Host Plant Resistance to Ascochyta Blight of Chickpea

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Abstract

Ascochyta blight (AB) caused by *Ascochyta rabiei* (Pass.) Labr. is an important and widespread disease of chickpea worldwide. The disease is particularly severe under cool and humid weather conditions. A large volume of literature is available on studies related to various aspects of AB including biology, epidemiology and management methods. In this bulletin, attempts have been made to briefly describe the distribution, economic importance, symptoms, causal organism, pathogen variability and host plant resistance. Information on recent advances in host plant resistance of AB and detailed descriptions of refined screening techniques developed at ICRISAT and elsewhere for identification of resistant sources are provided. This bulletin provides comprehensive information on screening methods for AB, and will be useful to all researchers involved in Ascochyta blight management through host plant resistance.

Cover: (From L to R) Stem girdling and breaking of branches, circular lesions on pods, close-up of lesion with pycnidial bodies arranged in concentric rings.

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Host Plant Resistance to Ascochyta Blight of Chickpea

S Pande, M Sharma, PM Gaur and CLL Gowda

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Foreword

I am very pleased to write a foreword for your publication on *Host plant resistance to Ascochyta blight of chickpea,* jointly compiled by plant pathologists and breeders from the International Crops Research Institute for the Semi-Arid Tropics. Ascochyta blight caused by *Ascochyta rabiei* (Pass.) Labr. is a serious problem in most chickpea environments where cool and humid weather prevails during the crop season. ICRISAT's research focus has mainly been on managing Ascochyta blight of chickpea through host plant resistance (HPR) that involves developing effective and repeatable resistance screening techniques, identifying sources of resistance and breeding for resistance through collaborative efforts of pathologists and breeders.

As climate change further increases climate variability, the risk of droughts and floods, diseases and pests, and threats to agricultural productivity and production will escalate. Hence the key to a sustainable future lies in improving crop productivity through ecologically friendly farming systems that are more effective in harnessing nature, and that will go a long way in enhancing the livelihoods of the poor. Therefore, developing appropriate strategies for disease management effective under these situations in the future are critical.

ICRISAT has developed several controlled environment and field screening techniques for Ascochyta blight and shared them with National Agricultural Research Systems (NARS) in India and elsewhere. These techniques have been used to screen a large number of germplasm accessions and breeding lines to identify resistance and enhance resistance breeding. Screening techniques have also been refined and made simpler. Currently, a number of chickpea lines are available with moderate to high levels of resistance to this disease. Several of the resistant and advanced breeding lines have been shared globally with chickpea researchers in both public and private institutions. Apart from describing the screening techniques developed at ICRISAT and elsewhere, this bulletin elucidates on recent developments in HPR of Ascochyta blight.

I am sure that this simple yet comprehensive compilation will serve as a useful guide to chickpea researchers and students, especially those who have interest in managing Ascochyta blight through HPR.

William D Dar
Director General, ICRISAT

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1. Introduction

Ascochyta blight (AB), caused by *Ascochyta rabiei* (Pass.) Labr. is an important foliar disease of chickpea (*Cicer arietinum* L.) worldwide that causes grain yield and quality losses up to 100% (Pande et al. 2005). The disease is devastating in areas where cool (15-25ºC) and humid weather (>150 mm rainfall) prevails during the crop season (Pande et al. 2005). The type of inoculum, inoculum concentration and physiological plant growth also affect the degree of infection and the amount of crop loss. Several epidemics of AB resulting in complete yield loss have been reported in the past (Pande et al. 2005). Currently, AB is the most important yield-limiting factor in Australia, Canada and USA, potentially affecting 95% of the area sown to chickpea (Knights and Siddique 2002, http://www.pulse.ab.ca/ascoch.pdf).

Using fungicides and/or cultural practices for the management of AB under environmental conditions favorable to disease development is uneconomical and difficult to carry out. Of the available management approaches, host plant resistance (HPR) is the most reliable, economical and effective method for managing AB. 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 AB resistant lines (Pande et al. 2005). This bulletin describes the advances in host plant resistance to AB and provides details of refined and repeatable screening techniques for identification of resistance sources and screening breeding materials. New sources of stable host plant resistance to AB based on controlled environment and field screening techniques are also reported.

2. Distribution and economic importance

The occurrence of AB of chickpea has been reported from 35 countries across six continents – 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 Ukraine); North America (Canada and USA); South America (Columbia and Mexico); and Australia (Nene et al. 1996, Pande et al. 2005, Knights and Siddique 2002). The economic importance of the disease is evident from the frequent occurrence of epidemics in several chickpea-growing areas of the world. More than 20 epidemics have been reported and most of these epidemics have occurred

in Pakistan, India and European countries. The disease occurred in epidemic form in 1981-1983 in the north-western states of India and in Pakistan, resulting in total loss of the crop (Singh et al. 1982, 1984). Severe epidemics of AB have also caused substantial yield loss in the Mediterranean region (Hawtin and Singh 1984). More than $1 million financial losses in the Palause region of the USA were reported by Kaiser and Muehlbauer (1988). The disease is currently the most important yield-limiting factor in Australia, potentially affecting 95% of the chickpea area (Knights and Siddique 2002). The 1998 Australian epidemic (Galloway and MacLeod 2003) devastated the chickpea industry and caused a drastic reduction in chickpea area from 105,000 ha in 1998 to 15,000 ha in 1999 in western Australia (Moore et al. 2004). Similarly, AB resulted in >70% yield losses in western Canada (http://www.pulse.ab.ca/ascoch.pdf).

3. Disease symptoms

Symptoms of AB can develop on all the above ground parts of the plant. AB is seed borne and can also spread through debris. In the field, the disease is usually seen around flowering and podding time as patches of blighted plants (Fig. 1). However, the disease can also appear at a very early crop growth stage under favorable environmental conditions. The initial symptoms appear

Fig. 1. Patches of Ascochyta blight infected plants.

as water-soaked lesions on the 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. 2). The spots on leaves and pods are circular, while on the stem and branches they are elongated (Fig. 3a & b). 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 (Fig. 4).

Fig. 2. Symptoms on aerial parts of the plant.

Fig. 3. (a) Elongated lesions on stem and (b) circular lesions on pods.

Fig. 4. Girdling and breaking of branches.

