



GENETIC DIVERSITY OF SOME RADISH (*RAPHANUS SATIVUS* L.) CULTIVARS REVEALED BY SEQUENCING METHOD

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Abstract

Radish is an important root vegetable due to its nutritional and medicinal role for human, and as a result of this importance and the lack of studies on this plant, in this study the genetic variations of some cultivars will be studied. Sequence is an important technique to show the genetic relationships among different plant species particularly economically important crops such as radish, which is considered from a medicinal and nutritious vegetable crop. Results revealed that highest genetic compatibility was between Syrian red radish cultivar and the sequence ID: FJ980407.1, (100%), then between both Locals red and white radish cultivars and the sequence ID: FJ980407.1, reached (99%). For Syrian red radish cultivar, the highest percentage similarity was between Syrian red radish cultivar and (radish) South Korea cultivar reached 100%, while the lowest percentage similarity was between Syrian red radish cultivar and both ((radish) Taiwan and (radish) Taiwan) cultivars reached 93%, and the lowest genetic distance was between Syrian red radish cultivar and (radish) South Korea cultivar reached (0.0), while the highest genetic distance was between Syrian red radish and (radish) Yanghua cultivar, reached 3.5. While the Local red radish cultivar, the highest genetic similarity was between Local red radish cultivar and (radish) South Korea cultivar, reached 99%, and the lowest genetic similarity was between Local red radish cultivar and both (radish) Taiwan and (radish) Germany cultivars, reached 91%, and the lowest genetic distance was between Local red radish cultivar and (radish) South Korea cultivar, reached (0.0), while the highest genetic distance was between Local red radish cultivar and (radish) USA cultivar, reached (7.2). For Local white radish cultivar, the highest genetic similarity was between Local white radish cultivar and (radish) South Korea cultivar, reached 99%, and the lowest genetic similarity was between Local white radish cultivar and both (radish) Taiwan and (radish) Germany cultivars, reached 91%, the lowest genetic distance was between Local white radish cultivar and (radish) South Korea cultivar reached (0.0), while the highest genetic distance was between Local white radish cultivar and both (radish) Kenya and (radish) Japan cultivars, reached (4.4). The study of genetic variations among radish cultivars is very important because it has a role in increasing the knowledge of the improvement and development of this plant.

Key words : Radish cultivars, Genetic variation, Sequence.

Introduction

Radish (*Raphanus sativus* L.) belong to the Brassicaceae family, grown and eaten all over the world and it is requested at high rates (Salerno *et al.*, 2005), and it is at first cultivated in China and Korea, and it is an important commercial vegetable crop (Kaneko and Matsuzawa, 1993). Radish is old and popular vegetables of tropical and temperate regions of the world, and it is great used as a root vegetable, soft leaves and green shoots (Alam *et al.*, 2010). Radish are commonly consume raw for their tender texture, pungent, peppery flavor and its content of ascorbic acid, phenolic acids, anthocyanins and glucosinolates, which can has a positive

effect on consumer's health (Giusti and Wrostand, 1996; Lu *et al.*, 2008; Jing *et al.*, 2012). It is a rich source of carbohydrates, protein and vitamins A, and vitamins C (Bakhsh *et al.*, 2006), and contains high amounts of anti-oxidants and glucosinolates (Malik *et al.*, 2010), and it is when consumed has many medical benefits such as prevent constipation, increase appetite, beneficial for jaundice, liver disorders and also beneficial in urinary complaints and piles (Brintha and Seran, 2009; Dhananjaya 2007). Radish is an open pollinated vegetable that has chromosome number $2n = 2x = 18$ (Muminovie *et al.*, 2005). Many of Phylogenetic studies have suggested that *Raphanus* species may have originated

