

## APPLICATIONS OF MOLECULAR GENETIC TO DETECT DNA DAMAGE CAUSED BY SOME ENVIRONMENTAL POLLUTANTS IN SOME PLANTS

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**E**nvironmental pollutants can have several deleterious properties on living organisms. They can induce acute toxicity, harming cells, tissues, and organs, at high concentrations or high activity. Nonetheless, bioaccumulation resulting from chronic toxification can also be extremely harmful. Plants, as biological indicators, can measure both the actual and potential effects of pollutants when they are used to measure the effects of pollution. Utilizing simple sequence repeats (SSR) and amplified fragment-length polymorphism (AFLP) markers, a "molecular marker fingerprinting" approach was implemented to assess the genotoxic impacts of pollution and determine the environmental risks related to the possible mutagenic effects on three plant species (lettuce, mint, and rocca). DNA from plants exposed to pollution displayed specific marker bands that were not detectable in the DNA of unexposed plants. The development of specific marker band products on the transcription and translation levels will allow for an entirely new understanding of the molecular basis of plants' toxic stress responses to pollution, which appear to reflect the genetic defense action and reaction against pollution. The distinctions between polluted and non-polluted plants seemed to be more pronounced for both markers. Finally, the three plants have a lot of potential to be used as markers of genotoxicity. Genotoxicity in the three plants can be precisely and affordably investigated using SSR and AFLP markers. Conservationists can manage and preserve Egypt's plants, both contaminated and non-polluted, by using the genetics molecular marker bands information from this study.

**Keywords:** pollution, genetic markers, SSR, AFLP, vegetative plants

## INTRODUCTION

Pollution is a serious of environmental problems in modern cities. Given their toxicity and long half-lives in the environment, metals are ranked among the most hazardous categories of anthropogenic environmental contaminants. Consequently, the assessment of metal deposition levels is essential for the evaluation of human exposure. Pollutants that are emitted from automobiles are very diverse. Fossil fuels contain many kinds of heavy metals that are emitted into the environment during combustion (Pecheyran et al., 2000). It is commonly known that cars cause a variety of additional pollutants in addition to heavy metal pollution Cu, Zn, and Pb (e.g., hydrocarbons, nitrogen oxides, carbon monoxide, and carbon dioxide) (Çiçek and Koparal, 2003).

In metropolitan areas, air pollution is caused by industrial sources (factories, refineries, power plants, etc.) and vehicles (cars, buses, trucks, etc.). Various methods have been developed to identify the origins and amounts of this pollution. Identifying and tracking air pollution using organisms is one of these techniques (Conti and Cecchetti, 2001). Many different plants have long been recognized as sensitive indicators of environmental conditions. Species show differing degrees of sensitivity to pollution. Numerous investigations have used the technique of transplantation to monitor pollution, and short-term transplantations could be an easy and economical method to monitor airborne pollutants (Canas et al., 1997). A large number of pollution-monitoring studies that have used biological and chemical methods have shown the ability of plant species to absorb elements directly and accumulate them in their tissues (Guidotti et al., 2009; Cansaran-Duman, 2011; Aslan et al., 2010 and 2011). After continuous contact with specific concentrations of heavy metals—including necessary ones—may produce genotoxicity, which can lead to damage nucleic acids such as additions, deletions, and point mutations. Mechanisms for mutation repair may be able to restore part of this harm, but some may still be there. In recent studies, it has been shown that changes in DNA due to genotoxic agents can be analyzed with DNA fingerprinting techniques, such as simple sequence repeats (SSR) and amplified fragment-length polymorphism (AFLP).

Metal buildup has long been bio indicated using plant species' DNA fingerprinting techniques, and physiological and chemical parameters have been used to evaluate environmental damage (Labra et al., 2003; Liu et al., 2005; Cenkçi et al., 2009; Aksoy-Körpe and Aras, 2011). However, several studies have also been published that have investigated the protective capacity against genotoxic agents, and genotoxicity indicator potential (Geyikoglu et al., 2007; Agar et al., 2010; Cansaran-Duman et al., 2011, 2012, 2014; Aras et al., 2012 and Güner et al., 2012).

Because they combine several benefits for practical applications—such as being co-dominant and multi-allelic, stably inherited, amenable to automation and high-throughput analysis, highly variable, and able to detect the highest level of polymorphism per locus—microsatellite or SSR marker are particularly helpful for studying the stress marker and genetic diversity (Röder et al., 2004).

