

CHICKPEA DISEASES

RESISTANCE-SCREENING TECHNIQUES



ICRISAT

Information Bulletin No. 10

INTERNATIONAL CROPS RESEARCH INSTITUTE FOR THE SEMI-ARID TROPICS

Abstract

Nene, Y.L., Haware, M.P., and Reddy, M.V. 1981. **Chickpea diseases: resistance-screening techniques.** Information Bulletin No. 10. Patancheru, A.P., India: International Crops Research Institute for the Semi-Arid Tropics.

More than 50 pathogens have been reported to affect chickpea (*Cicer arietinum* L.), an important legume crop in many parts of the tropics and subtropics. Several pathogens are of economic importance, particularly wilt, dry root rot, and *Ascochyta* blight. To assist plant breeders to develop disease-resistant material, techniques have been worked out and standardized at ICRISAT to screen germplasm and breeding material on a routine basis. These techniques are described in detail, with ten color illustrations, and a nine-point rating scale is given to facilitate the identification of resistant breeding material.

Resume

Nene, Y.L., Haware, M.P., Reddy, M.V. 1981. Chickpea, diseases: resistance-screening techniques. (**Maladies du pois chiche: techniques de criblage pour la resistance.**) Information Bulletin No. 10. Patancheru, A.P., India: International Crops Research Institute for the Semi-Arid Tropics.

Les rapports scientifiques mentionnent que le pois chiche (*Cicer arietinum* L.), une legumineuse qui occupe une place importante dans plusieurs regions des zones tropicales et subtropicales, est vulnérable a plus de 50 agents pathogenes. Certains sont d'une grande importance du point de vue économique, en particulier le flétrissement, la pourriture sèche des racines et l'antracnose due a l'*Ascochyta*. Des techniques ont été développées et uniformisées a l'ICRISAT, afin de cribler les ressources genetiques et le matériel de sélection. Ces techniques devraient être utilisées par les sélectionneurs pour leur permettre de créer du matériel résistant aux maladies. Ce bulletin donne une description détaillée de ces techniques et comprend dix illustrations en couleurs et une échelle d'évaluation sur neuf points, en vue de faciliter l'identification des lignées résistantes.

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is a nonprofit scientific educational institute receiving support from a variety of donors through the Consultative Group on International Agricultural Research. Major financial support for ICRISAT's work in 1981 was provided by governments and agencies of Australia, Belgium, Canada, Federal Republic of Germany, France, Japan, Mexico, the Netherlands, Nigeria, Norway, Sweden, Switzerland, United Kingdom, United States, and from the following international and private organizations: European Economic Community, International Development Research Center, International Fertilizer Development Center, the United Nations Development Programme, the Leverhulme Trust, and the International Fund for Agricultural Development. All responsibility for the information in this publication rests with ICRISAT; where trade names are used this does not constitute endorsement of or discrimination against any product by the Institute.

Correct citation: **NENE, Y.L., HAWARE, M.P., and REDDY, M.V.** 1981. Chickpea diseases: resistance-screening techniques. Information Bulletin No. 10. Patancheru, A.P., India: International CroDS Research Institute for the Semi-Arid Tropics.

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Resistance - Screening Techniques

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International Crops Research Institute for the Semi-Arid Tropics

