IDENTIFICATION OF DROUGHT TOLERANT FENUGREEK MUTANTS AT SEEDLING STAGE THROUGH MULTI-TRAIT GENOTYPE-IDEOTYPE DISTANCE INDEX (MGIDI)

Abstract

The top-performing mutants from the M_2 generation, induced by a combination of γ radiation and EMS treatment, underwent screening for drought tolerance at the seedling stage in the M_3 generation under controlled laboratory conditions. This investigation aimed to assess their potential for drought tolerance. The top 15 high-yielding mutants were assessed under both normal (control) and induced drought conditions (10% PEG solution). Utilizing the MGIDI method, the evaluation provided valuable insights into selection traits and identified mutants with potential drought tolerance. Under normal conditions, mutants M3-181 and M3-149 exhibited superior performance in seedling growth traits, with M3-149 being the top performer under induced drought conditions, followed by M3-181. Their proximity to the red-cutline suggests promising potential that warrants further investigation. Furthermore, these findings indicate that mutant lines displayed superior multi-trait performance compared to the parent cultivar. Specifically, mutants M3-181, M3-149, and M3-29 ranked higher than the parent cultivar under normal conditions, while mutants M3-149, M3-181, and M3-168 surpassed the parent cultivar RMt-1 under induced drought conditions. This underscores the effectiveness of mutation breeding, particularly through the induction of mutations using γ radiation and EMS, as an efficient strategy not only for generating genetic diversity but also for producing variants superior to the parent cultivar. To the best of our knowledge, this is first report made on mutant selection in fenugreek for drought tolerance at seedling stage, using the MGIDI approach.

Keywords: Fenugreek, M_3 mutants, Drought screening, MGIDI, Multi-trait selection

1. Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is a versatile crop valued for its foliage and seeds, used as a leafy vegetable and spice, respectively, and renowned for its medicinal properties (Basch *et al*., 2003). Due to the limited genetic variability in cultivated fenugreek germplasm, numerous efforts have been undertaken to enhance variability through mutation breeding. Freisleben and Lein coined the term "mutation breeding" to describe the intentional induction and development of mutant lines for agricultural enhancement (Lamo *et al*., 2017). Mutagenesis refers to the process of altering an organism's genetic information in a stable manner, which can occur naturally due to errors in DNA repair. Specifically, mutagenesis is the act of generating mutations, which leads to the creation of new gene variants (alleles). (Kawall, 2019). In many food crops, mutation breeding and plant mutagenesis are crucial for increasing genetic diversity to achieve desired traits. Induced mutagenesis is one of the most effective tools for identifying key regulatory genes and molecular pathways (Hall and Richards, 2013).

Globally, agricultural productivity is impacted by abiotic and biotic stresses, with abiotic stresses particularly hindering plant growth and productivity (Dutta and Bera, 2008). Among these, drought stress is a major abiotic stressor that restricts crop productivity due to limited water sources and irregular rainfall during the crop growth season (De *et al*., 2005). Drought-induced damage in plants primarily starts with the disruption of osmotic balance, along with associated metabolic and physiological disorders (Hasanuzzaman and Fujita, 2011). Climate change is expected to increase the frequency and severity of droughts in the future, posing a significant threat to global crop yields (Cutforth *et al*., 2007). Screening desirable genotypes is essential for successful breeding to develop elite lines with drought tolerance (Feller, 2006). Water potential can be managed using high molecular weight osmotic substances like polyethylene glycol (PEG 6000), commonly used to control water potential in seed germination studies (Hardegree and Emmerich, 1990). Osmotic adjustment is a major response exhibited by crop plants under water deficit conditions (Hsiao, 1973). Identifying genotypes that perform well under lower water potential helps in selecting plant genetic resources suitable for growing under water deficit conditions (Kaur *et al*., 2011). The objective of this investigation is to evaluate fenugreek mutants of M_3 generation for their ability to tolerate moisture stress during germination and seedling stages, which can then be used as genetic material in crop improvement programs to develop climate-smart fenugreek cultivars.

