Crop Protection 144 (2021) 105571

Contents lists available at ScienceDirect

Crop Protection

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

# Delineation of genotype-by-environment interactions for identification and validation of resistant genotypes in chickpea to *fusarium* wilt using GGE biplot

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ARTICLE INFO

*Keywords:*
Chickpea
*Fusarium* wilt
Multi-location evaluation
GGE Biplot
Stable resistance

ABSTRACT

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *ciceris,* is a major constraint to chickpea (*Cicer arietinum* L.) production and breeding for resistant cultivars is one of the most practical and economical strategies for managing this disease. The present study assesses elite chickpea breeding lines for *Fusarium* wilt resistance through multi-location evaluation in field sick plots over two years in India. The effects of genotype, environment and GE interaction for wilt incidence were highly significant with maximum variation caused by GE effect (82.09%) followed by genotype (11.16%) and environment effect (6.38%). GGE biplot analysis revealed that Rahuri and Indore locations were most discriminating locations and could differentiate the wilt resistant and susceptible chickpea genotypes while Dholi and Kanpur locations were least discriminating. Durgapura location was the most representative of average environment followed by Sehore while Rahuri and Indore locations were least representative. The genotypes GJG 0904, GJG 0906, GJG 1010, GJG 0814, GJG 0922, JAKI 9218 and GJG 1001 possessed high level of multiple race resistance against *Fusarium* wilt pathogen and can be exploited for disease resistance breeding in chickpea.

## 1. Introduction

Chickpea (*Cicer arietinum* L.) is the most important pulse crop in India accounting for 32.64% (11.90 million ha) of pulse acreage and 44.79% (11.38 million tonnes) of total pulse production in the country (FAOSTAT, 2018). The average yield of chickpea in India is only 956 kg/ha which is much below its potential yield (Dixit et al., 2019). Many factors contribute towards reducing the productivity including 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, Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceris* is the most serious problem in almost all chickpea growing areas of India. The disease is prevalent in the Indian subcontinent, West Asia, Africa, Southern Europe and the North American countries (Westerlund et al., 1974; Nene et al. 1989, 2012). The average annual losses to Fusarium wilt have been estimated to be 10–30% which may escalate to 90–100% depending upon varietal susceptibility and high soil temperature (>25 °C) (Jimenez-Diaz et al., 1993; Cortes et al., 2000; Landa et al., 2006, Navas-Cortés et al., 2007). The mode of action of the pathogen involves entering the host through roots and blocking the vascular vessel thereby resulting in progressive yellowing, wilting and

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https://doi.org/10.1016/j.cropro.2021.105571
Received 8 September 2020; Received in revised form 1 February 2021; Accepted 3 February 2021
Available online 6 February 2021

death of the plant. It has been reported that *F. oxysporum* is transmitted through seed as well as soil (Pande et al., 2007; Jendoubi et al., 2017). Fungus chlamydospores have been shown to persist in soil (Haware et al., 1996), seed hilum (Haware et al., 1978), cotyledons (Shakir and Mirza 1994) as well as in apparently healthy plants infected by the fungus (Haware and Nene 1982; Trapero-Casas and Jimenez-Diaz 1985). The pathogen can survive on plant debris, weeds etc over years even in absence of host plants (Landa et al., 2004; Singh et al., 2008; Castro et al., 2012). Hence the best strategy for management of this disease is to use the resistant cultivars (Sharma et al., 2005). Two pathotypes and eight races of pathogens have been reported (Haware and Nene 1982; Jimenez-Diaz et al., 1993). 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 (Sharma and Muehlbauer, 2007). Some reports suggest monogenic resistance against *F. oxysporum* f sp. *ciceris* wilt race 2 (Sharma et al., 2005) and race 3 (Sharma et al., 2005; Gowda et al., 2009) while others reported oligogenic resistance against race 1A (Upadhyaya et al., 1983; Singh et al., 2014), race 2 (Gumber et al., 1995; Kumar 1998) and race 4 (Tullu et al., 1999).

The All India Coordinated Research Project (AICRP) on Chickpea under the auspices of the India Council of Agricultural Research (ICAR), New Delhi coordinates applied chickpea research programmes in India. It tests improved technologies including chickpea genotypes through a network of 25–30 strategic centers' which are located across the country to represent specific agro-ecological regions. Advanced breeding line are evaluated for at least three years in multi-location evaluation trials for yield traits. Besides they are also evaluated for their reaction against different diseases either at specific hot spot locations in the country or under laboratory conditions. The best genotype in terms of yield and disease resistance over years is recommended for release as a variety for commercial cultivation. Screening at multiple locations not only helps to assess the performance stability of a genotype but also tests it for resistance against major biotic and abiotic stresses which show temporal and spatial variation in their complexity across the country.

