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Cytological and Molecular Effects of Silver Nanoparticles (AgNPs) on *Vicia faba* M1 Plants

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ABSTRACT

Silver nanoparticles (AgNPs) are among the most widely used nanoparticles and are found in various types of products. In the past few years, these nanoparticles have received significant attention as pesticides for agricultural applications. Utility of AgNPs as efficient pesticides would become a reality if the researches provided some understanding of toxicity of these nanoparticles. Evaluation of the potential genotoxic effects of AgNPs on *Vicia faba* M_1 plants was the main goal in this study. Seeds of *V. faba* (Sakha 1 variety) were treated with three concentrations of AgNPs (25, 50 and 75 ppm) and M_1 populations raised from these seeds were investigated at different levels. Results indicated that all the three concentrations were able to induce significant increase in seed germination percentage (G) and germination rate index (GRI) in addition to root length and seedling vigor index (SVI) compared to control, while no significant differences were detected among the three AgNPs concentrations and control for shoot length. The maximum germination percentage (99.00 %) was found at the 50 ppm concentration while the highest values of root length (15.57 cm) and seedling vigor index (43.77) were recorded at the 25 ppm concentration. Cytological analysis showed that only the concentration of 25 ppm significantly reduced mitotic index (83.47 %) compared to control (91.38 %). Moreover, all treatments caused significantly increase in the percentage of abnormal cells, while the lower concentration (25 ppm) induced the highest percentage of abnormalities (6.90 %) compared to the other treatments. On the molecular level, the effects of AgNPs on genomic template stability (GTS) were measured based on RAPD-PCR analysis using 16 arbitrary primers. All AgNPs concentrations caused reduction in GTS % values; compared to control, which showed decrease in GTS % values as AgNPs concentrations increased. These results indicated that AgNPs had toxic effects on *V. faba* M_1 plants at cytological and molecular levels.

Keywords: Silver nanoparticles, *Vicia faba*, cytological analysis and genomic template stability.

INTRODUCTION

Nanotechnology is one of the most promising approaches in all areas of science and technology. It produces and utilizes nano-sized particles ranges between 1 and 100 nm in at least one dimension (Nikalje, 2015). Nanoparticles exhibit completely new physical, chemical and biological properties compared to larger particles of bulk material and these novel properties have led to a versatile spectrum of applications for nano-sized particles.

In recent years, nanotechnology has a great application in the field of agriculture; especially in pest and disease managements. As suggested previously, it can be used in the preparation of new formulations like pesticides, insecticides, insect repellents, pheromones and fertilizers (Parisi *et al*., 2015 and Grillo *et al*., 2016). Nanotechnology has promising application in nanoparticle mediated gene transfer. It can be used to deliver DNA and other desired chemicals into plant tissues for protection of host plants against insect pests (Torney, 2009).

Silver nanoparticles (AgNPs) are among the most extensively used nanoparticles and are found in various types of products. Currently, most of nanotechnology products are based on nanoscale silver (Woodrow Wilson International Center for Scholars, 2011). These nanoparticles are applied primarily for their antimicrobial activity towards many pathogenic microbes (Sahayaraj *et al.*, 2015), then AgNPs have gained their popularity in different areas, such as plant biology, biotechnology, chemistry, agriculture, physics and medicine (Wijnhoven *et al.*, 2009).

In the past few years, AgNPs have received significant attention as pesticides for agricultural applications. AgNPs have been suggested as effective alternative pesticides in management of plant pests and diseases (Sahayaraj *et al.*, 2016).

Because of a wide range of applications and the utility as crop pesticides, AgNPs are showing unacceptable toxic effects on human health and the environment. The majority of toxicity data available on AgNPs are on bacteria species (Rai *et al.*, 2012) and cell lines (AshaRani *et al.*, 2009 and Foldbjerg *et al.*, 2011). A few *in vivo* studies have been performed to evaluate the toxic mode of action of AgNPs (Sahayaraj *et al.*, 2016). Results of both *in vitro* and *in vivo* studies have provided evidence for oxidative stress as an important mechanism that involved in inducing AgNPs toxicity in the test systems. In addition, silver ions released from nanoparticle surfaces contribute to the toxicity of AgNPs.

On the other hand, for the utility of AgNPs in agriculture, especially for plant protection and production, there are largely lack in research that evaluate the toxicity of AgNPs and their potential hazard on plant. In this respect, the present study aimed to investigate the potential genotoxic effects of AgNPs on *V. faba* plants which were considered as an excellent cellular model and an ideal plant for studying the genotoxicity and assessing several genetic endpoints that are induced by mutagens and chemicals (Kanaya *et al.*, 1994). A laboratory experiment was conducted to study the potential genotoxic effects of AgNPs on *V. faba* M_1 plants at different levels. M_1 populations were investigated for germination and seedling growth in addition to vigor index. Also, changes in mitotic activities and chromosomal abnormalities have been recorded. On the molecular level, the effect of AgNPs on genomic template stability (GTS) has been measured.

MATERIALS AND METHODS

The present study was conducted at the Laboratory of Genetics Department, Faculty of Agriculture, Kafrelsheikh University, Kafr El-Sheikh, Egypt.

Seed material:

Seeds of *V. faba* L.; Sakha 1 variety (2n = 12 chromosomes), were obtained from Food Legumes Research Section, Sakha Agricultural Research Station (SARS), Kafr El-Sheikh, Egypt.

