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Testing Water Quality on the Molecular Level Via Genetic Diversity Induced in Onion Genome Using Inter – Simple Sequence Repeats Technique (ISSR)

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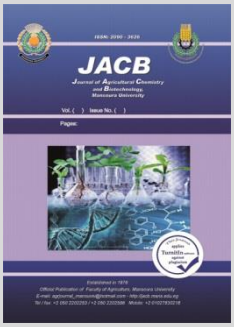

ABSTRACT

This study aimed to testing water quality on the molecular level using ISSR markers via genetic variation induced in the genome affected by the testing water sample. Inter–simple sequence repeats (ISSR markers) are the area in the onion genome flanked by microsatellite sequences. The amplification of these regions via PCR using a single primer produced multiple amplification products which could be used as a dominant multilocus genetic marker for studying the genetic diversity in various treatments. Microsatellites are the regions in the genome that consists of short DNA motifs as usually 2 – 5 nucleotides long which repeated linearly multiple times. In this study three water effluents collected from different water resources in addition to the control water from Nile River were used for testing their genetic effects on the onion genome. Six molecular markers were used in this study to achieve genetic modification induced among the four treatments of water effluents on onion roots. The amplified products varied in size from 150 – 1200 bp. ISSR – 3 primer showed that the poor quality water resulted from fertilizer industry which generated a lower number of bands followed by Menyet El Nasr and Kafr El – Sheikh drainage waters, respectively. The better quality water obtained from the Nile River which generated the highest number of bands in relation to other water effluents from different resources. This indicated that the total number of generated fragments was associated with better water quality.

Keywords *:* ISSR markers , testing water quality.

INTRODUCTION

Water quality for the irrigation purposes was affected on the quality and the yield of edible crops, as well as on the physical changes in the soil (FAO 1985). The quality of water used for irrigation purposes must be well within the permissible values. Water quality was affected by physical, chemical and biological characteristics of water. River water carrying a good quality with sediment load but can be use for irrigation as well. The characteristics of water quality were significant in water resources and management for drinking, industrial and irrigation purposes (Haydar *et al*. 2009). The development on water quality resulted to find out the level of acceptability. The possible factors affecting on water quality included nutrients, organic matter, heavy metals, suspended soilds, pesticides, drainage water effluents, human activities, chemical fertilizers and industrial chemicals, all of which have the main negative impacts on the water quality of most water sources (FAO 2013). Water quality is critical for the health of humans , animals , agricultural plants and food industry. In addition, proper management is prerequisite to meet water quality standards for balanced ecosystem. Due to use the poor water quality the characteristics of soil were affected and damage the yield of crops by several ways (Shakoor 2015). Poor water quality is one of the most important dangers threatening human and animal health, as well as, affecting on the ecological balance. All kinds of water effluents cause adverse effects on the soil and in turn on the living organisms in such areas. The use of wastewater in the irrigation of agricultural plants affects mitotic and meiotic divisions of plants. If these plants are consumed as food, it may affect human health adversely. The chemicals profile on the plants irrigated with wastewater could give rise to serious consequences such as allergy, respiratory disorders and cancer in middle ages (Sik *et al*. 2009).

To test the carcinogenic potentials of poor water quality such as drainage water and industrial wastewater, *in vitro* tests were used to measure the genotoxic effects to understand the relationship between mutagenic and carcinogenic potentials of these water effluents, one of these tests used for measuring the genotoxic effects was chromosomal aberrations and their frequencies (Magdaleno *et al* . 2008). Most studies on environmental quality focused on water quality management, because of its significance in maintaining human health and aquatic ecosystem. The addition of different kinds of pollutants and nutrients via the agricultural run off in the water rivers caused changes in physical and chemical properties of water . Agricultural applications releases residues which may decrease the quality of water resources.

Inter–simple sequence repeat (ISSR) is a technique of molecular marker that amplified inter–microsatellite sequence at various loci throughout the genome via a signle primer 16- 18 bp long composed of a repeated sequence by 2–4 arbitrary nucleotides. Each amplified band corresponds a unique DNA sequence delimited by two inverted micro – satellites leading to multilocus and highly polymorphic bands in which fragments are often polymorphic between various treatments (Zietkiewicz *et*

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al. 1994). Inter – simple sequence repeat ($ISSR_S$) are dominant genetic markers which could generate a large numbers of high informative and reproducible alleles (Nagaoka and Ogihara 1997). Molecular markers had overcome the limitations of using morphological and biochemical markers resulted from the effect of environment on the expression of genotypes. $ISSR_S$ analysis was considered as efficient molecular marker which appeared genetic variations in the populations (Ajal *et al*. 2014). It was used to evaluate the genetic diversity between treatments.

$ISSR_S$ is a good genetic marker to detect the effect of treatments on the genome which may be affected by water effluents via lacking genetic information (Ng and Tan 2015). Each band of $ISSR_S$ corresponds to DNA sequence demarcated by two inverted microsatellites (Abdel-Mawgood 2012). The advantages of ISSR markers are that the microsatellite sequences are highly different and universally distributed among the genome. ISSR is a significant genetic marker for detect genetic variations in the genome (Iruela *et al*. 2002). The present study was performed to assess the genetic variations induced by water effluents by evaluating their genetic effects on onion genome using molecular markers that will help the geneticist to detect water quality on the molecular level.

MATERIALS AND METHODS

Genetic materials

Onions bulbs (*Allium cepa* L., Family *Amaryllidaceae*) were collected commercially from the local market in Mansoura city, Dakahlia Governorate and sun-dried for two weeks. Thereafter, the healthy dry bulbs were used for the genetic test.

The results of the Allium test may moniter the cytotoxic or genotoxic components in the environment, which indicate the direct or indirect risks for all living organisms (El-Shahaby *et al*. 2003).

Study sites

This investigation was carried out on the effluent discharged from fertilizer industry in Dakhalia Governorate. Drainage water was also collected from the largest wells of leaching water effluent in Menyet El–Nasr center (Dakhalia Governorate) and Kafr El–Sheikh Governorate through July 2018. The control samples was the natural resource of water from the Nile River.

Preparation of solutions

Solutions that are needed in this study were prepared according to Payus *et al*. (2016).