The conventional AFLP methodology was tweaked to create a quick and accurate method for determining plant DNA genetic markers (Ranamukhaarachchi et al., 2000). Biological methods allow the direct evaluation of pollution's potential for genotoxicity, and considered sensitive and effective indicators of genotoxicity. Biological methods indeed provide a direct means of assessing the possible genotoxicity of pollutants. Genotoxicity is the term used to describe the capacity of some materials or agents to damage cells' genetic material (DNA), which can lead to mutations and possibly raise the risk of cancer and other detrimental health effects. However, biological methods provide direct evidence of genotoxicity by evaluating the consequences of contaminants on cells or living things. They offer valuable tools for environmental and occupational health assessments, helping to identify potentially hazardous substances and evaluate their impact on genetic material. However, it is important to note that these tests are part of a broader array of toxicological evaluations and should be interpreted together with additional relevant data to understand the overall risk posed by a specific pollutant.

The current study's goal was to assess the three species - lettuce, mint, and Rocca - that were used in this investigation of genotoxic potential in the governorate of Qalyubia under polluted conditions as well as their replication in non-polluted environments. The goal of the present study was to identify the alterations that have been taking place in SSR and AFLP profiles of the control species in order to expose the genetic variation patterns that have been impacted by pollution.

## **MATERIALS AND METHODS**

### **1. Plant Material**

Three plant species (lettuce, mint, rocca) were used in the present study under pollution area of the governorate of Qalyubia and their replication under non-polluted conditions.

### **2. DNA extraction**

Extraction of total DNA was performed using methods for medicinal and aromatic plants according to Anna et al. (2001). To eliminate RNA contamination, 10 mg/ml of RNase (Sigma, USA) was added to the DNA solution and incubated at 37°C for 30 min. Optical density was measured at

260 nm using the following equation to estimate the content of DNA in the different samples:

$$\text{Dilution factor (50} \times \times \text{OD260)} = \text{Conc. (ug/ml)}$$

### 3. Simple sequence repeats markers (SSRs) or microsatellites

Four microsatellite primers were developed on the basis of the associated treatments expression have been used for this study. They were on the average of 18-24 bp in length. Primers' sequences are listed in Table (1), genotyped markers were assigned using the Grain Genes database (<http://grain.jouy.inra.fr/cgi-bin/graingenes/browse.cgi>) (Kleinhofs and Graner, 2001).

**Table (1).** The SSRs primers related to treatments and their sequences.

| No. | Primers | PCR Sequences                                       |
|-----|---------|---|
| 1   | SSR1    | F: GTCGGGCTCCATTGCTCT<br>R: CCGGTACCCAGTGACGAC      |
| 2   | SSR2    | F: CTCCCATCACACCATCTGTC<br>R: GACATGGTTCCCTTCTTCTTC |
| 3   | SSR3    | F: ATGGTAGAGGTCCCAACTG<br>R : ATCACTGCTGTGCCTAGC    |
| 4   | SSR4    | F: CTAGCAACTTCCCAACCGAC<br>R: ATGCCTGTGTGTGGACCAT   |

F: forward R: Reverse

#### 3.1. PCR amplification and electrophoresis

PCR amplification was performed in a volume of 25 µl containing approximately 30 ng of template DNA, 1 µl of each forward and reverse primer, suitable quantity of dNTPs, MgCl<sub>2</sub> and Taq DNA polymerase and PCR buffer. Using an Eppendorf PCR machine (Germany), the reactions were run through 35 cycles of 94°C for 1 min, 54–56°C for 1 min, and 72°C for 2 min, culminating in a final extension at 72°C for 5 min. On a 3% agarose gel, the polymorphisms in the PCR reaction products were assessed. The gels were captured with a camera using the gel documentation equipment following a 60-min staining process with 8 µl of Nancy (reveal dye).

#### 3.2. Data scoring and statistical analysis

Carefully chosen bands from duplicated amplifications were used to guarantee an absence of artifacts (three times). Amplified bands based on its size in base pairs and primer code. Data recorded as discrete variables: 1 for the presence and 0 for the absence of a similar band. Only intense and reproducible bands appearing on the gel were scored. Band scoring was examined using SPSS version 16's Gene Tools gel analysis program.

#### 4. Amplified fragment length polymorphisms (AFLPs)

The PCR amplification method known as AFLP is based on the identification of genomic restriction fragments and is applicable to DNA of any origin or complexity. The fingerprints are produced, without any prior knowledge of sequence, using a limited set of genetic primers. Using the selective amplification of restriction fragments from a complete digest of genomic DNA, AFLP enables the identification of variance between genotypes that are closely related. The AFLP technique was used in accordance with Vos et al. (1995). The genomic DNA was cut using two restriction enzymes (EcoRI and MseI) to produce the samples, which were then ligated using double-stranded EcoR 1 and MseI adaptors. The overhanging sticky ends that the restriction enzymes produced were ligated onto the adaptors.

#### 5. Statistical Data Analysis

Scores of present (1) or absent (0) were assigned to each SSR and AFLP band. To compute genomic template stability (GTS %), the following formula was utilized:

$$GTS = (1 - a/n) \times 100n$$

with (a) being the average number of polymorphic bands found in each treated sample and (n) representing the total number of bands in the control (Savva, 1998 and Atienzar et al., 2000).

