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## FENUGREEK GERMPLASM DYNAMICS: DECIPHERING DIVERSITY, VARIABILITY AND GENOTYPE-ENVIRONMENT INTERACTION IN CENTRAL INDIA THROUGH MULTIVARIATE, AMMI AND GGE BIPLLOT ANALYSES

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### ABSTRACT

Despite India's status as the foremost fenugreek producer, the multitude of varieties it has generated has become obsolete over time, attributed to diminished performance, a decline in genetic diversity and reduced stability. This study focuses on assessing diversity and variability as well as exploring Genotype by Environment (G×E) interaction in fenugreek genotypes cultivated in the Kymore Plateau and Satpura Hill Agroclimatic region of Madhya Pradesh, India. Conducted from 2018 to 2021, the research involved 17 fenugreek germplasms under the AICRP on Spices initiative. Phenotypic data, encompassing plant height, flowering days, branches plant<sup>-1</sup>, pods plant<sup>-1</sup>, pod length and seed yield plant<sup>-1</sup> were collected. The principal Component 1 (PC1) emerges as the main contributor, capturing a substantial 61.291% of the total dataset variance. PC1's impact is underscored by highest positive loadings for days to first flowering, plant height, and pod length, collectively contributing to 61.291% of the variance. The PCA biplot illustrates distinctive genotype distributions, with MDM 120, RM 201, KFG-1 and UM-411 exhibiting heightened variability. Conversely, genotypes like Hisar Sonali and JFG-2013-18 show limited variability. Hierarchical clustering analysis, utilizing the "complete" method, forms two clusters, with RM-196 and RM-201 in Cluster I and the remaining genotypes in Cluster II. UPGMA clustering forms three clusters, with RM-201 exclusively in Cluster I and distinct genotypes in Clusters II and III. Both clustering methods align with PCA biplot analysis, emphasizing extensive diversity in the RM-201 genotype. The findings of the AMMI and GGE biplot analyses highlight the significant influence of genotype, environment, and their interaction on all traits (P < 0.05). AMMI analysis identified MDM 120, IFGS-11, Hisar Sonali, NDM 80, HM 355 and JFG-2013-16 as stable genotypes, with MDM-120 ranked as the most stable (ASV < 1). Integrating insights from AMMI, ASV and GGE biplot models, the study concludes that MDM 120, IFGS-11 and Hisar Sonali are prominent stable genotypes among the 17 considered.

**Key words :** Fenugreek, Principal Component Analysis, Hierarchical clustering, AMMI & GGE biplot analyses.

### Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is a diploid crop cultivated for both its foliage, utilized as a leafy vegetable, and its seeds, employed as a condiment. Its origin extends from Iran to Northern India (Smith, 1982). India holds a prominent position as both the leading producer and consumer of fenugreek. The primary

fenugreek-producing states in India include Rajasthan, Gujarat, Tamil Nadu, Andhra Pradesh, Uttar Pradesh, Himachal Pradesh and Haryana. The cultivation of fenugreek in India encompasses an extensive area of 1,69,000 hectares, yielding a production of 2,52,000 metric tons (Anonymous, 2022). Renowned for its distinct flavor, fenugreek is an indispensable component of Indian

culinary practices.

India, as the primary fenugreek producer, has introduced numerous cultivars over decades. Despite this, several cultivars are no longer in use due to inconsistent performance, diminished variability, and stability issues over the years. This research seeks to examine the variability, diversity and stability of genotypes cultivated in the Kymore Plateau and Satpura Hills region of Madhya Pradesh, India.

Hybridization followed by selection is a crucial approach in plant breeding for potential yield increase, emphasizing the importance of selecting appropriate parents (Islam, 2004). The investigation of numerous morphological parameters in germplasm aids in assessing population differences and breeding potential. To manage extensive data sets, Principal Component Analysis (PCA) is employed to exhibit the patterns and avoid redundancy (Adams, 1995; Amy and Pritts, 1991). Hotelling (1933) considers PCA an exploratory tool for identifying trends in multidimensional datasets where morphological and physiological variations are common in crop species.

The exploration of genetic diversity involves analyzing variations among genotypes through specific methods or combinations thereof. Employing cluster analysis models is a significant tactic for categorizing germplasm, organizing variability in numerous accessions and examining genetic relationships within germplasms. This is known for its advantages (Peeters and Martinelli, 1989), enables the integration of both qualitative and quantitative data, serving as an opportunity for selection and data reduction through similarity coefficients. Additionally, it offers insights into genetic diversity in crops, a versatile approach applied across diverse fields (Ibrahim *et al.*, 2011).

A cultivar exhibits substantial growth performance variation when cultivated in varied environments, an event known as genotype-environment interaction (GEI) (Dos *et al.*, 2003). The phenotype results from the combined influence of genes, the environment, and their interaction. Evaluating cultivar performance across multiple environments necessitates assessing both general and specific adaptation, involving the complex estimation of individual sources of variation. Over an extended period, various GEI models, such as analysis of variance (ANOVA), linear regression, principal component analysis (PCA), GGE biplots and additive main effect and multiplicative interaction (AMMI) models, have been employed for estimation. AMMI and GGE biplots are widely employed multivariate models for stability, adaptability, genotype ranking, and mega-environment

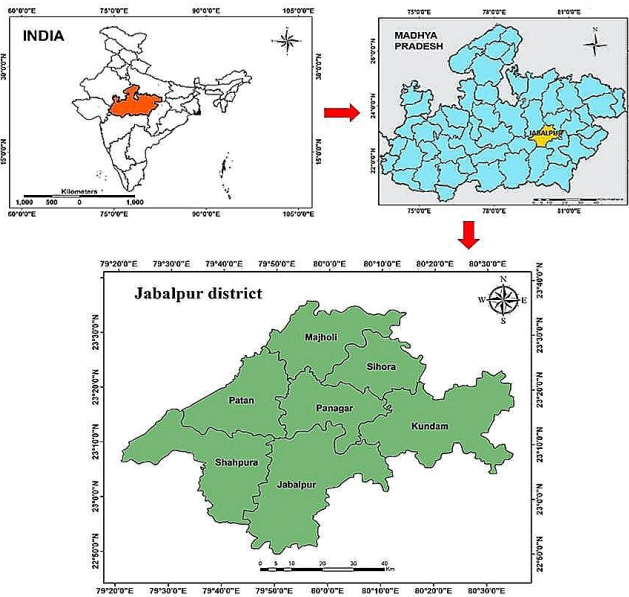
selection (Jamshidmoghaddam, Pourdad, 2013; Gauch, 1992; Yan and Tinker, 2006). These schemes consolidate principal component analysis (PCA) and biplot techniques to elucidate genotype-by-environment interaction ( $G \times E$ ). The AMMI model, incorporating ANOVA and PCA, is particularly effective for stability analysis in multi-environment trials (MET) datasets (Jamshidmoghaddam and Pourdad, 2013). AMMI, recognized as a crucial tool for GEI analysis, provides a comprehensive overview of various statistical models employed in GEI analysis (Zobel *et al.*, 1988). By amalgamating ANOVA, linear regression, and PCA, AMMI scrutinizes genotypic stability data, dissecting additive main effects and individual effects in the residual, enhancing analysis precision when both main effects and interactions are significant. Furthermore, AMMI discerns the impacts of genotypes and the environment in their interaction (Zobel *et al.*, 1988).

