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Assessment of Water Quality Via Genetic Diversity Induced in Onion Using Inter Simple Sequence Repeats (ISSR) Markers

Mervat I. Kamal*; A. H. Abd El – Hady; K. A. Zaied and A. S. Abd El- Mohsen

Depatment of Genetic , Faculty of Agriculture, Mansoura University.

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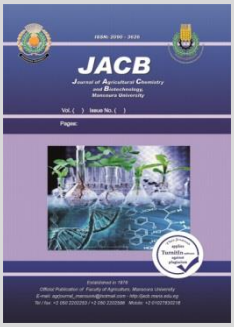

ABSTRACT

Inter simple sequence repeats (ISSR) or microsatellite markers were applied to assess genetic diversity induced in *Allium cepa* L. by drainage water and genetic relationship among five water resources which reflected water quality. Genomic DNA was extracted from fresh onion roots treated with drainage water from five resources. Six ISSR primers were initially used for screening water quality. Polymorphisms became evident through the presence and / or absence of DNA fragments in treated roots compared with the control treated with Nile River water. There was a distinct distance between the band profiles of treated roots and the control samples. When the cluster analysis was applied. The total number of bands generated by ISSR-3 molecular marker quality. This marker can be considered as a best reproducible primer to be used for screening water quality. This marker disappeared some bands depending on drainage water carrying toxicants. Similarity coefficients between different water resources was ranged from 0.76 to 0.98. The highest similarity coefficient was obtained between the drainage water from chemical fertilizer industry and oils and soap effluents. The lowest similarity was shown Nile River water and oils and soap industrial effluents. Number of polymorphic bands detected through six primers ranged from 3 to 10, whereas the unique fragments were ranged from 0 to 3. Finally , comparison between Nile River water treatment and treated genomes with drainage water shows that ISSR analysis can be used to evaluate the modifications induced in DNA structure in living organisms by drainage water carrying environmental pollution.

Keywords: ISSR markers , water quality

INTRODUCTION

Microsatellites or inter simple sequence repeats (ISSR) are short DNA sequence or short tandem repeats which are regions in the genome consisting of motifs of one to six nucleotides repeated multiple times in arrow, e.g….. AGC AGC AGC AGC…… Therefore, ISSRs are fragments of DNA that are flanked at both ends by such microsatellites sequences. A developed modification of ISSR– based marker is semi arbitrary markers amplified by PCR in the presence of one primer complemented to a target microsatellite (Meyer *et al.*1993). Using arbitrarily designed primers containing repetitive sequences complemented to microsatellite regions in the genome can be PCR – amplified which has been called microsatellite – primed PCR, or MP – PCR such amplification does not require informations about the genome sequence (Zietkiewicz *et al.* 1994). Primers containing repetitive sequences complemented to microsatellite location can be used as markers for genetic variation studies (Ng and Tan 2015). The alteration of a single nucleotide in a 10- base RAPD primer leading to a marked changed in the fingerprint of a given template. It is recognize that nucleotide changed in the genomic DNA must have the same effect on RAPD fingerprints as those as those shown for changing within the primer. The advantage of measuring the effect of genotoxic chemicals containing waste waters directely on DNA is mainly associated to the sensitivity of testing water quality. DNA modifications induced by drainage water were described enabling the generation of DNA fingerprints which have been used for screening DNA biodiversity related to water quality (Citterio *et al.* 2002). A large variety of DNA molecular markers can be used to assess the genotoxicity of drainage water which has been successfully used to screening the mutagenic effects of drainage water. Furthermore, Inter - simple sequence Repeat assay becomes more advantageous since it was described as a more reproducible technique. This tool becomes of great importance since screening of genotoxic effects involves the comparison of patterns generated from the control and treated plants (Vanijajiva 2011).

In particular, Polymerase Chain Reaction (PCR) is used to analyse the Inter- Simple Sequence Repeats (ISSR) to screening the genetic diversity induced among different treatments of drainage water. Therefore, this technique, due to high repeatability and polymorphism, as well as highly informative, is suitable for screening genetic diversity in different crops (Bornet and Branchard.2001). Biological assessment through biomarkers of exposed living organisms providing a tool for screening genotoxic pollutants that may cause damage to human health (Singh 2017). The most popular bioassays in genotoxicity screening was *Allium cepa* root tips (White and Claxton 2004). Plant, as biological indicators, can be used to mesure the potential effects of organic and inorganic pollutants and for assessment the effects of different compounds in the environment (Conte *et al.*1998). Molecular markers can be used not only for polymorphism studies but also for screening deleterious heavy metals effects on DNA by

