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 in each tray along with nine test entries. Trays with 10-day-old seedlings were transferred to CEF adjusted at 15±1°C and ~1500 Lux light intensity for 12 h a day, allowed to acclimatize for 24 h and inoculated with conidial suspension (3×10^5 spores ml^{-1}) of *B. cinerea*. After inoculation the plants were allowed to partially dry for 30 min and thereafter 100% RH was maintained till the end of experiment (Pande et al. 2002). The experiment was conducted in two replications with eight plants in each replication and repeated once.

In CTST-W, tender shoots of chickpea plants were cut from the actively growing chickpea plant (30–60 days after sowing) with a sharp edged blade in the evening. The lower portion of the detached twig was wrapped with a cotton plug and transferred to a test tube (15 × 100 mm) containing fresh water (Sharma et al. 1995), (Fig. 1B). The tubes were kept in CEF, allowed to acclimatize for 12–24 h and inoculated following standardized procedures (Pande et al. 2002).

In CTST-S, the detached twigs were planted into sterilized moist coarse sand-vermiculite medium in trays (Fig. 1C). Trays were kept in the CEF, allowed to acclimatize for 12–24 h and inoculated following standardized procedures as explained above. The experiment was conducted in two replications with eight twigs in each replication and repeated once.

To compare the CESTs and FST for BGM resistance, 10 chickpea lines selected from the International Botrytis Gray Mould Nursery (IBGMN) were evaluated under CEF at ICRISAT and in the field at hot spot locations in Pantnagar (India) and Ishurdi (Bangladesh). In FST test lines were sown in 2–3 m long rows spaced at 30 × 10 cm. Indicator-cum-infector rows of a susceptible cultivar H208/JG 62 were sown after every two-test row. At the onset of flowering, the trial was irrigated and plants were inoculated with a spore suspension (5×10^4 spores ml^{-1}) of 10 day old culture of *B. cinerea*. From the following day, sprinkler irrigation or perfo-irrigation was run every day for about 15 min after every 1 or 2 h from 9.00 to 17.00 h depending upon the environmental conditions (Fig. 2). Inoculation with spore suspension of *B. cinerea* was repeated twice at 10-day intervals after the first inoculation (Pande et al. 2002). The trial was replicated

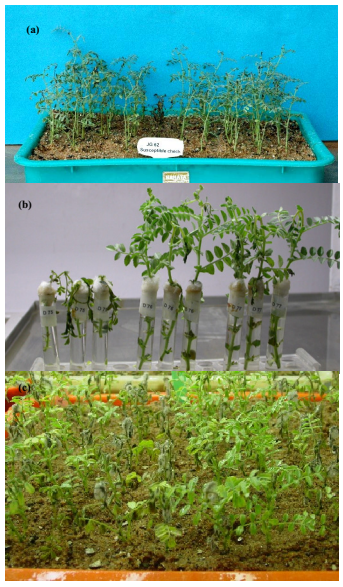

Figure 1. Controlled environment screening techniques at ICRISAT, Patancheru 502 324, Andhra Pradesh, India
(a) Whole plant (WPST) (b) Cut twig-water (CTST-W) (c) Cut twig-sand (CTST-S).

Figure 2. Field screening technique, Ishurdi, Bangladesh.

Table 1. Comparison of controlled environment and field screening techniques for Botrytis gray mold resistance.

	Disease score[1] (1–9 rating scale)[2]					
	Controlled environment			Field		
Entry	WPST[3]	CTST-W[4]	CTST-S[5]	Pantnagar	Ishurdi	Overall mean
ICC 8509	5.0	4.5	5.0	7.0	4.5	5.2
ICC 12339	4.5	4.0	5.5	5.0	4.5	4.7
ICC 89302	4.0	4.0	5.0	7.0	4.0	4.8
ICC 89303	6.0	6.0	6.5	7.0	6.0	6.3
ICC 89310	7.0	7.0	7.5	8.0	6.0	7.1
ICC 86215	6.0	5.5	4.0	6.0	5.5	5.4
ICC 86242	6.5	5.0	5.5	6.5	4.7	5.6
ICCX860030-BP-BP	6.0	6.0	7.0	7.0	6.0	6.4
ICCX860023-BP-BP-BP-3P-BH-IH-BH	6.0	6.0	7.0	8.0	6.6	6.7
ICCX880355-BH-BP-5H-BH	7.2	7.5	8.0	9.0	6.5	7.6
Susceptible check	9.0	9.0	9.0	9.0	9.0	9.0
Overall mean	6.1	5.9	6.3	7.2	5.8	
CD at 5%						
Techniques = 0.69						
Entry = 0.86						
Technique × Entry = 1.9						

1. Average of three replications.
2. Disease reaction was based on the disease score: 1 = asymptomatic; 1.1–3 = resistant; 3.1–5 = moderately resistant (MR); 5.1–7 = Susceptible; 7.1–9 = Highly susceptible (HS).
3. WPST = whole plant screening technique.
4. CTST-W = cut-twig screening technique in water.
5. CTST-S = cut-twig screening technique in sand.

twice at both the locations. Data on disease severity was recorded on a 1–9 rating scale after 20 days of inoculation (DAI) in WPST, 8 DAI in CTST-W and CTST-S and at the time of harvest in FST. Based on the mean disease score, individual chickpea line was categorized as asymptomatic (disease score 1.0), resistant (disease score 1.1–3), moderately resistant (disease score 3.1–5), susceptible (disease score 5.1–7) and highly susceptible (disease score 7.1–9).

Results obtained with CESTs i.e. WPST, CTST-W, CTST-S, and FST are comparable for BGM (Table 1). Analysis of variance revealed that there was no significant difference between the techniques except in the field screening at Pantnagar where disease pressure was marginally higher on a few test entries than the CESTs. However, the severity of BGM in susceptible check and in majority of test entries was uniform in all the techniques. Therefore, we can conclude that the CEST and FST are equally reliable, repeatable and economical. However, CTST-W and CTST-S are found to be rapid and economical and useful in screening segregating germplasm and breeding lines without destroying the plants and thus can be used to screen for other target traits and seed production.

References

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