



Molecular, morphological, palynological and biochemical characterization of six accessions of two *Mangifera indica* L cultivars (Keitt and Ewais) native to Egypt

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Abstract Accurate characterization of crops or fruits leads to successful improvements in breeding programs. Mango (*Mangifera indica* L.) is the second most common fruit in Egypt. It is a heterozygous plant; consequently, the possibility of incorrect characterization is significant. Therefore, this study used six accessions of two *M. indica* cultivars, ‘Keitt’ and ‘Ewais’, to produce precise molecular characterization and prove their genetic relatedness using inter-simple sequence repeat (ISSR) and start codon targeted (SCoT) markers. Pollen morphology was studied for the first time for these two Egyptian cultivars to determine their correlation. Morphological traits were quantitatively and qualitatively investigated. In addition, phytochemical analyses were performed to correlate the six mango accessions. One of these accessions (accession 3) was commercially identified as ‘Ewais’ cv., but the obtained result revealed that it does not belong to ‘Ewais’ cv. This finding was proved molecularly using ISSR and SCoT markers and morphologically. Similarly, phytochemical analyses revealed a difference between accession 3 and the other accessions under investigation. Polymorphic information content (PIC) and resolving

power (RP) data revealed that the SCoT technique was more informative than ISSR for studying these genotypes of mango. Accordingly, the present findings prove the necessity for molecular, morphological, palynological, and biochemical investigations for accurate characterization of mango genotypes and genetic relatedness for improving breeding programs.

Keywords *Mangifera indica*, ‘Keitt’ and ‘Ewais’ cultivars · Genetic relatedness · Molecular marker · Morphological approach · Palynology · Phytochemical analysis

Introduction

Mangifera indica L. (king of fruits) is an evergreen dicotyledonous angiosperm belonging to the family Anacardiaceae, order Sapindales (EL-Banhawy et al. 2020). Mango is characterized by its sweet taste, distinctive flavor and smell, a variety of cultivars, and large production. Almost all *Mangifera* species are tetraploid but cultivated mango is a diploid tree ($2n=40$, genome size 439 Mbp) (Singh et al. 2016). Kostermans and Bompard (1993) identified sixty-nine species of *Mangifera* based on flower morphology. The origin of *Mangifera indica* is the region between northwestern Myanmar, Bangladesh, and northeastern India (Kyaing et al. 2019), and then it has spread and been cultivated worldwide. According to The Food and Agriculture Organization’s

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(FAO) production yearbook, mangoes are the fifth in total production after *Citrus*, *Musa*, *Vitis*, and *Malus*. India leads the world in mango production, producing 22 million tons annually (43%) in 2019 (Farag et al. 2022). Additionally, Mexico, China, Indonesia, and Egypt were identified as the most important producers. *M. indica* has been cultivated in Egypt for at least 200 years (Abdelsalam et al. 2018). In 2020, the Egyptian Ministry of Agriculture and Land Reclamation reported that Egypt's total area for mango cultivation is 54,600,048 hectares with a yearly production of 1,203,743 tons, and total mango exports are 231 tons with more than 2 million pounds. *M. indica* is the most important fruit cultivated in Egyptian governorates. It is ranked third after *Citrus* and *Vitis*. Ismailia is the main *M. indica* supplying governorate, producing the best quality mango. Economically *M. indica* is an essential major tropical crop in Egypt (Serry et al. 2019).

Patil et al. (2019) reported that it is essential to use genotypes that are accurately identified, discriminated, recognized, and differentiated for successful mango breeding programs. Fruit qualities are not the only characteristics used to identify mango cultivars, but also morphological approaches such as tree length, immature stage, or fruiting season can be applied (de Oliveira et al. 2020). Morphology is a simple tool used to distinguish between closely related plant taxonomic groups. Mango cultivars at the early growing stage can be distinguished by the different morphological characteristics of the leaves. Breeders can quickly, objectively, and easily determine the type of mango tree by morphological characteristics to save time, money, and effort (EL-Banhawy et al. 2020).

Palynology is a technique used for answering some genetic and taxonomic issues (Azzazi 2011). Palynology is the study of the morphological structure of pollen and spores. Muniraja et al. (2020) studied the pollen ultrastructure of *M. indica* var. Beneshan.

Mango is heterozygous in nature and has seedling genotype diversity, which has demonstrated large genetic variation in shape, color, flavor, stage of development, pattern of bearing, yield, and resistance to deformity (Serry et al. 2019). In contrast to the morphological characteristics, the molecular markers are constant and cannot be affected by environmental conditions. The main objectives of mango breeding programs are to create better cultivars and improve

both plant and fruit characteristics. Therefore, molecular fingerprints are essential for the accurate identification of mango genotypes, cultivars, or hybrids to improve mango breeding programs and identify genetic resources (Patil et al. 2019).

Inter simple sequence repeat (ISSR) and start codon targeted (SCoT) techniques are PCR-based methods. ISSR- amplified DNA segment located between adjacent, oppositely oriented microsatellites (Ziêtkiewicz et al. 1994). While the SCoT marker has the advantages of extensive genetic data, high polymorphism, ease of application, repeatability, and specificity (Collard and Mackill 2009). Previously, 15 mango cultivars from Pakistan had been examined for molecular relationships depending on ISSR markers (Toili et al. 2017). Recently, the genetic diversity of some Indian mango cultivars was studied using ISSR and SCoT and other different fingerprint markers by Jena and Chand (2021). The SCoT marker has been widely used to study genetic diversity and phylogenetic evaluations of fruit trees such as mango (Gajera et al. 2014). Moreover, Zhou et al. (2020) discovered that most mangoes collected from different study areas were highly heterozygous, and the genetic variations observed using the SCoT marker were within mango groups, not between genotypes. Moreover, the genetic variation of sixteen mangos from Southern Vietnam was studied using SCoT marker (Vu et al. 2022). Additionally, the genetic variation and relatedness of twenty-three Egyptian mango genotypes were previously evaluated using ISSR and SCoT markers (Ghounim et al. 2022).

Another important tool used in the grouping of plant taxa is the identification and characterization of different phytochemical compounds. Different parts of mango trees have been phytochemically investigated. Different phytochemical groups have been reported in mango leaves, such as carbohydrates, proteins, phenolics, tannins, flavonoids, saponins, and steroids. Some of these groups have been used as effective pharmaceuticals and allelopathic agents (Lenucci et al. 2022). Mangiferin (xanthone glycoside) is the major secondary metabolite of *M. indica*. And has been used to distinguish between mango cultivars (Fitmawati et al. 2020).

Consequently, the aim of this study was to provide an accurate and valid correlation between six accessions of two mango cultivars at the molecular, morphological, palynological, and phytochemical

levels to produce useful data for mango breeders and breeding programs for improving Egyptian mango cultivars.

Materials and methods

Six accessions of two Egyptian *M. indica* cvs ‘Keitt’ and ‘Ewais’ (1,2 ‘Keitt’; 3,4,5,6 ‘Ewais’) were used in the present work. They were collected in four sequential seasons, from 2019 to 2022. Leaves and flowers of cultivated samples were collected from a private garden owned by Prof. Sahar A. Tawab, who obtained the mango tree from the Botanical Garden of the Egyptian Ministry of Water Resources and Irrigation, Wadi El-Natrun, Cairo, Egypt (30°17′16.4″N 30°22′20.3″E).

Morphological analysis

Six characteristics for tree, leaf, and inflorescence, including tree height, trunk girth, length of leaf, width of leaf blade, length of petiole, and length of inflorescence were quantitatively estimated in 10 replicates at the maturity stage. Moreover, nine characteristics were qualitatively investigated, such as color of trunk, shape of leaf, shape of leaf apex, shape of leaf margin, color of young leaf, color of mature leaf, shape of leaf blade base, color of inflorescence, and shape of inflorescence following the mango IPGRI descriptor Vieccelli et al. (2016).

Pollen grain structure analysis

Pollen grain structure was analyzed using a light microscope (LM). The air-dried pollen grains from each accession were placed in glycerin jelly on slides. Pollen grains were observed using a 100×oil immersion objective lens and 10×eyepieces from Carl Zeiss Jena (Nr.683454) microscope, Germany. True Chrome IIS Camera (Tucsen Photonics Co., Ltd., Gaishan Town, China) was used for photomicrography. The means of the polar axis diameter (P) and equatorial axis diameter (E) were measured, and the frequency of P/E was assessed (10 replicates for each specimen). The pollen description, identification, and terminology used follow Punt et al. (2007) for describing exine ornamentation. Ocular Stage Division (Erma) was used for the measuring process of

the pollen grains; the measuring unit was converted into μm .

For scanning electron microscopy (SEM) analysis, pollen grains were mounted on aluminum stubs covered with double-sided transparent tape and sputtered with technical gold (0.02 μm) in a sputter coater BAL-TEC SCD 005 (Capovani Brothers Inc., Scotia, NY, USA), as reported by Baum et al. (1970). Then samples were examined and photographed at 150 kV in a JOEL JSM 5200 SEM (JEOL, Tokyo, Japan) at the Scanning Electron Microscope Unit, Agriculture Research Center, Giza, Egypt.

