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Impact of Engineered Si Nanoparticles on Seed Germination, Vigour Index and Genotoxicity Assessment via DNA Damage of Root Tip Cells in *Lens culinaris*

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Abstract

Engineered nanoparticles are of great scientific interest due to their wide variety of potential applications in biomedical, agricultural, optical and electronic fields. The present study has been designed to study the effect of Si nanoparticle (SiNP) on plant growth parameters and to detect the possible genotoxicity induced by them. Seed germination results indicated that SiNP at lower concentration promotes seed germination, Vigour index and biomass; however, at higher concentrations they showed deviated results. To study toxicological end points microscopic examination of root tip cells were carried out. The result showed that exposure to the nanoparticle increase the number of chromosomal aberrations significantly. Dose-dependent decrease in Mitotic index (MI) in the treated populations was observed as compared to control. The result suggests potential of nanoparticle in causing genomic instability by impairing mitosis and altering DNA by inducing chromosomal anomalies.

Lower concentration of Si NP can induce positive results on germination and biomass of lentils Si Nanoparticles can penetrate the tissue system through seed or foliar dressing and interfere with the cell division cycle, inducing nanotoxicity.

Keywords: Nanoparticles; Genotoxicity; Chromosomal anomalies; SiNP

Introduction

The phytotoxicity study of ultrafine particles is an emerging issue to elucidate its potential impacts on plant system. Accidental or incidental release of commercial products like cosmetics and medicines which contain manufactured nanomaterials (MNMs) has become a real threat to the environment [1,2]. Significant increase in the consumption of nanoparticles in the recent years have raised safety concerns, regarding their potential effects [3,4] specially on plants and animals. Unique properties of NPs include very large specific surface area, high surface energy and quantum confinement [5]. Among wide uses of nanoparticles, the relationship between engineered nanoparticles and agriculture has been particularly attractive, considering the vital agricultural and environmental risks and their potential application as novel fertilizers. Si is the second most abundant element in the earth crust after oxygen and is known to be beneficial or even essential for plants especially under stress conditions. The toxicity of silica depends on the particle size, concentration and exposure duration. Relevant literature on the role of nano Si in generating cytotoxicity are absent; therefore, an effort has been made to study the role of Si nanoparticle (SiNP) in inducing phytotoxcity in lentils.

Presently many nanoparticles are being screened for biological and ecological toxicity. Response of these nanoparticles may vary greatly according to different plant species and there are reports of both positive and negative effects on the plants. The percentage of germination and growth was increased by Silica nanoparticles in braod bean [6]. Similarly, positive impact of Si nanoparticle on seed germination potential was observed in tomato [7]. Ghodake et al. [8] observed phytotoxicity in Allium cepa induced by application of Cobalt and ZnO NPS, possibly these nanoparticles penetrated radically so that they got adsorbed and accumulated in the root system and damage the cellular metabolism and stages of cell division. Therefore, overall profile of nanoparticles is quite unpredictable and poorly understood.

The radical of the seed emerges by rupturing the seed coat and is exposed to the test solution; therefore, toxicity test should be performed during seed germination and seedling elongation. Interaction of nanoparticles occurs at molecular level in the living cells and nano agriculture involves exploitation of these nanoparticles in agriculture with the aim that these particles impart some beneficial effects on the crop [9]. Mitotic studies in plants is thus considered as a reliable index in assessing genotoxicity in plants. Therefore, present study was conducted to find out the possible effects of NPs during plant germination and its role in causing genotoxicity by interfering with the normal mitosis. The test plant in the present study was Lentil. It is an important rabi crop in India because of its rich protein content. They are the source of inexpensive protein for vegetarian populations in many parts of the world, especially in West Asia and the Indian subcontinent. Seeds of lentils were treated with Silica NP and its impact on seed germination, seedling vigour and root tip cells were observed for induced genotoxicty studies.

Materials and Methods

The engineered nanoparticle that used here was Silica under the name of AEROSIL 300, were suspended in distilled water and dispersed by sonicator for 30 min. the low solubility and dispersibility of ENPS complicate the process of nanoparticle test solution, therefore

to avoid aggregation of the particles, small magnetic bars were placed in the suspension for constant stirring. Different doses of NP like 25, 50, 75, 100, 200, 300 µg/mL were prepared.