Presence of genetic variability in the gene pool of chickpea for *Fusarium* wilt resistance is a pre-requisite for breeding resistant varieties (Singh 1987; Salimath et al., 2007). However, the resistance breeding is not straightforward due to spatial and temporal variation in the wilt pathogen across the country (Sharma et al., 2009). 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). A way forward will be to screen the elite breeding materials for wilt resistance at different chickpea growing regions in the country for understanding their resistance potential to various races of *Fusarium* wilt existing in the region. Many researchers have identified elite genotypes through field screening of chickpea germplasm for resistance against *Fusarium* wilt (Halila and Strange 1997; 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 a particular region and the donor can be utilized for resistance breeding in that specific region only. Further, many germplasms from related species with high level of resistance against *F. oxysporum* are not suitable for their direct use in breeding programme due to their associated undesirable agronomic features. Hence, there is a need for a nation-wide mining of elite chickpea germplasm possessing stable resistance across different regions which can be readily utilized in chickpea wilt resistance breeding programme in India. The present study aims to screen elite breeding materials emanated from various chickpea breeding centers in India against complex races of *Fusarium* wilt through multi-location screening and evaluation at different wilt sick plots distributed at eight diverse locations in the country over two years for identification and validation of resistant genotypes in chickpea.

**Table 1**
Details of the chickpea breeding lines evaluated in multi-location trial during 2016 and 2017.

| S No. | Breeding line | Pedigree | Remarks[a] |
|---|---|---|---|
| 1. | AKG 1108 | JAKI 9218 X PG 96003 | Desi genotype tested in AVT-1 in SZ (Irrigated) |
| 2. | CSJK 74 | CSJK 25 X JGK 1 | Kabuli genotype tested in AVT-2 in SZ (Irrigated) |
| 3. | GAG 1107 | GJG 9807 X IPC 94-19 | Desi genotype tested in AVT-2 in WCZ (Irrigated) and AVT-1 in WCZ (Rainfed) |
| 4. | GJG 0814 | (JG 315 X GCP 9605) X JG 315 | Desi genotype tested in AVT-1 in CZ (Rainfed) |
| 5. | GJG 0831 | GJG 9807 X ICC 4958 | Desi genotype tested in AVT-1 in NEPZ (Irrigated-Late sown) |
| 6. | GJG 0904 | GJG 0105 X Phule G 92926 | Desi genotype tested in AVT-1 in CZ (Irrigated) |
| 7. | GJG 0906 | GJG 0105 X Phule G 92926 | Desi genotype tested in AVT-1 in CZ (Irrigated) |
| 8. | GJG 0907 | GJG 0105 X Phule G 92926 | Desi genotype tested in AVT-1 in SZ (Irrigated) |
| 9. | GJG 0922 | GJG 9920 X FG 703 | Desi genotype tested in AVT-1 in SZ (Irrigated-Late sown) |
| 10. | GJG 1001 | GJG 0105 X FG 711 | Desi genotype tested in AVT-1 in NEPZ (Irrigated) |
| 11. | GJG 1010 | GJG 0105 X GCP 9504 | Desi genotype tested in AVT-1 in NEPZ (Irrigated) |
| 12. | GJG 1012 | GJG 0105 X Phule G 92926 | Desi genotype tested in AVT-1 in SZ (Rainfed) |
| 13. | GJG 1013 | GJG 0105 X Phule G 92926 | Desi genotype tested in AVT-1 in NEPZ (Irrigated-Late sown) |
| 14. | GL 10006 | GG 1267 X GL 96010 | Desi genotype tested in AVT-1 in NEPZ (Irrigated) |
| 15. | GLK 28127 | GLK 28016 X FLIP 88-34C | Kabuli variety released for cultivation in NWPZ (Irrigated) |
| 16. | GNG 2144 | CSJ 901 X CSG 8962 | Desi variety released for cultivation in NWPZ (Irrigated-Late sown) |
| 17. | GNG 2226 | GNG 1581 X JG 11 | Desi genotype tested in AVT-1 in SZ (Rainfed) |
| 18. | H 09-96 | HC 5 X ICCV 96029 | Desi genotype tested in AVT-1 in NWPZ & NEPZ (Irrigated) |
| 19. | HK 09-206 | HK 00-297 X HK 00-301 | Kabuli genotype tested in AVT-1 in NWPZ (Irrigated) |
| 20. | HK 09-211 | HK 00-297 X HK 00-301 | Kabuli genotype tested in AVT-1 in NWPZ & WCZ (Irrigated) |
| 21. | IPCK 2009-164 | IPCK 305 X ICC 16144 | Kabuli genotype tested in AVT-1 in SZ (Irrigated) |
| 22. | JAKI 9218 | (ICCC 37 X GW 5/7) x ICCV 10 | Desi variety released for cultivation in SZ (Irrigated) |
| 23. | JG 16 | ICCC 42 x ICCV-10 | Desi variety released for cultivation in CZ (Irrigated) |
| 24. | NDG 11-24 | Udai X KWR 108 | Desi genotype tested in AVT-1 in NEPZ (Irrigated-Late sown) |
| 25. | PBG 5 | BG 257 x E 100Y | Desi variety released for cultivation in NHZ (Irrigated) |
| 26. | Phule G 0405 | Digvijay X WGC 2000-2 | Desi genotype tested in AVT-2 in WCZ (Irrigated) |
| 27. | Phule G 0408 | Rajas X ICC 95104 | Desi genotype tested in AVT-1 in WCZ (Irrigated) |
| 28. | Phule Vikram (Phule G 08108) | ICC 4958 X Annegiri | Desi variety released for cultivation in Maharashtra (Irrigated); suitable for machine harvesting. |

