Effect of nano zinc on cell division and chromosomal aberration in wheat plants: Cytological studies

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تأثير جسيمات الزنك متناهية الصغر على النقسام الخلوي والتشوهات الكروموسومية في القمح: دراسة سيتولوجية

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الملخص:

أجريت هذه الدراسة بقسم التقنيات الحيوية الزراعية بكلية الزراعة – جامعة الزيتونة – ترهونة – ليبيا خلال الفترة 2020– 2022 حيث تم دراسة التأثير السلبي لجسيمات الزنك متناهية الصغر وذلك بتركيزات وأزمنة تعرض مختلفة على معدل الإنقسام الخلوي وكذلك التشوهات الكروموسومية في القمح عن طريق الدراسات الخلوية. أوضحت النتائج أن معدل الإنبات تحت ظروف الكونترول تراوحت من 96.77 إلى 100% حيث تم فحص عدد خلايا تقريبا 2000 خلية حيث كانت أعلى نسبة في الخلايا المنقسمة تقع عند تركز 1.0 مل من تركيز الزنك الناني تحت زمن تعرض 8 ساعات بينما كانت أقل معدل إنقسام عند تركيز 2.0 وذلك عند زمن تعريض لمدة أربعة وعشرون ساعة من الزنك متناهية في الصغر إلى نسبة 54 % في عدد الخلايا المنقسمة. أوضحت النتائج أنه تحت وقت 16 ساعة من زمن التعرض كانت أعداد الخلايا الشاذة تتراوح 143 إلى 165 خلية شاذة منقسمة، وأخيراً عند 24 ساعة زمن تعرض كانت الخلايا الشاذة تتراوح 174 إلى 247 خلية شاذة منقسمة، فعليه نلاحظ أن أعلى عدد من الخلايا المنقسمة الشاذة والتي هي 247 خلية تم مشاهدتها عندما كانت الخلايا تتعرض لتركيز 2 مل عند زمن 24 ساعة وبنسبة 37.77% من الخلايا المنقسمة والبالغ عددها 700 خلية منقسمة، بينما كان أقل خلايا شاذة منقسمة سجلت تحت 0.5 مل/16 ساعة حيث كانت 128 خلية منقسمة شاذة ونسبة مئوية 13.47% من مجموع 654 خلية منقسمة. ويمكن القول بكل وضوح أنه بزيادة التركيز وكذلك وقت التعرض لمثل هذا النوع من جسيمات الزنك النانوية سببت بزيادة حالة النحر للكروموسوم في النويات وتعدد النويات مع وجود القطع الكروموسومية والتي سوف تزيد من فرصة نشاط التحلل للكروموسومات في التراكيز العالية وبزيادة فترة تعريض الجذور إلى جزيئات الزنك متناهية الصغر أدى ذلك إلى حدوث ضرر عام للخلية وتدمير محتوياتها الداخلية مما أثر على محتوى الجينوم الكلى لها. وتوصى هذه الدراسة بالمزيد من الدراسات الوراثية على استخدام المواد متناهية الصغر في المجال الزراعي لما لها من تأثير ضار على صحة الإنسان والبيئة.

الكلمات الدالة: *القمح – التشوهات الكروموسومية – الانقسامات الخلوية.*

Abstract:

Genotoxicity of nano zinc (Zn NPs) was evaluated on wheat root tips cells (*Triticum aestivum* L.) as cytogenetical to examine the impact of Zn NPs on mitotic cell division and chromosomal aberrations. Current research aimed to provide new knowledge about genotoxicity effect of Zn NPs on wheat plants. The current results showed that Zn NPs could enter the cells and interfere in cells normal function. Three various expose time and three Zn NPs concentrations were utilized. Three Zn NPs i.e., 1.0, 1.5 and 2.0 ml were completed to total volume of 1000 ml H_2O and root tips were treated after 8, 16 and 24 hours. The different Zn NPs concentrations were added for the tested wheat grains until the root length retched from 1.5 to 2 cm in length. Different mitosis division were observed and calculated in average 2000 cells, besides the mitotic index (%) were measured from the same divided and non-divided cells for each zinc nanoparticles concentrations. The treated root-tips showed numerous types of chromosomal aberrations as breaking of chromosomes, distortion in metaphysic, stickiness, precocious movement at metaphase and bridge, fragmentation, unequal separation, multiple bridge, fragmentation, nucleus elongation, gapping in chromosome type, different polars of anaphase stage, nucleus erosion, distribution in chromosome order and lagging chromosomes were observed in the current research.

