Published in Australasian Plant Pathology
Volume 40, Number 2, 149-156, http://dx.doi.org/10.1007/s13313-010-0024-8

Development of screening techniques and identification of new sources of resistance to Ascochyta blight disease of chickpea

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Running head: Screening techniques and sources of resistance to Ascochyta blight

Abstract. Effective controlled-environment and field screening techniques were developed and refined to identify resistance to Ascochyta blight (AB), caused by *Ascochyta rabiei* (Pass.) Labr. in chickpea. A controlled environment plant growth room facility developed for AB evaluation at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India was modified to evaluate chickpea genotypes for resistance to AB. Controlled environment screening techniques, such as a seedling screening technique using 10-day-old seedlings and cut-twig screening techniques using excised twigs (10–15cm long) were developed. Components of the screening techniques were optimized in the controlled environment-plant growth room. The controlled environment screening techniques were found to be rapid, reliable and reproducible and a positive correlation was found between the seedling and cut-twig screening techniques (r=0.94). The cut-twig screening technique was quicker than the seedling screening technique and is particularly useful in screening segregating breeding lines derived from wild *Cicer* spp. Results of the controlled environment screening techniques were compared with results of field screening trials carried out at Dhaulakuan and Ludhiana in India, where the pathogen is endemic. A significant positive correlation was found between results from the controlled environment and field screening techniques (r=0.88). Using these resistance screening techniques, 150 elite chickpea breeding lines were evaluated and 29 lines with high and stable resistance to AB were identified.

Additional keywords: *Ascochyta rabiei*, host plant resistance, screening techniques, resistant sources

Introduction

Chickpea (*Cicer arietinum* L.) is the third most important food legume worldwide, cultivated in 11.67 million ha producing 9.31 million tons of grain (FAO 2008). India accounts for approximately 64% of world chickpea production. Recently, chickpea has experienced an export-driven expansion in places such as Australia, Canada and USA. Despite the large area under chickpea cultivation, total production and productivity is quite low in most chickpea growing countries and there is a wide gap between potential yield (5 t ha^{-1}) and actual yield (0.8 t ha^{-1}). The primary cause of low yields in chickpea is its susceptibility to a number of biotic and abiotic stresses. Among biotic stresses, Ascochyta blight (AB) caused by *Ascochyta rabiei* (Pass.) Labr. is a widespread foliar disease that causes extensive crop losses (up to 100%) in most regions of the world where the crop is commonly grown (Pande *et al.* 2005). Several epidemics of AB causing complete yield loss have been reported in Pakistan, India, European countries and Mediterranean regions (Hawtin and Singh 1984; Singh *et al*. 1984; Kaiser *et al.* 1998; Pande *et al*. 2005). Currently, AB is the most important yield-limiting factor in Australia and Canada, potentially affecting 95% of the area sown to chickpea (Knights and Siddique 2002; Gan *et al.* 2006). AB has also been reported from Latin America (Kaiser *et al.* 2000) and north Africa (Akem 1999).

The occurrence and severity of AB in chickpea is weather dependent with devastating effects in areas where cool (15–25ºC), humid weather (>150 mm rainfall) prevails during the cropping season. The type of inoculum, inoculum concentration and physiological plant growth also affect the degree of infection and the amount of crop loss. Fungicidal management of AB is not economical and is hazardous to the environment as several applications of fungicides are required (Chang *et al.* 2007). Further, the use of fungicides having a site-specific mode of action such as QoI fungicides (azoxystrobin and

pyraclostrobin) increases the risk of fungicide resistance emerging in *A. rabiei* (Gossen *et al.* 2004; Wise and Gudmestad 2009). Therefore, host plant resistance, either alone or as a major component of integrated AB management is the most economical approach to manage this disease. A prerequisite for exploiting host plant resistance is the development of reliable and repeatable resistance screening techniques. A number of screening techniques under field and greenhouse conditions have been reported, but with variable reactions to AB (Nene *et al*. 1981; Singh *et al.* 1984; Sharma *et al*. 1995, Chen *et al*. 2005). Variation 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. A significant change in any of these components reduces the efficacy of the screening techniques resulting in failure of disease development. Therefore, the identification and standardization of various factors influencing AB infection and development are important to the development of effective field and greenhouse screening techniques for comparison internationally. In general, screening for AB resistance is usually carried out in the field in locations in northern India (Dhaulahuan and Ludhiana) where environmental conditions are favorable for AB development. However, consistency in AB development and resistance reaction in field screening technique depends on the existing environmental conditions leading to variable host reactions to AB. Therefore, at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India, a controlled environment plant growth room facility to screen chickpea genotypes for resistance to AB has been developed using sound epidemiology principles and requirements needed for AB development. Such a facility has advantages with regard to uniformity, repeatability, independence of season and reduced risk of disease spreading to the chickpea crop. The objectives of this study were to develop novel screening techniques to refine the existing

