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Biplot analysis of genotype × environment interactions and identification of stable sources of resistance to Ascochyta blight in chickpea (*Cicer arietinum* L.)

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Abstract Ascochyta blight (AB) caused by *Ascochyta rabei* (Pass.) Labr. is one of the most important constraints that limits the productivity of chickpea (*Cicer arietinum* L.). The absence of high levels of stable resistant sources to the pathogen has necessitated the continued search and identification of new sources of resistance. The main aim of this work was to identify new sources of resistance to AB and validate their stability across multi-environments. A collection of 424 elite chickpea genotypes were evaluated for AB resistance under controlled environmental conditions in 2005–2006 at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. Fifty-one genotypes with AB severity ≤3.0 (based on the 1–9 scale) were selected for a second round of evaluation in 2006–2007 at ICRISAT. Based on the results obtained during both years, an Ascochyta Blight Nursery (ABN) was established to evaluate the selected 29 chickpea genotypes, including 4 germplasm lines, 24 breeding lines and a highly susceptible line. The nursery was evaluated at 3 locations (Almora, Dhaulakuan and Ludhiana) in India over three crop seasons (2007–2008, 2008–2009 and 2009–2010) and under controlled environment conditions at ICRISAT to further confirm the stable performance of these genotypes. Analysis of variance revealed highly significant effects of year, location (year), genotype and genotype × location (year) interaction. The genotype and genotype × environment (GGE) biplot analyses of multi-environment data showed that resistance of five genotypes (EC 516934, ICCV 04537, ICCV 98818, EC 516850 and EC 516971) had mean disease severity ≤3.0 on the 1–9 scale and the reactions were consistent across the environments. Genotype EC 516934 was found resistant to AB at the seedling stage in the controlled environment at ICRISAT. The remaining genotypes showed moderately resistant reaction (3.0–5.0) to AB under controlled environment conditions. A significant positive correlation was found between the performance of the genotypes under controlled environment and field screening conditions (r=0.70; P<0.01). The resistant genotypes identified in the present study would be useful in breeding programs as stable resistant donors to evolve agronomically desirable AB resistant varieties.

Keywords Ascochyta blight · Chickpea · Genotype × environment interaction · Resistant sources

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Introduction

Chickpea (*Cicer arietinum* L.) is the third most important cool season food legume in the world. The seeds are used for both human and animal nutrition, as they contain a high level of proteins (20–23 %), carbohydrates (60.7 %), and dietary fibres

(17.4 %) (Jukanti et al. 2012). Chickpea also improves soil fertility by meeting up to 80 % of its nitrogen (N) requirement from symbiotic nitrogen fixation of atmospheric nitrogen (Saraf et al. 1998). In India, it is cultivated on 10.9 million ha annually producing 11.98 million tons of grain with an average productivity of 915 kg/ha (FAO 2012). The country ranks first and alone contributes about 68 % of global chickpea production. The other major chickpea producing countries include Australia, Pakistan, Turkey, Myanmar, Ethiopia, Iran, Mexico, Canada and the USA. Despite of a large area under cultivation, there exists a wide gap between potential (5 t/ha) and actual yield (0.8 t/ha) (Pande et al. 2011). The main reason for low productivity is susceptibility of commercially grown chickpea cultivars to biotic and abiotic constraints. Ascochyta Blight (AB) caused by the fungus *Ascochyta rabiei* (Pass.) Labr. is the major constraint limiting chickpea productivity worldwide (Gan et al. 2006). The occurrence of AB has been reported in more than 40 countries around the world (Pande et al. 2005, 2011). The build-up of inoculum in areas with intensive chickpea production or with short crop rotations has contributed to the severity of epidemics (Gan et al. 2006). This disease reduces chickpea seed yield and quality significantly, and in some circumstances yield losses for susceptible cultivars are as high as 100 % (Pande et al. 2005; Tivoli et al. 2006).

Control of AB is essential to ensure stable chickpea production. The use of fungicides does not give complete protection and is usually uneconomical (Pande et al. 2005). Availability of resistant cultivars is an essential component in the disease management strategy. A number of AB resistant sources have been identified and used in breeding programmes although none possess complete resistance (Pande et al. 2005; Tivoli et al. 2006). Developing chickpea varieties with high levels of resistance to AB has been a challenging proposition because of the following factors: (i) paucity of high levels of resistance in the primary genepool, (ii) complex genetic basis of resistance conferred by several quantitative trait loci (QTLs), (iii) a highly variable pathogen population, and (iv) the emergence of new pathotypes due to natural recombination through sexual reproduction in the AB life cycle (Pande et al. 2005). Variation in the pathogen population have been recognized (Basandrai et al. 2007; Varshney et al. 2009; Kaur et al. 2012), which has an important implication for breeding programmes for AB resistance and reinforces the need to search for additional sources of stable resistance. Increased efforts are needed to identify new sources of resistance and incorporate the resistance into commercial varieties. Furthermore, the stability of sources of resistance should be checked in time and space, requiring multi-location and multi-year experiments. This is of great importance in breeding programs, since large genotype (G) × environment (E) interactions result in discrepancies between expected and realized responses to selection and makes it difficult to predict the behaviour of the accessions in different situations. Genotype and genotype by environment (GGE) biplot analysis has been widely used in recent years to determine the stability of disease resistance through multilocation trials in order to identify stable resistant genotypes (Rubiales et al. 2012; Sibiya et al. 2012; Sharma et al. 2012). This study was therefore conducted to (i) identify the AB resistant genotypes from a chickpea germplasm collection and breeding lines for multi-location and multi-year testing and (ii) investigate the G × E interaction for the AB resistance in different environments (combination of location and year) to identify and confirm stable resistant sources.

