Phenotypic and Molecular Characterization of Mango Cultivars in Southwest Nigeria

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ABSTRACT
Morphological and microsatellites (SSR) markers are efficient tools for determining genetic relatedness among mango cultivars. Seventeen mango cultivars were used for this study. Eight fruit quantitative traits were collected and subjected to mean separation using One-Way ANOVA and correlation using Principal Component Analysis (PCA). Also, molecular analysis was done using PCR-based SSR markers. The resulting binary matrix was analyzed using Numerical Taxonomy and Multivariate Analysis System (NTSYSpc). Significant variation (p ≤ .05) among all the 17 mango cultivars was observed for the eight quantitative traits studied. The PCA showed that the fruits’ length, width, thickness weights, %pulp and %stone contributed to 98.73% of the variation observed in all the mango cultivars. A total of 21 alleles were detected from the seven polymorphic primers ranging from two to five alleles per locus with an average of 3.0 alleles per locus. The polymorphic information content (PIC) ranged from 0.52 to 0.80 with an average of 0.66. Both the morphological and molecular markers showed that the mango cultivars were diverse except for ‘Saigon’ and ‘Julie’ as well as ‘Harden’ and ‘Lipen’ which though appear morphologically distinct based on the understudied traits but showed strong similarity to each other through molecular analysis. Dendrogram constructed using the Unweighted Pair Group Method with Arithmetic distance (UPGMA) based on SSR markers revealed a similarity coefficient of 48–93% indicating high level of variability and the presence of outbreeding. Results of the morphological and microsatellite (SSR) analyses showed wide diversity among the mango cultivar used in this study.

Introduction

Mango (Mangifera indica L.) which is often referred to as ‘the King of fruits’ (Singh, 1996) because of its popularity and importance in the tropical world belongs to the genus Mangifera and the family Anacardiaceae. According to the Food and Agriculture Organization, the top mango-producing countries are India, China, Thailand, Indonesia and Mexico, with Nigeria ranking as the third in Africa (FAO, 2019). The flowering process of the crop is a complex process owing to the cool inductive temperatures which induce mango flowering under subtropical and upper-latitude tropical conditions (Ramirez and Davenport, 2010). Mango is heterogenous in nature and has a great diversity in seedling genotypes which have shown wide genetic diversity in terms of shape, color, bearing habits, maturity stage and yield (Serry et al., 2019). Most mango cultivars including some superior clones are hybrids, resulting from natural cross-pollination (Krishna and Singh, 2007). Several factors including selection, mutation, genetic drift and recombination provide sources of genetic diversity. Despite the level of genetic diversity that exists among mango landraces and
cultivars, the genome has been reported to be allopolyploid with chromosome counts of $2n = 40$, i.e. the diversity occurring in mango may be genomic structural variation (Begum et al., 2016; Krishna and Singh, 2007; Schiessl et al., 2018).

Genetic resources for potential crop improvement are invaluable in mango breeding programs, hence the need for collection, evaluation, characterization and documentation to increase their genetic base. Morphological characterization has been the traditional method of identification of many crops including mango. However, this method is influenced by environmental factors and the results are often biased and unreliable (Matthew and Oziegbe, 2016; Subedi et al., 2009). The ease and inexpensive nature of morphological characterization makes it a paramount technique in plant characterization and taxonomy. Mango varieties have been differentiated based on fruit characteristics that include size, shape, color and nutrient composition (Igbari et al., 2019). Arogundade et al. (2017) characterized some mango cultivars in Nigeria employing physicochemical and morphological properties of the fruits to show the diversity of the collected cultivars. Morphological traits in the characterization of mango cultivars create ambiguities in the identification of closely related cultivars (Begum et al., 2016). Also, the use of morphological markers and isozymes techniques in the characterization of mango cultivars often does not provide an accurate evaluation of diversity among mango cultivars, and this could lead to misidentification or duplication of the crop genotypes (Kumar et al., 2013). In the case of genetic erosion, where there is a need for conservation, a more in-depth classification and study is needed.