techniques, to examine the correlation between these techniques and to identify new and stable sources of resistance to AB.

Materials and methods

Controlled environment plant growth room

A controlled environment plant growth room facility (9.57m long × 6.23m wide × 2.72m high) developed at ICRISAT, Patancheru, India (Haware *et al.* 1995) was modified by the installation of fourteen aluminium racks each containing three shelves (Fig.1). Each rack is 1.35m long × 0.9m wide × 1.88m high. Temperature (15 – 30 ^{0}C), humidity and photoperiod were optimized for AB development in this growth room. To control temperature, an air-conditioning unit was installed with suitable ducting. Waterproof switches were installed to control the air-conditioning system. Four humidifiers (Model Defensor ABS2, AXAIR, a WHM Company, Switzerland) were kept in the growth room at four corners, 2m above floor level to maintain the relative humidity up to 100%. To provide photoperiod, four 28W fluorescent tubes were installed over each shelf. A timer was installed to automatically control the entire lighting system. The resistance screening techniques optimized for AB evaluation using the growth room facility are as follows.

Seedling screening technique

Raising of seedlings

Test genotypes (150 elite chickpea breeding lines) were grown in plastic trays (35×25×8 cm) filled with a mixture of sterilised river sand and vermiculite (10:1) in a greenhouse maintained at 25±1°C for 10 days. Ten rows (nine test lines and one susceptible check row) were sown in each tray and each row consists of eight seeds of one line. The trial was conducted in a completely randomized block design with three replications and repeated twice. In all, 24 seedlings per test line were screened.

Inoculum preparation

Ascochyta rabiei isolated from naturally-infected chickpea leaves collected from areas where pathogen is endemic by plating on Chickpea Dextrose Agar (CDA) medium was used in the study. Isolations were done from AB infected brown to black lesions on leaves/stems. Diseased tissues were cut in to 2-3mm pieces, surface sterilized with sodium hypochlorite solution (1%) for 1-2 minutes, washed three times with sterile distilled water and plated on CDA medium. The plates were incubated for 7 days at 20±1°C with a 12-h photoperiod. Single spore culture was done on 1/ 4 CDA following standard mycological procedures and pathogen identified according to Punithlingam and Holliday (1972). The *A. rabiei* culture has been deposited at Indian Type Culture Collection (ITCC), Indian Agriculture Research Institute, New Delhi, India (Accession No. ITCC 6651). The ITCC is registered with World Data Centre for Microorganisms (WDCM).

For mass inoculum preparation, kabuli chickpea seeds were soaked in water overnight, autoclaved at 121°C for 25 minutes, and inoculated with an actively growing culture of *A. rabiei.* Inoculated seeds were incubated at 20±1°C for 8 days with a 12-h photoperiod. The seeds were then soaked in water for 30 minutes and vortexed for 2–3 minutes to dislodge spores from seeds. The spore suspension was filtered through a double-layered muslin cloth; the spore concentration was adjusted to 5×10^4 conidia ml^{-1} using a haemocytometer.

Inoculation and incubation

Trays with 10-day-old seedlings were transferred to the plant growth room and maintained at 20±1°C with a 12-h photoperiod. Seedlings were adapted to these conditions for 24 h before inoculation. Test plants and known susceptible control plants were inoculated by spraying with the conidial suspension of *A. rabiei* (5×10^4 conidia ml^{-1}) until run-off. Inoculated seedlings were partially air dried for 30 minutes to avoid dislodgment of spores, then maintained at 20±1°C and continuous relative humidity of 100% for 96 h, then the

relative humidity was maintained 100% for 6–8 h per day until the end of the experiment. Uninoculated plants were used as a negative control.

