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RESEARCH ARTICLE

An approach to identify stable genotypes based on MTSI and MGDII indexes in okra [*Abelmoschus esculentus* (L.) Moench]

N. Sandeep* and B.M. Dushyantha Kumar

Abstract

Okra [*Abelmoschus esculentus* (L.) Moench] is a key vegetable crop in tropical and subtropical regions, exhibiting varying performance across different environmental conditions due to genotype-environment interactions. The present study was aimed at the identification of high performing and stable okra genotypes using the MultiTrait Stability Index (MTSI) and the Multi-Trait Genotype-Ideotype Distance Index (MGIDI). A total of 42 okra accessions were evaluated over different seasons for 12 morphological traits. MTSI and MGIDI analysis consistently identified four superior genotypes, namely, UAHS-8, UAHS-10, UAHS-11 and UAHS-19, that exhibited stability and high performance across seasons. Key traits contributing to the ideal genotypes included average fruit weight and fruit yield per plant. The molecular diversity analysis revealed significant genetic diversity among the genotypes, with 35 out of 45 SSR markers showing polymorphism and a high average Polymorphism Information Content (PIC) value of 0.69. The comparison between morphological and molecular dendrograms using the tanglegram shows there are overlapping clusters with a low cophenetic correlation, indicating environmental influence on phenotypic traits. Correlation analysis highlighted significant relationships among traits, such as a positive correlation between average fruit weight and fruit yield per plant (r = 0.52). The study underscores the efficacy of MTSI and MGIDI in identifying stable, high performing okra genotypes, providing a robust framework for improving genotype selection and breeding strategies. The combined use of phenotypic and genotypic data enhances the precision of genetic analyses, facilitating the selection of diverse and adaptable okra genotypes for future breeding programs.

Keywords: Okra, MTSI, MGIDI, multi-trait, stability.

Introduction

Okra (*Abelmoschus esculentus* L. Moench), a member of the Malvaceae family with a chromosome number of 2n = 8x = 72 or 144, originated in Southeast Asia (Sutar et al. 2013). It is widely adaptable due to its ease of cultivation, export potential, high monetary returns and suitability for regions with moderate rainfall. India ranks first in okra production and 12th in productivity, producing 6.37 mt from an area of five lakh hectares with a productivity of 11.6 t/ha (Anonymous 2020). Okra is a commonly used vegetable in culinary preparations, valued for its nutritional content. One of the constraints in increasing okra production is the lack of stability in high-yielding and widely adapted varieties or hybrids. Varietal adaptation to environmental fluctuations is crucial for stabilizing crop production. Fruit yield, being a complex polygenic trait, is influenced by several component characters that are polygenically inherited and highly susceptible to environmental variation; direct selection for yield may not be reliable. Emphasis should be placed on selecting yield attributes that are less influenced by the environment. Correlation studies provide an opportunity to study the magnitude and direction of the association of yield with its component characters and also among various component characters. Thus, evaluating genotypes in a single environment is not sufficient for the selection and identification of superior varieties (Shrestha et al. 2012).

Department of Genetics and Plant Breeding, College of Agriculture, Shivamogga, Keladi Shivappa Nayaka, University of Agricultural and Horticultural Sciences, Shivamogga 577 201, Karnataka, India.

***Corresponding Author:** N. Sandeep, Department of Genetics and Plant Breeding, College of Agriculture, Shivamogga, Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga 577 201, Karnataka, India, E-Mail: sandeepgowda.n@gmail.com

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Table 1. Genotype accessions taken in the study

S. No.	Genotype code	Genotype name	S. No.	Genotype code	Genotype name
1	G1	Arka Anamika	22	G22	VRO 106
2	G2	Pusa Swani	23	G23	UAHS- 1
3	G3	Varsha Uphar	24	G24	UAHS- 2
4	G4	Red Okra	25	G25	UAHS- 3
5	G5	Green Okra	26	G26	UAHS- 4
6	G6	Green Star	27	G27	UAHS- 5
7	G7	Anekombu	28	G28	UAHS- 6
8	G8	Local	29	G29	UAHS- 7
9	G9	Red Burgandy	30	G30	UAHS- 8
10	G10	Parbhani Kranti	31	G31	UAHS- 9
11	G11	Halubende	32	G32	UAHS- 10
12	G12	Kashi Vibhuthi	33	G33	UAHS- 11
13	G13	Kashi Satadri	34	G34	UAHS- 12
14	G14	Kashi Kirathi	35	G35	UAHS- 13
15	G15	Kashi Leela	36	G36	UAHS- 14
16	G16	Kashi Mangala	37	G37	UAHS- 15
17	G17	Pusa Makhmali	38	G38	UAHS- 16
18	G18	Pusa Utkarsh	39	G39	UAHS- 17
19	G19	VRO 103	40	G40	UAHS- 18
20	G20	VRO 109	41	G41	UAHS- 19
21	G21	VRO 178	42	G42	UAHS- 20

The best strategy is to evaluate genotypes in multiple environments for stable yield and average performance (Islam et al. 2015)

Genotype × environment interaction (GEI) is a major factor affecting the performance of vegetable and field crops under different environments. Understanding GEI and stability in crops serves as a decision tool, particularly at the final stage of variety introduction, for screening breeding lines and recommending released varieties (Yan and Kang 2003). By examining the phenotypic characteristics of crop cultivars, breeders can evaluate the adaptability and stability of genotypes to various environments with varying yield potentials. Therefore, it is necessary to understand the effect of the environment on genotype characters and their performance in specified conditions, which can be achieved by studying GEI along with stability analysis. In addition to considering GEI, breeders need to consider multiple traits simultaneously. Multi-trait selection indices facilitate the integration of several desirable attributes, allowing breeders to select cultivars according to their genetic superiority and phenotypic stability (Eberhart and Russell 1966). Recently proposed indexes, such as the Multi-Trait Stability Index (MTSI) (Olivato et al. 2019) and the Multi-Trait Genotype-Ideotype Distance Index (MGIDI) (Olivato and Nardino 2020), have emerged as novel tools for selecting superior genotypes that perform well across different environmental conditions with high yield stability and desirable traits.

The MTSI is a selection index that utilizes the mean performance and stability of the genotype for multi-trait selection (Authrapun et al. 2021). In this context, the current study proposes a framework for identifying suitable stable okra genotypes using MTSI and MGIDI indices. Therefore, the present study was conducted to identify ideal high-yielding okra genotypes that perform well under various environmental conditions through a multi-factorial, multi-trait stability analysis.

