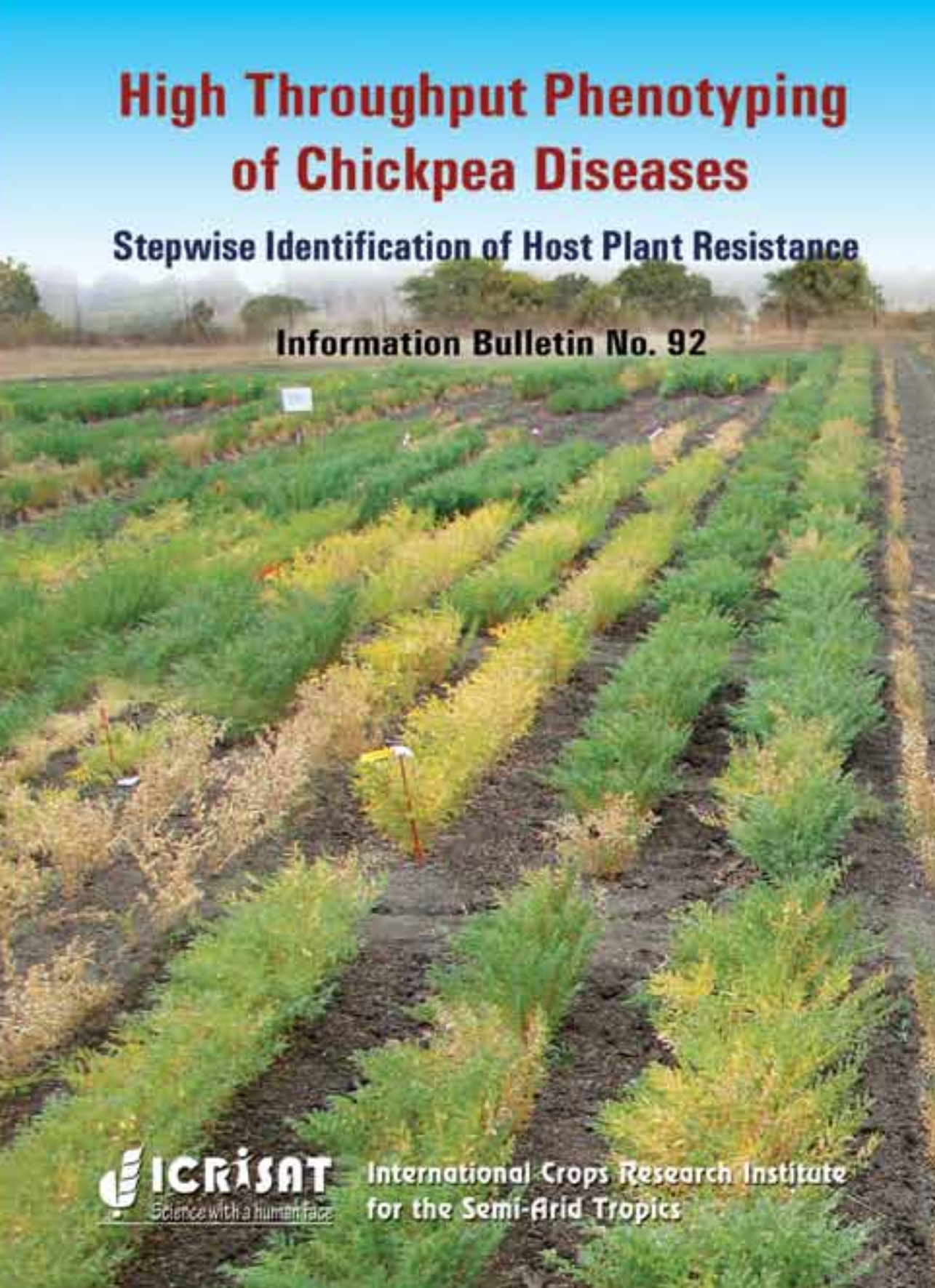
High Throughput Phenotyping of Chickpea Diseases

Stepwise Identification of Host Plant Resistance

Information Bulletin No. 92



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Abstract

Chickpea production is constrained by many fungal diseases. In general, soil borne diseases like fusarium wilt, dry root rot, collar rot, black root rot and foliar diseases like ascochyta blight and botrytis gray mold caused by different species of fungi are major consideration in all chickpea growing areas of the world. A large volume of literatures is available on studies related to various aspects of chickpea diseases including biology, epidemiology and management methods. In this bulletin, attempts have been made to briefly describe the distribution, economic importance, symptoms and causal organism of the above mentioned diseases of chickpea. The bulletin provides comprehensive stepwise information on screening methods for all the economically important diseases, and will be useful to all researchers involved in chickpea disease management through host plant resistance. Updated list of resistant sources has also been provided.

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Stepwise Identification of Host Plant Resistance

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Contents

Foreword

About 11 million hectares of chickpeas are cultivated annually around the world, with the subsistence farmers of semi-arid India producing more than 75% of the crop. Chickpea is a major source of protein for millions of people. To meet the ever-increasing demand for this legume crop, it is essential to manage the various stresses impacting crop yield. The major biotic stresses involve nearly 172 pathogens infecting chickpea from seedling stage till harvest. There are only a few diseases that are more destructive than others in different chickpea growing regions of the world. They not only reduce yield but also greatly impair the quality and stability of production year after year, undermining efforts to promote sustainable agriculture.

This Information Bulletin on High Throughput Phenotyping of Chickpea Diseases compiled by legume pathologists of ICRISAT, Patancheru, India, contains refined, repeatable and advanced host plant resistance (HPR) screening techniques developed at ICRISAT and transferred to National Agricultural Research System (NARS) scientists in Asia and Africa.

In general, screening techniques facilitate the culling out of ultra-susceptible genotype and identification of resistant material for further evaluation. Identification of resistant parents for the development and deployment of disease resistant chickpea lines has been instrumental in increasing production and productivity of chickpea in Asia and several countries in Africa.

In this bulletin, the authors have addressed globally important diseases of chickpea with greater focus on the screening techniques for critical identification of host plant resistance (HPR) that aid in disease resistance breeding programs. HPR is more effective, eco-friendly and economical than other disease management methods like fungicide application. HPR technology is easily adapted in diverse agronomical practices under different climatic zones. The efforts made by the authors in developing and adapting this technology in screening in-house and external germplasm accessions of chickpea against major diseases of chickpea are appreciable. I am sure that this publication will lead to faster identification of the various chickpea diseases and hasten the development of substantial resistant sources. New and young researchers in chickpea pathology will likewise find in this volume a most handy guide and reference.

William D Dar
Director General

Introduction

Nearly 172 pathogens have been reported to affect chickpea (*Cicer arietinum* L.) world-wide. Soil borne diseases such as wilt (*Fusarium oxysporum* f.sp. *ciceris*), dry root rot (R*hizoctonia bataticola*), black root rot *(Fusarium solani*), collar rot (*Sclerotium rolfsii*), and stem rot (*Sclerotinia sclerotiorum*) are important yield reducers. The ascochyta blight (*Ascochyta rabiei)* and botrytis gray mold (*Botrytis cinerea)* are the most destructive fungal foliar diseases causing substantial yield losses to chickpea in temperate and Mediterranean environments.

Chickpea diseases can be managed by developing and deploying resistant cultivars, using fungicides, cultural practices and biological control. Disease management using fungicides is uneconomical and not user friendly. Of the available management approaches, host plant resistant (HPR) is the most reliable, economical and effective method of managing chickpea diseases. Considerable efforts have been made by ICRISAT towards understanding the components of HPR such as biology and epidemiology, developing screening techniques, identifying resistance sources and utilizing these in breeding disease resistant lines in chickpea against the above mentioned diseases. The foremost step in developing resistant varieties is development of the reliable and repeatable screening techniques to identify resistance to diseases in germplasm and breeding lines.

In this bulletin, attempts have been made to collate, refine and develop a stepwise methodology to identify host plant resistance to several diseases of chickpea in the laboratory, greenhouse and under a field environment. Many chickpea accessions, which are maintained in national and ICRISAT genebank collections, have been evaluated at ICRISAT, and other locations in India have been listed. New sources of stable host resistance to wilt, dry root rot, Ascochyta blight, Botrytis gray mold diseases are also reported.