Materials and methods

Plant materials

Two sets of chickpea genotypes were evaluated for resistance to AB. The first set consisted of 424 genotypes including a germplasm collection and breeding lines obtained from E.J. Ted Knights, New South Wales Agriculture, Australia and were evaluated under controlled environment conditions at the seedling stage at ICRISAT in a preliminary screening during 2005–2006 (December–January). The resistant lines selected from the previous season were further evaluated under the same conditions during 2006–2007 season. Finally, a set of 29 genotypes were selected based on their low disease severity (≤3.0 on a 1–9 scale) over 2 years under controlled conditions and constituted a nursery called "Ascochyta Blight Nursery" (ABN) for multi-location and multi-year screening (Table 1). Simultaneously, the same nursery was also tested under controlled environment conditions at ICRISAT for 3 years. Cultivar ICC 4991, an old cultivar from Punjab (India) highly susceptible to AB was included as susceptible check.

Controlled environment evaluations

A controlled environment plant growth chamber protocol developed at ICRISAT was used by following standard seedling screening technique (Pande et al. 2011). Seedlings of the test genotypes along with a susceptible check (ICC 4991) were grown in 35×25×8 cm plastic trays filled with sterilized river sand and vermiculite mixture (10:1) in a greenhouse, maintained at 25±2 °C for 10 days. Ten genotypes with eight seeds/test row were planted in each tray (nine test genotypes and one susceptible check). The experiment was conducted in a completely randomized design with three replications and repeated once. Mass multiplication of a highly aggressive isolate, *A. rabiei* (Accessions No. ITCC 6651) achieved by growing the pathogen on sterilized kabuli chickpea seeds for 8 days at 20±1 °C with 12 h photoperiod. Seeds with profuse

Table 1 Pedigree of chickpea genotypes selected for resistance screening to Ascochyta blight in field disease nurseries in India

Entry no.	Genotype	Collection	Origin	Type	Pedigree
1	EC 516709	Breeding line	Australia	Desi	98180-1030
2	EC 516729	Breeding line	Australia	Desi	97020-1489
3	EC 516771	Breeding line	Australia	Desi bold	97039-1226
4	EC 516792	Breeding line	Australia	Desi	99067-1013
5	EC 516793	Breeding line	Australia	Desi	97020-1319
6	EC 516796	Breeding line	Australia	Desi	98314-1007
7	EC 516824	Breeding line	Australia	Desi	98047-1072
8	EC 516850	Breeding line	Australia	Desi	97020-1506
9	EC 516867	Breeding line	Australia	Desi	99315-1073
10	EC 516895	Breeding line	Australia	Desi	98047-1077
11	EC 516934	Breeding line	Australia	Desi	98176-1044
12	EC 516936	Breeding line	Australia	Desi bold	98047-1069
13	EC 516957	Breeding line	Australia	Desi	97020-1083-1001
14	EC 516967	Breeding line	Australia	Desi	99008-1004
15	EC 516971	Breeding line	Australia	Desi	99315-1104
16	EC 517003	Breeding line	Australia	Desi	99315-1009
17	EC 517011	Breeding line	Australia	Desi	97039-1012
18	EC 517012	Breeding line	Australia	Desi	98047-1079
19	EC 517023	Breeding line	Australia	Desi	97037-1465
20	EC 517025	Breeding line	Australia	Desi	97039-1644
21	EC 517039	Breeding line	Australia	Desi	97-139A × 34-99V4001
22	ICCV 04537	Breeding line	ICRISAT, Patancheru	Desi	ICCX-910028-32ABR-BP-4ABR-BP-BP (C 235 × NEC 138-2) × (FLIP 87-4C × ILC 4421)
23	ICCV 98815	Breeding line	ICRISAT, Patancheru	Desi bold	[(ICC 235 × NEC 138-2)-F1 × (FLIP 87-4C × ILC 4421)-F1]-46ABR-BP-5ABR-BP
24	ICCV 98818	Breeding line	ICRISAT, Patancheru	Desi bold	[(ICC 235 × NEC 138-2)-F1 × (FLIP 87-4C × ILC 4421)-F1]-42ABR-BP-26ABR-BP
25	ICC 607	Germplasm line	India	Desi	–
26	ICC 4181	Germplasm line	Iran	Desi	–
27	ICC 4200	Germplasm line	Iran	Desi	–
28	ICC 15989	Germplasm line	Syria	Desi	–
29	ICC 4991	Susceptible check	India	Desi	–

sporulation were soaked in water for 30 min, vortexed for 2–3 min to facilitate the release of conidia into water and filtered through a double-layered muslin cloth. The conidial concentration in the suspension was adjusted to 5×10^4 conidia/ml using a haemocytometer and this suspension was used as inoculum. Ten-day-old seedlings were transferred to the plant growth room maintained at 20±1 °C with 12 h photoperiod. Seedlings were adapted to these conditions for 24 h before inoculation. The seedlings were inoculated artificially by spraying the inoculum until the run-off on the foliage (~3 ml per plant) using a hand-operated atomizer. Inoculated plants were allowed to dry partially for 30 min to avoid dislodging of the spores and thereafter, maintained at 20±1 °C and continuous relative humidity (RH) of 100 % for 96 h, after 96 h the 100 % RH was maintained for 6–8 h per day for 7 days. Disease severity of individual genotype was assessed 10 days after inoculation (DAI) on a modified 1–9 rating scale, *i.e.* 1 = no visible symptoms and 9 = 100 % of the plants killed (Pande et al. 2011).

Field trials

The field trials were conducted at three locations (Almora, Dhaulakuan and Ludhiana) reported to have high AB severity and endemic pathogen populations for 3 years (2007–2008, 2008–2009 and 2009–2010) in India (Pande et al. 2011). The information on the test genotypes and environments is given in Tables 1 and 2 respectively.