A qualitative indicator of the genotoxic effect, changes in DNA variations in GTS may be interpreted as profiles caused by genotoxic exposure (Atienzar et al., 2000). The percentage amount of polymorphism found in the profiles of the SSR and AFLP bands was computed, taking into account band appearance, band disappearance, and band intensity variations relative to the control profile.

## RESULTS

### 1. Molecular Analysis

In the current study, SSR associated with environmental pollution-induced DNA damage against four SSR primers produced distinct banding patterns (Table 2 and Fig. 1). The four discriminatory primer pairs were successful in assessing the genetic stability value correlation among the three damaged plant species (lettuce, mint, and rocca) as well as the DNA damage caused by environmental pollution.

The primer SSR2 produced the most bands (18 bands), followed by SSR1 (16 bands), SSR3 (14 bands), and SSR4 (12 bands) for each of the three tested samples. Additionally, variations in band numbers were ascertained by contrasting every plant under non-pollution and pollution conditions. The maximum number of bands were detected at primer SSR1 (6 bands), which ranged from 227 to 688 bp, and primer SSR2 (5 bands), which

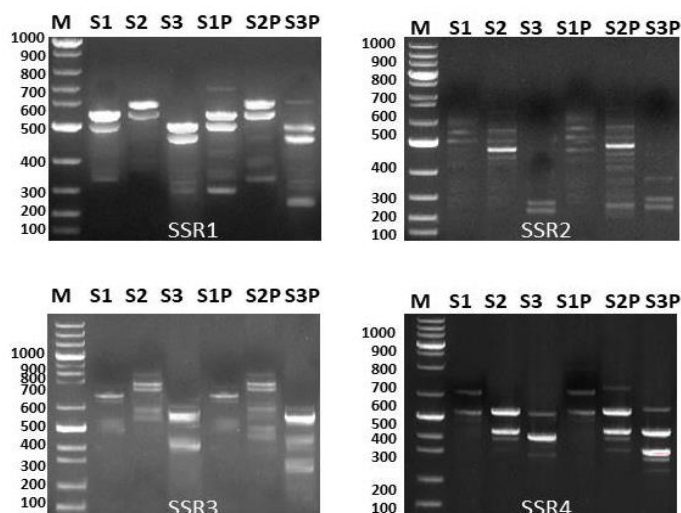
spanned from 246 to 584 bp, in lettuce plant (S1 and S1P). Primer SSR4 had four bands that spanned from 408 to 795 bp, while primer SSR3 had three bands that ranged from 481 to 733 bp. Although the highest number of bands in primer SSR3 (7 bands) ranged from 442 to 969 bp in mint (S2 and S2P), the lowest number of bands in primer SSR1 (5 bands) ranged from 263 to 596 bp. Additionally, the highest number of bands in primer SSR1 (5 bands) ranged from 206 to 606 bp in rocca (S3 and S3P). Primers SSR2, SSR3, and SSR4 had the fewest number of bands, with four bands that, as indicated in Table (2), spanned from 202 to 405 bp, 298 to 571 bp, and 245 to 481 bp, respectively. Conversely, as Table (3) shows, the appearance of certain markers' molecular weights varied among the three contaminated plants.

The results showed that 25 bands differed significantly and may be used as particular identifiers for each of the three contaminated plants, which are present in non-polluted areas but absent in polluted areas. Additionally, Table (3) shows the presence of certain molecular marker bands in the three contaminated plants. Mint plant (S2P) generated the most unique markers, 11 bands, which were arranged as follows: three markers (405, 322, and 263 bp) were generated by SSR1; five markers (5394, 331, 290, 243, and 209 bp) were estimated by SSR2; two markers (484 and 442 bp) were revealed by SSR3; and one marker (633 bp) was revealed by SSR4. In rocca plant (S3P), eight distinct markers were produced, two markers for each primer: SSR1 (606 and 206 bp), SSR2 (405 and 293 bp), SSR3 (323 and 298 bp), and SSR4 (277 and 245 bp). Lettuce (S1P) had the fewest particular markers (5 bands), which were distributed as follows: SSR1 had two markers (688 and 227 bp), SSR2 estimated two markers (340 and 246 bp), and SSR4 gave one marker (795 bp).

