RAPD Analysis of Genotoxic Effects of Nano-Scale SiO₂ and TiO₂ on Broad Bean (*Vicia Faba* L.)

GALAL, A. Ola

Department of Genetics, Faculty of Agriculture, Kafrelsheikh University

THABET, F. Ahmad

Department of Genetics, Faculty of Agriculture, Kafrelsheikh University

TUDA, Midori

Laboratory of Insect Natural Enemies, Institute of Biological Control, Faculty of Agriculture, Kyushu University

EL-SAMAHY, F.M. Magdy

Field Crop Pests Research Department, Plant Protection Research Institute, Agricultural Research Center

<https://doi.org/10.5109/2558893>

出版情報：九州大学大学院農学研究院紀要. 65 (1), pp.57-63, 2020-02. Faculty of Agriculture, Kyushu University

バージョン：

権利関係：

RAPD Analysis of Genotoxic Effects of Nano-Scale SiO₂ and TiO₂ on Broad Bean (*Vicia Faba* L.)

Ola A. GALAL^{1*}, Ahmad F. THABET^{1,2,3}, Midori TUDA^{2*} and Magdy F.M. El-SAMAHY³

Laboratory of Insect Natural Enemies, Institute of Biological Control, Faculty of Agriculture,
Kyushu University, Fukuoka 819–0395, Japan

(Received October 28, 2019 and accepted November 14, 2019)

Growing use of oxide nanoparticles has driven their access to the natural environment, including biological interactions within the ecosystem, despite a lack of knowledge about their genotoxic potential on exposed plant tissue. In this study, seeds of broad beans (*Vicia faba* L.), one of the major carbohydrate food sources as well as an ecotoxicological model plant, were treated with three concentrations (25, 50 and 75 mg/L) of two different types of nanoscale materials (n–), n–SiO₂ and n–TiO₂, to assess their safe use. Seeds were soaked in n–SiO₂ and n–TiO₂ each for 24 h, then germinated in peat moss for two weeks. DNA was isolated from leaves for RAPD (Random Amplified Polymorphic DNA) profile analyses. Results revealed a concentration dependent genotoxic effect for n–SiO₂ (however it seems to maintain genetic material stability) and high genotoxic effect for n–TiO₂.

Key words: broad bean, genotoxicity, GTS, Nano-scale materials, RAPD

INTRODUCTION

Nanoscale materials grew rapidly and have been used profusely not only in technological products but also in products for daily use, which stimulates some apprehension especially their influence on directly affected organisms. These materials can exert genotoxicity by direct and indirect mechanisms (Mehrian and Lima, 2016) showing a mutagenic effect upon eukaryotic (plant, animal and human) cells despite on-going debate on their toxic action (Golbamaki *et al.*, 2015). Since plants serve as producers, forming the basal, most critical trophic level of the food chain in the ecosystem, understanding how nanoscale materials affect them has become an urgent necessity. Due to the small size, large surface area and ability to generate reactive oxygen species (ROS) (Tumburu *et al.*, 2014; Elespuru *et al.*, 2018), nanomaterials could manipulate DNA resulting in different types of chromosomal aberrations (Kumari *et al.*, 2009; Singh *et al.*, 2009; Galal and El-Samahy, 2012). Nano-SiO₂ (n–SiO₂) and nano-TiO₂ (n–TiO₂) affect plant genetic material negatively depending on concentration, particle size and structure (Castiglione *et al.*, 2016; Khan and Ansari, 2018; Thabet *et al.*, 2019).

RAPD (Random Amplified Polymorphic DNA) is a PCR (Polymerase Chain Reaction) based technique and a relatively quick, easy, efficient, reliable and sensitive method that can identify a wide range of damaged DNA

and genetic mutations. Therefore, RAPD can be applied to studies of genotoxicity by detecting differences in genomic materials if they occur in primer specific DNA sequences (Williams *et al.*, 1990; Atienzar and Jha, 2006; Cencki *et al.*, 2009; Aboulila and Galal, 2019). Efficient RAPD analysis also depends on the purity of the template DNA (Sharma *et al.*, 2010). Here, to evaluate the genetic effects of n–SiO₂ and n–TiO₂, RAPD analysis was performed to detect DNA variations induced in *V. faba* cells after treatment with different concentrations (25, 50 and 75 mg/L) compared to untreated control.

