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A Trait Pyramiding Approach for Development of Non-Parametric Stability Models in Chrysanthemum (*Chrysanthemum Morifolium*) Crop Varietal Release

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SUMMARY

Plant breeding forms a vital part of horticulture, contributing significantly to the creation of new varieties with desirable traits. Diverse breeding methodologies have been adopted to achieve improvements in yield, biotic stress resistance, nutritional attributes, and adaptability to varying environments. In this pursuit, stability models are developed to release a promising line as a variety at the end of the evaluation trials. However, regular approach results in various stable line for various traits, making difficult for a holistic recommendation. Thus, through trait pyramiding concept, non-parametric stability models are developed to identify stable line(s) across all traits. This concept is demonstrated using various yield attributing traits of 15 Chrysanthemum lines evaluated at ICAR-Indian Institute of Horticultural Research, Bengaluru during 2023 to 2025. R-codes were developed for the analysis. Based on the Non-parametric stability models of Venugopalan indices, it was found that IIHR 4-8 and IIHR 2-16 are stable across all traits evaluated such as flower diameter (cm), number of flowers/plant, flower stalk length (cm), plant height (cm), plant spread (cm). These stable lines will be utilized effectively to release a variety holistically combining all desirable traits in crop hybridization trials, emphasizes the need to construct a non-parametric index for assessing the stability of a set of lines collectively based on various traits evaluated over multiple seasons or years in an experimental setup.

Keywords: Chrysanthemum; Non-parametric index; R-codes; Stability models; Trait pyramiding.

1. INTRODUCTION

Chrysanthemum (*Chrysanthemum morifolium*), belonging to the family Asteraceae, is one of the most important commercial floricultural crops, valued for its wide adaptability, diverse flower forms, and year-round market demand. In crop improvement studies, strong genotype × environment interactions often lead to variations in yield, flowering time, and floral quality across different locations and seasons, making the identification of stable and high-performing genotypes a major challenge in breeding programs. This complicates the development of a comprehensive recommendation for farmers that integrates all desirable traits into a single, stable line. In this context, non-parametric stability models offer a robust approach for evaluating Chrysanthemum genotypes without relying on strict distributional assumptions. By ranking genotypes across multiple environments and estimating stability based on rank consistency across evaluated traits, these models effectively identify lines that combine superior performance with wide adaptability. Such approaches are particularly valuable in ornamental crops like Chrysanthemum, where trait expression is highly influenced by environmental fluctuations.

Traditional parametric methods are commonly employed in plant breeding programs to evaluate the stability of genotypic performance, primarily through analysis of variance and related statistical measures. However, in many practical breeding scenarios, the relative ranking of genotypes is often more

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important than the absolute magnitude of variation. This underscores the importance of alternative nonparametric approaches, which rely on rank-based comparisons and offer an effective means of assessing the consistency of crop variety performance across different environments (Venugopalan *et al.,* 2025).

The second approach involves nonparametric methods that relate environments and phenotypes to biotic and abiotic factors without the need for specific modeling assumptions. Most breeding programs integrate both parametric and nonparametric approaches for practical decision-making (Becker and Leon, 1988). However, when key statistical assumptions - such as normality of errors and homogeneity of interaction effects - are violated, the performance of parametric stability methods may be compromised (Huehn, 1990). Consequently, parametric tests of variance significance and variance-based stability measures are sensitive to deviations from these assumptions. To address these limitations, nonparametric stability measures, which are less dependent on distributional assumptions, have been proposed as robust alternatives (Huhnand Nassar, 1989; Nassarand Huhn, 1987). In recent years, the application of nonparametric stability analyses has increased markedly in the evaluation of breeding materials, as demonstrated in china aster (Venugopalan *et al.* 2025), sunflower (Balalic *et al.* 2011; Tabrizi, 2012; Noruzi and Ebadi, 2015), soybean (Manjubala *et al.* 2018; Goksoy *et al.* 2019; Cubukcu *et al.* 2021), barley (Vaezi *et al.* 2018), faba bean (Arya and Verma, 2022), and oat (Kebede *et al.* 2023).

