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Research article

PHYSIOLOGICAL AND CYTOGENETIC RESPONSES OF WHEAT AND BARLEY TO SILVER NANOPRIMING TREATMENT

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ABSTRACT: This experiment was carried out to study the effect of silver-nanopriming on germination percentage, seedling growth and mitotic cell division. The results showed that silver nanoparticles (AgNPs) significantly increased the germination percentage. Mean comparison showed that the highest germination percentages (98, 92, 96 %) were observed in the pretreated wheat (Beni Sweif 1 and Gemmieza 9) and barley (Giza 130) seeds. The results clearly revealed that shoot length, fresh and dry weights were slightly promoted by AgNPs while in roots corresponding parameters were reduced compared with those of control. Photosynthetic pigments and chlorophyll fluorescence were affected to some extent by AgNPs priming. Cytological changes in root tips of 72h and 120h germinated seeds were observed by disturbed chromosomes at metaphase and anaphase. The main types of chromosomal aberrations are: chromosomes aneuploidy, binucleate cells, deletion chromosomes, deform nuclei, micronuclei, chromosomes fragment, and stickiness chromosomes. It was found that the mitotic index significantly increased in the three pretreated tested plants compared to the control. This study infers that AgNPs could penetrate plant system and might impair stages of cell division causing chromosomal aberrations.

Key words: Chlorophyll fluorescence, Chromosomal aberrations, Germination, Mitotic index, Nanoparticles.

INTRODUCTION

Nanoparticles (nano-scale particles = NSPs) are atomic or molecular aggregates with at least one dimension between 1 and 100 nm (Ball, 2002; Roco, 2003), that can drastically modify their physico-chemical properties compared to the bulk material (Nel *et al*., 2006). Nanotechnology is a new scientific field being developed since 1980s. Nano materials have a lot of different characters compared to the general materials with same components because of their small size effect, surface or interface effect, etc. With the development of new chemical or physical methods, the concern for environmental contaminations is also heightened as the chemical procedures involved in the synthesis of nano materials generate a large amount of hazardous byproducts. Thus, there is a need for clean, nontoxic and environment-friendly methods of nanoparticles synthesis (Mukherjee *et al*., 2001).

The noble metal nanoparticles such as gold (Au), platinum (Pt) and silver (Ag) nanoparticles have gained a considerable interest over the last decade owing to their important applications (Murray *et al*., 2001; Okuda *et al*., 2005). It is now well understood that the intrinsic characteristics of noble metal nanoparticles are dependent on their composition, size, crystallinity, shape, and structure (Xia *et al*., 2009). Moreover, they have been used in other various applications as antimicrobial, electrical conducting, and in sensing/optical applications (Babu and Prabu, 2011). Currently, AgNPs are used in more than 250 products (Fabrega *et al*., 2011).Silver nanoparticles have received much attention worldwide due to attractive physical and chemical properties. Silver nanoparticles can be synthesized using various methods: chemical, electrochemical (Vorobyova *et al*., 1999), γ-radiations (Chol *et al*., 2005), photochemical (Li *et al*., 2005), laser ablations (Tsuji *et al*., 2003). In protection of environment, nanotechnology is finding applications in photocatalysis, a process in which light promotes a reaction between compound such as pesticide residues and nanomaterial without the latter being consumed. Such a process would be useful in decomposition of water for agriculture and human safe. In food safety, photocatalysis could find uses in cleansing the surface of fresh fruits and vegetables with toxic agrochemical residues and in destroying bacteria on such produce (Raskar and Laware, 2014).

Changes in agricultural technology have been a major factor shaping modern agriculture. The development of nanodevices and nanomaterials could open up novel applications in plant biotechnology and agriculture (Zheng *et al*., 2005). The use of nanoparticles in the growth of plants and for the control of plant diseases is a recent practice (Zhang, 2003; Park *et al*., 2007). However, whether beneficial or harmful to plant growth is an unresolved issue, various studies had been carried out to understand the effect of nanoparticles on the growth of plants (Lu et *al*., 2002; Yang and Watts, 2005; Lee *et al*., 2008). Currently, the main thrust of research in nanotechnology focuses on applications in the field of electronics energy (Zhang, 2003), medicine and life sciences (Galbraith, 2007; Park *et al*., 2007). Experiences gained from these fields facilitate the development of genetically modified crops, plant protecting chemicals and precision farming techniques. Nanotechnology permits broad advances in agricultural research, such as reproductive science and technology, conversion of agricultural and food wastes to energy and other useful byproducts through enzymatic nanobioprocessing, disease prevention and treatment in plants using various nanocides (Yang and Watts, 2005). Seed priming is a technique of seed enhancements that improves germination or seedling growth. Seed priming enhances seed performance by rapid and uniform germination, normal and vigorous seedlings, which resulted in faster and better germination in different crops (Cantliffe, 2003).

The objectives of this study were to display the effect of silver nanoparticles on some crop plants as wheat *(Triticum durum* Desf. cv. Beni Sweif 1) (*Triticum aestivum* L. cv. Gemmieza 9) and barley (*Hordeum vulgare L.* cv. Giza 130). It was planned to study the influence of AgNPs on mitotic cell division, seed germination, seedling growth parameters, and photosynthesis activity.

MATERIALS AND METHODS

Synthesis of Ag Nanoparticles

Silver nanoparticles can be synthesized using the most popular method which is chemical reduction of silver nitrate ($AgNO_3$) using sodium borohydride ($NaBH_4$) according to the method of Sileikaite *et al*. (2006). Process involves total conversion of ions into particles in presence of stabilizing agents. Silver nitrate solution was reduced by sodium borohydride in distilled water in presence of Tween-20 (surfactant). The solution was heated on a hot water bath, in the temperature range 70-75ºC until the solution turned dark yellow to brown color indicating the formation of nanoparticles.

