Assessment of Genetic Variations of Taxodium Trees in Egypt

Ahmed E. Khalid 1, Fatama A. Hassan 2, Ahmed M. A. Mohamed 2 and Mohamed K. Gaber 3

1-Agricultural Botany Department, Faculty of Agriculture, Saba Bacha, Alexandria University
2- Department of Timber Trees, Horticultural Research Station, Sabahia, Alexandria, Egypt
3-Department of Plant Production, Faculty of Agricultural Saba Basha, Alexandria University

*E-mail: dr.a.khaled@alexu.edu.eg ; adrylink@yahoo.com

ARTICLE INFO
Article History
Received:21/3/2022
Accepted:5/6/2022
Available:9/6/2022

Keywords:
Taxodium, morphology, DNA barcoding

ABSTRACT
The current study was carried out at the Faculty of Agriculture, Saba Basha, Alexandria University, and the horticultural research station, Sabahia, Alexandria, Egypt. From 2018 to 2022 assessment of genetic variations of Taxodium trees in Egypt based on the morphological description and DNA barcoding. Four different localities were designated to survey the Bald cypress (T. distichum) samples. The results recorded that the tree length ranged from 21.22 m (Giza tree) to 11.89 m (El-Beheria tree), while the Alexandria tree was recorded 17.30 m forward by Qalyubia tree in a range of 12.61 m. The tree trunk diameter (cm) ranged from 36.40 cm (El-Beheria tree) to 115 cm (Alexandria tree), while Qalyubia tree was recorded 46.40 cm forward by El-Beheria tree in the range of 36.40 cm. The tree condition for all the trees was very good except the tree from Giza governorate was moderate. From these data, we can describe the different Taxodium trees from different locations in Egypt to be genetic resources that can be used in the future in the breeding program. The data of T. distichum El-Beheria governorate using rbcL gene showed that the G+C content was 43.96% and the A+T content was 56.04%. in addition, the nucleotide number and molecular percentage were as follows: A (148) 27.92%; C (113) 21.32%; G (120) 22.64% and T (149) 28.11%; while T. distichum Qalyubia Governorate, showed that the G+C content was 42.96% and the A+T content was 57.04%. in addition, the nucleotide number and molecular percentage were as follows A (148) 26.92%; C (113) 22.32%; G (120) 23.64% and T (149) 27.11%. The data of T. distichum Alexandria Governorate showed that the G+C content was 43.96% and the A+T content was 56.04%. in addition, the nucleotide number and molecular percentage were as follows: A (148) 27.92%; C (113) 21.32%; G (120) 22.64% and T (150) 28.11%. finally, the data showed that the G+C content was 43.96% and the A+T content was 56.04%. in addition, the nucleotide number and molecular percentage were as follows: A (149) 27.92%; C (120) 21.32%; G (111) 22.64% and T (132) 28.11%. The data was recorded to the Protein in T. distichum Giza Governorate using (rbcL) gene.
INTRODUCTION

The genus *Taxodium* belonged to the subfamily *Taxodiodeae* of the family *Cupressaceae* or cypress family. The literature showed that *Taxodiodeae* is a minor subfamily containing three botanic varieties (1) *Taxodium* in the United States (USA) and Mexico (2) *Glyptostrobus* is natural to China, and (3) *Cryptomeria* in Japan (Abdelsalam et al., 2016). The genetic difference inter the *Taxodium* genus has been understood to be either containing 3 separate types *T. distichum* (L.) Rich. var. *distichum* (Bald cypress), *T. distichum* var. *imbricarium* (Nutt.) (Pond cypress) and *T. distichum* var. *mexicana* (Montezuma cypress) as reported by (Abdelsalam et al., 2016).

The Cupressaceae family includes the genus *Taxodium* Rich. These plants are particularly appreciated for their root and high-resistant wood. In addition, the plants leave and cones, mainly high content of essential oils (EOs) as reviewed by (Hart, and Price 1990), besides *Taxodium* benefits, the plant leaves extracts could be considered as anti-microbial, anti-tumor, anti-termitic, anti-ispsmodic and bronchodilator activities.

