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Evaluation and Phylogeny Study for Elite Mango Strain Grown under Giza Conditions

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Abstract: An elite selected mango strain of seed origin and its grafted (six-year-old) were subjected to comparative study with the Alphonso cultivar. This comparative study was performed after phylogeny evaluation with four mango cultivars including Zebda, Ewais, Alphonso and Succari using two molecular markers, rbcL and ITS4 gens barcoding. The barcoding technique proved its ability to detect phylogeny within mango cultivars, as these techniques revealed that the Alphonso cultivar was highly related to the elite mango strain. Morphological and floral characteristics as well as physical and chemical fruit characteristics were evaluated. The results showed that the Elite Strain was earlier in the beginning of flowering (4th week of Feb. and 1st week of March), the end of flowering (1st week of May) and the date of harvest (3rd week of July) than the Alphonso cv., while the flowering period was the tallest (60.33 and 58.00 days) compared to the Alphonso cv. in both seasons, respectively. Elite Strain had a significantly lower malformation percentage (15.20 and 14.40%) than Alphonso cv. (35.10 and 39.30%) in both seasons, respectively. In regard to initial fruit set, fruit number per panicle, fruit number per tree and yield per tree, Elite Strain showed the highest values (12.40 and 15.10), (2.23 and 2.66 fruits/panicle), (112.67 and 129.33fruits/tree) and (38.66 and 43.66 kg) in both seasons, respectively, compared to Alphonso cv., which showed the lowest values. Moreover, the calculated biennial bearing index revealed that Elite Strain gave significantly lower percentage (6.05 %), while Alphonso cv. gained the higher percentage (27.42 %). The Elite Strain appeared to be significantly superior to the Alphonso cv. and gave the highest values in all studied physical and chemical fruit characteristics. These values were as follows: weight (342.33 and 340.67 g), volume (345.00 and 346.67 cm^3), length (11.60 and 11.76 cm) and diameter of fruit (7.77 and 8.07 cm) and weights of pulp (243.00 and 239.67 g), stone (40.67 and 41.00 g) and peel (58.67 and 60.00 g) and TSS % (18.00 and 17.67 %), reducing sugar (7.91 and 8.01 %), total sugar (19.82 and 20.41 %) and ascorbic acid content (48.80 and 50.47 mg/100g) in both seasons, respectively.

Key words: Mango (*Mangifera indica* L.) · Evaluation · Strain · Physical and chemical fruit characteristics · Fingerprint

INTRODUCTION

Mango (*Mangifera indica* L. (Anacardiaceae) is one of the most frequently cultivated horticultural crops in many tropical and subtropical regions. It has the fifth-largest global fruit market after citrus, banana, grapes and apple. Because of its excellent taste, varied flavors and aromas, high carotenoid content and high pro-vitamin A value, it is described as "the king of tropical fruits" in popular culture [1]. It is thought that the Assam-Burma region of eastern India is where cultivated mango first appeared and that the Mangifera genus is most diverse in South East Asia [2]. *Mangifera indica* L. has been identified in more than a thousand different varieties around the world [3]. Mango trees are currently one of the most common fruit trees in Egypt. After grapes and citrus, it comes in third. Mango production in Egypt is 1395244 tonnes, according to FAOSTAT [4].

The majority of mango cultivars in Egypt generally have lower productivity. Fruit attributes, particularly size, outer color and flavor, frequently fail to meet the demands of both the domestic and export markets. However, it is characterized by a few issues that have been determined to be the most severe ones that producers currently face: malformation, alternate bearing, low yield and a lack of pos-tharvest technologies.

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Therefore, there is a real need to select and evaluate promising strains having superior attributes.

Crop improvement and the preservation of plant genetic resources both depend on variety characteristics [5]. The physical fruit characteristics, such as shape, diameter, length, volume, weight, color and pulp weight, as well as chemical characteristics, such as total soluble solids, acidity, total sugars, vitamin C, flavor and aroma, all have a significant effect on the mango's quality.

