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Detection of Genotoxic Effect of Wastewater on *Vicia faba* L. by Using Biochemical Assay and RAPD Markers.

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MULTIPLE biomarker systems have been frequently used to measure the genotoxic effects of environmental pollutants on living organisms. In this study, faba bean seedlings were used (*Vicia faba*) as a bioindicator for water pollution. Five different sources of water: distilled water as a negative control, tap water, and polluted water from three different sources were used in irrigation of bean plants. All treatments showed great alterations in seed proteins, isozyme systems (α- esterase, β- esterase, peroxidase, and acid phosphatase) and DNA polymorphism detected by RAPD analysis. The detected variations between treatments included alterations in number (appearance and disappearance), staining intensity, thickness and relative mobility of bands. These results suggest that the irrigation by wastewater can cause genotoxic effects on plant and *V. faba* can be used as a bioindicator for evaluation of environmental pollution.

Keywords: Genotoxicity, Isozyme, Pollution, RAPD, SDS-PAGE, *Vicia faba*.

The contamination of water resources by genotoxic compounds is a worldwide problem (Buschini *et al*., 2004). Some of the cities around the world are having incomplete sewerage and therefore discharge its wastewater into large lakes, rivers, and canals or drains (Grover and Kaur, 1999). Many compounds in these wastes are genotoxic and can alter the genetic makeup in plants (Sabti and Kurelec, 1985). Heavy metals have long been recognized as one of the major sources of pollution in the aquatic and terrestrial environment (Arun-Kumar and Achyuthan, 2007). Chlorination is a common water disinfectant method which is able to reduce microbial water pollution, but can also produce genotoxic and toxic compounds if precursors are present in the water to be treated and the level of chlorine is high (Komulainen, 2004). So, we need to reduce irrigation with polluted water at least if we cannot prevent irrigation by it, or try to find out and improve crops of economic importance which can withstand these drastic conditions. Plants are good bioindicators of heavy metals because they play a significant role in food chain transfer and in defining habit.

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Numerous plants have been used as bioindicators of environmental pollution such as agricultural crops like *Allium cepa*, *Hordeum vulgaris*, *V. faba*, and *Zea mays* (Cabrera and Rodriguez, 1999, Nielsen and Rank, 1994, Grisolia *et al*., 2005 and Ma *et al*., 2005). Theoretically, the diversity of allozyme appears to be correlated with specific polluted environments (Ben-Shlomo and Nevo, 1988). Roa and Dubey (1990) reported that monitoring of antioxidant activities offers a useful tool in understanding the mechanism which makes plants relatively tolerant in field conditions. Mukherjee *et al.* (2004) suggested esterase variations to be a potential biomarker of heavy metal pollution. Recently, the isozymes could be used as a biochemical marker to study the tolerance of plant to stress (Zhang *et al*., 2013). Several studies have revealed that treated waste water often contains genotoxic substances that can not only injure the integrity of the genome of organisms but also negatively affect the expression of DNA directly or indirectly (Grisolia *et al*., 2005). RAPD method was successfully used to detect 'DNA effects' induced by metals such as lead, manganese, cobalt, and cadmium (Atienzar *et al*., 2001, Rancelis *et al* ., 2006 and Liu *et al*., 2009), heavy metals (Al-Qurainy, 2009) and gamma radiations (Hagger *et al*., 2005). Moreover electrophoretic techniques of protein are reproducible and rapid methods for quantifying, comparing and characterizing proteins (Bollag and Edelstein, 1993). Electrophoretic analysis of the protein provides information concerning the structural genes and their regulatory systems that control the biosynthetic pathways of that protein (Hassan, 2000).

The objectives of this study were to (i) study the risk of the genotoxicity of wastewater which used in irrigation on cultivated plants and (ii) indicate that RAPD analysis in conjunction with other biochemical parameters could be a powerful eco-toxicological tool in bio-monitoring pollution.

Material and methods

Plant material

V. faba L. cv Nubaria 1 seeds have been obtained from the Agricultural Research Station, Institute of Legumes, Sakha, Kafr El-Sheikh, Egypt.