MATERIALS AND METHODS

Nano-scale materials

Nano-scale silicon dioxide (n–SiO₂) and titanium dioxide (n–TiO₂) (anatase) were purchased from Nanotech Egypt Co., Egypt. For visualization purposes, osmium coating was applied to these nano-scale materials and observations were made under a scanning electron microscope (SEM) (SU8000, Hitachi Hitechnologies, Japan) at the Center of Advanced Instrumental Analysis, Kyushu University, Japan. The particle sizes measured of n–SiO₂ and n–TiO₂ were 119.1 ± 2.8 and 283.6 ± 15.9 nm, respectively (mean ± SD, n = 10 for each). Both n–SiO₂ and n–TiO₂ were dissolved in double distilled water by sonication for 30 min before being used to make concentrations of 25, 50 and 75 mg/L.

Preparation of plant samples

Vicia faba seeds were grown following Thabet *et al.* (2019). Briefly, seeds were surface sterilized with 2.5% sodium hypochlorite (NaOCl), then washed by distilled water three to four times followed by immersing in distilled water for 6 h. The seeds were then soaked for 24 h in n–SiO₂ and n–TiO₂, at three concentrations (25, 50 and 75 mg/L) each as well as in distilled water as a control (0 mg/L). After the treatment, the seeds were thor-

¹ Department of Genetics, Faculty of Agriculture, Kafrelsheikh University, Kafrelsheikh 33516, Egypt

² Laboratory of Insect Natural Enemies, Institute of Biological Control, Faculty of Agriculture, Kyushu University, Fukuoka 819–0395, Japan

³ Field Crop Pests Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki 12611, Egypt

* Joint corresponding authors (E-mail: ola.galal@agr.kfs.edu.eg or olagalal2002@yahoo.com and tuda@grt.kyushu-u.ac.jp)

oughly washed with distilled water. Ten seeds per replication (three replications per treatment) were allowed to grow in pots supplied with peat moss.

DNA extraction and RAPD-PCR

Individual plant samples were collected from each treatment and mixed to form a combined sample. Total genomic DNA (4–6 µg) was extracted from young healthy leaves by using the DNeasy Plant Mini Kit (QIAGEN GmbH, Germany). Polymerase chain reaction (PCR) was performed using 1 µl of the extracted genomic DNA in a 10 µl reaction mixture containing 5 µl 2X PCR Master mix solution [(i-Taq™) iNtRON Biotechnology, Shanghai, China], 1 µl (20 µM) of one of 14 decamer arbitrary random primers (Bio Basic Inc., Canada) (Table 1), and made up to 10 µl with sterile ddH₂O. The PCR amplification was performed according to Williams *et al.* (1990) in a thermal cycler (Perkin-Elmer, GeneAmp 2400, USA). PCR amplified products were differentiated on 1.5% agarose gel against a known DNA molecular marker: L1 (O'GeneRuler DNA Ladder Mix, Carlsbad, California, USA) or L2 (1Kb plus DNA ladder, TIANGEN, Taiwan).

Table 1. The nucleotide sequences of the primers used for RAPD analysis

Primer name	Sequence (5'→3')
OPA-09	GGGTAACGC
OPA-20	GTTGCGATCC
OPB-01	GTTTCGCTCC
OPB-05	TGCGCCCTTC
OPB-06	TGCTCTGCCC
OPB-07	GGTGACGCAG
OPB-08	GTCCACACGG
OPB-11	GTAGACCCGT
OPB-12	CCTTGACGCA
OPB-14	TCCGCTCTGG
OPH-01	GGTCGGAGAA
OPH-03	AGACGTCCAC
OPH-04	GGAAGTCGCC
OPH-05	AGTCGTCCCC

Estimation of total polymorphism and genomic template stability

DNA polymorphism was analyzed for each treatment as the percentage of polymorphic bands compared to control and genomic template stability (GTS) was calculated as follows:

$$\text{GTS (\%)} = (1 - \alpha/n) \times 100$$

where α is the number of polymorphic bands detected in each treatment and n is the number of total bands detected in the control (Cenkci *et al.*, 2010; Qari, 2010). By definition, GTS is the highest in the control (= 1). Polymorphism observed in the RAPD profile included disappearance of a band(s) and appearance of a new band(s) in each treatment compared to the control pro-

file (Atienzar *et al.*, 2000; Luceri *et al.*, 2000).

