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Original Research Paper

Non-parametric approach for multi-traits-based crop stability assessment in China aster [*Callistephus chinensis* (L.) Nees]

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ABSTRACT

An attempt has been made to construct non-parametric stability index, based on 36 different China aster lines evaluated during 2021-2023. Individual trait based parametric stability measures revealed differential ranking of lines across characters. Results based on the combined non-parametric stability measures computed, line 15-41-3, followed by 15-41-1 and 15-41-16 were identified as stable across three years for all the traits evaluated (plant height (cm), plant spread (cm), number of branches per plant, flower diameter (cm), number of flowers per plant, stalk length (cm), and vase life (days). This research calls for constructing non-parametric index for assessing the stability of set of lines collectively based on various traits, evaluated over seasons/years in an experimental set up to have a realistic assessment in studies related crop varietal release.

Keywords: China aster, non-parametric stability index, principal component analysis, venugopalan index

INTRODUCTION

The primary goal of improving crop productivity is to harness the genetic diversity present in available plant materials through various statistical analysis methods, and thus ultimately identifying stable varieties suitable for release at either institutional or multi-location levels. However, this process is complicated by the presence of genotype x environment (G×E) interactions, which make it challenging to accurately assess a variety's true genetic potential.

This interaction can result in situations where a particular variety might show high yield but fall short in quality or essential disease resistance characteristics, when compared to the benchmark standards set by control varieties that are targeted for improvement. Additionally, plant breeders typically prefer to recommend to farmers those entries that demonstrate consistent performance across all evaluated characteristics over multiple years, seasons, and locations, including the specific traits targeted for improvement, rather than entries that excel in only a limited number of characteristics. This situation emphasizes the necessity of implementing comprehensive stability analysis methods in crop improvement research programs based on trait pyramiding.

Traditional parametric stability analysis methods have been developed and widely used in horticultural crop improvement research since 1966. 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).

In addition to some limitations to this parametric approach, they evaluate each genotype's contribution to genotype x environment (G×E) interaction solely based on individual trait performance and stability across years. This approach doesn't align fully with breeders' needs, as they prefer to assess genotypes based on both their relative performance and stability over years, while, also considering performance across multiple traits simultaneously. This limitation of traditional parametric methods highlights the need for appropriate non-parametric approaches that can provide a more comprehensive evaluation of crop varieties. Hence, Venugopalan et al. (2020) introduced a method called venugopalan index for identifying the best okra line simultaneously based on crop stability across years collectively for several traits.

Traditional statistical methods (parametric approaches) for measuring plant performance stability are commonly employed in breeding programs, primarily focusing on analyzing variations and related statistical measures. However, in many breeding applications, what matters most is how varieties rank in comparison to each other. This makes a strong case for using alternative, non-statistical (non-parametric) approaches when evaluating how consistently different crop varieties perform.

These non-statistical methods offer several benefits compared to conventional statistical approaches. They don't require the data to follow specific statistical patterns, making them more flexible. They are less affected by unusual or extreme results that might skew the analysis. They're more straight forward to understand and apply in practice. Additionally, these methods remain reliable even when plant varieties are added to or removed from the analysis, as such changes don't significantly impact the overall findings (Truberg & Huhn, 2000; Adugna & Labuschagne, 2003; Kaya et al., 2003).

Here, we made an attempt to identify a best multi-variaty traits based stable line(s) across years in China aster genotypes and also to classify them based on their stability measurements across different methods.

MATERIALS AND METHODS

Database

Thirty six different China aster lines (15-41-12, 15-42-1, 15-42-2, 15-42-3, 15-52-1, 15-57-1, 15-57-3, 15-20-4, 15-39-6, 15-39-11B, 15-39-12, 15-41-7, 15-32-2, 15-32-1, 15-57-7, 15-40-1, 15-40-2, 15-41-5, 15-41-10, 15-39-4, 15-41-16, 15-39-14, 15-41-1, 15-41-3, 15-16-1, 15-36-1, 15-41-9, 15-41-18, 15-41-21, 15-57-5, 15-57-6, 15-31-1, 15-39-3, 15-57-2, 15-14-1, 15-36-2) evaluated during 2021-2023 at ICAR-Indian Horticultural Research, Bengaluru, India for different yield and yield attributing traits such as plant height (cm), plant spread (cm), number of branches per plant, flower diameter (cm), number of flowers per plant, stalk length (cm) and vase life (days) were considered.