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from hybridization between *Brassica rapa* (A genome)/oleracea (C genome) and *Brassica nigra* (B genome) lineages (Song *et al.*, 1990; Yang *et al.*, 2002). Until this time radish is not classified genetically at genetic and molecular levels despite its economic importance throughout the world (Rabbani *et al.*, 1998; Rabbani *et al.*, 2010). Wang *et al.*, (2011) was obtained the draft genome sequences of Chinese cabbage in *B. rapa*, and published, but nevertheless, there is a difficulty to use these sequence data as references to set the radish genome sequences due to the presence of a complex genome between *B. rapa* and *R. sativus* (Li *et al.*, 2011). The genetic diversity among plants species depends on several different factors, such as ecological, geographical, breeding system & anthropogenic effects (Saeed and Barozai, 2012), and can be determined it based on morphological, biochemical, and molecular kinds of information (Mohammadi & Prasanna 2003; Sudre *et al.*, 2007; Goncalves *et al.*, 2009). New technologies and the development of advanced bioinformatics tools have been used in many studies including both plant genomics and transcription. By using modern sequencing and bioinformatics several challenges have been overcome that emerged mainly in plant genomes with large size, high CG content, heterozygosity, transposable elements, repetitive DNA, and homopolymers or polyploidy, as with the most important crops (Turktas *et al.*, 2014). Botanists face numerous challenges, specially scientists working on crop production, such as increase in population, lowering in water and arable ground, changes in weather cases and predictability, so genome sequencing and re-sequencing can and should play a role in face all these challenges (Jackson *et al.*, 2011). Studying of genetic relationships and variation among plant cultivars and varieties is very important to overcome many of the problems and challenges they face.

Materials and Methods

DNA extraction from plant - Prepare lyophilized leaf sample

Leaves collection an after harvesting to allow storage at room temperature (15~20°C) and used liquid nitrogen to obtained plant to obtained plant material powder for Radish cultivars (Syrian red radish, Local red radish and Local white radish).

DNA isolation

Total genomic DNA was isolated from fully expanded leaves using the Kit, leave samples (5 mg) were ground to a fine powder in liquid nitrogen. DNA was extracted by using Genomic DNA Mini Kit (Geneaid\UK) or (PreMix kit (i-Taq). The extracted DNA (200 µl) was

stored at -20°C until use. Concentration, quality and quantity of DNA were determined by Nano drop-spectrophotometric at 260 nm. The analysis was conducted in the laboratory of Molecular Genetic in the university of Baghdad, genetic engineering and biotechnology institution.

DNA Electrophoresis

Amplification products were separated by electrophoresis (100 V) for (30 minutes) in 1.5% agarose gels and stained in ethidium bromide. A photographic record was taken under UV illumination.

PCR procedure

The RAPD primers were purchased from BIONEER \South Korea. A total of 6 decamer oligonucleotides of arbitrary sequence were tested for PCR amplification. Accup Power Gold Multiplex PCR premix (BIONEER \South Korea) was used to DNA amplification with RAPD primers and the thermal cycler conditions for PCR reactions were an initial denaturation cycle of 1 min and 30 s at 94°C was followed by 45 cycles comprising 1 min at 94°C, 1 min at 36°C and 2 min at 72°C. An additional cycle of 7 min at 72°C was used for final extension.

Agarose gel electrophoresis of DNA

Electrophoresis has been done to determine DNA pieces after the process of extraction or to detect the result of the interaction of PCR during the presence of the standard DNA to distinguish the bundle size of the outcome of the interaction of PCR on the Agarose gel. According to Sambrook *et al.*, (1989), the agarose gel has been made in 1.5% condensation by melting 1.5 g of agarose in 100 ml of previously made TBE buffer. Agarose has been heated to boil then left to cool down at (45-50°C). The gel has been poured in the pour plate in which the plate of agarose support has been prepared after fixing the comb to make holes that would hold the samples. The gel has been poured gently not to make air bubbles and left 30 minutes to cool down. The comb has been removed gently of the solid agarose. The plate has been fixed to its stand in the Electrophoresis horizontal unit represented by the tank used in the Electrophoresis. The tank has been filled with TBE buffer in which it covers the gel surface.

Preparation of sample

3 µl of the processor loading buffer (Intron/Korea) has been mixed with 5 µl of the supposed DNA to be electrophoresis (loading dye), the DNA loading in edge process and Electric current of 7 v\c2 has been exposed for 1-2 h till the tincture has reached to the other side of the gel. The gel has been tested by a source of the UV

with 336 nm after put the gel in pool contain on 30µl Red safe Nucleic acid staining solution and 500 ml from distilled water.

DNA Ladder (100bp)

The KAPA Universal Ladder Kit is designed for determining the approximate size and quantity of double-stranded DNA on agarose gel. KAPA Universal Ladder kits contain eighteen DNA fragments (in base pairs): 100, 150, 200, 300, 400, 500, 600, 800, 1000, 1200, 1600, 2000, 3000, 4000, 5000, 6000, 8000, and 10000. The KAPA Universal Ladder contains four reference bands (500, 1000, 1600, and 4000) for orientation.

