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Identification and multi-environment validation of resistance to *Fusarium oxysporum* f. sp. *ciceris* in chickpea

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ABSTRACT

Chickpea wilt incited by *Fusarium oxysporum* f. sp. *ciceris* is one of the most important constraints to chickpea production worldwide and best managed through host plant resistance. The aim of this work was to find new sources of resistance to wilt disease and validate their stability across different environments. One-hundred and twenty three lines with wilt incidence <10% were selected from preliminary evaluation of 948 lines including germplasm and breeding lines from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) for wilt resistance in the sick plot during 2003/2004 crop season at ICRISAT, Patancheru, India. Sixty lines were selected for second round of evaluation (2005/2006) and from those 57 lines were selected for third round of evaluation (2006/2007). In order to validate resistance stability, a Chickpea Wilt Nursery was constituted with 27 lines (7 germplasm accessions, 19 breeding lines and a highly susceptible check) and further tested in multi-location experiment for wilt resistance at 9 locations in India for three years (2007/2008–2009/2010). Variability in wilt incidence due to genetic differences among the genotypes, among the environments, and that due to genotype × environment interaction was highly significant ($P < 0.001$). Although complete resistance across the locations was not found, the genotype and genotype × environment (GGE) biplot analyses allowed the selection of three breeding lines (ICCV 05527, ICCV 05528 and ICCV 96818) and one germplasm accession (ICC 11322) with moderate level of disease resistance and stable performance across the environments. Genotype × environment (G × E) interaction contributed 36.7% of total variation of the multi-environment evaluation, revealing instability of the phenotypic expression across environments. The identified resistant sources should be useful to chickpea disease resistance breeding programs.

1. Introduction

Among the grain legumes, chickpea (*Cicer arietinum* L.) is unique because of the variety of food products that are prepared from it in different parts of the world. Chickpea is an important pulse crop grown in over 45 countries of Asia, Africa, the Americas, and Oceania, with annual production of 10.9 million tons from 11.98 million ha (FAOSTAT, 2010). South Asia is by far the largest producer of chickpea (76%) in the world with a share of more than 80% area followed by Middle East and North Africa (MENA) and Sub Saharan Africa (SSA). Among the South Asian countries, India is the largest chickpea growing country with annual production of 7.06 million tons from 7.54 million ha (FAOSTAT, 2010). The average global productivity of chickpea is about 0.8 t ha^{-1}, which is far below the actual yield potential, because the crop is subjected to a number of fungal diseases throughout the growing season (Reddy et al., 1990). *Fusarium* wilt, caused by *Fusarium oxysporum* Schlechtend. Emend Snyder. & Hans. f. sp. *ciceris* (Padwick) Snyder is one of the most important and widely distributed biotic stress of chickpea and has the potential to cause 10–100% yield losses depending on varietal susceptibility and agro-climatic conditions (Haware and Nene, 1980; Nene and Reddy, 1987; Jimenez-Diaz et al., 1989). The

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disease is prevalent in the Indian subcontinent, Ethiopia, Mexico, Spain, Tunisia, Turkey, and the United States (Westerlund et al., 1974; Nene et al., 1989; Halila and Strange, 1996). *F. oxysporum* f. sp. *ciceris* is a vascular pathogen that perpetuates in seed and soil, and hence is difficult to manage by the use of chemicals. Deployment of host plant resistance is the best means of combating disease problem and more relevant in a crop like chickpea, which is predominantly grown by resource poor farmers. Several sources of strong resistance to *Fusarium* wilt in chickpea has been identified (Haware and Nene, 1982; Nene et al., 1989; Pande et al., 2006; Sharma et al., 2010) and several are being utilized in breeding programs. However, resistance durability in these cultivars has short-lived because of the robust genetic variability in the pathogen population and rapid selection of virulence against specific host cultivars.

Pathogenic and genetic variability in the pathogen was characterized using differential lines and DNA markers (Haware and Nene, 1982; Rubio et al., 2003). The pathogen is reported to have eight races (Haware and Nene, 1982; Phillips, 1988; Jimenez-Diaz et al., 1989). Races 1A, 2, 3, and 4 have been reported from India, and races 0, 1A, 1B/C, 5, and 6, from the United States and Spain. Cultivar specialization (race) of *F. oxysporum* f. sp. *ciceris* was first reported in India (Haware and Nene, 1982) and resistance is governed by major resistance genes (Upadhyaya et al., 1983; Sharma et al., 2004). However, the actual number of races and their genetic variability at global level are unclear as no clear gene-for-gene relationship has been established for chickpea–*Fusarium* system. In order to develop effective strategies for management of this disease through host resistance, it is important to obtain information on multi-environment resistance stability. With this objective in mind, a collaborative Chickpea Wilt Nursery (CWN) was initiated to identify chickpea genotypes that exhibit stable resistance across the locations over years.

