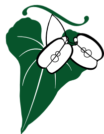
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Original Scientific Paper

Estimation of arsenic-induced genotoxicity in melon (*Cucumis melo*) by using RAPD-PCR and comet assays

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ABSTRACT:
In this study, arsenic (As)-induced genotoxicity in the roots and shoots of *Cucumis melo* (melon) seedlings were investigated by using the random amplified polymorphic DNA - polymerase chain reaction (RAPD-PCR) and comet assays. For this purpose, melon seedlings were exposed to arsenate [As(V)] at 0, 100, 200, 300, and 400 μM concentrations in the hydroponic system for 14 days to examine the level of As accumulation, alterations in growth parameters, and DNA damage. A reduction in growth with increasing As(V) concentration was observed in the melon seedlings. Total As accumulations in the shoot and root tissue increased in a dose-dependent manner; however, the level was higher in the roots than the shoots. In RAPD-PCR analysis, 26 primers gave reproducible and scorable results and produced a total of 128 bands in the control seedlings. Alterations in RAPD profiles, including the loss or appearance of new bands, were determined in the As-treated seedlings when compared to the control. The values of genomic template stability (GTS) were decreased by increasing the concentration of the As(V) in both tissue types. DNA strand breaks were observed in all the tested As(V) concentrations in the alkaline comet assay; furthermore, the loss of DNA integrity was higher with 300 and 400 μM As(V) treatments. The results clearly indicate that the combination of DNA-based molecular and cytogenetic techniques (e.g. the comet assay) may be proposed as a reliable evaluation of genotoxicity in plants after exposure to heavy metal pollution.

Keywords:
DNA damage, genomic stability, genotoxicity tests, heavy metal stress

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INTRODUCTION

Arsenic (As) is a metalloid naturally found in the environment generally in combination with elements such as tin (Sn), silver (Ag), gold (Au) and copper (Cu). The rising demand for these metals leads to widespread mining and causes As contamination in mining fields around the world (Islam *et al.* 2015). Arsenic can be found both in organic (dimethylarsinic and monomethylarsonic acid) and inorganic forms (arsenate and arsenite) in nature (Tu *et al.* 2003). Inorganic As is categorized as a Group 1 carcinogen (IARC 2004) and people are at risk due to its entry into the food chain (Chakraborti *et al.* 2003). Humans are exposed to environmental pollutants mainly through diet (Fries 1995) and approximately 30% of cancers can be related to long-term exposure to low-level carcinogenic pollutants found in the human diet as reported by Tricker & Preussmann (1990).

Arsenic accumulation disturbs the prooxidant-antioxidant balance, triggers signal transduction and leads to the generation of reactive oxygen species (ROS) such as superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$) in the cell which finally induces oxidative stress (Leterrier *et al.* 2012). Intensive oxidative stress causes DNA damage, altering its coding sequences and consequently affects cellular processes

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(Manova & Gruszka 2015). Random amplified polymorphic DNA (RAPD), a PCR-based technique, has been effectively used to detect DNA damage and mutations in plants induced by pollutants (Cenkci *et al.* 2010; Salarizadeh *et al.* 2015; Ciğerci *et al.* 2016). In this method, the potential genotoxic effects of contaminants are determined by comparing RAPD band patterns (e.g. the presence and absence of bands) between exposed and non-exposed samples (Manna & Bandyopadhyay 2017). Over the last two decades, a molecular cytogenetic-based assay, named the comet assay (also known as single cell gel electrophoresis, SCGE), has been utilized to investigate the response of plant cells to genotoxic agents (Santos *et al.* 2015; Abdelhaliem & Al-Huqail 2016; Na Roi-et *et al.* 2017). It is possible to determine single and double strand DNA breaks, alkali-labile sites and incomplete excision repair sites in the alkaline comet assay, and the DNA migration rate provides a measure of the extent of cellular DNA damage (Glei *et al.* 2016).

Melon is an important fruit plant grown in many countries, with a recently discovered potential role in the bioremediation of heavy metals (Gabrijel *et al.* 2009). However, basic studies on the biochemical and physiological response of melon to heavy metals are inadequate (Zhang *et al.* 2015). On the other hand, As toxicity has been studied either in the leaves (Ahmad *et al.* 2012; Ertürk *et al.* 2015) or roots (Duquesnoy *et al.* 2010; Kumari *et al.* 2017) of different plants and the detection of genotoxicity in both shoot and root tissues can provide useful information for a deeper understanding of As toxicity. In the current study, the melon seedlings were grown hydroponically in the presence of different As(V) concentrations and several endpoints were used to asses As toxicity.

