

Selection of superior and stable fodder maize hybrids using MGIDI and MTSI indices

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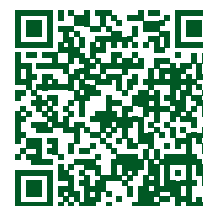
Abstract: Maize is recognized as an exceptional forage crop with superior forage quality among cereal forage crops. For forage breeders, the primary objective is to identify high-yielding and stable genotypes. The aim of this study was to select superior and stable fodder maize hybrids from 195 single-cross hybrids on the basis of 16 variables. The selection was carried out using the MGIDI index within each season, with a selection intensity of 5%. Genotypes G48, G47, and G79, which had the lowest MGIDI indices in the rainy 2022, winter 2022, and summer 2023 seasons, respectively, were identified as superior in the 16 variables studied. In addition, the MTSI index was used to evaluate genotype stability across multiple traits, identifying genotype G24 as having the lowest MTSI index. These robust statistical tools proved effective in identifying the most stable and high-performing genotypes among the 195 single-cross forage maize hybrids analyzed.

Keywords: Fodder maize, Hybrids, MGIDI, MTSI

INTRODUCTION


The Indian economy relies significantly on livestock, with approximately 8.8% of the Indian population employed in this sector (Singh et al. 2020). India's livestock population includes 65 million sheep, 102 million goats, 125 million other types of goats, and 198 million cattle. Annually, about 757 million tonnes of green fodder, 466 million tonnes of dry fodder, and 47.3 million tonnes of concentrated feed are utilized. However, there are deficits in fodder availability, with green fodder showing a 35.6% shortfall, dry matter a 10.95% deficit, and concentrated feeds a 44% shortage (IGFRI 2013). Current estimates show a shortfall of 28.9% in concentrate feed, 23.4% in dry forage, and 11.24% in green fodder (Hilli and Kapoor 2023). Increasing fodder production is essential to meet the growing demand from an expanding livestock population. Due to farmers prioritizing human food crops, livestock faces a shortage of feed, with green and dry fodder availability estimated at 734.2 million tonnes and 326.4 million tonnes, respectively - below the required levels of 827.19 million tonnes and 426.1 million tonnes (Roy et al. 2021). Fodder maize could help bridge this gap as a nutritious, high-yielding fodder source.

Maize (*Zea mays* L.) is a staple food crop globally, with diverse applications in biofuel production, animal feed, and as a raw material in various industries (Vathana et al. 2019). With its high starch (66.7%), protein (10%), fiber (8.5%),



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sugar (3%), and ash (7%) content, maize has excellent nutritional value for fodder (Kifayat et al. 2022). Therefore, identifying stable, high-yield fodder genotypes is a primary goal for forage breeders.

The breeding process involves extensive resources and effort, primarily in evaluating breeding materials at the final stages. Assessing genotypes across various yield trials is essential in crop breeding experiments. Selecting broadly adapted genotypes is challenging, as the relationship between genotype and phenotype varies with environmental interactions. Therefore, breeders aim to identify genotypes that perform well in specific environments while also being broadly adaptable. Using a selection index in breeding programs can help breeders identify stable, high-performing genotypes. Traditional selection indices, proposed by Smith (1936) and Hazel (1943), can be tedious, as they require specific weightings for each trait. These indices also depend on the inversion of phenotypic covariance and may encounter multicollinearity issues when handling multiple traits, ultimately reducing genetic gain estimates (Bizari et al. 2017). To address these limitations, Olivoto and Nardino (2020a) introduced the Multi-Trait Genotype-Ideotype Distance Index (MGIDI), a novel genetic technique for selecting superior genotypes based on multiple traits.

Identifying genotypes with minimal variation across environments is crucial for breeding stable genotypes (Yan and Kang 2003). To model these environmental interactions, breeders have developed stability analysis techniques (Olivoto et al. 2019a). Traditionally, stability estimates have relied on single-trait, univariate analysis, including mean and regression deviation calculations (Benakanahalli et al. 2021). The recently developed Multi-Trait Stability Index (MTSI) addresses limitations in univariate analysis and is effective for selecting stable genotypes (Olivoto et al. 2019b). MTSI is based on the Weighted Average Absolute Scores from BLUP for Yield (WAASBY) index, combining performance weighting (Y) and stability (Weighted Average of Absolute Scores [WAASB] index).