The main importance for *Taxodium* plants is wood, which has been economically appreciated due to its flexibility and heavily collected due to it is resistant to decay but is too soft, light, actual durable, and does not warp easily Wiemann, (2021), in addition, the author studied Characteristics and availability of commercially important woods. Currently, the *Taxodium* genus is separate in numerous ecosystems counting coastal wetlands and floodplains across the USA as reported by (Mitsch et al., 2013, McDonald et al., 2008, Barbuto et al., 2010). *T. distichum* (L.) (B. cypress) is typically wild rising in a riparian environment, or swamps (flooding) and is valuable as a landscape tree (Abdelsalam et al., 2019, Shachak et al., 2008, Schoen et al., 1991). The Bald cypress trees are growing from sixty to eighty feet in plant height and from three to six feet in stem diameter and spread from twenty-five to thirty-five feet.

Abou Dahab et al. (2010) stated that forests are vital renewable natural resources because they provide numerous important products, as well as fuel, timber, lumber, paper, and fodder. Forests are the most important wildlife habitat and provide other purposes such as recreation and air and watersheds. It regulates the level of rainfall necessary for the existence of vegetation on land. In Egypt, forests are important to raise the mass production of many woody trees by planting them in several arid and semi-arid regions. There are several tree species cultivated in different areas of the country, but the cultivation of woody trees (including *Taxodium*) in Egypt has been confronted with several problems, like a failure to provide the essential provision for plantation, and a lack of interest by farmers and crop growers.

The genetic tools represented the genetic changes among many individual living organisms and different species. Commonly, genetic markers don’t characterize the main target gene but performance as the flag and/or signs. There are three different genetic markers morphological, biochemical and DNA markers (Jones et al., 1981). The usage of sequencing or nucleotide sequence variances in one gene to examine the genetic relationship was commonly useful by Carl Woese as reported by (Berger and Fox 2011, and Matsen et al., 2011). The author documented that those sequences change in a conserved gene and rRNA, which can be ulitlize to conclude the phylogenetic relationship. Additionally, freshly the PCR or polymerase chain reaction tool has allowable sequence variety in any gene to be calculated. rRNA genes as evolve slowly, and frequently don’t differ among close organisms, (Woese, 2000). While the other genes (evolve rapidly) might overwrite the traces of early affinities. DNA barcodes or barcoding includes short sequences of DNA obtained from a particular region of a plant genome and comparison them among and within species to current a “barcode” for species documentation (Petit et al., 2002).
DNA barcoding as a new tool showed as a promising tool for species identification in all organisms as reviewed by (Hebert et al., 2004, Cowan et al., 2006). DNA barcodes have developed into a progressively significant tool for taxonomic investigations and species definition, besides for the discovery of new species as shown by (Hajibabaei et al., 2007). Besides, Jeanson et al. (2011) noticed that the initial DNA barcodes investigation in palms reached 92% success in identifying species by applying a combination of 3 cpDNA markers. Therefore, there is a major essential to examine the genetic background of Taxodium species as significant plant genetic resources and assess the genetic differences of these species in Egypt.

**MATERIALS AND METHODS**

The current experiments were carried out at the Faculty of Agriculture, Saba Basha (Agricultural Botany Department) Alexandria University, Egypt, and the horticultural research station (Department of timber trees), Sabahia, Alexandria, Egypt. These investigations were conducted from 2018 up to 2022 to assess genetic variations of Taxodium trees in Egyptian different localities, morphological description, DNA barcoding of Taxodium distichum (L.) Rich. in different localities in Egypt.

1. **Plant materials and Classification:**

   Bald cypress is a large, slow-growing tree but long-lived, a deciduous conifer, which frequently spreads to 100 to 120 ft in height and 3 to 6 ft in diameter. It grows knees that produce above water providing additional support. *Taxodium distichum*; Bald cypress; Taxodiaceae

2. **Collection and Survey:**

   Four, unlike localities, were designated to survey the Bald cypress (*Taxodium distichum*) samples from El-Beheria Governorate (Nubira City), Alexandria Governorate (Sabhiya region), Qalyubia Governorate and Giza Governorate. Ten tresses were selected from the above-mentioned governorates.