Pycnidia are arranged in concentric rings in the lesions, which is the characteristic symptom of the disease (Fig. 5a & b). On the seed coat, lesions formed often lead to seed infection through the testa and infected cotyledons. Infected seed may be discolored and possess deep, round or irregular cankers, sometimes bearing conidia visible with the naked eye. Infection during the pod formation stage results in shrivelled and infected seed (Fig. 6).

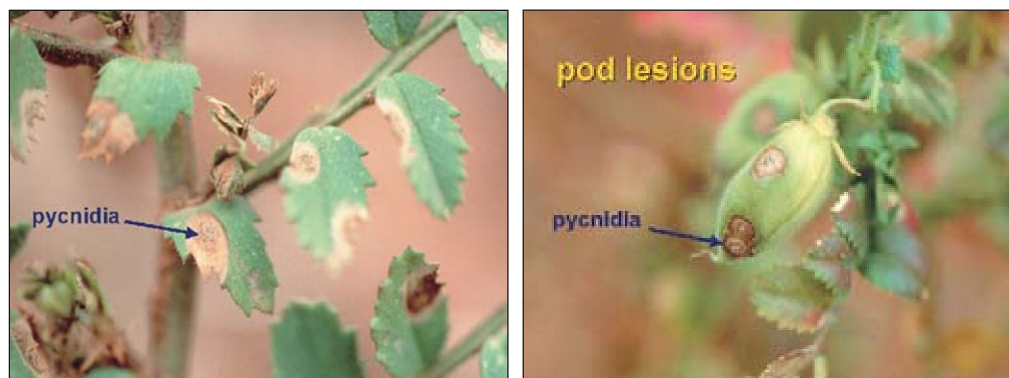

Fig. 5a. Pycnidial bodies arranged in concentric rings.

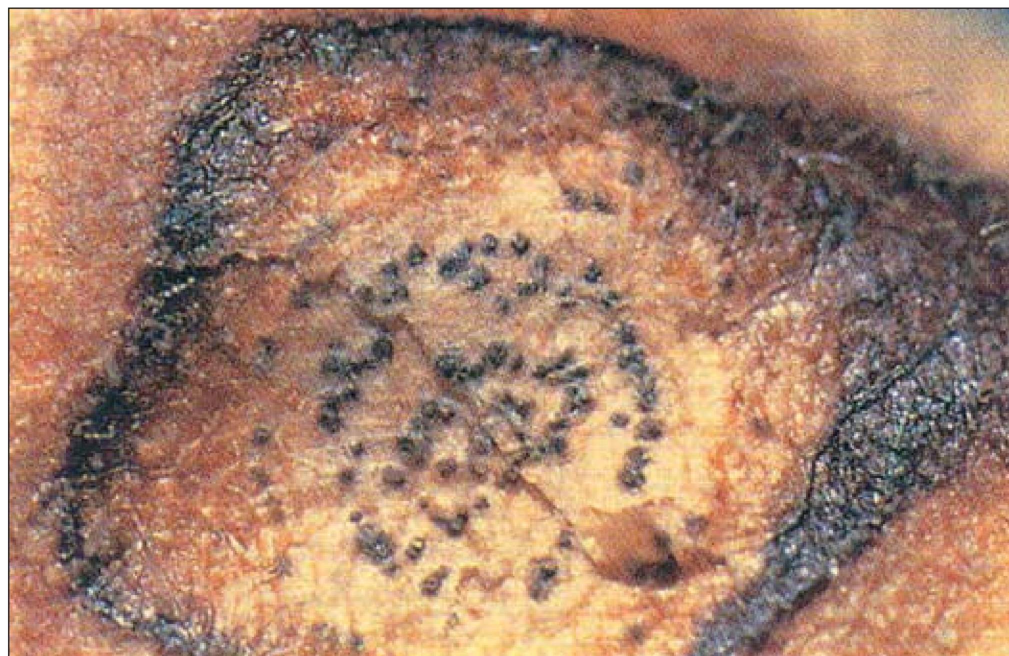

Fig. 5b. Close-up of lesion with pycnidial bodies arranged in concentric rings.

Fig. 6. (a) Healthy and (b) Ascochyta blight infected seeds.

4. Causal organism

Ascochyta rabiei (Pass.) Labr. (anamorph), the causal organism of AB of chickpea 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. Pycnidiospores are oval to oblong, straight or slightly bent at one or both ends and measure 6-12–4-6 µm (Punithalingam and Holliday 1972, Nene 1982). The fungus grows readily on a variety of nutrient media, the best being chickpea dextrose agar (Nene et al. 1981). *A. rabiei* generally produces a pale cream colored mycelium in which pale brown to black pycnidia are immersed (Fig. 7). Cultures vary in

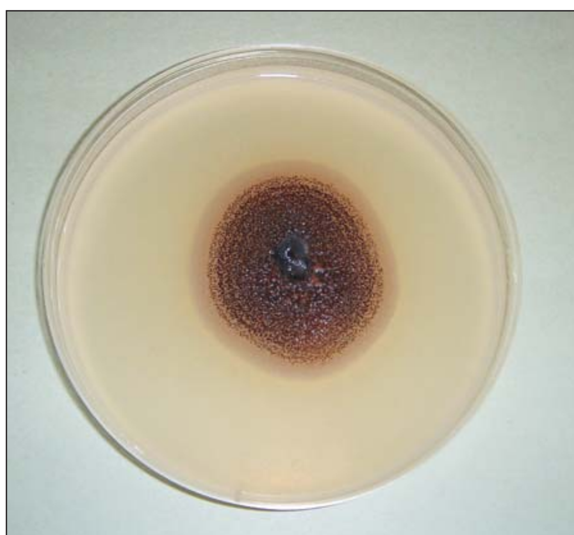

Fig. 7. Ascochyta rabiei *on chickpea dextrose agar medium.*

morphology and color, with isolates often producing unicellular conidia (Fig. 8) (CAB International 2000).

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. For successful sexual reproduction, the teleomorph requires pairing of 2 compatible mating types (MAT1-1 and MAT1-2), which are widely distributed in several major chickpea-growing areas of the world (Haware 1987, Kaiser 1997, Armstrong et al. 2001). However, Khan et al. (2002) suggested that *A. rabiei* is a heterothallic and the two mating types are not present in all chickpea growing areas. Pseudothecia are dark brown to black, subglobose, 120-270 μm in diameter, erupting from the host tissue and without a conspicuous ostiole. Binucleate asci are cylindrical to subclavate surrounded by paraphyses and contain 8 hyaline unequally bicellular ascospores. Ascospores are ellipsoid to biconic with a constriction at the septum and measure 9.5-16×4.5-7 μm.

