

Article



# A Framework for Identification of Stable Genotypes Based on MTSI and MGDII Indexes: An Example in Guar (*Cymopsis tetragonoloba* L.)

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Abstract: Guar, the most popular vegetable, is tolerant of drought and is a valuable industrial crop enormously grown across India, Pakistan, USA, and South Africa for pharmaceutically and cosmetically usable galactomannan (gum) content present in seed endosperm. Guar genotypes with productive traits which could perform better in differential environmental conditions are of utmost priority for genotype selection. This could be achieved by employing multivariate trait analysis. In this context, Multi-Trait Stability Index (MTSI) and Multi-Trait Genotype-Ideotype Distance Index (MGIDI) were employed for identifying high-performing genotypes exhibiting multiple traits. In the current investigation, 85 guar accessions growing in different seasons were assessed for 15 morphological traits. The results obtained by MTSI and MGIDI indexes revealed that, out of 85, only 13 genotypes performed better across and within the seasons, and, based on the coincidence index, only three genotypes (IC-415106, IC-420320, and IC-402301) were found stable with high seed production in multi-environmental conditions. View on strengths and weakness as described by the MGIDI reveals that breeders concentrated on developing genotype with desired traits, such as quality of the gum and seed yield. The strength of the ideal genotypes in the present work is mainly focused on high gum content, short crop cycle, and high seed yield possessing good biochemical traits. Thus, MTSI and MGIDI serve as a novel tool for desired genotype selection process simultaneously in plant breeding programs across multi-environments due to uniqueness and ease in interpreting data with minimal multicollinearity issues.

Keywords: guar; MTSI; MGIDI; yield; gum content; multi-trait;multi-environment

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# 1. Introduction

'Guar', a synonym of cluster bean (*Cyamopsis tetragonoloba* (L.) Taub), is one of the underutilized legumes that finds a place as a popular vegetable and industrial-oriented crop. Its drought tolerance ability and wide application in industrial sectors due to galactomannan gum content present in the seed endosperm proves the significance of this crop. Guar is native to the Indian subcontinent of Asia, and it can be cultivated in various kinds of environment with least possible inputs requirement [1–3]. About 80 percent of the world's guar production is contributed from India, USA, South Africa, and Pakistan [4]. Guar is extensively used for its gum content, called 'Galactomannan', which is a polysaccharide embedded in the endosperm of the seed and forms viscous gel even in cold water, and, due to its emulsifying and thickening, as well as stabilizing properties, this guar gum has been extensively used in food and other industries involving cosmetics, textiles, paper, oil drilling fracking, pharmaceuticals industries, and so on [5–7].

A seed of guar is comprised of nearly 14 to 17% of the seed coat, 35 to 42% of endosperm, and the remaining 43 to 47% is the germ content. The commercial component, guargum (galactomannan), will be in the endosperm of guar seeds up to an extent of 19–43%. The by-product obtained by processing of guar, known as Churi and Korma, has high protein content consisting of approximately 60% of the whole seed suitable for supplementing cattle and chicken fodder [8–10]. Indian and African people consume edible tender pods of guar as a vegetable because edible portion of tender pods contains 3.2g protein, 10.8 g carbohydrates, 1.4 g fat, 57 mg Calcium, 4.5 mg Iron, and Vitamin A and C with 16 kcal energy and 80g moisture per every 100 g [11,12]. Being a leguminous crop, guar can fix about 112.36 kg of N (Nitrogen) per hectare. There are reports that cotton rotated with guar has evidenced 15% increase in yield even without the application of nitrogen in cotton [13]. India occupies a major share in exporting different products of guar, like gum, splits, and meal, to many countries with 17.5% export share among the agricultural commodities during 2019–20 The country has exported guar of 169.54 MT to various countries of the world, including Germany, Canada, United States of America, Australia, Russia, etc., accounting to worth of Rs. 1406 crores ≈186 million US\$ in 2019–20 [14] (Figure 1). Santonoceto et al. [15] used a novel set of SSR markers to characterize the guar accessions having desirable morphological and productive, as well as genetic, traits. But studies on guar genotypes performing better under varied environmental situations with desirable qualitative traits are meager. Thus, in the present study, it has been attempted to use multivariate techniques which could result in the selection of guar genotypes with economical yield and exportable gum content.

Assessing the interaction between genotypes and environment resulting in high yielding potential is needed. The yielding potential of the genotypes will be greatly affected if grown under different environmental conditions. The crop which performs better in varied climatic situations will be an ideal one [16]. Adaptability and stability of genotypes to different kinds of environments with low, marginal, and average, as well as high, yielding potential can be assessed by studying phenotypic characters of the crop cultivar. Grain yield is more complicated phenomena determined by polygenes and environmental factors. Thus, it becomes necessary to understand the effect of environment on genotype characters and its performance in a specified environmental condition, which can be achieved by studying genotype and environment interactions (GEI), along with stability analysis. Sound knowledge on the degree and pattern of GEI helps in minimizing redundant replications of temporal and spatial yield trails which, in turn, reduces the cost for evaluating genotypes [17]. This selection can be made using multivariate selection indices and one such widely used index is SH (Smith-Hazel) index developed by Smith [18] and Hazel [19]. However, this SH index works using inversion of phenotypic covariance matrix which suffers from multicollinearity issues, leading to poor selection of desirable genetic traits across different environmental conditions [20–22]. To overcome these multicollinearity constraints, recently proposed indexes, viz. the Multi-Trait Stability Index (MTSI)[23] and the Multi-Trait Genotype-Ideotype Distance Index (MGIDI) [24], have emerged as novel tools for selecting superior genotypes which could perform better across different environmental conditions with high stability in yield and desirable traits. Based on this background, the current study proposes a framework to identify suitable stable genotypes of guar for cultivation in Karnataka by using MTSI and MGIDI indices for multi-factorial multi-trait stability analysis towards identifying ideal and high yielding genotypes of with higher galactomannan content across varied environmental conditions.



