

EFFECTS OF ULTRAVIOLET RADIATION ON CYTOLOGICAL AND MOLECULAR ASPECTS OF TOMATO

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Tomato (*Lycopersicon esculentum* L.) is considered as an important vegetable crop of special economic importance in the horticultural industry worldwide (Wang *et al.*, 2005). Tomato consumption is known to have health benefits due to its high content of phytonutrients. Recent studies suggest that high intake of fresh or processed tomato lowers the risk of prostate cancer (Campbell *et al.*, 2004).

Ultraviolet radiation (UV) is a kind of radiation that falls in a part of non-ionizing electromagnetic spectrum region which represents 8-9% of the total solar radiation (Frederic, 1993). UV is traditionally divided into three wavelength ranges: UV-A (320-400 nm), UV-B (280-320 nm) and UV-C (200-280 nm). UV-C is harmful to living organisms and can induce a variety of damaging effects in plants, while UV-A is the less hazardous part of UV radiation (Hollosoy, 2002).

During the past two decades, increased level of solar UV radiation have been measured at temperate and Polar Regions of the Earth surface as a consequence of the progressive reduction in the

stratospheric ozone layer (Madronich *et al.*, 1998; Hollosy, 2002).

Usually UV-C and most of the UV-B are reflected through stratospheric ozone, so only UV-A and a little of the UV-B reach the earth (Steeger *et al.*, 2001). Organisms on earth are adapted to UV-A, but may not be adapted to UV-B. Now and due to the reduction in atmospheric ozone, a great deal of attention has been focused on UV-B induced photo damage organisms. A 10% decline in the total stratospheric ozone would raise the amount of UV-B reaching the earth's surface by 20% (Misra *et al.*, 2002).

Increased UV-B radiation caused by reduced stratospheric ozone is expected to continue into the 21st century (Madronich *et al.*, 1998). A large number of studies have been conducted to evaluate the potential consequences of an increase in UV-B radiation on many plants (Zheng *et al.*, 2003). However, most of these studies addressed the effects of UV-B on the whole plant level. Less information on the UV-induced damage at the DNA level are found. Owing to its high energy level, the

impact of radiation on metabolic process can be very harmful. DNA, protein and photosynthetic apparatus are considered primary potential targets of UV radiation on plants (Hollosy, 2002; Santina *et al.*, 2008).

Cell irradiation with both UV-B and UV-C radiations results in various DNA photoproducts. These products may cause mutation during replication (Jiang and Taylor, 1993). The most common DNA photoproducts are cyclobutane-type pyrimidine dimers (CPDs) and the pyrimidine (6, 4) pyrimidone dimer. In addition, DNA protein cross-links, DNA strand breaks and deletion or insertion of base pairs can also be induced by UV-B exposure. Quaitte *et al.* (1992) found that (CPDs) represent approximately 75% of total DNA damage.

The production of reactive oxygen species (ROS) by UV has been also demonstrated (Mackerness and John, 2001) and the induction of antioxidative enzymes constitutes a defense response to these molecules (Santos *et al.*, 2004). The accumulation of flavonoids is also considered a defense mechanism against UV, as they protect the mesophyll tissue through epidermal screening of this radiation (Santina *et al.*, 2008).

Recently, molecular markers have been used to investigate DNA sequence variation(s) in and among the crop species and create new sources of genetic variation by introducing new and favorable traits from landraces and related crop species.

Among molecular markers, Randomly Amplified Polymorphic DNA (RAPD) is generally favored because of its sensitivity, simplicity and cost-effectiveness, coupled with the fact that DNA sequence information is not required for primer design, no radioisotope labeling is needed for sample detection, and only small amount of template DNA is required. In addition, this technique is reliable when assaying considerable number of primers and selecting few repeatable RAPD fragments (Nayak *et al.*, 2003; Lin *et al.*, 2009). RAPD is very useful in the study of biodiversity, hybridization, gene mapping and genetic map construction (Elham *et al.*, 2010)

Inter-simple sequence repeats (ISSRs) molecular markers amplify regions between microsatellite loci. This class of markers does not require any prior knowledge about the sequences to be amplified and shows high polymorphism in the material, being very useful in studies of genetic diversity, phylogeny, genomics and evolutionary biology (Reddy *et al.*, 2002). This type of markers provides highly effective plant fingerprinting (Joshi *et al.*, 2000; Adawy *et al.*, 2004).

The start codon targeted (SCoT) polymorphism is a novel, simple and reliable gene targeted marker technique based on the translation start codon (Collard and Mackill, 2009). Primers for SCoT marker analysis were designed based on the conserved region surrounding the translation initiation codon, ATG. Using a single 18-mer primer as a forward and reverse pri-

mer in the PCR, Collard and Mackill (2009) designed thirty-six primers that were used successfully for cultivar identification and genetic diversity analysis in many crops.

Therefore, the objectives of the present investigation are to detect the effects of the UV-B radiation on the mitotic division as well as on the genomic DNA of tomato using RAPD, ISSR and SCoT assays.

MATERIALS AND METHODS

Plant Material

Seeds of tomato (variety Castle Rock) were germinated in greenhouse at AGERI. At age of 3 weeks, plants were subjected to UV-B radiation for periods of 20, 40 and 60 minutes. Seeds of tomato (variety Castle Rock) were germinated in greenhouse at AGERI with 16 hr illumination per day. At age of 3 weeks, plants were subjected to UV-B radiation for periods of 20, 40 and 60 minutes.

UV-B irradiation

Seeds of *Lycopersicon esculentum* L. were germinated on moistened filter paper in Petri dishes with 16 hour illumination per day for three weeks till the shoots reached 1.5-2 cm long. The source of UV radiation (SunStudio) was placed at a distance of 30 cm from the plants. Irradiation in the UV-B wavelength (mainly 290-320 nm) was measured using a calibrated spectroradiator located at the Biophysics unit, Physics department of the

Faculty of Science, Ain Shams University. The level of UV-B radiation utilized approaches natural conditions found in certain regions like Italy (Meloni *et al.*, 2000). The radiation supplied a weighted irradiance of 6.3 KJ m² of biologically effective enhanced UV-B. Three different exposure periods of 20, 40 and 60 minutes were employed.

Cytological studies

Root tips (1-2 cm) were collected in bulk and fixed in Carnoy solution made of absolute ethanol:glacial acetic acid (3:1). After 24 hours root tips were transferred to 70% ethyl alcohol and stored in 14°C. The aceto-carmine staining method was used to stain the root tip cells as described by Fayed *et al.* (1985) and Sayed-Ahmed (1985). The fixed root-tips were washed thoroughly with distilled water and macerated by 1 N HCl at 60°C for 3 minutes and then washed with distilled water. The root tips were squashed on dry clean slides and stained with a small drop of fresh stain. Root tips were warmed gently in aceto-carmine for a short time. The cover slips were removed by the dry-ice method and the slides were immersed in a series of ethyl alcohol (50, 70 and 96 absolute alcohol: xylol (1:1) and then xylol for 5 minutes in each and then mounted in Canada balsam and dried. At least twelve prepared slides were used to determine the mitotic index and chromosomal aberrations.

The effects of UV-B radiation on the frequencies of chromosomal aberrations

tions and micronuclei were analyzed. All examinations were done with a light microscope at 300x and 400x magnifications. The mitotic index represented the percentage of dividing cells divided by the total cells examined. The numbers of chromosomal aberrations were estimated in dividing cells.

