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Impact of ecofriendly synthesized silver nanoparticles on yield parameters and molecular traits of pea (*Pisum sativum* L.)

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ABSTRACT

Pea (*Pisum sativum* L. cv Master B) plants were grown under normal conditions and regularly irrigated or sprayed, once a week, with 20, 40, 80, and 160 mg/L of ecofriendly prepared AgNPs solutions and tap water, as control, after two weeks from sowing until harvesting. Yield parameters (number of mature pods per plant, length of pods, and number of seeds per pod and weight of 100 seeds) were recorded for the first and second generations (M1 & M2). Seed protein SDS-PAGE (Sodium dodecyl sulphate-polyacrylamide gel electrophoresis) profiling of M2 seeds and ISSR (Inter-simple sequence repeats) profile of the M2 seedlings have been analyzed. The results showed that most yield parameters of M1 were increased but decreased in M2. The weight of 100 seeds which is the major indicator of yield productivity was enhanced in both generations. Seed protein profiling showed slight variations among the applied treatments as compared to the control. The concentration of 20 mg/L AgNPs solution showed more variations in the ISSR profile than other treatments. This study suggested that the genotoxic effect of AgNPs on parent pea plants (plants irrigated or sprayed) transmitted to the next generations and genetic variation can be induced using low concentration of AgNPs that may be useful in plant pre-breeding. However, further studies are recommended to fully understand the toxicity of AgNPs to plants.
Keywords: Crop yield; ISSR fingerprinting; Nanoparticles; Pea; Protein profile.

INTRODUCTION

In recent decades, the use of chemicals with anthropogenic activities and nanoparticles has been growing with great benefits for food production, human health and welfare. Furthermore, future agricultural practices are expected to use nanomaterials more frequently, exposing people and the environment to them on a larger scale (Devra, 2022). However, it is apparent from many reports that nanoparticles have various effects on plants, according to the properties of the nanomaterials, plant system used and techniques (Ali *et al*., 2021; Badr *et al*., 2021). Application of Fe_3O_4 (iron oxide nanoparticles) to leaves of *Ocimum basilicum* increased total carbohydrate and chlorophyll, oil levels, iron content, number of branches and leaves per plant, fresh and dry weights, and height of plants (El-Feky *et al*., 2013). Hernández *et al*. (2019) reported that Se NPs (selenium nanoparticles) enhanced the yield of tomato plants up to 21% and Wasaya *et al*. (2020) showed that a combination of foliar application of Zn and Ag nanoparticles improved growth and increased yield of *Vigna radiata*. Nano fertilizers can promote vegetative growth, pollination, and maturity, leading to increased production and improved product for vegetables and fruit trees (Rana *et al*., 2021), engineered nanoparticles affect plants at various growth stages by interventions, at the physiological and biochemical level (Rawat, 2021; Xalxo *et al*., 2021). Nanomaterials were also reported to cause variations in growth manner of plant shoot and root (Bhaskaran and Sahi, 2021) and can decrease food contamination during food processing and packaging in post-harvest of plants (Ali *et al*., 2021; Kale *et al*., 2021).

Nanoparticles are made of metals either by constructive or subversive methods (Salavati-niasari *et al*., 2008). The most important nanoparticles are silver nanoparticles (AgNPs) based on their strong uses in different fields of life. Elongation of root of *Eruca sativa* were stimulated and alteration in some proteins related to vacuoles and endoplasmic reticulum were indicated by treatment with 10-20 mg/L AgNPs (Vannini *et al*., 2013). The AgNPs also caused negative effect on germination, early growth and cell division and induced different chromosomal abnormalities in the roots of pea plants exposed to different doses at previous study of Labeeb *et al*. (2020). Lower concentrations of AgNPs stimulated the growth parameters of *Triticum aestivum* and banana (Yang *et al*., 2018; El-Mahdy *et al*., 2019) but higher concentrations of AgNPs reduced these growth parameters. Hasan *et al*. (2021) reported an inhibitory effect on seedling growth of lettuce at high concentrations of AgNPs but moderate concentrations of AgNPs induced a stimulatory effect.

Yield of many plants was affected by nanoparticles where nano-iron oxide increased some yield traits of soybean (Sheykhbaglou *et al*., 2010) and spraying plants of *Borage officinalis* and fenugreek with AgNPs improved yield (Seifsahandi *et al*., 2011; Sadak, 2019, respectively). Also, Razzaq *et al*. (2016) reported that irrigating wheat plants with low concentrations of AgNPs enhanced crop growth and yield, while higher concentrations caused negative effect on both yield parameters. Similar results were induced in pea (Mehmood and Murtaza, 2017). There are some studies showing positive effects of the nanoparticles on the metabolism of the next generations following exposure of nanoparticles application. Jangir *et al*. (2020) reported that nanopyrite (FeS_2) seed pre-treatment of wheat increased not only grain yield but also germination percentage of the second-generation seeds. On the other hand, CeO_2-NPs (cerium oxide nanoparticles) affected the seed quality and the seedlings growth of the second generation of tomato plants (Wang *et al*., 2013) and caused a reduction in seed production and seed quality of *Brassica rapa* over multigenerational exposures (Ma *et al*., 2016). AgNPs could delay flowering and decrease growth and yield in *Arabidopsis thaliana* (Ke *et al*., 2018) and these negative effects transferred to the offspring (Ke *et al*., 2020) and reduced germination rate of offspring over three generations (Geisler-Lee *et al*., 2014).