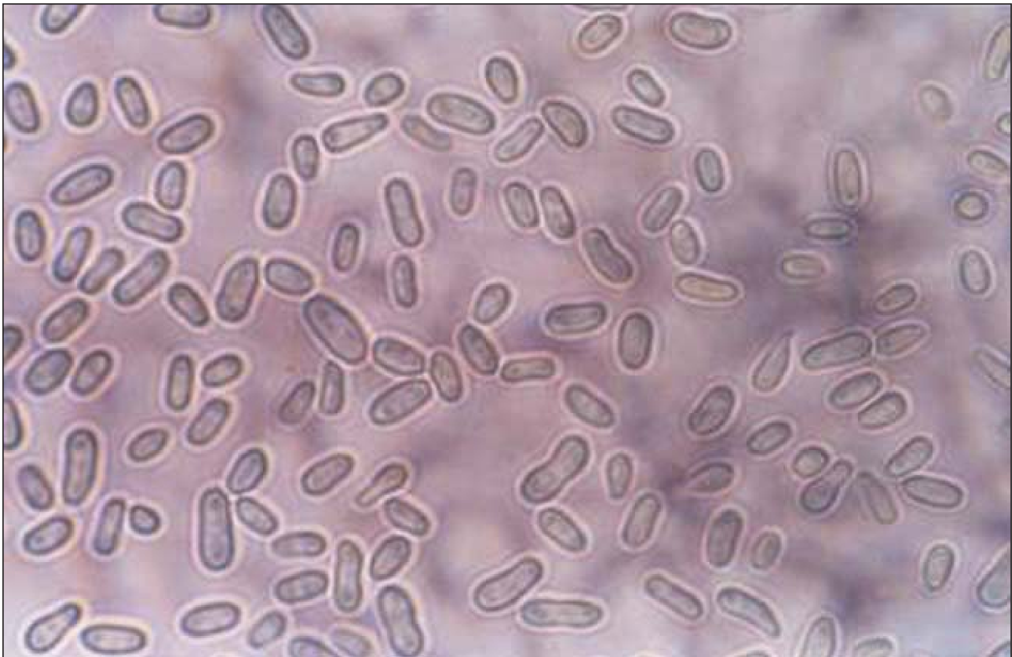

Fig. 8. Conidia of Ascochyta rabiei*.*

5. Pathogen variability

Existence of different races of *A. rabiei* has been reported due to the variations in host-pathogen interactions and breakdown of HPR in some cultivars at different locations. The presence of teleomorph (*D. rabiei*) in the *A. rabiei* life cycle contributes to variability within the pathogen population, which may generate new combinations of virulence genes and thus the development of new pathotypes. However, *A. rabiei* is heterothallic and the two mating types are not present in all chickpea growing areas (Khan et al. 2002). Based on the virulence of *A. rabiei* isolates on different genotypes, the existence of 2-12 races of *A. rabiei* has been reported by several researchers (Ambardar and Singh 1996, Singh and Sharma 1998, Porta-Puglia et al. 1996, Jan and Wiese 1991 and Chongo et al. 2004). However, in these studies, no definite relationships were observed between virulence of the isolates, host genotypes, their geographical origin, and morphological characteristics such as spore size, colony color and radial growth in vitro. A standard set of international differential lines, which can clearly distinguish all *A. rabiei* isolates from a broad geographical area, may help in the identification of different races of *A. rabiei*, if they indeed exist.

A few molecular genetic studies have been conducted to assess the variability in *A. rabiei* isolates in different parts of the world using DNA markers such as RAPD (Santra et al. 2001, Chongo et al. 2004), AFLP (Peever et al. 2004), SSR (Geistlinger et al. 2000) and SSR based oligo-fingerprinting (Jamil et al. 2000, Barve et al. 2004 and Varshney et al. 2009). However, little is known about the levels of genetic diversity of this pathogen in India. Therefore, it is necessary to monitor changes in the pathogen population to anticipate the breakdown of resistance in existing chickpea cultivars.

6. Host plant resistance

Host plant resistance (HPR) is most economical, either alone or as a major component of integrated AB management strategies. The preliminary step for exploiting HPR is the development of reliable and repeatable resistance screening techniques. A number of disease screening techniques under field and greenhouse conditions have been reported, but with variable results to AB (Nene et al. 1981, Singh et al. 1984, Sharma et al. 1995, Bretag and Meredith 2002). Variations in reactions to AB using these screening techniques were attributed to factors such as inoculum concentration, inoculation method, plant age at inoculation and environmental conditions such as temperature, humidity and photoperiod. Significant changes in any of these components

reduce the efficacy of the screening techniques resulting in failure of disease development. Therefore, identification and standardization of various factors influencing AB infection and development are essential to develop repeatable field and greenhouse screening techniques for identification of reliable and stable resistance sources. Major research efforts at ICRISAT, Patancheru, India, have been on the development of reliable screening techniques that clearly discern chickpea lines into different AB severity classes. Further, emphasis has been laid to develop and/or refine screening techniques that have reliability, uniformity and repeatability for the success of the AB resistance breeding program.

6.1 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 field, greenhouse and controlled environments. A controlled environment facility has been set up at ICRISAT, Patancheru that facilitates screening of chickpea lines against AB. Details of these screening techniques are described below.

6.1.1 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 were 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 (Singh et al. 1982; Reddy et al. 1984; Haware et al. 1995; Sharma et al. 1995; Pande et al. 2005, 2009). The major components of 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:

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

4. 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.
5. Arrange 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, 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. 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. Prepare chickpea flour-dextrose broth by mixing 40 g of chickpea flour and 20 g of dextrose in 1,000 ml of water. 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 as indicated above.
7. 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. 9).
8. Record disease severity when the susceptible check lines show the maximum disease rating of 9 on a 1-9 rating scale (Table 1).

Fig. 9. Field screening technique using sprinkler irrigation for Ascochyta blight development at Dhaulakuan, Himachal Pradesh.
Courtesy: Dr Ashwani Kumar, CSKHPKVV, Regional Research Station, Dhaulakuan, HP.