Total DNA extraction from irradiated tomato plants

Leaves of three weeks old irradiated plants were pooled in bulk and used to extract DNA with DNeasy Plant Mini Kit supplied from Qiagen Hilden Germany catalog number 69104. Tissues were ground using liquid nitrogen and 400 µl of AP1 buffer and 4 µl of RNase stock solution (100 mg/ml) were added to a maximum of 100 mg of ground tissue, the mixture was incubated for 10 min at 65°C. The tube was mixed 2-3 times during incubation, then 130 µl of AP2 buffer was added to the lysate, mixed and incubated for 5 min on ice. The lysate was applied to mini spin column (pink) and centrifuged for 2 min at 20,000 xg then the supernatant was transferred to a new tube and 1.5 volume of AP3 buffer was added and mixed by pipetting. The supernatant was transferred to mini spin column (white) and centrifuged for 1 min at 6000 xg then the column placed in a new 2 ml collection tube. A volume of 500 µl AW buffer was added to the column, centrifuged for 1 min at 6000 xg and the flow through was discarded then this step was repeated. The column was transferred to a new tube and 100 µl of AE buffer were added di-

rectly on the column then incubated for 5 min at room temp. The column was centrifuged for 1 min at 6000 xg to elute the DNA, which was stored at -20°C till further investigation

Molecular Marker Analysis

RAPD amplification was performed as described by Williams *et al.* (1990), using eleven 10-mer random primers (Table 2). ISSR assays were performed as described by Adawy *et al.* (2004) and Hussein *et al.* (2006). Nineteen ISSR primers (Table 4) were selected from different published papers to be employed in ISSR analysis. SCoT amplification was performed as described by Collard and Mackill (2009), using fifteen 18-mer primers (Table 6). These primers were selected from published papers (Collard and Mackill, 2009; Xiong *et al.*, 2011).

Data Analysis

The banding patterns generated by RAPD, ISSR and SCoT were examined to determine the level of polymorphism and the genetic relatedness among the four tested treatments and the control. The amplified fragments were scored as present (1) or absent (0). The genetic similarity among the control and three treatments was estimated according to Dice coefficient. Cluster analysis was based on similarity matrix obtained within-weighted pair group method using arithmetic average (UPGMA), and the relationships between the treatments were displayed as dendrogram.

RESULTS AND DISCUSSION

Tomato plants were subjected to both cytological studies and fingerprinting analysis using RAPD, ISSR and SCoT where five plants were used for each treatment.

I- Cytological Studies

Mitotic index (MI) was determined as a parameter of mitotic activity. The result obtained after exposure of tomato plants to UV-B radiation showed a slight effect on MI. This result shows that UV-B non-ionizing radiation with the chosen exposure times had no harmful effect on cell division. The mean values of summary data are shown in Table (1).

UV-B radiation induced both clastogenic and aneugenic effects. The frequency of mitotic abnormalities in response to UV-B treatment increased in a time-dependent manner. An increase in the frequency of mitotic abnormalities was observed after 20, 40 and 60 min, these periods were in accordance with Mostafaa *et al.* (2011) who studied the difference in the genetic background of irradiated freshwater leech; *Barbronia assiuti* for 30 min, 45 min and 60 min to UV-A and with El Tobgy *et al.* (2009) who studied the effect of laser radiation on anise and cum-in plants for 5, 10 and 20 min.

Mitotic abnormalities included lagging chromosomes, chromosome fragments and micronuclei (Fig. 1). Chromosome bridges and C-Metaphase have been noticed in very small numbers in the root

tips (Fig. 1e and 1h). The most pronounced increase in frequency of laggards was observed after one hour of exposure to UV (0.59%) as compared with control (0.03%) (Table 1 and Fig. 1). Laggards may be explained on the basis of abnormal spindle formation and failure of the spindle to carry the respective chromosomes to the poles. It has previously been reported that unsynchronized laggards might be due to the discrepancies in spindle formation. Laggards at anaphase can be attributed to the delayed terminalization or perhaps to stickiness of chromosomal ends. Behavior of laggard chromosomes is characteristic in that they generally lead to micronuclei formation (Pesnya and Romanovsky, 2013). These data indicate that UV-B radiation may damage the formation of spindle microtubules.

Effect of UV-B radiation on DNA is well represented by the production of chromosomal fragments/breaks. The frequency of chromosome fragments in the control group was 0.05% and the frequency of micronuclei was 0.22%. Exposure to UV-B radiation increased the frequencies of fragments and micronuclei in the root tips. The most increase in fragment frequency (0.42%) was recorded after one hour of exposure to UV-B radiation (Table 1). The highest increase in micronuclei frequency (0.61%) was observed after 40 min of exposure to UV (Table 1). Micronuclei were observed in previous studies at telophase I/II following gamma radiation and may be due to the association of fragments and/or lagging chromosomes which failed to reach the poles and got

included in the daughter nuclei (Jafri *et al.*, 2011). Micronuclei derived from lag-gard chromosomes have a higher probability to survive and undergo condensation in synchrony with the main nuclei than micronuclei derived from chromosome fragments. Micronuclei are mutagenic aspects which may lead to the loss of genetic material (Eckert *et al.*, 1997)

Our results are thus in agreement with previous reports and show that UV-B radiation can induce mitotic and chromosomal abnormalities in plant cells. We also concluded that an increase in radiation exposure time leads to an increase in the percentage of mitotic abnormalities, in accordance with Kravetz (2009) with a slight effect on mitotic index.

II- *Molecular studies*

RAPD analysis

The amplification profiles of the four tomato treatments (Control and three durations treatments) produced by the 11 selected primers revealed a total of 65 polymorphic bands out of 119 reproducible products (Table 2 and Fig. 2). This corresponds to a level polymorphism of 54.6%. The number of amplicons/primer ranged from 6 (OP-D20) to 15 (OP-A18) whereas the number of polymorphic bands per primer ranged from 1 (OP-D20) to 9 (OP-A18 and OP-A20).

The genetic similarity among the three tomato treatments and the control ranged from 71.7 to 83.3 (Table 3). The lowest genetic similarity (71.7) was be-

tween control and treatment No. 2 irradiated with UV-B for a period of 40 minutes. On the other hand, the highest genetic similarity (83.3) was between treatment No. 2 and treatment No. 3 irradiated with UV-B for periods of 60 minutes.

The dendrogram of genetic relationships from RAPD data separated the three tomato treatments and the control into two main clusters (Fig. 3). The first cluster comprised two treatments (Control and treatment No. 1) and the second cluster comprised the other two treatments (treatment No. 2 and treatment No. 3) irradiated with 40 min and 60 min, respectively.

The total polymorphic band between each treatment and control was 29, 47 and 33 in treatments 1, 2 and 3, respectively. These result revealed that the highest level of genetic variation was observed between the control and treatment no. 2 (40 min), which refelected the highest genomic changes. While, the lowest level of genetic variation was observed between the control and treatment no. 1 (20 min).

In this respect, El Sherif *et al.* (2011) used twenty-eight RAPD primers to investigate the effect of different doses of gamma irradiation on growth, yield, calyx extract and quality as well as molecular changes of Roselle plants. Six primers out of twenty-eight RAPD primers successfully amplified DNA fragments from *Hibiscus sabdariffa* L. DNA samples. The results indicated the occurrence of structural changes in treatments. Fifty-four

fragments were visualized across the six primers with percentage of polymorphism (38.4, 70, 50, 77.3, 54.5 and 38.4%). The result of RAPD analysis indicated that increasing doses of gamma irradiation caused severe effects on the plant development.

On the other hand, El-Fiky (2011) employed six RAPD decamer primers to identify irradiation-induced molecular marker/s in two genotypes of sesame [Giza-24(I) and Taka-3(I); irradiated with 100 Gy γ - rays] in contrast with five non-irradiated sesame genotypes [Shandaweel-3, Toshka-1, Giza-24 (C), Taka-1 and Taka-3(C)]. Results showed that five out of six primers exhibited polymorphic banding pattern between control and irradiated genotypes.