Materials and methods

A total of 42 genotypes, including advanced breeding lines from the Department of Genetics and Plant Breeding, College of Agriculture, Shivamogga, Karnataka, India, were used in this study (Table 1). The experiment was conducted at the Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka, India during *kharif* 2020, *rabi* of 2020–21, and *kharif* 2022. The site is located at 13˚55'N latitude, 75˚34'E longitude, and an

elevation of 640 meters. The station experiences an average annual rainfall of 900 mm, with red sandy loam soil. The experiment was designed as a randomized complete block design with two replications. Seeds of each genotype were sown with a spacing of 60 × 45 cm in 5 m long cropping rows. Twelve quantitative traits, namely, first flowering node (FFN), days to 50% flowering (DFF), plant height (PH), number of primary branches per plant (NPB), internodal length (IL), number of internodes (NIN), fruit length (FL), fruit diameter (FD), average fruit weight (AFW), number of fruits per plant (NOF), test weight (TW) and fruit yield per plant (FYPP) were assessed in this study.

DNA extraction and PCR

About 42 genotypes were utilized for the molecular diversity study. Young leaves from 20 to 25 day old seedlings were collected, sterilized, and stored at -20°C. DNA extraction employed a modified cetyl trimethyl ammonium bromide (CTAB) method (Murray and Thompson 1980), which involved grinding the leaves in liquid nitrogen, incubating them in extraction buffer and performing multiple phases of Phenol ChloroformIsoamyl alcohol extraction. The resulting nucleic acids were precipitated with isopropanol, washed with ethanol and dissolved in TE buffer, followed by RNA removal using RNase treatment. DNA quality was assessed through agarose gel electrophoresis, and concentrations were normalized to 25 ng/µL for PCR amplification.

For PCR, a reaction mixture containing Takara Master Mix, primers, and genomic DNA was prepared, with optimized amplification conditions. The PCR products were analyzed on a 3% agarose gel stained with ethidium bromide and visualized under UV light. Primers for simple sequence repeat (SSR) markers were selected based on prior research, as detailed in (Supplementary Table S1). Gel electrophoresis and subsequent visualization enabled the assessment of DNA banding patterns, enhancing the understanding of genetic diversity among the okra cultivars.

Statistical analysis

Morphological diversity analysis

Pooled analysis of variance and individual analysis of variance (ANOVA) was performed for each environment. The Mahalanobis D^2 statistic, introduced by Mahalanobis in 1936, was used to assess genetic divergence between different populations. This analysis was carried out using the data recorded on each germplasm. The 'biotools' package in R software (v 4.2) was utilized for the D^2 analysis. The Mahalanobis generalized distance (D^2) between any two populations is calculated using the following formula:

$$D^2 = \Sigma \lambda^{ij} \sigma^i \sigma^j$$

Where,

D^2 = Square of generalized distance

λ^{ij} = Reciprocal of the common dispersal index

$\sigma^i = \mu i1 - \mu i2$

$\sigma^j = \mu j1 - \mu j2$

μ = General mean

To simplify the computational procedure, the original correlated unstandardized character mean (Xs) was transformed into standardized uncorrelated variables (Ys). The D2 values were then obtained as the corresponding uncorrelated (Ys) values of any two uncorrelated germplasm (Rao 1952). Using all D^2 values, the genotypes were grouped into clusters using Tocher's method, as described by Rao (1952).

Marker diversity analysis

To measure the informativeness of the markers, the major allele frequency and the polymorphism information content (PIC) for each SSR marker were determined using PowerMarker Version 3.25 software. The PIC was calculated using the formula given by Botstein et al. (1980):

$$\text{PIC} = 1 - \sum_{i=1}^{n} P_i^2 - \sum_{i=1}^{n-1} * \sum_{j=i+1}^{n} 2P_i^2 P_j^2$$

Where, n = number of alleles pi and pj = allele frequency in population i and j, respectively

A binary data matrix (scored as '1' and '0') of SSR markers from 30 genotypes was subjected to cluster analysis. A dendrogram was constructed using the unweighted pair group method with arithmetic mean (UPGMA) based on similarity matrices calculated using the simple matching (SM) coefficient (Nei and Li, 1979).

Comparison of molecular and morphological diversity

To test the correlation between the morphological and molecular data, the two parallel matrices were compared using a Mantel test with 9999 permutations (Manco et al. 2020). The comparison of the two dendrograms was performed using a tanglegram plot. This analysis was conducted in R using the 'dendextend' (Galili 2015) and 'NbClust' (Charrad et al. 2014) packages.

Correlation coefficient analysis

The correlation coefficient among all possible character combinations at the phenotypic (rp) level was estimated using the formula provided by Al-Jibouri et al. (1958). This analysis was performed using the 'correlation' package in R (v 4.1.3).

$$\text{Phenotypic correlation} = r_{xy}(p) = \frac{\text{Cov}_{xy}(p)}{\sqrt{V_x(p) \times V_x(p)}}$$

Where,

Cov_{xy} (p) = Phenotypic covariance between x and y

V_x (p) = Phenotypic variance of character 'x'
V_y (p) = Phenotypic variance of character 'y'

The test of significance for the association between characters was done by comparing table 'r' values at n-2 error degrees of freedom for phenotypic and genotypic correlations with estimated values, respectively.

Multi-trait stability index

Stability analysis among genotypes was conducted using the 'metan' package developed by Olivoto and Lucio (2020). The MTSI/MGIDI index rescaled traits to a 0–100 value range, facilitating the definition of an ideotype. Most traits were assigned increased values, reflecting quantitative morphological traits of the okra crop correlated with yield. Traits like days to fifty flowering and first flowering node were assigned decreased values to identify genotypes with shorter crop cycles, potentially leading to higher fruit yield.

WAASBi (weighted average of absolute scores based on SVD of BLUP-interaction effects of the i^{th} genotype or environment) (Olivoto et al. 2019) is calculated by equation.

$$WAASB_i = \frac{\sum_{k=1}^{p} |IPCA_{ik} \times EP_k|}{\sum_{k=1}^{p} EP_k}$$

Where the interaction principal component axis ($IPCA_{ik}$) is the score of the i^{th} genotype (or environment) in the k^{th} IPCA; and EP_k is the amount of the variance explained by the k^{th}IPCA.

According to the multi-trait genotype-ideological distance index (MGIDI) proposed by Olivoto et al. (2020). The following formula was used to calculate the Euclidean distance between the genotype score and the ideal genotype as the MDIGI index:

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

In the formula, γij represents the scores of the ith genotype in the j^{th} factor (i = 1, 2,…, t; j=1, 2,…, f), where t and f represent the number of genotypes and factors, respectively, and γj represents the j^{th} scores for the ideotype. The genotypes with the lower MGIDI values are closer to the ideal genotype than other that exhibits all the desired values for the measured agronomic traits.

MTSI takes advantage of the weight between average performance and stability and, therefore, selects genotypes that are both stable and have a high performance. If the weights of all traits in the MTSI are completely assigned to the average performance, then the MTSI will become the MGIDI index. It should be noted that MGIDI is used to rank genotypes based on multiple traits but does not consider the stability of genotypes.

