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Multi-trait multi environment analysis for stability in MABC lines of Chickpea (*Cicer arietinum*)

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ABSTRACT

Fusarium wilt (*Fusarium oxysporum* f. sp. *ciceris*) is a major disease that can cause up to 90% yield loss in Chickpea (*Cicer arietinum* L.). The presence of 8 physiological races of *Foc* (0, 1A, 1B/C, 2, 3, 4, 5 and 6) makes it a complex task in the development of disease-resistant cultivar. Thus, Pyramiding of *Foc* races 1, 2, 3, 4 and 5 was undertaken using WR 315 as donor and Pusa 372 as recurrent parent through Marker assisted backcross (MABC) breeding approach. A total of 20 genotypes, including 17 MABC derived lines of Pusa 372 × WR 315, susceptible parent (Pusa 372), resistant check (WR 315) and national check (JG 16) were used. Multi-location testing of advanced MABC lines at 4 different regions (Amla, Badnapur, Sehore, IARI-New Delhi) was carried out using randomised block design (RBD) in two replications during 2020–21 winter (*rabi*) season. Usually, multi environment testing is performed involving a single trait, which provides lower reliability in selection of lines, compared to multi-trait analysis. The present study identifies highly stable Fusarium wilt resistant lines with higher yield advantage using MTSI (Multi trait stability index) and GGE (Genotype main effect and genotype × environment interaction) biplot methodology. From GGE biplot analyses the PC1 explains 84.97% and PC2 explains 8.96% of variability. MTSI results revealed that genotype (G) 1, 4 and 3 were stable for the multiple characters studied. But, based on GGE-mean stability value G 11, 12 and 3 were identified for higher yield and better stability values. Based on MTSI and GGE, G 3 may be considered as a stable line for multiple traits including yield superiority.

Keywords: Fusarium wilt, GGE, MABC, MTSI

Chickpea (*Cicer arietinum* L.) is a major source of protein for billions of people in developing and underdeveloped countries. Chickpea wilt (*Fusarium oxysporum* f. sp. *ciceris*) is the most serious constraint to production, can result in yield losses up to 90% (Sharma *et al*. 2019). India need 16–17.5 MT production from its current 10.5 m ha area to achieve self-sufficiency in production and nutritional security, and an average productivity of 15–17 q/ha to feed a growing world population of 10 billion by 2050 (Dixit *et al*. 2019). Chickpeas grown in India have a limited genetic base, which limits chickpea improvement by traditional approaches (Bharadwaj *et al*. 2011). The differences in genotype behaviour under the effect of G × E (Genotype × environment interaction) in multiple environments, can be used to determine genotype adaptability and stability, leading to the development of a wide and specifically adoptable genotype. G × E interaction has an impact on performance analysis with respect to a set of genotypes in a wide variety of environments (Gauch and Zobel 1996). This will lead to a better understanding of disease resistance and environmental influence on it. The presence of eight physiological races of *Foc* (0, 1A, 1B/C, 2, 3, 4, 5 and 6) (Haware and Nene 1982) makes developing disease-resistant cultivars a difficult challenge. As a result, employing marker assisted breeding with several pyramided genes to generate high yielding Fusarium wilt resistance lines with considerable yield advantage over conventional methodologies is possible (Mannur *et al*. 2018).

There is need of method that can choose stability and adaptability to select genotypes that are superior to yield governing traits and resistance to Fusarium wilt. This requires large number of genotypes to be evaluated in multi-environmental trials (MET). In general, MET analysis is performed using single trait, mainly grain yield (Bornhofen *et al*. 2018). To increase the reliability of these results, there is need to consider multiple traits for better accuracy of selection. Based on this concept, multi-trait index has been

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developed to consider several characters simultaneously for effective selection (Olivoto and Lucio 2020).

MATERIALS AND METHODS

Experimental material comprised of a total 20 chickpea genotypes among them, 17 MABC (BC_3F_3) lines, Pusa 372 susceptible recurrent parent, WR315 resistant non-recurrent parent, and JG 16 as national check variety for Chickpea (Table 1). The experiment was conducted in Randomised complete block design (RCBD) with two replications. Yield and other morphological characters were obtained under normal conditions in 4 diverse environments, viz. Amla (21.9283° N, 78.1308° E), Badnapur (19.8682° N, 75.7256° E), Sehore (23.2032° N, 77.0844° E) and ICAR-IARI, New Delhi (28°70′N, 76°58′E) during 2020–21.