Hybridization followed by selection is a crucial approach in plant breeding for potential yield increase, emphasizing the importance of selecting appropriate parents (Islam, 2004). The analysis of numerous morphological traits in germplasm aids in assessing population differences and breeding potential. To manage extensive data sets, Principal Component Analysis (PCA) is employed to express patterns and reduce redundancy (Adams, 1995; Amy and Pritts, 1991). Hotelling (1933) considers PCA an exploratory tool for identifying trends in multidimensional datasets where morphological and physiological variations are common in crop species.

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## Materials and Methods

The investigation took place during the Rabi main crop season spanning from 2018 to 2021 at the Vegetable Research Centre, Department of Horticulture, College of Agriculture, Jawaharlal Nehru Krishi Vishwavidyalaya, Adhartal, Jabalpur, Madhya Pradesh. Jabalpur district, situated along the Narmada River in central Madhya



**Fig. 1 :** Location of district Jabalpur, Madhya Pradesh, India (Shreesty, 2023).

Pradesh, covers an area from 22° 49' 42" N to 23° 37' 5" N and 79° 20' 56" E to 80° 35' 10" E, encompassing 5053 km<sup>2</sup> (Fig. 1). Characterized by a narrow plain bordered by highlands, the region exhibits black cotton soil predominantly in the western and southern parts. The experimental setup comprised 17 diverse fenugreek germplasms under the AICRP on Spices initiative, arranged in a Randomized Block Design (RBD), with each entry replicated thrice. Standard fenugreek cultivation practices were followed and phenotypic data, including plant height, days to flowering, number of branches plant<sup>-1</sup>, number of pods plant<sup>-1</sup>, pod length and seed yield plant<sup>-1</sup> were collected from plants chosen randomly in each plot. Stable genotypes were identified using the AMMI model following Gauch *et al.*'s (2008) approach and ASV was computed based on Purchase *et al.*'s (2000) recommendations. Additionally, GGE biplot analysis, as suggested by Yan *et al.* (2007), provided further insights into genotype performance. Statistical analyses, including ANOVA, AMMI, GGE biplot analyses, and multivariate analyses namely principal component analysis and hierarchical clustering analysis, were executed in R Studio using various packages (R Studio Team, 2015).

## Results and Discussion

The Table 1 presents an overview of means of various fenugreek genotypes based on key plant traits. Among the variables, UM-393 requires the longest time for first flowering at 53.00 days, while RM-196 has the shortest duration of 30.78 days. HM 273 exhibits the tallest plant height at 108.78 cm, with HM-444 having the lowest at

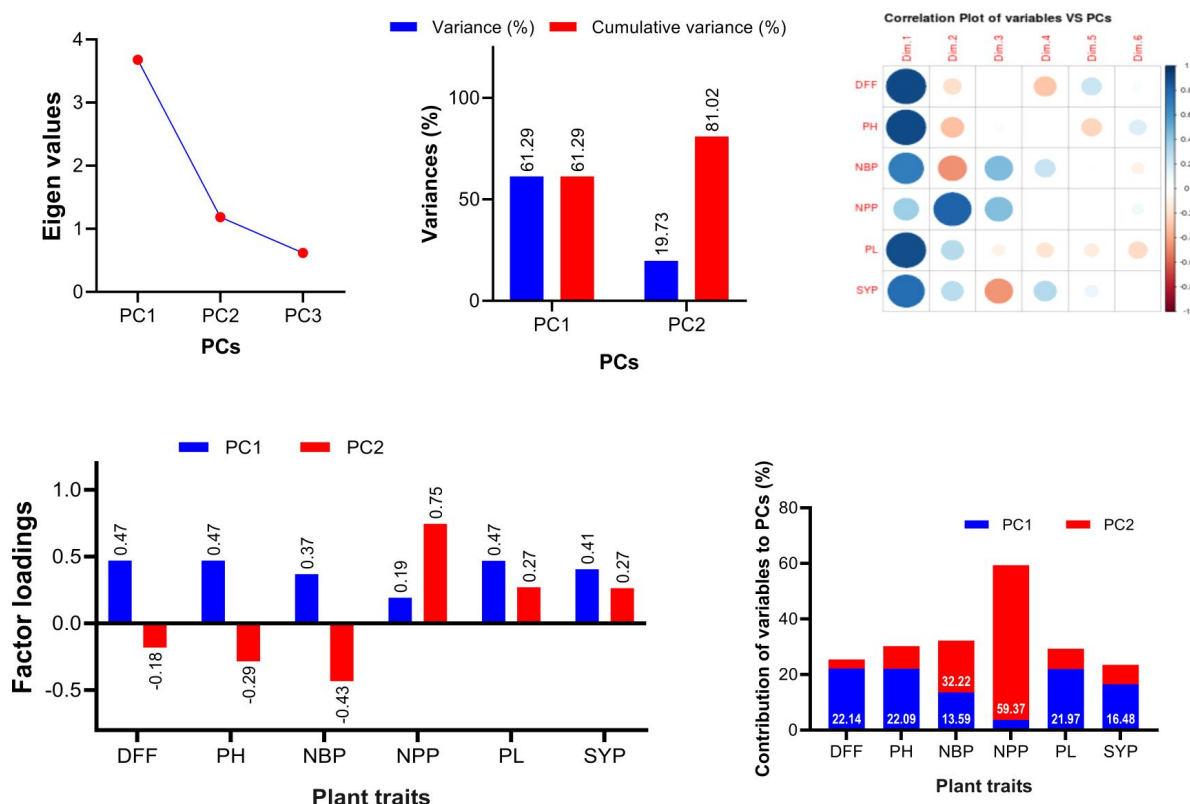
86.22 cm. JFG-2013-02 showcases the highest number of branches plant<sup>-1</sup> at 7.85, while RM-201 has the fewest branches at 4.10. NDM 80 leads in the number of pods plant<sup>-1</sup> with 31.01, whereas UM-393 has the lowest count at 24.53. MDM 120 has the longest pods at 13.14 cm, and AFG-06 records the shortest at 10.74 cm. KFG-1 displays the highest seed yield per plant at 20.54 g, while HM-444 records the lowest at 11.49 g. These extreme values provide valuable insights into the diverse range of traits among fenugreek genotypes, aiding in the assessment and selection of suitable candidates for breeding programs.