Silver nanoparticles:

Silver nanoparticles (AgNPs); 25±5 nm in size and spherical shape, have been synthesized by Nanotech Egypt for Photo-Electronics, City of 6 October, Al Giza, Egypt. These nanoparticles have been prepared by chemical reduction method as reported by Lee and Meisel (1982). The size and shape of AgNPs were performed on JEOL JEM-2100 high resolution transmission electron microscope (TEM) at an accelerating voltage of 200 kV.

Three different concentrations of AgNPs; 25, 50 and 75 ppm, were bio-assayed for genotoxicity against *V. faba* M_1 plants.

Experimental procedure:

Dry and healthy seeds of *V. faba*; presoaked in distilled water for 6 h., were treated with three different concentrations of AgNPs (25, 50 and 75 ppm) for 24 h. After treatment, seeds were thoroughly washed by distilled water to remove the residual amounts of AgNPs. Seeds soaked in distilled water were used as control.

The treated and control seeds were sown in four replicates for each in Randomized Complete Design to obtain M_1 generation which was investigated for germination, seedling growth, vigor index, mitotic index and chromosomal aberrations in addition to genome stability.

Germination, seedling growth and vigor index:

To evaluate the effect of AgNPs on germination, fifteen seeds per replication were allowed to germinate and grow in a 15 cm diameter Petri dish (four dishes/treatment) lined with Whatman No. 1 filter paper moistened with distilled water. Petri dishes were placed in a growth room at 25±1 °C and 12 h. of darkness. Seeds were observed for germination when the radicle was at least 3 mm length and the germinated seeds were counted every morning. Two different germination parameters; final germination percentage (G) and germination rate index (GRI), were assessed. The two parameters were determined as described by Al-Mudaris (1998). The value of G % was calculated after seven days for the treated and control seeds.

$$\mathbf{G}\ (\%) = \frac{\textbf{Number of germinated seeds}}{\textbf{Total number of seeds}} \times \mathbf{100}$$

$$\mathbf{GRI} = G1/1 + G2/2 + \cdots + Gi/i$$

Where **G1 is the germination percentage at the 1st day, G2 is the germination percentage at the 2nd day; and so on.**

Seedling growth; in terms of shoot and root lengths, were measured after four weeks as the mean of 10 seedlings per replicate (four replicates/treatment). The values for these two criteria were summed up to calculate the seedling vigor index (SVI) that reflects a collective measure of yield. The value of SVI was calculated according to Dahindwal *et al.* (1991) equation as follow:

$$\mathbf{SVI} = (\textbf{shoot length} + \textbf{root length}) \times \textbf{germination percentage}/\mathbf{100}$$

Cytological analysis:

Roots of *V. faba*; 1.5-2 cm length, of each replicate were harvested and fixed in a freshly prepared fixative composed of absolute ethanol and glacial acetic acid (3:1) for 24 h. The fixed roots were then stored in 70 % ethanol in refrigerator until use.

Slides were prepared according to Darlington and La Cour (1976) using chromosomal squash technique with 2 % aceto-carmine stain. About 800-1000 cells/replicate of each treatment and control were examined under high power of a light microscope. The mitotic index (MI) was determined as the ratio of the number of dividing cells to the total number of examined cells. Also, numbers and types of chromosomal aberrations were recorded and estimated as the percentage of cells showing chromosomal abnormalities relative to dividing cells.

Statistical analysis:

Data of germination parameters, seedling growth and SVI in addition to cytological analysis were subjected to One-Way Analysis of Variance to verify the effect of AgNPs treatments on *V. faba* M_1 plants. Values were expressed as mean±SE (standard error). The least significant differences (LSD) test was established to determine the differences between the mean values at $P < 0.05$ level of significance.

DNA extraction and RAPD-PCR condition:

Individual plant samples were collected from each treatment and mixed to form a combined sample. Total genomic DNA was extracted from young healthy leaves by using the DNeasy Plant Mini Kit (QIAGEN GmbH, Cat. No. 69104).

Polymerase chain reaction 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], 1 µl (20 µM) of decamer arbitrary random primers introduced from Bio Basic Inc., Canada (Table 1), and made up to 10 µl with sterile ddH_2O. The PCR amplification was performed according to Williams *et al.* (1990) in the PCR thermal cycler (Perkin-Elmer, Gene Amp. 2400). PCR amplified products were separated on 1.5 % agarose gel against a known DNA Ladder ready-to-use; L1: (Thermo Scientific O'GeneRuler DNA Ladder Mix, Cat-no: SM1173) or L2: (1Kb plus DNA ladder, TIANGEN).

Table 1. The used 16 primers and their nucleotide sequences.

Sr. No.	Primer name	Sequence (5′→3′)
1	OPA-09	GGGTAACGCC
2	OPA-14	TCTGTGCTGG
3	OPA-20	GTTGCGATCC
4	OPB-01	GTTTCGCTCC
5	OPB-06	TGCTCTGCCC
6	OPB-07	GGTGACGCAG
7	OPB-08	GTCCACACGG
8	OPB-10	CTGCTGGGAC
9	OPB-11	GTAGACCCGT
10	OPB-12	CCTTGACGCA
11	OPB-14	TCCGCTCTGG
12	OPB-17	AGGGAACGAG
13	OPH-01	GGTCGGAGAA
14	OPH-03	AGACGTCCAC
15	OPH-04	GGAAGTCGCC
16	OPH-05	AGTCGTCCCC

Estimation of genomic template stability:

Analysis of DNA polymorphism was conducted for each treatment and genomic template stability (GTS) value was calculated as follow:

$$\mathbf{GTS}\ (\%) = (\mathbf{1} - a/n) \times \mathbf{100}$$

Where; ***a* is the number of polymorphic bands detected in each treatment, and *n* is the number of total bands detected in the control.**

Polymorphism observed in RAPD profile included disappearance of a normal band and appearance of a new band in each treatment compared to control profile (Qari, 2010).

