Crop Protection 54 (2013) 106–113

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Multi-environment field testing for identification and validation of genetic resistance to *Botrytis cinerea* causing Botrytis grey mold in chickpea (*Cicer arietinum* L.)

M. Sharma [a,*], T. Kiran Babu [a], R. Ghosh [a], R. Telangre [a], A. Rathore [a], L. Kaur [b], K.P.S. Kushwaha [c], R. Das [a], S. Pande [a]

[a] International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502324, AP, India
[b] Department of Plant Pathology, Punjab Agricultural University (PAU), Ludhiana 141004, Punjab, India
[c] GB Pant University of Agriculture & Technology (GBPUA&T), Pantnagar 263145, Uttaranchal, India

ARTICLE INFO

Article history:
Received 4 April 2013
Received in revised form
31 July 2013
Accepted 31 July 2013

Keywords:
AMMI
Botrytis grey mould
Chickpea
Genotype × environment interaction
Host-plant resistance

ABSTRACT

Botrytis grey mould (BGM), caused by *Botrytis cinerea* Pers. Ex. Fr., is a destructive foliar disease of chickpea (*Cicer arietinum* L.) worldwide. Disease management through host-plant resistance is the most effective and economic option to manage this disease. The objective of this study was to identify new sources of resistance to BGM, validate their stability across environments and determine the magnitude of G × E interaction. One hundred and nine chickpea genotypes with moderate levels of resistance (BGM severity ≤5.0 on a 1–9 scale) were selected from the preliminary evaluation of 412 genotypes including germplasm and breeding lines under controlled environmental conditions in 2004–2005 at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. In order to validate resistance stability, an 'International Botrytis Grey Mould Nursery' (IBGMN) was constituted with 25 genotypes and tested in multi-environments for BGM resistance at two locations (Gurdaspur and Pantnagar) in India for 4 years and two locations (Tarahara and Rampur) in Nepal for 3 years. Additive main effects and multiplicative interaction (AMMI) analysis showed significant genotype (G), environment (E) and G × E interaction ($p < 0.0001$) with largest contribution by environment (47.36%). The first two principal component axes were significant, and contributed 48.21% to the total G × E interaction. The AMMI biplot analysis allowed the selection of five genotypes ICCV 96859, ICCV 96853, ICCV 05604, ICCV 96852 and ICCV 05605 with low BGM severity (between 3.7 and 4.7 on 1–9 scale) and moderate stability. Genotype ICCV 96859 having least disease severity and moderate stability could be highlighted and exploited in chickpea resistance breeding programmes.

1. Introduction

Chickpea (*Cicer arietinum* L.), is globally the third most important food legume crop of the world and the first most important pulse crop of India (http://www.icrisat.org/crop-chickpea.htm). The chickpea seeds are used for both human and animal consumption. It contains a high level of proteins (20–23%), carbohydrates (60.7%), and dietary fibres (17.4%) (Jukanti et al., 2012). South Asia is by far the largest producer and consumer of chickpea (FAOSTAT, 2010). The average global productivity of chickpea is about 0.8 t ha^{-1}, far below the actual yield potential, because the crop is subjected to large number of biotic and abiotic stresses. Among the biotic stresses, Botrytis grey mould (BGM, *Botrytis cinerea* Pers. ex. Fr.) is the second most important foliar disease after Ascochyta blight (*Ascochyta rabiei* (Pass.) Lab.). It is prevalent in South Asia (India, Bangladesh, Pakistan, Iran and Nepal), Australia and the Americas, and can cause complete yield loss in years with extensive rains and high humidity (Pande et al., 2006). The disease is the major cause for decline in chickpea area and production in northern and eastern India, Nepal (Pande et al., 2005) and Bangladesh (Bakr et al., 2004). Deployment of host plant resistance (HPR) is the best means of combating disease and more relevant in a crop like chickpea, which is predominantly grown by resource poor farmers. Germplasm with only moderate levels of resistance to BGM has been identified (Davidson et al., 2004; Pande et al., 2006) and integration of moderate levels of HPR with judicious use of fungicides as foliar application is reported for the sustainable chickpea production (Pande et al., 2005). In order to develop

* Corresponding author. Tel.: +91 40 30712493; fax: +91 40 30713074.
E-mail address: mamta.sharma@cgiar.org (M. Sharma).

0261-2194/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.cropro.2013.07.020

Table 1
Chickpea genotypes used in the IBGMN in India (2006–07 to 2009–10) and Nepal (2007–08 to 2009–10).

Serial no.	Genotype	Collection	Type	Pedigree
1	EC 516700	Breeding line	Desi	99315-1044
2	EC 516716	Breeding line	Desi	99039-1298
3	EC 516751	Breeding line	Desi	98003-1004
4	EC 516824	Breeding line	Desi	98047-1072
5	EC 516891	Breeding line	Desi	98063-1042
6	EC 516936	Breeding line	Desi bold	98047-1069
7	EC 516968	Breeding line	Desi	99142-1003
8	EC 516976	Breeding line	Desi	98047-1076
9	EC 517041	Breeding line	Desi	98314-1005
10	ICCV 04609	Breeding line	Desi	Dhanush × K 850
11	ICCV 05604	Breeding line	Desi bold	ICC 1069 × K 850
12	ICCV 05605	Breeding line	Desi bold	ICC 1069 × K 850
13	ICCV 88103	Breeding line	Desi	ICCC 13 × ICCC 18
14	ICCV 89332	Breeding line	Desi bold	JG 74 × K 850
15	ICCV 93928	Breeding line	Desi	CTCPS 50467 × ICCL 86233
16	ICCV 96817	Breeding line	Desi	(K 850 × ICCL 80074) × (LM 2100 × Dhanush)
17	ICCV 96852	Breeding line	Desi bold	(ICC 12237 × ICC 10690) × (L 132-1 × ICCL 85216)
18	ICCV 96853	Breeding line	Desi bold	ICCL 8000Y × L 132-1
19	ICCV 96859	Breeding line	Desi bold	[ICCV 89853 × E 100Y(M)] × C 235
20	ICC 14344	Accession	Desi	–
21	ICC 4063	Accession	Desi	–
22	ICC 4065	Accession	Desi	–
23	ICC 4074	Accession	Desi	–
24	ICC 4951	Accession	Desi	–
25	ICC 4954[a]	Released variety	Desi	–

