Screening for Ascochyta Blight Resistance in Chickpea Under Controlled Environment and Field Conditions

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ABSTRACT

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The occurrence and severity of Ascochyta blight of chickpea is influenced by environmental factors, which can complicate field screening. A controlled-environment plant growth room was developed at ICRISAT Asia Center to rapidly screen chickpea genotypes within 15 days for resistance to Ascochyta blight caused by *Ascochyta rabiei*. A positive correlation was observed between results from controlled-environment and field screenings in an area where the pathogen is endemic.

Ascochyta blight, a fungal disease of chickpea (*Cicer arietinum* L.) caused by *Ascochyta rabiei* (Pass.) Lab., can devastate chickpea crops over large areas if weather conditions favor infection and spread of the pathogen. The years 1979–80 and 1981–82, for instance, were disastrous for chickpea production in Pakistan and in the Punjab and Haryana states of India, where 40–50% crop losses were reported (2). Breeding for resistance to Ascochyta blight has been attempted in several countries (5). Screening for Ascochyta blight resistance is usually carried out in field nurseries, using debris from diseased plants as inoculum, keeping the humidity high by providing irrigation at least twice a day, and screening when weather conditions are naturally favorable to disease development (1,3). However, at places such as ICRISAT Asia Center, Patancheru, India (18° N, 78° E), where temperatures are relatively high and humidity low, Ascochyta blight field screening is not possible, and the only option is to screen under controlled environmental conditions. This method has advantages of uniformity, repeatability, independence of the season, and reduced risks of the disease spreading to chickpea crops. Ascochyta blight of chickpea is influenced by environment. In controlled-environment studies, it was concluded that the basic requirement for severe infection is a 17-hr wetness period in 2-wk-old seedlings at about 20 C (6). However, small temperature changes in near-saturated air will affect condensation on leaves. Therefore, to create a disease artificially, good control of temperature and humidity is very important for successful infection and disease development. This paper provides details of the construction and testing of a controlled-environment screening facility.

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MATERIALS AND METHODS

Physical arrangement. The plant growth room is 9.75 m long × 6.32 m wide × 3 m high. Sixteen mild steel (MS) painted racks, each containing four shelves, were installed in the room (Fig. 1). Each rack is 137 cm long × 122 cm

wide, and the space between shelves is 76 cm (Figs. 1 and 2).

Temperature and humidity. An air-conditioning unit (Model 10TR, 120,000 BTU, Westinghouse Electric, Pittsburgh, PA) was installed with suitable ducting arranged as shown in Figure 1. Three separate waterproof switches were installed to control the air-conditioning system within the plant growth room. The plant growth room can be maintained between 15 C and 30 C. A humidifier (Defenser-AG Type 13T, Welter Meier Holding AG, Switzerland) was mounted on a wall, 2 m above floor level. The unit functions between room temperatures 5 C and 35 C and maintains constant relative humidity (RH) at 80–85%, which allows continuous leaf wetness as long as required. A humidistat (HRK-1) supplied with the humidifier was installed on the opposite wall of the chamber to the humidifier to regulate the humidity in the plant growth room.

Lighting. On the top of each shelf, four 40-W fluorescent lights were provided, each fixed in a heavy-duty box made of 21 gauge sheet steel and painted to prevent rusting. In all, 256 fluorescent lamps were installed in these fixtures. The illumination was further enhanced by using polished stainless steel reflectors (irradiance of 54 W m^{-2}). A timer was installed to automatically control the entire lighting system.

Wiring. Each rack was supplied with a single phase 230-V AC 50-Hz supply via four 5A weatherproof switches so that lights on each shelf could be controlled individually. All of the 256 chokes were mounted together in an adjoining room so that they did not affect the temperature and humidity in the plant growth room. The wires between each fluorescent light and its choke were laid through a central wiring PVC duct connecting all racks. All electrical installations in the plant growth room were grounded through a 2-mm bare copper wire according to Indian Standard Specifications.

Screening method. Twenty chickpea lines and an Ascochyta blight susceptible control Pb 7 were grown in Ascochyta blight nurseries at Hisar in India. Each trial had at least two replicates. They were sown in October–November 1990 and 1992. Another set of 10 chickpea lines received from Islamabad, Pakistan, was sowed at Hisar in November 1991. Plot size in the nurseries was in 4-m rows, with plant spacings of 30 cm between and 10 cm within rows. In the field, disease incidence was enhanced by spreading debris from Ascochyta blight infested chickpea plants between plant rows, and by spraying spore suspensions onto the plants.