Estimation of band sharing index

Band sharing index (BSI) indicates resemblance between two samples using the following equation (Savva, 2000):

$$\text{BSI} = 2S / (A + B)$$

where S is the number of shared bands between two samples, A is the number of bands in the control and B the number of bands in the respective samples.

Estimation of primer polymorphism and polymorphic information content

The polymorphic information content was calculated as

$$\text{PIC} = 2fi \times (1 - fi)$$

where PIC is the polymorphic information content of the primer and fi is the frequency of band present (Roldan-Ruiz *et al.*, 2000; Aboulila *et al.*, 2019).

RESULTS

Total polymorphism

The number of amplified bands (a total of 113) in untreated control ranged from 4 (OPB-05) to 13 (OPA-09) bands (Fig. 1, Table 2). Also, the lowest number of polymorphic bands (17) detected at 25 mg/L n-SiO₂ recorded the lowest polymorphism (15.04%), whereas the highest number (47) detected at 25 and 50 mg/L n-TiO₂ corresponded to the highest polymorphism (41.59%). Nano-TiO₂ treatments generated higher numbers of new polymorphic bands whilst n-SiO₂ recorded noticeably the highest number of disappeared bands (33) at 75 mg/L compared to other treatments. These changes in band number illustrate that n-SiO₂ showed dose dependent increased polymorphism whereas n-TiO₂ showed equal (higher than other treatments) polymorphism at 25 and 50 mg/L.

Genomic template stability

Presence and absence of bands in a given sample was used to estimate GTS, as a qualitative measurement of DNA alterations in RAPD profile. With increasing n-SiO₂ concentration a decrease in *V. faba* GTS was observed, however n-TiO₂ at 25 and 50 mg/L recorded an equal effect on the genome stability (58.41%) (Fig. 2). This indicates that *V. faba* genome was more stable for n-SiO₂ than for n-TiO₂. Furthermore, n-SiO₂ increased numbers of both intensity-changed bands. It was noticeable that n-SiO₂ at 75 mg/L decreased both band numbers and intensity when the common event arising in the DNA patterns treated by n-SiO₂ was low intensity bands (Fig. 3).

Band sharing index

The band sharing index illustrates similarity among samples; here the first sample is the control. The high-

Fig. 1. Amplification pattern of *Vicia faba* DNA with 14 RAPD primers. L1 and L2: reference DNA ladders (molecular markers). Lane 1: control, lanes 2, 3 and 4: treated with n-SiO₂ (25, 50 and 75 mg/L, respectively), and lanes 5, 6 and 7: treated with n-TiO₂ (25, 50 and 75 mg/L, respectively).

Table 2. Number of polymorphic bands and polymorphism percentage deduced by RAPD profiles of *Vicia faba* after treatment with different concentrations of n-SiO₂ and n-TiO₂

Primer	Control bands	n-SiO ₂						n-TiO ₂					
		25 mg/L		50 mg/L		75 mg/L		25 mg/L		50 mg/L		75 mg/L	
		a	b	a	b	a	b	a	b	a	b	a	b
OPA-09	13	0	2	1	2	1	4	3	4	3	4	3	3
OPA-20	7	2	0	2	2	0	0	2	0	2	0	2	0
OPB-01	7	0	4	0	2	0	3	1	1	1	1	1	1
OPB-05	4	0	0	1	0	0	0	11	0	11	0	11	0
OPB-06	7	0	3	0	1	0	3	2	1	2	1	2	1
OPB-07	9	1	0	1	0	1	0	1	0	1	0	1	0
OPB-08	11	0	0	2	0	0	9	2	1	3	1	2	2
OPB-11	10	1	1	1	1	3	3	4	0	3	0	3	0
OPB-12	9	0	0	0	0	0	6	2	1	2	0	1	1
OPB-14	7	0	1	0	0	0	1	3	0	3	0	3	0
OPH-01	8	1	0	1	1	1	2	1	2	2	2	1	1
OPH-03	6	0	0	0	0	0	1	0	2	0	2	0	2
OPH-04	7	1	0	1	0	1	0	1	0	1	0	1	0
OPH-05	8	0	0	1	1	0	1	1	1	1	1	1	1
Total	113	6	11	11	10	7	33	34	13	35	12	32	12
Polymorphic bands (a+b)		17		21		40		47		47		44	
Polymorphism (%)		15.04		18.58		35.40		41.59		41.59		38.94	

a: appearance of new band(s) compared to the control, b: disappearance of band(s) compared to the control.