Non-parametric methods offer several distinct advantages over parametric models in varietal evaluation. These include (i) reduced sensitivity to outliers arising from environmental variability, (ii) the absence of assumptions regarding the normality of trait distributions, and (iii) the ability to identify consistent deviations and linear responses without imposing restrictive model requirements (Huhn, 1996). In this context, the present study evaluates various non-parametric approaches and introduces a novel index, supported by case studies from experiments conducted on okra at ICAR - IIHR, Bengaluru. The proposed framework can be effectively applied in varietal evaluation and release programs across diverse crop species. The study specifically aims to identify the most stable line(s) across years in Chrysanthemum, based on multiple traits, including flower diameter (cm), number of flowers per plant, flower stalk length (cm), plant height (cm), and plant spread (cm).

2. MATERIALS AND METHODS

2.1 Data base

A total of 15 Chrysanthemum (IIHR4-8, IIHR2-16, Fish Tail, Shanti, Jayanti, Jubilee, Maghi White, Himani, Kundan, NBRI Little Orange, NBRI Little Kusum, DFRC 4, Bidhan Chitra, Bidhan Manasi, Arka Pink Star (check)) lines/varieties were evaluated for flower quality and yield attributing traits such as flower diameter (cm), number of flowers/plant, flower stalk length (cm), plant height (cm) and plant spread (cm) at the experimental field of ICAR-Indian Institute of Horticultural Research, Bengaluru during three successive years from 2023 to 2025 (summer season).

2.2 Parametric approach of stability analysis

Traditional parametric stability analysis based on standard Eberhart-Russell and Freeman-Perkins method have been widely used in horticultural crop improvement research. These methods have been applied in various studies, such as those involving onions (Venugopalan & Veere Gowda, 2005), watermelon (Venugopalan & Pitchaimuthu, 2009), and chili peppers (Venugopalan & Reddy, 2010). However, regular approach results in various stable line for various traits, making difficult for a holistic recommendation. The limitations of parametric methods highlight the need for appropriate non-parametric approaches that provide a more comprehensive assessment of crop genotypes. This, in turn, emphasizes the importance of developing a combined stability measure that integrates all key traits, since biological traits are inherently interrelated. Consequently, the development of a common composite index becomes essential. As a next step, with a view to identify any such inherent interrelationships among traits, a well-known Principal Component Analysis (PCA) was employed to quantify the proportion of variability explained. (Venugopalan *et.al* 2025). This also justifies the necessity of developing a combined index based on all traits.

2.3 Nonparametric approach of stability analysis:

Several nonparametric indices have been developed to evaluate yield stability (Thennarasu, 1995; Nassar and Huhn, 1987). These methods rely on the relative ranking of genotypes across the test environments. The ranking is based on values of Y_{ij} with lowest Y_{ij} value receiving the rank 1, the next higher value 2 and so on. The non-parametric stability statistics derived from these genotype yield ranks across environments are described here.

$$NP_i^{(1)} = \frac{1}{n}\sum \left| r_{ij}^* - Md_i^* \right|$$

$$NP_i^{(2)} = \frac{1}{n}\left[\sum_{j=1}^{n} \left| r_{ij}^* - Md_i^* \right| \div Md_i \right]$$

$$NP_i^{(3)} = \frac{\sum \left(r_{ij}^* - \overline{r_{i.}^*} \right)^2 / n}{\overline{r_{i.}}}$$

$$S_i^{(1)} = 2\sum_{j}^{n-1} \sum_{j=j+1}^{n} \left| r_{ij} - r_{ij'} \right| \div \left[n(n-1) \right]$$

$$S_i^{(2)} = \sum_{j=1}^{n} \left(r_{ij} - \overline{r_{i.}} \right)^2 \div (n-1)$$

$$S_i^{(4)} = \sqrt{\sum_{j=1}^{n} \frac{\left(r_{ij} - \overline{r_{i.}} \right)^2}{n}}$$