Treatments

This study was carried out in one of the main agricultural governorates of the Nile Delta, Kafr El-Sheikh governorate. After the seeds were germinated, the seedlings were divided to separate groups. Each group was irrigated by only one source of water as the following: distilled water as a negative control, tap water, agricultural water from Meet yazeed branch canal in addition to agricultural polluted water from two different sources (Bitaytah darin which contained agricultural drainage water and Kitchener drain which contained agricultural water mixed with sewage and industrial wastes).

Protein analysis

The seeds of both offspring and mother plant were grinded in mortar after removing the coat to fine powder and then defatted with cold acetone with continuous stirring. The defatted powder (100mg) was mixed with 1000 µl 0.5 M Tris-borate buffer, pH 6.8 and 2% SDS, at 4ºC overnight. The extracted mixture

was centrifuged for 30 min. at 16000 rpm. The supernatant was kept at −20°C until use. One dimensional SDS-PAGE was carried out using 12% (w/v) polyacrylamide gel according to a protocol proposed by Laemmli (1970).

Enzyme analysis

Young leaves for three seedlings from 2-weeks-old of both offspring and mother genotype were used. 0.1 gm of each genotype was grinded in a mortar on ice bath in 1 ml sucrose 20%, transferred to Eppndorf tubes, left for two hours in the refrigerator with mild agitation from time to time and then centrifuged at 16000 rpm for 20 min. at 4ºC. The products were analyzed on 10% (w/v) polyacrylamide gels according to the procedures used by Manchenko (1994).

Four isozyme systems were examined which are α- and β-esterase, peroxidase and acid phosphatase. To detect esterase, EST (α- and β- naphthyl acetate), gels were incubated in 0.05 M phosphate buffer (pH 6.0) containing 0.15 gm fast blue B salt and α-naphthyl acetate (0.02 gm in 1 ml acetone). The gels were incubated in dark at 37 ºC until bands appeared then washed by distilled water and fixed in 3% acetic acid to reduce nonspecific background. In case of esterase, EST (β- naphthyl acetate); α-naphthyl acetate in the phosphate buffer was replaced by β-naphthyl acetate.

For detection of peroxidase, POX, gels were incubated in 0.05 M acetate buffer (pH 5.0) containing 0.065 gm benzidine dissolved in 1 ml of ethanol. 2 ml of 0.1 M $CaCl_2$ were added as co-enzyme. Finally, 2 ml of H_2O_2 were added as a substrate, incubated at 4^{o}C until dark brown bands appeared, washed by distilled water and fixed in 50 % glycerol (Soltis *et al*., 1983).

To recognize acid phosphatase**,** gels were incubated in 0.05 M acetate buffer (pH 6.0) for 30 min. and then replaced by the staining solution (1 % α- naphthyl acid phosphate sodium salt in 0.05 M acetate buffer (pH 5.0) and 0.05 gm black k-salt). Gels were incubated at 30ºC until the bands which represents the acid phosphatase activities appeared as dark brown colour.

DNA extraction

Total genomic DNA was extracted from seedling of the offspring and parent plant of *V. faba* L. by grinding 100 mg of each sample in liquid nitrogen to a fine powder. DNA extraction was performed by using Qiagen DNeasy™ Plant Minikit following the protocol of the manufacturer (Qiagen Inc, Valencia, CA).

RAPD analysis

RAPD was performed as described by Williams *et al.*, (1990) with minor modifications. Four Operon primers (A4, B1, B3 and B4) of 10 nucleotides were used for RAPD analysis based on their abilities to amplify *V. faba* genome and producing reproducible amplification patterns (Table 1). PCR reaction contained

20–40 ng genomic DNA, 5 Pico mole random primer, 0.2 mM dNTPs, 2.5 µl PCR buffer (10X), 2.0 mM MgCl, 0.5U *Taq* DNA polymerase in a total volume of 25 µl. The reaction was assembled on ice, overlaid with a drop of mineral oil. Amplifications were carried out using the following programs: 3 min at 95°C followed by 44 cycles of 2 min 92°C (Denaturation), 1 min 37°C (Annealing), and 2 min 72°C (Extension), with a final extension of 10 min at 72°C. The amplification products were analyzed on 1% (w/v) agarose gels after staining with 0.2 µg/ml ethidium bromide.

TABLE 1. Name and sequences of the selected random primers used in RAPD-PCR analysis.

