

Evaluation of chickpea (*Cicer arietinum* L.) accessions for *Fusarium* wilt resistance

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Abstract

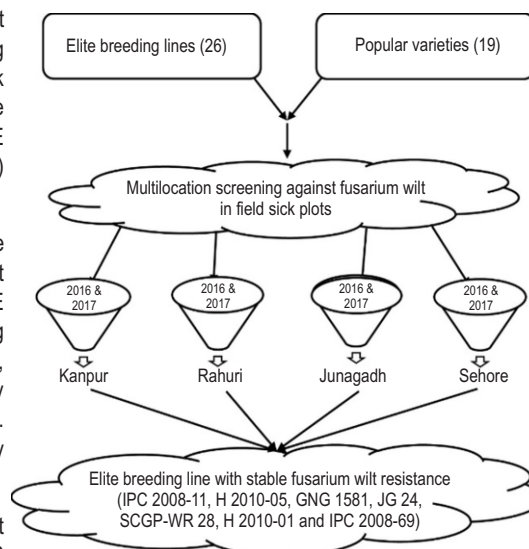
Aim: The present study was conducted to evaluate chickpea breeding lines and popular varieties for *Fusarium* wilt resistance through multi-location field evaluation in major chickpea growing states of India.

Methodology: Forty-five chickpea accessions were evaluated for *Fusarium* wilt resistance in field sick plots at Kanpur, Junagadh, Sehore and Rahuri in India during 2016 and 2017. Each accession was planted in three replications in a randomized block design. The data was subjected to analysis of variance (ANOVA) for testing the significance of variation due to accessions, environments and their interaction. GGE biplots analysis were constructed from the first two principal components (PC1 and PC2) using 45 genotypes and 8 environments using statistical software R, versions 2.15.

Results: The effects of genotype, environment and G x E interaction for wilt incidence were highly significant with maximum variation caused by G x E interaction effect (50.42%), followed by genotypic (46.92 %) and environmental effect (2.24%). GGE biplot analysis revealed that Rahuri and Junagadh locations were most discriminating locations and could differentiate the wilt resistant and susceptible chickpea accession, while Kanpur was least discriminating. Junagadh was most representative followed by Sehore and Kanpur while Rahuri was least representative of the average environment. On an average, the most severe wilt incidence was observed at Junagadh, followed by Sehore, Kanpur and Rahuri over the years.

Interpretation: Elite chickpea accessions possessing high level of fusarium wilt resistant at each location can be utilized for region specific breeding. The accessions IPC 2008-11, H 2010-05, GNG 1581, JG 24, SCGP-WR 28, H 2010-01 and IPC 2008-69 exhibited stable resistance over locations. These possessed resistance against multiple races of *Fusarium* wilt prevailing in the country and can be utilized as donors for disease resistance breeding.

Key words: Chickpea, *Fusarium* wilt, GGE biplot, Multi-location evaluation, Stable resistance



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Introduction

Chickpea (*Cicer arietinum* L.) has the largest share of area and production among all the pulse crops grown in India accounting for nearly 47% (10.90 MT) of the total pulse production during 2019-20. It has shown impressive growth in the country during last few decades, still it faces many challenges hampering its production (Dixit *et al.*, 2019). These include rainfed cultivation on poor soil, inadequate application of nutrients, narrow genetic base (Thudi *et al.*, 2016; Srivastava *et al.*, 2017) and various biotic and abiotic stresses affecting crop yield (Solh *et al.*, 1994). Among biotic stresses, wilt caused by *Fusarium oxysporum* sp. *ciceris* is the most serious problem in all chickpea growing areas in the country (Nene *et al.*, 2012). The average annual losses due to wilt have been estimated at about 10 to 30 %, which may escalate to 90-100% (Sankad with Sunkad, 2019) depending on varietal susceptibility and climatic conditions like high soil temperature (>25°C) (Cortes *et al.*, 2000; Jimenez-Diaz *et al.*, 2015).