Keywords: *nano zinc, wheat, chromosomal aberrations, root tips, cytological studies.*

Introduction:

Worldwide, nanotoxicology is an emerging discipline having increasing attraction. Nanotoxicity has been focused on several subjects, for instance, alumina, iron, silver, and zinc oxide nanoparticles have been applied to different plant species and cultivars based on different cytological testes (González-Melendi *et al.*, 2008; El-Naggar *et al.*, 2020; Fiordaliso *et al.*, 2022**)** who studied the toxicological effect of titanium dioxide nanoparticles and food-grade titanium dioxide (E171) on human and environmental health. The small size of nanoparticles (NPs) can modify the properties of the materials, which can lead to adverse biological effect on living cells (Gaidajis and Angelakoglou, 2009; Xu *et al.*, 2022**)**. Additional studies have been informed the positive and negative effects of NPs on higher plants based on its variable shape and size, it is difficult to expect the positive or negative result and its mode of action in the environment (Corredor *et al.*, 2009, Monica and Cremonini 2009, Ma *et al.*, 2015; Landa 2021).

Heavy metals (HMs) are commonly acknowledged to prevent seed germination, growth, and improvement of plants by disturbing their biochemical and physiological processes (Binhi, 2010 and Feng *et al.*, 2021). To understand the probable advantages of using nanotechnology to agriculture, the initial step should be to evaluate the level of penetration and transport of NPs in plants (Oberdörster *et al.*, 2007).

In the current research we have selected a monocot small size plant, short generation time and ability to grow well under controlled conditions (Lab experiment) under different concentrations of nano zinc (Zn NPs) to study the genotoxicity and its influence on seed germination and root tip cells of wheat.

Materials and Methods

Nano zinc preparation

Nano zinc (Zn NPs) was brought from "Bio Nano Tech" for fertilization development company, Egypt. The chemical composition of Zn NPS was as Amino acids (10%); Vitamins (1%); Zinc (6%) and the nano zinc (NPs) used in the present study were in colloidal form (25.6 –79.0 nm size).

Plant materials used in current study

The root-tip of Wheat (2n = 42) was used obtained from Ministry of Agriculture in Libya, Agriculture Research Center, Department of Cereals and Legumes, to treated with the different concentrations of zinc nanoparticles (Zn NPs).

Cytological studies

Mitotic studies:

Were carried out on root tips from germinated seeds. After treatments, the roots were collected as primary roots. The root tips were washed with distilled water and placed in 95% ethanol and glacial acetic acid (3:1) v/v for 24 hrs. at room temperature for killing and fixation. Root tips were removed from fixative solution and placed in 70% ethanol, stored in a refrigerator (4-5^{O}C) until examined (Samad *et al.* 1992; Abdelsalam *et al*., 2022). Slides were examined after preparation. Microscopic system (Made in USA) was used for taking photograph for the divided, undivided, and abnormal cells under the same magnification. All the mitosis stages were detected and determined under the different nanoparticle concentrations of Zn NPs.

Mitotic index (MI %):

Was calculated from ~ 2000 to 2500 cells for each zinc nanoparticles concentrations as Number of divided cell / numbers of observed cells. Also, all the chromosomal aberrations in the treated samples were noted.