Characterization of AgNPs

UV–Vis absorbance spectroscopy analysis

The reduction of pure Ag+ ions in silver nitrate ($AgNO_3$) into silver nanoparticles (AgNPs) was monitored periodically by UV–Vis spectroscopy (T80 UV–Vis spectrophotometer - double beam) after the dilution of the samples with deionized water (Raut *et al*., 2009). A UV–Vis spectrograph of the silver nanoparticles was recorded by using a quartz cuvette with water as reference. The UV–Vis spectrometric readings were recorded at a scanning speed of 200–800 nm (Leela and Vivekanandan, 2008).

Transmission electron microscope analysis of AgNPs

The suspension containing AgNPs was sampled by TEM analysis using (JEOL-TEM 100 CX) at the Electron Microscopic Unit, Faculty of Science, Alexandria University.

TEM samples were prepared by placing a drop of the suspension of AgNPs solutions on carbon-coated copper grids and allowing water to evaporate. The samples on the grids were allowed to dry for 4 min. The shape and size of silver nanoparticles were determined from TEM micrographs (Elavazhagan and Arunachalam, 2011).

Priming treatment

Seeds were purchased from Ministry of Agriculture, Egypt. Seeds were immersed in a 0.1% sodium hypochlorite solution for 5 min washed thoroughly several times with distilled water. For AgNPs-priming, the sterilized seeds were soaked in silver nanoparticles solution. The untreated seeds were soaked in distilled water. The seeds were immersed in priming media for 24 hours at a 24ºC. The treated seeds were rinsed several times with distilled water. Untreated seeds served as control.

Germination studies

Twenty seeds from each of the treatments were germinated in the Petri dishes which were containing two layers of Whatman No. 2 filter papers in 9 mm diameters with distilled water. Petri dishes were placed in a germination chambers in darkness. Germinated seeds were counted and removed when reached 72h and 120h for cytological analysis. A seed was considered to have germinated when the emerging ridicule longed 1 mm. Germination percentage = *Number of germinated seeds / Number of total seeds × 100.* Seedling Vigor Index = *Germination% × (mean shoot length + mean root length)*.After 120h seedlings were then propagated in pots containing soil and sand mixture (1:2) to complete growth for 10 days (15 days from beginning). The pots were irrigated each even day with half strength Hoagland nutrient solution (Hoagland and Arnon, 1950) to reach water field capacity. The pots (in triplicates) were placed in growth chamber under 16h light/ 8h dark cycle at 25±2°C during the light/dark period for 10 days.

Plant growth parameters

At harvest (15 days), the plants were divided into roots and shoots. Roots were rinsed twice with distilled water. Subsequently, growth and plant biomass was measured on fresh and dry weight basis. To obtain dry weight, roots and shoots were dried at 65°C until reaching a constant weight. Root and shoot lengths were measured.

Photosynthetic pigments

Chlorophyll a (Chl.a), chlorophyll b (Chl.b), and carotenoids were extracted and estimated according to the method of Lichtenthaler (1987). Pigments contents were calculated in mg g^{-1} FW.

Chlorophyll fluorescence

Measurements of Chl fluorescence was performed with OS-30P pulse modulated chlorophyll fluorimeter (Opti-sciences, Hudson, USA). Before each measurement, leaves were dark-adapted for 30 min with leaf-clips. To determine the minimal fluorescence (F_o), the weak measuring light was turned on and F_o was recorded. The leaves were then exposed to 0.1 s saturated flash of approximately 6000 µmol m^2 s. to obtain the maximal fluorescence yield (F_m). The ratio of variable to maximal fluorescence (F_v/F_m) was calculated automatically according to F_o and F_m measured [$F_v/F_m = (F_m - F_o)/F_m$].

Cytogenetic studies

Influence of nanoparticles on mitotic cell division and chromosomal aberrations

Root tips (1-2 cm) from 72h and 120h germinating seeds previously treated with Ag-nanoparticles and untreated (control), washed immediately and fixed in three parts of absolute alcohol and one part of glacial acetic during 24 hours in room temperature, then preserved in 70% alcohol and kept in refrigerator for future cytogenetic studies. For staining, root tips were hydrolyzed in 1.0 N Hydrochloric acid then rapidly squashed in a drop of 2% Acetocarmine (**Sharma and Sharma, 1980**) .Total 4000 cells were screened for calculations of the mitotic index and chromosomal together with nuclei abnormalities.

Statistical analysis

Based on the data obtained from the experiment, the results presented are the mean ± standard deviation (SD) gained from at least three replicate samples using Microsoft Office Excel 2007. Statistical analysis by the least significant difference (LSD) for multiple comparisons, taking $P \leq 0.05$ as significant, was calculated by SPSS 13.0.

RESULTS

UV-Visible spectroscopy:

The synthesis of the silver nanoparticles has been confirmed by measuring the UV-Vis spectra of the reaction mixture. As apparent from Figure 1A, the absorption peak appeared at about 430 nm is corresponding to the characteristic surface plasmon resonance of the resulting AgNPs.

TEM analysis of AgNPs

Figure 1B showed TEM image of the prepared silver- nanoparticles. The available AgNPs mostly were spherical and near spherical with a size of nearly 20 nm.