Molecular markers are valuable tools for the assessment of genetic variation (Varshney et al., 2005) and therefore assist in selection. Among the available molecular markers, polymerase chain reaction (PCR)-based markers are the most suitable for revealing genetic diversity and include RAPD, SSR, ISSR, AFLP and SNP. Among these, SSRs are receiving attention due to their multiallelic nature, reproducibility, hypervariability, codominant inheritance, comprehensive genome coverage, relative abundance and suitability for high-throughput genotyping (Parida et al., 2009; Rafalski et al., 1996). The use of SSR for genetic characterization was reported to serve two purposes that include the identification of genotypes and estimation of their genetic relatedness (Ravishankar et al., 2000). SSR markers have been effectively utilized for estimating genetic variability in mango (Honsho et al., 2005; Ravishankar et al., 2015; Shamili et al., 2005). Begum et al. (2016) showed that a high genetic variability exists among 90 mango cultivars using simple sequence repeat (SSR) and concluded that there is an increasing genetic erosion in their locality. Also, for clonal selection and improved breeding, there is a need for molecular evaluation. This study, therefore, investigated the genetic variability and relatedness among different mango cultivars using both morphological and SSR markers. These findings will be useful in mango genetic resource conservation and in breeding programs.

**Methods**

**Survey and Sampling**

An exploratory survey was conducted during the mango fruiting seasons following a selective sampling strategy so that mango cultivars already sampled were not repeated. Mango orchard of the National Horticultural Research Institute (NIHORT), Ibadan, comprising landraces and cultivars and some locations (Ogbomosho, Ikole and Ibadan) in southwest Nigeria were selected for this study. Seventeen mango varieties used for this study include Alfonso, Edward, Harden, John Bull, Julie, Kent, Lipen, Madoe, NG-25, NG-26, NG-27, Ogbomosho, Palmer, Peach, Saigon, Tommy Atkin and Uno.

**Morphological Characterization**

Morphological characterization was based on tree-ripened fruit characteristics. Fruit sampling was carried out thrice on 15 randomly selected mango fruits during the harvest season. Morphological characters of sampled fruits were recorded following mango descriptors (IPGRI, 2006). The fruit samples were evaluated for eight quantitative traits that include fruit length (cm), fruit width (cm), fruit thickness (cm), fruit weight (g), fiber length (mm), peel (%), pulp (%) and stone (%).
DNA Extraction and Quantification

Genomic DNA was extracted from young leaves using a modified Cetyltrimethylammonium Bromide (CTAB) procedure (Mignouna et al., 1998). The quality and quantity of DNA was assessed by gel electrophoresis using 1% agarose with known concentrations of undigested lambda DNA (Sigma, St. Louis, MO, USA). Quantification of DNA was done using a spectrophotometer (Beckman Coulter DU530) at 260 nm. Extracts were diluted with sterile water to obtain DNA concentrations of 25 ng/μL.

Polymerase Chain Reaction

In this study, a total of 15 simple sequence repeat (SSR) primer pairs were used. PCR for SSR reactions was conducted in a 20 μL volume in a 96-well microliter plate using an automated thermal cycler (model: Peltier Thermal Cycler 200). The reaction volume contained 25 ng of template DNA, 100 μM each of dNTP and 2.5 mM MgCl₂, 0.5 μM each of forward primer and reverse primer. 1X reaction buffer and 2 units of Taq DNA polymerase (Invitrogen) were carried out in a volume of 10 μL containing 1X reaction Buffer, 0.2 mM MgCl₂, 0.25 mM dNTP, 0.5 μM Primer, 1 Unit Taq DNA Polymerase, and Template DNA. The PCR for SSR was carried out with conditions, i.e., 94°C for 2 min, followed by 30 cycles of 94°C for 30 s, 55°C for 45 s and 72°C for 45 s, with a final extension step at 72°C for 7 min. PCR products were separated on 2% (w/v) agarose gels using 0.5X TBE Buffer at 90 V and stained with ethidium bromide for visualization under UV light.

Data Analysis

The quantitative data obtained on the mango fruits were subjected to principal component analysis (PCA) using PAST 3. Cluster analyses were carried out using unweighted pair group method arithmetic averages (UPGMA) to construct a dendrogram using Gower’s genetic distance, considering Eigenvalues of >0.3 in the PCA of the diversity study. Each genotype for the SSR primers was scored visually on the basis of their presence (1) or absence (0) separately for each cultivar. The sizes of fragments (molecular weight in base pair) were estimated using the DNA hyper-ladder 2 (Bioline) as base pair (bp) ladder that was run along with the amplified products. The scores obtained using all seven polymorphic primers for SSR were used for constructing the matrix. The statistical software NTSYSpc (Rohlf, 2005) and POWER MARKER software were used to construct a UPGMA dendrogram using hierarchical clustering. Using NTSYS software, a dissimilarity matrix was calculated utilizing Jaccard (1908) coefficient. Cluster analysis based on the dissimilarity matrix was performed using UPGMA (Sneath and Sokal, 1973) of the NTSYSpc version 2.2 (Rohlf, 2005).