Results and Discussion

Cell division and mitotic index

For the control in wheat grains, all the tested grains showed high germination percentage (almost = 100%). Mitosis division for wheat plants were assessed utilizing acetocarmine stains and all the mitosis stages were detected as found in **Table (1).** The obtained results demonstrated that, the total observed cells (TOCs) were ~1902 cells, in the examined cells, the results clearly suggested that the highest divided stage was the prophase stage by 701 cells while, the second mitosis stage metaphase was 302 cells; anaphase recorded 201 and Telophase 110 cells. Data showed that interphase was 564 cells and Mitotic index was 69.31%. in the control samples no abnormal stages were observed and the data showed the different mitosis stages.

Effect of Zn NPs on cell division and mitotic index (MI%)

The results showed that the total examined cells ranged from 1430 to 2123 cells by means was 1867.2 cells, inside the monitored cells data indicated that the greatest divided cells were noted to 1.0 ml under eight hours of treatments with Zn NPs solution by 1311 cells and the lowest number of divided cells were recorded to 2.0 ml after twenty four hours of treatments with Zn NPs solution by 654 cells as shown in Figure (1) and Table (2).

The general mean of divided cells where 997.9 cells by mitotic index was 54.0 %. Several mitosis stages were detected during the cell division for example prophase, metaphase, anaphase, and telophase in normal way, while abnormal stages were noted as

presented in Table (2). Regarding to prophase stage, results in Table (2) exhibited that the general mean was 644.9 cells comparing with metaphase 129.7 cells, anaphase 149.2 cells and finally telophase was 72.7 cells.

Table (1) Total observed cells and mitotic index for wheat root tips under control conditions.

Mitosis division	Mean
Number of the observed cells	1902 ± 14.22
Number Interphase cells	698 ± 11.20
Number of divided cells	1204 ± 45.65
Number of Prophase cells	701 ± 54.00
Number of Metaphase cells	302 ± 23.01
Number of Anaphase cells	201 ± 09.54
Number of Telophase cells	110 ± 12.20
Mitotic Index (%)	63.30 %

The results showed that there were many abnormal cells such as Nucleotide Deletion, Distributed Anaphase, Multi Polar Anaphase, Distributed Chromosome, Lagging Chromosome, C-metaphase Chromosome, Gap Chromosome, Fragments Chromosome, Bridge Chromosome, Uncloing Chromosome, Stickiness Chromosome, Ring Chromosome, Multinuclei, Elongation, Erosion as found in Table, 2 and Figures 1-3 For example, under 8 hours of treatment with Zn NPs the abnormal cells ranged from 153 to 165 cells, while, under 16 hours were 143 to 165 cells and finally, under 24 hrs. were 174 to 247 cells (Table, 2). The highest abnormal cells were recorded to the high concentration of Zn NPs under 24 hours. of treatment (274 cells) by 37.77% comparing with the divided cells (860 cells) and the lowest abnormal cells recoded to 1.0 ml under 16 hours (Zn NPs solution) 165 cells by 18.39% in compare with the divided cells 897 cells (Table 2). The data in Table (2) and Figures (1-3) clearly indicated that with increase in Zn NPs constrictions and time at expose, the number of abnormal cells increased and observed in different stages. The data clearly showed that high dose of Zn NPs and the highest time of expose caused in nucleus erosion beside the multinuclei with fragments, with the high dose of Zn NPs and 24 hours of treatment the cell wall burst, and all nucleus contents go out the cell to be described as a ghost cell. From the earlier data, it can be concluded that nowadays all farmers and producers used the Zn NPs without any roles just they aimed to increase the yield and plant production but with the high and excessive dose of these materials caused chromosomal aberrations and that mean decrease in the whole genome and may be transfer to the next generations. So, the governments and scientists should put the roles and dose of these materials to avoid the chromosol aberration. Current results in the same line with (Truta et al., 2013**)** who evaluated the amplitude of cytogenetic damage induced in *H. vulgare* L. during germination with different concentrations (10, 100, 250, 500 µM) of Zn^{+2}. They reported that the mitostimulatory effect was present at all concentrations of both zinc compounds. In addation, the rate of anaphase and telophase aberrations exceeded by 2 - 3 times comparing with the control, and the frequency of metaphase disturbances was 5.0-10.0 times higher than the control. Other findings reported that, zinc forms is stable with both