1. Fusarium wilt

1.1 Distribution and economic importance

Fusarium wilt is a major constraint to chickpea production worldwide. It has been reported from 32 countries on six continents, Asia (Bangladesh, China, India, Iran, Iraq, Myanmar, Nepal, Pakistan, Sri Lanka, Syria and Turkey); Africa (Algeria, Egypt, Ethiopia, Kenya, Malawi, Morocco, Sudan, Tunisia, Uganda and Zambia); Europe (Hungary, Italy, Spain and the former USSR); North America (USA); South America (Argentina, Chile, Columbia, Mexico and Peru); and Australia. The pathogen results in major economic losses ranging from 10-40% worldwide (Nene et al. 1984; Kaiser et al. 1994). It is estimated to cause 10-15% yield loss annually in India, but can result in 100% loss under specific conditions (Jalali and Chand 1992).

1.2 Disease symptoms

Fusarium wilt (FW) is a seed and soil-borne disease. The disease can affect the crop at any growth stage (Fig. 1). Infected plants show typical wilt symptoms, ie, progressive drooping and yellowing of petiole, rachis and leaflets and vascular discoloration (Fig. 2). The whole seedling collapses and lies flat on the ground. Vascular discoloration of internal tissues of the roots can be seen clearly in affected plants when split vertically from the collar region downward (Fig. 3). FW in chickpea may express symptoms in two

Fig. 1. Wilt symptoms in field.

Fig. 2. Drooping of plant.

Fig. 3. Xylem discoloration.

ways, i) severe chlorosis and flaccidity combined with vascular discoloration followed by plant death within 20 days of inoculation (wilt syndrome); ii) progressive foliar chlorosis with vascular discoloration followed by plant death within 40 days of inoculation (yellowing syndrome).

1.3 Causal organism

The disease is caused by *Fusarium oxysporum* f.sp*. ciceris* (Padwick) Matuo & K.Satô (Foc). Foc produces three types of asexual spores – macroconidia, microconidia and chlamydospores. Microconidia are oval to ellipsoid, mostly monocellular, and measure 2.5-3.5 × 5-11µm. Macroconidia are produced more sparsely than microconidia; they are 3-5 septate, fusoid, slightly curved and measure 3.5-4.5 × 25-65µm. Eight races of Foc (0, 1A, 1B/C, 2, 3, 4, 5 and 6) have been reported so far worldwide (Haware and Nene 1982, Jiménez-Díaz et al. 1993). Races 1A, 2, 3 and 4 have been reported from India, whereas races 0, 1B/C, 5 and 6 are found mainly in the Mediterranean region and the United States (California). The race 1A is more widespread and has been reported in India, California and the Mediterranean region. Recently, change in the race scenario of Foc has been found and many new races from India are suspected (unpublished information).

1.4 Screening techniques

A number of screening techniques have been developed and modified over time for resistance screening of chickpea genotypes against Foc in field, greenhouse and controlled environment. Nene (1981) gave a detailed account on developing different screening techniques for identifying resistance sources to FW in chickpea. Since then the components of screening techniques have been further refined and modified. Details of the refined screening techniques are as follows:

1.4.1 Field screening

Wilt sick plot

Detailed procedure for development of a wilt sick plot is as follows:

1. Select an isolated plot of adequate size to avoid spread of the fungus inoculum from this plot to others.
2. The plot should have had chickpea crop in the previous year, and at least traces of wilt incidence should have been observed.

3. Incorporate chopped small pieces of wilted plants collected from other fields uniformly in the surface soil of the selected plot.
4. Plant a sole crop of a highly susceptible cultivar (eg, JG 62) in this plot. Ensure a good plant population and carry out normal agronomic operations.
5. 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 dicing.
6. Repeat step 3; this will help in increasing the level of the inoculum to make the soil "sick".
7. Repeat steps 3 and 4 in the next season. By the end of this season, 90% wilt incidence should be recorded. If the incidence is less than 70%, repeat steps 3 and 4 one more time.
8. Initiate screening in the next season and 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. 4).
9. From the 4th or 5th year onwards, a susceptible check (eg, JG 62, early wilter) can be planted after every 4 test rows. Also include late wilter (K 850) and wilt resistant (WR 315) checks for comparison (Fig. 5).
10. Record disease incidence periodically at 30, 45, 60 days after sowing.

[Note: It must be emphasized that by following all these steps, a sick plot in which Foc will be the most predominant pathogen can be developed. However, the presence of other soil borne pathogens cannot be avoided.]

Fig. 4. Fusarium wilt sick plot.

Fig. 5. Comparision of chickpea lines resistant and susceptible to fusarium wilt.

1.4.2 Greenhouse screening

14.2.1 Root-dip

Raising of seedlings

- Chickpea seedlings of the test as well as susceptible genotypes are grown in polythene bags filled with a mixture of sterilized river sand in a greenhouse maintained at 25±1°C for 7 days (Fig. 6).
- Plastic bags are filled up to 2/3 of its volume with sterilized river sand.
- Each genotype is surface sterilized using 2% sodium hypochlorite for 2 minutes, rinsed in sterile water 2-3 times in order to wash off sodium hypochlorite, sow (5-6 seeds) in plastic bags and allow to grow for 8 days.

Inoculum preparation

- Obtain a culture of Foc from infected chickpea plants and purify by single spore isolation on potato dextrose agar (PDA) (Fig. 7a).
- For multiplication of inoculum, a 7 mm disk of actively growing culture of Foc is inoculated into 100 ml of potato dextrose broth (PDB) in 250 ml flasks (Fig. 7b).
- Incubate the inoculated flasks at 25°C in a shaker incubator at 125 rpm for 7 days.
- Dilute the entire contents of a flask with sterilized distilled water and adjust final inoculum concentration of 6.5×10^5 conidia/ml using haemocytometer.

Fig. 6. Raising of seedlings.

Fig. 7a. Foc culture on PDA.

Fig. 7b. Foc culture on PDB.

Inoculation and transplanting

- Eight day old seedlings grown in sterile sand are uprooted, and cleaned under running tap water (Fig. 8a).
- Lowermost portion of the roots is cut and the roots dipped for 1-2 min in inoculum taken in a beaker with a concentration as given above.
- Inoculated seedlings are transplanted in pre-irrigated and sterilized vertisol and sand (4:1) mixture filled in plastic pots (12 cm diameter) (Fig. 8b).
- The inoculated pots are incubated in a greenhouse maintained at 25 ± 3°C.
- Inoculated seedlings are observed for the incubation period, latent period and disease mortality up to 60 days after inoculation (Fig. 8c).
- The test genotypes should be evaluated in at least three replications with five seedlings in each pot.

Fig. 8a. Cleaning of roots.

Fig. 8b. Inoculation and transplanting.

Fig. 8c. Disease expression seen in plant on the right.

1.4.2.2 Sick pot

1. Obtain a pure culture of Foc from infected chickpeas in your area by following standard isolation procedures (Fig. 7a).
2. Prepare a sand-maize meal medium by placing 90 g of riverbed sand, 10 g of maize meal and 20 ml of distilled water in each 250 ml Erlenmeyer flask. Autoclave the medium in the flasks at 15 lb for 20 minutes (Fig. 9a).
3. Inoculate each flask with a bit of actively growing fungal culture and incubate at 25°C for 15 days.
4. Prepare a fungus-soil mixture by hand mixing contents of each flask with 2 kg of autoclaved field soil under hygienic conditions (Fig. 9b).

5. Fill large (15 cm diameter) earthen pots with the inoculated soil 2 kg per pot from step 4. Water the pots and wait for 4 days before proceeding to the next step.
6. Sow 5-7 seeds of a highly susceptible cultivar (eg, JG 62) in each pot at a 2-3 cm depth. Water adequately and regularly (Fig. 9c).
7. Most plants should show wilting after 10 days (Fig. 9d). Remove healthy plants after 30 days and chop and incorporate all the wilted plants into the soil.
8. Repeat steps 6 and 7 until 90% wilt is observed. These pots are then ready for screening.
9. For screening, either divide a pot into two sections and plant 5 seeds of a test line in one section and 5 seeds of a susceptible control in the other or use individual pot to evaluate one genotype.

Fig. 9a. Mass multiplication of Foc.

Fig. 9b. Mixing of inoculum into soil.

Fig. 9c. Growing seedlings.

Fig. 9d. Wilted plants in sick pots.

10. Uninoculated control (pots containing only sterilized soil without pathogen) is kept for comparison.
11. These pots can be used for several successive screenings.

[Note: Screening in pots requires only up to 60 days at any time of the year, compared with 4-6 months of the season in a sick plot].