Results

Morphological Variation in 15 Mango Cultivars

Significant differences (p < .05) were observed in all 17 mango cultivars evaluated for fruit size (length, width and thickness) and weight, percent peel, pulp, stone by weight and fiber length (Table 1). Fruit length varied from 8.4 cm (NG-25) to 18.8 cm (NG-27). Fruit width ranged from 1.8 cm (NG-25) to 3.5 cm (NG-27). Fruit thickness ranged from 5.3 cm (NG-25) to 10.7 cm (NG-27). Fruit weight ranged from 106.6 g (NG-25) to 893.7 g (Peach). NG-27 had significantly (p ≤ .05) higher fruit length and fruit weight than all the other mango cultivars. NG-27 had the highest fruit length, fruit width, fruit thickness, fruit weight and the lowest % stone. NG-25 had the lowest fruit length, fruit width, fruit thickness and fruit weight. The percent peel varied from 16.3 (Kent) to 23.5 (Saigon). The percent pulp varied from 46.9 (NG-25) to 74.3 (Julie). The percent stone varied from 7.2 (Edward) to 22.9 (NG-25). Fiber length ranged from 26 mm (Saigon) to 115 mm (Julie). The highest % pulp, fiber length and lowest % peel were observed in Julie. NG-25 had the highest % stone and lowest % pulp. Saigon had the highest % peel and lowest fiber length.
among (cm), and cultivars (PCA).

Table 1. Morphological evaluation of fruits of Mango cultivars.

