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Full Length Article

Impact of Biogenic Silver Nanoparticles on Some Physiological Attributes, Mitotic Index and Chromosomal Abnormalities of Wheat (*Triticum asetivum*) under Salt Stress

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Abstract

The present study was concerned with the biosynthesis of silver nanoparticles (AgNPs) via *Chrysthanthemum cornarium* leaf aqueous extract. Characterizations of prepared AgNPs were described using UV-Vis spectrophotometer, energy dispersive X-ray spectroscopy (EDX), scanning and transmission electron microscope (SEM and TEM). The biogenic AgNPs were spherical in shape with an average size ranging between 15–30 nm. The study also conducted to evaluate the effects of different concentrations (20, 40 and 80 mg L^{-1}) of the bio-based AgNPs as a priming agent (12 h) on germination, growth biomarkers, physiological attributes, cell activity and chromosome behavior of *Triticum aestivum* L. under salinity condition (150 mM). Results showed that 20 mg L^{-1} AgNPs without salt stress has insignificant effect, while the high concentrations of AgNPs-pretreatment significantly decreased germination percentage (GP), shoots and roots lengths and dry weights, as well as the photosynthetic pigments and the quantum yield of PSII **(**Fv/Fm) either with or without the existence of salt stress. Moreover, they significantly inhibited root meristems activity perceived by the mitotic index (MI) and induced various types of chromosomal aberrations such as c-metaphase, chromosomal bridges, sticky chromosomes, lagging chromosomes, chromosome fragments, disturbed anaphase and multipolar anaphase, as well, rare abnormalities for instance precocious chromosomes, abnormal orientation, multipolar, disturbance and multi groups were detected. In conclusion, the interactive effect of salinity and AgNPs was synergistic, implying that AgNPs caused toxicity to meristematic root cells, which can readily internalize AgNPs leading to interference with the normal cell functions, and reduction in wheat seedling growth.

Key words: Aberrations; Cytogenetic; Nanoparticles; Photosynthetic pigments; Salinity

Introduction

Salinity is one of the most intimidating abiotic stresses, negatively influences plants in different ways, depending on its extent and duration. It harmfully affects the morphological, physiological, and molecular responses of plant species (Shin *et al.* 2020; Giordano *et al.* 2021). Salt stress disrupts membrane permeability, water deficit and stomatal closure, oxidative damages, and nutritional imbalance thereby impairing vital cellular functions, as the reduction in photosynthetic pigments as well as rate, growth, and yield of many crop plants (Tang *et al.* 2017; Elsheery *et al.* 2020). Therefore, there is an incessant necessitate to extend novel approaches to alleviate the destructive results of these stresses on plants.

Application of nanotechnology in agriculture as nano-fertilizers and nano-pesticides had attested to be a pioneer field. Nanoparticles have tremendously small size; they have attained a number of particular characteristics, such as solubility, reactivity and surface area which formulate them different from their bulk counterparts. Numerous investigations have been reported on affirmative or harmful effects of nanoparticles on higher plants. Among nonmaterials, AgNPs participate an important part in the biology and medicine fields owing to their properties (Benakashani *et al.* 2016). These NPs were reported to overcome and improve the tolerance of crops to stresses. AgNPs modulate hormonal balance whilst enhanced the germination of seeds and salinity tolerance (Abou-Zeid and Ismail 2018).

Biological synthesis of AgNPs is an eco-friendly mode of synthesis using plant extracts as reducing and capping agent, the plant secondary metabolites take part in reducing the $AgNO_3$ into AgNPs in various redox chemical reactions (Javed and Mashwani 2020). Previously, it was recorded that *Chrysthanthemum cornarium* extract contain many phenolics and flavonoids compounds, for instance, 3,5-dicaffeoyl quinic

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acid, 4,5-dicaffeoyl quinic acid, gallic acid, chlorogenic acid, 2,5-dihydroxy benzoic acid, caffeic acid, cinnamic acid rutin and catechin (Abou-Zeid *et al.* 2014).

Nano-priming could be a beneficial technique to boost seed germination and beat growth reduction of plants in the salinized soils. Mahakham *et al.* (2017) findings were that seeds priming with green synthesized AgNPs (5 and 10 mg L^{-1}) enhanced germination and growth of rice plants. It was accounted that priming with 20% AgNPs positively affect *Thymus kotschyanus* germination and plant growth but the 60% AgNP caused venomous effects (Khalaki *et al.* 2016). The unconstructive impacts of nanoparticles on plants and environment should not be discarded. Several studies have reported a significant reactive oxygen species (ROS) production of in nano-primed seedlings (Gorczyca *et al.* 2015). Additionally, Fayez *et al.* (2017) reported that AgNPs damage chloroplasts, mitochondria and nucleus ultrastructure in barley plants.