Using DNA barcoding for molecular identification of cultivated plants can be tricky and challenging in terms of generating barcode data and analyzing this data to determine discrimination power [6]. In practice, the DNA barcoding technique is based on a short and unique DNA sequence for one locus or a few loci used together as a whole. The generating data from a unique species is used for fingerprinting and copyright protection for this species and marketplace regulation in general [7]. Mainly, to distinguish difficult taxa, DNA barcoding markers will be the best option for generating a phylogenetic tree [8].

Moreover, the plant barcode, such as rbcL and ITS, should be multi-locus, preferably comprising a conserved coding region or vice versa. This more rapidly evolving region is probably non-coding [9]. The rbcL (Ribulose -1, 5 – bisphosphate carboxylase/ oxygenase large subunit) gene that is coding for the large subunit of the enzyme RuBisCo is considered one of the most barcoding genes in the phylogeny of plants. With the consent of the consortium for the barcode of life (CBOL) in 2009, they considered matK (the chloroplast gene) and rbcL as the main barcodes of plant species, in addition to intergenic sequence and nuclear gene ITS as the addition barcodes [10]. Meanwhile, rbcL is well known for its comparability, universality and ease of amplification [11]. So, our study will try to find some genetic variations to build a clear phylogenetic relationship between some mango plants (*Mangifera indica* L. (Anacardiaceae) utilizing rbcL and ITS4 genes barcoding.

The objective of the present study is to evaluate some morphological and floral characteristics as well as physical and chemical fruit characteristics of a new seedling mango strain, which was selected based on its superior attributes and then it was compared with the Alphonso cultivar.

MATERIALS AND METHODS

This experiment was conducted over successive years (2019, 2020) in private mango orchard in Giza governorate on the grafted on Succari rootstock (six years old) of elite selected mango genotype (forty years old)

Table 1: Characteristics of the Elite Strain of selected mango tree

Character	Frist season	Second season
Trunk circumference (cm)	72	79
Tree height (m)	6.5	6.9
Tree canopy (m)	21.4	22
Date of initial flowering	25-Feb	1-Mar
Date of flowering end	20-Apr	22-Apr
Flowering duration (days)	53	51
Date of harvest	18-Jul	15-Jul
Malformation (%)	11.8	12.1
Initial fruit set	13.2	14.1
Fruit number/panicle at harvest	1.89	1.72
No. of fruit per tree at harvest	530	543
Yield/tree (kg)	192	208
Biennial bearing index	4	---
Fruit weight (g)	362	383
Fruit volume (cm^3)	365	375
Fruit length (cm)	11.8	12.1
Fruit diameter (cm)	7.9	8.1
Pulp weight (g)	274	292
Stone weight (g)	43	39
Peel weight (g)	45	52
Pulp (%)	75.69	76.24
Seed (%)	11.88	10.18
Peel (%)	12.43	13.58
TSS (%)	18.4	16.8
Acidity (%)	0.34	0.31
Ascorbic acid content (mg/100g pulp)	49	53
Embryo type	Monoembryonic	

Table (1), showed the characteristics of the elite mango genotype. At the beginning DNA barcoding techniques (rbcL and ITS4) were used against the elite mango genotype, Zebda, Ewais and Alphonso to identify the most related cultivar to the new elite genotype. The offspring of the elite mango genotype were compared with six years old (grafted on Succari rootstock) of Alphonso trees to evaluate the morphological and floral characteristics, as well as physical and chemical fruit characteristics. All trees were planted in light clay soil at a distance of 4x4 m and subjected to standard cultural practices.

Comparison Between the Elite Strain and Alphonso Cultivar in the Morphological, Floral and Physical and Chemical Fruit Characteristics

Morphological and Floral Characteristics: Morphological parameters such as trunk circumference (cm), tree height (cm) and tree canopy (m) were measured with the help of measuring tape. Regarding floral attributes, dates of initial flowering, flowering end and harvest time were recorded. Afterwards, flowering duration was calculated by days.

Malformation:

Malformation percentage was determined as follows:
Malformation (%) = No. of malformed panicles / Total no. of panicles × 100

Initial Fruit Set: The initial fruit set was calculated by labeling 24 healthy panicles on each replicate. Initial fruit set was determined as the number of setting fruits per panicle two weeks after petal fall [12].

