Jun 17, 2021

Chickpea Inoculation with *A. rabiei* for Ascochyta Blight Disease Assessment Under Controlled Conditions

Melody Christie[1], Dr Prabhakaran Sambasivam[1], Ido Bar[1], Ido Bar[1]

[1]Griffith University

dx.doi.org/10.17504/protocols.io.bvkwn4xe

GRDC Ascochyta rabiei research program

Melody Christie

ABSTRACT

This protocol describes the inoculation preparation, bioassay and disease assessment of *Ascochyta rabiei* isolates on a differential set of chickpea host genotypes.

DOI

dx.doi.org/10.17504/protocols.io.bvkwn4xe

PROTOCOL CITATION

Melody Christie, Dr Prabhakaran Sambasivam, Ido Bar, Ido Bar 2021. Chickpea Inoculation with A. rabiei for Ascochyta Blight Disease Assessment Under Controlled Conditions. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bvkwn4xe

CREATED

Jun 08, 2021

LAST MODIFIED

Jun 17, 2021

PROTOCOL INTEGER ID

50550

MATERIALS TEXT

Equipment for *A. rabiei* culture and inoculation work:

burner
ethanol bath for utensils
ethanol spray
petri dishes for dissection
tape
forceps
gloves
Kimwipes
lighter
media plates
para]lm
permanent lab marker
scalpel
scissors
pipette and tips
Tween 20
10cm diameter pots
premium potting mix
trays for bioassays (to fit 10 pots)
plastic containers with lids to fit 10 pots
spray bottles (500ml)
heamocytometer and cover slips

Inoculum preparation 30m

1 Approximately 2 weeks before the planned inoculation date, subculture isolates of *A. rabiei* onto V8 media agar which contains 30mg/L of antibiotic (Streptomycin/Penicillin [M]**30 mg/L**). Add antibiotic after media has been autoclaved and is cool enough to touch (approximately **45 °C**). Aseptically place one plug from the original culture in the middle of the plate and incubate at room temperature for 14 days. Include a positive control isolate with each batch per bioassay.

Sowing 30m

2 Autoclave soil (premium potting mix) before use (one day before sowing). Ensure pots have been cleaned and dried if previously used.

3 Into a 10 cm diameter pot sow five or six chickpea seeds, being careful not to sow too close to the side of the pot. Sow two pots of each chickpea genotype per isolate so that at least 10 plants can be inoculated. Sow ICC3996 and Kyabra one day earlier than the other genotypes. Fertilise with Osmocote (slow release) at the time of sowing and Aquasol once a fortnight.

4 Plants are grown under controlled environment conditions at approximately **23 °C** . When the chickpea plants are all at 4th node stage (or as many as possible without other varieties growing too big) inoculation should occur.

Inoculum preparation 30m

5 On the day of inoculation saturate the plates with distilled water and leave for **00:15:00** . 15m

Citation: Melody Christie, Dr Prabhakaran Sambasivam, Ido Bar, Ido Bar (06/17/2021). Chickpea Inoculation withÃÂ A. rabiei for Ascochyta Blight Disease Assessment Under Controlled Conditions. https://dx.doi.org/10.17504/protocols.io.bvkwn4xe

6 Using a wire loop or glass rod, gently scrape over the surface of the plate to dislodge spores.

7 Pour the solution into a **500 mL** flask (filter through a piece of tissue/gauze if required).

8 Using a heamocytometer adjust spore concentration to [M]**1*10^5 spores per ml** in a volume of Transfer suspension to a Preval hand held sprayer and add two to three drops of Tween 20 ([M]**0.02 % (v/v)**) per **100 mL** of spore suspension. Ensure the sprayer is labelled with the isolate code.

9 If for some reason the inoculum is not going to be used straight away, it can be placed in the dark in the fridge for a up to 2 hours.

Inoculation 30m

10 Place pots in groups of 10 (two pots of each genotype). Separate each pot away from the others for the inoculation step. Using a Preval hand held sprayer, spray inoculum onto chickpeas until runoff (approximately 15 - 20 ml per plant) and return to their original position.

11 Place pots inside the black plastic tubs and leave in dark for 24 hours

12 24 hours after inoculation remove plants from plastic tubs and place in a tray under grow lights.

13 Plants are misted until beading forms on leaves every 3 days and watered twice a week (or as required). Disease assessment (phenotyping) occurs after 21 days.

Disease Assessment

14 Disease assessments are made 21 days post inoculation using the following scales:

Citation: Melody Christie, Dr Prabhakaran Sambasivam, Ido Bar, Ido Bar (06/17/2021). Chickpea Inoculation withÃÂ A. rabiei for Ascochyta Blight Disease Assessment Under Controlled Conditions. https://dx.doi.org/10.17504/protocols.io.bvkwn4xe

Symptoms	Score	Description
	1	No leaf symptoms
	3	Pin prick lesions (sign of potential HR defence).
	5	Small, inconspicuous lesions present without pycnidia (evidence of potential initial biochemical defence/containment)
	7	Individual lesions developed with darker margins and some pycnidia (evidence of initial defence breakdown)
	9	Lesions with dark margin coalesced with pycnidia developed (evidence of defence breakdown as lesions are growing in size and the pathogen is able to reproduce).

Disease severity assessment scores 1-9 for leaf lesions.

Symptoms	Score	Description
	1	No stem symptoms.
	3	Pin prick lesions visible on the stem (sign of potential HR defence).
	5	Small, inconspicuous lesions present without pycnidia (evidence of potential initial biochemical defence/containment)
	7	Individual lesions developed with darker margins and some pycnidia, no stem breakage visible (evidence of initial defence breakdown)
	9	Lesions with dark margin coalesced with pycnidia developed and some stem breakage possible (evidence of defence breakdown as lesions are growing in size and the pathogen is able to reproduce).

Disease severity assessment scores 1-9 for leaf lesions

Separate notes should be taken for leaf drop of defoliation

Citation: Melody Christie, Dr Prabhakaran Sambasivam, Ido Bar, Ido Bar (06/17/2021). Chickpea Inoculation withÃÂ A. rabiei for Ascochyta Blight Disease Assessment Under Controlled Conditions. https://dx.doi.org/10.17504/protocols.io.bvkwn4xe