A randomized complete block design trial was conducted with two replications at each location. Forty seeds of each

Table 2 Description of environments (defined as a combination of location and year) of the trials for the multi-environment study during 2008 to 2010 in India

Location	Latitude (N)	Longitude (E)	Altitude (m)	Environment[a]	Season	Weather during the growing season					30 days before evaluation				
						Temperature (°C)		RH (%)		Rain (mm)	Temp. (°C)		RH (%)		Rain (mm)
						Max.	Min.	Max.	Min.		Max.	Min.	Max.	Min.	
Almora, Uttarakhand	29° 36′	79° 40′	1638	A08	2007/2008	21.3	2.2	94.8	39.8	36	19.7	1.0	95	41	5.5
				A09	2008/2009	23.3	2.7	95.8	39.1	80.5	22.7	1.7	94.9	34.6	50
				A10	2009/2010	22.5	2.9	94.5	39.8	96	20.9	2.5	92.7	43.3	67
Dhaulakuan, Himachal Pradesh	30°30′	77°20′	468	D08	2007/2008	23.6	5.8	91	44	25.3	21.4	4.5	92	47	7.2
				D09	2008/2009	25	7.7	94.6	37.1	33.8	25.1	7.5	96.6	33.6	23.8
				D10	2009/2010	24.1	7.2	91.9	44.5	59	23.4	6.4	93	45	33
Ludhiana, Punjab	30° 55′	75° 54′	255	L08	2007/2008	22.9	8.3	93.2	44.1	21.4	20.2	6.4	95	48.8	3.2
				L09	2008/2009	24.1	9.9	95.3	49.9	10.1	23.3	9.0	96	52	7.0
				L10	2009/2010	23	9.5	94.8	49.5	49.5	22.5	9.0	94	50	25

Climatic data are provided for the growing season and 30 days before evaluation

[a] Environment is denoted as first letter of the each location followed by year of screening (2007/2008=08; 2008/2009=09; 2009/2010=10)

genotype were sown in a 4-m-long test row with row to row spacing of 30 cm and plant to plant spacing of 10 cm. Susceptible cultivar ICC 4991 was included after every fourth test rows to serve as indicator/disease spreader. At the onset of flowering, AB-infected debris collected from the previous season was scattered over the field (3–4 kg per 100 m^2) at each location and season. Plants were also inoculated with a spore suspension of highly virulent isolate of *A. rabiei* (1×10^5 condia/ml) at each location in the evening (at a volume of 5 L of inoculum per 100 m^2). This was repeated 2–3 times at 10 days intervals if disease development was not sufficient. Sprinkler irrigation for 15 min per h from 1000 h to 1600 h was used to maintain high relative humidity on dry days to promote infection and disease development. The data on disease severity were recorded on 10 randomly selected plants on the 1–9 rating scale when susceptible check showed maximum rating of 9 and again at maturity (Pande et al. 2011). Data on weather variables, such as, temperature (minimum and maximum), relative humidity (minimum and maximum) and rainfall (mm) during the crop period were collected from the meteorological station of the respective locations in each year.

Statistical analysis

Data from field and controlled environment experiments were analysed separately using analysis of variance (ANOVA) to determine the contribution of year, location, genotypes and their interaction. The square-root transformation was applied for scale data of both the experiments before analysis to attain normality. ANOVA was carried out using the mixed procedure of the SAS software version 9.3 for Windows (SAS Institute Inc. 2011, Cary, NC). Broad-sense heritability from multi-environment trials was estimated to understand the extent to which the trait is influenced by the genotype as opposed to the environment (Singh et al. 1993).

Untransformed mean data of each genotype from field trials (2007–08, 2008–09 and 2009–10) were subjected to genotype and genotype × environment (GGE) biplot analyses (Yan et al. 2000; Yan 2001) to determine stability of resistance and to identify the stable AB reaction of the genotypes across environments. "Environment" was defined as the combination of "year" and "location" (each site in a given year was a separate environment, Table 2). The GGE biplot provided a visual depiction of the relationship among the genotypes and test environments, and was performed with GenStat 14 software (Payne et al. 2010) using a model based on singular value decomposition (SVD) of the first two principal components (Yan 2002). A scaling factor (*f*) of 0.5 (symmetric scaling) was chosen to carry out the singular value decomposition of the environment-centred data (Yan 2002) and to obtain the first two principal components (PC1 and PC2).

The abscissa of the "average environment coordination" (AEC) (single arrowed line) was drawn on the biplot as the line that connects the origin and the environmental average, *i.e.* the average of PC1 and PC2 coordinates across environments. It is an appropriate tool to compare genotypes by their average performance and stability. The projection of genotypes onto this axis represents the contribution of each genotype to G, so genotypes may be ranked along AEC abscissa, with arrow pointing to higher mean severity. The AEC ordinate (AEC_o), on the other hand, represents the contribution of each genotype to the interaction G × E, thus giving information about the genotypic stability or instability (consistency or inconsistency across environments). The most stable genotypes (most consistent across environments) will be those with lowest severity values (positive or negative). Each environment is characterised by its vector (the line that connects it with the origin of the biplot), the length of the vector was used to determine the discriminating ability of each of the test environments, with a shorter vector implying that the environment was not well represented by PC1 and PC2 (Yan et al. 2007). Angle between environment vectors were used to judge correlations (similarities/dissimilarities) between pairs of environments (Yan and Kang 2002). An angle of zero indicated a correlation of +1, while an angle of 90° or −90°, a correlation of zero, and an angle of 180°, a correlation of −1 (Yan 2002). The ideal environment should be that one showing a high projection value onto the AEC and a small absolute projection value onto the AEC ordinate (Yan et al. 2000). The association between field and controlled environment screening was determined in terms of Pearson's correlation coefficients using the correlation procedure in SAS (SAS Institute Inc. 2008. SAS/STAT® 9.2 User's Guide. Cary, NC).

Results

Preliminary screening under controlled environment

In the process of identification of new sources of resistance to AB, the preliminary screening of 424 germplasm and breeding lines under controlled environment conditions at ICRISAT during 2005–2006 revealed a broad range of responses among the tested material (Fig. 1a), which allowed the selection of 51 resistant lines (disease severity ≤3.0 on 1–9 scale) to be further evaluated. Of these, one was found resistant and the remaining were moderately resistant (3.1–5.0) to AB under controlled environment condition during the 2006–2007 cropping season (Fig. 1b). Based on 2 years of screening, a set of 29 genotypes including the susceptible check ICC 4991 were selected for further validation of their resistance under multi-location and multi-year screening.