Figure 1. Statistics of guar gum export from India to top ten countriesof the world in the year 2019–20.

# 2. Materials and Methods

# 2.1. Details of the Experimental Site and Study Environment

The genotypes stability experiment was set out in the campus of the University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka, India, which resides at latitude of 13°55′, longitude of 75°34′, and an elevation of 640m. The average annual rainfall of the station is 900mm with the soil type of red sandy loam. The study was undertaken during consecutive three environments/seasons, i.e., *kharif* season in 2013, summer season of 2013–14, and *kharif* season in 2014. The details of date of sowing and harvesting during the different seasons are given in Table 1.

Table 1. Various genotype accessions adopted in this study.

Deremators	Environments									
rarameters	E1 (Kharif:2013)	E2 (Summer:2013–14)	E3 (Kharif:2014)							
Date of sowing	15 August 2013	19 February 2014	18 August 2014							
Date of Harvesting	20 December 2013	28 June 2014	22 December2014							

## 2.2. Plant Material, Design of the Experiment, and Cultural Practices

Eighty-five genotype accessions were collected from NBPGR (National Bureau of Plant Genetic Resources), Regional Research Station situated at Jodhpur, Rajasthan. Details of the genotypes employed in the present work are given in Table 2.

ID	Accession No.	ID	Accession No.	ID	Accession No.
1	IC-421816	31	IC-370509	61	IC-415145
2	IC-421817	32	IC-370516	62	IC-415146
3	IC-311392	33	IC-370563	63	IC-415148
4	IC-311393	34	IC-373427	64	IC-415151
5	IC-311403	35	IC-373438	65	IC-415153
6	IC-311417	36	IC-373480	66	IC-415154
7	IC-311431	37	IC-373497	67	IC-415157
8	IC-311432	38	IC-373557	68	IC-415158
9	IC-311440	39	IC-402294	69	IC-415159
10	IC-311444	40	IC-402295	70	IC-415160
11	IC-311449	41	IC-402296	71	IC-415161
12	IC-323992	42	IC-402298	72	IC-415163
13	IC-324020	43	IC-402299	73	IC-415166
14	IC-324023	44	IC-402301	74	IC-415168
15	IC-325743	45	IC-402302	75	IC-420320
16	IC-325800	46	IC-402303	76	IC-420332
17	IC-325806	47	IC-402304	77	IC-420373
18	IC-325811	48	IC-415102	78	IC-421242
19	IC-325819	49	IC-415106	79	IC-421798
20	IC-325821	50	IC-415108	80	IC-421809
21	IC-325832	51	IC-415110	81	IC-421812
22	IC-325846	52	IC-415115	82	IC-421815
23	IC-329030	53	IC-415123	83	IC-421834
24	IC-329033	54	IC-415125	84	IC-421835
25	IC-329036	55	IC-415128	85	RGC1003
26	IC-329062	56	IC-415131		
27	IC-369838	57	IC-415135		
28	IC-370478	58	IC-415137		
29	IC-370490	59	IC-415138		
30	IC-370502	60	IC-415140		

Table 2. Various genotype accessions adopted in this study.

The design followed was a randomized complete block design (RCBD) to conduct the experiment in the field having three replications for all the 85 genotypes. The seeds of each genotype were sown at 30 × 10 cm spacing apart in five 1-m length cropping rows.

## 2.3. Traits Assessed

Ten quantitative traits and five biochemical parameters accounting for 15 traits in total were assessed.

# 2.3.1. Quantitative

Plant height represented as PH (cm), days to 50% flowering (DFF), days to maturity (DM), number of branches plant<sup>-1</sup> (BRAN), clusters plant<sup>-1</sup> (CP), pods plant<sup>-1</sup> (PP), grains pod<sup>-1</sup> (GP),Hundred seed weight as 100SW (g), and grain yield plot<sup>-1</sup> as GY (g) are the quantitative traits assessed in this study.

# 2.3.2. Biochemical

Gum percent (GP), crude protein (PROTEIN), crude fat (FAT), ash, and carbohydrate (CARB) percent were analyzed.

# Gum Percent

Estimation of guar gum involved galactomannan extraction followed by purification. A standardized procedure given by Das et al. [25], and then modified by Joshi [26] for gum extraction, was adopted in this study, and gum percent was estimated as follows:

Gum percent = Weight of gum powder obtained × 100 Initial weight of seeds taken.

Crude Protein Content, Crude Fat, Crude Fiber (FIBER), and Ash (ASH)

The seed samples of guar were grinded, sieved (1 mm size), and dried at  $135 \pm 2$  °C for 2 h. This dried sample was then subjected to incineration in an electric muffle furnace to determine ash content at 600 °C for two hours. Crude protein, crude fat, and crude fiber contents were also determined by the procedures given by AOAC [27].

#### Total Carbohydrates

The percentage of total carbohydrates present in guar seeds was assessed by the differential method of nitrogen-free extraction as described by McDonald et al. [28], and the total carbohydrate percentage was calculated using the equation as follows:

# % Carbohydrate = $100 - \sum$ (% crude protein + % crude fat + % crude fiber + % ash +% moisture).

#### 2.4. Analysis of Data with Statistical Method

Pooled analysis of variance and individual analysis of variance (ANOVA) was performed for each environment (E1, E2, and E3). The MTSI index was used to identify genotypes performing better and stable with multiple traits, thus exploiting wide adaptations. The MGIDI index was applied within each season to select genotypes that perform well in specific conditions, thus exploiting narrow adaptations. To identify the stable genotypes, stability analysis was carried out among the genotypes. In the present study, stability analysis over three environments by "metan" package, developed by Olivoto and Lúcio [29], was used. Since the data available under this study is limited, we propose here a framework for the identification of stable genotypes with multi-traits as an example by using Guar as test crop. This framework can be extended to other crops, as well as data sets, spread over several locations and seasons.

