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Applicability of Inter – Simple Sequence Repeats (Issr) for Testing Water Quality Through Cluster Analysis in Onion Genome Treated with Wastewaters



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ABSTRACT

In this investigation, three drainage water samples were collected from selected agricultural wells in Dakhalia and Kafr El-Sheikh Governorates. The drainage water samples were analyzed for physico – chemical properties. Six ISSR primers were used to assesst genetic diversity induced on the molecular level due to the treatments of onion roots with wastewaters from the different resources. This technique exhibits a great diversity in onion genotype particularly on the molecular level. The purpose of the present investigation was to evaluate the level of water quality on the molecular level using ISSR biomarkers, in addition to compare chemical composition of drainage water with the standards of WHO guidelines for drinking water quality. Onion roots , as biological indicator can be used to measure the genotoxic effects of water pollutants on DNA molecule via measuring the polymorphism of DNA fragments in treated roots. Results suggested that a qualitative measurement revealed changes in ISSR profiles. A distinct distance appeared between the band profile of treated roots and control samples. The comparison between treated and untreated genomes revealed that ISSR analysis could be used as a new tool to evaluate the level of water quality through how the drainage water modifies the structure of DNA molecule in living organisms. This study indicated that extreme application of chemical fertilizers had a severe impact on water quality. The physico-chemical analysis of drainage water quality used in this study revealed that the drainage water quality does not meet the WHO standards about direct reusing it in irrigation.

Keywords : ISSR markers , genotoxicity , drainage water .



INTRODUCTION

Agriculture sector is the largest user of fresh water on a popular basis. Most studies regarding to environmental quality focused on testing water quality because of the importance of water to human health and aquatic ecosystem. The addition of drainage water containing different kinds of pollutants through the agriculture leaching and surface run off to the water bodies brings a series of changes in the physical and chemical characteristics of water quality such as chemical fertilizers and pesticides. Agriculture applicability releases various kinds of pollutants which may reduce the quality of water resources. Agricultural practices degrade the surface waters which receive a high quantities of waste water from industrial and human activities, as well as, other resources. The first step in testing water quality is physicochemical analysis which is not enough in evaluating the toxic and genotoxic potential of the waters, because the complex mixtures of polluted substances, which may present at a too low concentrations to allow their analytical quantification. The applicability of genotoxicity assays was recommended in environmental monitoring because the genotype of the organism tested containing some regions in the genome may be differently sensitive to a broad spectrum of low concentrations from toxic chemicals which may not allow to analytical quantities (Radic *et al.* 2009). Water is a significant source to all forms of life, it makes up 50 to 97% of the plants and animal weight and

about 70% of the human body (Buchholz 1998). Despite the importance of water, it is the lowest managed resource over the world. The effect of industrial effluent on water quality of the rivers revealed that the analytical chemicals were above the allowable limits (Fakayode 2005). Some rivers in the urban areas are the end points of effluents discharged from industrial activities. If industrial effluents are not controlled and treated before discharging into the environment it can also pollute the other water resources such as ground water (Olayinka 2004). Therefore, both industrial waste waters and agriculture drainage water considered poor quality water in the affected areas (WB 2007). Unfortunately, there was no enough knowledge about the effluents released into the river. In this respect it is difficult to prevent the pollution of water which is important for surrounding living communities. Due to the increased water pollution and toxicological problems originated from the release of pollutants into the water resources, cheaper and reliable techniques are needed to evaluate the genotoxicity of contaminated poor quality water. Water quality affected its suitability to fulfill the requirement of the user for municipal purpose. Water quality determine the level of acceptability (FAO 2013). Thus the information about the quality of water is critical in the management of water productivity. The taste is a simple method of acceptability because the better tasting water is considered the preferred supply. There are a number of various water quality guidelines correlated with

