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# Genetic diversity of common figs (*Ficus carica*) cultivated in Thailand determined by 18S ribosomal RNA sequence

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**Abstract** The common fig (*Ficus carica*) is an economically important fruit crop widely consumed worldwide. In this study, the genetic diversity of 5 varieties of figs are commonly grown in Thailand as Longue d'Aout, Col De Dama Blanca, Weihai, Iraqi, and White Israel which showed highly similar 18S rRNA, and similar to 2 other related species of *Ficus palmata* and *Ficus johannis*. The only exception is shown in the Iraqi fig, which possessed 2 positions of single nucleotide substitutions (C to T). Interestingly, Iraqi is found to be a fig variety with distinct characteristics that facilitated rapid growth in the tropics e.g. single-lobed leaf, robust growth, and nematode-resistance, and is commonly used as a rootstock. In conclusion, this analysis is provided a fundamental starting point for further works on figs molecular genetics, which can facilitate fig commercialization and further breeding in Southeast Asia.

**Keywords:** Common fig, 18S ribosomal RNA gene, Genetic diversity

## Introduction

The common fig (*Ficus carica* L.) is a diploid species (2n=26), which belongs to family Moraceae that consists of more than 1400 species (Watson and Dallwitz, 1994). It is one of the 750 species of the genus *Ficus* (Berg, 1989) which also known as one of the most ancient tree (Mawa *et al*., 2013). It has considered that the domesticated common fig cultivated in Arabia (Storey, 1976) then diffused to Middle East, Asia and Mediterranean regions. Fig cultivation had been spread to Britain, China, Japan, Australia and South Africa, while its slower planting in Southeast Asia since the rainier climate (Aksoy, 1998). In Thailand, the first common fig was imported and planted by the Royal Project and Kasetsart University at Doi Ang Khang, Chiang Mai in 1981. Then, 10 common fig varieties were studied the best growth at 2 sites of Northern part of

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Thailand (Ban-Dong-Yen Model Field Crop of Queen Royal Project and Chiang Mai Royal Agricultural Research Center in 2008 (Wongmetha, 2008).

Fig fruit can be consumed fresh, dried or processed into jam. In ancient period, the dried fig fruit is consumable spared in a low crop production season. For nutritional value, fig fruit has a high dietary fiber, various vitamins and minerals such as calcium, iron, magnesium, manganese. Health benefits of fig fruit also known as healthy fruit because of it has the bioactive components, including flavonoids, phenolic acids, carotenoids, tocopherols, anthocyanin, and high fiber that used for health-promoting effects (Sandhu *et al*., 2023).

Several studies have characterized common fig cultivars by using morphological, pomological diversity (Gaaliche *et al*., 2012), biochemical markers such as isozymes, and molecular markers using RAPD, AFLP (Cabrita *et al.*, 2001) or microsatellite markers (Saddoud *et al.*, 2005). Chatti *et al.* (2010) compared the suitable markers and assessment the molecular polymorphisms in Tunisian fig cultivars using RAPD, ISSR, RAMPO and SSR markers for polymorphism study and genetic relationship among figs. Recently, genetic diversity of common figs in various countries have been reported by using several molecular markers such as Palestine (RAPD), Costa Rica (ITS regions, matK, and trnH-psbA), Italy (nuclear microsatellite markers), Iran (RAPD), Greek and Mediterranean countries (SSR) (Ali Shtayeh *et al.*, 2014; Baziar *et al*., 2018; Castro *et al*., 2015; Rodolfi *et al*., 2018; Sclavounos *et al.*, 2023).

There are about 90 types of fig tree in Thailand, mainly cultivated in the North since Ayutthaya and Rattana-Kosin for the Royal culture. Taxonomy and diversity of native fig (*Ficus* spp.) in Chiang Mai was reported 26 species in 2011(Tarachai *et al*., 2011). In 2012, genetic variation of 25 species of native figs (*Ficus* spp.) which were randomly collected at Sakhon Nakhon, Nakhon Phanom and Mukdahan, were analyzed by using High Annealing Temperature Random Amplified Polymorphic DNA (HAT-RAPD) technique (Phromthep, 2012). Ten varieties of common fig (*Ficus carica* L.), which were planted in Queen Royal project at Northern of Thailand, were examined the appropriate varieties and the best growth for the area by crown diameter, leaf and fruit characters (Wongmetha, 2008). Recently, common fig has been wildly cultivated in farm and home-garden. The basic varieties that are commonly grown are the BTM6, Brown Turkey, Australia. Italian White, longue d'aout (LDA) and Iraqi. Nevertheless, all common figs in Thailand are exotic species which may adapt their genetics for survival in tropical area and climate and they have not been studied at a molecular level. This study aimed to determine the genetic diversity of common figs the genetic diversity of 5 varieties of figs commonly grown in Thailand (Longue d'Aout (LDA), Col De Dama Blanca-Negra (CDDBN),

Weihai, Iraqi, and White Israel (WI)) by comparing the DNA sequences of the 18S ribosomal RNA gene.