Non-parametric approach of stability analysis

A number of non-parametric measures for assessing yield stability have been proposed (Nassar & Huhn, 1987; Thennarasu, 1995). These statistical measures are based on the ranks of the genotypes in each environment tested. The ranking is based on values of Yij (measured values of traits with respect to i^{th} genotype and j^{th} environment, respectively) with lowest Yij value receiving the rank 1, the next higher value 2 and so on. The non-parametric measures based on yield ranks of the genotypes in each environment are worked out are given here.

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

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

$$NP_i^{(3)} \quad \frac{\sqrt{\Sigma\left(r_{ij}^* - \bar{r}_{i.}^*\right)^2 / n}}{\bar{r}_{i.}}$$

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

$$S_i^{(2)} = \sum_{j=1}^{n}\left(r_{ij} - \bar{r}_{i.}\right)^2 / (n-1)$$

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

The rank rij is determined based on the rank of i^{th} genotype in j^{th} environment (r_{ij} is the rank values of the all traits across i^{th} genotype and j^{th} environment). The uncorrected Yij has the drawback that they may show significance even when there is no GE interaction. Hence, rank rij* is determined based on corrected phenotypic values Y*ij=[Yij], 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 by Ravi et al. (2013).

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

Non-parametric measures are well justified for evaluating the yield stability of crop varieties. Their key benefits include: (i) They do not require any assumptions about phenotypic data, (ii) They are less sensitive to measurement errors and outliers compared to parametric methods, (iii) The inclusion or exclusion of a few genotypes does not significantly alter their results, (iv) Since breeders are often interested in crossover interactions, stability estimates based on rank data are more meaningful, and (v) These methods are particularly valuable when parametric approaches fail due to substantial non-linear genotype-environment interactions. As a result, non-parametric techniques are widely used in crop variety selection, especially when crossover interactions are of primary concern (Raiger & Prabhakaran, 2001). However, it is well recognized that non-parametric methods are generally less powerful than parametric ones.

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

In the discussed non-parametric approaches for crop stability analysis, statistical measures rely on genotype rankings across different environments, derived from either average or median ranks. Each trait is analyzed separately based on genotype performance. However, researchers may prioritize specific traits lacking in existing varieties, and arbitrarily assigning weights to traits could influence final recommendations. From a practical perspective, breeders aim to identify lines that perform consistently well across all traits, environments, and seasons rather than excelling in only a few. Considering both positive and negative traits, a stability-based weighting approach was developed. This led to the proposal of a non-parametric index, the *Venugopalan Index* (Venugopalan et al., 2020), which evaluates genotype contributions to GE interaction based on relative performance and multi-trait stability in okra breeding. A step-by-step procedure is illustrated in a flowchart (Fig. 1).