MATERIAL AND METHODS

Plant material and treatments. The seeds of melon (*Cucumis melo* L.) cultivar Ananas were obtained from the local market. Homogeneous melon seeds were incubated between moistened filter papers (Whatman Grade 1) in petri dishes for 5 days (d). The seedlings were then transferred to a hydroponic culture system as described by Ramamurthy & Waters (2017). After acclimatization in hydroponic conditions for 24 h, the melon seedlings were cultured on a ½ Hoagland medium (Hoagland & Arnon 1938) containing 0 (control), 100, 200, 300, and 400 µM disodium hydrogen arsenate heptahydrate ($Na_2HAsO_4.7H_2O$) for 14 d. The used concentrations of As(V) were chosen based on previous studies (Duquesnoy *et al.* 2010; Leterrier *et al.* 2012). At the end of the 20-d culture period, the shoots (stems + leaves) and roots of the plants were harvested and stored at -80°C for further analysis. All of the experiments were performed under a 16 h / 28°C light and 8 h / 26°C dark photoperiod cycle, with an irradiance of 200 µmol m^{-2} s^{-1} at 65-70% humidity in the plant growth chamber. The hydroponic solutions were changed twice a week and the hydroponic culture system was continuously aerated by means of pumps. The experiments were carried out in 3 replicates with every set consisting of 15 plants.

Growth parameters. The lengths of the shoots and primary roots were measured using free software ImageJ analysis (https://imagej.nih.gov/ij/download.html) from photographs taken with an Olympus SC30 digital camera connected to an Olympus SZX7 stereoscopic microscope. The shoot and root dry weights were recorded after drying at 60°C for 48 h. The number of leaves on each plant were counted manually. Twenty seedlings per treatment were analyzed for the measurement of the growth parameters.

Total arsenic content. The roots and shoots were dried at 80°C for 24 h and ground using a stainless-steel mill (Isolab, Germany). The plant samples (0.5 g) were digested in a mixture of HNO_3 (67%) and H_2O_2 (30%) (3:1) by means of a microwave digester (Milestone Ethos-1, Australia) at 120°C for 30 min. After cooling, the final volume was adjusted to 25 mL with dH_2O. The total As in the extracts was analyzed in triplicate by inductively coupled plasma-optical emission spectrometry (ICP-OES) (Spectroblue, Germany) against blank and As standards (Merck, Germany).

Genomic DNA isolation and RAPD-PCR. Genomic DNA was extracted from the bulked shoot and root samples (0.2 g) using the previously described method (Doyle & Doyle 1990). The purities and concentrations of the extracted DNA samples were estimated by a microplate photometer (Thermo, Germany) and the integrity of the DNA samples was analyzed using 1% agarose gel. RAPD assay was carried out by using 26 decamer primers following the protocol suggested by Williams *et al.* (1990). Each 15 ml PCR mixture contained 0.25 µL Taq DNA polymerase, 1.5 mL 10X Taq buffer, 1 µL dNTP (10 mM), 2.4 mL $MgCI_2$ (25 mM), 1 mL primer (10 mM), 3.85 µL ddH_2O, and 50 ng genomic DNA as a template. The sequences of the primers used in this study are given in Table 1. The PCR conditions were as follows: 1 min at 94°C, 45 cycles of 1 min at 94°C, 1 min at 36°C, 2 min at 72°C and 10 min at 72°C following the last cycle. The products of the RAPD assay were examined on a 2% agarose gel along with a 1-kb DNA Ladder (Thermo, Germany). The reproducibility of the RAPD band profiles was verified by repeating all amplifications three times. The gel imaging was carried out using the Fusion FX7 imaging system (Vilber Lourmat, Germany) and the size of the amplification products was automatically determined by the Fusion-CAPT-Software (ver 16.07). For each primer, As-induced alteration in the RAPD band patterns was regarded as genomic template stabil-

Table 1. The primer list used for RAPD-PCR.