Genetic gain is crucial in plant breeding, guiding the direction of breeding programs. Focusing on a few traits for selection is insufficient, as it overlooks potential improvements in other important traits. Therefore, breeders aim to combine various desirable traits into a single genotype to boost overall performance. In crop improvement initiatives, the goal is often to develop an ideotype, a genotype that integrates multiple attributes for optimal performance (Donald, 1968). The aim of ideotype design is to enhance crop performance by considering multiple traits in genotype selection simultaneously (Olivoto and Nardino, 2021). Various selection indices have been developed for this purpose, but assigning realistic economic weights to effectively express the economic value of traits remains challenging. This often hinders breeders from identifying the optimal genotypes.

The Smith–Hazel (SH) index, a linear selection index commonly used by breeders for multi-trait selection, relies on phenotypic and genotypic covariance matrices. A vector of economic weights is used to determine the selection of index coefficients, maximizing the correlation between unknown genetic values and phenotypic values (Smith, 1936; Hazel, 1943). However, the SH index involves inverting the phenotypic covariance matrix, which can lead to poorly conditioned matrices and biased index coefficients when traits are highly correlated, a condition known as multicollinearity (Graham, 2003; Olivoto *et al*., 2017). This affects the accuracy of genetic gain estimates. Additionally, if the matrix is not positive definite, the SH index may have an infinite number of solutions, further complicating the process (Graham, 2003; Olivoto *et al*., 2017). Alongside multicollinearity, breeders also struggle with determining realistic economic weights for traits, which is essential for expressing their economic value (Bizari *et al*., 2017). This complexity adds to the challenges in optimizing the SH index for effective crop improvement.

To address these challenges, the multi-trait genotype–ideotype distance index (MGIDI) was introduced by Olivoto and Nardino (2021). This innovative multivariate selection index addresses the shortcomings of traditional indices, especially issues arising from multicollinearity. Unlike conventional methods, the MGIDI considers the inherent correlation among traits and effectively selects all variables in the evaluation process. This approach overcomes the limitations of poorly conditioned matrices and biased index coefficients seen with the SH index, facilitating the selection of superior genotypes. The focus on genotype–ideotype distance in the MGIDI aligns with the ideotype breeding concept, offering a more comprehensive and effective approach to crop improvement. Olivoto and Nardino (2021) highlight that MGIDI application results in significant genetic gain, representing a promising advancement in multivariate selection indices by assessing the strengths and weaknesses of tested genotypes. The main objective of this study is to identify rice accessions with high values for yield traits and early seedling vigor for future genetic improvement initiatives using the MGIDI. The MGIDI was computed according to Olivoto and Nardino (2020) to identify the best performing genotypes based on quantitative traits.

$$MGIDI_i = \left[\sum_{j=1}^{f} \left(\gamma_{ij} - \gamma_j\right)^2\right]^{0.5}$$

2. Materials and methods

The top-15 high yielding mutants from the M_2 generation, that were induced through combinational treatment of physical and chemical mutagens namely ϒ 60 Gy + EMS 0.30%, were selected. The doses of the mutagens were designed based on the previous reports (Sikder et al., 2017; Jyothsna et al., 2022). The seeds of the selected mutants were subjected to artificial drought condition with PEG-6000. Three replications of 10 seeds for each accession were counted and placed on two layers of paper towel (11 × 11 cm) previously moistened with water equal to three times the dry weight of the paper. The ends of the towels were tied with rubber bands and kept in a plastic tray containing 10% Polyethylene Glycol (PEG-6000). Germination percentage was recorded every 7 days. At the end of the 21st day, final germination percentage, germination rate, root length, shoot length, root dry weight, and shoot dry weight were recorded in PEG-6000, compared to those in distilled water. The paper sheets were rolled, placed vertically in a plastic beaker, covered with a polythene bag, and maintained at 25±1°C. The method recommended by Panchalingam (1983) and Babu *et al.* (1985) was used to screen the genotypes for moisture stress tolerance under laboratory conditions. A seed was considered germinated when the emerging radicle reached 1 mm in length. Radicle length, hypocotyl length, and seedling fresh and dry weights were measured as described by Uniyal *et al.* (1998). The promptness index (PI) was obtained by the method suggested by George (1967). The germination stress index (GSI) was calculated using the formula given by Maiti *et al.* (1994). The root length stress indexes (RLSI), vigor index (VI) and tolerance index (TI) were calculated as suggested by Kaur *et al.* (2011).