1.5 Disease incidence

Disease incidence is calculated using the following formula

$$\text{Disease incidence \%} = \frac{\text{No. of diseased plants}}{\text{Total no. of plants}} \times 100$$

Based on the disease incidence, test lines are categorized for their reaction to FW as follows (Table 1). Test lines showing disease rating <10% are considered acceptable for breeding programs; rating 10%-20% are acceptable only if lines with <10% disease incidence are not available.

Table 1. Disease reaction category based on disease incidence.

Disease incidence (%)	Disease reaction
0-10	Resistant
10.1-20.0	Moderately resistant
20.1-40.0	Moderately susceptible
40.1-100	Susceptible

1.6 Resistant sources

A large number of good sources of resistance to FW have been identified from India and worldwide (Haware et al. 1992a, Pundhir et al. 1998, Dua et al. 2001). Availability of easy and effective field screening methods has made breeding for resistance to FW an easy task. Using these techniques, over 150 wilt resistant lines have been identified at ICRISAT (details in Pande et al. 2010a). Recently, Sharma et al. (2012) have reported additional new and stable sources of resistance to FW (ICCV 05527, ICCV 05528 and ICCV 96818) and one germplasm accession (ICC 11322). A few wild chickpea genotypes resistant to wilt, multiple disease resistant chickpea lines and some new sources of resistance to FW are given in Table 2.

Table 2. Sources of resistance to FW in chickpea germplasm and breeding lines.

Genotypes	Disease reaction	Reference
IC 10149, ICC 9023, ICC 11550, ICC 902, ICCC 42, ICCV 10, Phule G 95007, KWR 108, GPF 2, IPC 99-13, IPC 99-1, IPC 2000-14, IPC 2000-41, IPC 99-10, IPC 2000-18, IPC 2000-52, IPCK 9-3, CPS 1, WR 315, JG 74, JG 1265, GL 8834, GL 87079, GL 91061, GL 86123, H 86-72, H 86-18, KPG 259-4	Resistant	Gaur and Chaturvedi 2004
Phule G-97311, 96331, 96325, 97315, Phule G-96007, 96005, 96112, 97022, 97116, 97128 92318, 97308, 97403, 95138, 97121, 95311, 910173-8, 96321, 96329, 97402, 96108, 93009, Vijay, Vishal, 95108, 95104, 96022, 95007, 95421, 92307, 95412, 93118, 95424, 95418, 97125, 97403, 910153-21, 92926 and AKG-9826	Resistant	Barbate et al. 2006
ICCs 67, 95, 637, 791, 867, 1164, 1205, 1356, 1392, 1398, 2065, 2072, 2210, 2629, 2990, 3218, 3230, 4495, 4533, 5639, 6279, 6571, 6811, 6816, 6874, 7184, 7554, 7819, 8058, 9848, 11584, 11664, 12028, 12155, 13219, 13441, 13599, 13816, 14402, 14669, 14815, 14831, 15868, 16207, 16374 and 16903 (chickpea mini-core)	Resistant	Pande et al. 2006a
ICCVs 05526, 05530, 05533, 04512, 04513, 05507, 05523, 05527, 05528, 05529, 05531, 05532, 05534	Resistant	Pande et al. 2007
ICs 327060, 327062, 327063, 327073, 327074, 327100, 327112, 327190, 327220, 327259, 327336, 327679, 327777, 327779 and 327975	Resistant	Dua et al. 2008
ICCV 09118, ICCV 09113, ICCV 09115, ICCX-030042-F4-P12-BP-BP, ICCX-030037-F4-P9-BP-BP, ICCX-030042-F4-P1-BP-BP, ICCV 09308, ICCV 09314	Resistant	Sharma et al. 2010
ICCV 05527, ICCV 05528, ICCV 96818	Stable sources of resistance	Sharma et al. 2012

2. Dry root rot

2.1 Distribution and economic importance

Dry root rot (DRR) is a potentially emerging disease of chickpea in rainfed ecologies worldwide. The disease is gaining importance under the changing scenario of climate particularly in the semi-arid tropics of Ethiopia and in central and southern India. It has also been reported from Egypt, Iran, Kenya, Lebanon, Mexico, Myanmar, Pakistan, Spain, Sri Lanka, Sudan, Syria, Tanzania, Turkey, Uganda, the USA and Zambia. It causes considerable yield losses that vary from 5% - 50% and may cause 100% losses in susceptible cultivars under favorable conditions.

2.2 Diagnostic Symptoms

The disease generally appears around flowering and podding stage. Most conspicuous symptom is sudden drying of the whole plant scattered in the field (Fig. 10). Symptoms include yellowing, drooping of the petioles and leaflets only on the tips. Leaves and stems of the affected plants are straw colored; in some cases, lower leaves turn brown (Fig. 11). The tap root turns black, shows signs of rotting, and is devoid of lateral and finer roots (Fig. 12). A grayish mycelium can sometimes be seen on the tap root. The dead roots are quite brittle and show shredding of bark and lateral root. Minute sclerotia can be seen on the exposed wood of the root and inner side of the bark or whenever split open at the collar region vertically.

Fig. 10. Dry root rot symptoms in field.

Fig. 11. Dry root rot affected plant.

Fig. 12. Rotting root system.

2.3 Causal organism

DRR is caused by necrotrophic fungus *Rhizoctonia bataticola* (Taub) Butler [Pycnidial stage – *Macrophomina phaseolina* (Tassi) Goid]. This species morphology is composed of hyphae and sclerotia (hyphal propagules). The pathogen has a predominant sclerotial stage and less frequently, a pycnidial stage in its life cycle. The sclerotia are viable for more than 12 months in the soil. It survives between crop seasons on infected plant debris.

2.4 Screening techniques

2.4.1 Field screening

The field screening techniques described for FW are also applicable for screening against DRR. The steps mentioned for the development of a wilt sick plot also hold good for DRR. The only difference is to plant a cultivar that is susceptible to *R. bataticola*, but resistant to *F. oxysporum* f. sp. *ciceris*, eg, BG 212. As pointed out under wilt screening techniques, 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 soil-borne pathogens. To confirm resistance specifically to *R. bataticola*, it would be necessary to follow the greenhouse screening techniques.

2.4.2 Greenhouse screening

2.4.2.1 Blotter paper technique

Raising of seedlings

- Grow chickpea seedlings of the test as well as susceptible genotypes in separate polythene bags in a greenhouse maintained at 25±1°C for 7 days (Fig. 13).

Fig. 13. Raising of seedlings.

- Fill plastic bags up to 2/3 of its volume with sterilized river sand.
- Surface sterilize each genotype using 2% sodium hypochlorite for 2 minutes, rinse in sterile water for 2-3 minutes in order to wash off sodium hypochlorite, sow (5-6 seeds) in plastic bags and allow to grow for 7 days.

Inoculum preparation

- Obtain a pure culture of *R. bataticola* (on PDA) from DRR infected chickpea plants in your area (Fig. 14a).
- Prepare PDB and place 100 ml of broth into one 250 ml flask and prepare as many flasks as needed. Autoclave at 15 lb for 20 minutes. One flask of inoculum will be needed to test 10 lines at once.
- Inoculate the sterilized PDB medium with the fungus and incubate for seven days at 25°C.
- Remove the mycelial mats from the flasks after seven days of incubation. Add two mycelial mats to 100 ml of sterile distilled water and macerate these in a blender for 1 minute (operate the blender intermittently) (Fig. 14b). Place this inoculum in a beaker of a suitable size.

Fig. 14a. R. bataticola culture.

Fig. 14b. R. bataticola inoculum.

Inoculation and incubation

- Uproot the seven day old seedlings of the test lines grown in polythene bags. Wash the root system in running water and rinse in sterile distilled water (Fig. 15a).

- Hold all seedlings of a test line in your hand and dip the roots into the inoculum for about 30 seconds (Fig. 15b). Remove excess inoculum by touching the edge of the beaker.
- Place 10 seedlings of the test line side by side on a blotter paper (size 45 × 25 cm with one fold; any color; thin) (Fig. 15c) 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 (Fig. 15d). Fold the blotter paper and moisten it adequately but not excessively. One folded blotter paper should have seedlings of one test line only.

Fig. 15a. Cleaning of roots.

Fig. 15b. Dipping of roots in the inoculum.

Fig. 15c. Arrangement of seedlings on blotter paper.

