Journal of Medicinal Plants and By-products (2025) 05: 400– 403

Estimation of Genetic Affinity between some Tissue-planted Date Palm Taxa and Natural-planted Date Palm Taxa in Iraq using the Nucleotide Sequence of the *Rbcl* Gene

Salah Abbas Zaidan, Ammar Ahmed Sultan* and Najem Abdullah Jumaa

Department Biology, College of Education for Pure Sciences, University of Diyala, Iraq

Article Info

Article Type
Original Article

Article History
Received: 03 November 2024
Accepted: 17 December 2024
© 2012 Iranian Society of Medicinal Plants.
All rights reserved.

***Corresponding author**
drammarmohamed@yahoo.com

ABSTRACT

The date palm is one of the most important early trees that man took care of because of its importance in many areas, including the economy, industry, and medicine. Due to the phenotypic similarities of date palm cultivars in several traits. This study's goal is to quantify the genetic affinity of 12 taxa of tissue-planted date palm and natural-planted date palm using specific primers for the *rbcl* gene, The *rbcl* gene's nitrogenous bases were aligned, As the results of paving the nitrogenous bases of the *rbcl* gene showed that there are two types of mutations, translocation and transformation, and the match rate between the study samples and the samples of the gene bank were (96%, 98%, 99%). The phylogenetic tree of the current study's findings revealed that there are three primary groupings, the K1 variety serving as their common ancestor. The variations K6 and K10, K2 and K7, K3 and K4, and K8 and K11 are the most closely related. The results of the phylogenetic tree of the current study varieties and samples of the gene bank and the ancestors of 3 main groups showed that the first group included the varieties (K3, K4, K12) and that the two varieties (K3) and (K4) were more closely related, while the second main group included the variety MG946856.1, and the third main group included There were 18 cultivars, and the most closely related cultivars were (MN216504.1 with MN216503.1), (KT454753.1 with K8), (K2 with K7), and (K6 with 10).

Keywords: Date palm, Genetic affinity, Phylogenetic tree, Rbcl

How to cite this paper
Zaidan S.A., Sultan A.A., Jumaa N.A. Estimation of Genetic Affinity between some Tissue-planted Date Palm Taxa and Natural-planted Date Palm Taxa in Iraq using the Nucleotide Sequence of the Rbcl Gene. Journal of Medicinal Plants and By-products, 2025; 14(05):400-403. doi: 10.22034/jmpb.2024.367573.1811

INTRODUCTION

The date palm, *Phoenix dactylifera* L., is a commercially significant fruit crop in the Middle East and a member of the Arecaceae family [1]. The date palm is a monocotyledonous perennial tree (2n = 36). Only the region between Mandali and Tikrit at 35 degrees north latitude and the city of Al-Faw at 30 degrees south latitude is used for palm planting in Iraq [2].There is no denying that palm cultivation is used to benefit other crop trees, including vegetables and fruits, especially in desert regions due to the low growth requirements [3,4], in addition to the fruits as a major source of income and basic food for the population of the countries [5]. According to EL-sohaimy and Hafez [6], dates have a high nutritional value due to the fruits' abundance of minerals like calcium, potassium, magnesium, iron, and selenium. Dates are also used in the food industry to make molasses, vinegar, and yeasts [7]. It also contains a lot of nutrients that are crucial for human nutrition and health, including proteins, lipids, amino acids, and carbohydrates [8]. Due to their chemical makeup and the oil they contain, date seeds are valuable as food for animals and are used in the production of cosmetics and pharmaceuticals [9,10]. Tissue culture is the cultivation of cells, tissues, or organs that have been isolated from the mother plant on artificial culture media using a variety of techniques and methods. It has practical purposes because there are ways to cultivate and manipulate plant tissues in tubes after they have been isolated from the soil, and their growth can be controlled in an organized or unorganized manner [11], Additionally, there are other requirements and procedures that must be followed in order to produce clones (individuals with the same genotype as the parent plant) in big numbers and quickly [12]. Since tissue culture calls for the use of high concentrations of growth regulators, particularly auxins, which are related to genetic variation that results in the occurrence of Somatic heterogeneity [13,14], the genetic indicator is important for detecting genetic variation in micropropagation by tissue culture. Because this region is characterized by universality and ease of analysis and amplification [15], the rbcl gene is a component of the DNA sequence found in chloroplast cpDNA and can be utilized to encode DNA [16]. Since the *rbcl* gene has a full length of 1428 bp [17] and exhibits a high degree of interspecies similarity [18], it has numerous properties that make it an ideal candidate for phylogenetic analysis. The *rbcl* gene is 664 bp in length, and this sequence is used to differentiate between different plant species and explain nucleotide differences as well as to determine the sequence length of the studied samples [19,20], to distinguish palm varieties based on their geographic distribution, and to do so [21]. Iraqi date palm were selected as they are economically viable and compared with international varieties therefore, the study aims to verify genetic diversity between tissue-propagated and vegetative date palm varieties using the *rbcl* gene.