**Table (2).** Changes of total bands of SSR primers, their amplified fragments of lettuce, mint and rocca plants under pollution and non-pollution.

| Primers | Total bands | Total size range (bp) | S1 & S1P    |                 | S2 & S2P    |                 | S3 & S3P    |                 |
|---------|-------------|-----------------------|-------------|-----------------|-------------|-----------------|-------------|-----------------|
|         |             |                       | Total bands | Size range (bp) | Total bands | Size range (bp) | Total bands | Size range (bp) |
| 1       | 16          | 206-688               | 6           | 227-688         | 5           | 263-596         | 5           | 206-606         |
| 2       | 18          | 202-584               | 5           | 246-584         | 8           | 209-560         | 4           | 202-405         |
| 3       | 14          | 298-969               | 3           | 481-733         | 7           | 442-969         | 4           | 298-571         |
| 4       | 12          | 245-795               | 4           | 408-795         | 6           | 245-633         | 4           | 245-481         |

Where: S1 = lettuce, S2= mint, S3= rocca, S1P= lettuce under pollution, S2P= mint under pollution and S3P= rocca under pollution



**Fig. (1).** Amplification bands of four SSR primers, their amplified fragments of the three plants under pollution and non-pollution, S1 = lettuce, S2= mint, S3= rocca, S1P= lettuce under pollution, S2P= mint under pollution and S3P= rocca under pollution.

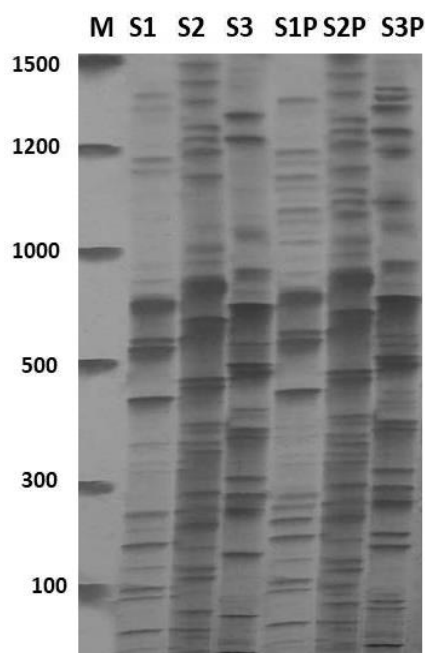
**Table (3).** Changes of total bands of SSR primers, the appearance of specific markers molecular weights (bp) of the three plants under pollution.

| Primers                                     | S1 & S1P<br>specific marker |              | S2 & S2P<br>specific marker |                           | S3 & S3P<br>specific marker |           |
|---|-----------------------------|--------------|-----------------------------|---------------------------|-----------------------------|-----------|
|   | S1P<br>bands no.            | M.w.<br>(bp) | S2P<br>bands no.            | M.w.<br>(bp)              | S3P<br>bands no.            | M.w. (bp) |
| 1   | 2                           | 688 & 227    | 3                           | 405, 322 & 263            | 2                           | 606 & 206 |
| 2   | 2                           | 340 & 246    | 5                           | 5394, 331, 290, 243 & 209 | 2                           | 405 & 293 |
| 3   | ---                         | ----         | 2                           | 484 & 442                 | 2                           | 323 & 298 |
| 4   | 1                           | 795          | 1                           | 633                       | 2                           | 277 & 245 |
| <b>Total marker bands of pollution (24)</b> |                             | 5            |                             | 11                        |                             | 8         |

Where: S1 = lettuce, S2= mint, S3= rocca, S1P= lettuce under pollution, S2P= mint under pollution and S3P= rocca under pollution.

## 2. AFLP associated with DNA damage brought on by environmental pollution

AFLP analysis using pairs of primers EcoR I- ACA and MseI – CTC provided total bands of 170 ranging from 110 to 1713 bp and were succeeded in evaluating DNA damage brought on by environmental pollution and genetic stability value association among the three polluted plant species (lettuce, mint and rocca) and their replicate under non-polluted conditions (Fig. 2). Bands were dispersed as follows: 25 bands ranged from 110 to 1610 bp in lettuce (S1), 29 bands from 109 to 1927 bp in mint (S2), 20 bands in rocca (S3), 34 bands in lettuce under pollution (S1P), 33 bands in mint under pollution (S2P), and 29 bands in rocca under pollution (S3P) between 112 and 1713 bp.



**Fig. (2).** Amplification bands of AFLP profile, the amplified fragments of the three plants under pollution and non-pollution, S1 = lettuce, S2= mint, S3= rocca, S1P= lettuce under pollution, S2P= mint under pollution and S3P= rocca under pollution.

Conversely, Table (4) estimates the molecular weights (bp) of the three plants that were being polluted based on variations in the appearance of certain markers. Thirty-one bands were found to be substantial differences and useful as specific identifiers for all three of the contaminated plants (S1P: lettuce, S2P: mint, and S3P: rocca), which were present in non-polluted areas but absent in polluted areas.



The largest number of specific markers were generated in lettuce plants (S1P) as 18 markers (1185, 1038, 832, 801, 771, 731, 331, 324, 265, 242, 231, 220, 204, 186, 166, 152, 125 and 112 bp). Nine specific markers were generated in rocca plant (S3P) as following: 1713, 1629, 1500, 1212, 970, 384, 366, 191 and 139 bp. The lowest number of specific markers were revealed in mint plants (S2P) with four markers (1023, 985, 920 and 852 bp).

