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## Identification of superior mutants in fenugreek through genotype-by-yield trait (GYT) graphical analysis

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### Abstract

The high yielding fenugreek mutants from M<sub>2</sub> population were selected through the genotype-yield trait biplot (GYT biplot). The GYT biplot analysis for both green herbage yield and seed yield indicates that all traits are positively correlated with these yields. Furthermore, green herbage yield and seed yield themselves are positively correlated. Among the 44 mutants analysed, M2-149, M2-181, M2-116, and M2-90 were identified as the top performers in terms of both green herbage and seed yield. These mutants should receive special attention in advanced generations to uncover significant findings on yield improvement and to explore other valuable traits. A distinctive aspect of this study is that it demonstrated the GYT biplot method as a comprehensive and effective tool for evaluating and selecting high-yielding mutants in the M<sub>2</sub> generation for fenugreek improvement.

**Keywords:** Fenugreek, mutants selection, GYT biplot analysis

### 1. Introduction

Mutagenesis involves the alteration of an organism's genetic material due to exposure to mutagens, leading to mutations (Oladosu *et al.*, 2016) [13]. These mutations can result in structural changes in proteins or a reduction or complete loss of protein expression. Such changes can be particularly detrimental to cells or organisms because a DNA sequence alteration impacts all copies of the corresponding protein (Drummond and Wike, 2008) [5]. Mutations can be artificially induced using mutagenic agents (Lamo *et al.*, 2017) [11]. These agents, which include both chemical and physical mutagens, increase the frequency of mutations. Induced mutations arise when genes are exposed to mutagens or other environmental factors. Chemical mutagens typically cause point mutations, while ionizing radiation leads to extensive chromosomal abnormalities (Auerback, 2013) [1]. Mutation breeding has been instrumental in developing crop varieties with novel characteristics and remains a valuable tool for breeders due to its efficiency and cost-effectiveness in generating new alleles and phenotypes. Fenugreek (*Trigonella foenum-graecum* L.), a versatile crop prized for its leaves and seeds used as a vegetable and spice, respectively, and known for its medicinal properties (Basch *et al.*, 2003) [2], has limited genetic variability in its cultivated forms. Consequently, numerous efforts have been made to enhance its genetic diversity through mutation breeding.

An ideal cultivar must exhibit superior performance across multiple target traits (breeding objectives). The challenge lies in the often unfavourable associations between these traits, where improvement in one trait can lead to a decline in another. To address this issue, two strategies have been proposed and utilized either independently or jointly: independent culling and index selection (Simmonds and Smartt, 1999; Yan and Frégeau-Reid, 2008) [20, 1]. Independent culling involves discarding a genotype if its performance for a specific trait falls below a set minimum threshold, regardless of its performance in other traits. Index selection, on the other hand, ranks genotypes based on an index, which is a linear combination of the target traits. The challenge with these methods is their high subjectivity, as breeders or researchers must assign weights for each trait in index selection and set truncation points for each trait in independent culling. These weights and truncation points can vary between researchers and even for the same researcher over time, potentially leading to significantly

different selection outcomes.

To address these limitations, this paper proposes the genotype by yield trait (GYT) biplot approach for evaluating genotypes across multiple traits. This method is based on the following principles:

- Yield is considered the most critical trait, with other traits being important only in combination with high yield.
- The superiority of a genotype should be assessed based on its performance in combining yield with other target traits, rather than its performance in individual traits.

In this approach, the genotype by trait (GT) two-way table from variety trials is transformed into a genotype by yield trait (GYT) two-way table, where each column represents the combination of yield and another trait. This GYT table is then visualized using a GYT biplot. The average tester coordination (ATC) view of the GYT biplot (Yan, 2001) [21] is employed to rank genotypes based on their overall superiority across yield-trait combinations and to display their trait profiles (i.e., strengths and weaknesses). This serves as the foundation for genotype evaluation and recommendation.