MATERIALS AND METHODS

DNA Extraction and Amplification of the Rbcl Gene

The study's date palm cultivars' leaf samples (Al-Khos) were gathered from three places in Iraq (Al-Rashidiya, Karbala, and Mandali), samples were collected from the middle of the leaf (frond) in three replications for each plant, and DNA was then extracted using the (ZR plant/Seed DNA Miniprep) methodology as follows:

Add up to 150 mg of finely cut plant or seed sample to a ZR BashingBead™ Lysis Tube. Add 750 µl Lysis Solution to the tube , Secure in a bead beater fitted with a 2 ml tube holder assembly and process at maximum speed for 10 minutes. Processing times may be as little as 40 seconds when using high-speed cell disrupters . See the manufacturer's literature for operating information. Centrifuge the Lysis Tube in a microcentrifuge at ≥10,000 x *g* for 1 minute. Transfer up to 400 µl supernatant to a Spin Filter in a Collection Tube and centrifuge at 7,000 rpm for 1 minute. Add 1,200 µl of Plant/Seed DNA Binding Buffer to the filtrate in the Collection Tube from Step 4 and mix. Transfer 800 µl of the mixture from to a Zymo-Spin™ IIC Column in a Collection Tube and centrifuge at 10,000 * *g* for 1 minute. Add 200 µl DNA Pre-Wash Buffer to the Column in a new Collection Tube and centrifuge at 10,000 x *g* for 1 minute. Add 500 µl Plant/Seed DNA Wash Buffer to the Column and centrifuge at 10,000 * *g* for 1 minute. Transfer the Column to a clean 1.5 ml microcentrifuge tube and add 50-100 µl (25 µl minimum) DNA Elution Buffer directly to the column matrix. Centrifuge at 10,000 x *g* for 30 seconds to elute the DNA.

Transfer the eluted DNA from Step 10 to a prepared Spin Filter (green top) in a clean 1.5 ml microcentrifuge tube and centrifuge at exactly 8,000 * *g* for 1 minute. The filtered DNA is now suitable for PCR and other downstream applications.

Polymerase Chain Reaction

Table 1 Mixture of the specific interaction for diagnosis gene

Taq PCR PreMix	5µl
Forward primer	10 picomols/µl (1 µl)
Reverse primer	10 picomols/µl (1 µl)
DNA	2 µl
Distill water	16 µl
Final volume	25µl

Table 2 The optimum condition of detection

1	Initial Denaturation	94 °C	3 min.	1 cycle
2	Denaturation -2	94 °C	1min.	35 cycle
3	Annealing	55 °C	1min.	
4	Extension-1	72 °C	1 min.	
5	Extension -2	72 °C	7 min.	1 cycle
1	Initial Denaturation	94 °C	3 min.	1 cycle