3. Results and discussion

This section discusses the results obtained from the analysis of MGIDI scores. The evaluation of MGIDI scores for mutants is presented separately for both control and induced-drought conditions.

3.1. Evaluation of mutant lines under control condition

The likelihood ratio tests on the analysed traits, using the BLUP approach, revealed significant genotypic effects across all traits under control conditions. This underscores the importance of genetic factors in driving variations in these traits. In the context of MGIDI, these significant genetic effects are vital as they contribute to the genetic diversity that MGIDI seeks to capture. For the mutants under control conditions, the first three principal components (PCs) had eigenvalues greater than 1 (Table 1). These three PCs accounted for 72.06% of the total variability present in the evaluated mutant lines. The PC1 recordings, representing a linear combination of the original predictor variables, captured the maximum variance in the dataset. Therefore, the selection of PC1 is highly critical for guiding the selection of mutant lines and their future utilization in breeding programs. Based on the eigenvalues of PCA, three factors from the factorial analysis are utilized to explain the total variance. Consequently, only these three factors are considered for MGIDI calculation. This focused approach ensures a targeted and effective investigation.

Table 1. Principal components analysis results for mutants evaluated under control and induced-drought conditions

PCs	**PCA for control condition**			**PCA for PEG-induced drought condition**		
	Eigen values	**Variance (%)**	**Cumulative variance (%)**	**Eigenvalues**	**Variance (%)**	**Cumulative variance (%)**
PC1	3.531	44.149	44.149	4.758	43.260	43.260
PC2	1.439	17.993	62.142	2.799	25.446	68.706
PC3	1.194	14.929	77.072	2.288	20.806	89.513
PC4	0.904	11.305	88.377	0.566	5.1530	94.666
PC5	0.509	6.3660	94.743	0.234	2.1362	96.802

The factor analysis scores and the selection differential parameters for the seedling traits evaluated under control conditions are presented in Table 2. FA1 is associated with the total seedling length, plumule and radicle lengths of the seedlings. FA2 is linked with seedling fresh and dry weight, while FA3 is associated with per cent germination, promptness index, and vigor index. The averages of communality and uniqueness are 77.07% and 22.93%, respectively. This confirms the effectiveness of factor analysis in identifying optimally selected traits (Olivoto and Nardino, 2021). The highest selection gain was identified for seedling fresh weight, followed by seedling dry weight. This underscores the effectiveness of MGIDI in enabling precise and advantageous trait selection for enhancing crop quality. It further highlights the importance of seedling fresh and dry weights in seedling development and the potential for improvement through plant breeding techniques. Moreover, both of these traits exhibited higher broad-sense heritability, indicating their suitability for selection programs aimed at screening for drought tolerance during the seedling stage.

Table 2. Factor analysis results for mutants under control conditions

Variables	**FA1**	**FA2**	**FA3**	**Communality**	**Uniquenesses**	**PSG**	**Sense**	**Goal**
Percent germination	-0.502	-0.225	0.725	0.829	0.170	0.517	Increase	100
Promptness index	-0.224	0.381	0.698	0.683	0.316	1.270	Increase	100

Plumule length	-0.921	0.017	0.020	0.849	0.150	1.790	Increase	100
Root length	-0.878	0.276	0.163	0.874	0.125	0.275	Increase	100
Total seedling length	-0.965	0.184	0.114	0.978	0.022	0.115	Increase	100
Fresh weight	-0.195	0.853	-0.046	0.768	0.232	7.820	Increase	100
Dry weight	-0.066	0.786	0.268	0.695	0.304	3.940	Increase	100
Vigor index	0.0842	0.096	0.684	0.485	0.514	0.090	Increase	100