Molecular analysis

DNA isolation, inter simple sequence repeat and start codon targeted polymerase chain reactions

Fresh mango leaves were used for the isolation of genomic DNA using DNeasy plant mini kit (Bio basic Com., Canada). DNA amount and purity were confirmed using NanoDrop 1000 (260/280 nm) and running on 1% agarose gel. The concentration of the DNA sample was diluted to 50 ng/ μL and stored at -80°C for ISSR and SCoT experiments.

Ten primers (14A, 44B, 49B, 89B, 98A, HB-08, HB-10, HB-11, HB-12, and HB-13) were used in the ISSR technique (Table 1). Primers were synthesized by Operon Biotechnologies, Inc., USA. ISSR primers are usually attached at the 3′ or 5′ end with 1 to 4 degenerate bases and continue into the flanking sequences as described by Ziêtiewicz et al. (1994). The volume of the PCR reaction mixture was 25 μL containing 2.5 μL of dNTPs (2.5 mM), 1.5 μL of MgCl_2 (25 mM), 2.5 μL of 10×buffer, 2.0 μL of primer (2.5 μM), 2.0 μL of template DNA (50 ng $\mu\text{L/L}$), 0.3 μL of Taq polymerase (5 U $\mu\text{L/L}$) and 14.7 μL of sterile ddH_2O .

Genomic DNA was used as a template for PCR amplification using 10 SCoT primers, including SCoT 1, SCoT2, SCoT 4, SCoT 5, SCoT 6, SCoT 7, SCoT 8, SCoT 9, SCoT 13, and SCoT 15 (Table 1). The SCoT primers were designed by Collard and Mackill (2009) and synthesized by Bio basic Com., Canada. The SCoT technique was performed as described by Xiong et al. (2011). The reaction mixture (25 μL) included 1.0 μL DNA (50 ng/ μL), 0.5 μL dNTP (10 mM), 2.5 μL 10×Easy Taq Buffer,

Table 1 Primer used for inter simple sequence repeat and start codon targeted analyses and their sequences

No	Primer for ISSR	Sequence (5'—3')	No	Primer for SCoT	Sequence (5'—3')
1	14A	CTC TCT CTC TCT CTC TTG	11	SCoT 1	ACG ACA TGG CGA CCA CGC
2	44B	CTC TCT CTC TCT CTC TGC	12	SCoT 2	CAACAATGGCTACCACCC
3	49B	CAC ACA CAC ACA GG	13	SCoT 4	CAA TGG CTA CCA CTA GCG
4	89B	CAC ACA CAC ACA GT	14	SCoT 5	CAA TGG CTA CCA CTA GCG
5	98A	CAC ACA CAC ACA CA	15	SCoT 6	CAA TGG CTA CCA CTA CAG
6	HB-8	GAG AGA GAG AGA GG	16	SCoT 7	ACA ATG GCT ACC ACT GAC
7	HB-10	GAG AGA GAG AGA CC	17	SCoT 8	CAACAATGGCTACCACGT
8	HB-11	GTG TGT GTG TGT TGT CC	18	SCoT 9	ACA ATG GCT ACC ACT GCC
9	HB-12	CAC CAC CAC GC	19	SCoT 13	ACC ATG GCT ACC ACG GCA
10	HB-13	GAG GAG GAG C	20	SCoT 15	CCA TGG CTA CCA CCG GCT

1.5 µL of MgCl₂ (25 mM) 1 µL each primer (10 µM), 0.25 µL Easy Taq DNA Polymerase (5 U/µL), and 19.75 µL ddH₂O.

Both reactions (ISSR and SCoT) were carried out in Techni TC-512 Thermal Cycler, Stafford, UK. The reaction was performed by 1 cycle at 94°C for 4 min, followed by 40 cycles of 1 min at 94°C, 1 min at annealing temperature 57°C, and 2 min at 72°C, followed by 72°C for 10 min. Amplified products were separated on a 1.5% agarose gel alongside a 100–3000 bp DNA Ladder from GeneDirex, Inc. (DNA Ladder H3 RTU Ready-to-Use). DNA banding patterns were analyzed using Gel Analyzer 3 software.

Data, dendrogram, and principal coordinate analyses

Clear bands (present 1 or absent 0) for each primer were used to form the binary data matrix. The number of total bands, polymorphic bands, monomorphic bands, polymorphic information content (PIC), resolving power (RP), and diversity index (DI), which is the mean of PIC were scored (Venkatesan et al. 2021).

In hierarchical clustering, the Dice similarity coefficient was calculated according to the Dice coefficient (Hegay et al. 2014).

Principal coordinate analysis (PCoA) is a powerful and popular multivariate analysis method that analyzes the proximity matrix, whether it is a dissimilarity matrix as a distance matrix or a similarity matrix as a correlation matrix. PCoA was performed depending on the data for ISSR and SCoT using PAST version 4.03 software (Hammer et al. 2001).

Phytochemical analyses

The leaves from each accession were washed with tap water and air-dried at room temperature. The dried leaves were powdered using a blender to coarse powder. Twenty gm of each powdered mango leaf was macerated in 70% ethanol (200 mL×4). Extracts were filtered, collected, and concentrated. The dry extracts were stored at −20 °C for further phytochemical investigations (Abeyasinghe et al. (2021)).

Phytochemical screening was performed by solubilizing the dry extract (0.1 g) of each accession in 25 mL of 70% ethanol. Preliminary phytochemical screening was performed according to Abeyasinghe et al. (2021) for the detection of alkaloids using Wagner's reagent, phenolics, tannins, phenols, flavonoids, steroids, and sterols using Salkowski's test, saponins, anthraquinones, carbohydrates, glycosides, proteins, and amino acids.

Quantitative estimation of some primary metabolites

For total carbohydrate estimation, 0.1 mL of 70% ethanolic extract was added to 3 mL of freshly prepared anthrone in a water bath (100° C) for 10 min. alongside different standard glucose solutions. The optical density of the cooled samples was read at 625 (Yemm and Willis 1954). The results were expressed as glucose equivalents.

Protein content was determined in fresh leaves from each accession. Fresh leaf samples (0.2g) were frozen in liquid nitrogen and grounded with 10 mL of 80% ethanol. All samples were centrifuged for 10 min at 6000 rpm at −5° C. Then 50 µL of sample was

added to 5 mL of the freshly prepared Bradford stain solution and incubated for 2 min alongside different standard bovine serum albumin solutions. The optical density was read at 595 nm (Bradford 1976). The results were albumin equivalents.

Quantitative determination of the secondary metabolites

The aluminum chloride colorimetric method was used to estimate the total flavonoid content in the ethanolic extract, according to Chang et al. (2002). A volume of 0.5 mL of each sample was taken along with different standard quercetin solutions. A volume of 1.5 mL 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of potassium acetate, and 2.8 mL of distilled H₂O were added. The absorbance of the reaction mixture was measured at 415 nm after 30 min of incubation at room temperature. The results were expressed as quercetin equivalents.

According to Siddhuraju and Becker (2003), the total phenolic content was determined. About 10 µL of the extract was added to 0.5 mL of Folin-Ciocalteu phenol reagent and 2.5 mL 20% sodium carbonate solution. After vortexing, the test tubes were placed in the dark for 40 min, and the optical density was scored at 725 nm against the reagent blank. The results were expressed as tannic acid equivalents. The tannins were estimated using the same extracts after adding 100 mg of polyvinyl polypyrrolidone (PVPP). The contents were vortexed and kept in the test tube for 4 h at 4° C, centrifuged, and the supernatant was collected. The tannin content of the sample was calculated as.

Tannin = total phenolics—non-tannin phenolics.

Mangiferin content was extracted by 50% methanol (100 mL×3) in 10 gm of mango leaves. Extracts were filtered, collected, and concentrated under vacuum at 40° C. Pure mangiferin was used to form solutions with concentrations of 100–500 µg/mL to form a mangiferin standard curve (Rasyid et al. 2020). A weight of 100 µg of each methanolic extract was dissolved in 1 mL methanol, and the optical density of each extract and standard solution was measured at a wavelength 257 nm.

Chlorophyll content was estimated from 0.1 g of finely cut fresh leaves. Fresh cut leaves from each accession were ground with 80% acetone (10 mL×3) overnight until the residue became colorless, then centrifuged at 6000 rpm for 5 min (Arnon 1949). The optical density of the solutions was read at 480, 645 and 663 nm against acetone (the blank).

Statistical analysis

Quantitative analyses were performed in triplicate, and the results were expressed as mean±standard deviation. One-way analysis of variance (ANOVA), F value, and Duncan's test were used for statistical comparisons, which were analyzed using SPSS software (SPSS ver. 26 for Windows). F- value was considered significant at ($P \leq 0.05$).