3. **Extraction of DNA:**

   Genomic DNA (gDNA) was obtained from *Taxodium* leaf tissue of all genotypes by means of the method described by Saghai-Maroof et al., (1984). *Taxodium* DNA samples were isolated using i-genomic plant extraction DNA Mini kit @ iNtron (Biotechnology) as follow:

   1- Young fresh leaves of Taxodium were collected from 10 tree Taxodium trees and washed with distilled water and stored at -20OC until usage.
   2- Leaves samples of Taxodium were then sliced into small pieces by the scissor.
   3- 0.5 gm of sliced leaves were grounded using mortar and pestle by adding liquid nitrogen and disrupting carefully until the samples were homogenized completely then the liquid nitrogen was allowed to evaporate.
   4- 10 mg of sample powder were transferred to a 1.5 tube by means of a spatula.
   5- 390 ml PG buffer, 7ml solution, 20ml proteinase k, and 5ml RNAse A solutions were added and vortex vigorously then the lysate was incubated for 30 min at 65 OC.
   6- 100ml buffer PPT was added to the lysate, mixed well, and incubated for 5 min on ice.
   7- The lysate was centrifuged for 5min at 13.000 rpm at room temperature.
   8- 200ml of supernatant was transferred into a new 1.5 ml tube.
   9- 650ml buffer PB was added to the lysate and mixed well by gently inverting five to six times or by pipetting.
   10- 650ml of the mixture were pipetted, including any precipitate that may have formed, into the spin column inserted in a 2.0ml ml collection tube was centrifuged for 1min at 13.000 rmp (RT) and the flow-through was discarded.
The last step was repeated with the remaining sample (maximum 200 ml) and the flow was discarded through and collection tube altogether.

12- The spin column was laced into a new 2.0ml collection tube (additionally supplied) and 700ml buffer PWA was added and centrifuged for 1 min at 13,000 rpm and the flow was discarded through and the collection tube was reused.

13- 700ml buffer PWB was added to the spin column and centrifuged for 1 min at 13,000 rpm and the flow was discarded through and centrifuged again for an additional 1 min to dry the membrane. The flow was discarded through and collection tube altogether.

14- The spin column was replaced with a new 1.5ml tube (not supplied) and 100 ml buffer PE was directly onto the membrane. The tubes were incubated for 1 min at room temperature and then centrifuged for 1 min at 13,000 rpm to elute.

4. PCR Purification:
   Polymerase chain reaction (PCR) protection was cleansed by using a Mini kit @ iNtron Biotechnology given to the manufacturer descriptions. Polymerase Chain Reaction was shown to confirm the presence of the genes of interest in the genomic DNA. 1 % Agarose was utilized for the resolution of the PCR products. 100 bp. DNA marker as a standard DNA was rummage-sale in the current study. The Rbcl primer was used in this study as F- 5'-AATGTCACCACAAACCAGAGACTAAAGC-3'; R- 5'-GTAAAATCA TAGTCCACCRCGC-3'.

5. Conserved Regions Analysis:
   The PROMALS, Clustal Omega server and BIOEDIT software were used to analyze the sequence alignments of the PCR products (Pei and Grishin, 2007; Sievers et al., 2011; Hall, 2011).

6. Statistical Analysis:
   Data were statistically analyzed using SAS (2001). Comparisons among the means were measured using LSD0.05.

RESULTS AND DISCUSSION

1. Morphological Studies:
   Results in Table 1 recorded the morphological explanation of Taxodium trees Collected from different localities in Egypt. According to the tree, length ranged from 21.22 m (Giza tree) to 11.89 m (El-Beheria tree), while the Alexandria tree was recorded 17.30 m forward by Qalyubia tree in a range of 12.61 m. Data in Table 1 recorded the morphological description of trunk diameter (cm) in Taxodium trees collected from unlike localities in Egypt. The tree trunk diameter (cm) ranged from 36.40 cm (El-Beheria tree) to 115 cm (Alexandria tree), while Qalyubia tree was recorded 46.40 cm forward by El-Beheria tree in the range of 36.40 cm. According to the shoot separate (m) in Table 1 and Figure 8 the pointed range is from 26.50 to 50.01 cm.