Fig. 2. Genomic template stabilities (GTS) in *Vicia faba* plants after treatment with different concentrations of n-SiO₂ and n-TiO₂.

Fig. 3. Changes in DNA-RAPD profile of *Vicia faba* treated with different concentrations of n-SiO₂ and n-TiO₂; a: appearance of new bands, b: disappearance of bands, c: increase in band intensity, d: decrease in band intensity.

est BSI value (0.92) was recorded at 25 mg/L n-SiO₂ (Table 3). Nano-SiO₂ maintained the similarity of bands whilst n-TiO₂ recorded lower values. Nano-SiO₂ showed a concentration dependent decreasing effect on BSI value. Generally, n-SiO₂ exhibited an alleviated effect compared to n-TiO₂.

Primer polymorphism and PIC value

Eight out of 14 primers used as RAPD markers (OPA-09, OPB-01, OPB-05, OPB-06, OPB-08, OPB-11, OPB-12 and OPH-01) can be selected for future studies

based on the high polymorphism (> 50%) and PIC (> 0.20) values. Results showed that 158 bands were amplified with an average of 11.29 bands per primer, 53.80% of them were polymorphic. The OPB-08 primer induced the highest polymorphism (87.50%), whereas the OPH-04 induced the lowest polymorphism (12.50%). The PIC values ranged from 0.02 (OPB-07) to 0.36 (OPB-05) with an average value of 0.20 (Table 4).

Table 3. Band sharing indices (BSI) in *Vicia faba* DNA after treatment with different concentrations of n-SiO₂ and n-TiO₂

Primer	A	n-SiO ₂									n-TiO ₂								
		25 mg/L			50 mg/L			75 mg/L			25 mg/L			50 mg/L			75 mg/L		
		S	B	A+B	S	B	A+B	S	B	A+B	S	B	A+B	S	B	A+B	S	B	A+B
OPA-09	13	11	11	24	11	12	25	9	10	23	9	12	25	9	12	25	10	13	26
OPA-20	7	7	9	16	5	8	15	7	8	15	7	9	16	7	9	16	7	9	16
OPB-01	7	3	3	10	5	5	12	4	4	11	6	7	14	6	7	14	6	7	14
OPB-05	4	4	4	8	4	5	9	4	4	8	4	15	19	4	15	19	4	15	19
OPB-06	7	4	4	11	6	6	13	4	4	11	6	8	15	6	8	15	6	8	15
OPB-07	9	9	10	19	9	10	19	9	10	19	9	10	19	9	10	19	9	10	19
OPB-08	11	11	11	22	11	13	24	2	2	13	10	12	23	10	13	24	9	11	22
OPB-11	10	9	10	20	9	10	20	7	10	20	9	13	23	10	13	23	10	13	23
OPB-12	9	9	9	18	9	9	18	3	3	12	8	10	19	9	11	20	8	9	18
OPB-14	7	6	6	13	7	7	14	6	6	13	7	10	17	7	10	17	7	10	17
OPH-01	8	8	9	17	7	8	16	6	7	15	6	7	15	6	9	17	7	8	16
OPH-03	6	6	6	12	6	6	12	5	5	11	4	4	10	4	4	10	4	4	10
OPH-04	7	7	8	15	7	8	15	7	8	15	7	8	15	7	8	15	7	8	15
OPH-05	8	8	8	16	7	8	16	7	7	15	7	8	16	7	8	16	7	8	16
Total	113	102	108	221	103	115	228	80	88	201	99	133	246	101	137	250	101	133	246
BSI		0.92			0.90			0.80			0.80			0.81			0.82		

A, number of bands in control. S, number of shared bands between control and treatment. B, number of bands in treatment.