## Materials and methods

### *Plant materials*

All five fig varieties (Weihai, col de dama blanca-negra (CDDBN), Longue d'Aout (LDA), White Israel (WI), and Iraqi) were cultivated in a home garden named "Farm-A-Jarn" located in Cha-am District, Phetchaburi, Thailand. Representative fruits and leaves were randomly collected from mature trees for morphological analysis. In this study, the leaf length (cm.), shape of leaf, shape of fruit, and fruit pulp internal color were determined. Fresh leaves were sampled from each variety for further DNA extraction.

### *DNA extraction*

Fig leaf samples were wiped with sterile water for cleaning and a 0.2-gram piece were cut and placed into a 1.5 ml centrifuge tube individually. DNA extraction started with small-pestle grinding of leaf piece in 200ul extraction buffer (0.2M Tris-HCl pH7.5, 0.25M NaCl, 25mM EDTA, 0.5% SDS and sterile water). Then, the homogenized plant samples were spin at 13200 rpm for 5 mins and 200 ul supernatant was transferred to new eppendorf tube containing 200 ul isopropanol. After gentle mixing, DNA was precipitated at room temperature for 5 mins and centrifuged at 13200 rpm for 5 mins. Next, supernatant was removed followed by pellet washing with 750 ul 75% EtOH and centrifuged at 13000 rpm for 10 mins at 4°C. Then, supernatant was discarded, and pellet was air-dried followed by pellet resuspend in 50 ul sterile $H_2O$. Finally, DNA pellet was spin again at 13000 rpm for 10 mins to get rid of undissolved pellets and transferred to a new tube. Total extracted DNA was measured using a Nanodrop spectrophotometer (OD260/280).

### *PCR reaction and DNA sequencing*

PCR primer was designed using the ITS region of ribosomal DNA (GenBank accession number KX572966), forward primer (FITS_F: 5'-CGTAGGTGAACCTGCGGAA-3') and reverse primer (FITS_R: 5'-TATGCTTAAACTCAGCGGG-3'). PCR products size was 715 base-pairs.

PCR reaction was carried out in a total volume of 50 ul including 1 ug DNA template, 25 ul One-Taq® Hot Start 2x Master Mix (New England Biolabs) 1 μM

of each primer, and nuclease-free water. The PCR was run in the thermocycler (Biometra® T-gradient, Germany) with the initial denaturation at 94°C (1 min), followed by 35 cycles of denaturation at 94°C (30 s), annealing at 55°C (30 s), and extension at 68°C (45 s). After final extension at 68°C (5 min), the PCR reaction was kept at 4°C. The PCR products were determined using 1.5% agarose gel electrophoresis with 1x TBE buffer and checked product size under UV-light by UV-transilluminator, Then, PCR products were purified using the GenepHlow™ Gel/PCR Kit (Geneaid Biotech Ltd., Taiwan) and sent to DNA sequencing using ABI Prism 3730XL DNA sequencer (U2Bio, Korea).

### *Data analysis*

Sequencing results from the ITS region were quality trimmed and analyzed using Geneious software version 9.0.4. All sequences were deposited to GenBank database using online BankIt program and established the accession number (663 bp).