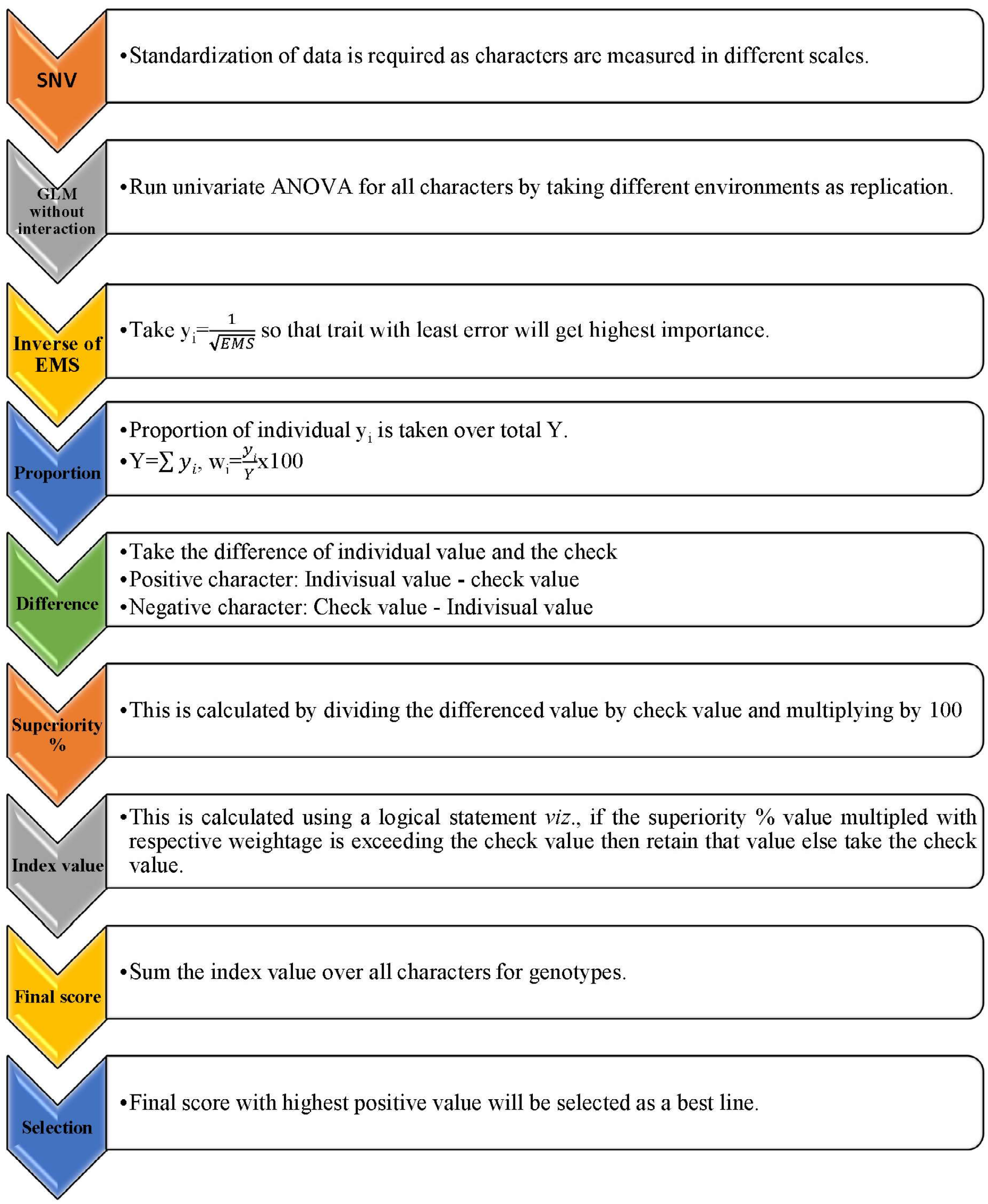

Fig. 1 : Flow chart of non-parametric method for crop varietal release

Grouping of lines based on stability measures China aster multi-location trials

Principle component analysis (PCA) was adopted to test the grouping of lines/genotypes of china aster based on seven stability measures (NPi_1, NPi_2, NPi_3, Si_1, Si_2, Si_4 and IIHR method).

RESULTS AND DISCUSSION

Non-parametric indices for varietal assessment based on multi-variate traits stability across locations

Multivariate approach was adopted for assessing the stability of 36 China aster lines (15-41-12, 15-42-1, 15-42-2, 15-42-3, 15-52-1, 15-57-1, 15-57-3, 15-20-4, 15-39-6, 15-39-11B, 15-39-12, 15-41-7, 15-32-2, 15-32-1, 15-57-7, 15-40-1, 15-40-2, 15-41-5, 15-41-10, 15-39-4, 15-41-16, 15-39-14, 15-41-1, 15-41-3, 15-16-1, 15-36-1, 15-41-9, 15-41-18, 15-41-21, 15-57-5, 15-57-6, 15-31-1, 15-39-3, 15-57-2, 15-14-1, 15-36-2) based on experimental trials conducted during years 2021-2023 for different yield and yield attributing traits.