The multi-trait stability index (MTSI) (Olivoto et al. 2019) was computed by equation.

$$MTSI_i = \left[\sum_{j=1}^{f} (F_{ij} - F_j)^2\right]^{0.5}$$

Where, the MTSI is the multi-trait stability index for the i^{th} genotype, F_{ij} is the j^{th} score of the i^{th} genotype, and F_j is the j^{th} score of ideotype. The genotype with the lowest MTSI is then closer to the ideotype and, therefore, presents a high mean performance and stability for all analyzed variables (Olivoto et al. 2019).

Results and discussion

The performance of agronomic traits of the genotypes is generally considered to be an important step in selecting genotypes suitable across different environments and with ideal agronomic traits, which can be used in future breeding programs to breed new and improved genotypes (Alipour et al. 2021). Multi-environmental testing (MET) serves as a tool that describes the adaptability, as well as stability, of genotypes across different environments (Allard and Bradshaw 1964). To develop a stable genotype that performs better under different environmental situations, the interaction between genotype and environment needs to be clearly understood. 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 (Kang and Pham 1991). The selected genotype should be amenable to crop management practices and soil fertility status, which serves as a precursor for increasing yield and yield-attributing traits of okra.

Genetic diversity present in the available germplasm has immense value for crop improvement. From the point of selecting the divergent parents for hybridization, the genetic distance is most important. Morphological markers, influenced by environmental factors, often reduce selection efficiency during cultivar development. In contrast, molecular markers are unaffected by environmental conditions, making them more reliable for genetic diversity assessment. Their determination is largely automated, minimizing human error. However, the use of molecular markers in okra is limited due to a scarcity of polymorphic markers and established genetic maps. Additionally, okra's allopolyploid nature and large chromosome number (2n = 56–196) add to the complexity (Lata et al. 2021).

The polymorphism level among okra cultivars was assessed by calculating the allele number, major allele frequency, and Polymorphism Information Content (PIC) values for each of the 45 SSR markers evaluated. Out of these, 35 markers were found to be polymorphic. Across 42 genotypes, a total of 78 alleles were detected at the loci of 35 microsatellite markers, highlighting the robustness of microsatellites in revealing polymorphism (Table 2). Eight markers (AeKVR-117, AeKVR-137, AeKVR-176, AeKVR-182, AeKVR-192, AeKVR-194, AVRDC OKRA 39, and Okra-137) produced three alleles each, while 27 markers produced

Table 2. Estimates of the number of alleles, major allele frequency and polymorphic information content (PIC) Values among 42 genotypes of okra

S. No.	Marker	No. of alleles	Major allele frequency	PIC	S. No.	Marker	No. of alleles	Major allele frequency	PIC
1	AeKVR-114	2	0.90	0.81	19	AVRDC OKRA 52	2	0.73	0.61
2	AeKVR-117	3	0.63	0.60	20	AVRDC OKRA 63	2	0.80	0.32
3	AeKVR-125	2	0.56	0.57	21	AVRDC OKRA 64	2	0.73	0.86
4	AeKVR-126	2	0.63	0.76	22	AVRDC OKRA 70	2	0.76	0.91
5	AeKVR-134	2	0.67	0.79	23	AVRDC OKRA 86	2	0.83	0.23
6	AeKVR-137	3	0.80	0.91	24	AVRDC OKRA 89	2	0.70	0.82
7	AeKVR-149	2	0.63	0.75	25	Okra-12	2	0.80	0.64
8	AeKVR-153	2	0.93	0.93	26	Okra-103	2	0.73	0.73
9	AeKVR-165	2	0.76	0.59	27	Okra-104	2	0.65	0.65
10	AeKVR-176	3	0.76	0.83	28	Okra-110	2	0.78	0.82
11	AeKVR-182	3	0.53	0.86	29	Okra- 111	2	0.80	0.84
12	AeKVR-192	3	0.60	0.75	30	Okra-119	2	0.82	0.82
13	AeKVR-193	2	0.63	0.80	31	Okra-125	2	0.66	0.66
14	AeKVR-194	3	0.76	0.41	32	Okra-128	2	0.84	0.85
15	AeKVR-195	2	0.60	0.54	33	Okra-137	3	0.64	0.64
16	AVRDC OKRA 9	2	0.66	0.79	34	Okra-141	2	0.67	0.67
17	AVRDC OKRA 21	2	0.86	0.19	35	Okra-148	2	0.83	0.78
18	AVRDC OKRA 39	3	0.76	0.39	36	Average	2.22	0.71	0.69

two alleles each. The major allele frequency ranged from 0.53 (AeKVR-182) to 0.93 (AeKVR-153), with a mean of 0.71.

High PIC values indicate more informative markers, whereas lower values suggest closer genetic relationships among the genotypes studied. PIC values, reflecting allele diversity and frequency, varied from 0.00 to 0.93, with an average of 0.69. The highest PIC value of 0.93 was recorded for marker AeKVR-153, followed by 0.91 for markers AeKVR-137 and AVRDC OKRA 70. These high PIC values demonstrate that these markers are particularly effective in differentiating okra cultivars, indicating greater diversity among the genotypes. These results align with the findings of Kumar et al. (2016) and Ravishankar et al. (2018), who observed similar polymorphism and PIC values among different okra genotypes. The ANOVA was computed for each environment, employing a randomized complete block design. Results showed statistical significance ($p \leq 0.05$) for all twelve traits across the studied environments. The dendrograms representing morphological and molecular diversity in okra genotypes, as illustrated in the tangle-gram (Fig. 1), reveal distinct clustering patterns that highlight the genetic variability and relationships among the studied genotypes. The morphological dendrogram on the left side and the molecular dendrogram on the right side both exhibit unique groupings that provide insights into the genetic diversity and stability of okra genotypes.

In the morphological dendrogram, genotypes such as VRO 109, UAHS-3, Varsha Upahar, UAHS-19, UAHS-10, UAHS-20, Halubende, UAHS-4, UAHS-11, UAHS-9, Kashi Vibhuti, UAHS-18, Kashi Kiranti, UAHS-8, UAHS-5 andUAHS-6 are clustered together, indicating their similarity based on phenotypic traits (Fig. 1), suggesting that these genotypes share common morphological characteristics. Additionally, genotypes like Pusa swani and Anekombu formed another distinct cluster, further supporting their phenotypic resemblance. Whereas, Local and Green stars formed solitary clusters. On the molecular side, the dendrogram reveals a different clustering pattern, reflecting the genetic diversity at the DNA level. For instance, the genotypes VRO 178, VRO 103, UAHS-5, UAHS-13, Kashi Satadri, UAHS-14, Green Okra, Pusa Swani, Parbhani Kranti, UAHS-11 and Kashi Kiranti are grouped together, suggesting a close genetic relationship that may not be evident from morphological traits alone. This molecular clustering provides a deeper understanding of the genetic makeup and can help in identifying genotypes with desirable traits such as disease resistance or stress tolerance. Other notable clusters include UAHS-2, Red Burgandy, UAHS-16 and UAHS-15, which indicates genetic similarity that could be leveraged for breeding programs aimed at improving genetic resilience.