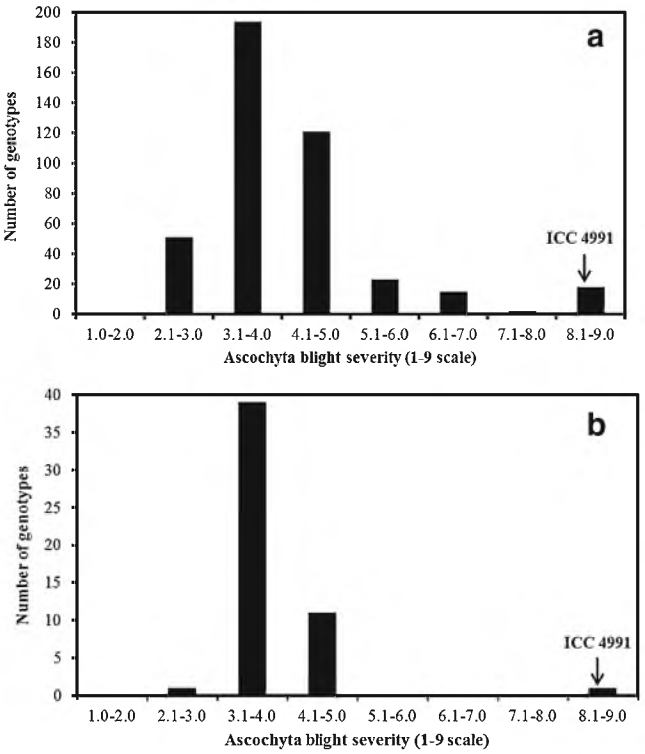

Fig. 1 Frequency distribution for Ascochyta blight severity of chickpea genotypes evaluated in controlled environment conditions at ICRISAT, Patancheru, **a** 424 genotypes during season 2005–2006, **b** 51 genotypes during season 2006–2007

Field studies

In the present study, AB severity of most of the chickpea genotypes varied greatly between environments as the performance of each genotype was not always stable across all environments, shown by the highly significant effects ($P<0.001$) of location (year), genotype and their interaction. Likewise, it showed that location (year), genotype and their interaction contributed most of the variability in AB observed (Table 3). The environmental effect was also evident in different patterns of frequency distributions (Fig. 2). AB severity was relatively high on the susceptible check ICC 4991 in all nine test environments ranging from 6.0 to 9.0 (Table 4 and Fig. 2). Among the nine environments, Ludhiana 2008 (L08) had highest mean AB severity (4.9) ranging from 2.0 to 8.0, whereas Almora 2008 (A08) had lowest mean AB severity (3.1) ranging from 2.0 to 7.0. Based on coefficient of determination, location, year, genotype and their interaction explained 95 % variance in AB severity in multi-environment experiments ($R^2=0.95$). The broad sense heritability estimate of AB nursery evaluated across nine environments under field condition was 0.82 indicating the involvement of genetic component in AB resistance.

When fitting the GGE biplot, the first two principal components (PCs) obtained by singular value decomposition of

Table 3 Analysis of variance for Ascochyta blight severity of 29 chickpea genotypes in nine environments under field conditions during 2007–08 to 2009–10

Source of variation	NDF	DDF	*F* value	*P*	Variation (%)[a]
Year	2	140	43.07	<.0001	1.60
Location (year)	6	103	44.72	<.0001	59.84
Replication (year × location)	9	95.8	1.71	0.0977	–
Genotype	28	211	27.68	<.0001	15.74
Year × genotype	56	175	5.21	<.0001	5.36
Location × genotype (year)	168	125	5.45	<.0001	17.48
Coefficient of variation (%)	10.34				
Coefficient of determination (R^2)	0.95				
Broad-sense heritability (*H*)	0.82				

NDF numerator degree of freedom; *DDF* denominator degree of freedom

[a]Relative percentage contribution of each source of variation to the total variance

environment-centered data explained 80 % (PC2=66 % and PC1=14 %) of total GGE variation for AB severity, which means that the biplot of PC1 and PC2 adequately explains the environment centered data. Results of the polygon view of the GGE biplot is presented in Fig. 3. The accessions that are furthest away from the biplot origin delimit the vertices of a polygon (dashed lines in Fig. 3). Vertex genotypes are those which contribute the most to the interaction, *i.e.* those which show the highest or the lowest severity to the disease. A line perpendicular to AEC (arrowed) had 16 genotypes on its left with a mean severity equal to or lower than 3.4 on the 1–9 scale across the environments. These were classified into three groups based on their levels of severity and stability in all environments. Group 1 consisting of one genotype EC 516934 (entry no. 11) with mean severity value of 2.7, showed consistently lowest levels of disease severity and stability across the tested environments. Group 2 included four genotypes (average severity in brackets), *i.e.* ICCV 04537 (2.8; entry no. 22), ICCV 98818 (2.9; entry no. 24), EC 516850 (2.9; entry no. 8) and EC 516971 (2.9; entry no. 15), having the lowest severities, but had moderate negative interaction with Dhaulakuan 2010 (D10) and Ludhiana 2010 (L10) and a positive interaction with rest of the environments. However, the genotypes in group 1 and 2 had disease severity ≤3.0. Group 3 is constituted of resistant genotypes with mean severity values of 3.1–3.4, which