Yield: The yield was calculated at the time of fruit harvesting and expressed in kg/tree as follows:

Yield/tree (kg) = No. of fruits × average fruit weight (g) /1000

Biennial Bearing Index: The biennial bearing index was calculated as reported by Serry [13] as follows:

Biennial bearing index = Difference between two yields / Sum of two yields × 100

Physical and Chemical Fruit Characteristics: At harvest time, samples of nine ripe fruits were taken from each tree (replicate) to estimate physical and chemical characteristics. The weight of the fruit, pulp, stone and peel was recorded using an electronic balance and expressed in grams. The length and diameter of fruit were measured using digital vernier calipers and were expressed in centimeters. The fruit volume was measured by the water displacement method and was expressed in cubic centimeter. The pulp percentage per fruit was calculated as follows:

Pulp (%) = Fruit pulp weight / Total fruit weight × 100

The stone percentage per fruit was calculated as follows:

Stone (%) = Seed weight / Total fruit weight × 100

The peel percentage per fruit was calculated as follows:

Peel (%) = Peel weight / Total fruit weight × 100

Total Soluble solids (TSS%) of fruit juice was determined by using a digital refractometer at room temperature, total acidity (%) in fruit pulp was determined as citric acid % (g/100 g) by titrating 10 g of fresh pulp sample against 0.1 N NaOH solution as described in A.O.A.C. [14] and ascorbic acid content (mg/100 g pulp) was estimated by using 2, 6dichlorophenolindophenol dye for titration as the method mentioned in [15]. Total and reducing sugars were determined as fresh weight according to the method described by Malik and Singh [16].

Statistical Data Analysis: The data were arranged in a complete randomized block design with four trees (replicates) (each replicate contained 9 fruits) and analyzed according to Snedecor and Cochran [17]. The differences among the means of data were compared by Duncan's multiple range test at 5 % level [18].

DNA Barcoding

Extraction and Purification of Genomic DNA: DNA was extracted from young leaves of the five mango genotypes by DNeasy Plant Mini Kit (Qiagen Santa Clarita, CA, Cat No. 69104). The quality of DNA was assisted using 0.8% agarose gel electrophoresis, visualized by pre-added SYBR™ Safe DNA Gel Stain (Invitrogen, ThermoFisher cat. No. S33102) (5ul /100ml) under UV light. The quantities and purities of DNA were assisted using a NanoDrop 2000 UV spectrophotometer at 260 nm and 280 nm (ThermoFisher Scientific inc, USA).

ITSn and rbcl Gene Amplification: PCR amplification of matK(ITS5-F:GGAAGTAAAAGTCGTAACAAGG;ITS4-R: TCCTCCGCTTATTGATATGC) Chatterton *et al*. [19] and rbcl (rbcl-F: ATGTCACCACAAACAGAGACTAAAGC; rbcl-R:GTAAAATCAAGTCCACCRCG) [20]. were carried out in 25 µL reaction containing 1.0U Taq DNA polymerase, 1mM dNTPs-Mix 10 mM, 1X Taq buffer, 2.5mM $MgCl_2$, 20mM of each amplification primer (10uM) and 10–50 ng of template DNA in a one-step PCR-program for ITS (94°C 5 min for 1 cycle; 94°C 30 s, 55°C 30 s, 72°C 1 min, 35cycles; 72°C 5 min for 1 cycle) and (94°C 3 min for 1 cycle; 94°C 30 s, 56°C 30 s, 72°C 50 s, 32 cycles; one cycle 72°C 7 min for rbcL. The amplified products were evaluated by electrophoresis in a 1.5% agarose gel buffered with 1X TAE. Gels were stained with SYBR™ Safe and bands were observed in the gel documentation system (BioRad).

PCR products were purified using a multifunction DNA purification kit (QIAquick PCR Purification Kit, Qiagen cat. No. 28104) The purified products containing 500 bp to 800 bp DNA segments were bidirectionally sequenced (PCR thermocycling conditions: 96°C for 15 s, 50°C for 15 s, 60°C for 4 min, 25 cycles) using a BigDye

Terminator v3.1 Cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and read using a SeqStudio™ Genetic Analyzer System cat no. A35644 (TheromFisher, Applied Biosystems, USA).