Two pathotypes and eight races of pathogens have been reported across the world; among these, races 1A, 2, 3 and 4 have been reported from India, while races 0, 1A, 1B/C, 5, and 6 from the United States and Spain (Sharma *et al.*, 2005). The host resistance against *Fusarium* wilt has been reported to be governed by single or multiple genes based on different races and resistance sources (Kumar 1998; Tullu *et al.*, 1999; Sharma *et al.*, 2007; Gowda *et al.*, 2009; Singh *et al.*, 2014). The pathogen *F. oxysporum* transmits through seed as well as soil (Jendoubi *et al.*, 2017) and can survive on plant debris, weeds, etc., over years even in the absence of host plants (Castro *et al.*, 2012). Hence, the best strategy to manage this disease is to use the resistant cultivars (Sharma *et al.*, 2005). Progress in resistance breeding in chickpea suffers due to variation in pathogen races over different locations as well as interaction with varying weather conditions prevalent over years (Sharma *et al.*, 2009; Soren *et al.*, 2016).

This is further accentuated due to climate change which may increase wilt severity due to rise in soil temperature necessitating pre-emptive breeding (Imtiaz *et al.*, 2011). Many researchers have identified elite genotypes through field screening of chickpea germplasm for resistance against *Fusarium* wilt (Dubey and Singh 2008; Saabale *et al.*, 2017). These studies are mostly based on evaluation of limited germplasm at one or few locations. As such, the resistance is often limited to wilt races prevalent in particular region and the donor can be utilized for breeding programs for that region only. Hence, there is a need for country-wide mining of elite chickpea germplasm possessing stable resistance across different regions which can be readily utilized in chickpea wilt resistance breeding programmes in the country. The present study aims to screen chickpea breeding lines emanated from various chickpea breeding centres in different geographical locations across the country and popular varieties against complex races of *Fusarium* wilt through multi-location screening and evaluation at different wilt sick plots distributed at four diverse locations in the country.

Materials and Methods

Plant material and evaluation locations: For the present study, 45 chickpea accessions including 26 advanced breeding lines and 19 released varieties were evaluated for *Fusarium* wilt resistance during 2016 and 2017. The details of these 45 accessions are provided in Table 1. The screening for *Fusarium* wilt resistance was done in wilt sick plots located at 4 different locations in the country viz., Kanpur (Kan) in the north and Junagadh (Juna), Sehore (Seh) and Rahuri (Rah) in Central India. These locations traverse from 19°23'33" to 26°26'59" N latitude and 70°27'23" to 80°19'54" E longitude varying from 90 to 515 m above mean sea level, representing the chickpea growing belt of India.

Data collection and analysis: Each accession was planted in three replications in a randomized block design with each replication comprising of 2 rows of 4m length at row and plant spacing of 30 x 10 cm. A highly susceptible cultivar JG-62 was included as infestor after every 2 rows of accessions under evaluation. JG-62 has been reported to be susceptible to all the races of *Fusarium* wilt except race 0 (Sharma *et al.*, 2005). Race 0 has not been reported in India till date hence, the variety JG 62 qualifies as highly susceptible control against *Fusarium* wilt in India. Data on wilt incidence was recorded from each replication at 10 days interval during entire crop season. Cumulative percent wilt incidence at all the stages for each genotype was used for data analysis. Percentage wilt incidence of each test genotype was calculated by the following formula (Nene *et al.*, 2012):

$$\text{Fusarium Wilt incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$$

Reaction of test genotypes was determined by following disease rating scale of Nene *et al.*, (2012) with little modification. Depending upon the range of wilt incidence, the test genotypes were grouped as resistant (<10.0 mortality), moderately resistant (10.1–20.0% mortality) and susceptible (>20.0 % mortality). The data was subjected to analysis of variance (ANOVA) for testing the significance of variation due to accessions, environments and their interaction for *Fusarium* wilt incidence as described by Gomez and Gomez (1984). Mean values were calculated and compared using F-test at 5% level of significance. Locations (4) and years (2) were combined to form 8 diverse environments. GGE biplots analysis was performed on *Fusarium* wilt incidence among 45 accessions using statistical software R, versions 2.15. Biplots were constructed using 45 genotypes and 8 environments.