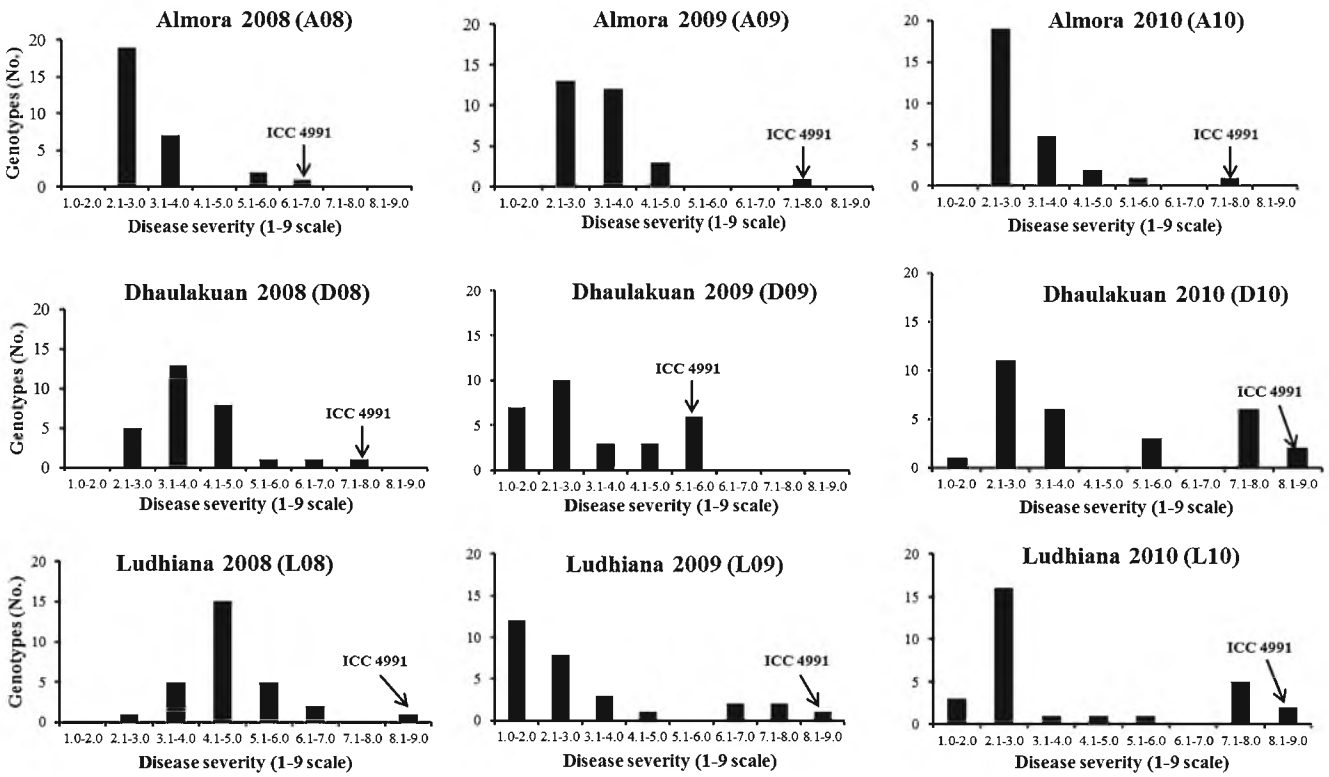

Fig. 2 Frequency distribution for Ascochyta blight severity (1–9 scale) of 29 genotypes of chickpea including a susceptible check ICC 4991 evaluated in nine environments based on field screening in India. Position of susceptible check is shown to facilitate comparisons across the environments

Table 4 Reaction of 29 chickpea genotypes to *Ascochyta rabiei* in controlled environment conditions at ICRISAT, Patancheru and field screening at Almora (A), Dhaulakuan (D) and Ludhiana (L) during 2007-08 to 2009-10