No	Primer name	Primer sequences (5′→3′)	No	Primer name	Primer sequences (5′→3′)
1	OPA-11	CAATCGCCGT	14	OPM-03	GGGGGATGAG
2	OPB-01	GTTTCGCTCC	15	OPM-04	GGCGGTTGTC
3	OPB-05	TGCGCCCTTC	16	OPM-05	GGGAACGTGT
4	OPB-07	GGTGACGCAG	17	OPM-07	CCGTGACTCA
5	OPB-08	GTCCACACGG	18	OPM-09	GTCTTGCGGA
6	OPB-10	CTGCTGGGAC	19	OPM-10	TCTGGCGCAC
7	OPB-13	TTCCCCCGCT	20	OPM-11	GTCCACTGTG
8	OPB-14	TCCGCTCTGG	21	OPM-12	GGGACGTTGG
9	OPB-15	GGAGGGTGTT	22	OPM-14	AGGGTCGTTC
10	OPB-18	CCACAGCAGT	23	OPM-15	GACCTACCAC
11	OPB-20	GGACCCTTAC	24	OPM-16	GTAACCAGCC
12	OPM-01	GTTGGTGGCT	25	OPM-17	TCAGTCCGGG
13	OPM-02	ACAACGCCTC	26	OPM-20	AGGTCTTGGG

ity (GTS) as determined by the formula GTS = (1- a / n) × 100, where "a" equals the polymorphic bands observed in each treated sample and "n" equals the total number of control bands.

Comet assay. A modified version of the alkaline comet assay protocol proposed by Gichner *et al.* (2008) was performed. For the isolation of nuclei, about 0.3 g of the root and shoot samples was placed in a 60 mm petri dish kept on ice containing cold 400 µL phosphate buffered saline (160 mM NaCl, 8 mM Na_2HPO_4, 4 mM NaH_2PO_4, pH 7.0). Using a fresh razor blade, the samples were gently sliced, and the plate was kept tilted in the ice so that the isolated nuclei would collect in the buffer. One day before the experiments, microscope slides were immersed in a 1% normal melting point (NMP) agarose solution and air-dried overnight. The nuclei suspension (50 µL) was added to 50 µL of low melting point (LMP, 1%) agarose and was mixed rapidly by pipetting at 40°C. The obtained mixture was put on a slide with a normal agarose layer and cooled for 5 min once covered with a coverslip. The slides were then placed in fresh ice-cold lysis solution (100 mM EDTA, 2.5 M NaCl, 10 mM Tris, 10% Dimethyl Sulfoxide, 1% Triton X-100, pH 10.0) for 1 h at 4°C, followed by settling horizontally in electrophoresis gel containing a cold buffer (1 mM Na_2EDTA, 300 mM NaOH, pH > 13.0) and incubated for 15 min for unwinding, prior to electrophoresis at 0.72 V cm^{-1} (26 V, 300 mA) for 25 min at 4°C. Subsequently, the slides were rinsed 4 times using a neutralization buffer (400 mM Tris buffer, pH 7.5), stained with 50 µL ethidium bromide (20 µg mL^{-1}) and immediately analyzed. An image analysis system connected to a camera-fluorescence microscope (Zeiss Axio Scope A1, Germany) system was used to examine the slides. For each experimental treatment, both the extent and distribution assessment of DNA damage exhibited thanks to comet analysis was carried out by analyzing 100 randomly selected cells on the slides (i.e. 300 cells per treatment). The obtained comets were categorized into five categories (0–IV) according to fragmentation intensity (Jaloszynski *et al.* 1997). The assessed amount of DNA damage for 100 comets, which ranged from 0 to 400, was calculated in accordance with the formula (Azqueta *et al.* 2009):

DNA damage index (arbitrary units) = $(1 \times n1) + (2 \times n2) + (3 \times n3) + (4 \times n4)$

(1) where $n1$–$n4$ are the number of comets in classes I–IV. The results were obtained on the basis of three independent experiments.

Statistical analysis. The results were reported as mean ± standard error (SE). One-way ANOVA followed by Duncan's multiple range test were used to assess whether the differences between the treatment groups were statistically significant ($p < 0.05$).