* **Corresponding author.**
E-mail address: mervat_y2007@yahoo.com
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comparing diversity in DNA banding profile between treated and control organisms. ISSR markers are highly polymorphic and considered efficient in the evaluation of genotoxic activity of heavy metals (Bernardes *et al*. 2015). PCR amplification of ISSRS by using a single primer produce multiple amplification products that can be used as a dominant marker in informating the genetic variation induced in the genome. In genetic diversity studies, a good marker must induce high genetic variations with the ability to generate multilocus data from the genome (Anne 2006). The production of ISSR biomarkers was significant advantage of microsatellite sequences that are variable and universally distributed among the genome (Shen *et al*. 2006). The clastogenic agents inducing lesions in the genome called as genotoxicity. DNA damage, mutations and chromosomal alternations are mainly evaluations of genotoxicity. Therefore, the genotoxic agents possess the ability of DNA alternation. The bioindicator system of *Allium cepa*, as a first screener of genotoxicity and amodel bioindicator to investigate the genotoxicity potentials of drainage water due to their safe and efficient use which also helping to prevent damage of human health (Tedesco *et al*. 2012). *Allium cepa* was chosen in this study because it was most popular plant test system for monitoring the genotoxicity of drainage water as a good indicator for testing water quality through mutagenic effects of waste waters. Mutagenic effects are widely used for the detection of harmful effects caused by different types of toxicants (White and Claxton 2004).

The aim of this study was to monitor drainage water genotoxicity from different residential zones used ISSR markers as a molecular tool for testing water quality. Special attention must be paid about the main concern in the management of the contaminated waste water and what possible genotoxic impact on the environment of the contaminated drainage water which may load via spread by leaching if not managed.

MATERIALS AND METHODS

Genetic materials

Onions bulbs (*Allium cepa* L) obtained from the local market in Mansoura city. These bulbs were sun-dried for three weeks. Though, the healthy dry bulbs were used for the genetic test.

Allium cepa test may appear the cytotoxic or genotoxic substances may present in the environment, indicated the direct or indirect harmful for all living organisms (El-Shahaby *et al*. 2003).

Study sites

This study were carried out on the waste water discharged from chemical fertilizer industry and Oils and Soap industry in Dakhalia Governorate. Agriculture drainage water was also obtained from Menyet El–Nasr center (Dakhalia Governorate) and Kafr El–Sheikh Governorate through January 2018. The control was expressed by the Nile river.

Preparation of solutions

Buffer solutions needed in this investigation were prepared according to Payus *et al*. (2016).

ISSR – PCR Reactions

A set of six primers name and sequence used in ISSR analysis are presented in Table 1.

Table 1. Primers used in this study.

Name	Sequence 5'-3'
ISSR- 3	5'-ACACACACACACACACYT-3'
ISSR-8	5'-AGACAGACAGACAGACGC-3'
ISSR- 10	5'- GACAGACAGACAGACAAT -3'
ISSR- 12	5'- ACACACACACACACACYC -3'
ISSR-15	5'- CTCTCTCTCTCTCTCTRG -3'
ISSR-16	5'- TCTCTCTCTCTCTCTCA -3'

A:adenine, T: Thymine, G: Guanine and C: Cytosine, Y: (C or T).

DNA extraction

The total genomic DNA was extracted from the meristem cells of onion roots grown in water effluents from different resources ; using DNeasy Tissue kits (Qiagene). The integrity of DNA_S were checked on agarose gel electrophoresis accoding to Payus *et al*. (2016) .

PCR amplification

This technique was performed according to Sambrook *et al*. (1989).

DNA fingerprinting

2 µl of DNA was running on agarose gel and 10 µl of DNA marker (100bp DNA Ladder) was used for comparison according to Attallah *et al.* 2014. Comparison between treatments was done based on the presence or absence of reproducible polymorphic DNA bands was conducted to appear the similarity coefficients by SPSS program version–18. using the unweight pair group tool with arithmetical average (UPGMA) according to Iruela *et al.* (2002).

Data Analysis

Banding patterns obtained from molecular marker analyses were compared amplification products scored as '1' for presence and '0' for absence of bands. Bands of the same Similarity coefficient between two genotypes was estimated according to Sneath and Sokal (1973).

The similarity matrix was used in the cluster analysis. Taxonomies according to Sneath and Sokal (1973).

Evaluation of basic parameters

The percentage of polymorphic bands was estimated by the following formula:

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

RESULTS AND DISCUSSION

ISSR-3 primer

The genotoxicity of metals- containing drainage water in plants is described as a cause of different damages in growth and nucleic acids. In the present study the application of ISSR markers was used to assess the genotoxicity of drainage water as a tool for estimating water quality.

As shown from the results presented in Figure 1 and Table 2 that issr-3 primer produced more number of bands in the treatment with the Nile river water. This primer produced 0.84 band frequency .However, the total number of bands was gradually decrease depending on drainage water containing toxicants. The results demonstrated that the water quality decreased from Kfr El sheikh drainage water, Menyet El Nasr drainage water , industrial waste water of fertilizer industry and liquid wastes of Oil and Soap industry, respectivity. ISSR-3 marker can be described as a more reproducible primer than other molecular markers in the analysis of water quality to confirm the reproducibility of each water resource, Previous results showed that

changes in DNA banding pattern reflect DNA alteration from single base changing to chromosomal rearrangements (Atienzar *et al.*2002). The alternation of DNA banding patterns such as loss or appearanace of some bands occur due to changes in oligonucleotide priming sites that affecting the activity and interaction of DNA polymerase with altered DNA, leading to apparent variations in the gel profiles of the ISSR products (Correia *et al.*2014). There by, drainage waste water such agriculture or industrial showed variations in loss of normal bands in the treatments with drainage water if compared with the bands appeared in control treatment.