To perceive the probable genotoxicity of AgNPs, we can refer to a variety of toxicological endpoints resembling mitotic activity, and chromosomal variations either in structure or number, that upshot from the impact of physical or chemical agents. that known as chromosomal abnormalities or aberrations such as breaks in DNA, bridges, and reduction in its synthesis. Patlolla *et al.* (2012) recorded cytotoxic and genotoxic effects on *Allium cepa* and faba bean root tips due to the application of AgNPs. Biosynthesized AgNPs (5 mg L^{-1}) induced a wide variety of mitotic disturbances within the onion root meristems (Abd-elhamed 2017). As well, AgNPs provoked cytotoxic effect on *D. polyantha* the tip meristematic cells and accountable for chromosomal aberrations which were found to be dose and duration dependent. Elevated degrees of AgNPs inhibit mitotic activity and augmented abnormalities of chromosomes and hence cell death (Daphedar and Taranath 2018). Therefore, the present investigation aimed to detect the consequences of seed priming with different concentrations of biogenic AgNPs under salt stress condition on germination, growth biomarkers, photosynthetic pigments, chlorophyll fluorescence, and the possible alterations of mitotic activity and chromosomal abnormalities of wheat meristematic cells.

Materials and Methods

Biosynthesis and characterization of AgNPs

Biosynthesis of AgNPs: Aqueous leaf extract *C. cornarium* was prepared by boiling powdered plant material (10 g) in Erlenmeyer flask with 100 mL of deionized distilled water for ten minutes at 100°C, afterward filtered through Whatman No 1 filter paper and the filtrate was stored at 4°C. For preparation of AgNPs, 10 mL of the prepared extract was added to 100 mL of 3 mM aqueous $AgNO_3$ solution and incubated for 2 h in a rotary shaker, then at room temperature for 24 h in the dark until the brownish color was developed which indicated the formation of AgNPs (Dwivedi and Gopal 2010).

Characterization of AgNPs: Visual observation of metal ions reduction was examined by the conversion of the pale yellow of the reaction mixture to brown colored solution. The surface Plasmon resonance absorbance peak of stabilized AgNPs was observed from UV-Vis spectrophotometer (T80 UV-Vis spectrophotometer–double beam) at a scanning speed of 200-800 nm, after the dilution of the samples with deionized water. TEM (JEOL-TEM 100CX) samples were prepared by placing a drop of the suspension of AgNPs solutions on carbon-coated copper grids, allowed to dry for 4min and the shape and size of AgNPs were determined from TEM micrographs. Energy dispersive X-ray (EDX) spectroscopy with SEM (JSM-IT200) has been used for elemental analysis (Elavazhagan and Arunachalam 2011). TEM and SEM were performed at the special unit of Electron Microscope, Faculty of Science, Alexandria University.

Experimental design

Wheat grains (*Triticum aestivum* L., cv. Sakha 61) were purchased from Agricultural Research Center, Giza, Egypt. Prior to germination, grains were first sterilized by 1% sodium hypochlorite solution for about 30 seconds, next, washed thoroughly with distilled water. Sterilized grains were soaked in different concentrations (0, 20, 40 and 80 mg L^{-1}) of the aerated priming AgNPs solution under dark conditions for 12 h on shaker. The primed-grains washed with distilled water, kept at room temperature in dark to dry-back. A factorial laboratory experiment of a complete randomized design with 4 replicates was carried out. Ten grains were allocated at random in Petri dishes lined with filter paper dampened with 10ml of either distilled water or salt concentration (150 mM NaCl) and incubated at natural environmental conditions for two weeks. The grains were considered germinated as the radicle reached 2 mm. Uniform 24 h radicals of germinating grains were selected in replicates for cytological analysis. 15-days old seedlings were collected; rinsed carefully in water and pressed gently between blotting paper, dissected to shoots and roots and saved for estimation of the growth parameters, photosynthetic pigments and photosynthetic efficiency.

Germination bioassay

The Germination percentage **(**GP) and Inhibition percentage (IP) were calculated as follows:

GP = number of actual germinated grains /total number of sown grains ×100,

$$IP = [(X - Y)/ X] \times 100$$

Where, X= Maximum number of germinated grains in control set, and Y= Maximum number of germinated grains in treated set.

Estimation of growth biomarkers

Shoots and roots lengths, dry weights (DW) were measured using appropriate procedures.

Estimation of photosynthetic pigments

Following the method described by Moran (1982) using N, N–dimethyl formamide, the chlorophyll a (Chl-a), chlorophyll b (Chl-b), total chlorophylls were determined and total carotenoids (Carot) calculated according to Wellburn (1994).

Estimation of quantum yield of PSII

Measurements of Chl-fluorescence was performed with OS-30P pulse modulated chlorophyll fluorimeter (Opti-sciences, Hudson, and USA) following the procedure described by Van Kooten and Snel (1990), before each measurement leaves were dark-adapted for 30 min with leaf-clips.