**Table (4).** Changes of total bands of AFLP profile, the specific markers molecular weights (bp) of the three plants under pollution and non-pollution.

| Sampl<br>es  | Names              | Size<br>range<br>(bp) | Total<br>bands | Specific<br>marker<br>no. | Specific molecular weights<br>(bp)  |
|--------------|--------------------|-----------------------|----------------|---------------------------|---|
| <b>S1</b>    | Lettuce            | 110-1610              | 25             | ---                       | -----   |
| <b>S2</b>    | Mint               | 109-1927              | 29             | ---                       | -----   |
| <b>S3</b>    | Rocca              | 112-1440              | 20             | ---                       | -----   |
| <b>S1P</b>   | Lettuce /pollution | 112-1610              | 34             | 18                        | 1185, 1038, 832, 801, 771, 731,<br>331, 324, 265, 242, 231, 220, 204,<br>186, 166, 152, 125 and 112 |
| <b>S2P</b>   | Mint/ pollution    | 109-1927              | 33             | 4                         | 1023, 985, 920 and 852  |
| <b>S3P</b>   | Rocca /pollution   | 112-1713              | 29             | 9                         | 1713, 1629, 1500, 1212, 970, 384,<br>366, 191 and 139   |
| <b>Total</b> | .....              | .....                 | 170            | 31                        | .....   |

Where: S1= lettuce, S2= mint, S3= rocca, S1P= lettuce under pollution, S2P= mint under pollution and S3P= rocca under pollution.

However, the genetic stability value association of the three contaminated plant species (lettuce, mint, and rocca) and their replication under non-polluted conditions was determined using GTS value, which was computed to assess DNA damage brought on by environmental pollution (Table 5).

According to Table (5), GTS values were generally higher in non-polluted plants (S1, S2, and S3) than in polluted plants (S1P, S2P, and S3P). The highest stability number (90%) was found in non-polluted (S2) mint plants when compared to polluted ones (S2P), while the lowest stability number (26%) was found in polluted (S1P) lettuce plants when compared to non-polluted (S1) lettuce plants, which produced (50%) GTS. Comparing rocca plant in the non-polluted (S3) to the polluted (S3P) conditions, this plant showed 70%. Certain bands that appeared on contaminated plants showed a clear correlation between pollution and genetic expression, action, and defense.

**Table (5).** GTS percentage value of the three plants under pollution and non-pollution against all molecular data.

| All molecular data | GTS value (%) |    |    |     |     |     |
|--------------------|---------------|----|----|-----|-----|-----|
|                    | S1            | S2 | S3 | S1P | S2P | S3P |
|                    | 50            | 90 | 70 | 26  | 82  | 60  |

Where: S1= lettuce, S2= mint, S3= rocca, S1P= lettuce under pollution, S2P= mint under pollution and S3P= rocca under pollution.

## DISCUSSION

It is true that environmental pollution can harm DNA. The term "DNA damage" describes changes or fractures in the structure of DNA, which can lead to genetic instability and mutations. Pollution from the environment can harm DNA, which can lead to a number of negative health effects, such as an increased risk of developing cancer and genetic abnormalities. Minimizing exposure to environmental pollutants through efficient pollution control measures, regulation, and adoption of healthier lifestyle choices is vital to lowering the risk of DNA damage and related health problems. Two molecular marker approaches that can be used to compare polluted and non-polluted plants and evaluate genetic variation are SSR and AFLP. Studies on pollution can benefit from this important knowledge regarding the genetic diversity and population structure of plants, which is provided by these techniques. By taking a snapshot of the genetic variety both within and between plant populations, these marker techniques enable researchers to determine genetic markers linked to pollution sensitivity or tolerance as well as evaluate the effect of pollution on plant genetic diversity (Mantiquilla et al., 2021).

Using SSR and AFLP patterns, the present study was able to assess the genetic stability values association of the three polluted plants (lettuce, mint, and rocca) and their replicate under non-polluted conditions. These patterns showed bands of DNA markers appearing at the molecular genetics level, which allowed us to identify the pollution action and changes by appearing of specific markers molecular weights through the three plants (lettuce, mint, and rocca) under pollution and absent in the other non-polluted plants. Significant differences were also found in the evaluation of the genetic damage caused by environmental pollution. Additionally, changes in GTS appeared high values in non-polluted plants (S1, S2, and S3) compared with polluted plants (S1P, S2P and S3P).

The emergence of certain molecular marker bands in the three plants under pollution conditions may indicate a direct correlation between pollution stress and the genetic defense mechanisms against it (Lin et al., 1998). Since molecular bands were freshly formed during stress in plants, it

appears that they are involved in the mechanism of stress defense, which enables the plant to adapt structurally and biochemically to cope with pollution-related stress circumstances.