- Keep uninoculated and inoculated seedlings of susceptible control (BG 212) separately with each batch of test seedlings.
- Keep the folded blotters, one on top of the other, in heaps of 10 in a tray.
- Place the trays in an incubator at 35°C for eight days. Provide 12 hours of artificial light. Moisten the blotters adequately every day (Fig. 15e).
- At the end of the incubation period (eight days), examine the seedlings for the extent of root damage, and score for the disease (Fig. 15f) (Table 3).

Fig. 15d. Wrapping of inoculated seedlings.

Fig. 15e. Incubation.

Fig. 15f. Disease expression.

2.4.2.2 Sick pot

Developing sick pot

- Obtain a pure culture of *R. bataticola* from infected chickpeas in your area by following standard isolation procedures (Fig. 14a).
- Prepare a sand-maize meal medium and place 100 g in 250 ml flask. Autoclave the medium in the flasks at 15 lb for 20 minutes. Inoculate each flask with a bit of actively growing fungal culturel and incubate at 30°C for 15 days (Fig. 16a).
- Prepare a fungus-soil mixture by hand mixing the pathogen @ 50 g/kg autoclaved soil (Fig.16b).
- Fill large (6 inches diameter) pots with the inoculated soil. Approximately 2 kg of soil will be required to fill each pot. Water the pots and wait for 4 days before proceeding to the next step.
- Sow 10-20 seeds of a highly susceptible cultivar (eg, BG 212) in each pot at 2-3 cm depth. Water adequately and regularly (Fig. 16c).

Fig. 16a. Mass multiplication of R. bataticola.

Fig. 16b. Mixing of inoculum with soil.

Fig. 16c. Susceptible cultivar grown in sick soil.

Fig. 16d. DRR sick pots.

- Remove healthy plants after 30 days. Chop and incorporate all the infected plants into the soil.
- Repeat steps 5 and 6 until >90% DRR is observed. These pots are then ready for screening. These pots can be used for several successive screenings (Fig 16d).

Screening using sick pot

- Sow 5 seeds of test as well as susceptible genotypes separately in sick pot.
- Allow them to grow for 10 days at 28±2 °C in a greenhouse.
- After 10 days, impose moisture stress (60% soil moisture content) and maintain the temperature at 35 °C in the greenhouse.

Fig. 17. Maintenance of soil moisture content.

- Determine soil moisture content (SMC) using the gravimetric method (amount of water held by soil particles and expressed as percent moisture on an oven-dry basis).
- The SMC in each pot is determined by maintaining constant weight (initial weight of pot and soil) by regular weighing and replacing the deficit in each pot by watering (Fig. 17).
- Plant of susceptible genotype grown in pathogen free soil at both 60 and 100% moisture content is used as control.
- Record disease incidence periodically up to 60 days after sowing (Fig. 18).

Fig. 18. Dry root rot expression (Inoculated and control).

2.5 Disease incidence

Disease incidence is calculated using the following formula, and based on disease reaction, test genotypes are categorized for their reaction as given in Table 3.

$$\text{Disease incidence \%} = \frac{\text{No. of diseased seedlings}}{\text{Total no. of seedlings}} \times 100$$

DRR severity was recorded on 1-9 rating scale as given in Table 3 below.

Table 3. Disease rating scale on 1-9 scale for dry root rot of chickpea.

Rating	Observation	Disease reaction
1	No infection on roots	Immune
>1 and <3	Very few small lesions on roots	Resistant
>3 and <5	Lesions on roots clear but small; new roots free from infection	Moderately resistant
>5 and <7	Lesions on roots; many new roots generally free from lesions	Susceptible
>7	Roots infected and completely discolored	Highly susceptible

2.6 Resistant sources

High level of resistance to DRR is not available so far. However, a few sources of resistance reported for DRR of chickpea is given in Table 4.

Table 4. Sources of resistance to DRR in chickpea germplasm and breeding lines.

Genotypes	Disease reaction	Reference
ICC 2867, 9023, 9032, 1003, 10803, 11550 and 11551	Resistant against DRR and FW	Nene et al. 1989
ICC 2644, 10384, 10630, 112244, 11332, ICCL 81002, 810810, ICC 12263, 12441 and ICCV 90254	Resistant	Gangwar et al. 2002
Phule G 9504, 96020, 96105, 96313 and GL 91059	Moderately resistant	Gangwar et al. 2002
Kranti (ICCC 37), Bharti (ICCV 10), JG 130 and Sadbhawana (WCG 1)	Tolerant	Dhar et al. 2004
ICCs 1710, 2242, 2277, 11764, 12328, and 13441	Moderately resistant	Pande et al. 2006a
GBM-2, GBM-6, GCP-101, ICCV-2	Resistant	Jayalakshmi et al. 2008
JG 2000-07, JSC 37, MPJG 89-11551, MPJG 89-9023, CSJ 592 and Rajas	Resistant	Gupta et al. 2012

3. Ascochyta blight

3.1 Distribution and economic importance

Ascochyta blight (AB) is the most devastating foliar disease of chickpea. It has been reported from 35 countries across six continents, ie, Asia (Bangladesh, China, India, Iran, Iraq, Israel, Jordan, Lebanon, Pakistan, Syria and Turkey); Africa (Algeria, Cyprus, Egypt, Ethiopia, Kenya, Libya, Morocco, Sudan, Tanzania and Tunisia); Europe (Bulgaria, France, Greece, Hungary, Italy, Portugal, Romania, Spain and the Ukraine); North America (Canada and the USA); South America (Columbia and Mexico); and Australia. The occurrence of severe epidemics of AB has caused substantial loss in yield in India, Pakistan and European countries and more than $1 million of financial losses in the Palause region of the USA. The disease is currently the most important yield-limiting factor, potentially affecting 95% of the chickpea area in Australia (Knights and Siddique 2002).

3.2 Diagnostic symptoms

Symptoms of AB can develop on all the above-ground parts of the plant. In the field, AB is seen around flowering and podding time as patches of blighted plant (Fig. 19). The initial symptoms appear as water soaked lesions on upper leaves. Later, these lesions become dark brown spots and spread rapidly on aerial parts of the plant: leaves, petioles, flowers, pods, branches and stem (Fig. 20, 21). Pycnidia are arranged in concentric rings in the lesions, which is the characteristic symptom of the disease (Fig. 22). The spots on leaves and pods are circular, while on the stem and branches they are elongated. The apical twigs, branches and stem often show girdling, and the plant parts above the girdled portion are killed or break off even before drying. On the seed coat, dark lesions are formed with pycnidia, which often lead to seed infection through testa and infected cotyledons. Night temperatures (10°C), day temperatures (20°C) and rain accompanied by cloudy days and excessive canopy favor the disease spread.

Fig. 19. Field showing large patches of AB infected plants.

Fig. 20. Lesions caused by AB on chickpea leaflets and pods.

Fig. 21. AB symptoms on aerial plant parts.

Fig. 22. AB pycnidial bodies arranged in concentric rings.

3.3 Causal organism

Ascochyta rabiei (pass.) Labr., the causal organism of AB, exists both as an anamorph and a teleomorph. The anamorph is characterized by the formation of spherical or pear shaped black fruiting bodies called pycnidia. A pycnidium contains numerous hyaline unicellular and occasionally bicellular pycnidiospores, or conidia, developed on short conidiophores (stalks) embedded in a mucilaginous mass. The teleomorph, *Didymella rabiei* (Kovacheski) var. Arx (Syn. *Mycosphaerella rabiei* Kovacheski) is characterized by pseudothecia developing on chickpea crop residues that have overwintered in the field.

3.4 Screening techniques

A number of different screening techniques have been developed and modified over time for resistance screening of chickpea genotypes against *A. rabiei* in controlled environment and field. Details of the most commonly used screening techniques are described below.

3.4.1 Field screening

Field screening of chickpea genotypes for AB resistance is done at hot spots worldwide. In India, Dhaulakuan in Himachal Pradesh, Hisar in Haryana, Ludhiana and Gurdaspur in Punjab are identified as hot spots where the environmental conditions are favorable for the development of disease. Nene et al. (1981) gave a detailed account on developing screening techniques for chickpea germplasm against AB. Since then the components of these techniques have been further refined and modified (Haware et al. 1995, Sharma et al. 1995, Pande et al. 2011). The major components of modified field screening techniques (planting of test material, infector/indicator rows, relative humidity and disease rating scale) and steps involved in establishing disease on test and indicator chickpea lines are as follows:

- Collect debris of infected chickpea plants and store it under dry conditions for use in the following season. For 1 ha, six bags (100 × 75cm) of debris should be sufficient.
- Plant 2-4 rows of test lines (3-5 m long rows), spaced 40 cm apart following normal agronomic operations.
- Include a highly susceptible cultivar such as ICC 4991 as indicator cum spreader row after every 4 rows of test lines.