The Components of the Maxima PCR PreMix kit (i-Taq) Which contains 1- i-Taq DNA Polymerase (v/2.5U), 2- DNTPs (v/2.5mM), 3- Reaction buffer (10X) (v/ 1X) and 4- Gel loading buffer (v/ 1X). Detection of Gene *ITS* by Using PCR: Detection of *ITS* gene was conducted by using primers for amplification. A fragment 560 bp of *ITS* was amplified using a forward primer (*ITS1* F: 52 - TCCGTAGGTGAACCTGCGG -32) and a reverse primer (*ITS4* R:52 TCCTCCGCTTATTGATATGC-32) (Primers set supplied by IDT (Integrated DNA Technologies company, Canada.). The PCR amplification was performed in a total volume of 25µl containing 1.5µl DNA, 5 µl Taq PCR PreMix (Intron, Korea), 1µl of each primer (10 pmol) then distilled water was added into tube to a total volume of 25µl. The thermal cycling conditions were done as follows: Denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 45s, 52°C for 1 min and 72°C for 1min with final incubation at 72°C for 7 min using a thermal Cycler (Gene Amp, PCR system 9700; Applied Biosystem). The PCR products were separated by 1.5% agarose gel electrophoresis and visualized by exposure to ultraviolet light (302nm) after red stain staining (Intron Korea)

Sequencing and Sequence Alignment

The PCR products were separated on a 2% agarose gel electrophoresis and visualized by exposure to ultra

violat light (302 nm) after ethidium bromide or Red Stain staining. Sequencing of gene was performed by national instrumentation center for environmental management (nicem) online at (http://nicem.snu.ac.kr/main/?en_skin=index.html), biotechnology lab, machine is DNA sequencer 3730XL, Applied Biosystem), Homology search was conducted using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online at ([http:// www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) and BioEdit program.

Results

The table (1) showed the genetic compatibility among radish cultivars used in the study (Syrian red radish, Local red radish and Local white radish) and the sequence ID: FJ980407.1. Where showed genetic compatibility 100 % between Syrian red radish cultivar and the sequence ID: FJ980407.1, and 99% between Local red radish cultivar and the sequence ID: FJ980407.1, and 99% between Local white radish cultivar and the sequence ID: FJ980407.1. The table 1 showed also that there substitution in the site 192 bp as a transition between (T-C), fig. 1, and appear substitution as a transition between (A-G) and (A-G) in the two sites 280 and 277 bp respectively, (fig. 3, 4).

Fig. 2 represents results of PCR product for radish cultivars used in this study (Syrian red radish, Local red radish and Local white radish), which has been separated by electrophoresis (100 V) for (30 minutes) in 1% agarose gels and stained in ethidium bromide.

Fig. 5 explained that cultivars divided in two groups, genetic similarity between them 99%. The first group included radish cultivars ((radish) Japan, (radish) Yanghua and (radish) Canada), while the second group included ((radish) Taiwan, (radish) Germany, (radish) USA, (radish) Syrain, (radish) South Korea, (radish) China, and (radish) Kenya). And observed that the highest percentage similarity was between Syrian red radish cultivar which cultivated in Iraq and (radish) South Korea cultivar

Table 1: Represents Genetic compatibility among radish cultivars used in the study (Syrian red radish, Local red radish and Local white radish) depending on identity (ID).

No. of sample	Type of substitution	Location	Nucleotide	Range of nucleotide	Sequence ID	Identities	Source
Syrian red radish			66 to 692	ID: FJ980407.1	100%	<i>Raphanus sativus</i>
Local red radish	Transversion	192	G>T	65 to 692	ID: FJ980407.1	99%	<i>Raphanus sativus</i>
	Transition	277	G>A				
	Transition	280	A>G				
Local white radish	Transversion	192	G>T	66 to 692	ID: FJ980407.1	99%	<i>Raphanus sativus</i>
	Transversion	243	C>A				
	Transition	277	G>A				

