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Full Length Research Article

Identification of Palm Varieties Based on the DNA Barcoding using *mat K* Gene

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Abstract

Throughout the world, the family Palmae (palm tree) is composed of over 230 genera and about 3000 species. Because there are over 3000 species of palm trees, identifying palm tree varieties based on morphological characteristics can be difficult. Our aim is to collect three varieties of palm plants, i.e., *Borassus flabellifer, Latania lontaroides*, and *Phoenix dactylifera,* and find out morphological characters like fronds, trunk, and size. The genomic DNA was isolated using the *CTAB* method, amplified with *the matK* protein-coding gene, and sequenced. The Amplicon has a size of 700-750 bp. The phylogenetic tree obtained by MEGA 9.0 suggests that the palmate-shaped leaves of palm varieties are closely related to each other when compared to pinnate-shaped leaves. *Borassus flabellifer* and *Latania lontaroides* are closely related, and this was identified by a distance matrix, which shows the minute distance between *Borassus flabellifer* and *Latania lontaroides*. From this, we conclude that among the 3000 species of palm varieties, palmate-shaped leaves are closely related, while pinnate-shaped leaves are also closely related.

***Key words*:** *Borassus flabellifer, Latania lontaroides, Phoenix dactylifera, MatK* gene, Phylogenetic tree, MEGA 7.0, Distance matrix

Palms are an evergreen, mostly tropical or subtropical monocotyledonous plant in the family *Arecaceae* (*Palmatae).* Worldwide, the family *Palmae* (palm tree) is composed of over 230 genera and about 3000 species. Palms are the most extensively cultivated plant families; they have been important to humans throughout their lives. Many food products are derived from palm trees, and palms are widely used in landscaping, making them one of the most economically important plants [1]. The palm family is an economically important pantropical plant family with over 3000 species; it can be challenging to identify a palm tree. Therefore, we found a new comparative morphological trait in palm plants [2-3]. It is challenging to classify and identify species of palms using herbarium specimens since some species are tall, have big leaves, or are thorny [4-6].

DNA barcoding may be a particularly useful method for verifying the identification of palm species. There have only been a few studies that have used DNA barcoding to clarify species relationships in the palm family, despite the family's abundance in species and economic or cultural significance. Rates of species discrimination based on DNA barcoding vary among studies and genera.

Maturase K (matK) is a plant plastidial gene. The protein it encodes is an intron maturase, a protein that splices introns [7]. Amongst other maturase, this protein retains only a well conserved domain X and remnants of a reverse transcriptase domain. Universal matK primers can be used for DNA barcoding of angiosperm [8]. MatK as the standard plant barcode based on assessments of recoverability, sequence quality and levels of species discrimination. Recently, several investigators have used rbcL and matK sequences for barcoding or species identification [9-12] as well as for phylogenetic analysis [13-16]. MatK (66.6%) and can be potentially used as a standard barcode to discriminate the species of Palmae [17-18].

Our primary task was to gather the chosen palm kinds from the St. Joseph's College campus in Trichy and analyse them using morphological characteristics. Next, extract the genomic DNA from a few different types of palm trees. After that, amplify and sequence the amplicons from the MatK Chloroplast DNA region. DNA sequences can be aligned using the pair-wise technique using clustal W to determine their distance from one another. Finding the best morphological features and building the phylogenetic tree of DNA sequences.

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MATERIALS AND METHODS

Sample collection and morphological characterization

The three samples were collected from the St. Joseph's College campus in Trichy. The morphological characters are identified. The leaf samples were individually placed in plastic pouches and transported to a laboratory, where all the samples were stored at -80 ºC until processed for DNA extraction [19-20].

Molecular characterization

Plant genomic DNA isolation

DNA extraction steps

The leaf samples were immersed in liquid nitrogen and crushed using sterile mortar and pestle to get a fine powder. The DNA was extracted by CTAB method. Quality of the extracted DNA was determined using gel electrophoresis Isolated plant genomic DNA was preserved at -20 °C.

Table 1 Primer sequence used

Primers	Direction	Sequence
MatK	Forward	5'-CGATCTATTCATTCAATATTTC-3'
MatK	Reverse	5'-TCCGATAAATCGATCCAGACC -3'

Constituents colume of PCR reaction

17.5X Taq Buffer	:	5µl
10mM dNTP mix	:	1µl
50mM $MgCl_2$	:	0.75µl
Primer (forward and reverse)	:	5µl
Taq Polymerase (1 unit)	:	0.3µl
Template DNA	:	2µl
Sterile MilliQ water	:	35.95µl
Total volume	:	50µl

PCR conditions

PCR amplification was performed in 50µl volume containing 50mM Kcl, 10mM Tris HCl at pH 8.3, 2.5mM $MgCl_2$, 1µM of each primer, and 200nM of each dNTPs and 1.5U of Taq polymerase (GenTec biotech). Amplification was performed in an Eppendorf Thermal Cycler, Germany. PCR cycles are as follows: Initial denaturation at 94 ºC for 4 minutes, 35 cycles of 45 seconds in 94 ºC for denaturation, 45 seconds at 50 ºC for annealing, 45 seconds at 72 ºC for elongation and final extension at 72 ºC for 10 minutes.