The Primers Used in the Interaction

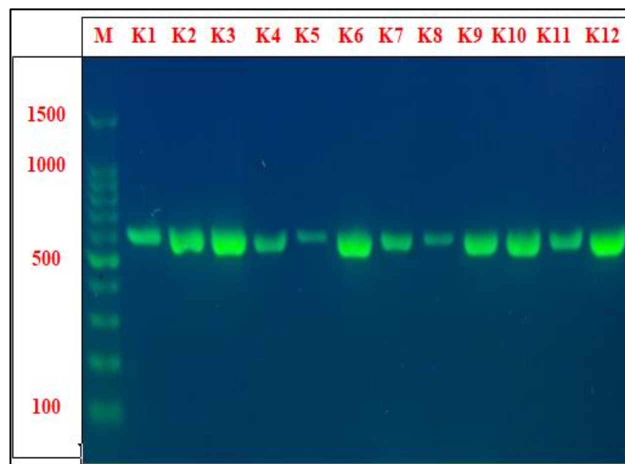

Fig. 1 shows the result of rbcl gene amplification for the samples used in the current study, which was carried out on an agarose gel at a concentration of 2% for an hour and a half under a 90 volt/min electric potential difference, ethidium bromide staining, and ultraviolet photography. K1: Merhaje (tissue), K2: Kalas international (tissue), K3: Sakaai international (tissue), K4: Koronfuly (tissue), K5: Um Alden (tissue), K6: Majhool international (tissue), K7: Barhe (free), K8: Barhe (tissue), K9: Merhaje (free), K10: Koronfuly (free), K11: Abomaan international (tissue), K12: Um Alden (free).

Table 3 The specific primer for the rbcl gene [22].

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5′-ATGTCACCACA AACAGAGACTA AAGC -3′	57.2	42%	500-650 base pair
Primer	Sequence	Tm (°C)	GC (%)	Product size

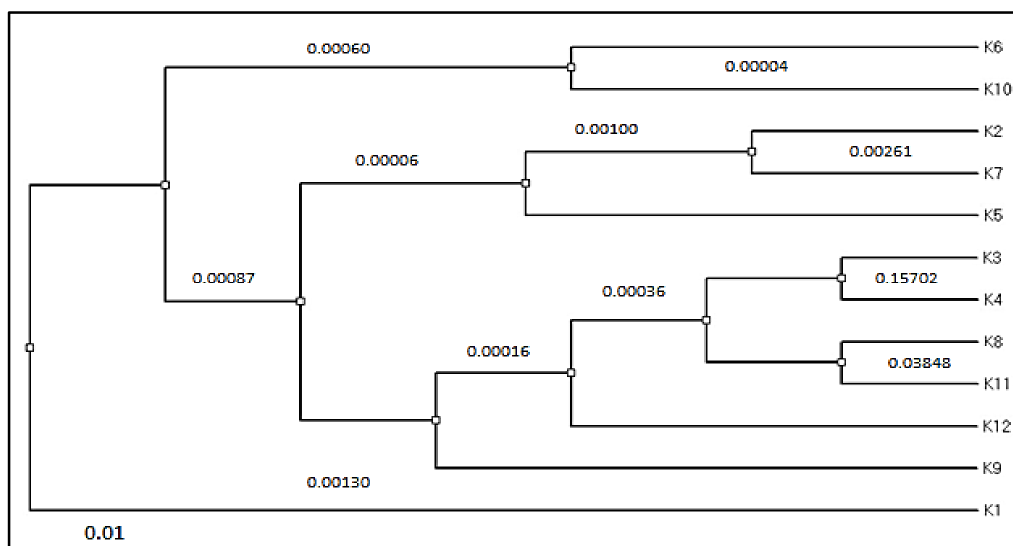

Fig 2 The phylogenetic tree of the current study samples was based on the nucleotide sequence of the rbcl gene. K1: Merhaje (tissue), K2: Kalas international (tissue), K3: Sakaai international (tissue), K4: Koronfuly (tissue), K5: Um Alden (tissue), K6: Majhool international (tissue), K7: Barhe (free), K8: Barhe (tissue), K9: Merhaje (free), K10: Koronfuly (free), K11: Abomaan international (tissue), K12: Um Alden (free).