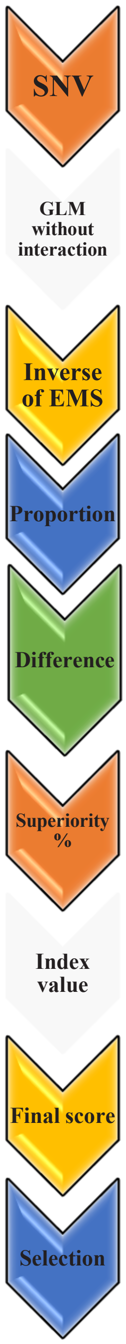
The rank r_{ij} is determined based on the rank of i^{th} genotype in j^{th} environment (Y_{ij}). The uncorrected Y_{ij} has the drawback that they may show significance even when there is no GE interaction. Hence, rank r_{ij}*is determined based on corrected phenotypic values Y*ij= $\left(Y_{ij} - \overline{Y_{i.}} \right)$, $\left(\overline{Y_{i.}} \right)$ being the mean performance of ith genotype. The corrected values depend only on the GE interaction and error components. Mdi* is the median ranks for adjusted values. These measures are widely used to assess the stability for different characters individually in crop improvement research. A detailed study from practical point of view is discussed in Ravi *et al.* (2013).

Strengths and limitations of the non-parametric approach in stability analysis

Nonparametric measures are highly suitable for assessing the yield stability of crop varieties. Their main advantages include: (i) They do not rely on assumptions about phenotypic data, (ii) They are more robust to measurement errors and outliers than parametric methods, (iii) The inclusion or exclusion of a few genotypes has minimal impact on the results, (iv) Since breeders often focus on crossover interactions, stability estimates based on rank data provide greater relevance, and (v) These methods are particularly useful when parametric approaches fail due to strong non-linear genotype-environment interactions. Consequently, nonparametric techniques are widely applied in crop variety selection, especially when crossover interactions are a key consideration (Raiger and Prabhakaran, 2001).

2.4 Non-parametric method for crop variety release developed at ICAR-IIHR

In non-parametric approaches to crop stability analysis, statistical indices are typically derived from the ranking of genotypes across multiple environments using mean or median rank values. Genotype performance is generally evaluated independently for each trait. However, breeders often prioritize specific traits that may be lacking in existing varieties, and assigning arbitrary weights to such traits can influence the final recommendations. In practice, the objective is to identify genotypes that exhibit consistent performance across all traits, environments, and seasons, rather than those excelling in only a few aspects. To address both desirable and undesirable traits, a stability-based weighting approach was proposed. This led to the development of a non-parametric measure, the Venugopalan Index (Venugopalan *et al.,* 2020), which evaluates genotype contributions to genotype × environment (GE) interactions based on relative performance and multi-trait stability in okra breeding. The step-by-step procedure is illustrated through a flow chart.



- Standardization of data is required as characters are measured in different scales.
- Run univariate ANOVA* for all characters by taking different environments as replication.
- Take $y_i = \frac{1}{\sqrt{EMS}}$ so that trait with least error will get highest importance.
- Proportion of individual y_i is taken over total Y.
- $Y = \sum y_i$, $w_i = \frac{y_i}{Y} x100$
- Take the difference of indivisual value and the Grand mean
- Positive character: Indivisual value - Grand mean value
- Negative character: Grand mean value - Indivisual value
- This is calculated by dividing the differenced value by grand mean value and multiplying by 100
- This is calculated using a logical statement viz, if the superiority % value multipled with respective weightage is exceeding the grand mean value then retain that value else take the grand mean value.
- Sum the index value over all characters for each genotypes.
- Final score with highest positive value will be selected as a best line genotypes.

Fig. 1. Schematic representation of the non-parametric approach for crop variety release developed at ICAR-IIHR

**All trait values were transformed using the Standard Normal Variate (SNV) method prior to analysis to standardize the data and improve adherence to normality assumptions. This transformation ensures that the data more closely meet the requirements of parametric ANOVA, thereby justifying its application in the present study.*

2.5 Correlation analysis among stability measures

Pearson's correlation analysis was performed among the non-parametric stability measures to evaluate the degree of association and agreement between different indices. This analysis helps in identifying redundancy among measures that provide similar information, as well as distinguishing indices that capture different aspects of genotype stability. Understanding these relationships is essential for selecting appropriate stability parameters and justifies the use of a composite index for integrated stability assessment.