Many improved chickpea lines were developed by chickpea breeding program at ICRISAT that contributed significant increase in chickpea productivity in semi-arid regions of Asia and Africa. Plant pathologists evaluate and test genotypes in multiple locations and years to determine the stability of resistance and monitoring the virulence changes in the pathogen population. Since large variability can exist both within the host and within the pathogen, understanding the host-by-pathogen interaction patterns for a particular host–pathogen system can be difficult and challenging. Several methods have been proposed to analyze the genotype × environment (GE) interaction (Finlay and Wilkinson, 1963; Eberhart and Russell, 1966). Often with large number of genotypes evaluated across a number of environments and years, it is difficult to determine the genotype response across environments and years without the help of graphical representation of data. Recently, biplot analysis of genotype × environment data has been advanced such that many important questions can be graphically addressed using a "GGE biplot" (Yan et al., 2000; Yan, 2001). These questions include the which-won-where pattern, mega-environment investigation, mean performance and stability of genotypes, discriminating ability and representativeness of environments, etc. The objective of this work was to identify stable sources of wilt resistance in chickpea germplasm accessions and breeding lines and validate the resistance through multi-year and multi-location field experiments in India.

2. Materials and methods

2.1. Plant material

Two sets of chickpea genotypes were evaluated for resistance to *Fusarium* wilt at ICRISAT, Patancheru. The first set consisted of 948 germplasm accessions and breeding lines from ICRISAT chickpea breeding program, whereas the second set-chickpea wilt nursery (CWN) of 27 breeding and germplasm lines was constituted from subsequent evaluation of resistant breeding and germplasm lines based on the wilt reaction (≤10% wilt incidence) in 2004/2005–2005/2006. The summary of the pedigrees for the breeding lines for the selected genotypes used in this study is presented in Table 1.

Table 1
Chickpea genotypes used in the Chickpea Wilt Nursery (CWN) during 2007/2008–2009/2010 in India.

S. no.	Genotype	Type	Pedigree
1	ICC 95	Accession	–
2	ICC 2072	Accession	–
3	ICC 11322	Accession	–
4	ICC 11324	Accession	–
5	ICC 14364	Accession	–
6	ICC 14386	Accession	–
7	ICC 15996	Accession	–
8	ICCV 93217	Breeding line	ICCL 86237 × ICC 693
9	ICCV 96818	Breeding line	(K-850 × ICCL 80074) × ICC 1069) × (JM-2100 × Dhanush)
10	ICCV 96851	Breeding line	(ICC 12237 × ICC 1069) × (L 132–1 × ICCL 85216)
11	ICCV 04104	Breeding line	(ICCV 93001 × JAKI 9218) × BG 256
12	ICCV 04107	Breeding line	(ICCV 92065 × ICCV 88202) × KW 118
13	ICCV 04108	Breeding line	(ICCV 92065 × ICCV 88202) × KW 118
14	ICCV 04113	Breeding line	GL 84099 × ICCC 37
15	ICCV 04311	Breeding line	(L 550 × ICC 14196) × ICCV 92329
16	ICCV 04312	Breeding line	(L 550 × ICC 14196) × ICCV 92329
17	ICCV 04314	Breeding line	ICCV 2 × ICCV 92325
18	ICCV 05107	Breeding line	ICC 4958 × ICCV 92311
19	ICCV 05110	Breeding line	GL 84099 × ICCC 37
20	ICCV 05112	Breeding line	ICCV 2 × PDG 84–16
21	ICCV 05309	Breeding line	ICCV 2 × ICCV 92311
22	ICCV 05310	Breeding line	ICCV 2 × ICCV 92325
23	ICCV 05527	Breeding line	H 75–35 × [G 130 × (K 1189 × Chaffa)]
24	ICCV 05528	Breeding line	H 75–35 × [G 130 × (K 1189 × Chaffa)]
25	ICCV 05529	Breeding line	Pant G-114 × ICC 3935
26	ICCV 06106	Breeding line	GL 84099 × ICCC 37
27	ICC 4951[a]	Released variety	

[a] ICC 4951 is a susceptible cultivar

2.2. Field trials

A process of screening and selection was carried out for 4 years (2003/2004–2006/2007) including preliminary screening aiming to identify genotypes with resistance to wilt disease. A CWN was constituted and submitted to a multi-environment screening at nine locations for three years (2007/2008–2009/2010). Information for the tested environments is given in Table 2. The scheme of this process was as follows.