| Accession | Fruit length (cm) | Fruit width (cm) | Fruit thickness (cm) | Fruit weight (g) | Peel (%) | Pulp (%) | Stone (%) | Fiber length (mm) |
|-----------|-------------------|------------------|----------------------|-----------------|----------|----------|-----------|------------------|
| Alfonso   | 11.7<sup>b</sup>  | 2.3<sup>bcd</sup> | 7.2<sup>bcd</sup>    | 258.3<sup>c</sup> | 17.5<sup>abc</sup> | 67.0<sup>de</sup> | 17.2<sup>d</sup> | 35.0<sup>ab</sup> |
| Edward    | 13.3<sup>cde</sup> | 3.0<sup>def</sup> | 8.6<sup>efg</sup>    | 570.0<sup>b</sup> | 18.4<sup>bcd</sup> | 57.4<sup>bc</sup> | 7.2<sup>a</sup>  | 113.0<sup>ab</sup> |
| Harden    | 11.7<sup>b</sup>  | 2.8<sup>bdef</sup> | 8.1<sup>cde</sup>    | 383.3<sup>c</sup> | 22.1<sup>efgh</sup> | 67.0<sup>de</sup> | 8.3<sup>ab</sup> | 69.0<sup>ab</sup>  |
| John bull | 17.3<sup>ef</sup> | 2.9<sup>bdef</sup> | 9.5<sup>fgh</sup>    | 850.1<sup>ef</sup> | 22.9<sup>gh</sup>  | 63.9<sup>de</sup> | 9.6<sup>b</sup>  | 38.0<sup>b</sup>   |
| Julie     | 14.1<sup>d</sup>  | 2.9<sup>bdef</sup> | 8.4<sup>def</sup>    | 485.1<sup>f</sup>  | 15.2<sup>a</sup>  | 76.3<sup>b</sup> | 7.2<sup>ab</sup> | 115.0<sup>ab</sup> |
| Kent      | 16.1<sup>e</sup>  | 3.2<sup>def</sup> | 10.2<sup>gh</sup>    | 663.3<sup>h</sup>  | 16.3<sup>ab</sup> | 72.9<sup>de</sup> | 8.6<sup>ab</sup> | 36.0<sup>bc</sup>  |
| Lipen     | 13.9<sup>cd</sup> | 3.3<sup>ef</sup> | 9.9<sup>fgh</sup>    | 683.3<sup>h</sup>  | 18.3<sup>bc</sup> | 65.5<sup>d</sup> | 11.2<sup>c</sup> | 59.0<sup>d</sup>   |
| Madoe     | 11.6<sup>b</sup>  | 2.4<sup>bcd</sup> | 7.2<sup>gh</sup>     | 303.0<sup>d</sup>  | 23.2<sup>gh</sup> | 63.9<sup>d</sup> | 11.4<sup>c</sup> | 75.0<sup>c</sup>   |
| NG-25     | 8.4<sup>a</sup>   | 1.8<sup>a</sup>  | 5.3<sup>a</sup>      | 106.6<sup>d</sup>  | 19.3<sup>cde</sup> | 47.3<sup>a</sup> | 22.8<sup>e</sup> | 50.0<sup>cd</sup>  |
| NG-26     | 12.3<sup>bc</sup> | 2.4<sup>bcd</sup> | 7.8<sup>cde</sup>    | 290.0<sup>d</sup>  | 21.5<sup>efgh</sup> | 57.8<sup>b</sup> | 18.7<sup>d</sup> | 57.0<sup>d</sup>   |
| NG-27     | 18.8<sup>a</sup>  | 3.5<sup>ab</sup> | 10.7<sup>h</sup>     | 825.0<sup>f</sup>  | 20.4<sup>cdegh</sup> | 68.5<sup>cde</sup> | 7.5<sup>ab</sup> | 42.0<sup>bc</sup>  |
| Ogbomoso  | 12.5<sup>cde</sup> | 2.2<sup>bcd</sup> | 6.8<sup>abcd</sup>   | 221.1<sup>b</sup>  | 22.6<sup>gh</sup> | 51.6<sup>b</sup> | 22.8<sup>e</sup> | 35.0<sup>ab</sup>  |
| Palmer    | 16.7<sup>bc</sup> | 2.7<sup>bcdef</sup> | 8.1<sup>cde</sup>    | 533.8<sup>g</sup>  | 21.3<sup>defgh</sup> | 63.7<sup>d</sup> | 9.2<sup>b</sup>  | 35.0<sup>ab</sup>  |
| Peach     | 15.9<sup>ef</sup> | 2.8<sup>bcdef</sup> | 8.2<sup>de</sup>     | 893.7<sup>ij</sup> | 23.0<sup>ghi</sup> | 64.2<sup>d</sup> | 9.2<sup>b</sup>  | 39.0<sup>b</sup>   |
| Saigon    | 12.5<sup>cde</sup> | 2.1<sup>abc</sup> | 6.0<sup>ab</sup>     | 218.6<sup>bc</sup> | 23.5<sup>h</sup>  | 65.7<sup>d</sup> | 12.7<sup>c</sup> | 26.0<sup>c</sup>   |
| Tommy     | 15.3<sup>de</sup> | 2.7<sup>bcd</sup> | 7.6<sup>cde</sup>    | 314.9<sup>bc</sup> | 23.5<sup>gh</sup> | 61.4<sup>d</sup> | 14.5<sup>cd</sup> | 18.3<sup>a</sup>   |
| Atkin     | 18.6<sup>abc</sup>| 2.1<sup>abc</sup> | 9.6<sup>b</sup>      | 182.4<sup>abc</sup> | 19.8<sup>cdef</sup> | 66.6<sup>cde</sup> | 12.1<sup>c</sup> | 39.0<sup>b</sup>   |

Means followed by the same letter(s) are not significantly different at P < .05 using the Duncan’s multiple range test.

**Principal Component Analysis**

PCA took into account the first two PCA at cumulative variance of 99.91% of the total variance (Table 2). The first PCA, i.e., PC 1, showed that fruit length (cm), fruit width (cm), fruit thickness (cm), fruit weight (g), %pulp and %stone contributed 98.73% of the total variation in fruit morphology among the mango cultivars evaluated, while the PC 2 took into account fruit length (cm) and %pulp, which contributed 1.18% of the total fruit morphological variation (Table 2).