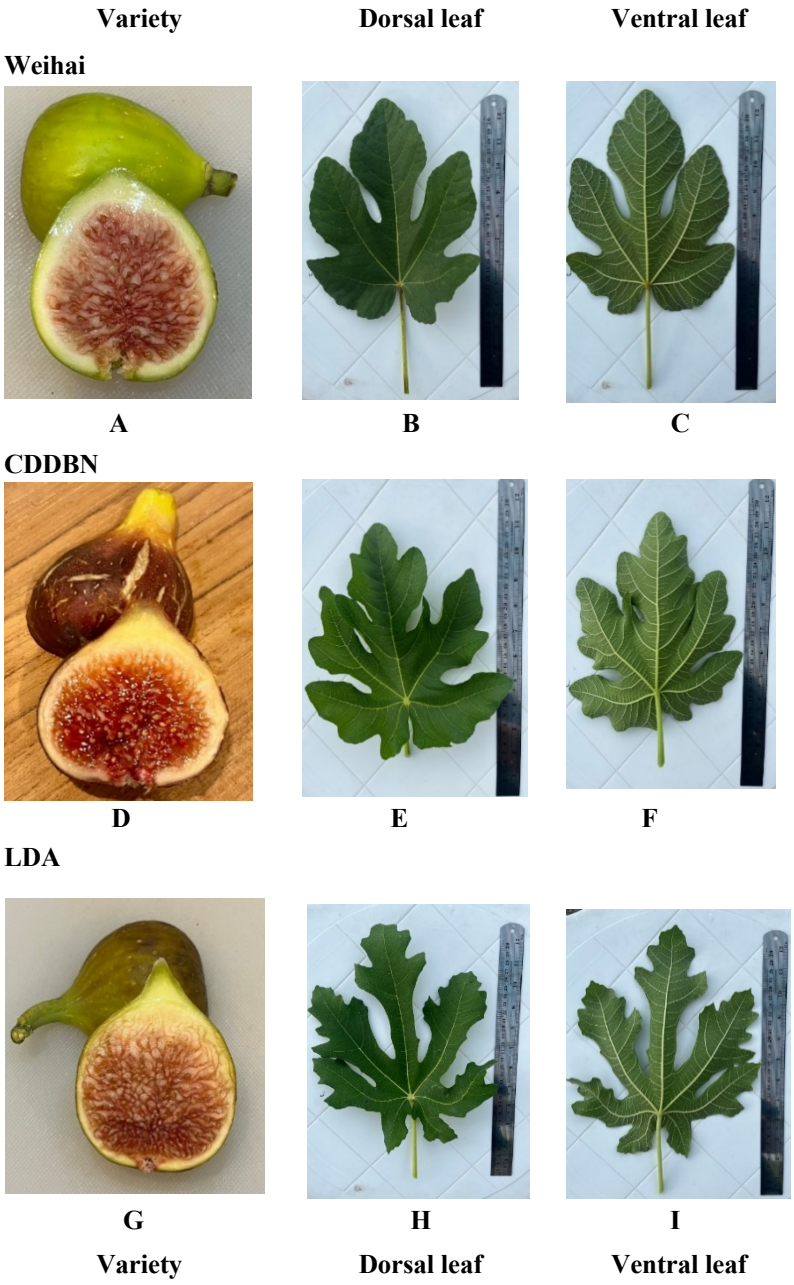
Multiple sequence alignments were performed by Geneious software using the global alignment with free end gaps and 93% similarity cost matrix. Phylogenetic tree was constructed using the Neighbor-Joining method, Jukes-Cantor genetic distance model within the Geneious software. *Ficus religiosa* ITS region (MT227788.1) was used as an outgroup.

## Results

### *Morphological characteristics*

Five varieties of common fig fruits, dorsal and ventral side of leaves were presented in Figure 1. All fruits and leaves were randomly collected from mature trees for morphological study in leaf length (cm.), shape of leaf, shape of fruit, and fruit pulp internal color. All fruit characters had the same in pulp color and shape as were found in each variety. Weihai varity which originate from China, its ripe green fruit with sweet red filling (Figure 1; A). CDDBN fruit is a small to medium in size with a dark red pulp, a thick jammy texture, an intense berry flavour (Figure 1; D). Longue d'Aout (LDA) is a widely grown fig throughout Europe and USA, its fruit color has yellow skin with grey and purple overtones with red pulp (Figure 1; G), while WI is a variety with excellent flavour, yellow-green fruit pulp (Figure 1; J). Iraqi fig is a small to medium-sized fruit with dark purple to black skin (Figure 1; M) and the interior flesh is sweet and moderate seed crunch. The leaves of the Iraqi fig are heart-shaped (Figure 1; N-O), while

other varieties in this study showed a large, palmate-shaped with three to five lobes that are up to 8 inches-length (Figure 1).