Entry no.	Genotype	Ascochyta blight severity (1–9 scale)													
		Controlled[a]				Field[b]									Mean
		2008	2009	2010	Mean	A08	A09	A10	D08	D09	D10	L08	L09	L10	
1	EC 516709	3.0	4.5	5.0	4.2	2.5	2.5	3.0	3.0	3.0	8.0	5.0	5.0	9.0	4.6
2	EC 516729	3.5	5.0	4.5	4.3	2.5	3.0	2.5	4.5	6.0	8.0	4.5	8.5	8.0	5.3
3	EC 516771	3.5	4.5	5.0	4.3	3.0	2.5	2.5	6.5	5.0	7.5	4.5	7.0	5.5	4.9
4	EC 516792	4.0	4.5	3.5	4.0	2.5	4.5	3.5	3.5	2.5	2.5	5.0	2.0	4.5	3.4
5	EC 516793	3.5	4.5	5.0	4.3	3.5	2.0	3.0	5.0	6.0	8.0	4.5	7.0	8.0	5.2
6	EC 516796	4.5	4.5	4.0	4.3	2.5	3.5	4.0	4.0	4.5	5.5	3.5	3.0	2.5	3.7
7	EC 516824	5.5	5.5	3.0	4.7	2.5	3.5	2.5	4.0	4.0	5.5	6.0	3.5	3.0	3.8
8	EC 516850	4.5	4.5	4.0	4.3	3.5	3.0	3.0	3.0	2.5	2.5	4.5	1.5	3.0	2.9
9	EC 516867	4.0	3.5	4.5	4.0	2.0	3.5	2.5	3.5	3.0	2.5	5.0	2.5	3.0	3.1
10	EC 516895	4.0	4.0	4.5	4.2	3.5	3.5	3.0	3.0	5.0	8.5	6.0	8.0	9.0	5.5
11	EC 516934	3.5	2.5	3.0	3.0	2.0	2.5	3.0	4.0	2.0	2.0	3.5	2.5	2.5	2.7
12	EC 516936	4.0	4.5	3.5	4.0	2.0	3.5	3.0	3.5	3.5	2.5	4.5	3.0	3.0	3.2
13	EC 516957	5.0	5.0	4.5	4.8	3.5	5.0	3.0	4.5	5.5	8.5	6.0	3.5	8.0	5.3
14	EC 516967	5.0	5.0	4.5	4.8	3.5	3.5	3.0	4.5	3.5	2.5	5.5	1.5	2.5	3.3
15	EC 516971	5.0	5.0	3.5	4.5	3.0	3.0	4.5	3.0	2.0	2.5	4.5	1.5	2.5	2.9
16	EC 517003	3.5	4.5	5.0	4.3	2.5	3.0	3.5	4.0	5.5	8.0	4.0	2.0	8.0	4.5
17	EC 517011	4.0	5.5	3.5	4.3	2.5	3.5	2.5	4.0	2.5	5.5	4.5	1.5	2.5	3.2
18	EC 517012	4.5	4.5	3.5	4.2	5.5	2.5	5.0	3.5	2.0	3.5	6.5	3.5	2.5	3.8
19	EC 517023	4.5	5.0	4.0	4.5	3.5	3.0	3.0	4.0	2.5	4.0	3.5	2.0	3.0	3.2
20	EC 517025	3.5	4.5	3.5	3.8	3.0	3.0	2.5	6.0	5.5	3.5	5.5	2.5	3.5	3.9
21	EC 517039	4.5	5.0	3.5	4.3	3.0	3.5	4.0	5.0	3.0	3.5	3.5	2.0	2.5	3.3
22	ICCV 04537	4.0	3.5	3.5	3.7	3.0	3.0	3.5	4.0	2.0	2.5	3.0	2.5	2.0	2.8
23	ICCV 98815	3.5	4.5	4.0	4.0	3.5	3.5	3.0	4.5	2.0	3.5	4.5	2.0	2.5	3.2
24	ICCV 98818	3.5	4.5	4.0	4.0	2.5	3.0	3.5	4.0	2.0	3.0	4.5	2.0	2.0	2.9
25	ICC 607	4.5	3.5	4.0	4.0	2.5	4.0	3.0	4.0	3.0	2.5	5.0	2.5	3.0	3.3
26	ICC 4181	4.0	3.5	4.0	3.8	2.5	3.5	3.0	5.0	2.5	2.5	5.0	3.0	3.0	3.3
27	ICC 4200	3.5	3.5	4.5	3.8	2.5	3.5	3.0	5.0	2.0	3.5	6.5	2.0	2.0	3.3
28	ICC 15989	5.0	6.5	3.0	4.8	5.5	5.0	5.5	3.0	2.5	3.0	4.5	1.0	3.0	3.7
29	ICC 4991[c]	9.0	9.0	9.0	9.0	7.0	7.5	7.0	7.5	6.0	8.0	9.0	8.0	8.0	7.6
	Mean	4.3	4.6	4.2	4.4	3.1	3.5	3.4	4.2	3.5	4.6	4.9	3.3	4.2	3.8
	SE (m)±	0.4	0.4	0.3	0.3	0.4	0.4	0.3	0.7	0.5	0.8	0.5	0.7	0.7	0.2
	CV (%)	12.3	13.1	11.6	12.3	19.0	15.4	13.2	23.8	20.3	24.5	14.4	28.1	23.0	20.0
	LSD ($P<0.05$)	1.1	1.2	1.0	0.8	1.2	1.1	0.9	2.1	1.4	2.3	1.4	1.9	2.0	0.5

[a] Disease severity based on the mean of three replications under controlled environment conditions at ICRISAT for 3 years

[b] Disease severity based on the mean of two replications in 9 environments in India

[c] Susceptible check (SC)

showed a proportional response across all the environments, that is, environments with more stable lines [EC 516936 (entry no. 12), EC 517011 (entry no. 17) and EC 517023 (entry no. 19)] with mean AB severity value of 3.2 and less stable lines [EC 516967 (entry no. 14) and ICC 4200 (entry no. 27)] with mean severity of 3.3. The susceptible check line ICC 4991 (entry no. 29) was consistently more susceptible, hence was farthest on the right side of the origin of biplot and had a high positive PC1 value.

The length of an environment vector (line connecting it with the origin) is proportional to the standard deviation of cultivar means in the environment, which is a measure of the discriminating power of the environment. All the test environments had positive PC1 scores indicating good AB discriminative

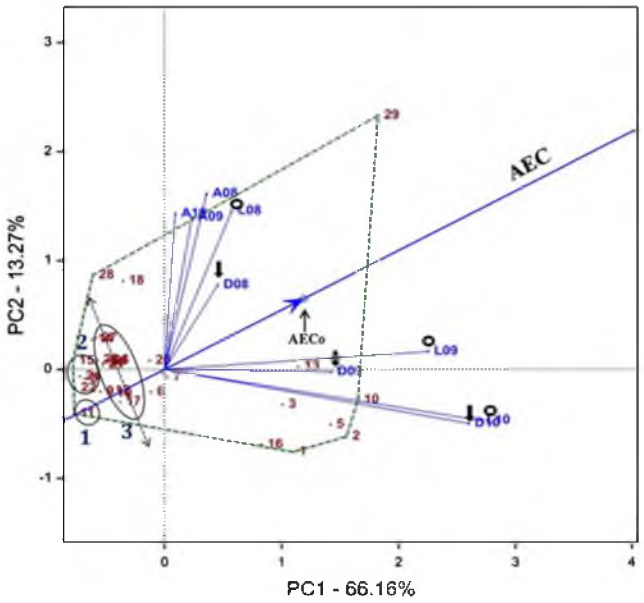

Fig. 3 GGE biplot of first and second principal components (PC1 and PC2, respectively) based on Ascochyta blight severity of 29 genotypes of chickpea including a susceptible check ICC 4991 in 9 environments during 2008 to 2010. The environments are shown in *bold and upper-case, with first letter abbreviating the location followed by season* (A08 = Almora 2007–2008, A09 = Almora 2008–2009, A10 = Almora 2009–2010; D08 = Dhaulakuan 2007–2008, D09 = Dhaulakuan 2008–2009, D10 = Dhaulakuan 2009–2010; L08 = Ludhiana 2007–2008, L09 = Ludhiana 2008–2009, L10 = Ludhiana 2009–2010). Refer to Table 1 for full names of genotypes