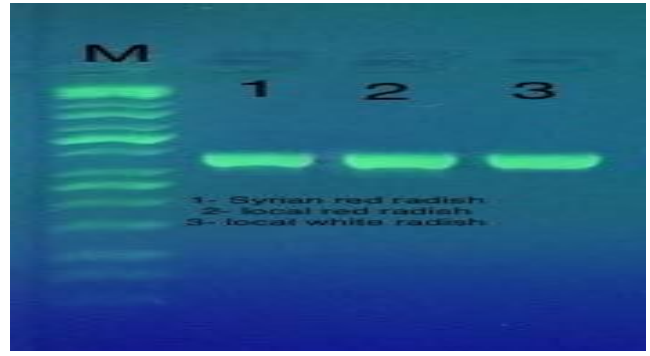
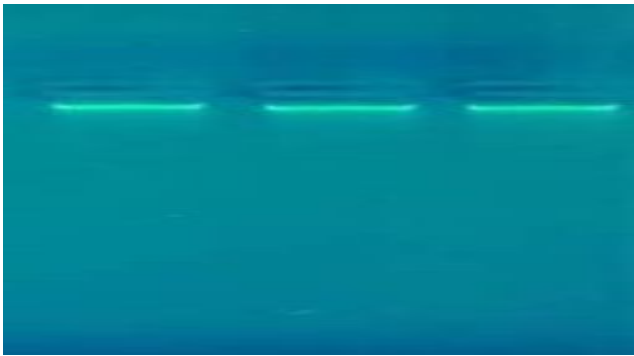


Fig. 1: DNA extraction of radish cultivars used in this study (1- Syrian red radish, 2- Local red radish, 3- Local white radish).

Fig. 2: PCR product with its primer with radish cultivars (1- Syrian red radish, 2- Local red radish and 3- Local white radish).

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Query 121 TTCCGGATATCACCAAACCCCGGCACGAAAAGTGTCAAGGAACATGCAACTAAACAGTAT
180      |||
Sbjct 185 TTCCGGAGATCACCAAACCCCGGCACGAAAAGTGTCAAGGAACATGCAACTAAACAGTCT
244
Query 181 GCTTTCGCCAACCCGGAACGGTGTGTTGTTTCGAAAGCAGTTCTGAAATGTAAAGTCTATA
240      |||
Sbjct 245 GCTTTCGCCAACCCGGAACGGTGTGTTGTTTCGAAACAGTTCTGAAATGTAAAGTCTATA
304
    
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Fig. 3: Sequence of Local red radish cultivar and areas of substitution and comparison with the cultivated radish cultivars in world.

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Query 121 TCCGGATATCACCAAACCCCGGCACGAAAAGTGTCAAGGAACATGCAACTAAACAGTATG
180      |||
Sbjct 186 TCCGGAGATCACCAAACCCCGGCACGAAAAGTGTCAAGGAACATGCAACTAAACAGTCTG
245
Query 181 CTTTCGCCAACCCGGAACGGTGTGTTGTTTCGAAAACAGTTCTGAAATGTAAAGTCTATAA
240      |||
Sbjct 246 CTTTCGCCAACCCGGAACGGTGTGTTGTTTCGAAAACAGTTCTGAAATGTAAAGTCTATAA
305
    
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Fig. 4: Sequence of Local white radish cultivar and areas of substitution and transition comparison with the cultivated radish cultivars in world.

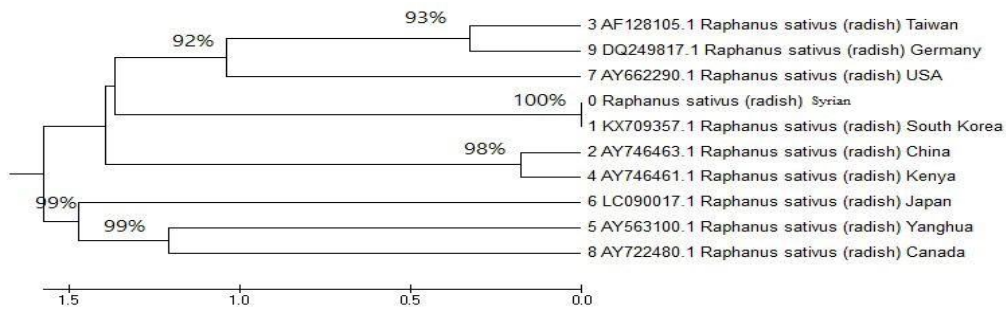


Fig. 5: Dendrogram for Syrian red radish cultivar and its comparison with the cultivated radish cultivars in world.

reached 100%, and between Syrian red radish cultivar and both [(radish) Kenya and (radish) China] cultivars reached 98%. While the lowest percentage similarity was between Syrian red radish cultivar and both [(radish) Taiwan cultivar and (radish) Taiwan] cultivars reached 93%. Table 2 shows the percentage similarity between Syrian red radish cultivar and the cultivated radish cultivars in world according to its identity (ID).

