JJBS

Volume 13, Number 1,March 2020
ISSN 1995-6673
Pages 13 - 17

Jordan Journal of Biological Sciences

Impact of Nanoparticles on Genetic Integrity of Buckwheat (*Fagopyrum esculentum* Moench)

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Received April 7, 2019; Revised April 16, 2019; Accepted April 24, 2019

Abstract

Nanomaterials are increasingly produced over the last decades and are expected to play an increasing role in future science, technology and medicine. These Nanoparticles (NPs) are particles between 1 and 100 nm in size. The present paper deals with the effect of copper and silver NPs on somatic cells of *Fagopyrum esculentum* Moench. For this, graded concentrations of silver and copper NPs *viz*., 18µg/mL, 36µg/mL and 54µg/mL were used for treatment along with control. Mitotic study was observed to be quite normal in case of control. An inverse relationship between the active mitotic index (AMI %) and concentrations of NPs was scored. However, as a result of the treatment on root tip cells, various chromosomal anomalies were induced such as stickiness, fragmentation, precocious movement, C-metaphase, bridge and unorientation, etc. The stickiness was found to be the predominant abnormality in both treatments. The total abnormality percentage (TAB %) was recorded higher in copper NPs as compared to silver NPs. Root length was also measured, which depicted a substantial effect of NPs on the root growth. On the basis of this result, it could be concluded that the copper NPs are more cytotoxic than silver NPs.

Keywords: AMI%, Cytotoxity, *Fagopyrum esculentum* Moench, Nanoparticles, TAB%,

1. Introduction

Buckwheat (*Fagopyrum esculentum* Moench) belongs to family polygonaceae, and contains a glucoside called rutin, a phytochemical that strengthens capillary walls. A dried buckwheat leaf has been manufactured in Europe under the brand name "Fagorutin" for use as herbal tea. Similar effects are associated with the inclusion of resistant starch in diet, which help to prevent colon cancer. These beneficial traits may be positively regulated using nanotechnology because this science has opened new vistas in the field of plant sciences, and interest for its exploration is increasing nowadays. Nanotechnology has large potential to provide an opportunity for the researchers of plant science and other fields, and to develop new tools for incorporation of NPs into plants that augment existing function and new ones (Cossins 2014). Nanoparticles (NPs) are wide class of materials that include particulate substances, which have one dimension less than 100 nm at least (Laurent *et al*., 2010). Pesticides and herbicides are usually used in agriculture to get better crop yield and efficiency. But nowadays, severe debate is going on the negative effect of conventional pesticides and herbicides on the environment. Random usage of pesticides increases pathogen and pest resistance, reduces soil biodiversity, decreases nitrogen fixation, contributes to bioaccumulation of pesticides, leads to pollinator decline, and destroys habitat for birds. When NPs are applied with herbicide, low amount of herbicide is required to achieve the weed eradication. NPs owing to their size can freely enter into the cells and can imply significant influences on the cellular function. These unique features make them highly attractive for implementation in products for wide application (Benn *et al*., 2010). NPs have unique physiochemical properties and the potential to boost the plant metabolism (Giraldo *et al*., 2014). The literature on the ecotoxicity of NPs and Nanomaterials as well as the chemistry of both manufactured and natural NPs is summarised in recent reports (Handy *et al*., 2008). Treatment of *Arabidopsis thaliana* plants with 1 or 2.5 mg/L of AgNPs was found to increase seedling biomass, whereas treatment with higher concentrations was found to decrease seedling biomass (Kaveh *et al*., 2013). Another study by Zafar *et al*., (2016) demonstrated effects of NPs on germination and shoot growth of *Brassica nigra.* Compared to fine particles, NPs are highly insoluble in water and culture media and show strong genotoxicity in the aqueous environment (Horie *et al*., 2013).