3. RESULTS AND DISCUSSION

Before attempting for constructing stability models based on non-parametric approaches, average performance of evaluated lines individually for different traits across the years are presented along with the rank based on the mean performance (Table 1).

It is noticed that differential ranking of various genotypes across traits. Since, the biological traits are inter-related, a composite index by capturing the trait association is required. To understand such an inter-relationship among traits, principal component analysis was performed, to determine how much variability was explained. The results based on the scree plot (Fig. 2) revealed that PC1 explained the highest proportion of variance (approximately 51%, as indicated on the y-axis).

Table 1. Mean performance of different Chrysanthemum lines (2023-25 summer)

Genotype	Flower diameter (cm)		Plant height (cm)		No. of flowers/plant		Flowering duration (days)		Plant spread (cm)	
	Value	Rank	Value	Rank	Value	Rank	Value	Rank	Value	Rank
IIHR 4-8	9.15	16	4.75	3	67.38	**1**	14	14	3.45	7
IIHR 2-16	9.92	8	4.77	2	64.91	6	9	9	3.30	13
Fish Tail	19.74	7	4.79	**1**	60.94	15	11	11	3.45	8
Shanti	19.85	6	4.31	7	64.28	10	13	13	3.39	11
Jayanti	19.86	5	4.54	5	66.53	2	12	12	3.38	12
Jubilee	21.32	3	4.26	9	63.82	12	3	3	3.57	5
Maghi White	21.53	2	4.09	12	65.86	3	6	6	3.61	3
Himani	21.72	**1**	4.09	12	64.86	8	7	7	3.45	8
Kundan	20.29	4	4.49	3	63.37	13	8	8	3.21	16
NBRI Little Orange	9.51	11	4.56	4	52.65	16	4	4	3.58	4
NBRI Little Kusum	9.30	13	4.14	10	61.06	14	16	16	3.90	**1**
DFR C-4	9.54	10	4.49	7	64.19	11	15	15	3.23	15
Bidhan Chitra	9.60	9	4.11	11	65.07	5	2	2	3.45	6
Bidhan Manasi	9.16	15	4.41	8	64.89	7	1	**1**	3.72	2
Arka Pink Star (Check)	9.22	14	4.51	6	65.09	4	5	5	3.44	10