Moreover, Danylchenko and Sorochinsky (2005) investigated the possible mutation changes in alfalfa seedlings DNA resulting from exposure to UV-B and γ -ray using the RAPD technique. The authors concluded that the appearance of polymorphic bands between control and irradiated plants can be explained as the result of different DNA structural changes (breaks, transpositions, deletions etc). They also showed that single nucleotide substitutions in 10-mer primers indeed can be reflected on the amplicons profiles.

Cuadra *et al.* (2004) revealed that treating *Jaborosa magellanica* with UV-B radiation caused changes in plant growth, plant chemistry and by using random primers and PCR amplification procedure, a high degree of polymorphism was detect-

ed when treated plants were compared to non-irradiated plants. These biochemical changes may be interpreted as plant response to UV-B radiation stress and as an indicator of DNA damage.

Hegazi and Hamid Eldin (2010) studied the effect of different gamma irradiation doses and water soaking on seeds of two okra varieties (Sabahia and Balady) using RAPD and SDS-PAGE. The RAPD results showed variation in DNA profile in response to gamma irradiation treatments. The percentage of polymorphism observed was 47.37% in var. Sabahia while, it was 50% in var. Balady. The relatively high doses of gamma irradiation (400 and 500 Gy) induced more changes in genomic DNA pattern than the low dose (300 Gy). In addition, the authors mentioned that these results are in good correspondence with Wendt *et al.* (2001) who used the RAPD markers to study the effect of gamma radiation on potato.

ISSR analysis

The 19 ISSR primers used in the present study produced good reproducible and scorable patterns (Fig. 4). As shown in Table (4), a total of 130 fragments were generated by the 19 primers with an average of 6.9 fragments/primer. Primer H2 yielded the highest number of products (13 amplicons), while primers (H5, 7, 9, 11 and 13) revealed the lowest number (4 amplicons). The number of polymorphic markers also varied between primers, ranging from 0 in primers (H5, 6, 7, 9 and 11) to 9 in primers (H2 and H4). This corresponds with a level polymorphism of

35.4%. The average number of polymorphic fragments/primer among the UV treatments and control was 2.4.

As shown in Table (5), the estimated similarities among the control and 3 UV-B treatment durations ranged from 83.8 to 93.8. The highest genetic similarity (93.8) was between treatment No. 1 and treatment No. 3. While, the lowest genetic similarity (83.8) was between control and treatment No. 2. These results are in good agreement with our expected arrangement of these treatments.

The dendrogram of genetic relationships from ISSR data clearly separated the control in a separate cluster while, all treated treatments (treatment No. 1, 2 and 3) were separated in the second cluster (Fig. 5).

The total polymorphic bands between each treatment and control was 31, 35 and 24 in treatments 1, 2 and 3, respectively. These results revealed that the highest level of genetic variation was observed between the control and treatment No. 2 (40 min), reflecting the highest genomic changes. While, the lowest level of genetic variation was observed between the control and treatment 3 (60 min).

In this respect, Cuadra *et al.* (2010) analyzed the effects of UV-B radiation on *Gnaphalium*'s DNA and on UV-absorbing compounds using five ISSR primers. Among these primers; the four ISSR primers selected for this analysis generated a total of 189 fragments. The dendrograms

obtained using these markers efficiently separated plants from different treatments. The authors observed linear relationship between UV-B dose and percentage of dissimilarity which may be related to DNA damage caused by the different UV-B treatments.

On the other hand, Pestana *et al.* (2011) evaluated the genetic variability in putative banana mutants irradiated with gamma ray, using a set of agronomical and ISSR markers. The ISSR analysis using 19 primers yielded a total of 186 polymorphic bands, where, 74 were monomorphic with an average of 9.8 total bands and 5.8 polymorphic bands, respectively. The authors concluded that these results can be used successfully in the genetic breeding program of banana aiming to develop new varieties in a short time.

Meanwhile, Khatri *et al.* (2011) used 11 ISSR primers to study genetic variation among the radiated population in banana. ISSR primers produced 37 bands in all the clones studied (two monomorphic and 35 polymorphic bands). The number of fragments produced by the primers ranged from 2-5, with an average of 3.45 fragments. Genetically most similar genotypes were G-215MP3 and G-215MP4 (89.8%) while, most dissimilar genotypes were Basrai and G-215MP2 (38.5%). Based on similarity matrix, clones were divided into three clusters and two groups.

In addition, Mudibu *et al.* (2011) aimed to determine the effect of irradiation

tion with gamma rays on the genetic variability using ISSR markers in different soybean varieties. The results showed that the level of polymorphic loci among the soybean varieties varied from 70 to 90%. The authors noticed that the increasing of gamma rays dose consequently increased the level of polymorphism in progenies by 10%.

SCoT analysis

In the present investigation, eighteen SCoT primers were employed to investigate the genetic polymorphism among the three tomato treatments and the control. As shown in Table (6) and Fig. (6), all of the 18 primers detected polymorphic patterns and revealed a total number of 182 amplicons with 43.0% level of polymorphism. The number of amplicons per primer ranged from 6 to 18 with an average of 12.1 amplicons per primer. The number of monomorphic amplicons varied from 3 to 12 with an average of 6.9 monomorphic amplicons per primer. However, the number of polymorphic amplicons varied from 1 to 12 with an average of 5.2 polymorphic amplicons per primer.

As shown in Table (7), the estimated similarities among the three tomato treatments ranged from 79.8 to 89.9. The highest genetic similarity (89.9) was between sample No. 1 (20 min exposure to UV-B) and sample No. 3. (60 min exposure to UV-B) while the lowest genetic similarity (79.8) was between control and sample No. 1. (20 min exposure to UV-B).

The dendrogram of genetic relationships from SCoT data clearly separated the control in a separate cluster from all treatments (Sample No. 1, 2 and 3) which are placed in a second cluster (Fig. 7).

In this respect, since SCoT marker was recently innovated by Collard and Mackill (2009), till now, up to our knowledge, there is no published paper reporting the use of SCoT marker to assess the genetic changes that happened after plant irradiation.

However, many studies employed SCoT as a novel molecular marker technique to evaluate and study the genetic relationships between closely related genotypes. In this manner, Xiong *et al.* (2011) used start codon targeted polymorphism technique to study genetic diversity and relatedness among 20 accessions of four major botanical varieties of peanut. Out of 36 primers screened, 18 primers could produce unambiguous and reproducible bands. All 18 primers generated a total of 157 fragments, with a mean of 8.72 ranging from 4 to 17 per primer. Of 157 bands, 60 (38.22%) were polymorphic. One to seven polymorphic bands were amplified per primer, with 3.33 polymorphic bands on average. Polymorphism per primer ranged from 14.29 to 66.67%, with an average of 36.76%.

On the other hand, Luo *et al.* (2011) employed two molecular marker systems (SCoT and ISSR) in order to identify and detect the genetic relatedness between 23 of mango germplasms. Using 18 selected

SCoT primers, 158 bands were generated, of which 104 (65.82%) were polymorphic.

Genetic relationships among the different tomato treatment plants as revealed by combined data

The scoring data derived from the three marker types (RAPD, ISSR and SCoT) were combined and computed to generate more accurate relatedness among the three tomato treatments. The range of pair similarity coefficient between them ranged from 79.5 to 89.5 (Table 8). The lowest similarity coefficient (79.5) was observed between control and sample No. 2. (40 min exposure to UV-B) while, sample No. 1 (20 min exposure to UV-B) and sample No. 3 (60 min exposure to UV-B) had the highest similarity value (89.5).