[a] ICC 4954 is susceptible cultivar.

effective strategies for management of BGM, it is important to obtain information on stability of resistant genotypes across a range of environments. Several methods have been proposed to analyse performance of genotypes across the environments (Finlay and Wilkinson, 1963; Eberhart and Russell, 1966; Gauch and Zobel, 1997). Two frequently used models for statistical analyses of genotype × environment data have been the genotype and genotype × environment interaction (GGE) model (Yan et al., 2000; Yan, 2001) and the additive main effects and multiplicative interaction (AMMI) model (Gauch et al., 2008). Recently many review articles have compared both these models thoroughly listing their advantages and disadvantages (Gauch, 2006; Gauch et al., 2008; Yan et al., 2007). The GGE model has been utilized to identify breeding lines and cultivars that are resistant to various diseases in chickpea and faba bean (Villegas-Fernandez et al., 2009, 2011; Rubiales et al., 2012; Sharma et al., 2012; Pande et al., 2013). Use of AMMI in analysing the multi-environment disease data to identify stable sources of resistance has also been reported (Forbes et al., 2005; Shagol and Tad-awan, 2008). Genotype × environment (G × E) interactions are important in the development and evaluation of stable disease resistant varieties. Therefore, the present study was conducted with the objective to identify BGM resistant genotypes in chickpea germplasm and breeding lines, and to validate the resistance stability through multi-year and multi-location field evaluations for further use in breeding programmes.

2. Materials and methods

2.1. Plant material and locations

A set of 412 chickpea genotypes including germplasm accessions and breeding lines was evaluated for BGM resistance during 2004–05 under controlled environmental conditions. Selected genotypes were subsequently evaluated under the same conditions during 2005–06 at ICRISAT. Based on the two years of controlled environmental evaluation, a set of 25 chickpea genotypes with high levels of resistance was selected and an International Botrytis Grey Mould Nursery (IBGMN) constituted (Table 1).

The nursery was evaluated for BGM resistance at two locations (Gurdaspur and Pantnagar) in India and two locations (Tarahara

Table 2
Description of environments (combination of location and season) of the IBGMN in India (2006–07 to 2009–10) and Nepal (2007–08 to 2009–10).

Location	Latitude (N)	Longitude (E)	Altitude (m)	Environment[a]	Season	Weather during the growing season[b]				
						Max. *T* (°C)	Min. *T* (°C)	Max. RH (%)	Min. RH (%)	Rain (mm)
Gurdaspur, India	32°03′	75°52′	242	GURD 07	2006–07	19.2	10.9	100.0	77.4	29.9
				GURD 08	2007–08	18.9	10.3	97.2	80.2	9.70
				GURD 09	2008–09	20.5	11.9	99.6	72.2	16.4
				GURD 10	2009–10	16.1	14.6	94.0	68.1	10.0
Pantnagar, India	30° 55′	75° 54′	255	PANT 07	2006–07	26.9	6.7	92.4	41.2	34.0
				PANT 08	2007–08	26.0	7.2	90.2	39.4	3.30
				PANT 09	2008–09	27.1	8.7	90.6	41.6	3.90
				PANT 10	2009–10	27.9	7.7	90.2	38.4	11.1
Tarhara, Nepal	26°42′	87°16′	200	TARA 08	2007–08	26.0	11.7	87.8	64.6	31.2
Rampur, Nepal	27°50′	86°34′	286	RAMP 09	2008–09	26.1	16.5	91.2	68.1	0.35
				RAMP 10	2009–10	25.9	15.7	92.8	65.2	2.20

[a] Environment is denoted as first four letter of the each location followed by year of screening (2006/2007 = 07; 2007/2008 = 08; 2008/2009 = 09; 2009–10 = 10).
[b] Climatic data are provided for the growing season (Max. *T*, maximum temperature; Min. *T*, minimum temperature; Max. RH, Maximum relative humidity; Min. RH, minimum relative humidity).

and Rampur) in Nepal. These locations were reported to have high BGM severity under favourable environmental conditions (Chaurasia and Joshi, 2001; Pande et al., 2005). In India, the nursery was evaluated for four crop seasons (2006–07, 2007–08, 2008–09 and 2009–10) and in Nepal for three crop seasons (2007–08 in Tarahara; 2008–09 and 2009–10 in Rampur) (Table 2).

2.2. Controlled environment evaluation

Seedlings of the test genotypes along with a susceptible check ICC 4954 were grown in 45 × 30 × 5 cm^3 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 (including the check) with eight seeds/test row were sown in each tray. The experiment was conducted in a completely randomized design (CRD) with three replications and repeated once.

Mass multiplication of the most representative isolate of *B. cinerea* was done by growing the pathogen on autoclaved flowers of *Tagetes erecta* (marigold) for 8 days at 25 °C and 12 h photoperiod. Conidia from the profusely sporulating culture were harvested into sterile distilled water and a conidial suspension at the concentration of 3 × 10^5 conidia/ml was used as inoculum. Twenty-four hours before inoculation, 10 day-old seedlings grown in plastic trays were transferred to the plant growth room maintained at 15 ± 1 °C with a 12 h photoperiod for acclimatization. The seedlings were inoculated artificially by spraying the inoculum on the foliage until run-off using a hand-operated atomizer. Inoculated plants were dried for 30 min to avoid dislodging of the spores and, thereafter, the growth room was maintained at 15 ± 1 °C and 95–100% relative humidity (RH) with a 12 h photoperiod of 2500–3000 lux intensity. The severity of the disease in all the test genotypes was recorded after 20 days of inoculation using a 1–9 rating scale where, 1 = no infection on any part of the plant and 9 = extensive soft rotting, fungal growth on more than 70% of the leaves, branches, and stems (Pande et al., 2012). Based on mean BGM severity, test genotypes were categorized as resistant (1.0–3.0 rating), moderately resistant (3.1–5.0 rating), susceptible (5.1–7.0 rating) and highly susceptible (7.1–9.0 rating).