Markers validation in independent genotypes of different genetic background is essential in figuring out the effectiveness and reliability of the markers to predict phenotypic (Koyama et al., 2001; Cakir et al., 2003 and Collins et al., 2003). It suggests that frequent screening using marker-assisted selection (MAS) should use SSR markers. To validate the markers, it is also necessary to test for their existence on a range of cultivars and other noteworthy genotypes. Therefore, genotype resistant specific marker-assisted selection for stress tolerance may exist, indicating that highly informative SSR markers could be a useful screening method for brewing genotypes (Qian et al., 2011). An effective method for studying mutational change analysis is to use PCR-based SSR and AFLP techniques. These techniques can be used to identify the impacts of pollution and both organic and inorganic genotoxic agents on various species in terms of their ability to cause mutations (Labra et al., 2003). These techniques are helpful because they enable fast comparisons between polluted and non-polluted samples at the same time, especially in pollution research (Liu et al., 2005). To get genotoxicity data on the range of contaminants detected, plant samples near pollution sources were compared in this investigation. The heightened levels of environmental pollution in the area are confirmed by the findings of a prior investigation that was carried out in tandem and found significant levels of pollutant deposition in the same samples (Çiçek et al., 2008).

The GTS is a qualitative metric used to quantify variations in the AFLP and SSR profiles. DNA replication and repair effectiveness as well as the degree of DNA damage may have an impact on the GTS (Atienzar et al., 1999). This might account for the emergence and vanishing of bands. Variations in SSR and AFLP band patterns may also be caused by mutations, rearrangements, and other DNA lesions, according to earlier research. It has been shown that modifications to the GTS could be considered as a result of changes in molecular data profiles caused by pollution. Therefore, as stated by Atienzar et al. (2000), because DNA damage occurs frequently and inhibits DNA replication and repairs, a high probability of DNA damage reduces the stability of the genomic template. Therefore, in an SSR and AFLP test, even a one-band alteration or a small drop in the GTS may be significant the genotoxic consequences of environmental contaminants on living things in their natural habitats. In this sense, proof of pollution's potential for genotoxicity may be discovered in the production of DNA band alterations in response to a particular type of stressor (Aras et al., 2010). The best way to assess environmental genotoxicity may be to directly test the genotoxic effects of pollutants on living organisms. The three plants -

lettuce, mint, and rocca - have quantities of potentially harmful compounds that are revealed and are appropriate for genotoxicity detection.

## CONCLUSION

There is great potential for the three plants - lettuce, mint, and rocca - to serve as genotoxicity indicators. SSR and AFLP markers can be used to precisely and economically investigate genotoxicity in the three plants. The genetics molecular marker bands information from this study can be used by conservationists to manage and safeguard Egypt's plants, both polluted and non-polluted. This knowledge aids in our comprehension and leads us to the conclusion that one way genetics protects against pollution is by coding some resist genes against pollution action as a defensive pathway, which increases transcription and translation rate under pollution stress. However, it is important to keep in mind that these SSR and AFLP techniques provide indirect evidence of the effects of pollution on plants by detecting changes in the genetic diversity or structure. To demonstrate a clear link between pollution and genetic changes, more investigation is required. The truth is that these studies will be more fruitful in the future if they employ Next-Generation Sequencing (NGS) technology, which compares and analyses the genetic composition and gene expression profiles of contaminated and unpolluted plants. Researchers can identify genes that are differentially expressed and crucial for the response to pollution stress by contrasting the transcriptome and structural genome of plants that are contaminated and those that are not. Knowing which molecular pathways and activities are either triggered or suppressed in contaminated plants can help us understand how plants respond to pollution. Plans to increase plant resilience, decrease the effects of pollution, and guide environmental management and monitoring programs can all benefit from this understanding.

## REFERENCES

- Agar, G., M. Gulluce, A. Aslan, S. Bozari et al. (2010). Mutation preventive and antigenotoxic potential of methanol extracts of two natural lichen. *Journal of Medicinal Plants Research*, 4: 2132-2137.
- Aksoy-Körpe, D. and S. Aras (2011). Evaluation of copper-induced stress on eggplant (*Solanum melongena* L.) seedlings at the molecular and population levels by use of various biomarkers. *Mutation Research*, 719: 29-34.
- Anna, M.P., M. Hirsikorpi, T. Kamarainen, L. Jaakola and A. Hohrola (2001). DNA isolation methods for medicinal and aromatic plants. *Plant Molecular Biology Reporter*, 19: 273a-f.
- Aras, S, Ç. Kanlıtepe, D. Cansaran-Duman, M.G. Halıcı et al. (2010). Assessment of air pollution genotoxicity by molecular markers in the exposed samples of *Pseudevernia furfuracea* (L.) Zopf in the Egyptian J. Desert Res., **74**, No. 1, 1-16 (2024)