[a] AVT-2: Advanced Varietal Trial-2; AVT-1: Advanced Varietal Trial-1; NHZ: Northern hill zone (Jammu & Kashmir, Himachal Pradesh, Uttarakhand and North Eastern Himalayan region); NEPZ: North east plain zone (Eastern Uttar Pradesh, West Bengal, Jharkhand, Bihar and North East states); NWPZ: North west plain zone (North West Rajasthan, Punjab, Haryana, Western Uttar Pradesh, Uttarakhand and Delhi); CZ: Central Zone (Gujarat, Madhya Pradesh, Chhattisgarh, Maharashtra, and Southern Rajasthan); SZ: South Zone (Karnataka, Andhra Pradesh & Tamil Nadu).

**Table 2**
Description of eight Indian locations for evaluation of chickpea genotypes against *Fusarium* wilt resistance in chickpea.

| Location | Zone | Latitude (N) | Longitude (E) | Altitude (m) | Crop Duration | Temperature | | Annual Rainfall (mm) |
|---|---|---|---|---|---|---|---|---|
| | | | | | | Max | Min | |
| Durgapura, Rajasthan | North West Plain Zone | 26° 55′ 19 ′′ | 75° 46′ 43 ′′ | 432 | November to March | 32.2 | 19.2 | 564.8 |
| Kanpur, Uttar Pradesh | North East Plain Zone | 26° 26′ 59 ′′ | 80° 19′ 54 ′′ | 133 | November to April | 32.2 | 18.7 | 959.6 |
| Dholi, Bihar | North East Plain Zone | 26° 07′ 17 ′′ | 85° 22′ 07 ′′ | 59 | November to April | 26.1 | 13.6 | 1046 |
| Junagadh, Gujarat | Central Zone | 21° 30′ 55 ′′ | 70° 27′ 23 ′′ | 90 | November to March | 30.5 | 15.8 | 827 |
| Indore, Madhya Pradesh | Central Zone | 22° 43′ 10 ′′ | 75° 51′ 27 ′′ | 550 | November to March | 31.8 | 16.9 | 1062 |
| Jabalpur, Madhya Pradesh | Central Zone | 23° 10′ 07 ′′ | 79° 55′ 54 ′′ | 403 | November to March | 31.9 | 17.9 | 1277 |
| Sehore, Madhya Pradesh | Central Zone | 23° 11′ 54 ′′ | 77° 05′ 42 ′′ | 498 | November to March | 26.3 | 14.0 | 1218 |
| Rahuri, Maharashtra | Central Zone | 19° 23′ 33 ′′ | 74° 38′ 55 ′′ | 515 | November to March | 31.2 | 17.4 | 1123 |

**Table 3**
Distribution of *Fusarium* wilt races at eight locations in India.

| Locations | Predominant Fusarium Wilt races |
|---|---|
| Durgapura | Race 1,2,4 |
| Kanpur | Race 2 |
| Dholi | Race 2 |
| Junagadh | Race 1 |
| Indore | Race 2,4 |
| Jabalpur | Race 2,4 |
| Sehore | Race 2,4 |
| Rahuri | Race 3 |

## 2. Materials and methods

### 2.1. Plant material and evaluation locations

A panel of 126 chickpea genotypes including advanced breeding lines and released varieties were evaluated for *Fusarium* wilt resistance for at least two years during 2011–2015 at 12 field sick plots in India. A preliminary screening among these lines led to selection of 28 elite genotypes which were found to be superior in their resistance against *Fusarium* wilt at multiple screening locations. These 28 elite genotypes (Table 1) were further screened for *Fusarium* wilt resistance at eight different field sick plots in three agro-climatic zones of India namely Central zone (Junagadh, Indore, Jabalpur, Sehore and Rahuri locations), North west plain zone (Durgapura location) and North east plain zone (Kanpur and Dholi locations) during 2016 and 2017. These locations traverse from 19° 23′ 33 ″ to 26° 55′ 19 ″ N latitude and 70°27′23″ to 85°22′07″ E longitude representing the chickpea growing belt of India (Table 2). These field sick plots have been maintained by artificially adding the predominant race of the Fusarium wilt prevalent in the region to have adequate inoculum load for screening against the disease and have different predominant race of Fusarium wilt (Table 3). The predominant race of Fusarium wilt at each location was ascertained by observing disease development in a common set of chickpea differential (Haware and Nene 1982) every year.