Seed germination and seedling growth parameters

The results showed that the effect of AgNPs priming was significant on germination percentage, the highest germination percentages (98 ,92 ,and 96%) was achieved after 24h in wheat " Beni Sweif 1 "and " Gemmieza 9" and barley "Giza 130" respectively, compared with those percentages of control (85,80,85%) respectively (Figure 2A). The results showed that use of nanoparticles can increase -to some extent- seedling vigor index in all studied plants compared to the control (Figure 2B). The effect of silver nanoparticles on shoot and root fresh and dry weights are shown in Table 1. It was observed that there was a positive response in shoots, while slight inhibition was noticed in roots in those priming with Ag-nanoparticles than those priming in water in all tested plants.

Table 1: Effect of silver nanoparticles priming on growth parameters of wheat and barley.

Plant	Treatment	FW (g)		DW (g)	
		Shoot	Root	Shoot	Root
Wheat Beni Sweif 1	H_2O	5.02±0.12	4.50±0.21	0.462±0.03	0.260±0.02
	AgNPs	6.82±0.23	3.32±0.06	0.482±0.04	0.210±0.02
Wheat Gemmieza 9	H_2O	5.12±0.14	3.60±0.12	0.563±0.01	0.476±0.01
	AgNPs	5.73±0.21	3.31±0.15	0.571±0.03	0.386±0.02
Barley Giza 130	H_2O	5.07±0.11	2.60±0.09	0.80±0.08	0.513±0.04
	AgNPs	6.76±0.14	2.26±0.11	0.92±0.04	0.487±0.07

Each value is the mean of triplicates ±SD.

The percentage inhibition in roots dry weights were 19% and 19% for wheat (Beni Sweif 1 and Gemmieza 9) and 5% for barley "Giza 130" respectively compared with water primed plants. The effect of silver nanoparticles on shoot and root lengths of wheat and barley are shown in (Figure 3). Shoot length of wheat and barley showed positive effect of AgNPs, while root length for all tested plants was inhibited in those priming in Ag- nanoparticles in wheat " Beni Sweif 1 " and barley "Giza 130", where " Gemmieza 9" showed no difference in root length compared to the control plant. Among the application of silver-nanoparticles the results in Figure 2B showed slight higher values for seedling vigor index for all plants compared to the control.

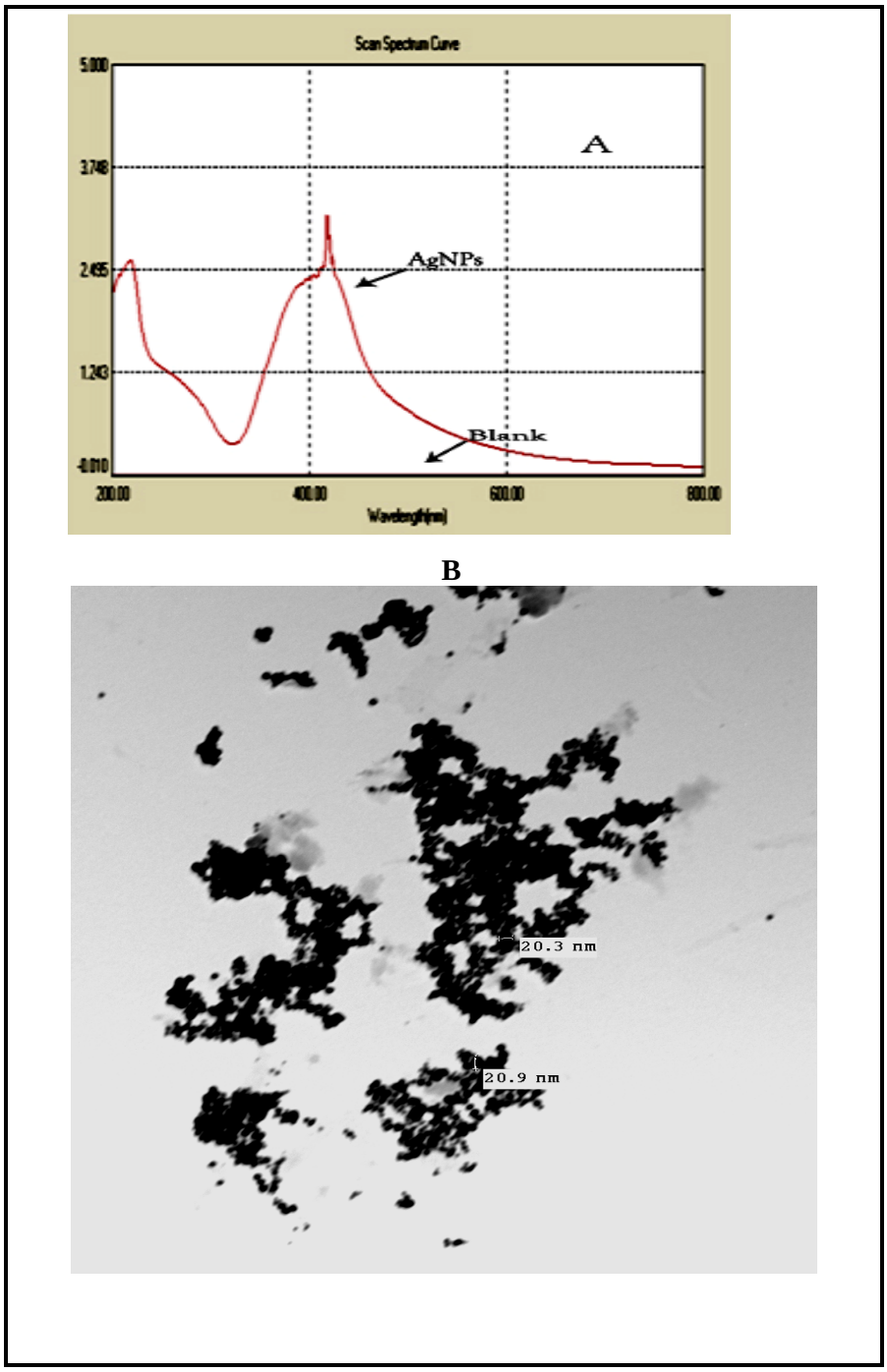

Figure 1: (A) UV-Visible absorptions spectrum (at 430 nm) of silver nanoparticles, and (B) TEM image of silver nanoparticles formed during preparations by chemical reductions methods.