Table 1. Disease rating scale for Ascochyta blight.		
Rating	Symptoms	Resistant 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

6.1.2 Greenhouse screening

6.1.2.1. Isolation plant propagator

Nene et al. (1981) gave a detailed account of this technique for screening chickpea germplasm against AB (Fig. 10). The procedure is described below:

1. Use an isolation plant propagator.
2. Whenever necessary, operate evaporative coolers around the propagators to maintain temperatures below 30°C.
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 and vermiculite (3:1).
5. Grow at least 10 seedlings of one accession in one pot. In addition, raise seedlings of a susceptible control in one pot in each sub-section of the propagator.
6. Obtain a pure culture of an aggressive isolate of *A. rabiei* on chickpea flour-dextrose broth (Nene et al. 1981).
7. Remove fungal growth from the flasks and dilute with sterilized distilled water to 2×10^4 conidia/ml.

Fig. 10. Isolation plant propagator screening technique for Ascochyta blight.

8. Use a hand sprayer to spray inoculate the two-week-old seedlings with the fungus inoculum. Cover plants with a plastic cover for 10 days.
9. Record the incubation period, percentage infection and percentage mortality.
10. Score the disease severity on a 1-9 rating scale twice, once when the susceptible check shows a rating of 9 (Table 1), and again 10 days later.

6.1.2.2. Pot culture

1. Chickpea plants are grown in polythene pots (15 cm) in the greenhouse.
2. The pots containing one-month-old test plants and susceptible controls are placed in 8-10 cm deep circular pit.
3. Water is added to the pots before inoculation.
4. The plants are inoculated by spraying spore suspension (1×10^5 conidia/ml) and covering with a moist muslin cloth chamber.
5. Moist muslin cloth chambers are removed after 48 hours, and the plants are kept wet during the daytime by spraying water daily for up to 13 days from 10 AM to 4 PM.
6. Leaf wetness is maintained for 21 days to ensure maximum disease severity (Singh et al. 1982).
7. Disease severity is recorded on a 1-9 rating scale (Table 1).

6.1.3. Controlled environment screening

A controlled environment facility (CEF) with adjustable temperature, humidity and photoperiod has been developed at ICRISAT, Patancheru, India for screening chickpea germplasm and breeding lines for AB resistance (Fig. 11). The physical arrangements, temperature and humidity controlling devices in CEF have been discussed by Haware et al. (1995). Later, scientists modified the components of the CEF and developed resistance screening techniques with controlled weather variables required for penetration, infection, colonization and development of AB. Details of the modified screening techniques using CEF are as follows.

Fig. 11. Controlled environment facility for Ascochyta blight screening at ICRISAT, Patancheru.

6.1.3.1 Whole plant screening technique (WPST)

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. 12a).
- Ten genotypes including nine test lines (eight seedlings/line) and one susceptible check (ICC 4991) are sown in each tray (Fig. 12b).

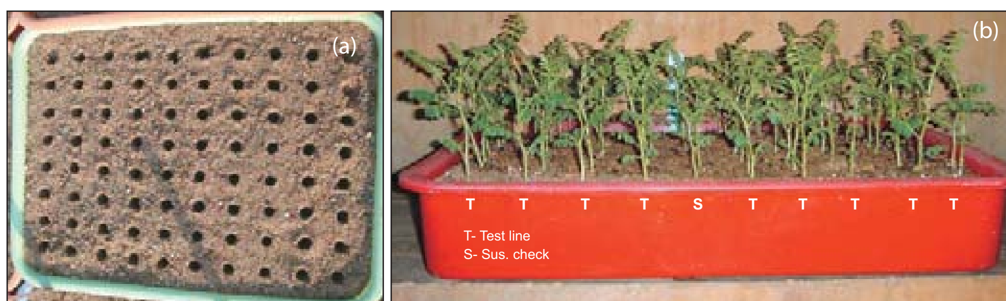

Fig. 12. Raising of seedlings (a) Sowing of seeds, (b) Ten-day-old seedlings.

Inoculum multiplication

- Single conidial isolate of *A. rabiei* isolated from naturally infested chickpea leaves from hot spot location for AB on 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. 13a).
- 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. 13b).
- Filter spore suspension through a double-layered muslin cloth and adjust spore concentration to 5×10^4 conidia/ml using a haemocytometer.

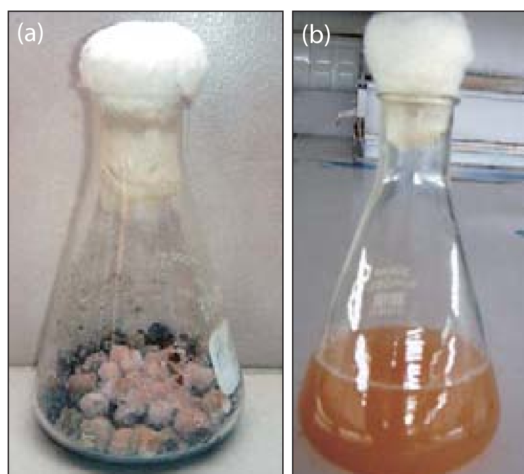

Fig. 13. Inoculum preparation (a). A. rabiei *infested grains (b). Spore suspension.*

Inoculation of the test entries

- 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 run-off (Fig. 14).

Fig. 14. Spraying of spore suspension on test genotypes.

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. 15).

Fig. 15. Incubation conditions in controlled environment facility at ICRISAT.

- Record disease severity on a 1-9 rating scale (Table 1) when the susceptible check shows a rating of 9 (Fig. 16 & 19).