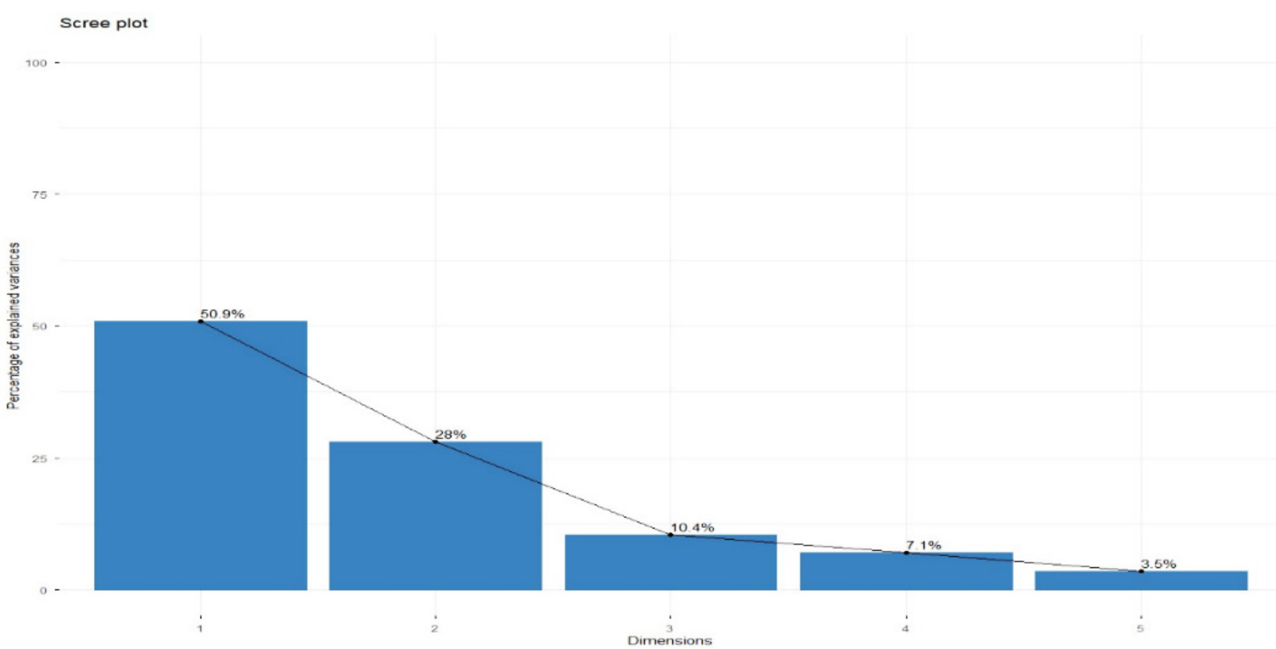


Fig. 2. Scree Plot

PC2 and PC3 each explain around 28% and 10.4%, respectively. Sharp drop-off after PC3, with PC4-PC5 contributing minimally (under 7 and 3.5% each). Based on the component selection, on the "elbow" rule, selecting 3 principal components would be reasonable. These first 3 PCs together explain roughly 80-89% of total variance. Fig. 3 indicated that trait plant spread followed by no. of flower/plant, flowering diameter showing the more contribution. Data structure implications revealed that the steep initial slope suggests strong correlation (association among the evaluated traits) structure in the original data. Hence, there is a need to assess the stability of the evaluated line based on all-traits information, moving away from individual trait based studies.

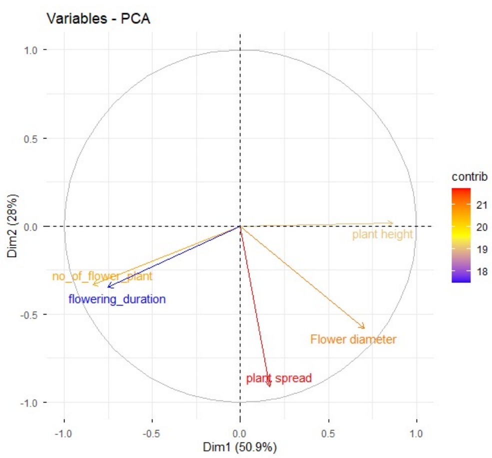


Fig. 3. PCA variables biplot

3.1 Non-parametric stability approaches

Analysis using seven stability parameters (Si_1, Si_2, Si_4, NPi_1, NPi_3 and IIHR method) evaluated five key characteristics in Chrysanthemum is presented in Table 2. The results showed that based on the combined nonparametric stability measures (for 15 lines) computed, IIHR 4-8, followed by IIHR 2-16 are stable across all traits evaluated i.e. flower diameter (cm), number of flowers/plant, flower stalk length (cm), plant height (cm) and plant spread (cm) across three years (2023 to 2025), which will be utilized by the breeder in Chrysanthemum improvement trials (last column in Table 2).

The correlation analysis among non-parametric stability measures revealed (Table 3) distinct patterns of association, indicating both redundancy and complementarity among the indices. Strong and highly significant positive correlations were observed among the Si measures, particularly between $S_i^{(1)}$ and $S_i^{(2)}$ ($r = 0.890$, $p < 0.01$) and between $S_i^{(2)}$ and $S_i^{(4)}$ ($r = 0.778$, $p < 0.01$), suggesting that these indices provide largely similar information and may be redundant in stability assessment. Likewise, the NP_i measures exhibited moderate to very strong positive correlations, with $NP_i^{(2)}$ and $NP_i^{(3)}$ showing the highest association ($r = 0.908$, $p < 0.01$), indicating a high degree of agreement within this group. Notably, the IIHR method exhibited low and non-significant correlations with all individual