Sequence Quality and Recoverability: Sequence quality was assessed using Sequence Scanner version 2.0 software (Applied Biosystems) with two quality metrics, trace score (TS) and contiguous read length (CRL). TS, calculated as the average base call quality value of bases in the post-trim sequences, ranged from 0 to 100 and was defined using three levels: low quality (TS, 0–20), medium quality (TS, 21–34) and high quality (TS, 35–100). CRL is the longest uninterrupted stretch of bases with a quality higher than 20 QV (represents an error rate of basecall at 1 in 100 and a call accuracy of 99%) in a window size of 20 bp. Sequence success rate was examined on the basis of the ratio of sequence traces with TS 35 and CRL 200 bp to the total number of PCR products. Sequence traces were trimmed, assembled and manually edited using CLC genomic workbench version 2.0 (Qiagen, USA) to obtain high-quality bidirectional sequences.

Five quality control criteria were sequentially implemented: 1) the sequence trace should have a CRL 200 bp and a TS 35; 2) heterozygous sites were indicated by the second peak .40% of the first peak; 3) both 59 and 39 ends of the sequence were trimmed until less than three bases with quality scores, 25 (or ambiguities) in a 25-base window; 4) assembled contigs should have a minimum overlap of 80% in the alignment of forward and reverse reads with a minimum match percentage of 98% and 5) all of the heterozygous sites (mixed bases) were manually checked and edited based on bidirectional reading chromatograms.

Phylogenetic Analysis: The concatenated SNPs of rbcL of 5 mango genotypes were imported into BioNumerics version 7.1 (Applied Maths, Saint-Martens-Latem, Belgium) and an ST number was assigned to each distinct combination of SNPs. Phylogenetic analysis was performed using the Maximum Likelihood method to generate a dendrogram based on pairwise similarity. All taxa with zero inter-taxon distance were identified. The root position in the tree was assigned to the deepest branch, measured by maximum branch length. Bootstrap values were conducted with 1000 random additions.

Multiple Sequence Alignment: The Multiple sequence alignment of sequenced fragments for ITS and rbcL were generated using clustal omega tools from EMBL-EBI [21].

RESULTS AND DISCUSSION

Tree Morphological, Floral and Yield Parameters of the Elite Strain and Alphonso Cultivar: Data in Table 2 showed substantial differences in trunk perimeter between the selected strain and the Alphonso cultivar. The Elite Strain recorded trunk perimeter significant values that were higher than those of the other cultivar in both seasons (38.333 and 42.00, respectively), while there was no discernible variation in tree height between the Selected Strain and Alphonso cultivar in the first season, the second season revealed a discernible difference between the Elite Strain and Alphonso cultivar. While the two varieties of canopy trees did not significantly differ from one another.

Also, the observed dates for the beginning and end of flowering, as well as for the blooming duration and dates of harvest are shown in (Table 2). When it came to flowering dates throughout the two seasons, the Elite Strain was 4th February and 1st March in the two seasons, respectively, earlier than Alphonso cv. that was the 2nd week of March in the two seasons. In the same, Elite Strain was the earliest of both seasons with regard to the end of flowering (first week of May) and harvest dates (third week of July) than Alphonso cv. where the end of flowering was (2nd week of May) and harvest dates was (first weeks of August). The data also showed that, over the two seasons, Alphonso cv. had the least flowering period value (53–53.66 days) and the Elite Strain had the largest blooming period value (60.33 and 58 days). Genetic characteristics and climatic conditions may be responsible for the variance in flowering behavior. These results are in close association with El- Agamy *et al*. [22] and Serry [13].

The findings revealed in Table 2 and Fig. 1 that the selected strain was (15.20-14.4 %) significantly less malformation % than the Alphonso cultivar (35.10-39.30%) in both seasons. It is worth noting that Kumar *et al*. [23] mentioned that mango cultivars vary widely in their susceptibility to malformation, with temperature, tree age, time and other factors playing a role. In comparison to late-blooming varieties, most early and mid-season cultivars show a decreased incidence of the illness.