   The highest shoot separate was recorded for Giza tree and the lowest one was the Alexandria tree as found in Table 1. Leaf length (cm) and leaf width (mm) results are recorded in Table 1. For Alexandria tree the data were 1.56 cm and 1.13 mm; El-Beheria was 1.24 cm and 0.88 mm; Giza tree was 1.40 cm and 1.28 mm and Qalyubia was 1.10 cm and 0.66 mm, respectively as found in Table 1. The tree condition for all the trees was very good except the tree from Giza governorate was moderate. From these data, we can describe the different Taxodium trees from different locations in Egypt to be genetic resources that can be used in the future in the breeding program.
Table 1. Morphological characterization *Taxodium* plants Collected from Unlike locations in Egypt.

<table>
<thead>
<tr>
<th>Morphological characteristic</th>
<th>Alexandria</th>
<th>El-Beheria</th>
<th>Giza</th>
<th>Qalyubia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tree Length (m)</td>
<td>17.30b</td>
<td>11.89c</td>
<td>21.22a</td>
<td>12.61c</td>
</tr>
<tr>
<td>Trunk Diameter (cm)</td>
<td>115a</td>
<td>36.40d</td>
<td>73.89b</td>
<td>46.40c</td>
</tr>
<tr>
<td>Shoot Separate (m)</td>
<td>26.50c</td>
<td>11.00d</td>
<td>50.01b</td>
<td>11.61d</td>
</tr>
<tr>
<td>Leaf Length (cm)</td>
<td>1.56b</td>
<td>1.24c</td>
<td>1.40b</td>
<td>1.16c</td>
</tr>
<tr>
<td>Leaf Width (mm)</td>
<td>1.13bc</td>
<td>0.88cd</td>
<td>1.28b</td>
<td>0.66d</td>
</tr>
<tr>
<td>Cone Length (cm)</td>
<td>2.61b</td>
<td>2.10ed</td>
<td>1.80d</td>
<td>2.55bc</td>
</tr>
<tr>
<td>Seed Number/Cone</td>
<td>25.3b</td>
<td>21.0c</td>
<td>18.66c</td>
<td>31.2b</td>
</tr>
<tr>
<td>Seed Length (mm)</td>
<td>0.44d</td>
<td>0.55c</td>
<td>0.35d</td>
<td>0.81b</td>
</tr>
<tr>
<td>Condition of tree</td>
<td>Normal</td>
<td>Normal</td>
<td>Moderate</td>
<td>Normal</td>
</tr>
<tr>
<td>Age of tree (y)</td>
<td>44.0</td>
<td>28.0</td>
<td>144</td>
<td>24.0</td>
</tr>
<tr>
<td>Type</td>
<td>B. cypress</td>
<td>B. cypress</td>
<td>B. cypress</td>
<td>B. cypress</td>
</tr>
</tbody>
</table>

2. DNA Barcoding of Taxodium Species:

The data showed the nucleotide composition of *RbcL* barcode gene of *Taxodium distichum* for El-Beheria governorate trees which detected the length of 530 base pairs and the molecular weight = 160687.00 Daltons, for the single-stranded, while was = 321679.00 Daltons, in the double-stranded. The data showed that the G+C content was 43.96% and the A+T content was 56.04%. In addition, the nucleotide number and molecular percentage were as follows: A (148) 27.92%; C (113) 21.32%; G (120) 22.64% and T (149) 28.11%. The data was recorded to the Protein in *Taxodium distichum* El-Beheria Governorate using (*rbcL*) gene, partial cds; chloroplast, which detected length = 177 amino acids and the molecular weight = 19800.80 Daltons. The amino acids, number and molecular percentage were as follow: Ala A, (4) 2.26; Cys C, (3) 1.69; Asp D, (5) 2.82; Glu E, (6) 3.39; Phe F, (4) 2.26; Gly G, (5) 2.82; His H, (2) 1.13; Ile I, (11) 6.21; Lys K, (13) 7.34; Leu L, (37) 20.90; Met M, (6) 3.39; Asn N, (7) 3.95; Pro P, (10) 5.65; Gln Q, (6) 3.39; Arg R (8) 4.52; Ser S (8) 4.52; Thr T (11) 6.21; Val V (12) 6.78; Trp W (5) 2.82 and Tyr Y (6) 3.39.