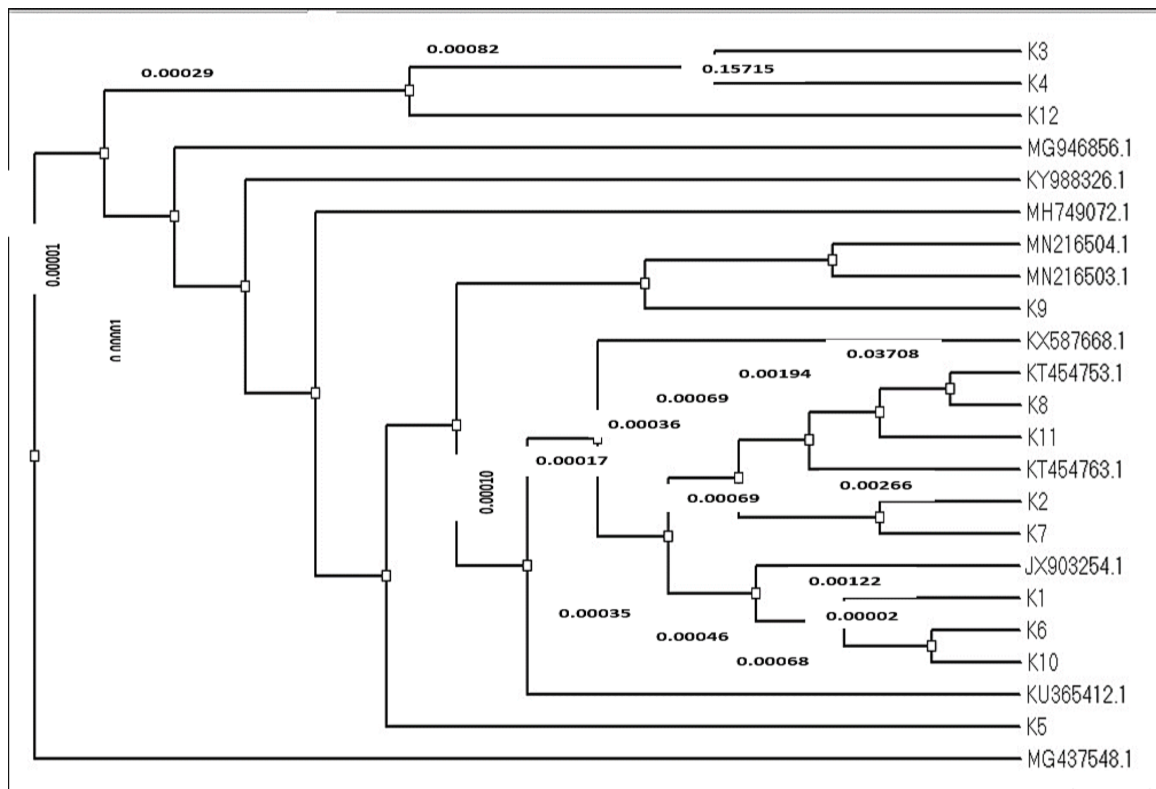

Fig. 3 The phylogenetic tree of the samples of the current study and the samples of the Genome Bank, were based on the nucleotide sequence of the rbcl gene. K1: Merhaje (tissue), K2: Kalas international (tissue), K3: Sakaai international (tissue), K4: Koronfuly (tissue), K5: Um Alden (tissue), K6: Majhool international (tissue), K7: Barhe (free), K8: Barhe (tissue), K9: Merhaje (free), K10: Koronfuly (free), K11: Abomaan international (tissue), K12: Um Alden (free),MG496856.1, KY988326.1, MH749072.1, MN216504.1, MN216503.1, KX587668.1, KT454753.1, KT454763.1, JX903254.1, KU365412.1 and MG437548.1: Genbank samples

Phylogenetic Analysis

The alignment of the nitrogenous bases in the rbcl gene between the samples from the current investigation and the samples from GenBank were compared using the Bioedit tool. The phylogenetic tree was coordinated and constructed using the common neighbor (NJ) approach using the MeGA5 tool. The percentage of the current study samples' rbcl gene sequences that matched the reference sequence was also determined using the BlASTn software. It is kept in a gene bank.

RESULTS AND DISCUSSION

the *rbcl* gene amplification results for some tissue-grown local date palm cultivars, some naturally occurring date palm cultivars, as well as some tissue-grown and naturally occurring international date palm cultivars. The amplification results revealed that the molecular weight of the resulting bundles is 650 base pairs for all local and international cultivars, and there is no difference.

The results of two types of mutations (transition and transformation) in the alignment of the nitrogenous bases between the samples from the current study and the samples from GenBank. The match rate was 96% in the K4 and K8 varieties, 98% in the K2, K7, and K12 varieties, and 99% in items k1, k3, k5, k6, k9, k10, and k11.

