Pathology

Evaluation of Wild *Cicer* Species for Resistance to Ascochyta Blight and Botrytis Gray Mold in Controlled Environment at ICRISAT, Patancheru, India

S Pande[1,*], D Ramgopal[2], GK Kishore[1], N Mallikarjuna[1], M Sharma[1], M Pathak[1] and **J Narayana Rao[1]** (1. ICRISAT, Patancheru 502 324, Andhra Pradesh, India; 2. Acharya NG Ranga Agricultural University, College of Agriculture, Rajendranagar, Hyderabad 500 030, Andhra Pradesh, India)
*Corresponding author: s.pande@cgiar.org

Chickpea (*Cicer arietinum* L.) is the third most important food legume crop grown over 45 countries across five continents. It maintains soil fertility through biological nitrogen fixation and contributes to the sustainability of cropping systems in cereal-legumes rotation.

Ascochyta blight (AB, caused by *Ascochyta rabiei*) and Botrytis gray mould (BGM, caused by *Botrytis cinerea*) are destructive fungal foliar diseases of chickpea (Davidson et al. 2004; Pande et al. 2004 and Pande et al. 2005) that can cause up to 100% yield losses. Cool and wet weather favour these diseases and their epidemic development. Management of AB and BGM rely on fungicides, but these are not effective when the disease pressure is high. Deployment of resistant genotypes could be an effective way to minimize yield losses due to AB and BGM. Since adequate levels of disease resistance are not available in the cultivated chickpea germplasm, wild *Cicer* spp. have been identified as good sources of resistance to these diseases and there is a potential to transfer resistance genes from these species into cultivated *C. arietinum* species (Singh et al. 1992 and Haware et al. 1992). Therefore, in our quest to identify durable levels of resistance to AB and BGM, we initiated a large-scale screening of wild *Cicer* accessions under optimal disease development conditions at ICRISAT.

Ascochyta blight. Following the controlled environment screening technique (CEST), 148 wild accessions belonging to seven *Cicer* spp. *viz.*, *C. bijugum*, *C. cuneatum*, *C. echinospermum*, *C. judaicum*, *C. pinnatifidum*, *C. reticulatum* and *C. yamashitae* were evaluated for AB resistance. Eight seedlings each of the test entry and a susceptible genotype were raised in rows in plastic trays filled with sand-vermiculite mixture (4:1) in a greenhouse. Nine test entries and a susceptible check Pb7 were sown in each tray. These trays with 12-day-old seedlings were transferred to controlled environment facility (CEF) maintained at 20±1°C and ~1500 Lux light intensity for 12 h a day, allowed to acclimatize for 24 h and inoculated with the conidial suspension (5×10^4 conidia ml^{-1}) till runoff. The *A. rabiei* conidia were produced on the autoclaved seeds of chickpea and harvested into sterile distilled water to prepare the conidial suspension for inoculation. After inoculation, the seedlings were allowed to dry partially for 30 min; thereafter 100% relative humidity (RH) was maintained till the end of the experiment. Disease severity was recorded on a 1–9 rating scale 10 days after inoculation (Pande et al. 2005). The experiment was repeated once. Based on the mean disease score of two repetitions (16 seedlings), individual chickpea lines were categorized as asymptomatic (disease score 1.0), resistant (disease score 1.1–3.0), moderately resistant (disease score 3.1–5.0), susceptible (disease score 5.1–7.0) and highly susceptible (disease score 7.1–9.0).

Out of 148 accessions evaluated, five accessions of *C. judaicum* (ICC 17211, IG 69986, IG 70030, IG 70037 and IG 70038) were resistant. Of the remaining lines, 55 accessions were moderately resistant, 61 were susceptible and 27 were found to be highly susceptible to AB infection (Table 1).

Botrytis gray mold. One hundred and forty-eight wild *Cicer* accessions belonging to seven *Cicer* spp. *viz.*, *C. bijugum*, *C. cuneatum*, *C. echinospermum*, *C. judaicum*, *C. pinnatifidum*, *C. reticulatum* and *C. yamashitae* were raised similar to AB resistance screening procedures in the greenhouse and tested for BGM resistance in CEF. There were eight seedlings of each of the nine test genotypes and a BGM susceptible line (JG 62 as indicator) in each tray. Trays with 12-day-old seedlings were transferred to CEF adjusted at 15±2°C and ~1500 Lux light intensity for 12 h a day, allowed to acclimatize for 24 h and inoculated with the conidial suspension (3×10^5 conidia ml^{-1}) till runoff. After inoculation, the seedlings were allowed to dry for 30 min; thereafter 100% RH was maintained till the end of experiment. The *B. cinerea* inoculum was multiplied on autoclaved petals of marigold (*Tagetus erecta)* flowers for 8 days at 25°C and 12 h photoperiod. Conidia from the profusely sporulating culture were harvested into sterile distilled water and used for inoculations. The experiment was repeated once. Disease severity was recorded on a 1–9 rating scale as

done for AB at 20 DAI, and based on the mean disease score of two repetitions (16 seedlings) individual chickpea lines were categorized as asymptomatic, resistant, moderately resistant and susceptible or highly susceptible.