- Identify the normal time of infection. In many countries, favorable (cool and wet) weather is common around flowering time. At such a time, scatter the infected plant debris (step 1) all over the designated plot.
- Arrange a sprinkler irrigation system as a standby. This must be used if dry weather prevails at the normal time of infection.
- If the disease development is not uniform, at the flowering stage, spray spore suspension (1×10^5 conidia ml^{-1}) prepared either from infected plants from the field itself or from a pure culture of the fungus grown on chickpea flour-dextrose broth.
- Prepare chickpea flour-dextrose and pour 30 ml of broth into each flask. Autoclave at 15 lb for 20 minutes. Inoculate with a pure culture of *A. rabiei* and incubate for 10 days with 12 hours of light at 20–25°C. Dilute inoculum as indicated above.
- For a 1 ha plot, prepare 25-40 flasks (250 ml) of inoculum; 150-250 L of diluted inoculum will be enough to spray 1 ha.
- High RH (>85%) is maintained by running a sprinkler system for 10-15 minutes every hour from 10 AM to 4 PM daily (if the RH goes below 85%) (Fig. 23).
- Record disease severity when the susceptible check lines show the maximum disease rating of 9 on a 1-9 rating scale (Table 5).

Fig. 23. Field screening technique for AB. (a) Sprinlkler irrigation, (b) Disease expression.

Courtesy: Dr Ashwani Basandrai CSKMPKVV, Regional Research Station, Dhaulakanan, HP.

3.4.2 Controlled environment screening techniques (CEST)

A controlled environment facility (CEF) with adjustable temperature, humidity and photoperiod has been developed at ICRISAT for screening chickpea germplasm and breeding material for AB resistance (Fig. 24). The physical arrangements, temperature and humidity controlling devices in CEF have been provided in detail by Pande et al. (2011). Several screening techniques were developed and standardized using this CEF and are explained below.

Fig. 24. Controlled environment facility for AB screening at ICRISAT.

3.4.2.1 Seedling screening

Raising of seedlings

- Chickpea seedlings of the test genotypes are grown in plastic trays (35×25×8 cm) filled with a mixture of sterilized river sand and vermiculite (10:1) in a greenhouse maintained at 25±1°C for 10 days. (Fig. 25a & b).
- Ten genotypes including nine test lines (eight seedlings/line) and one susceptible check (ICC 4991) are sown in each tray.

Fig. 25a. Sowing of seed.

Fig. 25b. 10-day-old seedlings.

Inoculum multiplication

- Single conidial isolate of *A. rabiei* isolated from naturally infested chickpea leaves from hot spot location for AB on chickpea flour-dextrose agar (CDA) medium is used in the study.
- For mass multiplication, soak kabuli chickpea seeds in water overnight, drain the water, fill-in with approximately 70 g of soaked seeds of kabuli chickpea and autoclave at 121°C for 25 minutes in 250 ml conical flasks.
- Cool the autoclaved conical flasks with chickpea grains at room temperature for 12 h.
- Inoculate these flasks with actively growing culture of *A. rabiei* grown on CDA (Fig. 26a).
- Incubate the inoculated seeds at 20±1°C for 8 days with a 12-h photoperiod.
- Prepare spore suspension by soaking *A. rabiei* infected kabuli chickpea seeds in sterilized distilled water for 30 minutes, vortex for 2-3 minutes to dislodge the spores from the seeds (Fig. 26b).
- Filter spore suspension through a double-layered muslin cloth and adjust spore concentration to 5 × 10^4 conidia/ml using a Haemocytometer.

Fig. 26a. A. rabiei infested grains.

Fig. 26b. AB spore suspension.

Inoculation

- Transfer trays with 10 day old seedlings to CEF maintained at 20±1°C and 12 h photoperiod.
- Allow seedlings to acclimatize for 24 hours.
- After 24 hours, spray spore suspension of *A. rabiei* (5 × 10^4 conidia/ml) on the test genotypes as well as on susceptible check until runoff (Fig. 27).

Fig. 27. Inoculation.

Incubation

- After inoculation, allow the seedlings to partially dry for 30 minutes to avoid dislodging of spores.
- Adjust air temperature (20±1°C), relative humidity (100% continuously for 96 h and thereafter 6-8 h a day till the completion of experiment) and photoperiod (12h, ~1500 lux light intensity provided with fluorescent lights) throughout the experiment (Fig. 28).
- Record disease severity on a 1-9 rating scale (Table 5) when the susceptible check shows a rating of 9 (Fig. 29).

Fig. 28. Incubation.

Fig. 29. AB reaction.

3.4.2.2 Cut-twig screening

The cut-twig screening technique was standardized to facilitate screening of segregating germplasm and breeding lines without destroying the plants so that the same plant can be used for other target traits and seed production. This method of screening is very useful in an inter-specific hybridization program, where every plant may be valuable for other target traits and seed production. Resistant plants identified by this method can be further used in crosses in the same crop season. Details of the steps involved in standardization of cut-twig screening are as follows.

3.4.2.2.1 Cut-twig screening using water as support medium

Excised twigs

- Cut about 10-15 cm long tender shoots of test as well as susceptible chickpea genotypes (30-60 days) with a sharp edged blade and immediately immerse in water (Fig. 30a).

- Wrap lower portion of each excised twig with a cotton plug and transfer to a test tube (15 × 100 mm) containing fresh water (Fig. 30b).

Inoculation and incubation

- Place test tubes with excised twigs in a test tube stand and transfer to the CEF maintained at 20±1°C and ~1500 lux light intensity (12 hours a day).
- Allow excised twigs to acclimatize for 24 hours.
- Inoculate by spraying a spore suspension (5×10^4 conidia/ml) of *A. rabiei* (Fig. 30c).
- Inoculation method and incubation conditions are similar to that mentioned in seedling screening technique.
- Record disease severity on a 1-9 rating scale (Table 5) when the susceptible check shows a rating of 9 (Fig. 30d).

Fig. 30a. Excised twigs.

Fig. 30b. Wrapping with cotton plug.

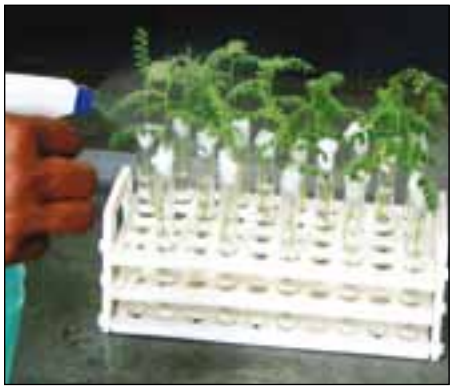

Fig. 30c. Inoculation.

Fig. 30d. AB expression.

3.4.2.2.2. Cut-twig screening using sand as support medium

The technique is a modification of cut-twig screening-water. In cut-twig screening-water, only a single twig can be evaluated per test tube. Therefore, the technique was modified and instead of water in test tubes, sand and vermiculite mixture in trays was used to support the excised twigs. The advantage of cut-twig screening-sand over cut-twig screening-water is that more twigs (about 60-70 twigs/tray) can be screened at one time and it is also more economical and easier to handle. Steps involved in screening with this technique are as follows:

- Plant detached twigs in sterilized moist sand filled in plastic trays (35 × 25 × 8 cm) (Fig. 31a).
- Place twigs of susceptible cultivars along with test entries in each tray for comparison.
- Transfer trays to the CEF, maintain at 20±1°C, allow to acclimatize for 24 hours.
- Inoculation method and incubation conditions are similar to those used for seedling screening technique.
- Score disease severity on a 1-9 rating scale (Table 5) when the susceptible check shows a rating of 9 (Fig. 31b).

Fig. 31a. Planting of excised twigs in sand.

Fig. 31b. AB reaction.

3.5 Disease rating scale

The rating scale for AB on chickpea seedlings is given in Table 5. Based on the disease score, the test lines are categorized for their reaction to AB infection as follows: 1 = Asymptomatic (A); 1.1-3 = resistant (R); 3.1-5 = moderately resistant (MR); 5.1-7 = susceptible (S); and 7.1-9 = highly susceptible (HS). Test lines showing rating 1-3 are considered acceptable

for breeding programs; rating 3.1-5 are acceptable only if lines with rating 1-3 are not available; rating 5.1-9 are not acceptable.

Table 5. Disease rating scale (1-9 scale) for Ascochyta blight.