RESULTS AND DISCUSSION

Effects of AgNPs on germination, seedling growth and vigor index:

The effect of different concentrations of AgNPs on the seed germination parameters (G % and GRI), shoot and root lengths, and SVI of *V. faba* M_1 generation are presented in Table 2. Results showed that control treatment exhibited the lowest significant value for G %, GRI, root length and SVI compared to the three different concentrations of AgNPs. On the other hand, no significant differences were detected between the three different concentrations of AgNPs and the control treatment for shoot length.

Table 2. Effect of AgNPs on germination percentage (G) and germination rate index (GRI), shoot and root lengths as well as seedling vigor index (SVI) of *V. faba* M_1 plants.

Treatment	G (%)	GRI	Shoot length (cm)	Root length (cm)	SVI
Control	92.0±2.83^{b}	31.81±2.66^{b}	26.47±0.46^{a}	11.26±0.41^{c}	35.84±1.42^{b}
25 ppm	97.0±1.91^{ab}	50.00±0.00^{a}	29.43±1.58^{a}	15.57±0.58^{a}	43.77±2.36^{a}
50 ppm	99.0±1.00^{a}	50.00±0.00^{a}	26.60±1.12^{a}	14.24±0.54^{ab}	38.28±2.43^{ab}
75 ppm	97.0±1.91^{ab}	48.33±0.68^{a}	26.70±2.41^{a}	13.40±1.01^{b}	40.35±2.38^{ab}

Means in each column with the same letter are not significantly differed at *P* < 0.05 level of significance.

Data in Table 2 revealed also that the maximum G % value (99 %) was found at the 50 ppm concentration and this then decreased up to 97 % at the highest concentration of AgNPs (75 ppm), which did not differ significantly from 25 ppm treatment. The reduction in G % value may be attributed to the effect of the highest concentration of AgNPs (75 ppm) on the delay or inhibition of physiological and biological processes necessary for seed germination, which include enzyme activity (Chrispeeds and Varner, 1976), hormonal imbalance (Khan and Al-Quainy, 2009) and the inhibition of mitotic process (Ananthaswamy *et al.*, 1971). This was in accordance to studies of Jung *et al.* (2008) and Rai *et al.* (2012), they demonstrated that AgNPs may interact with cellular structures and biomolecules such as proteins, lipids and DNA causing damage and inactivation of proteins and enzymes and, therefore, cellular damage.

Regarding to the effect of AgNPs on the GRI, it was clear that all AgNPs concentrations induced significantly increase in the GRI relative to the untreated control. On the other hand, all the three different concentrations did not differ significantly from each other.

For shoot length, AgNPs did not cause any noticeable significantly differences on *V. faba* M_1 plants compared to the untreated control. Unlike shoot length, root length differed significantly among all treatments. The application of 25 ppm significantly increased root length to 15.57 cm comparing with control treatment (11.26 cm), however, root length was significantly reduced with the concentration increasing and reached to 13.40 cm at the highest concentration of AgNPs. Generally, all AgNPs concentrations increased root length than the untreated control. Improvement of growth and yield following exposure to mutagens have been widely reported by Borzouei *et al.* (2010) and Mudibu *et al.* (2011).

With respect to the SVI, data in Table 2 showed that the highest value (43.77) was recorded at the lowest AgNPs concentration (25 ppm), while at the highest concentrations (50 and 75 ppm) it was significantly reduced to 38.28 and 40.35, respectively, which did not differ significantly.

The measurements of growth parameters clearly indicate that all the AgNPs concentrations led to increase root length and SVI, however the low concentration of 25 ppm was more effective in enhancing these parameters. The reduction in root length and SVI with increasing the concentration is related to the mechanism of action of AgNPs. These nanoparticles may inhibit an energy supply system resulting in the inhibition of mitosis which can be associated with seedling growth depression. Similar observations indicated that AgNPs may lead to inhibition of ATP synthesis (Raffi *et al.*, 2008 and Mirzajani *et al.*, 2011).

Cytological effects of AgNPs:

a. Effect on mitotic index:

The effects of the applied AgNPs concentrations on root tip cells of M_1 plants are shown in Table 3. A decrease in MI (83.47 %) was recorded at the concentration of 25 ppm compared to the control (91.38 %), while at 50 and 75 ppm concentrations, the MI did not differed significantly from control treatment.

It has been recognized that the improvement in growth and yield in plants is associated with increased mitotic activity in the roots, and the reduction in growth and yield is associated with reduced mitotic activity. This was not in agreement with our results; where the application of 25 ppm concentration significantly reduced MI, while the highest values of root length and SVI were recorded at the same concentration.

Table 3. Percentage of mitotic phases, mitotic index (MI), types and percentage of abnormalities in the root tip cells of *V. faba* M_1 plants treated with three different concentrations of AgNPs.