In addition, the performance of two mango types, Elite Strain and Alphonso, was shown to be noticeably different between the two varieties. During the two seasons of study, Elite Strain had significantly higher performance (12.40 and 15.10 initial fruit set), (2.23 and 2.66 fruit number/panicle at harvest), (112.67 and 129.33 no. of fruit per tree), (38.66 and 43.66 yield kg) than the

Table 2: Tree morphological, floral and yield parameters of the Elite Strain and Alphonso cultivar

	Frist season		Second season	
Character	Elite Strain	Alphonso	Elite Strain	Alphonso
Trunk perimeter (cm)	38.33 A	34.66 B	42.00 A	39.00 B
Tree height (cm)	268.67 A	256.67 A	285.33 A	272.67 B
Canopy (m)	8.48 A	8.06 A	11.03 A	9.68 A
Beginning of flowering	4th week of Feb.	2nd week of March	1st week of March	2nd week of March
Flowering end	1st week of May	2nd week of May	1st week of May	2nd week of May
Flowering period (day)	60.33 A	53.66 B	58.00 A	53.00 B
Malformation %	15.20 B	35.10 A	14.40 B	39.30 A
Initial fruit set	12.4 A	9.1 B	15.1 A	8.6 B
Fruit number/panicle at harvest	2.23 A	0.96 B	2.66 A	1.26 A
No. of fruit per tree	112.67 A	71.67 B	129.33 A	124.33 A
Yield (kg/tree)	38.66 A	13.66 B	43.66 A	24.00 B
Biennial bearing index	6.05 B	27.42 A		
Harvest	3th week of July	1st week of Aug.	3th week of July	1st week of Aug.

Means followed by the same letter(s) in each column are not significantly different at ($P < 0.05$) using Duncan's multiple range test

Table 3: Physio-chemical properties of the Elite Strain and Alphonso cultivar

	Frist season		Second season	
Character	Elite Strain	Alphonso	Elite Strain	Alphonso
Fruit weight (g)	342.33 A	190.33 B	340.67 A	193.33 B
Fruit volume (cm^3)	345.00 A	193.33 B	346.67 A	193.33 B
Fruit length (cm)	11.60 A	9.17 B	11.78 A	9.23 B
Fruit diameter (cm)	7.77 A	7.33 B	8.07 A	7.17 B
pulp weight (g)	243.00 A	118.33 B	239.67 A	117.33 B
stone weight (g)	40.67 A	27.67 B	41.00 A	29.00 B
Peel weight (g)	58.67 A	44.33 B	60.00 A	47.00 B
Pulp %	71.03 A	62.21 B	70.40 A	60.74 B
Stone %	11.87 B	14.54 A	12.033 B	14.96 A
Peel %	17.10 B	23.25 A	17.567 B	24.30 A
Total Soluble solids %	18.00 A	16.83 B	17.667 A	15.67B
Reducing sugar %	7.91 A	6.87 B	8.01 A	6.05 B
Total sugar %	19.82 A	17.20 B	20.41 A	17.03 B
Ascorbic acid content (mg/100g)	48.803 A	40.90 B	50.47 A	40.78 B
Acidity (%)	0.33 A	0.38 A	0.33A	0.37A
Embryo type	Monoembryonic	Monoembryonic	Monoembryonic	Monoembryonic

Means followed by the same letter(s) in each column are not significantly different at ($P < 0.05$) using Duncan's multiple range tests

Alphonso variety (9.10 and 8.60 initial fruit set), (71.67 and 124.33 no. of fruit per tree), (13.66 and 24.00 yield kg). These results were in harmony with those stated by El-Khawaga and Maklad [24].

Moreover, the calculated biennial bearing index revealed that Elite Strain gave a significantly lower percentage (6.05 %), while Alphonso cv. gained a higher percentage (27.42 %). This means that the two mango cultivars under study were regular in bearing according to Serry [13] and El-Agamy *et al*. [22], since the tree is in regular bearing (on-year) if the index is less than 50%, whereas the tree is in alternate bearing (off-year) if the index is more than 50%. Nevertheless, Elite Strain variety was more regular than the Alphonso cultivar.