The data showed the nucleotide composition of *RbcL* barcode gene of *Taxodium distichum* for Qalyubia governorate trees which detected the length of 530 base pairs and the molecular weight = 160687.00 Daltons, for the single-stranded, while was = 321679.00 Daltons, in the double-stranded. The data showed that the G+C content was 42.96% and the A+T content was 57.04%. In addition, the nucleotide number and molecular percentage were as follows: A (148) 26.92%; C (113) 22.32%; G (120) 23.64% and T (149) 27.11%. The data was recorded to the Protein in *Taxodium distichum* Qalyubia Governorate using (*rbcL*) gene, partial cds; chloroplast, which detected length = 177 amino acids and the molecular weight = 19800.80 Daltons. The amino acids, number and molecular percentage were as follow: Ala A, (4) 2.26; Cys C, (3) 1.69; Asp D, (5) 2.82; Glu E, (6) 3.39; Phe F, (4) 2.26; Gly G, (5) 2.82; His H, (2) 1.13; Ile I, (11) 6.21; Lys K, (13) 7.34; Leu L, (37) 20.90; Met M, (6) 3.39; Asn N, (7) 3.95; Pro P, (10) 5.65; Gln Q, (6) 3.39; Arg R (8) 4.52; Ser S (8) 4.52; Thr T (11) 6.21 and Val V (12) 6.78.

The data showed the nucleotide composition of *RbcL* barcode gene of *Taxodium distichum* for Giza governorate trees (Pond cypress) which detected the length of 530 base pairs and the molecular weight = 160687.00 Daltons, for the single-stranded, while was = 321679.00 Daltons, in the double-stranded. The data showed that the G+C content was 43.96% and the A+T content was 56.04%. In addition, the nucleotide number and molecular percentage were as follows: A (148) 27.92%; C (113) 21.32%; G (120) 22.64% and T (150) 28.11%. The data was recorded to the Protein in *Taxodium distichum* Alexandria Governorate using (*rbcL*) gene, partial cds; chloroplast, which detected length = 177 amino acids and the molecular weight = 19800.80 Daltons. The amino acids, number and molecular percentage were as follow: Ala A, (4) 2.26; Cys C, (3) 1.69; Asp D, (5) 2.82; Glu E, (6) 3.39; Phe F, (4) 2.26; Gly G, (5) 2.82; His H, (2) 1.13; Ile I, (11) 6.21; Lys K, (13) 7.34; Leu L, (37) 20.90; Met M, (6) 3.39; Asn N, (7) 3.95; Pro P, (10) 5.65; Gln Q, (6) 3.39; Arg R (8) 4.52; Ser S (8) 4.52; Thr T (11) 6.21 and Val V (12) 6.78.
acids and the molecular weight = 19800.80 Daltons. The amino acids, number and molecular percentage were as follow: Ala A, (4) 2.26; Cys C, (3) 1.69; Asp D, (5) 2.86; Glu E, (6) 3.39; Phe F, (4) 2.26; Gly G, (5) 2.86; His H, (2) 1.13; Ile I, (11) 6.26; Lys K, (13) 7.34; Leu L, (37) 20.90; Met M, (6) 3.39; Asn N, (7) 3.96; Pro P, (10) 5.65; Gln Q, (6) 3.39; Arg R (8) 4.56; Ser S (8) 4.52; Thr T (11) 6.26 and Val V (12) 6.76.

The data showed the nucleotide composition of Rbcl barcode gene of Taxodium distichum for Alexandria governorate trees (blad cypress) which detected the length of 530 base pairs and the molecular weight = 160687.00 Daltons, for the single-stranded, while was = 321679.00 Daltons, in the double-stranded. The data showed that the G+C content was 43.96% and the A+T content was 56.04%. in addition, the nucleotide number and molecular percentage were as follows: A (149) 27.92%; C (120) 21.32%; G (111) 22.64% and T (132) 28.11%. The data was recorded to the Protein in Taxodium distichum, Giza Governorate using (rbcL) gene, partial cds; chloroplast, which detected length = 152 amino acids and the molecular weight = 19800.80 Daltons. The amino acids, number and molecular percentage were as follow: Ala A, (4) 2.26; Cys C, (3) 1.69; Asp D, (5) 2.86; Glu E, (6) 3.39; Phe F, (4) 2.26; Gly G, (5) 2.86; His H, (2) 1.13; Ile I, (11) 6.26; Lys K, (13) 7.34; Leu L, (37) 20.90; Met M, (6) 3.39; Asn N, (7) 3.96; Pro P, (10) 5.65; Gln Q, (6) 3.39; Arg R (8) 4.56; Ser S (8) 4.52; Thr T (11) 6.26 and Val V (12) 6.76.