The GGE biplots were constructed from the first two principal components (PC1 and PC2) that were derived by subjecting mean values to singular-value decomposition (Frutos *et al.*, 2014). For testing the mean performance and stability of an accession, the biplots were drawn using Mean vs Stability function with no scaling (Scale = 0), Tester Centered G + GE (Centering = 2) with genotype focused (Row metric preserving)

singular-value partitioning (SVP = 1). For testing the environments, the Discriminability vs Representativeness function was utilized with no scaling (Scale = 0), Tester Centered G + GE (Centering = 2) with environment focused (Column metric preserving) singular-value partitioning (SVP = 2).

Results and Discussion

ANOVA based on evaluation of chickpea genotypes at 8 diverse environments indicated significant genotypic,

environmental and genotypic x environment (G x E) interaction effect (Table 2). Among these three sources of variation, the G x E interaction effect showed maximum contribution towards total variation (50.42%) followed by genotypic effect (46.92%) and environmental effect (2.24%). High contribution of GxE interaction indicates high level of variability in the environment *i.e.*, variable pathogen races at different locations and effect of variation in local weather conditions over years on *Fusarium* wilt incidence (Kulkarni and Chopra, 1982). None of the accession showed resistant reaction against wilt at all the locations.

Table 1: Details of chickpea accessions evaluated in multi-location trial during 2016 and 2017

Genotypes	Pedigree	Remarks
BCP 10	-	Breeding Line
BCP 60	BDNG 33 X No. 404 X BDNG 9-3	Breeding Line
CSJK 96	KAK 2 X CSJK 25	Breeding Line
GJG 0921	GJG 9920 X FG 703	Breeding Line
GJG 1004	GJG 0105 X GCP 9504	Breeding Line
GJG 1316	ICCV 03112 X JAKI 9218	Breeding Line
GJG 1320	ICCV 03112 X JAKI 9218	Breeding Line
GLK 08-104	-	Breeding Line
GNG 2207	C 235 X SAKI 9516	Breeding Line
GNG 2261	GNG 1581 X Pusa 1103	Breeding Line
GNG 2263	GNG 1581 X Avroddhi	Breeding Line
H 2010-01	HC 1 X GL 94022	Breeding Line
H 2010-05	HC 1 X GL 94022	Breeding Line
HK 2013-109	HK 92-94 x HK 1	Breeding Line
IPC 2010-134	GNG 469 X FG 711	Breeding Line
IPC 2012-98	JGK 1 x JG 315	Breeding Line
IPC 2007-28	DCP 92-3 X JG 16	Breeding Line
IPC 2008-11	DCP 92-3 X GNG 469	Breeding Line
IPC 2008-69	ICCV 96030 X C. Pinnatifidum	Breeding Line
IPCK 2006-56	(H 92-94 X HK 95-97) X HK 89-131	Breeding Line
JG 24	(JG 74 x ICC 4958)-21	Breeding Line
JG 74315-2	[(JG 74 x WR 315) x JG 74]-2010-1-3-5-11-15-10-2]	Breeding Line
Phule G 12110	ICCV 03112 X JAKI-9218	Breeding Line
Phule G 13107	ICCV 03112 x JAKI 9218	Breeding Line
SCGPWR 28	SAGL-109 x JG 315	Breeding Line
SCGPWR 32	JG 315 x RVSSG 2	Breeding Line
BDG 128	ICCV 2 x ICCV 5	Released Variety
CSJ 515	FG 712 X CSJ 146	Released Variety
DCP 92- 3	Sel. from germplasm	Released Variety
GCP 101	GCP 2 x ICCV 2	Released Variety
GCP 105	ICCL 84224 x Annegiri 1	Released Variety
GNG 1581	GPF 2 x H 82-2	Released Variety
HK 2	(H 82-2 x E 100 ym.) x Bhima	Released Variety
HK 4	HK 92-94 x HK 1	Released Variety
ICCV 10	PI 231 x P1265	Released Variety
IPCK 2002- 29	L 144 x H 82-2	Released Variety
IPCK 2004-29	[(ICCV 2 X ICCV 88507) X ICCV 42] X ICC 7344	Released Variety
Kripa	Selection from local GP	Released Variety
Pant G 186	ILL 613 x Pant G 114	Released Variety
PUSA 372	P 1231 x P 1265	Released Variety
PUSA 547	Mutant of BG 256	Released Variety
RSG 888	RSG 44 x E 100ym.	Released Variety
RSG 963	RSG 524 x PD 84-10	Released Variety
Vihar	(ICCC 32 x ICCL 8004) x (ICCC 49 x FLIP 82-8C) x ICCV 3)	Released Variety
Vijay	P 127 x Annegiri 1	Released Variety