### 2.2. Data collection and analysis

Each genotype was planted in four replications in a randomized block design with each replication comprising of two rows of 4m length at row and plant spacing of 30 x 10 cm. A highly wilt susceptible cultivar JG 62 was included as infector row after every two rows of genotypes under evaluation. JG 62 has been reported to be susceptible to all the races of *F. oxysporum* f sp *ciceris* except race 0 (Sharma et al., 2005). Since race 0 has not been reported in India till date, the variety JG 62 qualifies as highly susceptible control against *Fusarium* wilt in India. Data on Fusarium wilt incidence was recorded from each replication at 10 day intervals during the entire crop season. Cumulative percent of Fusarium wilt incidence at all the stages for every genotype was used for data analysis. Percentage wilt incidence of each test genotype was calculated by following formula:

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

The reaction of test genotypes was determined following the disease rating scale of Sharma et al. (2012) with minor modification. Depending upon the range of Fusarium wilt incidence, the test genotypes were grouped as resistant (R, <10.0% mortality), moderately resistant (MR, 10.1–20.0% mortality) and susceptible (S, >20.0% mortality).

The data was subjected to analysis of variance (ANOVA) for testing the significance of variation due to genotypes, environment and their interaction to *Fusarium* wilt incidence as described by Gomez and Gomez (1984) considering genotype, environment and replication as random effects. Mean values were calculated and compared using F-test at 5% level of significance. Locations (eight) and years (two) were combined to form 16 diverse environments. The genotype main effect and genotype by environment interaction (GGE) Biplot analysis was performed on mean *Fusarium* wilt incidence among the 28 chickpea genotypes over 16 environments using statistical software R, versions 2.15 (R Core Team, 2013). 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 utilizing statistical package "GGEBiplotGUI" (Frutos et al., 2014). For testing the mean performance and stability of genotype, 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 Discriminativeness 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).

## 3. Results

The average wilt incidence in *Fusarium* wilt susceptible check variety "JG 62" ranged from 84.9% to 100% with mean value of 97.2% in field sick plots across all the locations. Since the disease incidence was well above 80% at every test location, there was adequate disease pressure for screening of chickpea lines in the wilt sick plots at these locations.

The ANOVA of mean Fusarium wilt incidence showed that the effect due to genotype was significant at all the test locations (P = 0.01). This indicated that the breeding lines varied in their resistance reaction against *Fusarium* wilt at different test locations. The effect due to year was also significant (P = 0.01) indicating variation in disease reaction over years necessitating to do disease screening over multiple seasons. The interaction effect among genotypes and years was also significant at all the locations. Thus, the genotypes responded differentially over years without any specific pattern. Hence each location acted independently over years and can be assumed as an independent environment. Subsequently, the individual location and year combinations were assigned as separate environment so that eight locations and two years data formed 16 independent environments. This data was analyzed for partitioning the effect of genotypes (n = 28), environments (n = 16) and

**Table 4**
Analysis of variance for *Fusarium* wilt incidence in 28 chickpea lines evaluated at 16 environments (eight locations and two years) in India.

| Source of Variation | d. f. | Sum of Square | Mean Sum of Square | Variation (%) |
|---|---|---|---|---|
| Replication | 3 | 7.93 | 2.64$^{NS}$ | |
| Environment (E) | 15 | 56833.21 | 3788.88** | 6.38 |
| Genotype (G) | 27 | 102219.31 | 3785.90** | 11.16 |
| G x E | 405 | 705102.75 | 1740.99** | 82.09 |
| Error | 1341 | 2552.04 | 1.90 | |

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

their interaction using Gomez and Gomez (1984). The effects of genotype, environment and the genotype × environment (GxE) interaction for wilt incidence were all highly significant (P < 0.001) (Table 4). Maximum variation was caused due to G × E interaction (82.09%) followed by genotypes (11.16%) and environment effect (6.38%).

The disease reaction of chickpea genotypes at various locations identifies location specific Fusarium wilt resistant genotypes (Table 5). At Junagadh (predominance of fusarium wilt race 1), eight genotypes (GAG 1107, GJG 0814, GJG 0904, GJG 0906, GJG 0922, GJG 1013, JAKI 9218, Phule G 0408) showed resistant reaction during both the years while seven genotypes (GJG 0907, GJG 1010, JG 16, Phule G 0405, Phule G 08108, GJG 0831, GNG 2226) showed resistance and/or moderately resistance reaction against Fusarium wilt. Similarly, at Rahuri (predominance of fusarium wilt race 3), eight genotypes (GAG 1107, GJG 0831, GJG 0904, GJG 0906, GJG 1013, GNG 2226, HK 09–211, Phule G 0408) showed resistant reaction during both the years while seven genotypes (GJG 0814, GJG 0907, GJG 1001, GJG 1012, JG 16, Phule G 08108, GJG 0922) showed resistance and/or moderately resistance reaction against Fusarium wilt. Fusarium wilt pathogen race 2 is prevalent at both Kanpur and Dholi locations. Only two genotypes (GLK 2827 and HK 09–211) showed resistance and/or moderately resistance reaction against Fusarium wilt during both the years at each location while seven genotypes (GJG 0814, GJG 0906, GJG 1010, HK 09–206, JAKI 9218, Phule G 0408, Phule G 08108) showed resistance and/or moderately resistance reaction against Fusarium wilt in three out of four environments. Fusarium wilt pathogen race 2 has been reported to be more virulent than race 1 (Sharma et al., 2019). Fusarium wilt pathogen races 2 and 4 were predominant at Indore, Jabalpur and Sehore locations. The genotype GJG 0904 was resistant during both the years at each environment while GNG 2144 showed resistant or moderately resistance reaction in five out of six environments. At Durgapura, races 1 2 and 4 were prevalent and only one genotype GJG 0906 showed resistance and/or moderately resistance reaction during both years.