Table 2. Non-parametric stability measures computed for Chrysanthemum

Measure of stability/ Lines	$S_i^{(1)}$		$S_i^{(2)}$		$S_i^{(4)}$		$NP_i^{(1)}$		$NP_i^{(2)}$		$NP_i^{(3)}$		IIHR method	
	Value	Rank	Value	Rank	Value	Rank	Value	Rank	Value	Rank	Value	Rank	Value	Rank
IIHR 4-8	1.40	6	1.39	3	7.17	2	8.67	9	1.24	10	1.42	8	1996.66	**1**
IIHR 2-16	1.33	7	0.66	13	1.67	14	13.00	1	2.21	6	1.96	4	1751.41	**2**
Fish Tail	2.94	1	3.05	1	8.50	1	10.33	6	2.11	7	1.38	9	170.91	7
Shanti	2.11	2	1.29	4	2.33	12	5.33	14	2.26	5	1.01	13	1166.71	4
Jayanti	1.02	11	0.93	9	3.67	7	9.00	7	1.11	12	1.31	10	170.91	7
Jubilee	1.65	3	1.01	8	2.83	10	12.67	2	4.30	2	4.41	1	170.91	7
Maghi White	1.04	10	0.69	12	2.67	11	10.33	5	3.21	4	1.63	5	170.91	7
Himani	1.50	5	1.48	2	3.83	6	8.67	8	4.91	1	4.13	2	170.91	7
Kundan	1.51	4	1.26	5	7.00	3	7.67	10	1.26	9	1.59	6	170.91	7
NBRI Little Orange	1.13	8	1.01	7	4.67	5	6.33	12	0.78	15	0.71	15	455.83	5
NBRI Little Kusum	0.43	15	0.32	14	1.83	13	7.33	11	1.04	13	1.23	12	170.91	7
DFR C-4	0.82	12	0.74	11	2.83	9	3.00	15	0.78	14	0.75	14	170.91	7
Bidhan Chitra	1.06	9	1.17	6	3.67	8	6.00	13	1.34	8	1.29	11	219.47	6
Bidhan Manasi	0.63	13	0.84	10	5.17	4	11.67	3	4.19	3	3.97	3	170.91	7
Arka Pink Star (Check)	0.55	14	0.29	15	1.17	15	11.33	4	1.17	11	1.44	7	1227.14	3

stability measures, highlighting its independence and reinforcing its role as a composite index that integrates multiple stability aspects. These findings justify the use of a combined non-parametric index, as it effectively minimizes redundancy while capturing complementary information from diverse stability measures, leading to a more holistic and reliable identification of stable genotypes.

Table 3. Pearson correlation coefficients among non-parametric stability measures

Measures	$S_i^{(1)}$	$S_i^{(2)}$	$S_i^{(4)}$	$NP_i^{(1)}$	$NP_i^{(2)}$	$NP_i^{(3)}$	IIHR method
Si(1)	1	0.89**	0.54*	0.06	0.19	0.02	0.05
Si(2)	0.89**	1	0.77**	-0.01	0.14	0.02	-0.12
Si(4)	0.54*	0.77**	1	-0.02	-0.03	-0.01	-0.10
NPi(1)	0.06	-0.01	-0.02	1	0.52*	0.58*	0.19
NPi(2)	0.19	0.14	-0.03	0.52*	1	0.90**	-0.22
NPi(3)	0.02	0.02	-0.01	0.58*	0.90**	1	-0.21
IIHR method	0.05	-0.12	-0.10	0.19	-0.22	-0.21	1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Hence, in view of differential ranking of entries if assessed trait wise for stability, combined index was employed for capturing the reality. Weightage for each trait was also computed for use as selection indices (Fig. 4) in the ongoing crop hybridization trails.

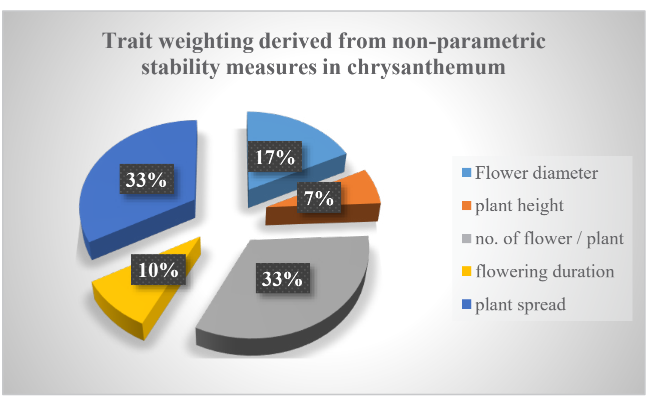


Fig. 4. The weightage of various traits computed (for combined non-parametric index) Chrysanthemum

Accordingly, a newly formulated index was employed, which involved assigning derived weights to each trait (Fig. 4) and performing an overall ranking by integrating all traits. Thus, the figure (Fig. 4) clearly reveals that plant spread and number of flowers per plant contribute more significantly than plant height and flowering duration. This indicates that these two traits play a greater role in enhancing the overall yield of Chrysanthemum.