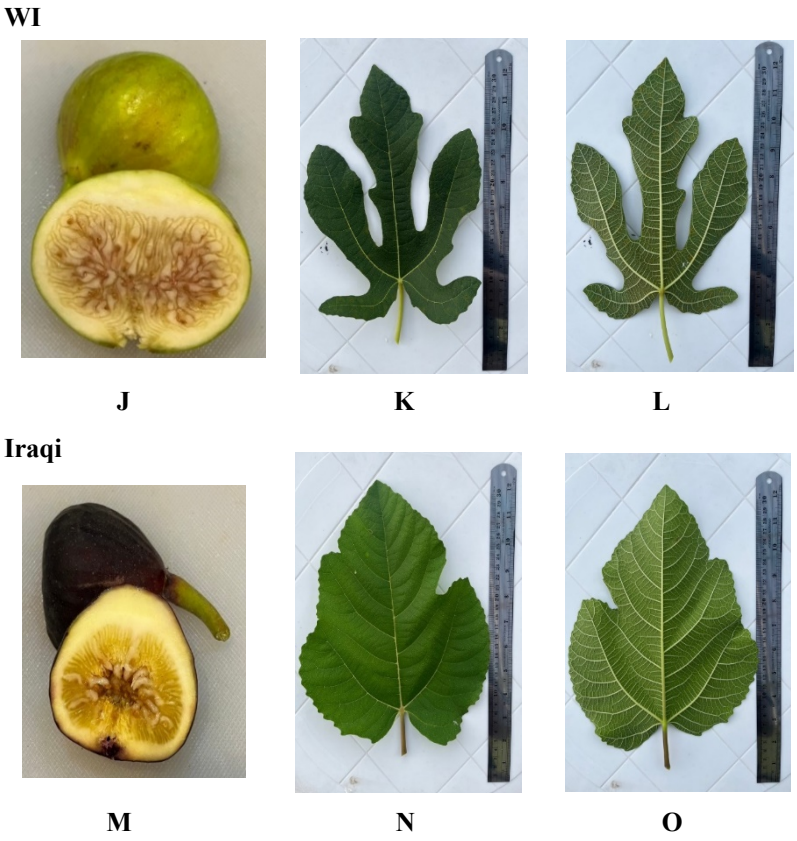



**Figure 1.** Fruits and leaves of five varieties of common fig were Weihai (A, B, C), col de dama blanca-negra (CDDBN) (D, E, F), Longue d'Aout (LDA) (G, H, I), White Israel (WI) (J, K, L), and Iraqi (M, N, O)

### *Genetic variation analysis*

Five fig ITS sequences were submitted to GenBank database by using online BankIt program: the accession number PQ182243-PQ182247.

All 5 commercial fig varieties show identical sequences within the ITS region, and identical to the reference *Ficus carica* (Figure 2). The only exception is the Iraqi variety, which has a C > T single nucleotide substitution at position 521, and a C > T single nucleotide substitution at position 654 (Figure 2 and 3). Both of these substitutions are unique to Iraqi variety and is not present in any other varieties or related fig species.

Genetic distance of three fig varieties (Weihai, LDA, and WI) showed 100% similarity to the references *Ficus caria*, wheras Iraqi and CDDBN were 99.68% and 99.84%, respectively (Table 1).

**Table 1**. Genetic distance of five fig varieties

| | MT227788.1 | F8-Whitelsrael_FF.ab1 | F7-Iraqi_FF.ab1 | F6-LDA_FF.ab1 | F5-CDDBN_FF.ab1 | F4-Weihai_FF.ab1 | EU091637.1 | AY730125.1 |
|---|---|---|---|---|---|---|---|---|
| MT227788.1 | | 95.15 | 94.82 | 95.15 | 94.98 | 95.15 | 95.15 | 95.13 |
| F8-Whitelsrael_FF.ab1 | 95.15 | | 99.68 | 100.00 | 99.84 | 100.00 | 100.00 | 100.00 |
| F7-Iraqi_FF.ab1 | 94.82 | 99.68 | | 99.68 | 99.51 | 99.68 | 99.68 | 99.68 |
| F6-LDA_FF.ab1 | 95.15 | 100.00 | 99.68 | | 99.84 | 100.00 | 100.00 | 100.00 |
| F5-CDDBN_FF.ab1 | 94.98 | 99.84 | 99.51 | 99.84 | | 99.84 | 99.84 | 99.84 |
| F4-Weihai_FF.ab1 | 95.15 | 100.00 | 99.68 | 100.00 | 99.84 | | 100.00 | 100.00 |
| EU091637.1 | 95.15 | 100.00 | 99.68 | 100.00 | 99.84 | 100.00 | | 100.00 |
| AY730125.1 | 95.13 | 100.00 | 99.68 | 100.00 | 99.84 | 100.00 | 100.00 | |

**Figure 2.** Multiple sequence alignment between the 5 fig varieties (Weihai, CDDBN, LDA, Iraqi, and WI) and the references *Ficus caria*, a related species *Ficus religiosa*, and *Ficus palmata* as an outgroup