Wilt sick plot evaluation and Disease incidence calculation: Disease incidence was obtained for each genotype, which were evaluated in well-established sick plots with sufficient load of the pathogen inoculum (5-6 × 10^6 conidia/ml/g of soil). Visual observations were taken at 60 days after sowing (DAS) based on mortality rate as per the classification of Sharma *et al*. (2019), as highly resistant (less than 10% plant mortality), moderately resistant (10.1–20.0% plant mortality), susceptible (20.1–40.05% plant mortality), and highly susceptible (more than 40.0% plant mortality).

Table 1 Chickpea genotypes with their line number and mean yield across environments and average disease incidence across environments, including yield significance between genotypes and critical difference (CD)

Genotype	Plant no.	Average yield (kg/ha) and yield significance between genotypes	Average disease incidence (0–100%)
G1	P372*WR315-3	2486^{cd}	7.40
G2	P372*WR315-5	2244^{ghi}	12.12
G3	P372*WR315-8	2527^{abc}	6.97
G4	P372*WR315-9	2480^{cd}	8.18
G5	P372*WR315-11	2314^{efg}	10.97
G6	P372*WR315-15	2486^{cd}	6.97
G7	P372*WR315-18	2183^{hi}	14.46
G8	P372*WR315-19	2353^{ef}	10.32
G9	P372*WR315-21	2407^{de}	9.62
G10	P372*WR315-24	2393^{de}	8.96
G11	P372*WR315-25	2628^{a}	4.68
G12	P372*WR315-27	2594^{ab}	5.87
G13	P372*WR315-28	2400^{de}	9.65
G14	P372*WR315-32	2265^{fgh}	13.21
G15	P372*WR315-34	2498^{bcd}	6.85
G16	P372*WR315-37	2315^{efg}	10.75
G17	P372*WR315-38	2304^{efg}	10.42
G18	Pusa 372	2019^{j}	29.91
G19	WR315	1891^{k}	2.67
G20	JG16	2140^{i}	18.42
CD (at 95% level of significance) = 106.7			

$$\text{Per cent Disease incidence} = \frac{\text{Total number of infected plants}}{\text{Total number of plants}} \times 100$$

Traits assessed: Traits such as yield (per hectare), days to 50% flowering (DFF), days to maturity (DM), 100-seed weight (HSW) along with disease incidence (DI) were considered in this study.

Anova and Multi-trait analysis: For each environment, an individual analysis of variance and a combined analysis of variance (ANOVA) was performed using the entire environment for all characters. Multi trait stability analysis (MTSI) was employed for simultaneous selection considering mean performance and stability in the analysis of MET using both fixed and mixed effect model. Data from the traits and genotype were used to assess the genotypic stability of multi-trait using the WAASB index (lower is better) (Benakanahalli *et al*. 2021). Further, the data was analysed for multi-trait stability index WAASB (Weighted Average of Absolute Scores) as proposed by Olivoto *et al.* (2019) and calculated as:

$$WAASB_i = \frac{\sum_{k=1}^{p} |IPCA_{ik} - EP_k|}{\sum_{k=1}^{p} EP_k}$$

where $WAASB_i$, weighted average of absolute scores of the i^{th} genotype; $IPCA_{ik}$, score of i^{th} genotype in the k^{th} interaction principal component axis (IPCA); EP_k, the amount of the variance explained by the k^{th} IPCA. The genotype with the lowest WAASB value is considered the most stable.

Statistical analysis: All analyses were performed in the R software. Preliminary variability study was performed with *Trait Stats*: R package, developed by Nitesh *et al*. (2021); while the GGE Biplots and MTSI analysis was performed with *metan*: R package, developed by Olivoto and Lucio (2020).

RESULTS AND DISCUSSION

Outcome by analysis of variance (ANOVA): Based on the five traits under study, ANOVA was performed and significant differences ($P<0.05$) for both factor [Genotypes (G) and Environments (E)] and their G × E interaction (GEI) were analysed for all traits, which exhibited diversity in response of the genotypes under study in 4 diverse environments. The coefficient of phenotypic variation (CV), which shows experimental precision, was below 16.67%, showing high homogeneity of the experimental conditions (Table 2). Descriptive statistics indicated that the Days to 50% flowering (DFF) ranged from 49–79 days while Days to maturity (DM) ranged from 98–26 days. The disease incidence (DI) was highest (100%) for susceptible check (JG 62) while the recurrent parent (Pusa 372) had 53% DI.