ISSR – PCR Reactions

A set of six primers ISSR (Table 1) was used in the detection of polymorphism. The amplification reaction was carried out in 25 µl reaction volume containing 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 µM primer, 1 U Taq DNA polymerase and 30 ng template DNA.

Table 1. Primers name and sequences used in ISSR analysis.

Primer Code	Sequence 5'-3'
ISSR- 2	5'-AGAGAGAGAGAGAGAGYG-3'
ISSR- 3	5'-ACACACACACACACACYT-3'
ISSR- 4	5'-ACACACACACACACACYG-3'
ISSR- 5	5'-GTGTGTGTGTGTGTGTYG-3'
ISSR-6	5'-CGCGATAGATAGATAGATA-3'
ISSR-8	5'-AGACAGACAGACAGACGC-3'

A: adenine; T: thymine; G: Guanine, C: cytosine and Y: (C or T).

DNA extraction

Total genomic DNA was extracted from the onion roots grown in water effluents from three resources ; Kafr El–Sheikh drainage water, Menyet El–Nasr drainage water , water effluents of fertilizer industry, as well as that grown in natural water of Nile river as a control, by using DNeasy Tissue kits (Qiagene). The integrity of DNA_S obtained from these four treatments were checked on agarose gel electrophoresis accoding to Sambrook *et al*. (1989) and Payus *et al*. (2016) .

Thermocycling Profile

PCR amplification was performed in a Perkin-Elmer/GeneAmp® PCR System 9700 (PE Applied Biosystems) programmed to fulfill 35 cycles after an initial denaturation cycle for 5 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 50 Sec. an annealing step at 45ºC for 50 Sec. and an elongation step at 72ºC for 1min. The primer extension segment was extended to 7 min at 72ºC in the final cycle.

ISSR fingerprinting

Run 2 µl of DNA samples on agarose gel in comparison to 10 µl of a DNA size marker (100bp DNA Ladder). (Attalah *et al*. 2014). The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TBE buffer at 95 volts. A 100bp DNA ladder was used as a molecular size standard. PCR products were visualized on UV light and photographed using a Gel Documentation System (BIO-RAD 2000). The comparison between treatments based on the presence or absence of reproducible polymorphic DNA bands was conducted to show the similarity coefficients by SPSS program version–18. A dendrogram was designed based on similarity cofficients by the unweight pair group method with arithmetical average (UPGMA) according to Iruele *et al*. (2002).

Data Analysis

The banding patterns generated by ISSR-PCR marker analyses were compared to determine the genetic relatedness of the samples under study. Clear and distinct amplification products were scored as '1' for presence and '0' for absence of bands. Bands of the same mobility were scored as identical. The genetic similarity coefficient (GS) between two genotypes was estimated according to Dice coefficient (Sneath and Sokal 1973) as follow:

Dice formula: Gsij = 2a/(2a+b+c)

Where, Gsij is the measure of genetic similarity between individuals i and j, a is the number of bands shared by i and j, b is the number of bands present in i and absent in j, and c is the number of bands present in j and absent in i.

The similarity matrix was used in the cluster analysis. The cluster analysis was employed to organize the observed data into meaningful structures to develop taxonomic affinity. At the first step, when each accession represents its own cluster, the distances between these accessions are defined by the chosen distance measure (Dice coefficient). However, once several accessions have been linked together, the distance between two clusters is calculated as the average distance between all pairs of accessions in the two different clusters. This method is called unweighted pair group method using arithmetic average (UPGMA) (Sneath and Sokal 1973).

Estimation of basic parameters

A band scored in ISSR can also be termed a locus, each ISSR band is separately considered as one locus. Meanwhile, the number of polymorphic bands in the number of ISSR bands that showed variation, i.e. the bands is present in some samples and absent in the other ones. The percentage of polymorphic bands was calculated by the formula as follows:

% of polymorphic bands = Number of polymorphic bands / Total number of bands x 100.

RESULTS AND DISCUSSION

Primer ISSR – 2

As shown from the results presented in Figure 1 and Tables 2 and 3 the average number of polymorphic bands per primer 2 was 9.0. The percentage of polymorphism for this primer was 69%. The fragment sizes ranged from 160 to 830 bp . The mean of band frequency was 0.53. The ability of this primer to differentiate between the effects of water effluents on onion genotype was assessed using the number of bands according to Prevost and Wilkinson (1999). Whereby the number of bands appeared varied from 6 under the effect of irrigated water from the Nile River to 10 under the effect of fertilizer industry effluents. These results indicated that under the effect of water effluents some bands appeared, while some other bands disappeared. Molecular markers have a significant impact to assess the effect of environmental pollution due to irrigated water containing factory effluents which affect on the performance of plant genotypes. A wide range of molecular markers has been used to estimate genetic diversity of plant genotypes from different parts of the world. The results obtained in this study agreed with Hasnaoui *et al.* (2010) , who showing different ranges of genetic polymorphism in the genotypes of pomegranate using SSR markers. In addition, Ajal *et al*. (2014) reported that inter – simple sequence repeats (ISSR) analysis was considered as efficient molecular marker, showing genetic diversity in the wild populations of pomegranate studied in the Western Himalaya region. Meanwhile, Sheidai and Noormohammadi (2005) decided that cytological and DNA markers (RAPD, AFLP, SSR and ISSR) had been used to assess the genetic diversity of Iranian pomegranates. These studies demonstrated the occurrence of high genetic variations among Iranian genotypes at both cytogenetic and molecular levels including RAPD , AFLP and SSR markers.

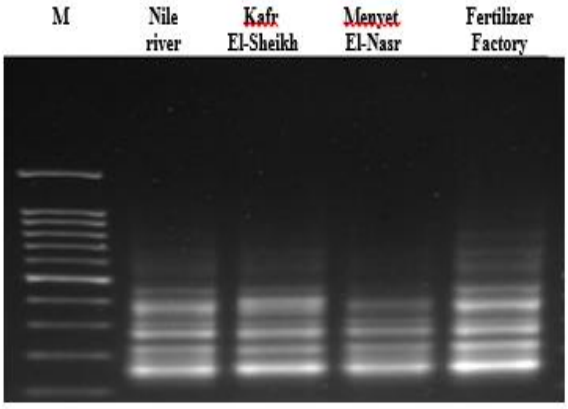

Figure 1 . ISSR profiles of four water samples treated onion roots as revealed by primer ISSR – 2.