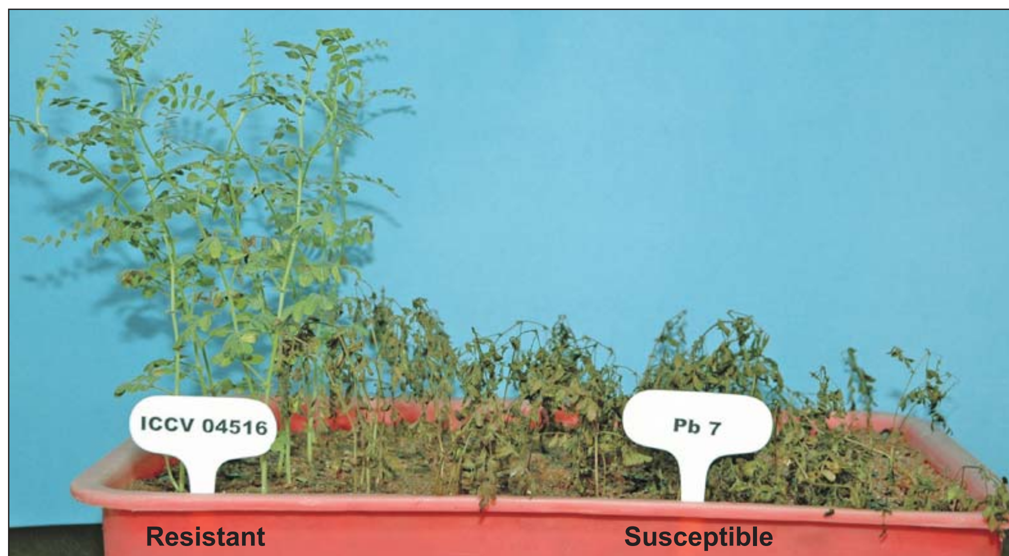

Fig. 16. Ascochyta blight reaction using whole plant screening technique.

The advantage of this technique is that it is rapid, durable, easy to handle and economical as about 1,000 genotypes (in three replications) can be screened in one cycle. A large number of chickpea genotypes have been screened using this technique at ICRISAT (Pande et al. 2005).

6.1.3.2 Cut-twig screening techniques (CTST)

The cut-twig screening technique (CTST) 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 hybridisation 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. Originally the technique was developed by Sharma et al. (1995) using excised twigs from the test chickpea plant and placing them in test tubes containing water and incubating in moist – muslin – cloth chamber. However, the technique has been further modified using the CEF at ICRISAT. Details of the steps involved in standardization of CTSTs are as follows.

6.1.3.2.1 Cut-twig screening technique using water as support medium (CTST-W)

Excised twigs

- Cut about 10-15 cm long tender shoots of test chickpea genotypes (30-60 days) with a sharp edged blade and immediately immerse in water (Fig. 17a).
- Wrap lower portion of each excised twig with a cotton plug and transfer to a test tube (15×100 mm) containing fresh water (Fig. 17b).

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) (Fig. 15).
- Allow excised twigs to acclimatize for 24 hours.
- Inoculate by spraying a spore suspension (5×10^4 conidia/ml) of *A. rabiei*.
- Inoculation method and incubation conditions are similar to that mentioned in WPST.
- Record disease severity on a 1-9 rating scale (Table 1) when the susceptible check shows a rating of 9 (Fig. 17c).

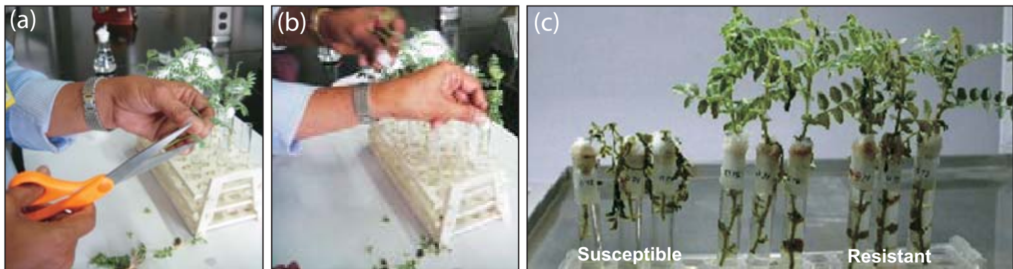

Fig.17. Cut-twig screening technique – water (a) excised twigs (b) wrapping with cotton plug (c) Ascochyta blight reaction.

6.1.3.2.2 Cut-twig screening technique using sand as support medium (CTST-S)

The technique is a modification of CTST-W. The resources required are more in CTST-W since only a single twig can be evaluated per test tube. Therefore, CTST-W was modified and instead of water in test tubes, sand and vermiculite mixture in trays (CTST-S) was used to support the excised twigs. The advantage of CTST-S over CTST-W 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 CTST-S are as follows:

- Detached twigs are planted in sterilized moist sand filled in plastic trays (35×25×8 cm) (Fig. 18a).
- Twigs of susceptible cultivars along with test entries are also kept in each tray for comparison.
- Trays are transferred to the CEF, maintained at 20±1°C, allowed to acclimatize for 24 hours (Fig. 15).
- Inoculation method and incubation conditions are similar to those used for WPST.
- Score disease severity on a 1-9 rating scale (Table 1) when the susceptible check shows a rating of 9 (Fig. 18b).

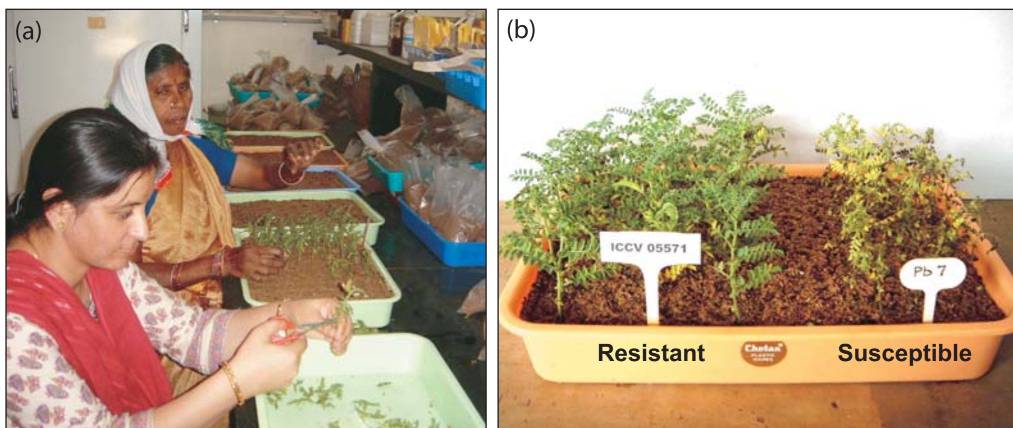

Fig.18. Cut-twig screening technique – sand. (a) planting of excised twigs in sand, (b) Ascochyta blight reaction.