Rating	Symptoms	Resistance class
1	No symptoms	Asymptomatic
2	Minute lesions prominent on the apical stem	Resistant
3	Lesions up to 5 mm in size and slight drooping of the apical stem	Resistant
4	Lesions obvious on all plant parts and clear drooping of apical stem	Moderately resistant
5	Lesions obvious on all plant parts; defoliation initiated; breaking and drying of branches slight to moderate	Moderately resistant
6	Lesions as in 5; defoliation; broken, dry branches common; some plants killed	Susceptible
7	Lesions as in 5; defoliation; broken, dry branches very common; up to 25% of the plants killed	Susceptible
8	Symptoms as in 7 but up to 50% of the plants killed	Highly susceptible
9	Symptoms as in 7 but up to 100% of the plants killed	Highly susceptible

3.6 Resistant sources

Several sources of resistance to AB have been identified in different chickpea growing areas of the world and a few sources are released as cultivars in different countries (details in Pande et al. 2010b). New sources and stable resistance identified at ICRISAT for AB are given in Table 6.

Table 6. Sources of resistance to AB in chickpea germplasm and breeding lines.		
Genotype	**Disease reaction**	**Reference**
ICCV 04524, 04525, 04526, 04537,98811, 98816, 04523, 05571, 04052, 04530, 05546, 05514, 04505,05502, 05512, 04509, 05551, 05503, 05511, 05513, 05515, 05523, 98818, 04512, 05530, 04513, 05531.	Resistant	Pande et al. 2010b
ICC 76, ICC 607, ICC 652, ICC 1069, ICC 1400, ICC 1468, ICC 3932, ICC 4033, ICC 4181, ICC 4200, ICC 4936, ICC 6304, ICC 6373, ICC 6945, ICC 8923, ICC 12952, ICC 12961,ICC 14911, ICC 14912, ICC 14917, ICC 15628, ICC 15973, ICC 15975, ICC 15976, ICC 15978, ICC 15979, ICC 15980, ICC 15982, ICC 15988, ICC 15989, ICC 15990, ICC 15991, ICC 16953, ICC 16955, ICC 17000, ICCV 04530, ICCV 04537, ICCV 98815, ICCV 98818, ICCX-810800, EC 516729, EC 516709, EC 516771, EC 516792, EC 516793, EC 516796, EC 516824, EC 516850, EC 516867, EC 516878, EC 516895, EC 516916, EC 516934, EC 516936, EC 516957, EC 516967, EC 516971, EC 516974, EC 517003, EC 517011, EC 517012, EC 517023, EC 517025, EC 517039, FLIP 81-70, FLIP 82-52C.	Stable resistant sources	Pande et al. 2010b
Wild species *Cicer judiacum* (ICC 17211, IG 69986, IG 70030, IG 70037 and IG 70038)	Resistant	Pande et al. 2005, 2006b

4. Botrytis gray mold

4.1 Distribution and economic importance

Botrytis gray mold (BGM) is an important foliar disease of chickpea in the South Asian countries, Bangladesh, Nepal, India and Pakistan. It has also been reported from Argentina, Australia, Canada, Columbia, Hungary, Mexico, Myanmar, Spain, Turkey, the USA and Vietnam. The disease may cause 100% yield loss in susceptible cultivars under conducive conditions.

4.2 Diagnostic symptoms

BGM symptoms appear on all aerial parts of the plant. The growing tips and flowers are most vulnerable (Fig. 32). Symptoms appear first as water-soaked lesions on stems (Fig. 33), branches, leaves, flowers and pods. The lesions then turn grey or dark brown and are covered with erect, hairy sporophores and masses of hyaline conidia (Fig. 34). Stem lesions are 10-30 mm long and may girdle the stem completely. Affected leaves and flowers turn into a rotting mass. On thick and hard stems, the mold growth gradually transform in to a dirty grey mass containing dark green to black sporodochia. Sometimes, tiny black sclerotia are formed on dead tissue. When disease affects pods, no seed or only shrivelled seeds are formed. Occasionally, grayish white mycelia can be seen on immature seeds.

Fig. 32. BGM infected branches, leaves and flowers.

Fig. 33. Lesions caused by B. cinerea on stem.

Fig. 34. Fungal sporulation on pod.

4.3 Causal organism

BGM is caused by *Botrytis cinerea* Pers. ex Fr., (teleomorph: *Botryotinia fuckeliana* (de Bary) Whetzel), necrotrophic pathogen. The fungus grows profusely on dead flowers. It forms white and cottony appearance colonies on PDA. The mycelium is septate and brown. One celled hyaline, oval conidia are formed in clusters on short sterigmata present at the tips of the conidiophore. The sporodochia formed on host surface may turn into hard sclerotial masses. The teleomorphic state is formed after fertilization of sclerotia with uninucleate microconidia followed by exposure to low temperature. The teleomorphic state of fungus infects chickpea.

4.4 Screening techniques

Techniques to screen chickpea germplasm and breeding material for BGM resistance have been developed by different research institutes. The various screening techniques, viz. field and controlled environment used for screening germplasm and breeding material for BGM resistance are as follows.

4.4.1 Field screening

- This technique is efficient for large-scale screening of germplasm and breeding material in segregating generations.
- The test lines are sown in 2-3 m long rows spaced at 30 × 10 cm. Indicator-cum-infector rows of a susceptible cultivar, H 208 or JG 62, are sown after every two test rows.
- When the plants are 70-80 days old (at the onset of flowering), the field is irrigated in the morning and plants are inoculated by spraying a spore suspension (50,000 spores/ml) of 10-day old culture of *B. cinerea*.
- From the morning of the following day, sprinkler irrigation or the perfo-spray system is run every day for about 15 minutes every 1 or 2 hours from 9 am to 5 pm depending upon the environmental conditions (Fig. 35).
- The inoculation of plants is repeated twice at 10 day intervals after the first inoculation. The disease severity is scored on a 1-9 rating scale in mid-February, and in the first and last weeks of March (Table 7) (Pande et al. 2002).

Fig. 35. Field screening for BGM. (a) Sprinkler irrigation, (b) Disease expression.

4.4.2 Controlled environment screening

Using the CEF as mentioned for AB, the following screening techniques have been developed for screening against BGM.

4.4.2.1 Seedling screening

Raising of seedlings

- Chickpea seedlings of the test genotypes are grown in plastic trays (35×25×8 cm) filled with a mixture of sterilized river sand and vermiculite (4:1) in a greenhouse maintained at 25±1°C for 10 days.

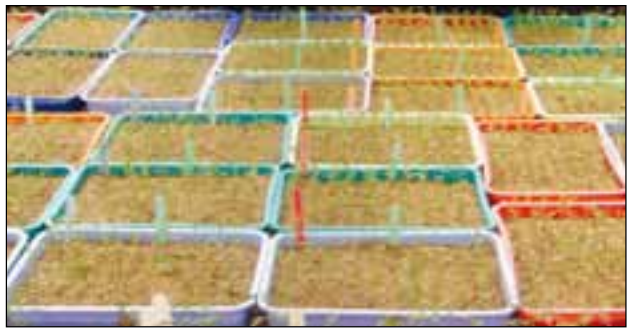

Fig. 36. Raising of seedlings.

- A susceptible control, JG 62/H 208, is sown as an indicator in each tray along with nine test genotypes (Fig. 36).

Inoculation multiplication

- Single conidial isolate of *B. cinerea* isolated from naturally infested chickpea leaves from hot spot location for BGM on PDA medium is used in the study (Fig. 37a).
- For mass multiplication, take mature flower petals of *Tagetus erecta* L. (marigold), dry them in shade and wash the petals using sterilized distilled water (SDW). Drain the water, take 20 g of petals in a 150 ml conical flask, add 1% dextrose solution (1-2 ml) to it and autoclave at 15 lb for 15 minutes.
- Inoculate above-prepared marigold petals medium with 1 cm disc of actively growing culture of *B. cinerea* grown on PDA medium (Fig. 37b).
- Incubate at 15°C for 8 days with a 12 hour photoperiod in an incubator.
- Harvest the conidia produced on marigold petals into sterile distilled water.

Fig 37a. Botryis cinerea.

- Filter spore suspension through a cheese cloth and adjust spore concentration to 3×10^5 ml^{-1} using a heamocytometer.

Inoculation

- Transfer trays with 10 day old seedlings to CEF maintained at 15±1°C and ~1500 lux light intensity for a 12 h photoperiod.
- Allow seedlings to acclimatize for 24 hours.
- After 24 hours, spray spore suspension of *B. cinerea* (3×10^5 conidia/ml) on the test genotypes as well as on the susceptible check until runoff (Fig. 37c).