Multi-environmental evaluation of MABC lines for

Table 2 Analysis of joint variance for the trait yield (per hectare), days to 50% flowering (DFF), days to maturity (DM), 100 seed weight (HSW) and disease incidence (DI) evaluated in 20 genotypes in 4 different environment

		Mean sum of square				
Sources of variance	DF	Yield	DFF	DM	100SW	DI
E	3	19160314***	3620.31***	2239.77***	99.37***	70.14***
G	19	280883***	28.68***	36.004***	19.99***	269.03***
GE	57	20408***	8.13***	8.286***	4.84**	29.94***
RESID	76	5068***	1.13***	0.694***	2.91***	3.02***
CV (%)		3.03	1.76	0.734	8.17	16.67

, significant at P≤0.01; *, significant at P≤0.001.

disease score, revealed that lines G1, G3, G4, G6, G9, G10, G11, G12, G13, G15 were highly resistant and G2, G5, G7, G8, G14, G16, G17 were moderately resistant (Table 1). While the recurrent parent of the MABC lines, Pusa 372 was susceptible in Badnapur, Amla and New Delhi, but was highly susceptible in Sehore, and national check (JG 16) was moderately resistant at all the locations. Also, WR 315 was highly resistant in all races which is the resistance donor. Thus, in general MABC lines were resistant compared to its parent Pusa 372.

GGE biplot

1) Mean vs Stability: As illustrated in Fig 1**,** GGE biplot analysis exhibited a total of 93.93% variation, the horizontal axis (PC1) accounted for 84.97% of the total variation (main effect of genotypes), whereas the vertical axis (PC 2) accounted for 8.96% of total variation and showed the impact of G × E interaction (Yan and kang 2002).The average environment coordinate (AEC) axis is a single arrow line that runs from the biplot origin to the average environment depicted by a dotted circle. On the vertical axis, genotypes (lines) to the right of the AEC indicated higher yield than average yield, and vice versa. The biplot organized the yield performance as G11>G12>G3>G15>G1>G4. The AEC vertical axis displayed genotype yield stability (lines). The genotypes yield stability was considered stronger, if the line length perpendicular to the horizontal AEC axis was shorter (Lakew *et al*. 2014, Harish *et al.* 2020). The genotype (G) 11 and 3 were the most stable and high yielding as they were farthest from origin with shortest vector length.

2) Which-Won-Where pattern analysis: The polygon view was generated by interconnecting the points of the genotypes that were farthest from the biplot origin with straight lines, resulting in markers of all genotypes being encompassed in the polygon (Supplementary Fig 1). The biplot was divided into various sectors, lines perpendicular to the extensions were drawn from the biplot origin. The peak genotype in each sector was the best cultivar for traits found in that environment, on the other hand, genotypes found inside the polygon and near the biplot's origin were less sensitive to environmental changes (Baxevanos *et al*. 2008). The genotypes positioned at the greatest distance from the biplot origin were the best or the worst genotypes