6.1.3.3. Detached leaf technique

Detached leaf technique involves the following steps:

- Collect leaflets from the most recent fully expanded leaves from 15-day-old chickpea plants.
- Allow the detached leaflets to float, lower surface facing downwards in tap water inside 90 mm petri dishes.
- Inoculate upper surfaces of the leaflets with 5 μl of *A. rabiei* suspension.
- Incubate the leaflets for 14 days at 20±1°C, with a 12-hour photoperiod.
- Record disease severity based on the number of leaflets infected and lesion size (Dolar et al. 1994).

6.2 Disease rating scale

Rating scale for AB on chickpea seedlings is given in Table 1 and Fig. 19. 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.

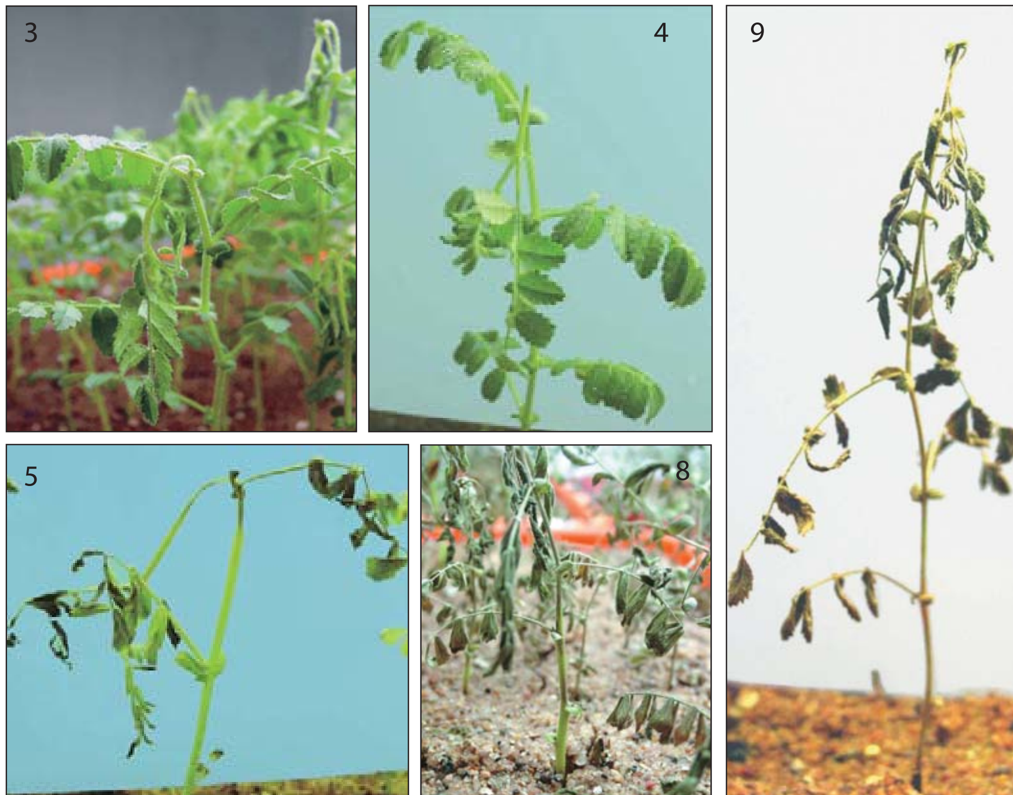

Fig. 19. Disease severity of Ascochyta blight infection on 1-9 rating scale.

6.3 Comparison of screening techniques

A significant positive correlation was found between CESTs (WPST, CTST-W, CTST-S) and FST. Significant positive correlation was found between WPST (10-day-old seedlings) and CTST ($r=0.94$, $P<0.0001$) and FST ($r=0.89$, $P<0.0001$). Similarly, CTST and FST were highly correlated ($r=0.88$, $P<0.0001$) (Pande et al. 2009). High positive correlation between controlled environment and field screening techniques suggests that AB severity ratings at seedling

stage could be adequate to discriminate chickpea lines for resistant/susceptible reactions. Positive correlations between greenhouse and field screening techniques for AB have been observed by others as well (Haware et al. 1995, Sharma et al. 1995).

7. Sources of resistance

Several sources of resistance to AB have been identified in studies conducted in different chickpea-growing areas of the world (Table 2). Some of the resistance sources were also released as cultivars (Table 3). This includes chickpea lines screened for AB resistance at the International Centre for Agricultural Research in the Dry Areas (ICARDA), Syria. At ICARDA, >25,000 chickpea lines have been screened for AB resistance and 14 stable sources of resistance have been identified. ILC 200, ICC 4475, ICC 6328, ILC 6482 and ICC 12004 were found to be resistant to 6 races of *A. rabiei* in repeated field and greenhouse evaluations (Singh and Reddy 1992). In total, 1,584 AB resistant chickpea lines were developed with a range of maturity, plant height, and seed size not previously available to growers in the blight epidemic areas in the Mediterranean region. These included 92 lines resistant to 6 races of *A. rabiei* (Singh and Reddy 1996).

New sources of resistance to AB have been identified at ICRISAT (Table 4). A high level of resistance (≤3.0 on 1-9 scale) has been identified in 29 chickpea breeding lines with a range of maturity (early, medium and late). Stable sources of resistance to AB have been identified through a multilocation, multiyear evaluation of chickpea lines at five locations in India (Dhaulakuan, Ludhiana, Gurdaspur, Hisar and ICRISAT, Patancheru), which could be used in breeding location specific AB resistant varieties (Table 5).

High level of AB resistance has also been identified among wild *Cicer* species. Resistance against AB has been identified in *C. judiacum, C. pinnatifidum, C. echinospermum* and *C. reticulatum* (Singh et al. 1981; Singh and Reddy 1991; Collard et al. 2001 and Pande et al. 2005, 2006).

At ICRISAT, Patancheru, 148 accessions belonging to seven *Cicer* species (*C. bijugam*, *C. cuneatum, C. echinospermum, C. judiacum, C. pinnatifidum, C. reticulatum* and *C. yamashitae*) were evaluated in the CEF. Of the 148 accessions, five accessions of *C. judiacum* were resistant and 34 moderately resistant. Thirteen accessions of *C. pinnatifidum*, seven of *C. bijugam*, and one accession of *C. cuneatum* were moderately resistant to AB (Pande et al. 2006) (Table 6).