The first two principal components (PC1 and PC2) of the GGE Biplot, derived from subjecting environment centered wilt incidence data i.e. *Fusarium* wilt variation due to GGE to singular value decomposition explained about 45.09% of the total variation in multi-environment trial (Fig. 1). Based on Mean vs Stability function of GGE Biplot analysis, chickpea genotype GJG 0904 followed by GJG 0922, GJG 0906 and GJG 0814 showed lower disease incidence with higher stability (Fig. 1). These can be utilized as donors for transferring stable *Fusarium* wilt resistance to agronomically superior lines. In the "Discrimitiveness vs representativeness" biplot, the length of environmental vector acts as a measure of discriminating ability of an environment (Fig. 2). All the environments plotted at far distance from biplot origin indicate that they were all able to discriminate between genotypes. However, they vary in their vector length indicating difference in their discriminating ability. Thus, Rahuri and Indore locations were the most discriminating locations while Dholi was least discriminating. All the locations formed small angle with AECa and were most representative of the average environment. However, Durgapura location was most representative followed by Sehore while Rahuri and Indore locations were least representative of the average environment. The cosine of angle between two environment vectors approximates the correlation between them. If the angle between two environment axis is less than 90̊, the correlation is positive while an angle more than 90̊ indicates negative correlation between environments. Presence of right angle between two

**Table 5**
Fusarium wilt resistance status of chickpea genotypes at various locations in India during 2016 and 2017.

| S No. | Genotypes | Durgapura | | Kanpur | | Dholi | | Junagadh | | Indore | | Jabalpur | | Sehore | | Rahuri | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 | 2016 | 2017 |
| 1 | AKG 1108 | S | S | MR | MR | S | S | MR | MR | R | R | R | MR | S | S | S | R |
| 2 | CSJK 74 | S | S | S | R | S | MR | S | MR | MR | S | R | R | S | MR | S | R |
| 3 | GAG 1107 | R | S | S | S | S | S | R | R | S | S | S | S | S | S | R | R |
| 4 | GJG 0814 | S | S | MR | R | MR | S | R | R | R | MR | S | S | R | S | R | MR |
| 5 | GJG 0831 | S | S | R | MR | S | S | MR | MR | S | S | S | S | S | S | R | R |
| 6 | GJG 0904 | MR | S | S | MR | MR | S | R | R | R | MR | MR | R | R | MR | R | R |
| 7 | GJG 0906 | R | R | S | R | MR | MR | R | R | R | MR | S | R | S | MR | R | R |
| 8 | GJG 0907 | S | S | S | S | S | S | MR | R | R | MR | S | MR | S | R | R | MR |
| 9 | GJG 0922 | MR | MR | S | R | MR | S | R | R | S | S | MR | R | MR | MR | MR | MR |
| 10 | GJG 1001 | S | S | MR | R | S | S | S | MR | R | S | R | S | MR | S | R | MR |
| 11 | GJG 1010 | S | MR | S | R | R | MR | MR | R | MR | MR | MR | S | S | MR | S | S |
| 12 | GJG 1012 | MR | R | MR | R | S | S | S | MR | S | S | S | S | MR | MR | MR | R |
| 13 | GJG 1013 | S | S | S | MR | MR | S | R | R | R | S | R | S | S | MR | R | R |
| 14 | GL 10006 | R | S | R | MR | S | S | S | S | S | S | S | S | S | MR | S | S |
| 15 | GLK 28127 | S | S | R | R | R | MR | S | MR | S | S | S | R | S | MR | S | MR |
| 16 | GNG 2144 | S | S | MR | R | S | S | MR | S | R | R | MR | MR | S | R | S | S |
| 17 | GNG 2226 | MR | MR | S | MR | S | S | MR | MR | S | R | S | S | S | S | R | R |
| 18 | H 09-96 | R | S | S | S | MR | R | S | S | MR | S | S | S | S | S | S | S |
| 19 | HK 09-206 | S | S | MR | MR | S | R | S | S | S | MR | R | R | S | S | R | S |
| 20 | HK 09-211 | S | S | MR | R | MR | MR | S | S | S | R | S | S | S | S | R | R |
| 21 | IPCK 2009-164 | MR | S | MR | MR | S | S | S | S | MR | MR | S | MR | S | R | S | S |
| 22 | JAKI 9218 | S | MR | MR | R | S | MR | R | R | R | S | S | R | MR | MR | S | R |
| 23 | JG 16 | MR | S | R | S | R | S | MR | R | S | R | S | MR | S | S | MR | R |
| 24 | NDG 11-24 | R | MR | S | S | MR | MR | R | S | R | S | S | S | S | S | S | R |
| 25 | PBG 5 | S | S | R | MR | S | S | R | S | S | R | S | R | S | S | MR | S |
| 26 | Phule G 0405 | S | MR | R | MR | S | S | R | MR | S | S | S | MR | S | MR | S | S |
| 27 | Phule G 0408 | S | S | R | MR | MR | S | R | R | MR | S | S | S | S | S | R | R |
| 28 | Phule G 08108 | S | S | R | MR | MR | S | R | MR | R | S | R | S | S | MR | MR | R |

*R: Resistant; MR: Moderately Resistant; S: Susceptible.