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October 1981

Introduction

Chickpea (*Cicer arietinum* L.) is grown mainly in the Indian subcontinent, West Asia, North and East Africa, southern Europe, and South and Central America. More than 50 pathogens have been reported to affect chickpeas, and several of them are of economic importance. These include wilt [*Fusarium oxysporum* Schlecht emend. Snyd. and Hans f. sp. *ciceri* (Padwick) Snyd. & Hans], several root and stem rots [*Rhizoctonia bataticola* (Taub.) Butler; *R. solani*/ Kuhn.; *Fusarium solani* (Mart.) Sacc.; *Sclerotium rolfsii* Sacc.; *Operculella padwickii* Kheswalla, *Pythium ultimum* Trow; *Sclerotinia sclerotiorum* (Lib) de Bary]; etc., blights [*Ascochyta rabiei* (Pass.) Labrousse; *Stemphylium sarciniforme* (Cav.) Wilts, grey mold (*Botrytis cinerea* Pers. ex Fr.), rust (*Uromyces ciceris-arietini* (Grogg.) Jacz. & Boyer], and viral diseases (e.g., stunt caused by pea leaf roll virus). In the late 1970s ICRISAT pathologists have focused their attention on wilt, dry root rot (*R. bataticola*), *Ascochyta* blight, and stunt. To assist the breeders develop disease-resistant material, techniques have been developed and standardized to screen germplasm and breeding material efficiently and effectively. We have already reported most of these techniques briefly (Reddy and Nene 1978, 1979; Nene and Haware 1980; Singh et al. 1981). However, since procedural details of these techniques were not described in these papers, we considered it worthwhile to bring together this detailed description of the techniques in this bulletin. We hope this information will be useful to chickpea researchers all over the world. Also, principles that have been used to develop these techniques should be useful in working out techniques for other diseases elsewhere. A field-screening technique for resistance to stunt is still in the process of development and is therefore not included in this bulletin.

We use field techniques for large-scale screenings and glasshouse/net house/labora-

tory techniques to confirm resistances identified in the field screening as well as to carry out inheritance and race identification studies.

Wilt (*Fusarium oxysporum* f. sp. *ciceri*)

The disease has been observed in Bangladesh, Burma, Ethiopia, India, Malawi, Mexico, Pakistan, Peru, Sudan, USA, and Tunisia. It causes substantial losses and can be devastating (Fig. 1). The fungus is soilborne and survives in the residual stubble for over 3 years. It may also spread through seed unless the seed is treated with a mixture of benomyl and thiram.

Sick Plot

1. Select a plot of adequate size and ensure that it is isolated from other chickpea fields to avoid spread of the fungus inoculum from this plot to others. The plot should have been cropped in the previous year with chickpea, and at least traces of wilt incidence should have been observed.
2. Collect as many wilted plants from other fields as possible, chop them into small pieces, and incorporate these uniformly in the surface soil of the plot.
3. Plant a sole crop of a highly susceptible cultivar (e.g., JG-62 or T-3) in this plot. Ensure a good plant population and carry out normal agronomic operations.
4. By the end of the season, at least 20% of the plants should show wilt symptoms. After harvesting and threshing, scatter the debris uniformly all over the plot and incorporate it by discing. Also repeat step 2; this will help increase the level of the inoculum to make the soil "sick."
5. Repeat steps 3 and 4 in the next season.



Figure 1.

By the end of this season, you should see more than 90% wilt incidence. If the incidence is less than 70%, repeat steps 3 and 4 once more.

6. Initiate screening in the next season. Plant a susceptible cultivar after every two test rows in the whole field. These rows will serve as checks, and will help in monitoring and maintaining the wilt sickness of the plot. The susceptible check rows should show more than 90% wilt (Fig. 2).
7. From the 4th or 5th year onwards, plant every fifth row as a susceptible check. This will provide for more breeding material and at the same time maintain the level of sickness.
8. Planting any other crop in this plot is not recommended.

It must be emphasized that by following all these steps a sick plot in which *F. oxysporum* f. sp. *ciceri* will be the most predominant pathogen can be developed. However, the presence of other soilborne pathogens cannot be avoided.

Pot Screening

1. Obtain a pure culture of *F. oxysporum* f. sp. *ciceri* from infected chickpeas in your area by following standard isolation procedures.
2. Prepare a sand-maize meal medium by placing 90 g riverbed sand, 10 g maize meal, and 20 ml distilled water in each 250 ml Erlenmeyer flask. Autoclave the medium in the flasks at 15 lb for 20 min. Inoculate each flask with a bit of fungus



Figure 2.

