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ASSESSMENT OF GENETIC RELATEDNESS AMONG SOME SPECIES OF AQUATIC PLANTS USING RAPD MARKERS

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ABSTRACT

The current study was performed using the RAPD markers to detect the Genetic relationship, Genetic diversity, and DNA fingerprint of twelve categories of the aquatic plants genotypes. Isolating Genomic DNA from aquatic plants of fresh leaves, and an amount of DNA was extracted, 966 Nano gram/microliter, with a purity 1.8. Twelve primers were tested showing varied multiplication results between the studied categories. These primers showed 101 contrastive bands from the original 114amplified bands. The highest number of amplified bands was 13 obtained through all genomes by the OP-M05 and OP-L05 primers and the lowest number of the amplified bands 4 by the OP-M20 primer. The difference in the number of bands resulting from the use of each primer is different from the difference in the molecular weights of these bands, which depends on the number and locations of the sequence of primers on the DNA bands.

***Keywords*:** Aquatic plants, RAPD markers, Genetic variations.

Introduction

Environmental problems are one of the most important dangers threatening to human, animal health and the ecological balance. Most of the water system (river and lack) are commonly used for purposes, such as irrigation of fields, landscape, public parks and also as drinking water. On the other hand, it is reported that the use of wastewater for the irrigation of agricultural fields harms the mitotic division of plant and in turn wipes out the plant due to some substances contained within this water. If these plants are consumed as food, it may influence human health adversely. The chemicals profile of plants grown on such fields could give rise to serious consequences such as allergy at early ages, respiratory disorders, coronary and cancer in middle ages (Carita and Marin-Morales, 2008).

The RAPD technique is a selective and sensitive assay for DNA analysis in eco-genotoxicology. A recent advance in molecular biology, the RAPD is used to evaluate the variation at the DNA level. It is a reliable and reproducible assay and has the potential to detect a wide range of DNA damage as well as mutations caused by heavy metal stress and therefore, it can be applied to study genotoxicity (Atienzar and Jha, 2006).

The RAPD technique is suitable for the analysis of DNA extracted from any organism because of its rapidity, applicability to any organism and its ability to detect a wide range of DNA damages and mutation. Previous studies have shown that changes in DNA band patterns observed reflects DNA alteration in genome from single base changes (point mutation) to complex chromosomal rearrangements, and that DNA fingerprinting offers a useful biomarker assay in assessment of genotoxicity (Baeshin *et al*., 2009; Ozakca and Silah, 2013; Hassan and Yassein, 2014). To the best of our knowledge, there is no documented information on the phytogenotoxicity evaluation of bilge water usingmolecular markers. Consequently, the RAPD assay was employed in this study to detect possible DNA damage in *Allium cepa* root cells exposed to bilge water to ascertain the toxicological implications of the wastewater on plant and animal life.

Materials and Methods

The collected samples of *Ceratophyllum demersum*, *Hydrilla verticillata* and *Lemna* sp. from Euphrates River in Al-Kufa city; for RAPD-PCR fingerprinting were washed several times by tape water, then by distilled water, frozen by liquid nitrogen and kept at - 20°C.Using twelve RAPD primers detailed in table no.1. DNA was extracted using Genomic DNA Mini Kit from leaves resulting in DNA concentration 145 µg/ml and purity 1.9, PCR amplification conducted using Thermo cycler (Agilent technology sure cycler 8800) in PCR pre mix master mix containing (250 µM of each dNTP, 1Unit of Taq DNA polymerase and 1X reaction buffer, with 1.5 mM MgCl2) and programmed according to Muhammad *et al.* (2017), Sofalian *et al*. (2008) and Abou-Deif *et al*. (2013). Scoring data as 1, 0 for presence and absence of amplification product and data then entered into PAST statistic vital program, Version 62.1. Electrophoresis done using 1.5 % agarose gel at 70 Volts for 3 hours.

