

Original Research Article

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Isolation and Validation of Dry Root Rot causing pathogen *Rhizoctonia bataticola* in Chickpea (*Cicer arietinum* L.)

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ABSTRACT

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India is the largest producer and consumer of chickpea, contributing an elephant share (65 per cent) towards the global chickpea production. However, in recent past the changing climate has led to the emergence of Dry root rot (DRR) disease in chickpea growing areas, which frequently encounters a prolonged dry spells coupled with high temperature. The disease is posing serious threat to chickpea cultivation by pronounced effect of devastation in susceptible cultivars. To combat this disease, identification of resistant cultivars would be pre-requisite. Thus our study was focused on isolating the DRR pathogen *Rhizoctonia bataticola* from infected chickpea plots of Kalyana Karnataka region and its morphological characterization followed by screening the identified pathogen to validate the genotypes conferring resistant/tolerance to DRR. A total of nine genotypes were screened by using paper towel method. JSC-37 showed resistant reaction to DRR (which is a nation check and donor for the DRR by IIPR Kanpur). Followed by genotypes MABC-WR-SA-1, A-1 and JG-11 showed moderate resistant/ tolerant reaction.

Introduction

Chickpea is an important food legume commonly known as Bengal gram or Garbanzo (*Cicer arietinum* L.). It is cultivated and consumed across the globe including semiarid tropics. India is the largest producer and consumer of chickpea accounting to 65 per cent of global production. The productivity of chickpea is diminishing and is unstable in India due to several biotic and abiotic stresses (Mannur *et al.*, 2015). In recent past due to changing climatic scenario, chickpea cultivation is getting vulnerable to

dry root rot (DRR) disease caused by (*Rhizoctonia bataticola* (Taub.) Butler. In southern parts of India the crop is primarily grown under rainfed ecosystem which are characterized by high temperature and low soil moisture for prolonged duration thus favoring DRR (Pande and Sharma, 2010; Gupta *et al.*, 2015).

Rhizoctonia bataticola (Taub.) Butler is a very important soil-inhabiting, fungus posing a serious threat to a wide range of crops. It is known to incite different types of diseases viz., stem blight, seedling blight, leaf blight,

seedling decay, root rot, stalk rot, fruit rot, and charcoal rot in crop plants (Dhingra and Sinclair, 1978). The crop losses due to DRR have been estimated around 10-25% (Pandey and Singh, 1990). A critical analysis of weather data over the years revealed that incidence of DRR is high in areas where average temperature exceeds 33°C (Sharma *et al.*, 2010). DRR as an acute-emerging disease that occurs irregularly, both temporally and spatially and may cause massive disruptions in system performances and whose range is expanding to new areas (Savary *et al.*, 2011). when the diseased plants were uprooted, the tap root appears black in color and lower portion generally remains in soil, while the number of lateral and fine roots reduces. The dead root is quite fragile and appears shredding of the bark. The dark tiny sclerotial bodies get exposed or remain inside the wood or bark (Nene *et al.*, 1991).

Rhizoctonia bataticola (Taub.) Butler belongs to order Cantharellales and family Ceratobasidiaceae. The pathogen produces hyphae and sclerotia (hyphal propagules). *Rhizoctonia* means “root killer” hence this pathogen is known to cause different root rots like wet root rot and dry root rot etc in many crops. *R. bataticola* is important member in the genus that causes seedling blight and root rot in many legumes(chickpea, green gram soybean *etc.*) when the plants are weakened due to some other stress factors (Hawang *et al.*, 2003). when the fungi produces pycnidia then the perfect stage or sexual stage is known as *Macrophomina phaseolina* (Tassi) Goid. Due to the presence of only sclerotial phase in chickpea, the pathogen is referred as *Rhizoctonia bataticola* (Taub.) Butler (Mamta Sharma *et al.*, 2015).

Zonal agricultural research station, Kalaburagi encompassing the zone 1 and 2 of agro climatic zones where temperatures are high and soil moisture is less hence, we focused on isolating DRR pathogen from

Kalyana Karnataka region particularly Kalaburagi districts and screened chickpea genotypes to validate the resistant sources for DRR.

Materials and Methods

The study consists of isolation of pathogen from the infected plant from Kalaburagi districts and making fungal suspension and artificial inoculation to chickpea seeds of different genotypes and scoring them according to rating scale depending on the level of disease symptom expression on seedlings of chickpea by paper towel method.