Table 2: Analysis of variance for *Fusarium* wilt incidence for 45 chickpea lines evaluated at 8 environments (4 locations and two years) in India

Source of variation	d. f.	Sum of square	Mean sum of square	Variation (%)
Replication	2	9.13	4.57NS	
Environment (E)	7	13072.58	1867.51**	2.24
Genotype (G)	44	305231.56	6937.08**	46.92
G x E	308	287726.37	934.18**	50.42
Error	718	1857.84	2.59	

^{NS}Non-significant, ^{**}Significant at P=0.01

Table 3: Classification of chickpea accessions based on their resistance against *Fusarium* wilt at different locations

Locations with R or MR	No. of accessions	Chickpea accessions
8	8	H 10-05, IPC 2008-11, GNG 2263, IPC 2008-69, H 10-01, JG 74315-2, JG 24, SCGPWR 28
7	10	IPC 07-28, CSJK 96, GNG 2261, GNG 1581, GNG 2207, SCGPWR 32, IPC 12-98, GCP 105, GJG 1320, IPCK 06-56
6	8	HK 4, GJG 1316, CSJ 515, GCP 101, IPC 10-134, GLK 08-104, Phule G 12110, Phule G 13107
4-5	6	BCP 60, GJG 1004, BCP 10, GJG 0921, HK 2, PG 186
1-3	9	HK 13-109, Vihar, ICCV 10, Vijay, DCP 92-3, Kripa, IPCK 04-29, PUSA 372, PUSA 547

*R: Resistant; MR: Moderately Resistant

However, at each location, highly resistant accessions were identified. At Kanpur, the accessions H10-05, H 10-01, GNG 263, GCP 105 and HK 4 exhibited resistant reaction. Similarly, highly resistant accessions were identified at Junagadh (BCP 10, SCGPWR 28, H 10-05, GLK 08-104, IPC 2008-11), Rahuri (CSJ 515, H 10-05, IPC 2008-11, IPC 10-134, GNG 1581) and Sehore (IPC 7-28, JG 74315-2, H 10-01, PG 186, GJG 1316). These accessions can be utilized for region specific breeding for respective states. The frequency distribution of different wilt resistant categories at each location indicated that maximum resistant accessions were reported from Junagadh, followed by Rahuri, Kanpur and Sehore (Fig.1). Maximum moderately resistant accessions were found at Kanpur, followed by Sehore, Rahuri and Junagadh. Thus, most severe wilt incidence was observed at Junagarh, followed by Sehore, Kanpur and Rahuri.

Previous reports on race distribution of *Fusarium* wilt in different parts of the country have indicated presence of a particular race in many locations as well as multiple races in single location. However, at each location, a predominant race was present at higher frequency (Dubey et al., 2012). This implies that a genotype having a particular race specific resistant gene will exhibit high resistance in location where *Fusarium* wilt race is predominant, however, the presence of other races, albeit in lesser frequency will hinder in expression of immune reaction. Further, the difference in frequency of different races alone or in combination with crop micro-climatic conditions may affect the expression of resistance of a genotype over years leading to increase or decrease in its resistance response (Sharma et al., 2009). Thus, a genotype showing high resistance in one year may become moderately resistance in other year solely due to

variation in race distribution (Sharma et al., 2019) and other weather parameters like high soil temperature (>25°C) and less soil moisture (Rafiq et al., 2020). The accessions H 10-05, IPC 2008-11, GNG 2263, IPC 2008-69, H 10-01, JG 74315-2, JG 24, SCGPWR 28 exhibited moderate to high resistance against wilt at all 8 locations, while IPC 07-28, CSJK 96, GNG 2261, GNG 1581, GNG 2207, SCGPWR 32, IPC 12-98, GCP 105, GJG 1320, IPCK 06-56 exhibited resistance at 7 locations (Table 3). These accessions showed stable resistance across locations. Other accessions showed susceptible reaction at more than one location. When genotype is tested in multi environment testing (MET) trials, shifts in relative ranking of genotype-by-environment interaction have been reported (Sharma et al., 2012; Parihar et al., 2017). Thus, GGE biplot provides a better graphical representation of true worth of an accession as it simultaneously assesses stability of genotypes, mean performance, discriminating ability, representativeness of environment, etc. (Jeberson et al., 2019).