Of the 148 wild accessions evaluated, 29 accessions were found to be resistant. Out of 29 resistant accessions 23 were from *C. judaicum* (ICC 17194, ICC 17205, ICC 17149, ICC 17148, ICC 17204, IG 69977, IG 70033, IG 72931, IG 72932, IG 17150, IG 69959, IG 69969, IG 70032, IG 70038, ICC 17151, ICC 17190, ICC 17192, ICC 17195, IG 69943, IG 69997, IG 69998, IG 70034 and IG 70037); three from *C. bijugum* (IG 69981, IG 70023 and IG 70006) and three from *C. reticulatum* (IG 72959, IG 72933 and IG 72941). The remaining 107 were categorized as moderately resistant (50), susceptible (51) and highly susceptible (6) to BGM (Table 2). Twelve lines did not germinate.

Ascochyta blight and Botrytis gray mold. Five AB resistant accessions belonging to *C. judaicum* (ICC 17211, IG 69986, IG 70030, IG 70037 and IG 70038) were separately evaluated for AB and BGM twice in the CEF to identify combined resistance to both the diseases. Procedures for raising the seedlings, inoculum preparation, inoculations, and disease scoring were similar to AB and BGM evaluations explained earlier. Two accessions (IG 70037 and IG 70038) were found to be resistant (≤3.0, on 1–9 scale) to both the diseases and the remaining three (ICC 17211, IG 69986 and IG 70030) were moderately resistant (Table 3). These wild *Cicer*

Table 1. Evaluation of wild *Cicer* accessions for resistance to Ascochyta blight in controlled environment.

Cicer species	No. of lines tested	Reaction to Ascochyta blight infection[a]				
		A	R	MR	S	HS
C. bijugum	30	–	–	7	20	3
C. cuneatum	3	–	–	1	2	–
C. echinospermum	4	–	–	–	3	1
C. judaicum	47	–	5	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 = asymptomatic (A); 1.1–3.0 = resistant (R); 3.1–5.0 = moderately resistant (MR); 5.1–7.0 = susceptible (S); 7.1–9.0 = highly susceptible (HS).

Table 2. Evaluation of wild *Cicer* accessions for resistance to Botrytis gray mold in controlled environment.

Cicer species	No. of lines tested[b]	Reaction to Botrytis gray mold infection[a]				
		A	R	MR	S	HS
C. bijugum	28	–	3	18	7	–
C. cuneatum	3	–	–	3	–	–
C. echinospermum	2	–	–	1	–	1
C. judaicum	45	–	23	18	4	–
C. pinnatifidum	26	–	–	4	20	2
C. reticulatum	27	–	3	6	18	–
C. yamashitae	5	–	–	–	2	3
Total	136	–	29	50	51	6

a. Based on the disease score the wild accessions were categorized for their reaction to Botrytis gray mold infection as follows: 1 = asymptomatic (A); 1.1–3.0 = resistant (R); 3.1–5.0 = moderately resistant (MR); 5.1–7.0 = susceptible (S); 7.1–9.0 = highly susceptible (HS).

b. 12 lines did not germinate.

Table 3. Identification of combined resistance to Ascochyta blight and Botrytis gray mold diseases in controlled environment.

	Disease reaction (on 1–9 rating scale)					
	Ascochyta blight			Botrytis gray mold		
Accession No[1]	Test 1	Test 2	Mean	Test 1	Test 2	Mean
ICC 17211	2.7	2.0	2.3	4.0	3.0	3.5
IG 69986	2.5	3.5	3.0	4.5	2.5	3.5
IG 70030	3.5	2.5	3.0	4.5	2.5	3.5
IG 70037	2.0	4.0	3.0	4.0	2.0	3.0
IG 70038	2.7	3.0	2.8	3.5	2.0	2.8

1. All accessions belong to *Cicer judaicum.*

accessions, found resistant to AB, BGM and or to both the diseases, can be used in the chickpea foliar disease resistance breeding programs as resistant donor parents.

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Comparison of Greenhouse and Field Screening Techniques for Botrytis Gray Mold Resistance

S Pande*, M Sharma, M Pathak and **J Narayana Rao**
(ICRISAT, Patancheru 502 324, Andhra Pradesh, India)
*Corresponding author: s.pande@cgiar.org

Botrytis gray mold (BGM), caused by *Botrytis cinerea* Pers. ex. Fr., is the most destructive foliar disease of chickpea in eastern India, Bangladesh, Nepal, and western Australia. Cool wet weather favors the development of BGM and can cause upto 100% yield loss. Host plant resistance (HPR) is the most economical and eco-friendly means of management of BGM. For exploitation of HPR, reliable field and controlled environment screening techniques are essential. In general, field screening techniques (FST) are used for large-scale screening of germplasm and breeding material, and controlled environment screening techniques (CESTs) are used to confirm field resistance, screening against different pathotypes/races and to carry out inheritance and race identification studies.

Several CESTs, such as whole plant screening technique (WPST), cut-twig screening technique in water (CTST-W) and cut-twig screening technique in sand (CTST-S) were standardized in a controlled environment facility (CEF) at ICRISAT, Patancheru. Components of CESTs such as optimum temperature, relative humidity, and photoperiod for BGM were identified. This study attempts to compare CESTs with FSTs.

In WPST, seedlings of the test material were grown in rows in plastic trays filled with a mixture of sterilized sand and vermiculite (4:1) in a greenhouse (Fig. 1A). One row of a susceptible cultivar JG 62 was planted as indicator