Table 3 represents genetic distance between Syrian red radish cultivar which cultivated in Iraq and the cultivated radish cultivars in world. The lowest genetic distance was between Syrian red radish cultivar and (radish) South Korea cultivar reached (0.0), while the highest genetic distance was between Syrian red radish and (radish) Yanghua cultivar, reached (3.5).

Table 2: Genetic distance between Syrian red radish cultivar and the cultivated radish cultivars in world.

S.No.	Accession	Source	Country	Source	Compatibility
1.	ID: KX709357.1	<i>Raphanus sativus</i> (radish)	South Korea	<i>Raphanus sativus</i>	100%
2.	ID: AY746463.1	<i>Raphanus sativus</i> (radish)	China	<i>Raphanus sativus</i>	99%
3.	ID: AF128105.1	<i>Raphanus sativus</i> (radish)	Taiwan	<i>Raphanus sativus</i>	99%
4.	ID: AY746461.1	<i>Raphanus sativus</i> (radish)	Kenya	<i>Raphanus sativus</i>	98%
5.	ID: AY563100.1	<i>Raphanus sativus</i> (radish)	Yanghua	<i>Raphanus sativus</i>	99%
6.	ID: LC090017.1	<i>Raphanus sativus</i> (radish)	Japan	<i>Raphanus sativus</i>	99%
7.	ID: AY662290.1	<i>Raphanus sativus</i> (radish)	USA	<i>Raphanus sativus</i>	99%
8.	ID: AY722480.1	<i>Raphanus sativus</i> (radish)	Canada	<i>Raphanus sativus</i>	99%
9.	ID: DQ249817.1	<i>Raphanus sativus</i> (radish)	Germany	<i>Raphanus sativus</i>	92%

Fig. 6 shows that cultivars divided in two groups, genetic similarity between them 99%. The first group included (radish) Japan cultivar, while the second group included ((radish) Iraq (Local red radish), (radish) South Korea, (radish) Yanghua, (radish) Canada, (radish) China, (radish) USA, (radish) Taiwan, and (radish) Germany. From this figure found that the highest genetic similarity was between Local red radish cultivar and (radish) South Korea cultivar, reached 99%, and the lowest genetic similarity was between Local red radish cultivar and both ((radish) Taiwan and (radish) Germany) cultivars, reached 91%. Table 4 shows the percentage similarity between

Local red radish cultivar and the cultivated radish cultivars in world according to its identity (ID).

Table 5 explained the genetic distance between Local red radish cultivar and the cultivated radish cultivars in world. The lowest genetic distance was between Local red radish cultivar and (radish) South Korea cultivar, reached (0.0), while the highest genetic distance was between Local red radish cultivar and (radish) USA cultivar, reached (7.2).

Fig. 7 shows that cultivars divided in two groups, genetic similarity between them 99%. The first group

Table 3: Genetic distance between Syrian red radish cultivar which cultivated in Iraq and the cultivated radish cultivars in world.

	1	2	3	4	5	6	7	8	9	10
1. 0 <i>Raphanus sativus</i> (radish) Syrian										
2. 1 KX709357.1 <i>Raphanus sativus</i> (radish) South Korea	0.0									
3. 2 AY746463.1 <i>Raphanus sativus</i> (radish) China	2.8	2.8								
4. 3 AF128105.1 <i>Raphanus sativus</i> (radish) Taiwan	2.9	2.9	2.4							
5. 4 AY746461.1 <i>Raphanus sativus</i> (radish) Kenya	2.8	2.8	0.4	3.0						
6. 5 AY563100.1 <i>Raphanus sativus</i> (radish) Yanghua	3.5	3.5	2.9	2.2	3.0					
7. 6 LC090017.1 <i>Raphanus sativus</i> (radish) Japan	3.3	3.3	4.5	2.9	3.0	2.4				
8. 7 AY662290.1 <i>Raphanus sativus</i> (radish) USA	2.5	2.5	2.7	2.0	3.0	3.2	3.4			
9. 8 AY722480.1 <i>Raphanus sativus</i> (radish) Canada	2.9	2.9	3.5	3.3	3.4	2.4	3.4	2.9		
10. 9 DQ249817.1 <i>Raphanus sativus</i> (radish) Germany	2.8	2.8	3.2	0.7	2.4	2.7	2.9	2.2	2.9	