The aim of this study is to identify the most superior fodder maize hybrids across various environments by using MGIDI. Additionally, hybrids that demonstrate both exceptional performance and stability across environments are selected using MTSI.

MATERIAL AND METHODS

Experiment materials and study environment

The environment includes all the circumstances that affect an individual or a population and influence phenotypic variation. By using years or seasons as environmental factors in genotype selection, we can identify genotypes that consistently perform well under different conditions, thus optimizing the adaptability of each genotype. This study was conducted at the Tamil Nadu Agricultural University (lat 11° 1' 24.384'' N, long 76° 55' 25.7664'' E, alt 427 m asl), Tamil Nadu, India. Genotype stability was assessed over three consecutive seasons: rainy 2022, winter 2022-2023, and summer 2023. A total of 195 single-cross fodder maize hybrids were developed using a line × tester hybridization method with 13 lines and 15 testers (Supplementary Table 1). Assessment was conducted by using a randomized block design with two replications, a spacing of 30 cm × 15 cm, and a planting density of 50 plants per 2.4 m².

Traits measured

Twelve quantitative traits and four biochemical parameters were evaluated for all hybrids. Quantitative traits included Days to 50% Flowering (DFF), Plant Height (PH), Cob Placement Height (CH), Leaf Length (LL), Leaf Breadth (LB), Number of Leaves (NL), Number of Nodes (NN), Stem Girth (SG), Internode Length (IL), Leaf-Stem Ratio (LSR), Dry Matter Yield (DMY), and Green Fodder Yield (GFY). The biochemical parameters - Crude Protein (CP), Crude Fiber (CF), Acid Detergent Fiber (ADF), and Neutral Detergent Fiber (NDF) - were analyzed by using Near Infrared Spectrophotometry (NIR) (Model: SpectraAlyser; Make: ZEUTECH, Germany).

Phenotypic variance analysis

Variance components for all studied variables were estimated to partition total phenotypic variance into genotypic variance, genotype × environment (G×E) variance, and residual components, using the gamem_met model in the *metan* package (version 1.18.0).

Selection of genotypes within season based on MGIDI

Superior genotypes for each of the three environments were selected by using the MGIDI, which involves four steps: rescaling variables, performing factor analysis, designing ideotypes, and calculating the distance between genotype and ideotype.

Rescaling variables: Each variable was rescaled according to the equation:

$$rX_{ij} = \frac{\eta_{nj} - \varphi_{nj}}{\eta_{oj} - \varphi_{oj}} \times (\theta_{ij} - \eta_{oj}) + \eta_{nj}$$

where η_{nj} and φ_{nj} are the rescaled maximum and minimum values of variables, η_{oj} and φ_{oj} are the maximum and minimum original values of traits, and θ_{ij} is the original value of the i^{th} variable for the j^{th} genotype. For traits with desired positive gain, $\eta_{nj} = 100$ and $\varphi_{nj} = 0$.

Factor analysis: Factor analysis was performed on the rescaled values (rX_{ij}) to address correlation and reduce data dimensionality, grouping correlated variables into single factors (FAs). A rescaled matrix based on the Best Linear Unbiased Prediction (BLUP) for genotype was used. Factorial scores for each genotype were estimated using the formula:

$$X = \mu + Lf + \xi$$

where μ , f , and ξ are $p \times 1$ vectors of standardized mean, common factors, and residues, respectively, $L = p \times f$ is the factor loading matrix, p and f are number of variables and retained common factors. Factors with eigenvalues greater than one were initially selected, with final loadings obtained by *varimax* rotation.

Ideotype planning and MGIDI estimation: A different morpho-physiological variables take account into a single genotype with the aim of improving the productivity is referred as ideotype. The concept of ideotype design is improving the performance of crop by simultaneous selection of multiple traits. In this study, the ideotype has the maximum of rescaled value and equal weight was considered for all the studied variables in the selection process. The final one is estimation of MGIDI index by using the following formula

$$MGIDI_i = \sqrt{\sum_{j=1}^f (F_{ij} - F_j)^2}$$

where F_{ij} is the score of the i^{th} genotype score for the j^{th} factor ($i = 1, 2, \dots, g; j = 1, 2, \dots, f$), with g and f representing the total number of genotypes and factors, respectively. Genotypes with the lowest MGIDI values are closest to the ideotype, indicating optimal values for all traits in each environment. Analyses were performed in R Studio (version 4.2.1) using the *metan* package (version 1.18.0) (Olivoto and Lúcio 2020b) with the functions *gamem* and *mgidi*.