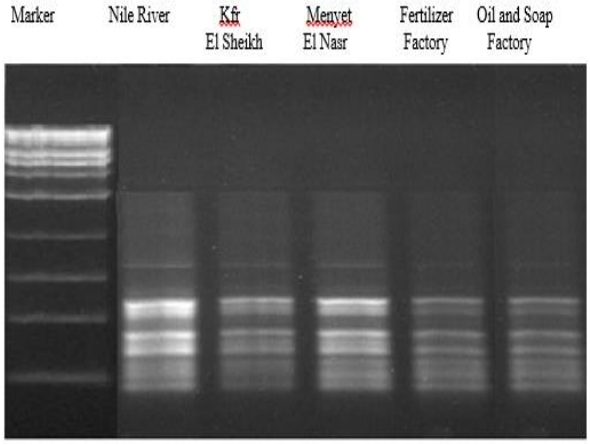

Figure 1 . ISSR profiles of five water resources treated onion roots as generated by ISSR-3 primer.

Table 2. Number of bands and their molecular sizes generated from onion roots treated with drainage water based on ISSR-3 primer.

M	Nile River	Kfr El Sheikh	Menyet El Nasr	Fertilizer Factory	Oils and Soap Factory	Frequency
1500	1	1	1	1	1	1.0
800	1	1	1	1	1	1.0
600	1	1	1	1	1	1.0
550	1	1	1	1	1	1.0
400	1	1	1	1	1	1.0
350	1	1	1	1	1	1.0
300	1	1	1	1	1	1.0
280	1	1	0	0	0	0.4
250	1	1	1	0	0	0.6
220	1	0	0	0	0	0.2
200	1	1	1	1	1	1.0
Total bands	11	10	9	8	8	0.84

The genotoxic effects of drainage water were also evaluated using cluster analysis as shown in Figure 2 which appear comparisons among treated and control plants. The dendogram revealed two main groups with the first one subdivided in three clusters including Menyet El – Nasr drainage water and industrial waste waters from both industrial activities. The second group representing the Nile river water as a control and Kfr El Sheikh drainage water.

The results obtained from this study indicated that DNA polymorphism detected by ISSR analysis seems to be a good technique to evaluate drainage water induced DNA damage (Correia *et al.*2014).

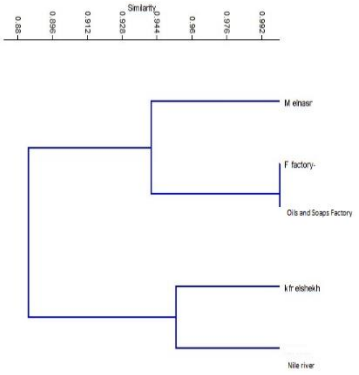

Figure 2. Dendrogram illustrated water quality dependent on Jacquards similarity coefficient generated from ISSR analysis using ISSR -3 primer.

ISSR-8 Molecular Marker

As shown from the results presented in Figure 3 and Table 3 the total number of generated bands from the treatment with the Nile River water was II. However, the generated bands from the treatment with drainage water from Menyet El- Nasr were exceeded that generated from the Nile water with one band. The generated bands from the treatment with factory effluents from both industrial activities were decreased with one band in relation to the control from the Nile River water treatment. Considering the number of bands that appear and disappear, it clear that this may be due to genotoxic effects leading to DNA damage by drainage water from Menyet El- Nasr and industrial effluents. Modifications of DNA fingerprinting are due to changes in nucleotide priming sites that affect the activity of DNA polymerase with damaged DNA, leading to induced variations in DNA pattern of the ISSR products (Correia *et al.*2014).In addition, there was no band changing for the treatment with drainage water from Kfr El- Sheikh Governorate. The results obtained herein agreed with Poyraz *et al.* (2018), who reported that the DNA damage leading to band changing. Sunar *et al.*(2009) found that *Zea mays* L. seeds treated with *Verbascum speciosum* extract induced changing in band intensity, loss of some bands and generating of new bands if compared with the control.

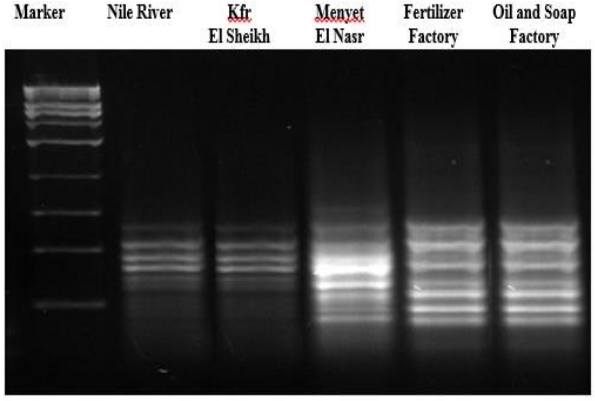

Figure 3. ISSR profiles of five water resources treated onion roots as generated by primer ISSR- 8.

Table 3. Number of bands and their molecular sizes generated from onion roots treated with drainage water based on ISSR-8 primer.