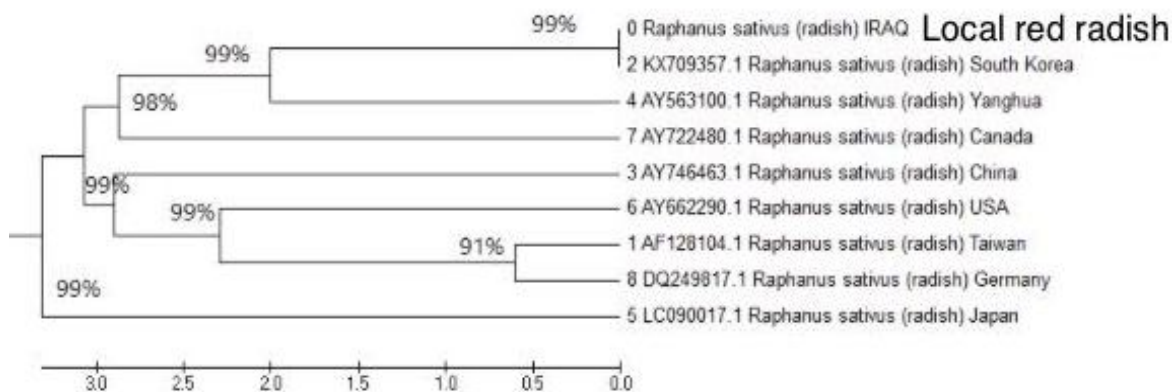
**Fig. 6:** Dendrogram for Local red radish and its comparison with the cultivated radish cultivars in world.

Table 4 : Genetic distance between Local red radish cultivar and the cultivated radish cultivars in world.

S.No.	Compatibility	Country	Source	Accession
1	ID: AF128104.1	<i>Raphanus sativus</i> (radish)	Taiwan	99%
2	ID: KX709357.1	<i>Raphanus sativus</i> (radish)	South Korea	99%
3	ID: AY746463.1	<i>Raphanus sativus</i> (radish)	China	99%
4	ID: AY563100.1	<i>Raphanus sativus</i> (radish)	Yanghua	99%
5	ID: LC090017.1	<i>Raphanus sativus</i> (radish)	Japan	99%
6	ID: AY662290.1	<i>Raphanus sativus</i> (radish)	USA	99%
7	ID: AY722480.1	<i>Raphanus sativus</i> (radish)	Canada	98%
8	ID: DQ249817.1	<i>Raphanus sativus</i> (radish)	Germany	91%

included (radish) Japan cultivar, while the second group included [(radish) Taiwan, (radish) Germany, (radish) USA, (radish) Iraq (Local White radish), (radish) South Korea, (radish) China, (radish) Kenya, (radish)] Canada. From this figure found that the highest genetic similarity was between Local white radish cultivar and (radish) South Korea cultivar, reached 99%, and the lowest genetic similarity was between Local white radish cultivar and both [(radish) Taiwan and (radish) Germany] cultivars, reached 91%. Table 6 shows the percentage similarity between Local white radish cultivar and the cultivated

radish cultivars in world according to its identity (ID).

Table 7 shows the genetic distance between Local white radish cultivar and the cultivated radish cultivars in world. The lowest genetic distance was between Local white radish cultivar and (radish) South Korea cultivar reached (0.0), while the highest genetic distance was between Local white radish cultivar and both ((radish) Kenya and (radish) Japan) cultivars, reached (4.4).