The cluster analysis using the different marker systems revealed three dendrograms (Figs. 3, 5 and 7). All dendrograms clearly discriminated between the control and irradiated treatments except RAPD dendrogram. Therefore, to obtain a more accurate cluster analysis based on large genome coverage, a dendrogram was constructed using the combined data obtained from the different types of markers (RAPD, ISSR and SCoT). As shown in Fig. (8), this dendrogram, based on the combined data, clearly clustered the control and studied tomato treatments into two main clusters. The first cluster comprised only the control while, the second cluster comprised the rest of the three irradiated durations. This could be attributed to the higher level of genome coverage achieved by combining

data of three types of markers than when using each marker separately.

Similar conclusion was drawn by Adawy *et al.* (2004), Agrama and Tuinstra (2003) and Hussein *et al.* (2006) who indicated that the combined dendrogram can clearly discriminate among close genotypes. Thus, combined analysis of RAPD, ISSR, and SCoT data provide a quick, reliable and highly informative system for DNA fingerprinting and also allows establishing genetic relationships in many crops. In conclusion, induced mutations using physical mutagens is one way to create genetic variation resulting in new varieties with better characteristics. The genetic variability caused by mutations has been used in plant improvement programs that enable plant breeders to select new genotypes with improved characteristics such as abiotic and biotic tolerance, grain yield and quality. In order to understand the significance of change due to increased UV-B radiation, future research on plant effects should focus on basic research including biochemical and physiological mechanisms conducted in the laboratory, also it is advisable to stop man made contamination of the stratospheric ozone to keep living organisms safe on earth.

SUMMARY

Measurement of ozone level proved that stratospheric ozone layer is being depleted as a result of contamination with man-made chlorofluorocarbon. One component of global climate change is the loss of stratospheric ozone which protects the earth from UV radiation. UV

is traditionally divided into three wavelength ranges: UV-A, UV-B and UV-C. UV-C radiation is completely absorbed by atmospheric gases. UV-B is additionally absorbed by stratospheric ozone and thus only a very small proportion is transmitted to the earth's surface, whereas UV-A radiation is hardly absorbed by ozone.

Organisms on earth are adapted to UV-A, but may not be adapted to UV-B. Now and due to the reduction in atmospheric ozone, a great deal of attention has been focused on UV-B induced photo damage organisms. A 10% decline in the total stratospheric ozone would raise the amount of UV-B reaching the earth's surface by 20%.

UV radiation has been shown to be harmful to living organisms, damaging DNA, lipids, protein and membrane. Plants, which use sunlight for photosynthesis, cannot avoid exposure to increased levels of UV radiation so they are at higher risk. In order to understand the significance of change of climate to increased UV-B radiation. Our work has demonstrated the severe effect of UV on tomato cytological parameters as well as on the level of DNA using different molecular marker assays.

Tomato seeds (Castle Rock variety) were germinated, then at age of three weeks; they were exposed to UV-B radiation during 0, 20, 40 and 60 min periods. Mitotic abnormalities and mitotic index were analyzed. Exposure to UV-B radiation slightly affected the mitotic index but

induced both clastogenic and aneugenic effects. UV-B radiation increased the frequency of mitotic abnormalities in a time-dependent manner. Changes in DNA fingerprint was monitored in UV irradiated seedlings using RAPD, ISSR and SCoT molecular marker assays.

The DNA of the irradiated tomato plants and control were assessed using 11 RAPD, 19 ISSR and 15 SCoT primers. These primers produced 119, 130 and 182 amplicons, respectively. The numbers of polymorphic amplicons were 65, 46 and 78; corresponding to a level of polymorphism 54.6, 35.4 and 43.0%, respectively. The dendrograms obtained using these marker types efficiently separate different duration irradiated plants and control in two clusters except RAPD dendrogram. The data scored from RAPD, ISSR and SCoT were combined and computed to generate more accurate relationships based on large and versatile genome coverage. The combined dendrogram clearly separated irradiated plants and control ones into two separate clusters; which is in a good agreement with both ISSR and SCoT dendrograms.

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REFERENCES

- Adawy, S. S., Ebtissam A. Hussein, M. M. Saker and Hanaiya A. El-Itriby (2004). Intra- and Inter-varietal variation of Upper Egypt date palm cultivars (*Phoenix dactylifera* L.): I. As revealed by RAPD and ISSR markers. Proceed. Int. Conf. Genet. Eng. & Appl., Sharm El-Sheikh, South Sinai, Egypt (April, 8-11, 2004): 165-179.
- Agrama, H. D. and M. R. Tuinstra (2003). Phylogenetic diversity and relationships among sorghum accessions using SSRs and RAPDs. *Afri. J. of Biotech.* 2: 334-340.
- Collard, B. C. Y. and D. J. Mackill, (2009). Start Codon Targeted (SCOT) polymorphism: A simple novel DNA marker technique for generating gene-targeted markers in plants. *Plant Mol. Bio.*, 27: 86-93.
- Campbell, J. K., K. Canene-Adams, B. L. Lindshield, T. W. Boileau, S. K. Clinton and J. W. Jr Erdman (2004). Tomato phytochemicals and prostate cancer risk. *J. Nutr.*, 134: 3486S-3492S.
- Cuadra, P., H. Raul and F. Victor (2004). Effects of UV-B radiation on the Patagonian *Jaborosa magellanica*. *J. Photochemistry and Photobiology B: Biology*, 76: 61-68.
- Cuadra P., V. Daniela, F. Victor and H. Raul (2010). Effects of UV-B radiation in morpho-genetic characters of *Gnaphaliumluteo-album*. *J. Photochemistry and Photobiology B: Biology*, 101: 70-75.
- Danylchenko, O. and B. Sorochinsky (2005). Use of RAPD assay for the detection of mutation changes in plant DNA induced by UV-B and γ -rays. *BMC Plant Biology*. 5 (Suppl 1):S9, doi:10.1186/1471-2229-5-S1-S9.
- Eckert, I., W. Caspary, M. Nusse, M. Liechty, L. Davis and H. Stopper (1997). Micronuclei containing whole chromosomes harbouring the selectable gene do not lead to mutagenesis. *Mutagenesis*, 12: 379-382.
- El-Fiky, Z. A. (2011). Comparative analysis of RAPD marker sequences in relation to gamma irradiation in sesame. *Egypt. J. Genet. Cytol.*, 40: 239-251.
- El Sherif, Fadia, K. Salah, G. Ezzat, S. Nashwa and R. Khaled (2011). Effect of gamma irradiation on enhancement of some economic traits and molecular changes in *Hibiscus Sabdariffa* L. *Life Sci. J.*, 8: 220-229.
- Elham, A. A. H., A. A. H. Atef, R. A. H. Nagwa and A. R. Aida (2010). Phylogenetic diversity and relationships of some tomato varieties