M	Nile River	Kfr El Sheikh	Menyet El Nasr	Fertilizer Factory	Oils and Soap Factory	Frequency
710	0	0	1	0	0	0.2
630	1	1	1	0	0	0.6
600	1	1	1	1	1	1.0
560	0	0	0	1	1	0.4
510	1	1	1	1	1	1.0
450	1	1	1	0	0	0.6
420	1	1	1	1	1	1.0
360	1	1	1	1	1	1.0
340	1	1	0	0	0	0.4
320	1	1	1	1	1	1.0
300	1	1	1	0	0	0.6
280	1	1	1	1	1	1.0
250	1	1	1	1	1	1.0
240	0	0	0	1	1	0.4
200	0	0	1	1	1	0.6
Total bands	11	11	12	10	10	0.72

Dendrogram (Figure 4) revealed two main groups, the first group subdivided in two clusters including industrial drainage water from fertilizer factory and oils and soap industry. The second group subdivided in two clusters, the first one included the drainage water from Menyet El Nasr and the second included the drainage water from Kfr El-Sheikh Governorate and the Nile water treatment as a control. The DNA polymorphism detected by ISSR analysis reflected DNA damage due to interfere of drainage water carrying pollutants with DNA integrity (Correia *et al.* 2014).

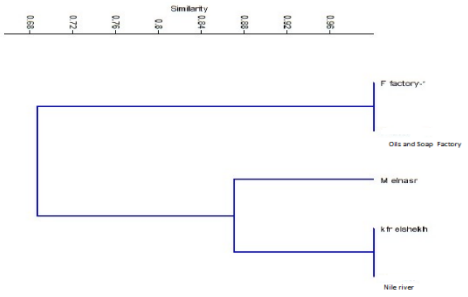

Figure 4 . Dendrogran illustrated water quality dependent on jacquards similarity coefficient generated from ISSR analysis using ISSR – 8 primer.

ISSR – 10 molecular marker

As shown from the results presented in Figure 5 and Table 4 the more number of generated bands (13) was generated from the treatments with the Nile water and Kfr El- Sheikh drainage water. The treatment with drainage water from Menyet El- Nasr center generated 12 bands with a loss of one band than the control. In addition, the treatment with factory effluents disappearing two bands than the control. This primer reflected water quality from different resources. The frequency of bands was equal 0.92. The dendrogram shown in Figure 6 appeared the same trend as shown in Figure 4. The results appeared that the genetic markers used in this study can recognized variations on DNA level e.g., nucleotide changes, deletion, duplication, inversion and insertion (Hibbett *et al.*2007). ISSR-10 molecular marker do not unique bands, but some polymorphic bands were generated. Unique band means that the appear of band in one treatment and absence of it with the same size in all other treatments (Raghunathachari *et al*. 2000).

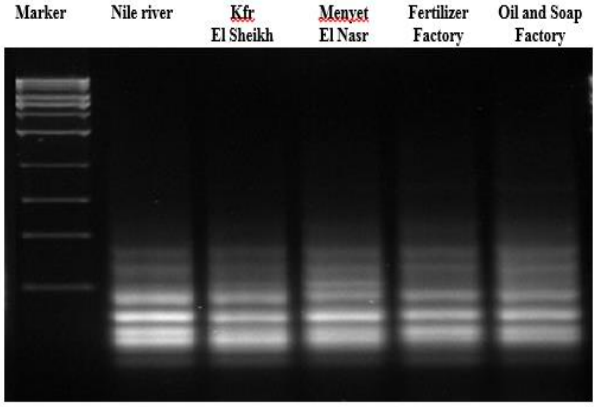

Figure 5. ISSR profiles of five water resources treated onion roots as generated by ISSR – 10 primer.

Table 4. Number of bands and their molecular sizes generated from onion roots treated with drainage water based on ISSR- 10 primer.

M	Nile river	Kfr El Sheikh	Menyet El Nasr	Fertilizer Factory	Oils and Soap Factory	Frequency
500	1	1	0	0	0	0.4
450	1	1	1	0	0	0.6
420	1	1	1	1	1	1.0
370	1	1	1	1	1	1.0
350	1	1	1	1	1	1.0
330	1	1	1	1	1	1.0
300	1	1	1	1	1	1.0
280	1	1	1	1	1	1.0
230	1	1	1	1	1	1.0
200	1	1	1	1	1	1.0
190	1	1	1	1	1	1.0
170	1	1	1	1	1	1.0
150	1	1	1	1	1	1.0
Total bands	13	13	12	11	11	0.92

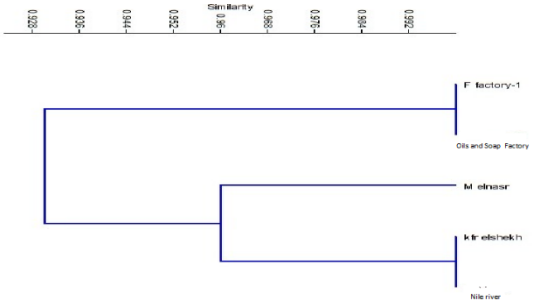

Figure 6. Dendrogram illustrated water quality dependent on Jacquards similarity coefficient generated from ISSR-10 primer.