The two NPs selected for the present appraisal are silver and copper NPs. Cupric oxide II is an important inorganic compound with the formula CuO. A black solid. it is one of the two stable oxides of copper, the other being Cu_2O or cuprous oxide. Copper (II) oxide belongs to the monoclinic crystal system. The copper atom is coordinated by 4 oxygen atoms in an approximately square planar configuration. Halder *et al*., (2015) reported that copper (Cu) and cadmium sulphide (CdS) NPs induce stable and heritable phenotypic changes in *Macrotyloma uniflorum* (Lam.) Verdc (Family: Leguminosae). The bio uptake of copper NPs into the cell causes generation of reactive oxygen species (ROS). Silver nitrate is an inorganic compound with chemical formula $AgNO_3$. It was once called lunar caustic with antiseptic activity. Silver nitrate can potentially be used as antifungal and antibacterial agent. The penetration of silver NPs causing chromosomal aberrations in *Allium cepa* root tips was reported by Kumari *et al*., (2009) and Panda *et al*., (2011). Within the cell, the integration of silver creates a low molecular weight region

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where the DNA condenses (Sobha *et al*., 2010). Studies performed in a variety of organisms indicate AgNP toxicity, which may be ascribed to different mechanisms, including the disruption of the integrity of the cell membrane and binding of the proteins and DNA (Arora *et al*., 2009).

There is a scarcity of scientific data describing the dose-response relationship with respect to their cytogenetic toxicity of NPs in plant systems. Plants are the important component in ecological system and may serve as a potential pathway for NPs transport and a route for bioaccumulation into the food chain (Zhu *et al*., 2008). The present study aims to investigate the mutagenecity and genotoxicity of silver and copper NPs as a function of mitotic index, chromosomal aberrations and mitotic behaviour. The testing material used in this study is *Fagopyrum esculentum* Moench (2n =16), commonly known as Buckwheat. It is categorized as a pseudocereal; the crop is not a cereal, but the seeds (strictly achenes) are usually classified among the cereal grains because of their similar usage.

As a result of detailed literature study, this work represents a study on this subject being an implication of NPs on buckwheat that will prove to be useful for future studies.

2. Material and Methodology

2.1. Seed procurement

The seeds of *Fagopyrum esculentum* Moench variety-VL-7 were collected from National Bureau of Plant Genetics Resources (NBPGR), Shimla, Phagli, India.

2.2. Seed treatment

Fresh seeds of buckwheat were pre-soaked in distilled water for 12 hours. Then the seeds were treated with various concentrations of NPs i.e. 18µg/mL, 36µg/mL and 54µg/mL, suspension for 3 hrs along with control by dilution method. After treatment, the seeds were washed and left for recovery. These seeds were placed in sterilized Petriplates, kept in seed germinator for germination at 25±2°C with humidity 60-80%. After seed germination, the root length was measured at various concentrations.

Treated germinated seeds of each concentration were fixed in Carnoy's fixative [glacial acetic acid: absolute alcohol, (1:3)] along with control set. After 24 hours, all of them were removed from fixative and transferred into bottles containing only 90% alcohol.

2.3. Mitotic preparation

The control and treated root tips were excised, processed and hydrolysed in 1N HCl by adjusting water bath at 60°C for 1-2 minutes so that tissues of root tips get soften. Then they were washed under running water to remove excess HCl and allowed to keep on blotting paper for dehydration. Dried root tips were stained with 2% acetocarmine for 30 minutes. By using squash technique, mitotic slides were prepared and observed cells were snapped under Nikon Research Electron Microscope using Olympus PCTV Vision Software. Approx. 10 microscopic field views were recorded from each slide.

2.4. Formula used for scoring of data

The array of mitotic indices and various abnormalities were screened out by applying the formula below:

Active mitotic index (AMI) % = (Total number of dividing cells/Total number of observed cells)*100

Total abnormality percentage (TAB) % = (Total number of abnormal cells/Total number of observed cells)*100

2.5. Statistical analysis

The data obtained was analysed using statistical software, SPSS 16 and means were compared using Duncan's Multiple Range Test (DMRT) ($P \leq 0.05$). All the results were expressed in form of Mean ± Standard Error. The graph was plotted by using Sigmaplot 10.00 software.