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Biochemical and molecular markers have been increasingly used in genetic diversity and plant breeding research. Protein electrophoresis is a rapid method for characterizing and comparing proteins (Bollag and Edelstein, 1993). Numerous DNA markers have been developed and applied in plant genetic diversity research (Bhandari *et al*., 2017). A biomarker for examining the genotoxic impact of contaminants on plants is DNA fingerprinting (Badr *et al*., 2021). The inter simple sequence repeats developed by Bornet *et al*. (2001) involve amplification of genomic segments flanked by inversely oriented and closely spaced microsatellite sequences by a single primer or a pair of primers based on SSRs (simple sequence repeats) anchored 5' or 3' with 1-4 purine or pyrimidine residues. The distinctions of genetic resources, cultivar characterization, and marker-assisted breeding programmes have all used the ISSR markers polymorphism. Examples include the genetic diversity among chosen *Medicago sativa* cultivars combined with DNA bar-coding (Badr *et al*., 2020), the fingerprinting of the forage legume types alfalfa and Egyptian clover (Bondok, 2019), and variations and hybrid lines of pea (Badr *et al*., 2015). In a previous study (Labeeb *et al*., 2022), considerable variations were recorded in the ISSR fingerprinting indicating point mutations induced by different treatments of AgNPs in pea (*Pisum sativum*). ISSR marker also recorded variation following exposure of *Chrysanthemum* AgNPs-treated-plants (Tymoszuk and Kulus, 2020). Therefore, the main objective of this study is to determine the impacts of various ecofriendly silver nanoparticle treatments on a number of yield traits of two generations (M1 and M2) of pea plants treated with AgNPs, as well as the protein profile in M2 seeds using SDS-PAGE and ISSR fingerprinting in M2 pea seedlings.

MATERIALS AND METHODS

Silver nanoparticles preparation

In this study, silver nanoparticles were ecofriendly synthesized as described in previous study done by Labeeb *et al*. (2020).

Plant material

Seeds of pea *(Pisum sativum* L. cv Master B) were kindly provided by the Horticultural Department, Faculty of Agriculture, Kafrelsheikh University, Kafrelsheikh city, Egypt. The seeds were sterilized by soaking in 5% sodium hypochlorite for 10 min, and then rinsed three times in sterilized distilled water. Four pots (24 cm × 24 cm) were used for each treatment; seven seeds were sown in each pot. Soil composed of clay, sand and peat moss in a ratio of 2:1:1, respectively. Two weeks after germination, the pots were split into two groups with Group 1 receiving weekly irrigations of 20, 40, 80, and 160 mg/L of AgNPs solutions. At the same time as group 1 was being irrigated, group 2 was sprayed with the identical solutions. Tap water was used to irrigate sprayed and control plants simultaneously. At maturity, some yield parameters (number of mature pods per plant, length of the pod, number of seeds per pod, the weight of 100 seeds) were recorded. Seeds of the first generation (M1) were collected, sterilized and grow to maturity. Yield components of the second generation (M2) were recorded.

For ISSR- PCR study, seeds of the second generation were soaked in distilled water for two hours, after sterilization, germinated on moistened filter papers in sterilized Petri-dishes and supplied with distilled water, germinated in the dark until emergence of seedling then grown under normal conditions at 22±1°C for fourteen days. Plants were watered regularly for 14 days. Young leaves were collected for further studies.

Protein extraction and SDS-PAGE

Seeds of M2 generation were ground and total protein was extracted in 0.125 M Tris/borate buffer pH 8.9. The extracts were centrifuged at 10000 g for 20 min and supernatants were stored at -20ºC until use. Extracts were denatured before being loaded on acrylamide gel by heating at 100ºC with 5% β-mercaptoethanol for 5 min. The SDS-PAGE of protein was performed using 12.5% (w/v) polyacrylamide gel (Laemmli, 1970).

DNA extraction and amplification of ISSR-PCR products

Genomic DNA was extracted and purified from young leaves of 14 days M2 pea seedlings using CTAB (cetyltrimethyl ammonium bromide) method developed by Rogers and Bendish (1985). The amplification of ISSRs was carried out using 18 ISSR primers (Table 1). The amplification reactions were done in Primus 25 advanced® cycler machine in 20 µl reaction volume containing 1µM of the primer, 2 µl genomic DNA (20 ng), 10 µl Dream Taq Green PCR Master MIX (Thermo Fisher Scientific, Inc.) and 7 µl dd.H_2O. The PCR reactions were done by initial denaturation at 95°C for 5 min, 40 cycles of denaturation at 95°C for 1 min, annealing at 45°C for 40 sec, extension at 72°C for 1 min and a final extension at 72°C for 5 min. DNA was visualized by loading 10 µl from the PCR products on 1.6% agarose and electrophoresis in TBE (Tris/-Borate/EDTA) buffer with ethidium bromide at 100 V for 1 hr and photographed by the Gel Documentation system (WiseDoc®, WGD-30, DATHAN Scientific, Co., Ltd.).