Treatment	No. of examined cells	No. of dividing cells	No. of abnormal cells	Mitotic phase (%)				Chromosomal aberration (%)							MI (%)	Abnormalities (%)
				Prophase	Metaphase	Anaphase	Telophase	C-metaphase	Disturbance	Stickiness	Bridge	Laggard	Fragments	Breaks		
Control	3276	2984	20	87.77	2.95	3.55	5.06	5.00	40.0	10.0	0.00	35.0	10.0	0.00	91.38±1.56a	0.65±0.17d
25 ppm	3765	3141	148	89.81	1.02	0.45	3.88	12.16	18.24	36.49	14.86	11.49	0.68	6.08	83.47±2.39b	6.90±0.48a
50 ppm	3461	3277	102	91.94	1.07	0.37	3.51	2.94	22.55	34.31	20.59	14.71	0.00	4.90	94.73±0.55a	3.49±0.44c
75 ppm	3416	3113	140	90.52	1.03	0.42	3.24	6.43	24.29	33.57	11.43	15.71	7.14	1.43	91.31±2.32a	5.05±0.15b

Means in each column with the same letter are not significantly differed at *P* < 0.05 level of significance.

b. Effect on percentage and type of chromosomal abnormalities:

Data in Table 3 revealed that the effect on chromosomal abnormalities varied strongly among AgNPs concentrations. A highly significant increase; compared to control, was observed at all concentrations. The highest significant increase in chromosomal abnormalities (6.90 %) was scored at the concentration of 25 ppm. The lowest percentages of abnormalities were recorded at 50 ppm (3.49 %) and 75 ppm (5.05 %) compared to the concentration of 25 ppm. Thus, the lowest concentration induced a higher percentage of abnormalities compared to higher concentrations of AgNPs.

The foregoing results showed significant reduction in mitotic activity in the root tips of *V. faba* M_1 plants following seed treatment with 25 ppm of AgNPs. On the other hand, no significant differences were observed in MI values; compared to control, following application of higher concentrations of AgNPs (50 and 75 ppm). In this respect, De Veylder *et al.* (2003) reported that; in plant root tips, arrest in cell cycle progression is caused by check points that mediate the entry of cells into S-phase and mitosis. On the other side, the cell may spontaneously continue cycle progression but this is often followed by genome instability, allowing cell survival at the cost of tolerating mutation (Hartig and Beck, 2006).

In the current study, cells in plants grown from seeds treated with lower concentration, and in which lower MI values were scored, also harbored a higher frequency of chromosomal abnormalities. When the AgNPs concentrations were increased to 50 and 75 ppm, lower frequencies of chromosomal abnormalities were scored. Similar results were reported by Badr *et al.* (2014) in cowpea root cells; a lower proportion of chromosomal abnormalities was scored as the γ-radiation dose increased.

Types of abnormalities produced in *V. faba* M_1 plants are given in Table 3 and illustrated in Figure 1. Chromosomal stickiness, where the chromosomes appear clumped together (Figures 1c and h), followed by disturbed configurations (Figures 1a, d and e), appeared to be the most common abnormalities that were induced in the root cells at all AgNPs concentrations; 25, 50 and 75 ppm. Stickiness was attributed to partial dissociation of the nucleoproteins and alterations in their pattern of organization caused by mutagenic treatment (Kumar *et al.*, 2003).

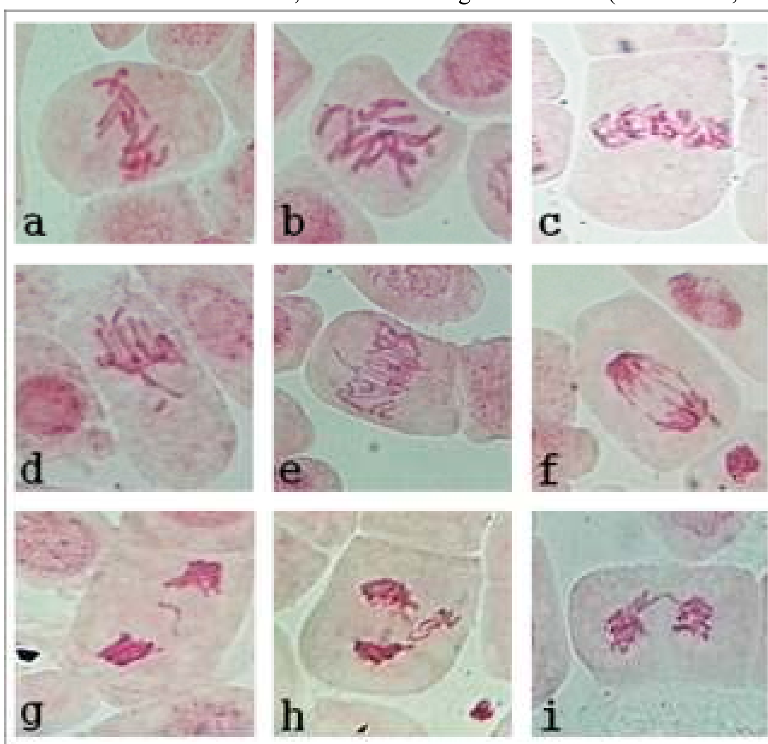

Figure 1. Types of abnormalities observed in *V. faba* root tips treated with three different concentrations of AgNPs. a) Disturbed metaphase with chromosome breakage, b) C-metaphase, c) Sticky metaphase, d) Disturbed metaphase with fragment, e) Disturbed anaphase, f) Anaphase bridge with chromosome breakage, g) Telophase with lagging chromosome, h) Stickness at telophase with bridge and laggards, i) Telophase bridge.