2.2.1. Identification of plant material for the multi-environment screenings

A total of 948 germplasm accessions and breeding lines were screened for wilt resistance in sick-plot under artificial epiphytotic conditions at ICRISAT, Patancheru in a preliminary screening during 2003/2004 season (October–February). A randomized complete block design (RCBD) with two replications was employed. Each genotype was sown along a 4 m-long row being 60 cm apart with known susceptible (JG 62) and resistant (WR 315) checks inserted every eight rows. Every year, the resistant accessions were selected (wilt incidence <10%) from the previous season of screening and reevaluated in sick plot at Patancheru. This process was continued for 3 years (2004/2005–2006/2007).

2.2.2. Multi-environment screenings

Based on results obtained from consecutive screening for 3 years at Patancheru, a set of 27 genotypes with consistent and higher levels of resistance were selected and constituted CWN for a multilocation experiment from 2007/2008 to 2009/2010. The nursery consisting of 27 genotypes with 7 germplasm accessions, 19 breeding lines and one susceptible check (ICC 4951). Seed stocks of test genotypes were increased and maintained at ICRISAT, Patancheru and sub-sampled for supplying to the collaborators at key locations in the major chickpea growing areas. Nine sites were selected in India based on the availability of wilt sick-plot (Fig. 1). These sites represented a wide diversity in latitude from 17°53′ at Patancheru to 30°55′ at Ludhiana, and longitude from 70°36′ at Junagadh to 80°24′ at Kanpur. Seeds of the test lines from CWN were sown in wilt sick plot at 9 locations in 8 states of India during the 2007/2008 to 2009/2010 crop seasons. The nursery was laid out in a RCBD with two replications. Each genotype was grown in one row of 4 m length with row-to-row spacing of 60 cm and plant-to-plant spacing of 10 cm within the row. In order to increase the disease pressure in the nursery, the susceptible check variety (ICC 4951) was planted on every 5th row. Disease pressure in nurseries was considered adequate for wilt evaluation when a susceptible control line had above 80% disease incidence.

2.3. Data collection and analysis

Data on disease incidence was collected from each replication in the RCBD in the field experiments during 2007/2008–2009/2010. Diseased and total plants were counted at seedling, flowering, pod filling, and at near maturity stages of the crop and percentage of

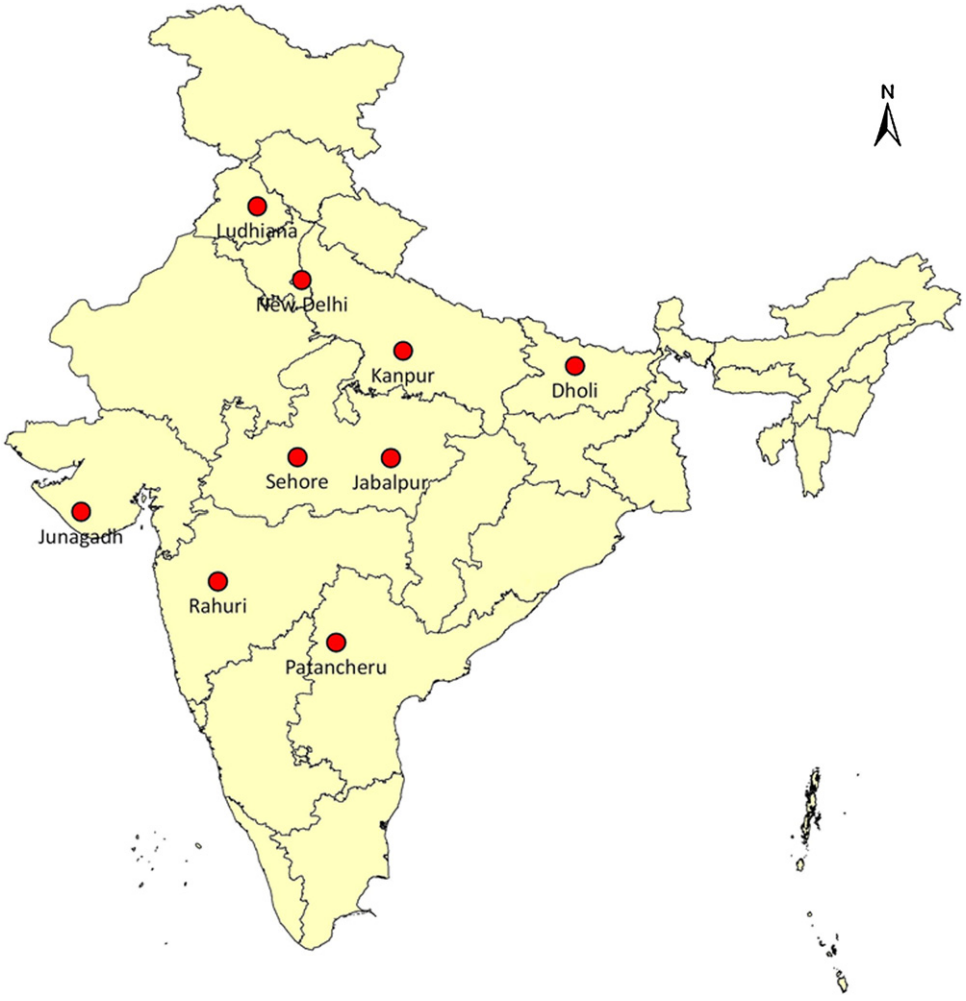

Fig. 1. Test locations of Chickpea Wilt Nursery (CWN) in India, during 2007/2008–2009/10.

plants infected in each genotype was calculated. Cumulative wilt incidence data for each genotype in each location was used in data analysis.