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the use of waste water in agriculture sector (Tak *et al.* 2010). Special measures are must be taken to asses water quality to avoid the potential threat of different toxicants that it may be containing. Biological monitoring through molecular biomarkers of exposed organism provides a tool for testing water quality (Conte *et al.* 1998) that may threat human health (Singh 2017). The effective popular tools of bioassays which could be used for genotoxicity testing to assess water quality are *Salmonella* mutagenicity test, the anaphase aberration test in *Allium cepa* root tips and the micronucleus test (White and Claxton 2004). Characterization and grouping of water resources based at first on taste testing which are influenced by environmental variations. Then the molecular markers were preferred because of co-dominance, high reproducibility, easy and fast assay and easy to exchange the data between water filtration laboratories (Joshi *et al.* 1999). A molecular marker is DNA sequence that is easily detected and can be monitored easily. For diversity analysis ISSR markers are widely used to assess genetic diversity induced due to their simplicity and low cost. It combines with PCR, therefore it is highly specific, gives higher number of bands and covers higher locations in the genome. The information about the genetic diversity generated due to the reduction in water quality helps in the efficient management of water filtration and selection of the best resource of water. ISSR_s exhibit the specificity of microsatellite markers, so they require no sequence knowledge for primer synthesis thus enjoying the advantage of random markers (Joshi *et al.* 2000). Grant (1994) suggested that the higher plants provide a good genetic system for characterization and monitoring environmental pollutants. They are useful indicators for testing water quality via the cytogenetic and mutagenic effects (Constantin and Owens 1982). In this investigation, chemical analysis of drainage water from different resources was evaluated in comparison with the standard of World Health Organization Guidelines (WHO) for drinking – water quality in fourth edition (2011). This study aimed to apply ISSR molecular markers to detect DNA modification on onion genome caused by drainage water which are related to water quality.

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 reveal 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 sample was the natural resource of water from the Nile river.

Preparation of solutions

Reagents 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 MgCl₂, 0.2 mM dNTPs, 1 µM primer, 1 U Taq DNA polymerase and 30 ng template DNA.

DNA extraction

The 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 DNAs obtained from these four treatments were checked on agarose gel electrophoresis according to payus *et al.* (2016) and Sambrook *et al.* (1989).

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'-GTGTGTGTGTGTGTGYG-3'
ISSR-6	5'-CGCGATAGATAGATAGATA-3'
ISSR-8	5'-AGACAGACAGACAGACGC-3'

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

Gel electrophoresis

Amplification products of ISSR were separated on 1.5 % agarose gels in 1X TEA buffer solution against DNA ladder 100bp. The bands were detected by staining with ethidium bromide. Meanwhile, the PCR products were checked by UV – transilluminator, as well as photographed by gel documentation system with image lab software according to Attallah *et al.* (2014). 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 coefficients by the unweight pair group method with arithmetical average (UPGMA) according to Iruela *et al.* (2002).

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 showed variation , i.e. the bands present in some samples and absent in the other ones. The percentage of polymorphic bands was calculated by the formula as follows:

$$\% \text{ of polymorphic bands} = \frac{\text{Number of polymorphic bands}}{\text{Total number of bands}} \times 100.$$

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 94°C. Each cycle consisted of a denaturation step at 94°C for 50 Sec. an annealing step at 45°C for 50 Sec. and an elongation step at 72°C for 1min. The primer extension segment was extended to 7 min at 72°C in the final cycle.

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 monomorphic. The genetic similarity coefficient (GS) between two genotypes was estimated according to Dice coefficient (Sneath and Sokal 1973) as follows, Dice formula:

$$G_{sij} = 2a / (2a + b + c)$$

Where, G_{sij} 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 taxonomies. 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).

Chemical Analysis

A total of 25 elements were assayed in each sample of waste water in addition to the control. All the water samples were chemically assessed in the central laboratory of Faculty of Agriculture, Mansoura University. The concentrations were measured by ppm to be compared with the standards of World Health Organization

Guidelines (WHO) for drinking water quality in fourth edition (2011) and council of the European Union (1998).

RESULTS AND DISCUSSION

ISSR analysis

The heavy metals - containing water drainage caused genotoxicity and also generate oxidative stress in the cells through interfering with antioxidant defense system (Gratao *et al.* 2005). In this study, ISSR markers mostly dominant genetic markers were used. They usually produce multiple fragments of DNA (each of which is defined a locus) in a single reaction, which generated a high number of loci across the genome of each treatment. These dominant markers were used in this study as initial steps for testing water quality through the genetic modification induced in the genome grown in drainage water. As shown from the results presented in Table (2) a total of 49 bands were scored from the six primers used with the treatments of high quality water from the Nile River. Accordingly, the poor quality water resulted from Kafr El Sheikh, as well as, Menyet El-Nasr drainage waters and fertilizer industry effluents generated 49, 43 and 42 bands, respectively. The size of amplified bands was differed from primer to the other. The lowest 150bp and the largest 1200 bp were amplified by the same primer ISSR - 4. Meanwhile, the smallest size of bands (220 - 350 bp) was amplified by the primer ISSR - 6. The mean of fragment size among all the six primers detected about 186 - 740bp.