The MGIDI scores and the mutants exhibiting superior performance across multiple traits under control conditions are depicted in Fig. 1 (right). Out of the 15 mutants analysed, two mutants, namely M3-181 and M3-149, were recommended for further consideration. Following the methodology proposed by Olivoto and Nardino (2021), both mutants fall on the red cutline, suggesting intriguing characteristics worthy of further investigation. The strengths and weaknesses of the mutant lines under normal conditions provide a comprehensive evaluation of their performance, as illustrated by the contribution of each factor to MGIDI (Fig. 1, left). Mutants associated with FA1, such as M3-85, M3-108, and M3-29, exhibited greater total seedling length, plumule length, and radicle length. However, these mutants did not excel in other traits. Mutants within FA2, including the parent cv. RMt-1, M3-118, M3-181, and M3-175, demonstrated higher seedling fresh and dry weights. Mutants identified in FA3, such as M3-29, M3-181, M3-118, M3-140, and M3-85, displayed elevated per cent germination, promptness index, and vigor index. Among these mutants, only M3-181 exhibited superiority across multiple traits, warranting further attention.

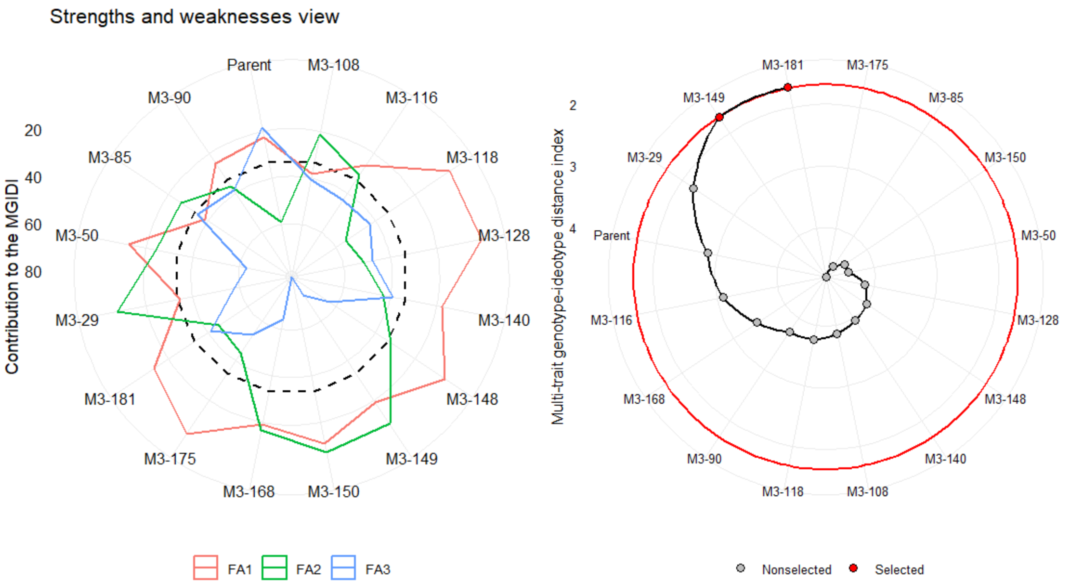

Fig. 1. Strength and weakness view (left) and MGIDI plot (right) for the mutants evaluated under normal conditions

3.2. Evaluation of mutant lines under induced-drought condition

The likelihood ratio tests conducted on the analysed traits using the BLUP approach demonstrated significant genotypic effects across all traits under induced-drought conditions. Principal Component Analysis (PCA) results for mutants under induced-drought conditions revealed the first three principal components (PCs) with eigenvalues exceeding 1 (Table 1).