A combined dendrogram based on genotypic and phenotypic data improves precision in genetic analyses of

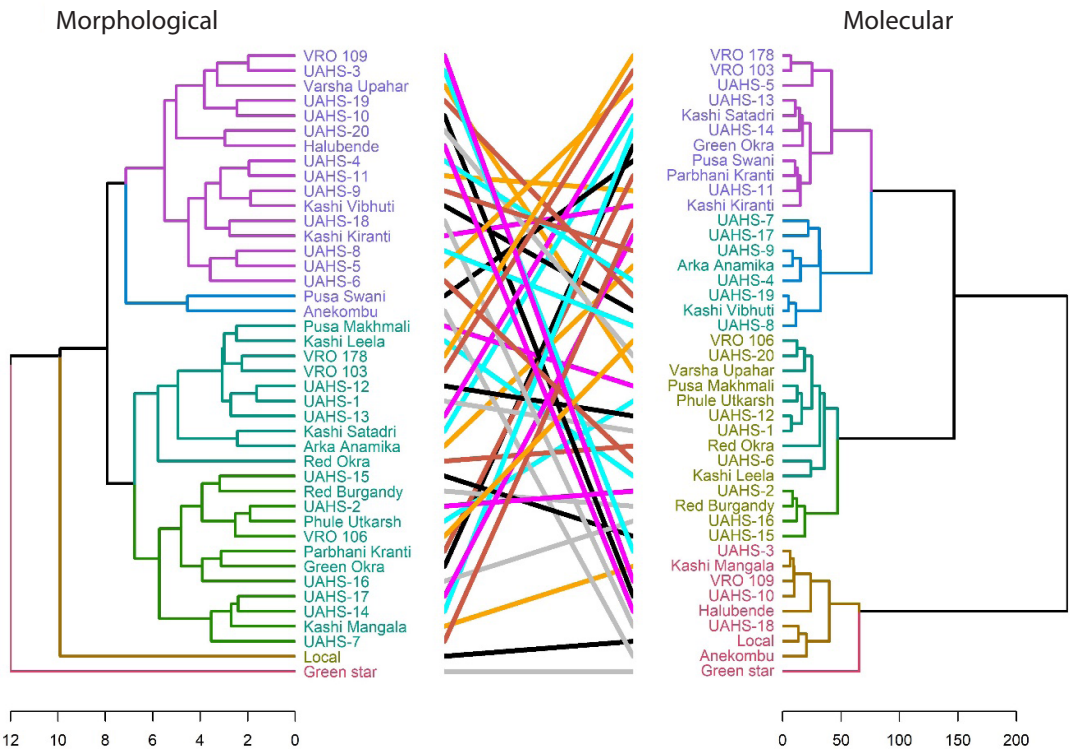

Fig. 1. Tanglegram of dissimilarity of 42 okra (*Abelmoschus esculentus* L.) accessions

germplasm. The hierarchical clusters based on phenotypic and genotypic data revealed that the genotypes could be clustered into heterogeneous groups. The tangle-gram comparing phenotypic and genotypic clustering revealed that these clusters were largely independent (Fig. 1). The cophenetic correlation between the dendrograms (r = 0.37) was not significant, indicating that the resemblance of morphological traits with SSR markers did not strongly correlate. The inconsistency between phenotypic and genotypic clusters is likely caused by environmental variance. Genotype × environment interaction confounds phenotypic performance, which reduces the correlation between genotype and phenotypic expression. The genotypes used in this study consisted of diverse types with different adaptations, which led to deviations from their genetic potential. Inconsistencies between genotype and phenotype expressions have been reported previously in okra by Massucato et al. (2019) and Nkhata et al. (2020) in common beans. The differential clustering of genotypes in the combined dendrogram can be used for a more informative analysis of diversity in the population.

The integration of morphological and molecular data through this analysis aids in the identification of stable genotypes that are not only phenotypically desirable but also genetically robust. This dual approach enhances the precision of breeding programs, enabling the selection of genotypes that are likely to perform well under varying environmental conditions. The insights gained from such analyses are pivotal for the development of improved okra varieties that combine high yield with stability and resilience, thereby contributing to sustainable agricultural practices.

The correlation matrix (Fig. 2) displays the relationships among twelve quantitative traits in okra, providing a comprehensive overview of the phenotypic associations between these traits.

Significant correlations were observed among several traits. For instance, a strong positive correlation (r = 0.52) was observed between AFW and FYPP, indicating that genotypes with heavier fruits tend to have higher yields. Similarly, PH showed significant positive correlations with the number of internodes (r = 0.66) and internodal length (r = 0.44), suggesting that taller plants generally have more and longer internodes. Additionally, the first flowering node exhibited a strong positive correlation with the number of primary branches per plant (r = 0.58), implying that early flowering genotypes tend to develop more primary branches. A notable negative correlation was found between the number of fruits and average fruit weight (r = -0.55), indicating that larger fruits tend to be lighter. Furthermore, days to 50% flowering negatively correlated with fruit yield per plant (r = -0.18) and plant height (r = -0.12), suggesting that early flowering genotypes may produce lower yields. Several trait combinations exhibited non-significant correlations, highlighting the complex nature of trait interactions in okra. For example, internodal length and fruit diameter (r = 0.096) showed a weak and