Table 2. Number of bands and their molecular sizes obtained from molecular analysis of four treatments of onion roots with different water samples based on ISSR – 2 primer.

Molecular marker	Nile river	Kafr El-Sheikh	Menyet El-Nasr	Fertilizer Factory	Frequency
830	0	0	0	1	0.3
670	0	0	0	1	0.3
540	0	0	0	1	0.3
410	1	1	0	1	0.8
360	0	1	0	0	0.3
340	0	0	1	1	0.5
330	1	0	0	0	0.3
280	1	1	1	1	1.0
250	1	1	1	1	1.0
210	1	1	1	1	1.0
180	0	0	0	1	0.3
170	0	1	0	0	0.3
160	1	1	1	1	
Total bands	6	7	5	10	1.0

Table 3. Analysis of genetic polymorphism obtained by ISSR-2 primer from four different water samples treated onion roots.

Banding pattern	Number
Monomorphic bands	4
Polymorphic (without Unique)	2
Unique bands	7
Polymorphic (with Unique)	9
Total number of bands	13
Polymorphism (%)	69%
Mean of band frequency	0.53

As shown from the results presented in Figure 2, cluster analysis assess the level of polymorphism between the four treatments of water samples treated onion roots. The genetic distances shown among the four samples ranged from 0.64 to 0.90 based on ISSR profiles. The dendrogram demonstrated three distinic major groups. The first group includes one cluster which treated with the Nile river water, as well as, Kafr El – Sheikh Governorate drainage water, both shown some similarity between each other . The second group includes the treatment of irrigated water effluents of Menyet El – Nasr Center, Dkhalia Governorate. The third group includes the treatment of fertilizer industry effluents. The second and the third groups showed low genetic similarity. This may be due to the higher genotoxicity effect of each source of water effluents on the genotype of onion. The number of amplified fragments varied from 5 (Water effluents of Menyet El–Nasr) to 10 (Water effluents of fertilizer industry) across the samples in relation to the number of fragments in the control which equal six. ISSR primers showed a high percentage of polymorphic bands reached to 69 %. These markers could be used for characterization the genetic effect of water effluents on the genotype of plants grown under the irrigation of water drainage. The results also shown a high degree of genetic diversity between the waste water from different resources. Hence, despite the relative degree of diversity, the onion genotype represents a degree of similarity. The results obtained in this study agreed with Attallah *et al*. (2014), who found similarity index among nine *Beauvaria* species ranged from 0.531 to 0.876 (RAPD) and 0.625 to 0.967 (ISSR) .

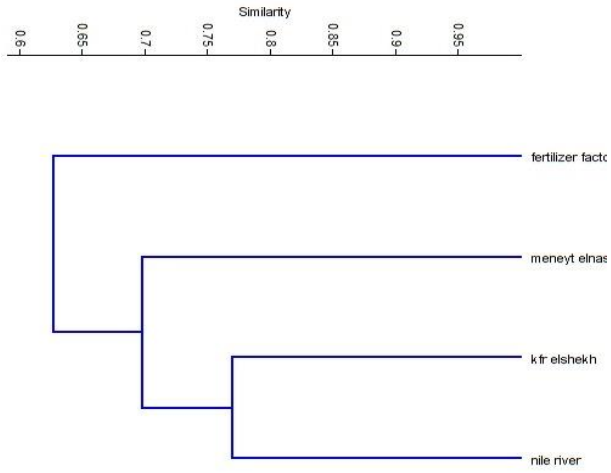

Figure 2 . Dendrogram illustrated the distribution of four water samples based on water quality using UPGMA dependent on Jacquards similarity coefficient obtained from ISSR analysis using the primer ISSR – 2 .

Primers ISSR – 3

As shown from the results presented in **Figure 3** and **Tables 4 and 5** the higher number of bands (9) appeared with the treatment of water from the Nile river, followed by Kafr El – Sheikh drainage water (8), followed by Menyet El–Nasr drainage water (7), as well as , the water effluents produced from fertilizer industry produced the lower number of bands (6). The results indicated that the number of bands ranged between 9-6. This attributed to the poor quality of water effluents which appeared lower number of bands due to the treatment with fertilizer industry effluents, followed by Menyet El Nasr and Kafr El – Sheikh drainage waters, respectively. The better quality of Nile river water produced the highest number of bands in relation to the other treatments of water effluents. The fragment sizes of bands were ranged between 200 – 550 bp.

The total number of generated fragments as shown by primer ISSR–3 showed response with better water quality . This primer produced 30 bands across the four water samples. The percentage of polymorphism reached 33% , as well as the mean of band frequency was equal 0.83.

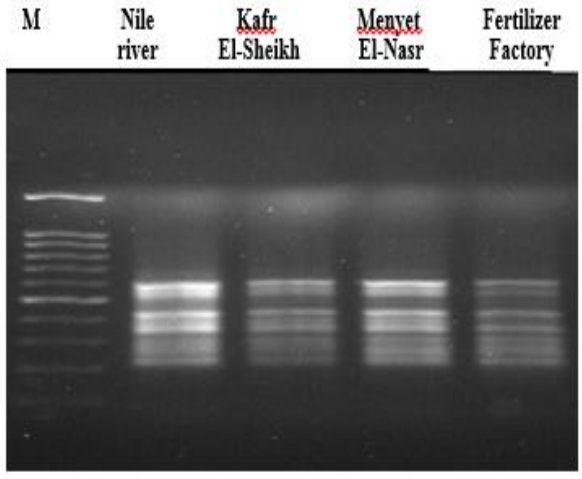

Figure 3 . ISSR profiles of four water samples treated onion roots as revealed by primer ISSR – 3.

Table 4. Number of bands and their molecular sizes obtained from molecular analysis of four treatments of onion roots with different water samples based on ISSR – 3 primer.

Molecular marker	Nile river	Kafr El-Sheikh	Menyet El-Nasr	Fertilizer Factory	Frequency
550	1	1	1	1	1.0
470	1	1	1	1	1.0
360	1	1	1	1	1.0
300	1	1	1	1	1.0
260	1	1	1	1	1.0
250	1	1	0	0	0.5
240	1	1	1	0	0.7
220	1	0	0	0	0.3
200	1	1	1	1	
Total bands	9	8	7	6	1.0

Table 5. Analysis of genetic polymorphism obtained by ISSR-3 primer from four different water samples treated onion roots.