- growth from tubes and incubate at 25°C for 15 days.
3. Prepare a fungus-soil mixture by hand-mixing contents of each flask with 2 kg of nonautoclaved field soil. The soil must come from a chickpea field where wilt normally occurs.
 4. Fill large (30-cm diameter) earthen pots with the inoculated soil from step 3. Approximately 10 kg of soil will be required to fill each pot. Water the pots and wait for 4 days before proceeding to the next step.
 5. Sow 40-50 seeds of a highly-susceptible cultivar in each pot at 2-3 cm depth. Water adequately and regularly. Most plants should show wilting after 10 days.
 6. Remove healthy plants after 30 days. Chop and incorporate all the wilted plants into the soil.
 7. Repeat steps 5 and 6 until over 90% wilt is observed. These pots are then ready for screening.
 8. Divide a pot into two sections. Plant ten seeds of a test line in one section and ten seeds of a susceptible check in the other (Fig. 3).
 9. These pots can be used for several successive screenings.
- Note: Screening in pots requires up to only 60 days at any time of the year, compared with 4-6 months of the season in a sick plot.

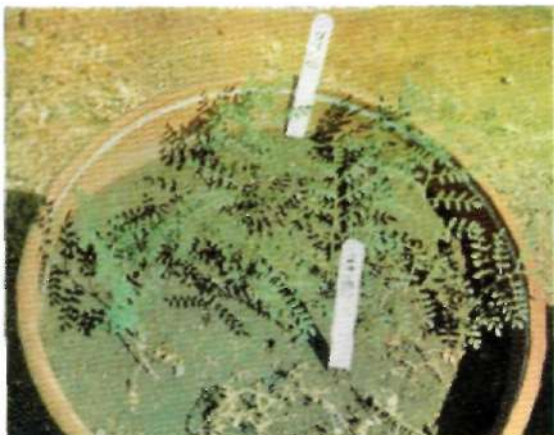


Figure 3.

Water Culture

1. Obtain a pure culture of *F. oxysporum* f. sp. *ciceri* from infected chickpeas in your area.
2. Sow 15 surface-sterilized (5 min in 2.5% sodium hypochlorite) seeds of the test as well as susceptible check lines in autoclaved riverbed sand placed in 15-cm pots. Use one pot for each line. Approximately 1 kg of sand will be required for each pot. Nurse the seedlings until these are required for transplanting.
3. Prepare potato-dextrose broth (peeled and sliced potato, 200 g; dextrose, 20 g; distilled water, 1000 ml). Place 100 ml broth in one 250-ml flask and prepare as many flasks as needed. One flask of inoculum will be sufficient for testing 18 lines (10 seedlings per line). Autoclave at 15 lb for 20 min.
4. Inoculate the medium (step 3) with a bit of the fungus growth from tubes. Incubate on a shaker (8 hr each day) at room temperature (25-30°C) for 10 days.
5. Dilute entire contents of a flask with sterilized distilled water to get the final inoculum dilution of 2.5% (usually about 3.5 liters of water will be needed to attain the desired dilution of the contents

of one flask). This will ensure approx. 6.5×10^5 spores/ml.

6. Pour 20 ml of inoculum into each sterilized 150 x 15 mm glass tube (step 5).
7. Remove the 10-day-old (from sowing) seedlings from sand (step 2). Wash the root system in running water and then rinse in sterilized distilled water.
8. Transplant one seedling into each tube (step 6) and hold it in position by a cotton plug.
9. Add sterilized distilled water to the tubes after every 2 days to make up the loss.
10. Use ten seedlings for each line. With each batch of test line, use seedlings of a susceptible line (e.g., JG-62) to serve as check. Also keep for each line a non-inoculated seedling as another check.
11. Keep tubes in a specially designed box (Fig. 4).
12. The susceptible check usually wilts in 7-10 days. Record data 15 days after inoculation (step 8). Noninoculated seedlings should remain green up to 3 weeks.

Note: This technique is particularly well-suited for race studies.

Dry Root Rot (*Rhizoctonia bataticola*)

Dry root rot has been reported from Australia, Ethiopia, India, Iran, and USA. The disease is serious in areas where the ambient day temperatures are around 30°C at the seedling or at the flowering stage. In India this disease occurs mainly in the central and southern states where a lot of mortality at the flowering and podding stages is due to this disease.