Data analysis

The data were statistically analyzed using one way ANOVA and the experimental values were compared to the control and expressed as mean values ± SE by Graph-Pad prism version 9.1.0.(221). Protein and DNA-ISSR bands size and polymorphism were determined by Lab-image software version 7.1.3 (Kapelan, 2019). In binary matrices, bands were scored as 1 for presence and 0 for absence. Similarity between plants exposed to various Ag NP concentrations was calculated using Dice coefficient of similarity (Dice, 1945) using the NTSYS-pc software version 2.02 (Rohlf, 2002). Making of distance trees for protein and DNA-ISSR data explaining the distance among the studied treatments achieved using the unweighted pair group way by the arithmetic average (UPGMA) (Sokal and Mickener, 1958) as applied in the NT-SYS-pc.

RESULTS

Yield traits of M1 from parents treated with AgNPs

In irrigated plants (Table 2), the number of pods/plant was significantly decreased ($p \leq 0.05$) at the highest AgNP concentrations (80 and 160 mg/L) with values of 1.46 ±0.15 and 1.69 ±0.21, respectively, in comparison to the

Table (1): List of 18 ISSR primers used in the current study and their sequences.

No	ISSR primers	Sequences (bp)	No.	ISSR primers	Sequences (bp)
1	UBC 807	$(AG)_8T$	**10**	UBC 842	$(GA)_8YG$
2	UBC 810	$(GA)_8T$	**11**	UBC 844	$(CT)_8RC$
3	UBC 811	$(GA)_8C$	**12**	844 A	$(CT)_8AC$
4	UBC 825	$(AC)_8T$	**13**	UBC 845	$(CT)_8RG$
5	UBC 834	$(AG)_8YT$	**14**	UBC889	$DBD(AC)_7$
6	UBC 835	$(AG)_8YC$	**15**	UBC 898	$(CA)_6RY$
7	UBC 836	$(AG)_8YA$	**16**	HB 11	$(GT)_6CC$
8	UBC 840	$(GA)_8YT$	**17**	M1	$(AC)_8CG$
9	UBC 841	$(GA)_8YC$	**18**	M13	$(AGC)_4Y$

control (2.60 ±0.37). Meanwhile, a non-significant increase in pod length was observed for all treatments, with the exception of 160 mg/L for irrigated plants (6.16±0.27 cm) compared to controls (6.47±0.22 cm). The number of seeds per pod was significantly high at 80 mg/L for irrigated plants and 40 mg/L in sprayed plants (4.42±0.22 and 4.00±0.37, respectively) compared to controlled plant (2.78±0.14). In a similar manner, all irrigated plants showed significant ($p \leq 0.01$) increase in weights of 100 seeds/pod, whereas sprayed plants displayed the highest weights at AgNP concentration of 40 mg/L with value of 16.35±0.47 gm compared to control (11.56±0.24 gm).

Yield traits of M2 pea plants

The number of mature pods/plant (Table 3) was comparable in the control and in all treatments, with the exception of 20 mg/L of AgNPs for irrigated plants, which showed a significant increase (1.67±0.33 pods/plant). Meanwhile, the mean length of the pod showed a decrease in M2, it was not statistically significant, with the exception of the treatment with 20 mg/L for sprayed plants in which the mean length was slightly increased (6.56±0.29 cm) in comparison to the control (6.35±0.96 cm). By increasing concentrations of AgNPs-irrigated plants, there is a significant decrease in the number of seeds per pod of M2. With the exception of the high dosage of 160 mg/L, the average weight of 100 seeds from M2 pea plants increased. The concentration of AgNPs at 40 mg/L resulted in the highest significant weight recording15.61 15.61±0.31 gm for M2 plants and concentration of 20 mg/L for sprayed plants recording 14.18±0.46 gm compared to control (11.71±0.18 gm).

SDS-PAGE protein pattern in M2 pea seeds

SDS-PAGE electrophoretic banding pattern of seed proteins for the M2 generation of pea treated previously with the different concentrations of AgNPs solutions is illustrated in Table (4, supplementary) and Figure (1). Protein profiles showed variations in the number of bands, thickness, and their intensity depending on the concentration of AgNPs solution when compared with the control. In total, 32 bands were observed ranging from 190.32 to 12.22 KDa. Three bands with molecular weights of 85.20, 60.66 and 57.18 KDa disappeared in M2 seeds previously irrigated with the highest concentration (160 mg/L) and were expressed in other treatments and control.