Banding pattern	Number
Monomorphic bands	6
Polymorphic (without Unique)	2
Unique bands	1
Polymorphic (with Unique)	3
Total number of bands	9
Polymorphism (%)	33%
Mean of band frequency	0.83

As shown in Figure 4 a dendrogram obtained from ISSR data illustrated that the four treatments of water effluents produced two distinct groups. The first group includes one cluster of Nile river water and Kafr El–Sheikh Governorate drainage water which are closly related distance. The similarity matrix was ranged between 0.94 – 0.99. Meanwhile, the second group includes the treatment of water effluents of fertilizers industry and Menyet El – Nasr drainage water which are closely related distance. The similarity matrix of this group was ranged between 0.92– 0.99. These results indicated that the second cluster was placed as poor quality water in relation to the first cluster. It may be due to the reason that the water effluents in these regions were chemically and physically changed because of high pollutants that may altered water quality. These results agreed with Madadi *et al.* (2017), who obtained genetic differences within each cultivar of pomegranate as one of the oldest edible fruits of horticultural crops in Iran, and these variation may be due to different environmental conditions. In addition, Anne (2006) reported that a good genetic marker have the ability to generate multilocus data in the genome under investigation. The ISSR markers use microsatellite sequences that are highly variable and ubiquitously distributed across the genome which achieving high reproducibility in relation to RAPDs. This makes ISSR markers an ideal genetic marker for genetic diversity studies (Shen *et al.* 2006). The results showed that the primers varied greatly in their ability to resolve the variability between many the treatments of water effluents. Some primers produced more number of bands, while the others appeared a less number. The results obtained herein appeared a high genetic variation among the treatments of water effluents which indicated variations in water quality from the four resources. These results reflected that the genetic variations induced in onion genotype was related to

water quality. The variation in the number of bands amplified by variable primers affected by different factors as primer structure and genome annealing sites (Kernodle *et al*. 1993). In this study different primer structures were used to reveal the genetic diversity among the treatments which showed a high rate of diversity between them. The intensity of the bands was not considered because the concentration of DNA was not controlled exactly at 50 ng for each sample. This means that the number of DNA template copies was not similar among all the samples that may be affected the intensity of the observed bands. In this investigation, the unique band and the polymorphic bands were observed. The unique band defines as the presence of a band in a specific treatment and absence the same band of the same size in all other treatments (Raghunathachri *et al*. 2000).

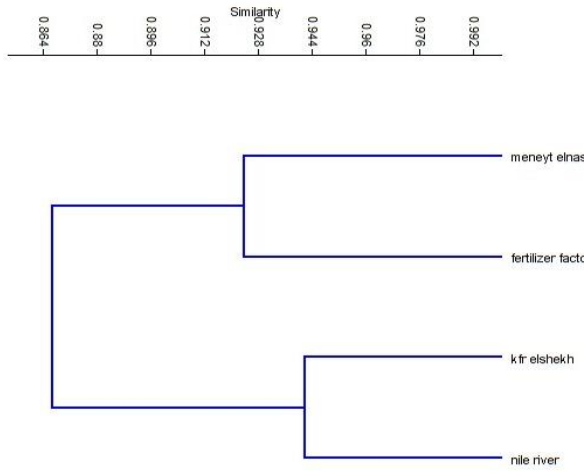

Figure 4. Dendrogram illustrated the distribution of four water samples based on water quality using UPGMA dependent on Jacquards similarity coefficient obtained from ISSR analysis using the primer ISSR – 3 .

Primer ISSR – 4

As shown from the results presented in Figure 5 and Table 6 and 7 the ISSR–4 primer produced a total of 12 amplified fragments, four of which were monomorphic and eight were polymorphic.

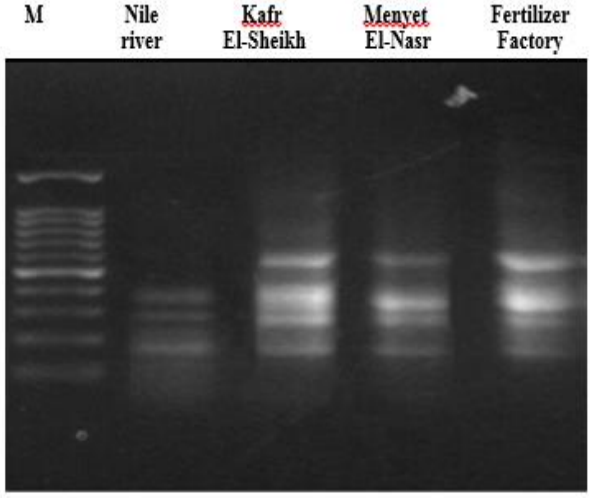

Figure 5. ISSR profiles of the four water samples treated onion roots as revealed by primer ISSR – 4.

Table 6. Number of bands and their molecular sizes obtained from molecular analysis of four treatments of onion roots with different water samples based on ISSR – 4 primer.

Molecular marker	Nile river	Kafr El-Sheikh	Menyet El-Nasr	Fertilizer Factory	Frequency
1200	0	1	0	0	0.3
1150	0	0	0	1	0.3
920	1	1	0	0	0.5
780	1	1	1	1	1.0
740	1	1	1	0	0.3
520	0	1	1	1	0.8
340	1	1	1	1	1.0
280	0	1	1	1	0.8
260	1	0	0	0	0.3
230	0	1	1	1	0.8
210	1	1	1	1	1.0
150	1	1	1	1	
Total bands	7	10	8	8	1.0

Table 7. Analysis of genetic polymorphism obtained by ISSR-4 primer from four different water samples treated onion roots.

Gel Polymorphism	Number
Monomorphic bands	4
Polymorphic (without Unique)	4
Unique bands	4
Polymorphic (with Unique)	8
Total number of bands	12
Polymorphism (%)	67%
Mean of band frequency	0.67

The mean number of band frequency was 0.675. The percentage of polymorphism was 67 % . Atotal of 33 bands produced among the four treatments of different water samples , out of which 8 were polymorphic bands. The major part of genetic variations shown by the ISSR– 4 primer occurred within the treatment of Kafr El– Sheikh drainage water which produced 10 bands, followed by Meneyt El–Nasr drainage water and fertilizer industry effleunts, all of which produced eight bands in relation to natural water resource of Nile river which produced seven bands. The treatments with fertilizer industry effluents , as well as Meneyt El–Nasr drainage water were generated variably less than Kafr El – Sheikh drainage water.