Physio-chemical Properties of the Elite Strain and Alphonso Cultivar: The quality of mango depends significantly on the physical properties of the fruit. Fruit physical parameters of Elite Strain. Mango strain and Alphonso cv. are shown in (Table 3). The results revealed that Elite Strain was superior in compare to Alphonso cv., generally, all fruits quality values of Elite Strain were significantly higher than those of Alphonso [fruit weight (342.33-340.67g), (190.33- 193.33 g) fruit volume (345.00-346.67 cm^3), (193.33- 193.33 cm^3), fruit length (11.60 –11.78 cm), (9.17-9.23 cm), fruit diameter (7.77 – 8.07 cm), (7.33 -7.17 cm), pulp weight (243.00 – 239.67 g), (118.33- 117.33 g), stone weight (40.67- 41.00 g), (27.67- 29.00 g), peel weight (58.67- 60.00 g), (44.33-47.00g),

Fig. 1: Some stages of the Elite Strain.
a: The full bloom of the tree of the Elite Strain, aged 6-year-old.
b: Close-up view of panicles; note that full-bloom panicles.
c: Fruit set after one month of full bloom.
d: The yield of the Elite Strain before harvest.
e, f: Fruit shape and color of peel and pulp of the Elite Strain.

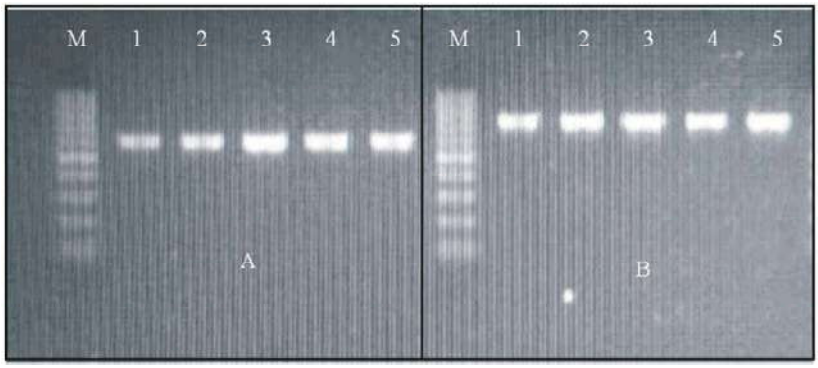

Fig. 2: The result of amplification PCR with the utilization of ITS4 (A) rbcL (B)
M: 100pb ladder, 1: Alphonso, 2: Elite Strain, 3: Succari, 4: Ewais, 5: Zebda

pulp % (71.03 – 70.40), (62.21-60.74), Total Soluble solids % (18.00-17.67), (16.83-15.67), reducing sugar % (7.91 - 8.01), (6.87-6.05), total sugar % (19.82 – 20.41), (17.20-17.03) and ascorbic acid content (mg/100g), (48.80-50.47), (40.90-40.78)] respectively during two season. Except stone percentage which was lower than stone percentage of Alphonso (11.87 -12.03), (14.54 - 14.96), peel percentage which was lower than peel percentage of Alphonso (17.10 and 17.57), (23.25- 24.30) while, acidity percentage is no significant difference between Elite Strain and Alphonso cultivar (0.33-0.33), (0.38-0.37) respectively during two season.

The color of the fruit of the selected strain at ripening was orange tinged with red, the pulp of the strain was orange; and the texture was buttery (Fig. 1). In addition, the fruit has a monoembryonic seed.

The variations of fruit length, width and weight have been reported by Bora *et al.* [25]; El-Agamy *et al.* [22] while evaluating different mango cultivars. Genetic or physiological influences may be responsible for this variance. The observations of Ahmed *et al.* [26]; Bora *et al.* [25]; are also in line with the current findings of those who studied mango fruit quality and its correlation with physio-chemical parameters of fruits.