The correlation among the mango cultivars and the separation of PC 1 and PC 2 showed dispersion in all the quarters for the morphological characters (Figure 1). Figure 2 shows the agglomerative hierarchical clustering dendrogram that illustrates the relationship among the cultivars using the unweighted pair group method with arithmetic mean (UPGMA). The cluster analysis classified the 15 cultivars into different clusters with Gower similarity index ranging from 0 to 0.45. At a genetic distance of 0.3, the dendrogram was divided into four branches. At 0.3 similarity coefficient, Julie and Edward were grouped into Cluster I; Kent, Lipen and NG-27 were grouped into Cluster II; and ED-20 and NG-25 were grouped into Cluster III. Cluster IV had the highest number of cultivars (NG-26, Ogbomoso, Alfonso, Uno, Saigon, Madoe, Harden and Palmer) grouped together (Figure 2).

Table 2. Principal components, eigen values, and proportion of variation of the Mango cultivars.

| Characters      | PC 1     | PC 2     | PC 3     |
|-----------------|----------|----------|----------|
| Fruit length    | 0.0092   | −0.0229  | 0.0529   |
| Fruit width     | 0.0016   | 0.0034   | 0.0122   |
| Fruit thickness | 0.0050   | 0.0058   | 0.0364   |
| Fruit weight    | 0.9998   | 0.0087   | −0.0162  |
| %Peel           | −0.0011  | −0.0440  | −0.1117  |
| %Pulp           | 0.0115   | 0.0300   | 0.9152   |
| %Stone          | 0.0140   | −0.0576  | −0.3775  |
| %Fiber          | −0.0077  | 0.9966   | −0.0533  |
| Eigenvalue      | 63860.30 | 760.71   | 47.93    |
| % Variance      | 98.73    | 1.18     | 0.07     |
| Cumulative % variance | 98.73 | 99.91 | 99.98 |
Figure 1. Morphological diversity among the Mango cultivars using PCA.

Figure 2. Dendrogram constructed based on the quantitative characters of the 17 Mango cultivars using Gower genetic distant. Lane M = 1 kb DNA ladder, lanes 1–17 = Mango cultivars in the order; Saigon, Julie, Edward, Madoe, Ogbomosho, Harden, Palmer, Lipen, Alfonso, Uno, Kent, Peach, Tommy Atkin, Johnbull, NG-25, NG-26 and NG-27.
Genetic Variation in 15 Mango Cultivars

Fifteen (15) SSR primers (Ajayi et al., 2019) were used for generating banding profiles (Figure 3), out of which seven primers (SSR20, EF592206, EF592216, EF59210, EF592198, EF592211 and EF592197) gave clear polymorphic bands for all the cultivars under study. The numbers of alleles detected varied from 2 (EF592206, EF59210 and EF592198) to 5 (EF592211). The average number of alleles per primer pair was 3. The allele size ranged from 120 (EF592206) to 300 bp (SSR 20).

All the SSR markers used in the study showed a high level of polymorphism (Table 3). The polymorphism information content (PIC) ranged from 0.5215 to 0.8032, while the gene diversity ranged from 0.5813 to 0.8235. In this study, the highest PIC and gene diversity were recorded for the marker EF59210, while the lowest PIC and gene diversity were recorded for the markers EF592211 and EF592206. The cluster analysis separated almost all the cultivars into separate groups at a similarity coefficient of 0.82 (Figure 4). At 0.93 similarity coefficient, ‘Saigon’ and ‘Julie’ were grouped as identical cultivars as well as ‘Harden’ and ‘Lipen’ cultivars. ‘Edward’ was distinctly separated as a different cultivar from the SSR Markers.

Table 3. Characteristics of polymorphic SSR markers used in molecular analysis of 17 Mango cultivars.

| Microsatellite name | 5' to 3' Primer sequence | No. of Alleles | PIC    | Gene diversity |
|---------------------|--------------------------|----------------|--------|----------------|
| SSR 20              | F: CGCTCTGTGAGAATCAAATGGT R: GGACTCTTATTAGCCAATGGGATG | 4               | 0.664  | 0.713          |
| EF592206            | F: GCGAAAGAGGAGAGTGCAG   R: TCTATAAGTGCACCCCTCACC | 2               | 0.529  | 0.581          |
| EF592216            | F: TCTATAAGTGCACCCCTCACC R: ACTGCCACCGTGGGAAGTAG | 3               | 0.584  | 0.657          |
| EF59210             | F: AGCTATGGCAGCAGCAATATCA R: GTCTCTCTTGCCGCAAC    | 2               | 0.803  | 0.824          |
| EF592198            | F: TCTGACCTCACCTCTTCTCA R: ATACTCTTGCCCTGCTCTGTTG | 2               | 0.752  | 0.782          |
| EF592211            | F: TCTGTTAGGCGGCTGTTTG   R: CACCTCCCTCCTCCTCCTT | 5               | 0.522  | 0.602          |
| EF592197            | F: GCTGCTCTCAACTGAGACC R: GCAGTAATGCTCGGAGAAGAC | 3               | 0.632  | 0.664          |
| Mean                |                          | 3               | 0.641  | 0.689          |
Discussion