The plants showing the symptoms of drooping of petioles and leaflets which are confined to top of the plants are uprooted and roots are observed and the roots which were showing the symptoms such as dark brown to black discoloration and signs of rotting and is devoid of most of its lateral and finer roots and also shredding of bark which comes out in the form of flakes (Haware, 1990) are taken to the lab for isolation of Dry root rot (DRR) causing fungi *Rhizoctonia bataticola*.

The roots were washed in a running tap water and air dried in a normal room temperature later in a laminar air flow chamber the roots were cut into 1-5 mm length and dipped in 0.1 % sodium hypochlorite solution for one minute and washed 3 times subsequently in sterile distilled water and kept on tissue paper to dry later these root bits were placed on the PDA (potato dextrose agar) media in the center of the Petri plates and plates were incubated at 27 ± 2 °C for 7 days to allow the fungi to grow fully in Petri plates. A small portion of *R. bataticola* colony from the previous plate was taken from the periphery of a 90 mm diameter Petri dish, spread onto other Petri dishes containing potato dextrose agar media and incubated in the dark at 27 ± 2 °C for 7 days to get the pure culture. To store the pure cultures of *R. bataticola*, the

5mm pathogen discs from 7 days old fully grown pure culture plates were transferred to test tube slants containing PDA media and incubated in the dark at 27 ± 2 °C for 6-7 days till the pathogen is grows fully in test tubes. The culture tubes were labelled and stored at 4 °C.

The fungal suspension was isolated by flooding the 7 days old pure culture plate with sterilized distilled water later these different chickpea genotypes seeds were dipped in fungal suspension for 10 mins and later seeds were placed on two layers of moist germination paper, which were placed on a polythene paper and rolled carefully to avoid any excess pressure on seeds. These rolled paper towels were incubated in seed germinator at 20 ± 2 °C for 14 days. All the seedlings were carefully observed for the disease symptoms and are Ratings were given based on their disease severity levels (Nene *et al.*, 1981).

Coarsely grinded sorghum seeds were soaked in water for over-night, next day air-dried under room temperature, filled in conical flasks and flasks were sealed with non absorbent cotton plugs, wrapped in aluminum foil, and autoclaved at 15 psi (121° C) for 20 minutes. After cooling, the seeds in flasks

were inoculated with 4-5 mm mycelial discs from a 7-day old pure culture of *R. bataticola* and incubated at 27 ± 2 °C for 15 days. autoclaved sand was mixed with ground sorghum seeds to ensure that sorghum seeds don't stick together which also helps in shaking of flask. The flasks were shaken at alternate days for uniform colonization of the grains. After 15 days the pathogen development on the surface of the grains can be seen and this inoculum thus produced was used in developing the sick plot. The sick plot at ZARS, Kalaburagi is bordered with thick wall so that inoculum is confined to the designated plot. The inoculum was repeatedly applied for 3 times to the field.

Results and Discussion

The fungi were fully grown after 7 days. The fungi in petriplate was fluffy aerial mycelium of grey to black colour as high as to touch the lid cover of the culture plates later lie flat to bottom of petriplate as it ages (Plate 1a). When the fungus is observed under microscope, mycelium shows characteristic features of *R. bataticola* as right angle branching of the mycelium and constriction of hyphae near the point of origin (Plate 1b) (Table 1 and 2).

Table.1 Reaction of chickpea varieties against DRR disease caused by *Rhizoctonia bataticola* (Taub.) Butler by Paper towel method

Sl. No.	Variety	Disease severity Grade	Disease reaction
1	JSC - 37 (Resistant genotype)	3	R
2	MABC 66-266	7	S
3	JG – 11	5	MR
4	MABC WR SA - 1	5	MR
5	MABC WR SA – 2	7	S
6	WR 315	7	S
7	A – 1	5	MR
8	GBM – 2	7	S
9	WRC 411-111	7	S

Table.2 The level of resistance and/or susceptibility for each variety was determined by using 1-9 rating scale (Nene *et al.*, 1981)

Scale	Description	Disease reaction
1 -3	No infection on roots to very few lesion on roots	Resistant
4-5	lesions on roots clear and small & new roots free from infection	Moderately resistant
6-7	lesions on roots many & new roots or secondary roots free from infection	Susceptible
8-9	Roots infected and completely discolored	Highly susceptible