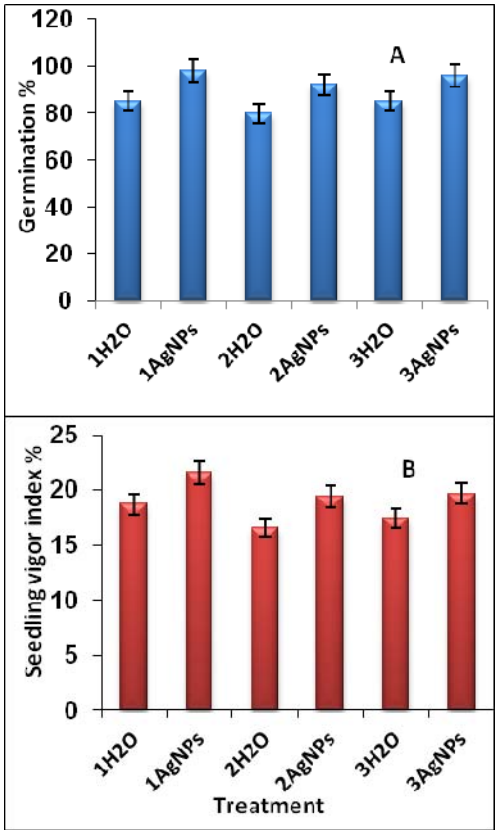

Figure 2: Germination percentage and seedling vigor index of wheat "Beni Sweif 1" (1) "Gemmieza 9" (2), and (3) barley "Giza 130" under silver nanoparticles application. Each value is the mean of triplicates ±SD

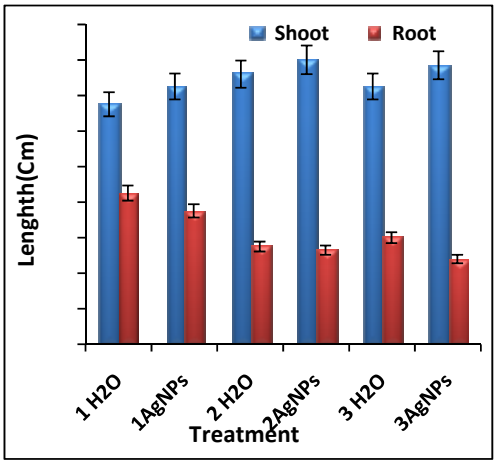

Figure 3: Shoot and root lengths of wheat "Beni Sweif 1" (1), "Gemmieza 9" (2), and (3) barley "Giza 130" under silver nanoparticles application. Each value is the mean of triplicates ±SD.

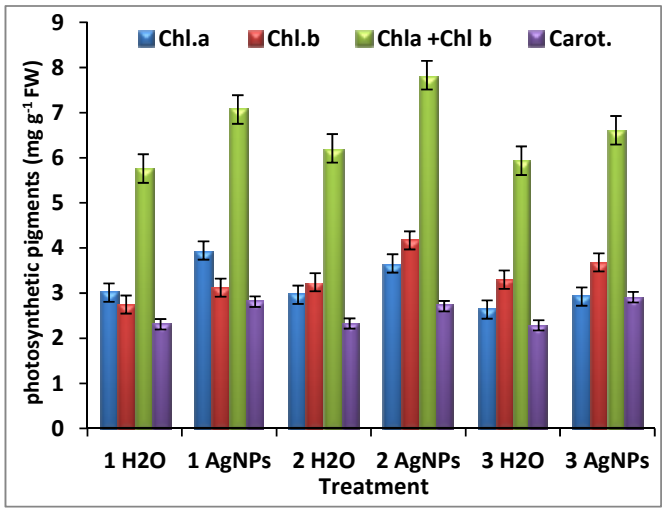

Figure 4: Photosynthetic pigments content of wheat "Beni Sweif 1" (1)," Gemmieza 9" (2), and (3) barley "Giza 130" under silver nanoparticles application. Each value is the mean of triplicates ±SD.

Photosynthetic pigments:

The intensity of photosynthesis considerably depends on the operation and quantity of photosynthetic pigments. In the course of our examinations, we recorded the changes of chlorophylls (a and b) contents in leaves as a result of priming with 20nm Ag-nanoparticles. Effect of silver nanoparticles on photosynthetic pigments content (chlorophyll a and b, total chlorophylls and carotenoids) of wheat " Beni Sweif 1 ", "Gemmieza 9", and barley "Giza 130" showed significantly increase above control as shown in Figure 4. In leaves treated with 20nm of silver nanoparticles total chlorophylls increased in wheat by 23% and 26% (Beni Sweif 1, Gemmieza 9) respectively and 11% for barley compared to the control.

Chlorophyll fluorescence:

The results indicated that Fv/Fm values of leave treated with Ag-nanoparticles treatment were higher than those of the control treatment, Beni Sweif 1 showed 2.4%, and Gemmieza 9 increase percent was 2.9%, and barley was 3.2% (Table 2).