Primer Name	Sequence (5'→3')	% GC
OPA-04	AATCGGGCTG	60%
OPB-01	GTTTCGCTCC	60%
OPB-03	CATCCCCCTG	70%
OPB-04	GGACTGGAGT	60%

Results

Protein-banding pattern

Protein profiles had great variations (disappearance and appearance of new bands, thickness, intensity and relative mobility) depending on the source of irrigation when compared with the control (Fig. 1). There were four new bands induced by irrigation with some water sources compared to control. A new band with a molecular weght of 85 KDa was expressed in tap water (lane 2) and in water from Meetyazed canal of (lane 4) which was more thickness and intensity in case of tap water. At molecular weight of about 50 kDa, a second new band was appeared in irrigation with tap water (lane 2), water from Bitaytah drain (lane 3), water from Meetyazed canal (lane 4) and water from Kitchener drain (lane 5). The third new band (~ 40KDa) was present under the irrigation with water from Bitaytah drain (lane 3) and water from Meetyazed canal (lane 4). The last new band is faint and present in case of irrigation with tap water (lane 2), by water from Bitaytah drain (lane 3) and by water from Meetyazed canal (lane 4) of molecular weight of about 30 kDa. At about 100 kDa and between 100 and 85 kDa, there were two induced bands of the widest thickness, highest intensity with relative mobility during the irrigation with water collected from Bitaytah drain (lane 3).

There was a band of molecular weight between 70 and 60 kDa that expressed in all tested plants under irrigation with water from Bitaytah drain (lane 3) with highest intensity, but it had relative mobility in the irrigation with tap water (lane 2), water from Bitaytah drain (lane 3), water from Meetyazed canal (lane 4) and it had low intensity and thickness at the irrigation with water from Kitchener drain (lane 5). At molecular weight between 60 and 50 kDa, a unique band was absent which characteristic for irrigation water from Bitaytah drain (lane 3). At about 50 kDa in all examined plants, a band was induced except for the control and it increased in intensity and thickness in case the irrigation with water from Bitaytah drain (lane 3). The protein bands from plants irrigated by water from

both Meetyazed canal (lane 4) and Kitchener drain (lane 5) had low thickness and intensity band of molecular weight between 50 and 40 kDa compared to other protein. Plants irrigated with water from Bitaytah drain and from Meetyazed canal have a characteristic band of molecular weight of 40 kDa, lanes (3&4) respectively, with more thickness and intensity in plants irrigated with water from Bitaytah drain (lane 3). All sources of water which used in irrigation induced a band of molecular weight between 40 and 30 kDa with the widest thickness and relative mobility with the irrigation from Bitaytah drain water (lane 3).