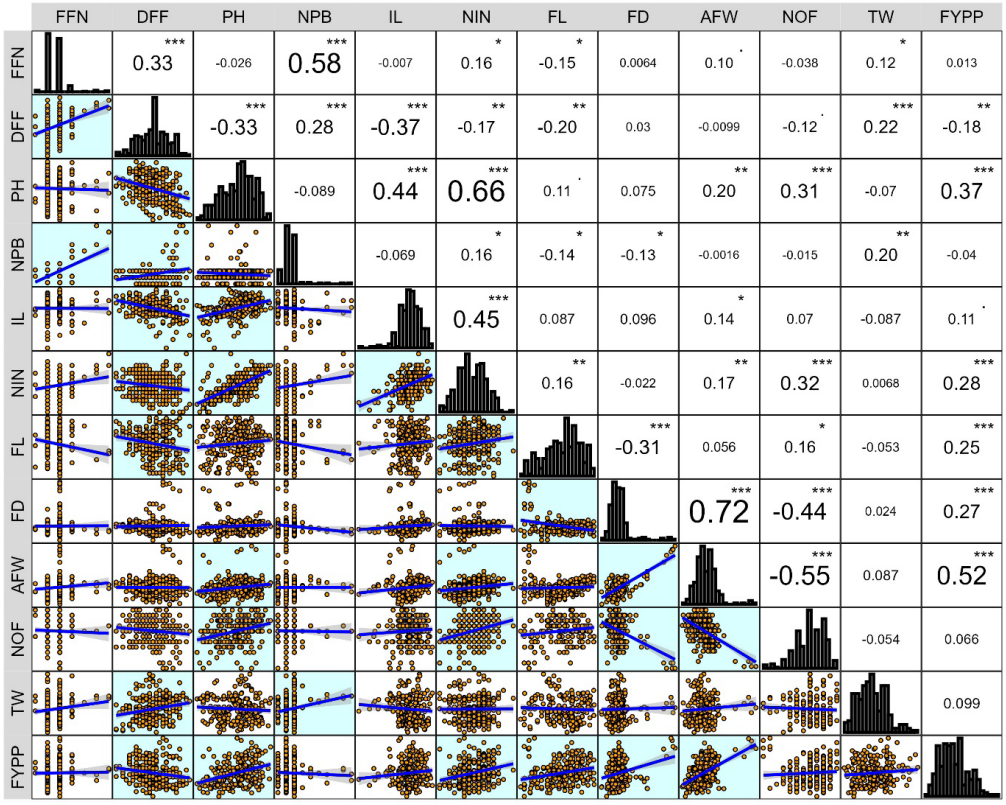

Fig. 2. The correlation matrix among twelve quantitative traits in okra

non-significant correlation, suggesting that these traits may vary independently.

The correlation analysis provides crucial insights into the relationships among key agronomic traits in okra. The strong positive correlation between average fruit weight and fruit yield per plant underscores the importance of selecting genotypes with heavier fruits to enhance overall yield. This finding aligns with the results of similar studies in other crops, where fruit weight is a key determinant of yield (Kumar et al. 2020). The significant positive correlation between plant height, number of internodes, and internodal length suggests that taller plants with more internodes are likely to perform better in terms of growth and yield attributes. The present findings are consistent with earlier findings in other studies that highlighted the role of plant architecture in yield performance.

Negative correlations, such as between fruit diameter and average fruit weight, indicate potential trade-offs in breeding programs. Breeding for larger fruit size may inadvertently reduce fruit weight, affecting yield potential. Thus, careful consideration of these trade-offs is essential in developing balanced breeding strategies (Islam et al. 2015). The non-significant correlations among certain traits highlight the complex genetic architecture of okra, suggesting that some traits may not directly influence each other and could be independently selected for improvement.

The MTSI evaluation system has unique and easy-to-understand characteristics. MTSI has many practical applications in breeding practices. For example, obtaining multiple agronomic traits and selection for mean performance and stability can be performed simultaneously (Evans 1993). The MGIDI index and the MTSI index use the same rescaling process to select genotypes. This rescaling program places all the agronomic traits in the range of 0–100, which contributes to the definition of ideotype; thereby, all identical ideotype values for the evaluated agronomic traits are expressed as 100. This is only possible from the perspective of the selection direction required by the rescaling process. Future METs studies will have to rescale the evaluated traits by the breeders to define the value of the new maximum and minimum value of the agronomic trait after rescaling, respectively (Rocha et al. 2018).

Table 3 presents the factor analysis performed with the WAASBY index (MTSI). Factors with eigenvalues greater than 1 were retained, resulting in four factors. The 12 traits under study were grouped into these four factors (FA). The first four-factor components (FA) had eigenvalues greater than one and accounted for 64.60% of the total variance among the traits. FA1 clustered four traits, including PH, NIN, NOF, and FFN, while FA2 grouped DFF, NPB, and FD traits (Table 4). The MTSI index analysis assigned ranks to all 42 studied genotypes based on the desired value of the trait (Fig.3). A

Table 3. Factor loadings explained variance and communalities resulted after superposition by factor analysis (MTSI)

Variable	FA1	FA2	FA3	FA4	Communality	Uniqueness
FFN	0.15	-0.10	-0.05	0.77	0.63	0.37
DFF	0.29	-0.68	0.16	0.03	0.57	0.43
PH	-0.72	0.28	0.19	0.14	0.66	0.34
NPB	-0.11	-0.68	-0.08	-0.29	0.56	0.44
IL	-0.20	0.79	0.19	-0.08	0.71	0.28
NIN	-0.11	0.11	0.72	-0.06	0.55	0.44
FL	0.06	0.26	0.68	-0.15	0.56	0.44
FD	0.67	0.01	-0.05	0.37	0.63	0.37
AFW	0.84	-0.14	0.32	0.11	0.85	0.15
NOF	-0.86	0.04	0.17	0.11	0.78	0.22
TW	0.12	-0.25	0.07	-0.79	0.71	0.29
FYPP	-0.05	-0.24	0.70	0.07	0.55	0.45
Eigenvalues	3.06	1.80	1.74	1.16		
Variance (%)	25.50	15.00	14.50	9.67		
Accumulated (%)	25.50	40.50	55.00	64.60		

selection pressure of approximately 15% was used to identify the top six genotypes, which included G7 (Anekombu), G33 (UAHS-11), G32 (UAHS-10), G41 (UAHS-19), G26 (UAHS-4), and G30 (UAHS-8), and these were utilized to determine selection differentials (SDs). The selection differential is the difference in mean phenotype between selected individuals and the population mean, quantifying the change in a population's mean trait value between pre and post-selection.

The MTSI index provided the desired selection differential for 7 (PH, NOF, FFN AFW, FL, FYPP and IL) out of the 12 studied traits with a success frequency of 58% in selecting desired traits. The selection differential percent for traits ranged from -7.74% (NIN) to 16.4% (FL). Traits with high heritability included the number of internodes per plant (0.99), fruit length (0.98), and the number of fruits per plant (0.98) (Table 4).

The role of each factor in the MTSI index was used to identify the strengths and weaknesses of genotypes. The less involvement of a factor (FA), the closer the characters within that factor are to the ideotype. Fig. 4 depicts the strengths and weaknesses of the stable accessions identified across three consecutive growing seasons. Factors that contributed the most were placed toward the center, while those that contributed less were drawn near the plot edge. The strengths and weaknesses of accessions showed that the six selected genotypes performed better for the majority of traits in FA3, such as AFW, FL, and FYPP. The weakness of G3 (Anekombu), however, was related to FA2 traits, such as DFF, NPB, and FD. Factors FA1 and FA2 showed weaknesses in most selected genotypes.

The factor loading analysis revealed that the MGIDI explained 77.30% of the total variation in the traits. We retained the top five main components (Table 5). In this study, the 12 evaluated traits were divided into five factors. PH, NIN, and NOF belonged to FA1; FA2 characteristics included FFN, DFF, and NPB; FA3 included traits FD, AFW, and FYPP; FA4 comprised fruit length, and the remaining three traits IL and TW were classified as FA5 (Table 6).