Cytogenetic studies

Mitotic division and chromosomal behavior were normal in control roots. Results in Tables 3-6 showed the numbers and percentages of abnormal cells under different treatments with AgNPs in the three tested plants. These results showed, significant increase in the percentage of total abnormal cells in the seeds primed with AgNPs compared with those primed with water (control groups).The highest percentage of abnormal cells, recorded in roots of wheat " Beni Sweif 1 " and " Gemmieza 9" than barley "Giza 130" at 72h and 120h of germination compared to control roots. AgNPs induced different types of mitotic abnormal cells in the roots of the three tested plants. The main types of simple chromosomal aberrations identified in the 4000 analyzed cells are: chromosomes aneuploidy, binucleate cells, deletion chromosomes, deform nuclei, micronuclei, chromosomes fragment, and stickiness chromosomes. Results in tables 7-10 showed mitotic index for 4000 examined cells, the mitotic index was used to determine the rate of cell division. It must be noted that, the slides prepared for the assessment of chromosomal aberrations were used also for calculating the mitotic index. It was found that the mitotic index significantly increased in the three tested plants primed in AgNPs compared to the control.

Table 2: Photochemical efficiency in wheat and barley under silver nanoparticles application.

Plant	Treatment	F_0	Fv	Fm	Fv/Fm	Fv/F_0
Wheat Beni Sweif 1	H_2O	116	357	473	0.754	3.077
	AgNPs	151	514	663	0.772	3.403
Wheat Gemmieza 9	H_2O	132	414	546	0.758	3.136
	AgNPs	163	578	741	0.780	3.546
Barley Giza 130	H_2O	127	400	527	0.759	3.149
	AgNPs	119	431	550	0.783	3.621

Table 3: Effect of silver nanoparticles priming on chromosomal aberrations and nuclei deformations of wheat and barley after 72 hours.

Plant	Treatment	Observation result (from 4000 observed cells)								
		Replicates	Aneuploidy	Binucleate	Deform nuclei	Micronuclei	Deletion	Fragment	stickiness	Total
Wheat Beni Sweif 1	Priming in H_2O	REP 1	2	9	12	11	7	3	1	45
		REP 2	6	7	21	21	2	4	3	64
		REP 3	8	9	19	11	4	3	0	54
	Priming in Ag Nanoparticles	REP 1	111	114	234	88	172	128	43	890
		REP 2	121	165	239	85	198	181	49	1038
		REP 3	105	134	198	79	174	159	49	898
Wheat Gemmieza 9	Priming in H_2O	REP 1	6	14	8	11	4	7	3	53
		REP 2	8	123	5	12	6	6	11	171
		REP 3	9	121	7	14	11	9	7	178
	Priming in Ag Nanoparticles	REP 1	146	122	87	181	167	120	76	899
		REP 2	177	109	65	129	186	152	69	887
		REP 3	142	156	93	120	153	120	76	860
Barley Giza 130	Priming in H_2O	REP 1	10	21	9	6	3	6	1	56
		REP 2	13	19	3	8	2	5	2	52
		REP 3	8	12	5	8	2	4	0	39
	Priming in Ag Nanoparticles	REP 1	29	98	152	118	34	119	41	591
		REP 2	93	76	101	76	56	128	28	558
		REP 3	63	118	89	69	69	193	29	630

Table 4: Effect of silver nanoparticles priming on percentage chromosomal aberrations and nuclei deformations of wheat and barley after 72 hours.

Plant	Treatment	Percentage of abnormal cell (from 4000 observed cells)								
		Replicates	Aneuploidy	Binucleate	Deform nuclei	Micronuclei	Deletion	Fragment	stickiness	Total
Wheat Beni Sweif 1	Priming in H_2O	REP 1	0.05	0.225	0.3	0.275	0.175	0.075	0.025	1.125
		REP 2	0.15	0.175	0.525	0.525	0.05	0.1	0.075	1.6
		REP 3	0.2	0.225	0.475	0.275	0.1	0.075	0	1.35
	Priming in Ag Nanoparticles	REP 1	2.775	2.85	5.85	2.2	4.3	3.2	1.075	22.25
		REP 2	3.025	4.125	5.975	2.125	4.95	4.525	1.225	25.95
		REP 3	2.625	3.35	4.95	1.975	4.35	3.975	1.225	22.45
Wheat Gemmieza 9	Priming in H_2O	REP 1	0.15	0.35	0.2	0.275	0.1	0.175	0.075	1.325
		REP 2	0.2	3.075	0.125	0.3	0.15	0.15	0.275	4.275
		REP 3	0.225	3.025	0.175	0.35	0.275	0.225	0.175	4.45
	Priming in Ag Nanoparticles	REP 1	3.65	3.05	2.175	4.525	4.175	3	1.9	22.48
		REP 2	4.425	2.725	1.625	3.225	4.65	3.8	1.725	22.18
		REP 3	3.55	3.9	2.325	3	3.825	3	1.9	21.5
Barley Giza 130	Priming in H_2O	REP 1	0.25	0.525	0.225	0.15	0.075	0.15	0.025	1.4
		REP 2	0.325	0.475	0.075	0.2	0.05	0.125	0.05	1.3
		REP 3	0.2	0.3	0.125	0.2	0.05	0.1	0	0.975
	Priming in Ag Nanoparticles	REP 1	0.725	2.45	3.8	2.95	0.85	2.975	1.025	14.78
		REP 2	2.325	1.9	2.525	1.9	1.4	3.2	0.7	13.95
		REP 3	1.575	2.95	2.225	1.725	1.725	4.825	0.725	15.75

Table 5: Effect of silver nanoparticles priming on chromosomal aberrations and nuclei deformations of wheat and barley after 120 hours.

