Dry Root Rot Disease Assays in Chickpea: a Detailed Methodology

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Dry Root Rot Disease Assays in Chickpea: a Detailed Methodology

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Abstract

Dry root rot (DRR) disease is an emerging biotic stress threat to chickpea cultivation around the world. It is caused by a soil-borne fungal pathogen, *Rhizoctonia bataticola*. In the literature, comprehensive and detailed step-by-step protocols on disease assays are sparse. This article provides complete details on the steps involved in setting up a blotting paper technique for quickly screening genotypes for resistance to DRR. The blotting paper technique is easy and less expensive. Another method, based on the sick pot approach, is a mimic of natural infection and can be applied to study the interacting components—plant, pathogen, and environment—involved in the disease triangle.

Contents

Introduction

- Dry root rot (DRR) is one of the economically significant diseases in chickpea (Nene et al., 1981)

- DRR infected plants lack lateral roots and possess brittle taproots and yellow foliage (Nene et al., 1981; Sharma et al., 2015; Sinha et al., 2019)

- DRR under drought stress has been reported to be an emerging threat to chickpea cultivation (Sharma et al., 2015; Sinha et al., 2019)

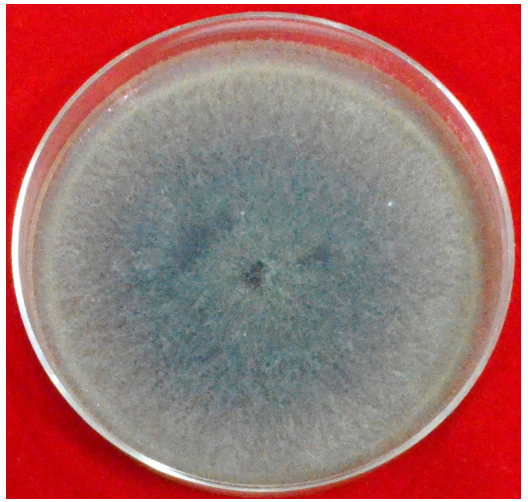
- Moreover, DRR incidence is reported to be aggravated under drought stress in field conditions (Sinha et al., 2019).

- DRR is more prevalent in rainfed areas than in irrigated fields (Nene et al., 1981; Sharma et al., 2015; Sinha et al., 2019).

- It is a root-specific disease caused by *Rhizoctonia bataticola* (teleomorph, *Macrophomina phaseolina*) (Nene et al., 1981)