GGE biplot was constructed by the two principle components (PC1 and PC2) derived from subjecting environment centered wilt incidence data, i.e., *Fusarium* wilt variation due to GGE to singular value decomposition. The first two principal components explained about 76% of total variation in multi-environment trial (Fig 2). This indicated the presence of complex interaction among genotypes and environments. The performance and stability of a genotype can be visualized graphically in GGE biplots by utilizing the average environment coordination (AEC) method (Yan, 2002). The line passing through biplot origin and marker for average environment is termed AEC abscissa (AECa) and it points toward higher mean value.

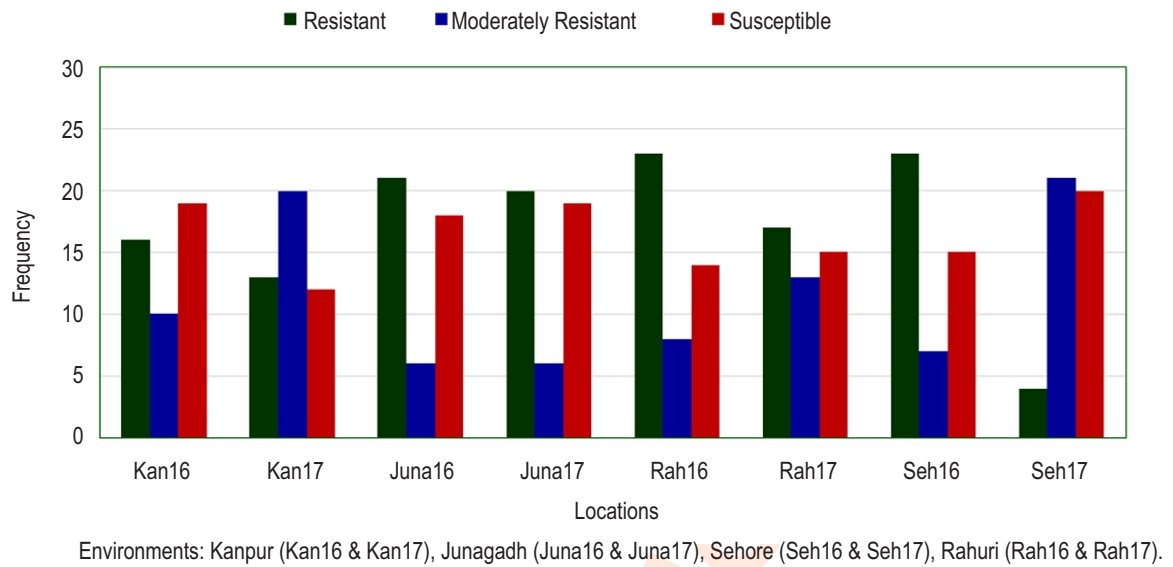


Fig. 1: Frequency distribution of 45 elite chickpea accessions based on *Fusarium* wilt reaction at 8 environments.