Plant	Treatment	Observation result (from 4000 observed cells)								
		Replicates	Aneuploidy	Binucleate	Deform nuclei	Micronuclei	Deletion	Fragment	stickiness	Total
Wheat Beni Sweif 1	Priming in H_2O	REP 1	0	23	49	47	23	6	1	149
		REP 2	10	11	42	31	0	9	36	139
		REP 3	12	7	40	21	9	7	0	96
	Priming in Ag Nanoparticles	REP 1	129	147	339	91	221	274	47	1248
		REP 2	129	278	409	97	248	309	99	1569
		REP 3	169	198	488	119	201	218	87	1480
Wheat Gemmieza 9	Priming in H_2O	REP 1	10	14	11	31	26	9	1	102
		REP 2	9	123	24	47	9	8	4	261
		REP 3	38	121	28	41	39	11	5	317
	Priming in Ag Nanoparticles	REP 1	261	145	107	221	182	147	87	1150
		REP 2	218	181	149	163	223	275	86	1295
		REP 3	211	248	159	156	249	341	78	1442
Barley Giza 130	Priming in H_2O	REP 1	11	21	10	7	6	7	0	62
		REP 2	16	19	17	32	8	6	2	91
		REP 3	14	12	19	23	9	8	1	86
	Priming in Ag Nanoparticles	REP 1	51	103	173	128	205	161	33	854
		REP 2	107	96	122	87	113	211	39	775
		REP 3	112	126	176	78	98	167	43	800

Table 6: Effect of silver nanoparticles priming on percentage chromosomal aberrations and nuclei deformations of wheat and barley after 120 hours.

Plant	Treatment	Replicates	percentage of abnormal cell (from 4000 observed cells)							
			Aneuploidy	Binucleate	Deform nuclei	Micronuclei	Deletion	Fragment	stickiness	Total
Wheat Beni Sweif 1	Priming in H_2O	REP 1	0	0.575	1.225	1.175	0.575	0.15	0.025	3.725
		REP 2	0.225	3.075	0.6	1.175	0.225	0.2	0.1	5.6
		REP 3	0.95	3.025	0.7	1.025	0.975	0.275	0.125	7.075
	Priming in Ag Nanoparticles	REP 1	3.225	3.675	8.475	2.275	5.525	6.85	1.175	31.2
		REP 2	3.225	6.95	10.225	2.425	6.2	7.725	2.475	39.225
		REP 3	4.225	4.95	12.2	2.975	5.025	5.45	2.175	37
Wheat Gemmieza 9	Priming in H_2O	REP 1	0.25	0.35	0.275	0.775	0.65	0.225	0.025	2.55
		REP 2	0.225	3.075	0.6	1.175	0.225	0.2	0.1	5.6
		REP 3	0.95	3.025	0.7	1.025	0.975	0.275	0.125	7.075
	Priming in Ag Nanoparticles	REP 1	6.525	3.625	2.675	5.525	4.55	3.675	2.175	28.75
		REP 2	5.45	4.525	3.725	4.075	5.575	6.875	2.15	32.375
		REP 3	5.275	6.2	3.975	3.9	6.225	8.525	1.95	36.05
Barley Giza 130	Priming in H_2O	REP 1	0.275	0.525	0.25	0.175	0.15	0.175	0	1.55
		REP 2	0.4	0.475	0.425	0.8	0.2	0.15	0.05	2.5
		REP 3	0.35	0.3	0.475	0.575	0.225	0.2	0.025	2.15
	Priming in Ag Nanoparticles	REP 1	1.275	2.575	4.325	3.2	5.125	4.025	0.825	21.35
		REP 2	2.675	2.4	3.05	2.175	2.825	5.275	0.975	19.375
		REP 3	2.8	3.15	4.4	1.95	2.45	4.175	1.075	20

Table 7: Effect of silver nanoparticles on mitotic index together with cells percentage on different phases of mitotic division (Prophase, Metaphase, Anaphase and Telophase) of wheat and barley after 72 hours.

Plant	Treatment	% of dividing cell observation after examined 4000 cells					
		Replicates	Mitotic index	% of cells in prophase	%of cells in metaphase	% of cells in anaphase	%of cells in telophase
Wheat Beni Sweif 1	Priming in H_2O	REP 1	9.7	3.775	1.9	2.225	1.8
		REP 2	9.825	3.95	2.325	1.925	1.625
		REP 3	9.575	3.85	2.175	1.825	1.725
	Priming in Ag Nanoparticles	REP 1	10.325	4.125	1.975	2.325	1.9
		REP 2	10.575	4.375	2.1	2.375	1.725
		REP 3	11.15	4.45	2.175	2.425	2.1
Wheat Gemmieza	Priming in H_2O	REP 1	9.35	3.325	2.45	1.975	1.6
		REP 2	8.125	2.525	2.075	2.175	1.35
		REP 3	7.45	2.275	2.225	1.475	1.475
	Priming in Ag Nanoparticles	REP 1	9.3	3.85	2.275	1.625	1.55
		REP 2	10.3	4.675	2.175	1.775	1.675
		REP 3	9.65	4.425	1.975	1.725	1.525
Barley Giza 130	Priming in H_2O	REP 1	10.15	3.9	2.05	2.8	1.4
		REP 2	9.8	3.2	2.025	2.4	2.175
		REP 3	10.125	3.1	2.45	2.275	2.3
	Priming in Ag Nanoparticles	REP 1	11.25	4.15	2.2	2.45	2.45
		REP 2	11.775	4.275	2.45	2.775	2.275
		REP 3	10.575	3.975	2.3	2.125	2.175

Table 8: Effect of silver nanoparticles on mitotic index together with cells percentage on different phases of mitotic division (Prophase, Metaphase, Anaphase and Telophase) of wheat and barley after 120 hours.