Cytogenetic study

Four random roots from each treatment were fixed in a fixative solution (ethanol: acetic acid -3:1 v/v), and the conventional Feulgen squash technique was used to prepare permanent slides of root meristems according to Sharma and Sharma (1980). Slides examined and photographed using light microscope (Olympus-Japan) at 400 X magnification. The frequency of dividing cells per root (mitotic activity) and the rate of aberrations were recorded. Mitotic index, phase index, and the percentage of aberrant-dividing and non-dividing cells were determined in AgNPs-treated and untreated root tips in presence or absence of salt stress according to the following formula:

$$\text{Mitotic index (MI\%)} = \frac{\text{Number of DCs}}{\text{Number of total cells}} \times 100$$

$$\text{Phase index \%} = \frac{\text{Number of cells in each phase}}{\text{Total number of dividing cells}} \times 100$$

$$\text{\% aberrant dividing cells} = \frac{\text{Number of aberrant dividing cells}}{\text{Total number of dividing cells}} \times 100$$

$$\text{\% aberrant nondividing cells} = \frac{\text{Number of aberrantnon-dividing cells}}{\text{Total number of non-dividing cells}} \times 100$$

Statistical analysis

The data in completely randomized design with four replicates were tested for significance using one-way ANOVA test following the method of Sokal and Rohlf (1995). Statistical analysis was carried out according to Duncan's multiple range tests using SPSS20. Differences between treatment-means were considered statistically significant at $P \leq 0.05$.

Results

Biosynthesis and characterization of AgNPs

The color of reaction mixture changed from pale yellow to brown color indicating the formation of AgNPs, the absorbance peak of stabilized AgNPs was observed at 410 nm (Fig. 1A). The EDX analysis confirmed the presence of Ag element, and the highest characteristic absorption band of the elemental was observed at 3.21 kEV, while the intensity of the Ag signal was very high (Fig. 1B). TEM and SEM images (Fig. 1C–D) indicated that the AgNPs had a uniform spherical shape with average size ranging from 15–30 nm.

Germination, growth biomarkers, photosynthetic pigments and quantum yield of PSII

The results of the present study depicted that the effects of seed priming with different concentrations of the biogenic AgNPs (0, 20, 40 and 80 mg L^{-1}) on wheat growth under salinity stress (150 mM NaCl) provoked a significant suppression in wheat growth as reflected by the GP, the shoots and roots lengths, dry weights (DW) as well as the photosynthetic pigments and the maximum quantum yield of PSII (Fv/Fm). From Fig. 2A, an adverse effect was noted under high concentrations of AgNPs (40 and 80 mg L^{-1}), the inhibition of germination was significantly high and was about 30 and 40%, respectively (Fig. 2B). The measurements of growth parameters clearly indicated that all the AgNPs concentrations led to reduction in shoots and roots lengths either in presence or absence of NaCl (Fig. 2C). Synergistic effects of AgNPs and NaCl were documented by the declines of the shoots and roots DW, the reduction reached about 53 and 56% for shoots and 60, and 74% for roots, respectively, compared to the water primed controls (Fig. 2D).

The amounts of photosynthetic pigments decreased as the concentrations of AgNPs increased in the priming solution with salt stress, the reduction in Chl-a, Chl-b, total chlorophylls and carotenoids content was dose-dependent and the higher concentration (80 mg L^{-1}) reduced them by 42, 25 ,50 and 39%, respectively (Fig. 2E). The Fv/Fm ratio which reflects the quantum efficiency for photochemistry of PSII decreased significantly as the concentration of AgNPs increased under salt stress condition, the reduction percentage at high AgNPs-treated ones was about 35% with respect to the control (Fig. 2F). Nonetheless, 20 mg L^{-1} AgNPs-primed grains in absence of salt showed insignificant variations on the formerly mentioned parameters in comparison with controls.

Cytological study

The root tips of germinated grains (24 h) were subjected to cytological studies of mitotic division. Untreated wheat root

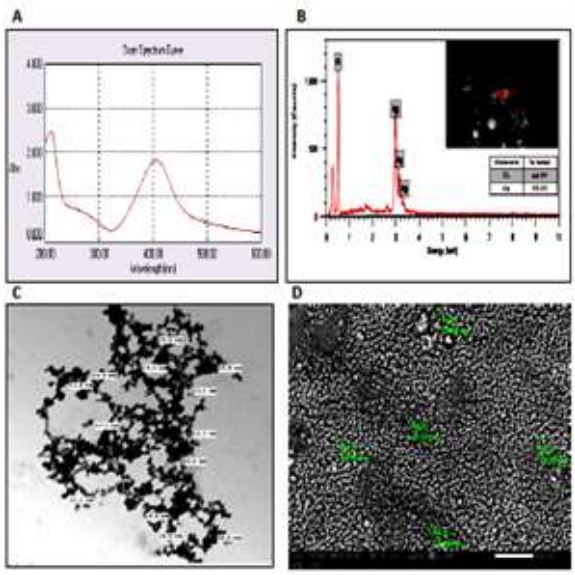

Fig. 1: Characterization of biogenic AgNPs, (A): UV–Vis absorption spectrum, (B): EDX spectra, (C): Transmission electron micrograph, and (D): Scanning electron micrograph