3. Results

3.1. Effect of NPs on the germination & root growth of the plant

NPs are found very effective for the germination of plant. At 54 µg/ml concentration of silver NPs, the seed germination of buckwheat was found quite well (Figure 1 A, B). Efficacy of NPs depends on their concentration and varies from plant to plant. According to Suriyaprabha *et al*. (2012), silicon NPs increased seed germination in maize by providing better nutrients availability, optimum pH and conductivity to the growing medium. The silver NPs are more efficiently utilized by plants than the copper NPs. In copper NPs, the seed germination was comparatively less, indicating that copper NPs are more toxic than silver NPs. In silver NPs treated seeds, the root length was also recorded to be increased in comparison to control.

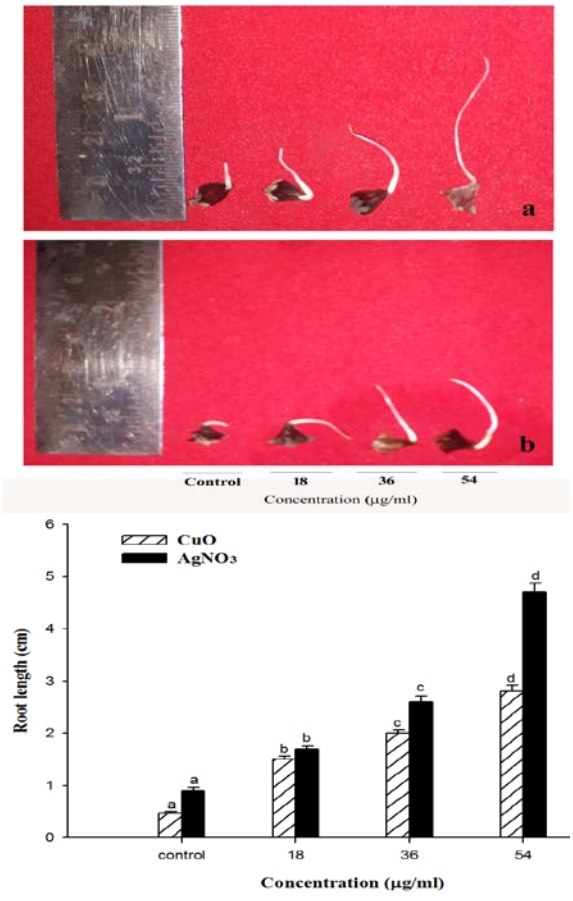

Figure 1A and B. Effect of Nanoparticles on root length of *Fagopyrum esculentum* Moench (a- $AgNO_3$ & b-CuO)

3.2. Effect of NPs on AMI% & TAB%

The data scored after NPs treatment was documented in form of Table 1. The computed AMI% for varying copper and silver concentrations divulges an inverse association between respective concentrations and AMI%. AMI% in case of control was calculated to be 11.94±0.18[a]. In comparison to control, AMI% was seen to decline with respect to increase in both the NPs. The reduced AMI% may occur due to breakdown of plant self-protection system and further inhibition of cell DNA replication, transcription and protein synthesis (Liu *et al*., 2015). The reduction is also because of slower transition of cells from S phase to M phase of cell cycle. In case of silver NPs, it declines from 11.57±0.12[b] to 8.90±0.05[d], whereas in case of copper NPs the decline from 10.80±0.57[b] to 7.85±0.22[d] was observed. On the other hand, it was observed that the TAB% shows direct correlation with increasing concentration of NPs. As at the highest concentration i.e. 54µg/ml, the TAB% was increased up to 4.50±0.57 ($AgNO_3$) and 7.16±0.33 (CuO). But it was comparatively lower in case of silver NPs treatment set; henceforth, higher dose of NPs has proved to be more chromotoxic and mitodepressive. It was also observed that the metaphasic and anaphasic abnormalities are almost equal at 18µg/ml (Figures 2 and 3), but as the concentration increases the anaphasic abnormalities were found to be more as compared to metaphasic abnormalities in copper NPs treatment. But in case of silver NPs metaphasic abnormalities were found more than the anaphasic abnormalities. Induction of various chromosomal aberrations also occurred. Kumari *et al*., (2011) reported that ZnO NPs exert cytotoxic and genotoxic effects, including lipid peroxidation, decreasing the mitotic index, increasing micronuclei and chromosomal aberration indices on root cells of *Allium cepa*.