Based on a range of wilt incidence, genotypes were characterized as resistant (1–10% mortality), moderately resistant (11–20%), susceptible (21–50%) and highly susceptible (>50% mortality). The arcsine transformation (Gomez and Gomez, 1984) was applied for per cent wilt incidence data before analysis to make residual normal. Year wise analysis of variance was done to determine the contribution of location, genotypes and their interaction using the mixed model analysis in GENSTAT statistical package (version 14.0; Rothamsted Experiment Station, Herpenden, Herts AL52JQ, UK), considering location and genotypes as fixed and replications as random. In order to pool the data across the locations and to make the error variance homogeneous, individual location variances were estimated and modeled to error distribution using residual maximum likelihood (REML) procedure in GenStat.

Data obtained from 9 locations over 3 years (27 environments, *i.e.* combination of location and year) were used for GGE biplot analysis except Rahuri location for the year 2009 due to non-availability of two genotypes data. To determine stability and identify superior lines across environments, genotype and genotype × environment (GGE) biplot analyses were conducted (Yan et al., 2000) using GenStat software (Payne et al., 2010) comprising seasons 2007/2008–2009/2010. GGE biplot is a method of graphical analysis of multi-environment data, it displays the main genotype effect (G) and the genotype × environment (G × E) interaction of multi-environment tests. It is also appropriate because it can eliminate environmental factor from the analyses. "Environment" was defined as the combination of "season" and "location" (each site in a given year was a separate environment, Table 2). This biplot was constructed by plotting the first two principal components (PC1 and PC2, also referred to as primary and secondary effects, respectively) derived from arcsine transformed values of the environment centered data (Yan, 2002). Singular value portioning is achieved by providing a scaling factor *f* to obtain alternative accessions and environment scores. In order to assay the stability of accessions, the average environment coordinate (AEC) is found by taking the mean of the PC1 and PC2 scores for environments. A performance line passing through the origin of the biplot is used to determine mean performance of a genotype. The arrow on the performance line represents increasing mean disease severity (*i.e.*, increase susceptibility to wilt). A genotype distanced farther from the biplot origin on either side on the stability line represents relatively lower stability. A genotype closer to the performance line is considered more stable than the one placed farther. GGE biplot analysis was also used to examine the relationship among environments over the years. Each environment is characterized by its vector (the line that connects it with the origin of the biplot), the length of the vector represents the genotypic variability in that environment. The cosine of the angle between the vectors of two environments approximates the correlation coefficients between them. Two environments are positively or negatively correlated if the angles between their vectors are <90° and >90°, respectively. The two environments are independent if the angle between them is near 90°. The environments with longer vectors are more discriminative of the genotypes; short vectors are less discriminative. An ideal test location should be that one showing a high projection value onto the AEC abscissa (more discriminating of principal effects of genotypes) and a small absolute projection value onto the AEC ordinate (more representative of all the tested environments) (Yan et al., 2000).

Table 3
Analysis of variance showing Wald Statistics and their percentage of total variation for *Fusarium* wilt incidence of 27 chickpea lines evaluated in 9 locations during 2007/2008–2009/2010 seasons in India.

Source of variation	NDF	DDF	Wald statistic	P[a]	Variation (%)[b] F
Environment (E)	25	19.2	1307.1	***	8.9
Genotype (G)	26	333.9	7960.5	***	54.4
G × E	650	233.6	5368.7	***	36.7

NDF, numerator degree of freedom; and DDF, denominator degree of freedom.
[a] *P*, *F*-test probability of Wald statistic, *** $P < 0.001$.
[b] Fraction of Wald statistic associated to each term or interaction.

3. Results

Preliminary screening performed on 948 chickpea genotypes in wilt sick plot during 2003/04 at Patancheru, India revealed a broad range of response to wilt disease among tested material, which allowed the selection of 123 promising genotypes (≤10% incidence) to be further validated (*data not shown*). These were subsequently evaluated in same location during 2004/2005 season and sixty genotypes were found resistant with disease incidence ≤10%. In 2005/2006 season, 57 out of 60 genotypes found resistant to wilt at Patancheru and these were subsequently reevaluated during 2006/2007 at same location. Finally, a CWN consisting of 27 genotypes (7 germplasm accessions, 19 breeding lines and a susceptible check) were selected and constituted to determine the stability of resistance across 9 locations over 3 years (2007/2008–2009/2010) in India.