Results

Morphological characteristics

The results in Table 2, 3 and Figs. 1, 2 represent the variation in quantitative and qualitative

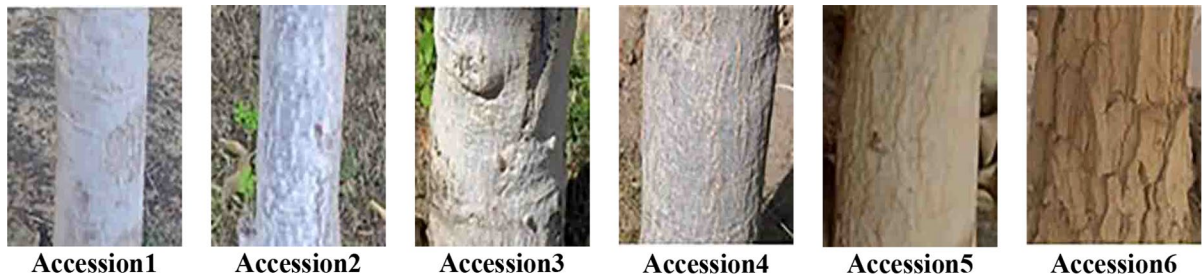
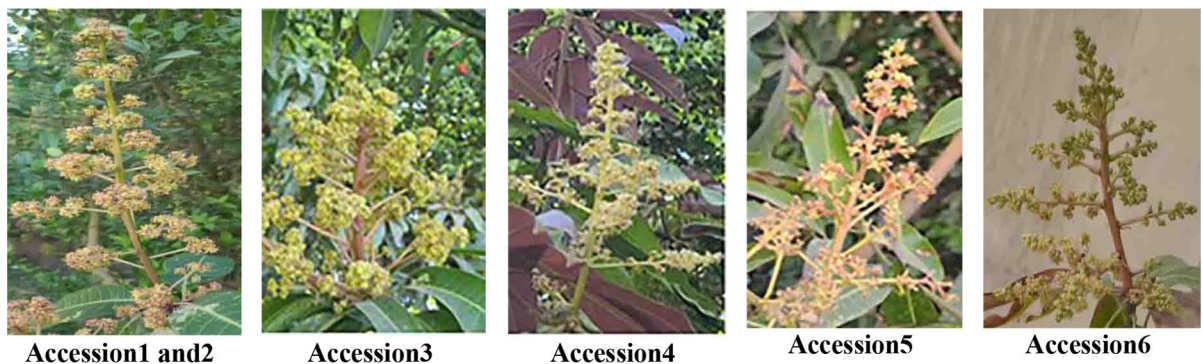
Table 2 Quantitative results of 6 morphological characters (cm) of the six accessions of 'Keitt' and 'Ewais' cultivars

Name of accession	Tree height	Trunk girth	Length of leaf	Width of leaf blade	Length of petiole	length of inflorescence
Accession1	260±0.03 ^c	20±0.17 ^d	17.16±0.23 ^b	5.06±0.04	2.70±0.35	28.4±0.09 ^a
Accession2	183±0.01 ^f	19±0.51 ^d	17.04±0.15 ^b	5.36±0.08	2.86±0.11	27.1±0.13 ^{a,b}
Accession3	470±0.09 ^a	29±0.08 ^c	21.84±0.27 ^b	4.55±0.13	2.88±0.18	12.0±0.14 ^d
Accession4	302±0.07 ^c	30±0.11 ^{b,c}	22.54±0.58 ^{a,b}	5.62±0.05	2.68±0.20	24.5±0.07 ^{c,b}
Accession5	308±0.03 ^b	37±0.46 ^a	22.62±0.19 ^a	4.35±0.12	3.38±0.16	22.3±0.04 ^c
Accession6	280±0.21 ^d	33±0.01 ^b	22.44±0.62 ^a	5.75±0.06	2.84±0.13	23.0±0.15 ^c
F- value	267.30 ^{***}	153.60 ^{***}	4.47 ^{**}	Non-significant	Non-significant	101.77 ^{***}

Values with non-similar letters signify significant differences (Duncan's test) ***: $P < 0.001$, **: $P < 0.01$

Table 3 Qualitative results of nine morphological characters of the six accessions of ‘Keitt’ and ‘Ewais’ cultivars

Name of accession	Color of trunk	Shape of leaf	Shape of leaf apex	Shape of leaf margin	Color of young leaf	Color of mature leaf	Shape of leaf blade base	Color of Inflorescence	Shape of Inflorescence
Accession1	Grey	Elliptic	Acute	Entire	Light green	Dark green	Obtuse	yellowish green	Pyramidal
Accession2	Grey	Elliptic	Acute	Entire	Light green	Dark green	Obtuse	yellowish green	Pyramidal
Accession3	Greyish brown	Narrow elliptic	Acute	Slightly wavy	Light brick red	Pale green	Acute	Light red	Broad pyramidal
Accession4	Greyish brown	Lanceolate	Acute	Entire	Light brick red	Green	Acute	Light red	Pyramidal
Accession5	Pale brown	Lanceolate	Acuminate	Slightly wavy	Light brick red	Green	Acute	Light red	Pyramidal
Accession6	Pale brown	Lanceolate	Acuminate	Entire	Light brick red	Dark green	Acute	Light red	Pyramidal

**Fig. 1** Bark color of the six accessions of ‘Keitt’ and ‘Ewais’ cultivars**Fig. 2** Shape of leaves and inflorescence of the six accessions of ‘Keitt’ and ‘Ewais’ cultivars

morphological features of tree, leaf, and inflorescence shapes of six accessions of mango cultivars under investigation.

Tree height indicates the growth and quality of the mango tree. Among the mango accessions, accession 3 (Ewais cv.) and accession 5 (Ewais cv.) exhibited

the maximum tree height (4.70 m) and trunk girth (37 cm), respectively. However, the minimum height (1.83 m) and trunk girth (19 cm) were observed in accession 2 (Ewais cv.). The maximum length of leaf (22.62 cm), width of the leaf blade (5.75 cm), and length of petiole (3.38 cm) were observed in accession 5, accession 6 (Ewais cv.) and accession 5 (Ewais cv.), respectively, while the minimum length of leaf (17.04 cm) and width of leaf (4.35 cm) was detected in accession 2 (Keitt) and accession 5 (Ewais), respectively. The length of the petiole (2.68 cm) was found to be the lowest length in accession 4 (Table 2). The trunk color differed from grey in two accessions of Keitt cv., to grayish brown in accession 3 and accession 4 ('Ewais' cv.), and pale brown in accession 5 and accession 6 ('Ewais' cv.) (Table 3, Fig. 1).

Leaf shape varied from elliptic in two accessions of 'Keitt' cv., narrow elliptic in accession 3 ('Ewais' cv.), and lanceolate in the reminder accessions of 'Ewais' cv. The leaf margin was wavy (undulate) in accession 3 and accession 5 ('Ewais' cv.); however, it was entire in accession 4, accession 6 ('Ewais' cv.), and the two accessions of 'Keitt' cv. Leaf apex shape was predominantly acute in accession 1, accession 2 ('Keitt' cv.), and accession 3 and 4 ('Ewais' cv.), but the remaining accessions ('Ewais' cv.) were acuminate. Table 3 shows that leaf base shape was predominantly acute in all accessions of 'Ewais' cv., while obtuse in 'Keitt' cv. Young leaf color was observed as light green in accessions of 'Keitt' cvs. and light brick red in all accessions of 'Ewais' cvs. and gained a pale green to dark green color when it became mature.

The mango inflorescence bears both hermaphrodite and male flowers in the same panicle. The color and

shape of the inflorescence (panicle) differed among the accessions and all accessions formed a pentamerous flower type at the terminal position. The color of the inflorescence varied from yellowish green to light red (Fig. 2). Accession1 and accession2 ('Keitt' cv.) showed a yellowish-green inflorescence, whereas all accessions of 'Ewais' cv. showed a light red color inflorescence. From Table 3, it was observed that accession 3 among the six mango accessions exhibited broadly pyramidal-shaped inflorescence, and all reminder accessions ('Ewais' and Keitt cvs.) showed pyramidal-shaped inflorescences.

Additionally, the results indicated that the maximum length (28.4 cm) of the inflorescence was recorded in accession 1, followed by accession 2 (27.1 cm) ('Keitt' cv.), whereas the minimum length (12 cm) of the inflorescence was recorded in mango accession 3 ('Ewais' cv.) (Table 2).

Pollen grain structure

This study is the first one to deal with the pollen morphology of the two Egyptian cultivars ('Keitt' and 'Ewais'). The palyno-morphological characteristics of the six accessions belonging to two cultivars ('Keitt' and 'Ewais') were examined using light and scanning electron microscopy. Morphological pollen characteristics and their microphotographs are listed in Table 4 and Fig. 3.