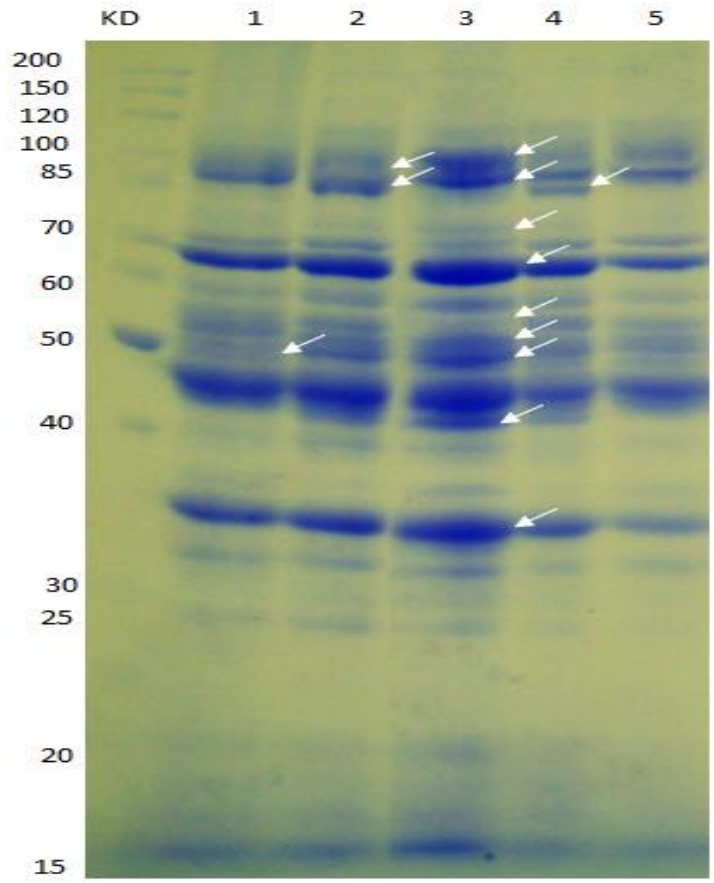

Fig. 1. Protein banding pattern of *V. faba* irrigated by different sources of water: Lane (1) distilled water, Lane (2) tap water, Lane (3) water from Bitaytah drain, Lane (4) water from Meetyazed canal and Lane (5) water from Kitchener drain.

Isozyme polymorphism

The electrophoretic profile of α-Esterase (EST) exhibited two zones of activity (Fig. 2). These zones of activity were interpreted as product of two loci (α-EST-1 and α-EST-2). All tested plants; α-EST-2 showed one band varied in thickness and intensity. The thickness of the bands in case of tap water irrigation was the widest between all plants under study. The intensity of the band under the irrigation by water from Kitchener drain (lane 5) was the lowest between all irrigated plants. While for α-EST-1 locus were exhibited four bands. The control includes three bands (1, 2 and 4) but they were very faint (lane 1) and tap water irrigation (lane 2) is characterized by unique band (band 2). Plants were irrigated with water from Bitaytah drain water, Meetyazeed canal and from Kitchener drain had band number 3 (lanes 3, 4 and 5) respectively, but their intensity was slightly more in case of irrigation by water from Kitchener drain (lane 5).

For β-Esterase (EST), there are two loci (β-EST -1 and β-EST -2) where β-EST-1 locus exhibited three alleles (Fig. 2). Plants which irrigated with tap water (lane 2) and that irrigated from Meetyazeed canal had two faint bands (1ane 3). The treatment by water from Kitchener drain included two faint bands (2 and 3) but the treatment by water from Bitaytah drain is characterized by high intensity band number 2. β-EST-2 locus showed two alleles, one allele was detected in all treatments and varied in thickness, density, and moving distance. The highest intensity and thickness was recorded under irrigation by tap water (lane 2) while it was faint in case of irrigation from Kitchener drain (lane 5). The second allele was observed only in case of tap water (lane 2).

The electrophoretic profile of peroxidase (POX) (Fig. 2), exhibited two zones of activity and were interpreted as products of two loci (POX-1 and POX-2). Under all treatments, POX-1 showed one band varied in thickness, intensity and moving distance. It shows more intensity and high thickness under the irrigation from Bitaytah drain and from Meetyazeed canal (lanes 3 and 4), while it had slightly long distance movement with irrigation from Kitchener drain (lane 5). Interestingly, it was faint under both distilled water and Kitchener drain (lanes 1 and 5). POX-2 locus showed one band in all treatments which was very faint in case of irrigation from Kitchener drain (lane 5) and was very thick and has more intensity during the irrigation from Bitaytah drain (lane 3).

Acid phosphatase profile exhibited three loci (ACP-1, ACP-2 and ACP-3). ACP-1 locus showed one band in all treatments, it has more thickness and intensity in tap water treatment and was very faint in other treatments. ACP-2 locus showed one band in all treatments but disappeared under the treatment from Meetyazeed canal (lane 4). ACP-3 locus showed one band in all treatments and had more thickness and intensity under the treatment by water from Bitaytah drain and Kitchener drain (lanes 3 and 5).