Rhizoctonia bataticola

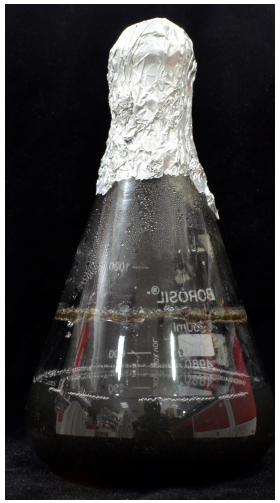
A



4 days old

Culture in PDA plate

B



5 days old

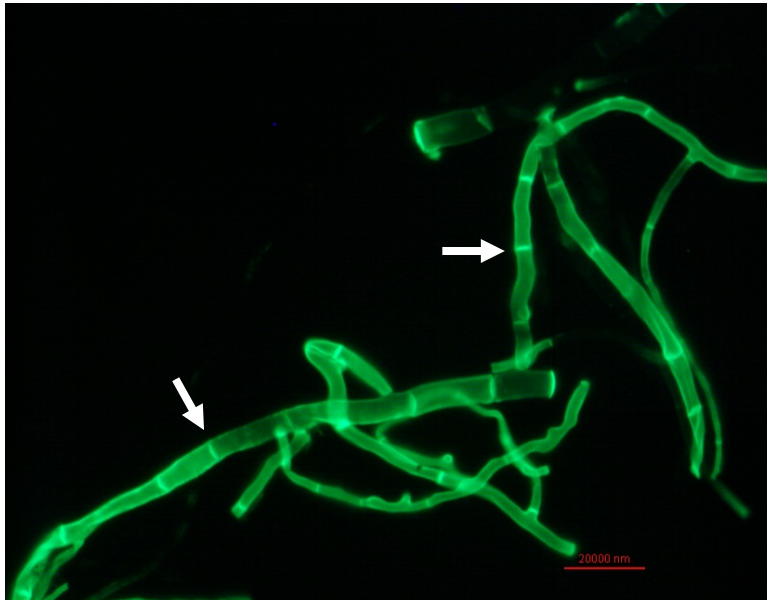
Broth culture in PDB

C

10 days old

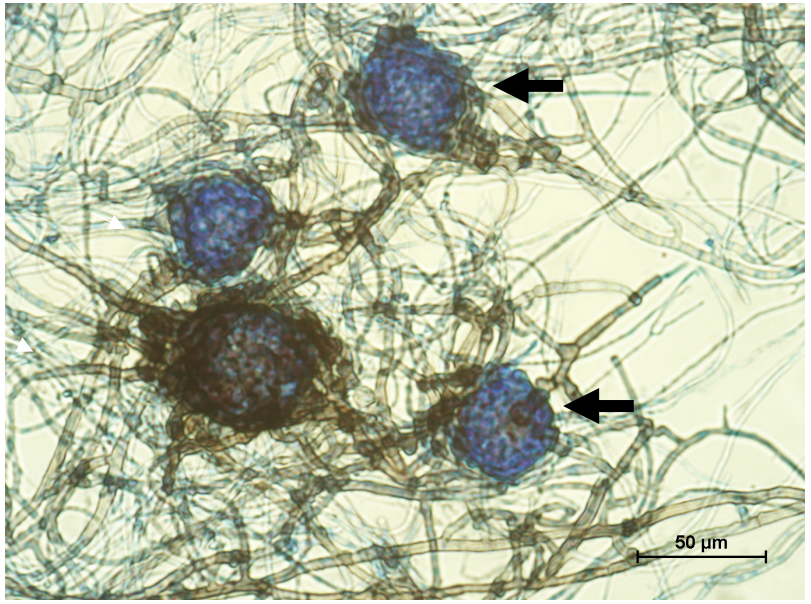
PDA slant

D



Septate mycelia

E



Microsclerotia

Focus of the methodology

- Robust, easy, and cost-effective disease assays are essential to investigate *R. bataticola* infection patterns in chickpea.

- Screening and identification of resistant/susceptible genotypes are critical for molecular breeding for crop improvement.

- Chickpea germplasm available across the globe harbors genetic variation for the trait

- The utilization of resistant varieties is the way to overcome the disease and circumvent fungicide use

Methodologies

- Blotting Paper Technique
- Sick Plot Technique

Blotting Paper Technique

- The primary disease assay used to observe the response of chickpea genotypes to *R. bataticola* infection is the blotting paper technique.

- It is a simple technique and can be executed using liquid fungal inoculum, seedlings with roots, and sterile blotting paper.

- However, this technique has not been utilized to its maximum because no step-by-step protocol is available in the literature.

Blotting Paper Technique- Flowchart

Step 1	Surface sterilize the chickpea seeds with 2% sodium hypochlorite
Step 2	Sow the seeds in 15-cm height pot containing Soilrite
Step 3	Uproot the 8 days old seedlings, wash under running tap water and rinse with sterile water twice
Step 4	Inoculate Potato dextrose broth with *R. bataticola* and incubate at 28°C for 5-days with 180 rpm in a shaker
Step 5	Dip only the roots in inoculum and remove the excess inoculum
Step 6	Place pathogen inoculated and mock (water) inoculated plants in separate blotter paper
Step 7	Keep the trays with plants at 28±2°C for eight days with 16 h artificial light and relative humidity at approximately 70% and moisten the plants every day with adequate water
Step 8	Inspect for root damage on 8 days after inoculation

Blotting Paper Technique- Steps

Step 3
Nursery

Step 4
Uprooted plants

Step 5
Fungal inoculum

Step 6-
Dip inoculation

i.

ii.

iii.

iv.

Step 7-
Blotting paper set up

Blotting Paper Technique- Results

A

B

Shoot

Root

Sick Plot Technique

- Sick pot technique involves the preparation of a potential sick culture and the imposition of drought stress.
- Hence, drought stress aggravates DRR disease incidence, it is essential to study the plant-pathogen interaction under drought stress.
- The sick pot technique provides the platform for such a simultaneous study, promoting better possibilities for germplasm screening and understanding the mechanistic basis of the interaction.
- Pathomorphological changes such as an increase in root length and reduction in lateral root number—inherent to DRR disease—can be addressed using the sick pot technique.