Table 4. Number of total amplified bands, polymorphic bands, percentage of polymorphism and polymorphic information content (PIC) values of the primers used for RAPD profile

Primer name	Number of bands		Polymorphism (%)	PIC value
	Total	Polymorphic		
OPA-09	17	10	58.82	0.27
OPA-20	11	5	45.45	0.15
OPB-01	8	5	62.50	0.23
OPB-05	15	11	73.33	0.36
OPB-06	9	5	55.56	0.25
OPB-07	10	1	10.00	0.02
OPB-08	16	14	87.50	0.28
OPB-11	16	9	56.25	0.21
OPB-12	11	8	72.73	0.24
OPB-14	10	5	50.00	0.20
OPH-01	12	7	58.33	0.24
OPH-03	6	2	33.33	0.16
OPH-04	8	1	12.50	0.03
OPH-05	9	2	22.22	0.10
Total	158	85	53.80	2.74
Average	11.29	6.07		0.20

DISCUSSION

Exposure to nanoscale materials involves phytotoxicity (Ghosh *et al.*, 2010; Hatami *et al.*, 2014) and genotoxicity (López-Moreno *et al.*, 2010; Thabet *et al.*, 2019) in plants which is related to the ability to cause oxidative stress. Nano-SiO₂ (Tripathi *et al.*, 2017) and n-TiO₂ (Song *et al.*, 2013; Laware and Raskar, 2014; Tumburu *et al.*, 2014; Koce, 2017) uptake into the plant cell often involves free radical (unstable molecules with free outer electrons) development that ends up in the oxidative

destruction of macromolecules such as proteins and nucleic acids generating DNA modifications and/or enzyme disruption (Droge, 2002). Plants exposed to nanoscale materials show cytotoxic and genotoxic effects, including a change in mitotic index and increase in chromosomal aberration (Kumari *et al.*, 2009; Yang *et al.*, 2015; Khan and Ansari, 2018), indicating genomic damage.

Nano-SiO₂ had a concentration dependent toxic effect on the genetic material as recorded in *Glycine max* (Elsadany *et al.*, 2015) at 250–450 mg/L and in *V. faba* at 75–225 mg/L (Thabet, 2015) and at 25–75 mg/L (Thabet *et al.*, 2019). Nano-SiO₂ is also known to regulate expression of genes involved with salt stress on *Solanum lycopersicum* (Almutairi, 2016) and increases lignin gene expression in *Avena sativa* (Asgari *et al.*, 2018). A study reporting the interaction of n-SiO₂ with algae showed that n-SiO₂ coated alumina were less toxic to *Pseudokirchneriella subcapitata* than bare n-SiO₂ (Van Hoecke *et al.*, 2008).

Our results demonstrate that n-TiO₂, by inducing new amplified bands, generated high polymorphism and low GTS, indicating that n-TiO₂ had a genotoxic effect on *V. faba* DNA. A genotoxic effect of n-TiO₂ has been recorded with *Arabidopsis thaliana* at 100 mg/L (Landa *et al.*, 2012), *Cucurbita pepo* at 50 mg/L (Moreno-Olivas *et al.*, 2014), *Triticum aestivum* at 5–150 mg/L (Silva *et al.*, 2016), *Zea mays* at 1000–3000 mg/L (Mutlu *et al.*, 2018) and *V. faba* at 25–75 mg/L (Thabet *et al.*, 2019). Nano-TiO₂ can activate antioxidant enzymes (Xue *et al.*, 2010) or regulate genes involved mainly in responses to biotic and abiotic stress (Landa *et al.*, 2012). Furthermore, n-TiO₂ has been linked to mutagens as it could induce DNA breakage (Petković *et al.*, 2011; Moreno-Olivas *et al.*, 2014). Also, structure and

concentration controls its genotoxic effect as anatase/rutile structure had a dose-dependent response and was more genotoxic than an anatase structure at lower concentrations (Silva *et al.*, 2016).

Generally, mutations are responsible for the appearance of new PCR product visible on agarose gel if they occur at the same locus in at least 10% of cells (Atienzar *et al.*, 2000) and/or large deletions. Disappearance of bands may be associated with point mutations and/or complex chromosomal rearrangements caused by genotoxic chemicals (Atienzar and Jha, 2006). Changes observed in DNA profiles such as modifications in band intensity and loss of bands may be due to changes in oligonucleotide priming sites leading to genomic rearrangements, and less likely due to point mutations or DNA damage in the primer binding sites which can block or reduce the efficiency of DNA polymerization in PCR reaction (Liu *et al.*, 2005; Gao *et al.*, 2010).

CONCLUSION

This work demonstrated toxic effects of n-TiO₂ and n-SiO₂ on *V. faba* DNA and these are in line with our previous cytological and developmental studies. Data on uptake and translocation mechanisms of nano-scale materials are needed to understand mechanism and to inhibit potential toxic influences of such materials on the plant.