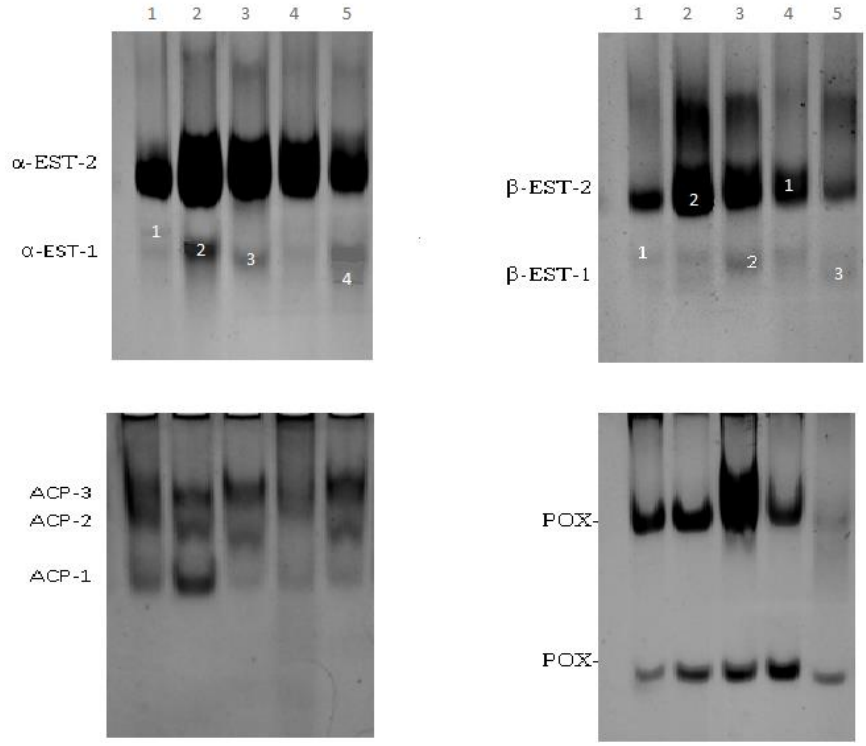

Fig. 2. α-Estrase, β-Estrase, Peroxidase and acid phosphatase zymograme from *V. faba* seedlings irrigated by water from different sources: Lane (1) distilled water, Lane (2) tap water, Lane(3) Bitaytah drain, Lane (4) Meetyazed canal, and Lane (5) Kitchener drain

RAPD analysis

Regared to use primer A4 (Fig. 3), the control had two specific fragments with molecular weights of about 1000 bp and 700 bp (lane 1). Fragment with molecular weight 700-800 bp was found in all treatments except for both control (lane 2) and Bitaytah drain water treatments (lane 3), while it has relative mobility in case of irrigation with tap water and Meetyazeed canal water (lanes 2 and 4, respectively). Bitaytah drain water treatment was characterized by disappearance of a fragment with molecular weight 400-500 bp (lane 3) which has more intensity in tap water treatment (lane 2). Faint fragment with molecular weight 300-400 bp was characteristic for control (lane 1), while fragment with molecular weight of about 200 bp was characteristic for Bitaytah drain and Meetyazeed canal water treatments.