The pollen grains of all accessions examined in this study were isopolar, monads, radially symmetrical, and tricolporate, displaying the presence of three colpi along the polar axis with porate endoaperture. Colpus was narrow and long-extending to poles in all accessions except in accession 5 and accession 6

Table 4 Pollen characteristics of the six accessions of 'Keitt' and 'Ewais' cultivars

Name of accession	Polar diameter mean (P) (μm)	Equatorial diameter mean (E) (μm)	P/E	Pollen shape	Exine sculpture	Mean exine thickness (μm)
Accession1	28.388 ± 0.01	23.42 ± 0.1 ^a	1.26 ± 0.01 ^b	Subprolate	Striate	2.419 ± 0.10 ^a
Accession2	28.227 ± 0.16	22.74 ± 0.11 ^a	1.24 ± 0.06 ^b	Subprolate	Striate	2.419 ± 0.14 ^a
Accession3	25.969 ± 0.31	16.12 ± 0.0 ^c	1.61 ± 0.03 ^a	prolate	Striate-perforate	1.89 ± 0.07 ^{a,b}
Accession4	26.450 ± 0.24	21.77 ± 0.1 ^b	1.21 ± 0.05 ^b	Subprolate	Striate-perforate	1.61 ± 0.01 ^b
Accession5	25.160 ± 0.23	20.64 ± 0.11 ^b	1.22 ± 0.01 ^b	Subprolate	Striate- reticulate	1.61 ± 0.09 ^b
Accession6	25.646 ± 0.19	21.29 ± 0.21 ^c	1.20 ± 0.09 ^a	Subprolate	Striate- reticulate	2.25 ± 0.12 ^b
<i>F value</i>	Non-significant	99.4 ^{***}	8.11 ^{**}			4.96 ^{**}

Values with non-similar letters signify significant differences (Duncan's test), ***: $P \leq 0.001$, **: $P \leq 0.01$

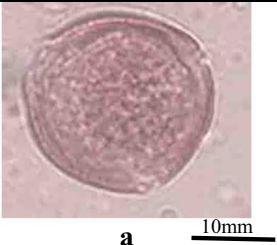
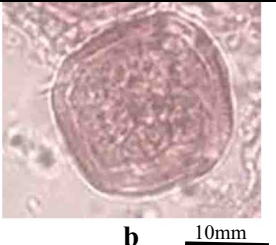
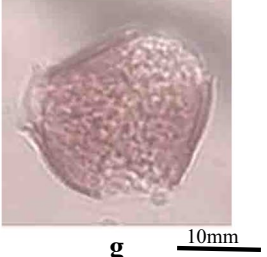
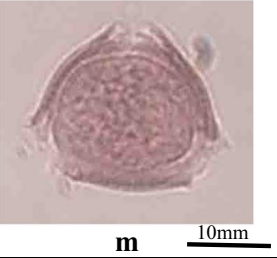
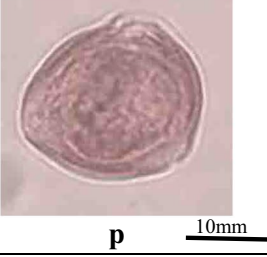
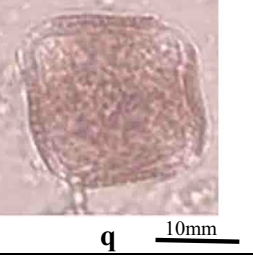
Accession1	 a 10mm	 b 10mm	 c 10mm
Accession2	 d 10mm	 e 10mm	 f 10mm
Accession3	 g 10mm	 h 10mm	 i 10mm
Accession4	 j 10mm	 k 10mm	 l 10mm
Accession5	 m 10mm	 n 10mm	 o 10mm
Accession6	 p 10mm	 q 10mm	 r 10mm

Fig. 3 Light microscope microphotographs of mature pollen grains of six accession belonging to two cultivars of *Mangifera indica* ‘Keitt’ and ‘Ewais’ **a, b**: polar and **c**: equatorial view of accession 1; **d**: polar and **e, f**: equatorial view of accession 2; **g** polar and **h, i**: equatorial view of accession 3; **j**: polar and **k, l**: equatorial view of accession 4; **m**: polar and **n, o**: equatorial view of accession 5; **p, q**: polar and **r**: equatorial view of accession 6

(‘Ewais’ cv.), which had broad and long-extending poles (Fig. 4). The outline of the polar view of pollen grain was mostly subcircular or subtriangular with obtuse apices and more rarely elliptic, whereas the outline of pollen in the equatorial view was mostly elliptic and rarely circular (Fig. 3). The exine ranged from 1.6 to 2.4 μm thick. The pollen size classification of all accessions was medium-sized (25.1–50 μm); the shortest polar diameter mean (P) was found for pollen grains of accession 5 (25.16 μm) and the longest for accession 1 (28.38 μm). The shortest mean (E) diameter occurred in the pollen of accession 3 (16.12 μm), while the longest was in accession 1 (22.4 μm). The range of P/E varies from 1.20 to 1.61. The pollen diameter averaged from 25.16 to 28.38 μm . The pollen grain shape of all accessions was subprolate except accession 3, which was prolate (Table 4 and Fig. 3). Pollen grains of all accessions had three apertures, while accession 1 and 6 had three and rarely four apertures (Fig. 3 b, q).

Under SEM, the pollen of *M. indica* cultivars in the present study can be divided into three types mainly based on the features of exine ornamentation; striate type (striae parallel to colpus) in accession 1 and accession 2 (Keitt cv.) (Fig. 4 c, f), and striate-perforate type in accession 3 (Fig. 4 i) (smooth striae parallel to colpus) and accession 4 (coarse striae inclined to colpus) (Fig. 4 l) (‘Ewais’ cv.) and striate-reticulate type in accession 5 and accession 6 (‘Ewais’ cv.) (Fig. 4 o, r).

Using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) agglomeration methods, a dendrogram was made on all morphological and palynological characteristics as the basis for the clustering of mango accessions (Fig. 5). The main tree possessing all accessions was divided into two clusters at a similarity coefficient of 0.24. Cluster I contained accessions 1 and 2 (‘Keitt’ cv.), which were closely related at a 0.95 similarity coefficient. Cluster II included all accessions of ‘Ewais’ cv. which were further segregated into two sub-clusters at a similarity

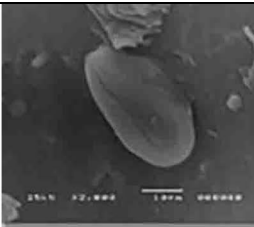
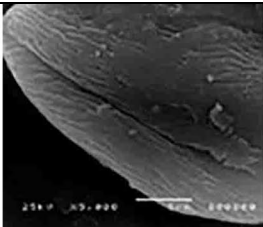
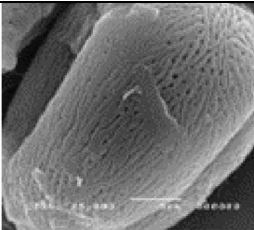
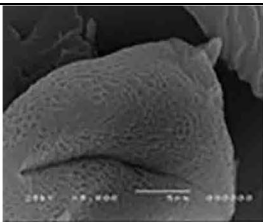
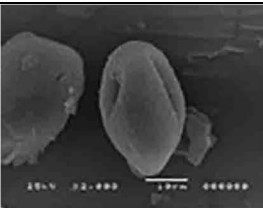
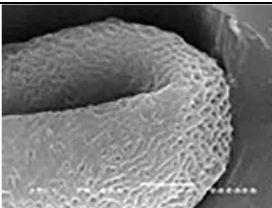
coefficient of 0.47. Subcluster I was constituted with accession 3, which split from the other ‘Ewais’ cv. at a similarity coefficient of 0.47. All accessions of ‘Ewais’ cv. constituted the second subcluster. The two accessions 5 and 6 are closely related at a similarity coefficient 0.85.

The results of the cluster analysis derived from morphological and palynological data showed that although accession 3 was recognized as ‘Ewais’ cv, it split away from the other ‘Ewais’ accessions. The obtained result revealed that the most important variables to separate the two mango cvs. in this study are the P and E diameter, the ratio of P/E (pollen shapes), exine ornamentation, leaf length, leaf width, leaf color, and length and shape of the inflorescence.

Molecular relationship

Twenty loci (10 loci for ISSR and 10 loci for SCoT) were used in this investigation. The twenty loci yielded 97 amplified and reproducible alleles. Among the six accessions, 43 alleles were produced from ISSR with sizes ranging from 180 to 1400 bp, and 54 alleles were generated by SCoT with sizes ranging from 1300 to 1710 bp (Table 5 and Fig. 6). The maximum number of fragments was 10, generated by locus SCoT 9, while the minimum number of fragments was 2, generated by locus SCoT 2. Eighteen alleles produced in ISSR from 43 were monomorphic, and 11 alleles were unique. Additionally, 24 alleles from 54 generated by SCoT were monomorphic, and 13 alleles were unique. Almost all the 20 loci were polymorphic, and the polymorphism percentage of ISSR ranged from 0 to 100%, while the polymorphism percentage of SCoT ranged from 0 to 80% (Table 5). These polymorphism percentages displayed genetic diversity among all mango accessions.