### Assessment of diversity and variability through multivariate analyses

#### Exploring variability through principal component analysis

The Fig. 2 presents the outcomes of principal component analysis (PCA) for examined data, offering insights into the distribution of variance among different principal components (PCs). Principal Component 1 (PC1) emerges as the dominant contributor, with an eigenvalue of 3.677, elucidating a substantial 61.291% of the total variance. This suggests that PC1 captures a significant portion of the underlying patterns or structures within the data, signifying its crucial role in explaining the dataset's variability. The cumulative percentage of variance up to PC1 is 61.291%, emphasizing its substantial impact on the overall dataset. Moving to Principal Component 2 (PC2), it possesses an eigenvalue of 1.184, accounting for 19.731% of the total variance. While not as dominant as PC1, PC2 makes a substantial contribution to the genotypes' variability. The cumulative percentage of variance up to PC2 reaches 81.023%, indicating that the combined influence of PC1 and PC2 explains over 81% of the total variance. The subsequent principal components, PC3 to PC6, contribute gradually decreasing percentages of variance, with PC6 finalizing the cumulative percentage at 100%. This comprehensive analysis of principal components provides a nuanced understanding of the dataset's structure and the contribution of each component to its overall variability.

The factor loadings of the principal components (PCs) provide valuable insights into the relationships between plant traits and each PC (Fig. 2), helping to discern the patterns and contributions of individual traits to the overall variability. In PC1, the highest positive loading is observed for days to first flowering (0.471), indicating that this trait strongly influences the variation captured by PC1. Additionally, plant height and pod length exhibit substantial positive loadings, contributing to the overall variability



**Fig. 2 :** Results from PCA explaining the eigen values, variances, correlation between plant traits and PCs, factor loading of plant traits and contribution of plant traits to the PCs.

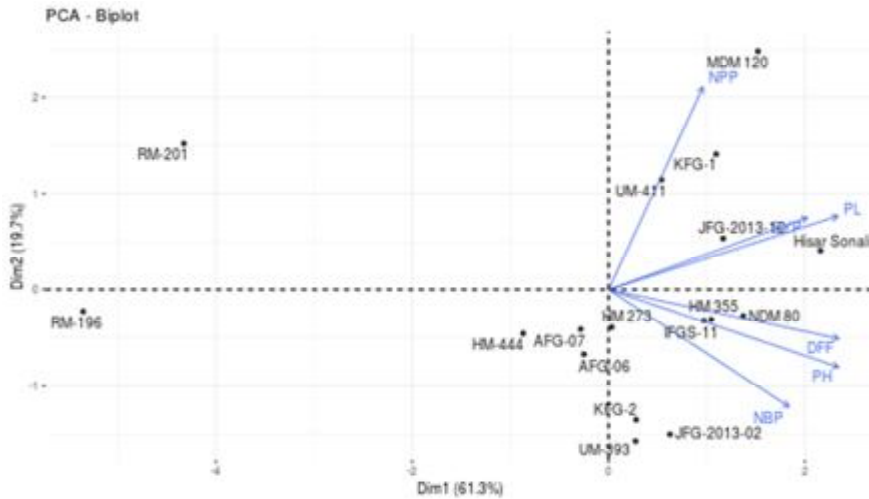
**Table 1 :** Mean performance of fenugreek genotypes evaluated during 3 seasons.

| Genotypes    | Days to first flowering | Plant height (cm) | Number of branches plant <sup>-1</sup> | Number of pods | Pod length (cm) | Seed yield plant <sup>-1</sup> (g) |
|--------------|-------------------------|-------------------|--|----------------|-----------------|------------------------------------|
| RM-196       | 30.78                   | 72.83             | 4.46                                   | 24.56          | 6.57            | 9.54                               |
| RM-201       | 32.33                   | 64.62             | 4.10                                   | 29.45          | 8.60            | 13.19                              |
| HM 273       | 46.22                   | 99.24             | 5.30                                   | 21.99          | 11.47           | 18.15                              |
| HM 355       | 47.44                   | 108.78            | 6.53                                   | 29.08          | 11.68           | 16.47                              |
| NDM 80       | 48.00                   | 103.34            | 7.46                                   | 31.01          | 11.33           | 17.58                              |
| MDM 120      | 49.89                   | 100.41            | 5.31                                   | 41.14          | 13.14           | 17.62                              |
| KFG-1        | 47.00                   | 96.75             | 5.96                                   | 34.40          | 11.47           | 20.54                              |
| KFG-2        | 49.00                   | 105.94            | 5.73                                   | 19.95          | 11.46           | 16.03                              |
| UM-393       | 53.00                   | 102.41            | 6.66                                   | 24.53          | 9.72            | 14.69                              |
| UM-411       | 48.89                   | 92.38             | 5.68                                   | 33.29          | 12.25           | 16.63                              |
| IFGS-11      | 49.11                   | 98.25             | 6.61                                   | 26.67          | 11.74           | 18.06                              |
| AFG-06       | 46.78                   | 98.59             | 6.30                                   | 26.88          | 10.74           | 14.03                              |
| AFG-07       | 46.33                   | 98.63             | 6.38                                   | 29.14          | 9.69            | 15.28                              |
| JFG-2013-16  | 46.44                   | 106.56            | 6.34                                   | 32.82          | 12.51           | 16.35                              |
| JFG-2013-02  | 46.22                   | 104.51            | 7.85                                   | 26.16          | 10.53           | 15.22                              |
| HM-444       | 48.56                   | 86.22             | 6.56                                   | 29.53          | 10.52           | 11.49                              |
| Hisar Sonali | 49.78                   | 106.93            | 7.45                                   | 35.40          | 12.91           | 16.72                              |

