Original Research Article

First Report of Common Bean (Phaseolus vulgaris L.) Root Rot Caused by Rhizoctonia solani in Jammu and Kashmir

ABSTRACT

Root rot of common bean (*Phaseolus vulgaris*) is one of the most serious disease, causes unadorned damage to crop health and leads to the heavy yield losses. In the present investigation, *Rhizoctonia solani*, a new root rot pathogen was found pathogenic and cause of beans root rot in the region of Jammu and Kashmir, India. The pathogen was characterized by morpho-cultural as well as through molecular sequencingusing ITS primer for establishing the proper identification of isolated pathogen. Based on the pathogenicity test carried on common bean cv. Shalimar Bean-1 and available literature, it was recorded as the first report of root rot in common beans caused by *Rhizoctonia solani* in Jammu and Kashmir.

Key words: ITS primers, pathogenicity, *Phaseolus vulgaris, Rhizoctonia solani*, root rot,

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is a regularly consumed crop, thereby occupying a prime position among the legumes in the world including India. Diseases are becoming more frequent encountered constraints in the successful production of common bean. Among important diseases, soil borne pathogenic fungi causing root rot/damping off are widely distributed and causing serious damage to the crop that led to heavy yield losses (Panth *et al*., 2020). The main soil borne pathogens *viz*., *Fusarium solani, Pythium ultimum, Rhizoctonia solani* (Singh and Schwartz 2010; Scandiani *et al.,* 2011; Vural and Soylu, 2012) and *Macrophomina phaseolina* (Clare *et al*., 2010), which are responsible for fusarium root rot (FRR), pythium seed rot/damping off, rhizoctonia root rot and macrophomina charcoal rot respectively, and have been reported world-wide. In Kashmir, root rot of beans has been reported to be caused mainly by *Fusarium solani* (Dar *et al.,* 1997; Badri *et al*., 2014). However, in present study *Rhizoctonia solani* was found pathogenic and cause beans root rot.

2. Materials and Methods

2.1 Symptomatology

The root rot samples of common bean were collected from various districts *viz*., Anantnag, Baramulla and Pulwama of Kashmir valley of UT of Jammu and Kashmir. The symptoms of the infected plants showed dwarfing and yellowing on above ground plant and irregular to circular sunken scaled lesions with reddish brown to black borders on roots and hypocotyl region. The infected root system was devoid of lateral roots. The pathogen destroyed the pith which resulted in a brick-red discoloration of interior part of stem near the soil line (Fig. 1A and 1B).

Fig. 1. Symptoms of root rot observed under field condition; A: Rotting symptoms developed on roots, B: Above ground plant symptoms due to root rot

2.2 Isolation and purification

The pathogen was isolated from the symptomatic roots samples using tissue bit isolation technique of Sicard *et al*. (1997). The suspected tissue bits were inoculated on potato dextrose agar plates and were incubated at 25±1°C for seven days. Hyphal tip technique was used to obtain the axenic culture of pathogen (Rangaswami, 1972).

2.3 Pathogenicity test

To ascertain ability of the isolate to cause root rot in bean plants, pathogenicity tests was carried on beans cv. Shalimar Bean-1.Sick-soil method was employed for performing the pathogenicity test (Dubey *et al.,* 2012). Plastic pots (15 cm height) were filled with sterilized soil (1.5 kg pot^{-1}) and inoculated with mass culture of the pathogen @ 10g kg^{-1} soil, five days prior to sowing. One un-inoculated pot filled with sterilized soil of same quantity was kept as a control. Three apparently healthy bean seeds, first surface sterilized by 1 per cent sodium hypochlorite for two minutes followed by 3 to 4 washings with sterile distilled water, were sown in each pot. Both the inoculated and un-inoculated pots were kept under poly-house for observations on disease development.