A new band of 81.14 KDa formed when M2 seedlings were previously irrigated with 20, 40, or 80 mg/L of AgNPsA. This band wasn't present in either the control or the other treatments. A second new band with a molecular weight of 16.34 KDa appeared in M2 seedlings that had previously been irrigated with 20 mg/L of water or sprayed with 20 and 40 mg/L of water. Neither the control nor the other treatments had this band. In the meantime, a band with a molecular weight of 12.22 KDa was detected in the control and seeds that had previously been irrigated with 40 and 80 mg/L AgNPs solutions, but it disappeared from seeds that had received other treatments. Electrophoretic data were used for cluster analysis (Fig. 2) using the arithmetic average (UPGMA) method. The dendrogram grouped M2 seeds previously irrigated by 20 mg/L and sprayed with 20 and 40 mg/L AgNPs in one group and separated the highest concentration 160 mg/l.

ISSR Fingerprinting in M2 pea seedlings

A total of 110 bands including 56 polymorphic and 54 monomorphic markers were produced by 16 of the 18 tested ISSR primers in seedlings of the second-generation

Table (2): Effects of AgNPs on yield traits of pea plants (M1) treated with different concentrations of AgNPs solutions.

Treatments†			Measured parameters			
			Number of mature pods plant^{-1}	Pod length (cm)	seed numbers pod^{-1}	Weight of 100 seeds (gm)
Control			2.60 ±0.37	6.47 ±0.22	2.78 ±0.14	11.56 ±0.24
Treated plant with AgNps (mg L^{-1})	**Irrigated plants**	**T1**	2.33 ±0.28	6.62 ±0.26	3.56 ±0.41	13.91 ±0.30***
		T2	2.38 ±0.261	6.72 ±0.23	3.40 ±0.22	13.43 ±0.33**
		T3	1.46 ±0.15*	6.66 ±0.22	4.42 ±0.22***	15.34 ±0.25***
		T4	1.69 ±0.21*	6.16 ±0.27	3.40 ±0.26	14.80 ±0.55***
	Sprayed plants	**T1**	2.17 ±0.16	6.67 ±0.15	2.50 ±0.18	10.23 ±0.12*
		T2	2.09 ±0.16	6.47 ±0.17	4.00 ±0.37*	16.35 ±0.47***
		T3	2.50 ±0.31	6.93 ±0.28	3.55 ±0.24	11.77 ±0.29
		T4	2.36 ±0.20	6.97 ±0.22	3.69 ±0.34	11.73 ±0.31

†Treatment with AgNps at different concentrations (mg/L):T1, 20; T2, 40; T3, 80 and T4, 160. Data per column with asterisks are significantly different at level $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ for *, ** and ***, respectively.

Table (3): Effects of silver nanoparticles on yield traits (M2) of pea plants previously treated with different concentrations of Ag NPs solutions.

Treatments†			Measured parameters			
			Number of mature pods/ plant	Pod length (cm)	seed numbers pod^{-1}	Weight of 100 seeds (gm)
Control			1.00±0.00	6.35±0.96	4.67±0.33	11.71±0.18
Treated plant with AgNps (mg L^{-1})	Irrigated plants	T1	1.67±0.33*	5.53±0.37	4.00±0.25	13.53±0.73
		T2	1.00±0.00	5.50±0.35	1.750±0.47**	15.61±0.31***
		T3	1.00±0.00	4.80±0.87	2.00±0.57**	11.76±0.53
		T4	1.00±0.00	4.67±0.44	2.00±0.58**	11.68±0.57
	Sprayed plants	T1	1.00±0.00	6.56±0.29	4.00±0.57	14.18±0.46**
		T2	1.00±0.00	6.30±0.367	3.50±0.42	13.51±0.51*

†Treatment with AgNps at different concentrations (mg/L):T1, 20; T2, 40; T3, 80 and T4, 160. Data, per column with asterisks, are significantly different at level $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ for *, ** and ***, respectively.

and recorded 50.90 % polymorphism (Figure 3). Four ISSR fingerprinting profiles produced by 4 primers are illustrated, as examples, in Figure. (4). The highest percentage of polymorphism (81.82 %) was recorded by primer UBC 840 with bands size range of 257 - 1135 bp. New ISSR markers were induced in the M2 plants that were absent in the control. These include a band amplified by primer UBC 834 with a size of 718 bp and two bands by primer UBC 836 with size of 636 bp and 819 bp. On the other hand, bands present in the control are absent in the ISSR profiles of M2 plants, as primer UBC807 with a size of 777 bp, two bands by UBC 811