Sick Plot

The steps mentioned for the development of a wilt sick plot will also hold good for dry root rot. The only difference will be to plant

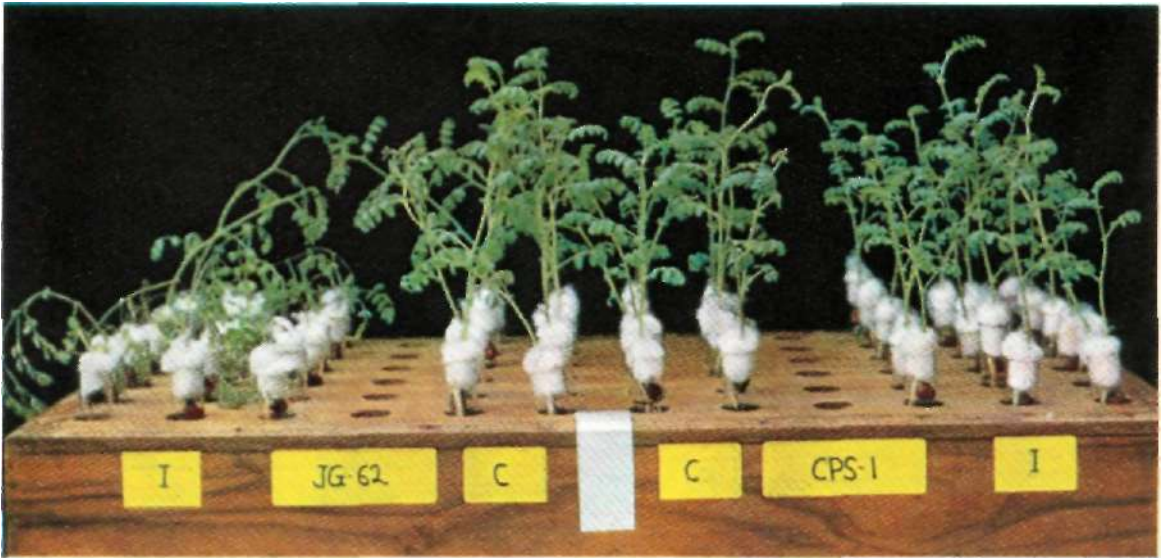


Figure 4.

a cultivar that is susceptible to *R. bataticola*, but resistant to *F. oxysporum* f. sp. *cicert*. Examples of such cultivars are BG-212 and ICC-229.

As pointed out in step 9 under wilt, it will not be possible to have a sick plot only for *R. bataticola*. We can, however, encourage its multiplication to ensure its predominance over other soilborne pathogens.

To confirm resistance specifically to *R. bataticola*, it would be necessary to follow the blotter paper technique described below.

Blotter Paper Technique

1. Obtain a pure culture of *R. bataticola* from infected chickpeas in your area.
2. Sow 20 surface-sterilized (5 min in 2.5% sodium hypochlorite) seeds of the test lines in autoclaved riverbed sand placed in 15-cm pots. Use one pot for each line. Also sow seed of check lines. Approximately 1 kg of sand will be required for one pot. Nurse the seedlings till these are 5 days old from sowing.
3. Prepare potato-dextrose broth (peeled and sliced potato, 200 g; dextrose 20 g; distilled water, 1000 ml). Place 100 ml broth in one 250-ml flask and prepare as many flasks as needed. Autoclave at 15 lb for 20 min. For testing ten lines at one time, one flask of inoculum will be needed.
4. Inoculate the medium (step 3) with the fungus. Incubate for 5 days at 25°C.
5. Remove the mycelial mats from the flasks at the end of the incubation period. Add two mycelial mats to 100 ml sterilized distilled water and macerate these in a Waring blender for 1 min (operate the blender intermittently). Place this inoculum in a beaker of a suitable size,
6. Uproot the 5-day-old seedlings of the test lines (step 2). Wash the root system in running water and rinse in sterilized distilled water.
7. Hold all seedlings of a test line in a hand and dip the roots of these in the inoculum (step 5) with an up and down movement for about 30 seconds (Fig. 5). Remove