The selection pressure of approximately 15% identified the top genotypes (Fig. 5). The MGIDI index provided the desired selection differential (SD) for 7 out of 12 studied traits with a success frequency of 58% in selecting desired traits. The selection differential (SD) percent for traits ranged from -32.90% (NPB) to 17.00% (FFN). Traits with high heritability included FL (0.95) and FD (0.94) (Table 6). Assuming a selection intensity of 15%, different genotypes can be screened. Through the MGIDI index, genotypes G33(UAHS-11), G41(UAHS-19), G32(UAHS-10), G27(UAHS-5), G30(UAHS-8) and G3(Varsha Uphar) were very close to the red cutting point, indicating that these genotypes are expected to have an excellent phenotype (Fig. 5).

Fig. 6 depicts the genotype's strengths and weaknesses. Factor FA3 showed greater strength towards the most selected genotypes. FA5 and FA4 groups were found less in selecting genotypes. However, FA1's contribution was only for a few selected genotypes, such as Varsha Uphar and UAHS-10. Comparing the best linear unbiased predictors (BLUPs) of fruit yield showed that Green Star, UAHS-11, Kashi Kiranthi, Varsha Uphar, and UAHS-18 had higher predictions than the overall grain yield and, therefore, were superior genotypes. Kashi Leela had the lowest predicted grain yield (Fig. 7).

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

Variables	Factor	Xo	Xs	SD	SD Percent	h^2	Indicators
PH	FA1	122.00	130.00	8.10	6.65	0.89	increase
NIN	FA1	1.66	1.54	-0.13	-7.74	0.99	increase
NOF	FA1	20.90	22.50	1.62	7.77	0.98	increase
FFN	FA1	18.10	18.40	0.31	1.71	0.97	decrease
DFF	FA2	47.10	47.50	0.39	0.84	0.89	decrease
NPB	FA2	1.52	1.53	0.01	0.52	0.96	increase
FD	FA2	7.39	7.00	-0.39	-5.33	0.96	increase
AFW	FA3	16.80	18.10	1.33	7.95	0.93	increase
FL	FA3	16.70	19.40	2.74	16.4	0.98	increase
FYPP	FA3	366.00	391.00	24.90	6.80	0.95	increase
IL	FA4	1.63	1.83	0.21	12.7	0.95	increase
TW	FA4	6.42	5.98	-0.44	-6.83	0.95	increase

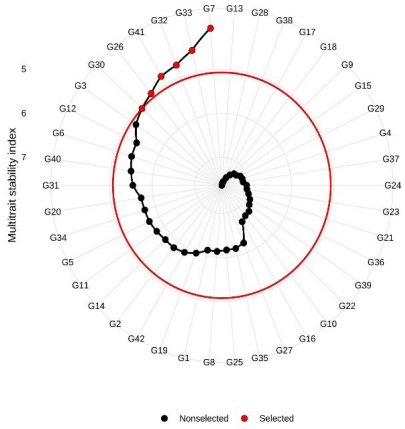

Fig. 3. Ranking of genotypes based on MTSI index

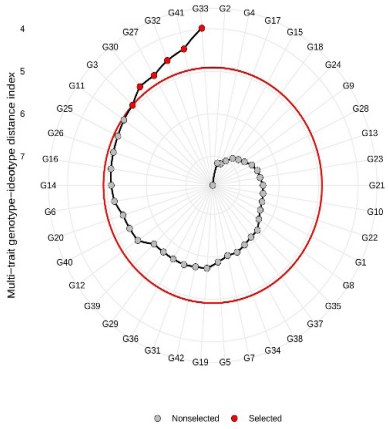

Fig. 5. Ranking of genotypes based on MGIDI index

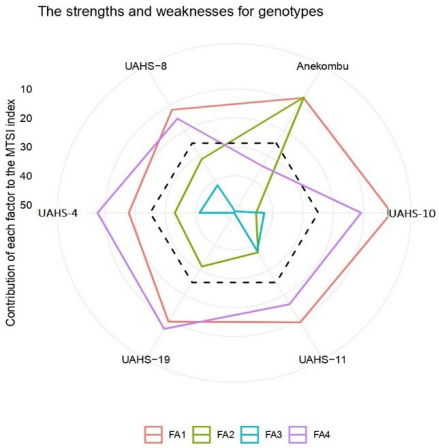

Fig. 4. Strength and weakness view of stable genotypes identified on the computed MTSI index

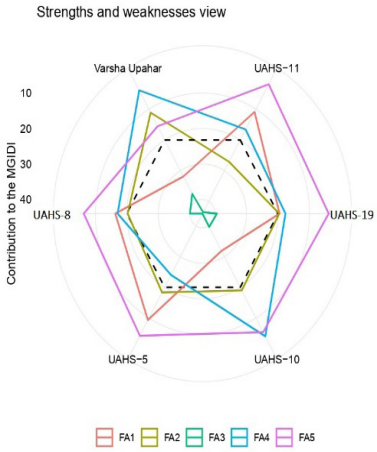

Fig. 6. Strength and weakness view of stable genotypes identified on the computed MGIDI index

Table 5. Factor loadings explained variance and communalities resulted after superposition by factor analysis (MGIDI)

Variable	FA1	FA2	FA3	FA4	FA5	Communality	Uniqueness
FFN	0.03	-0.9	-0.06	0.08	0.00	0.81	0.19
DFF	-0.03	-0.57	-0.16	0.26	-0.45	0.62	0.38
PH	-0.89	-0.25	-0.05	-0.02	-0.02	0.87	0.13
NPB	0.05	0.87	-0.13	0.00	0.13	0.80	0.20
IL	0.01	0.03	-0.12	0.10	-0.81	0.68	0.32
NIN	-0.79	0.31	-0.07	0.16	-0.15	0.77	0.23
FL	-0.07	-0.14	-0.08	0.90	-0.05	0.85	0.15
FD	0.12	-0.08	0.85	-0.35	-0.01	0.86	0.14
AFW	0.18	0.07	0.94	0.14	0.11	0.96	0.04
NOF	-0.66	-0.06	-0.58	0.10	-0.02	0.79	0.21
TW	0.13	0.22	-0.04	0.12	0.65	0.51	0.49
FYPP	-0.37	-0.04	0.58	0.51	0.14	0.76	0.24
Eigenvalues	3.03	2.28	1.80	1.12	1.05		
Variance (%)	25.20	19.00	15.00	9.37	8.76		
Accumulated (%)	25.20	44.20	59.20	68.50	77.30		

Table 6. Factors linked to correlated traits, selection differential, heritability and indicators (MGIDI)

Variables	Factor	Xo	Xs	SD	SD Percent	h^2	Indicators
PH	FA1	122.00	128.00	5.80	4.76	0.42	increase
NIN	FA1	16.80	17.90	1.16	6.94	0.63	increase
NOF	FA1	18.10	18.30	0.27	1.52	0.92	increase
FFN	FA2	1.63	1.90	0.27	17.00	0.88	decrease
DFF	FA2	47.10	47.4	0.25	0.52	0.68	decrease
NPB	FA2	1.52	1.02	-0.50	-32.9	0.89	increase
FD	FA3	1.66	1.57	-0.09	-5.49	0.94	increase
AFW	FA3	20.90	22.30	1.44	6.91	0.82	increase
FYPP	FA3	366.00	395.00	29.10	7.97	0.86	increase
FL	FA4	16.70	19.60	2.91	17.4	0.95	increase
IL	FA5	7.39	7.46	0.07	0.93	0.62	increase
TW	FA5	6.42	6.17	-0.25	-3.82	0.93	increase

The genotypes above and below the BLUPs are indicated by blue and red circles, respectively. Horizontal error bars present 95% confidence intervals of BLUPs in a two-tailed test.