Based on the ideotype concept, the MTSI/MGIDI index the traits were rescaled by assigning 0–100 value for all the traits, where 0 defines the least valued trait, and 100 defines the most valuable/desired trait, which facilitates to define ideotype [30]. In the present investigation, most of the traits were assigned with increased values defining quantitative morphological traits of guar crop, which directly or indirectly correlates to the yield and quality of the gum content. Days taken to 50% flowering and days taken to maturity were assigned with decreased trait value to identify the short crop cycle, leading to high seed yield coupled with high gum content.

# 2.4.1. Mean Performance and Stability of Multiple Traits

## Rescaling the Traits

The first step was to compute a stability analysis and obtain the WAASB index that is the Weighted Average of Absolute Scores from the singular value decomposition of the matrix of BLUPs for the genotype-environment interaction effect [31] for each genotype. Then, we rescaled both the mean performance (e.g., grain yield) and stability (WAASB index) to a 0–100 range, in which 0 is the most undesired value and 100 the most desired value [31]. The rescaled value of a given trait for the *i*<sup>th</sup> genotype for both mean performance (*rY*<sub>i</sub>) and stability (*rW*<sub>i</sub>) is given by Equation (1):

$$rY_{i} = rW_{i} = \frac{n_{nj} - \varphi_{nj}}{n_{oj} - \varphi_{oj}} \times (\theta_{ij} - \eta_{oj}) + \eta_{nj}, \tag{1}$$

where:  $\eta_{nj}$  = New maximum value for the *j*<sup>th</sup> trait after rescaling;  $\varphi_{nj}$  = New minimum value for the *j*<sup>th</sup> trait after rescaling;  $\eta_{oj}$  = Original maximum value of the *j*<sup>th</sup> trait after rescaling;  $\varphi_{oj}$ = Original minimum value of the *j*<sup>th</sup> trait after rescaling;  $\theta_{ij}$  = Original value for the *j*<sup>th</sup> trait of the *i*<sup>th</sup> genotype/treatment.

The values for  $\eta_{nj}$  and  $\varphi_{nj}$  for the traits in which negative gains are desired and for the stability of all traits (lower WAASB values are desired) would be  $\eta_{nj} = 0$  and  $\varphi_{nj} = 100$ , whereas the traits in which positive gains are desired, then, the values will be vice-versa, i.e.,  $\varphi_{nj} = 100$  and  $\eta_{nj} = 0$ . In the rescaled two-way table ( $rY_{ior} rW_i$ ), each column will have 0–100 range that considers the desired sense of selection (increase or decrease) and maintains the correlation structure of the original set of variables representing that the ideal treatment will be having a value of 100 for almost all the traits after rescaling.

# The WAASBY Index

To account for the mean performance and stability of individual traits, the WAASBY index [31] was then computed.

$$WAASBY_i = \frac{(rY_i \times \theta_Y) + (rW_i \times \theta_W)}{\theta_Y + \theta_W},$$
(2)

where *WAASBY*<sub>*i*</sub> is the superiority index for the *i*thgenotype;  $rY_i$  and  $rW_i$  are the rescaled values (0–100) for the response trait (Y) and the stability (WAASB), respectively; and  $\theta_y$ 

and  $\theta_W$  are the weights for the mean performance (e.g., grain yield) and stability (WAASB), respectively. In our study, we considered a higher weight for mean performance at the expense of stability. A two-way table containing the WAASBY index of each genotype in each trait ( $rX_{ij}$ ) was then obtained and used in the following procedure.

# Factor Analysis

The third main step is to compute an exploratory factor analysis with rXijto group correlated traits into factors and then estimate the factorial scores for each genotype as follows:

$$\mathbf{X} = \mathbf{\mu} + \mathbf{L}\mathbf{f} + \mathbf{\epsilon},\tag{3}$$

where: **X** =  $p \times 1$  vector of observations; **µ** =  $p \times 1$  vector of standardized means; **L** =  $p \times f$  matrix of factorial loadings; **f** =  $p \times 1$  vector of common factors; and  $\varepsilon = p \times 1$  vector of residuals, being p and f the number of traits and commonfactors retained, respectively.

The eigen values and eigen vectors were obtained from the correlation matrix of  $\mathbf{r}\mathbf{X}_{ij}$ . The initial loadings were obtained considering only factors with eigenvalues higher than one. Then, the *varimax* rotation criteria [32] was used for estimating final loadings and for the analytic rotation. Then, the genotypes were scored following the equation:

F

$$= \mathbf{Z} \left( \mathbf{A}^{\mathrm{T}} \, \mathbf{R}^{-1} \right)^{\mathrm{T}},\tag{4}$$

where:  $\mathbf{F} = g \times f$  matrix with the factorial scores;  $\mathbf{Z} = g \times p$  matrix with the (rescaled) standardized means;  $\mathbf{A} = p \times f$  matrix of canonical loadings;  $\mathbf{R} = p \times p$  correlation matrix between the traits; and *g*, *f*, and *p* refers to the number of genotypes, factors retained, and analyzed traits, respectively.

#### Ideotype Planning and the MTSI Index

By definition (Equation (1)), the ideotype has the highest rescaled value (100) for all the analyzed desirable traits. Thus, the ideotype can be defined by a  $l \times p$  vector I such that **I** = [100,100...,100]. The scores for I is also estimated according to Equation (3).