Agarose gel electrophoresis

1.5% Agarose gel was prepared using 1X TBE buffer and 5µl of each amplified sample were loaded in each well after mixing it with 3µl of 6X gel loading dye. DNA ladders of uniform sizes from 100bp to 1000bp were used to determine the size of the PCR amplicons. Gel were run at 50V for 1 hour on a submarine Agarose gel unit and visualized under UV transilluminator and photographed.

Data analysis

The new DNA sequences were identified at genus and species level using BLAST software tool (NCBI, USA). The DNA sequences that shown maximum similarity to query sequences were selected and analysed using MEGA 7.0 (Molecular Evolutionary Genetics Analysis) for phylogenetic analysis. The phylogenetic tree was generated based on Neighbour Joining Method [21].

RESULTS AND DISCUSSION

Morphological characterization

Morphological characterization is identified and mentioned in (Table 1).

Table 1 Identification of palm plant based on morphological character

Fronds	Pinnate/feather shape - *Phoenix dactylifera* Palmate/fan shape- *Borassus flabellifer, Latania lontaroides*
Trunk	Solitary trunk - *Borassus flabellifer, Phoenix dactylifera* Multi trunk - *Latania lontaroides*
Size	Tall - *Borassus flabellifer, Phoenix dactylifera* Small - *Latania lontaroides*
Colour	Green - *Borassus flabellifer, Phoenix dactylifera* Red - *Latania lontaroides*

DNA isolation, purification and quantification

DNA isolation from three different palm samples was carried out successfully using the CTAB method and purified using a sodium acetate-ethanol precipitant. At 0.8%, the isolated genomic DNAs were quantified in agarose gel electrophoresis. The genomic DNA of three different palm samples shows that the genome is likely to be similar in length, and the DNA sequencing was performed by a Eurofins Genomics India Private Limited laboratory.

Distance matrix of three different palm sample

The distance between *Borassus flabellifer* and *Latania lontaroides* is 0.003, and the distance between *Phoenix dactylifera* and *Latania lontaroides* is 0.024. Finally, the distance between *Phoenix dactylifera* and *Borassus flabellifer* is 0.026.

Table 2 Distance matrix of three different palm sample phylogenetic tree for three different species

Species 1	Species 2	Dist
AJ03matK Phoenix Dactylifera	*AJ02matK Latania lontaroides*	0.024
AJ03matK Phoenix Dactylifera	*AJ01matK Borassus Flabellifer*	0.026
AJ02matK Latania lontaroides	*AJ01matK Borassus Flabellifer*	0.003

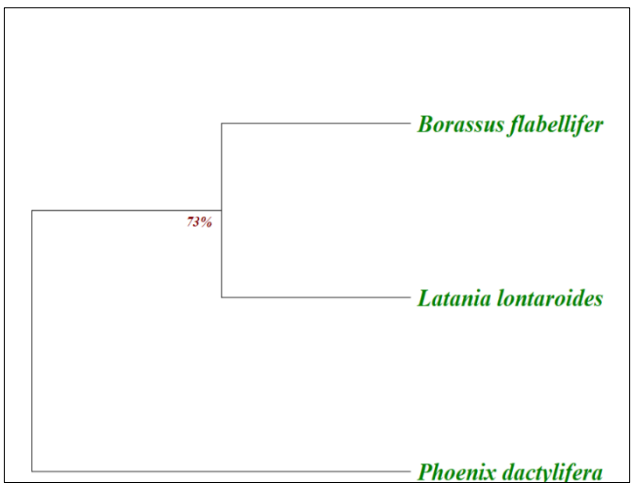

Fig 1 Evolutionary relationships of taxa

The phylogenetic tree obtained using MEGA 7.0 for three different species of palm plants is based on MatK gene sequences. The tree was generated based on the NJ method, and it is separated into three species and two clades. Borassus flabellifer and *Latania lontaroides* formed a first clade; the

second clade came from Phoenix dactylifera. It belongs to a distinct clade. Even though three plants belong to the palm variety, the constructed phylogenetic tree shows wide variation among them.

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.02655106 is shown (Next to the branches). The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. The analysis involved 3 nucleotide sequences. All positions containing gaps and missing data were eliminated. There was a total of 711 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

CONCLUSION

The palm samples were identified based on morphological characteristics. Then the genomic DNA was isolated by the CTAB method. The *mat K* gene of chloroplast DNA regions was amplified and sequenced for three palm plants, namely: a) *Borassus flabellifer*; b) *Latania lontaroides;* and c) *Phoenix dactylifera*. The amplicon sizes ranged approximately from 700 to 750 bp in all three species and sequences obtained and analyzed. The sequence's similarities were identified, and distances were found between the species. The phylogenetic tree obtained by MEGA 7.0 suggests that the two species, *Borassus flabellifer* and *Latania lontaroides*, which have palmate-shaped leaves, are closely related, while *Phoenix dactylifera*, which has a solitary trunk, is in a different group. *Borassus flabellifer* and *Latania lontaroides* are closely related, and they were identified by distance matrices. The distance matrix shows the minute distance between *Borassus flabellifer* and *Latania lontaroides*. Since the *mat K* region shows that there is a lot of variation among the plants, this could be a good candidate region for discriminating the palm plants and being very reliable.

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