In order to investigate genetic relationships among the treatments of water effluents cluster analysis based on Dice coefficients of genetic distance and UPGMA algorithm were pointed out as shown in Figure 6 . According to the clustering pattern obtained by ISSR, the four treatments of water from different sources classified into two distinct groups. The first group including one cluster which contained all poor water quality as fertilizer industry effluents, Kafr El–Sheikh and Meneyt El–Nasr drainage water. The genetic similarity of this cluster ranged from 0.82 – 0.95. This indicated that the water effluents from these resources were less quality and may have a genotoxic effects on the genotypes, as well as ecosystem grown in this environment. The second group includes the treatment of Nile river which is a natural resource of water with a good quality than the other resources examined in this study. Based on ISSR dendrogram, the first group consists of water effluents from three resources and the second including the natural resource of water. The dendrogram obtained showed the best clustering pattern.

The genetic analysis using this primer would be more useful for collecting all water resources with poor quality in one cluster and the natural resources of good water quality in another cluster. Information about the degree and distribution of genetic variations generated by the water effluents has a significant effects on water quality testing on the molecular level. ISSR marker was an efficient technique for estimating the genetic variations resulted from poor water quality. Additionally, the higher polymorphic fragment percentage and the number of unique and polymorphic bands obtained in this study indicated the power of ISSR molecular markers in fingerprinting and genetic diversity analyses to assess water quality on the molecular level. Also, the results obtained in this study revealed genetic diversity among the treatments of onion with water effluents, which can be used for testing water quality on the molecular level. Thus should focus on testing water quality on the molecular level before reuse to be improving before using its if they are poor quality via development of treatment to these resources. These results are in harmony with El–Kawokgy *et al*. (2018), who obtained 97 sourced bands of PCR products in *Bacillus* via three primers, out of which 80 were polymorphic bands and 12 were unique bands. These agreed with the number of markers generated by RAPD and ISSR methodology in rice (Kaushik *et al*. 2003), as well as in *Agrobacterium* (EL-Shaer *et al*. 2014). The fragment sizes of bands obtained were ranged between 150 – 1200 bp. This clearly indicated that the treatment with water effluents harbours broad spectrum of genetic diversity induced by poor water quality and therefore ISSR technique shall be used as parental candidates in testing water quality programe aiming toward improvement of water resources before reuse of water effluents.

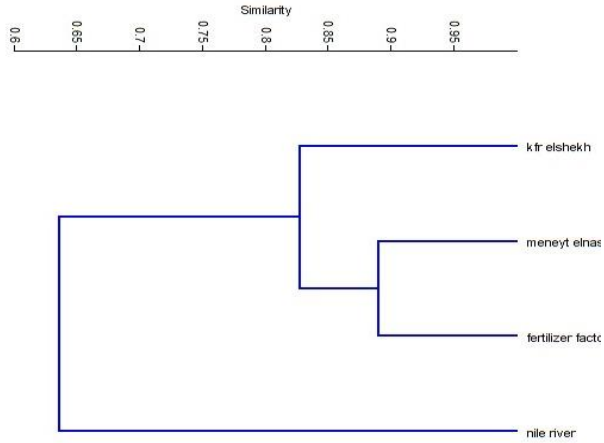

Figure 6. Dendrogram illustrated the distribution of four water samples based on water quality using UPGMA dependent on Jacquards similarity coefficient obtained from ISSR analysis using the primer ISSR – 4 .

Primer ISSR – 5

The results presented in Figure 7 and Tables 8 and 9 showed that the ISSR–5 primer produced a total of 12 amplified fragments of which seven were polymorphic bands. The percentage of polymorphism was 58%. The mean of band frequency per this primer was 0.7. The fragment sizes of bands obtained were ranged between 160 – 690 bp. The less number of bands resulted from the treatment with fertilizer industry effluents which generated seven bands in relation to the control which generated 10 bands. This indicated that some bands were disappeared in the treatment with fertilizer industry effluents. This may be due to the higher genotoxic effects of these effluents on the genotype in relation to other water effluents which appeared the same number of 10 bands as seen in the control treatment. This indicated that the genotoxic effect may generated some bands disappeared as seen in the treatment with fertilizer industry effluents. Out of 12 bands generated by ISSR- 5 primer, 5 were monomorphic and 7 were polymorphic, thus generating 58 % polymorphism.

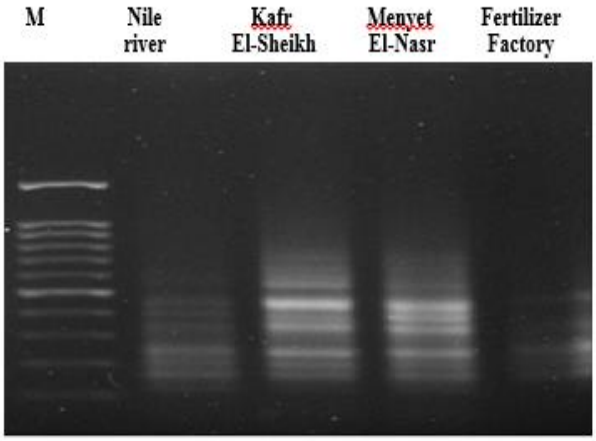

Figure 7. ISSR profiles of four water samples treated onion roots as revealed by primer ISSR – 5.

Table 8. Number of bands and their molecular sizes obtained from molecular analysis of four treatments of onion roots with different water samples based on ISSR – 5 primer.

Molecular marker	Nile river	Kafr El-Sheikh	Menyet El-Nasr	Fertilizer Factory	Frequency
690	1	1	1	0	0.8
580	1	1	0	0	0.5
480	1	1	1	0	0.8
400	0	0	0	1	0.3
380	1	1	1	0	0.8
340	1	1	1	1	1.0
330	1	1	1	0	0.8
290	0	0	1	1	0.5
280	1	1	1	1	1.0
210	1	1	1	1	1.0
180	1	1	1	1	1.0
160	1	1	1	1	
Total bands	10	10	10	7	1.0

Table 9. Analysis of genetic polymorphism obtained by ISSR-5 primer from four different water samples treated onion roots.