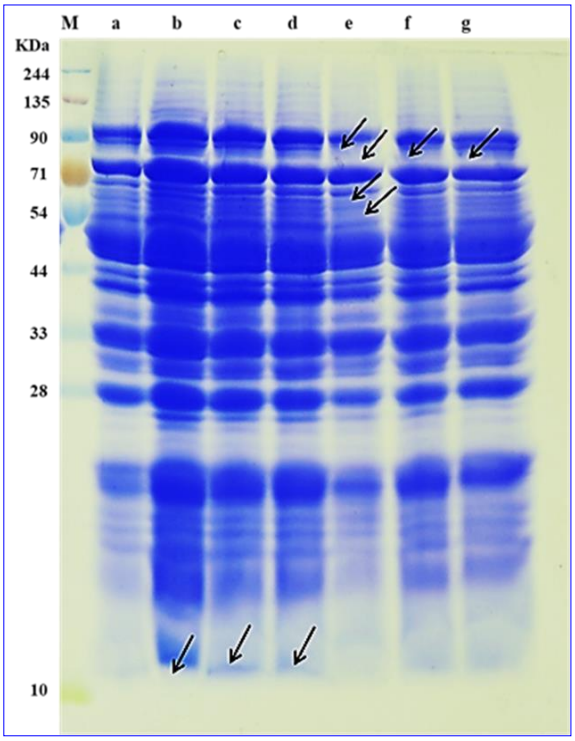


Figure (1): Protein banding pattern by SDS-PAGE analysis of pea M2 seeds previously irrigated with different concentrations of AgNPs (mg/L): a, 0; b, 20; c, 40; d, 80 and e, 160; f-g, sprayed plants with 20 and 40 mg/L of AgNPs solutions, respectively. M = Standard protein marker.

with size of 693 bp and 952 bp and one small band (290 bp) by primer UBC 835.

Markers produced by some primers such as UBC 840 with size of 438 bp and UBC 841 with size of 284 bp were absent in M2 plants treated previously with high concentrations of AgNPs solutions. Other bands that were absent in ISSR profile of plants previously exposed to low concentrations of AgNPs were recorded at high concentrations such as bands amplified by primer UBC 840 with size of 345, 397, 518, 564, 588, 920 and 1135 bp. and two bands by primer M 13 with size of 633 bp and 878 bp.

The effects of AgNPs treatments on ISSR fingerprinting of M2 of pea plants previously irrigated with 0, 20, 40, 80 and 160 mg/L of AgNPs solutions and sprayed with 20 mg/L and 40 mg/L AgNPs solutions showing the presence (a), and absence (b) of bands compared to control are illustrated in Figure **(**5, supplementary). The highest number of newly bands (12 bands) was recorded in the ISSR profile of M2 seedlings previously irrigated by 20 mg/L AgNPs, while those previously sprayed with 20 mg/L recorded the highest number of absent bands (31 bands). So, the concentration 20 mg/L resulted in more variations in the ISSR profile than other treatments. A cluster analysis constructed using the arithmetic average (UPGMA) shows the distinction of M2 plants previously irrigated by 20 mg/L AgNPs from control and other samples and to some extend M2 plants previously sprayed with 20 m/L (Fig. 6).

DISCUSSION

In the current study, yield data of M1 plants either irrigated or sprayed with AgNPs solutions generally, increased for all parameters studied, except number of pods per plant which showed non-significant reduction in both irrigated and sprayed plants compared to control. The changes in the number of seeds per pod and weight of 100 seeds are consistent with the results recorded by Mehmood and Murtaza (2017) on *Pisum sativum* and Sadak (2019) in

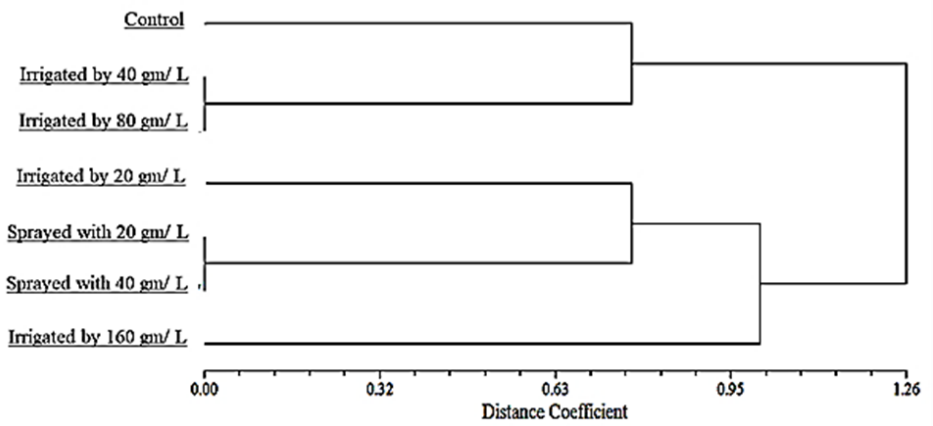


Figure (2): Dendrogram of pea M2 seeds previously irrigated or sprayed with different concentrations of AgNPs solutions and control based on SDS-PAGE protein analysis.