- by electrophoretic protein and RAPD analysis. *J. American Sci.*, 6: 434-441.
- Fayed, A. H., A. S. Mondour, A. A. Mahmoud and M. A. Ismail (1985). Effect of colchicine treatment and Rhizobium inoculation on cell division and micronuclei formation in meristems of *Vicia faba*. *Egypt. J. of Envir. Muta. Terat. and Carcin.*, 1: 21-36.
- Frederic, J. E. (1993). Ultraviolet sunlight reaching the Earth's surface. A review of recent research. *Photochem. Photobiol.*, 57: 175-178.
- Gulfishan, M., A. H. Khan, I. F. Jafri and T. A. Bhat (2012). Assessment of mutagenicity induced by MMS and DES in *Capsicum annum* L. *Saudi J. of Bio. Sci.*, 19: 251-255.
- Hegazi, A. Z. and N. Hamid Eldin (2010). The effect of gamma irradiation on enhancement of growth and seed yield of okra [*Abelmoschu sesculentus* (L.) Monech] and associated molecular changes. *Journal of Horticulture and Forestry*, 2: 38-51.
- Hollosy, F. (2002). Effects of ultraviolet radiation on plant cells. *Micron.*, 33: 179-197.
- Hussein, Ebtissam H. A., Amina A. Mohamed, S. Attia, S. S. Adawy (2006). Molecular characterization and genetic relationships among cotton genotypes I- RAPD, ISSR and SSR analysis. *Arab J. of Biotech.*, 9: 313 -328.
- Jafri, I. F., A. H. Khan and M. Gulfishan (2011). Genotoxic effects of 5-bromouracil on cytomorphological characters of *Cichorium intybus* L. *Afri J. of Biotech.*, 10: 10595-10599.
- Jiang, N. and J. S. Taylor (1993). *In vivo* evidence that UV-induced C-T mutations at dipyrimidine sites could result from the replicative bypass of cis-syn cyclobutane dimers or their deamination products. *Bio.*, 32: 472-481.
- Joshi, S. P., V. S. Gupta, R. K. Aggarwal, P. K. Ranjekar and D. S. Brar (2000). Genetic diversity and phylogenetic relationship as revealed by inter-simple sequence repeat (ISSR) polymorphism in the genus *Oryza*. *Theor. Appl. Genet.*, 100: 1311-1320.
- El Tobgy, K., M. K., Y. A. H. Osman and E. A. El Sherbini (2009). Effect of laser radiation on growth, yield and chemical constituents of anise and cumin plants *J. of Appl. Sci. Res.*, 5: 522-528.
- Khatri A., D. Saji, U. D. Mahummad, A. K. Imtiaz and S. N. Ghulam (2011). *In vitro* mutagenesis in banana and variant screening through ISSR. *Pak. J. Bot.*, 43: 2427-2431.

- Kravetz, E. A. (2009). Cellular and tissue mechanisms of recovery processes in *Hordeum distichum* L. under irradiation. *Cytology and Genetics*, 43: 9-17.
- Lin, K. H., Y. C. Lai, H. C. Li, S. F. Lo, L. F. O. Chen and H. F. Lo (2009). Genetic variation and its relationship to root weight in the sweet potato as revealed by RAPD analysis. *Scientia Horticulturae*, 120: 2-7.
- Luo, C., H. Xin-hua, C. Hu, O. Shi-jin, G. Mei-ping, S. B. C. James, T. Tondo and J. S. Raymond (2011). Genetic diversity of mango cultivars estimated using SCoT and ISSR markers. *Bioch. Systematics and Eco.*, 10: 5-23.
- Mackerness, S. A. H. and B. John (2001). Early signaling components in ultraviolet-B responses: distinct roles for different reactive oxygen species and nitric oxide. *FEBS Lett.*, 489: 237-242
- Madronich, S., R. L. McKenzie, L. O. Bjorn and M. M. Camdwell (1998). Changes in biologically active ultraviolet radiation reaching the earth's surface. *J. Photochem. Photobiol.*, 46: 5-19.
- Meloni, D., G. R. Casale, A. M. Siani, S. Palmieri and F. Cappellani (2000). Solar UV dose patterns in Italy. *Photochem. Photobiol.*, 71: 681-690.
- Misra, R. B., G. S. Babu, R. S. Ray and R. K. Hans (2002). Tubifix: a sensitive model for UV-B- induced phototoxicity. *Ecotoxicol. Environ. Saf.*, 52: 288-295.
- Mostafa, M. A., N. El-Shimy, Z. El-Bakry and H. Sabi (2011). Impact of Ultraviolet Radiation on the Genetic Background of Egyptian Freshwater Leech; *Barbronia assiuti*. *World Appl. Sci. J.*, 12: 1974-1980.
- Mudibu, J., K. K. C. Nkongolo, M. Mehes-Smith and A. Kalonji-Mbuyi (2011). Genetic analysis of a soybean genetic pool using ISSR marker: effect of gamma radiation on genetic variability. *Int. J. Plant Breed. Genet.*, 5: 235-245.
- Nayak, S., B. K. Debata, V. K. Srivastava and N. S. Sangwan (2003). Evaluation of agronomically useful somaclonal variants in Jamrosa (a hybrid *Cymbopogon*) and detection of genetic changes through RAPD. *Plant Sci.*, 164: 1029-1035.
- Pesnya, D. S. and A. V. Romanovsky (2013). Comparison of cytotoxic and genotoxic effects of plutonium-239 alpha particles and mobile phone GSM 900 radiation in the *Allium cepa* test. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 750: 27-33.
- Pestana, R. K. N., E. P. Amorim, C. F. Ferreira, V. B. O. Amorim, L. S.

- Oliveira, C. A. S. Ledo and S. O. Silva (2011). Genetic dissimilarity of putative gamma-ray-induced 'Preciosa - AAAB-Pome type' banana (*Musasp*) mutants based on multivariate statistical analysis. *Genet. and Mol. Res.*, 10: 3976-3986.
- Quaite, F. E., B. M. Sutherland and J. C. Sutherland (1992). Action spectrum for DNA damage in alfalfa lowers predicted impact of ozone depletion. *Nature*, 358: 576-578.
- Reddy, P. M., N. Sarla and E. A. Siddiq (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica*, 128: 9-17.
- Santina, Z., S. Irene, L. R. Nicoletta and G. Rossella (2008). Effects of UV-B radiation on antioxidant parameters of iron-deficient barley plants. *Env. and Exp. Bot.*, 63: 71-79.
- Santos, I., F. Fidalgo, J. M. Almeida and R. Salema (2004). Biochemical and ultra structural change in leaves of potato plants grown under supplementary UV radiation. *Plant Sci.*, 167: 925-935.
- Sayed-Ahmed, Mahassen, S. (1985). Genetical and Cytogenetical Studies in Broad Bean (*Vicia faba* l.). M. Sc. Thesis, Fac. Agric, Zagazig University, Egypt.
- Steeger, H. U., J. F. Freitage, S. Michl, M. Wiemer and J. Paul (2001). Effects of UV-B radiation on embryonic, larva and juvenile stages of northern sea plaice (*Pleuronectes platessa*) under simulated ozone hole condition. *Helgo. Mar. Res.*, 55: 56-66.
- Wang, X. F., R. Knoblauch and N. Leist (2005). Varietal discrimination of tomato (*Lycopersicon esculentum* L.) by ultrathin-layer isoelectric focusing of seed protein. *Seed Sci. Technol.*, 28: 521-526.
- Wendt, S. N., A. P. José, C. O. Antônio, L. Vera, L. C. Fernando, S. M. Cristina and L. V. Isabel (2001). Plant regeneration and molecular characterization of potato cultivar Macaca, obtained from gamma-irradiated explants, *J. of New Seeds*, 3: 17-37.
- Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V. Tingey (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.*, 18: 6531-6535.
- Xiong, F., Z. Ruichun, H. Zhuqiang, J. Jing, H. Liangqiong, Z. Weijian and T. Ronghua (2011). Start codon targeted polymorphism for evaluation of functional genetic variation and relationships in cultivated peanut (*Arachis hypogaea* L.) genotypes. *Mol. Biol. Rep.*, 38: 3487-3494.

Zheng, Y., W. Gao, J. R. Slusser, R. H. Grant and C. Wang (2003). Yield and yield formation of field winter wheat in response to supplemental

solar ultraviolet-B radiation. *Agr. and Forest Meteorology*, 120: 279-283.