Table 1. A Comparative account of Cytological abnormalities induced by Nanoparticles ($AgNO_3$ and CuO) in root meristems of *Fagopyrum esculentum* Moench

Treatment	Conc. (µg/ml)	AMI (%) (Mean±S.E.)	Metaphasic Abnormalities (%) (Mean±S.E.)					Anaphasic Abnormalities (%) (Mean±S.E.)					TAB (%) (Mean±S.E.)
			Cm	Sc	Pr	St	Un	Br	Lg	St	Un	Oth	
	Control	11.94 ± 0.18[a]	-	-	-	-	-	-	-	-	-	-	-
Silver Nanoparticle ($AgNO_3$)	18	11.57 ± 0.12[b]	0.25 ± 0.12	0.35 ± 0.20	-	0.74 ± 0.03	-	-	-	0.62 ± 0.13	0.11 ± 0.10	0.25±0.12	2.30 ± 0.10
	36	10.58 ± 0.83[c]	0.52 ± 0.01	-	-	0.51 ± 0.28	-	-	0.17 ± 0.17	0.69 ± 0.15	-	1.05±0.30	2.76 ± 0.37
	54	8.90 ± 0.05[d]	0.17 ± 0.16	0.13 ± 0.13	0.47 ± 0.03	0.65 ± 0.20	0.47 ± 0.29	0.51 ± 0.30	0.82 ± 0.20	-	0.30 ± 0.15	0.47±0.03	4.50 ± 0.57
	Control	11.94 ± 0.18[a]	-	-	-	-	-	-	-	-	-	-	-
Copper Nanoparticle (CuO)	18	10.80 ± 0.57[b]	0.49 ± 0.26	0.13 ± 0.13	0.40 ± 0.69	0.67 ± 0.06	0.90 ± 0.97	-	-	0.51 ± 0.20	0.26 ± 0.20	0.34 ± 0.18	3.30 ± 0.58
	36	9.11 ± 0.28[c]	0.63 ± 0.15	0.59 ± 0.74	-	0.59 ± 0.31	0.36 ± 0.18	-	-	1.63 ± 0.48	0.12 ± 0.13	0.74 ± 0.80	4.67 ± 0.12
	54	7.85 ± 0.22[d]	0.33 ± 0.26	0.82 ± 0.20	0.66 ± 0.21	1.25 ± 0.39	-	0.61 ± 0.12	1.42 ± 0.44	0.53 ± 0.53	0.45 ± 0.24	0.44 ± 0.24	7.16 ± 0.33

Where:**Conc-** Concentration, **Cm-** C- metaphase, **Sc-** Scattering, **Pr-** Precocious movement **, St-** Stickiness, **Un-** Un-orientation, **Br-** Bridge formation, **Lg-** Laggard formation, **Oth-**Others.

Means followed by lowercase letter are statistically significant at $p < 0.05$ in Duncan's Multiple Range Test

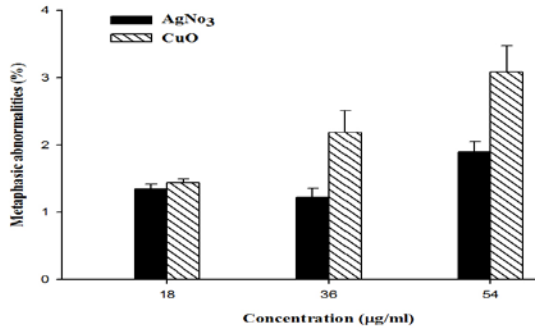

Figure 2. Comparative account of metaphasic abnormalities (%) induced by Nanoparticles ($AgNO_3$ and CuO) in the root meristems of *Fagopyrum esculentum* Moench.