Table 2 . Total number of bands obtained by six ISSR primers among the treatments of four water samples from different resources.

Primers	Nile River water (NRW)	Kafr El Sheikh drainage water (KEDW)	Menyet El Nasr drainage water (MEDW)	Fertilizer industry effluents (FIE)	Mean of band frequency	Fragment sizes bp	Mean number of bands
ISSR - 2	6	7	5	10	0.530	160- 830	7.00
ISSR- 3	9	8	7	6	0.830	200- 550	7.50
ISSR - 4	7	10	8	8	0.675	150- 1200	8.25
ISSR - 5	10	10	10	7	0.700	160 - 690	9.25
ISSR - 6	3	3	3	3	0.800	220- 350	3.00
ISSR - 8	14	11	10	8	0.770	230- 820	10.75
Total / Mean	49	49	43	42	0.72	186 - 740	7.63

However, the mean frequency of bands detected about 0.72. The two primers agreed in their results among the six markers detected are ISSR-3 and ISSR-8. Both primers generated a lower number of bands from the treatment with poor quality water as fertilizer industry effluents. In contrast, they generated a higher number of bands from the treatment with high quality water from the Nile River. Additionally, moderate number of bands were obtained from the less quality water as Menyet El-Nasr drainage water and Kafr El-Sheikh drainage water.. It seems that the natural resources of water as the Nile river present considerable DNA bands some of which disappeared in the treatments with water effluents due to genotoxicity of these effluents in the genome. This may be due to genetic alteration in the microsatellites, inter simple sequence repeats (ISSR_s) leading to dis matched with the marker. In most genetic studies, a better marker was identified by a high genetic diversity induced and the ability to produce multilocus data from the genome treated (Anne 2006). At the same time the good quality water from the Nile river achieving higher reproducibility of bands than the less or poor quality water from the other resources. In this study, six primers were used to achieve the best

ones may be used for testing water quality on the molecular level through genetic diversity induced among the genome tested. This study attempt to fill the knowledge gap between genetic and its applicability for testing water quality, that enable the users in water filtration industry or stations have regarding the genetic practical usage of ISSR markers in their techniques used for testing water quality, before reuse by human populations or before reuse in agriculture sector. Furthermore, this work studied the polymorphism information which measures the allelic diversity in a locus by the different types of molecular markers. Interestingly, it is worth to note that ISSR-3 and ISSR-8 were the better primers for testing water quality because they are targeted the genetic sequences which are more affected by the quality of water. This suggest the advantages of these molecular markers to be used in laboratories of the filtration of water stations. The genotoxic effect of drainage water was quantified using ISSR_s analysis by comparing DNA from onion roots treated with drainage water from different resources with the control treated with the water from the Nile river (Table 3). Out of initially screened ten ISSR primers , six primers were found to be polymorphic and produced clear and

reproducible amplification pattern. All the six primers generated 63 distinct reproducible amplicons with the mean of 10.5 bands per primer. ISSR – 8 primer generated the largest number of monomorphic bands in contrast with the primer ISSR – 6 which produced the lowest number of monomorphic bands. The percentage of polymorphism ranged between zero by the ISSR – 6 primer to 69% by the primer ISSR – 2.

Table 3. Summary of the gains and losses of bands in ISSR – amplified products.

Marker	TNB	PB	MB	P %	F
ISSR-2	13	9	4	69	0.5
ISSR-3	9	3	6	33	0.8
ISSR-4	12	8	4	67	0.6
ISSR-5	12	7	5	58	0.7
ISSR-6	3	0	3	0	1.0
ISSR-8	14	6	8	43	0.8
Total	63	33	30	-	-
Average	10.5	5.5	5	52	0.7

TNB: Total number of bands; PB: Polymorphic bands; MB: Monomorphic bands; P% : Polymorphism percentage and F: Frequency.