2.3. Multi-environment evaluations

The IBGMN was laid out in a randomized completed block design (RCBD) with two replications. Susceptible cultivar (ICC 4954) was sown after every two-test rows to serve as an indicator and infector row. Forty seeds of each 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. At the onset of flowering, BGM infected debris was scattered over the field (3–4 kg per 100 m^2) in each location and season. Plants were also inoculated with a spore suspension of location-specific *B. cinerea* (5 × 10^4 conidia/ml) at each location and repeated 2–3 times at 10-day intervals to ensure uniform disease development and to avoid escape (Pande et al., 2012). Sprinkler irrigation was used to maintain high RH on the dry days for 15 min per hour from 1000 to 1600 h to promote infection and disease development. The data on disease severity was recorded on 10 randomly selected plants on a 1–9 rating scale (Pande et al., 2012).

2.4. Statistical analysis

Data for individual environments was analysed using analysis of variance (ANOVA) for BGM disease severity. Square root transformation was applied before combined analysis to attain normality of residuals. Further to test presence of G × E interactions, data across 11 environments with 25 genotypes was subjected to ANOVA. Homogeneity of error variances across environments was tested using Bartlett test and found significant. Hence, ANOVA was carried out using the mixed model procedure of the SAS software version 9.3 for Windows (SAS Institute Inc. 2011, Cary, NC) to model environment error variances. Genotypes, environments and G × E interactions were declared significant at 5% ($p < 0.05$) level.

Stability of genotypes was determined numerically and graphically using the AMMI model (Zobel et al., 1988). The following AMMI model was used to determine the stability of genotypes across 11 locations:

$$y_{ij} = \mu + g_i + e_j + \sum_{n=1}^{N} \lambda_n \zeta_{in} \eta_{jn};\ \ i = 1,2,\ldots,v;\ \ j = 1,2,\ldots,s.$$

where, y_{ij} is the mean BGM severity of the ith genotype ($i = 1, 2,\ldots v$) in the jth environment ($j = 1, 2\ldots s$); μ is the grand mean; g_i is the effect of the ith genotype; e_j = mean deviation/effect of the jth environment; λ_n = eigen value of the nth IPCA axis; ζ_{in} = genotypic score of the ith genotype on the nth IPCA; η_{jn} = environment score of the jth environment on the nth IPCA, n = number of IPC axes retained in the model.

After selecting the AMMI model, a study of phenotypic stability of the biplot graphic was designed. This graphic was obtained by the combinations of the orthogonal axis of the IPCAs. The biplot term refers to a type of graphic that contains two categories of points or markers. In this study, it refers to genotypes and

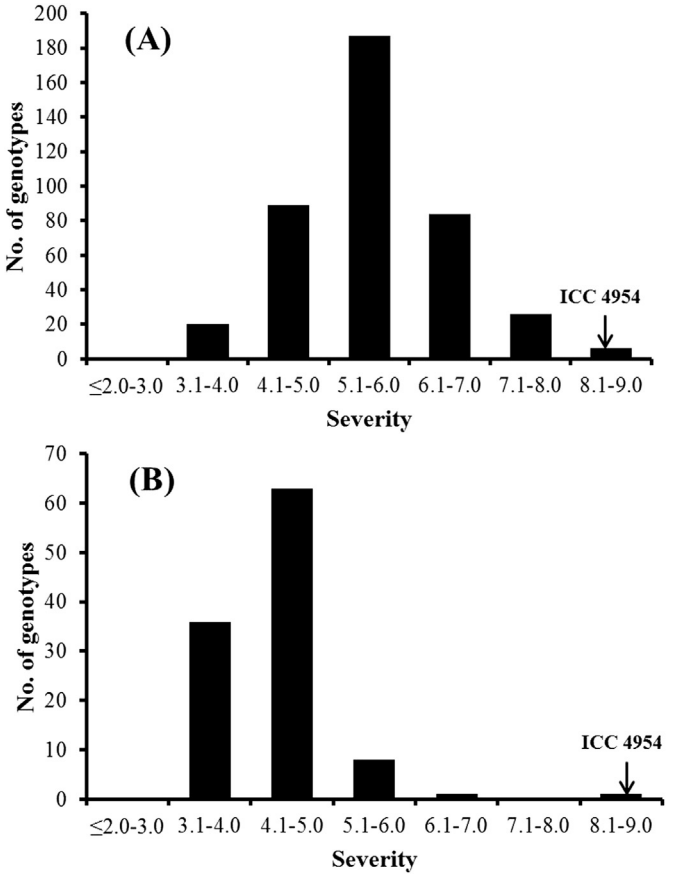

Fig. 1. Frequency distribution for BGM severity of chickpea genotypes together with a susceptible check (ICC 4954) evaluated in controlled environment at ICRISAT, Patancheru. (A) 412 genotypes during 2004–05 season, (B) 109 selected genotypes in second round of evaluation (2005–06 season).

environments. The AMMI1 biplot was drawn based on the variation caused by the main additive effects of genotype and environment, and the multiplicative effect of the G × E interaction. The abscissa represents the main effects (overall average of the variables of the genotypes evaluated) and the ordinate is the first interaction axis (IPCA1). The ideal genotype is one with low disease severity and IPCA1 values close to zero. Stability of genotypes was also studied using AMMI2 biplot generated using genotypic and environmental scores of the first two IPCA components. An undesirable genotype has low stability associated with high disease. Finally, the predictive averages were estimated according to the selected model. Also, an AMMI stability value (ASV) was calculated in order to rank genotypes in terms of stability using the formula suggested by Purchase et al. (2000). The AMMI model and biplots were generated using agricolae package available in R software (R Core Team, 2013). Broad-sense heritability (h^2_b, defined as the ratio of genetic variance to the sum of genetic variance and environmental variance) was estimated for BGM severity across all locations (Aruna et al., 2011).

3. Results

3.1. Controlled environment screenings

In the process of identification of new sources of resistance to BGM, the preliminary screening of 412 germplasm and breeding lines under controlled environment at ICRISAT during 2004–2005 revealed a broad range of responses among the tested material (Fig. 1A). This allowed the selection of 109 resistant lines (score 3.1–5.0 on a 1–9 scale) for further evaluation. Of these, 99 genotypes were found to have moderate resistance to BGM under the same conditions during the 2005–2006 season (Fig. 1B). Based on two years of controlled-environment screening, a set of 25 genotypes (5 germplasm accessions, 19 breeding lines and a susceptible check) were selected and IBGMN constituted (Table 1) to determine the stability of resistance across 11 environments in India and Nepal (Table 2).