Polymorphic information content (PIC) ranged from 0.03–0.98 for ISSR and 0.06–0.98 for SCoT, with averages of 0.336 and 0.552 (Table 5). The average RP values of 5.0125 and 5.179 for ISSR and SCoT, respectively, proved the efficiency of the ISSR and SCoT markers in differentiating mango accessions. Table 5 demonstrates the discriminative ability of ISSR and SCoT techniques. The percentage of diversity index (DI %), and RP% of ISSR were 0.336 and 5.012, respectively, while DI%, and RP% of SCoT were 0.55 and 5.179, respectively.

Accession1	 a 10mm	 b	 c
Accession2	 d 10mm	 e	 f
Accession3	 g 10mm	 h	 i
Accession4	 j 10mm	 k	 l
Accession5	 m 10mm	 n	 o
Accession6	 p 10mm	 q	 r

◀Fig. 4 Photomicrographs and scanning electron micrographs of mature pollen grains of two cultivars ('Keitt' and 'Ewais') with six accessions (SEM scale bar 5 μ m and 10 μ m). **a–c** accession 1: a LM pollen photo in polar view, **b, c** SEM photos: striate sculpture; **d–f** accession 2: d LM pollen photo in polar view, **e, f** SEM photos: striate sculpture; **g–i** accession 3: g LM pollen photo in polar view, **h, i** SEM photos: striate-perforate sculpture; **j–l** accession 4: j LM pollen photo in polar view, **k, l** SEM photos: striate-perforate sculpture; **m–o** accession 5: m LM pollen photo in polar view, **n, o** SEM photos: striate-reticulate sculpture; **p–r** accession 6: p LM pollen photo in polar view, **q–r** SEM photos: striate-reticulate sculpture

Genetic similarities matrix of the six mangifera indica accessions

The genetic similarity coefficient and identity between the six mango accessions produced by the combination of ISSR and SCoT data are presented in Table 6. The average similarity coefficient was 0.825. The highest genetic identity (0.891) was recorded between accessions 5 and 6 (two 'Ewais' cv.), followed by 0.861 recorded between accessions 1 and 2 (two 'Keitt' cv), while the lowest genetic identity (0.759) was noted between accessions 2 and 3.

Cluster and principal coordinate analysis

A dendrogram based on genetic distance using the UPGMA mean was constructed using ISSR data (Fig. 7a) and SCoT data (Fig. 7b). The ISSR dendrogram split into two major clusters at 0.759 similarity coefficient. The first cluster enclosed accession 3 only, and the second cluster included the rest of accessions. The second cluster was divided into two subclusters at 0.816 similarity coefficient. The first subcluster contained the two accessions 5 and 6 at the same distance. While the second subcluster is divided into two sub-sub clusters, one of them contained accession 4 only, and the other sub-sub cluster includes accessions 1 and 2.

The dendrogram based on SCoT data split into two major clusters with a 0.814 similarity coefficient. The first cluster enclosed accession 3 only, and the second cluster included the rest of the accessions. The second cluster was divided into two subclusters with a 0.857 similarity coefficient. The first subcluster contained the two accessions 4 and 5 at the same distance (0.911). While the second subcluster is divided into two sub-sub clusters. One of them contained

accession 6 only, and the other sub-sub cluster includes accessions 1 and 2.

The combination of data from ISSR and Scot produced a dendrogram using the UPGMA presented in Fig. 8. The dendrogram was split into two major clusters at a 0.808 similarity coefficient. The first cluster enclosed accession 3 only, and the second cluster included the remaining accessions. The second cluster was divided into two subclusters with a 0.824 similarity coefficient. The first subcluster contained the two accessions 1 and 2 at the same distance. While the second subcluster is divided into two sub-sub clusters at 0.850, one of them contained accession 4 only, and the other sub-sub cluster includes accessions 5 and 6. These results revealed that accessions 1 and 2 ('Ewais' cv.) are very close to each other at a similarity coefficient of 0.861; as well as, accessions 5 and 6 ('Ewais' cv.) are very closely related to each other at a similarity coefficient 0.891, and they are closely related to accession 4, which is also 'Ewais' cv. Therefore, the three previous dendrograms proved that accession 3 does not relate to any studied cultivars ('Ewais' cv and 'Keitt' cv.).

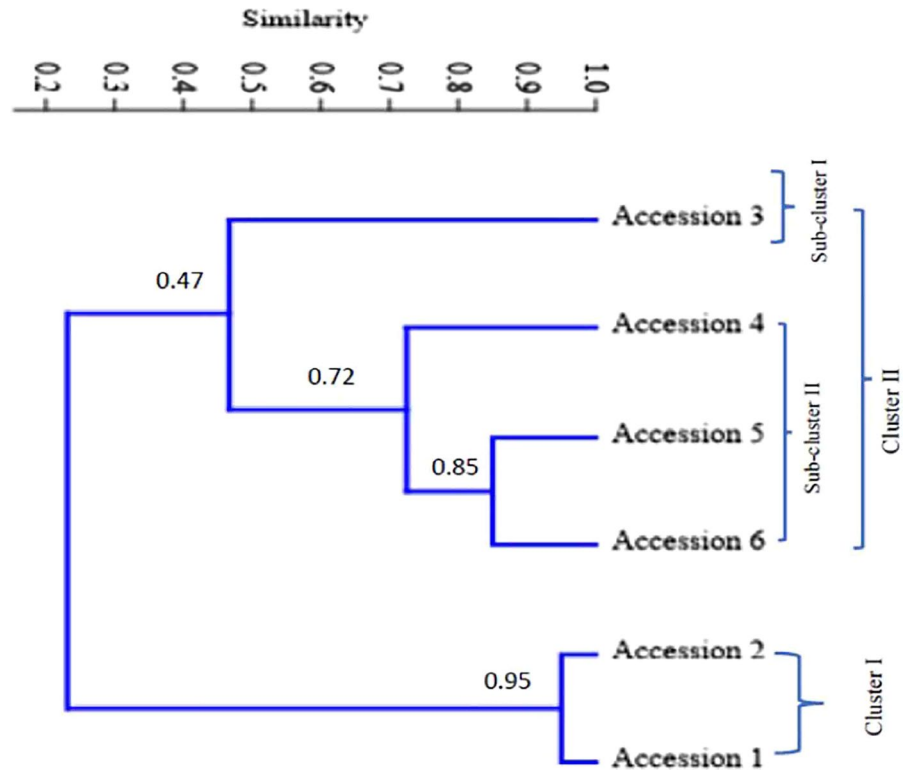
Figure 9 shows the scatter plots for principal coordinate analysis (PCoA) produced from ISSR and SCoT marker results. The PCoA result was similar to the clustering constructed by UPGMA. The PCoA result grouped the six mango accessions into four groups. Group 1 comprises 2 accessions (1 and 2) of 'Keitt' cv., group 2 includes 2 accessions (5 and 6) of 'Ewais' cv., group 3 contains accession 4 of 'Ewais' cv., and the last group 4 contains accession 3 of 'Ewais' cv.

Phytochemical analyses

The phytochemical screening of *M. indica* ethanolic extract demonstrated the existence of most biologically active phytochemical compounds and the absence of anthraquinones, phenols, and saponins. Alkaloids were present in accession 3 only.

Quantitative analysis of primary metabolite showed that carbohydrate content ranged from 25.40 to 27.99 g% and total protein content ranged from 12.4 to 15.6 g%. The two 'Keitt' accessions 1 and 2 contain almost the same amount of carbohydrates and are significantly higher than the amount of carbohydrates among 'Ewais' cv. Moreover, the present findings showed that accession 3 ('Ewais'

Fig. 5 Dendrogram of six accessions of two Egyptian *Mangifera indica* cultivars ‘Keitt’ and ‘Ewais’ based on morphological and palynological criteria. (accession 1 and 2 ‘Keitt’ cv.); (accession 3–6 ‘Ewais’ cv.)



cv.) differs from other ‘Ewais’ accessions. Its carbohydrate and protein contents were 23.11 and 7.70 g/100 gm, respectively (Table 7).

The phytochemical composition of secondary metabolites revealed the presence of total flavonoids in the mango accessions in the range of 96.95 to 109.43 mg quercetin/g, except accession 3, which contains 90.78 mg/g. Accessions 1 and 2 showed a significantly higher content of total flavonoids than those of ‘Keitt’ accessions. Total phenolics ranged from 126.11 to 184.16 mg tannic acid/g. Total tannins ranged from 124.35 to 180.33 mg tannic acid/g. The most important phytochemical present in mango is mangiferin, which was present in all investigated accessions in the range of 247 to 271 mg/g. Once more, accession 3 was different as it contained a higher amount of mangiferin (305 mg/g) than the other five accessions (Table 7).