The contribution of the j^{th} factor (ω_{ij}) to the MGIDI of the i^{th} genotype was calculated to highlight each genotype's strengths and weaknesses, as follows:

$$\omega_{ij} = \frac{\sqrt{D_{ij}^2}}{\sum_{j=1}^f \sqrt{D_{ij}^2}}$$

where D_{ij} is the distance between the i^{th} genotype and the ideotype for the j^{th} factor.

Selection across seasons based on MTSI

The main distinction between the MGIDI and MTSI indices lies in the factor analysis approach. MTSI utilizes the "WAASBY" index for rescaled values, whereas MGIDI is based on BLUP values. The WAASBY index incorporates both stability (through the WAASB index) and mean performance of variables. WAASB stands for the Weighted Average of Absolute Scores, derived from the singular value decomposition of the BLUP matrix for genotype-by-environment interaction (GEI) effects. Genotypes with lower WAASB scores are considered more stable. Thus, WAASBY accounts for both the average performance and stability across all studied variables.

The WAASBY index is calculated as follows:

$$WAASBY_i = \frac{(rY_i \times \theta_y) + (rW_i \times \theta_w)}{\theta_y + \theta_w}$$

where rY_i and rW_i represent the rescaled values for the response variable (Y) and stability (WAASB), respectively, and θ_y and θ_w are the weights assigned to mean performance and stability. In this study, equal weighting was given to both performance and stability. The MTSI analysis was conducted using the *metan* package (version 1.18.0) with the *mtsi* function (Olivoto and Lúcio 2020b).

RESULTS AND DISCUSSION

Selection of genotypes based on MGIDI using the BLUP method

Variance components for genotype (G), genotype-by-environment interaction (G×E), and residuals are presented in Supplementary Figure 1. Eight variables showed a greater proportion of G×E variance than genotypic variance, indicating that GEI significantly impacts genotype performance across different environments. This finding supports the need to perform genotype selection within each specific environment.

Multivariate techniques, such as principal component analysis and linear discriminant analysis, outline the relationships between traits but pose challenges for selecting genotypes based on multiple trait values (Olivoto et al. 2022). To address the limitations of linear selection, the MGIDI selection method was applied in this study. Selection of single-cross fodder maize hybrids based on the mean performance of 16 fodder traits, using MGIDI for different seasons, is depicted in Supplementary Figures 2–4.

A selection intensity (SI) of 5% was applied to target the most superior hybrids. In Supplementary Figures 2–4, the green circle line marks genotypes selected based on this intensity. With a 5% SI, MGIDI values for selected genotypes across the three seasons were analyzed (Table 1). A lower MGIDI value indicates proximity to the ideotype, meaning genotypes with the lowest MGIDI scores are considered ideal, excelling across all studied variables in their respective environments (Al-Ashkar et al. 2023). This method identified hybrid genotypes G48 in rainy 2022, G47 in winter 2022, and G79 in summer 2023 as having the lowest MGIDI values, marking them as ideal performers for the forage traits studied. Seasonal environmental factors may cause a genotype that performs well in one season to perform poorly in another. Therefore, hybrids identified as ideal for a specific season are considered best adapted to that season’s conditions.