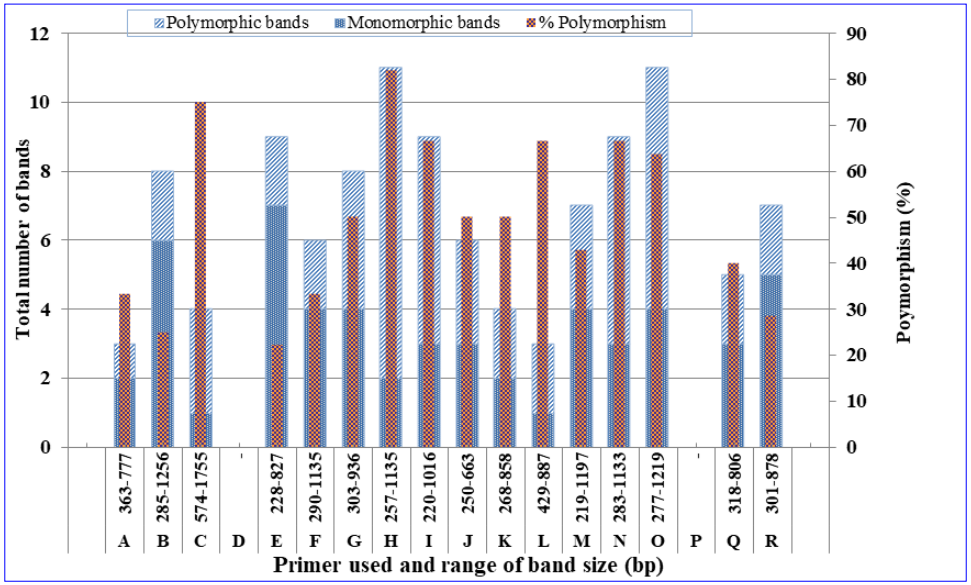
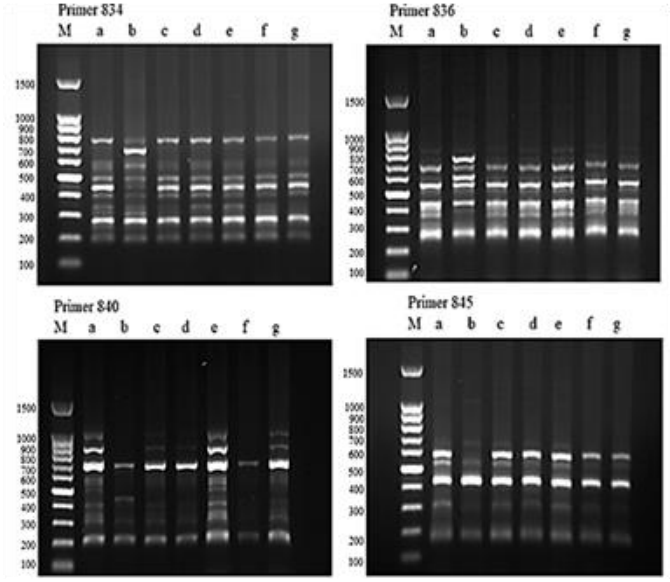


Figure (3): Bands generated by 16 of 18 ISSR primers, with ranged size, showing monomorphic and polymorphic bands and the percentage of polymorphism of pea M2 seedlings grown from seeds previously treated with the AgNPs concentrations. Primer used: A, UBC 807; B, UBC 810; C, UBC 811; D, UBC 825; E, UBC 834; F, UBC 835; G, UBC 836; H, UBC 840; I. UBC 841; J. UBC 842 ; K, UBC 844; L, 844 A; M, UBC 845; N, UBC889; O, UBC 898; P, HB 11; Q, M1 and R, M13.



Figure(4): ISSR profile of pea M2 seedlings previously irrigated by different concentrations of AgNPs (mg/L): a, 0; b, 20; c, 40; d, 80 and e, 160. f-g, sprayed with 20 mg/L and 40 mg/L AgNPs solutions .M, Standard DNA molecular size marker (Expressed in bp).

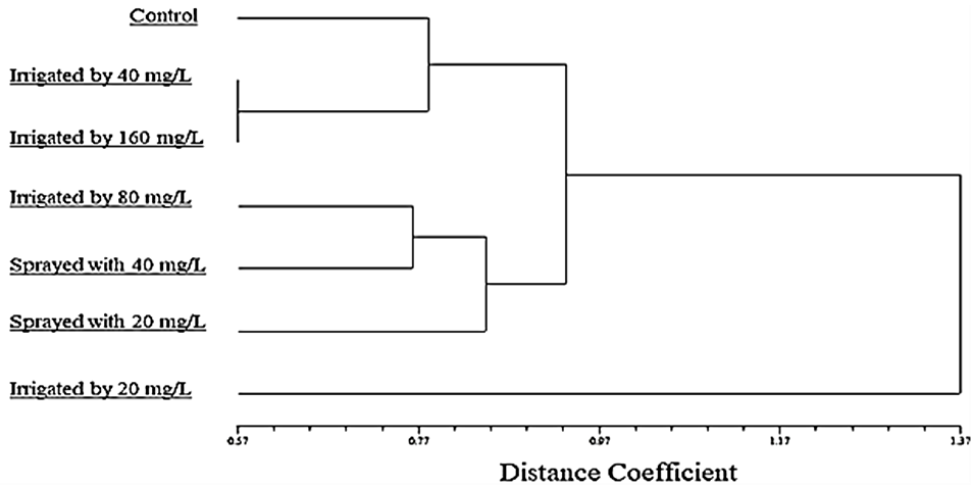


Figure (6): Dendrogram illustrating differential cluster of M2 pea seedlings, previously irrigated with AgNPs solutions at different concentrations (mg/L). :0, 40, 80 and 160 verses to sprayed with 20 mg/L and 40 mg/L AgNPs solutions, based on ISSR fingerprinting