Plant	Treatment	% of dividing cell observation after examined 4000 cells					
		Replicates	Mitotic index	% of cells in prophase	% of cells in metaphase	% of cells in anaphase	% of cells in telophase
Wheat Beni Sweif 1	Priming in H_2O	REP 1	8.5	3	1.775	1.975	1.75
		REP 2	8.025	2.75	2.05	1.75	1.475
		REP 3	7.575	2.55	2.075	1.675	1.275
	Priming in Ag Nanoparticles	REP 1	9.3	2.425	2.9	2.1	1.875
		REP 2	10.3	2.25	3.975	2.05	2.025
		REP 3	9.8	2.825	3.125	2.25	1.6
Wheat Gemmieza 9	Priming in H_2O	REP 1	7.45	2.775	1.775	1.75	1.15
		REP 2	6.925	2.125	1.925	1.65	1.225
		REP 3	6.85	2.2	1.975	1.4	1.275
	Priming in Ag Nanoparticles	REP 1	7.925	2.1	2.3	1.975	1.55
		REP 2	8.7	1.9	2.925	2.125	1.75
		REP 3	8.65	2.025	2.575	2.55	1.5
Barley Giza 130	Priming in H_2O	REP 1	9.125	3.65	2	2.45	1.025
		REP 2	9	2.9	1.9	2.225	1.975
		REP 3	9.625	2.875	2.875	2.2	1.675
	Priming in Ag Nanoparticles	REP 1	11.25	3.025	2.775	2.775	2.675
		REP 2	11	3.125	2.875	2.525	2.475
		REP 3	10.95	2.875	3.025	2.85	2.2

Table 9: Effect of silver nanoparticles on mitotic cell division together with cells in different phases of mitotic division (Prophase, Metaphase, Anaphase and Telophase) of wheat and barley after 72 hours.

Plant	Treatment	Number of cell observation after examined 4000 cells					
		Replicates	Number of cells in mitotic division	Number of cells in prophase	Number of cells in metaphase	Number of cells in anaphase	Number of cells in telophase
Wheat Beni Sweif 1	Priming in H_2O	REP 1	340	120	71	79	70
		REP 2	321	110	82	70	59
		REP 3	303	102	83	67	51
	Priming in Ag Nanoparticles	REP 1	372	97	116	84	75
		REP 2	412	90	159	82	81
		REP 3	392	113	125	90	64
Wheat Gemmieza 9	Priming in H_2O	REP 1	298	111	71	70	46
		REP 2	277	85	77	66	49
		REP 3	274	88	79	56	51
	Priming in Ag Nanoparticles	REP 1	317	84	92	79	62
		REP 2	348	76	117	85	70
		REP 3	346	81	103	102	60
Barley Giza 130	Priming in H_2O	REP 1	365	146	80	98	41
		REP 2	360	116	76	89	79
		REP 3	385	115	115	88	67
	Priming in Ag Nanoparticles	REP 1	450	121	111	111	107
		REP 2	440	125	115	101	99
		REP 3	438	115	121	114	88

Table 10: Effect of silver nanoparticles on mitotic cell division together with cells in different phases of mitotic division (Prophase, Metaphase, Anaphase and Telophase) of wheat and barley after 120 hours.

Plant	Treatment	Number of cell observation after examined 4000 cells					
		Replicates	Number of cells in mitotic division	Number of cells in prophase	Number of cells in metaphase	Number of cells in anaphase	Number of cells in telophase
Wheat Beni Sweif 1	Priming in H_2O	REP 1	388	151	76	89	72
		REP 2	393	158	93	77	65
		REP 3	383	154	87	73	69
	Priming in Ag Nanoparticles	REP 1	413	165	79	93	76
		REP 2	423	175	84	95	69
		REP 3	446	178	87	97	84
Wheat Gemmieza 9	Priming in H_2O	REP 1	374	133	98	79	64
		REP 2	325	101	83	87	54
		REP 3	298	91	89	59	59
	Priming in Ag Nanoparticles	REP 1	372	154	91	65	62
		REP 2	412	187	87	71	67
		REP 3	386	177	79	69	61
Barley Giza 130	Priming in H_2O	REP 1	406	156	82	112	56
		REP 2	392	128	81	96	87
		REP 3	405	124	98	91	92
	Priming in Ag Nanoparticles	REP 1	450	166	88	98	98
		REP 2	471	171	98	111	91
		REP 3	423	159	92	85	87

DISCUSSION

It has been long known that one of the main merits of priming treatments is to increase germination and emergence rate (Heydecker and Coolbear, 1977). The results of the present study showed that the effect of AgNPs seed priming (24h) was significant on germination percentage than the control ones. According to Sadeghi *et al*. (2011) completion of pre-germination metabolic activities during seed priming, making the seed ready for soon germination after planting compared with unprimed seeds. Increased emergence rate due to seed priming may be due to increased rate of cell division in the root tips of seedlings from primed seeds as reported by Farooq *et al*. (2005) in tomato plants. In the present study the 20nm AgNPs priming caused increase in mitotic index, and the appearance of chromosomal aberrations and nuclei deformations. Kumari *et al*. (2009) reported that the impacts of AgNPs impaired the stages of cell division and caused cell disintegration in root tips of onion. Moreover, Patlolla *et al*. (2012) stated that AgNPs exposure significantly increased the number of chromosomal aberrations, micronuclei, and decreased the mitotic index in exposed groups compared to control.