Disease scoring

The disease reaction of individual genotypes was recorded 10 days after inoculation (DAI) on a 1–9 rating scale (modified from Jan and Wiese 1991), where 1 = no visible symptoms; 2 = minute lesions prominent on the apical stem; 3 = lesions up to 5 mm in size and slight drooping of apical stem; 4 = lesions obvious on all plant parts and clear drooping of apical stem; 5 = lesions on all plants parts, defoliation initiated, breaking and drying of branches slight to moderate; 6 = lesions as in 5, defoliation, broken, dry branches common, some plants killed; 7 = lesions as in 5, defoliation, broken, dry branches very common, up to 25% of plants killed; 8 = symptoms as in 7 but up to 50% of the plants killed and 9 = symptoms as in 7 but up to 100% of the plants killed. Based on the disease score, test lines were categorized for their reaction to AB 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); and 7.1–9.0 = highly susceptible (HS) (Pande *et al*. 2006) (Fig. 2).

Cut-twig screening technique

This technique was earlier developed by Sharma *et al.* (1995), and has been further modified using the plant growth chamber at ICRISAT. Methods for evaluating cut-twigs were standardized using two different support mediums; water and sand and are described below.

Excised twigs

About 10–15 cm long tender shoots of test chickpea genotypes were cut with a sharp edge disposable sterilized surgical blade (Feather Industry Ltd., Tokyo, Japan) in the evening and lower part (about 5cm) immediately immersed in water. The lower portion of each excised twig was wrapped in a cotton plug and transferred to a test tube (15×100 mm)

containing fresh water. Excised twigs of susceptible genotypes along with test genotypes were kept for comparison.

Inoculation and incubation

Test tubes with the excised twigs were transferred to the growth room maintained at 20±1°C and ~1500 Lux light intensity (12-h photoperiod). The excised twigs were adapted to the conditions for 24 h before inoculation. The twigs were inoculated by spraying with a conidial suspension (5×10^4 conidia ml^{-1}) of *A. rabiei*. The inoculation method and post-inoculation incubation conditions were similar to seedling screening technique. Disease severity was recorded on 1–9 rating scale when the susceptible check showed a rating of 9. The disease scoring system was similar to the seedling screening technique as symptom expression and development were the same in both techniques.

This cut-twig screening technique using water as a support medium was further modified by placing the excised twigs in a slanting manner in sterilised moist sand in plastic trays (35×25×8 cm) instead of water. Excised twigs of a susceptible genotype were included in each tray for comparison. The rest of the procedure for inoculation, incubation and disease scoring is similar to the cut-twig screening technique using water as a support medium.

Field screening technique

The field trial was conducted at two hot spot locations in India - Dhaulakuan and Ludhiana, where AB is endemic. Trials were conducted for two seasons at both the locations (2004-05 and 2005-06 at Ludhiana and 2007-08 and 2008-09 at Dhaulakuan). Components of the field screening technique such as planting of test material; indicator/infector rows, inoculation stage; maintenance of humidity required for infection, colonization and development of AB were standardized as described below.

Planting of test material

A randomized complete block design trial was conducted with two replications. One hundred and fifty elite chickpea breeding lines were planted in a plot size of 100 m^2 with a spacing of 30 cm between the rows and 10cm between plants in the same row. A highly susceptible cultivar to AB (ICC 4991) was included between every four-test rows to serve as indicator/infector rows.

Inoculation and disease scoring

At the onset of flowering, AB-infected plant debris collected from the previous season was scattered over the field (3-4kg per 100m^2). Plants were also inoculated with a spore suspension of *A. rabiei* (1×10^5 spores ml^{-1}) in the evening (For 100 m^2 plot, 5 L of inoculum was sprayed). Inoculation was repeated 2-3 times at 10-day intervals, if disease development was not uniform. Following inoculation, the field was sprinkler-irrigated every day for 10–15 minutes per hour from 1000–1600 h to maintain high relative humidity during dry weather. Data on disease severity was recorded on a 1–9 rating scale when susceptible check show maximum rating 9 and again at close to maturity (Nene et al 1981).