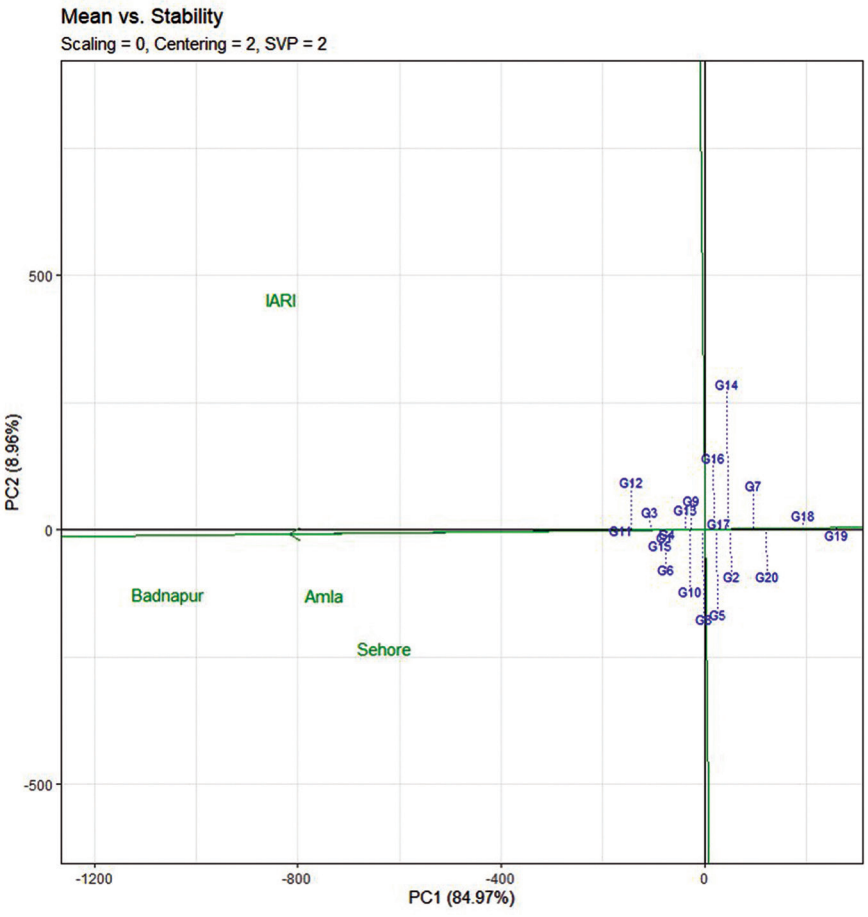

Fig 1 Average-environment coordination (AEC) view showing the mean yield performance and stability of different genotypes based on genotype and genotype interaction (GGE)-biplot analysis.

in some or all environments. G11 performed superior in Amla, Sehore and Badnapur, whereas G12 performed better under New Delhi condition.The evaluation of testing locations based on discrimination power vs representativeness clearly identified a positive association between the length of the location vector and the ability to discriminate between locations, but a negative correlation between the angle of the location vector with the ideal location and the location's representativeness of the target environment. An ideal location needs to be highly distinctive and also be representative of the target location simultaneously (Yan 2010). The ability of a location to maximize the diversity among potential genotypes in a study is referred to as discrimination (Blanche and Myers 2006). The ability to represent explains that the study included an environment that was indicating the conditions in other environments (Mohammadi and Amri 2012). An ideal environment will be able to identify genotypes with high and stable yields at the same time. The angle between Badnapur, Amla and Sehore was less than 90^0, indicating positive correlation between environments and similar results can be expected. Such correlation may be because of differences in geographical location and prevalent race complexity (Badnapur in Maharashtra, Amla and Badnapur in Madhya Pradesh, which are all part of central India). New Delhi, had the longest environmental vectors among the test environments, making it the most "discriminating location" with the potential to distinguish different genotypes. As, New Delhi belongs to North India and presence of distinct climatic conditions may be reason for distinct location characteristic compared to other locations