The highest number of monomorphic bands (8) was generated by the primer ISSR-8 followed by the primer ISSR-3 and ISSR-5, respectively. Additionally, the lowest number of monomorphic bands were generated by the primer ISSR-6.

Both primers ISSR-3 and ISSR-8 generated the higher number of monomorphic bands, they are the better primers that could be used for testing water quality. The presence of genetic changes obtained in ISSR profiles for the exposed plant roots depended on the water quality testing. DNA profiles generated by the molecular markers revealed genetic diversity between control and the treatments with drainage waters in the number of polymorphic and monomorphic bands. ISSR patterns generated by drainage water exposed onion roots were varied from those obtaining using control DNA. ISSR primers revealed the gain or loss of a band. In some profiles, alteration of a single amplification band was observed, however in others, more variations of profiles of gains and losses of bands appeared. The ISSR patterns exhibited bands size between 150–1200 bp in length. The results obtained in this study agreed with DeWolf *et al.* (2004), who found that reproducible DNA patterns were generated from a range of aquatic invertebrates, bacterial cells and plants and successfully were used to determine the genotoxin – induced DNA alteration. In this study, DNA damage was assessed using ISSR patterns that reflect water quality through DNA modification in onion roots treated with drainage water from different resources. Similarly, DNA damage induced by water effluents reflected the genotoxicity of wastewaters because of alteration appeared in ISSR profiles via disappearance of some bands and gain of new PCR products occurred in the patterns generated in exposed roots. The results obtained herein are in harmony with Welsh *et al.*(1991), who found that changes in band profiles shown in DNA fingerprinting reflect DNA changes from single base (point mutations) to a complex chromosome rearrangements. The exposure of onion roots to drainage water containing genotoxic chemical effluents can induce DNA damage as seen in ISSR profiles.

Genetic similarity and cluster analysis

The genetic similarity between four water resources was quantified as seen in Table (4). The low level of similarity was shown between fertilizer industry effluents

and natural water of Nile river, followed by Menyet El-Nasr and Kafr El-Sheikh drainage water, respectively. These results reflected that fertilizer industry drainage water has the poorest water quality followed by Menyet El-Nasr and Kafr El-Sheikh drainage water, respectively. There was a distinct distance between the high quality water from the Nile River and three water effluents from the different resources.

Table 4. Genetic similarity between four water resources using ISSR data as revealed by Dice coefficient.

Water resources	Nile River	Kafr – El sheikh	Menyet- El Nasr	Fertilizer Factory
Nile River	100			
Kafr El - Sheikh	84	100		
Menyet El - Nasr	82	86	100	
Fertilizer Factory	74	84	76	100

The level of genetic similarity between fertilizer industry effluents and Kafr El – Sheikh drainage water was 84 %. On the other hand, the level of genetic similarity between drainage waters from Menyet El-Nasr and Kafr El-Sheikh was 86 %. Additionally, there is a distinct distance was found between fertilizer industry effluents and Menyet El-Nasr drainage water which showed 76 % similarity . Polymorphic bands obtained in this study were employed in the assessment of genetic distance matrices to be used for genetic similarity reconstruction. The polluted water resources showed high similarity with each other. The water drainage from different resources revealed concordance between polymorphism data. This indicated that drainage waters from different resources had a different access to DNA molecule and induces various effects with fewer point mutation events if compared with the natural water resource from the Nile river. Through the data obtained from the six types of molecular biomarkers indicated genetic diversity among the amplification patterns of the exposed and unexposed onion roots to the water effluents. The results revealed the tendency of source – dependent (water quality-dependent) band loss and gain resulting from the exposure of onion roots to the tested water effluents as in the most water quality tests (Bernardes *et al.* 2015). In this study ISSR analysis confirms that biomolecular markers technique can be applied to evaluate the water quality and how the environmental pollutants may modify the structure of DNA molecule in the test organism ISSR biomarkers can be used to detect water quality via a wide range of genetic damage induced on the DNA level. This study confirm that drainage water must be filtration before reuse in agriculture sector because polluted water is harmful to the plants and is potentially hazardous to human populations. Thus, special care must be considered into clearing and filtration of effluents water resources before reuse to avoid the ecological risks that may happen in the ecosystems. Hence, this study highlights the importance of testing water quality using molecular biomarkers tools together with chemical analysis to correct the plan of sustainable development of contaminated resources to assure that contaminations are eliminated.