3.2. Multi-environment evaluations of IBGMN

The BGM disease severity in most of the chickpea genotypes varied greatly between eleven environments as depicted by their severity values (Table 3). This is also shown by the frequency distribution of genotypes in each experiment suggesting a genotype × environment interaction (Fig. 2). Mean severity ratings of the susceptible genotype (ICC 4954) at different environments ranged from 7.0 to 9.0 indicating the high disease pressure in all test environments (Table 3).

The subsequent AMMI analysis of variance for the multi-environment experiment indicated that the environment, genotype and their interaction for BGM severity were significant implying a substantial variation among the genotypes as well as environments. Among the three sources of variation (genotype, environment and genotype × environment), the largest portion of variability for BGM severity was accounted for by the environment (47.36%), followed by G × E (32.55%) and genotype (20.07%) (Table 4). The AMMI analysis also showed that the first two principal components (IPCA1 and IPCA2) together accounted for large portions of the G × E sum of squares ~48.41% (26.38% and 22.03% for IPCA1 and IPCA2, respectively, Table 4). Low heritability was observed for BGM disease severity (29.7%) across the locations.

3.2.1. Stability of genotypes and environments

Effect of genotype (G) and environment (E) was explained by the AMMI1 (IPCA vs. means of genotype and environment) (Fig. 3) and AMMI2 (IPCA2 vs. IPCA1) biplots (Fig. 4). In AMMI1 (Fig. 3), the X-coordinate indicates the main effects (means of G × E) and the *Y*-coordinate indicates the effects of interaction (IPCA1). Values closer

Table 3
Average BGM severity (1–9 scale) and ASV of 25 chickpea genotypes together with susceptible check (ICC 4954) in the eleven environments in India (2006–07 to 2009–10) and Nepal (2007–08 to 2009–10).

Entry no.	Genotype	BGM severity (1–9 rating scale)												ASV[a]
		GURD 07	PANT 07	GURD 08	PANT 08	TARA 08	GURD 09	PANT 09	RAMP 09	GURD 10	PANT 10	RAMP 10	Mean	
1	EC 516700	3.0	8.0	3.5	7.0	5.5	2.5	5.0	5.0	4.5	6.5	7.0	5.2	0.494
2	EC 516716	2.5	5.0	3.5	7.0	6.0	3.5	7.0	6.5	4.0	5.0	7.0	5.2	0.896
3	EC 516751	3.0	7.0	3.5	7.0	6.0	3.5	5.0	7.5	4.0	5.0	7.5	5.4	0.934
4	EC 516824	3.5	7.0	3.5	7.0	7.0	5.0	5.0	7.5	4.5	7.0	4.5	5.6	0.703
5	EC 516891	3.0	6.0	3.5	9.0	6.0	3.5	6.0	5.0	5.0	9.0	6.5	5.7	0.372
6	EC 516936	4.0	7.0	3.5	7.0	6.0	4.0	5.5	5.5	4.0	8.0	7.0	5.6	
7	EC 516968	3.0	8.0	2.0	7.0	6.5	2.5	3.0	6.0	2.5	7.0	6.5	4.9	1.301
8	EC 516976	4.0	7.0	3.5	5.0	6.5	2.5	7.0	7.0	5.5	7.0	7.0	5.6	0.371
9	EC 517041	4.0	6.0	3.0	7.0	7.5	3.0	9.0	5.0	4.0	8.0	6.5	5.7	0.527
10	ICCV 04609	3.5	6.0	2.5	7.0	5.5	5.0	5.0	3.0	4.5	5.0	5.5	4.8	0.907
11	ICCV 05604	3.0	3.0	2.5	7.0	6.5	1.5	5.0	5.0	3.0	5.0	7.0	4.4	0.755
12	ICCV 05605	2.5	5.0	2.5	5.0	7.5	2.5	7.0	5.5	3.5	5.0	6.0	4.7	0.671
13	ICCV 88103	2.0	6.0	1.0	7.0	6.5	4.5	3.0	5.0	5.5	9.0	5.0	5.0	1.458
14	ICCV 89332	3.0	6.0	3.0	9.0	6.0	3.5	9.0	5.0	4.0	6.5	7.0	5.6	0.740
15	ICCV 93928	6.5	8.5	2.0	7.0	8.0	5.5	7.0	4.0	5.5	5.5	5.0	5.9	1.385
16	ICCV 96817	2.0	5.0	2.5	7.0	6.0	2.0	7.0	6.0	2.5	5.0	8.0	4.8	1.323
17	ICCV 96852	2.5	6.0	3.0	6.5	5.5	3.0	6.5	3.5	3.5	3.5	7.0	4.6	0.795
18	ICCV 96853	3.0	3.0	4.0	5.0	4.0	4.0	5.0	3.0	4.0	3.0	4.5	3.9	1.230
19	ICCV 96859	2.0	5.0	2.5	5.0	4.5	2.5	3.0	5.5	2.5	3.0	5.5	3.7	0.571
20	ICC 14344	5.0	6.0	2.5	5.0	9.0	5.0	7.0	5.5	5.0	7.5	5.5	5.7	0.883
21	ICC 4063	3.0	9.0	3.5	9.0	7.0	3.0	7.0	8.0	3.0	7.5	6.0	6.0	1.137
22	ICC 4065	7.0	9.0	4.5	9.0	7.0	4.5	7.0	7.5	5.5	7.5	6.0	6.8	0.496
23	ICC 4074	3.5	7.5	3.0	7.0	6.5	3.5	5.0	8.0	3.5	4.5	7.0	5.4	1.000
24	ICC 4951	7.0	8.0	4.5	9.0	8.0	6.5	7.0	4.0	6.0	8.5	6.5	6.8	1.509
25	ICC 4954	7.0	7.0	7.0	9.0	7.5	7.0	8.5	7.5	7.0	9.0	7.0	7.6	0.835
	Mean	3.7	6.4	3.2	7.1	6.5	3.7	6.1	5.6	4.3	6.3	6.3	5.4	–
	SE m±	0.4	0.5	0.7	0.3	0.6	0.7	0.3	0.9	0.4	0.4	0.7	–	–

[a] AMMI stability value.