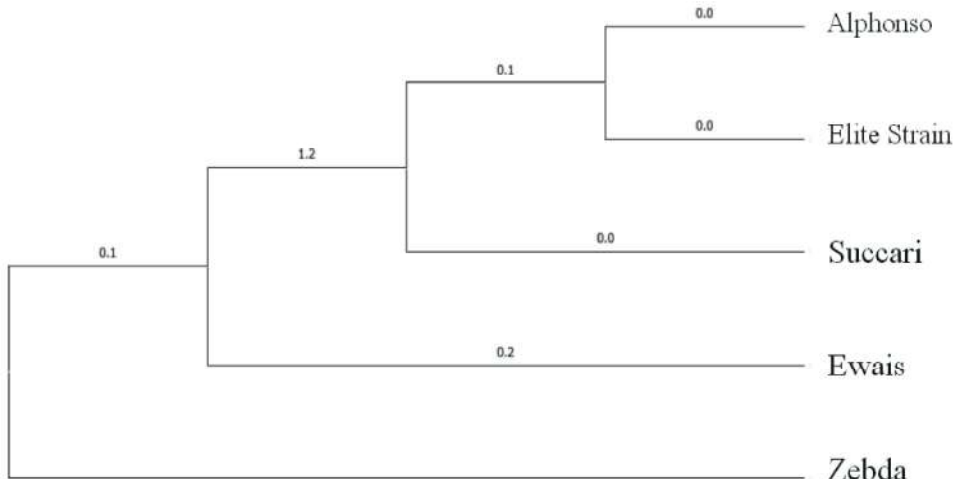

Fig. 3: Phylogenetic tree of 5 Mango genotypes constructed by the Maximum Likelihood method

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Succari          - - GACCCGTGAACTTGTTGTTAACGCCGGGGACGYGCGGGCCTTGTGCTTGCGCGCCCTC
Zebda            - - GACCCGTGAACTTGTTGTTAACGCCGGGGACGCGCGGGCCTTGTGCTTGCGCGCCCTC
Ewais            ACGACCCGTGAACTTGTTGTTAACGCCGGGGACGCGCGGGCCTTGTGCTTGCGCGCCCTC
Alphonso         - CGACCCGTGAACTTGTTGTTAACGYCGGGGACGCGCGGGCCTTGTGCTTGCGCGCCCTC
Elite Strain     - CGACCCGTGAACTTGTTGTTAACGCCGGGGACGCGCGGGCCTTGTGCTTGCGCGCCCTC
                   ********************** ******** **************************

Succari          GCTCGCGTCGCGTTGGGCTTTCGTTGCGCGTGCACCCCTGCGTGTGCGTGCCCGTTCGCC
Zebda            GCTCGCGTCGCGTTGGGCTTTCGTTGCGCGTGCACCCCTGCGTGTGCGTGCCCGTTCGCC
Ewais            GCTYGCGTCGCGTTGGGCTTTCGTTGCGCGTGCACCCCTGCGTGTRCGTGCCCGTTCGCC
Alphonso         GCTYGCGTCGCGTTGGGCTTTCGTTGCGCGTGCACCCCTGCGTGTRCGTGCCCGTTCGCC
Elite Strain     GCTYGCGTCGCGTTGGGCTTTCGTTGCGCGTGCACCCCTGCGTGTGCGTGCCCGTTCGCC
                 *** ****************************************** ***************

Succari          CCTCGCGGTGCCTTAACCAACCCCGGCGCGAATTGCGCCAAGGACTTGTTAACGAGAGGG
Zebda            CCTCGCGGTGCCTTAACCAACCCCGGCGCGAATTGCGCCAAGGACTTGTTAACGAGAGGG
Ewais            CCTCGCGGTGCCTTAACCAACCCCGGCGCGAATTGCGCCAAGGACTTGTTAACGAGAGGG
Alphonso         CCTCGCGGTGCCTTAACCAACCCCGGCGCGAATTGCGCCAAGGACTTGTTAACGAGAGGG
Elite Strain     CCTCRCGGTGCCTTAACCAACCCCGGCGCGAATTGCGCCAAGGACTTGTTAACGAGAGGG
                 **** ********************************************************