Sick Pot Technique- Flowchart

Substrate
Soak chickpea seeds overnight and autoclave

Inoculum
10 days old actively growing *R. bataticola*

Sick culture
Inoculate the substrate (100 g) with five 5 mm fungal discs and incubate at 30°C for 15 days

▼

Sick pot
Mix 50 g of sick culture with 100 g of soilrite uniformly

▼

Sow the susceptible genotype seeds in sick pot and allow them to grow

▼

Assess the symptoms in roots and foliar

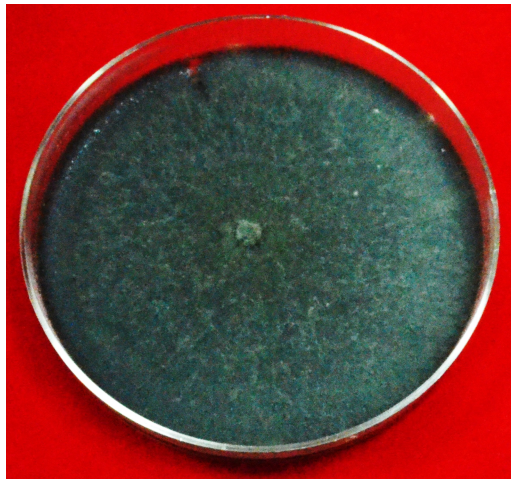
▼

Chop down infected plants and assimilate into the same sick pot

▼

Use the sick pot which is showing 90% of infection for further experiment

Sick Pot Technique- steps

i. 

Inoculum

ii. 

Substrate

iii. 