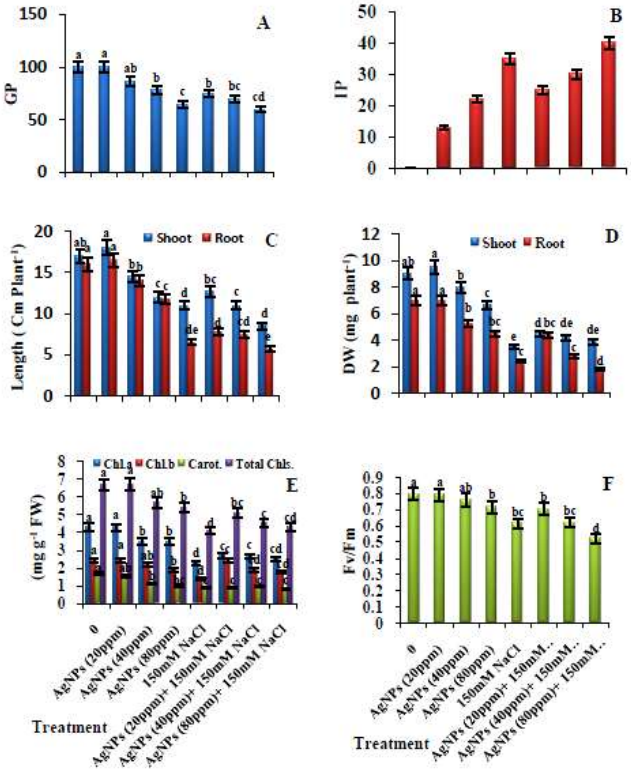

Fig. 2: Effects of the interaction between salinity and seed-priming with AgNPs on (A): GP, (B): IP, (C): Shoots and roots lengths, (D): Dry weights, (E): Photosynthetic pigments and (F): Maximum yield of PSII in 15-day-old wheat seedlings. Different letters indicate significant difference by Duncan's multiple range tests ($p \leq 0.05$). Values are means ± SE (n= 4)

tips showed all mitotic stages (Fig. 3), application of AgNPs in presence or absence of salt caused a significant decrease in the MI of root meristems. Treating the grains with 80 mg L^{-1} AgNPs reflected significant mito-depressive effect symbolized in 67% reduction in MI under salt stress, compared with the control (Table 1). Moreover, it was found that the highest metaphase/prophase (M/P) ratio generally coincided with the low mitotic activity, where the

Table 1: Effects of the interaction between salinity and seed-priming with AgNPs on mitotic phase indices of wheat root tip cells. Values are means ± SE (n= 4)

Nanoparticle level (mg L^{-1})	TC	Mitotic Phases%					
		P	M	A	T	M/P	A+T
Control	5150±28.9	42.09±3.50	22.56±1.01	15.12±0.98	20.23±1.20	0.54	35.35
AgNPs (20 mgL^{-1})	4776±21.5	33.91±2.33	20.98±1.60	19.54±1.01	25.57±1.09	0.62	45.11
AgNPs (40 mgL^{-1})	4500±12.0	19.0±1.20	24.54±0.88	25.33±2.10	31.13±2.20	1.29	56.46
AgNPs (80 mgL^{-1})	4000±16.8	11.4±3.50	26.58±2.20	29.96±3.03	32.07±2.08	2.33	62.03
150mM NaCl	4301±19.2	42.48±3.3	19.33±1.00	13.6±1.09	24.58±1.09	0.46	38.18
AgNPs (20 mgL^{-1}) + 150mM NaCl	4390±21.1	29.44±2.22	21.26±2.20	26.05±2.34	23.35±0.88	0.72	49.4
AgNPs (40 mgL^{-1}) + 150mM NaCl	4341±18.4	16.15±0.88	25.21±0.98	23.23±2.20	35.41±2.33	1.56	58.64
AgNPs (80 mgL^{-1}) + 150mM NaCl	3800±11.5	9.92±1.20	32.23±1.09	32.64±1.80	25.21±1.09	3.25	57.85

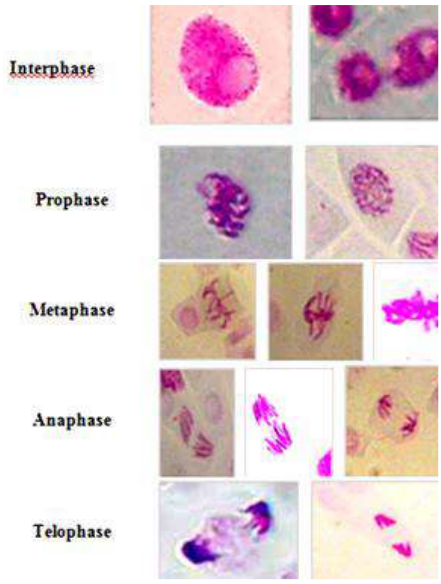

Fig. 3: Representative examples of normal mitotic phases in wheat root tips

M/P ratio were 2.33 and 3.25% compared to that of the MI value 11.1 and 4.41% found at 80 mg L^{-1} AgNPs-primed under water or NaCl. The lowest M/P ratio 0.46 was recorded for NaCl stressed plants in absence of AgNPs. The highest percentage of anaphase + Telophase (A+T) was recorded for 40 and 80 mg L^{-1} AgNPs in absence and presence of salt stress (Table 1).