Banding pattern	Number
Monomorphic bands	5
Polymorphic (without Unique)	6
Unique bands	1
Polymorphic (with Unique)	7
Total number of bands	12
Polymorphism (%)	58%
Mean of band frequency	0.7

Clustering pattern as depicted by the dendrogram shown in Figure 8 indicated that the application of ISSR

assay classified the four treatments of water into two distinct groups. The first group includes one cluster which contained only the treatment of fertilizer industry effluents which appeared the less water quality because of mainly physical and chemical changes of this water resource. The second group includes the treatments with the natural resource of water from the Nile river, Kafr El – Sheikh and Meneyt El–Nasr drainage waters. The second cluster further divided into two sub cluster, one of which included the treatment of Meneyt El–Nasr drainage water, while the second sub cluster included Kafr El–Sheikh drainage water and the water of Nile river. This indicated that the water effluents of fertilizer industry, as well as Meneyt El–Nasr drainage water followed by Kafr El – Sheikh drainage water were less water quality if compared with the water from the Nile river which is high quality water in relation to other water resources. In this study cluster analysis was done to assess the level of polymorphism between the treatments of water from different resources. A dendrogram was constructed using a distance matrix using the UPGMA method based on genetic distances from ISSR marker analysis which showed two main groups were observed. These results agreed with Attallah *et al*. (2014), who found that the microsatellite markers were highly polymorphic and so important for genetic analysis of *Beauvaria sp*. The major highlight of this study is the application of ISSR – based analysis for testing genetic diversity induced among the treatments of water effluents. This analysis provided important knowledge about the effect of water drainage on the ecosystem grown in the environment affected by industrial, as well as drainage water which showing better response towards the generation of genetic diversity. In addition, the ISSR data UPGMA analysis clearly separated the treatments of water effluents into distinct groups. The results obtained in this study agreed with El–Kawokgy *et al*. (2018), who used four ISSR primers detected 55 amplicons among which 92.696 % bands were polymorphic. The genetic similarity values obtained by the same authors among *Bacillus* species based on ISSR profiles were ranged between 0.50 to 0.83.

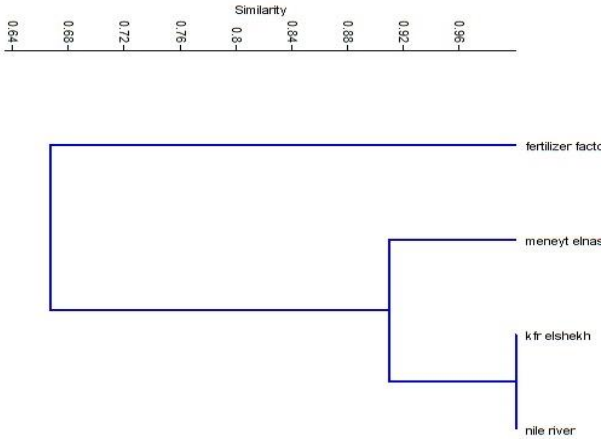

Figure 8. Dendrogram illustrated the distribution of four water samples based on water quality using UPGMA dependent on Jacquards similarity coefficient obtained from ISSR analysis using the primer ISSR – 5 .

Cluster analysis was performed in this study to construct a dendrogram of water quality tested to reveals the genetic diversity among the treatments of water effluents from different resources. It can be concluded that each molecular marker approaches of DNA analysis could define the variations between the treatments of water effluents from the different resources which possess a high degree of genetic diversity. The variations recognized on the DNA level are mainly reflected the water quality which is a better biotechnology for assessment the quality of water tested.

Primer ISSR – 6

The results presented in Figure 9 and Tables 10 and 11 showed that primer ISSR – 6 produced a total of 3 amplified fragments without any percentage of polymorphism. The fragments obtained were monomorphic bands. The fragment sizes of these bands were ranged between 220 – 350 bp. This primer do not show any differences between the four treatments of water resources on the molecular level. This indicated that this primer was not recommended to be used for detection of genetic variations between treatments of water effluents . Based on this marker analysis it can not be used for testing water quality because it can not be defined any genetic variations among the treatments detected .This marker was not considered as a perfect genetic marker for treatment of water effluents with lacking genetic information (Ng and Tan 2015). However, PCR amplification of ISSRS sites using a single primer produced multiple amplification products which could be used as a dominant multi locus marker in achieving the genetic variations generated by different water effluents from variable resources. This primer was not complementary to a target microsatellite. This regard to every band corresponds to a sequence of DNA demarcated by two inverted microsatellites (Abdel–Mawgood 2012) .

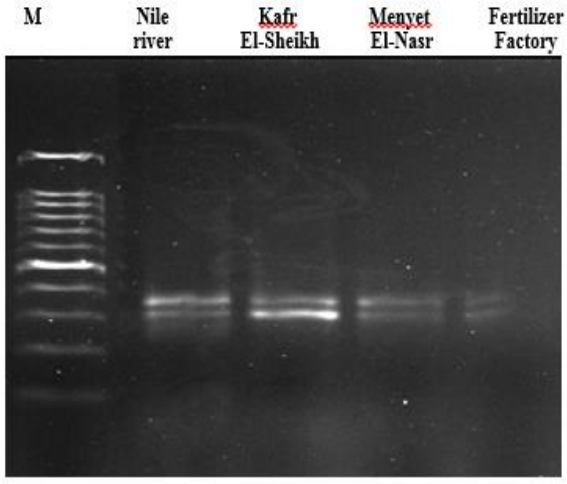

Figure 9. ISSR profiles of four water samples treated onion roots as revealed by primer ISSR – 6.

Table 10. Number of bands and their molecular sizes obtained from molecular analysis of four treatments of onion roots with different water samples based on ISSR – 6 primer.

Molecular marker	Nile river	Kafr El-Sheikh	Menyet El-Nasr	Fertilizer Factory	Frequency
350	1	1	1	1	1.0
270	1	1	1	1	1.0
220	1	1	1	1	0.3
Total bands	3	3	3	3	

Table 11. Analysis of genetic polymorphism obtained by ISSR-6 primer from four different water samples treated onion roots.