A potential limitation of the GT biplot method is its inability to explain most of the variation in large datasets, which may result in an inadequate display of data patterns, especially when main effects are small and interactions are complex. Additionally, the GT biplot method cannot differentiate the influence of all traits on yield combinations. The GYT biplot method was recently developed to address these shortcomings. The GYT biplot method has been introduced as an effective and comprehensive approach that graphically highlights the strengths and weaknesses of each genotype and provides a superiority index (SI) for evaluating genotypes based on the combination of all traits with yield (Yan and Frégeau-Reid, 2018) [22]. This method has been utilized to evaluate and select genotypes based on multiple traits combined with seed yield in various environments, including durum wheat (Kendal, 2019) [10], sesame (Boureima and Abdoua, 2019) [3], barley (Hudzenko *et al.*, 2021) [8], and rapeseed (Gholizadeh *et al.*, 2022) [7]. Notably, prior to this study, the GYT biplot method had not been applied to assess the relationships between agronomic characteristics or to select mutants based on multiple traits in fenugreek.

## 2. Materials and Methods

In the M<sub>2</sub> generation, 2557 plants were maintained that were induced by the treatment of mutagens, γ 60 Gy + EMS 0.30%. The mutagenic doses were fixed based on the earlier reports (Sikder *et al.*, 2017; Jyothsna *et al.*, 2022) [19, 9]. The 44 mutants that had higher yields than the parent cv. RMt-1 were selected and subjected to GYT biplot analysis to explore the relationship among the plant traits and mutants, in response with the yield. The plant characters analysed were days to first flowering (DFF), days to maturity (DM), plant height at flowering (PHF), plant height at harvest (PHH), number of primary branches (NPB), number of secondary branches (NSB), leaves plant<sup>-1</sup> (LP), pods plant<sup>-1</sup> (PP), pod length (PL), seeds pod<sup>-1</sup> (SP), thousand seed weight (TSW), chlorophyll content index (CCI), green herbage yield (GHY) and seed yield (SY). The statistical analysis was conducted using the package 'metan' with the

function 'gytb()' in RStudio (Olivoto and Lúcio, 2020; RStudio Team, 2022) [14, 15].

## 3. Results and Discussion

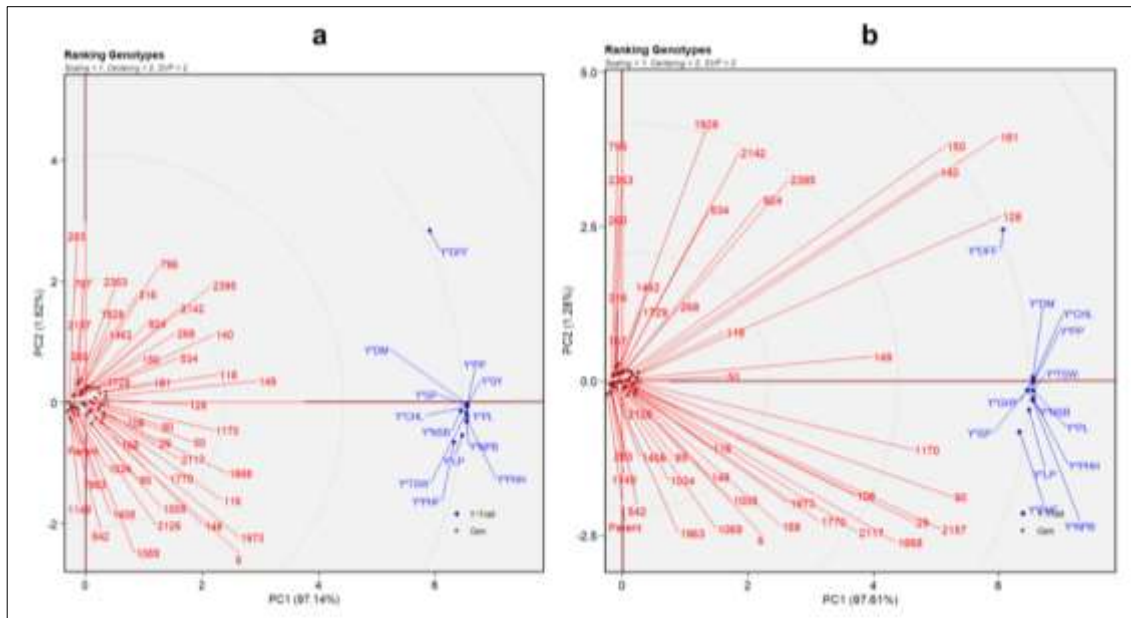
### 3.1. GYT biplot analysis based on the green herbage yield