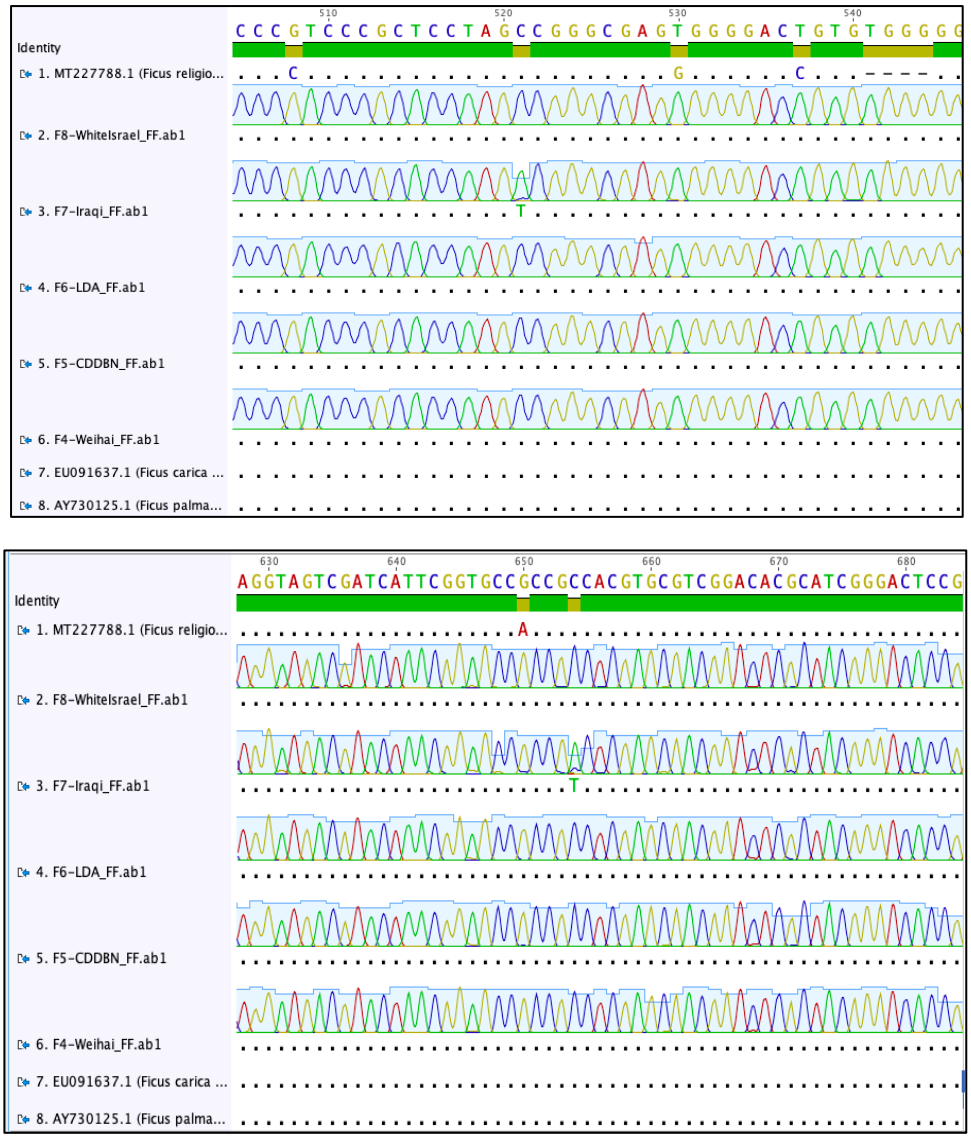



**Figure 3.** Chromatogram DNA sequence alignment of five fig varieties (Weihai, CDDBN, LDA, Iraqi, and WI) compared with the 3 reference species (*Ficus religiosa*, *Ficus carica* and *Ficus palmata*)