The fourth step of the analysis is to estimate the multi-trait stability index [23] using the Equation (5):

MSTI<sub>i</sub>= 
$$\left[\sum_{j=1}^{f} (F_{ij} - F_j)^2\right]^{0.5}$$
, (5)

where the MTSI is the multi-trait stability index for the *i*<sup>th</sup> genotype,  $F_{ij}$  is the *j*<sup>th</sup> score of the *i*<sup>th</sup> genotype, and  $F_j$  is the *j*<sup>th</sup> score of ideotype. The genotype with the lowest MTSI is then closer to the ideotype and, therefore, presents a high mean performance and stability (MPE) for all variables analyzed. The functions waasb and mtsi of the "metan" package [29] were used to compute the MTSI index.

## 2.4.2. Mean Performance of Multiple Traits within Environments

The multi-trait genotype-ideotype distance index (MGIDI) proposed by Olivoto and Nardino [24] was used to identify genotypes that perform well for most of the traits within each environment. The MGIDI has the same theoretical foundation as the MTSI (rescaling the trait, computing the factor analysis, and the distance from each genotype to the ideotype), with a key difference that the re-scaled matrix used to compute the factor analysis in the MGIDI is obtained with the BLUP for genotype (mean performance), rather than the WAASBY (mean performance and stability), in the MTSI. It is important to note that, if the weight for the mean performance ( $\theta_Y$ ) in the MTSI is 100 for all traits under study, the genotype classification by the MTSI will become identical to the MGIDI since stability will not take into account. The genotype with the lowest MGIDI is then closer to the ideotype representing desired values for all the assessed traits within each environment.

The proportion of the MGIDI index of the i<sup>th</sup> genotype explained by the  $j^{th}$  factor ( $\omega_{ij}$ ) was used to show the strengths and weaknesses of genotypes within each environment, and it was computed as:

$$\omega i j = \frac{\sqrt{D_{ij}^2}}{\sum_{j=1}^f \sqrt{D_{ij}^2}}$$
(6)

where  $D^{2ij}$  is the distance between the i<sup>th</sup> genotype and the ideotype for the j<sup>th</sup> factor. Factor with low contributions represents that the traits will be closer to ideotype. The functions gamem and mgidi of the "metan" package [29] were used to compute the MGIDI index. The analyses were conducted in collaboration with colleagues from King Saud and Princess Nourabint Abdulrahman Universities.

# 2.4.3. Estimation of Heritability and Selection Differential

# Heritability

Estimation of heritability in broad-sense heritability (h<sup>2</sup>) of all traits was calculated according to the formula as described by Allard and Bradshaw [33]:

h<sup>2</sup> (Heritability)= 
$$[(\sigma^2 G)/(\sigma^2 P)] \times 100$$
,

where:  $h^2$  = heritability;  $\sigma^2 G$  = Genotypic variance;  $\sigma^2 P$  = Phenotypic variance.

#### Selection Differential(S)

It is computed as the difference between the mean of the selected parents (PS) and mean of the population before selection (PO) and is symbolized as (S). It is a measure of the phenotypic superiority of the selected parents over the population from which the parents were selected [34].

# 3. Results

# 3.1. Outcome by Analysis of Variance (ANOVA)

Typical multi-environment trials data was used to compute ANOVA by the function anova\_ind for each environment with a randomized complete block design [35]. The outcome of ANOVA depicts that all the fifteen traits were statistically significant ( $p \le 0.05$ ) in each of the environments studied (E1, E2, and E3). The variation in traits for 85 genotypes is depicted in Figure 2 for grain yield, and the remaining 14 traits in Figure 3 as box plots. Box plots represent the information on the range, mean, and variation concerning the trait it represents. Irrespective of the seasons and genotypes, the grain yield (GY) plant<sup>-1</sup> ranged from 75 to 780 g with a coefficient of variation (CV) of 37.37 percent. Maximum variability is noticed for traits, like pods per plant and plant height. Meanwhile, the variation is least for gum percent and 100 seed weight. The CV was highest for the number of branches plant<sup>-1</sup> (67.14%) with a range of 0 to 22, followed by clusters plant<sup>-1</sup> (36.53%), carbohydrates (11.08), and the variation was least for days taken for maturity (3.97) with a range of 87–110 days. This indicates the variability of genotypes for different traits considered in this study.



Figure 2. Box plots depicting the variation in grain yield (GY) for individual genotypes over seasons. The dashed line shows the overall mean.



Figure 3. Box plot depicting the variation in different characters (traits) of genotypes over seasons used in this study.

# 3.2. Selection Across the Seasons with MTSI

Linear Correlation between Traits, Loadings, and Factor Delineation

The traits, such as pods plant<sup>-1</sup> with plant height, pods plant<sup>-1</sup> with clusters plant<sup>-1</sup>, number of branches with pods plant<sup>-1</sup>. No. of branches and grain yield with grains per pod, and gum trait with 100 seed weight, were positive and strongly correlated (r > 0.75 and p < 0.001), whereas the plant height with days to fifty percent flowering were negatively correlated.

The factors with eigen values >1 were retained (six factors). Table 3 depicts that these six factors account for about 68 percent out of the total variation recorded among the traits. Therefore, there are possibilities to reduce the dimensionality of data by keeping high explanatory strength. The 15 traits under study were grouped into the six factors (FA) as follows:

Factors	Traits Considered
FA1	Days taken to 50% flowering, Clustersplant <sup>-1</sup> , Pods plant <sup>-1</sup> , and carbohydrates
FA2	Plant height, Grains per pod <sup>-1</sup> , Grain Yield
FA3	Days taken to maturity and Fiber traits
FA4	Number of branches plant <sup>-1</sup>
FA5	Protein andFat
FA6	100 seed weight, Gum and Ash

Orthogonal rotation results to factor loadings which range from -1 to +1 and are the correlation coefficients among the traits and the factor.