The results of photosynthetic pigment concentrations in the six mango accessions leaves are presented in Table 8. Total chlorophyll ranged from 1.56 to 2.04 mg/g. Carotenoid content ranged from 0.6 to 0.9 mg/g. Results of Table 8 revealed the difference in chlorophyll a and b and carotenoid

contents in leaves of accession 3 compared to the other accessions.

Similarly, phytochemical analysis revealed the difference between accession 3 and the other five accessions.

Discussion

Accurate molecular, morphological, palynological, and biochemical characterization of mango cultivars is essential for breeding programs. A similar study by Sankaran et al. (2018) studied the relationships among six *Mangifera* species using morphological, molecular, cytological, and palynological research. Their findings were similar to the present work findings. They reported that, to determine the phylogenetic grouping and biogeographical localities of *Mangifera* species, it is important to integrate data from morphological, molecular, cytological, and palynological research.

In the present study, the morphological characteristics of the studied *M. indica* accessions of the two cultivars (1,2 Keitt, 3,4,5,6 Ewais) were evaluated

Table 5 Inter simple sequence repeat and start codon targeted fragments, for 20 loci assessed from the six accessions of two Egyptian *Mangifera indica* cultivars ‘Keitt’ and ‘Ewais’

Analysis	Primer	T.B	M.B	P.B	U.B	Polymor- phism (%)	RP	PIC
ISSR-PCR	14A	3	3	0	0	0.00	12	0.31
	44B	3	3	0	0	0.00	12	0.31
	49B	8	0	8	4	100	6.26	0.03
	89B	8	2	6	1	75	4.64	0.19
	98A	3	1	2	1	66.66	2.66	0.06
	HB-8	3	2	1	1	33.33	1.66	0.31
	HB-10	4	1	3	0	75	3.96	0.98
	HB-11	4	3	1	1	25	1.98	0.69
	HB-12	3	2	1	1	33.33	1.66	0.31
	HB-13	4	1	3	2	75	3.3	0.17
	Total	43	18	25	11		50.12	3.36
	Mean	4.3	1.8	2.5	1.1	58.14	5.012	“DI value” 0.336
	SCoT-PCR							
SCoT-PCR	SCoT 1	4	3	1	1	25	4.66	0.31
	SCoT 2	2	2	0	0	0.00	1.66	0.35
	SCoT 4	7	3	4	1	57.14	12	0.98
	SCoT 5	3	2	1	1	33.33	3.66	0.31
	SCoT 6	3	1	2	0	66.66	4.64	0.53
	SCoT 7	6	4	2	1	33.33	2.99	0.06
	SCoT 8	4	1	3	2	75	4.32	0.63
	SCoT 9	10	2	8	3	80	8.92	0.95
	SCoT 13	9	4	5	4	55.55	3.96	0.66
	SCoT 15	6	2	4	0	66.66	4.98	0.74
Total		54	24	30	13		51.79	5.52
Mean		5.4	2.4	3.0	1.3	55.56	5.179	“DI value” 0.552

T.B, Total band; M.B, Monomorphic band; P.B, Polymorphic band; U.B, Unique band; RP, Resolving power; PIC, Polymorphic information content; DI, Diversity index

using 15 morphological features and it was demonstrated that mango accessions showed some variability in tree height, trunk color, sizes and shapes of leaves, color, length, and shape of inflorescence, resulting in reliable distinguishing characteristics. The current results revealed that the morphological characteristics of the cultivars expressed little difference from each other except in tree height, trunk color, size and shape of leaves, color, length, and shape of the inflorescence. However, leaf margin, leaf apex, and other aspects showed little difference and cannot be used as a discriminating tool for the two cultivars of *M. indica* used in this study. The variation in plant height between cultivars in the present study could be due to genetic character differences under different environmental condition and the dissimilarity between the cultivars for length and width of the leaf could be due to the genetic characteristics of the cultivar and the effect of environmental factors (Zulhairil et al. 2015). Variation in the leaf width

of mango was previously observed (Majumder et al. 2011). The results of the present investigation on ‘Ewais’ cv. showed close conformity with the findings of EL-Banhawy et al. (2020), in which the length of leaf, width, and petiole length ranged from 16 to 23 cm, 4.1 to 5.7 cm, and 2.9 to 4.5 cm, respectively. Additionally, the trunk had three colors; gray, grayish brown, and pale brown, which agrees with the results of Khadivi et al. (2022). In addition, the leaf margin ranged from wavy to entire, as reported by Rajwana et al. (2011). The results of Bhamini et al. (2018), who studied 24 mango cultivars were consistent with the results here. The leaf apex ranged from acute to acuminate, and the leaf color was similar to that reported by Vieccelli et al. (2016). Furthermore, this study showed variability in the color of inflorescence (yellowish green, light red, yellowish-green, and light red), which is similar to the findings reported by Saheda et al. (2019). The difference in the shape and size of the panicles of *M. indica* cultivars in this

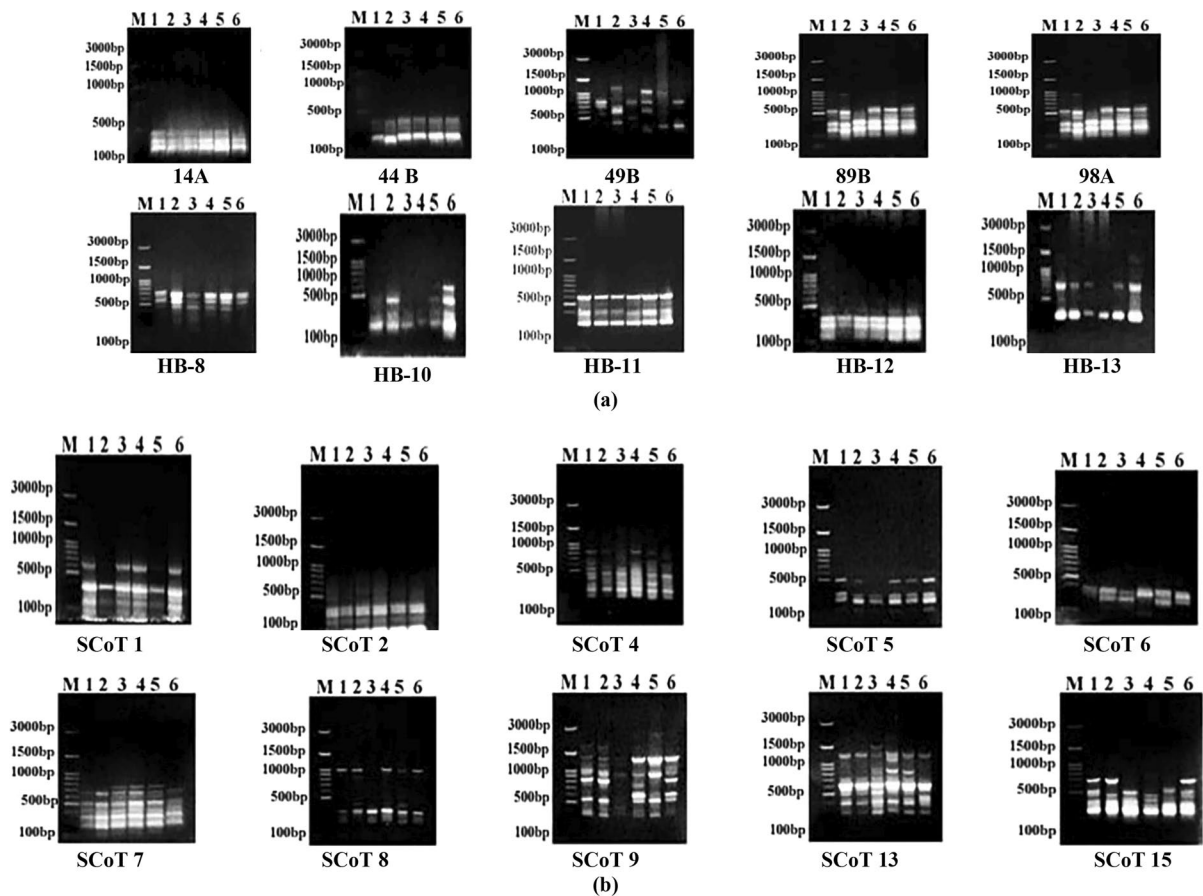


Fig. 6 Genetic polymorphism of six accessions of two Egyptian *Mangifera indica* cultivars 'Keitt' and 'Ewais' based on inter simple sequence repeat analysis (a) start codon targeted

analysis (b) M: Marker; 1,2: Accessions 1 and 2 of *M. indica* 'Keitt' cv; 3,4,5,6: Accessions 3, 4, 5 and 6 of *M. indica* 'Ewais' cv

Table 6 Genetic similarity matrix among six accessions of two Egyptian *Mangifera indica* cultivars 'Keitt' and 'Ewais' according to Dice's coefficient