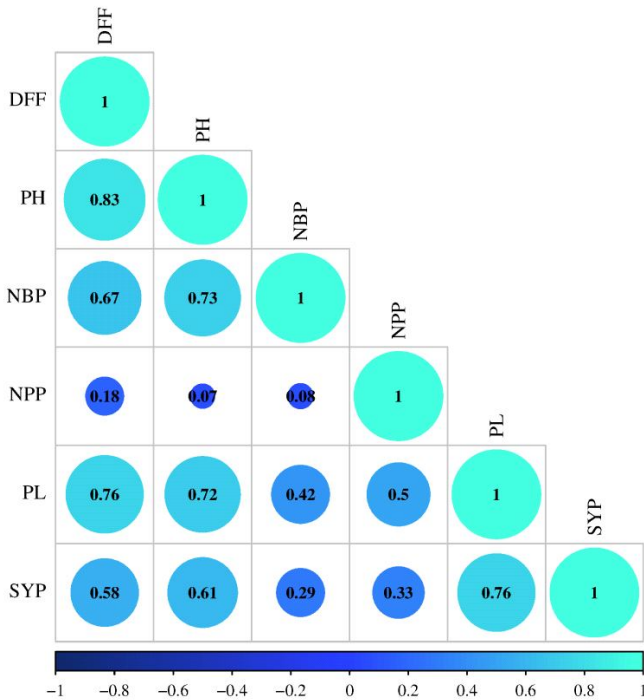
explained by PC1. This indicates that these traits show wide variations than the other traits namely number of pods plant<sup>-1</sup> and number of branches plant<sup>-1</sup>. In PC2, number of pods plant<sup>-1</sup> exhibits the highest positive loading (0.746), indicating a strong association between this trait and the variability captured by PC2. Moreover, the plant height and seed yield plant<sup>-1</sup> also display notable positive loadings, contributing significantly to the variability explained by PC2. In PC3, the number of branches plant<sup>-1</sup>

and seed yield plant<sup>-1</sup> stand out with positive loadings of 0.576 and 0.575, respectively, suggesting their influential roles in shaping the patterns captured by PC3. Conversely, number of pods plant<sup>-1</sup> displays a substantial negative loading in PC3. PC4 is characterized by a strong positive loading for days to first flowering (0.561), while PC5 and PC6 show distinct patterns with several traits contributing both positively and negatively to their respective variabilities. Overall, these factor loadings provide an understanding of how each plant trait contributes to the principal components' variations, aiding in the interpretation of the complex relationships within the dataset.

Fig. 2 represents the percentage contribution of each plant trait to the respective principal components (PCs). In PC1, days to first flowering, plant height, pod length, and seed yield plant<sup>-1</sup> exhibit substantial contributions, with days to first flowering and plant height playing particularly significant roles in explaining the primary source of variation, as they collectively contribute to 61.291% of the variance in PC1. Moving to PC2, number of pods



**Fig. 3 :** PCA biplot illustrating the distribution of variables and genotypes and their relations.



**Fig. 4 :** Correlation plot showing the correlation among the variables and correlation coefficients.

plant<sup>-1</sup> stands out as the dominant contributor, making a substantial contribution of 55.643%. This underscores that PC2 primarily represents variability associated with the number of pods plant<sup>-1</sup>, with additional positive contributions from plant height and seed yield plant<sup>-1</sup>. In PC3, number of branches plant<sup>-1</sup> emerges as a central contributor, making a substantial contribution of 33.199%. This highlights that the variability explained by PC3 is strongly influenced by the number of branches plant<sup>-1</sup>, with additional positive contributions from number of pods plant<sup>-1</sup> and seed yield plant<sup>-1</sup>. These findings indicates how each plant trait contributes to the distinct patterns

observed in the principal components, offering valuable information for discerning the key factors driving variability in the dataset.

The PCA biplot (Fig. 3) illustrates distinct genotype distributions in the positive regime, with MDM 120, RM 201, KFG-1 and UM-411 exhibiting heightened variability compared to other genotypes. Notably, MDM 120 demonstrates exceptional variability, suggesting its potential for promising performance in future breeding endeavors due to its extensive genetic divergence. In contrast, Hisar Sonali and JFG-2013-18 display limited variability, specifically in pod length and

seed yield plant<sup>-1</sup>. Genotypes HM 335, NDM 80, IFGS-11, HM-273, KFG-2, JFG-2013-02, and UM-393 are closely related, indicating a narrow range for days to first flowering, plant height and number of branches plant<sup>-1</sup>. This discernment suggests caution in utilizing these lines collectively to exploit the benefits of heterosis, given their close genetic relationship.

The correlation between various plant traits and Pearson correlation coefficients are depicted in Fig. 4. Seed yield plant<sup>-1</sup> exhibits positive correlations with all the observed characters, with the most significant correlation observed with pod length, ensuing plant height and days to 50% flowering. Pod length demonstrates a stronger correlation with both days to 50% flowering and plant height. Additionally, plant height as well as the days to 50% flowering exhibit a robust mutual correlation. Conversely, the number of branches plant<sup>-1</sup> and number of pods plant<sup>-1</sup> display weaker correlations with all the aforementioned traits that positively correlate with seed yield plant<sup>-1</sup>. Consequently, traits such as early maturity, greater pod length (potentially indicating a higher seed number per pod) and increased plant height exhibit enhanced seed yield. Hence, these characteristics warrant attention in fenugreek improvement initiatives aimed at augmenting seed yield.

Choudhary *et al.* (2022) employed principal component analysis to investigate the variability in plant characters *viz.*, days to 50% flowering, days to maturity, plant height, number of branches plant<sup>-1</sup>, pod length, number of seeds pod<sup>-1</sup>, seed test weight, and seed yield plant<sup>-1</sup>. Of these traits, seed yield plant<sup>-1</sup> exhibited the highest contribution (22.78%) to the total variability, pursued by the number of pods per plant (20.39%) and

**Table 2 :** AMMI ANOVA for fenugreek genotypes evaluated during 3 seasons.

| Source of variation    | Degrees of freedom | Mean squares            |                   |  |                                    |                 |                                    |
|------------------------|--------------------|-------------------------|-------------------|--|------------------------------------|-----------------|------------------------------------|
|                        |                    | Days to first flowering | Plant height (cm) | Number of branches plant <sup>-1</sup> | Number of pods plant <sup>-1</sup> | Pod length (cm) | Seed yield plant <sup>-1</sup> (g) |
| Environment            | 2                  | 577.69*                 | 11233.49*         | 42.08*                                 | 1232.16*                           | 8.70*           | 753.97*                            |
| Replication            | 6                  | 2.14                    | 214.20*           | 0.14                                   | 8.11*                              | 3.05*           | 7.59*                              |
| Genotype               | 16                 | 302.46*                 | 1309.53*          | 9.07*                                  | 249.38*                            | 24.22*          | 62.62*                             |
| Genotype × Environment | 32                 | 277.41*                 | 1118.23*          | 5.12*                                  | 383.79*                            | 21.71*          | 86.13*                             |
| IPCA1                  | 17                 | 508.10                  | 1963.38           | 9.08                                   | 650.46                             | 37.32           | 124.94                             |
| IPCA2                  | 15                 | 15.95                   | 160.39            | 0.64                                   | 81.55                              | 4.02            | 42.15                              |
| Residuals              | 96                 | 4.46                    | 54.94             | 0.27                                   | 5.31                               | 1.91            | 4.15                               |
| CV (%)                 |                    | 4.57                    | 7.65              | 8.38                                   | 7.90                               | 12.61           | 12.95                              |

\*Significant at  $P_{0.005}$

**Table 3 :** Environmental means and scores.

| Environments | Mean  | IPCAe(1) | IPCAe(2) |
|--------------|-------|----------|----------|
| 2018-19      | 11.33 | -1.51    | -2.90    |
| 2019-20      | 18.38 | -2.65    | 2.42     |
| 2020-21      | 17.51 | 4.16     | 0.49     |

plant height (16.13%). The cumulative contribution of the first three principal components was 66.20% of the total variability. In other experiment, the PCA revealed that genotypes, specifically NS 2006-1, UM 353 and JFg 244, demonstrated superiority and genetic diversity. These genotypes hold promise for seed yield and other yield-related traits such as days to 50% flowering, number of seeds per pod, test weight, and harvest index (Maloo *et al.*, 2020).