Comparison of screening techniques

To compare the controlled environment and field screening techniques for AB evaluation, ten chickpea lines were evaluated using both the controlled environment screening techniques (seedling, cut-twig water and cut-twig sand) at ICRISAT and the field screening technique in the field at Ludhiana and Dhaulakuan in 2008-09 crop season. Data on disease severity recorded on a 1–9 scale both from the controlled environment and the field were compared and correlation coefficients calculated.

Statistical analysis

Data recorded on disease severity from different experiments were subjected to statistical analysis. Analysis of variance (ANOVA) and correlation coefficient were computed using

the GENSTAT 12th Edition computer programme. After ANOVA, the least significant difference (l.s.d.) was calculated for different factors to compute the smallest significant difference between the means. Probability values were calculated to indicate the significance of the results.

Results

Effectiveness of screening techniques

Based on the mean of three years data, of the 150 breeding lines evaluated in the controlled environment technique at ICRISAT, 38 lines were found to be resistant (AB score 2.0–3.0), 79 lines were moderately resistant (AB score 3.1–5.0), 15 lines were susceptible (AB score 5-7) and 18 were highly susceptible (AB score 7-9). The known susceptible line ICC 4991 had a disease rating of 9. In the field screening at Ludhiana, based on the mean disease score of two years, 50 lines were found to be resistant, 60 were moderately resistant, 22 were susceptible and 17 were highly susceptible to AB. At Dhaulakuan, of the 150 lines evaluated, 55 lines were found to be resistant, 53 were moderately resistant, 15 were susceptible and 17 were highly susceptible to AB. Twenty nine lines were found to be highly resistant to AB (AB score 2-3) both in the controlled environment and in the field in all the years of evaluation (Table 1). In general, AB severity under field conditions at both the locations was comparatively less than in the controlled environment.

Among the controlled environment screening techniques, the seedling screening technique using 10-d old seedlings is easy to handle and economical as about 1000 genotypes (in three replications) can be screened in one cycle. This technique is routinely used to screen chickpea germplasm and breeding material for AB resistance at ICRISAT. The cut-twig screening technique was found to be more rapid than seedling screening technique. However, the disadvantage of the cut-twig screening technique using water as support medium is that it can accommodate only one seedling in one test tube, so large

scale screening by this method is not economical. However, the use of sand as a support medium allows more excised twigs per tray (60–70) and is economical. There was a positive correlation (r=0.94) between the results of the cut twig and the seedling screening techniques.

Comparison of screening techniques

In the ten lines evaluated for comparing the field and controlled environment screening techniques for AB evaluation, analysis of variance revealed no significant difference ($P<0.0001$) in AB severity between the controlled environment and field screening techniques (Table 2). The known susceptible line ICC 4991 showed a disease rating of 9 in all the techniques. There was a significant and positive correlation between the controlled environment and field screening techniques. The seedling screening technique was highly correlated with the field screening technique (r=0.89). Similarly, the cut-twig and field screening techniques were highly correlated (r=0.88). AB severity ratings were slightly higher in a few lines in the controlled environment than in the field. At Ludhiana, the AB severity was comparatively more in year 2004-05 as compared to 2005-06 whilst at Dhaulakuan, the AB severity was slightly more in 2007-08 as compare to 2008-09.

Discussion

In the present study, a controlled environment plant growth room facility earlier developed by Haware et al. (1995) at ICRISAT was modified to provide conditions conducive to the development of AB. Using this growth room facility, components of controlled environment screening techniques (seedling screening technique and cut-twig screening technique) for AB evaluation were optimized. Large number of chickpea genotypes have been screened using this seedling screening technique at ICRISAT and currently the technique is being extensively used for AB resistance evaluation (Pande *et al*. 2005; 2006). A cut-twig screening technique using sand as a support medium was found to be rapid and

reliable. The technique is used for screening wide-hybridization crosses and the segregating material derived from these crosses. Pande *et al*. (2006) reported five accessions of *C. judiacum* (ICC 17211, IG 69986, IG 70030, IG 70037 and IG 70038) resistant to AB under controlled environment at ICRISAT. Sharma *et al*. (1995) used the cut-twig method of screening for resistance to AB in order to test the wide-hybridization crosses to incorporate resistance from wild *Cicer* species into cultivated genotypes. Chen and Muehlbauer (2003) developed a mini-dome technique for pathogenicity assay and screening for AB resistance and this technique is in use at Pullman, USA. The purpose of mini-domes is to form a uniform high level of humidity to promote disease development and they found technique to be useful where growth chamber facilities are not available.