**Table 3.** Factorial loadings obtained using varimax rotation and communalities resulted during the factor analysis.

Variable	FA1	FA2	FA3	FA4	FA5	FA6	Communality	Uniqueness
DFF	-0.50	-0.09	-0.14	-0.43	-0.34	0.07	0.58	0.42
DM	-0.20	0.22	-0.57	-0.02	-0.14	0.09	0.44	0.56
PH	-0.31	-0.69	-0.12	0.07	-0.12	0.14	0.63	0.37
СР	-0.87	-0.06	0.02	0.14	-0.04	0.07	0.79	0.21
PP	-0.85	-0.16	-0.10	0.05	0.05	-0.04	0.76	0.24
GP	0.20	-0.49	0.33	-0.41	0.25	0.10	0.63	0.37
Branches	-0.02	-0.20	0.13	0.83	-0.15	-0.03	0.77	0.23
100SW	-0.26	0.33	0.17	0.36	0.14	0.49	0.59	0.41
GY	0.02	-0.82	0.12	0.12	0.16	0.00	0.74	0.26
Gum	0.08	-0.09	0.10	-0.15	0.00	0.87	0.81	0.19
Protein	0.10	-0.09	0.10	-0.02	0.75	0.34	0.71	0.29
Fat	-0.01	0.03	0.10	0.10	-0.86	0.07	0.77	0.23
Fiber	-0.20	0.11	0.74	0.15	-0.13	0.04	0.65	0.35
Ash	-0.08	0.14	0.39	-0.04	-0.16	-0.74	0.75	0.25
Carbohydrates	-0.60	0.18	0.20	-0.12	-0.14	-0.25	0.54	0.46

FA: Factors analysis, DFF: Days taken to 50%flowering, DM: Days taken to Maturity, PH: Plant Height, CP: Clusters plant<sup>-1</sup>, PP: Pods plant<sup>-1</sup>, GP: Grains pod<sup>-1</sup>, 100SW:Hundred Seed Weight, GY: Grain Yield.

MTSI index analysis assigns rank to all the 85 studied genotypes based on desired value of the trait (Figure 4). The selection pressure (~15%) was used to identify the top thirteen genotypes, which included IC-415106, IC-373480, IC-420373, IC-420332, IC-402299, IC-415131, IC-421798, IC-402301, IC-415115, IC-420320, IC-415158, IC-415123, and IC-415151, and which were further used to compute selection differentials. The selection differential is the difference in mean phenotype between individuals selected and the population mean.



Figure 4. Ranking of genotypes based on MGIDI index.

The MTSI Index provided desired selection differential (SD) for 12 out of 15 studied traits with success frequency of 80% in selecting desired traits. The remaining three traits with undesired SD were Days to fifty percent flowering (SD= -2.11), Protein (SD= -0.12), and Ash (SD= -0.03). The top thirteen genotypes across the environment showed desired values for most of the guar traits. The selection differential (SD) percent for traits ranged from -5.80% (Days to 50% flowering) to 39.71% (Grain yield). The traits with high heritability were Grain yield (99%), followed by Protein and Gum (94%). The next best heritability percentage was observed in Days taken to 50% flowering (93%) and Fat, as well as the Carbohydrates (90%). The remaining traits showed little less heritability coupled with a high value of selection differential (Table 4).

Variables	Factor	Хо	Xs	SD	SD Percent	ercent h <sup>2</sup> Ind	
DFF	FA 1	36.43	34.32	-2.11	-5.80	0.93	Decrease
СР	FA 1	16.35	21.36	5.01	30.62	0.60	Increase
PP	FA 1	74.64	91.95	17.31	23.19	0.77	Increase
Carbohydrates	FA 1	44.52	45.90	1.39	3.12	0.91	Increase
PH	FA 2	70.26	78.75	8.49	12.08	0.68	Increase
GP	FA 2	4.95	5.42	0.47	9.58	0.72	Increase
GY	FA 2	377.39	527.27	149.88	39.71	0.99	Increase
DM	FA 3	98.99	99.24	0.26	0.26	0.87	Decrease
Fiber	FA 3	5.92	6.25	0.32	5.47	0.90	Increase
Branches	FA 4	5.00	6.46	1.45	29.07	0.77	Increase
Protein	FA 5	28.99	28.86	-0.12	-0.42	0.94	Increase
Fat	FA 5	3.76	3.90	0.14	3.80	0.93	Increase
100SW	FA 6	3.44	3.59	0.15	4.27	0.86	Increase
Gum	FA 6	27.84	28.93	1.09	3.93	0.94	Increase
Ash	FA 6	4.79	4.76	-0.03	-0.66	0.91	Increase

Table 4. Factors linked to correlated traits, selection differential, heritability, and indicators.

# 3.3. Selection Within Seasons with MGIDI

Table 5 shows 13 superior guar genotypes selected in three different environments, i.e., E1, E2, and E3, based on the MGIDI index.

E1	E2	E3	Common Genotypes to All Seasons
IC-373480	IC-415106	IC-421798	IC-415106
IC-420373	IC-402299	IC-402301	IC-420320
IC-415106	IC-373480	IC-415158	IC-402301
IC-402299	IC-420373	IC-415106	
IC-420320	IC-311431	IC-421242	
IC-402301	IC-420320	IC-421834	
IC-415146	IC-402301	IC-415125	
IC-373497	IC-324020	IC-420320	
IC-324020	IC-311449	IC-421835	
IC-370516	IC-415146	IC-420332	
IC-415158	IC-415115	IC-402302	
IC-415115	IC-373497	IC-402304	
IC-420332	IC-415123	IC-415135	

**Table 5.** Genotypes selected in the individual environment and across the environments.

# 3.3.1. Coincidence Index

The coincidence index was computed based on comparing the genotypes selected within each environment. Three genotypes were found common across the environments, i.e., IC-415106, IC-420320, and IC-402301, as indicated in Venn plot (Figure 5 and Table 6).