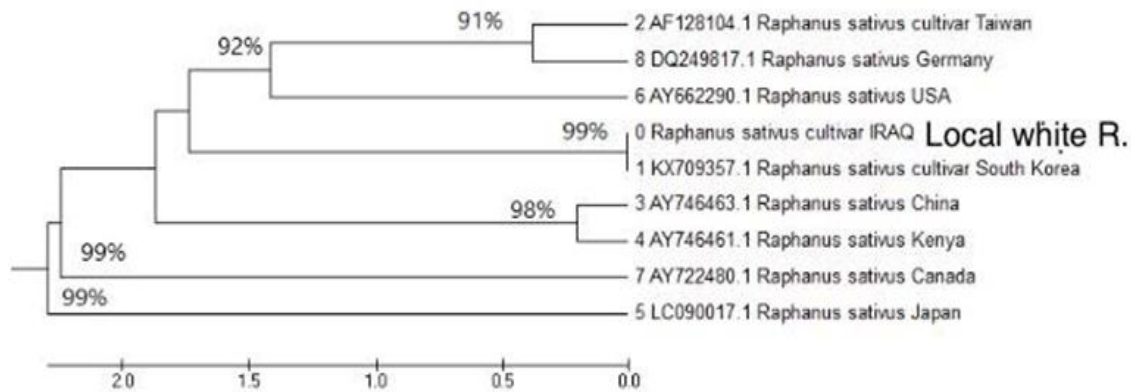


Fig. 7: Dendrogram for Local white radish cultivar and its comparison with the cultivated radish cultivars in world.

Table 5: Genetic distance between Local red radish cultivar and the cultivated radish cultivars in world.

Local red radish	1	2	3	4	5	6	7	8	9
1. 0 <i>Raphanus sativus</i> (radish) IRAQ radish									
2. 1 AF128104.1 <i>Raphanus sativus</i> (radish) Taiwan	5.3								
3. 2 KX709357.1 <i>Raphanus sativus</i> (radish) South Korea	0.0	5.2							
4. 3 AY746463.1 <i>Raphanus sativus</i> (radish) China	5.8	5.1	5.6						
5. 4 AY563100.1 <i>Raphanus sativus</i> (radish) Yanghua	4.0	4.6	4.0	6.2					
6. 5 LC090017.1 <i>Raphanus sativus</i> (radish) Japan	6.2	6.0	6.5	7.8	5.5				
7. 6 AY662290.1 <i>Raphanus sativus</i> (radish) USA	7.2	4.6	7.0	5.9	7.0	7.4			
8. 7 AY722480.1 <i>Raphanus sativus</i> (radish) Canada	6.0	7.1	6.2	7.4	5.1	7.3	6.3		
9. 8 DQ249817.1 <i>Raphanus sativus</i> (radish) Germany	5.9	1.2	6.1	6.4	5.9	6.3	4.6	6.0	

Table 6: Genetic distance between Local white radish cultivar and the cultivated radish cultivars in world.

S. No.	Accession	Source	Country	Compatibility
1.	ID: KX709357.1	<i>Raphanus sativus</i> (radish)	South Korea	99%
2.	ID: AF128104.1	<i>Raphanus sativus</i> (radish)	Taiwan	99%
3.	ID: AY746463.1	<i>Raphanus sativus</i> (radish)	China	99%
4.	ID: AY746461.1	<i>Raphanus sativus</i> (radish)	Kenya	98%
5.	ID: LC090017.1	<i>Raphanus sativus</i> (radish)	Japan	99%
6.	ID: AY662290.1	<i>Raphanus sativus</i> (radish)	USA	99%
7.	ID: AY722480.1	<i>Raphanus sativus</i> (radish)	Canada	99%
8.	ID: DQ249817.1	<i>Raphanus sativus</i> (radish)	Germany	91%

Table 7: Genetic distance between Local white radish cultivar and the cultivated radish cultivars in world.

Local white	1	2	3	4	5	6	7	8	9
1. 0 <i>Raphanus sativus</i> cultivar IRAQ radish.									
2. 1 KX709357.1 <i>Raphanus sativus</i> cultivar South Korea	0.0								
3. 2 AF128104.1 <i>Raphanus sativus</i> cultivar Taiwan	4.3	4.6							
4. 3 AY746463.1 <i>Raphanus sativus</i> China	2.3	3.6	3.2						
5. 4 AY746461.1 <i>Raphanus sativus</i> Kenya	4.4	4.3	4.1	0.4					
6. 5 LC090017.1 <i>Raphanus sativus</i> Japan	4.4	4.4	4.0	5.1	4.4				
7. 6 AY662290.1 <i>Raphanus sativus</i> USA	3.4	3.5	2.8	4.1	4.3	5.1			
8. 7 AY722480.1 <i>Raphanus sativus</i> Canada	4.1	4.1	4.8	4.9	4.9	4.9	4.6		
9. 8 DQ249817.1 <i>Raphanus sativus</i> Germany	2.7	2.3	0.8	4.0	3.0	4.5	2.8	3.9	