### Investigating divergence of the genotypes through clustering analyses

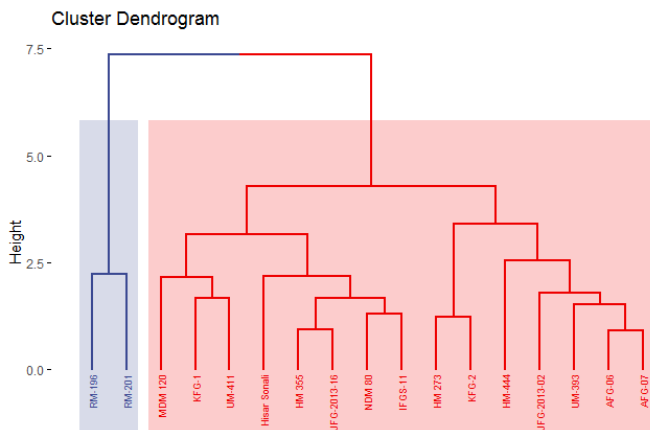
The exploration of genetic divergence among 17 genotypes employed a hierarchical clustering approach utilizing the “complete linkage” and “UPGMA” methods, incorporating Euclidean distances. As depicted in Fig. 5, the “complete linkage” method yielded a cluster pattern dividing the 17 genotypes into two clusters. Specifically, Cluster I encompasses RM-196 and RM-201, while Cluster II comprises the remaining 15 genotypes. Notably, this clustering outcome aligns with the findings of the PCA biplot analysis, as illustrated in Fig. 3. Nonetheless, UPGMA clustering organizes the 17 genotypes into three distinct clusters. Cluster I exclusively contains RM-201, while Cluster II encompasses UM-411, MDM-120, KFG-1, IFGS-11, MDM 80, JFG-2013-02, HM 335 and Hisar

Sonali. Cluster III includes HM 273, KFG-2, HM-444, JFG-2013-02, UM 393, AFG-06, and AFG-07 (Fig. 6). Remarkably, the outcomes of both clustering methods align with the PCA biplot analysis, emphasizing the extensive diversity inherent within the genotype RM-201, as illustrated in Figs. 3, 5 and 6.

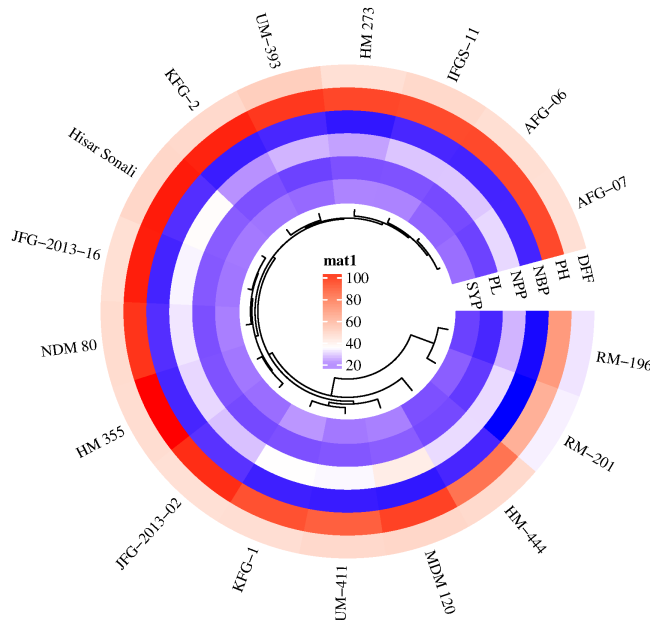
Choudhary *et al.* (2017) conducted an investigation into the genetic divergence of 40 genotypes from Rajasthan, India, utilizing hierarchical clustering analysis. The analysis resulted in the formation of five clusters, with clusters 2 and 5 exhibiting the greatest distance. This observation suggests that genotypes within these clusters can serve as distinct parents for hybridization and subsequent selection. The identified divergence also presents an opportunity for the emergence of transgressive segregants in the progenies of these distinct parents, contributing valuable variability for potential cultivar improvement. In a parallel study, Maloo *et al.* (2020) evaluated the genetic diversity of fenugreek genotypes from Rajasthan, India, employing both morphological observations and molecular markers. Their research involved 20 genotypes and the morphological traits, including plant height, number of pods plant<sup>-1</sup>, test weight, biological yield plot<sup>-1</sup> and harvest index, were meticulously observed and recorded. The subsequent clustering analysis, stemming from the morphological characterization, grouped the 20 genotypes into 2 clusters. This analysis highlighted that genotypes such as NS 2006-1, UM 353 and JFg 244 exhibited superiority and genetic diversity, suggesting their promise for seed yield.

### Assessment of stability of the genotypes

Fig. 1 illustrates the geographical position of Jabalpur district in Madhya Pradesh, India. The comprehensive



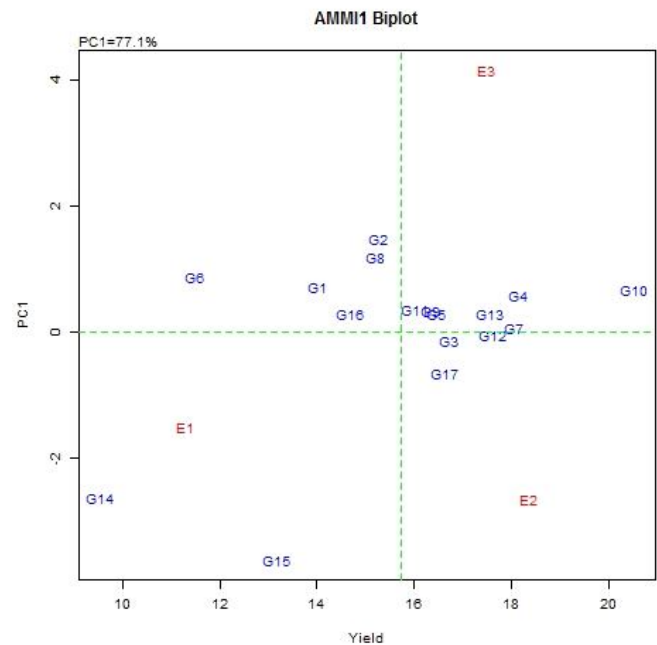
**Fig. 5 :** Dendrogram from hierarchical clustering analysis using the “complete linkage” method.



