



**Faculty of Agriculture (Saba Basha)
Agricultural Botany Department**

**Assessment of genetic variations of
Taxodium trees in Egypt**

**A Thesis submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy**

In

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(Genetics)**

Presented by

Ahmed Mohamed Abdelsalam Mohamed

(B.Sc. Science, Faculty of Agriculture, Alexandria University, 2000)

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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

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 superior log likelihood value. The tree is drawn to scale, with branch heights 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.

Figure (42):

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The evolutionary history was inferred using the UPGMA method. The tree is drawn to scale, with branch heights 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.

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List of Abbreviations

Bp	Base pairs
LSD (P = 0.05)	Least Significant Difference
dNTPs	Deoxy nucleotide triphosphate
NS	Not Significant
PCR	Polymerase chain reaction
ppm	Parts per million
rpm	Revolutions per minute
SEm (\pm)	Standard Error (of the means)
UPGMA	Unweight Pair Group Method
%	Percent
$^{\circ}\text{C}$	Degree Celsius
μl	Microliter

The genus *Taxodium distichum* belonged to the subfamily Taxodioideae of the family Cupressaceae or cypress family. The literature showed that Taxodiodeae is a minor subfamily containing three botanic varieties are (1) *Taxodium d.* in United States (USA) and Mexico (2) *Glyptostrobus* is natural to China, and (3) *Cryptomeria* in Japan.

The genetic difference inters the *Taxodium distichum* genus has been understood to be either containing of 3 separate types are *T. distichum* (L.) Rich. var. *distichum* (Bald cypress), *T. distichum* var. *imbricarium* (Nutt.) (Pond cypress) and *T. distichum* var. *mexicana* (Montezuma cypress).

The main importance for *Taxodium d.* 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, in addition the author studied Characteristics and availability of commercially important woods.

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 flag and/or signs. There are three different genetic markers including the morphological, biochemical and DNA markers.

The usage of sequencing or nucleotide sequence variances in a one gene to examine the genetic relationship was commonly useful by Carl Woese, 2000. The author documented those sequences change in a conserved gene and rRNA, can be utilize to conclude phylogenetic relationship.

DNA barcodes or barcoding includes short sequences of DNA obtained from a particular region of plant genome and comparison them among and within species to current a "barcode" for species documentation.

DNA barcoding as new tools showed as promising tool for species identification in all organisms. DNA barcodes has developed a progressively significant tool for taxonomic investigations and species definition, besides for the discovery of new species.

Therefore, there is a major essential to examine the genetic background of *Taxodium distichum* species as significant plant genetic resources and also assessment the genetic differences of these species in Egypt.

The current experiments were carried out at Faculty of Agriculture, Saba Basha (Agricultural Botany Department) Alexandria University, Egypt and horticultural research station (Department of timber trees), Sabahia, Alexandria, Egypt.

These investigations were conducted during 2018 up to 2022 to assessment of genetic variations of *Taxodium* trees in Egypt in different localities, morphological description, DNA barcoding of *Taxodium distichum* (L.) Rich. in different localities in Egypt.

Bald cypress is a large, slow-growing tree but long-lived, 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

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

Data recorded the morphological description of *Taxodium* trees Collected from different localities in Egypt. According to the tree height ranged from 21.22 m (Giza tree) to 11.89 m (El-Beheria tree), while Alexandria tree recorded 17.30 m forward by Qalyubia tree in range 12.61 m.

Data recorded the morphological description of stem diameter (cm) in *Taxodium* trees Collected from different localities in Egypt. The tree stem diameter (cm) ranged from 36.40 cm (El-Beheria tree) to 115 cm (Alexandria tree), while Qalyubia tree recorded 46.40 cm forward by El-Beheria tree in range 36.40 cm.

According to the shoot separate (m) pointed range from 26.50 to 50.01 cm. The highest shoot separate was recorded to Giza tree and the lowest one was Alexandria tree.

Leaf height (cm) and leaf width (mm) results for Alexandria tree the data were 1.56 cm and 1.13 mm; El-Beheria were 1.24 cm and 0.88 mm; Giza tree were 1.40 cm and 1.28 mm and Qalyubia were 1.10 cm and 0.66 mm, respectively.

The tree condition for all the tree was very good expect the tree from Giza Governorate was moderate. Form these data we can describe the different *Taxodium* trees from different locations in Egypt to be as genetic resources can be used in the future in the breeding program

The data showed the nucleotide composition of *Rbcl* barcode gene of *Taxodium distichum* for El-Beheria Governorate trees which detected the height 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 follow: A (148) 27.92%; C (113) 21.32%; G (120) 22.64% and T (149) 28.11%.

The data recorded to the Protein in *Taxodium distichum*_El-Beheria Governorate using (*rbcL*) gene, partial cds; chloroplast, which detected height = 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 height 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 follow: A (148) 26.92%; C (113) 22.32%; G (120) 23.64% and T (149) 27.11%.

The data recorded to the Protein in *Taxodium distichum*_ Qalyubia Governorate using (*rbcL*) gene, partial cds; chloroplast, which detected height = 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 height 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 follow: A (148) 27.92%; C (113) 21.32%; G (120) 22.64% and T (150) 28.11%.

The data recorded to the Protein in *Taxodium distichum*_ Giza Governorate using (*rbcL*) gene, partial cds; chloroplast, which detected height = 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) 2.86; 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 height 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 follow: A (149) 27.92%; C (120) 21.32%; G (111) 22.64% and T (132) 28.11%.

The data recorded to the Protein in *Taxodium distichum*_ Alexandria Governorate using (*rbcL*) gene, partial cds; chloroplast, which detected height = 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 showed that the smallest segment height (actual for each sequence) is 15 and the maximum average entropy was 0.2; the maximum entropy per position: 0.2 and the gaps limited to 2 per segment, finally the contiguous gaps limited to 1 in any segment

Four *Taxodium* varieties were distinguished into individual clades. as follows: Group I belong to *Taxodium distichum* making different isolate included *Taxodium distichum* El-Beheria Governorate, *Taxodium distichum* Alexandria Governorate, *Taxodium distichum* Qalyubia Governorate which are closely to each other. Otherwise, Group II included one variety *Taxodium distichum* var. *imbricarium* (Pond cypress) Giza Governorate