Incubation

- After inoculation, the seedlings are allowed to partially dry for 30 minutes to avoid dislodging of spores; temperature at 15±1°C and 100% RH is maintained for 96 h; thereafter, RH is reduced to 95% and run for 7-8 h a day until the end of the experiment (Fig. 37d).
- Disease severity is scored on a 1-9 rating scale at 14 and 20 DAI (Fig. 37e) and Table 7.

Fig. 37b. Mass multiplication of B. cinerea.

Fig. 37c. Inoculation.

Fig. 37d. Incubation in controlled environment growth room.

Fig. 37e. Disease expression.

4.4.2.2 Cut-twig screening

The cut-twig screening technique (CTST) proved very effective and efficient for screening breeding material derived from a wide range of hybridization programs, particularly for back crossing, even though this technique may affect plant chemistry and, thus, resistance as mentioned above. Details of the techniques are given below.

4.4.2.2.1 Cut-twig screening using water as support medium

Excised twigs

- Cut about 10-15 cm long tender shoots of test as well as susceptible chickpea genotypes (30-60 days) with a sharp edged blade and immediately immerse in water (Fig. 38a).
- Wrap lower portion of each excised twig with a cotton plug and transfer to a test tube (15 × 100 mm) containing fresh water (Fig. 38b).

Inoculation and incubation

- Place test tubes with excised twigs in a test tube stand and transfer to the CEF maintained at 15±1°C and ~1500 lux light intensity (12 hours a day).
- Allow excised twigs to acclimatize for 24 hours. Inoculate by spraying a spore suspension (3×10^5 conidia/ml) of *B. cinerea* as explained in the above section (Fig. 38c). Twigs of a susceptible variety (H 208, G 543 or L 550) are used as susceptible controls.

Fig. 38a. Excised twigs.

Fig. 38b. Wrapping with cotton.

- Post-inoculation and incubation conditions are similar to that mentioned in seedling screening technique.
- Record disease severity on a 1-9 rating scale (Table 7) when the susceptible check shows a rating of 9 (Fig. 38d).

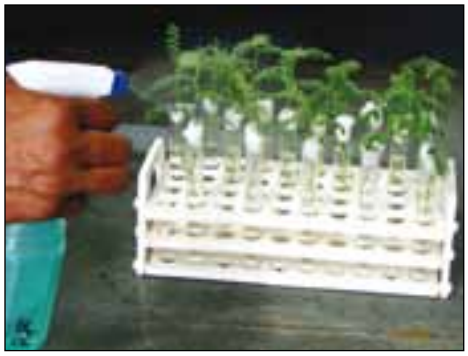

Fig. 38c. Inoculation.

Fig. 38d. Disease expression.

4.4.2.2.2 Cut-twig screening using sand as support medium

The technique is a modification of cut-twig screening using water. Steps involved in screening with this technique are as follows

- Plant detached twigs in sterilized moist sand filled in plastic trays (35 × 25 × 8 cm) (Fig. 39a).
- Place twigs of susceptible cultivars along with test entries in each tray for comparison.
- Transfer trays to the CEF, maintain at 15±1°C, and allow to acclimatize for 24 hours.
- Inoculation method and incubation conditions are similar to those used for seedling screening technique.
- Score disease severity on a 1-9 rating scale (Table 7) when the susceptible check shows a rating of 9 (Fig. 39b).

Fig. 39a. Planting of excised twigs.

Fig. 39b. Disease expression.

4.5 Disease rating scale

A 1-9 rating scale used for scoring BGM is given in Table 7.

Table 7. Rating scale of BGM of chickpea for field and controlled environment screening.

Rating	Field screening	Controlled environment screening
1	No infection on any part of the plant	No infection on any part of the plant
2	Minute lesions on lower leaves, flowers and pods covered under dense plant canopy; usually not visible	Minute water soaked lesions on emerging tender leaves; usually not seen
3	Lesions on <5% of the leaves, flowers and pods covered under dense plant canopy	Minute water soaked lesions on 1-5% of emerging and uppermost tender leaves; usually seen after careful examination
4	Lesions and some fungal growth (conidiophores and conidia) can be seen on up to 15% of the leaves, flowers, pods and branches covered under dense plant canopy	Water soaked lesions on 6-10% of uppermost tender leaves and tender shoots
5	Lesions and slight fungal growth on up to 25% of the leaves, flowers, pods, stems and branches covered under dense plant canopy	Water soaked lesions; soft rotting of 11-25% of tender leaves and shoots
6	Lesions and fungal growth on up to 40% of the leaves, flowers, pods, stems and branches; defoliation; 25% of the plants killed	Water soaked lesions; soft rotting of 26-40% of top leaves and shoots
7	Large lesions and good fungal growth on up to 60% of the leaves, flowers, pods, stem and branches; defoliation common; drying of branches; 50% of the plants killed	Soft rotting; fungal growth on 41-55% of the leaves and branches
8	Large lesions and profuse fungal growth on up to 80% of the leaves, flowers, pods, stems and branches; severe defoliation; drying of branches; 75% of the plants killed	Soft rotting; fungal growth on 56-70% of the leaves, branches and stems
9	Large lesions; very profuse fungal growth on up to 100% of the flowers, pods, stems and branches; almost complete defoliation; drying of plants; 100% of the plants killed	Extensive soft rotting; fungal growth on >70% of the leaves, branches and stems

4.6 Resistant sources

Host plant resistance available to BGM infection in chickpea, determined by screening programs conducted at various locations in India and other countries, is given in Table 8.

Table 8. Sources of resistance to BGM resistance in chickpea.

Resistance	Genotype	Reference
Moderately resistant	ICCVs 04052, 04503, 04506, 04509, 04510, 04512, 04513, 04514, 04515, 04601, 04602, 04607, 04608, 04609, 05501, 05502, 05505, 05506, 05508, 05509, 05510, 05516, 05520, 05521, 05522, 05524, 05525, 05526, 05527, 05528, 05529, 05530, 05531, 05532, 05533, 05535, 05543, 05546, 05547, 05553, 05555, 05560, 05565, 05566, 05571, 05572, 05573, 05603	Pande et al. 2007
Resistant	ICC 12483, ICC 1069, ICCV 41, BG 276	Sunil Kumar et al. 2006
Moderately resistant	ICC 13252, ICC 7612, ICCV 2, Pusa 209, GNG 146, BG 261, Pusa 244, GNG 663, GPL 14	Sunil Kumar et al. 2006
Resistant (Wild species)	ILWC 35/S-1 (*C. echinospermum*) and ILWC 9/S-1 (*C. pinnatifidum*)	Singh et al. 1991
Resistant (Wild species)	*C. judaicum* 182, *C. judaicum* ILWC 19-2, *C. pinnatifidum* 188, *C. pinnatifidum* 189, *C. pinnatifidum* 199, *C. pinnatifidum* ILWC 9/S-1, *C. bijugum* ILWC 9/S-1, *C. bijugum* ILWC 7/S-1, *C. echinospermum* ILWC 35/S-1 and *C. echinospermum* ILWC 39	Singh et al. 1998

5. Collar rot

5.1 Distribution and economic importance

Collar rot (CR) of chickpea is prevalent in areas with high soil moisture and warm temperature. The disease is widespread and is of economic importance in Bangladesh, Colombia, Egypt, Ethiopia, India, Kenya, Mexico, Nepal, Pakistan, the Philippines, Sudan, Syria, Uganda and Zambia.

5.2 Diagnostic symptoms

The disease usually occurs at the seedling stage (up to 6 weeks after sowing) particularly in wet soil conditions (Fig. 40). The affected plants turn yellow and show signs of rotting at the collar region (Fig. 41). Whitish mycelial strands can be seen on wilted plant parts (Fig. 42). On seedlings uprooted from wet soil in the early stages of infection, rapeseed-like sclerotia (1 mm diameter) can be observed. The infected seedlings are usually seen as small patches in field.

Fig. 40. Field symptoms of collar rot.

Fig. 41. Yellowing of leaves and constriction at the collar region.

Fig. 42. White mycelial strands of S. rolfsii on an infected chickpea plant.