ISSR- 12 molecular marker

As shown from Figure 7 and Table 5 the treatment with Nile River water and Kfr El – Sheikh drainage water generated nine bands for each one. The treatments with

drainage water from Menyet El- Nasr and fertilizer industry generated eight bands, while the factory effluents from oils and soap industry generated the lower number of bands which reached to seven bands. On the other hand, the dendrogram presented in Figure 8 appeared the same trend shown in Figure 2 with ISSR-3 primer. The results indicated that treatment with the Nile water Kfr El- Sheikh drainage water generated the highest number of bands, but the lowest number of bands was generated by the factory effluents released from oils and soap industry. It can be concluded that each molecular marker approaches of DNA analysis could identify the variation between the Nile water treatment and all of the water resources carrying pollutants. This agreed with El- Kawokgy(2018), who used ISSR technique to detect genetic variation between the parents and fusants of Bacillus thuringiensis.

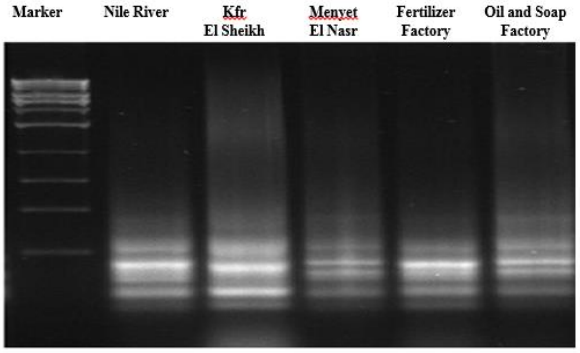

Figure 7.ISSR profiles of five water resources treated onion roots as generated by ISSR – 12 primer.

Table 5. Number of bands and their molecular sizes generated from onion roots treated with drainage water based on ISSR- 12 primer.

M	Nile River	Kfr El Sheikh	Menyet El Nasr	Fertilizer Factory	Oils and Soap Factory	Frequency
450	0	0	1	1	1	0.6
400	1	1	0	0	0	0.4
320	1	1	1	1	1	1.0
300	1	1	1	1	1	1.0
260	1	1	1	1	1	1.0
240	1	1	0	0	0	0.4
220	1	1	1	1	1	1.0
210	1	1	1	1	0	0.8
190	1	1	1	1	1	1.0
180	1	1	1	1	1	1.0
Total bands	9	9	8	8	7	0.82

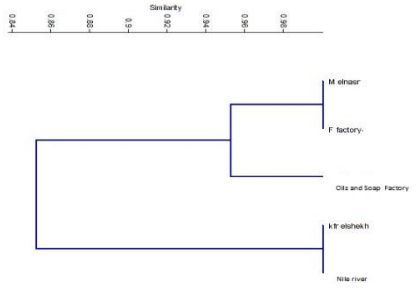

Figure 8. Dendrogram illustrated water quality dependent on Jacquards similarity coefficient generated fromISSR-15 primer.

ISSR- 15 molecular

As shown from Figure 9 and Table 6 the Nile River water generated eight number of bands, but the higher number of bands was generated from the treatment with factory effluents which generated ten number of bands with two bands increased than the control. On the other hand, Kfr El-Sheikh drainage water generated seven bands with a decrease of one band than the control. Meanwhile, Menyet El- Nasr drainage water generated nine number of band with an increase of one band than the control. These results illustrated that ISSR markers have been proved to be useful in genetic variations studies because of their reproductivity and great power for the detection of polymorphism (Sofalian *et al.*2009). The results showed that there is a genetic variations among the treatments of water from the different resources.

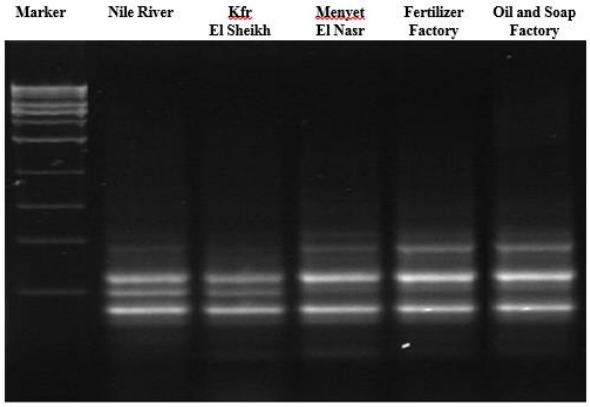

Figure 9. ISSR profiles of five water resources treated onion roots as generated by ISSR-15 primer.

Table 6. Number of bands and their molecular sizes generated from onion roots treated with drainage water based on ISSR- 15 primer.