Data reported multiple sequence analysis of Rbcl gene of query four of Taxodium distichum varieties submitted to the GenBank database by direct submission on DDBJ with database similarity sequence. And data recorded the multiple sequence analysis of Rbcl gene of Taxodium distichum using MEGA11 software. The data in Tables 2-4 presented that the minimum segment length is 15 and the maximum average entropy was 0.2; the maximum entropy/ positions: is 0.2 and the gap is limited to 2/segment, finally the contiguous gap is limited to 1 in any segment. The likelihood of rejecting the null hypothesis that the sequence has changed with the same pattern of substitution is judged by the extent of changes in base composition biases between sequences (Tables 2 & 3). There was a total of 534 positions in the final dataset.

**Table 2:** The Predicted Conserved region of Rbcl gene using BioEdit for the Taxodium distichum under study.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Region</th>
<th>Position</th>
<th>Segment Length</th>
<th>Average entropy (Hx)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Taxodium distichum</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>El-Beheria</td>
<td>15 to 85</td>
<td>51</td>
<td>0.0793</td>
<td></td>
</tr>
<tr>
<td>Qalubiya</td>
<td>138 to 191</td>
<td>54</td>
<td>0.0764</td>
<td></td>
</tr>
<tr>
<td>Giza</td>
<td>206 to 231</td>
<td>26</td>
<td>0.0762</td>
<td></td>
</tr>
<tr>
<td>Alexandria</td>
<td>233 to 268</td>
<td>36</td>
<td>0.0757</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3:** Maximum likelihood approximation of substitution matrix.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>T/U</th>
<th>C</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-</td>
<td>10.11</td>
<td>7.52</td>
<td>6.50</td>
</tr>
<tr>
<td>T/U</td>
<td>9.97</td>
<td>-</td>
<td>6.07</td>
<td>7.97</td>
</tr>
<tr>
<td>C</td>
<td>9.97</td>
<td>8.16</td>
<td>-</td>
<td>7.97</td>
</tr>
<tr>
<td>G</td>
<td>8.12</td>
<td>10.11</td>
<td>7.52</td>
<td>-</td>
</tr>
</tbody>
</table>

The estimation T/Transversion bias (R) is 0.39. Substitution pattern and rates were assessed under the Kimura (1980) 2-parameter model. The nucleotide frequency is A = 25.00 %, T / U = 25.00 %, C = 25.00 %, and G = 25.00 %.
Table 4: Maximum compound likelihood approximation of the pattern of nucleotide substitution.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>T</th>
<th>C</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-</td>
<td>4.89</td>
<td>3.64</td>
<td>0.12</td>
</tr>
<tr>
<td>T</td>
<td>4.82</td>
<td>-</td>
<td>27.86</td>
<td>3.86</td>
</tr>
<tr>
<td>C</td>
<td>4.82</td>
<td>37.46</td>
<td>-</td>
<td>3.86</td>
</tr>
<tr>
<td>G</td>
<td>0.15</td>
<td>4.89</td>
<td>3.64</td>
<td>-</td>
</tr>
</tbody>
</table>

The number of base substitutions/sites from averaging over all sequence pairs is 3.75. Analyzes were conducted using the Maximum Compound Likelihood model. This analysis involved 61 nucleotide sequences. Note that even when the substitution patterns are homogeneous among lineages, the compositional distance will correlate with the number of differences between sequences (Table 4). This analysis involved 61 nucleotide sequences. There was a total of 534 positions in the final dataset.