2.4 Genomic DNA extraction

Genomic DNA was extracted by using modified CTAB (Cetyltrimethyl ammonium bromide) method (Murray and Thompson, 1980). Two ITS primers *viz*., ITS1F (5`TCCGTAGGTGAACCTGCG`3) and ITS4R (3`TCCTCCGCTTATTGATATGC`5) were used for sequence analysis of the root rot pathogens. The PCR cycle for ITS primers with initial denaturation at 94°C for 5 minute, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 1 minute, extension at 72°C for 2 minutes with final elongation of 72°C for 10 minutes was performed. After PCR amplification, 40 to 45 µl PCR product of root rot pathogen was sent for custom sequencing (Bio Kart India Pvt. Ltd. Bengaluru, Karnataka, India). ITS sequence of pathogen was retrieved from chromatograms using BioEdit version 7.0 (Hall, 1999) and the sequenced product was subjected to BLASTn in NCBI (National Centre of Biotechnology Information) database and identification of pathogen was established.

3. Result and Discussion

The pathogen was identified by morpho-

cultural as well as through molecular sequencing. The pure culture of *R. solani* produced a profuse white aerial mycelium which turned to brown to dark brown in colour after seven days of incubation (Fig. 2A and 2B). Hyphal branches of pathogen were formed at right angle from the main hyphae with prominent constriction and a septum at point of origin (Fig. 2C and 2D). The diameter of hyphae varied from 6.60 to 7.78µm with an average of 7.01µm. Sclerotia were small to large in size and developed on upper surface of media in circular manner (target board) after five days of incubation. These were 95 to 160 x 62 to 145µm with an average of 81.5 to 107µm in size, spherical/oval, dirty white and later turning to dark brown. Monilliod cells were formed either singly or in chains, barrel shaped, thick walled, initially hyaline and then turned to brown in colour and measuring 18.01 to 23.45 x 8.2 to 13.7 µm in size. On the basis of mycelial characters, sclerotia and monillioid cells, the fungus was identified as *Rhizoctonia solani* (Parmeter and Whitney, 1970).

To confirm the precise identity of the pathogen, the PCR amplification was carried out for Internal Transcribed Spacer (ITS) region of the ribosomal DNA with primers ITS1/IST4 (White *et al*., 1990). The sequence was retrieved from the chromatogram and was analyzed through Basic Local Alignment Search Tool-Nucleotide (BLASTn) programme in NCBI (National Centre of Biotechnology Information) database (ncbi.nlm.nih.gov/genbank) and identification of pure cultures was established. The sequence of the isolated pathogen showed 98 per cent similarity with reference *Rhizoctonia solani* sequences in GenBank (Fig. 3). The sequences were submitted in the GenBank under accession no: MZ708012.

Pathogenicity of isolated pathogen was carried on common bean cv. Shalimar Bean-1 under pot culture experiment by adopting the sick soil method (Dubey *et al.,* 2012). These pots were maintained under greenhouse with 12 hours of photoperiod at 25±1°C.The pathogen inoculated plants showed the development of characteristic symptoms *viz*., yellowing and drying of leaves after 16 days of inoculation and the roots showed irregular to circular sunken lesions with reddish brown-black margins on roots, rotted similar to those originally detected under field conditions. No symptoms development was observed in controlled plants (Fig. 4). The pathogen was also reported to cause disease in other crops (Venegas**,** 2008; Gonzalez- Aliferis and Jabaji, 2012).But to our knowledge and on the basis of available literature, this is the first report of common beans root caused by *Rhizoctonia solani* in Kashmir. Thus it becomes important to study the impacts of this new disease on common bean production for the potential infestation of the pathogen in Kashmir region.