Chromosome bridges (Figures 1f, h and i) and lagging chromosomes (Figures 1g and h) also appeared in a considerable proportion of cells at all AgNPs concentrations. The bridges might be attributed to the breaking and reunion of the chromosomes or to the stickiness of the chromosomes at metaphase. The lagging chromosomes may be due to abnormal spindle formation, and as a result, spindle fibers fail to carry the respective chromosomes to the poles (Badr *et al.*, 2013). The behavior of laggards is a characteristic in that they generally lead to micronuclei formation (Kumar and Rai, 2006).

Moreover, C-metaphase configurations (Figure 1b), where the chromosomes are scattered in the cytoplasm as a result of complete inhibition of spindle fiber formation, were frequently observed at 25 ppm concentration, while lower proportions of C-metaphase were scored at 50 and 75 ppm. Fragments (Figure 1d) and breaks (Figures 1a and f) also were appeared at lower frequency following treatment with AgNPs at all different concentrations.

Datta (2002) revealed that cytological effects cannot be considered as the factors that directly lead to the development of abnormal plant parts and reduction in plant height. These findings are in line with our results, that the applied AgNPs concentrations produced positive effects on root length and SVI. This view may be supported by the occurrence of a higher percentage of abnormalities in plants exposed to AgNPs concentration; particularly the low concentration (25 ppm), and can be understood in the light of the view of Datta *et al.* (2011) who reported that a positive correlation exists between chromosomal abnormalities and antioxidant enzymes related to the defense mechanism of cells.

Effect of AgNPs on RAPD profile and genomic template stability:

RAPD technique is a reliable and sensitive method that can identify a wide range of damaged DNA and genetic mutations and therefore can be applied to genotoxicity and carcinogenesis studies (Atienzar and Jha, 2006 and Cenkci *et al.*, 2009).

To evaluate the genetic effects of AgNPs, RAPD analysis was performed to detect DNA variations induced in *V. faba* cells treated with different AgNPs concentrations (25, 50 and 75 ppm) compared to untreated control.

As presented in Figure 2 and summarized in Table 4, sixteen oligonucleotide primers were utilized and yielded a total of 138 amplified bands in untreated control ranged from 6 bands (primers OPB-06 and OPH-03) to 13 bands (primer OPA-09). RAPD profiles generated by these primers revealed discriminate differences between control and the different AgNPs treatments as reflected by changes in bands number (appearance of new bands or loss of normal bands) and bands intensity (increase or decrease the intensity of amplified bands).

Table 4. Changes in DNA-RAPD profile of *V. faba* treated with different concentrations of AgNPs.

Sr. No.	Primer	No. of bands in control	AgNPs concentrations											
			25 ppm				50 ppm				75 ppm			
			a	b	c	d	a	b	c	d	a	b	c	d
1	OPA-09	13	3	4	1	2	6	3	1	6	4	5	1	1
2	OPA-14	8	0	2	4	0	0	2	4	0	0	2	3	0
3	OPA-20	7	3	0	2	4	4	0	2	2	3	1	2	2
4	OPB-01	7	0	0	3	0	0	3	0	1	0	5	0	1
5	OPB-06	6	0	0	0	4	0	0	0	3	0	0	0	3
6	OPB-07	9	1	0	8	0	2	0	8	0	1	0	8	0
7	OPB-08	11	4	1	2	0	4	2	1	1	5	2	1	2
8	OPB-10	10	0	0	0	0	0	0	0	0	0	3	1	1
9	OPB-11	10	1	0	7	0	2	3	1	1	2	3	1	1
10	OPB-12	9	0	1	0	1	0	1	0	2	0	1	0	2
11	OPB-14	8	0	1	1	2	0	1	0	3	0	1	1	2
12	OPB-17	11	5	4	1	0	0	6	0	2	4	3	2	1
13	OPH-01	8	1	1	6	0	1	2	4	0	1	0	3	0
14	OPH-03	6	0	0	1	0	0	0	1	1	0	0	1	1
15	OPH-04	7	2	1	1	0	2	1	1	0	2	1	1	0
16	OPH-05	8	0	1	1	0	0	1	1	0	0	2	1	0
Total		138	20	16	38	13	21	25	24	22	22	29	26	17
a+b			36				46				51			
GTS (%)			73.91				66.67				63.04			

a: appearance of new band, b: disappearance of normal band, c: increase in band intensity, d: decrease in band intensity, a+b: polymorphic bands and GTS: genomic template stability.

Generally, among all differences, the increase in band intensity was the major event arising in the DNA patterns treated with 25 ppm concentration (38 bands), while treatments of 50 and 75 ppm showed the highest disappearance of normal bands (25 and 29 bands, respectively) compared to control.

Concerning differences in bands number (presence and/or absence), most used primers generated at least one of these two alterations in a given sample as a result of AgNPs treatment. The maximum number of appearing and disappearing bands (51 bands) was found in *V. faba* DNA treated with the highest concentration of AgNPs (75 ppm), while the lowest change in bands number (36 bands) was recorded for the lowest concentration (25 ppm).