Table (1): % of Total mitotic abnormalities, mitotic index and mitotic abnormalities in the meristematic root cells of tomato plants.

| Group | % of total mitotic abnormalities | % Mitotic index | Percentage of types of mitotic abnormalities | | |
|----------------|----------------------------------|-----------------|--|----------------------|-------------|
| | | | Laggards | Chromosome fragments | Micronuclei |
| Control | 0.30 | 16.230 | 0.03 | 0.05 | 0.22 |
| No.1 (20 min) | 0.54 | 18.383 | 0.20 | 0.07 | 0.27 |
| No. 2 (40 min) | 1.43 | 15.910 | 0.51 | 0.31 | 0.61 |
| No. 3 (60 min) | 1.60 | 17.361 | 0.59 | 0.42 | 0.59 |

Table (2): Total number of amplicons, monomorphic amplicons, polymorphic amplicons and percentage of polymorphism as revealed by RAPD markers among the control and three tomato treatments.

| Primer | Sequence 5' → 3' | Total No. of amplicons | Monomorphic amplicons | Polymorphic amplicons | % of polymor- phism |
|---------|---------------------|---------------------------|--------------------------|--------------------------|------------------------|
| OP A07 | GAAACGGGTG | 8 | 2 | 6 | 75.0 |
| OP A08 | GTGACGTAGG | 13 | 8 | 5 | 38.5 |
| OP A12 | TCGGCGATAG | 7 | 3 | 4 | 57.0 |
| OP A13 | CAGCACCCAC | 14 | 12 | 2 | 14.0 |
| OP A18 | AGGTGACCGT | 15 | 6 | 9 | 60.0 |
| OP A19 | CAAACGTCGG | 14 | 6 | 8 | 57.0 |
| OP A20 | GTTGCGATCC | 12 | 3 | 9 | 75.0 |
| OP B01 | GTTTCGCTCC | 10 | 3 | 7 | 70.0 |
| OP C06 | GAACGGACTC | 11 | 4 | 7 | 63.6 |
| OP C08 | TGGACCGGTG | 9 | 2 | 7 | 77.7 |
| OP D20 | ACCCGGTCAC | 6 | 5 | 1 | 16.7 |
| Total | | 119 | 54 | 65 | 54.6 |
| Average | | 10.8 | 4.9 | 5.9 | |

Table (3): Genetic similarity matrices among three tomato treatments and the control as computed according to Dice coefficient from RAPDs.

| | C | 1 | 2 |
|---|------|------|------|
| 1 | 80.0 | | |
| 2 | 71.7 | 74.8 | |
| 3 | 78.1 | 82.8 | 83.3 |

Table (4): Total number of amplicons, monomorphic amplicons, polymorphic amplicons and percentage of polymorphism as revealed by ISSR markers among the control and three UV treatments.

| Primer | Sequence | Total No. of amplicons | Monomorphic amplicons | Polymorphic amplicons | % of polymorphism |
|---------|----------|------------------------|-----------------------|-----------------------|-------------------|
| H1 | (TA)10G | 10 | 5 | 5 | 50.0 |
| H2 | (CG)8A | 13 | 4 | 9 | 69.2 |
| H3 | (CA)9A | 7 | 6 | 1 | 14.3 |
| H4 | (CA)9G | 12 | 3 | 9 | 75.0 |
| H5 | (AG)9G | 4 | 4 | 0 | 0.0 |
| H6 | (AT)9A | 6 | 6 | 0 | 0.0 |
| H7 | (AG)9A | 4 | 4 | 0 | 0.0 |
| H8 | (AG)9T | 6 | 5 | 1 | 16.7 |
| H9 | (AG)9C | 4 | 4 | 0 | 0.0 |
| H10 | (AC)9T | 5 | 3 | 2 | 40.0 |
| H11 | (AC)9C | 4 | 4 | 0 | 0.0 |
| H12 | (GA)9G | 9 | 5 | 4 | 44.4 |
| H13 | (AC)9G | 4 | 3 | 1 | 25.0 |
| H14 | (GA)9A | 6 | 3 | 3 | 50.0 |
| H15 | (GA)9T | 5 | 4 | 1 | 20.0 |
| H16 | (GA)9C | 5 | 4 | 1 | 20.0 |
| H17 | (TA)5GT | 10 | 6 | 4 | 40.0 |
| H18 | (CA)6T | 7 | 5 | 2 | 28.6 |
| H19 | (TAA)4C | 9 | 8 | 3 | 33.3 |
| Total | | 130 | 86 | 46 | 35.4 |
| Average | | 6.9 | 4.5 | 2.4 | |

Table (5): Genetic similarity matrices among three tomato treatments and the control as computed according to Dice coefficient from ISSRs Where C represents the control, 1, 2 and 3 represent 20 min, 40 min and 60 min exposure to UV-B, respectively.

| | C | 1 | 2 |
|---|------|------|------|
| 1 | 85.2 | | |
| 2 | 83.8 | 88.9 | |
| 3 | 88.5 | 93.8 | 92.2 |

Table (6): Total number of amplicons, monomorphic amplicons, polymorphic amplicons and percentage of polymorphism as revealed by SCoT markers among the three tomato treatments and the control.

| Primer | Sequence 5' → 3' | Total No. of amplicons | Mono- morphic amplicons | Polymor- phic amplicons | % of poly- morphism |
|---------|---------------------|------------------------------|-------------------------------|-------------------------------|------------------------|
| SCoT-1 | ACGACATGGCGACCACGC | 14 | 8 | 6 | 42.9 |
| SCoT-2 | ACCATGGCTACCACCGGC | 11 | 5 | 6 | 54.5 |
| SCoT-3 | ACGACATGGCGACCCACA | 10 | 8 | 2 | 20.0 |
| SCoT-4 | ACCATGGCTACCACCGCA | 16 | 12 | 4 | 25.0 |
| SCoT-5 | CAATGGCTACCACTAGCG | 18 | 6 | 12 | 66.7 |
| SCoT-6 | CAATGGCTACCACTACAG | 15 | 7 | 8 | 53.3 |
| SCoT-7 | ACAATGGCTACCACTGAC | 13 | 12 | 1 | 7.7 |
| SCoT-8 | ACAATGGCTACCACTGAG | 14 | 8 | 6 | 42.9 |
| SCoT-9 | ACAATGGCTACCACTGCC | 8 | 4 | 4 | 50.0 |
| SCoT-10 | ACAATGGCTACCACCAGC | 13 | 5 | 8 | 61.5 |
| SCoT-11 | ACAATGGCTACCACTACC | 6 | 3 | 3 | 50.0 |
| SCoT-12 | CAACAATGGCTACCACCG | 12 | 7 | 5 | 41.7 |
| SCoT-13 | ACCATGGCTACCACGGCA | 11 | 7 | 3 | 27.3 |
| SCoT-14 | ACCATGGCTACCAGCGCG | 10 | 5 | 5 | 50.0 |
| SCoT-15 | CCATGGCTACCACCGGCT | 11 | 6 | 5 | 45.5 |
| Total | | 182 | 103 | 78 | 43.0 |
| Average | | 12.1 | 6.9 | 5.2 | |

Table (7): Genetic similarity matrices among three tomato treatments as computed according to Dice coefficient from SCoTs where 1, 2 and 3 represent 20 min, 40 min and 60 min. exposure of plants to UV-B, respectively and C represents the control plants.

| | C | 1 | 2 |
|---|------|------|------|
| 1 | 79.8 | | |
| 2 | 80.9 | 84.5 | |
| 3 | 81.1 | 89.9 | 89.4 |

Table (8): Genetic similarity matrices among the control and three tomato treatments as computed according to Dice coefficient from combined data of RAPD, ISSR and SCoT.