- Province of Kayseri (Central Anatolia). Journal of Environmental Monitoring, 12: 536-543.
- Aras, S., T. Beyaztaş, D. Cansaran-Duman and E. Gökçe (2012). Evaluation of genotoxicity of *Pseudevernia furfuracea* (L.) Zopf by RAPD analysis. Genetics and Molecular Research, 10: 3760-3770.
- Aslan, A., G. Apaydın, K. Yazıcı, E. Cengiz et al. (2010). Analysis of trace element concentrations of some lichens of Turkey. Asian Journal of Chemistry, 22: 389-400.
- Aslan, A., A. Çiçek, K. Yazıcı, Y. Karagöz et al. (2011). The assessment of lichens as bioindicator of heavy metal pollution from motor vehicles activities. African Journal of Agricultural Research, 6: 1698-1706.
- Atienzar, F.A., M. Conradi, A.J. Evenden, A.N. Jha et al. (1999). Qualitative assessment of genotoxicity using random amplified polymorphic DNA: comparison of genomic template stability with key fitness parameters in *Daphnia magna* exposed to benzo[a]pyrene. Environmental Toxicology and Chemistry, 18: 2275-2282.
- Atienzar, F.A., B. Cordi, M.E. Donkin and A.J. Evenden (2000). Comparison of ultraviolet-induced genotoxicity detected by random amplified polymorphic DNA with chlorophyll fluorescence and growth in a marine macroalgae, *Palmaria palmata*. Aquatic Toxicology, 50: 1-12.
- Cakir, M., S. Gupta, G.J. Platz, G.A. Ablett, R. Loughman, L.C. Emebiri and R. Appels (2003). Mapping and validation of the genes for resistance to *Pyrenophora teres* f. *teres* in Barley (*Hordeum vulgare* L.). Australian Journal of Agricultural Research, 54: 1369-1377.
- Canas, M.S., L. Orellana and M.L. Pignata (1997). Chemical response of the lichens *Parmotrema austrosinense* and *P. conferendum* transplanted to urban and non-polluted environments. Annales Botanici Fennici, 34: 27-34.
- Cansaran-Duman, D. (2011). Study on accumulation ability of two lichen species (*Hypogymnia physodes* (L.) Nyl and *Usnea hirta* (L.) Weber ex F.H. Wigg) at iron-steel factory site, Turkey. Journal of Environmental Biology, 32: 839-844.
- Cansaran-Duman, D., T. Beyaztaş, O. Atakol and S. Aras (2011). Assessment of the air pollution genotoxicity by RAPD in *Evernia prunastri* L. Ach. province of iron steel factory in Karabük, Turkey. Journal of Environmental Sciences, 23: 1171-1178.
- Cansaran-Duman, D., S. Aras, O. Atakol and I. Atasoy (2012). Accumulation of trace elements and the assessment of the genotoxicity in the lichen *Pseudevernia furfuracea* transplanted to a polluted site in Ankara. Ekoloji, 21: 1-14.

- Cansaran-Duman, D., E. Altunkaynak S. and Aras (2014). Heavy metal accumulation and genotoxicity indicator capacity of the lichen species, *Ramalina pollinaria* collected from around the iron-steel factory in Karabük, Turkey. Turkish Journal of Botany, 38: 477-490.
- Cenkçi, S., M. Yıldız, İ.H. Cigerci, M. Konuk et al. (2009). Toxic chemicals-induced genotoxicity detected by random amplified polymorphic DNA (RAPD) in bean (*Phaseolus vulgaris* L.) seedlings. Chemosphere, 76: 900-906.
- Çiçek, A. and A.S. Koparal (2003). The assessment of air quality and identification of pollutant sources in the Eskişehir region Turkey using *Xanthoria parietina* (L.) Th. Fr. (1860). Fresenius Environmental Bulletin, 12: 24-48.
- Çiçek, A., A.S. Koparal, A. Aslan and K. Yazıcı (2008). Accumulation of heavy metals from motor vehicles in transplanted lichens in an urban area. Communications in Soil Science and Plant Analysis, 39: 168-176.
- Collins, H.M., F. Panozzo, S.J. Logue, S.P. Jefferies and A.R. Barr (2003). Mapping and validation of chromosome regions associated with high malt extract in barley (*Hordeum vulgare* L.). Australian Journal of Agricultural Research, 54: 1223-1240.
- Conti, M.E. and G. Cecchetti (2001). Biological monitoring: lichens as bioindicators of air pollution assessment - a review. Environmental Pollution, 114: 471-92.
- Geyikoglu, F., H. Turkez and A. Aslan (2007). The protective roles of some lichen species on colloidal bismuth subcitrate genotoxicity. Toxicology and Industrial Health, 23: 487-492.
- Guidotti, M., D. Stella, C. Dominici, G. Blasi et al. (2009). Monitoring of traffic-related pollution in a province of central Italy with transplanted lichen *Pseudevernia furfuracea*. Bulletin of Environmental Contamination and Toxicology, 83: 852-858.
- Güner, A., H. Turkez and A. Aslan (2012). The *in vitro* effects of *Dermatocarpon intestiniforme* (a lichen) extracts against cadmium induced genetic and oxidative damage. Ekoloji, 21: 38-46.
- Kleinhofs, A. and A. Graner (2001). An Integrated Map of the Barley Genome. In: 'Phillips, R.L. and I.K. Vasil (Eds.)'. DNA-based Markers in Plants (2<sup>nd</sup> Ed.). Dordrecht: Kluwer Academic Publishers, pp. 187-200.
- Koyama, M.L., L. Aurora, M. .K. Robert, J.F. Timothy and R.Y. Anthony (2001). Quantitative trait loci for component physiological traits determining salt tolerance in rice. Plant Physiology, 125: 406-422.