Field screening techniques for AB evaluation were optimized at the two hot spot locations Dhaulakuan and Ludhiana in India. At Ludhiana, AB severity was greater in 2004-05 then in 2005-06 and is attributed to the high rainfall (>150mm) during the 2004-05 crop season as compare to only 50mm in 2005-06 crop season. Moreover, the maximum temperature was more favorable (for 8 weeks during crop season) for disease development in 2004-05 (18ºC-20 ºC) as compared to 2005-06 (19ºC-28ºC). Differences in AB severity were also noted for the two years of the trial at Dhaulakuan. Disease severity was slightly greater in 2007-08 in comparison to 2008-09. This is also attributed to more favourable environmental conditions for AB development in the year 2007-08. In general, AB severity recorded was less under field conditions at both the locations Ludhiana and Dhaulakuan compared to the controlled environment at ICRISAT. High disease scores in the plant growth rooms may be attributed to uniform and favourable temperatures and relative humidity for AB development. Similar observations were also reported by Haware *et al.* (1995) and Basandrai *et al.* (2007). AB resistance screening under field conditions has

been described by several researchers worldwide (Nene *et al*. 1981; Riahi *et al*. 1990; Weising *et al*. 1991).

A significant positive correlation was found between the controlled environment and field screening techniques. Positive correlations between greenhouse and field screening techniques for AB have also been observed by others (Haware *et al*. 1995; Sharma *et al*. 1995). These results indicated that the controlled environment plant growth room can be more useful not only for practical screening but also for studying the genetics of AB resistance. Moreover, large-scale screening of segregating breeding populations at the seedling stage for AB resistance under controlled environment is more economical, faster and independent of season compared with field screening.

The present study reports 29 new sources of resistance to AB with very high levels of resistance in desi chickpea breeding lines in both field and controlled environment screening tests in all years of the evaluation. Breeding of chickpea for resistance to AB is an important goal worldwide but is often limited due to the absence of high levels of resistance in chickpea germplasm which along with the highly variable pathogen, has precluded the development of varieties with both high and durable resistance (Knights and Siddique 2002; Pande *et al*. 2005; Chen *et al*. 2004, Tivoli *et al*. 2006). The ICARDA scientists have developed more than 3000 lines with moderate resistance to AB (Malhotra *et al*. 2003), but the frequency of highly resistant lines to AB is generally low (Iqbal *et al*. 2002; Atanasova and Mihov 2009).

The highly resistant AB lines identified in the present study can be exploited in breeding programs as resistant donors to evolve agronomically desirable AB-resistant varieties. At ICRISAT, it was found that most of these AB resistant lines have a wide range of maturity (112-142 days) and acceptable seed size (data not published). These lines are being further evaluated for agronomic performance and adaption in different

environments. In conclusion, the development of well-established controlled environment and field screening techniques has allowed the recognition of useful sources of resistance to AB in several germplasm and breeding collections of cultivated and wild chickpea. The controlled environment facility is presently being used successfully to screen chickpea germplasm accessions and breeding material.

Acknowledgements

This work was supported by a grant from the Council of Grain Growers Organization (COGGO), Western Australia under the research project CAN 091 122 039.

Fig. 1. Controlled environment plant growth room facility for Ascochyta blight screening at ICRISAT, Patancheru, India.