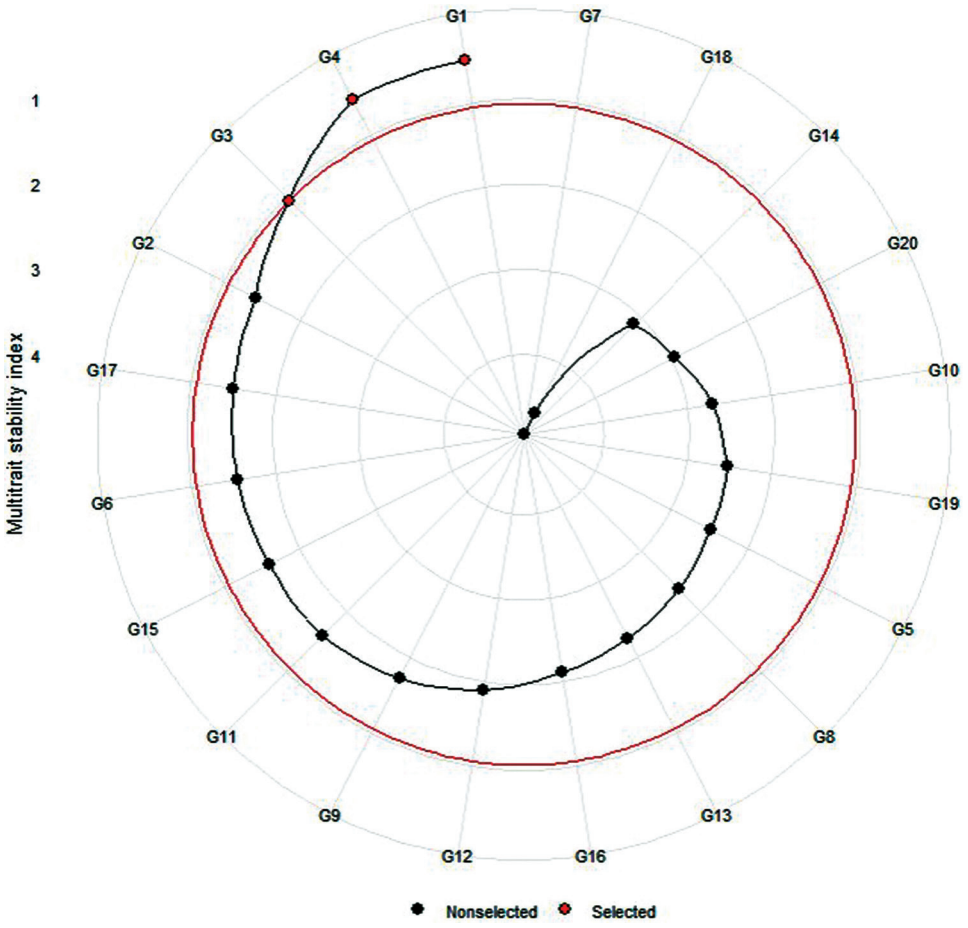

Fig 2 Genotypes selected by multi-trait stability index applied on yield (per hectare), days to 50% flowering (DFF), days to maturity (DM), 100 seed weight (HSW) and disease incidence (DI).

3) Multi Trait Stability Index (MTSI): Based on multiple traits information, the genotypes were classified as desired or undesired, which revealed that genotype 1, 4 and 3 were the most desired genotypes in multiple environments. These genotypes showed WAASBI index values below 1.042. Also, genotypes G2, G17 and G6 were near the cut-off point and close to the circle, can be potential lines which may need further investigation in future (Fig 2). Such, multi environment testing of genotypes acts as a potential tool to study adaptability, stability across environments (Allard and Bradshaw 1964). For identification of stable genotype across environment, G × E interaction study is essential (Comstock *et al.* 1963).This interaction involves both genetic and non-genetic factors. Therefore a stable genotype with high yielding potential is highly suitable for selection. Presence of multiple races for Fusarium wilt resistance complicates the process of selection. Also, there is differential response of genotypes in different location owning to race complexity and environmental influence in disease development that affect the yield. Thus, in divergent environmental conditions, it is a great challenge to select genotypes that remain stable. Even though if a variety is superior with respect to yield, but if it is unstable, then it is less useful in breeding programme. Traditional methodology employees univariate methods and analysis on mean, regression and deviation from regression which may not be sufficient to access the mean performance of multiple traits. Multivariate methodology can assess genotypic stability across different environment, which an univariate methodology is incapable (Dehghani *et al.* 2006). Modern tools such as multi-trait selection index can help in multiple trait selection at a time for greater precision and has recently been highly reliable in different crops (Olivoto *et al*. 2019, Zuffo *et al*. 2020, Benakanahalli *et al*. 2021). Development

of wilt resistant cultivar by MABC is an emerging strategy as a part of integrated disease management. Stable performance of traits is crucial for selection of elite lines. Analysing the G × E interaction can identify lines that are stable and broad based for multiple location and utilization of MTSI for multiple trait selection. This is the first paper that we are aware of uses the WAASB stability model to analyze genotype-environment interactions and stability analyses for MABC lines in chickpea.

Comparative stability analysis of GGE biplot and MTSI, showed that MABC lines G3 was the most stable and also one among the top high yielder across different environment under study. Similarly the MABC lines G11 and G12 also had higher mean yield as compared with other MABC lines hence can be selected for best performing across the environments. These lines can be recommended for further AICRP trails. These lines also recorded superior selection differential along with high heritability for traits under study, hence has the potential to isolate superior lines for development of MABC variety.

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