The genetic tree was divided into three main groups, and the first main group contained two varieties, K6 and K10, as The distance between them was 0.00004, as shown in Figure 2. The results show that the common ancestor of the study samples is the K1 variety, and the genetic tree was divided into three main groups, and as shown in Figure 2. While variations K5, K7, and k2 comprised the second main group, the genetic distance between the k7 variety and the k2 variety was 0.00261, and the distance between the k5 variety and the k2 variety was 0.00100. While the third group contained six varieties (K3, K4, K8, K11, K12, and K9), the two cultivars K3 and K4 were more closely related with a genetic distance of 0.15702; the two cultivars K8 and K11 were close with a distance of 0.00032; and the cultivar K12 was further away from the varieties (K3, K4, K8, K11) with a distance of 0.00036. In this regard, there are other studies conducted on date palms to know the genetic diversity of cultivars taken from different regions, as genetic diversity and genetic distance between cultivars were known [16,23,24]. The results indicate that the cultivar found in the genebank samples MG437548.1 is the common ancestor of the study samples and the genebank samples, and Figure 2 shows the genetic tree Phylogeny tree of the *rbcl* gene amplification product for the samples of the current study and the samples of the genebank, Figure 3. findings showed that the genetic tree It was classified into three main groups. The first main group contained three taxa (K3, K4, and K12), of which K3 and K4 were genetically closer to one another than K12, with a distance of 0.15715 between them, a single variety was included in the second major group. It is one of the gene bank samples, MG946856.1. The second was divided into two secondary sub-branches, the first of which included the cultivar MH749072.1, while the third main group included two subgroups, the first of which featured the cultivar KY988326.1. The two cultivars were MN216504.1 and MN216503. 1 within one group and the most genetically similar to cultivar K9 of the second group, which contained 16 cultivars from the current study and the gene bank cultivars. While the two cultivars K8 and KT454753.1 were in the same ring (closer genetically) and were

genetically closer to cultivars K11 and KT454763.1 with a genetic distance of 0.03708. Figure 3. of the findings demonstrates that the two cultivars K2 and K7, which are in the same ring, are genetically closer with a genetic distance of 0.00266. There was also a genetic affinity between the two cultivars K6 and K10, which are closer to the two cultivars K1 and JX903254.1, with a genetic distance of (0.00002). According to Figure 3. findings, the cultivars KU365412.1, MH749072.1, and K5 were genetically more distinct from one another than the other cultivars in the third main group [25].

CONCLUSION

A distinction between some naturally grown date palm cultivars and some tissue-grown cultivars of the same cultivar was made using the nucleotide sequence of the *rbcl* gene, along with one between local date palm cultivars and foreign date palm cultivars. A phylogenetic tree of the current study cultivars was also drawn, and point mutations were discovered between one naturally grown variety and the tissue grown.