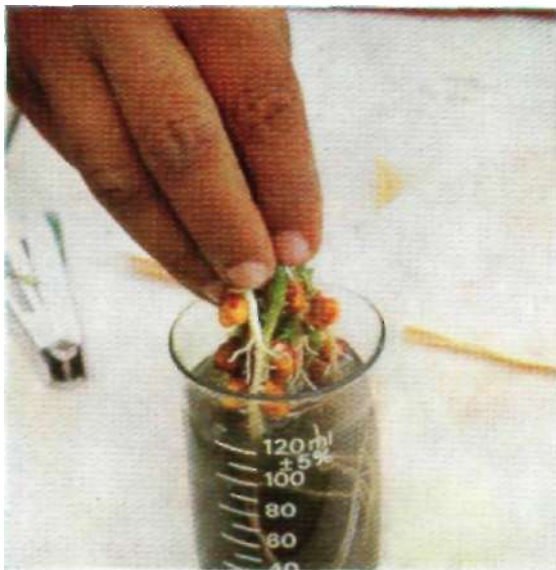


Figure 5.

Figure 6.



excess inoculum by touching the edge of the beaker.

8. Place 20 seedlings of the test line side by side on a blotter paper (size 45 cm X 25 cm with one fold; any color; thin) so that only the cotyledons and roots are covered and the green tops of the seedlings remain outside the blotter paper after it is folded. Fold the blotter paper **and** moisten it adequately but not excessively (Fig. 6). One folded blotter paper will have seedlings of one test line.
9. Inoculate seedlings of a susceptible check (e.g., BG-212) with each batch of test seedlings.
10. Keep folded blotters, one on top of the other, in heaps of ten in a tray (Fig. 6). One of these ten blotters should have the

seedlings of the susceptible check (step 9).

11. Place the trays in an incubator at 35°C for 8 days. Provide 12-hr artificial light. Moisten the blotters adequately every day.
12. At the end of the incubation period (8 days), examine the seedlings for the extent of root damage, and score for the disease (Fig. 7).

Ascochyta Blight

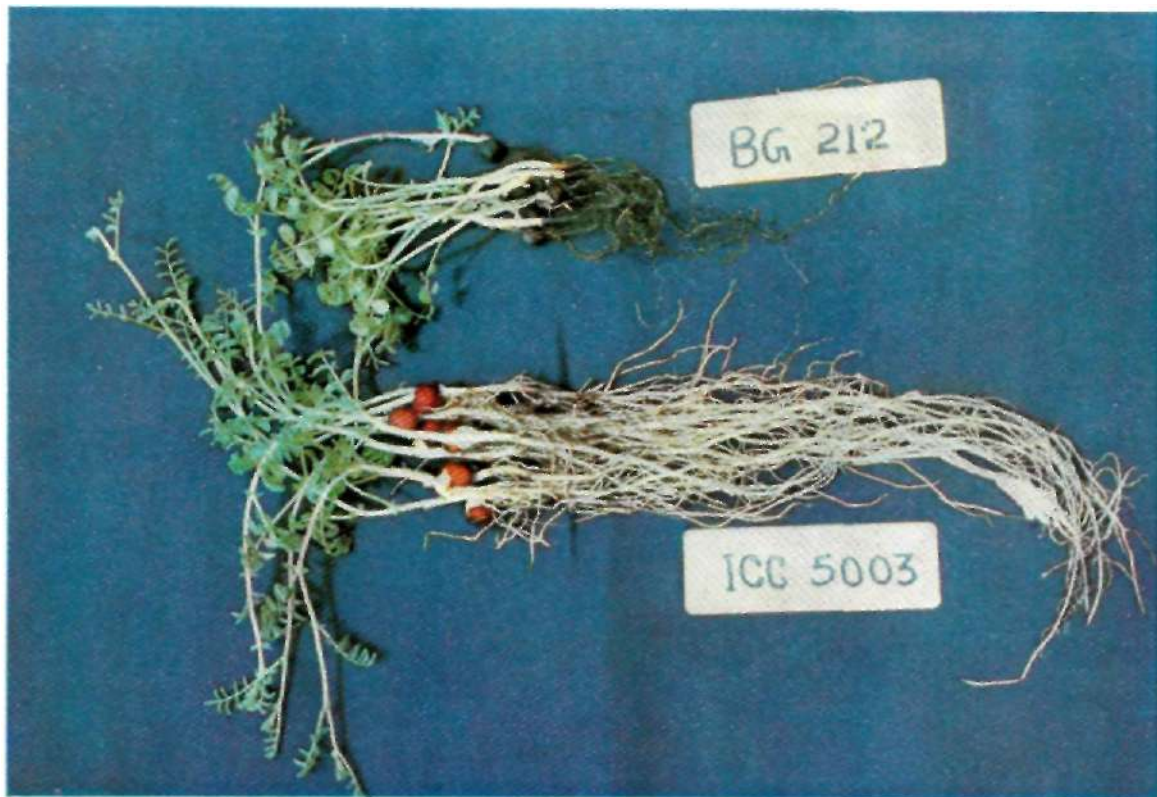
Ascochyta blight [*Ascochyta rabiei* (Pass.) Labrousse] is a serious disease of chickpeas in countries around the Mediterranean, West Asia, southern USSR, Pakistan, and northern India. It is capable of causing complete loss in some years.