For the plant growth room studies, seeds were sowed in 80 plastic trays (35 × 25 × 8 cm) in sterilized river sand. There were 10 seedlings in each line in three replicates. Trays were transferred to the plant growth room approximately 2 wk after sowing. A monoconidial isolate of *A. rabiei* from a chickpea field at Hisar was used in the experiments. The cultures were stored on potato-dextrose agar at 5 C. The spores were produced on chickpea seed, prepared by autoclaving 100 g of chickpea seed in 50 ml of water for 30 min in a flask. The seeds were inoculated from a 7-day-old culture of *A. rabiei* and incubated for 10 days at 20 C. The spore suspension was made by soaking infected seed in sterile distilled water for 30 min, stirring with a glass rod, and passing the suspension through double-layered muslin cloth. The suspension was adjusted to the required spore concentration using a hemacytometer. Seedlings were inoculated after transfer to the plant growth room by spraying spore suspensions (2 × 10^6 spores per milliliter) of a Hisar isolate of *A. rabiei*. Air temperature was maintained at 20 C (±1 C) in the plant growth room. Leaf wetness was maintained for 72 hr using the humidifier. Relative humidity in the room was maintained between 65 and 70% during the subsequent 12 days. In the plant growth room, the fluorescent lights provided a 12-hr photoperiod.

Disease scores were recorded at podding in the field nurseries and 2 wk after inoculation in the plant growth room. Plants were scored on a scale of 1–9 as described by Nene et al (2), where 1 = no symptoms and 9 = plants killed. The