In the current work the treatment of wheat grains with AgNPs and NaCl caused different kinds and rates of aberrant dividing cells (ADCs). The stages of mitosis dividing cells were observed, and various chromosomal abnormalities were recorded (Table 2). The lowest rate of ADCs (2.39%) was recorded for the control, while the highest one (34.09%) was recorded for the salt treatment accompanied with high AgNPs-treatment. Moreover, it was found that the percentage of ADCs significantly differences of the control and water primed-NaCl stressed plants. It was considerably lower than that of the AgNPs-primed ones even in water or salt treatments. However, no significant differences were found among concentrations for the same treatments, as well as when the mean values of aberrant dividing cells of all concentrations were compared with different treatments (F= 1.83 at P> 0.05). More or less one kind of aberration was found per cell, the highest value (1.14) was recorded in 80 mg L^{-1} AgNPs-primed with stress of NaCl, which decreased to 0.49 per cell in control. Simple linear regression obtained by plotting treatment versus ADCs achieved values of coefficient of determination (R^2) for data of about 0.833 and 0.958 for water and NaCl, respectively (Fig. 4). However, the interaction between the effect of different concentrations on the rates of aberrations was highly significant (F = 140.27) at P≤ 0.05). It was found that there was a significant negative correlation between the MI and ADCs (Fig. 4).

The cytotoxic effect of AgNPs was manifested by the appearance of several types of chromosome abnormalities as C-metaphase, chromosome bridges, lagging chromosome, stickiness, chromosome fragments, disturbed Anaphase, multipolar anaphase and micronucleus, beside those, there are rare abnormalities appeared with as precocious chromosomes, abnormal orientation, multipolar, disturbance and multi groups (Table 2; Fig. 5). The highest frequency of chromosomal aberrations was detected in the cells exposed to 80 mg L^{-1} AgNPs and NaCl stress. The pooled effects of mean concentrations indicated that both stickiness and bridges were the highest types of aberrations

Table 2: Effects of the interaction between salinity and seed-priming with AgNPs on chromosome aberrations of wheat root tip cells. Values are means ± SE (n= 4)

Treatment	Chromosome Aberrations %								Aberrations/cell
	CM	Bridge	Lagging	Stickiness	Fragment	Micronucleus	Disturbed anaphase	Multipolar	
Control	0.24±0.03	0.95±0.01	0.72±0.08	0.0±0.00	0.48±0.01	0.24±0.01	0.0±0.00	0.0±0.00	0.49
AgNPs 20 mgL^{-1}	0.0±0.00	6.80±0.20	2.27±0.08	5.95±0.1	2.27±0.03	0.0±0.00	0.0±0.00	0.28±0.08	1.00
AgNPs 40 mgL^{-1}	1.72±0.04	6.21±0.40	2.07±0.06	9.31±0.09	0.34±0.01	0.34±0.05	0.34±0.03	0.0±0.00	1.02
AgNPs 80 mgL^{-1}	1.80±0.06	5.39±0.09	2.99±0.02	9.88±0.09	1.80±0.08	0.30±0.01	0.30±0.01	0.0±0.00	1.05
150mM NaCl	0.70±0.08	1.86±0.01	0.23±0.01	0.23±0.01	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	1.00
AgNPs 20 mgL^{-1} + 150mM NaCl	0.38±0.01	8.81±0.20	2.68±0.05	9.58±0.05	5.75±0.07	1.15±0.04	0.0±0.00	0.0±0.00	1.04
AgNPs 40 mgL^{-1} + 150mM NaCl	0.41±0.09	5.79±0.10	1.24±0.02	21.90±0.03	0.83±0.01	0.0±0.00	0.0±0.00	0.0±0.00	1.06
AgNPs 80 mgL^{-1} + 150mM NaCl	0.0±0.00	7.73±0.06	2.27±0.05	18.64±0.02	4.55±0.03	1.82±0.08	0.0±0.00	0.45±0.06	1.14