5.3 Causal organism

Collar rot is caused by *Sclerotium rolfsii Sacc*. Sclerotia serve as the principal over-wintering structure and primary inoculum for disease. Sclerotia (spherical and 0.05-1.0 mm in diameter) are formed laterally from main hyphal strands and are initially white, becoming light brown to dark brown at maturity. The mycelium is septate, hyaline and branched at acute angles. Mature mycelium is in cord-like strands with clamp connections between hyphal cells. Newly developed mycelium is slender, lacks clamp connections, and is snow white with a silky luster. Hyphal cells are binucleate to multinucleate. The basidial stage is rarely found in nature.

5.4 Screening techniques

5.4.1 Greenhouse screening

Inoculum preparation

- Isolate a culture of *S. rolfsii* from CR infected chickpea plants following standard isolation procedures and purify by single sclerotial culture on PDA (Fig. 43a).
- For multiplication of *S. rolfsii*, prepare a groundnut (*Arachis hypogaea*) shell medium (soak partially broken groundnut shells in water for 2 hours separately and autoclave at 121°C for 45 minutes). Inoculate each flask with a mycelial bit (1 cm^2) from a 10 day old culture of *S. rolfsii* grown on PDA and incubate at 25 ± 1°C with a 12 hour photoperiod for 20 days (Fig. 43b).

Fig. 43a. S. rolfsii culure on PDA.

Fig. 43b. Mass multiplication of S. rolfsii on groundnut shell.

Sick bed preparation

- Prepare the potting medium by mixing groundnut shell impregnated with *S. rolfsii* with autoclaved soil (medium vertisol) at a rate of 100 g of inoculum per 4 kg of soil.
- Fill the potting medium in metal trays (70 × 30 × 16 cm) and mix thoroughly (Fig. 43c).

Fig. 43c. Sick bed preparation.

Sowing test lines

- Sow 10 seeds of each cultivar (surface sterilized with 0.1% clorox solution, before sowing) in infested soil at 2-3 cm depth, keeping 2 inches row to row and 1.5 inches plant to plant distance. Water adequately and regularly (Fig. 43d).
- Incubate the trays in the greenhouse at 28-30°C.

Fig. 43d. Raising of test lines in sick soil.

5.5 Disease incidence

Record observations on collar rot incidence as seed rot and seedling mortality (Fig. 43e) and calculate disease incidence as follows,

Fig. 43e. Disease expression.

$$\text{\% seed rot} = \frac{\text{Number of ungerminated seeds}}{\text{Total number of seeds sown}} \times 100$$

$$\text{\% seedling mortality} = \frac{\text{Number of infected seedling}}{\text{Total no. of seedlings}} \times 100$$

$$\text{Total mortality} = (\text{\% seed rot} + \text{\% seedling mortality})$$

5.6 Resistant sources

No sources of resistance are available to CR so far. However, efforts are being made and preliminary screening of chickpea genotypes suggests low levels of resistance to collar rot in germplasm accessions ICC 1696, ICC 4709 and ICC 14391 (SD Singh, personal communication); breeding lines RSG 130, 132 and 191 (Chitale et al. 1990); and cultivar SAKI 9516 (Dua et al. 2001).

6. Black root rot

6.1 Distribution and economic importance

Black root rot (BRR) is a minor disease reported from Argentina, Nebraska, Chile, India, Mexico, Spain, Syria and the USA.

6.2 Symptoms

The disease can occur at any stage but more often at the seedling stage. The affected plants turn yellow, wilt and prematurely dry (Fig. 44). Dead plants are seen scattered in the field. The root system is rotted, most of the finer roots are destroyed, while the taproot remains intact, but becomes dark and necrotic (Fig. 45). Affected plants dry prematurely but may go on producing new roots if sufficient moisture is available. Excessive moisture and moderately high temperatures (25°–30°C) encourage disease development.

Fig. 44. Field symptoms of black root rot.

Fig. 45. Rotting of taproot and lateral roots (right).

6.3 Causal organism

The causal organism of BRR is *Fusarium solani.* It is a filamentous fungus and the anamorph is *Haematonectria haematococca*. It produces microconidia, macroconidia and chlamydospores. The microconidia are produced on long monophilides in false head, this distinguishes *F. solani* from *F. oxysporum*.

6.4 Screening techniques

6.4.1 Greenhouse screening

- *F. solani* isolated from chickpea roots is multiplied on PDB (100 ml in a 250 ml flask) for seven days at 25°C in a shaker at 125 rpm (Fig. 46a).
- Seedlings are raised in plastic pots (15 cm) in autoclaved sand soil (vertisol) mixture (1:1) for seven days (Fig. 46b).
- The inoculum is diluted by adding 100 ml of sterilized water and mixed thoroughly.

Fig. 46a. Mass multiplication of F. solani on PDB broth.

Fig. 46b. Raising of seedlings seen in plant on the right.

- For inoculation, about 3 cm of soil around the seedlings is removed and 5 ml of inoculum is poured near the collar region. The soil surface is re-levelled. The soil is kept moist before and after inoculation (Fig. 46c).
- 25 days after inoculation, the seedlings are carefully removed from each pot and the soil is washed from the roots (Fig. 46d). Data is recorded on a 1-9 rating scale.

Fig. 46c. Inoculation.

Fig. 46d. Disease expression.

6.4.2 Root-dip technique

The root-dip technique described for fusarium wilt is also applicable for screening against this disease. The steps mentioned for growing the seedlings and preparing inoculum are the same. The only difference is that the culture used is *F. solani* and the disease score is recorded on a 1-9 rating scale based on root blackening.

6.5 Disease rating scale

The severity of BRR is recorded on a 1-9 rating scale as shown in Table 9.

Table 9. Disease rating for BRR of chickpea.	
Disease rating	Symptoms
1	Plant healthy; no root infection
3	Plant healthy; slight infection in hypocotyl region along with restricted lesions on a few roots
5	Plant stunted; black root rotting on 50% of roots
7	Plant stunted accompanied by yellowing of leaves; 75% of roots affected
9	Plants with severe stunting and yellowing of leaves; completely rotted roots

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Appendix

1. Potato dextrose broth

Peeled potato = 200 g
Dextrose = 20 g
Distilled water = 1000 ml

Preparation: Boil peeled and sliced potatoes in distilled water and pass the mixture through a muslin cloth. Collect the extract, add dextrose to it and stir well. Make the final volume 1000 ml and distribute 100 ml in a 250 ml Erlenmeyer conical flask. Autoclave it at 121.6°C at 15 lb pressure for 20 minutes.

2. Potato dextrose agar

Peeled potato = 200 g
Dextrose = 20 g
Distilled water = 1000 ml

Preparation: Boil peeled and sliced potatoes in distilled water and pass the mixture through a muslin cloth. Collect the extract, add dextrose and agar. Then stir well. Make the final volume 1000 ml and distribute 100 ml in a 250 ml Erlenmeyer conical flask. Autoclave it at 121.6°C at 15 lb pressure for 20 minutes.

3. Chickpea extract dextrose broth medium

Chickpea seed granules = 40 g
Dextrose = 20 g
Distilled water = 1000 ml

Preparation: Boil chickpea seed granules for 10 minutes and pass through muslin cloth. Collect the filtrate, and make the final volume 1000 ml. Add dextrose and mix thoroughly. Pour 100 ml solution in a 250 ml Erlenmeyer conical flask and autoclave at 15 lb pressure for 20 minutes.

4. Chickpea flour dextrose broth medium

Chickpea flour = 40 g
Dextrose = 20 g
Distilled water = 1000 ml

Preparation: Mix all the ingredients thoroughly and make the final volume 1000 ml. Pour 100 ml solution in a 250 ml Erlenmeyer conical flask and autoclave at 15 lb pressure for 20 minutes.

5. Sand maize meal medium

Sand = 90 g
Maize granules= 10 g
Distilled water = 20 ml

Preparation: Mix sand and maize granules in a 250 ml Erlenmeyer conical flask. Add distilled water and shake well. Autoclave the medium at 15 lb for 45 minutes.

About ICRISAT

The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) is a non-profit, non-political organization that conducts agricultural research for development in Asia and sub-Saharan Africa with a wide array of partners throughout the world. Covering 6.5 million square kilometers of land in 55 countries, the semi-arid tropics have over 2 billion people, and 644 million of these are the poorest of the poor. ICRISAT and its partners help empower these poor people to overcome poverty, hunger, malnutrition and a degraded environment through better and more resilient agriculture.

ICRISAT is headquartered in Hyderabad, Andhra Pradesh, India, with two regional hubs and four country offices in sub-Saharan Africa. It belongs to the Consortium of Centers supported by the Consultative Group on International Agricultural Research (CGIAR).

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