Wilt incidence of most of the chickpea genotypes varied greatly between locations and years. Performance of each genotype was not always stable through all environments. This was also confirmed by the different frequency distributions of genotypes in each location over the 3 years suggesting a genotype × environment interaction (Fig. 2). The subsequent analysis of variance of wilt incidence showed that the effects of genotype, environment and the genotype × environment interaction for wilt incidence were all highly significant ($P < 0.001$) (Table 3). Among the three sources of variation (genotype, environment and genotype × environment), the largest portion of variability for wilt incidence was accounted by genotypes (54.4%), followed by genotype × environment interaction (36.7%) and environment (8.9%) (Table 3). Although, we found

Table 2
Description of environments (combination of location and season) of the Chickpea Wilt Nursery during 2007/2008–2009/2010 in India.

Location	State	Environment[a]	Latitude (N)	Season	Longitude (E)	Altitude (m)
Dholi	Bihar	Dhol07, Dhol08, Dhol09	24°9′	November–March	72°1′	52.2
Jabalpur	Madhya Pradesh	Jaba07, Jaba08, Jaba09	23°10′	November–March	79°59′	411
Junagadh	Gujarat	Juna07, Juna08, Juna09	21°31′	November–March	70°36′	106
Kanpur	Uttar Pradesh	Kanp07, Kanp08, Kanp09	26°28′	November–March	80°24′	125
Ludhiana	Punjab	Ludh07, Ludh08, Ludh09	30°55′	October–February	75°54′	255
New Delhi	New Delhi	NewD07, NewD08, NewD09	28°35′	November–March	77°12′	239
Patancheru	Andhra Pradesh	Pata07, Pata08, Pata09	17°53′	October–February	78°27′	522
Rahuri	Maharashtra	Rahu07, Rahu08, Rahu09	19°23′	October–February	74°42′	258
Sehore	Madhya Pradesh	Seho07, Seho08, Seho09	23°12′	November–March	77°00′	457

[a] Environment is denoted as first four letters of the each location followed by year of screening.

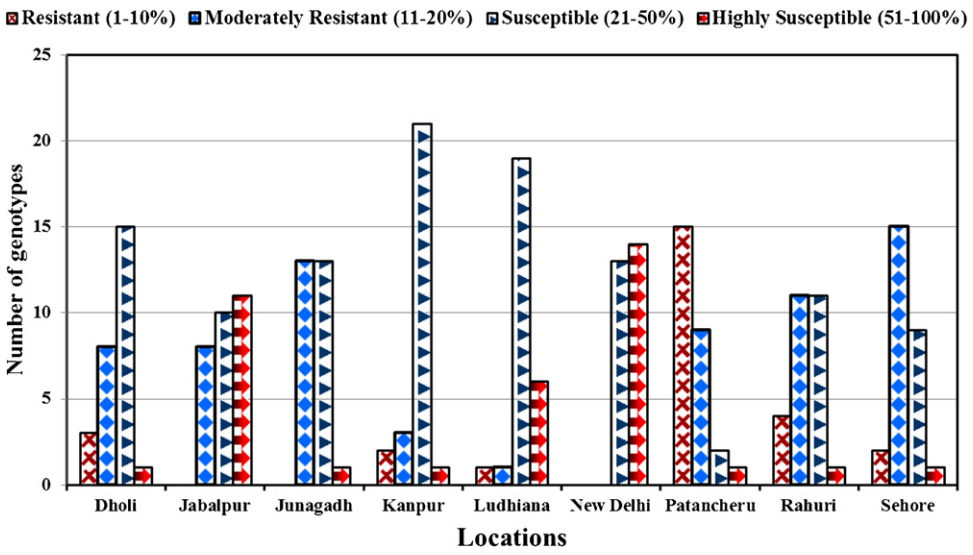

Fig. 2. Frequency distribution for *Fusarium* wilt incidence of 27 chickpea genotypes in 9 locations over 3 years (27 environments). The different patterns of point to genotype × environment (G × E) interaction.

significant G × E interaction, the average disease incidence of each location over three season were presented in Table 4.