Control
No fungal growth

Inoculated
Fungal growth

Sick Pot Technique- results

A

Control

B

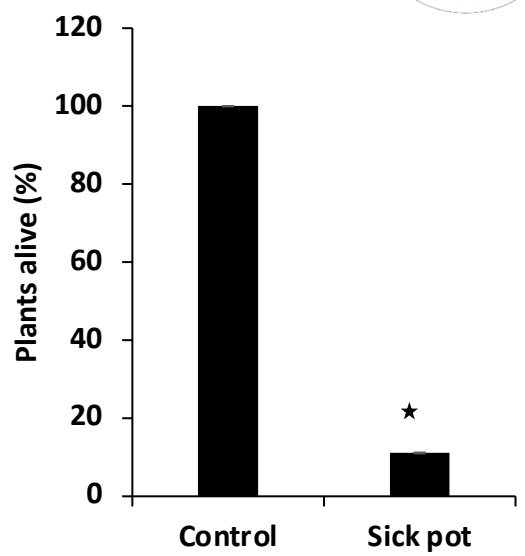
Sick pot

C

Mock Control

DRR Infected

D



E

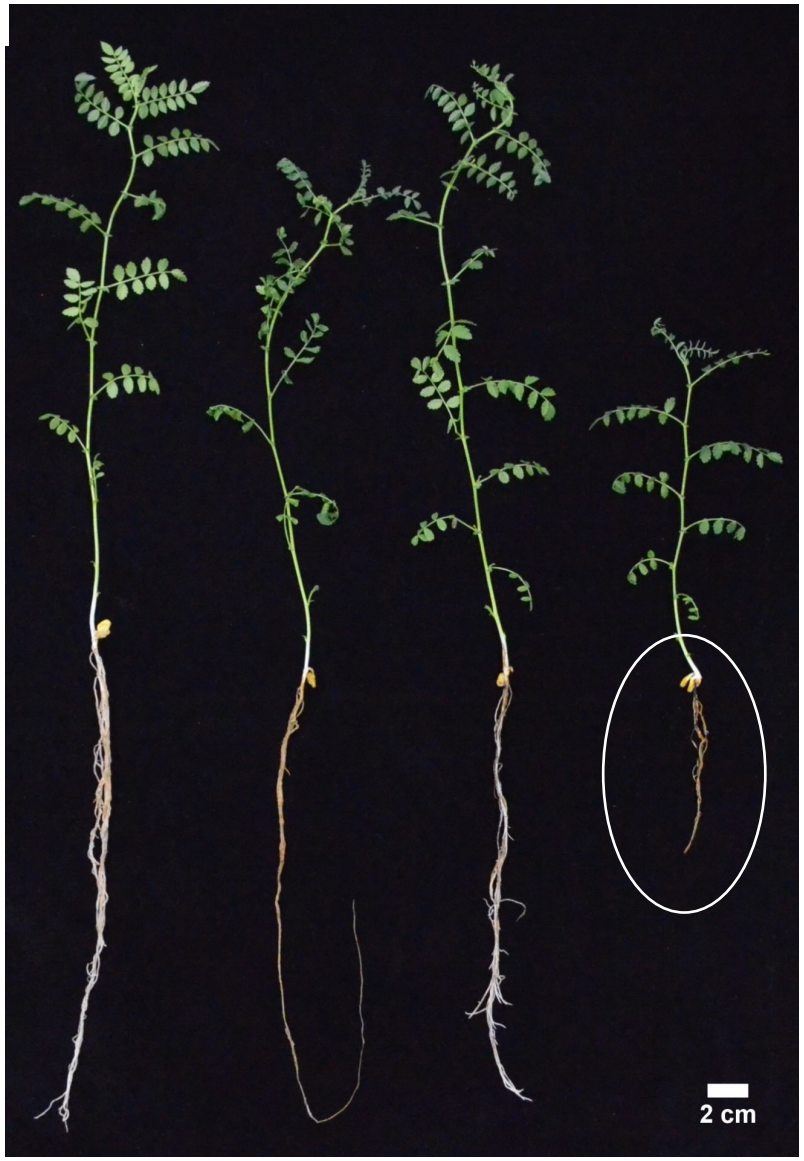
Control

F

Sick pot

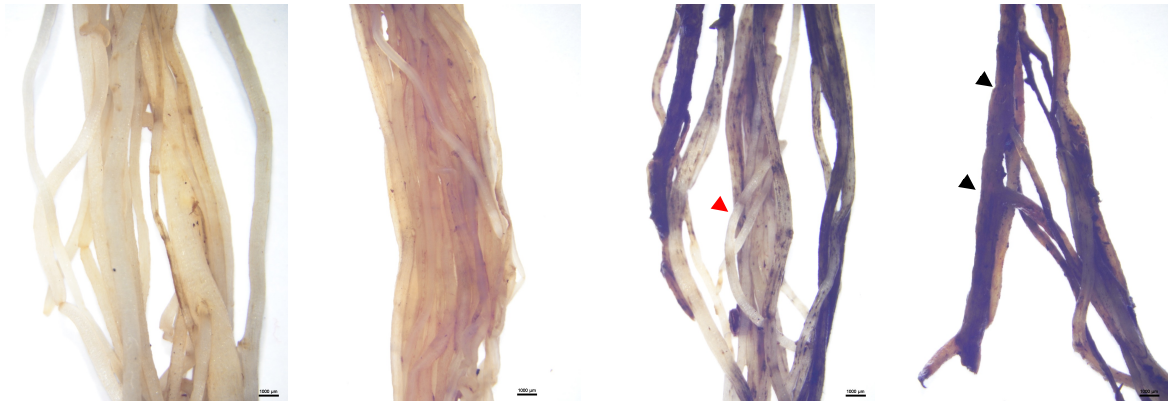
Sick Pot Technique- under drought stress

A



Control Drought Pathogen Combined stress

B



Control Drought Pathogen Combined stress

More infection under drought stress

Blotting Paper Technique: Advantages

- Screen chickpea genotypes under laboratory conditions.
- Dip inoculation enables the investigation of interaction on a temporal basis with easy control over inoculum load and facilitates in vitro screening.
- Furthermore, even young seedlings can be used.
- Five-day-old fungal culture can yield enough inoculum to infect the plants.
- Liquid inoculum contains both mycelia and microsclerotia.
- Root rot symptoms can be used to score the disease and identify resistant genotypes.

Blotting Paper Technique: Disadvantages

- Drought stress imposition is impossible
- Screening with this technique will not reflect natural responses

Sick Plot Technique: Advantages

- Interactions among plants, pathogens, and drought stress.
- Plants show typical DRR symptoms in the sick pot method.
- In a sick pot, drought stress can be imposed at any age of the plant and screen the plants.
- Plants subjected to combined drought and pathogen infection showed severe root rot as compared to pathogen-only treatment.
- Screen the genotypes under combined drought and pathogen stress to identify resistant genotypes.
- Several studies were attempted earlier to screen the genotypes but by using blotting paper technique.
- Besides, field screening has also been conducted but without imposing drought stress.
- It is crucial to impose drought stress during different stages of chickpea and assess the genotype response.

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