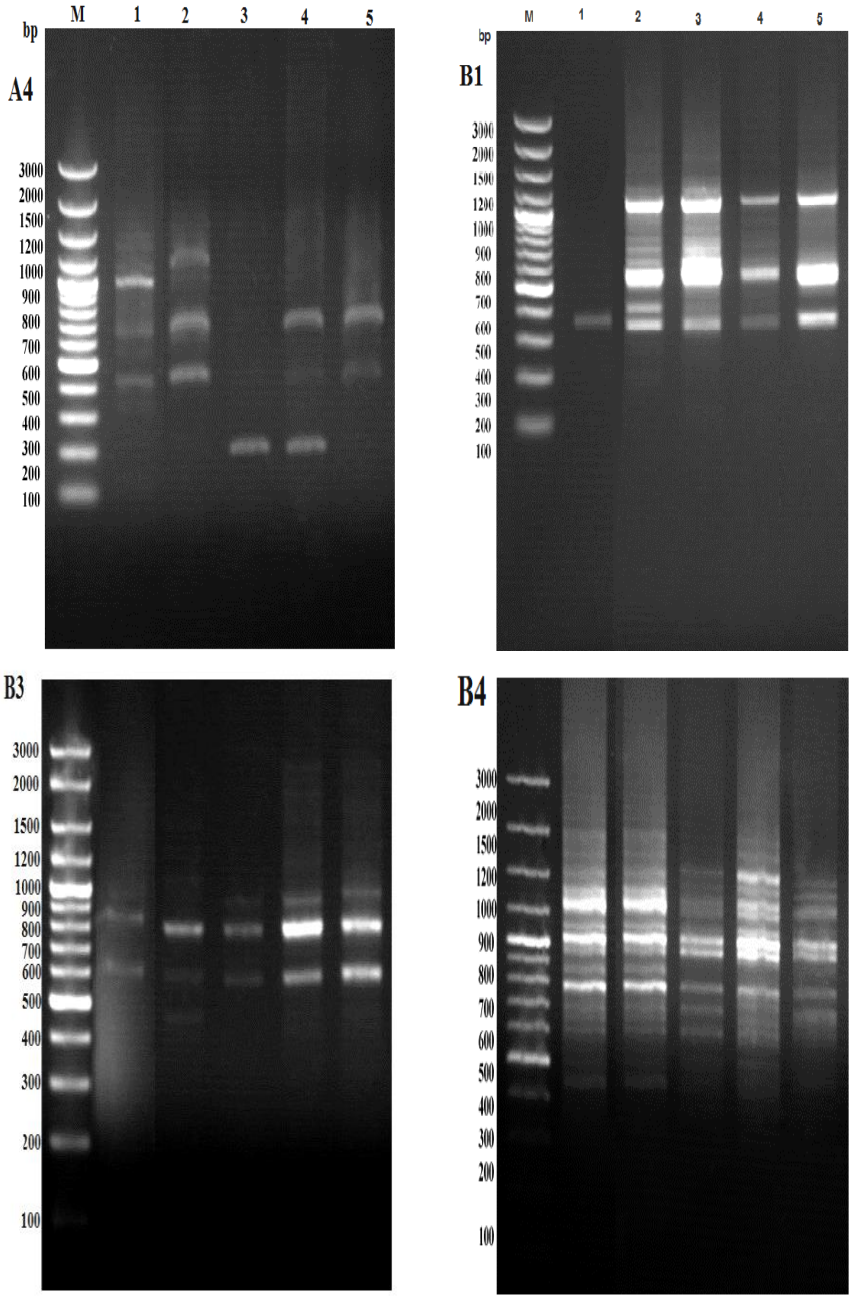

Fig.3. PCR product amplification of DNA from offspring of *V. faba* irrigated with different water source by using A4, B1, B3 and B4 primers: Lane (1) Distilled water, Lane (2) Tap water, Lane(3) Bitaytah drainr, Lane(4) Meet yazed canal and Lane (5) Kitchener drain.

Primer B1(Fig. 3) showed faint fragment with molecular weight of about 1500 bp for tap water and Bitaytah drain water treatments (lanes 2 and 3) respectively. All treatments have 1200 bp fragment except control (lane 1). This fragment has low intensity in Meetyazeed canal water treatments, more thickness and high mobility in Bitaytah drain water and tap water treatments. There was a characteristic fragment with molecular weight 900 bp for Bitaytah drain water treatment (lane 3). Also, there was a fragment with molecular weight about800 bp overall treatments.

The intensity of this fragment vary from high for Bitaytah drain water (lane 3) followed by Kitchener drain water (lane 5) to moderate for both tap water (lane 2) and Bitaytah drain water (lane 3) and less in case of Meetyazeed canal water (lane 4). When the water from Meetyazeed canal was used in irrigation, a fragment with molecular weight about 700 bp was present (lane 4), while it disappeared in case of control, tap water and Meetyazeed canal water treatments (lanes 2, 3 and 5) respectively. Tap water has two specific bands with molecular weights of 600-700 bp and 400-500 bp. Fragment with molecular weight of about 600 bp was found in all treatments except control. It has more thickness in Bitaytah drain water treatment, less intensity in Meetyazeed canal water treatment and high relative mobility in tap water treatments. Fragment with molecular weight 300-400 bp was found in all treatments with different intensities.

Faint fragments with molecular weights of about 1200 and 1000-1200 were found in Meetyazeed canal water and tap water treatments with using primer B3 (Fig.3). Primer B3induced fragment with 1000 bp in all treatments except in tap water (lane 2) and was faint in control and Bitaytah drain water treatments (lanes 1 and 3) respectively. Fragments with molecular weights of about 900 bp and 600 bp were found in all treatments with more intensity and thickness in Meetyazeed canal water (lane 4) and Kitchener drain water (lane 5) treatments. A faint fragment with molecular weight 400-500 bp was characteristic for tap water (lane 2).