Wilt incidence of the susceptible check (ICC 4951) ranged between 84.1% and 100% with the mean of 92.9% in all 9 test locations indicating the adequate disease pressure in sick plot across the locations and years. Among the 9 locations, New Delhi (51.4%) and Ludhiana (42.2%) recorded highest average wilt incidence over 3 years, while the mean wilt incidence was lowest at Rahuri (24.4%) and Sehore (24.1%) (Table 4). Of the 26 test lines, ICC 11322 and ICCV 05527 although had mean wilt incidence of 18.4% and 18.9%, respectively, their responses to wilt infection at different locations varied from 8.4% (Rahuri) to 33.5% (New Delhi) for ICC 11322 and 4.6% (Dholi) to 34.5% (New Delhi) for ICCV 05527 (Table 4). Significant differences in wilt resistance were observed among the 26 genotypes. Fifteen genotypes were resistant at Patancheru, 4 at Rahuri, 3 at Dholi, each 2 genotypes at Kanpur and Sehore, one at Ludhiana. None of the genotypes were found resistant at Jabalpur, Junagadh and New Delhi (Fig. 2). Differential disease reactions across locations were evident in many test genotypes. For instance, ICCV 05527 was resistant at Dholi, Patancheru and Sehore, and moderately resistant at Junagadh and Rahuri, and susceptible at remaining locations.

The first two principal components obtained by singular value decomposition of the GGE model explained 65.47% of the total

Table 4
Average *Fusarium* wilt incidence (%) on tested chickpea genotypes at different locations in India during 2007/2008–2009/2010.

S. no.	Genotype	Location and wilt incidence (%)									
		Dholi	Jabalpur	Junagadh	Kanpur	Ludhiana	New Delhi	Patancheru	Rahuri	Sehore	Mean
1	ICC 95	18.5	18.8	12.8	41.9	46.3	39.1	11.6	21.3	13.0	24.8
2	ICC 2072	14.7	13.4	11.8	30.8	36.9	40.1	9.6	37.9	21.5	24.1
3	ICC 11322	24.1	15.9	11.5	30.3	17.8	33.5	10.1	8.4	14.2	18.4
4	ICC 11324	10.7	31.7	17.0	25.2	4.3	23.2	16.6	43.0	20.7	21.4
5	ICC 14364	28.3	54.4	30.2	26.8	32.6	29.6	7.9	25.3	10.7	27.3
6	ICC 14386	32.5	19.1	15.6	14.1	38.9	66.7	8.7	5.6	18.5	24.4
7	ICC 15996	30.1	64.3	30.2	43.7	41.8	34.4	31.8	9.6	34.6	35.6
8	ICCV 93217	23.9	52.0	39.4	27.8	40.5	69.3	7.8	14.2	21.4	32.9
9	ICCV 96818	18.8	17.5	15.2	7.1	32.0	54.0	7.9	19.2	15.4	20.8
10	ICCV 96851	19.6	21.8	20.8	26.6	42.9	86.6	6.7	33.8	13.8	30.3
11	ICCV 04104	17.6	32.7	22.4	49.0	41.4	54.3	11.0	27.8	14.1	30.0
12	ICCV 04107	16.7	17.8	15.9	41.6	35.8	58.7	11.8	11.7	16.7	25.2
13	ICCV 04108	39.2	15.5	19.5	24.2	37.5	59.5	9.8	13.2	23.1	26.8
14	ICCV 04113	21.7	16.3	13.7	45.3	59.7	40.2	7.7	7.5	17.6	25.5
15	ICCV 04311	41.9	76.6	38.3	46.1	44.9	43.4	13.5	31.9	47.1	42.6
16	ICCV 04312	30.9	65.5	40.0	15.0	57.0	56.9	13.7	30.4	29.9	37.7
17	ICCV 04314	21.6	48.6	42.5	26.6	60.0	57.5	9.0	42.5	43.0	39.0
18	ICCV 05107	28.1	59.6	35.7	26.2	46.5	56.8	8.6	17.5	19.0	33.1
19	ICCV 05110	27.8	22.2	19.1	46.0	63.3	79.2	9.8	13.9	17.8	33.2
20	ICCV 05112	17.3	36.0	27.7	40.9	46.8	42.2	6.8	21.2	12.0	27.9
21	ICCV 05309	34.0	69.0	50.2	49.2	60.3	60.4	47.7	46.9	48.0	51.7
22	ICCV 05310	34.4	83.1	44.9	50.4	44.7	69.8	15.4	19.3	33.7	44.0
23	ICCV 05527	4.6	28.8	17.5	28.6	27.3	34.5	9.7	11.7	7.3	18.9
24	ICCV 05528	17.3	22.6	20.7	7.2	23.2	46.3	14.9	15.6	12.5	20.0
25	ICCV 05529	10.2	36.9	23.6	36.1	29.8	23.2	16.8	15.8	13.3	22.9
26	ICCV 06106	32.5	23.5	26.8	18.0	42.2	39.1	9.5	13.6	11.2	24.0
27	ICC 4951 (Sus. Check)	93.0	86.0	95.8	96.6	84.1	88.7	91.9	100.0	100.0	92.9
	Mean	26.3	38.9	28.1	34.1	42.2	51.4	15.8	24.4	24.1	31.7

