Pathology

An Improved Growth Medium for the Multiplication of *Ascochyta rabiei* in the Laboratory

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Ascochyta blight, caused by *Ascochyta rabiei* (Pass.) Lab., is a major disease of chickpea. For successful artificial screening of chickpea germplasm for resistance to ascochyta blight in the greenhouse, large amounts of inoculum are required. Some current methods for the preparation of medium in the laboratory are very labor intensive and expensive. Therefore, we conducted a comparative study of nine media for the production of inoculum with the following compositions.

1. Potato dextrose broth: 200 g peeled potatoes, 20 g sucrose, and 1000 mL distilled water.
2. Chickpea extract dextrose broth: 40 g chickpea granules, 20 g sucrose, and 1000 mL distilled water.
3. Modified chickpea seed meal medium: 20 g chickpea granules, 0.4 g $Ca(NO_3)_2$, 0.15 g $MgSO_4$, 0.15 KH_2PO_4, 0.06 g KCl, 15 g dextrose, 0.5 g yeast extract, and 1000 mL distilled water as suggested by Khalil and Khan (1986).
4. Chickpea seeds (desi): 20 g dry seeds soaked in water for 15 h and autoclaved without adding water.
5. Chickpea seeds (kabuli): 20 g dry kabuli chickpea seeds soaked in water for 15 h and autoclaved without adding water.
6. Chickpea seeds (kabuli) boiled: 20 g dry kabuli seeds boiled for 30 min and autoclaved without adding water as suggested by Alam et al. (1987).
7. Chickpea seeds (kabuli) + sucrose: 20 g chickpea seeds soaked for 15 h and 400 mg sucrose solution added and autoclaved.
8. Chickpea seeds (kabuli) + yeast extract: 20 g chickpea seeds soaked in water for about 15 h and 200 mg yeast extract solution added and autoclaved.
9. Chickpea seeds (kabuli) + sucrose + yeast extract: 20 g chickpea seeds soaked in water for 15 h and 400 mg sucrose and 200 mg yeast extract solutions added and autoclaved.

There were three 150 mL flasks, representing three replications for each treatment and 50 mL medium was poured in each flask in case of liquid media. The media were inoculated with 0.1 mL spore suspension from a 10-day old culture on potato dextrose agar and the inoculated flasks were incubated at 20° ± 1°C for 10 days.

In case of liquid media, the mycelial mat was taken out and churned in 100 mL sterilized water. In case of seed-medium, all the contents from the flask were taken out and churned in 100 mL sterilized water. The necessary serial dilutions were made for each replication of each treatment and the spores were counted with the help of a haemocytometer. The number of spores mL^{-1} obtained from each medium are given in Table 1.

Table 1. Effect of different media on sporulation of *Ascochyta rabiei*.

Treatment	Mean number of spores mL^{-1} in millions	
	Hisar isolate	Delhi isolate
Potato dextrose broth	4.8	7.2
Chickpea extract dextrose broth	8.0	10.2
Modified chickpea seed meal medium (broth)	9.9	15.2
Desi chickpea seeds — soaked in water for 15 h	249.7	230.3
Kabuli chickpea seeds — soaked in water for 15 h	354.7	391.7
Kabuli chickpea seeds boiled	353.7	382.0
Kabuli chickpea seeds + sucrose	359.0	400.3
Kabuli chickpea seeds + yeast	323.7	380.7
Kabuli chickpea seeds + yeast + sucrose	275.7	389.7
SE	±7.92	±10.36
CV (%)	6.4	7.3

The 'Delhi' isolate produced more spores in all the media used than the 'Hisar' isolate. The media containing chickpea seeds produced large number of spores than the liquid media. The maximum number of spores were observed in both the isolates in kabuli chickpea seeds + sucrose medium followed by kabuli chickpea seeds (soaked and autoclaved) medium. Hence chickpea seeds + sucrose and even kabuli chickpea seeds media are economical, easy to prepare, and a large quantity of inoculum can be prepared within 10 days.

References

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Alam, S.S., Strange, R.N., and **Qureshi, S.H.** 1987. Isolation of *Ascochyta rabiei* and a convenient method for inoculum production. Mycologist 21(1):20.

Effect of *Ascochyta rabiei* Inoculum Age on Disease Development

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For successful screening of chickpea for ascochyta blight [*Ascochyta rabiei* (Pass.) Lab.] resistance, the use of viable inoculum plays an important role in disease development under artificial conditions.

To study the effect of age of the inoculum on disease development, the 'Delhi' isolate of *Ascochyta rabiei* was used. The fungus was multiplied on autoclaved kabuli chickpea seeds in a 250 mL conical flask (20 g kabuli chickpea seeds soaked in water for 15 h and autoclaved) and incubated at 20°C for 10, 15, 20, 30, 40, 50 and 60 days. The susceptible chickpea line Pb 7 was sown in plastic trays (40 × 34 × 8 cm) in three replications (30 seeds per replication) for each treatment. Chickpea seedlings were raised in a greenhouse for 7 days and moved to a plant growth room on the day of inoculation.

Chickpea seeds with pycnidia were kept in water for about 30 min and stirred with a clean glass rod, and the spore suspension was then passed through a double-folded muslin cloth. The concentration of spores was adjusted to 200×10^4 mL^{-1} for all the treatments. Seven-day old seedlings were inoculated with the inoculum of different ages separately, using a hand sprayer.

The plastic trays with seedlings were placed on shelves fitted with four cool fluorescent lamps. Temperature around 20°C and relative humidity above 90% were maintained throughout the experiment.

The data on the disease intensity in the chickpea seedlings are given in Table 1. The disease incidence was rated on a 1–9 point scale (where 1 = healthy plants and 9 = completely dead plants) 10 days after inoculation. High disease intensity was observed on the seedlings inoculated with 10- and 15-day old inoculum. Disease severity decreased with an increase in the inoculum age. The disease intensity was only 3.3 when 60-day old inoculum was used.

Table 1. Effect of age of inoculum of *Ascochyta rabiei* on disease incidence under controlled conditions.

Inoculum age (days)	Disease rating on 1–9 scale[1]
10	8.0
15	8.0
20	7.6
30	6.6
40	5.0
50	4.0
60	3.3
SE	±0.26
CV (%)	7.5

1. Scored on a 1-9 scale, where 1 = healthy, and 9 = dead.

Host Range Studies with the Ascochyta Blight Pathogen of Chickpea

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The host range of *Ascochyta rabiei*, the causal agent of ascochyta blight of chickpea is considered to be confined to *Cicer* species. Zachos et al. (1963) were unable to infect lentil (*Lens culinaris*), pea (*Pisum sativum*), and vetch (*Vicia* sp) with *A. rabiei*. In inoculation studies with *A. rabiei*, Sprague (1930) failed to infect several plant species, including lentil, pea, and bean (*Phaseolus vulgaris*). Tripathi et al. (1987) inoculated over 40 species of crops and weeds with a Pantnagar isolate of *A. rabiei* but no infection resulted. In greenhouse inoculation studies at the International Center for Agricultural Research in the Dry Areas (ICARDA) in Syria, *A. rabiei* infected cowpea, bean, and peas (Nene and Reddy 1987).

Ascochyta blight was first detected on chickpea in the US Pacific Northwest in 1983 and reached epidemic proportions in 1987 (Kaiser and Muehlbauer 1988). In May