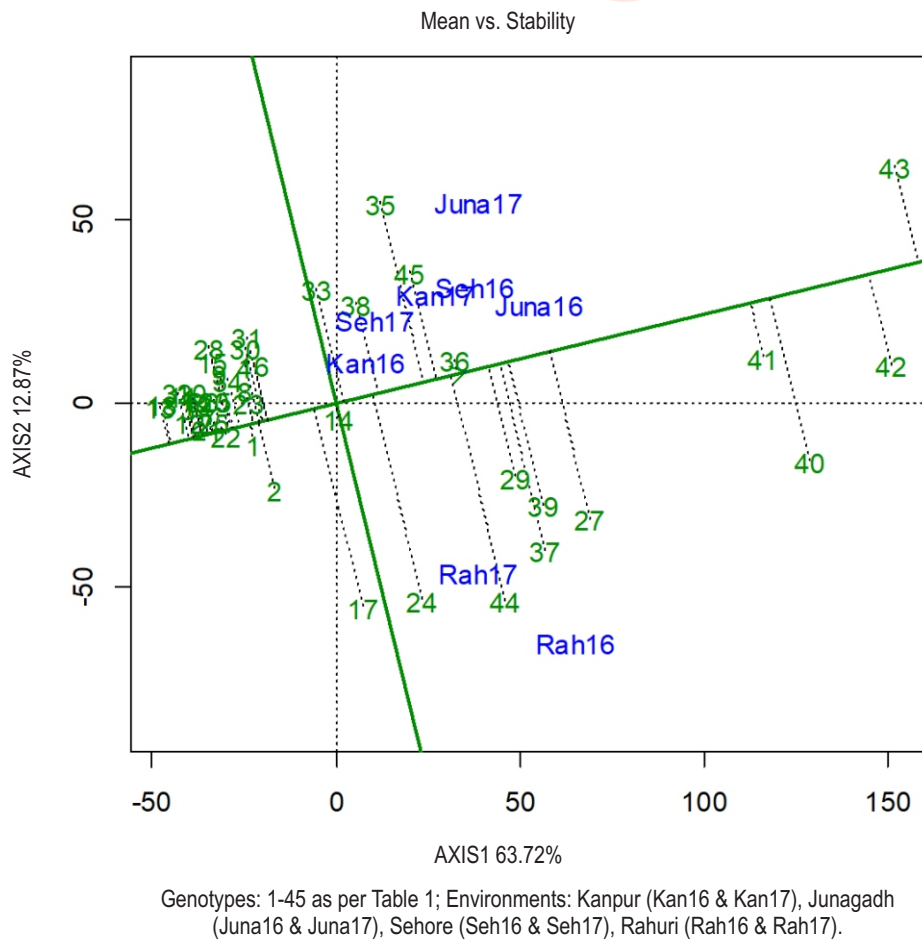


Fig. 2: GGE biplot based on 1st and 2nd Principle Components showing ranking of 45 chickpea accessions based on both mean *Fusarium* wilt incidence and stability over 8 environments in India.