ability. However, environments greatly differed in their discriminative ability as shown by their different vector lengths. Of the nine test environments, three environments [Ludhiana 2009 (L09), Ludhiana 2010 (L10) and Dhaulakuan 2010 (D10)] supported higher disease expression and discrimination (high positive PC1 scores, longest vectors) than others. In contrast, the test environment Dhaulakuan 2008 (D08) had a short vector compared to the rest of the environments, meaning that all genotypes performed similarly in it. The environments could be ranked as follows in terms of disease expression and discrimination of genotypes: Ludhiana 2010 (L10) > Dhaulakuan 2010 (D10) > Ludhiana 2009 (L09) > Dhaulakuan 2009 (D09) > Almora 2008 (A08) > Ludhiana 2008 (L08) > Almora 2009 (A09) > Almora 2010 (A10) > Dhaulakuan 2008 (D08). The location Dhaulakuan (D08, D09 and D10) showed diversity in different years as represented by their position at different points and different vector lengths in the biplot indicated by a black arrow (⬇). The location Dhaulakuan in 2008 (D08) had the shorter vector length and was less discriminative of genotypes than the same site in 2009 (Dhaulakuan 2009) and 2010 (Dhaulakuan 2010). Rainfall data also showed relatively less rains during 30 days before evaluation in Dhaulakuan 2008 (D08) compared to same location environments Dhaulakuan 2009 (D09) and Dhaulakuan 2010 (D10). Similarly, the site Ludhiana (L), differed in the 3 years for both vector length and association indicated by circle (⭕). In contrast to that, vectors for the 3 years (2008, 2009 and 2010) for the Almora location (A08, A09 and A10) had a similar length and co-ordinates, hence were not discriminating. In addition to exhibiting a high level of discrimination, an ideal test location should also be representative of the target growing region. The cosine of the angle between two environmental vectors provided an estimate of their correlation coefficient and the vectors with the smallest angles with to the AEC axis will be the most representative (Yan 2002). The angles between all the nine environments except Ludhiana 2010 (L10) and Dhaulakuan 2010 (D10) in this study were less than 90°, indicating positive associations among them. All the locations had a similar distance from the AEC axis, so no particular environment was more representative of the overall target environment.

Controlled environment studies

The ANOVA exhibited significant ($P<0.0001$) variation among the 29 genotypes for AB resistance in individual years (2007–08, 2008–09 and 2009–10; *data not shown*) as well as in combined data (Table 5). Although, there was significant interaction between genotype and years, the variance (%) for genotypes was very high, indicating the variation in the AB severity were mainly contributed by genotypes (Table 5). Disease severity in the susceptible check ICC 4991 was high (9.0 rating on the 1–9 scale) in all the 3 years (Table 4). Based on the mean severity over 3 years, one genotype, EC 516934, was found resistant whereas the remaining genotypes were moderately resistant to AB. The broad sense heritability estimate of AB nursery evaluated under controlled environmental conditions over 3 years was 0.82.

Resistance in both field and controlled evaluations

In general, AB severity under field conditions in nine environments was comparatively less than in the controlled environment at ICRISAT. However, a significant positive correlation was found between the performance of the genotypes from the controlled environment and field screening ($r=0.70$, $P<0.01$). AB severity of 29 genotypes ranged from 3.0 to 9.0 with a mean of 4.4 in controlled conditions and 2.7–7.6 with a mean of 3.8 in field screening (Table 4). Among the 29 genotypes, five genotypes (EC 516934, ICCV 04537, ICCV 98818, EC 516850 and EC 516971) were found to be resistant to AB with mean severity ≤3.0 in field screening across the nine environments. Under controlled environment screening, genotype EC 516934 was resistant to AB over the 3 years and the remaining genotypes showed moderate resistant reaction.

Table 5 Analysis of variance for severity to Ascochyta blight of 29 chickpea genotypes in controlled environment screening at ICRISAT, Patancheru, India for 3 years (2007–2008, 2008–2009 and 2009–2010)

Source of variation	NDF	DDF	*F* value	*P*	Variation (%)[a]
Year	2	55	9.85	0.0002	4.08
Replication (year)	3	45.2	0.40	0.7571	–
Genotype	28	82.4	11.86	<.0001	63.79
Year × genotype	56	53.4	3.08	<.0001	32.14
Coefficient of variation (%)	6.54				
Coefficient of determination (R^2)	0.86				
Broad-sense heritability (*H*)	0.75				

NDF numerator degree of freedom; *DDF* denominator degree of freedom

[a] Relative percentage contribution of each source of variation to the total variance

Discussion

Large scale evaluation of a genetically diverse chickpea germplasm collection and breeding lines against biotic stresses in multi-location is an expensive process impeding effective utilization. Therefore, a larger collection needs to be reduced to a meaningful and manageable number of lines for evaluation of traits of economic importance such as disease resistance. In the present study, screening of 424 germplasm and breeding lines under controlled conditions during 2005–2006 was a preliminary evaluation to cull out the highly susceptible genotypes. The additional evaluations performed under controlled conditions during 2006–2007 allowed further refinement of selection of lines for multi-location and multi-year testing. Multi-environment evaluation revealed that some of the genotypes showed consistent resistance (stable) reaction across the environments, whereas some of the genotypes showed variations in disease reaction across the environments. Differential reactions of the chickpea genotypes to AB in multi-environments can be attributed to the differential virulence in the pathogen population (Chen et al. 2004; Vail and Banniza 2008; Kaur et al. 2012; Atik et al. 2013). Significant effects of location (year) suggested that the weather conditions were more conducive to the disease at some locations over others and that locations varied between years. The relative effects of the weather were minimized by conducting the experiment at locations known for higher levels of disease severity, and natural inoculum was supplemented with artificial inoculation supported by sprinkler irrigation. Differences in AB severity among the locations could also be due to existence of variable pathogen population at these locations. Other researchers have reported similar variations in AB severity at the different locations and years (Basandrai et al. 2007; Pande et al. 2011). Disease severity in a few genotypes was variable among the environments, but the severity level on the susceptible check ICCV 4991 indicated high and adequate disease pressure in all the environments. Average severity was lower at Almora (A08, A09 and A10) than at the other two locations (Dhaulakuan and Ludhiana), suggesting a lower levels of virulence of the Almora pathogen populations confirming previous reports of higher virulence of Dhaulakuan and Ludhiana isolates (Basandrai et al. 2005; Pande et al. 2011; Kaur et al. 2012). The prevalence of higher mean severity at Dhaulakuan and Ludhiana has also been reported (Basandrai et al. 2007; Kaur et al. 2012). At Almora, mean AB severity was greater in 2009 and 2010 than in 2008, which may be attributed to the high rainfall during the growing season (>80 mm) and 30 days before evaluation (≥50 mm) as compared to 36 mm during 2008 and 5.5 mm in 30 days before evaluation. This underlines the role of weather conditions on the development of AB in chickpea, even with misting irrigation (Jhorar et al. 1997; Basandrai et al. 2007; Pande et al. 2011).