At the first instance, individual trait-based stability measures developed, revealed differential ranking of entries across locations and when summed up revealed that none of these 36 lines was consistently ranked in the same order for every trait. However, the non-parametric approach based on combined index of all traits & stability over locations revealed that 15-41-3, 15-41-1, 15-41-16 (in the same order) as superior with the combined index for these entries worked out being 2592.60, 2537.97 and 2292.13, respectively. Hence, in view of differential ranking of entries if assessed trait wise for stability, combined index may be employed for capturing the reality.

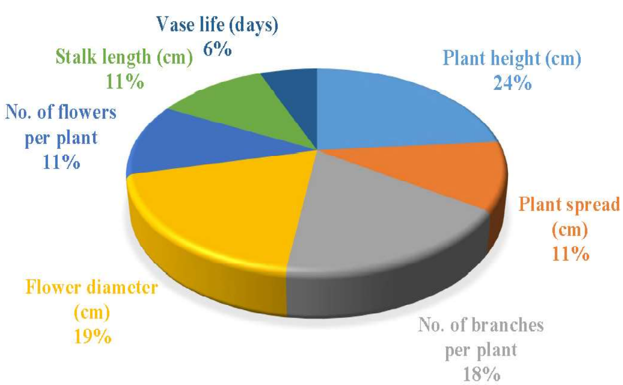

Fig. 2 : Weightage of various traits computed (for combined non-parametric index) in China aster

Accordingly, new index as discussed was adopted which was based on assigning derived weights (Fig. 2) for all the traits and collective ranking based on all the traits.

Principal component analysis

To observe the grouping of genotypes/line based on the different measures of stability principal component analysis were used and the results of the PCA were depicted below. The results based on scree plot revealed that (Fig. 3) PC1 explains the highest amount of variance (approximately 45-50% based on the y-axis).

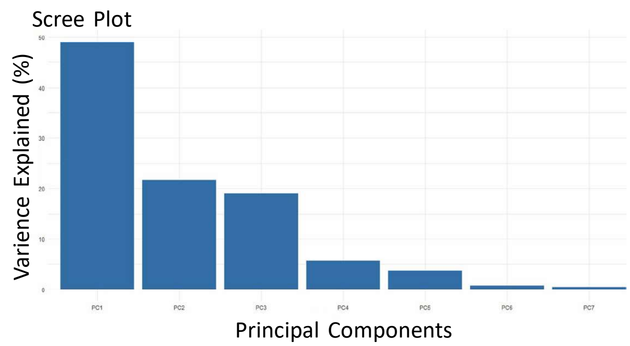

Fig. 3 : Scree plot

PC2 and PC3 each explain around 20% and 18%, respectively. Sharp drop-off after PC3, with PC4-PC7 contributing minimally (under 10% each). Based on the component selection, on the “elbow” rule, selecting 3 principal components would be reasonable. These first 3 PCs together explain roughly 80-85% of total variance. Additional components (PC4-PC7) provide diminishing returns

Data structure implications revealed that the steep initial slope suggests strong correlation structure in the original data. Most of the meaningful patterns can be captured in 3 dimensions. The flat tail (PC4-PC7) likely represents noise or very minor variations.

While considering both Fig. 3 and Table 1, it is clearly proved that PC1 contribute more variance explained and in that also IIHR method (Venugopalan Index) being the superior one for identifying the multiple genotypes/lines across the seasons/locations of China aster.

Table 1 : PCA loading

Stability measures	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Si_1	-0.46	0.00	0.32	0.43	-0.40	-0.53	0.21
Si_2	-0.42	-0.45	0.15	0.00	-0.33	0.52	-0.47
Si_4	-0.28	-0.62	-0.15	-0.53	0.19	-0.28	0.35
NPi_1	-0.39	-0.07	-0.44	0.56	0.58	0.06	-0.06
NPi_2	-0.43	0.44	-0.11	-0.19	-0.13	0.48	0.56
NPi_3	-0.40	0.45	-0.18	-0.43	0.04	-0.35	-0.54
IIHR method	-0.15	0.09	0.78	-0.09	0.58	0.09	-0.01

The grouping of genotypes based on the stability measures are presented in Fig. 4.