4. CONCLUSION

Based on the combined non-parametric stability indices computed for 15 lines, IIHR 4-8 was identified as the most stable genotype, followed by IIHR 2-16, across all evaluated traitsi.e. Flower diameter (cm), number of flowers per plant, flower stalk length (cm), plant height (cm), and plant spread (cm), over three years (2023–2025). These stable lines will be utilized by breeders in Chrysanthemum crop improvement trials as stability rankings varied when traits were assessed individually, the use of a combined stability index is recommended to better capture the overall performance of genotypes. Additionally, trait-specific weightages were estimated and can be applied as selection indices in breeding programs. This study calls for employing non-parametric stability methods for identifying potential lines for release as varieties by considering the combined performance and stability for all the commercially important traits in any crop improvement study.

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ANNEXURE

R code for combined Index

STEP 1: Load your data

```
> library(readxl)
# export your data set
> data <- give your file as per what you have uploaded in excel format for the analysis
# Check structure:
> head(data)
>str(data)
# Expected structure:
Genotype    Trait1     Trait2
Trait3      ...
If the first column is genotype names:
>rownames(data) <- data[,1]
> data <- data[,-1]
```

STEP 2: Compute Thennarasu Stability (N1–N4)

```
>thennarasu<- function(data) {
ranks <- apply(data, 2, rank) # rank within each environment
mean_rank<- rowMeans(ranks)
N1 <- apply(abs(ranks - mean_rank), 1, mean)
N2 <- apply((ranks - mean_rank)^2, 1, mean)
N3 <- apply(abs(ranks - mean_rank), 1, max)
N4 <- apply((ranks - mean_rank)^2, 1, max)
```

```
out <- data.frame(
Mean = rowMeans(data),
N1 = N1,
N2 = N2,
N3 = N3,
N4 = N4
)
round(out, 2)
}
>th_result<- thennarasu(data)
>th_result
```

STEP 3: Nassar&Hühn Stability (S1–S6)

```
>nahu<- function(data) {
r <- apply(data, 2, rank)
S1 <- apply(abs(r - rowMeans(r)), 1, mean)
S2 <- apply((r - rowMeans(r))^2, 1, mean)
S3 <- apply(r, 1, var)
S6 <- apply(abs(r - rowMeans(r)), 1, max)
round(data.frame(S1, S2, S3, S6), 2)
}
>nh_result<- nahu(data)
>nh_result
```

STEP 4: Combine All Stability Measures

```
>combined_stability<- cbind(th_result, nh_result)
>combined_stability
```

STEP 6: Trait Weight Calculation

Step 6.1 — Fit ANOVA for each trait

```
>mse<- sapply(data, function(x) {
model <- aov(x ~ 1)
summary(model)[[1]]$"Mean Sq"[1]
})
```

Step 6.2 — Convert to weights

```
> weights <- (1 / sqrt(mse))
> weights <- weights / sum(weights) * 100
> weights
```

STEP 8: Compute Superiority Index

```
> check <- data["CHECK_NAME", ]
> superiority <- matrix(NA, nrow=nrow(data), ncol=ncol(data))
>colnames(superiority) <- colnames(data)
> for (j in seq_along(data)) {
if (colnames(data)[j] %in% positive_traits) {
superiority[,j] <- (data[,j] - check[j]) / check[j] * 100
} else {
superiority[,j] <- (check[j] - data[,j]) / check[j] * 100
}
}
```

STEP 9: Weighted Index (FINAL SCORE)

```
>final_score<-rowSums(sweep(superiority, 2, weights, "*"))
>final_result<- data.frame(
Genotype = rownames(data),
Score = final_score,
Rank = rank(-final_score)
)
>final_result[order(final_result$Rank), ]
```