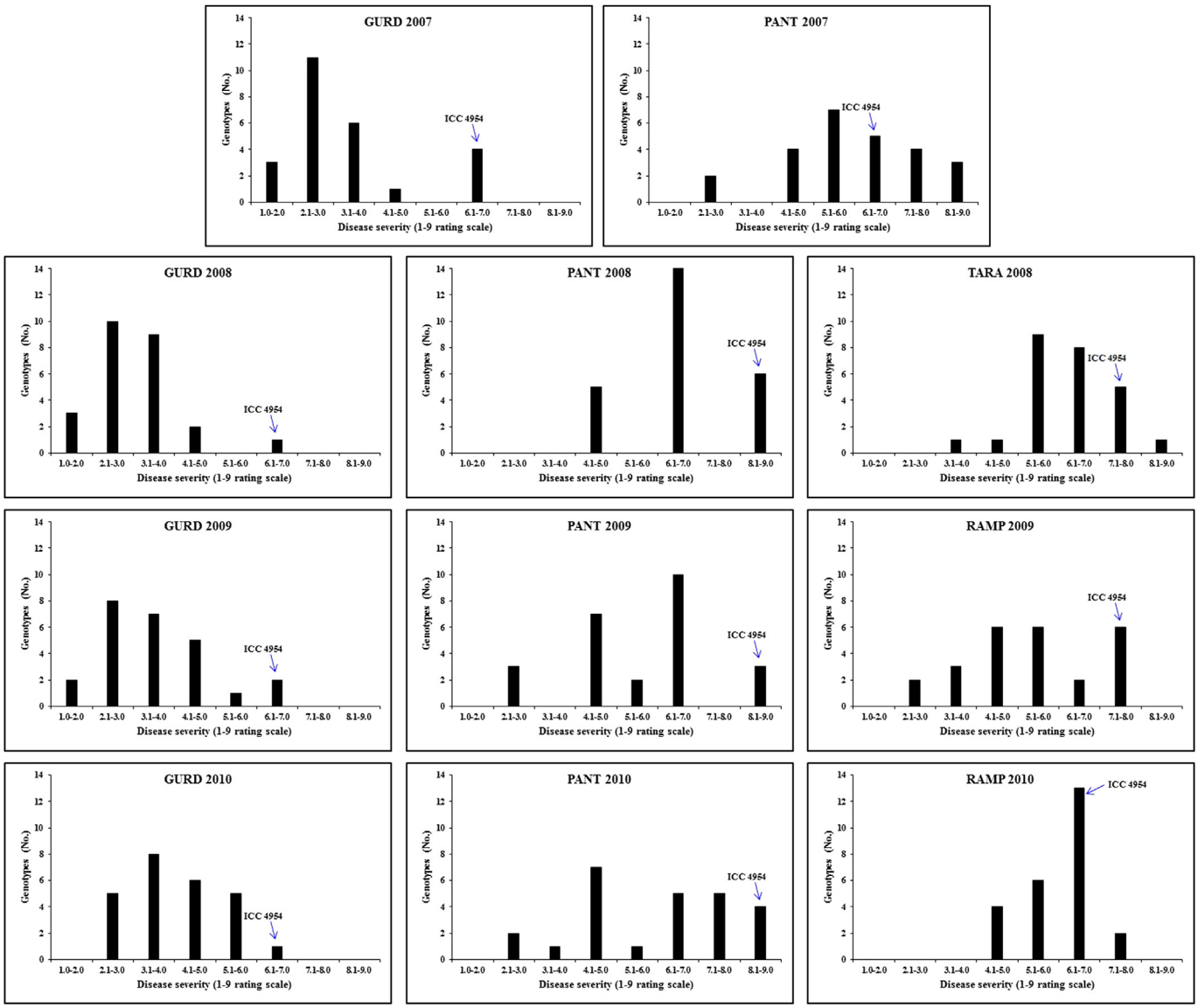

Fig. 2. Frequency distribution for BGM severity of 25 chickpea genotypes together with a susceptible check (ICC 4954) in the 11 environments in India and Nepal. The different patterns point to genotype × environment interaction. Position of susceptible check (ICC 4954) is shown by arrow.

to the origin of the axis (IPCA1) contributed less to the interaction than those that were further away. Genotypes G4 (EC 516824), G5 (EC 516891), G6 (EC 516936) and G9 (EC 517041) showed greater stability; however, BGM severities for these genotypes were higher than the overall average (5.4 on 1–9 scale). Genotype G25 (ICC 4954), the susceptible check, showed maximum disease severity (7.6) and was placed far from the origin of the axis. Genotype G24 (ICC 4951) was the most unstable and had BGM severity of 6.8. The genotypes with low severity and moderate stability were G19 (ICCV 96859, BGM score 3.7), G18 (ICCV 96853, BGM score 3.9), G11 (ICCV

Table 4
Analysis of variance and partitioning of the G × E interaction for BGM severity of 25 chickpea genotypes evaluated in 11 environments in India (2006–07 to 2009–10) and Nepal (2007–08 to 2009–10) by the AMMI method.

Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F^a value	*P* value	Explained %	Accumulated %
Genotypes (G)	24	401.3	16.72*	24.85	<0.0001		
Environments (E)	10	946.7	94.67*	35.42	<0.0001		
G × E	240	650.8	2.71*	4.03	<0.0001		
Treatments	274	1998.8	7.29*	10.84	<0.0001		
Block	11	29.4	2.67*	3.97	<0.0001		
IPCA1	33	171.7	5.20*	7.73	<0.0001	26.38	26.38
IPCA2	31	143.4	4.63*	6.88	<0.0001	22.03	48.41
Residuals	176	335.7	1.91*	2.84	<0.0001		
Error	264	177.6	0.67*	–	–		

[a] *F* tests were done using the error mean square as a denominator.