Seeds of lentil were procured from National Seed Corporation, India. The average germination rate in control was found to be 95%. Seeds were surface sterilized with 5% NaOCl for 10 min and Whatmann No.1 filter paper was then placed into each Petri dish and 10 mL of different concentrations of the nanoparticle suspensions were added in each plate. The seeds were then transferred to the Petri dish, with 50 seeds per dish and kept in incubator at controlled temperature of 25 ± 1°C. The parameters such as seedling height, root length and vigour index were assessed after 14 days of germination. Vigour index was calculated by the procedure as described by Abdul Baki and Anderson. The data was analyzed statistically.

Root tip cells of the treated populations were used to study the cytological endpoints such as mitotic index (MI), chromosomal aberrations (CA) and micronucleus induction (MN). The root tips of lentils were fixed in Aceto alcohol (3 alcohol: 1 acetic acid) and hydrolyzed in 1 N HCl at 50°C for 5 min and were stored in 70% alcohol. Root tips were then squashed and stained in a 2% aceto carmine stain. Abnormalities were counted in each phase of mitosis. Mitotic aberrations scored were chromatid breaks, fragments, bridges lagging chromosomes, stickiness, C-mitosis etc.

Data was analysed statistically by software SPSS 17 for Windows 7. One-way analysis of variance (ANOVA) with p-values less than 0.05 were considered as statistically significant.

Characteristics of nano-silica colloids

Samples of commercially available hydrophilic fumed Silica nanoparticle were obtained under the name of AEROSIL 300, manufactured by Evonik industries (Germany). According to manufacturer's data, the physical characteristics of the particles include specific surface area/BET (Brunauer-Emmett-Teller Theory) surface area 300 ± 30 with a PH value of 3.7-4.5.

Results

The commercial application of nanoparticles has increased greatly in recent years and had become a matter of great concern, particularly when the impacts of nanoparticles on the environment are unknown. Mechanism of interactions of nanoparticles and biological system at molecular level are yet under study, therefore in present study, we investigated the impact of SiNP application on seed germination, Vigour Index and Mitotic Index of Lentil. Germination of SiNP dressed lentil seeds started from 3rd day after sowing in control. Minimum germination (88.26%) was recorded at 300 ppm of NP, where the germination was delayed from third day to 6th day. The results showed that seed germination and seedling growth decreased with increasing concentrations of Si. The highest germination percentage (96.08%) was recorded at 25 µg/mL. SiNPs had a toxic effect on lentil seedlings at higher concentrations as shown in the root length values. Significant decrease in the root length was observed at 200 and 300 µg/mL, resulting into lengths of 2.25 and 2.01 cm respectively. Similar significant decreasing trend for shoot length was also recorded at higher concentrations. SINP has a tendency to inhibit the fresh weight except at lower concentrations (25 and 50 µg/mL), where it increased over control (Table 1). The mean performance of seedling vigour index (I) with respect to seedling length ranged from 1840.89 to 1060.00. Maximum seedling vigour index mass with respect to dry weight (Vigour Index (II) was recorded at 25 and 50 µg/mL of NP (Table 1).

Conc.	Germination (%)	Root length	Shoot length	Seedling Wt(fresh)	Seedling Wt(Dry)	VI (I)	VI (II)
Control	95.72	2.83 ± 0.51	14.0 ± 3.74	1.16	0.78	1610.96	74.66
25 µg/mL	96.08	3.50 ± 0.89	15.66 ± 1.86	1.58	0.97	1840.89	93.19
50 µg/mL	96.00	3.33 ± 0.87	14.80 ± 2.28	1.34	0.86	1740.48	82.56
75 µg/mL	95.11	2.50 ± 0.44	12.33 ± 2.25	0.99	0.65	1410.48	61.82
100 µg/mL	94.21	2.25 ± 0.27	12.30 ± 3.66	0.97	0.61	1370.75	57.46
200 µg/mL	90.24	2.25* ± 0.21	11.80* ± 2.50	0.89	0.5	1267.87	45.12
300 µg/mL	88.26	2.01*± 0.25	10.00* ± 1.37	0.8	0.39	1060	34.42
VI=Vigor Index							

Table 1: Effect of SINP on germination percentage, seedling length and vigour index in Lentil. Asterisks indicate significant differences from the control. P<0.05 in One-way ANOVA.