Stampoulis *et al*. (2009) reported that the seed germination and root growth of zucchini plants in hydroponic solution amended with AgNPs showed no negative effects whereas a decrease in plant biomass and transpiration was observed on prolonging their growth in presence of AgNPs. In the present work the shoots fresh and dry weights were both higher than those of the control plants, while roots in the three tested plants showed slight decline compared to the control. Decrease in root length and root biomass could be due to the silver nanoparticles which directly provoked alterations of membranes and other cell structures and molecules, as well as protective mechanisms.

Babu and Prabu (2011) reported that cell walls thickness of about 5 to 20 nm functions as natural sieves which transports small particles passes through large pores to enter in the protoplasm. One of the pathways was reported where particle size of 20 nm silver nanoparticles may be transported inside the cells through plasmodesmata. Additionally Mazumdar and Ahmed (2011) stated that particles must be entered through cell wall and plasma membrane of root cells. Xylem is one of the main passages of uptake and transportations to shoot and leaves of plant. Damaged in intracellular level may be due to surface area or size of particles interacts with plant roots. Adriano (2001) stated that plants are able to take up silver, although this element has no biological functions, the typical level of Ag in plant tissue is <1 ppm. When the ionic form of Ag occurs in low concentrations in the soil, it accumulates evenly throughout the whole plant. At much higher concentrations, Ag accumulation increases in the plant roots, but it is poorly translocated to the shoots **(**Klein *et al*., 1975). Recently Mazumdar (2014) revealed that once silver nanoparticles enter inside the cells, it may cause damage to the vacuoles and cell walls integrity and probably affect other cell organelles too. Also, retardation of growth during seedling stage was due to considerable absorption of silver nanoparticles by the root cells.

It has been reported that Ag is able to displace other cations from electropositive sites located on the cell walls, membranes and DNA molecules. In the present study the limited toxic effects observed in the root tissue are probably due to the ability of the plants to 'block' and store AgNPs at the membrane level. On the other hand Aubert *et al*. (2012) reported that, nanosized individuals, translocated to the upper levels of the plant, resulted in a higher toxicity, as already reported for other metal-based nanoparticles. Kuamri *et al.* (2011) confirmed that there could be uptake of nano-silver through seeds and this could in turn affect germination percent, root length and protein concentration. This could have been due to the entry of nano-silver into the cell and could have caused damage to DNA (Hackenberg *et al*., 2011); or it might have also been due to the inhibition of DNA synthesis at S-phase (Mamta *et al*., 2009). In present work different types of structural chromosomal aberrations and nuclei deformations were observed in the three plants treated with silver nanoparticles. The increase in the induction of structural chromosomal aberrations was detected in pretreated root-tips (72h and 120h germination) compared to the control. Out of all types of aberrations, chromosomes aneuploidy, binucleate cells, deletion chromosomes, deform nuclei, micronuclei, chromosomes fragment, and stickiness chromosomes were the predominant forms of chromosomal aberrations observed. These results were in agreement with those reports of Mamta *et al*. (2009) and Kumari *et al*. (2011).Increases in percentage of aberrations in root meristems indicates genotoxic effects of test chemicals (Smaka-Kincl *et al*.,1996). Number of factors can be contributing to the increased chromosomal aberrations; the most important one is due to the interference of chemicals during DNA repair. Different types of chromosomal aberrations by the chemicals/nanoparticles represent their clastogenicity. The chromatid breaks, which represent the DNA double strand breaks that may not have undergone the G_2 repair. Any such irreversible DNA damages will lead to the chromosomal aberrations (Kihlman and Anderson, 1984).

Recently, Kumar (2014) reported that the significant toxic effect of metal oxides nanoparticles was observed on growth parameters like germination percentage, root length, shoot length and vigor index of maize plants. Results of the present work showed that the effect of silver nanoparticles on photosynthetic pigments content (chlorophyll a and b, total chlorophyll and carotenoids) of wheat and barley showed significantly increase above control as shown in Figure 4.

These results are confirmed by results obtained from other study (Karthick and Chitrakala, 2011), they demonstrated that chlorophyll a content was significantly increased by Ag nanoparticles in green gram and sorghum plants. In other study (Racuciu *et al*., 2009) response of *Cucurbita pepo* seedlings exhibited a slight increase of chlorophyll contents. It has been shown that a parameter derived from chlorophyll fluorescence, the ratio of variable/maximum fluorescence, Fv/Fm, is a quantitative measure of the photochemical efficiency of photosystem II (Maxwell and Johnson, 2000).Results of the present work showed slight increase in Fv/ Fm values in the three plants treated with AgNPs compared with the control, the Fv/Fm values were ranged from 0.7 and 0.8. Bolhàr-Nordenkampf *et al*. (1989) reported that when the plant keeps its photosynthetic complex intact, the ratio Fv/Fm must vary between 0.75 and 0.85 while a decrease in this ratio indicates the presence of photoinhibition in the reaction centers of PSII.

CONCLUSION

The present study demonstrated the effect of silver nanoparticles on crop plant species wheat " Beni Sweif 1 ", " Gemmieza 9", and barley "Giza 130". Silver nanoparticles could penetrate plant systems and might interfere with intracellular components, attributed the accumulation and uptake of AgNPs by the roots otherwise translocation to the shoot system impairing various stages of the cell division causing chromosomal aberrations. AgNPs influenced growth parameters as well as photosynthetic pigments and chlorophyll fluorescence.

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