nucleic acids, and its can caused negative impact, and cussing high errors in the structure of genetic information system (Patra *et al*., 2004). The interaction between Zn and DNA is little known in the light of its involvement in carcinogenesis. Also, micronuclei were also reported at high Zn^{2+} doses in *Vicia faba* (Kumari *et al*., 2012). The observations of genotoxicity of Zn compounds showed on different woody plant species several responses, based on the zinc concentrations, time of exposure, species of plants, and zinc compounds, and treatment with unary or binary solutions (Ince *et al*., 1999; Marcato-Romain *et al*., 2009; Patra *et al*., 2004; Steinkellner *et al*., 1998), but also on the number of somatic and metacentric chromosomes or on the length of the diploid complement (Ma *et al*., 1995). Some reports state that high Zn concentrations are not strongly genotoxic (Codina *et al*., 2000; Gómez-Arroyo *et al*. 2001; Marcato-Romain *et al*., 2009). The aneugenic and clastogenic action of Zn was also evidenced in other species like wheat, black cumin, onion, sugarcane (El-Ghamery *et al*., 2003; Jain *et al*., 2010; Shaymurat *et al*., 2012; Somesh *et al*., 2005), but a connection between Zn concentration and aberration frequency was not always noticed. Similar studies were carried out by Bin Hussein *et al*. (2002) on the toxicology of Al_2O_3, SiO_2, ZnO, and Fe_3O_4 on *Arabidopsis thaliana*. The results showing that ZnO nanomaterials at $400mgL^{-1}$ capable of inhibiting germination. Several researchers described the key role of Zn/ZnO nanomaterials for plant growths and yield (Bin Hussein *et al*. 2002). For example, higher plant mostly absorbs Zn as a divalent cation (Zn^{+2}), which acts either as a functional, structural, or as the metal component of enzymes or are gulatory cofactor of numerous enzymes.

Table (2) The impact of different Zn NPs concentrations on mitotic index and chromosomal aberrations.

Type of aberrations	Concentrations of Zn NPs and time of treatments									
	8 hours			16 hours			24 hours			General mean
	1.0	1.5	2.0	1.0	1.5	2.0	1.0	1.5	2.0	
Number of Observed Cells	2010	1901	1846	1998	1430	1631	1921	2123	1945	1867.2
Number of Divided Cells	1311	1299	1217	1000	897	902	860	841	654	997.9
Number of Prophase Cells	820	841	764	621	585	602	559	601	411	644.9
Number of Metaphase Cells	185	169	155	123	120	115	99	106	95	129.7
Number of Anaphase Cells	230	188	181	135	135	127	129	107	111	149.2
Number of Telophase Cells	110	91	86	77	63	67	66	50	44	72.7
Mitotic Index (%)	65.22	68.33	65.93	50.05	62.73	55.30	44.77	39.61	33.62	54.0
Number of Abnormal	153	165	154	143.5	165	155.9	174	203	247	173.4
Percentage of aberration	11.67	12.70	12.65	14.35	18.39	17.28	20.23	24.14	37.77	18.8
Nucleotide Deletion	1	2	6	0	6	8	8	10	10	5.7
Distributed Anaphase	9	8	9	7	8	7	10	11	16	9.4
Multi Polar Anaphase	4	8	4	1	0	0	4	7	4	3.6
Distributed Chromosome	1	1	1	1	3	1	9	10	12	4.3
Lagging Chromosome	2	0	0	1	4	5	9	10	12	4.8
C-metaphase Chromosome	11	7	9	11	14	14	14	20	25	13.9
Gap Chromosome	0	3	0	1	2	0	0	2	1	1.0
Fragments Chromosome	18	19	22	22	14	25	20	24	50	23.8
Bridge Chromosome	0	3	8	8	8	7	3	7	9	5.9
Uncloing Chromosome	30	22	18	20	25	21	3	5	9	17.0
Stickiness Chromosome	23	26	33	30	27	23	20	22	24	25.3
Ring Chromosome	0	2	3	0	2	4	9	9	0	3.2
Multinuclei	25	30	27	22	29	22	25	25	22	25.2
Elongation	11	5	0	1	2	1	5	7	8	4.4
Erosion	9	17	2	5	5	2	15	10	12	8.6