The fungus survives on infected plant debris and is also transmitted through seed. The disease spreads rapidly under cool, wet, and windy conditions.

Field Screening

1. Collect sufficient debris of infected chickpea plants and store it dry for use in the next season. For 1 ha, 6 bags (100 x 75 cm) should be sufficient.
2. Plant two to four rows of a susceptible cultivar (e.g., ICC-460, Syrian Local, PB-7) all around the plot.
3. Plant test lines following normal agronomic operations. Ensure that a susceptible cultivar is planted after every two to four test rows. These rows will serve as indicator-cum-spreader rows.

Figure 7.



4. Identify the normal time of infection. In many countries favorable (cool and wet) weather is common around flowering time. At such time scatter the infected plant debris (step 1) all over the plot.
5. Arrange for a sprinkler irrigation system as a standby. This must be used if dry weather prevails at the normal time of infection.
6. If the disease development is not uniform, spray spore suspension prepared either from infected plants from the field itself or from a pure culture of the fungus. For a 1-ha plot, prepare 25-40 flasks (250 ml) of inoculum; 150-250 liters of diluted inoculum will be enough for 1 ha. (Prepare chickpea flour-dextrose broth by mixing 40 g chickpea flour and 20 g dextrose in 1000 ml water. Pour 30 ml broth in each flask. Autoclave at 15 lb for 20 min. Inoculate with a pure culture of *A. rabiei* and incubate for 10 days

with 12-hr light at 20-25°C. Dilute as indicated above).

7. Record disease rating when the susceptible check lines show the maximum rating (9 on the 1-9 scale). Record the disease rating again when the crop is close to maturity (Fig. 8).

Note: Closer spacings with rows across the normal wind direction enhance uniform disease development.

Isolation Plant Propagator

1. Use units of the Isolation Plant Propagator (Burkard Manufacturing Co. Ltd., Rickmansworth, Herts, England).
2. Whenever necessary, operate evaporative coolers around the propagators to maintain temperatures below 30°C (Fig. 9).
3. Arrange for artificial light (cool daylight; eight 120-cm tubes, 40 W each) in the lower sections of the propagators.
4. Fill pots with autoclaved fine riverbed sand + vermiculite (3:1).
5. Grow at least ten seedlings of one accession in one pot. Also raise seedlings of a susceptible check (e.g., ICC-460) in one pot in each subsection of the propagator.
6. Obtain a pure culture of an aggressive isolate of *A. rabiei*. Prepare chickpea flour-dextrose broth (40 g chickpea flour and 20 g dextrose in 1000 ml distilled water) and place 30 ml in each of the 250-ml flasks. Autoclave at 15 lb for 20 min. Inoculate the medium with the fungus. Incubate at 20-25°C for ten days with 12-hr artificial light per day.
7. Remove fungus growth from the flasks and dilute with sterilized distilled water to 2×10^4 spores per ml.
8. Use a hand sprayer to spray-inoculate the 2-week-old seedlings with the fungus inoculum. Cover plants with the plastic cover for 10 days.

Figure 8.