AUTHOR CONTRIBUTIONS

O. A. Galal and M. F. M. El-Samahy conceived the original idea. O. A. Galal designed and directed the experiment, and supervised the work. O. A. Galal and A. F. Thabet contributed to the experiments, the data analysis, preparation of the figures and tables, and the interpretation of the results. A. F. Thabet wrote the manuscript and conducted the literature search. Both O. A. Galal and M. Tuda did critical revision of the earlier versions of the manuscript. All authors approved the final version.

REFERENCES

- Aboulila, A. A. and O. A. Galal 2019 Evaluation of silica nanoparticles (SiO₂NP) and somaclonal variation effects on genome template stability in rice using RAPD and SSR markers. *Egypt. J. Genet. Cytol.*, **48**: 1–16
- Aboulila, A. A., O. A. Galal and M. I. Abo-Youssef 2019 Molecular characterization of drought tolerance in nine Egyptian rice genotypes using RAPD, SCOT and SSR markers. *Egypt. J. Genet. Cytol.*, **48**: 17–37
- Almutairi, Z.M. 2016 Effect of nano-silicon application on the expression of salt tolerance genes in germinating tomato (*Solanum lycopersicum* L.) seedlings under salt stress. *POJ* **9**: 106–114
- Asgari, F., A. Majd, P. Jonoubi and F. Najafi 2018 Effects of silicon nanoparticles on molecular, chemical, structural and ultrastructural characteristics of oat (*Avena sativa* L.). *Plant Physiol. Bioch.*, **127**: 152–160
- Atienzar, F. A. and A. N. Jha 2006 The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: a critical review. *Mutat. Res.*, **613**: 76–102
- Atienzar, F. A., B. Cordi, M. B. Donkin, A. J. Evenden, A. N. Jha and M. H. Depledge 2000 Comparison of ultraviolet-induced genotoxicity detected by random amplified polymorphic DNA with chlorophyll fluorescence and growth in a marine macro algae *Palmaria palmata*. *Aquat. Toxicol.*, **50**: 1–12
- Castiglione, M. R., L. Giorgetti, L. Bellani, S. Muccifora, S. Bottega and C. Spano 2016 Root responses to different types of TiO₂ nanoparticles and bulk counterpart in plant model system *Vicia faba* L. *Environ. Exp. Bot.*, **130**: 11–21
- Cenkci, S., M. Yildiz, I. Cigerici, M. Konuk and A. Bozdog 2009 Toxic chemical induced genotoxicity detected by random amplified polymorphic DNA (RAPD) in bean (*Phaseolus vulgaris*) seedlings. *Chemosphere*, **76**: 900–906
- Cenkci, S., I. H. Cigerici, M. Yildiz, C. Ozay, A. Bozdog and H. Terzi 2010 Lead contamination reduces chlorophyll biosynthesis and genomic template stability in *Brassica rapa* L. *Environ. Exp. Bot.*, **67**: 467–473
- Droge, W. 2002 Free radicals in the physiological control of cell function. *Physiol. Rev.*, **82**: 47–95