Discussion

Radish it is an important commercial vegetable crop, because its effect on consumer's health. The dilation of sequence information in a growing number of species has a role to comparative studies and application of molecular breeding and biotechnology approaches for crops improvement (Green, 2001 and Liu *et al.*, 2013). Bolger, *et al.*, (2014) were concluded that crop genome sequences had great effect on crop research and its improvement in a relatively short time, even at the current scales of perfection. Sequence as a technique is very important for radish, where find (Warwick and Black, 1991; Inaba and Nishio, 2002) that phylogenetic analyses of Brassicaceae species by using DNA markers or nucleotide sequences of genes have detected that *R. sativus* belongs to the *rapa/oleracea* lineage not to the *nigra* lineage. He, *et al.*, (2015) showed by using low-coverage sequencing on Radish to analyze repeat elements, and they detected the genome structure of radish and find that satellite repeats are most commanding elements, which is differ from most reported species in which LTR (long terminal repeat) retrotransposons are the most ample element of the genome. Markers SSR (Simple sequence repeats) and SNP (single nucleotide polymorphisms) are have been vastly used for construction of high- intensity genetic maps and for identification of

QTLs (Quantitative trait locus) which related with economically important indicators in radish (Budahn *et al.*, 2009; Tsuru *et al.*, 2005; Shirasawa *et al.*, 2011, and Wang *et al.*, 2012). The results obtained were showed that the high genetic compatibility was between Syrian red radish cultivar and the sequence ID: FJ980407.1, reached (100%), compare with between tow cultivars (Local red and white radish) reached 99% (Table 1), and differences in percentage similarity among cultivars (Syrian red radish, which cultivated in Iraq, and Local red and white radish) and the cultivated radish cultivars in world (Tables 2, 4, 6), in addition to variations in genetic distance among cultivars (Syrian red radish, which cultivated in Iraq, and Local red and white radish) and the cultivated radish cultivars in world (Tables 3, 5, 7). The reason for these genetic differences may be due to the mutation, the hybridization, the gauge of its dispersal, the agricultural operations and domestication considered factors that effect on variance of cultivated plant species (Yamane, *et al.*, 2009; Heiser, 1988). Loveless and Hamrick (1984) explained that genetic differences of plant inhabitation is affected largely by many factors such as mating system, genetic driftage, evolutionary history and life history, and also Saeed and Barozai (2012) explained that genetic variances among plants species depends on different factors, like ecological, geographical, breeding method and anthropogenic interventions. The

degree genetic variations and its importance are affected by many factors such as: consanguinity degree, quality of chromosomal replication, chromosomal behavior whether bilaterally, quadruple or hexagonal, type of multiplication, nature of gene number, chromosome number, genetic homogeneity, and environmental factors during multiplication (Whelan *et al.*, 1983), and the long-dated artificial selection led to many *R. sativus* landraces with different sizes, shapes, colors, and flavors of the edible organs, beyond these morphological variance, *R. sativus* also gave wealthy multiformity in nuclear genome (Huh and Ohnishi, 2002; Lü *et al.*, 2008; Nakatsuji *et al.*, 2011), mitochondrial (Yamagishi and Terachi, 2003) and chloroplast genomes (Yamane *et al.*, 2005; Yamane *et al.*, 2009, and Yamagishi *et al.*, 2009). Several of *R. sativus* cultivars were suggested after its domestication through different regional origination, based on the polymorphism of the mitochondrial and chloroplast genomes (Yamagishi and Terachi, 2003; Yamagishi *et al.*, 2009). This agree with Naseeruddin *et al.*, (2011) when he evaluated twenty genotypes of radish to detection the genetic differences, where the variance analysis shows more differences among genotypes for almost all attributes. and also agree with Khokar *et al.*, (1987), where they find significant diversity between the cultivars, through evaluated eight radish cultivars for time taken to gain edible size, root length, breadth of plant root per hectare.

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