his study on *Trigonella foenumgraecum*. The M1 plants sprayed with 40 mg/L AgNPs, which was the most effective treatment in affecting the number of seeds per pod and weight of 100 seeds per plant agree with the results of Sadak (2019). Spraying plants of *Borage officinalis* with AgNPs improved yield by increasing concentration of AgNPs from 20 ppm to 60 ppm (Seifsahandi *et al*., 2011). Also, Razzaq *et al*. (2016) reported that AgNPs in soil increased the number of grains per spike and the 100-grain weight at 25 and 50 ppm AgNPs treatments.

Yield increase might have been attributed to several physiological processes which enhance plant growth and flowering. These may include increasing growth parameters such as, photosynthetic pigments and IAA (Indole-3-Acetic Acid) as suggested by (Sadak, 2019). In this respect, Wagi and Ahmed (2019) proposed that nanoparticles might interact with plant hormones and antioxidants and promote plant growth. They also suggested that AgNPs promotes root exudates production that may facilitate plant microbes' interactions which in turn improve plant growth. Increasing yield might also be through blocking the action of ethylene in responses like senescence, abscission, and growth retardation (Beyer, 1976). These views are congruent with the view of Sharon *et al*. (2010) that AgNPs enhance crop yield because silver is considered as an excellent growth stimulator for plants.

Silver nanoparticles appear to have a specific effect on increasing crops yield. On the other hand, AgNPs showed phytotoxicity to wheat resulting in shorter plant height, and lower grain weight as well as the quality of wheat grain traits following exposure in a life cycle study (Yang *et al*., 2018). Ke *et al*. (2018) noticed late flowering and growth and yield reduction of offspring in *Arabidopsis thaliana* from parents treated with AgNPs and Ke *et al*. (2020) recorded negative effects on floral development. Reproductive toxicity was also recorded in a life history study on the uptake and transport of AgNPs in *Arabidopsis thaliana (*Geisler-Lee *et al*., 2014). Saleeb *et al*. (2019) reported that plants can take and accumulate silver in roots and shoots and this in turn affects crop health and yield. Yield of plants is affected by other types of nanoparticles. For example, nano-iron oxide at the concentration of 0.75 g/L increased leaf and pod dry weight of soybean and at 0.5 g/L increased grain yield but other measured traits were not affected (Sheykhbaglou *et al*., 2010). Singh and Kumar (2018) reported radish plants treated with CuO and ZnO NPs (copper oxide and zinc oxide nanoparticles) produced smaller M1 seed weight and reduced some growth parameters in M1 seedlings.

The impact of AgNPs on the second-generation plants was carried out through yield components and DNA-ISSR fingerprinting. The present results showed reduction in the number of pods per plant and length of pod for all treatments except 20 mg/L AgNPs. The number of seeds per pod decreased at high concentrations significantly and non-significantly at low concentrations but the weight of 100 seeds which is the major indicator of yield productivity significantly increased at all treatments except the of high concentration 160 mg/L compared to control. It is obvious that there are negative effects of silver nanoparticles on the second-generation seed production especially the M2 treated with high concentrations of AgNPs. Geisler-Lee *et al*. (2014) recorded reduction in germination rate of offspring over three generations in *Arabidopsis thaliana* treated with AgNPs but no significant effects were recorded on in floral development. Nanopyrite wheat seeds pre-treatment induced enhancement in seed vigor and germination capacity of the second-generation seeds (Jangir *et al*., 2020). Greater reductions in plant growth, seed quality and seed production over multigenerational exposures in *Brassica rapa* (Ma *et al*., 2016) and treated second generation seedlings with CeO2-NPs were smaller and weaker than control in tomato (Wang *et al*., 2013). The previous study of Labeeb *et al*. (2022) indicated considerable variations in genomic DNA that may affect the physiology and development of offspring plants. Thus, the changes in the M2 generations plants may be attributed to the genetic diversity created by the AgNPs exposure of the parent plants.

SDS-PAGE profile for M2 pea seeds proteins from treated seeds showed slight variations. The highest treatment of 160 mg/L showed absence of many bands compared to the control and other treatments. Vannini *et al*. (2013) reported that AgNPs induced alteration of some proteins in *Eruca sativa* related to the endoplasmic reticulum and vacuole suggesting that they may be targets of the AgNPs action. When released into the environment, silver nanoparticles have the potential to oxidise and change into ionic form and plants can consume silver in both its particulate and ionic forms, which can alter the expression of genes and proteins involved in membrane transport and oxidative response (Noori *et al*., 2021). When compared to other forms of silver, the ionic form of silver had the greatest impact on the expression of genes and proteins. Cluster analysis using the UPGMA method of the protein electrophoretic data grouped M2 plants previously irrigated by 20 mg/L and sprayed with 20 and 40 mg/L AgNPs in one group and separated the highest concentration 160 mg/L from the other treatments and the control.