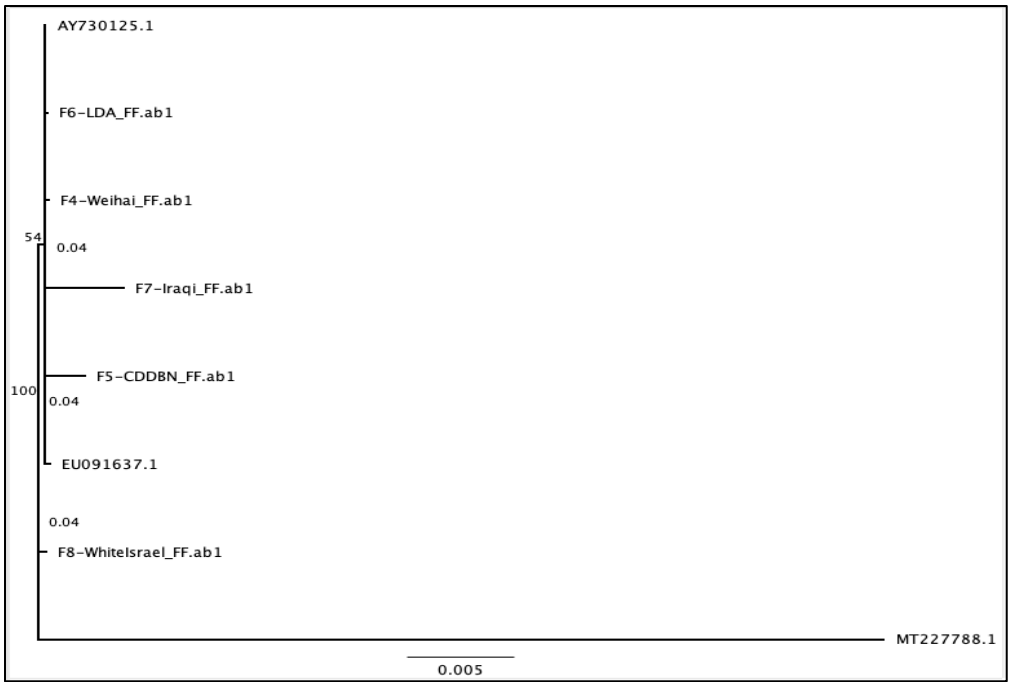



**Figure 4.** Phylogenetic tree construction using five fig sequences and the references *Ficus caria,* a related species *Ficus religiosa* (MT227788.1)*,* and *Ficus palmata* as an outgroup

## Discussion

Varieties of *Ficus carica* have been analyzed in both morphological characters and molecular markers. In 2006, a simple classification of fig variety is fruit characters such as fruit skin color (Solomon *et al*., 2006). The color of fig fruit skin varies from yellow to blue or dark purple. This study, five fig varieties were commercial planting and most consume in Thai-healthy people market. All five figs can cultivate and bear fruit in Phetchaburi province, which is tropical area, the quantity of rainfall during summers surpasses that of winters. The fig fruit characters revealed the same as the originate size, fruit skin, and pulp color. Weihai ripe fruit skin has green color while WI has yellow-green fruit pulp. LDA fruit color has yellow skin with grey and purple overtones with red pulp whereas CDDBN has a dark red pulp. Iraqi fig is a dark purple to black skin and yellow pulp. However, all five varieties in this research have not been observed in Thai cultivation although the ten fig varieties had reported for finding the best growth in Northern, Thailand since 2008 (Wongmetha, 2008).

Study of fig diversity by morphological parameters may affect from various factors such as growth condition and environment. Molecular tools may be the better technology for genetic diversity analysis with a high precision determination. The RAPD markers combined with pomological traits were used to identified the Tunisian, Palestinian and Persian fig (*Ficus carica*) cultivars for the genetic diversity and relationship among varieties (Ali Shtayeh *et al*., 2014; Baziar *et al*., 2018; Chatti *et al*., 2010). The internal transcribed spacer (ITS) region is used for genetic variation study, which is located between the 18S and 28S rRNA genes and have advantages in molecular markers since its high degree of variation among closely related species. Castro *et al.*, 2015 utilized ITS region combined with others two regions as DNA barcodes for F. carica genotype classification (Castro *et al*., 2015). In this study, we selected the ITS region of F. carica (accession number KX572966) for primer design and PCR. Then, sequencing results were analyzed and Phylogenetic tree was constructed using Geneious software. Result revealed that all 5 varieties had a highly similar 18S rRNA, and similar to 2 other related species (*Ficus palmata* and *Ficus Johannis*). Iraqi was suspected to be a hybrid between Ficus carica and Ficus palmata. The C > T substitution is not present in palmata, so it is probably not related to palmata. Interestingly, Iraqi is a unique variety that performs well in hot and humid tropical climate. This variety is frequently used as a rootstock for grafting other more fragile varieties. These mutations may play a role in the unique characteristics of this variety. Future studies should identify the genetic basis of its phenotypes, which may help facilitate future fig breeding.

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