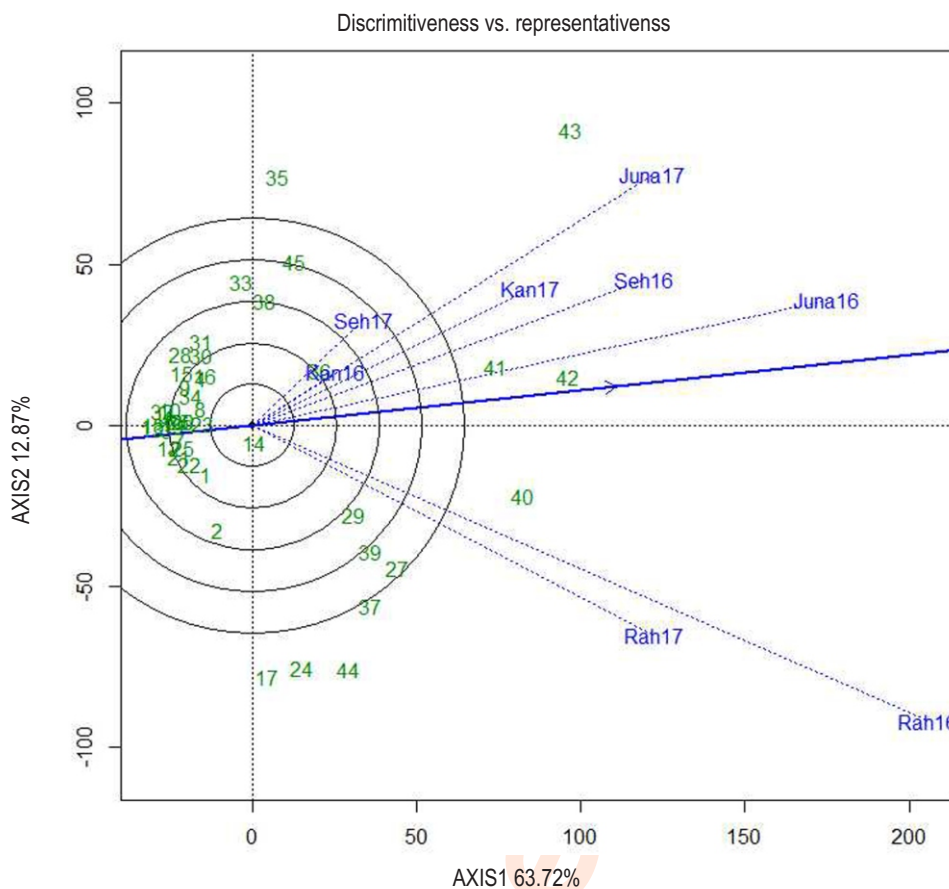


Fig. 3: GGE biplot based on 1st and 2nd Principle Component showing discriminating ability and representativeness of 8 test environments based on mean *Fusarium* wilt incidence of 45 chickpea accession in India.

The perpendicular line to AEC passing through the biplot origin is termed as AEC ordinates and points to greater variability (poor stability) in either direction. In the present context, the best genotypes would be having lowest wilt severity and the highest stability. Graphically, the genotype showing highly stable reaction against *Fusarium* wilt should show higher negative projection on AECa and it should be located closer to AECa, *i.e.*, its projection on AECa should be closed to zero (Yan, 1999). Based on Mean vs Stability function of GGE biplot analysis, chickpea accession IPC 2008-11, H 2010-05, GNG 1581, JG 24 and SCGPWR 28 showed lower disease incidence with high stability (Fig. 2). Chickpea genotypes H 2010-01 and IPC 2008-69 showed moderate resistance against fusarium wilt with moderate stability.

These accessions are expected to provide stable resistance against *Fusarium* wilt due to presence of multiple resistance genes against different pathogen races prevalent in the northern and central parts of country (Sharma *et al.*, 2014). These can be utilized as donors for transferring stable *Fusarium*

wilt resistance to agronomically superior lines. In the “Discrimitiveness vs representativens” biplot, the length of environmental vector acts as a measure of discriminating ability of an environment (Fig. 3). All the environments plotted at far distance from biplot origin indicating they all were able to discriminate between genotypes. However, they varied in their vector length, indicating difference in their discriminating ability. Thus, Rahuri (Rah16) and Junagadh (Juna16) were the most discriminating locations, while Kanpur (Kan16) was least discriminating. All the locations formed small angle with AECa and were most representative of average environment. However, Junagadh (Juna 16) was most representative followed by Sehore (Seh16) and Kanpur (Kan17), while Rahuri (Rah17) was least representative of the average environment. Test environments that are discriminative but non-representative are useful for selecting specifically adapted genotypes in target environments. Hence, Rahuri can be used to screen material having specific resistance against wilt races prevalent in the region. Non-discriminating test environments are less useful as they provide

less discriminating information about the genotypes. The cosine of angle between two environment vectors approximates the correlation between them. If the angle between two environment axis $<90^\circ$, the correlation is positive, while an angle $>90^\circ$ indicates negative correlation between environments. Presence of right angle between two environment axis indicates absence of correlation. All the angles between environment axis were acute ($<90^\circ$), indicating positive correlation among test environments. Thus, the genotypes identified as having stable resistance against *Fusarium* wilt can be utilized as donors at all these test locations.

The present study provides a feasible approach to screen and identify chickpea accessions possessing resistance against multiple races of *Fusarium* wilt through multi-location testing. The accessions IPC 2008-11, H 2010-05, GNG 1581, JG 24, SCGPWR 28, H 2010-01 and IPC 2008-69 possessed high level of resistance against *Fusarium* wilt prevailing in the country. These can be utilized as donors for disease resistance breeding.

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Add-on Information

Authors' contribution: A.K. Srivastava, G.P. Dixit: Conceptualization, Analysis, Resources, Paper writing, Supervision; N.P. Singh: Review and finalization of paper, Supervision; D.R. Saxena, P.R. Saabale, K.S. Raghuvanshi, V.P. Anandani: Investigation, Conduct of trial, Data recording.

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