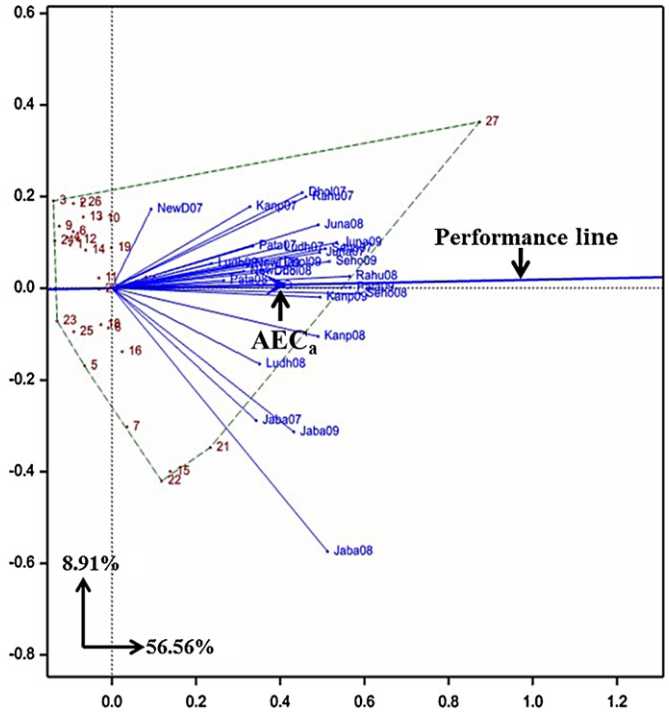

Fig. 3. GGE biplot of first and second principal components (PC1 and PC2, respectively) based on *Fusarium* wilt incidence of 27 chickpea genotypes together with a susceptible (ICC 4951) check in 26 environments (combination season–location) in India. The environments are shown in initial four letters abbreviating the location followed by season (2008 = 08, 2009 = 09 and 2010 = 10) and their vectors portrayed as solid lines. Those genotypes contributing to the most interaction delimit the vertices of a polygon (dashed lines) comprising the rest genotypes. AEC_a is the abscissa of the "Average Environment Coordination" axis, which connects the origin with the environmental average. (Refer to Tables 1 and 2 for names of the genotypes and environments.)

variation of the environment-centered data implying complex interaction between genotypes and environments. Performance of genotypes within a single locality in different years was rather constant as shown by proximity of vectors (for instance, JAB07, JAB08 and JAB09) in the GGE biplot (Fig. 3). A polygon has been drawn on the biplot (dashed lines in Fig. 3), vertices being the genotypes that contribute the most to the interaction, that is, those which show the highest or the lowest wilt incidence. The "average environment coordination" axis (AEC) is the appropriate tool to compare genotypes by their average performance and stability. It is drawn on the biplot GGE, its abscissa (AEC_a) being the line that connects the origin and the environmental average, that is, the average of PC1 and PC2 coordinates across environments. The best genotype would be that with the lowest severity (higher negative projection on AEC_a) and the highest stability, *i.e.*, projection on AEC_a close to 0 (Yan, 1999).

The GGE biplot analysis of the 27 germplasm accessions and breeding lines revealed that ICCV 11322, ICCV 96818, ICCV 05527 and ICCV 05528 had moderate level of stability with low level of wilt incidence (Fig. 3). One breeding line ICCV 05309 and susceptible check ICC 4951, were consistently the more susceptible by being farthest on the right side of the origin of the biplot on the performance line. All the environments were discriminative for genotypes as shown by the position of the environment–year combinations away from the biplot origin. However, environments differed in their discriminative ability as shown by their different vector length. For instance, the location in Jabalpur in 2008 (Jaba08) with long vector length was highly discriminative of genotypes than the same location in 2007 (Jaba07) and 2009 (Jaba09). GGE biplot analysis showed that most of the environments form smallest angle with AEC_a and were most representative as they had a near-zero projection on AEC_a (Fig. 3). The angles between all the twenty-seven environments were less than 90°, indicating the high correlations amongst them. However, some of the correlations with Jaba08, Jaba09, Jaba07, Ludh08, Kanp08, Kanp09 and Seho08 were only moderate. Frequently, a given site/environment showed diversity in different years as represented by their position at different points and different vector lengths in the biplot.