M	Nile River	Kfr El Sheikh	Menyet El Nasr	Fertilizer Factory	Oils and Soap Factory	Frequency
530	0	0	1	1	1	0.6
460	1	1	1	1	1	1.0
430	0	0	1	1	1	0.6
350	1	1	1	1	1	1.0
330	1	1	1	1	1	1.0
310	0	0	1	1	1	0.6
290	1	1	0	0	0	0.4
270	0	0	0	1	1	0.4
250	1	1	1	1	1	1.0
230	1	1	1	1	1	1.0
200	1	0	0	0	0	0.2
160	1	1	1	1	1	1.0
Total bands	8	7	9	10	10	0.73

The genotoxic effects were also evaluated using cluster analysis(Figure 10) which revealed two main groups with the first group subdivided in two clusters, the first one included Menyet El- Nasr drainage water and fertilizer industry effluents, however, the second one included industrial effluents from oils and soaps industry. The second group including Kfr El- Sheikh drainage water and the Nile River water. The results indicated that the exposure of organisms to drainage water carrying genotoxic substances can induce DNA damage. These results agreed with Enan (2008), who demonstrated that mutations that inhibit primer binding or interfere with the amplification can lead to the

variation in DNA fingerprinting. The cluster analysis is one of the most effective technique in numerical computation. It can estimate the distances between the control and drainage waters. Adendrogram construction based on the average linkage between treatments can appear the relationship between every pair of entities.

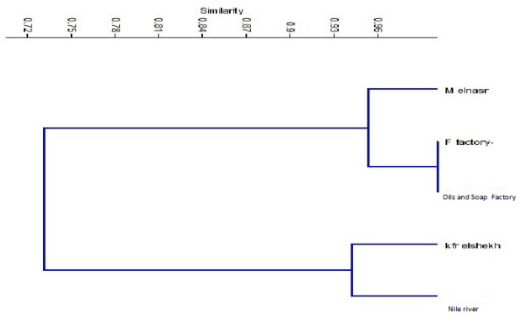

Figure 10. Dendrogram illustrated water quality dependent on Jacquards similarity coefficient generated from ISSR analysis using ISSR-3 primer.

ISSR-16 molecular marker

As shown from Figure 11 and Table 7 the treatment with drainage water appeared and disappeared of some bands in relation to the control treatment with Nile water which generated six bands. The highest number of bands generated from the treatment with drainage water from Kfr El- Sheikh Governorate which revealed eight bands, but the lowest number of bands was obtained from the treatment with liquid industrial wastes of fertilizer factory which generated five bands. The mean frequency of total bands appeared was reached to 0.53 . The disappearance of some bands may be attributed to DNA photoproducts(e.g.pyrimidine dimer,6-4 photoproducts), which may block or reduce the polymerization of DNA in PCR reaction. Asingle point mutation within the primer site can induce significant changes in RAPD patterns. (Enan 2008). The fingerprinting pattern was affected by the loss orgain of priming sites due to DNA mutations.

Dendrogram construction (Figure 12) showed the same trend which illustrated before in Figure 10. The results indicated that treatments with drainage water induced damaged on the molecular level. These results agreed with Zhiyi and Haowen (2004) , who demonstrated that there is an obvious distance between the fingerprinting from Zebrafish treated with chemical tested and the normal samples.

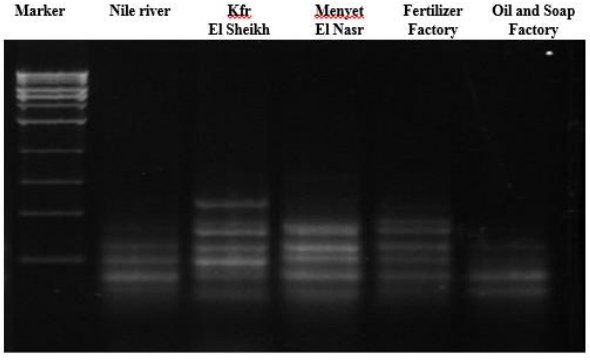

Figure 11. ISSR profiles of five water resources treated onion roots as generated by ISSR-16 primer.

Table 7. Number of bands and their molecular sizes generated from onion roots treated with drainage water based on ISSR-16 primer.

M	Nile River	Kfr El Sheikh	Menyet El Nasr	Fertilizer Factory	Oils and Soap Factory	Frequency
570	0	1	0	0	0	0.2
440	0	1	1	1	1	0.8
410	0	0	1	1	1	0.6
400	1	1	0	0	0	0.4
350	1	1	1	1	1	1.0
310	0	0	1	1	1	0.6
290	1	1	0	0	0	0.4
250	1	0	0	0	0	0.2
240	1	1	1	1	1	1.0
220	1	0	0	0	0	0.2
210	0	1	1	0	1	0.6
200	0	1	1	0	0	0.4
Total bands	6	8	7	5	6	0.53

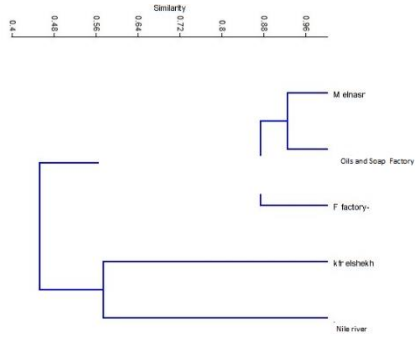

Figure 12. Dendrogram illustrated water quality dependent on Jacquards similarity coefficient generated from ISSR analysis using ISSR-16 primer.

Molecular marker analysis

ISSR markers were analysed for polymorphism changed in onion genome after exposure to water drainage from different resources (Table 8).