**Fig. 6 :** Dendrogram from UPGMA clustering analysis.

examination of various plant traits across 17 genotypes revealed a notable impact of the environment, specifically the season, as indicated by the significant effect presented in Table 1. The amalgamated analysis of variance (ANOVA) along with AMMI ANOVA is detailed in Table 2. The mean squares of environmental interaction for all examined plant traits were proven to be statistically significant at  $p < 0.05$ . This signifies that each characteristic under investigation is subject to influence by the prevailing environmental conditions.

Upon examination of the information presented in Table 3, an analysis of the average yield and IPCA scores across three distinct environments is evident. Notably, the 2019-20 seasons exhibited the highest mean yield, succeeded by the 2020-21 season, which, however, displayed the highest IPCA scores. Across all years, consistent findings revealed that IPCA 2 scores were

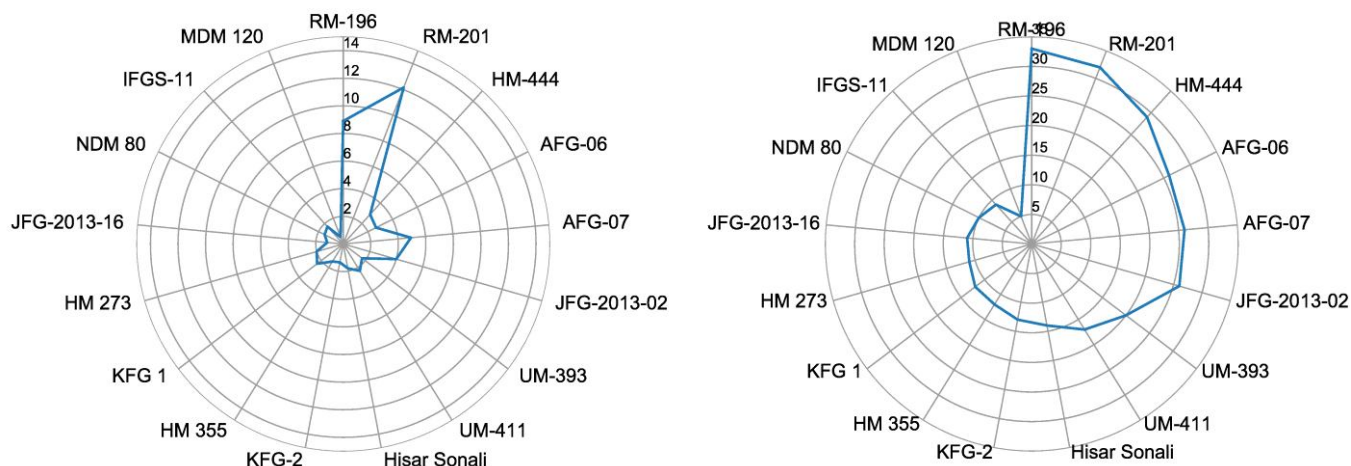


**Fig. 7 :** AMMI biplots for yield of fenugreek genotypes. G1 - RM 196; G2 - RM 201; G3 - HM 273; G4 - HM 355; G5 - NDM 80; G6 - MDM 120; G7 - KFG 1; G8 - KFG 2; G9 - UM 393; G10 - UM 411; G11 - IFGS 11; G12 - AFG 06; G13 - AFG 07; G14 - JFG-2013-16; G15 - JFG-2013-02; G16 - HM 444; G17 - Hisar Sonali.

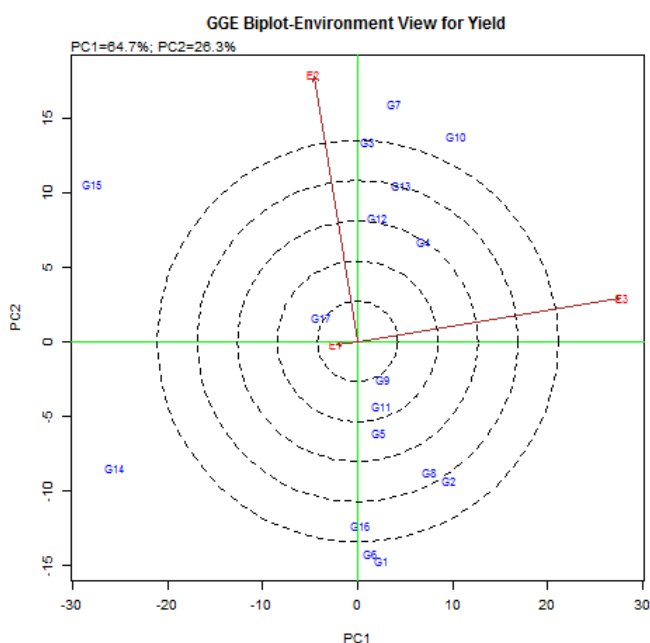
lower than IPCA 1 scores. Specifically, the 2020-21 seasons demonstrated the highest IPCA scores, with a ratio of IPCA 1 to IPCA 2 as 4.16:0.49. This suggests that genotypes cultivated during this particular year exhibited the highest yields, showing casing their genuine genetic potential with minimal environmental influence. A heightened IPCA 1 score further indicates that the environmental productivity during the 2020-21 seasons was notably elevated.

Fig. 7 illustrates the distribution of fenugreek genotypes based on yield characteristics. The AMMI analysis biplots visually represent the magnitudes of genotypes and environmental contributions to the interaction. The graph highlights the stability of genotypes at specific locations, suggesting superior yield performance under favorable conditions. Genotypes positioned closer to the zero axis (IPCA1) indicate smaller interactions, with MDM 120, IFGS-11, Hisar Sonali, NDM 80, HM 355 and JFG-2013-16 exhibiting low IPCA1 scores (Table 3) and being considered the most stable based on this model. Additionally, these genotypes are identified as high yielders (Table 1). In contrast, genotypes located farther from the zero axis are deemed unstable.