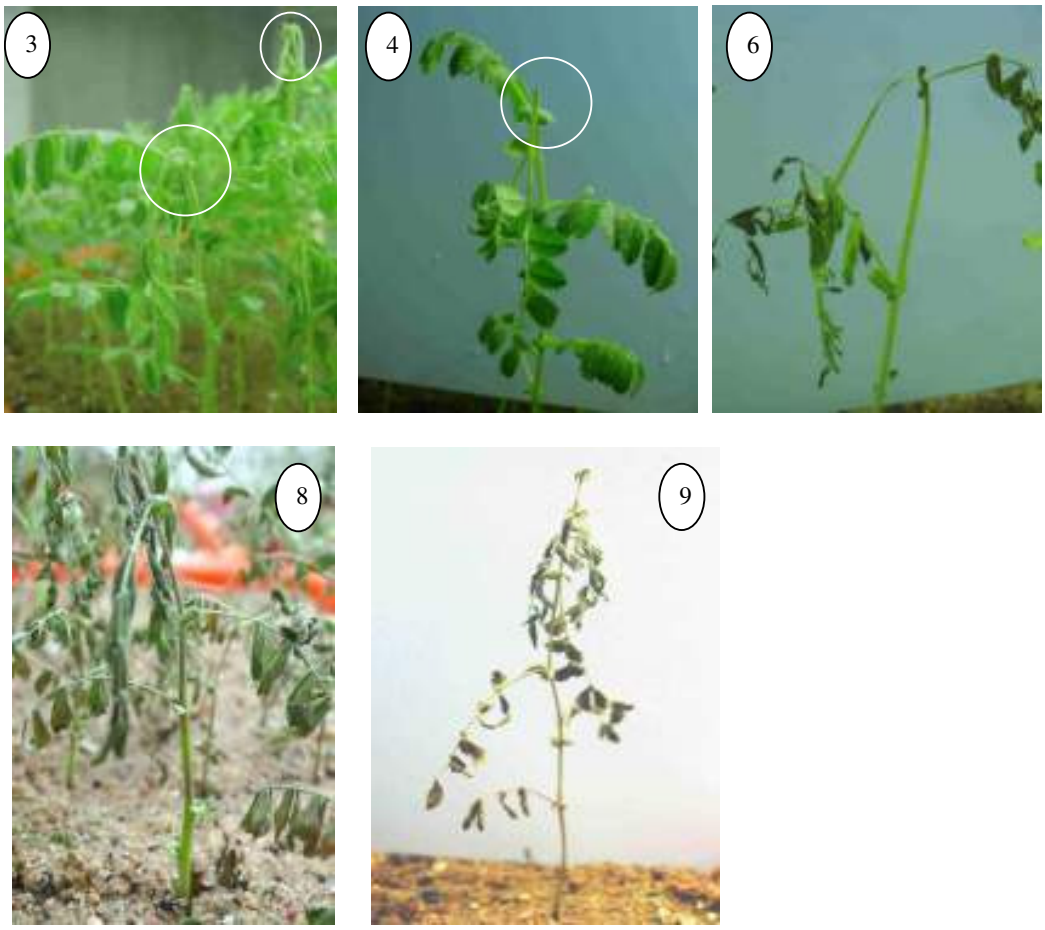

Fig. 2. Disease rating scale for Ascochyta blight in chickpea.

3=Slight drooping of the apical stem; 4=Clear drooping of the apical stem; 6=Breaking and drying of branches initiated; 8=Breaking and drying of branches common and >50% plant mortality and 9=Complete drying of the branches and 100% plant mortality.

Table 1. Ascochyta blight reaction of 29 resistant breeding lines to *Ascochyta rabiei* in controlled environment and field screening.

Breeding lines	Ascochyta blight reaction (1-9 scale)[A]									
	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
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	
l.s.d. (5%)	0.71	0.71	0.74		0.73	0.89		0.81	0.71	

[A] Disease reaction is based on the mean of two replications.

– Data not available.

Table 2. Comparison of controlled environment and field screening techniques for evaluation against *Ascochyta rabiei* causing Ascochyta blight of chickpea.

	Disease score (1–9 rating scale)[A]			
	Controlled environment screening techniques			Field screening
Genotype	Seedling	Cut-twig water	Cut-twig sand	technique[B]
ICC 4033	2.0	2.5	2.3	2.4
ICC 6304	3.3	3.8	3.6	4.1
ICC 12968	3.0	2.3	3.0	4.0
ICCV 05530	3.0	3.0	3.0	3.0
ICCV 05511	3.0	3.3	3.5	3.0
ICCV 05513	3.0	3.0	3.0	3.0
ICC 15996	7.0	7.5	8.0	7.0
ICCV 05602	9.0	9.0	9.0	9.0
ICCV 93704	7.7	7.0	7.5	8.7
ICC 4991 (Sus. check)	9.0	9.0	9.0	9.0
l.s.d. (5%)				
Technique = 0.69; Genotype = 0.86; Technique × Genotype = 1.9				

[A]Average of three replications.

[B]Average of disease score from two locations

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