The study highlights the importance of multi-trait selection indices, specifically the WAASBY index (MTSI) and MGIDI index, in identifying stable and high-yielding okra genotypes across different seasons. The MTSI index proved effective in identifying genotypes with desirable traits and high heritability, such as the number of internodes per plant, fruit length, and the number of fruits per plant. Similarly, the MGIDI index was successful in selecting genotypes that exhibit superior phenotypic traits, demonstrating the utility of these indices in breeding programs (Rocha et al. 2018). These novel methods are currently being used in breeding programms to identify stable genotypes for yield and other desirable traits in various crops such as barley (Pour-Aboughadareh et al. 2021), cassava (Koundinya et al.2021), sweet corn (Patel et al. 2023), and forage sorghum (Behera et al. (2024) and other crops for different environments.

Factor analysis played a crucial role in understanding the clustering of traits and their contributions to the overall performance of the genotypes. By retaining factors with eigenvalues greater than 1, we accounted for a significant portion of the total variance, ensuring a robust selection process (Evans 1993). The grouping of traits into

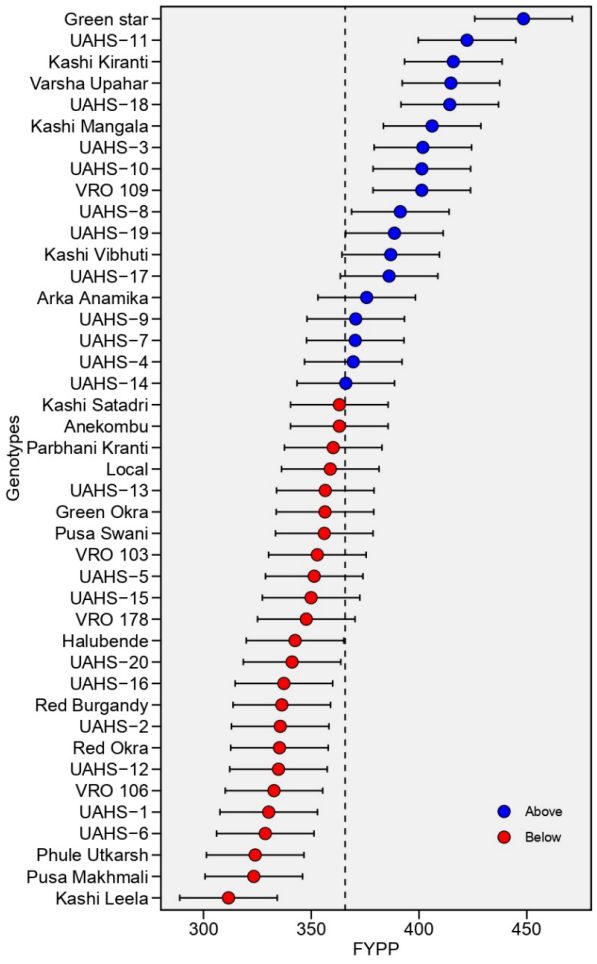

Fig. 7. Best linear unbiased prediction (BLUPs) of fruit yield per plant for 42 okra genotypes

factors allowed for a more targeted approach in selecting genotypes with the desired characteristics.

The selection differentials (SDs) and heritability estimates provided insights into the genetic potential and stability of the selected genotypes. High heritability values for traits like the number of internodes per plant and fruit length indicate that genetic factors largely govern these traits and can be effectively incorporated into breeding programs for developing superior genotypes (Alipour et al. 2021). The strengths and weaknesses analysis of the selected genotypes further emphasized the importance of multi-trait selection indices. By identifying the traits that contribute most to the overall performance, breeders can focus on improving these traits in future breeding efforts. The stability and adaptability of the selected genotypes across different growing seasons underscore their potential for use in diverse environmental conditions (Kang and Pham 1991). The use of multi-trait selection indices, such as the MTSI and MGIDI indices, in combination with factor analysis and selection differentials and correlation studies provides a comprehensive approach for identifying and developing stable and high-yielding okra genotypes (Olivato et al. 2019; Yan and Kang 2003). These methods enable breeders to select genotypes that not only perform well in specific environments but also exhibit stability and adaptability across different conditions, thereby contributing to the development of superior okra varieties (Allard and Bradshaw, 1964). Our selection based on the multi traits may assemble genotypes with superior adaptability across prevailing weather conditions of immense significance for hybridization programs. The genotypes selected from the present research are best utilized in a breeding program for the development of superior genotypes to perform better in diverse environmental conditions.

Supplimentary material

Supplimentary material Table S1 is presented and which can be accessed at www.isgpbb.org

Authors' contribution

Conceptualization of research (NS, BMDK); Designing of the experiments (NS, BMDK); Contribution of experimental materials (BMDK); Execution of field/lab experiments and data collection (NS); Analysis of data and interpretation (NS); Preparation of the manuscript (NS, BMDK).

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Supplementary Table S1. List of SSR primers and their sequences