Changes in bands number as revealed by appearance or disappearance of bands could be used to estimate GTS % for each treatment, which considered as a qualitative measurement of DNA alterations in RAPD profile. Data presented in Table 4 revealed that all AgNPs treatments caused reduction in GTS % values compared to control. The highest value of GTS (73.91 %) was recorded for 25 ppm concentration, while 75 ppm treatment exhibited the lowest value (63.04 %). Thus, values of GTS % were decreased as AgNPs concentrations increased.

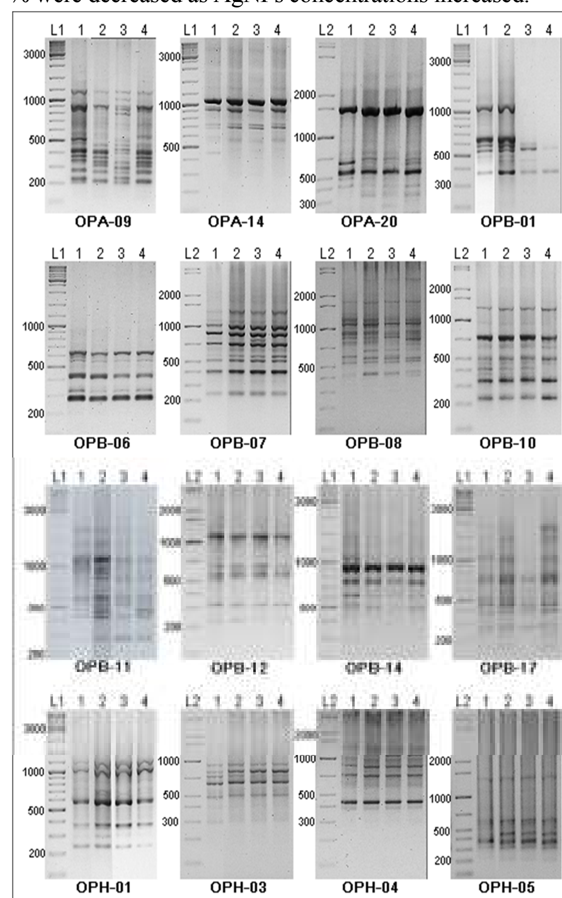

Figure 2. Amplification pattern of *V. faba* M_1 plants with 16 RAPD markers. L1 and L2: DNA Ladders, and lanes 1-4: the treatments of AgNPs (control, 25, 50 and 75 ppm, respectively).

Changes in bands number obtained in the present study reflected DNA alterations ranged from single base (point mutations) to complex chromosomal rearrangements (Atienzar and Jha, 2006 and Ozturk *et al.*, 2010). 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). On the other hand, damage caused to genomic DNA would induce the modification of the binding sites which can lead to alterations of electrophoretic PCR patterns which implies a disappearance of band (depending on the extent of DNA damage) (Atienzar and Jha, 2006 and Rocco *et al.*, 2012). Thus, the new bands could be attributed to mutations while the disappeared bands could be attributed to DNA damage.

Changes in DNA patterns as a result of AgNPs applications may be induced by direct and/or indirect interaction with genomic DNA causing genotoxicity. Several studies demonstrated that AgNps primarily cause their toxicity by enhancing intracellular levels of reactive oxygen species (ROS) (AshaRani *et al.*, 2009 and Eom and Choi, 2010). These ROS; which include radicals containing oxygen, subsequently produce cellular damage such as disrupting membrane integrity and damag of proteins and DNA (AshaRani *et al.*, 2009 and Foldbjerg *et al.*, 2011). On the other hand, findings of Luk *et al.* (1975) reported that free silver ions that released from nanosilver bind to DNA molecules, specifically purine and pyrimidine bases. Farther, they documented that association of silver with DNA molecules is not formed by an interaction with phosphate groups. However, the binding to DNA is probably by replacing the hydrogen bond between complementary base pairs.

CONCLUSION

Results of the present study revealed that all the three concentrations of AgNPs induced positive effects on G %, GRI, root length and SVI compared to untreated control, while no significant differences were detected between the three AgNPs concentrations and the control for shoot length. The highest values of root length and SVI were recorded at the low level of AgNPs (25 ppm) which significantly reduced MI and also harbored a higher frequency of chromosomal abnormalities. At the molecular level, all AgNPs treatments caused reduction in GTS % value; compared to control, which were decreased as AgNPs concentrations increased. Thus, it is suggested that the GTS % may be the most sensitive parameter compared to the other obtained parameter in the detection of AgNPs genotoxicity.

Based on the obtained results, AgNPs may be used for improving germination and seedling growth, while at cytological and molecular levels, these nanoparticles showed toxic effects on mitotic activity and chromosomes in addition to genomic DNA. To better predict and prevent potential toxic impacts of AgNPs on plant that resulting from their utilization in agriculture, more data on plant uptake, translocation in addition to potential biological effects are needed at various cellular and molecular levels.

REFERENCES

Al-Mudaris, M. (1998). Notes on various parameters recording the speed of seed germination. Der Tropenlandwirt, 99: 147-154.

Ananthaswamy, H.M.; V.K. Vakil and A. Shrinivas (1971). Biological and physiological changes in gamma irradiated wheat during germination. Radiation Botany, 11: 1-12.

AshaRani, P.V.; G. Low Kah Mun; M.P. Hande and S. Valiyaveettil (2009). Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano., 3(2): 279-290.

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 macroalgae Palmaria palmate. Aquat. Toxicol., 50: 1-12.