RESULTS

Growth parameters and As accumulation. Treatment with As(V) at 100, 200, 300 and 400 µM caused a reduction in shoot length of 11.03%, 39.14%, 56.93%, and 60.67%, respectively, compared to the control samples (Table 2). Arsenate applications reduced the primary root length in all the tested concentrations, while the maximum decrease was determined subsequent to 400 µM As(V) treatment when compared to the control group (Table 2). Arsenate significantly decreased the shoot and root dry weight of the melon seedlings in a concentration-dependent manner (Table 2). While 100 µM of As(V) application did not change the number of leaves, this number significantly ($p < 0.05$) decreased in those seedlings exposed to 200, 300, and 400 µM of As(V) (Table 2).

Arsenate treatments at 100, 200, 300 and 400 µM increased the As accumulation in the shoots by 108.59%, 148.80%, 216.83% and 355.83%, respectively, as com-

Table 2. The effect of various concentrations of As(V) on primary root and shoot length (cm), root and shoot dry weight (mg) and leaf number (per plant) of melon (*C. melo*).

Growth parameters	Control	As(V) concentrations (µM)			
		100	200	300	400
Shoot length (cm)	5.62 ± 0.26 [a]	5.00 ± 0.32 [a]	3.42 ± 0.25 [b]	2.42 ± 0.18 [c]	2.21 ± 0.15 [c]
Primary root length (cm)	13.12 ± 0.76 [a]	11.50 ± 0.56 [b]	7.76 ± 0.62 [c]	5.26 ± 0.43 [d]	2.78 ± 0.23 [e]
Shoot dry weight (mg)	35.85 ± 0.83 [a]	31.62 ± 0.77 [b]	26.07 ± 0.58 [c]	16.21 ± 0.44 [d]	13.78 ± 0.54 [e]
Root dry weight (mg)	14.17 ± 0.75 [a]	11.56 ± 0.39 [b]	8.69 ± 0.20 [c]	5.21 ± 0.16 [d]	4.07 ± 0.24 [e]
Leaf number (per plant)	3.25 ± 0.16 [a]	2.87 ± 0.29 [a]	2.38 ± 0.14 [b]	2.21 ± 0.09 [b]	2.07 ± 0.07 [b]

Values represent mean ± SE (n=20). Different small superscripts (a, b, c, d, and e) in the same row are significantly different at $p < 0.05$, as assessed by Duncan's multiple range test.

Table 3. Molecular sizes (bp) of appeared (+) /disappeared (–) bands and genomic template stability (GTS) values in the shoots of melon seedlings exposed to As(V) at different concentrations for 14 days.

Primers	**Control**	**As(V) concentrations (µM)**			
		100	**200**	**300**	**400**
A11	6	ND	ND	ND	ND
B01	4	ND	+ 1030	ND	+ 1030
B05	4	– 1580	– 2515	+ 3030	– 1580, + 1150
B07	5	– 1970, – 955	ND	– 955	ND
B08	4	ND	ND	ND	ND
B10	6	– 1650, – 1490	– 1650, – 1490	– 1650	– 2950, – 545
B13	4	– 1795, + 885	– 1795, – 815	– 1795, + 885	– 815
B14	4	– 975	– 975	– 975	– 975
B15	2	ND	ND	ND	ND
B18	6	ND	– 1380, – 1260, + 250	– 1380, – 1260, + 250	– 1380, – 1260, + 250
B20	5	ND	– 1410	– 1410, – 660	– 660
M01	7	ND	ND	ND	ND
M02	5	+ 1910	– 842	+ 1910	+ 1910, + 1350
M03	8	ND	– 1425, – 620	– 1850, – 1425, – 800, – 685	– 1425, – 800, – 685
M04	6	ND	ND	ND	– 900, + 595
M05	4	ND	ND	ND	ND
M07	5	ND	ND	– 745, – 450	ND
M09	4	– 1750, + 625	– 1750, + 2455, + 625	– 1750, + 2455, + 625	– 1750, + 2455, + 1225
M10	8	ND	ND	ND	ND
M11	7	ND	ND	ND	ND
M12	4	ND	ND	ND	+ 1400, + 1200
M14	5	– 1090, + 2350	– 1090, + 2350, + 1310	– 1090, + 2350, + 1230, + 520	+ 1310, +1230, + 780, + 520
M15	4	ND	ND	ND	– 3100
M16	4	ND	+ 980	+ 980	+ 980
M17	3	ND	ND	ND	ND
M20	4	ND	ND	ND	ND
Total average (GTS, %)	128 (100)	9 (–), 4 (+) (89.84)	14 (–), 7 (+) (83.59)	16 (–), 10 (+) (79.68)	14 (–), 15 (+) (77.34)

ND: Not determined.