Table 6. Common genotypes between the environments based on coincidence index.

Variable-1	Variable-2	Index	<b>Common Genotypes</b>
			IC-373480,IC-420373,
			IC-415106,IC-402299,
MGIDI-Kharif-2013	MGIDI-Kharif-2014	72.85	IC-420320,IC-402301,
			IC-415146,IC-373497,
			IC-324020,IC-415115
			IC-415106,IC-420320,
MGIDI-Kharif-2013	MGIDI-Summer-2013–14	27.60	IC-402301,IC-415158,
			IC-420332
MGIDI-Kharif-2014	MCIDI Summar 2012, 14	0 50	IC-415106,IC-420320,
	MGIDI-Summer-2013–14	9.50	IC-402301



Figure 5. Common genotypes (Table 6) shared across the environments shown in Venn chart.

## 3.3.2. Selection Gains Within the Seasons

MTSI index provided selection gains within the seasons, and the results revealed that most of the traits under study contributed towards the selection of desired guar genotype during E1 and E2. Traits, viz. protein, DM, and DFF during E1 and gum content and protein, as well as DFF during E2,was found least contributing in the selection gain process. The selection differential percent in both the seasons was found higher for the grain yield and lower for the protein content (–5.16 and 37.21% in E1; and –6.88 and 31.39% in E2, respectively. However, the heritability percentage (h<sup>2</sup>) was highest for the grain yield, i.e., 97 and 96% in E1and E2, respectively, followed by protein content, with 94% during both seasons. In the case of E3, the SD percent varied from –10.33 (DFF) to 46.35 percent (Branches). The h<sup>2</sup> was highest for grain yield (0.99%), followed by pods per plant (0.97%) (Table 7).

VAD	CD Demonst (E1)	$\mathbf{h}^2$	SD Percent	h²	SD Percent	$\mathbf{h}^2$
VAK	SD Percent (E1)	(E1)	(E2)	(E2)	(E3)	(E3)
PH	12.35	0.91	13.64	0.94	18.15	0.91
СР	29.36	0.79	17.71	0.82	24.10	0.89
PP	21.06	0.91	10.12	0.90	28.48	0.97
GY	37.21	0.97	31.39	0.96	4.55	0.99
Gum	0.54	0.93	-2.39	0.87	-0.82	0.69
Protein	-5.16	0.94	-6.88	0.94	-3.18	0.73
Ash	3.94	0.82	4.09	0.82	1.47	0.46
CARB	2.91	0.71	4.10	0.71	2.77	0.53
DM	-0.95	0.70	0.15	0.68	0.89	0.87
Fat	5.74	0.88	5.87	0.88	2.29	0.54
DFF	-1.06	0.85	-0.84	0.89	-10.33	0.92
Branches	20.22	0.93	21.81	0.73	46.35	0.80
100 SW	1.33	0.36	1.92	0.36	6.56	0.81
GP	6.31	0.65	9.16	0.66	0.00	0.00
Fiber	8.24	0.81	10.25	0.81	1.97	0.36

Table 7. Selection gains within the seasons assessed by MTSI index.

## 3.3.3. View on Strengths and Weakness of the Genotypes

Figure 6a–c depicts the genotype's strengths and weaknesses. Factor's contribution towards MGIDI is categorized into two as less and more contributing factors. The factors which contribute more were plotted close to the center, whereas lesser contributing factors were plotted towards the edge.

A view on strengths and weakness during E1 reveals that 13 selected genotype's performance was more towards majority of traits in FA1 group, such as plant height, pods plant<sup>-1</sup>, and clusters plant<sup>-1</sup>, as well as the grain yield. Out of 13 genotypes, ten showed strength towards gum, protein, ash, and carbohydrates grouped under FA2. Days to maturity and fat (FA3) were found more contributing factors towards the selection of most of the genotypes, except IC-415158 and IC-415146. FA4 group was found less contributing in selecting genotypes. However, FA5 and FA6 groups showed strength towards the selection of genotypes in environment 1 (Figure 6a).

Figure 6b reveals that the selected genotypes in E2 had strength towards FA4, which was the most contributing factor for most of the genotypes selection. FA2 strength towards the selection of the genotypes IC\_373497 and IC\_415115. Most of the genotypes performed well in FA3 group, except IC-402301, IC-311431, and IC-402299. FA5 group stands out for the selection of genotypes, viz. IC\_415106 and IC\_402299, in the corresponding environment, whereas FA1 and FA6 showed little strength to the selected genotypes.

The strength and weakness view during E3isdepicted in the Figure 6c. Factor FA1 showed greater strength towards the most selected genotypes in the corresponding environment; however, IC-415125, IC-415106, and IC-402301 performed weakly. The factors

FA2 and FA3 have weakness towards most selected genotypes. Most of the selected genotypes performed well within FA4. However, FA5 contribution was only for few selected genotypes, viz. IC-402302, IC-415135, and IC-415106 (Figure 6c).

The data pertaining to the traits of selected genotypes in comparison with the overall mean traits of all the genotypes, along with the coefficient of variation and the mean, as well as the range, is presented in the Table 8. It can be inferred that the selected genotypes were found to be superior for most of the traits compared to the overall mean traits of all the genotypes across the seasons. This clearly indicates the ability of the presented methodology in identifying the superior genotypes that can be employed as ideotypes for further breeding of guar towards high grain yield with superior biochemical traits.



Figure 6. *Strengths* and *weakness* view of the stable genotypes identified in E1 (a), E2 (b), and E3 (c).