Table 8. Gains and losses in ISSR – amplified products from onion roots treated with drainage water.

Marker	TNB	PB	MB	P %	F
ISSR-3	11	3	8	27	0.8
ISSR-8	15	8	7	53	0.7
ISSR-10	13	2	11	15	0.8
ISSR-12	10	4	6	40	0.8
ISSR-15	12	6	6	50	0.7
ISSR-16	12	10	2	83	0.5
Total	73	33	40	-	-
Aveage	12.2	5.5	6.7	44	0.7

Notes: TNB: Total number of bands, PB: polymorphic bands, MB: monomorphic bands, % p: percentage polymorphism and F: Frequency revealed by ISSR marker.

Six ISSR primers amplified 73 bands in total, of which 33 were polymorphic bands were employed for the generation of genetic distance which used for genetic relationship reconstruction. The percentages of polymorphism were ranged between 15(ISSR-10) to 83 (ISSR-16).These results agreed with Correia *et al*.(2014), who found that ten ISSR primers produced polymorphic bands which produced in *Plantago almogravensis* a total of

257 and 258 in roots and 255 and 265 bands in leavels in the presence and absence of Al,respectively. Furthermore, Madadi *et al*.(2017) found that ISSR markers produced 114 amplification products in pomegranate as one of the most important horticultural crops, out of which 97 were polymorphic (83.23%).

Genetic diversity analysis

To assessment genetic diversity induced in the same genotype of onion by drainage water, genetic similarity coefficients were calculated (Table 9). Similarity coefficients ranged from 0.76- 0.98. The highest similarity coefficient was obtained between the drainage water from fertilizer industry, oils and soap effluents. In addition, the lowest similarity was obtained between the Nile water, oils and soap industrial wastes. These results illustrated that ISSR markers were appropriate for detecting relationships between drainage water from the different resources. These results agreed with Vanijajiva (2012), who applied ISSR markers to assess genetic diversity and genetic relationships among 15 accessions of pineapple one of the most important fruits in Thailand, and found three clusters based on the dendrogram which separated with similarity coefficients raining from 0.316- 0.968.

Table 9. Genetic similarity between different water resources using ISSR data as revealed by Dice coefficient.

M	Nile river	kfr El-Shekh	Menyet El-Nasr	Fertilizer factory	Oils and Soap Factory
Nile river	1.00				
kfr Elshekh	0.92	1.00			
M ElNasr	0.80	0.86	1.00		
Fertilizer factory	0.78	0.80	0.94	1.00	
Oils and Soap Factory	0.76	0.80	0.94	0.98	1.00

Jacard's similarity coefficient dendrogram obtained from ISSR analysis was shown in Figure 13.

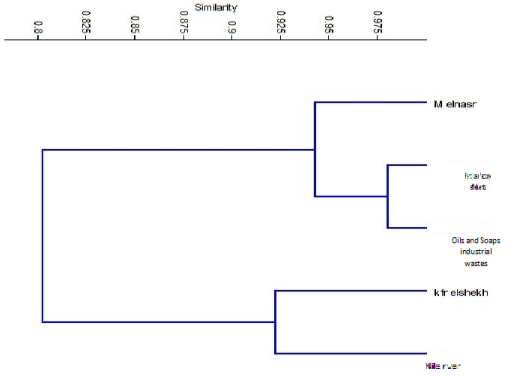

Figure 13. Dendrogram illustrated water quality dependent on Jacquards similarity coefficient generated from ISSR analysis using six primers.

ISSR dendrogram divided into two subclusters, the first one including Menyet El- Nasr drainage water which placed alone in a group of subcluster, the second one including both industrial efflents from different industrial activites. The second group including the Nile water and Kfr El- Sheikh drainage water. These results agreed with Al-Qurainy (2010), who constructed dendrogram to evaluate the genetic distance generated among *Eurca sativa* treated with various heavy metals with different concentrations. These results clearly indicates that drainage water from different resources present in these clusters harbouring broad spectrum for inducing genetic diversity which differed from one source to another. The clustering pattern obtained in this study revealed that there was relation between the drainage water containing substances and genotypic diversity induced. This disagreed with Malhotra *et al*.(1974) who reported that geographic diversity cannot be used as an index of genetic diversity in different crops.

Molecular data analysis

Diversity analysis based on ISSR gel polymorphism was shown in Table 10. The number of polymorphic bands ranged from 3 to 10, whereas polymorphism percentage ranged from 27 to 83. Diversity analysis based on ISSR fingerprinting showed number of bands detected through six primers ranged from 10 to 13. The bands generated were primer dependent and were in mean frequency range from 0.5 to 0.8 DNA from onion roots treated with drainage water displayed polymorphic fragments which may not detectable in DNA of control sample. The unique fragments ranged from 0 to 3 with four out of six primers.These results agreed with Mengoni *et al*.(2001), who determined genetic diversity of heavy metal tolerant populations of *Silene paradoxa* (L.) using chloroplast microsatellite analysis. In addition, Al- Qurainy (2010) demonstrated that fourteen primers out of sixteen revealed monomorphic banding patterns which reflected a high degree of homogeneity in rocket plants. The same outher also found that out of 20 primers screened, only two revealed polymorphic bands, indicating its reproducibility. Furthermore, Liu *et al*.(2005) found that the changes occurring in RAPD profiles of *Eruca sativa*(L .)root tips folloing Cd treatment included variation in loss of normal bands and appearance of new bands compared with the normal seedlings.