Cluster analysis shown in Figure 1 illustrate that all water types were classified into three groups. The first group included the cluster of fertilizer industry effluent as a source of poorest quality water. The second group included the cluster of drainage water from both

resources Kafr El-Sheikh Governorate and Menyet El-Nasr Center, Dkhalia Governorate. Additionally, the third group included the natural water resources of the Nile river. The first cluster of fertilizer industry effluents distantly related from the other clusters because of low genetic similarity level which do not exceed 77 %. The second cluster includes the drainage water from Kafr El-Sheikh and Menyet El-Nasr Center which are closely related with a similarity level reached up to 85 %. The comparison between untreated and treated genomes showed that ISSR analysis could be used to assess how the less quality water modify the structure of DNA molecule in living organisms. On the basis of this study the results suggested that ISSR technique is a powerful technique for measuring genotoxic activity of water resources due to environmental pollutants which reflect the level of water quality. This method could be applicable on a wide level of bioindicators. The results obtained in this investigation agreed with Al-Qurainy (2010), who constructed the dendrogram to evaluate the genetic distance generated by the seedlings treated with heavy metals. The same author found that similarity matrix was ranged between 42.8 to 100 % which showed wide genetic variability among the seedlings treated with different concentrations of heavy metals. However, the level of heavy metals increased in the industrial regions due to industrial and human activities, such may be harmful to the life on the earth. Therefore, it is significant to evaluate the genotoxic effect of drainage water in these sites via DNA biomarker analysis. The results obtained in this study agreed with Attallah *et al.* (2014) , who demonstrated that microsatellite biomarkers were polymorphic and so important for genetic analysis. The results obtained indicated that treated onion roots with drainage water showed damage at the molecular level after grown in waste water effluents.

Physico – chemical characteristics

The industrial wastewater effluents were collected from the major discharge points from the three locations described in this study in comparison to the control water from the Nile River. Physical and chemical properties of the drainage waters in comparisons to Nile river water and WHO standards are shown in Table (5). The results revealed that titanium was present in all water effluents with a high concentration in drainage water of Menyet El-Nasr followed by drainage water of Kafr El-Sheikh but it was not present in Nile water . Aluminum was also present in all water effluents with a high rate in fertilizer industry waste water followed by drainage waters of Menyet El-Nasr and Kafr El-Sheikh, respectively. The level of aluminum concentration was zero in natural water resource of Nile River. The concentration of aluminum was beyond the maximum permissible limits required by World Health Organization (WHO 2011) and Council of the European Union (EU)1998 . Among all four water resources , mercury , boron , gallium , lithium and lead were found only in drainage water collected from Kafr El – Sheikh Governorate with a concentration beyond the maximum permissible limits required by WHO and EU. However, barium concentration was found in fertilizer industry effluents with a level above that in all other water resources. Potassium was present in the drainage water collected from Menyet El-Nasr center with a concentration beyond the maximum permissible limits required by WHO. Furthermore, strontium was present in all three water effluents with a concentration beyond the maximum permissible limits required by WHO. A high concentration of strontium was found in Kafr El-Sheikh drainage water followed by fertilizer industry effluents. The concentration of strontium in Nile river water was below the maximum permissible levels required by WHO. Meanwhile, zinc was present in all three water effluent resources but it was absent in the Nile river water . Although elevated levels of ammonium was shown to exceed the maximum permissible limits required by WHO in all four water resources, but higher levels were shown in fertilizer industry waste water followed by drainage water of Menyet El-Nasr and Kafr El – Sheikh, respectively. The concentration of ammonium in the Nile River water was beyond the maximum permissible limits required by WHO but it was less than that in all waste water effluents used in this study. It is well known that the waste water effluents from the different resources in a complex mixture of organic chemicals, as well as variants of unidentified toxicants which are named as non-conventional pollutants, which may cause risks of unknown magnitude to human populations and ecosystem grown in industrial regions.