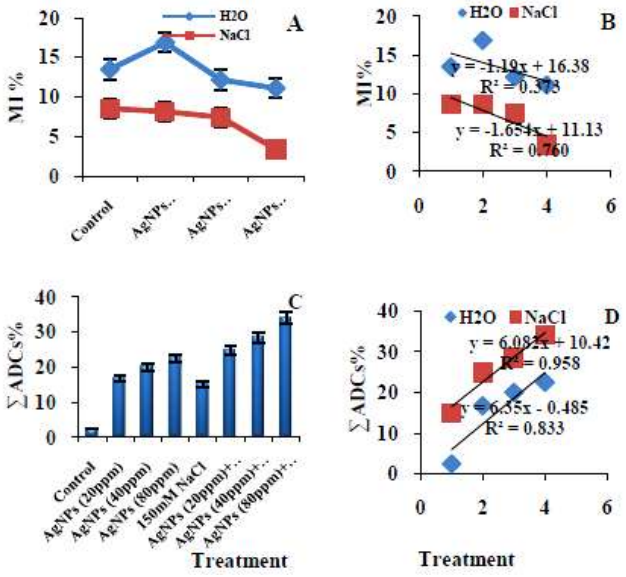

Fig. 4: Effects of the interaction between salinity and seed-priming with AgNPs on (A): Mitotic index, (B): Regression analysis for MI, (C): ∑ADCs% and (D): Regression analysis for ∑ADCs% of wheat root tip cells. Values are means ± SE (n= 4)

(Fig. 5). Stickiness represented about 75.49% of the total percentage of mitotic aberrations. The highest stickiness and bridge percentages were 50.35 and 43.54%, respectively were detected for salt treatment than water-treated ones that reached 25.14 and 19.35%, respectively (Table 2).

The highest mean percentages of bridges (5), fragments (1) and laggards (2) were found in salt treatments. The mean percentage of C-metaphase ranged from 0.24 for control to 1.80 for 80 mg L^{-1} AgNPs in absence of NaCl stress. The highest percentage of micronuclei (2.97) was for salt stress, and the highest mean percentages value of disturbed anaphase was found in 40 mg L^{-1} AgNPs water irrigated grains, moreover, the highest percentage for multipolar anaphase was found in 80 mg L^{-1} AgNPs-salt treatment (Table 2; Fig. 5).

Discussion

Leaf aqueous extract *C. cornarium* of acts as a reducing agent for AgNPs biogenesis and its results are in line with those obtained by Gamboa *et al.* (2019). Salt stress causes severe diminution in the yield and quality of stressed crop plants. Under the prevailing experimental conditions, the inhibitory effects of salinity stress on wheat growth is consistent with other reports (Yanyan *et al.* 2018; Shin *et al.* 2020). GP, growth characteristics, photosynthetic pigments, and chlorophyll fluorescence were significantly reduced in plants treated with 150 mM NaCl (Fig. 2). Thus, the impaired growth of wheat seedlings could be due to disorder in the integrity of the plasma membrane, the poor root growth, and Na^+ and Cl^- toxicity appeared to modify the enzyme activity involve in nucleic acid and protein metabolism and reduced the use of food reserved in seeds together with inhibition of cell division and/or restriction of cell elongation, impaired photosynthesis, nutrient imbalances, stomatal conductance, variations in chloroplasts ultrastructure and hormonal inequity. Furthermore, photosynthesis is associated with chlorophyll a and b accompanied by carotenoids, which form the light-harvesting a/b protein complex, and many genes are known to affect the plastid pigments (Sofy *et al.* 2020; Arif *et al.* 2020). The results revealed significant reduction in the