S. No.	Primer name	Primer Sequence	Reference
1	AeKVR-106	**F**:TAGCGAAGAAATCACAGTTCACA **R**:CCCAAAACGGGTAACGTAAAG	Ravishankar *et al.* (2018)
2	AeKVR-114	**F**:TGAGAAGCGATGTTCTAGCGATT **R**:ATGAGGAAATAACTGACCCAGC	Ravishankar *et al.* (2018)
3	AeKVR-117	**F**: TACGTTCCGTACCTTACTTCGG **R**:GTTACGACGAGGTTTACCAAGG	Ravishankar *et al.* (2018)
4	AeKVR-125	**F**:TCGTATCGTTGAAGAAGGAAACA **R**:TGAATCGTCCGTGATATAAACC	Ravishankar *et al.* (2018)
5	AeKVR-126	**F**:GTACGGATACTCAAACGAAGGC **R**:GTAGGTGCAGTGTTGTTACCGA	Ravishankar *et al.* (2018)
6	AeKVR-131	**F**:TATTCATAAACAGGGATGAGCAG **R**:TCTTCAGTCCGATACAGCACTT	Ravishankar *et al.* (2018)
7	AeKVR-134	**F**:TCTTATCGACTGATGTTGAGGCA **R**:TGTGGGAAACTATAACCGGAAC	Ravishankar *et al.* (2018)
8	AeKVR-137	**F**:TAAAGGGTATAATGCAGCCATGT **R**:TCGTGGTTAGTTTCGTTTTCCT	Ravishankar *et al.* (2018)
9	AeKVR-149	**F**:TCACCAGGCTCGACCACTC **R**:GTACGTCGGGTACGACCG	Ravishankar *et al.* (2018)
10	AeKVR-153	**F**:TGGAGTGTGCTGCCAAGTTTAT **R**:TTGTTTATGCTGTGATGCTGAC	Ravishankar *et al.* (2018)
11	AeKVR-165	**F**:TAGCAAAAGCGATGATTGTCTG **R**:CCCCTAAACCCTAATCCTGACT	Ravishankar *et al.* (2018)
12	AeKVR-176	**F**:TCCGTTTTATTTCGACCGTTACC **R**:TAACCGAACCCGAACCGTA	Ravishankar *et al.* (2018)
13	AeKVR-182	**F**:TAAAGCGAGGTGGTCTACATGAC **R**:ATTGGGTGAAAGACAGAAAGGA	Ravishankar *et al.* (2018)
14	AeKVR-183	**F**:TGGTTTAGGGTTTACCGACTACG **R**:TAAGTTCGGGTTTAGGGTACGA	Ravishankar *et al.* (2018)
15	AeKVR-187	**F**:TCCGAGATTCAAGCGGATTATAG **R**:ACGACCACGCAACCGTAT	Ravishankar *et al.* (2018)
16	AeKVR-192	**F**:TCGTGACCGTGGACTCGTAGGTA **R**:ACGACCGACCGACCGAAC	Ravishankar *et al.* (2018)
17	AeKVR-193	**F**:TAACGCAAACTGAACTCTCGTTA **R**:ACCTAACCCTAACCCTAAACCG	Ravishankar *et al.* (2018)
18	AeKVR-194	**F**:TCGAACCCCTGAACTTGGTATT **R**:CACCACCGTAATAACCTAACCC	Ravishankar *et al.* (2018)
19	AeKVR-195	**F**:TCGTAACCCGTATAATGCAACAG **R**:AACGTAACCTAACCCTAAACCCT	Ravishankar *et al.* (2018)
20	AVRDC OKRA 1	**F**: ATGGAGTGATTTTTGTGGAG **R**: GACCCGAACTCACGTTACTA	Ouedraogo *et al.* (2018)
21	AVRDC OKRA 9	**F**: ACCTTGAACACCAGGTACAG **R**: TTGCTCTTATGAAGCAGTGA	Ouedraogo *et al.* (2018)
22	AVRDC OKRA 21	**F**: TCATGTCTTTCCACTCAACA **R**: CCAAACAAAATATGCCTCTC	Ouedraogo *et al.* (2018)
23	AVRDC OKRA 28	**F**: CCTCTTCATCCATCTTTTCA **R**: GGAAGATGCTGTGAAGGTAG	Ouedraogo *et al.* (2018)
24	AVRDC OKRA 39	**F**: TGAGGTGATGATGTGAGAGA **R**: TTGTAGATGAGGTTTGAACG	Ouedraogo *et al.* (2018)

25	AVRDC OKRA 52	**F**: AACACATCCTCATCCTCATC **R**: ACCGGAAGCTATTTACATGA	Ouedraogo *et al.* (2018)
26	AVRDC OKRA 57	**F**: CGAGGAGACCATGGAAGAAG **R**: CGAGGAGACCATGGAAGAAG	Ouedraogo *et al.* (2018)
27	AVRDC OKRA 63	**F**: GTGTTTGAAAGGGACTGTGT **R**: CTTCATCAAAACCATGCAG	Ouedraogo *et al.* (2018)
28	AVRDC OKRA 64	**F**: AAGGAGGAGAAAGAGAAGGA **R**: ATTTACTTGAGCAGCAGCAG	Ouedraogo *et al.* (2018)
29	AVRDC OKRA 70	**F**: GTAGCTGAACCCTTTGCTTA **R**: CTATCATGGCGGATTCTTTA	Ouedraogo *et al.* (2018)
30	AVRDC OKRA 78	**F**: CTCCGACAATTCAAGAAAAG **R**: CACCCAATCAAGCTATGTTA	Ouedraogo *et al.* (2018)
31	AVRDC OKRA 86	**F**: ATGCAAACAAGCTAGTGGAT **R**: ATTCTCTTCAGGGTTTCCTC	Ouedraogo *et al.* (2018)
32	AVRDC OKRA 89	**F**: TTTGAGTTCTTTCGTCCACT **R**: GTATTTGGACATGGCGTTAT	Ouedraogo *et al.* (2018)
33	Okra-12	**F**: AATGAAGTTGGAGTCGACAG **R**: CAATACTCGTTGTTGTGGTG	Kumar *et al.* (2016)
34	Okra-103	**F**: GAATTCGATTCCAATACAGG **R**: TCGTCGTCTTCATTTCTCTT	Kumar *et al.* (2016)
35	Okra-104	**F**: CGGTAAATCTTGTCTCTTGC **R**: TATAGGAAAACCCCCAAGAT	Kumar *et al*. (2016)
36	Okra-110	**F**: GGCAACAACAGTTCTCCTT **R**: AATTGGGGTTAGTGACGATA	Kumar *et al.* (2016)
37	Okra- 111	**F**: CATTTTAAGGAGCGAGTGTC **R**: CTCTTCCTCAACAAAACCAG	Kumar *et al*. (2016)
38	Okra-119	**F**: GCAGCGGTAGAAATAAATGT **R**: GGAGGGTTTAGGTATGGTTT	Kumar *et al.* (2016)
39	Okra 121	**F**: CTAAATGCAAACTCGAACCT **R**: TAATCTATGCCTGAACCGTC	Kumar et al. (2016)
40	Okra-125	**F**: CCCCTTCCTCTAGATCTCAT **R**: GACGGTGGAGATTGAACTT	Kumar *et al*. (2016)
41	Okra-128	**F**: GAACTTCTGTGCGGTATGAT **R**: ACCGTTATTTTCTGCCTCTT	Kumar *et al.* (2016)
42	Okra 135	**F**: GTGATTATGGTTGCCTGAAT **R**: CCCACTGACAGCTTATTGAA	Kumar *et al.* (2016)
43	Okra-137	**F**: GAGAGAGATTGCTTCGACTG **R**: TAAACTTTAAACTCAGCGGC	Kumar *et al.* (2016)
44	Okra-141	**F**: TATCCCGATACTTTCTCCAA **R**: TTAGCCTCTAAGGGGAAAAG	Kumar *et al.* (2016)
45	Okra-148	**F**: TGCTTATTCATGCTGACCTA **R**: AGCACTTGATATCCAAGGAA	Kumar *et al.* (2016)