Table 4. Molecular sizes (bp) of appeared (+) /disappeared (–) bands and genomic template stability (GTS) values in the roots of melon seedlings exposed to As(V) at different concentrations for 14 days.

Primers	Control	As(V) concentrations (µM)			
		100	200	300	400
A11	6	+ 1310	+ 1310	+ 1310, + 995	+ 1310, + 995
B01	4	ND	+ 1030	+1510, + 1030	+ 3080, + 1510, + 1030
B05	4	– 1580	ND	– 1580, + 620	– 1580, + 1150, + 620
B07	5	– 1970, –– 955	– 955	– 955, + 580	– 955, + 580
B08	4	+ 355	+ 355	+ 355	+ 355
B10	6	– 1650	– 1650	– 1650, –– 1490	– 1650, – 1490, – 545
B13	4	ND	ND	ND	ND
B14	4	ND	ND	ND	ND
B15	2	+ 435	+ 435	+ 435, + 330	+ 435, + 330
B18	6	– 2350, – 1260	– 2350, – 1380, – 1260, – 620, + 250	– 2350, – 1380, – 1260, – 620, + 250	– 2350, – 1380, – 1260, – 620, + 250
B20	5	ND	ND	ND	ND
M01	7	– 240, + 480	– 240, + 1785, + 480	– 240, + 1785, + 480	– 240, – 435, + 1785, + 480
M02	5	– 842	– 842	+ 1910, + 1350	+ 1910, + 1350
M03	8	+ 582	– 1850, – 800	– 1850, – 800	– 1850, – 800
M04	6	+ 1300, +755	+ 1300, +755	– 900, +1300, + 755	– 1805, – 900, + 1300 , + 755
M05	4	– 890, – 480	– 890, – 480, + 989	+ 1480, + 989, + 825	+ 1480, + 989, + 825
M07	5	ND	– 745, – 450	– 745, – 450	– 745, – 450
M09	4	ND	ND	ND	ND
M10	8	– 2434, – 1960	– 1960, – 1522	– 1960, – 1522, + 550	– 2434, – 1960, – 1522, – 880, – 678, + 550
M11	7	ND	– 1150, – 445	– 1150, – 820, – 445	– 1190, – 1150, – 1030, – 820, – 445
M12	4	+ 1400	+ 1400	+ 1400, + 850	+ 1400, + 850
M14	5	+ 2350	+ 2350	+ 2350, + 1230, + 520	+ 2350, + 1230, + 780
M15	4	+ 720	+ 720	+ 720, + 450	– 3100, – 1650, + 720
M16	4	– 1150, + 705	+ 705, + 480, + 455	+ 980, + 705, + 455	+ 980, + 705, + 455
M17	3	+ 1505	+ 1505	+ 1505, – 1345	+ 1505, – 1345
M20	4	ND	ND	ND	ND
Total average (GTS, %)	128 (100)	13 (–), 12 (+) (80.46)	18 (–), 17 (+) (72.65)	20 (–), 31 (+) (60.15)	30 (–), 32 (+) (51.56)

ND: Not determined.

pared to the control samples, however, As content in the root tissue was greater than that in the shoots in all the As(V) concentrations (Fig. 1). A similar level of As accumulation (302.66 and 373.58 µg g^{-1} DW, respectively) was found in the roots as a result of 100 and 200 µM As(V) treatments. Treatment with 300 and 400 µM As(V) caused a 3 - 4 fold greater increase in As accumulation compared to 100 and 200 µM As(V) applications in the root tissue (Fig. 1).

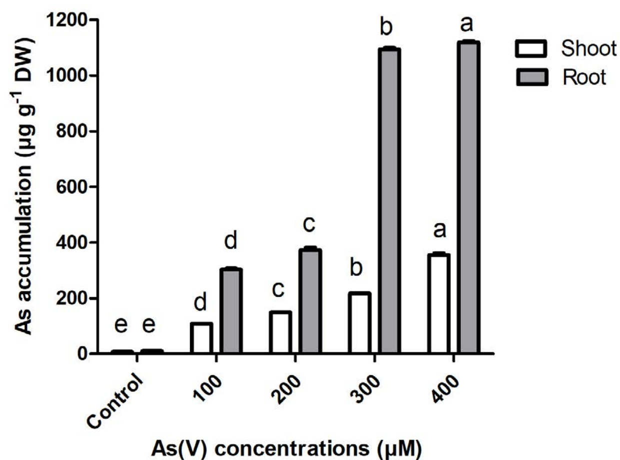

Fig. 1. Arsenic accumulation in the roots and shoots of melon seedlings after 14 days of exposure. The values are the means of three independent measurements (± SE). The different letters above the bar show the statistical differences according to Duncan's multiple range test ($p < 0.05$) between the treatments for each tissue.