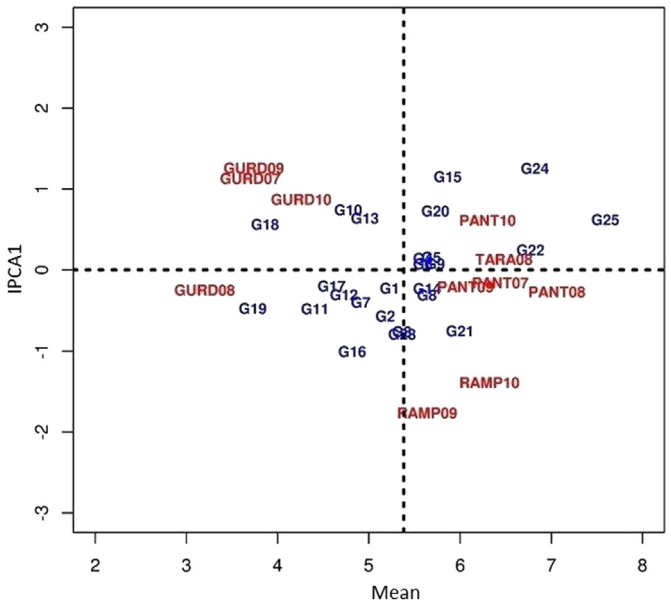

Fig. 3. AMMI1 biplot showing the ICPA1 vs. means for the BGM severity of 25 chickpea genotypes evaluated in 11 environments in India and Nepal. Environments are shown as the first four letters of each location followed by year of screening and genotypes were represented by G followed by their serial number. Refer to Tables 1 and 2 for full names of genotypes and environments, respectively.

05604, BGM score 4.4), G17 (ICCV 96852, BGM score 4.6) and G12 (ICCV 05605, BGM score 4.7).

For the environments, differences can be seen both in their main effect and interaction effect (Fig. 3). In the main effect, GURD 08 had the lowest mean rating which means low BGM severity (3.2), followed by GURD 07 and GURD 09 each with a disease score of 3.7 and GURD 10 (4.3). In these locations, average disease severity recorded was below the overall average (5.4), indicating that these were favourable environments for expression of resistance. The environments RAMP 10, PANT 10, GURD 07 and GURD 09 contributed significantly to the interactions, however the environment with least contribution to the interaction was TARA 08 (Fig. 3).

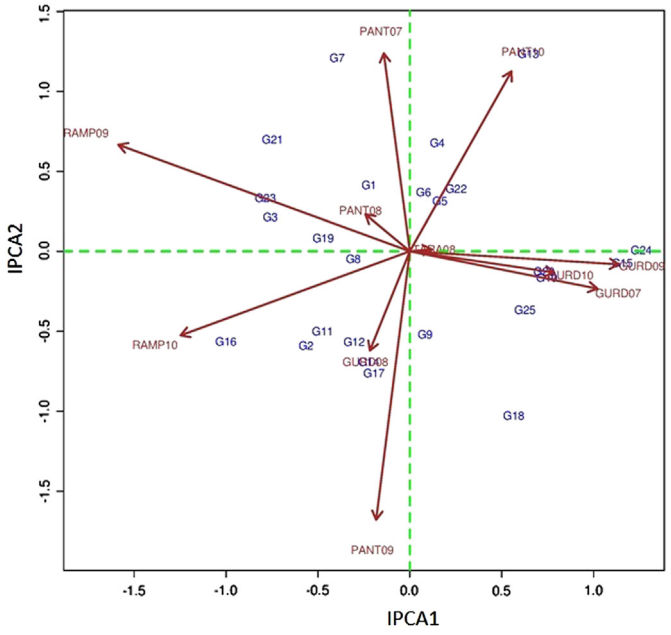

Fig. 4. AMMI2 biplot showing the first two principal axes of interaction (ICPA2 vs. ICPA1) for the BGM severity of 25 chickpea genotypes evaluated in 11 environments in India and Nepal. Environments are shown as the first four letters of each location followed by year of screening and genotypes were represented by G followed by their serial number. Refer to Tables 1 and 2 for full names of genotypes and environments, respectively.

AMMI2 biplot analysis (Fig. 4) indicated that genotypes G8 (EC 516976), G5 (EC 516891) and G6 (EC 516936) were the most stable; as these genotypes were positioned near the origin of the biplot and contributed least to the G × E interactions. The other stable genotypes found adjoining were G19 (ICCV 96859), G1 (EC 516700) and G22 (ICC 4065) (Fig. 4). The stability of these genotypes was also indicated by their low ASV values (Table 4). The G × E interaction was highest in genotypes G24 (ICC 4951) and G15 (ICCV 93928) as they were placed far from the biplot origin.

The biplot also indicated that environment TARA 08 was the largest contributor to the phenotypic stability of genotypes (Fig. 4). In this environment, no difference ($p > 0.001$) was found in BGM severity among genotypes via the individual ANOVA. On the other hand, environments RAMP 09, PANT 09, GURD 09, RAMP 10, PANT 07, and PANT 10 mostly contributed to the G × E interaction as they were positioned far from the origin in the AMMI2 biplot. Genotypes and environments positioned close to each other in the biplot have positive interaction e.g. G17 with GURD 08, G13 with PANT 10, G15 and G24 with GURD 09, G20 and G10 with GURD 10 (Fig. 4).

4. Discussion

Developing chickpea cultivars with high levels of BGM resistance has been challenging due to the lack of sources of high levels of resistance in chickpea (Davidson et al., 2004; Pande et al., 2006; Isenegger et al., 2011). Stability of resistance to *B. cinerea* is crucial for the success of a breeding programme. Multi-year and multi-location evaluations are important to identify stable sources of disease resistance. In the present study, 412 genotypes were screened under controlled environmental conditions during 2004–05 as a first step to discard the highly susceptible genotypes. Further evaluations performed under the same conditions during 2005–06 allowed refinement of selection of genotypes for multi-environment and multi-year evaluation. Individual analysis of field trials at different locations revealed differences in the response of the genotypes to BGM. Variations in frequency distributions of genotypes showed that it is necessary to check the stability of the genotypes through multi-location and multi-year testing.

An understanding of the G × E interactions is essential for the implementation of efficient evaluation and selection of stable sources of resistance. Currently, studies involving the interpretation of stable resistant sources in chickpea using G × E interactions are limited. In this study, the AMMI model was used to evaluate the phenotypic stability of 25 chickpea genotypes to BGM across 11 environments in India and Nepal. The AMMI analysis showed that variation in disease severity was mainly contributed by environment (47.36%) indicating that the locations were diverse. This was also reflected by low broad sense heritability for BGM severity (29.7%) across locations (Aruna et al., 2011). The first two principal component axes were significant and both sums contributed 48.21% to the total G × E interaction. The greater effect of environment followed by G × E interaction has also been reported by Shagol and Tad-awan (2008) and Aruna et al. (2011). The inconsistency in phenotypic expression among environments is frequently encountered in BGM resistance evaluations (Villegas-Fernandez et al., 2009, 2011) due to pathotype variation, cultivar specificity of the different genotypes and other factors like weather,

soil properties or agricultural practices (Singh and Bhan, 1986; Rewal and Grewal, 1989; Isenegger et al., 2008; Pande et al., 2010).