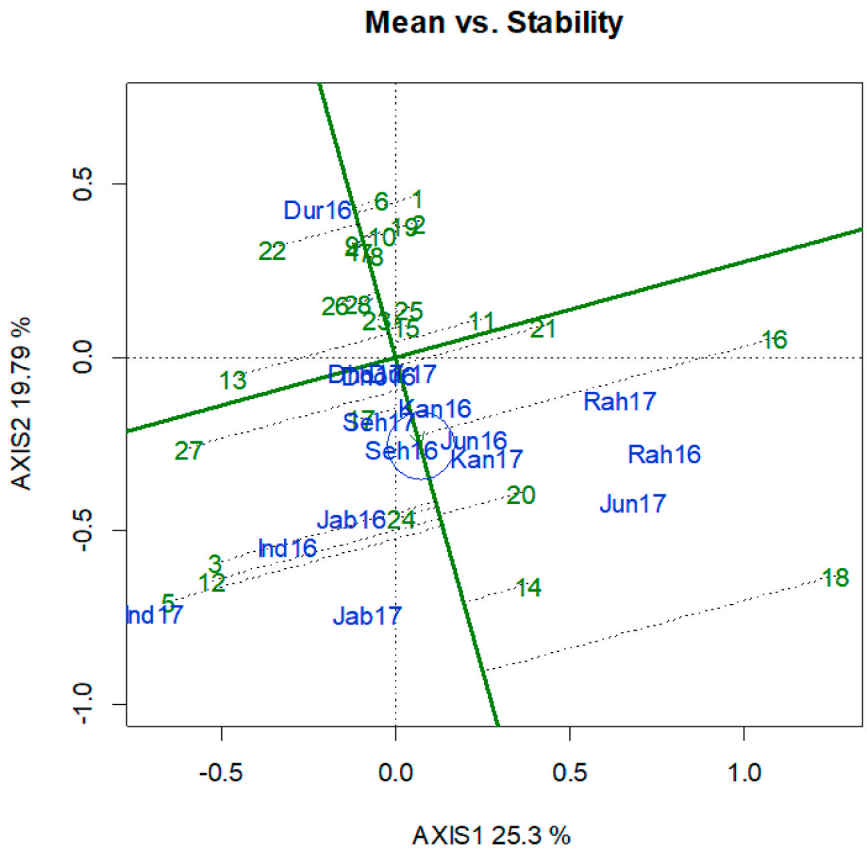



**Fig. 1.** GGE Biplot based on 1st and 2nd principal components showing ranking of 28 chickpea genotypes based on both mean *Fusarium* wilt incidence and stability over 16 environments in India.
Genotypes: 1–28 as per Table 1; Environments: Durgapura (Dur16, Dur 17), Kanpur (Kan16, Kan17), Dholi (Dho16, Dho17), Junagadh (Jun16, Jun17), Indore (Ind16, Ind17), Jabalpur (Jab16, Jab17), Sehore (Seh16, Seh17), Rahuri (Rah16, Rah17).

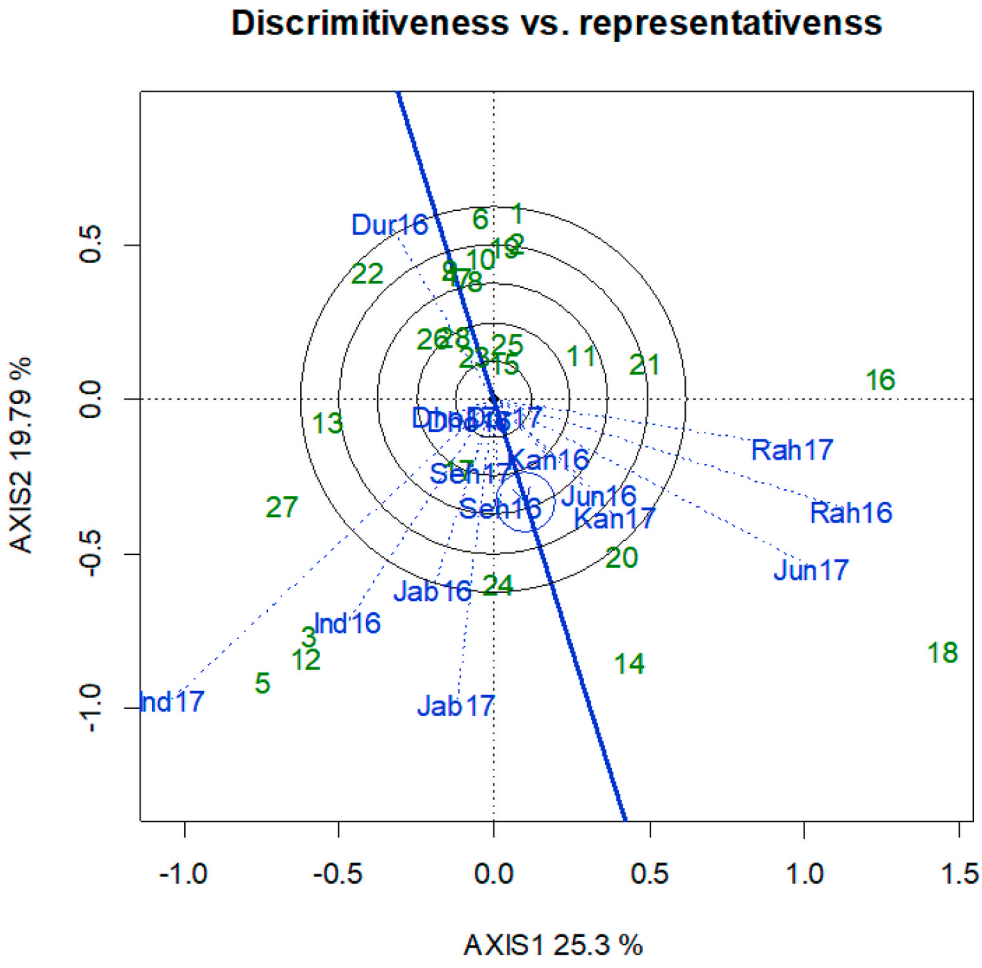
Genotypes: 1-28 as per Table 1; Environments: Durgapura (Dur16, Dur 17), Kanpur (Kan16, Kan17), Dholi (Dho16, Dho17), Junagadh (Jun16, Jun17), Indore (Ind16, Ind17), Jabalpur (Jab16, Jab17), Sehore (Seh16, Seh17), Rahuri (Rah16, Rah17).