Banding pattern	Number
Monomorphic bands	3
Polymorphic (without Unique)	0
Unique bands	0
Polymorphic (with Unique)	0
Total number of bands	3
Polymorphism (%)	0%
Mean of band frequency	0.8

In genetic diversity experiments, a better marker have appeared a high genetic variations with the ability to induce multi locus results among the studied genome (Anne 2006). So this marker tack disadvantage to assess microsatellite sequences that are much variable and universally distributed among the genome. For these reasons this marker was not successfully used to assess the variations of water quality from different resources. In addition, this primer was not successfully to detect polymorphic loci among the different water samples – treated onion roots. The results obtained by this primer dis – agreed with El – kawokgy *et al.* (2018), who found high genetic variation among the parents and their fusants of *Bacillus*. Furthermore, Hibbett *et al.* (2007) demonstrated that differentiation in the number of bands amplified by various primers affected by different factors as primer structure and the annealing regions in the genome. Though this study used different primer structures to assess the genetic diversity generated by different water resources among the genome. Then the primer used herein do not succeeded to characterize the effect of water effluents on the molecular level.

Primer ISSR – 8

The results presented in Figure 10 and Tables 12 and 13 appeared that the ISSR–8 primer generated a total of 14 amplified fragments. Eight of which were monomorphic and six were polymorphic fragments. The percentage of polymorphism reached 43 % , although the mean frequency of bands was equal 0.77. The fragment sizes of these bands were ranged between 230–820 bp. This marker well differentiate between the treatments of water effluents from the different resources. This primer could be considered in discovering the polymorphism between the effect of different water effluents. It was revealed that the natural water from the Nile river, Kafr-El Sheikh drainage water, Meneyt El – Nasr drainage water and fertilizer industrial effluents generated 14, 11, 10 and 8 amplified fragments, respectively. This indicated that the lower number of fragments may respond to the high genotoxicity effect on the genotype. Consequently, the poor water quality may generated the lower number of bands if compared with the better water quality which generated the higher number of bands. Thus, the primer ISSR–8 was identified as the better primer for distinguishing the water quality through genetic diversity induced among the treatments of different water resources. This indicated that the lower number of fragments may respond to the high genotoxicity effect on the genotype. Consequently, the poor water quality may generated the lower number of bands if compared with the better water quality which generated the higher number of bands. Thus, the primer ISSR–8 was identified as the better primer for distinguishing the water quality through genetic diversity induced among the treatments of different water resources. Thus the major part of generated bands was occurred within the treatment with better quality water from the Nile river, meanwhile the lower number of generated bands was induced from the treatment with fertilizer industry effluents followed by Meneyt El–Nasr and Kafr-El Sheikh drainage waters, respectively. A summary of genetic diversity indices in four different treatments of water resources appeared that treatments of water resources appeared that water quality was varied from one resource to another which is the best in Nile river water and lowest in fertilizer industry effluent. Moreover, the polymorphism information content among the treatments of water effluents which represents a measure of allelic diversity at a locus matches with a primer ISSR–8 showed 43 % polymorphism. In this respect, Botstein *et al.* (1980) decided that the polymorphism information content (PIC) index can be used to estimate the level of gene variation. Therefore, the results obtained in this study suggested that the primer ISSR- 8 is one of the most informative marker type among the makers used in this study. Interestingly, this primer can be used to achieve the target genetic sequences which can reflect the water quality tested. The results indicated that ISSR–8 primer successfully produced unique positive or negative markers. It was succeeded to characterize the four treatments of water effluents which related to water quality testing. The number of unique bands matched with this primer was 6 bands. These results agreed with Alsamman *et al.* (2017), who found that a variety Maraki of olive was characterized by the highest number of unique markers, moreover the variety Manzanillo was characterized via the fewer number of unique markers. Interestingly, the same authors found that the variety calamata was identified by six positive unique markers. However, Besnard *et al.* (2001) successfully characterize an olive cultivar by three different unique RAPD markers. The $ISSR_S$ markers proved to be a reliable tool for testing water quality via the genetic diversity distinguish closely the different water resources which cover a large genomic area. In addition, the potential use of ISSR markers to distinguish water quality on the molecular level by unique and polymorphic markers could be useful for the detection of genetic diversity induced by the water effluents. This suggesting the importance of testing water quality before reuse in agriculture sector.

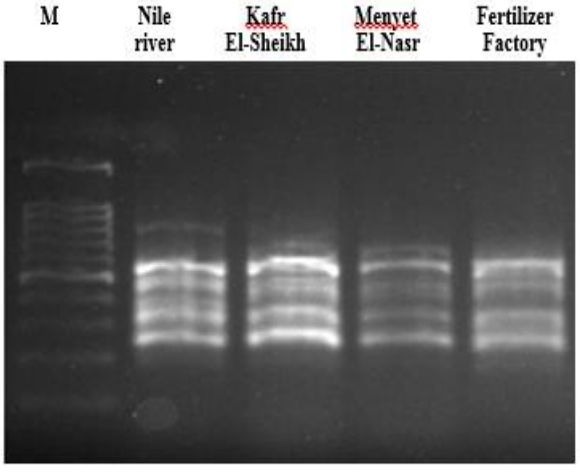

Figure 10. ISSR profiles of four water samples treated onion roots as revealed by primer ISSR – 8.

Table 12 . Number of bands and their molecular sizes obtained from molecular analysis of four treatments of onion roots with different water samples obtained from different resources based on ISSR – 8 primer.

Molecular marker	Nile river	Kafr El-Sheikh	Menyet El-Nasr	Fertilizer Factory	Frequency
820	1	0	0	0	0.3
650	1	1	1	0	0.8
560	1	1	1	1	1.0
520	1	1	1	1	1.0
450	1	1	1	0	0.8
430	1	1	1	1	1.0
420	1	0	0	0	0.3
400	1	1	0	0	0.5
360	1	0	0	0	0.3
340	1	1	1	1	1.0
300	1	1	1	1	1.0
270	1	1	1	1	1.0
250	1	1	1	1	1.0
230	1	1	1	1	
Total bands	14	11	10	8	1.0

Table 13. Analysis of genetic polymorphism obtained by ISSR-8 primer from four different water samples treated onion roots.