Primer B4 (Fig. 3) showed faint fragments with molecular weights of about 2000 bp characteristic for control, tap water and Meetyazeed canal water treatments (lanes 1, 2 and 4) respectively. Fragment with molecular weight of about 1200 bp disappeared in Bitaytah drain water (lane 3) and Kitchener drain water treatments (lane 5), but it had more intensity and relative mobility in Meetyazeed canal water treatment (lane 4).

Fragments with molecular weights of 1000 bp, 900 bp, 800-900 bp and 700-800 bp were found in all treatments with differences in intensity, and mobility. Fragment with molecular weight 900-1000 bp was characteristic for control, tap water and Meetyazeed canal water treatments (lanes 1, 2 and 4) respectively. Fragment with molecular weight of about 800 bp was characteristic to control

(lanes 1) and tap water treatments (lane 2). 600-700 bp fragment was found under control treatment (lane 1), tap water (lane 2) and Meetyazeed canal water treatments (lane 3). Moreover a fragment with molecular weight of about 600 bp was also found in all treatments except Kitchener drain water treatment (lane 5). Faint fragment with molecular weight of about 500 bp was characteristic for Meetyazeed canal water treatment (lane 4).

Discussion

In the present study, the profiles of total seed proteins of all treatments showed a great variation. This variation was observed in number of bands, staining intensity, thickness and relative mobility. The variation may be attributed to the mutagenic potential of polluted water where, electrophoretic analysis of the protein has been used to evaluate mutagenic potential produced through pollution in the environment (Barakat and Hassan, 1997). Each band in the protein banding pattern of an organism reflects a separate transcriptional event (Hussein and Salam, 1985). The appearance of new bands could be explained on the basis of mutational events at the regulatory genes that either suppress transcription or activate unexpressed gene(s). This conclusion is fortified with the data of Al-Muraikhi (2000) and El-Nahas (2000). It was reported that new bands of protein appeared in Cd-treated fronds of *Lemna trisulca* (Prasad *et al*., 2001) and rice seedlings exposed to acute Cd toxicity (Ahsan *et al*., 2007). Also, there were changes in band intensity in all treatments. Some bands were characterized by dark appearance, while others had faint or moderate appearance. Changes in band intensity may be due to induction of gene mutation at the regulatory system which modulates, attenuate or enhances transcription rate of a particular structural gene. This leads to the production of faint or over expressed protein bands (Barakat and Hassan, 1997 and Shehab *et al*., 2000). The increase in band intensity may also be interpreted on the base of gene duplication (Gamal El-Din *et al*., 1988). Moreover, some treatments showed an increase in bands relative mobility. The changes in bands relative mobility could be attributed to the occurrence of point mutation in the concerned structural genes that create stop codon prior or post the original. They gave rise to the production of shorter or longer polypeptide chains (El-Nahas, 2000). Also, agricultural drainage water treatment recorded very high number of bands with high intensity (10 bands) and more thickness (9 bands) compared with the other treatments and control. It induced three new bands which showed the high effect on protein profile of plants irrigated with it. This obvious effect may be due to high content of herbicides in agricultural drainage water. Quantitative and qualitative differences were recorded in seed storage protein composition upon exposure of wheat to herbicides (Kumar, 2012).

In α-esterase zymogram, there was a polymorphism in the two loci, α- Est-1 and α- Est-2, appeared as a variation in thickness, staining intensity and disappearance or appearance of new bands. In addition, β-estrase isozyme patterns of some treatments exhibited disappearance or appearance of new bands in β- Est-1 locus. This variation may be attributed to the pollutants like heavy metals found in these effluents. Mukherjee *et al*. (2004) reported that esterase