Badr, A.; H. Zaki; M.O. Germoush; A.Q. Tawfeek and M.A. El-Tayeb (2013). Cyto-physiological impacts of Metosulam herbicide on *Vicia faba* plants. Acta Physiol. Plant, 35: 1933-1941.

Badr, A.; M. Halawa and H.H. El-Shazly (2014). Cytological effects of gamma radiation and its impact on growth and yield of M1 and M2 plants of cowpea cultivars. Cytologia, 79(2): 195-206.

Borzouei, A.; M. Kafi; H. Khazaei; B. Naseriyan and A. Majdabdi (2010). Effects of gamma radiation on germination and physiological aspects of wheat (Triticum aestivum L.) seedlings. Pak. J. Bot., 42: 2281-2290.

Cenkci, S.; M. Yildiz; I. Cigerci; M. Konuk and A. Bozdag (2009). Toxic chemical induced genotoxicity detected by random amplified polymorphic DNA (RAPD) in bean (*Phaseolus vulgaris*) seedlings. Chemosphere, 76: 900-906.

Chrispeeds, M.J. and J.E. Varner (1976). Gibberelic acid induced synthesis and release of α-amylase and ribonuclease by isolated barley aleurons layers. Plant Physiol., 42: 346-406.

Dahindwal, A.S.; B.P.S. Lather and J. Singh (1991). Efficacy of seed treatment on germination, seedling emergence and vigor of cotton (*Gossypium hirsutum*) genotypes. Seed Research, 19: 59-61.

Darlington, C.D. and L.E. La cour (1976). The Handling of Chromosomes, 6th ed. Allen and Unwin, London.

Datta, S.K. (2002). Parameters for detecting effects of ionizing radiations on plants. In: Tripathi, R.D., Kulshreshtha, K., Agarwal, M., Ahmad, K.J., Varshney, C.K., Krupa, S. and Pushpangadan, P. (eds.). Plant Responses to Environmental Stress. International Book Distributing Co., Lucknow. pp. 257-265.

Datta, B.; D. Chakrabarty and A. Dhar (2011). Identification of unknown roundwater pollution sources using classical optimization with linked simulation. Journal of Hydro-Environment Research, 5: 25-36.

De Veylder, L.; J. Loubès and D. Inzé (2003). Plant cell cycle transitions. Curr. Opin. Plant Biol., 6: 536-543.

Eom, H.J. and J. Choi (2010). p38 MAPK activation, DNA damage, cell cycle arrest and apoptosis as mechanisms of toxicity of silver nanoparticles in Jurkat T cells. Environ. Sci. Technol., 44(21): 8337-8342.

Foldbjerg, R.; D.A. Dang and H. Autrup (2011). Cytotoxicity and genotoxicity of silver nanoparticles in the human lung cancer cell line, A549. Arch. Toxicol., 85: 743-750.

Grillo, R.; P.C. Abhilash and L.F. Fraceto (2016). Nanotechnology applied to bio-encapsulation of pesticides. Journal of Nanoscience and Nanotechnology, 16(1): 1231-1234.

Hartig, K. and E. Beck (2006). Crosstalk between auxins, cytokinins and sugars in the plant cell cycle. Plant Biol., 8: 1-8.

Jung, W.K.; H.C. Koo; K.W. Kim; S. Shin; S.H. Kim and Y.H. Park (2008). Antibacterial activity and mechanism of action of the silver ion in *Staphylococcus aureus* and *Escherichia coli*. Appl. Environ. Microbiol., 74(7): 2171-2178.

Kanaya, N.; B.S. Gill; I.S. Grover; A. Murin; R. Osiecka; S.S. Sandhu and H.C. Andersson (1994). Vicia faba chromosomal aberration assay. Mutat. Res., 310: 231-247.

Khan, S. and F. Al-Qurainy (2009). Mutagenic effect of sodium azide on seed germination of *Eruca sativa* (L.). Aust. J. Basic. Appl. Sci., 3(4): 3081-3087.

Kumar, G. and P. Rai (2006). Partial genome elimination through micronuclei in soybean (*Glycine max*). Nat. Acad. Sci. Lett., 29: 417-421.

Kumar, G.; S. Kesarwani and V. Sharma (2003). Clastogenic effect of individual and combined treatment of gamma rays and EMS in Lens culinary. J. Cytol. Genet., 4: 149-154.

Lee, P.C. and D. Meisel (1982). Adsorption and surface-enhanced raman of dyes on silver and gold sols. Journal of Physical Chemistry, 86: 3391-3395.

Luk, K.F.S.; A.H. Maki and R.J. Hoover (1975). Studies of heavy-metal binding with polynucleotides using optical detection of magnetic-resonance. Silver (I) binding. J. Am. Chem. Soc., 97: 1241-1242.

Mirzajani, F.; A. Ghassempour; A. Aliahmadi and M.A. Esmaeili (2011). Antibacterial effect of silver nanoparticles on *Staphylococcus aureus*. Res. Microbiol., 162: 542-549.

Mudibu, J.; K.K. 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.

Nikalje, A.P. (2015). Nanotechnology and its applications in medicine. Med. chem., 5: 81-89.

Ozturk, F.; F. Duman; Z. Leblebici and R. Temizgul (2010). Arsenic accumulation and biological responses of watercress (Nasturtium officinale R. Br.) exposed to arsenite. Environm and Experimental Botany, 69: 167-174.

Parisi, C.; M. Vigani and E. Rodríguez-Cerezo (2015). Agricultural nanotechnologies: What are the current possibilities?. Nano Today, 10(2): 124-127.