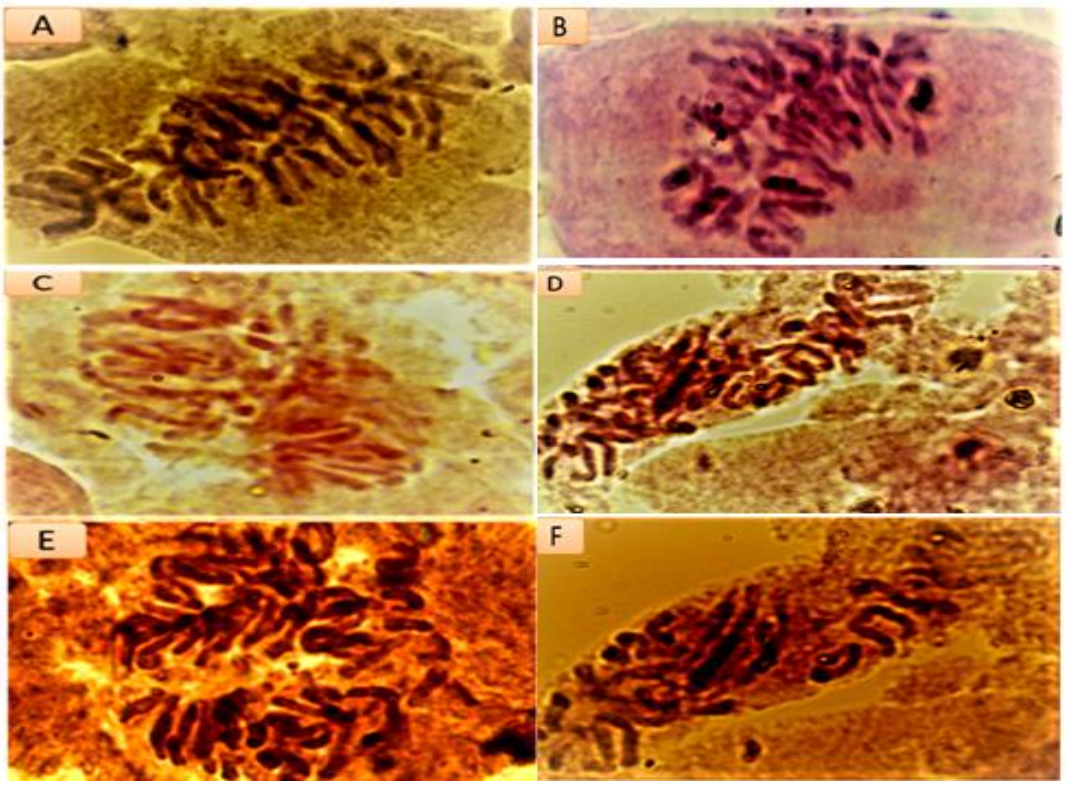

Fig. (1) The effect of different nano zinc (Zn NPs) concentrations on chromosomal aberrations of wheat plants i.e., 2.0 ml after 8 hrs. (a & b) abnormal metaphase with c-phase, chromatids deletion and fragments; 1.5 ml after 16 hrs. (c &d) showing ring chromosome, sticky ends, fragments and lagging chromosome; 2.0 ml after 24 hrs. (e &f) showing distributed anaphase, fragments, chromatids deletion, lagging chromosome and C-phase.