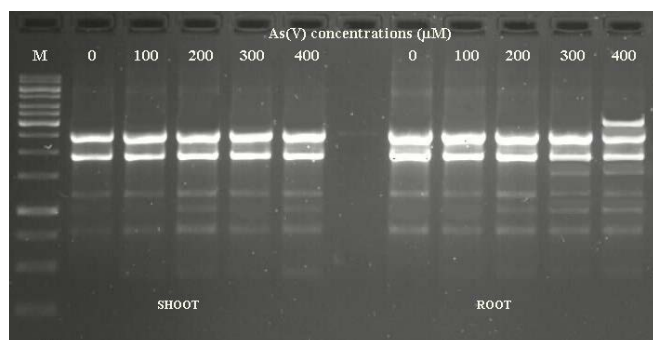

Fig. 2. RAPD profiles of B01 primer of genomic DNA extracted from the shoots and roots of the melon seedlings exposed to As(V) at different concentrations for 14 days. M: DNA molecular size marker (1.0-kb).

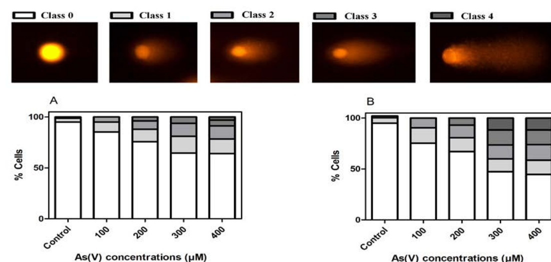

Fig. 3. Evaluation of DNA damage in melon seedlings exposed to increasing concentrations of As(V) for 14 days by the alkaline comet assay. The percentage of comets observed after As(V) exposure in the shoots (A) and roots (B). The cells were assessed visually and ranked into classes from 0 (undamaged) to 4 (maximally damaged), according to the size and shape of the tail. The values represent the average for the data obtained from three independent experiments. The images for the all comet classes were taken using the ×20 lens, with the exception of Class 4 (×40 lens was used).

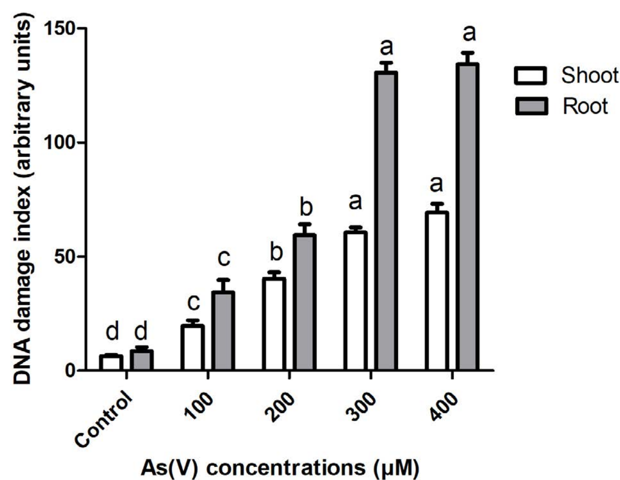

Fig. 4. Extent of DNA damage (arbitrary units) assessed by the comet assay in the roots and shoots of melon seedlings after As(V) exposure for 14 days. The values are the means of three independent measurements (± SE). The different letters above the bar show the statistical differences according to Duncan's multiple range test ($p < 0.05$) between the treatments for each tissue.