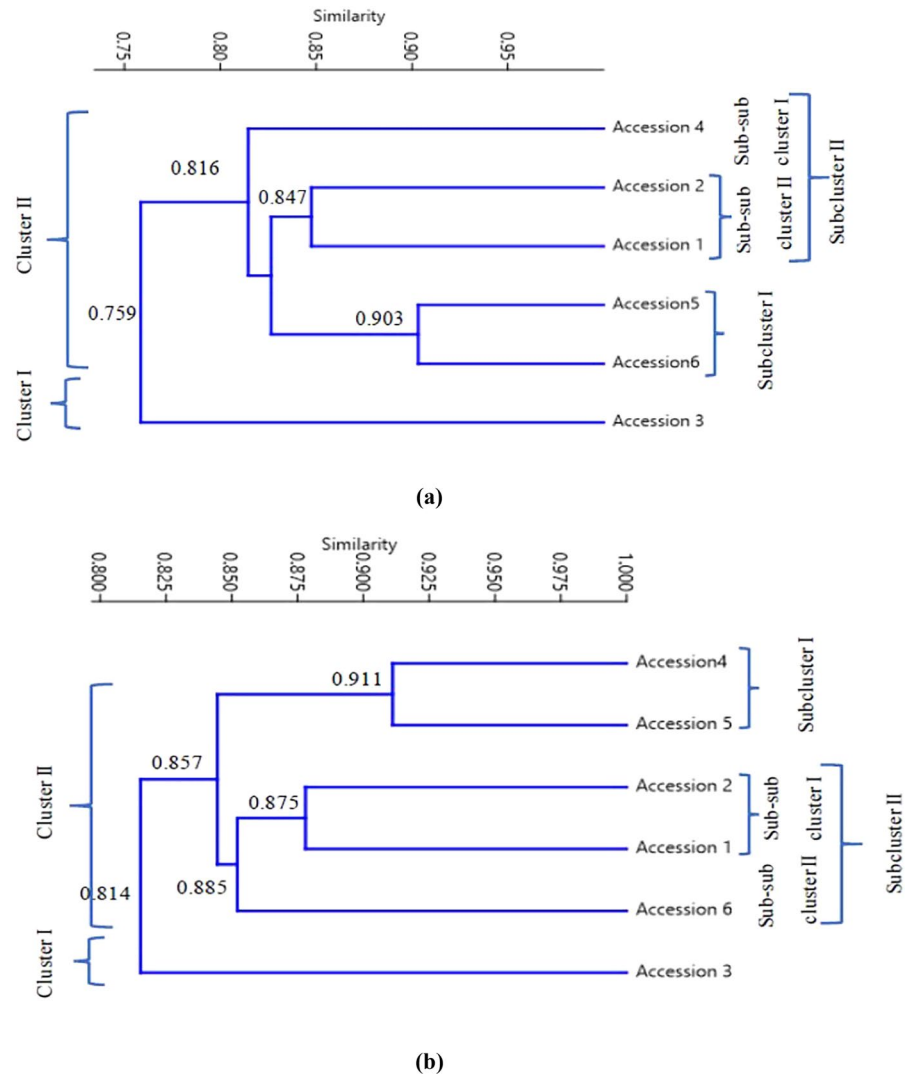
	Accession1	Accession2	Accession3	Accession4	Accession5	Accession6
Accession1	1.000					
Accession2	0.861	1.000				
Accession3	0.812	0.759	1.000			
Accession4	0.841	0.765	0.826	1.000		
Accession5	0.841	0.832	0.826	0.880	1.000	
Accession6	0.853	0.789	0.808	0.824	0.891	1.000

study may be caused by the genetic and physiological composition of the shoot from which the panicle rises. Moreover, variations in the inflorescence length of different mango cultivars were previously reported (Majumder et al. 2011). Previous research on mango cultivars revealed that the panicle length of 'Keitt' cv. is the longest one and varies through the different

years. Mohamed et al. (2016) stated that the panicle length of 'Keitt' cv. grown in the Aswan region was 34.97 and 34.07 cm during the 2012 and 2013 seasons, respectively.

In the current study, the micromorphological features of pollen grains displayed alteration in pollen shape and exine sculpturing, which can be used as

Fig. 7 Dendrogram of six accessions of two Egyptian *Mangifera indica* cultivars ‘Keitt’ and ‘Ewais’ based on genetic similarity produced by inter simple sequence repeat (a) and start codon targeted (b)



taxonomic characteristics for appropriate classification. The pollen size classification of all mango accessions was medium sized which agreed with Erdtman's (1952) classification. The pollen shape in this study was in harmony with Smitha and Samuel (2018) and Muniraja et al. (2020), who reported that pollen grains of *M. indica* accessions were tri-zonocolporate and their shapes ranged from sub-prolate to prolate-spheroidal, whereas Rocha et al. (2021) stated that the pollen grains of mango were medium-sized and had a sub-prolate to prolate shape.

Under SEM, the pollen of *M. indica* cultivars in the present study can be divided into three types, based on the features of exine ornamentation. In accordance with the findings of Smitha and Samuel

(2018), exine ornamentation showed two different sculptures: striate reticulate and striate elongate. In addition, Rocha et al. (2021) was consistent with the result herein, where exine sculpture was striate-perforate, while Muniraja et al. (2020) stated that mango pollen grain exine had reticulate perforated ornamentation. In contrast, 3-porate pollen grains were documented in *M. indica*. In addition, tricolpate pollen was previously reported at anther dehiscence (Lora and Hormaza 2018), differing from the evidence found in the results of Ramirez and Davenport (2016) and Shixiong et al. (2018) and consistent with the results in the present study, where colporate grains are common. On the other hand, the striate-reticulate ornamentation observed

Fig. 8 Dendrogram of six accessions of two Egyptian *Mangifera indica* cultivars ‘Keitt’ and ‘Ewais’ based on genetic similarity produced from combined data of inter simple sequence repeat and start codon targeted

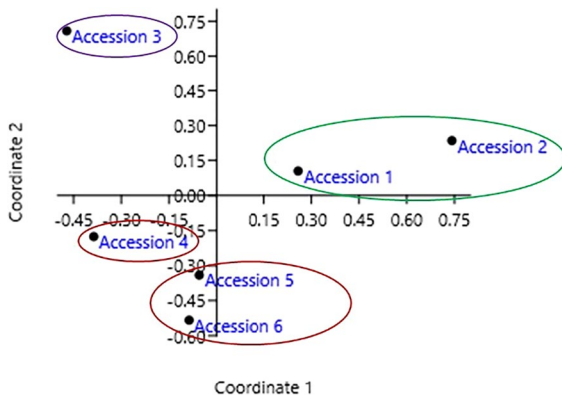
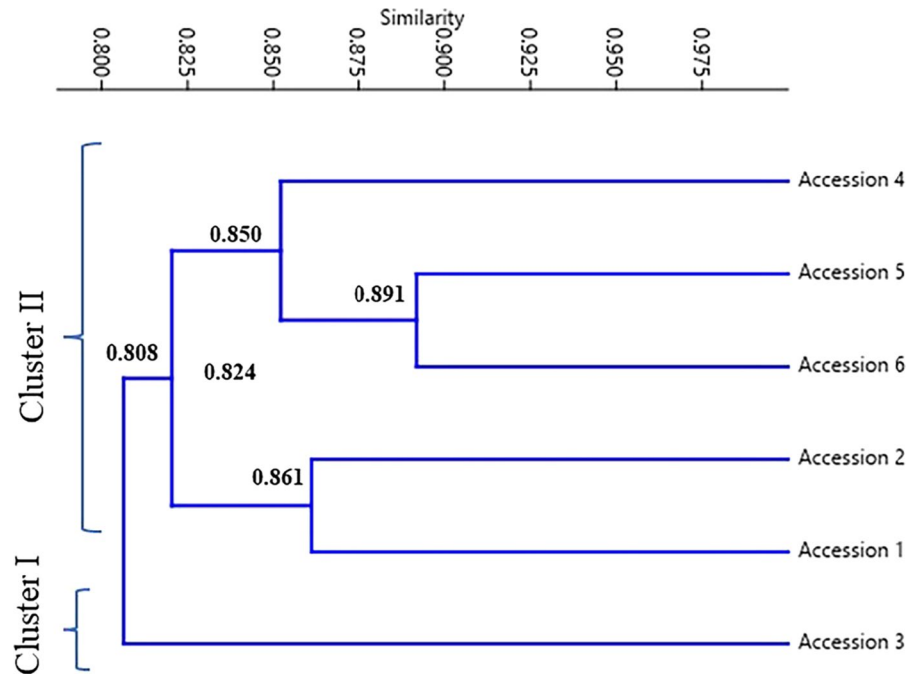


Fig. 9 Scatter plot of principal coordinate analysis (PCoA) of 6 accessions of *Mangifera indica*

in this study differs from the standard striate pattern described previously in the pollen grains of some cultivated trees and the finely reticulate pattern reported by Shixiong et al. (2018). Tricolporate pollen grain which was found in the studied cultivars of *M. indica* is considered to have an advanced status, as confirmed by Yu et al. (2018), who stated that all taxa with tricolporate pollen grains or pollen with more pores or colpi reveal their advanced status, whereas taxa with a lower number of pollen

apertures exhibit a primitive status on the evolutionary scale.