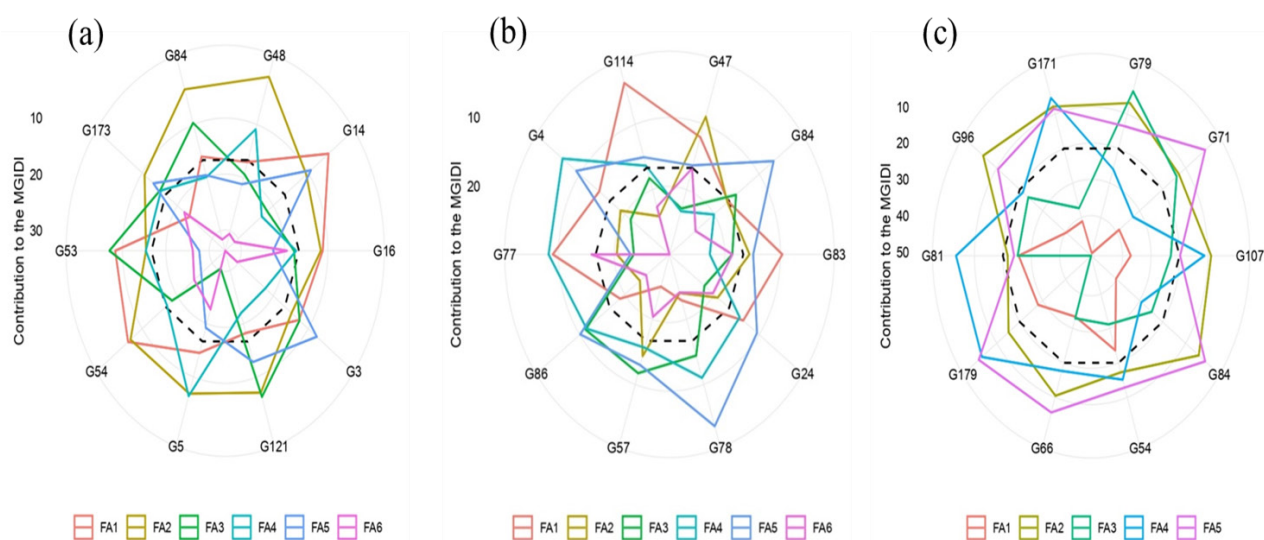


Figure 1. Strength and weakness of selected hybrids based on MGIDI (a) rainy 2022, (b) winter 2022, (c) summer 2023

This approach for selecting superior genotypes has also been employed by Olivoto et al. (2022) in strawberries, Pour-Aboughadareh et al. (2021) in barley, Benakanahalli et al. (2021) in guar, Maranna et al. (2021) in soybean, and Uddin et al. (2021) in maize.

The selection gains for various traits in hybrids across all three seasons are presented in Table 2. The highest gains were recorded for cob height (15.20) in the rainy season of 2022, green fodder yield (17.20) in winter 2022, and leaf-stem ratio in summer 2023. Most fodder-related traits exhibited positive and substantial selection gains across seasons. Similar study on selection gains using the MGIDI index in soybean was conducted by Maranna et al. (2021). Among the traits, LSR showed the highest heritability (0.98) in rainy 2022, ADF in winter 2022, and CP, NDF, and LSR (0.93) in summer 2023. Identifying the strengths and weaknesses of selected genotypes provides insight into the traits that contribute most to effective selection. Figures 1a-1c present the strengths and weaknesses of selected hybrids across the three seasons. In the MGIDI index, the most and least contributing factors can be differentiated, with lines closer to the plot center representing higher-contributing factors and those further out representing lesser contributions (Olivoto and Lúcio 2020b). In the hybrids, the leaf-stem ratio (FA6) was the most significant factor for selecting top genotypes during rainy 2022, while leaf-stem ratio and crude protein (FA6) were crucial in winter 2022. In summer 2023, days to

Table 1. Selected single cross hybrids with low MGIDI in different seasons and MTSI

Rainy 2022		Winter 2022		Summer 2023		MTSI	
Hybrids	MGIDI	Hybrids	MGIDI	Hybrids	MGIDI	Hybrids	MTSI
G48	5.28	G47	4.73	G79	3.39	G24	6.72
G14	5.34	G84	5.01	G71	3.88	G44	6.79
G16	5.41	G83	5.03	G107	4.12	G81	6.81
G3	5.63	G24	5.25	G84	4.16	G7	6.91
G121	5.75	G78	5.26	G54	4.23	G84	7.00
G5	5.76	G57	5.27	G66	4.27	G74	7.05
G54	5.78	G86	5.71	G179	4.36	G75	7.11
G53	5.85	G77	5.79	G81	4.54	G78	7.2
G173	5.85	G4	5.87	G96	4.58	G79	7.27
G84	5.87	G114	5.89	G171	4.59	G49	7.31