Table 1 : Primers and their sequences used as RAPD markers

Primer	Sequence 5'-3'	Primer	Sequence 5'-3'
OP-H01	GGTCGGAGAA	OP-I02	GGAGGAGAGG
OP-M06	CTGGGCAACT	OP-M14	AGGGTCGTTC
OP-R06	GTCTACGGCA	OP-V19	GGGTGTGCAG
OP-M05	GGGAACGTGT	OP-E20	AACGGTGACC
OP-R12	ACAGGTGCGT	Op-P04	GTGTCTCAGG
OP-M20	AGGTCTTGGG	OP-L05	ACGCAGGCAC

Results and Discussion

Isolating Genomic DNA from aquatic plants of fresh leaves, and an amount of DNA was extracted, 966 Nano gram/microliter, with a purity 1.8 Using the Bio Drop device, it was estimated that between 260 and 280 wavelengths. The estimated molecular sizes of the DNA were250-2000bp, and were determined using 1% of the agarose Gel and electric signals.

Twelve primers were tested showing varied multiplication results between the studied categories. These primers showed 101 contrastive bands from the original 114amplified bands. The highest number of amplified bands was 13 obtained through all genomes by the OP-M05 and OP-L05 primers and the lowest number of the amplified bands 4 by the OP-M20primer (Figures 1, 2 and 3).The difference in the number of main and amplified bands is mainly due to the primer structure and that some primers recognize a large number of link locations which are more useful than those recognizing a lower number of these locations, giving better chance of detecting DNA polymorphisms among individuals (Williams *et al*., 1990 and Tahir, 2014).

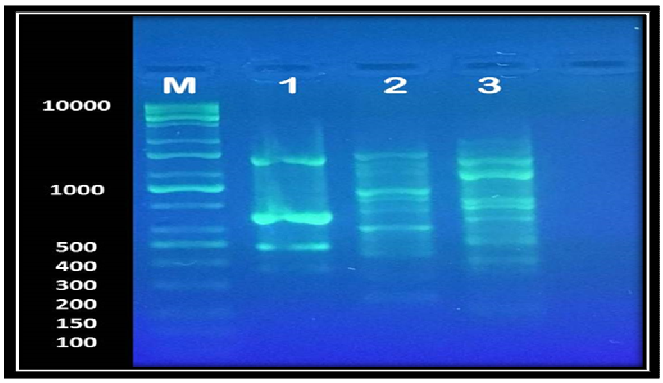

Fig. 1: OP-M05 primer multiplication results on 1% agarosegel with standard of 30-ampere and 70-volt volumetric index for *Ceratophyllum demersum*, *Hydrilla verticillata* and *Lemna* sp.

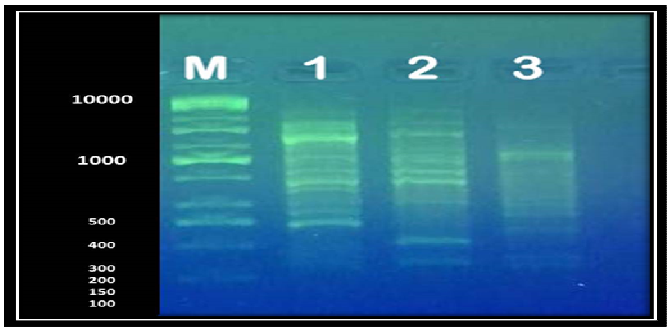

Fig. 2 : OP-L05primer multiplication results on 1% agarose gel with standard of 30-ampere and 70-volt volumetric index for *Ceratophyllum demersum*, *Hydrilla verticillata* and *Lemna* sp.

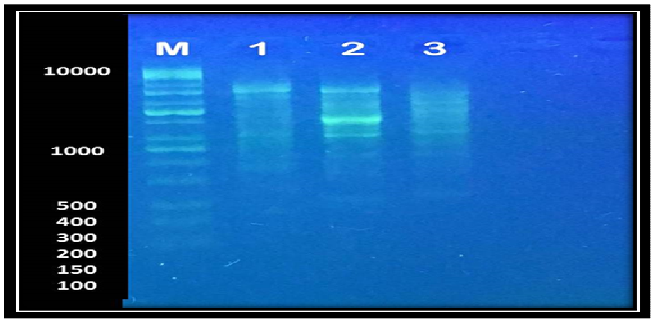

Fig. 3 : OP-M20primer multiplication results on 1% agarose gel with standard of 30-ampere and 70-volt volumetric index for *Ceratophyllum demersum*, *Hydrilla verticillata* and *Lemna* sp.