| | C | 1 | 2 |
|---|------|------|------|
| 1 | 81.0 | | |
| 2 | 79.5 | 83.4 | |
| 3 | 82.8 | 89.5 | 88.7 |

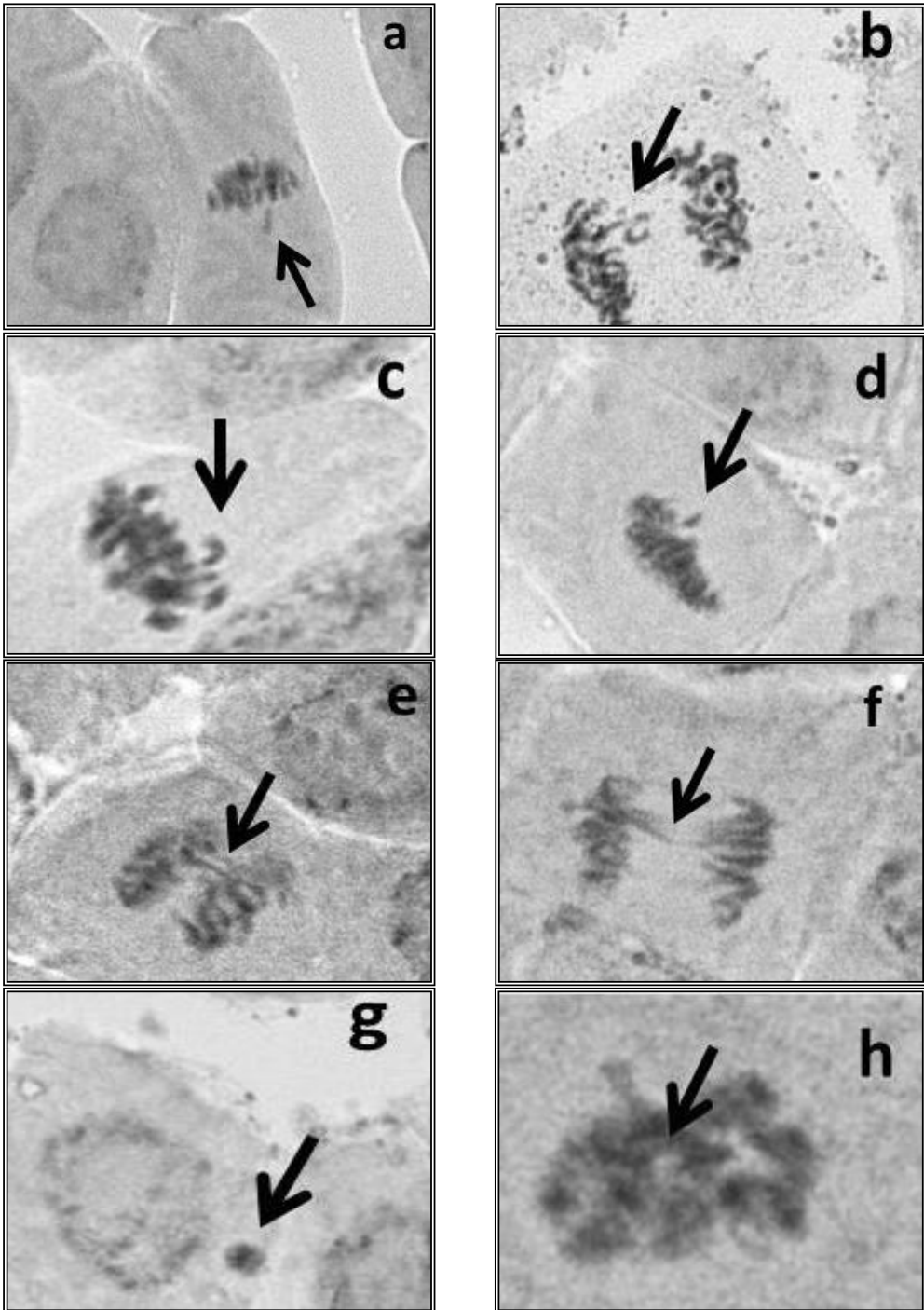


Fig. (1): Some examples of mitotic abnormalities observed after 1 hour exposure of tomato root tips to UV radiation. Arrows mark the abnormalities: laggard at metaphase (a), laggard at anaphase (b), chromosome fragments at metaphase (c and d), chromosome bridge (e), broken bridge (f), micronucleus (g) and C-Metaphase (h).

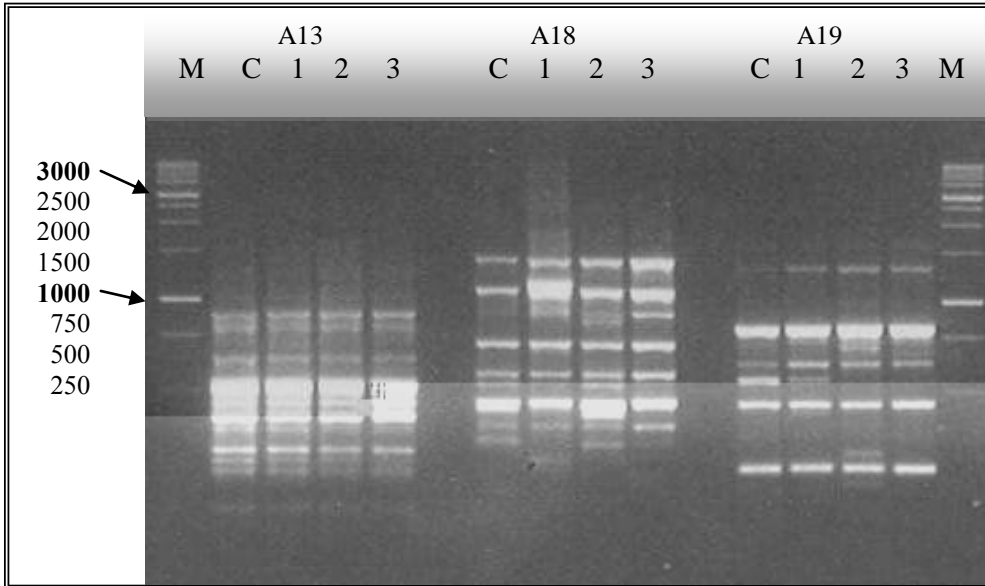


Fig (2): The separation pattern of the RAPD products on 1.5% agarose gel using the primers: OP-A13, OP-A18 and OP-A19. M: 1 kb ladder DNA marker. Where C represents the control and 1, 2 and 3 represent 20 min, 40 min and 60 min exposure to UV-B, respectively.

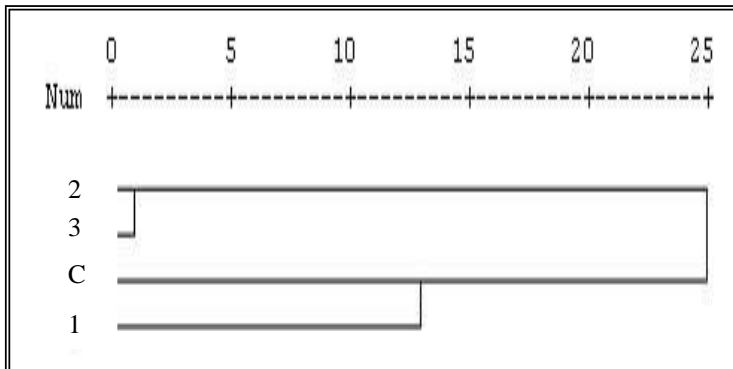


Fig. (3): Dendrogram for the three tomato treatments and the control constructed from the RAPD data using Unweighed Pair-group Arithmetic average (UPGMA) and similarity matrices computed according to Dice coefficient.