These three PCs collectively explained 89.52% of the total variability observed in the studied traits under induced-drought conditions. Table 3 presents the factor analysis scores and selection differential parameters for the 15 mutants and their respective traits under induced drought conditions. Factor Analysis 1 (FA1) exhibited strong associations with the tolerance index (0.995), total seedling length (0.992), and root length stress index (0.919), highlighting the critical importance of these traits for drought tolerance, as reported by various researchers. FA2 displayed close associations with seedling dry weight (0.798), vigor index (0.941), and seedling fresh weight (0.894), indicating their significance for drought tolerance at the seedling stage. FA3 exhibited higher scores for root length stress index (0.911), germination stress index (0.911), and promptness index (0.852), underscoring their importance in assessing drought tolerance. The average communality and uniqueness were calculated as 89.51% and 10.48%, respectively, further confirming the effectiveness of factor analysis in identifying optimal traits for screening genotypes for drought tolerance at the seedling stage. The selection differential parameters, including broad-sense heritability and selection gain, showed higher scores for germination stress index and promptness index, indicating their efficacy in selection programs targeting drought tolerance.

Table 3. Factor analysis results for mutants under control conditions

Variables	**FA1**	**FA2**	**FA3**	**Communality**	**Uniquenesses**	**PSG**	**Sense**	**Goal**
Percent germination	0.280	0.090	-0.877	0.857	0.142	0.406	Increase	100
Germination stress index	-0.04	-0.084	-0.949	0.911	0.088	0.481	Increase	100
Promptness index	0.018	-0.066	-0.920	0.852	0.147	0.870	Increase	100
Plumule length	0.841	-0.034	-0.100	0.719	0.280	6.220	Increase	100
Root length	0.903	0.164	0.043	0.845	0.154	1.030	Increase	100
Total seedling length	0.992	0.090	-0.020	0.994	0.005	0.212	Increase	100
Root length stress index	0.919	0.168	-0.227	0.926	0.073	0.081	Increase	100
Fresh weight	0.192	0.894	-0.142	0.857	0.142	0.111	Increase	100
Dry weight	-0.086	0.978	0.128	0.981	0.018	5.830	Increase	100
Vigor index	0.111	0.941	0.085	0.905	0.094	2.900	Increase	100
Tolerance index	0.995	-0.046	-0.033	0.994	0.005	3.390	Increase	100

Fig. 2 presents the MGIDI score plot and the strengths and weaknesses of the 15 mutants assessed under induced-drought conditions. Mutant lines M3-149 and M3-181 exhibited superior performance compared to other mutants and the parent cultivar RMt-1 in tolerating induced-drought stress at the seedling stage, as they were positioned near or above the red-cutline (Fig. 2, right). This underscores their multi-trait superiority and highlights the need for further investigation into their potential. Interestingly, mutant M3-181, which demonstrated superiority under normal conditions, ranked second under induced-drought conditions, while the second superior mutant under normal conditions excelled under induced-drought conditions. This indicates the adaptability of M3-149 to perform well under moisture-deficit conditions. Moreover, M3-181, positioned near the red-cutline, warrants further exploration of its capabilities. The strengths and weaknesses of the 15 mutants under induced-drought conditions are visualized in Fig. 2 (left). Mutants closely associated with FA1, including the parent cultivar RMt-1, M3-168, M3-116, and M3-118, exhibited higher tolerance index, total seedling length, and root length stress index. In FA2, mutants M3-181, M3-29, M3-148, M3-149, M3-150, M3-168, M3-175, M3-116, and M3-168 demonstrated higher seedling fresh and dry weights and vigor index. FA3 scores indicated that mutants M3-108, M3-140, M3-149, and M3-175 displayed higher root length stress index, germination stress index, and promptness index. Notably, the superior mutant under induced-drought conditions, M3-149, scored in both FA2 and FA3, exhibiting higher seedling fresh and dry weights, vigor index, root length stress index, germination stress index, and promptness index, contributing to its superior MGIDI score.