RAPD-PCR and GTS. To determine the genotoxic effect of As(V), RAPD-PCR assay was carried out on the genomic DNA extracted from the roots and shoots of melon seedlings exposed to various As(V) concentrations for 14 d. Out of the 30 RAPD primers screened, 26 primers generated stable and reproducible results producing a total of 128 bands in the roots and shoots of the control group. Alterations were observed in the number of new bands and/or the loss of normal bands as matched with the RAPD profiles of the untreated seedlings with the roots and shoots of the As(V)-treated seedlings (Tables 3, 4). The RAPD profiles generated by the B01 primer in the root and shoot samples are presented in Fig. 2. The total number of new bands appeared and bands disappeared in the shoot samples was 13, 21, 26 and 29 as a result of 100, 200, 300 and 400 µM As(V) treatments, respectively (Table 3). Genomic template stability (%) was estimated to be 89.84% at 100 µM As(V), 83.59% at 200 µM As(V), 79.68% at 300 µM As(V) and 77.34% at 400 µM As(V) treatments in the seedling shoots (Table 3). An increase in the number of polymorphic bands occurred with the increase of As(V) concentration in the root samples (Table 4). The value of GTS was 80.46%, 72.65%, 60.15% and 51.56% for 100, 200, 300 and 400 µM As(V) treatments in the roots (Table 4).

Comet assay. Treatments with As(V) induced the DNA strand breaks showing the typical comet structure in which DNA damage appears in the head of the comet with a consequent increment of DNA in the tail (Fig. 3). The number of comets obtained through their visual count and classification were applied in Eq. (1) to estimate the DNA damage index (Fig. 4). A dose-related response after exposure to As(V) was clearly observed in the comet assay of the root and shoot samples.

DISCUSSION

Arsenic toxicity is known to affect plants both morphologically and molecularly. Some studies previously revealed that As induces the generation of ROS in different plants (Kanwar *et al.* 2015; Marsa *et al.* 2015; Surgun-Acar 2019; Surgun-Acar Y & Zemheri-Navruz 2019). The excessive presence of ROS can cause the non-specific oxidation of organic compounds such as lipids, proteins, and carbohydrates in addition to generating steady-state levels of unpaired cellular DNA damage (Gill & Tuteja 2010). This DNA damage has potential genotoxic and cytotoxic consequences on the cells and the gradual accumulation of such damage can bring about genomic instability and/or stress (Tuteja *et al.* 2009; Kumar *et al.* 2016).

In this study, the growth parameters were found to significantly decrease in those melon seedlings subjected to As(V) treatments. Comparable changes in growth-related traits have been reported in wheat (Li *et al.* 2007) and rice seedlings (Ahmad *et al.* 2012) under As stress. Plant growth reduction can be due to the inhibition of ATP synthesis caused by competitive interactivity of As with phosphate leading to inadequate energy flow in the cells (Farooq *et al.* 2015).

Arsenic accumulation in plants is related to the availability of this metal in the soil and depends on the physiological characteristic of the plant (Kumar *et al.* 2015). The translocation of As from the root to the shoot and its consequent redistribution in the plant tissues is carried out by xylem (Meharg & Macnair 1992). In the present study, a greater As accumulation was found in the roots when compared to the shoots and this increase in As concentration occurred in a dose-dependent manner. The absorption of toxic metals by plants triggers a tolerance mechanism that restricts upward movements leading to an increased accumulation in the root system (Srivastava *et al.* 2005). The results of the present study are in conformity with those reported by Pita-Barbosa *et al.* (2019) in *Arabidopsis thaliana* and Gupta *et al.* (2019) in *Vicia faba* under As stress. Carbonell-Barrachina *et al.* (1997) reported limiting As transport to the shoots and increasing As accumulation in the root system of *Lycopersicum esculentum*, however, in *Phaseolus vulgaris* plants As is easily transferred to the shoots and accumulates in high concentrations in the leaf tissue. These differences in the absorption and translocation of As are the most likely cause of the variation in plant tolerance to As toxicity (Carbonell-Barrachina *et al.* 1997).

RAPD-PCR assay represents a quick, easy, and yet effective technique to study genotoxicity (Atienzar & Jha 2006; Manna & Bandyopadhyay 2017). Changes in RAPD patterns such as band loss and/or gain are enhanced by increased As(V) concentration in the roots and shoots of melon seedlings. The accumulation of ROS generated by As produces DNA damage by attacking the DNA bases and sugar moieties that generate consequent DNA fragmentation and alteration in RAPD profiles (Chandrakar *et al.* 2017). DNA damage induced by As was previously determined by applying the RAPD technique in the roots and shoots of *Trifolium repens* (Ghiani *et al.* 2014) and *Oryza sativa* cultivars (Majumder *et al.* 2019, 2020). Another finding of this study is that As(V) treatments cause a greater reduction in GTS rates in the roots than in the shoots. Similarly, Pandey & Gupta (2015) found that changes in RAPD profiles were higher in the roots when compared to the shoots in rice seedlings exposed to As(III) and the changes in RAPD profiles correlated with stress indicators (growth parameters, total chlorophyll, protein, MDA content) and modulators (proline and cysteine content).