Table 2: The primer, the size of the pieces, the number of main bands, the number of amplified bands, the number of monomorphic bands, the number of polymorphic bands, uniqe bands, polymorphism, primer efficiency and discrimination value of each RAPD primer in this study.

No.	Primer	No. of amplified bands	No. of polymorphic bands	Polymorphism%	Discrimi-natory value %	Primer efficiency
1	OP-H01	9	8	7.017544	7.894737	7.920792
2	OP-M06	12	10	8.77193	10.52632	9.90099
3	OP-R06	9	9	7.894737	7.894737	8.910891
4	OP-M05	13	13	11.40351	11.40351	12.87129
5	OP-R12	7	7	6.140351	6.140351	6.930693
6	OP-M20	4	4	3.508772	3.508772	3.960396
7	OP-I02	11	8	7.017544	9.649123	7.920792
8	OP-M14	11	8	7.017544	9.649123	7.920792
9	OP-V19	6	4	3.508772	5.263158	3.960396
10	OP-E20	9	8	7.017544	7.894737	7.920792
11	OP-P04	10	9	7.894737	8.77193	8.910891
12	OP-L05	13	13	11.40351	11.40351	12.87129

The difference in the number of bands resulting from the use of each primer is different from the difference in the molecular weights of these bands, which depends on the number and locations of the sequence of primers on the DNA bands. The very light bands are ignored and this corresponds to (Barone *et al*., 1999; Swoboda and Bhalla, 1997). In addition, the number of DNA binding sites depends on the type of genome and the extent to which the enzyme is defined on those sites (Caetano – Anolles, 1997). Either the variance based on the differences in the intensity of the bands. The bands shine as a result of the appearance of some bands amplified together in the same molecular weight and appear in the form of a single dense band is actually more than a band may be the result of homozygosity case where the same site is multiplied by the other allele and has the same molecular weight So the amplified pieces are grouped together in those locations. The increase in the concentration of the template DNA leads to a repetition of the number of copies of the target DNA, which leads to the same multiplication of the site more than once. Since it is difficult to determine the precise concentration of DNA for several factors, the difference in the thickness of the resulting bandscannot be used as a measure of genetic variation. RAPD is one of the markers of complete sovereignty. Thus, it is not possible to estimate the number of alleles per site (AL-Hassani, 2002). This is consistent with what does not rely on the intensity of bands glazing as a measure of contrast due to the difficulty of controlling the precise concentration of DNA(Vogt *et al*., 1997)

The existence of joint bands between some species only, without the rest of the varieties, can be invested by linking them to specific traits common to these varieties such as resistance to pathogens or inappropriate environment. In addition, the presence of common bands makes the RAPD markers more appropriate than the other DNAmarkers. (1996) Powell *et al*., when compared to RAPD with other markers such as SSR and RFLP. RAPD was found to bemore accurate for studying the genetic relationship between the presence of co-beams between the studied species compared with SSR and RFLAP which is usually characterized by the appearance of a band or tow band.

The most important fact to be taken into account is that variations in the level of polymorphism can be the result of distinct areas of the aquatic plants genome that have been evaluated by selected indicators or differences between the materials used (Sun *et al*., 2001). The number of primers required to identify selected speciesis dependent on discrimination power of each tested primers which means its ability to finding unique pattern for the studied aquatic plants. Discrimination power of a primer will increased by increasing the number of specific varieties using the specific primers (Arif *et al*., 2010)

Of the other important criteria to be analyzed, the efficiency of the primer and Discriminatory capacity determined by the materials and methods of all the primers tested in this study. The primer efficiency range may show the primer's ability to give a large proportion of the polymorphic bands according to a number of multiplication ranges. Thus, the primer efficiency is not the primer which gives the largest number of multiplied packs, but the ability to show differences between the studied species (Newton and Graham, 1997). In our current study and previous studies, the rate of variation was 66-67.4% (Srivastava *et al*., 2009). The same principle was true with other primers OPA-03, OPA-04, OPA-10 (Sarikar Roy *et al*., 2012; AL-Tamimi, 2014) OPE-02 (AL-Badeiry, 2013) others are certainly related to the germplasm used.

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