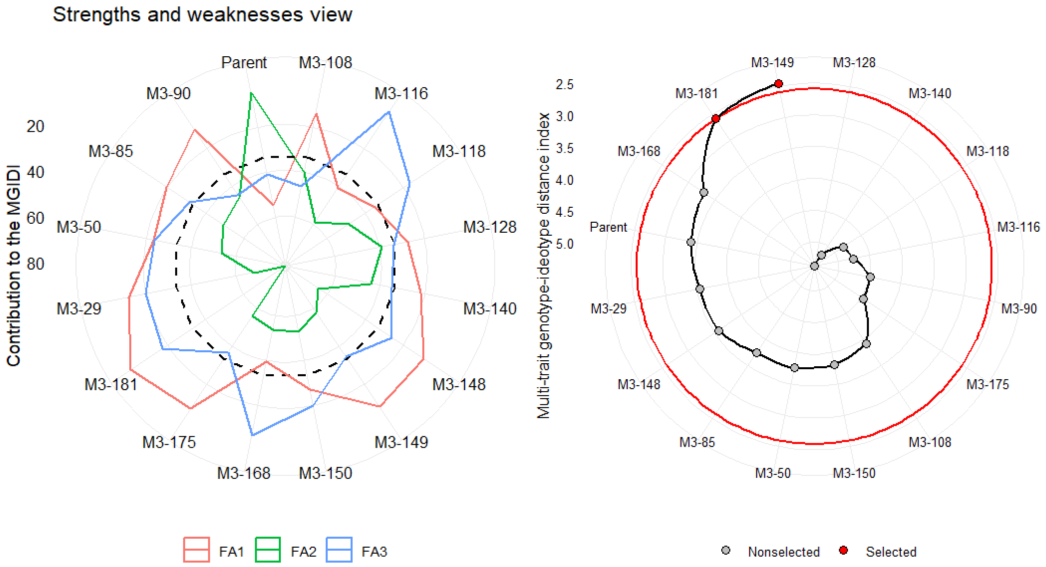

Fig. 2. Strength and weakness view (left) and MGIDI plot (right) for the mutants evaluated under induced-drought conditions

Mamum *et al*. (2022) utilized the MGIDI to identify novel EMS-induced mutants in rice, successfully identifying 10 superior lines among 100 mutant plants. The versatility of the MGIDI model is evident from its effective application in evaluating optimal yield and yield-related traits across various crops, including Bush Yam (Adewumi *et al*., 2023), Maize (Palaniyappan *et al*., 2023), Wheat (Meier *et al*., 2021), Eggplant (Uddin *et al*., 2021), Guar (Cymopsis tetragonoloba) (Benakanahalli *et al*., 2021), and soybean (Woyann *et al*., 2019; Volpato *et al*., 2019). These diverse studies collectively highlight the efficacy of multivariate selection indices for simultaneous trait selection. Additionally, Olivoto and Nardino (2021) have emphasized the superiority of the MGIDI as the most efficient index for selecting genotypes with desired characteristics, further solidifying its relevance and effectiveness in crop improvement endeavours. Jalalifar *et al*. (2023) have also underscored the promising prospects of selected rice genotypes identified through MGIDI in their study, emphasizing their careful selection as valuable resources for breeding programs. These selected genotypes lay the groundwork for generating recombinant populations through strategic crosses, fostering maximum genetic diversity for the development of new rice lines. This innovative approach aligns with sustainable and effective crop improvement strategies.

4. Conclusion and future prospects

The top-performing mutants from the M_2 generation, induced by a combination of ϒ 60 Gy + EMS 0.30%, underwent screening for drought tolerance at the seedling stage in the M_3 generation under controlled laboratory conditions. This investigation aimed to assess their potential for drought tolerance. The top 15 high-yielding mutants were assessed under both normal (control) and induced drought conditions (10% PEG solution). Utilizing the MGIDI method, the evaluation provided valuable insights into selection traits and identified mutants with potential drought tolerance. Under normal conditions, mutants M3-181 and M3-149 exhibited superior performance in seedling growth traits, with M3-149 being the top performer under induced drought conditions, followed by M3-181. Their proximity to the red-cutline suggests promising potential that warrants further investigation. Furthermore, these findings indicate that mutant lines displayed superior multi-trait performance compared to the parent cultivar. Specifically, mutants M3-181, M3-149, and M3-29 ranked higher than the parent cultivar under normal conditions, while mutants M3-149, M3-181, and M3-168 surpassed the parent cultivar under induced drought conditions. This underscores the effectiveness of mutation breeding, particularly through the induction of mutations using γ radiation and EMS, as an efficient strategy not only for generating genetic diversity but also for producing variants superior to the parent cultivar.

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