sequencing and phylogenetic analysis revealed that isolates of *R. bataticola* shared greater than 99% sequence similarity. Kuninaga *et al.* [18] also detected differences in ITS regions length fragments of AG-1 1A isolates. We observed lower similarity among the isolates in ITS1 region than in ITS2 region, observed by Kuninaga *et al.* [18]. As suggested by Kuninaga *et al.* [18], the ITS1 region may be more useful for characterization of the isolates.

Although rRNA is an immensely powerful chronometer for elucidating phylogenetic relationships, its high level of sequence conservation seems to limit its value for measuring and resolving close relationships. From the study it appears that ITS is a potent tool for the taxonomic study, with minute amount of DNA required and the high reproducibility making it an ideal method for studying population heterogeneity. It is pertinent to note that despite some variations in ITS sequences, it is clearly possible to identify conserved regions in both the spacers which can be used for species delineation. Such region could also be used for rapid species identification and designing of primers and probes.

www.IndianJournals.com
Members Copy, Not for Commercial Sale
Downloaded From IP - 61.247.228.217 on dated 27-Jun-2017

References

1. **Carling D. E., Kuninaga S. and Brainard K. A.** 2002a Hyphal anastomosis reactions, rDNA-internaltranscribed spacer sequences, and virulence levels among subsets of *Rhizoctonia solani* anastomosis group-2 (AG-2) and AG-B1. Phytopathology, **92**: 43-50.
2. **Kuninga S. and Yokozava R.** 1984. DNA sequence homology in *Rhizoctonia solani* Kuhn. VI. Genetic relatedness within AG-4. Annals of the Phytopathological Society of Japan, **50**: 346-352.
3. **Carling D. E. and Kuninaga S.** 1990. DNA based-sequence homology in *Rhizoctonia solani* Kuhn: inter-and-intra-group relatedness of anastomosis group-9. Phytopathology, **80**: 1362-1364.
4. **Jabaji-Hare S. H., Meller Y., Gill S. and Charest P. M.** 1990. Investigation of genetic relatedness among anastomosis groups of *Rhizoctonia solani* using cloned DNA probes. Can. J. Pl. Pathol., **12**: 393-404.
5. **Sande van der C. A. F. M., Kwa M., Nues van R. W., Heerikhuizen van H., Raue H. A. and Plant R. J.** 1992. Functional analysis of internal transcribed spacer 2 of *Saccharomyces cervisiae* ribosomal DNA. J. Mol. Biol., **223**: 899-910.
6. **Musters W. K., Boon K., Sande van der C. A. F. M., Heerikhuizen van H. and Plant R. J.** 1990. Functional analysis of internal transcribed spacers of yeast ribosomal DNA. EMBO J., **9**: 3989-3996.
7. **Carling D. E., Baird R. E., Gitaitis R. D., Brainard K. A. and Kuninga S.** 2002b. Characterization of AG-13, a newly reported anastomosis group of *Rhizoctonia solani.* Phytopathology, **92**: 893-899.
8. **Monga D., Rathore S. S., Kumar R., Kumar M. and Chakraborty P. K.** 2005. Rapid preparation of DNA from *Rhizoctonia solani* and *Rhizoctonia bataticola* fungi causing root rot infection. J. Cotton Res. and Development, **19**: 84-87.
9. **White T. J., Bruns T., Lee S. and Taylor J. W.** 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In*: PCR protocols : A guide to methods and applications, Innis M A., Gelfand D. H., Sinky J. J. and White T. J. (eds.). Academic Press, Inc., New York. Pp. 315-322.
10. **Monga D., Kumar M., Kumar R., Saini N. and Chakraborty P. K.** 2007. Analysis of genetic diversity in Cotton root rot pathogens *Rhizoctonia solani* and *Rhizoctonia bataticola* using RAPD markers. Indian J. Phytopathol., **60**: 259-263.
11. **Sambrook J. E., Fritsch F. and Maniatis.** 2001. Molecular cloning: a laboratory manual, Third edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
12. **Kumar S., Tamura K. and Nei M.** 2004. MEGA3: Integrated Software for Molecular Evolutionary Genetics Analysis and Sequence Alignment. Briefings in Bioinformatics, **5**: 150-163.
13. **Welsh J. and McClelland M.** 1990. Fingerprinting genomes using PCR with arbitary primers. Nucleic Acids Res., **18**: 7213-7218.
14. **Williams J. G. K., Kubelik A. R., Livak K. J., Rafalski J. A. and Tingey S. V.** 1990. DNA polymorphisms amplified by arbitary primers. Nucleic Acids Res., **18**: 6531-6535.
15. **Bruns T. D., White T. J. and Taylor J. W.** 1991. Fungal molecular systemics. Annu. Rev. Ecol. Syst., **22**: 525-564.
16. **Leavy L., Castleburry L. A., Carris L. M., Meyer R. J. and Pimentel G.** 2001. Internal Transcribed Spacer Sequence-Based Phylogeny and Polymerase Chain Reaction-Restriction Fragment Length Polymorphism Differentiation of *Tilletia walkeri* and *T. indica.* Phytopathol., **91:** 935-940.
17. **Fenille, Roseli C. *et al.*** 2003. Identification of *Rhizoctonia solani* associated with soybean in Brazil by rDNA-ITS sequences. Fitopatol. bras., **28**: 413-419.
18. **Kuninga S., Natsuaki T., Takeuchi T. and Yokosawa R.** 1997. R. sequence variation of the rDNA- ITS regions within and between anastomosis group in *Rhizoctonia solani.* Current Genetics, **32**: 237-243.