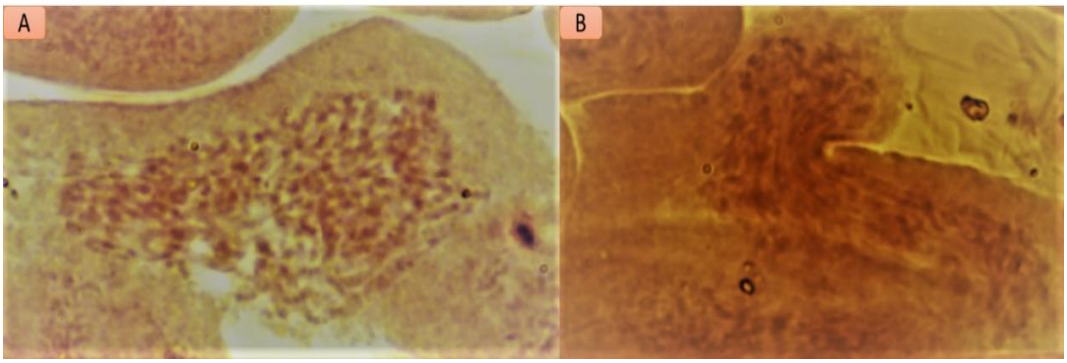

Fig. (2) Explosion in cell wall under high Nano-amino zinc (Zn NPs) concentrations (2,0 ml after 24 hrs.) of Iraqi wheat.

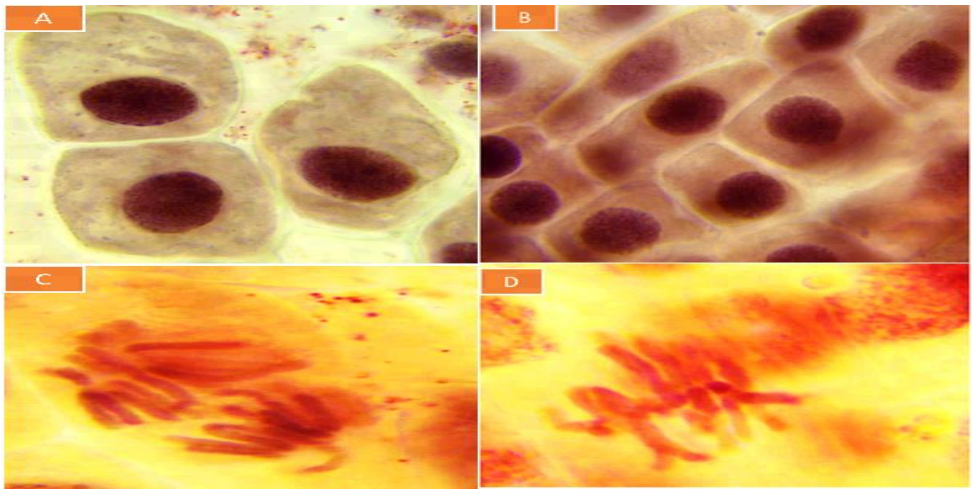

Figure (3) The effect of different nano zinc (Zn NPs) concentrations on chromosomal aberrations of wheat plants i.e., 1.0 ml after 8 hrs. (a & b) normal prophase; 1.5 ml after 24 hours (c &d) showing ring chromosome, sticky ends, fragments and lagging chromosome and chromatids deletion, lagging chromosome and C-phase.

Conclusion:

Nano zinc (Zn NPs) could enter freely into the cells and interfere in cells normal function. The treated root-tip cells exhibited several types of chromosomal aberrations as Nucleotide Deletion, Distributed Anaphase, Multi Polar Anaphase, Distributed Chromosome, Lagging Chromosome, C-metaphase Chromosome, Gap Chromosome, Fragments Chromosome, Bridge Chromosome, Uncloing Chromosome, Stickiness Chromosome, Ring Chromosome, Multinuclei, Elongation, Erosion.

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