A UPGMA dendrogram was made on all morphological and palynological characteristics for clustering mango accessions. All accessions were divided into two clusters. Cluster I contained accessions 1 and 2 (‘Keitt’ cv.) and Cluster II included all accessions of ‘Ewais’ cv., whereas accession 3 split from the other ‘Ewais’ cv. The present study revealed that palynological features reinforced the morphological data in establishing the variability of cultivars.

The data from ISSR and SCoT indicated the validity of both ISSR and SCoT analyses to assess the genetic diversity among the six mango accessions. ISSR uses microsatellite sequences as primers to produce multi-locus bands. SCoT is beneficial for analyzing genetic diversity, phylogeny, and evolutionary biology (Reddy et al. 2002). While SCoT is a predominant marker that targets the short, conserved region of genes containing the ATG (initiation codon) by a single primer in PCR analyses (Collard and Mackill 2009). In the present study, ISSR and SCoT markers amplified 97 reproducible alleles with a high polymorphism percentage. This result is in harmony with the result of Luo et al. (2010), who stated that the SCoT marker is a very important tool to assess the genetic link between various *M. indica* cvs and

Table 7 Phytochemical composition of some primary and secondary metabolites of the six accessions of *Mangifera indica* extract

Name of accession	Carbohydrate (g /100g fw)	Total protein (g 100/g fw)	Total flavonoids (mg/ g dw)	Total phenolics (mg /g dw)	Tannins (mg/ g dw)	Mangiferin (mg/ g dw)
Accession1	27.99±0.82 ^a	15.0±0.25 ^a	109.43±0.73 ^a	136.11±0.53 ^d	133.85±0.81 ^d	253±1.02 ^e
Accession2	27.57±0.57 ^b	15.6±0.46 ^a	103.88±0.95 ^b	126.11±0.66 ^f	124.35±0.70 ^f	259±0.87 ^d
Accession3	23.11±0.71 ^f	7.7±0.18 ^b	90.78±0.36 ^f	167.22±0.57 ^b	165.53±0.97 ^b	305±0.91 ^a
Accession4	25.40±0.12 ^e	14.7±0.26 ^b	98.52±0.64 ^d	158.33±0.59 ^c	155.44±0.73 ^c	271±0.69 ^b
Accession5	25.59±0.54 ^d	12.4±0.37 ^b	96.95±0.60 ^e	133.98±0.28 ^e	129.38±0.28 ^e	264±0.93 ^c
Accession6	26.11±0.39 ^c	14.7±0.14 ^a	99.33±0.53 ^c	184.16±0.41 ^a	180.33±0.86 ^a	247±0.22 ^f
<i>F value</i>	91.60 ^{***}	4.6 ^{**}	12.43 ^{***}	152.38 ^{***}	15.84 ^{***}	127 ^{***}

Values with non-similar letters signify significant differences (Duncan's test). ***, $P \leq 0.001$, **, $P \leq 0.01$

Table 8 Quantitative estimation of chlorophyll and carotenoid content of the six accessions of *Mangifera indica* extract (mg/g fw)

Name of accession	Wavelengths (nm)			Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoid
	633	645	480				
Accession1	1.083	0.554	0.834	1.226±0.04 ^{a,b}	0.761±0.09 ^b	1.986±0.17 ^b	0.9±0.01
Accession2	0.676	0.322	0.531	1.044±0.06 ^c	0.516±0.02 ^d	1.560±0.05 ^d	0.6±0.05
Accession3	0.909	0.411	0.683	0.772±0.01 ^d	1.054±0.04 ^a	1.829±0.22 ^c	0.3±0.02
Accession4	1.144	0.552	0.855	1.304±0.12 ^a	0.729±0.06 ^b	2.032±0.14 ^a	0.9±0.01
Accession5	0.994	0.471	0.735	1.136±0.09 ^{c,b}	0.613±0.07 ^c	1.749±0.09 ^c	0.7±0.04
Accession6	1.175	0.547	0.841	1.345±0.07 ^a	0.702±0.01 ^{b,c}	2.046±0.02 ^a	0.9±0.01
<i>F value</i>				33.44 ^{***}	48.6 ^{***}	122 ^{***}	Non-significant

Values with non-similar letters signify significant differences (Duncan's test). ***, $P \leq 0.001$

showed a high level of polymorphism. Additionally, considerable genetic diversity was reported among 28 Egyptian mango cvs with genetic polymorphisms ranging from 0.71% to 100% (Abdelsalam et al. 2018). Moreover, the mean polymorphism frequencies in the present study were similar to the mean polymorphism percentage among 23 Egyptian mango cultivars reported by Ghounim et al. (2022), which were equal to 44.44% for SCoT and 58.82% for ISSR.

The high value of PIC reported in this study indicates the efficacy of ISSR and SCoT markers for differentiating genetic diversity among mango accessions. A PIC value of more than 0.5 is considered a high informative value; a value below 0.25 is considered a low value; and a range between 0.25 and 0.5 is considered informative. Moreover, RP can strongly correlate closely related mango accessions (Zhou et al. 2020). Furthermore, the discriminative ability results of ISSR and SCoT techniques proved the higher discriminative potency of the SCoT technique than ISSR because SCoT markers afforded more

revealing data than ISSR markers and reinforced the usage of SCoT to estimate the genetic diversity among the mango cultivars more than ISSR markers. This result matches the results of Etminan et al. (2016) in *Hordeum* and Gorji et al. (2011) in *Solanum*. They also proved that the SCoT marker was more efficient than the ISSR marker. However, Abd El-Aziz et al. (2019) demonstrated that the combination of ISSR and SCoT data was more useful when assessing the genetic diversity among apricot strains.

Additionally, the average similarity coefficient among the six accessions indicated genetic differences among them, demonstrating that these genotypes are genetically distinct from one another and have the greatest genetic distance. This may be due to some somatic mutations accumulated over many years or may be due to the polyembryonic nature of mango, germplasm exchange, high hybridization, the significance of genetic segregation in mangoes following cross-pollination, or the selection process made by the breeders to enhance mango qualities,

such as climatic tolerance and improvement of fruit properties. These reasons could explain the production of various genetically distinct lines of mango that have come from the same cultivar (Arogundade et al. 2022).

In the current study, a dendrogram using UPGMA and scatter plots for PCoA were used to evaluate the genetic diversity between the six mango accessions to help the breeders prove any evolutionary linkages. The UPGMA and PCoA results showed a large genetic distance between accession 3 and the other accessions, suggesting a difference in the genetic composition of this accession and also suggesting that it may belong to different cultivars. Therefore, UPGMA and PCoA results prove that ISSR and SCoT markers are beneficial for identifying mango cultivars and analyzing their genetic diversity (Patil et al. 2019; Zhou et al. 2020). However, there are only a few molecular genetic research on mangoes, and these investigations have the potential to overcome critical challenges in mango production and to develop significant breeding techniques. Therefore, it is crucial to increase the genetic base of cultivated mango to improve the harvest and overcome mango vulnerability to different infections and insects by integrating features from genotypes with different ecological adaptations.

Phytochemical analyses revealed that carbohydrate content and total protein content are compatible with the results of Ali et al. (2020) and Kumar et al. (2021). Besides, total phenolics content was like the result of Laullo et al. (2018). The contents of total tannins and mangiferin (the most important phytochemical present in mango) were in accordance with those reported by Ali et al. (2020). These findings revealed that mango accessions under investigation contain high concentrations of carbohydrates, which are considered osmotically active compounds. The presence of flavonoids, phenolic compounds, tannins, and mangiferin in mango extracts demonstrated their beneficial properties. The existence of these secondary metabolites provides mango anticancer, antioxidant, antibacterial, anti-inflammatory, and immunomodulatory potential (Maldonado-Celis et al. 2019).

The mango leaves of the six accessions contain high levels of carotenoids, which are essential for plant defense because of their antioxidant potential. In addition, carotenoids are vital in their role as precursors of

important vitamins such as vitamin A (Kumar et al. 2021).

Conclusion

Combining genetic, morpho-biochemical, and palynological characterization among the six mango accessions in this study may provide valuable evidence for mango selection and identification. Both genetic polymorphism and phytochemical traits showed significant variability. Likewise, palynological features reinforced the morphological data in establishing the variability. The results of this study demonstrated that although classified as an 'Ewai' cv., accession 3 displayed significant diversity and variation. Therefore, evaluation of the genetic, morphological, palynological, and biochemical features of mango is essential for improving preservation, identifying genetic relatedness among mango accessions, and improving breeding programs. In addition, these findings demonstrate that both ISSR and SCoT markers can be used to identify mango cultivars, but the SCoT marker is more efficient for analyzing the genetic diversity among mango cultivars than ISSR.

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Declarations

Competing interests The authors have no competing interests to declare.

Ethical statement No humans or animals were involved in the experimental procedure of this study.

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