For seven different yield and attributing traits (plant height, plant spread, number of branches per plant, flower diameter, number of flowers per plant, stalk length and vase life) identified three groupings (group 1: 15-41-7,15-41-18,15-57-3, 15-41-21, and 15-41-12; group 2: 15-41-1,15-41-16, 15-36-1, 15-41-3,15-57-6,15-57-7, and 15-41-10; group 3: 15-32-1, 15-39-11B, 15-40-1, 15-39-14,15-31-1 and 15-39-6) and the descriptive statistics for each group has given in Table 2.

Longer vectors indicate stronger influence on the variation between samples. Hence, flower diameter, plant height, stalk length, plant spread, number of flowers and branches per plant showed very strong influence on the variation between the genotypes, and plant height, stalk length and plant spread pointing in similar direction and it suggesting positive correlation between the traits. Then, flower diameter with number of branches and flowers per plant showing negative correlation. With respect to flower diameter with vase life, plant spread with number of branches per plant and stalk length with number of flowers per plant showing right angle direction indicates that there is no correlation between the traits.

Environmental variability poses a significant challenge in crop improvement programs, as genotype-by-environment (G x E) interactions can diminish selection efficiency and reduce the accuracy of genetic predictions. When evaluating crop varieties or hybrids, assessing stability based on just one or a few traits is often inadequate, since breeders aim to develop varieties that demonstrate consistency across multiple desirable characteristics.