Fig. 2. Morphological and cultural characterization of *Rhizoctonia solani* causing root rot of common bean. (A) Front view of *Rhizoctonia solani* culture, (B) Back view of *Rhizoctonia solani* culture, (C) Branches from the main hyphae formed at

right angle with prominent constriction at point of origin (D) Monilioid cells formed in chains

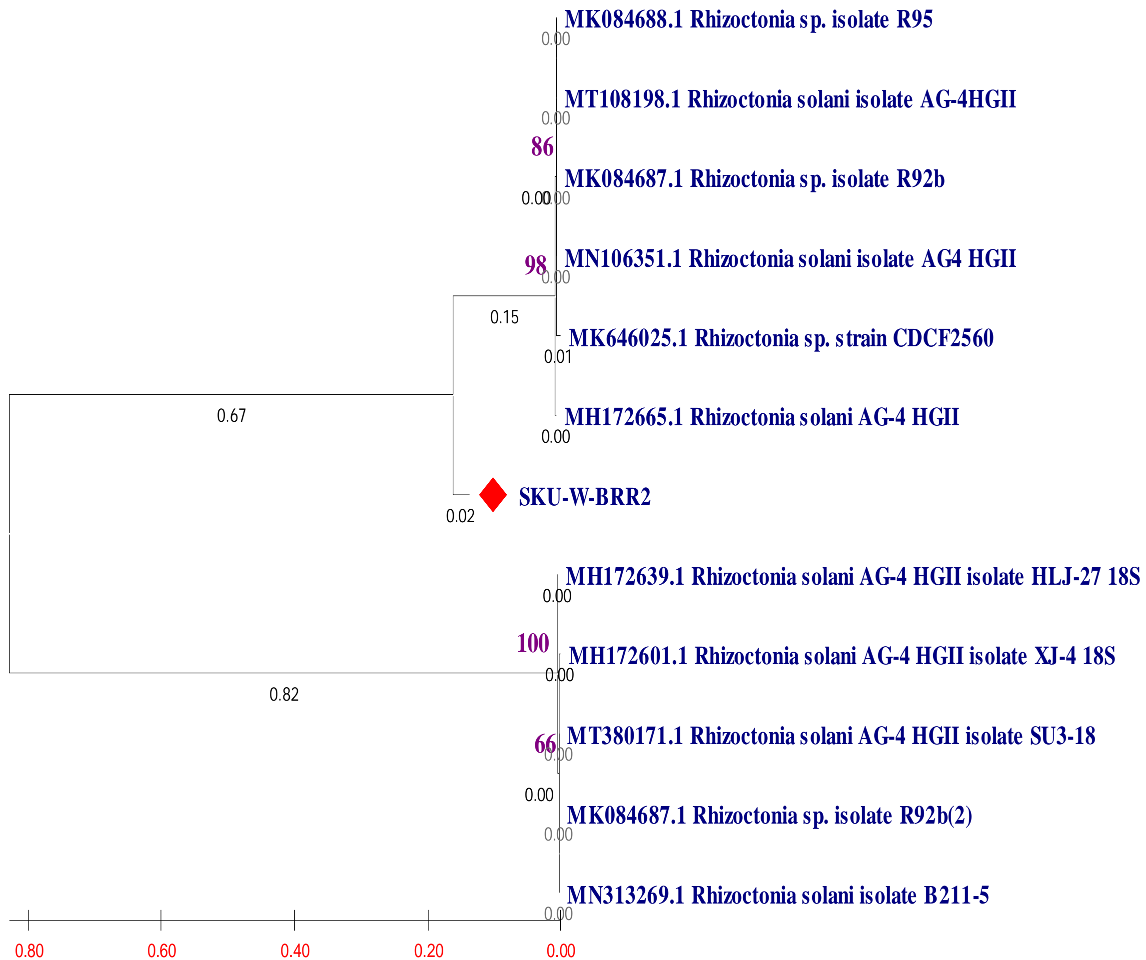

Fig. 3. Phylogenetic analysis of the original isolate by using the Maximum Likelihood method and Kimura 2-parameter mode on Internal Transcribed Spacer (ITS sequence).The position of bean root rot pathogen *Rhizoctonia solani* (SKU-W-BRR2) is depicted with red ( ) colour

Fig. 4. Pathogenicity test of isolated fungal pathogen *Rhizoctonia solani* on common bean cv. Shalimar Bean-1 under pot culture.

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