The comet assay is a sensitive technique for evaluating DNA damage, repair, and apoptosis for various cell types without the need for a preliminary study of cell turnover rate or karyotype (Jha 2008). It is quite clear that the most serious damage that can occur at the molecular level in terms of various kinds of DNA lesions that often comprise strand breaks are easily and rapidly detected by the comet assay (Fadeel *et al.* 1999). This study reported the remarkable migration of DNA fragments depending on the dose in the As(V) treated melon seedlings. Conversely, low As(V) accumulation in the shoots might provide an explanation for lower levels of As(V)-induced DNA damage in the shoots compared with the roots. Lin *et al.* (2008) used the comet assay to show that As-induced oxidative stress causes DNA damage in *Vicia faba*. The spectroscopic (infrared and near-infrared spectroscopy) and genotoxicity (comet assay) techniques demonstrated molecular modifications primarily associated with chemical interactions of As with biomolecules such as nucleic acids, carbohydrates, lipids, and proteins (Boccia *et al.* 2013). A significant increase in the tail of the comet, dependent on the concentration of mercury (Hg) (between 0-100 μM), was determined by Azevedo *et al.* (2018).

CONCLUSION

Recent developments in molecular biological techniques have led to the successful application of several sensitive DNA-based assays in the field of eco-genotoxicity. In the

present study, the genotoxicity of As(V) was evaluated in the shoots and roots of *C. melo* seedlings by means of a combined RAPD-PCR and comet assay approach. The research indicated that As(V) treatments can cause substantial phytotoxicity and the growth parameters were found to gradually decrease with increasing As(V) concentrations. The obtained data show that As-treatment caused major DNA damage in the roots as displayed by the more abundant As accumulation found in them compared to that located in the shoots. In conclusion, it is possible to state that RAPD analysis together with physiological parameters and the comet assay could be effective eco-toxicological techniques in biomonitoring genotoxic agents.

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REZIME

Procena genotoksičnosti dinje (*Cucumis melo*) izazvane arsenom pomoću RAPD-PCR i komet testa

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U ovoj studiji, genotoksičnost indukovana arsenom u korenima i izdancima sadnica *Cucumis melo* (dinje) ispitivana je pomoću RAPD-PCR i komet testa. U tom cilju, sadnice dinje bile su izložene različitim koncentracijama arsenata [As(v)] u hidroponskom sistemu tokom 14 dana da bi se ispitao nivo akumulacije As, promena parametara rasta i oštećenja DNK. Kod sadnica dinje primećeno je smanjenje rasta sa povećanjem koncentracije As(V). Ukupna akumulacija As u nadzemnom i podzemnom delu povećavala se u zavisnosti od doze, međutim nivo je bio veći u korenu u poređenju sa nadzemnom delom. U RAPD-PCR analizi, 26 prajmera je dalo ponovljive i pouzdane rezultate i proizvelo ukupno 128 traka u kontrolnim sadnicama. Promene u RAPD profilima, uključujući gubitak ili pojavu traka, utvrđene su u tretiranim sadnicama u poređenju sa kontrolama. Vrednosti stabilnosti genomskih obrazaca (GTS) su smanjene sa povećanjem koncentracije As (V) u oba tipa tkiva. Prekidi DNK lanaca primećeni su u svim testiranim koncentracijama As(V) u alkalnom komet testu; pored toga, gubitak integriteta DNK bio je veći u tretmanu sa 300 i 400 μm As(V). Ovi rezultati jasno ukazuju da kombinacija molekularnih tehnika zasnovanih na DNK i citogenetičkih tehnika (npr. komet test) može koristiti za pouzdanu procenu genotoksičnosti biljaka nakon izlaganja teškim metalima.

Ključne reči: DNA oštećenja, stabilnost genoma, testovi genotoksičnosti, stres teškim metalima