Succari          CTCGCTCCCGTCGCCCCCGGACACGGTGCGTGCGTGCGGGACGCGACGCCTCCTTTCATT
Zebda            CTCGCTCCCGTCGCCCCCGGACACGGTGCGTGCGTGCGGGACGYGACGCCTCCTTTCATT
Ewais            CTCGCTCCCGTCGCCCCCGGACACGGTGCGTGCGTGCGGGACGCGACGCCTYCTTTCATT
Alphonso         CTCGCTCCCGTCGCCCCYGGACACGGTGCGTGCGTGCGGGACGCGACGCCTYCTTTCATT
Elite Strain     CTCGCTCCCGTCGCCCCYGGACACGGTGCGTGCGTGCGGGACGCGACGCCTYCTTTCATT
                 ***************** ************************ ******* ********

Succari          ATCTATAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGWAGWACGTAGCGA
Zebda            ATCTATAACGACTCTCGGCAACGGATATCTCGGCTCTCGCWTCGATGAARAACGTASCGA
Ewais            ATCTATAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGA
Alphonso         ATCTATAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGA
Elite Strain     ATCTATAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATGAAGAACGTAGCGA
                 ****************************************** ****** *  ***** ***

Succari          AATGCGATACTTGGTGTGAATTGCAGWATCCCGTGAACCATCGAGTCTTTGAACGCAAGT
Zebda            AATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGT
Ewais            AATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGT
Alphonso         AATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGT
Elite Strain     AATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGT
                 ************************** **********************************

Succari          TG
Zebda            TG
Ewais            TG
Alphonso         TG
Elite Strain     TG
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Fig. 4: Multiple sequence alignment of five mango cultivars for ITS region using clustal Omega tool (https://www.ebi.ac.uk/Tools/msa/clustalo/)

Table 4: Estimates of Evolutionary Divergence between Sequences

	Alphonso	Elite Strain	Succari	Ewais	Zebda
Alphonso	0				
Elite Strain	0.0163	0			
Succari	0.0491	0.0403	0		
Ewais	1.5140	1.5140	1.4529	0	
Zebda	1.2395	1.1743	1.0618	1.2879	0

DNA Barcoding: As shown in Fig. (2), both barcoding primers (ITS4 and rbcL) amplified the five mango cultivars successfully. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model [27]. The tree with the highest log likelihood (-1822.10) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with a superior log likelihood value. The analysis involved 5 nucleotide sequences. Codon positions included were $1^{st}+2^{nd}+3^{rd}$+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 415 positions in the final dataset.

The phylogenetic data (Fig. 3) and Evolutionary Divergence (Table 4) showed that the Elite Strain selected cultivar was highly related to Alphonso and both were related to the Succari cultivar. The sequencing data showed no clear reads in rbcL markers which is not suitable for barcoding in mango cultivars under study. Vice versa, ITS4 generates high-quality reads distinguished between the cultivars under investigation.

As shown in Fig. 4 and Table 4, the number of base substitutions per site from between sequences is shown. Analyses were conducted using the Maximum Composite Likelihood model [28]. This analysis involved 5 nucleotide sequences. Codon positions included were $1^{st}+2^{nd}+3^{rd}$+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 482 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 [29, 30].

CONCLUSION

According to a fingerprint technique utilizing two molecular markers, rbcL and ITS4 genes barcoding, Alphonso cultivar was highly related to Elite Strain. The Elite Strain was earlier in the beginning of flowering (4^{th} week of Feb. and 1^{st} week of March), the end of flowering (1^{st} week of May) and the date of harvest (3^{rd} week of July) and malformation % was (15.20 and 14.40%). In addition, initial fruit set, fruit number per panicle, fruit number per tree and yield per tree, Elite Strain showed values of (12.40 and 15.10%), (2.23 and 2.66 fruits/panicle), (112.67 and 129.33 fruits/tree) and (38.66 and 43.66 kg) in both seasons, respectively. Moreover, the calculated biennial bearing index revealed that Elite Strain gave a significantly lower percentage (6.050 %), while Alphonso cv. gained a higher percentage (27.427 %). Generally, all fruits quality values of Elite Strain were significantly higher than those of Alphonso except stone percentage which was lower than stone percentage of Alphonso peel percentage and acidity percentage during two seasons.

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