Table 2. Selection gain of forage related traits based on MGIDI index in hybrids

Rainy 2022				Winter 2022				Summer 2023			
Trait	FA	SG	h ²	Trait	FA	SG	h ²	Trait	FA	SG	h ²
DFF	FA1	2.20	0.94	DFF	FA1	0.97	0.84	DFF	FA1	0.19	0.90
CH	FA1	15.20	0.94	NL	FA1	4.06	0.76	CH	FA1	2.60	0.78
PH	FA1	7.46	0.87	NN	FA1	3.65	0.74	PH	FA1	2.63	0.71
NL	FA1	3.84	0.90	CF	FA2	5.94	0.75	NL	FA1	4.3	0.79
NN	FA1	3.16	0.84	ADF	FA2	3.90	0.98	NN	FA1	3.38	0.78
CF	FA2	5.6	0.72	NDF	FA2	8.45	0.95	LL	FA1	1.80	0.74
ADF	FA2	2.24	0.90	DMY	FA3	6.85	0.63	GFY	FA1	-2.21	0.89
NDF	FA2	4.47	0.91	GFY	FA3	17.2	0.88	CP	FA2	-0.03	0.93
DMY	FA3	6.62	0.91	LB	FA4	2.61	0.65	CF	FA2	3.35	0.92
GFY	FA3	4.45	0.85	SG	FA4	1.79	0.58	ADF	FA2	2.79	0.88
LL	FA4	6.96	0.84	CH	FA5	4.95	0.79	NDF	FA2	1.93	0.93
IL	FA4	7.83	0.79	PH	FA5	3.58	0.80	LB	FA3	4.51	0.69
CP	FA4	2.67	0.97	LL	FA5	1.73	0.59	SG	FA3	5.47	0.60
LB	FA5	3.64	0.80	IL	FA5	0.84	0.64	LSR	FA4	14.9	0.93
SG	FA5	7.47	0.81	LSR	FA6	13.2	0.95	DMY	FA4	-7.76	0.92
LSR	FA6	9.94	0.98	CP	FA6	6.28	0.91	IL	FA5	0.07	0.57

MGIDI - Multi-trait Genotype Ideotype Distance Index, FA - Factor analysis, SG - Selection gain, h² - Heritability, DFF - days to fifty per cent flowering, PH - plant height, CH - Cob placement height, LL - Leaf length, LB - Leaf breadth, NL - Number of leaves, NN - Number of nodes, SG - Stem girth, IL - Internode length, LSR - Leaf stem ratio, CP - Crude protein, CF - Crude fiber, ADF - Acid detergent fiber, NDF - Neutral detergent fiber, DMY - Dry matter yield, GFY - Green fodder yield.

fifty percent flowering, plant height, cob height, number of leaves, leaf length, and number of nodes were the most contributing traits, grouped in FA1. Hybrid G48, with the lowest MGIDI index, showed strength in FA2 and FA4 during the rainy season of 2022. Similarly, G47 exhibited strength in FA2 during winter 2022, while G79 showed strength in FA3 in summer 2023. This approach to assessing genotype strengths and weaknesses has also been applied by Pour-Aboughadareh et al. (2021), Benakanahalli et al. (2021) and Olivoto et al. (2022).