The results also showed considerable variations in the profile of DNA-ISSR fingerprinting which proved the genotoxic effect of AgNPs on parent pea plants (plants irrigated or sprayed) and their transmission to the next generations in agreement with Ke *et al*. (2020) who recorded the negative effects of AgNPs on floral development transferred to the offspring in *Arabidopsis thaliana*. After cultivating tobacco plants for 8 weeks in soil contaminated with AgNPs of various sizes and concentrations, DNA-damaging effects were noted (Lovecká *et al.,* 2021). The variability between the samples caused by the presence or lack of DNA loci may be the result of point mutations caused by the interaction of AgNPs with the phosphorus in the DNA

molecule, which may have damaged the DNA (Li *et al*., 2013).

Additionally, Bello-Bello *et al*. (2018) concluded that exposure to AgNPs enhances ISSR polymorphism, which may be helpful in promoting *Vanilla planifolia*'s genetic diversity. As was previously stated, NPs have the potential to harm DNA directly or indirectly through the production of reactive oxygen species (ROS) and the creation of oxidative stress. The rise in ROS, which increases genomic DNA damage by inducing point mutations, may be the cause of the changes in ISSR profiles, which result in ISSR polymorphism. The highest number of new bands (12 bands) was recorded in M2 seedlings previously irri-gated with 20 mg/L AgNPs, while those previously sprayed with the same concentration recorded the highest number of absent bands (31). Thus, the concentration 20 mg/L results in more variations in the ISSR profile of the M2 seedlings than other treatments which is consistent with Tymoszuk and Kulus (2020) in *Chrysanthemum* plants treated with 20 mg/L AgNPs, where gain or loss of loci was observed using ISSR markers.

The cluster analysis constructed using the UPGMA method showed the distinction of M2 seedlings previously irrigated by 20 mg/L AgNPs from control and other samples and to some extend M2 seedlings previously sprayed with 20 mg/L. These results in M2 seedlings are consistent with data of previous study of Labeeb *et al*. (2022) during germination of pea seeds for two weeks in AgNPs solutions. Thus, the current study may confirm the use of low concentration of AgNPs (20 mg/L) to induce genetic variation that may be used in plant pre-breeding to induce mutations where Tymoszuk and Kulus (2022) reported silver nanoparticles (AgNPs) could be used as a mutagen in *chrysanthemum* breeding.

CONCLUSION

In conclusion, low AgNPs concentrations have a significant positive impact on pea yield in first-generation plants (M1) than they do on second-generation plants (M2) when high concentrations are applied to produce seed. The protein profiles of M2 plant seeds varied somewhat across treatments compared to controls, however low AgNPs concentrations in the ISSR fingerprinting of M2 plants induced significant differences. This study showed that the genotoxic effects of AgNPs on irrigated or sprayed, parent pea plants were passed down to the next generations, and it somewhat suggested using low concentrations of AgNPs (20 mg/L AgNPs) to create genetic variation that may be useful in plant pre-breeding. To trace the effects of nanomaterials on succeeding generations and to ascertain their long-term effects on plants, more research is necessary.

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تأثير جسيمات الفضة النانونية على الإنتاجية و السمات الجزيئية لنبات البسلة

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الملخص العربـــــي

تمت زراعة نبات البسلة في ظروف طبيعية، وتم معالجتها بتركيزات مختلفة من جسيمات الفضة النانونية (20 و 40 و 80 و 160 ملجم / لتر) سواء بالري أو بالرش بانتظام كل أسبوع بعد أسبوعين من الزراعة حتى الحصاد، وتم استخدام ماء الصنبور للمجموعة الضابطة. كذلك تم تسجيل معاملات المحصول (عدد القرون الناضجة لكل نبات، طول القرون، عدد البذور لكل قرن ووزن 100 بذرة) لنباتات الجيل الأول (M1) والجيل الثاني (M2). تم دراسة تأثير جسيمات الفضة النانونية على البروتين لبذور البسلة ومعرفة تأثيرها أيضا على DNA باستخدام تقنية مابين التكرارات البسيطة المتكررة ISSR للبادرات في الجيل الثاني. أظهرت النتائج أن معظم معاملات محصول M1 قد زادت، ولكنها انخفضت في M2. تم تسجيل زيادة في وزن 100 بذرة في كلا الجيلين والذي يعدّ مؤشرا رئيسيا لزيادة الإنتاجية في النبات. أظهر وصف البروتين في البذور اختلافات طفيفة بين المعاملات المختلفة مقارنة بالمجموعة الضابطة. كما طرأت تغيرات على ال DNA باستخدام تقنية مابين التكرارات البسيطة المتكررة، وأظهر تركيز 20 ملجم / لتر اختلافات أكثر من المعالجات الأخرى. قد تشير هذه الدراسة إلى أنه يمكن إحداث التباين الجيني باستخدام تركيز منخفض من جسيمات الفضة النانونية. ومع ذلك، يوصى بإجراء مزيد من الدراسات لفهم التأثيرات السامة لجسيمات الفضة النانونية على النبات.