The AMMI biplot analysis is a useful tool in explaining the specific patterns of main effects and G × E interactions of genotypes and environments simultaneously (Crossa et al., 1990). In the AMMI1 biplot, genotype EC 516824 (G4), EC 516891 (G5), EC 516936 (G6) and EC 517041 (G9) showed greater stability, since the coordinates on the axis were the lowest ICPA1; however their BGM severity was high. Genotype ICCV 96859 (G19) could be highlighted as this had the lowest BGM severity (3.7) and moderate stability. Shagol and Tad-awan (2008) reported that the environment placed far towards the left has the lowest mean rating and vice-versa as indicated in location GURD 08, that had the lowest BGM severity (3.2), while PANT 08 had the highest mean disease severity (7.1). Locations RAMP 09, PANT 09 and RAMP 10 positioned far from the origin were shown to exert strong interactive forces on BGM severity. These environments are more discriminating which means the genotypes reacts in various degrees in these environments.

In the present study, we found genotypes EC 516976 (G8), EC 516891 (G5) and EC 516936 (G6) were more stable and positioned near the origin of the biplot and contributed least to the G × E interactions. The statistically stable genotypes and environments were represented by points near to the origin in the AMMI2 biplot (Guerra et al., 2009). The other stable genotypes found adjoining were ICCV 96859 (G19), EC 516700 (G1) and ICC 4063 (G22). An AMMI stability values (ASV) also indicated that EC 516891 (G5) and EC 516936 (G6) had the lowest value and more stability, while genotypes ICCV 88103 (G13) and ICC 4951 (G24) were least stable with a larger ASV value. Naroui Rad et al. (2013) and Purchase et al. (2000) also revealed that the lower the ASV value, the more stable the genotypes. Guerra et al. (2009) observed that genotypes and environments with IPCA scores with the same sign had specific positive interaction (RAMP 09 with G23) and those with the opposite sign had specific negative interaction (RAMP 09 with G18 and PANT 09 with G9 and G19).

In environments with high stability, genotypes with general adaptability tend to perform well and can be selected with greater safety. On the other hand, environments with high G × E interaction (high instability) such as RAMP 09, PANT 09, RAMP 10, PANT 07 and PANT 10 should be avoided in the preliminary stages, because the tendency is to select genotypes with specific adaptability to these sites. The order of the genotypes in a stable environment is more reliable, because the classification is determined by genotypic effects (where the G × E interaction is zero) (Duarte and Vencovsky, 1999).

It was possible to estimate the phenotypic responses of each genotype in a given environment by the AMMI2 biplot (Fig. 4). Strong positive interaction was found between G17 and GURD 08, G13 with PANT 10, G15 and G24 with GURD 09 and G20 and G10 with GURD 10. This implies that BGM severity seems to be favoured when these genotypes are grown in these environments.

From this study, it is concluded that, the AMMI model facilitated the selection of five genotypes ICCV 96859, ICCV 96853, ICCV 05604, ICCV 96852 and ICCV 05605 with moderate BGM severity and stability. Genotype ICCV 96859 having least BGM severity and moderate stability could be exploited in chickpea resistance breeding programmes.

Acknowledgements

The authors are thankful to Dr Nabin Dangal, Regional Agricultural Research Station, Tarahara, Nepal and Dr Subash Subedi, National Grain Legume Research Program, Rampur, Nepal for providing the BGM disease score and weather data.

References

Aruna, C., Bhagwat, V.R., Sharma, V., Hussain, T., Ghorade, R.B., Khandalkar, H.G., Audilakshmi, S., Seetharama, N., 2011. Genotype × environment interactions for shoot fly resistance in sorghum (*Sorghum bicolor* (L.) Moench): response of recombinant inbred lines. Crop Prot. 30, 623–630.

Bakr, M.A., MacLeod, W.J., Johansen, C., et al., 2004. International collaboration to develop integrated management and resistant varieties to minimise the impact of Botrytis grey mould in chickpea. In: Fischer, T., Turner, N., Angus, J. (Eds.), Proceedings of the 4th International Crop Science Congress, Brisbane, Australia. http://www. cropscience.org.au/icsc2004/poster/3/7/3/1181_bakrma.html.

Chaurasia, P.C.P., Joshi, S., 2001. Management of Botrytis Grey Mould (*Botrytis cinerea* Pers. Ex. Fr.) of chickpea at Tarahara, Nepal. Nepal Agric. Res. J. 4 & 5, 37–41.

Crossa, J., Gauch, H.G., Zobel, R.W., 1990. Additive main effects and multiplicative analysis of two international maize cultivar trials. Crop Sci. 30, 493–500.

Davidson, J.A., Pande, S., Bretag, T.W., Lindbeck, K.D., Kishore, G.K., 2004. Biology and management of *Botrytis* spp. in legume crops. In: Elad, Y., Williamson, B., Tudzynski, P., Delen, N. (Eds.), Botrytis: Biology, Pathology and Control. Kluwer Academic Publishers, Netherlands, pp. 295–318.

Duarte, J.B., Vencovsky, R., 1999. Interacao genotipos × ambientes: uma introducao a analise AMMI. In: Serie Monografias. Sociedade Brasileira de Genetica, Ribeirao Preto, p. 60.

Eberhart, S.A., Russell, W.A., 1966. Stability parameters for comparing varieties. Crop Sci. 6, 36–40.

FAOSTAT, 2010. Food and Agriculture Organization of the United Nations. Rome http://faostat.fao.org.

Finlay, K.W., Wilkinson, G.N., 1963. The analysis of adaptation in a plant-breeding program. Aust. J. Agric. Res. 14, 742–754.

Forbes, G.A., Chacón, M.G., Kirk, H.G., Huarte, M.A., et al., 2005. Stability of resistance to *Phytophthora infestans* in potato: an international evaluation. Plant Pathol. 54, 364–372.

Gauch, H.G., 2006. Statistical analysis of yield trials by AMMI and GGE. Crop Sci. 46, 1488–1500.