environment axis indicates absence of correlation. Most of the angles were acute (<90̊) indicating positive correlation among test environments. The angle between Rahuri, Indore and Durgapura locations was obtuse (>90̊) indicating negative correlation due to the presence of relatively large GE interaction effect.

## 4. Discussion

Breeding varieties for disease resistance depends on availability of donors with high level of stable resistance as well as efficient phenotyping techniques (Saeed and Panguluri 2013). *Fusarium* wilt is the most devastating disease of chickpea affecting all crop growing areas in India. The disease is notoriously persistent in soil and also spreads through seeds and infested soil. Four races of the pathogen had been reported in India with presence of more than one race at some locations (Table 3). The changing climate has aided to rapid evolution of newer and complex forms of the pathogen resulting in rapid breakdown of disease resistance in the newly released varieties (Vadez et al., 2011; Gautam et al., 2013). Hence, the quest for developing new varieties having resistance against multiple races of pathogen is always the choice of plant breeders (Singh and Reddy 1991). Although a number of *Fusarium* wilt resistant chickpea varieties have been developed by different national and international research centers, they do not provide stable resistance across environments due to presence of local races (Infantino et al., 1996; Singh et al., 2008). Developing multi-race resistance in chickpea varieties is the most viable strategy for managing Fusarium wilt. Theoretically, this can be achieved through stepwise breeding for resistance for individual race of *Fusarium* wilt and combining resistance against different races together at a later stage. Practically this approach is tedious, time consuming and puts enormous strain on available resources as screening for multiple races of pathogen can be an arduous task. A practical approach could be to combine the breeding effort of individual breeders at different centers of the country, pool their elite breeding lines followed by multi-location screening at different sick plots varying in their race composition.

The All India Coordinated Research Project on Chickpea in India provides such a platform for researchers in the country. Chickpea breeders located in different parts of the country develop new lines possessing resistance against the race of wilt pathogen prevalent in their respective region. The lines from different regions represent the sum total of elite breeding lines present in the country. These are evaluated for yield traits and disease resistance at multiple locations. High contribution of GE 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 (Kulakarni and Chopra, 1982). Among the 28 elite genotypes evaluated at eight different field sick plots located in three zones namely Central zone (five test locations), North east plain zone (two test locations) and North west plain zone (one test location), none showed consistent resistance response at all the locations, indicating the complexity of the pathogen races in the country. The frequency of resistant genotypes was more at locations with single race of pathogen as compared to those locations having multiple races. Thus, at Junagadh (race 1), Rahuri (Race 3), Kanpur and Dholi (race 2) many resistant genotypes could be found while at Indore, Jabalpur, Sehore and Durgapura, relatively smaller number of genotypes showed resistant reaction. In the North west plain zone (Durgapura), genotypes GJG 0906 showed resistant reaction while NDG 11–24, GJG 1012 and GJG 0922 showed moderate resistance against *Fusarium* wilt while GLK 28127, HK




Genotypes: 1-28 as per Table 1; Environments: Durgapura (Dur16, Dur 17), Kanpur (Kan16, Kan17), Dholi (Dho16, Dho17), Junagadh (Jun16, Jun17), Indore (Ind16, Ind17), Jabalpur (Jab16, Jab17), Sehore (Seh16, Seh17), Rahuri (Rah16, Rah17)

**Fig. 2.** GGE Biplot based on 1st and 2nd principal component showing discriminating ability and representativeness of 16 test environments based on mean *Fusarium* wilt incidence of 28 chickpea genotypes in India.
Genotypes: 1–28 as per Table 1; Environments: Durgapura (Dur16, Dur 17), Kanpur (Kan16, Kan17), Dholi (Dho16, Dho17), Junagadh (Jun16, Jun17), Indore (Ind16, Ind17), Jabalpur (Jab16, Jab17), Sehore (Seh16, Seh17), Rahuri (Rah16, Rah17).