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.

Raffi, M.; F. Hussain; T.M. Bhatti; J.I. Akhter; A. Hameed and M.M. Hasan (2008). Antibacterial characterization of silver nanoparticles against *E. Coli ATCC-15224*. J. Mater. Sci. Technol., 24: 192-196.

Rai, M.K.; S.D. Deshmukh; A.P. Ingle and A.K. Gade (2012). Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. J. Appl. Microbiol., 112(5): 841-852.

Rocco, L.; G. Frenzilli; G. Zito; A. Archimandritis; C. Peluso and V. Stingo (2012). Genotoxic effects in fish induced by pharmacological agents present in the sewage of some Italian water- treatment plants. Inc. Environ. Toxicol., 27: 18-25.

Sahayaraj, K., M. Roobadevi, S. Rajesh and S. Azizi (2015). Vernonia cinerea (L.) Less silver nanocomposite and its antibacterial activity against cotton pathogen. Research on Chemical Intermediates, 41(8): 5495-5507.

Sahayaraj, K.; M. Madasamy and S. Anbu Radhika (2016). Insecticidal activity of bio-silver and gold nanoparticles against *Pericallia ricini* Fab. (Lepidaptera: Archidae). Bionanoparticles for pest management J. Biopest, 9(1): 63-72.

Torney (2009). Nanoparticle mediated plant transformation. Emerging technologies in plant science research. Inter departmental plant physiology major fall seminar physics. p. 696.

Wijnhoven, S.W.P.; W.J.G.M. Peijnenburg; C.A. Herberts; W.I. Hagens; A.G. Oomen; E.H.W. Heugens; B. Roszek; J. Bisschops; I. Gosens; D. Van De Meent; S. Dekkers; W.H. De Johg; M. van Zijverden; A.J.A.M. Sips and R.E. Geertsma (2009). Nano-Silver: a review of available data and knowledge gaps in human and environmental risk as sessment. Nanotoxicology, 3: 109-138

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.

Woodrow Wilson International Center for Scholars (2011). The project on emerging nanotechnologies. http://www.nanotechproject.org/inventories/consumer/analysis_draft/.

التأثيرات السيتولوجية والجزيئية لجزيئات الفضة النانومترية (AgNPs) على نباتات الجيل الطفري الأول للفول البلدي

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تعد جزيئات الفضة النانومترية (AgNPs) من بين الجزيئات النانومترية الأكثر استخداما وقد وجدت فى أنواع مختلفة من المنتجات. في السنوات القليلة الماضية, حظيت هذه الجزيئات النانومترية باهتمام كبير كمبيدات في التطبيقات الزراعية. استعمال هذه الجزيئات (AgNPs) بكفاءة كمبيدات يمكن أن يصبح فعال إذا قدمت الأبحاث بعض الفهم عن سميتها. كان تقييم تأثيرات السمية الوراثية المحتملة على نباتات الجيل الطفري الأول (M_1) فى الفول البلدي هو الهدف الأساسي فى هذه الدراسة. تمت معاملة بذور الفول البلدي (صنف سخا ١) بثلاثة تركيزات من AgNPs (٢٥ , ٥٠ , ٧٥ جزء في المليون) وتم فحص عشائر الجيل الطفري الأول (M_1) الناتجة عن هذه البذور على مستويات مختلفة. أشارت النتائج الى أن التركيزات الثلاثة كانت قادرة على إحداث زيادة معنوية في نسبة إنبات البذور (G %) ودليل معدل الإنبات (GRI) بالإضافة الى طول الجذر ودليل النشاط للبادرة (SVI) مقارنة مع المعاملة الضابطة، ولم يتم تحديد إختلافات معنوية بين تركيزات AgNPs الثلاثة والمعاملة الضابطة بالنسبة لطول الساق. تم العثور على أعلى قيمة لنسبة الإنبات (٩٩,٠ %) عند التركيز ٥٠ جزء في المليون في حين تم تسجيل أعلى قيم لطول الجذر (١٥,٥٧ سم) ودليل نشاط البادرة (٤٣,٧٧) عند التركيز ٢٥ جزء في المليون. أظهر التحليل السيتولوجي أن التركيز الأقل فقط (٢٥ جزء في المليون) أدى الي إنخفاض الدليل الميتوزي بشكل كبير (٨٣,٤٧ %) مقارنة بالمعاملة الضابطة (٩١,٣٨ %). وعلاوة على ذلك ، أحدثت جميع المعاملات زيادة معنوية في النسبة المئوية للخلايا غير الطبيعية, في حين أن التركيز الأقل (٢٥ جزء في المليون) أدى إلى حدوث أعلى نسبة من الشذوذات الكروموسومية (٦,٩٠ %) مقارنة بالمعاملات الأخرى. على المستوى الجزيئي, تم قياس تأثيرات AgNPs على ثبات القالب الجينومي (GTS) على أساس تحليل RAPD-PCR بإستخدام ١٦ بادئ عشوائي. وتسببت جميع تركيزات AgNPs في إنخفاض قيم % GTS مقارنة بالمعاملة الضابطة التي أظهرت إنخفاضا في قيم % GTS مع زيادة تركيزات AgNPs. أشارت هذه النتائج إلى أن AgNP لها تأثيرات سامة على نباتات الجيل الطفري الأول (M_1) للفول البلدي على المستويات السيتولوجية والجزيئية.