To test genotoxicity potential of nano-Si on test plan, root tip squashes stained with acetocarmine were prepared to study mitotic divisions in root tip cells. Microscopic observations of root tip cells of control plant revealed a large number of dividing cells showing normal mitosis in control; however, the treated plants showed abundant abnormalities. The mitotic abnormalities which were frequently observed in the treated populations were stickiness, c mitosis, laggards, Anaphasic Bridges and micronuclei (Figure 1).

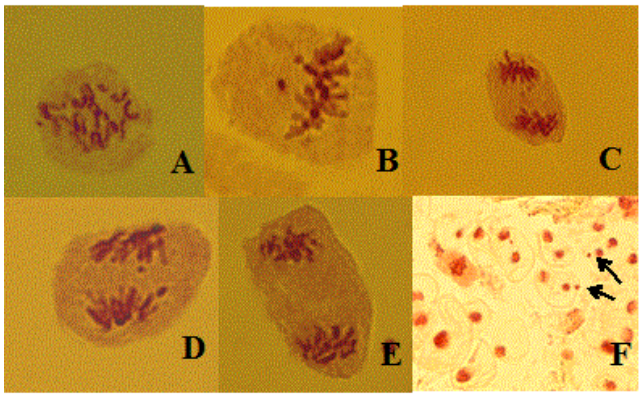

Figure 1: A. C-Mitosis, B. Stray Chromosomes at metaphase, C. Laggards at anaphase, D. Multipolar Anaphase, E and F, Micronucleus. A. C- Mitosis B. Stray Chromosomes at metaphase C. Laggards at anaphase D. Multipolar Anaphase E, F. Micronucleus

These abnormalities are significantly higher at Anaphase and Telophasic stages of treated cells at a concentration of 200 and 300 µg/mL. The study revealed frequencies of anomalies at different stages of mitosis which are listed in Table 2.

Mitotic index was used to determine the rate of cell division. The slides prepared for the assessment of structural chromosomal anomalies were used for calculating the mitotic index (Table 2). The mitotic indexes obtained from control and treated plants with nanoparticles are presented in Figure 2.

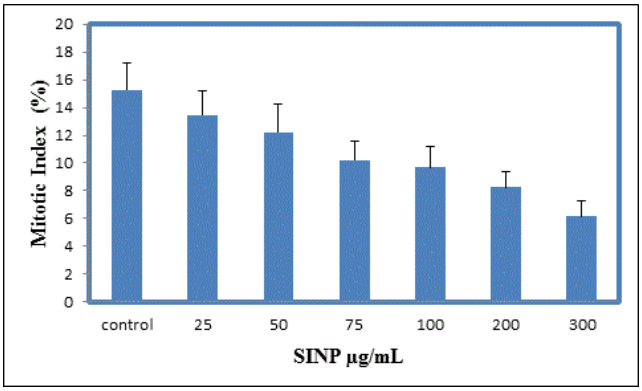

Figure 2: Effect of Si NP on Mitotic Index in root tip cells of *Lens culinaris*. Data represents Mean ± SD.

Results indicate that mitotic index values tend to decrease with the increasing concentrations of NP (Table 2).

Concentrations	Prophase	Metaphase	Anaphase	Telophase	Total cells	Dividing Cells	MI (%)
(mg/ml)	N ± SD A ± SD	N ± SD A ± SD	N ± SD A ± SD	N ± SD A ± SD			
Control	92 ± 0.29 00	86 ± 0.34 4 ± 0.28	64 ± 0.21 2 ± 0.11	80 ± 0.20 00	898	218	24.27
25	78 ± 0.31 4 ± 0.28	72 ± 0.86 5 ± 0.33	53 ± 0.14 8 ± 0.28	70 ± 0.54 10 ± 0.85	858	285	33.52
50	69 ± 0.18 7 ± 0.64	65 ± 0.52 5 ± 0.08	40 ± 0.23 15 ± 0.21	60 ± 0.21 1 2± 0.72	874	290	33.18
75	58 ± 0.24 8 ± 0.23	60 ± 0.12 6 ± 0.53	28 ± 0.52 2 5± 0.63	39 ± 0.10 18 ± 0.19	915	286	31.25
100	3 4 ± 0.16 10 ± 0.27	4 8 ± 0.16 8 ± 0.62	18 ± 0.25 27 ± 0.71	28 ± 0.23 21 ± 0.14	864	245	28.35
200	21 ± 0.41 18 ± 0.14	24 ± 0.74 11 ± 0.74	12 ± 0.22 38 ± 0.12	11 ± 0.70 27 ± 0.21	798	298	37.34

Table 2: Frequency of Abnormalities induced by Si Nanoparticle on different phases of Mitosis.