09–211, GJG 1010, GJG 1001 were found promising in North east plain zone (Kanpur and Dholi). In the central zone (Junagadh, Indore, Jabalpur, Sehore, Rahuri), chickpea genotypes GJG 0904, GJG 0906, GJG 1013, JAKI 9218 and GJG 0814 showed better resistance against wilt. These lines can be utilized as wilt resistant donors in respective breeding programmes of the zones. The disease reaction of individual genotypes varied from one year over other at individual locations as well as over locations. There was no set pattern in such deviation in disease reaction of genotypes which indicates the complex nature of wilt pathogen, resistance potential of the genotypes as well as their interaction with environmental conditions. Fusarium wilt resistance against different races in chickpea have been reported to be governed by single or multiple genes and often resistance is governed by recessive genes. Absence of resistant gene or presence of multiple races of pathogen and/or their interaction with suitable environmental condition can alter the expression of resistance in a genotype. Many researchers have reported the presence of multiple races of Fusarium wilt together at a location as well as individual race in different parts of the country (Dubey et al., 2012; Durai et al., 2012). However, at each location only one or few races predominate. This prevents expression of immune response by a genotype at any location due to presence of many races, albeit in less frequency. Further, difference in frequency of multiple races at different location in combination with mico-climatic condition prevalent during crop season causes differential expression of resistant reaction in a genotype at multiple locations. Thus, it becomes imperative to screen the genotypes over years to assess their true resistance potential.

In several cereal and legumes resistant breeding programmes, GGE biplot analysis has been extensively utilized for identifying genotypes with low disease incidence and high stability (Sharma and Duveiller 2007; Twizeyimana et al., 2008; Sharma et al. 2012, 2016; Das et al., 2019). 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 abcissa (AECa) and it points toward higher mean value. 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 Fusarium wilt incidence 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 the AECa

i.e. its projection on AECa should be closed to zero (Yan 1999). Seven genotypes namely GJG 0904, GJG 0906, GJG 1010, GJG 0814, GJG 0922, JAKI 9218 and GJG 1001 were common among top ten most stable and disease resistant chickpea lines based on the frequency distribution of *Fusarium* wilt resistant, moderately resistant and susceptible genotypes at diverse locations over two years and GGE biplot analysis. There was some shift in ranking of the lines for *Fusarium* wilt resistance by these two methods. Overall, the genotype GJG 0906 performed better than GJG 0922 in terms of resistance reaction at several locations (Table 5) but the biplot indicated GJG 0922 with better stability and disease resistance. This change in ranking can be observed for other genotypes also. This may be due to the fact that biplot analysis takes into account the absolute performance of genotypes at all the locations. Although few genotypes may show resistance or moderate resistance reaction at several locations, their performance at other locations are too poor which nullifies the higher resistance rating at other place. This leads to change in ranking of the genotypes in terms of disease resistance and stability. When a genotype is tested in multi environment testing trials, shifts in relative ranking of genotype-by-environment interaction have often been reported (Ceccarelli et al., 1995; Sharma et al., 2012; Parihar et al., 2017). Thus, GGE biplot provides a better graphical representation of the true worth of a genotype. These lines also included seven commercial varieties. Among these, five varieties are released for cultivation in Central zone (JAKI 9218, JG 16, Phule G 0405), NEPZ (GNG 2144) and NWPZ (GLK 28127) while other two are state releases for cultivation in Punjab (PBG 5) and Maharashtra (Phule G 08108). Although these do not rate very high for wilt resistance but are performing very well on yield parameters. Thus, there is a need to strike balance between these desirable but partly incompatible opposing goals i.e. maximizing yield and minimizing the cost of protection (Brown and Rant 2013).

## 5. Conclusion

The present study provides a feasible approach to screening and identifying chickpea genotypes possessing resistance against multiple races of *Fusarium* wilt through multi-location screening of elite breeding material. The breeding lines GJG 0904, GJG 0906, GJG 1010, GJG 0814, GJG 0922, JAKI 9218 and GJG 1001 possessed high level of stable resistance against *Fusarium* wilt and can be exploited for *Fusarium* wilt disease resistance breeding in chickpea.

## Credit author contribution statement

GP Dixit and AK Srivastava: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization. DR Saxena, PR Saabale, KS Raghyvanshi, VP Anandani, RK Singh, OP Sharma, AR Wasinikar and S Sahni: Investigation, Recording observations. NP Singh and RK Varshney: Writing - review & editing, Visualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. conflict of interest.

## Acknowledgments

The authors acknowledge the collaborators of All India Coordinated Research Project (AICRP) on Chickpea for sharing the data and support from Indian Council of Agricultural Research (ICAR), New Delhi, India.

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