REFERENCES

1. Moussouni S., Pintaud J.C., Vigouroux Y., Bouguedoura N. Diversity of Algerian oases date palm (Phoenix dactylifera L., Arecaceae): Heterozygote excess and cryptic structure suggest farmer management had a major impact on diversity. PLoS One. 2017;12:e0175232.
2. Ibrahim A.O.B., Zayed A.W. Palm cultivation and date quality between environmental factors and service and care programs. Khalifa Int Award for Date Palm and Agric Innov. 2019;6-107-37 9948-978.
3. Rhouma S., Dakhlaoui-Dkhil S., Ould Mohamed Salem A., Zehdi-Azouzi S., Rhouma A., Marrakchi M., *et al.* Genetic diversity and phylogenetic relationships in date-palms (Phoenix dactylifera L.) as assessed by random amplified microsatellite polymorphism markers (RAMPOs). Sci Hortic. 2008;117(1):53–7.
4. Fernández-López J., Viuda-Martos M., Sayas-Barberá E., Navarro-Rodríguez de Vera C., Pérez-Álvarez J.A. Biological, nutritive, functional and healthy potential of date palm fruit (*Phoenix dactylifera* L.): Current research and future prospects. Agron. 2022;12(4):876.
5. Chao C.T., Krueger R.R. The date palm (Phoenix dactylifera L.): Overview of biology, uses and cultivation. HortSci. 2007;42:1077–82.
6. El-Sohaimy S, Hafez E. Biochemical and nutritional characterizations of date palm fruits (Phoenix dactylifera L.). J Appl Sci Res. 2010;6(8):1060–7.
7. Abd Rabou F.A., Radwan E.S. The current status of the date palm (Phoenix dactylifera) and its uses in the Gaza Strip, Palestine. Biodiversitas. 2017;18(3):1047–61.
8. Assirey E.A. Nutritional composition of fruit of 10 date palm (Phoenix dactylifera L.) cultivars grown in Saudi Arabia. J Taibah Univ Sci. 2015;9:75–9.
9. Abdessalam M., Jimenez-Araujo A., Guillen-Bejarano R., Rodriguez-Arcos R., Sindic M. Date seeds: A promising source of soil with functional properties. A Review. Foods. 2020;9:787.
10. Laghouiter O., Benalia M., Gourine N., Djeridan A., Bombarda I., Yousfi M. Chemical characterization and in vitro antioxidant capacity of nine Algerian date palm cultivars (*Phoenix dactylifera* L.) seed oil. J Nutr Metab. 2019;11(2):103–17.
11. Rai A.C., Kumar A., Modi A., Singh M. Advances in plant tissue culture, current developments and future trends. Academic Press; 2022.
12. Al-Barky F.R. Plant breeding and improvement. Najaf Al-Ashraf Press; 2020. p. 245.
13. Phillips P.L., Kaeppler S.M., Othoft P. Genetic instability of plant tissue cultures: Breakdown of normal controls. A review. Proc Natl Acad Sci U S A. 1994;91:5222–6.
14. Cultis C., Radoman S., Kunert K.J. Method for finding genetic markers of somaclonal variation. International publication number WO 99/53; 1999.
15. Newmaster S.G., Fazekas A.J., Ragupathy S. DNA barcoding in land plants: Evaluation of rbcL in a multigene tiered approach. Can J Bot. 2006;84:335–41.
16. Hagar A.H., Gadalla E.G., Haggag S.Z., AbuEl-Maaty S., Hussein M.H. Identification of some cultivars of Egyptian date palm *(Phoenix dactylifera* L.) using DNA barcoding. Plant Arch. 2020;20(Suppl 1):1807–13.
17. Patwardhan A., Ray S., Roy A. Molecular markers in phylogenetic studies—a review. J Phylogenetics Evol Biol. 2014;2:131.
18. Kellogg E.A., Juliano N.D. The structure and function of RuBisCO and their implications for systematic studies. Am J Bot. 1997;84:413–28.
19. Alaklabi A., Ahmed A., Al Qthanin R.N., Arif T.A., Panneerselvan A., Al-Khulaidi A. Molecular characterization of endangered endemic plant Aloe pseudorubroviolaceae using chloroplast matK and plastid rbcL gene. Saudi J Biol Sci. 2021;28:1123–7.
20. Eglal M.S., Hassan M.E. DNA barcodes in Egyptian olive cultivars (*Olea europaea* L.) using the rbcL and matK coding sequences. J Crop Sci Biotechnol. 2023.
21. Saboori S., Noormohammadi Z., Sheidai M., Marashi S. Date palm (*Phoenix dactylifera* L.) cultivar relationships based on chloroplast genotyping. Iran J Sci Technol Trans Sci. 2021;45:833–40.
22. Maloukh L., Alagappan K., Jarrar M., Salehi J., El-wakil H., Rajya L. Discriminatory power of rbcL barcode locus for authentication of some UAE native plants. 2017.
23. Ahmad I., Khan S., Naeem M., Hayat M., Ahmed S., Murtaza G., *et al*. Molecular identification of ten palm species using DNA fingerprinting. Int J Pure Appl Biosci. 2019;7(1):46–51.
24. Raeisi F., Fahmideh L., Fakheri B.A., Kikhasaber M. Studying of genetic diversity of rbcL gene in some date palm cultivars in Sistan and Baluchistan province, Iran. Plant Genet Res. 2021;8(1).
25. Yao G., Zhang Y., Barrett C., Xue B., Bellot S., Baker W., *et al*. A plastid phylogenomic framework for the palm family (Arecaceae). BMC Biol. 2023;21:50.