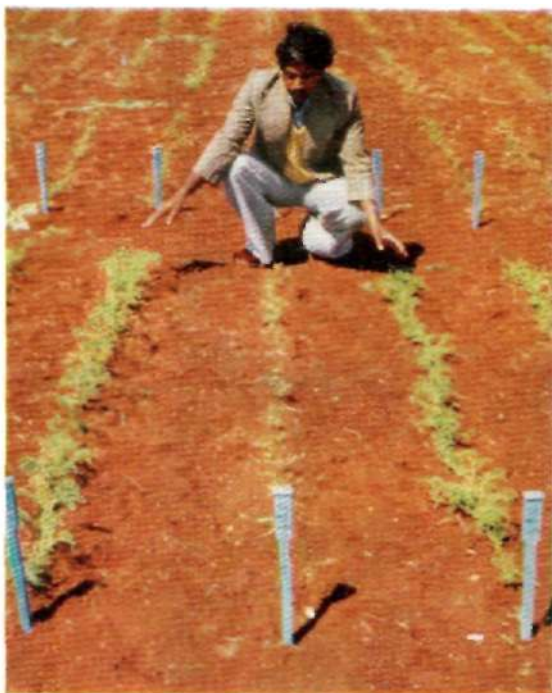




Figure 9.

9. Record the incubation period, percentage infection, and percentage mortality.
10. Score the disease severity on a 9-point scale (1-9) twice; once when the susceptible check shows 9 rating and again 10 days later (Fig. 10).

Rating Scale

A 9-point scale is used at ICRISAT for easy scoring. Interpretation of the scale is as follows: (1) resistant; (2-3) moderately resistant; (4-5) tolerant; (6-7) moderately susceptible; (8-9) susceptible (Table 1).

Test lines showing ratings 1-3 are considered acceptable for the breeding program; ratings 4-5 are acceptable only if lines with ratings 1-3 are not available; ratings 6-9 are not acceptable.

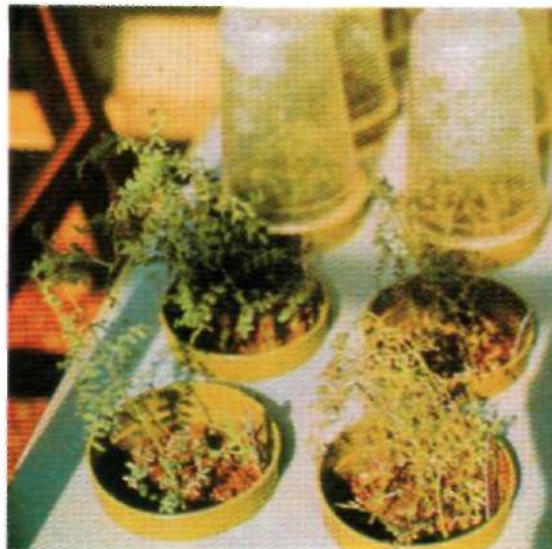


Figure 10.

Table 1. Description of the rating scale for scoring.

Rating	Wilt	Dry root rot		Ascochyta blight	
		Sick plot	Blotter paper	Field	Propagator
1	No mortality	No mortality	No infection on roots	No lesions	No lesions
2	—	—	—	—	Lesions on some plants; usually not visible
3	10% or less mortality	10% or less mortality	Very few small lesions on roots	Lesions visible on a few plants upon careful examination, no stem girdling	Few scattered lesions; usually seen after careful examination
4	—	—	—	—	Lesions and defoliation on some plants; not damaging
5	11-20% mortality	11-20% mortality	Lesions on roots clear but small; new roots free from infection	Lesions visible on many plants with stem girdling on few plants; little damage	Lesions common and easily observed on all plants but defoliation/damage not great
6	—	—	—	—	Lesions and defoliation common; few plants killed
7	20-50% mortality	20-50% mortality	Lesions on roots many; new roots generally free from lesions	Lesions present on almost all plants; 50% plants with stem girdling; death of a few plants; damage conspicuous	Lesions very common and damaging; 25% plants killed
8	—	—	—	—	All plants with extensive lesions causing defoliation and drying of branches; 50% plants killed
9	51% or more mortality	51% or more mortality	Roots infected and completely discolored	Lesions very common on all plants; stem girdling on more than 50% plants; death of most plants; damage more than 75%	Lesions extensive on all plants; defoliation and drying of branches; more than 75% plants killed

The above rating scale is used only if the total number of plants of a test line varies between 20 and 50. The rating scale will be different if plant number is more than 50.

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