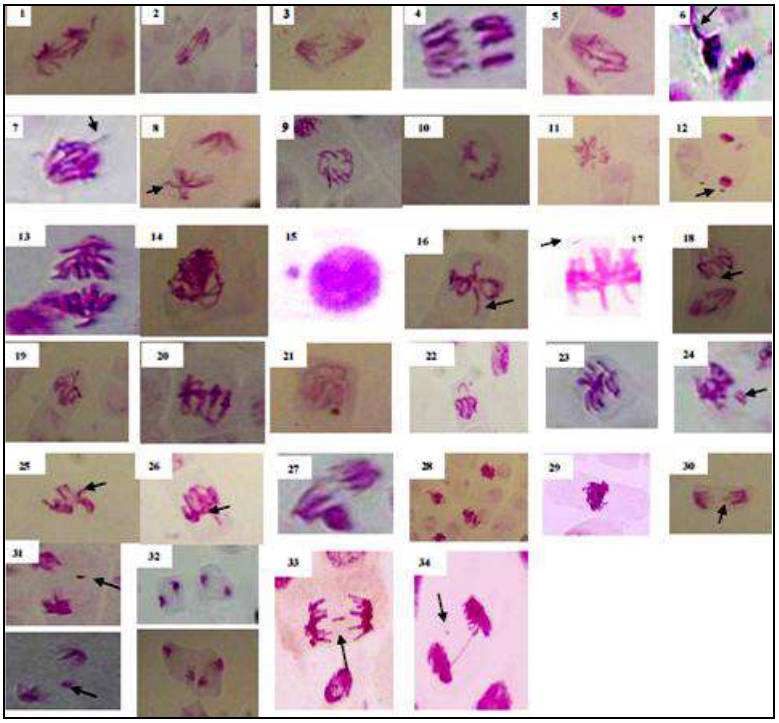

Fig. 5: Representative examples of abnormal cell divisions in wheat root tips after treatment with AgNPs with or without salt stress 1: Anaphase with precocious chromosome and one bridge, 2: Anaphase with bridge,3: Anaphase with abnormal chromatids orientation,4: Multipolar anaphase,5: Anaphase with Multiple bridges, 6: Anaphase with precocious,7: Anaphase with precocious chromosome and multiple bridges, 8: Anaphase with precocious chromosomes,9,10and11: C-metaphhase,12: Telophase with micronucleus, 13: Disturbed anaphase, 14: Disturbed prophase, 15: Interphase with micronucleus, 16 and 17: Metaphase with fragment, 18: Metaphase with fragment, Anaphase with bridge,19: Metaphase with micronucleus, 20: Multigroup metaphase, 21: Prophase with micronucleus, 22: Metaphase with precocious chromosome and micronucleus, 23: Metaphase with two groups, 24: Metaphase with laggard chromosome, 25: Metaphase with fragment,26: Metaphase with precocious chromosome, 27: Sticky anaphase, 28and 29: Sticky metaphase, 30: Telophase with fragment, 31: Telophase with micronucleus,32: Telophase with abnormal chromosomes orientation, 33: Telophase with laggard chromosome, 34: Telophase with bridge and micronucleus

content of photosynthetic pigments in response to different concentrations of AgNPs in absence or presence of salt (Fig. 2). This may be due to the structural disruption of the chloroplasts, pigment-protein complex, which can result in oxidation of chlorophyll thus disturb plant growth and development as documented in *Phaseolus vulgaris* (Bargaz *et al.* 2016) and mango (Elsheery *et al.* 2020).

Nanoparticles (NPs) effects on plants can be either promotive or preventive depending on the plant species, kind and concentrations of NPs applied (Pooja *et al*. 2019). Contrary to this study, previous researchers have reported that seed priming with AgNPs mitigates the adverse impacts of NaCl stress on *Triticum aestivum* and *Satureja hortensis* plants (Wahid *et al.* 2020; Nejatzadeh 2021). In concurrence with this study several researchers showed that NPs exert negative effects such as suppression of plant growth, inhibition of physiological features (Goswami *et al*. 2019). AgNPs affect GP, root development, and cellular compartments, thylakoid membrane, photosynthesis, metabolism and plant growth (Goswami *et al*. 2019; Abbas *et al.* 2020). In this study, treatment with AgNPs did not counteract the inhibitory effect of salinity on wheat plants. There was a significant diminution in GP and seedling growth, photosynthetic pigments and quantum yield of PSII at higher concentrations (40 and 80 mg L^{-1}) of AgNPs. A highest GP was measured in water-primed and the lowest one was noticed in plants treated with 80 mg L^{-1} under salt stress (Fig. 2). Thabet *et al.* (2020) reported a noxious effect of AgNPs on GP, seedling development of maize plants at higher concentrations, since can affect cell growth and metabolism. AgNPs were deposited on the surface of cell as well as within the organelles and resulted in cell oxidative stress through the induction and accumulation of ROS that sequentially cause lipid peroxidation, RNA, DNA and protein damages (Gorczyca *et al.* 2015). It is known that reduction in the photosynthetic pigment content was a general effect of metal-based NPs in *Brassica* sp. (Vishwakarma *et al.* 2017) and *Lycopersicon esculentum* (Noori *et al.* 2020).

The AgNPs induce strong cytotoxicity in broad spectrum of plants (Hafez and Fouad 2020; Mwando *et al.* 2020). Treating wheat grains for 12 h with different concentrations of AgNPs (20, 40 and 80 mg L^{-1}) grown under salinity stress caused a significant decrease in MI,

which is a sign of mito-depression and/or cytotoxicity (Table 1; Fig. 4). Smaka-Kinel *et al.* (1996) reported that mito-depressive and cytotoxic effects might de due to modified protein and/or DNA contents. This is usually accompanied by a rise in the cells fraction with c-mitosis, multi-groups, sticky and abnormal chromosome orientation. In the present study, a decrease in the MI was found to be significant with all treatments of NaCl combined with AgNPs or not which indicates the cytotoxic effect. Also, the suppression of MI was probably due to either the blocking of G_1, or G_2 preventing cells from entering mitosis (Table 1).