Discussion

Recent studies have shown the physiological responses of plant seedlings to nanoparticles during germination, but the effect of nanoparticle on seedling growth and germination varied significantly among the plants [10]. Mechanism of nanotoxicity is still unknown; however, possibly it could be due to the chemical composition, structure, size and surface area of the nanoparticles [11]. It is also believed that nanoparticles interact with the plant body through surface adsorption or traversing through small openings in the plants [12], presence of nanoparticles on the root surface interferes with the surface chemistry of the roots in such a way that it effects the interaction of roots with their environment. Moreover, small sized nanoparticles cause toxicity even at lowest concentration due to its easy uptake and translocation inside the plant [13]. Toxicity of nanoparticles may also be attributed to two different actions: (1) chemical toxicity based on the chemical composition, e.g., release of (toxic) ions; and (2) stress or stimuli caused by the surface, size and shape of the particles [14,15].

Seed germination and seedling elongation are frequently used for phytotoxicity test with several advantages such as sensitivity, simplicity, low cost and suitability for unstable chemicals or samples [16,17]. In the present investigation, SiNPs stimulated the growth of roots and shoots at lower concentrations (25 and 50 µg/mL) showing that relatively low concentrations could promote the seedling growth. These positive responses could be due to the amplified uptake of water and nutrients by the seedlings at lower concentration [18]. However higher

concentrations as selected randomly showed a decrease in seedling germination, seedling length, and biomass (both fresh and dry). It is clear that the physicochemical properties, as well as the structure and morphology of nanomaterials have a high influence on toxicity [19]. Since the size of nanoparticles are so small, after being released in the environment, they interact with air and start reacting (photolysis and oxidation) with other components [20]. Ultimately releasing toxic by-products, which is harmful to the living beings and environment.

To better characterize the toxicity potential of SiNP on the test plant, mitotic studies of root tip cells were performed as a reliable index of genotoxicity. Different types of chromosomal anomalies were observed with different concentrations of Si nanoparticles, which increased with the increasing concentrations, showing their clastogenic effect.

Roots tips of treated cells showed abnormalities such as stickiness, laggards, bridges, fragments, C mitosis and micronuclei (MN). Frequent anomalies were observed at 300 µg/mL suggesting its greater tubergenic effect as compared to control. Occurrence of these abnormalities suggests their clastogenic, aneugenic and tubergenic effects. Stickiness at prophase and metaphase appears as a consequence of improper folding of chromatin fibres or due to altered pattern of organisation of nucleoproteins and depolymerisation of DNA [21]. The formation of chromatin bridges at anaphase were attributed to unequal exchange of chromatin material, resulting into the formation of dicentric chromosomes which are pulled equally to both poles at anaphase [22]. MNs were formed as a result of chromosome breakage (clastogenic agent) or whole chromosomes (aneugenic agent) that were not included into the main nucleus during the cell division cycle [23].

It was hypothesised that occurrence of root tip abnormalities might be due to the fact that the root cells were the first target tissue to encounter nanoparticles therefore they show abnormal mitotic division. SiNP at higher concentration encourages production of ROS through disruption of mitochondrial respiratory chain which can lead to changes in DNA, these changes induces genotoxicity and could cause alteration in the encoded protein and gene expression.

Conclusion

The present study has been designed to evaluate the supposed role of NP in sustainable application of nanotechnology. The result indicates that Si nanoparticles can possibly penetrate the plant system and interfere with the cell division cycle, ultimately alter the DNA and proteins synthesis related to it and inducing mutations. Lower concentrations of NP increases seedling germination and vigour index as evident from the results. This indicates that nanoparticles can be supplied to the crops either through foliar application or by seed dressing with monitored doses to get the positive results. The results suggest a need for administration of a safe dose of nanoparticle in the environment and toxicity focused research. These observations will help to further understand the role of nanoparticles in inducing phytotoxicity, their sustainable use and disposal and at which concentrations they are useful.

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