Banding pattern	Number
Monomorphic bands	8
Polymorphic (without Unique)	3
Unique bands	3
Polymorphic (with Unique)	6
Total number of bands	14
Polymorphism (%)	43%
Mean of band frequency	0.77

Today, molecular genetic markers were used increasingly to address the genetic diversity induced in the genotype. In this study molecular markers are used to address the water quality from different resources. Furthermore, microsatellites are undoubtedly still the marker of select for many studies on genetic diversity tested that require primers with high resolution, hypervariability and co – dominance (Kalia *et al*. 2011).

The present study illustrated the phylogenetic tree Figure 11 which appeared that the four water resources had divided into two major groups. The first group included the high water quality from the Nile river .

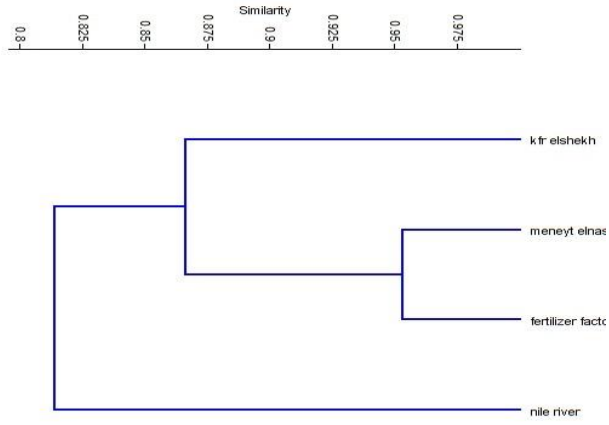

Figure 11 . Dendrogram illustrated the distribution of four water samples based on water quality using UPGMA dependent on Jacquards similarity coefficient obtained from ISSR analysis using the primer ISSR – 8 .

However , the second groups subdivided into two sub – cluster , the first one included the poor water quality from Meneyt El–Nasr drainage water and fertilizer industry effluents, the second sub – cluster included the drainage water from Kafr-El Sheikh Governorate which is less poor water quality than the other resources of water effluents used in this study.The water quality was responding to banding pattern. These results indicated that ISSR primers were successfully assessed the genetic diversity induced by drainage water from different resources. Additionally, ISSR markers can be developed for population genetic analysis to generate PCR or amplicons products to achieve water quality from the drainage resources before reuse in agricultural sector. The dendrogram discussed herein showed that the four water resources were divided into three major groups of water quality. These results agreed with Attallah *et al*. (2014), who found that microsatellite markers were highly polymorphic for genetic analysis.

In conclusion, ISSR markers known as random amplified microsatellites can be used for assessment genetic diversity induced multiple DNA fragments allowing the generation of a high number of sites among the genome. This leading these genetic markers to be used for evaluating the level of water quality to avoid the potentially harmful to human populations. Thus, it was necessary to be used for identification of water quality on the molecular level because it is powerful procedure to survey the genotoxic effects of water effluents. The use of more ISSR molecular markers may improve the reliability of this tool for identifying the water quality on the molecular level. Genetic similarity measured through cluster analysis of each primer revealed variation degree of genetic relatedness among the drainage water belonging to different resources. ISSR molecular markers varied in their ability to characterize water quality and for detecting polymorphisms among the treatments of drainage water, but they can complement each other.

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إختبار جودة المياه علي المستوى الجزيئى من خلال الإختلافات الوراثية المستحدثة في جينوم البصل بإستخدام التتابعات القصيرة المتكررة داخل الجينوم (ISSR)

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تهدف هذه الدراسة إلى إختبار جودة المياه علي المستوى الجزيئ بإستخدام تقنية ISSR لإظهار الإختلافات الوراثية المستحدثة داخل الجينوم الذي إمتص عينة المياه محل الدراسة. إن التتابعات الوراثية القصيرة داخل الجينوم المعروفة بالـ ISSR هى مناطق محصورة داخل جينوم البصل تحتوى علي تكرارات من التتابعات القصيرة للقواعد النيتروجينية . عملية تضخيم هذه المناطق بإستخدام تكنيك تفاعل البلمرة المتسلسل المعروف بالـ PCR بإستخدام بريمر واحد ينتج عنه عديد من النسخ المعظمة والتى يمكن إستخدامها كعلامات وراثية سائدة متعددة المواقع لدراسة الإختلافات الوراثية في المعاملات المختلفة . التتابعات القصيرة هي المناطق من الجينوم التى تتكون من قطع قصيرة من DNA يتراوح طولها من ٢- ٥ نيوكليتيدات والتى تتكرر داخل الجينوم عدة مرات بشكل خطى . تم إستخدام ثلاث عينات من المخلفات المائية التى تم جمعها من ثلاثة مصادر مختلفة للمياه بالإضافة إلى عينة المياه القياسية من نهر النيل وذلك لإختبار الأثر الوراثي لهذه المصادر المائية علي جينوم نبات البصل . تم أيضاً إستخدام ست مرقمات جزيئية ISSR لإظهار الإختلافات الوراثية المستحدثة على مستوى المعاملات الأربعة للمصادر المائية المختلفة علي جذور البصل. أوضحت النتائج إختلاف حجم الشظايا المعظمة من DNA تراوحت ما بين ١٥٠- ١٢٠٠ زوج من القواعد النيتروجينية . أظهر المرقم الجزيئى ISSR–3 أن الجودة المنخفضة لمياه الصرف الصناعى الناتجة عن صناعة الأسمدة الكيماوية قد أنتجت أقل معدل من عدد الحزم يليها المعاملات بمياه الصرف الزراعي بمنية النصر بمحافظة الدقهلية ثم مياه الصرف الزراعي بمحافظة كفر الشيخ ، علي الترتيب . بينما نتج عن مياه نهر النيل كمصدر مائي ذو جودة مرتفعة فى قطاع الزراعة وأعطى أعلي معدل من عدد الحزم بالمقارنة بالمعاملات الثلاثة الآخرى كمصادر لمياه الرى. تعكس النتائج المتحصل عليها من هذه الدراسة أن العدد الكلي للحزم التى تم إظهارها علي الجل كان متصلاً بالجودة العالية للمياه المستخدمة فى المعاملات المختلفة.