Understanding the genetic diversity among plant species provides useful information for varietal development, conservation and management (Romero et al., 2009). Determination of genetic relatedness in mango cultivars will provide important information for varietal selection and conservation in mango breeding programs (Ravishankar et al., 2000). Identification of genotypes using molecular markers can help in the selection of desired traits in tree crops; this will also minimize the risk of mix-ups in orchards (Struss et al., 2001).

The mango classification was done using 17 cultivars that include three cultivars, NG-25, NG-26 and NG-27. The results from the morphological and microsatellite characterizations showed that most of the mangoes were diverse. Karihaloo et al. (2003) reported that high diversity occurred among cultivated and wild mangoes studied.

The fruit length and fruit thickness fall within the range reported by Begum et al. (2016) except cultivar NG-27 which also happens to be the longest and thickest of all the studied cultivars. NG-27 could be a new cultivar owing to the diversity in some of the traits studied. Pandey (1998) and Krishna and Singh (2007) reported that the outbreeding ability of mango would have resulted in the genetic diversity within the crop. The morphological traits showed that the fruits length, width, thickness weights, %pulp and %stone contributed to the diversity in the first principal component. The separation of PC values showed that mango cultivars were dispersed in all quarters, indicating a high level of genotypic variation among the mango cultivars in southwestern Nigeria. The dendrogram showed that although NG-25, NG-26, NG-27 and Ogbomoso are all local cultivars, they were genetically diverse. NG-27 belonged to a different clade. Krishna and Singh (2007) reported that agro-climatic conditions contributed to the high diversity in mango cultivars. This report was later corroborated by the study of Begum et al. (2016) which showed that there is high genetic erosion among the local mango cultivars and each cultivar possesses its distinct characters which contributed to both economic and cultural values of the crop. Fruit length, width, thickness, weight, %pulp and % stone showed significant variation suitable for morphological characterization of mango fruit using PCA as shown by the study.

A morphology-based method is sufficient for plant characterization (Patwardhan et al., 2014); however, the use of molecular markers in conjunction with the classical phylogenetic approach is more reliable. Microsatellites have become the markers of choice for use in the characterization of many plant species due to their codominant, highly polymorphic and reproducible nature (Gupta and Varshney, 2000). All the seven markers used in this study were polymorphic, showing high allelic

![Figure 4. UPGMA (unweighted pair-group with arithmetic mean) dendrogram showing genetic relationships among 15 Mango cultivars.](image-url)
variations in the DNA of the 15 mango cultivars, with marker EF59210 being the most polymorphic possessing a PIC value of 0.80 and gene diversity of 0.82. These results confirm the effectiveness of microsatellites when used in genetic diversity studies. Filho et al. (2010) and Parthiban et al. (2018) reported that such discriminating power and high polymorphism primers can be used effectively in constructing genetic linkage maps. UPGMA cluster analysis in this study revealed significant genetic diversity as evident from the Jaccard’s similarity coefficient ranging from 0.48 to 0.93 for SSR markers. This is similar to the work of Shamili et al. (2005) who found 35–100% genetic similarity among 41 mango cultivars using 16 SSR markers. Kumar and Narayanaswamy (2001) reported Jaccard’s similarity in the range of 61–95% in 50 Indian cultivars, and Fitmawati et al. (2010) observed 69–98% similarity in 82 cultivars of mango from Indonesia.

**Conclusion**

The morphological and microsatellite (SSR) results obtained for the mango cultivars showed valuable diversity among the mango cultivars studied, which may be due to the outcrossing ability of the mango. This study will provide information on interesting traits unique to the mango cultivars studied that will suit diverse consumer preferences and industrial uses.

**Disclosure Statement**

No potential conflict of interest was reported by the author(s).

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