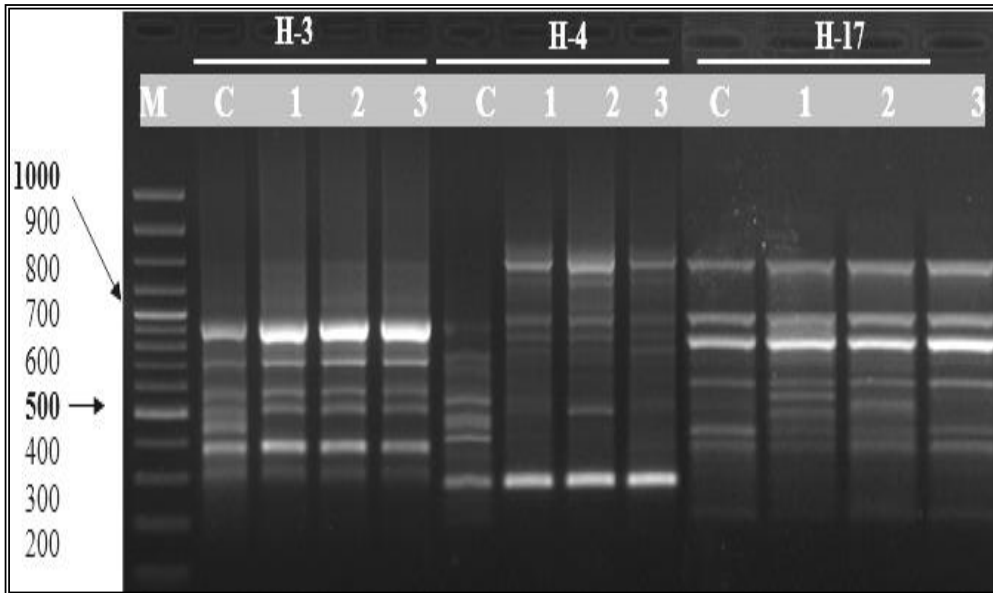


Fig. (4): The separation pattern of the ISSR products on 1.5% agarose gel using the primers: H-3, H-4 and H-17. M: 100 bp ladder DNA marker, where C represents the control and 1, 2 and 3 represents 20 min, 40 min and 60 min exposure to UV-B, respectively.

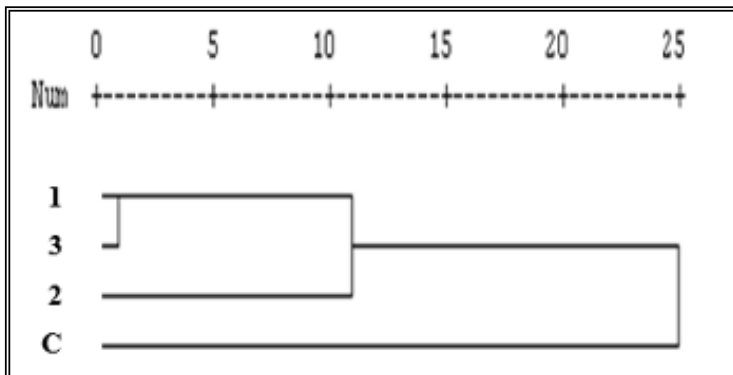


Fig. (5): Dendrogram for the three tomato treatments and the control constructed from the ISSR data using Un-weighted Pair-group Arithmetic average (UPGMA) and similarity matrices computed according to Dice coefficient.

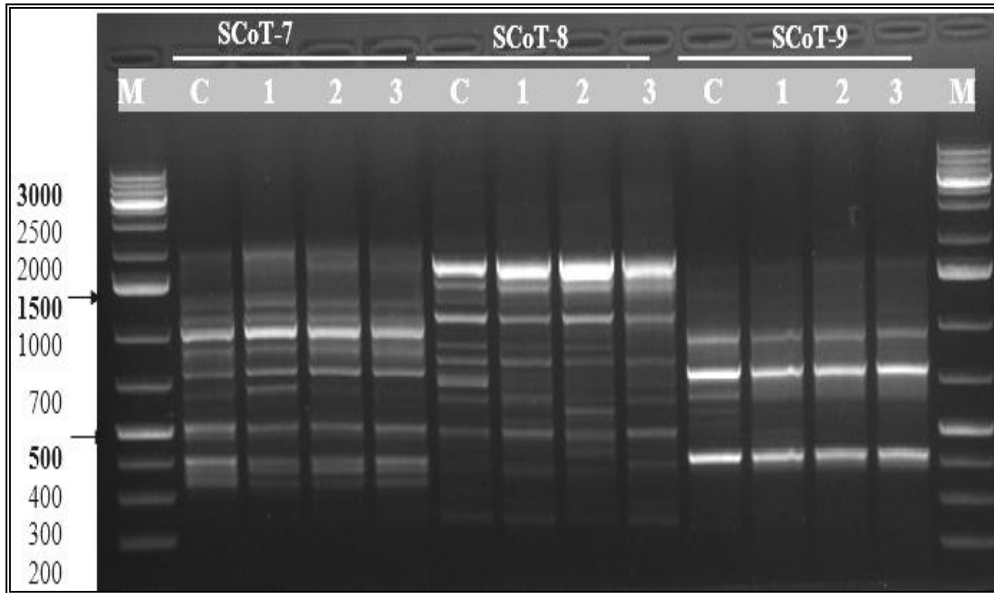


Fig (6): The separation pattern of the SCoT products on 1.5% agarose gel using the primers: SCoT-7, SCoT-8 and SCoT-9. M: 100bp ladder DNA marker. Where C represents the control and 1, 2 and 3 represent 20 min, 40 min and 60 min exposure to UV-B, respectively.

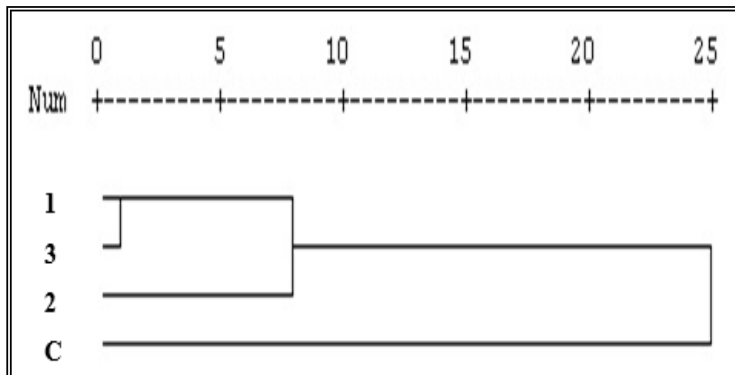


Fig. (7): Dendrogram for the three treatments of tomato constructed from the SCoT data using Un-weighted Pair-group Arithmetic average (UPGMA) and similarity matrices computed according to Dice coefficient. Where C represents the control and 1, 2 and 3 represent 20 min, 40 min and 60 min. exposure of plants to UV-B, respectively.

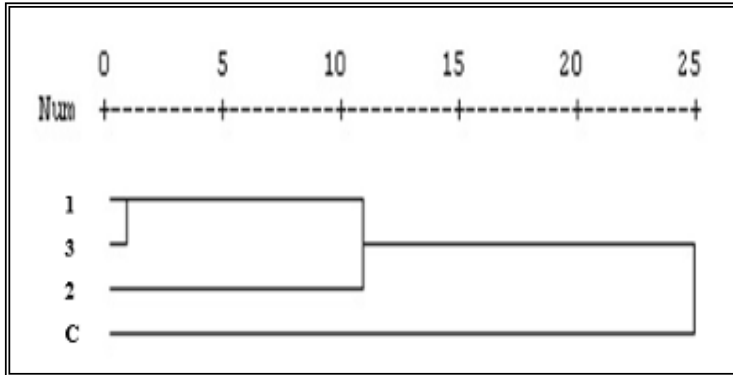


Fig. (8): Dendrogram for the control and three tomato treatments constructed from the combined data of RAPD, ISSR and SCoT using Un-weighted Pair-group Arithmetic average (UPGMA) and similarity matrices computed according to Dice coefficient.