Genotype		DFF	DM	PH	СР	PP	GP	BRAN	100SW	GY	GUM	PROTEIN	FAT	FIBER	ASH	CARB
IC-415106	Mean	33.33	97.00	66.88	22.83	103.37	6.23	10.80	3.77	676.69	21.41	22.97	4.62	6.67	6.53	50.64
	CV	5.59	2.16	19.54	19.46	7.39	22.54	10.48	3.76	9.73	8.81	9.08	11.79	11.73	12.02	4.33
	MIN	31.00	94.00	44.00	18.00	90.40	4.00	9.00	3.62	622.00	18.96	20.20	4.04	5.37	5.65	47.65
	MAX	36.00	100.00	79.50	30.00	110.00	7.40	12.00	4.00	779.96	24.00	25.40	5.60	7.40	7.75	53.60
IC-420320	Mean	34.33	102.67	82.02	28.33	81.45	5.97	7.10	4.02	547.51	29.35	31.53	2.67	6.26	4.71	43.26
	CV	4.76	3.36	4.32	11.31	9.31	22.38	53.85	2.45	10.63	16.06	10.73	38.34	13.21	21.57	7.31
	MIN	33.00	98.00	76.60	24.00	71.50	4.00	2.00	3.90	486.40	25.64	27.15	1.85	5.03	3.25	38.85
	MAX	37.00	108.00	86.50	32.00	90.00	7.40	11.60	4.10	652.83	38.20	36.40	4.50	7.40	6.15	47.30
IC-402301	Mean	31.67	95.83	79.48	25.93	112.28	4.13	8.10	3.40	355.72	22.10	23.22	4.30	6.69	5.48	49.79
	CV	4.31	2.03	18.84	23.11	9.70	30.40	34.35	4.92	12.38	9.80	9.22	7.72	12.24	19.73	3.65
	MIN	30.00	93.00	55.00	20.00	102.40	2.80	5.60	3.20	315.00	18.96	20.40	3.90	5.37	4.00	47.65
	MAX	33.00	99.00	92.50	36.00	132.80	5.60	12.00	3.60	423.00	24.50	25.40	4.80	7.80	6.95	52.20
Not selected	Mean	36.45	98.99	70.17	16.34	74.59	4.94	5.00	3.44	376.86	27.81	28.98	3.76	5.92	4.79	44.50
	CV	11.18	3.97	25.94	36.53	29.00	21.67	67.14	10.30	37.37	18.95	11.64	21.02	19.65	19.66	11.08
	MIN	24.00	87.00	19.00	4.00	14.40	2.00	0.00	2.10	75.00	12.84	19.35	1.85	2.69	2.80	21.85
	MAX	49.00	110.00	115.00	40.00	146.00	8.50	22.00	4.20	779.96	42.40	36.40	5.60	8.55	8.75	55.00

Table 8. Comparison of traits of selected genotypes with the average values of the not selected genotypes.

## 4. Discussion

Guar/clusterbean is one of the most popular vegetable and commercially important crop extensively grown in semi-arid regions of India. Its drought tolerating capacity and nitrogen-fixing ability have made this crop suitable to drought-prone areas of India [12,13]. Among many leguminous crops grown in India, guar stands as a unique crop due to its gum content commercially known as galactomannan. The gum and its derivatives extracted from guar have significant role in various forms of industries, like textiles, cosmetics, paper, food, oil fricking and drilling, pharmaceuticals, and so on. Due to its wider applications in different industrial sectors, many countries import guar gum from India, contributing maximum towards foreign exchange earnings [14]. This urges a need to develop genotype/cultivar of guar with optimum yielding potential and qualitative gum content even when grown under varied environmental conditions. In this context, MET (Multi-Environmental Testing) serves as a tool that describes the adaptability, as well as stability, of genotypes across different environments [33]. To develop a stable genotype that performs better under different environmental situations, interaction between genotype and environment needs to be clearly understood [36]. Genotype-environment interaction is a very complicated process involving genetic and non-genetic factors. The genotype which outrages all the climatic vagaries and performs better at both congenial, as well as in unfavorable, environments is termed as 'stable genotype'. Despite the good yielding potential, if the cultivar is not stable, that is of no use [37–39]. The selected genotype should be amenable towards crop management practices and soil fertility status which serves as a precursor for increasing yield and yield attributing traits of guar, along with qualitative commercially important gum content.

Genotypes cultivated under different environmental conditions will not respond similarly due to fluctuations in seasons, locational heterogeneity, and their complex interactions [40]. Thus, it is a great challenge to identify genotypes that remain stable across divergent environmental conditions [41]. Even though many researchers have identified genetic variation towards the adaptability process, inbreeding variation of the genotypes has not been exploited due to difficulties in describing and measuring phenotypic characters that determine stability [42]. Scientists also attempted to examine genotypic behavior under diverse climatic conditions, and a few such examples are: Comstock and Robinson [43], who studied interaction between genotype and environment involving genotype means at different locations in different years; and Finlay and Wilkinson [44], who measured phenotypic stability using statistical approach that was proved efficient in determining the genotype performance. They also showed that the relationship between genotypeenvironment would be linear if both the environment and genotypic factors follow similar scale of measurement. These results were based on two parameters they studied, i.e., means of genotypes and coefficients of regression. Highly fluctuating environmental conditions in the targeted area lead to bad performance of genotypes [45]. Blum [46] reported that yield trait should not be considered in selection procedure as it is highly influenced by environmental factors, leading to misinterpretation during estimation of genetic basis.