variations and metal contamination of the environments are interrelated. Furthermore, the irrigation by waste water affected peroxidase isozyme pattern, particularly. This isozyme is routinely used as oxidative stress marker and an indicator of metal toxicity (Radotic *et al.*, 2000). SOD, CAT, GPX, APX, and EP isoenzymes had the most sensitive responses in the seedlings of *V. faba* under Pb and Lanthanum contamination (Wang *et al*., 2009). Under stress conditions, organisms activate nonenzymatic and enzymatic defense systems to quench ROS. The enzymatic defense responses comprise superoxide dismutases, catalases, peroxidases, glutathione reductase and several NADP+ reducing enzymes (Diaz *et al*., 2001, Scandalios, 2002, Mittler, 2002, and Singh and Agrawal, 2010). Regard to acid phosphatase isozyme profile, it showed little variation between the three loci: ACP-1, ACPp-2 and ACP-3. The effect of some treatment on the expression of acid phosphatase was very pronounced in the three loci. ACP-1 and ACP-3 loci with some treatments showed a high thickness and intensity bands. Novicoff *et al*. (1961) suggested that acid phosphatase is hydrolytic in nature and helps in the autolysis of the cell after its death. It could be used as indicator for studying cell mortality due to intoxication. Babu and Devaraj (2008) suggested that higher level of acid phosphatase activities in plant increases its tolerance to stress. Transcription activity of acid phosphatases tends to increase in plants with high P-stress (Li *et al*., 2002).

In the present study, RAPD profiles showed a great variation in banding patterns, particularly in intensity, number, thickness and mobility of the generated DNA fragments by the used primers. This variation was attributed to the effect of the waste water on the genetic materials of *V. faba*. The appearance of new PCR products in the DNA profiles with all used primers may be occurred because some oligonucleotide priming sites could become accessible to oligonucleotide primers because some changes in DNA sequence have occurred due to mutations (resulting in new annealing events), and/or deletions and/or homologous recombination (Atienzar *et al*., 1999). These data confirm the results of Pietrasanata *et al*. (2000) and Enan (2006). The disappearance of some DNA fragments in the DNA profiles might be due to the structural rearrangement in DNA caused by different types of DNA damage. This may correlate with the level of products in DNA template after exposure to pollution which can change the number of binding sites of taq polymerase (Guida *et al*., 2010).

The increase in the intensity of some DNA fragments in the DNA profiles might be due to the structural rearrangement in DNA caused by different types of DNA damage. Moerover, the increase in the thickness of some DNA fragments might be due to the presence of heavy metals (Enan, 2006). Rancelis *et al.* (2006) found a high and unique expression of individual plant polymorphism after exposure of seeds of *V. faba* to cobalt excess. The change in the mobility of some DNA fragments in the DNA profiles might be due to Single Strand Conformational Polymorphism (SSCP). SSCP reveals differences in

electrophoretic mobility between normal and mutant single strands of DNA (Orita *et al*., 1990). So, RAPD analysis could used as a useful biomarker assy for detection of genotoxic effects as previously reported by Liu *et al*. (2009) in barly, and Ahmed *et al*. (2012) in rice.

In conclusion, the irrigation by wastewater can cause genotoxic effects on plant as previously stated (Swaileh *et al*., 2008 and Al-Quarainy, 2009) and *V. faba* can be used as a bioindicator for evaluation of environmental pollution.

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الكشف عن تأثير السمية الوراثية للمياه الملوثة على نبات الفول باستخدام العلامات البيوكيميائية والتكبير العشوائي متعدد الأشكال (RAPD)

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هناك العديد من الانظمة الحيوية التى تسخدم فى قياس تأثير الملوثات البيئية على الكائنات الحية. وفى هذه الدراسة تم استخدام بادرات نبات الفول كمؤشر حيوي لقياس تلوث مياه الرى فى محافظة كفر الشيخ. استخدمت خمسة مصادر مختلفة من المياه في ري بادرات نباتات الفول (الماء المقطركمعالجة سالبة ، ماء الحنفية ، ومياه رى ملوثة من ثلاثة مصادرمختلفة). أظهرت جميع المعاملات تغيرات واضحة في البروتين الكلى للبذور، وأنظمة الأيزوزيم (استريز-α ، استريز-β ، البيروكسيديز، والفوسفاتيزالحمضي). كما طرأت تغيرات على DNA والتي تم الكشف عنها بواسطة تحليل التكبير العشوائى متعدد الاشكال للدنا (RAPD). وقد أظهرت المعالجات المختلفة تغييرات في ظهورواختفاء بعض الحزم ،والتغير فى كثافة وسمك الحزم ، بالاضافة للحركة النسبية للحزم. وتعبر هذه النتائج عن امكانية حدوث السمية الوراثية نتيجة استخدام المياه الملوثة فى الري وكذلك امكانية استخدام نبات الفول كمؤشر حيوى لقياس هذه السمية الوراثية.