Framing about the standards of water quality means to provide safe drinking water to the citizen. WHO provided general guidelines about drinking water quality based on scientific research experiments. Many countries design their own water quality standards according to the WHO standards. The excess levels of these metals as described by WHO caused diseases and it is dangerous to the health of human populations.

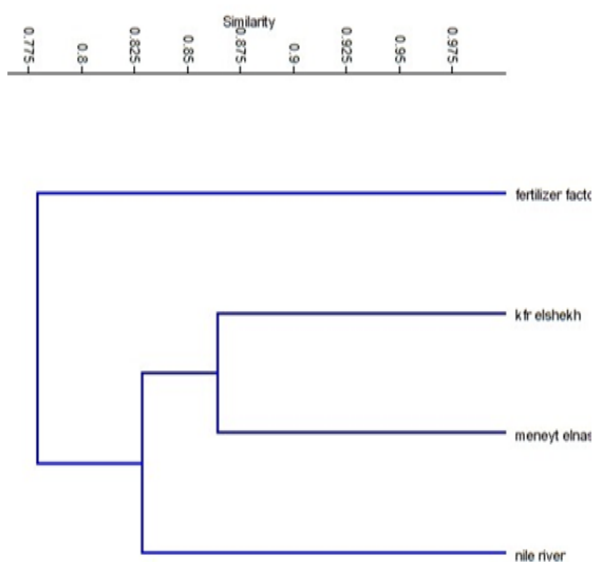


Figure 1 . Dendrogram of four water quality collected from different resources constructed data using unweighted pair-group arithmetic average (UPGMA) and similarity matrices computed according to Dice coefficients.

Table 5. Physico- chemical characteristics of industrial wastewater and drainage water analyzed by ppm.

Metal	Drinking water directives (EU) mg/L	WHO guidelines drinking water mg/L	Nile Revier water	Drainage water from kafer Elsheikh Governorate	Drainage water from Menyet El Nasr Center	Fertilizer Factory effluents
Titanium (Ti)			0.0	5.635	6.923	2.769
Aluminum (Al)	0.2	0.2	0.0	0.260	1.587	5.338
Bismuth (Bi)			.028	.014	0.0	0.0
Mercury (HG)	0.001	0.006	0.0	.018	0.0	0.0
Silver (AG)		0.05	0.020	0.004	0.002	0.0
Boron (B)	1.0	2.4	0.0	0.022	0.0	0.0
Barium (BA)		0.7	0.034	0.024	0.034	0.041
Calcium (CA)		75	39.526	24.127	27.974	31.781
Cadmium (CD)	0.005	0.003	0.0	0.0	0.0	0.0
Cobalt (CO)			0.031	0.003	0.002	0.003
Chromium (CR)	0.05	0.05	0.0	0.007	0.008	0.004
Copper (CU)	2.0	2.0	0.091	0.018	0.013	0.015
Iron (FE)	0.2	0.3	0.039	0.030	0.026	0.024
Gallium (GA)			0.0	0.034	0.0	0.0
Indium (IN)			0.0	0.105	0.224	0.0
Potassium (K)		12	8.167	5.836	28.359	6.481
Lithium (LI)			0.0	0.001	0.0	0.0
Magnesium (MG)		30	16.050	22.423	19.185	13.562
Manganese (MN)	0.5	0.4	0.0	0.028	0.098	0.041
Nickel (NI)	0.02	0.07	0.0	0.0	0.001	0.0
Lead (PB)	0.01	0.01	0.0	0.007	0.0	0.0
Strontium (SR)		178.75	0.0362	0.299	0.323	0.311
Zinc (ZN)		3.0	0.0	0.003	0.046	0.010
Nitrate (NO ₃)	50	50	0.59	5.89	11.79	35.37
Ammonium (NH ₄)	0.5	.05	0.74	5.89	17.07	19.75

Therefore, water quality must be tested regularly against all the parameters defined by WHO prior to use for drinking or irrigation in agriculture sector. The level of population load in discharged wastewater effluents exceeded the maximum limits determined by WHO which considering these water resources have a significant impact on population load. The results revealed that fertilizer industry effluents are the major sources of ammonia load discharged to the river water. The level of ammonia load in fertilizer effluents was greater than the load of total ammonia in drainage waters, which had a significant impact on river water quality. The results obtained in this study agreed with Islam *et al.* (2010), who suggested that polluted water in the river with ammonia discharged from fertilizer factories was not good for human consumption. It is therefore recommended that the disposal of drainage water must be stopped to the river to save water quality necessary for human health. As seen from the chemical analysis conducted in this study the quality of drainage water does not meet the local standards about the direction of reuse drainage water in irrigation sector. Therefore, it is recommended to mix drainage water with fresh irrigation water to improve their quality via reducing the excessive levels of toxicants.