Plate.1a Pure culture of *Rhizoctonia bataticola*

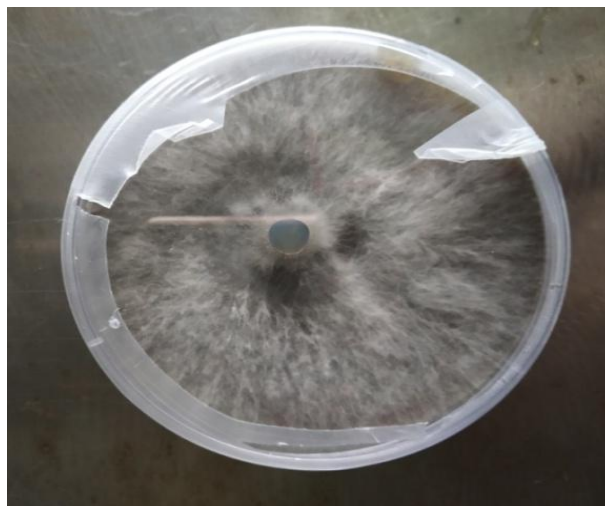


Plate.1b Young hyphae showing right angle branching

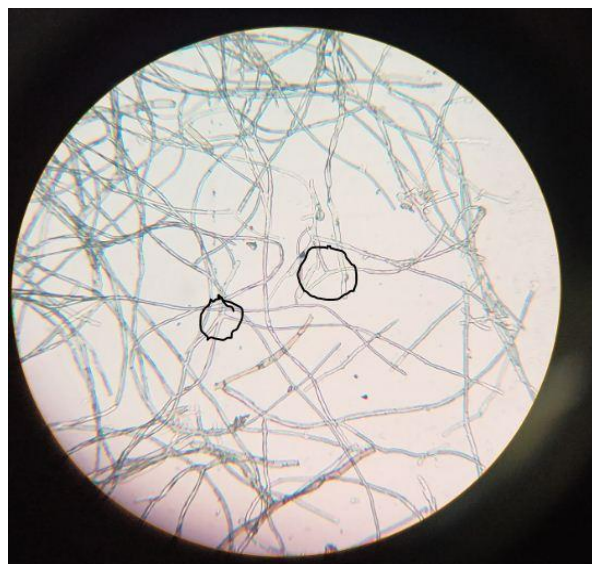


Plate.1c 40X objective microscopic view of sclerotia produced in old culture

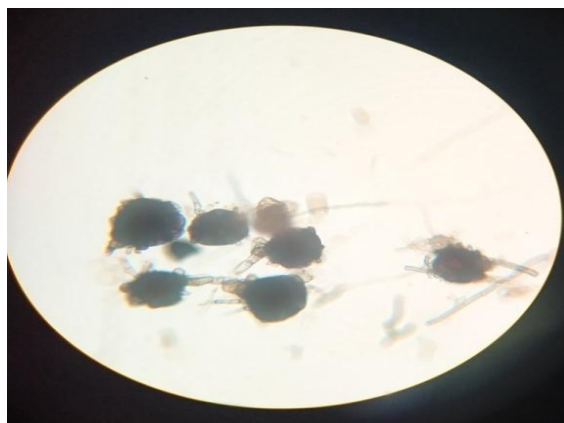


Plate.1d Highly susceptible genotype showed roots infected and discolored



As the fungi ages mycelium taken from old Petri plates also shows sclerotia, which are black to brown, varying from spherical, oblong to irregular shapes (Plate 1c). The colony characters and morphological characters of mycelium and sclerotia were in agreement with the descriptions of Ram and Singh (2018). Thus, the fungus under present investigation was identified as *Rhizoctonia bataticola* (Taub.) Butler.

The study was attempted during 2019 in plant pathology laboratory at zonal agricultural research station Kalaburagi in order to check the disease reaction of 9 chickpea genotypes including resistant check against DRR. Among the genotypes, the national resistant donor showed resistant reaction to DRR

disease, three genotypes showed moderate resistant, and five genotypes showed susceptible reaction respectively. The resistant genotype recorded no infection on roots, moderately resistant genotypes showed lesions on roots that were clear and small and new roots were free from infection, susceptible genotypes showed large and many number of lesions on roots and new roots or secondary roots were free from infection. While the highly susceptible genotypes show roots infection and completely discolored. however none of our genotypes were highly susceptible (Plate 1d).

The resistant and moderately resistant varieties/genotypes can be used in chickpea breeding programs to impart resistance for

DRR. Further, it was found that these moderately resistant genotypes were well adopted for local climate and can be used to develop disease resistant cultivars for Kalyana Karnataka region.

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