4. Discussion

Deployment of resistant cultivars remains the most viable strategy to manage chickpea wilt disease in India and other countries in the developing world. Screening germplasm and breeding lines for disease resistance is a comprehensive task, which encompasses different approaches. Among the options available, field trails are regarded as powerful tools to identify sources resistance as they reflect the natural conditions to which the selected material will be eventually subjected to. Nevertheless, field trails have drawback of variability of pathogen population and also environments, so plant responses can differ from one location to another (Flor, 1971; Kulakarni and Chopra, 1982; Venderplank, 1984). Since this is not desirable, phenotypic stability becomes one the main issues in these process of selection. This has been rational in this work. Starting with a relatively large number of genotypes, the first objective was to identify the wilt resistant lines with consistent performance at ICRISAT, Patancheru for further validation under multilocation and multiyear trials. Trial in season 2003/2004 at Patancheru was just an preliminary evaluation to discard ultra-susceptible material and selected genotypes were subsequently evaluated at same location from 2004/2005 to 2006/2007 to identify the genotypes with stable performance for multiyear testing at different locations in India. In this study, chickpea genotypes were evaluated in different locations to identify genotypes that have stable and broad based resistance to *Fusarium* wilt across geographical locations in India. Multi-environment testing (9 locations × 3 years) provided differential reactions to wilt in the genotypes tested. Wilt incidence on these genotypes were significantly affected by the environment (location) and their interaction. Significant effects of interaction between chickpea genotype and environment suggested that the pathogen populations, in term of virulence genes, varied across different geographical locations although the possibility that the different genotypes could also respond differentially to different environmental conditions cannot be excluded (Kulakarni and Chopra, 1982). However, the relative effect of environment factor on wilt incidence was minimized by screening the genotypes under *Fusarium* wilt sick plot.

Disease incidence in chickpea lines was quite variable at different locations, but the incidence levels on the susceptible control ICC 4951 indicated high and adequate disease pressure in all tests. Some locations, such as New Delhi and Ludhiana had much higher average wilt incidence on almost all tested chickpea genotypes over 3 years than other locations. In contrast, average wilt incidence was the lowest at Patancheru. The differences in wilt incidence among the locations might arise either from the differences in virulence of pathogen population or from differences among the dominant genotypes in the pathogen populations or combination of both factors. The largest number of lines (24 out of 26) among the test entries was resistant and moderately resistant at Patancheru location and none of them at New Delhi, and remaining locations fall between these two locations confirming that isolate from New Delhi is more virulent than remaining location isolates. The resistance in largest number of genotypes at Patancheru location isolate may be for the reason that these lines have been bred for their resistance using the Patancheru isolate over several seasons.

Multi-environment evaluation of genotypes for resistance to *Fusarium* wilt in 9 locations allowed to fine-tune selection for particular locations and also to study the variability of the pathogen population. Analysis of the stability of chickpea genotypes for wilt resistance using GGE biplot showed that three breeding lines (ICCV 05527, ICCV 05528 and ICCV 96818) and one germplasm accession (ICC 11322) by being farthest to the left of the biplot origin and near to the performance line could be considered from stable to moderate stable for wilt resistance across the environments. Among these, ICCV 05527 and ICCV 05528 have also been found moderately resistant to Botrytis gray mold and Ascochyta blight (*Personal communication*). All these breeding lines could be valuable for chickpea breeding programs attempting to improve chickpea wilt resistance. Two test lines ICCV 04107 and ICCV 04108 developed from similar genetic background responded to *F. oxysporum* f. sp. *ciceris* similarly and were placed closely on GGE biplot. In contrast, ICCV 04311 and 04312, ICCV 05527 and 05528 exhibiting similar overall levels of resistance showed varying resistance pattern across environments and years and thus placed differently on GGE biplot. Identification of genotypes that possess high stability for low disease severity is a key component that ensures the selection of useful sources of high resistance for breeding programs (Sharma and Duveiller, 2007). A regular stability analysis often does not provide relative ranking of superior entries, which results in a subjective judgment when selecting a cultivar (Yan and Kang, 2002). The GGE biplot approach used in this study could help breeders better prioritize genotypes to use in breeding programs. The combined visual assessment of the level of resistance and its stability is a big advantage, and adds confidence in the decision to promote a superior genotype. This GGE biplot analysis has recently been widely used in selection of superior genotypes that have low and stable resistance to spot blotch in wheat (Sharma and Duveiller, 2007), soybean rust (Twizeyimana et al., 2008), Botrytis gray mold (Villegas-Fernandez et al., 2009), rust and chocolate spot (Villegas-Fernandez et al., 2011), and ascochyta blight in Faba bean (Rubiales et al., 2012).

In conclusion, this work has allowed to identify sources of resistance to *Fusarium* wilt of chickpea with great potential for use in breeding programs. All of them present a moderate resistance that, at least in some cases, may have a multi-gene basis, which should facilitate obtaining a durable resistance in the final material (Gururani et al., 2012). The scheme of field trials could be used as a model for future screenings. Further work ought to be oriented toward confirming resistance under controlled conditions.

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