Table 2. Sources of resistance to Ascochyta blight in chickpea germplasm.

Genotype	Remarks	Reference
ICC 3634, ICC 4200, ICC 4248, ICC 5124, ICC 6981, ILC 196, ILC 3346, ILC 3956 and ILC 4421	-	Reddy and Singh 1984
ILC 72, ILC 191, ILC 3279 and ILC 3856	Resistant in eight chickpea-growing countries (including India, Pakistan and the Mediterranean region)	Singh et al. 1984
ICC 76, ICC 187, ICC 607, ICC 1121, ICC 1136, ICC 1416, ICC 1754, ICC 1762, ICC 1903, ICC 7773, ILC 236, ILC 482, ILC 484, ILC 2548 and ILC 2956	ILC 482 and ICC 1903 were always rated 1 on a 1-9 scale	Katiyar and Sood 1985
ICC 4000 and 4014	Both foliage and pods of ICC 4000 were resistant to Ascochyta blight	Singh and Kapoor 1985
ILC 3864, ILC 3870 and ILC 4421	-	Pal and Singh 1990
ILC 190, ILC 201, ILC 202, ILC 2506, ILC 3856, ILC 5928, ICC 3996 and FLIP 83-48	Resistant to 3 to 6 races of *A. rabiei*	Singh and Reddy 1990
ILC 5586, ILC 5894, ILC 5926, ILC 6482, ILC 7795, ICC 4475, ICC 6328 and ICC 12004	Resistant both in greenhouse and field	Singh and Reddy 1992
ILC 3287	Rate-reducing phenomenon of Ascochyta blight observed	Reddy and Singh 1993
CG 715, ACC 76, H 86-8, H 86-100 and HK 86-120	-	Singh and Pal 1993
ILC 3896, ICC 7514, NEC 123, P 1279-2 and P 4268-1	-	Gaur and Singh 1996
ICC 8161	-	Shukla and Pandya 1988
ICC 1278, ICC 1284, ICC 1285 and ICC 1304	-	Wadud and Riaz 1988
FLIP 92-262C, FLIP 92-110C and FLIP 92-154C	-	Toker et al. 1999

Table 3. Ascochyta blight resistant chickpea lines/varieties/cultivars released in different countries.				
Accession	Country of origin	Country of release	Released name	Year of release
ILC 72	n.a.	Italy	Califfo	1990
ILC 72	n.a.	Spain	Fardan	1985
ILC 195	USSR	Egypt	Giza 195	1995
ILC 195	USSR	Morocco	ILC 195	1986
ILC 195	USSR	Turkey	ILC 195	1986
ILC 200	USSR	Spain	Zegri	1985
ILC 202	USSR	China	ILC 202	1988
ILC 237	Spain	Oman	ILC 237	1988
ILC 411	Iran	China	ILC 411	1988
ILC 464	Turkey	Cyprus	Kyrenia	1987
ILC 482	Turkey	Algeria	ILC 482	1988
ILC 482	Turkey	France	TS 1009	1988
ILC 482	Turkey	Iran	ILC 482	1995
ILC 482	Turkey	Iraq	Rafidain	1992
ILC 482	Turkey	Jordan	Jubeiha 2	1990
ILC 482	Turkey	Lebanon	Janta 2	1989
ILC 482	Turkey	Morocco	ILC 482	1986
ILC 482	Turkey	Syria	Ghab 1	1986
ILC 482	Turkey	Turkey	Guney Sarisi 482	1986
ILC 484	Turkey	Libya	ILC 484	1993
ILC 533	Egypt	Georgia	Elixir	2000
ILC 915	Iran	Sudan	Jebel Marra-I	1994
ILC 1335	Afghanistan	Sudan	Shendi	1987
ILC 2548	USSR	Spain	Almena	1985
ILC 2555	Ethiopia	Spain	Alcazaba	1985
ILC 3279	USSR	Algeria	ILC 3279	1988
ILC 3279	USSR	China	ILC 3279	1988
ILC 3279	USSR	Cyprus	Yialosa	1984
ILC 3279	USSR	Iran	ILC 3279	1995

...Continued

Table 3. Contd.				
Accession	Country of origin	Country of release	Released name	Year of release
ILC 3279	USSR	Iraq	Dijla	1992
ILC 3279	USSR	Italy	Sultano	1990
ILC 3279	USSR	Jordan	Jubeiha 3	1990
ILC 3279	USSR	Syria	Ghab 2	1986
ILC 3279	USSR	Tunisia	Chetoui	1987
ILC 6188	France	Italy	Ali	1998

n.a.- Not available
Source: ICRISAT (2002)

Table 4. Ascochyta blight reaction of 29 resistant breeding lines in controlled environment and field screening.

Breeding lines	Ascochyta blight reaction (1-9 scale)									
	Controlled environment				Field					
	Patancheru				Ludhiana			Dhaulakuan		
	2005	2006	2007	Mean	2005	2006	Mean	2008	2009	Mean
ICCV 04524	2.0	2.0	2.0	2.0	3.0	3.0	3.0	2.0	3.0	2.5
ICCV 04525	2.3	2.0	2.6	2.3	3.0	3.0	3.0	3.0	2.0	2.5
ICCV 04526	2.3	2.6	2.0	2.3	2.3	2.7	3.0	3.0	2.0	2.5
ICCV 04537	2.3	2.0	2.6	2.3	3.0	3.0	3.0	3.0	2.0	2.5
ICCV 98811	2.7	2.5	2.9	2.7	3.0	3.0	3.0	3.0	2.0	2.5
ICCV 98816	2.3	2.6	2.3	2.3	2.7	2.7	2.7	-	2.0	2.0
ICCV 04523	2.7	3.0	2.4	2.7	2.0	2.0	2.0	2.0	2.0	2.0
ICCV 05571	2.8	3.0	2.6	2.8	3.0	3.0	3.0	3.0	2.0	2.5
ICCV 04052	3.0	2.0	4.0	3.0	3.0	3.0	3.0	-	-	-
ICCV 04530	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	-	3.0
ICCV 05546	3.7	3.0	2.3	3.0	2.7	2.3	3.0	3.0	-	3.0
ICCV 05514	3.0	2.3	3.7	3.0	3.0	3.0	3.0	2.0	2.0	2.0
ICCV 04505	3.3	3.0	2.7	3.0	2.7	2.3	3.0	3.0	2.0	2.5
ICCV 05502	3.0	3.3	2.7	3.0	3.0	3.0	3.0	3.0	2.0	2.5