- Labra, M., T. Di Fabio, F. Grassi, S.M. Regondi et al. (2003). AFLP analysis as biomarker of exposure to organic and inorganic genotoxic substances in plants. *Chemosphere*, 52: 1183-1188.
- Lin, H., S. Yanagihara and J. Zhuang (1998). Identification of QTL for salt tolerance in rice via molecular markers. *Chinese Journal of Rice Science*, 12 (2): 72-78.
- Liu, W., P. Li, X.M. Qi, Q. Zhou et al. (2005). DNA changes in bar-ley (*Hordeum vulgare*) seedlings induced by cadmium pollution using RAPD analysis. *Chemosphere*, 61: 158-167.
- Mantiquilla, J.A., M.S. Shiao, H. Shih, W. Chen and Y. Chiang (2021). A review on the genetic structure of ecologically and economically important mangrove species in the Indo-West Pacific. *Ecological Genetics and Genomics*, 18: 100078.
- Pecheyran, C., B. Lalere and O.F.X. Donard (2000). Volatile metal and metalloid species (Pb, Hg, Se) in a European urban atmosphere (Bordeaux, France). *Environmental Science and Technology*, 34: 27-32.
- Qian, G., J. Ping, D. Wang, Z. Zhang and S. Luo (2011). Malt genotypic screening of polymorphism information content (PIC) of PCR-based marker in barley, based on physiological traits. *Molecular Biology*, 1: 101-106
- Ranamukhaarachchi, D.G, M.E. Kane, C.L. Guy and Q.B. Li (2000). Modified AFLP technique for rapid genetic characterization in plants. *BioTechniques*, 29 (4): 858-9, 862-6
- Röder, M.S., X.Q. Huang and M.W. Ganai (2004). Wheat Microsatellites: Potential and Implications. In 'Lörz, H. and G. Wenzel (Eds.)', *Biotechnology in Agriculture and Forestry: Molecular Marker Systems*. Springer Verlag, pp. 255-266.
- Savva, D. (1998). Use of DNA fingerprinting to detect genotoxic effects. *Ecotoxicology and Environmental Safety*, 41: 103-106.
- Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee et al. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, 23: 4407- 4414.

## تطبيقات الوراثة الجزيئية لتحديد تحطم الحامض النووي ببعض الملوثات البيئية في بعض النباتات

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يمكن أن يكون للملوثات البيئية العديد من الصفات الضارة بالكائنات الحية عند التركيزات العالية أو النشاط العالي لتلك الكائنات، ومن الممكن أن تسبب سمية حادة وتلحق الضرر بالخلايا والأنسجة والأعضاء. ومن ناحية أخرى، يمكن أن تسبب حالات التسمم العالية أضرارًا جسيمة بسبب التراكم الحيوي. يمكن أن تستخدم النباتات كمؤشرات بيولوجية، ومنها قياس التأثيرات الفعلية والمحتملة للملوثات عند استخدامها لقياس آثار التلوث. لقد تم تطبيق تقنيات "البصمة الوراثية" استنادًا إلى واسمات SSR و AFLP لتقييم درجة السمية وتأثيرها على الجينات، من أجل تحديد المخاطر البيئية المرتبطة بتأثيراتها الطفوية المحتملة في ثلاثة أنواع نباتية (الخس، والنعناع، وروكا). أظهر الحمض النووي من النباتات المعرضة للتلوث نطاقات محددة لا يمكن اكتشافها في الحمض النووي للنباتات غير المعرضة. يبدو أن كلا العلامتين تظهران اختلافات أكثر أهمية بين النباتات الملوثة وغير الملوثة، مما سيوفر رؤية جديدة لفهم أفضل للأساس الجزيئي لاستجابات الإجهاد السام للتلوث في النباتات، والتي تعكس عمل الدفاع الجيني ورد الفعل وراثيًا من خلال ظهور بعض العلامات المميزة للمنتجات على مستويي النسخ والترجمة.