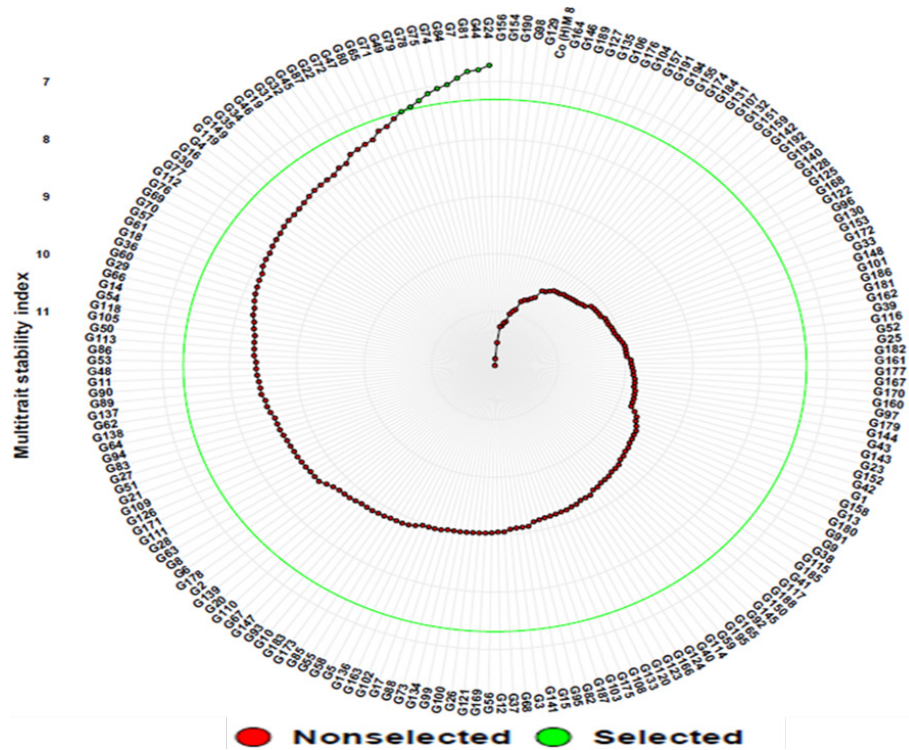


Figure 2a. MTSI index for selection of stable hybrids based on multi traits

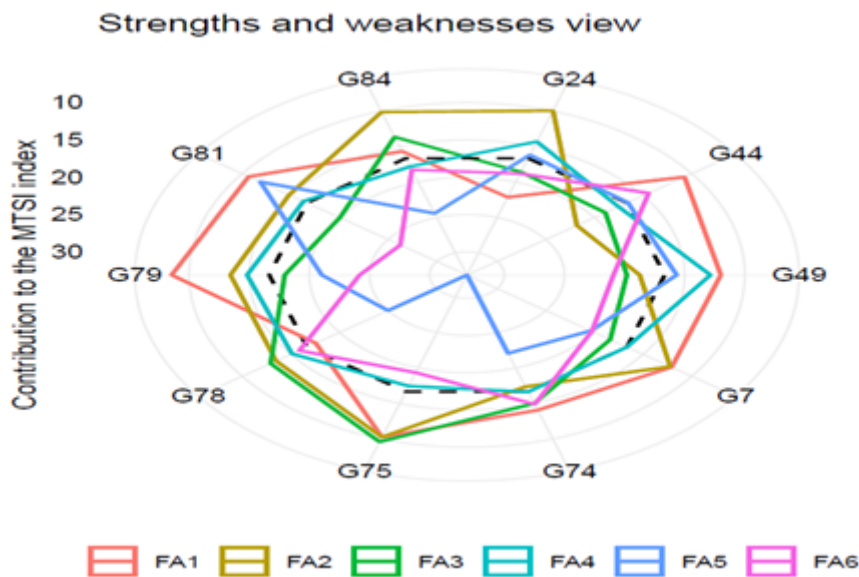


Figure 2b. Strength and weakness of selected hybrids based on MTSI

GENOTYPE SELECTION ACROSS ENVIRONMENTS BASED ON MTSI USING THE WAASBY METHOD

Selecting stable, superior genotypes is a primary objective for plant breeders and all those involved in crop improvement. Traditionally, genotype stability has been assessed using first-degree statistics, such as mean, regression, and regression deviation. However, these univariate approaches do not capture stable performance across multiple traits and may lead to suboptimal selections (Dehghani et al. 2006). Using the MTSI genetic tool across environments provides a more comprehensive approach to identifying stable performers.

The MTSI analysis of 16 variables showed that six interactive principal components (IPCA) had eigenvalues greater than one, cumulatively accounting for 66.30% of the variance (Supplementary Table 2). The first two principal components (PCs) explained 33.10% of the variance, with PC1 accounting for 21.50% and PC2 for 11.60%. Factor analysis grouped the 16 variables into six factors based on associations (Table 3): FA1 included DFF, NL, and NN; FA2 included CF, ADF, and NDF; FA3 included LL, DMY, and GFY; FA4 included LB, SG, and CP; FA5 included LSR; and FA6 included CH, PH, and IL.

A 5% selection intensity was applied to identify the top ten stable performers. The selected hybrids - G24, G44, G81, G7, G84, G74, G75, G78, G79, and G49 (Figure 2a) - were determined to be the most stable among the 195 single-cross hybrids evaluated based on multiple traits. Genotype G24 had the lowest MTSI value (6.72), followed closely by G44 (6.79), indicating that these genotypes are highly stable and superior across all studied variables (Olivoto et al. 2019a). Notably, hybrid G84 was identified as a common genotype between MGIDI and MTSI selections, as shown in the Venn diagram (Supplementary Figure 5). The ranking of genotypes was based on the WAASBY index, which serves as a reliable indicator for identifying stable and superior genotypes across diverse environments with multiple traits (Olivoto et al. 2019b). The WAASBY index provided a desirable selection differential for all variables, ranging from 7.57 (for LSR) to 30.90 (for GFY). Selection gains for mean performance were achieved for each variable, with values from 0.19 (for CP) to 11.70 (for GFY). The mean values for all investigated variables in the selected genotypes (X_s) exceeded the original population mean (X_o), indicating environmental influences on trait expression and supporting this method as an effective tool for identifying stable genotypes in varying environments (Hussain et al. 2021). Traits such as NL (0.81), DFF (0.79), NN (0.77), PH (0.74), CH (0.71), and LL (0.63) showed higher broad-sense heritability compared to others. This suggests that including these high-heritability traits in the selection criteria could maximize the expected genetic gain.