3. Phylogenetic Analysis of Collected Taxodium Plants:

Many sequences alignment showed that there are mutable numbers of Indels in the RbcL gene. The evolutionary distances for the 4 Taxodium varieties were illustrious into individual clades, as follows: Group I belong to Taxodium distichum making different isolate including Taxodium distichum El-Beheria Governorate, Taxodium distichum Alexandria Governorate, Taxodium distichum Qalyubia Governorate which are close to each other. Otherwise, Group II included one variety Taxodium distichum var. imbricarium (Pond cypress) Giza Governorate (Figs 1-3). The nucleotides frequencies are 28.03% (A), 28.42% (T/U), 21.13% (C), and 22.41% (G). The T/transversion rate ratios are $k_1 = 0.03$ (purines) and $k_2 = 7.658$ (pyrimidines). The overall T/transversion bias is $R = 1.848$. This analysis complicated 61 nucleotide sequences.

Fig. 1. The evolutionary history was inferred using the Neighbor-Joining method. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. This analysis involved 87 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion choice). There was a total of 534 positions in the final dataset.
Fig. 2. Evolutionary analysis by Maximum Likelihood method. The tree with the highest log likelihood (-2936.00) 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 Tamura-Nei model, and then selecting the topology with a superior log-likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 87 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There was a total of 534 positions in the final dataset.

Fig. 3. The evolutionary history was inferred using the UPGMA method. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [2] and are in the units of the number of base substitutions per site. This analysis involved 87 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion choice). There was a total of 534 positions in the final dataset.
Conclusion
The current study aimed to assess the genetic variations of Taxodium trees in Egypt from different localities based on morphological characteristics and DNA barcoding. The results recorded different variations in morphological characters between the tested samples for tree length, and tree trunk diameter (cm) and tree condition. Also, from the data, it could be described the different Taxodium trees from different locations in Egypt to be as genetic resources which can be used in the future in the breeding program. The DNA barcoding data of T. distichum El-Beheria governorate, T. distichum Qalyubia Governorate, T. distichum_ Giza Governorate and T. distichum_ Alexandria Governorate using rbcL gene showed differences in G+C content and the A+T content, in addition, the nucleotide number and molecular percentage.

REFERENCES


تقييم الاختلافات الوراثية لأشجار التاكسوديوم في مصر

أحمد السيد خالدا 1، فاطمة عبد العزيز حسن 2، أحمد محمد عبد السلام 3، محمد قدرى جابر 4

1 كلية الزراعة سابا باشا - قسم النبات الزراعى - جامعة الاسكندرية - مصر
2 قسم الأشجار الخشبية - معهد بحوث البساتين - مركز البحوث الزراعية - الصحية - الاسكندرية
3 كلية الزراعة سابا باشا - قسم الانتاج النباتى - جامعة الاسكندرية - مصر

أقيمت هذه الدراسة بكلية الزراعة سابا باشا ومركز البحوث الزراعية بالصبحية خلال الفترة الزمنية من عام 2018 حتى عام 2022. وهدفت هذه الدراسة إلى تقييم الاختلافات الوراثية لأشجار التاكسوديوم في مصر وكانت خطوات البحث كالاتي: عمل تجميع للاصول الوراثية النباتية لنبات التاكسوديوم في مناطق مختلفة من مصر، عمل التوصيف الوراثى لانواع التاكسوديوم المنزرعة في مصر، إجراء التميز أو التشفير الوراثى barcoding لأشجار التاكسوديوم الوراثي لأنواع التاكسوديوم المنزرعة في مصر، إجراء التميز أو التشفير الوراثى barcoding لأشجار التاكسوديوم الوراثي لأنواع التاكسوديوم المنزرعة في مصر، استخدام معلمات وراثية مختصة، عمل خريطة تصنيفية كاملة لأشجار التاكسوديوم المنزرعة في مصر اعتماداً على المعلمات المتخصصة. أوضحت النتائج أن هناك إثبات كلاً من التاكسوديوم مختلف عن بعضها في الصفات المورفولوجية وكذلك التركيب الوراثي وبناءً على النتائج تم وجود الاشجار المجمعة من كل من محافظة الاسكندرية والبحيرة والقليوبية معاً في مجموعة واحدة والأشجار الموجودة في محافظة الجيزة في مجموعة منفصلة. وتوصى الدراسة بالحفاظ على الاصول الوراثية النباتية المصرية عن طريق عمل التوصيف المورفولوجي والوراثي لها.