Molecular evidence reveals presence of *Rhizoctonia solani* AG 1-IB on *Tagetes patula* in India

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Abstract *Rhizoctonia solani* was isolated from marigold (*Tagetes patula*) showing web blight symptoms in Meghalaya, India. Fluorescence microscopy was used to confirm its multinucleate character. Molecular characterization using ITS1-5.8-ITS2 region of nrDNA proved that it belonged to AG1-IB subgroup. To our knowledge, this is the first record of AG1-IB on marigold from India.

Keywords *Rhizoctonia solani* · *Tagetes patula*, AG1-IB

French marigold (*Tagetes patula* L.) is an important flower crop grown on commercial scale in India. Leaf and flower extracts have medicinal properties. Carotenoids are used in cancer therapy (Naik et al. 2003). Dye is used in textile industry and colours in food industry. Essential oil has been reported to possess anticandidal activity (Dutta et al. 2007). Marigold flowers of Indian variety contain lutein ester which is a natural antioxidant (Bhattacharyya et al. 2010). Reports regarding the use of *T. patula* in phytoremediation have also been published (Sun et al. 2011).

During July 2009, marigold plants with severe web blight symptoms were observed in Umiam. Even mycelial aggregates were visible on the infected plants (Fig. 1). Isolations from infected plants were attempted on PDA amended with antibiotic streptomycin (100 ppm). *Rhizoctonia* like colonies were further multiplied on PDA without antibiotics. DAPI (2-(4-amidinophenyl)-1H-indole-6-carboxamide) (Roche, Germany) staining was used for ascertaining number of nuclei per cell (Kulik and Dery 1995). Nikon Eclipse 80i equipped with camera Nikon DS Ri1-U2 and NIS-Elements AR imaging software was used for fluorescent microscopy. Thirty cells were observed randomly and nuclei counted (av. ~7). Fluorescence microscopy revealed the isolate to be multinucleate (Fig. 2). Light microscopy was conducted using a Leitz Dialux 22 microscope. Hyphae were branched at 90° with lateral branches having constriction at the point of origin. Branching was present near the distal septum in case of young hyphae. Sclerotia were not differentiated into cortex and medulla. Hyphal diameter was 6–9 µm. These characters putatively identified the isolate as *Rhizoctonia solani* Kühn [teleomorph = *Thanatephorus cucumeris* (A. B. Frank) Donk] (Sneh et al. 1991). A living culture has been deposited in MACS Agharkar Research Institute, Pune, India (NFCCI No. 2606).

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Fig. 1 Symptoms of web blight on *Tagetes patula*

At present, *R. solani* contains 13 AG groups and AG BI (Bridging isolate) (Carling et al. 2002). In addition to AG grouping, other methods like pectic zymograms (Cruickshank 1990; Macnish et al. 1993), PCR-RFLP (Guillemaut et al. 2003), fatty acid profiles (Priyatmojo et al. 2002) and rDNA ITS sequencing have also been used for classifying this fungus group (Sharon et al. 2006). We attempted AG determination based on ITS 1, 2 and 5.8 S sequence of rDNA. DNA was isolated from the mycelium, multiplied on potato dextrose broth for 4 days using standard phenol-chloroform extraction methods. PCR was conducted using universal primers

Fig. 2 Multinucleate hyphae of *Rhizoctonia solani* observed using DAPI staining

ITS 1 and 4 (White et al. 1990). Reaction volume was 50 μ l containing template DNA (120 ng), Taq (2.5 U), dNTP's (200 μ M each), buffer with $MgCl_2$ (5 μ l) (Roche, Germany) and primers (1 μ M each). Cycling conditions were 5 min initial denaturation at 94 $^{\circ}C$, denaturation at 94 $^{\circ}C$ for 1 min, 58 $^{\circ}C$ for annealing (30 s) and 72 $^{\circ}C$ (1 min) for extension and final extension step of 7 min at 72 $^{\circ}C$. PCR was done in a Thermal Cycler 2,720 (Applied Biosystems). PCR amplified product was checked through electrophoresis using ethidium bromide and band identification and validation under UV transillumination. PCR amplified product was sequenced and the DNA sequence deposited in Genbank (JN426850). Blast search revealed 99 % to 100 % similarity with AG 1-IB.

To confirm our identification, phylogenetic analysis was also conducted using MEGA 5.03 (Tamura et al. 2011) and alignment was done in MAFFT v.6 (Katoh 2008). Phylogenetic analysis was conducted using the Minimum Evolution (ME) method and evolutionary distance was calculated using Tamura 3-parameter. The rate variation among sites was modeled with a gamma distribution. The ME tree was searched using the Close-Neighbor-Interchange (CNI) algorithm. The Neighbor-joining algorithm was used to generate the initial tree (Tamura et al. 2011 and references included therein for analysis). ITS sequence of *Athelia rolfsii* (AY684917.1) was used to root the tree. Our sequence clustered together with the AG 1-IB clade (Bootstrap support 96 %, 1,000 replicates). The Maximum Parsimony (MP) tree was obtained using the Close-Neighbor-Interchange algorithm in which the initial trees were obtained with the random addition of sequences (Felsenstein 1985; Tamura et al. 2011 and references included therein for analysis). MP analysis also placed our isolate in AG 1-IB clade with Bootstrap support 100 %, 1,000 replicates, consistency index (0.56), retention index is (0.88), composite index is (0.56) (Fig. 3).

Koch's postulate were proved by placing mycelial discs of 5 mm diameter of 8 days old culture (PDA) on detached healthy leaves of *T. patula* kept in petri dishes, which were lined with moistened filter paper, non-inoculated leaves served as control. Inoculated leaves developed symptoms whereas non inoculated leaves remained healthy. *R. solani* was also reisolated from the inoculated leaves.

To our knowledge this is the first record of *R. solani* subgroup AG 1-IB on *T. patula* from India. *T. patula* might be serving as a reservoir of AG 1-IB and spreading it to other host species.

Fig. 3 Minimum evolution tree (Except AG 1 all the daughter nodes have been collapsed to improve clarity). Evolutionary distance was calculated using Tamura 3-parameter. The rate variation among sites was modeled with a gamma distribution. ITS sequence of *Athelia rolfsii* (AY684917.1) was used to root the tree

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