- Elespuru, R., S. Pfuhler, M. J. Aardema, T. Chen, S. H. Doak, A. Doherty, C. S. Farabaugh, J. Kenny, M. Manjanatha, B. Mahadevan, M. M. Moore, G. Ouedraogo, L. F. Stankowski and J. Y. Tanira 2018 Genotoxicity assessment of nanomaterials: recommendations on best practices, assays, and methods. *Toxicol. Sci.*, **164**: 391–416
- Elsadany, M. F. I., A. A. Aboulila, T. M. Abo-Sein and R. I. E. Magouz 2015 Effect of silica nano-particles in control of mite, *Tetranychus cucurbitacearum* (Sayed) and agronomic traits of soybean plants and qualitative assessment of its genotoxicity using total protein and RAPD analysis. *J. Agric. Chem. Biotechn. Mansoura Univ.*, **6**: 529–544
- Galal, O. A. and M. F. M. El-Samahy 2012 Genetical effects of using silica nanoparticles as biopesticide on *Drosophila melanogaster*. *Egypt. J. Genet. Cytol.*, **41**: 87–106
- Galal, O. A. and A. F. Thabet 2018 Cytological and molecular effects of silver nanoparticles (AgNPs) on *Vicia faba* M1 plants. *J. Agric. Chem. Biotechn. Mansoura Univ.*, **9**: 269–275
- Gao, Y., P. Zhou, M. Liang, E. Z. Yue and J. S. Wan 2010 Assessment of effects of heavy metals combined pollution on soil enzyme activities and microbial community structure: modified ecological dose response model and PCR-RAPD. *Environ. Earth Sci.*, **60**: 603–6012
- Ghosh, M., M. Bandyopadhyay and A. Mukherjee 2010 Genotoxicity of titanium dioxide (TiO₂) nanoparticles at two trophic levels: plant and human lymphocytes. *Chemosphere*, **81**: 1253–1262
- Golbamaki, N., B. Rasulev, A. Cassano, R. L. M. Robinson, E. Benfenati, J. Leszczynski and M. T. D. Cronin 2015 Genotoxicity of metal oxide nanomaterials: Review of recent data and discussion of possible mechanisms. *Nanoscale*, **7**: 2154–2198
- Hatami, M., M. Ghorbanpour and H. Salehiarjomand 2014 Nanoanatase TiO₂ modulates the germination behavior and seedling vigour of some commercially important medicinal and aromatic plants. *J. Biol. Environ. Sci.*, **8**: 53–59
- Khan, Z. and M. Y. K. Ansari 2018 Impact of engineered Si nanoparticles on seed germination, vigour index and genotoxicity assessment via DNA damage of root tip cells in *Lens culinaris*. *J. Plant Biochem. Physiol.*, **6**: 5243–5246
- Koce, J. D. 2017 Effects of exposure to nano and bulk sized TiO₂ and CuO in *Lemna minor*. *Plant Physiol. Bioch.*, **119**: 43e49
- Kumari, M., A. Mukherjee and N. Chandrasekaran 2009 Genotoxicity of silver nanoparticles in *Allium cepa*. *Sci. Total Environ.*, **407**: 5243–5246
- Landa, P., R. Vankova, J. Andrlova, J. Hodek, P. Marsik, H. Storchova, J. C. White and T. Vanek 2012 Nanoparticle-specific changes in *Arabidopsis thaliana* gene expression after exposure to ZnO, TiO₂, and fullerene soot. *J. Hazard. Mater.*, **241–242**: 55–62
- Laware, S. L. and S. Raskar 2014 Effect of titanium dioxide nanoparticles on hydrolytic and antioxidant enzymes during seed