Fig. 1a shows the GYT biplot based on the green herbage yield in response to other plant traits for the mutant under evaluation. The parameters with their effects on yield, namely the days to maturity, seed yield, thousand seed weight, pods plant<sup>-1</sup>, seeds pod<sup>-1</sup>, number of primary and secondary branches, chlorophyll content index, pod length, leaves plant<sup>-1</sup>, plant height at flowering and harvest were too closely related with each other, when compared to days to first flowering. The mutants namely M2-90, M2-50, M2-1668, M2-149, M2-148, M2-128 showed higher association with the days to first flowering. Since M2-181, M2-118, M2-149 are the top yielders and they hint at the days to first flowering, it is interpreted that the days to first flowering is a major component driving the green herbage yield. Fig. 2a shows the polygon illustrating the which-won-where/what view of the mutants under study. The mutants placed on the vertex of the polygon such M2-767, M2-1928, M2-181, M2-149, M2-283, M2-29, M2-116, M2-168, M2-85, M2-1149, M2-562 and the parent cultivar RMt-1 showed maximum values for the traits under which their corresponding zones fall for the traits. The mutants, M2-149 and M2-181 showed maximum values for days to first flowering, chlorophyll content index, pods plant<sup>-1</sup> and seed yield, indicating the role of these traits on the yield. Mutants M2-1130 and M2-90 showed higher scores for thousand seed weight, plant height at harvest, number of secondary branches plant<sup>-1</sup> and leaves plant<sup>-1</sup>. Fig. 3a shows the rank plots of genotypes based on their overall performance, since all the parameters studied are positively correlated with the yield. The mutants are ranked based on the superiority index (SI) and the most favourable mutants are M2-181 > M2-149 > M2-118 > M2-116 > M2-90. The mutants *viz.*, M2-1449, M2-1963, M2-542 were among the last, with performance nearer to that of the parent cv. RMt-1. Selecting and forwarding the superior mutants would be indeed beneficial but discarding or ignoring the least performers at this stage of mutation breeding is not recommendable. To explore the potentials of these lines, they were forwarded to the M<sub>3</sub> generation.

### 3.2. GYT biplot analysis based on the seed yield

The GYT biplot for seed yield is presented in the Fig. 1b. All the traits were found to positively correlated with seed yield, showing a similar trend that of green herbage yield. This reveals that the green herbage yield is also positively correlated with the seed yield, therefore a plant giving higher green herbage yield can be expected to give superior seed yield as well. Similar to that of GYT biplot based on green herbage yield, the mutants M2-149, M2-181, M2-118 and M2-128 showed higher inclination towards days to first flowering than to other traits, reaffirming that the days to first flowering is highly correlated with the seed yield. The polygon in the which-won-where/what view (Fig. 2b) shows the superior mutants and the traits underlying their superiority. The mutant M2-181 showed superiority for most the traits favouring the seed yield namely days to first flowering and maturity, chlorophyll content index, pods plant<sup>-1</sup>, seeds pod<sup>-1</sup> and pod length, revealing their importance in yield improvement. Other mutants namely





**Fig 3:** Rank plot of genotypes based on superiority indices upon green herbage yield (a) and seed yield (b)

#### 4. Summary and future prospects

The GYT biplot analysis carried out for both green herbage yield and seed yield shows that all the traits are positively correlated with both the green herbage and seed yields. Indeed, green herbage yield and seed yield were also positively correlated. The mutants M2-149, M2-181, M2-116 and M2-90 were the best among the 44 mutants in terms of both green herbage yield and seed yield. Special focus on these mutants in the advanced generations are warranted to bring out intriguing findings on yield improvement as well as exploration on other useful traits. The unique feature of this study is that it showed that GYT biplot method can be used as a comprehensive and effective method for the evaluation and selection of high-yielding mutants in M<sub>2</sub> generation for fenugreek improvement.

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