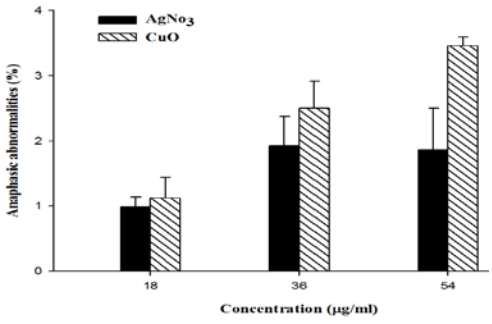

Figure 3. Comparative account of anaphasic abnormalities (%) induced by Nanoparticles ($AgNO_3$ and CuO) in the root meristems of *Fagopyrum esculentum* Moench.

3.3. Cytological impact of NPs

The cytological appraisal of the NPs shows a noteworthy impact on the cytology of root meristems of *Fagopyrum esculentum* Moench. Control seeds showed a normal mitotic behaviour, perfect alignment of chromosomes on equatorial plate at metaphase (2n=16) (Figure 4A) and 16:16 pole ward separation during anaphase (Figure 4B). However, the root tips treated with silver and copper NPs solution showed various types of chromosomal abnormalities such as metaphasic plate distortion, unorientation at metaphase, breaking of chromosomes, fragmentation, spindle dysfunctioning, stickiness, scattering, precocious movement at metaphase, laggard, bridge formation, unequal segregation and tripolarity, etc. NPs are capable of entering the nucleus, and directly or indirectly interacting with nuclear material, leading to alterations in DNA integrity (Kruszewski *et al*., 2011; Asare *et al*., 2012).

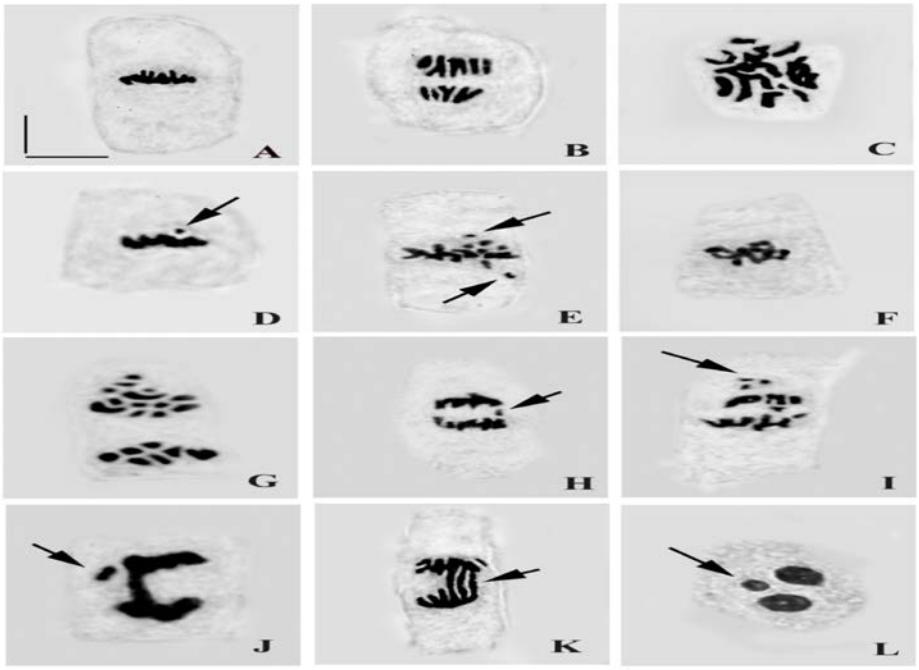

Figure 4. Different types of chromosomal aberrations induced by Nanoparticles in *Fagopyrum esculentum* Moench**:** A. Normal Metaphase (2n=16) B. Normal anaphase (16:16 separation), C. C-metaphase, D. One precocious chromosome with sticky metaphase, E. Two Precocious chromosome at metaphase, F. Loop formation at metaphase G. Scattering at anaphase, H. Laggard formation at anaphase, I. Laggard with forward movement at anaphase, J. Laggard with bridge formation at anaphase, K. Triple Bridge formation with forward movement at anaphase, L. Micronuclei formation at telophase [Scale bar: Length 6.28µm, Width- 7.52µm]