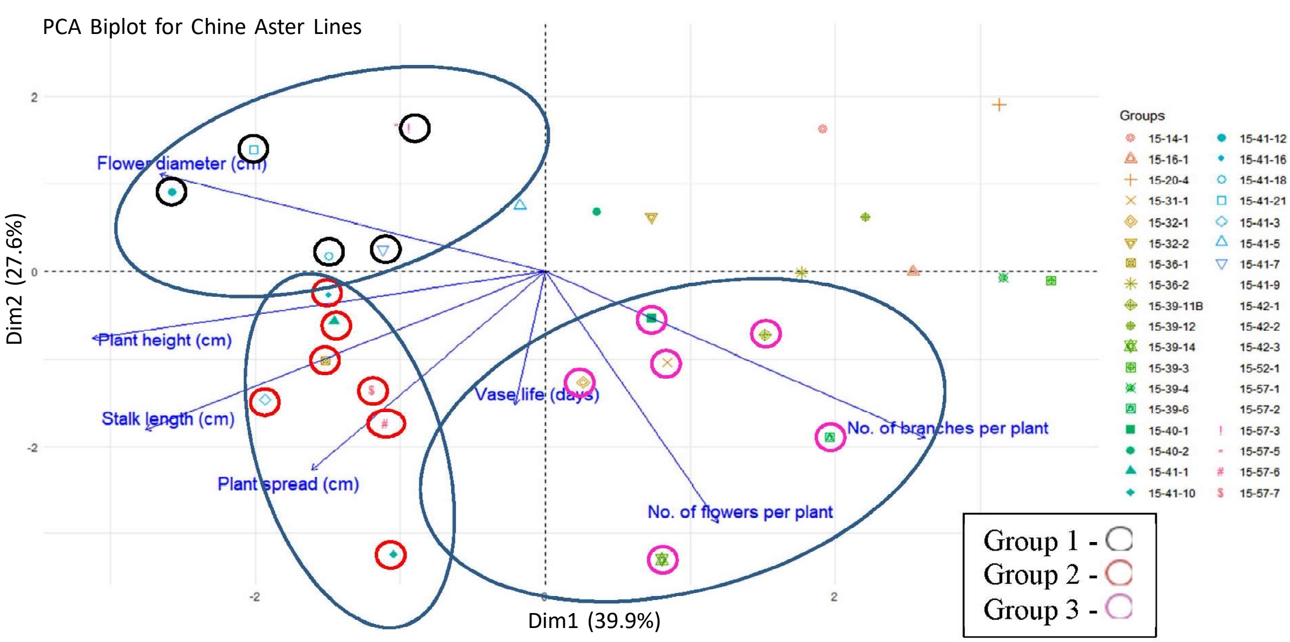

Fig. 4 : PCA biplot for China aster lines

Table 2 : Cluster group descriptive statistics

Parameter	Plant height (cm)	Plant spread (cm)	No. of branches/ plant	Flower diameter (cm)	No. of flowers/ plant	Stalk length (cm)	Vase life (days)
Group 1 (15-41-12, 15-57-3, 15-41-7, 15-41-18, 15-41-21)							
Mean	67.15	37.92	9.27	6.84	41.82	49.46	8.27
Standard error	2.09	1.70	0.83	0.26	2.12	1.60	0.40
Median	68.86	36.50	9.50	6.48	43.83	49.00	8.33
Standard deviation	4.66	3.80	1.85	0.58	4.75	3.57	0.90
Sample variance	21.76	14.44	3.42	0.34	22.52	12.77	0.80
Kurtosis	2.60	0.37	-1.90	-0.64	3.71	-2.64	1.74
Skewness	-1.52	1.07	-0.38	1.06	-1.90	0.23	-1.18
Range	12.11	9.47	4.34	1.34	11.72	7.94	2.34
Group 2 (15-57-7, 15-41-10, 15-41-16, 15-41-1, 15-41-3, 15-36-1, 15-57-6)							
Mean	71.98	44.33	13.69	6.45	54.29	51.99	8.36
Standard error	1.82	1.93	0.43	0.12	2.99	0.86	0.16
Median	73.67	42.83	13.83	6.42	52.67	52.11	8.33
Standard deviation	4.80	5.11	1.14	0.33	7.92	2.27	0.44
Sample variance	23.08	26.10	1.31	0.11	62.66	5.16	0.19
Kurtosis	-0.76	4.20	-1.09	1.45	-0.11	-1.08	-1.95
Skewness	-0.69	1.77	0.48	0.74	0.69	-0.10	-0.15
Range	12.83	16.33	3.00	1.05	23.17	6.33	1.00
Group 3 (15-39-6, 15-39-11B, 15-32-1, 15-40-1, 15-39-14, 15-31-1)							
Mean	57.07	39.83	18.47	5.99	58.92	47.19	8.53
Standard error	1.65	1.29	1.14	0.18	3.52	1.79	0.15
Median	56.42	41.00	18.83	6.09	57.33	46.75	8.67
Standard deviation	4.05	3.17	2.80	0.43	8.61	4.38	0.37
Sample variance	16.40	10.05	7.81	0.19	74.20	19.16	0.14
Kurtosis	-0.76	0.93	1.19	2.50	2.71	-2.96	-1.78
Skewness	0.51	-1.24	-0.98	-1.30	1.37	0.10	-0.64
Range	10.92	8.50	8.00	1.28	25.34	9.28	0.83

A non-parametric ranking approach has been developed to identify superior lines across multiple growing seasons, considering overall performance across all traits rather than focusing solely on one or two characteristics. This method determines trait importance by assigning weights based on demonstrated stability both across years and within replicated trials, rather than using arbitrary weighting systems. The approach also accounts for traits where either increase or decrease is desirable relative to existing cultivars.

This comprehensive evaluation methodology is recommended for variety identification and release programs, particularly in multi-location trials (MLT). The system offers a more holistic approach to variety assessment and selection. Analysis using seven stability parameters (Si_1, Si_2, Si_4, NPi_1, NPi_3 and IIHR method) evaluated seven key characteristics in China aster: plant height, plant spread, branch count per plant, flower size, flower count per plant, stalk length, and vase life duration. This analysis revealed three distinct clusters of plant lines. The first cluster comprised lines 15-41-7, 15-41-18, 15-57-3, 15-41-21, and 15-41-12. The second cluster included lines 15-41-1, 15-41-16, 15-36-1, 15-41-3, 15-57-6, 15-57-7, and 15-41-10. The third cluster consisted of lines 15-32-1, 15-39-11B, 15-40-1, 15-39-14, 15-31-1 and 15-39-6 respectively.

CONCLUSION

The present study concluded that based on the combined non-parametric stability measures (for 36 lines) computed 15-41-3, followed by 15-41-1 and 15-41-16 are stable across all traits evaluated plant height, plant spread, number of branches per plant, flower diameter, number of flowers per plant, stalk length and vase life across three years (2021, 2022 and 2023), which will be utilized by the breeder in the ongoing hybridization trials.

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