The AMMI analysis discerns primary genotypic and environmental effects, scrutinizes main components, and further delineates Genotype-Environment Interaction



**Fig. 8 :** **A-** AMMI stability value of the genotypes; **B-** Yield stability index of the genotypes.



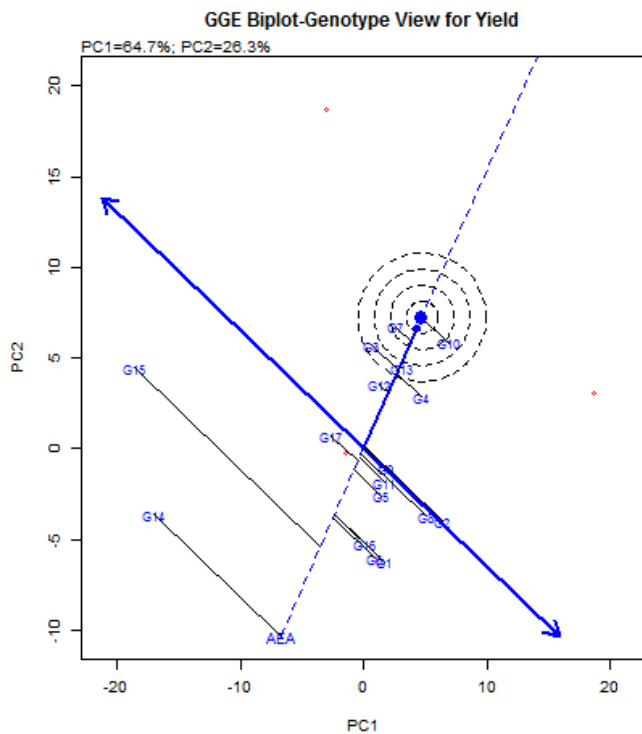
**Fig. 9 :** Biplot of fenugreek genotypes against average yields in three environments. G1 - RM 196; G2 - RM 201; G3 - HM 273; G4 - HM 335; G5 - NDM 80; G6 - MDM 120; G7 - KFG 1; G8 - KFG 2; G9 - UM 393; G10 - UM 411; G11 - IFGS 11; G12 - AFG 06; G13 - AFG 07; G14 - JFG-2013-16; G15 - JFG-2013-02; G16 - HM 444; G17 - Hisar Sonali.

(IPCA) (Roostaei *et al.*, 2014). Genotypes exhibiting specific adaptability manifest distinct responses based on their environment, implying that these genotypes attain higher yields in environments to which they are adapted, but not necessarily in others. Such genotypes can realize their genetic potential for high yields when cultivated in conducive environments. Stability analysis endeavors to select genotypes that demonstrate stability and specificity to certain environments, facilitating the identification of interactions between genotypes and their respective environments.

Fig. 7 does not provide rankings for the stability of the studied genotypes; rather, it solely presents the visual distribution of genotypes. Another method to assess genotype stability is the AMMI Stability Value (ASV), which identifies stable genotypes based on the smallest value range (Purchase *et al.*, 2000). According to the results in Table 3, the genotypes are ranked based on their ASV. Notably, the genotype MDM 120, with ASV values less than 1 (055), is identified as the most stable genotype (Fig. 8). Table 4 provides details on the average yield for each genotype, along with IPCA1, IPCA2 values, AMMI Stability Value (ASV) and yield stability index (YSI), as recommended by Zobel *et al.* (1998) and Purchase *et al.* (2000). Notably, KFG-1 exhibited the highest mean grain yield among genotypes across the three seasons, reaching 20.54 q ha<sup>-1</sup>, followed by HM-273 (18.15 q ha<sup>-1</sup>) and IFGS-11 (18.06 q ha<sup>-1</sup>), while RM-196 recorded the lowest yield at 9.54 q ha<sup>-1</sup>. Genotypes with lower ASV values were considered stable, and breeders favored those with seed yields surpassing the mean grand yield (Fig. 8). The outcomes of the AMMI analysis indicated relatively low positive and negative values for IPCA1 and IPCA2, suggesting a greater influence of environmental factors on yield compared to genetic factors. This aligns with findings reported by de Oliveira *et al.* (2014) in the context of passion fruits.

The graphical representation of average yield biplots for fenugreek genotypes (environment view) is depicted in Fig. 9. The length of the genotype vector illustrates the variation of a genotype from the average genotype, considering the effects of genotype, phenotype and their interaction. Genotypes positioned near the biplots axis center exert minimal influence on genotype and genotype-environment interaction (GEI). Stable genotypes are characterized by smaller vector distances from the center (Yan *et al.*, 2007). Notably, IFGS-11, KFG 1 and NDM

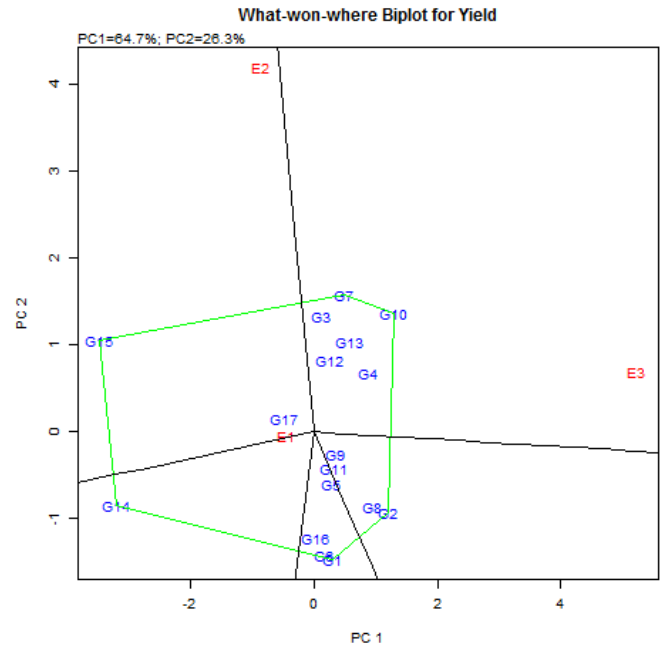




**Fig. 10 :** Biplot of average yield and stability of fenugreek genotypes in three environments. G1- RM 196; G2 - RM 201; G3- HM 273; G4 - HM 355; G5 - NDM 80; G6 - MDM 120; G7 - KFG 1; G8 - KFG 2; G9 - UM 393; G10 - UM 411; G11 - IFGS 11; G12 - AFG 06; G13 - AFG 07; G14 - JFG-2013-16; G15 - JFG-2013-02; G16 - HM 444; G17 - Hisar Sonali.

80 emerge as the most stable genotypes, while RM-196 and RM-201, exhibiting the longest vectors, are identified as the most unstable genotypes. The unstable genotypes can be considered adapted to specific locations, necessitating development in an appropriate environment. The length of the environmental vector indicates the magnitude of influence from genotypic factors, environment, and GEI. A longer environmental vector signifies higher GEI, with Environment 1 displaying the longest vector and hence the highest GEI. Genotypes KFG-2, JFG-2013-16, and HM 355 are positioned at the periphery of the circle in Figure-9, indicating their performance in marginal environments and adaptation to such conditions. The distance and angle between two genotype vectors reveal the dissimilarity between genotypes; larger vector distances and angles indicate substantial differences, while closely plotted genotypes with smaller angles have similar yield potential (Li and Xu, 2014).