In this study, high M/P ratio induced by AgNPs might be due to ability of cells to enter mitosis giving fewer cells at prophase and therefore, mitotic division was delayed at metaphase (Table 1). It is known that the mitotic cell cycle is controlled by an advanced pattern of protein phosphorylation mediated by the cyclin-dependent kinases (Cdks) and reversed by protein phosphate that interacts with different cyclins to endorse diverse cell-cycle transition points (Sumner 2003). It is suggested that AgNPs prevent or suppress the Cdks activity, preventing cell from entering mitosis and causing mitotic depression and high values of M/P ratio, since a decrease in MI was correlated with an increase in M/P ratio. Also, M-phase promoting factor, as a checkpoint, is degraded at the metaphase-anaphase transition (Rahal and Amon 2008). Herein, metaphase-anaphase transition was delayed resulting in the raise of cells at metaphase. This might be due to the inactivation of mitotic kinase and MPF, or due to disassembling of the spindle microtubules. In addition, anaphase promoting complex is the next checkpoint protein, which is required as cells pass through anaphase and telophase and complete mitosis (Daphedar and Taranath 2018).

Our results showed that treatments of wheat grains with AgNPs of sizes ranging from 15-30 nm caused significantly higher chromosomal aberrations in comparison with the control indicating genotoxic effects. Previously, it is reported that priming with AgNPs causing chromosomes aneuploidy, binucleate cells, deletion chromosomes, deform nuclei, micronuclei, chromosomes fragment, and stickiness chromosomes in wheat and barley seedlings (Abou-Zeid and Mostafa 2014). Debnath *et al.* (2018) reported that AgNPs of 1-10 nm damaged DNA causing genotoxic effect. Daphedar and Taranath (2018) held that AgNPs showed dose-dependent reduction of MI and the higher AgNPs concentration inhibited MI and caused abnormalities in the chromosomes of *Allium cepa* roots. NPs could penetrate root cells and cause considerable changes in intracellular components hence damaged the cell division (Table 1).

In the present study, several chromosome abnormalities appeared as c-metaphase, chromosome bridges, lagging chromosome, stickiness, chromosome fragments, disturbed anaphase, multipolar anaphase and micronucleus, beside those, there are also rare abnormalities as precocious chromosomes, abnormal orientation, multipolar, disturbance and multi groups (Table 2; Fig. 5). Stickiness and bridges (mostly sticky bridges) were the most frequent kind of aberrations, which increased with an increase in AgNPs concentration, a common mark of toxic effect on chromosomes (Table 2). Chromosome stickiness was due to instantaneous reactions with DNA during its reticence period causing inter-and intra-chromosomal cross links involving both DNA-DNA and DNA-protein (Kovaleva 2008). However, Patil and Bhat (1992) suggested that stickiness is a type of physical adhesion involving mainly the matrix of chromatin material. Cuylen *et al.* (2016) reported that cells lacking high positively charged chromosome protein coat (ki-67) showed a sever defect in the separation of chromosomes causing stickiness. In this study, inhibition of root growth in treated wheat seedling was connected with distinctive errors in cell division and the behavior of chromosome for e.g., micronuclei, bridge, multiple breaks, and early chromosome separation, as reported by Abdelsalam *et al.* (2018) and Daphedar *et al.* (2021).

Fragments were found in the present study in 20 mg L^{-1} AgNPs-treated grains germinated in water; however, they were detected in both low and high-AgNPs-primed grains germinated under NaCl stress (Table 2; Fig. 5). This may be owing to DNA breakage by endonuclease, or as the result of changes in the levels of DNA methylation (Kaeppler and Rhee 2000). This work showed that AgNPs could pierce plant system and might harm cell division stages causing the aberrations of the chromosome. The production of ROS caused by AgNPs concentrations may resulted from many harmful effects to plant cells and may boost DNA damage and augment gene expression of death receptor. The increase in lysosomal ROS induced by AgNPs may cause DNA point mutations or provoked single or double stranded breaks (Singh *et al.* 2009).

In the present study, laggards, C-metaphase, disturbed A-T, multipolar cells increase in water and salt treated of 40 and 80 mg L^{-1} AgNPs-primed grains. These kinds of aberrations were suggested to indicate that the spindle formation was adversely affected or could be due to disorder in the spindle apparatus (Kumari *et al.* 2009). Thus, it can be suggested that AgNPs of the present study caused the disturbance in the structure and function of spindle microtubules, indicating its mutagenicity. Application of AgNPs also caused fewer of micronuclei which is true mutagenic aspect concerning as a result of lagging chromosomes or chromosome fragments, and loss of genetic material (Table 2; Fig. 5).

Conclusion

AgNPs and salinity significantly decreased GP, growth biomarkers, photosynthetic pigments, chlorophyll florescence, inhibit mitotic index and increased the frequency of chromosomal abnormalities such as c-metaphase, chromosomal bridge, sticky chromosomes, lagging chromosomes, fragment chromosomes,

disturbed anaphase and multipolar. Consequently, higher concentrations of AgNPs may persuade momentous inhibition in the activity of root meristems and hence wheat growth.

Author Contributions

Both the authors equally contributed to planning, execution of the experiments, and write-up and improvement of the manuscript.

Conflicts of Interest

Authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

This work does not involve animals hence ethics approval is not required.

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