4. Discussion

Studies conducted to increase information on genotoxic risks related to exposure to emerging nanomaterials are of increasing interest (Landsiedel *et al*., 2009). Ghosh *et al.* (2010) have shown that TiO2 NPs (~100 nm) are able to induce significant increases in genetic damage in *Allium cepa* and *Nicotiana tabacum* when they used the comet assay for testing genotoxic effect. The effect of ZnO-NPs at elevated concentrations (10–2000 mg/L) revealed a biomass drop, damaged root surface cells, and induced abnormal defence system against ROS (Lee *et al*., 2016).

Disruption of spindle fibers causes scattered condensed chromosome leads to the formation of C-metaphase (Figure 4 C). Precocious movement (Figure 4 D,E) of chromosomes observed during the present study might have occurred due to disturbed homology for chromosome pairing, disturbed spindle mechanism or inactivation of spindle mechanism (Agarwal and Ansari, 2001). The loop formation (Figure 4 F) might have originated due to failure of kinetochores to attach with spindles and leading to the joining of ends forming loops (Kumar and Pandey 2016). Unorientation and scattering of chromosomes at anaphase (Figure 4.G) may be due either to the inhibition of spindle formation or destruction of spindle fibres formed (Kumar and Rai 2007). Laggards (Figure 4 H, I) in the present study have been attributed to delayed terminalisation and /or failures of chromosomal movement following spindle fiber discrepancies. The fragments which appeared on the breakage of bridges as a result of spindle fibers functioning to pull the chromosomes towards poles, formed laggards (Kumar and Gupta, 2009). Stickiness was found in both metaphase and anaphase of mitosis. Chromosome stickiness (Figure 4 J) leads to inactivation of DNA replication, increases chromosomal contraction and condensation or nucleoproteins probably leading to cell death (Khanna and Sharma, 2013). Stickiness was found to be most dominant chromosomal aberration recorded in the metaphase and anaphase of mitosis. At the higher concentrations, different abnormalities were observed viz. bridges, micronuclei etc. which was not found at the lower concentration. According to Liu *et al*.(2015), the chromosome bridge might have resulted due to enhanced activity of UV-B radiations, making chromosome breaks, and then the two chromosome sides are respectively healed, producing with double centromere chromosomes, i.e. “chromosome bridges” (Figure 4 J, K). Micronuclei (Figure 4 L) may arise mostly from acentric fragments or lagging chromosome (Fenech 2000). Cellular interaction of NPs, which leads to the generation of ROS, has been shown to be related to the physicochemical characteristics of NPs: size, coating, shape and surface charge (Carlson *et al*., 2008; Kim and Ryu 2013).

5. Conclusion

Based on previous study, it has been reported that Zn and ZnO NPs affected the growth of radish, rape, and ryegrass, but neither supernatant from centrifugation nor

filtrated Zn and ZnO solutions showed significant phytotoxic effects (Lin and Xing 2007). The present study clearly shows the chromotoxic effect of NPs. The lower concentration of NPs is beneficial for plant, but at the higher concentration it shows chromosomal aberrations. It is evident from compiled information that effect of NPs depends on their mode of application, size, and concentrations. So, in the near future NPs act as a light for farmers to induce or enhance the productivity of crops, but when its fine or good concentration is used.

Acknowledgement

The author is thankful to Laser Spectroscopy and Nanomaterials Laboratory, Department of Physics, University of Allahabad for providing NPs and is also highly obliged to National Bureau of Plant Genetics Resources (NBPGR), Shimla, Phagli, India for providing the inbred seeds of buckwheat.

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