Fig. 10 presents biplots illustrating the average yield and stability of fenugreek genotypes. The X-axis represents the average yield for each genotype, while the Y-axis denotes yield stability. Genotypes positioned



**Fig. 11 :** Which-won-where view of GGE biplots analysis of fenugreek genotypes. G1- RM 196; G2 - RM 201; G3- HM 273; G4 - HM 355; G5 - NDM 80; G6 - MDM 120; G7 - KFG 1; G8 - KFG 2; G9 - UM 393; G10 - UM 411; G11 - IFGS 11; G12 - AFG 06; G13 - AFG 07; G14 - JFG-2013-16; G15 - JFG-2013-02; G16 - HM 444; G17 - Hisar Sonali.

to the right of the Y-axis exhibit greater yields than the overall average, whereas those on the left display lower yields. Genotypes positioned far from the X-axis are considered unstable. Notably, IFGS-11, Hisar Sonali, NDM 80, MDM 120 and UM-411 are identified as the most stable genotypes with higher yields. Additionally, Figure-11 illustrates a which-won-where plot for the fenugreek genotypes under study. Six sectors are identified, with JFG-2013-02, RM-196, RM-201, IFGS-11, KFG 1, and AFG-07 genotypes recognized as the superior for each sector. The environment is parted into three sectors, with UM-411 well adapted to environment 2 and HM 273, NDM 80, Hisar Sonali, and MDM 120 well adapted to environment 3. Notably, no genotype is identified as adapted to environment 1. AMMI models effectively described stable genotypes, while GGE biplots provided additional informative insights by elucidating genotype stability based on low and high yields. Combining the insights from AMMI, ASV, and GGE biplots models, we conclude that MDM 120, IFGS-11 and Hisar Sonali are the stable genotypes among the 17 genotypes under investigation.

Meena *et al.* (2015) conducted an extensive investigation into the stability of 30 Indian fenugreek genotypes, exploring their performance across four distinct

**Table 4 :** Ranking of 17 fenugreek genotypes based on seed yield, AMMI stability value (ASV) and yield stability index (YSI).

| Genotypes    | Average yield | Rank(A) | IPCAG (1) | IPCAG (2) | ASV   | ASV rank (B) | YSI (A+B) | YSI rank |
|--------------|---------------|---------|-----------|-----------|-------|--------------|-----------|----------|
| RM-196       | 9.54          | 17      | -2.64     | -0.66     | 8.90  | 16           | 33        | 1        |
| RM-201       | 13.19         | 15      | -3.62     | 0.16      | 12.15 | 17           | 32        | 2        |
| HM-444       | 11.49         | 16      | 0.86      | 0.18      | 2.90  | 13           | 29        | 3        |
| AFG-06       | 14.03         | 14      | 0.71      | -1.18     | 2.66  | 12           | 26        | 4        |
| AFG-07       | 15.28         | 11      | 1.47      | -0.24     | 4.94  | 15           | 26        | 5        |
| JFG-2013-02  | 15.22         | 12      | 1.19      | -0.33     | 4.00  | 14           | 26        | 6        |
| UM-393       | 14.69         | 13      | 0.30      | -1.39     | 1.71  | 7            | 20        | 7        |
| UM-411       | 16.63         | 7       | -0.66     | -0.64     | 2.29  | 10           | 17        | 8        |
| Hisar Sonali | 16.72         | 6       | -0.13     | 1.74      | 1.79  | 8            | 14        | 9        |
| KFG-2        | 16.03         | 10      | 0.36      | -0.58     | 1.35  | 3            | 13        | 10       |
| HM 355       | 16.47         | 8       | 0.28      | -1.16     | 1.49  | 4            | 12        | 11       |
| KFG 1        | 20.54         | 1       | 0.68      | 0.68      | 2.37  | 11           | 12        | 12       |
| HM 273       | 18.15         | 2       | 0.58      | 0.50      | 2.01  | 9            | 11        | 13       |
| JFG-2013-16  | 16.35         | 9       | 0.32      | -0.45     | 1.17  | 2            | 11        | 14       |
| NDM 80       | 17.58         | 5       | 0.29      | 1.17      | 1.51  | 5            | 10        | 15       |
| IFGS-11      | 18.06         | 3       | 0.06      | 1.69      | 1.71  | 6            | 9         | 16       |
| MDM 120      | 17.62         | 4       | -0.05     | 0.52      | 0.55  | 1            | 5         | 17       |

IPCA(1) score - interaction principal component axis one score; IPCA(2) score - interaction principal component axis two score; ASV - AMMI stability value; YSI - yield stability index.

environments created through variations in sowing dates and irrigation regimes in Rajasthan, India. The utilization of the AMMI analysis allowed for a nuanced delineation of genotype and environment effects. The findings of the study highlighted UM-137, UM-128, UM-322 and RMt-1 as exhibiting remarkable stability in seed yield. In a parallel study, Yadav *et al.* (2020) undertook a comprehensive experiment aimed at computing stability using the AMMI model in Haryana, India. Their research involved the evaluation of 60 elite genotypes under six diverse environmental conditions. The findings of this report confirmed a significant level of diversity within the genotypes, with noteworthy stability observed in seed yield across all environments for genotypes JFg 240, HM 346, HM 258, NDM 20, NDM 13 and RM 28.

### Conclusion and Summary

Although, India leads in fenugreek production, numerous varieties have become obsolete due to performance issues, decreased genetic diversity and stability reduction. This research delves into evaluating diversity and variability coupled with Genotype by Environment (G×E) interaction exploration, in fenugreek genotypes cultivated in the Kymore Plateau and Satpura Hill Agroclimatic region of Madhya Pradesh, India. IN PCA, PC1 dominates, explaining 61.291% variance, with highest loadings for flowering time, plant height and pod

length. Genotype distributions in PCA biplot reveal heightened variability in MDM 120, RM 201, KFG-1, and UM-411, while Hisar Sonali and JFG-2013-18 display limited variability. Hierarchical clustering forms two clusters (RM-196, RM-201; others), while UPGMA forms three clusters aligning with PCA biplot. AMMI and GGE analyses emphasize significant genotype, environment, and interaction impact on traits ( $P < 0.05$ ). Stable genotypes identified include MDM 120, IFGS-11, and Hisar Sonali.

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### Contribution

**RN** - conceptualization, supervision, investigation, resources, writing - review and editing; **JJ** - investigation, data curation, formal analysis, writing - original draft, review, editing, visualization; **AS, PU, R**- investigation, data curation, formal analysis.

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## 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.

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