The traditional methods of stability analysis employed based on univariate and analyzed on mean, regression, and deviation from regression. Multivariate techniques were used to assess genotypic stability across divergent environments as univariate method proved inefficient in stability assessment [47]. As advanced in breeding techniques adoption of multi-trait selection index [18,19], most of the plant breeders are not interested in adopting this index either for initial trials [48] or advanced plant breeding trials [49] that are usually demonstrated in diverse environmental conditions [23,50–52]. As the efficiency of multivariate techniques to solve the multicollinearity issues seems to be high when dealing with multi-traits [21,23,53], therefore, to bridge the gaps in traditional methods of stability analysis, Olivoto and Nardino[24] developed an index that can overcome the defects of all the traditional methods, wherein all the selected traits favorably satisfy the breeding applications [54]. In this context, multivariate novel techniques, viz. MTSI and MGIDI indexes, were employed for identifying stable genotypes with multiple traits suitable for wider adaptations.

## 4.1. Analysis of Variance

The ANOVA being an additive technique fairly describes the main effects in stability analysis [55]. All the fifteen traits in each environment were found significant ( $p \le 0.05$ ), and these results corroborate with the data reported in the literature of Jain and Patel [56]. They carried out an experiment to identify stable guar genotypes with phenotypic traits based on the performance of seed yield and stability index of thirteen guar genotypes at two stations for two years, wherein the variance shown significant for studied traits.

#### 4.2. Genotypes Selection Based on Multivariate Approach

The results of the present investigation suggest that selection of stable guar genotypes based on multivariate analysis is effective and that most of the productive and adopted guar genotypes were influenced by most of the climatic factors, edaphic factors, and genetic factors, as well as with genetic and environmental interactions. Rashidi et al. [57] performed Additive Main Effects and Multiplicative Interaction (AMMI) technique to study chickpea grain yield as influenced by diverse environmental conditions, and the outcome revealed that the yield was mainly determined by genotype, environment, and their interaction. They also indicated nearly 82 percent variation in genetic traits and high fluctuating environment lead to larger differences in grain yield of bengalgram. The data analyzed in the present stability assessment study recommended that the three genotypes', IC-415106, IC-420320, and IC-402301, performance was stable under divergent environmental conditions with desired values for the selected traits, such as plant height, grain yield, clusters plant<sup>-1</sup>, pods plant<sup>-1</sup>, and biochemical traits. These findings corroborate with Arunkumar et al.'s[58]report, where they conducted the multi-location trials on yield significance and a stability index based on the Eberhart and Russel [59] model and were also able to identify two genotypes out of ten genotypes shown stable performance across five locations to corresponding traits. The major issue is that the seeds of the guar crop become black for rain at the time of harvest. The selected guar genotypes are shown tolerance to discoloration for excess moisture at harvest.

View on the strengths and weaknesses (Figure 6) provided by MGIDI made an easy identification of genotype's strengths, as well as weaknesses, depending on multiple trait framework. The selected genotypes (IC-415106, IC-420320, and IC-402301) had the high productive capability not only with grain yield also with gum, protein, fiber, and ash contents of the guar crop. A similar study was performed by Gabriel et al. [60] and Olivoto and Nardino [24], who studied 13 strawberry cultivars, wherein the strengths were reported towards high productivity, TSS, and flesh firmness for the Albion cultivar employing MGIDI index R-based package.

Most of plant breeders employed traditional stability indices analysis based on firstdegree statistics. The decision to select the stable genotype on mean, regression, and deviation from regression parameters may be not sufficient to provide a simple interpretation of mean performance and stability of multiple traits. Therefore, the MTSI technique as "metan" R package is an advanced quantitative genetic tool to exploit suitable varieties in all crop species. These findings were similar to the work of Koundinya et al. [61], who tried to assess twenty-five cassava genotypes using MTSI to analyze genotype-environment interaction involving the traits, viz. leaf area index, yield per plant, harvest index, dry matter, and starch yield per plant. Out of 25 genotypes, four cassava genotypes namely, 8S501, Sree Athulya, CR43-7, and 9S127 stand out as desirable genotypes with better mean performance and stability under multi-environment conditions.

The importance/promptness of the MGIDI in evaluating field or commercial crop cultivars is known to expand extensively, and it is called adopting to Speed breeding. The essential point needs to be understood, and the ultimate goal of plant breeders is to explore the application of multivariate techniques in selecting suitable genotype with effective productivity performing better in varied environmental conditions. In the present investigation, the genotypes were categorized as desired and undesired based on multiple traits information (Figure 4). The genotype's strengths and weakness assessment (Figure 6) serves as a novel tool to formulate better crop management strategies. Adoption of MTSI and MGIDI indexes in future stability assessment studies are known to minimize unnecessary calculations; thus, the recommendation of superior cultivars in plant breeding studies concerning field/commercial crops will be at ease.

# 5. Outcome of the Study

The multi-trait framework provided by the multi-trait stability index (MTSI) provides an easy way to select high-performance and stable cultivars in plant breeding experiments with guar (*Cyamopsis tetragonoloba* L.) that perform better at varied environmental conditions. In the present investigation, the genotypes selected by the MTSI index presented desired values for 13 out of 15 productive and biochemical traits. The view on genotype's strengths and weakness assessed by selection procedure performed within environments using multi-trait genotype-ideotype distance index (MGIDI) indicated the importance of an ideal genotype with improved morphological quantitative traits, such as clusters plant<sup>-1</sup>, pods cluster<sup>-1</sup>, grains pod<sup>-1</sup>, branches plant<sup>-1</sup>, and plant height. In addition, the strengths of selected genotypes are due to productive crude protein, fiber, and gum. Overall, MTSI to identify wide adaptations, and MGIDI to identify superior genotypes within environments, provides a new framework of multivariate techniques to carry out stability assessment that will optimize the use of resources, as well as time, thus contributing to the sustainability of guar breeding programs worldwide.

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