Table 10. Gel polymorphism generated from five water resources treated onion roots as obtained from six primers.

Molecular traits	ISSR -3	ISSR -8	ISSR-10	ISSR -12	ISSR -15	ISSR -16
Monomorphic bands	8	7	11	6	6	2
Polymorphic bands without unique	2	7	2	5	4	7
Unique bands	1	1	0	1	0	3
Polymorphic bands with unique	3	8	2	6	4	10
Total number of bands	11	15	13	12	10	12
Polymorphism %	27	53	15	50	40	83
Mean of band frequency	0.8	0.7	0.8	0.7	0.8	0.5

In conclusion, drainage water containing pollutants creates mutations. The comparison between Nile water treatment and treated genomes with drainage water showed that ISSR analysis can be used to evaluate how the environmental pollutants modify the structure of DNA in living organisms which reflected water quality. On the basis of these considerations ISSR technique is a powerful tool for measuring genotoxicity which reflected water quality. Onion have been used as bio- indicators to assess the genotoxicity of environmental pollution including drainage water, which considered as a good bio- indicator sensitive to

health hazard substances. Conflicts of interest.The outhors declare that they have no conflicts of interest.

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تقدير جودة المياه من خلال الإختلافات الوراثية المستحدثة فى البصل بإستخدام المعلمات الجزيئية للتتابعات القصيرة المتكررة (ISSR)

ميرفت إبراهيم كمال ، أشرف حسين عبد الهادى ، خليفه عبد المقصود زايد و عبدالله سمير عبد المحسن

قسم الوراثة – كلية الزراعة – جامعة المنصورة

تم فى هذه الدراسة تطبيق تقنية التتابعات القصيرة المتكررة (ISSR) داخل الجينوم المعروفة بالتتابعات الدقيقة Microsatellite لتقدير الإختلافات الوراثية المستحدثة فى البصل المعامل بواسطة مياه الصرف الزراعى والصناعى وعلاقة القرابة الوراثية على مستوى المصادر الخمسة المستخدمة من مياه الصرف الزراعى والتى ستعكس جودة المياه. تم إستخلاص DNA من جذور البصل الحية المعاملة بمياه الصرف تحت الدراسة من مصادرها الخمسة. تم أيضاً إستخدام ستة معلمات جزيئية للتتابعات القصيرة المتكررة داخل الجينوم لتوصيف جودة المياه تحت الدراسة . كما تم إستخدام تكنيك طباعة الـ DNA للتتعات القصيرة داخل الجينوم لتقدير الضرر الواقع على جزئ DNA فى جذور البصل المعاملة بمياه الصرف. لقد أصبح تعدد الأنماط الوراثية لحزم DNA على الجيل دليل يتمثل فى وجود أو غياب شظايا الـ DNA فى الجذور المعاملة مقارنة بتلك الموجودة فى تجربة المقارنة المعاملة بمياه النيل. أوضحت النتائج وجود مسافة واضحة بين أنماط حزم DNA للجذور المعاملة بمياه الصرف وتلك المعاملة بمياه النيل العادية وذلك عندما تم تطبيق التحليل العنقودى. حدث إنخفاض تدريجى فى عدد الحزم الكلية المتكونة بفعل المنقب الجزيئ ISSR-3 معتمداً على وجود مياه الصرف المستخدمة . لذا يعتبر المنقب الجزيئ ISSR-3 هو أفضل منقب يمكن إستخدامه من بين المنقبات المختبرة فى الكشف عن جودة المياة المختبرة تحت الدراسة . يعمل هذا المنقب الجزيئ على إخفاء بعض الحزم معتمداً على المواد السامة التى تحملها مياه الصرف. تراوحت درجة التشابه الوراثي بين مصادر المياه تحت الدراسة ما بين ٠،٧٦ – ٠،٩٨ . تم الحصول على أعلى قيمة فى معامل التشابه بين مياه الصرف الخاصة بمصنع الأسمدة الكيماوية ومياه الصرف الخاصة بمصنع الزيوت والصابون . كما ظهرت أقل قيمة من درجة التشابة بين مياة النيل ومياه الصرف الزراعى الناتجة عن مصنع الزيوت والصابون . تراوح معدل الحزم متباينة الأنماط من خلال ٦ معلمات جزيئية ما بين ٣-١٠ ، بينما تراوح معدل تكون شظايا DNA الفريدة ما بين صفر إلى ٣. وفى النهاية أظهرت المقارنة بين المعاملة بمياة النيل العادية والمياة بمخلفات مياه الصرف الزراعى أو الصناعى أن تحليل التتابعات القصيرة المتكررة (ISSR) يمكن إستخدامه فى تقييم التحورات الوراثية المستحدثة بفعل مياه الصرف التى تحمل الملوثات البيئية فى تركيب جزيئ DNA للكائنات الحية.