Multi-environment testing revealed not only significant genotypic effects but also significant effects of the environment and the genotype × environment interaction for AB severity. The effect of environment might be ascribed to different virulence in the local populations of the pathogen. The widely varying reports of races and pathotypes of *A. rabiei* (Ambardar and Singh 1996; Porta-Puglia et al. 1996; Chen et al. 2004; Basandrai et al. 2005; Kaur et al. 2012) have been problematic based on differences in experimental design and interpretation of results, and the classification into races is still controversial (Chen et al. 2004; Vail and Banniza 2008). The presence of a teleomorph (*Didymella rabiei*) in the *A. rabiei* life cycle contributes to variability within the pathogen population, which may generate new combination of virulence genes and the development of new pathotypes (Pande et al. 2005).

Multi-year and multi-location evaluations are important to identify stable sources of disease resistance. Individual analysis of each field trial revealed differences in the response of the genotypes to the disease, with some showing various degrees of resistance. However, the variations in frequency distributions of genotypes and the highly significant G × E (Genotype (year) × location) interaction in the ANOVA showed that it is

necessary to check the stability of disease reactions across environments. Diversity both in time and space of the nine trials was considered to be sufficient for this type of study. This justifies selection of the site regression method to generate a GGE biplot as the appropriate method for analysing the data from multi-environment trials (Crossa and Cornelius 1997). Besides environment, genotype and G × E interaction also contributed to variability in AB severity. This means that there were substantial differences in the response of the genotypes in the tested environments. This inconsistency of phenotypic expression across environments is a problem frequently encountered in AB resistance screening (Rubiales et al. 2012), whose explanation may include: (i) different pathogen races, that is specific virulence in the local pathogen populations matching specific resistance genes in the plant material (Basandrai et al. 2005; Kaur et al. 2012); (ii) particular sensitivities of the different genotypes to the overall disease severity level (Bhardwaj et al. 2010); (iii) or other factors like weather, soil properties or agricultural practices.

In GGE biplot analysis, the PC1 and PC2 accounted for 80 % of the total variation for G and GE suggesting that this biplot is a good approximation of the mean performance and stability (Yang et al. 2009; Yan et al. 2010). Thus, the biplots may be interpreted as effective graphical representation of the variability in the multi-location nurseries data. Disease resistant genotypes are those with very low PC1scores (low severity scores) and low absolute PC2 scores (highly stability) (Yan et al. 2007). In this context, the best genotypes (entry number in bracket) would be EC 516934 (20), ICCV 04537 (1), ICCV 98818 (3), EC 516850 (16) and EC 516971 (23) with a disease severity ≤3.0 on the 1–9 scale (low PC1 score) and showing high stability (low PC2 scores). In addition, group 3 (EC 516936, EC 517011, EC 517023, EC 516967 and ICC 4200) may also be considered for further studies, but taking into account their performance and consistency across the environments. High broad-sense heritability estimate under field and controlled conditions indicates that selection of resistant genotypes can be useful for exploitation in future advanced breeding programs for chickpea.

The purpose of the multi-environment evaluation was to identify stable resistant genotypes and confirm their resistant stability across these environments. Among the nine environments, three environments, Ludhiana 2009 (L09), Ludhiana 2010 (L10) and Dhaulakuan 2010 (D10) were characterised by high disease severity and were discriminative (long vector). The angles between all of the nine environments except Ludhiana 2010 (L10) and Dhaulakuan 2010 (D10) were less than 90°, indicating the high correlations amongst them. It would be interesting then to test the most divergent genotypes against isolates from these locations under controlled conditions. Acute angles among vectors associated with Almora in 2008, 2009 and 2010 are indicative of higher similarities among the environments meaning that the same information could be obtained from these environments (Yan and Tinker 2006). It was observed that similar vector length of environments Almora 2009 (A09) and Almora 2010 (A10) compared to Almora 2008 (A08) could be attributed to higher rainfall 30 days before evaluation in Almora 2009 (A09) and Almora 2010 (A10). Other localities tested in 2007–2008 (Dhaulakuan 2008 and Ludhiana 2008) were less discriminating of genotypes than those in 2008–2009 (Dhaulakuan 2009 and Ludhiana 2009) and 2009–2010 (Dhaulakuan 2010 and Ludhiana 2010). This seems to be related to the level of disease pressure reached in each environment.

Controlled environment evaluation of chickpea genotypes provided comparatively more uniform results (with low coefficient of variations) and high AB severity over 3 years compared to the field evaluation. However, we found positive correlation (r=0.70; P<0.01) between the controlled environment and field screening techniques, which confirms the findings of the field trials and stability of resistant reactions of genotypes in different environments. Significant positive correlations between field and controlled environment screenings are well demonstrated for AB (Haware et al. 1995; Sharma et al. 1995; Pande et al. 2011). This supports the evaluation for AB resistance using 10-day-old-seedlings in a controlled environment and adult plant field screening at locations known for high levels of AB incidence (Pande et al. 2011). However, controlled environment screening is more economical, faster and independent of season compared with field screening.

The absence of high levels of genetic resistance to the pathogen dictates the continued search and identification of new sources of resistance to AB. In this study, we identified five breeding lines with high levels of resistance to AB that can be useful to chickpea disease breeding programs.

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