The role of individual factors containing correlated variables in MTSI serves as an effective criterion for assessing the strengths and weaknesses of selected stable genotypes across environments (Figure 2b). FA1, for instance, contributed

Table 3. Selection gain of 16 traits across the seasons assessed by MTSI

Traits	Selection differential for the WAASBY index				Selection gain for the mean of the variables			
	Factor	X_o	X_s	SD (%)	X_o	X_s	h^2	SG (%)
DFF	FA1	62.3	74.8	20.1	58.50	59.90	0.79	1.97
NL	FA1	56.7	67.7	19.3	14.20	15.10	0.81	4.96
NN	FA1	59.1	70	18.5	14.70	15.50	0.77	4.60
CF	FA2	61.7	74.5	20.7	25.80	27.00	0.33	1.55
ADF	FA2	64.1	72.9	13.7	32.50	33.60	0.37	1.26
NDF	FA2	61.9	74.4	20.1	52.70	54.60	0.33	1.21
LL	FA3	57.1	61.8	8.25	93.00	97.70	0.63	3.18
DMY	FA3	57.7	65.4	13.3	11.90	13.10	0.38	3.90
GFY	FA3	58.7	76.8	30.9	52.40	62.70	0.60	11.70
LB	FA4	54.1	64.8	19.7	8.36	8.67	0.54	2.00
SG	FA4	59.9	67.2	12.2	5.27	5.55	0.41	2.22
CP	FA4	59.9	68.7	14.8	12.70	12.70	0.32	0.19
LSR	FA5	55.9	60.1	7.57	0.27	0.27	0.19	0.34
CH	FA6	62.1	69.3	11.5	122.00	139.00	0.71	9.56
PH	FA6	66.6	79.1	18.8	247.00	265.00	0.74	5.24
IL	FA6	61.6	69.1	12.2	18.10	19.50	0.36	2.81

FA - Factor analysis, SG - Selection gain, h^2 - Heritability, X_s - Mean for selected genotypes, X_o - Original population mean, DFF - days to fifty per cent flowering, PH - plant height, CH - Cob placement height, LL - Leaf length, LB - Leaf breadth, NL - Number of leaves, NN - Number of nodes, SG - Stem girth, IL - Internode length, LSR - Leaf stem ratio, CP - Crude protein, CF - Crude fiber, ADF - Acid detergent fiber, NDF - Neutral detergent fiber, DMY - Dry matter yield, GFY - Green fodder yield.

most significantly to hybrids G79 and G75. Similarly, hybrids G84, G24, and G75 showed strength in FA2; G49 and G24 had strength in FA4; and FA6 contributed significantly to the stability of G74 and G44. Among the stable genotypes, all but G81 displayed a weakness in FA5. This approach, previously used by Zuffo et al. (2020) to select stable soybean genotypes under drought and salinity stress, has also been effectively applied by Sellami et al. (2021) and Hussain et al. (2021) for selecting stable genotypes based on multiple traits in lentil and chickpea, respectively.

CONCLUSION

This study aimed to identify superior and stable fodder maize genotypes using multi-trait selection indices. The MGIDI index was used for genotype selection within individual environments, while the MTSI index facilitated selection across multiple environments. In the environment-specific selections, hybrids G48 in rainy 2022, G47 in winter 2022, and G79 in summer 2023 were identified as ideal genotypes. Genotype G24 was recognized as a stable and superior performer overall. Notably, hybrid G84 was the only genotype commonly selected by both MGIDI and MTSI indices, indicating its consistent performance across environments.

DATA AVAILABILITY

The datasets generated and/or analyzed in this study are available from the corresponding author upon reasonable request.

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