- germination in onion. *Int. J. Curr. Microbiol. App. Sci.*, **3**: 749–760
- Liu, W., P. J. Li, X. M. Qi, Q. X. Zhou, L. Zheng, T. H. Sun and Y. S. Yang 2005 DNA changes in barely (*Hordeum vulgare*) seedlings induced by cadmium pollution using RAPD. *Chemosphere*, **61**: 158–167
- López-Moreno, M. L., G. de la Rosa, J. A. Hernández-Viezas, H. Castillo-Michel, C. E. Botez, J. R. Peralta-Videa and J. L. Gardea-Torresdey 2010 Evidence of the differential biotransformation and genotoxicity of ZnO and CeO₂ nanoparticles on soybean (*Glycine max*) plants. *Environ. Sci. Technol.*, **44**: 7315–7320
- Luceri, C., C. Filippo, G. Caderni, L. Gambacciani, M. Salvadori, A. Giannini and P. Dolara 2000 Detection of somatic DNA alterations in azoxymethane-induced F344 rat colon tumors by random amplified polymorphic DNA analysis. *Carcinogenesis*, **21**: 1753–1756
- Mehrian, S. K. and D. R. Lima 2016 Nanoparticles cyto and genotoxicity in plants: mechanisms and abnormalities. *Environ. Nanotech. Monit. Manag.*, **6**: 184–193
- Moreno-Olivas, F., V. U. Gant Jr, K. L. Johnson, J. R. Peralta-Videa and J. L. Gardea-Torresdey 2014 Random amplified polymorphic DNA reveals that TiO₂ nanoparticles are genotoxic to *Cucurbita pepo*. *J. Zhejiang Univ.-Sci. A (Appl. Phys. Eng.)*, **15**: 618–623
- Mutlu, F., F. Yurekli, B. Mutlu, F. B. Emre, F. Okusluk and O. Ozgl 2018 Assessment of phytotoxic and genotoxic effects of anatase TiO₂ nanoparticles on maize cultivar by using RAPD analysis. *Fresen. Environ. Bull.*, **27**: 436–445
- Petković, J., B. Zegura, M. Stevanović, N. Drnovšek, D. Uskoković, S. Novak and M. Filipič 2011 DNA damage and alterations in expression of DNA damage responsive genes induced by TiO₂ nanoparticles in human hepatoma HepG2 cells. *Nanotoxicology*, **5**: 341–353
- Qari, S. H. M. 2010 DNA-RAPD fingerprinting and cytogenetic screening of genotoxic and antigenotoxic effects of aqueous extracts of *Costus speciosus* (Koen.). *JKAU: Sci.*, **22**: 133–152
- Roldán-Ruiz, I. J. D., E. Van Bockstaele, A. Depicker and M. De Loose 2000 AFLP markers reveal high polymorphic rates in Ryegrasses (*Lolium spp.*). *Mol. Breed.*, **6**: 125–134
- Savva, D. 2000 The use of arbitrarily primed PCR (AP-PCR) fingerprinting to detect exposure to genotoxic chemicals. *Ecotoxicology*, **9**: 341–353
- Sharma, K. K., N. W. Zaidi, V. S. Pundhir and U. S. Singh 2010 Study of genetic diversity in rhizospheric *Trichoderma* isolates from Uttarakhand. *Ann. Pl. Protec. Sci.*, **18**: 403–410
- Silva, S., H. Oliveira, S. C. Craveiro, A. J. Calado and C. Santos 2016 Pure anatase and rutile + anatase nanoparticles differently affect wheat seedlings. *Chemosphere*, **151**: 68–75
- Singh, N., B. Manshian, G. J. S. Jenkins, S. M. Griffiths, P. M. Williams, T. G. G. Maffei, C. J. Wright and S. H. Doak 2009 Nanogenotoxicology: The DNA damaging potential of engineered nanomaterials. *Biomaterials*, **30**: 3891–914
- Song, U., M. Shin, G. Lee, J. Roh, Y. Kim and E. J. Lee 2013 Functional analysis of TiO₂ nanoparticle toxicity in three plant species. *Biol. Trace. Elem. Res.*, **155**: 93–103
- Thabet, A. F. 2015 Efficiency and safety of silica nanoparticles in controlling the main insect pests on faba bean and soybean. M. Sc. thesis, *Economic Entomology Department, Faculty of Agriculture, Kafrelsheikh Univ., Egypt*, 100 pp.
- Thabet, A. F., O. A. Galal, M. F. M. El-Samahy and M. Tuda 2019 Higher toxicity of nano-scale TiO₂ and dose-dependent genotoxicity of nano-scale SiO₂ on the cytology and seedling development of broad bean *Vicia faba*. *SN Appl. Sci.*, **1**: 956
- Tripathi, D. K., Shweta, S. Singh, S. Singh, R. Pandey, V. P. Singh, N. C. Sharma, S. M. Prasad, N. K. Dubey and D. K. Chauhan 2017 An overview on manufactured nanoparticles in plants: uptake, translocation, accumulation and phytotoxicity. *Plant Physiol. Bioch.*, **110**: 2–12
- Tumburu, L., C. Andersen, P. Rygiewicz and J. Reichman 2014 Phenotypic and genomic responses to titanium dioxide and cerium oxide nanoparticles in *Arabidopsis* germinants. *Environ. Toxicol. Chem.*, **34**: 70–83
- Van Hoecke, K., K. A. De Schampelaere, P. Van der Meeren, S. Lucas and C. R. Janssen 2008 Ecotoxicity of silica nanoparticles to the green alga *Pseudokirchneriella subcapitata*: importance of surface area. *Environ. Toxicol. Chem.*, **27**: 1948–1957
- Williams, K., A. Kubelik, K. Livak, J. Rafalski and V. Tingey 1990 DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.*, **18**: 6531–6535
- Xue, C., J. Wu, F. Lan, W. Liu, X. Yang, F. Zeng and H. Xu 2010 Nano titanium dioxide induces the generation of ROS and potential damage in HaCaT cells under UVA irradiation. *J. Nanosci. Nanotech.*, **10**: 8500–8507
- Yang, Z., J. Chen, R. Dou, X. Gao, C. Mao and L. Wang 2015 Assessment of the phytotoxicity of metal oxide nanoparticles on two crop plants, maize (*Zea mays* L.) and rice (*Oryza sativa* L.). *Int. J. Environ. Res. Public Health*, **12**: 15100–15109