Gauch, H.G., Piepho, H.P., Annicchiarico, P., 2008. Statistical analysis of yield trials by AMMI and GGE: further considerations. Crop Sci. 48, 866–889.

Gauch, H.G., Zobel, R.W., 1997. Identifying mega-environments and targeting genotypes. Crop Sci. 37, 311–326.

Guerra, E.P., Oliveira, R.A., Daros, E., Zambon, J.L.C., Ido, O.T., Bespalhok Filho, J.C., 2009. Stability and adaptability of early maturing sugarcane clones by AMMI analysis. Crop Breed. Appl. Biotechnol. 9, 260–267.

Isenegger, D.A., Ford, R., Taylor, P.W.J., 2011. Disease reaction of chickpea (*Cicer* spp.) genotypes to Botrytis grey mould. Australas. Plant Pathol. 40, 583–590.

Isenegger, D.A., Macleod, W.J., Ford, R., Taylor, P.W.J., 2008. Genotypic diversity and migration of clonal lineages of *Botrytis cinerea* from chickpea fields of Bangladesh inferred by microsatellite markers. Plant Pathol. 57 (5), 967–973.

Jukanti, A.K., Gaur, P.M., Gowda, C.L.L., Chibbar, R.N., 2012. Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.). Brit. J. Nutr. 108, S11–S26. http://dx.doi.org/10.1017/S0007114512000797.

Naroui Rad, M.R., Abdul Kadir, M., Raffi, M.Y., Jaffar, H.Z.E., Naghavi, M.R., Ahmadi, F., 2013. Genotype × environment interaction by AMMI and GGE biplot analysis in three consecutive generations of wheat (*Triticum aestivum*) under normal and drought stress conditions. Aus. J. Crop Sci. 7 (7), 956–961.

Pande, S., Sharma, M., Gaur, P.M., Basandrai, A.K., Kaur, L., Hooda, K.S., Basandrai, D., Kiran Babu, T., Jain, S.K., Rathore, A., 2013. Biplot analysis of genotype × environment interactions and identification of stable sources of resistance to ascochyta blight in chickpea (*Cicer arietinum* L.). Australas. Plant Pathol.. http://dx.doi.org/10.1007/s13313-013-0219-x.

Pande, S., Galloway, J., Gaur, P.M., Siddique, K.H.M., Tripathi, H.S., Taylor, P., MacLeod, M.J., Basandrai, A.K., Bakr, A., Joshi, Kishore, G.K., Isenegger, D.A., Rao, J.N., Sharma, M., 2006. Botrytis grey mould of chickpea: a review of biology, epidemiology, and disease management. Aust. J. Agric. Res. 57, 1137–1150.

Pande, S., Sharma, M., Nagavardhini, A., Rameshwar, T., 2012. High throughput phenotyping of chickpea diseases. In: Information Bulletin No. 92. International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324, Andhra Pradesh, India, ISBN 978-92-9066-552-6. Order Code: IBE 092.

Pande, S., Stevenson, P., Rao, J.N., Neupane, R.K., Chaudhary, R.N., Grzywacz, D., Bourai, V.A., Kishore, G.K., 2005. Reviving chickpea production in Nepal through integrated crop management, with emphasis on Botrytis grey mould. Plant Dis. 80, 1252–1262.

Pande, S., Sharma, M., Kishore, G.K., Shivram, L., Mangala, U.N., 2010. Characterization of *Botrytis cinerea* isolates from chickpea: DNA polymorphisms, cultural, morphological and virulence characteristics. Afr. J. Biotechnol. 9 (46), 7961–7967.

Purchase, J.L., Hating, H., Deventer, C.S., 2000. Genotype × environment interaction of winter wheat (*Triticum aestivum*) in South Africa: Stability analysis of yield performance. S. Afr. J. Plant Soil 17 (3), 101–107.

R Core Team, 2013. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org/.

Rewal, N., Grewal, J.S., 1989. Differential response of chickpea to grey mould. Indian Phytopathol. 42, 265–268.

Rubiales, D., Avila, C.M., Sillero, J.C., Hybl, M., Narits, L., Sass, O., Flores, F., 2012. Identification and multi-environment validation of resistance to *Ascochyta fabae* in faba bean (*Vicia faba*). Field Crop Res. 126, 165–170.

Shagol, C.C., Tad-awan, B.A., 2008. Genotype × environment interaction in potato grown organically in six different locations of Benguet. Res. J. 16, 40–50.
Sharma, M., Kiran Babu, T., Gaur, P.M., Ghosh, R., Rameshwar, T., Chaudhary, R.G., Upadhyay, J.P., Gupta, Om, Saxena, D.R., Kaur, L., Dubey, S.C., Anandani, V.P., Harer, P.N., Pande, S., 2012. Identification and multi-environment validation of resistance to *Fusarium oxysporum* f. sp. *ciceris* in chickpea. Field Crop Res. 135, 82–88.
Singh, G., Bhan, L.K., 1986. Physiological races of *Botrytis cinerea* causing grey mould of chickpea. Plant Dis. Res. 1, 69–74.
Villegas-Fernandez, A.M., Sillero, J.C., Emeran, A.A., Flores, F., Rubiales, D., 2011. Multiple-disease resistance in *Vicia faba*: multi-environment field testing for identification of combined resistance to rust and chocolate spot. Field Crop Res. 124, 59–65.
Villegas-Fernandez, A.M., Sillero, J.C., Emeran, A.A., Winkler, J., Raffiot, B., Tay, J., Flores, F., Rubiales, D., 2009. Identification and multi-environment validation of resistance to *Botrytis fabae* in *Vicia faba*. Field Crop Res. 114, 84–90.
Yan, W., 2001. GGE biplot – a windows application for graphical analysis of multi-environment trial data and other types of two way data. Agronomy Journal 93, 1111–1118.
Yan, W.K., Hunt, L.A., Sheng, Q.L., Szlavnics, Z., 2000. Cultivar evaluation and mega-environment investigation based on the GGE biplot. Crop Sci. 40, 597–605.
Yan, W.K., Kang, M.S., Ma, B., Woods, S., Cornelius, P.L., 2007. GGE biplot vs. AMMI analysis of genotype-by-environment data. Crop Sci. 47, 641–653.
Zobel, R.W., Wright, M.S., Gauch, H.G., 1988. Statistical analysis of a yield trial. Agronomy Journal 80, 388–393.