In conclusion, onion is a good plant bioindicator for studying genetics, as well as testing water quality. ISSR analysis is a technique which could be used for detecting mutants induced due to low water quality. The treated samples generated a polymorphic differences in DNA profile. The ISSR method has been successfully used as a good tool for detecting drainage water-induced DNA damage and showed satisfactory potential as a reliable tool for genotoxicity assays. It has been used as a new tool for testing water quality. The results indicated that treated onion roots are damaged of the molecular level after exposure to drainage waters. The level of damage reflected the range of water quality.

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تطبيق تقنية التتابعات القصيرة المتكررة التي تتخلل الجينوم في اختبار جودة المياه من خلال التحليل العنقودي لجينوم البصل المعامل بالمخلفات المائية ميرفت إبراهيم كمال قسم الوراثة – كلية الزراعة – جامعة المنصورة

تم في هذه الدراسة جمع ثلاث عينات من مياه الصرف الصناعي والزراعي من مصارف زراعية منتخبة بمحافظة الدقهلية وكفر الشيخ. تم تحليل عينات مياه الصرف بالإضافة إلى المياه الطبيعية من نهر النيل بالنسبة لخصائصها الطبيعية والكيميائية. استخدم في هذه الدراسة أيضاً ست مرقمات جزيئية للتتابعات القصيرة المتكررة داخل الجينوم (ISSR) لتقييم الاختلافات الوراثية المستحدثة على المستوى الجزيئي نتيجة معاملة جنور البصل بالمخلفات المائية المختبرة من المصادر المختلفة. تعمل تقنية ISSR على إظهار الاختلافات الوراثية المستحدثة في جينوم البصل نتيجة هذه المعاملات وخصوصاً على المستوى الجزيئي. لذا تهدف هذه الدراسة إلى تقييم مستوى جودة المياه على المستوى الجزيئي باستخدام تقنية المرقمات الجزيئية ISSR، هذا بالإضافة إلى مقارنة التركيب الكيميائي لمياه الصرف بالمعايير القياسية الإسترشادية الصادرة عن منظمة الصحة العالمية بالنسبة لجودة مياه الشرب. تم في هذه الدراسة استخدام جنور نبات البصل كأداة بيولوجية لقياس السمية الوراثية للمخلفات المائية المختبرة على جزيئي DNA من خلال قياس معدل الاختلاف في الأنماط الوراثية لحزم DNA في الجنور المعاملة. أوضح التقدير الكمي ظهور اختلافات وراثية مستحدثة في البصمة الوراثية للـ DNA باستخدام تقنية ISSR. هذا بالإضافة إلى ظهور مسافات وراثية واضحة بين أنماط حزم المادة الوراثية في الجنور المعاملة بمياه الصرف وتلك المعاملة بمياه النيل الطبيعية. أوضحت المقارنة بين جينوم البصل المعامل والغير معامل بمياه الصرف أن تحليل ISSR يمكن استخدامه كطريقة حديثة لتقييم مستوى جودة المياه من خلال كيف تحور مياه الصرف من تركيب جزيئي DNA في الكائنات الحية. لقد عكست نتائج هذه الدراسة أن الاستخدام المتزايد للأسمدة الكيميائية سوف يكون له تأثيرات سلبية على جودة المياه. أوضحت نتائج التحليل الكيميائي والفيزيائي لجودة مياه الصرف المستخدمة في هذه الدراسة أن جودة مياه الصرف المختبرة لا تتفق مع المعايير القياسية لمنظمة الصحة العالمية حول الاستخدام المباشر لهذه المياه في رى الزراعة الحقلية.