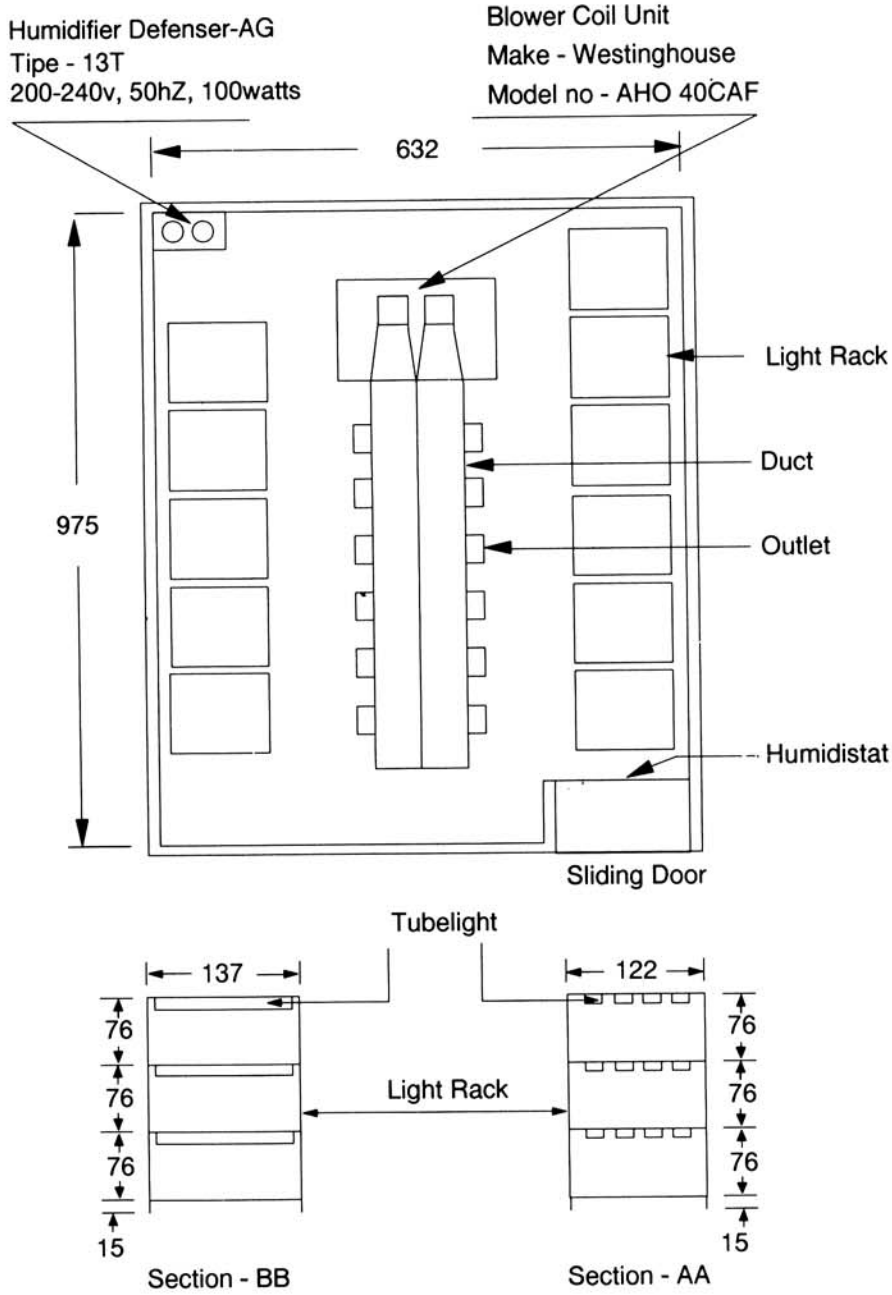

Fig. 1. Plant growth chamber at ICRISAT Asia Center, Patancheru, India. All dimensions are in centimeters.

repeatability of the plant growth room screening was investigated by testing chickpea lines at least twice. Correlation coefficients were calculated for the relationship between disease scores from the Ascochyta blight nursery at Hisar and the plant growth room at ICRISAT Asia Center.

RESULTS AND DISCUSSION

The results of screening materials in the plant growth room at ICRISAT Asia Center and under field conditions at Hisar are given in Tables 1 and 2. The scores were highly correlated. The 10 chickpea lines from Pakistan (Table 2) were resistant to Ascochyta blight when field screened at Islamabad in 1989–90 and 1990–91 (B. A. Malik, *personal communication*). The higher disease scores in the plant growth room were apparently more due to uniform and favorable temperatures and relative humidity. The disease reaction at the seedling stage in the plant growth room and disease assessment at podding in the field were

Table 1. Reaction of 21 chickpea lines to Ascochyta blight in the Ascochyta blight nursery (ABN) at Hisar and in the plant growth room at ICRISAT Asia Center, Patancheru during 1990–91[a]

	Disease score[b]	
Chickpea line	ABN	Plant growth room
ICC 607	9.0	9.0
ICC 1065	9.0	9.0
ICC 1400	5.0	6.0
ICC 1472	9.0	9.0
ICC 12967	9.0	9.0
ICC 13416	9.0	9.0
ICC 13816	5.5	5.5
ICC 14911	6.0	7.0
ICCL 86446	5.5	6.0
ICCL 86447	5.0	5.0
ICCV 89445	5.0	7.0
ICCX 790151	4.5	5.0
ICCX 800839	7.0	7.0
ICCX 800859	4.0	5.0
ICCX 810457	6.0	7.0
ICCX 810737-1	7.0	9.0
ICCX 810737-2	5.5	7.0
ICCX 810800	4.5	6.0
ICCX 810974	4.0	6.0
ICCX 830677	5.5	5.0
Pb 7[c]	9.0	9.0

[a] $r = 0.9067$; $R^2 = 0.82$.
[b] Disease score: 1 = no symptoms to 9 = killed by the pathogen.
[c] Susceptible check.

Table 2. Reaction of chickpea lines received from Pakistan to Ascochyta blight in the Ascochyta blight nursery (ABN) at Hisar and in the plant growth room at ICRISAT Asia Center, Patancheru during 1991–92[a]

	Disease score[b]	
Chickpea line	ABN	Plant growth room
NARC 9001	4.0	4.6
NARC 9002	5.0	5.0
NARC 9003	5.0	4.6
NARC 9004	4.0	4.6
NARC 9005	5.0	6.0
NARC 9006	4.0	6.3
NARC 9007	5.0	5.3
NARC 9008	3.0	4.0
NARC 9009	4.0	5.3
NARC 9010	5.0	5.3
Pb 7[c]	9.0	9.0

[a] $r = 0.874$; $R^2 = 0.76$.
[b] Disease score: 1 = no symptoms to 9 = killed by the pathogen.
[c] Susceptible check.

Fig. 2. Plant growth room showing screening of chickpea seedlings for Ascochyta blight resistance.

significantly correlated (Table 2).

Ascochyta blight resistance screening under field and partially controlled environment conditions has been described by several researchers (1,3,4,7), but their objectives were to screen chickpea in the field or screenhouse. These results are more adaptive, with a fundamental need for strong correlation between the results from the plant growth room and those of the target area of crop improvement. The results reported here and others not yet reported give reasons for confidence that the present controlled-environment plant growth room can serve a useful purpose, not only for practical screening, but also for studying the genetics of Ascochyta blight resistance. Limited germ plasm screening of 500 chickpea lines in the Ascochyta blight nursery at Hisar and their reactions to disease in the plant growth room also supported the correlation between field and plant growth room screening. The facility is presently being used successfully to screen germ plasm accessions and breeding material.

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