...Continued

Table 4. Contd.										
Breeding lines	Ascochyta blight reaction (1-9 scale)									
	Controlled environment				Field					
	Patancheru				Ludhiana			Dhaulakuan		
	2005	2006	2007	Mean	2005	2006	Mean	2008	2009	Mean
ICCV 05512	2.7	4.0	2.3	3.0	3.0	3.0	3.0	3.0	3.0	3.0
ICCV 04509	2.3	4.0	2.7	3.0	3.0	3.0	3.0	3.0	2.0	2.5
ICCV 05547	3.7	3.0	2.3	3.0	3.0	3.0	3.0	3.0	-	3.0
ICCV 05551	3.7	3.0	2.3	3.0	3.0	3.0	3.0	3.0	3.0	3.0
ICCV 05503	2.0	4.0	3.0	3.0	3.0	3.0	3.0	3.0	-	3.0
ICCV 05511	2.3	4.0	2.7	3.0	3.0	3.0	3.0	3.0	2.0	2.5
ICCV 05513	2.7	3.0	3.3	3.0	2.3	3.7	3.0	3.0	2.0	2.5
ICCV 05515	3.0	3.3	2.7	3.0	3.3	2.7	3.0	3.0	2.0	2.5
ICCV 05523	3.0	3.0	3.0	3.0	4.0	2.0	3.0	3.0	2.0	2.5
ICCV 05532	2.7	3.3	3.0	3.0	3.3	2.7	3.0	3.0	2.0	2.5
ICCV 98818	3.0	3.3	2.7	3.0	3.0	3.0	3.0	3.0	3.0	3.0
ICCV 04512	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	2.0	2.5
ICCV 05530	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	2.0	2.5
ICCV 04513	3.0	3.7	2.3	3.0	3.0	3.0	3.0	3.0	2.0	2.5
ICCV 05531	3.0	3.3	2.7	3.0	3.0	3.0	3.0	2.0	2.0	2.0
ICC 4991 (Sus. check to AB)	9.0	9.0	9.0	9.0	9.0	9.0	9.0	9.0	7.0	8.5
SEM	0.25	0.25	0.26		0.25	0.31		0.28	0.34	
SED	0.35	0.35	0.36		0.36	0.44		0.38	0.42	
CV (%)	13.95	12.71	14.48		13.67	16.19		14.75	15.84	
LSD (5%)	0.71	0.71	0.74		0.73	0.89		0.81	0.71	

- data not available.

Table 5. Stable sources of resistance to Ascochyta blight from 2002-2008.		
AB score[1]	No. of lines	Chickpea lines[2]
≤5	66	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,

[1]AB score on 1-9 scale, where ≤5= resistant to moderately resistant reaction.
[2]Based on the field evaluation at four locations in India (Hisar, Dhaulakuan, Gurdaspur, Ludhiana), one in Pakistan and in controlled environment at ICRISAT, Patancheru.

Table 6. Reaction of wild *Cicer* species to Ascochyta blight in controlled environment screening at ICRISAT, Patancheru, India.

Wild *Cicer* species	No. of accessions screened	AB score (1-9 scale)[a]			
		R	MR	S	HS
C. bijugam	30	-	7	20	3
C. cuneatum	3	-	1	2	-
C. echinospermum	4	-	-	3	1
C. judiacum	47	5[b]	34	8	-
C. pinnatifidum	27	-	13	13	1
C. reticulatum	31	-	-	15	16
C. yamashitae	6	-	-	-	6
Total	148	5	55	61	27

[a] Based on the disease score, the wild accessions were categorized for their reaction to Ascochyta blight infection as follows: 1.0-3.0 = resistant (R), 3.1-5.0 = moderately resistant (MR), 5.1-7.0 = susceptible (S) and 7.1-9.0 = highly susceptible (HS).

[b]ICC 17211, IG 69986, IG 70030, IG 70037 and IG 70038.

8. Genetic basis of host-pathogen interaction

Detailed information on the number, nature and diversity of genes conferring resistance is a prerequisite for exploiting a particular genotype in resistance breeding programs. Initial studies suggested that AB resistance of chickpea is due to either a single dominant or a recessive gene (Singh and Reddy 1991). Depending on the mode of inheritance of resistance to AB in F_1 and F_2 generations, Singh and Reddy (1983) concluded that the resistance in ILC 72, ILC 183, ILC 200 and ILC 4935 was due to a single dominant gene, and in ILC 191 to a single recessive gene. Allelic studies by Tewari and Pandey (1986) indicated the presence of 3 independently segregating dominant genes for resistance in P 1215-1, EC 26446 and PG 82-1, and a recessive gene in BRG 8. However, 2 dominant complementary genes were reported to have control over disease resistance: Arc_1 and Arc_2 in genotype GLG 84038, and Arc_3 and Arc_4 in GL 84099. Similarly, the resistance in ICC 1468 is reported to be controlled by 1 dominant gene ($Arc_{5(3,4)}$) and 1 recessive gene (Arc_1). In these 3 genotypes, inter-allelic interactions, additive gene effects and dominance influenced the resistance (Dey and Singh 1993).

Recent studies on RILs suggest that several QTLs are involved in controlling resistance to AB. Three sets of RILs derived from 2 intraspecific crosses, PI 359075(1) × FLIP 84-92C(2) and Blanco Lechoso × Dwelley, and 1 interspecific cross, FLIP 84-92C(3) × *C. reticulatum* (PI 489777), were developed at ARS-USDA, Pullman, WA (http://www.nps.ars.usda.gov/). Evaluation of disease response in these RILs indicated that 3 recessive complementary major genes with some modifiers conferred AB resistance. Absence of 1 or 2 of the major genes confers susceptibility while presence of the modifiers determines the degree of resistance (Tekeoglu et al. 2000). These different estimates of the genetic basis of AB resistance result from the use of different fungal isolates and host genotypes. Clearly, AB resistance breeding is a complex endeavor, as any new cultivar needs to carry resistance genes effective against a range of AB isolates. However, these studies seem to suggest that there is a range of different sources of resistance. Pyramiding of different resistance genes may facilitate building up the level of resistance and increasing the durability of that resistance.

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