Research Article

Higher toxicity of nano-scale TiO_2 and dose-dependent genotoxicity of nano-scale SiO_2 on the cytology and seedling development of broad bean *Vicia faba*

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Abstract
Since nanotechnology entered the field of agriculture, its safety impact on crops has been a high priority interest. Here, we aimed to evaluate the effect of two different types of nanoparticles (n-), n-SiO_2 and n-TiO_2, on the above- and below-ground growth and the root-tip cell mitosis of broad beans (*Vicia faba* L.), one of the major carbohydrate food sources as well as an ecotoxicological model plant. Seeds were soaked in n-SiO_2 and n-TiO_2 each at different concentrations (25, 50 and 75 mg/L) for 24 h. Nano-TiO_2 decreased vigor index, reflecting shorter shoots at all concentrations studied. By contrast, germination percentage and root length were not affected by any treatments. Cytological analysis suggested no significant difference in mitotic index (index for cell division activity) from the control. However, total chromosomal aberrations (%) were increased dose-dependently by n-SiO_2 and dose-independently by n-TiO_2. Also, different types of chromosomal abnormalities were induced by the nanomaterials; n-SiO_2 induced bridges at 50 and 75 mg/L, whereas n-TiO_2 induced breaks at 50 mg/L. In addition, cells in prophase were more frequently observed and those in anaphase less frequently seen with decreasing n-SiO_2 concentrations. We concluded that n-TiO_2 was more toxic than n-SiO_2 for broad bean chromosomes and early plant development at the concentrations studied. Finally, our review indicates the lack of evidence of germination enhancement by n-TiO_2 in Poaceae, a large monocotyledon family, which may require further attention.

Keywords Chromosomal aberration · Eco-toxicology · Genotoxicity · Mitotic index · Nano fertilizer · Nano particle · Root and shoot length · Seed germination · Silica · Titanium dioxide · Vigor index

1 Introduction

Use of nano-scale materials (1–100 nm) has been widespread especially in the last half-century and grew rapidly through all fields involving food as well as various industrial products (e.g., cosmetics, toothpaste, paints, electronics, and pharmaceuticals) [34] which lead to daily human exposure. Since they have unknown characteristics and the data on their potential toxicity is still limited [57], their toxic effect including genotoxicity must be studied. Since these materials entered the field of agriculture as nanofertilizers and nanopesticides, the need to study their effect on plants which are the easiest models to study nano-scale material toxicity (where the interaction between both of them is an effective aspect to assess their predictable dangerous side effects) has been paramount.

Nano-scale materials such as nano-SiO_2 (n-SiO_2) and nano-TiO_2 (n-TiO_2) can affect early plant development

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(Tables S1 and S3). Concentration, particle size and structure of nanomaterials are known to affect plant growth differently. Also, these materials can exert genotoxicity by direct and indirect mechanisms [33]. They can penetrate plant cells [26] to interrupt cell division causing chromosomal abnormalities and cell degeneracy, for example in *Lens culinaris* (lentil) and *Allium cepa* (onion) [24, 36, 38, 51], and can generate reactive oxygen species (ROS) which cause DNA damage and cell death [37]. Nano-scale materials show high toxicity and are believed to be more toxic than their bulk material [16]. Plant root-tip cells treated by such materials exhibited many types of chromosomal abnormalities, such as breaks, lagging, disturbance, spindle dysfunction, stickiness, fragments, gaps and multipolarity in mitotic and meiotic cells [1, 9, 40].

Nano-SiO_2 which is promising for biological applications due to its excellent biocompatibility and large-scale synthetic availability could stimulate plant growth (Table S1) and is believed to enhance it at low to medium (50–800 mg/L, [3] or high (2000–14,000 mg/L, [48] concentrations. However, this may involve a genotoxic effect on the plant cell mitosis and chromosomal abnormalities [50] (Table S2).

Nano-TiO_2 has both positive and negative effects on plant growth [8, 22, 62] (Table S3). Therefore, it must be used gingerly until relevant data enable its safe utilization [52]. Its genotoxicity depends on its particle size and crystalline structure [8, 29] (Table S4); Exposure to the n-TiO_2 is capable of inducing genotoxicity in the plant systems even at a low concentration (12.5 mg/L) due to the internalization of the particles and the oxidative stress [36]. Bulk materials of TiO_2, however, showed higher toxicity on *V. faba* than its nano-forms [7], which may need further tests.

Broad bean (*Vicia faba* L.) (Fabaceae) is one of the most important model plants for ecotoxicology studies [39] and also an important crop as a carbohydrate food source. This work was established to test.

- n-SiO_2 and n-TiO_2 effects on *V. faba* growth through seed germination, root and shoot lengths as well as vigor index.
- the genotoxic effects of n-SiO_2 and n-TiO_2 on different types of chromosomal aberrations for six homogeneous chromosomes of *V. faba* root-tip cells as well as mitotic cell division phases.

2 Materials and methods

2.1 Nano-scale materials

Nano-scale silicon dioxide (n-SiO_2) and titanium dioxide (n-TiO_2) (anatase) were purchased from Nanotech Egypt Co., Egypt. For visualization purpose only, osmium coating was applied to these nano-scale materials and observation was made under a scanning electron microscope (SEM) (SU8000, Hitachi Hitechnologies) at the Center of Advanced Instrumental Analysis, Kyushu University. Both n-SiO_2 and n-TiO_2 were suspended in double distilled water by sonication for 30 min before use to make concentrations of 25, 50 and 75 mg/L.

2.2 Seed material

Seeds of commercial broad bean (*V. faba*) variety Sakha 1 were obtained from Food Legumes Research Section, Sakha Agricultural Research Station, Egypt.

2.3 Experimental procedure

Seeds were surface sterilized with 2.5% sodium hypochlorite (NaOCl) for 3 min, and then were rinsed 3–4 times with distilled water followed by immersing in distilled water for 3 h. The seeds were then soaked for 24 h in three different concentrations 25, 50 and 75 mg/L each of n-SiO_2 or n-TiO_2 solution, or in distilled water as the control (0 mg/L). After treatment, the seeds were thoroughly washed at least three times with distilled water. The randomized complete block design was used in three replications for both materials. Experiments were carried out at the Laboratory of Genetics Department, Faculty of Agriculture, Kafrelsheikh University, Egypt.

2.3.1 Germination, seedling lengths and vigor index

To study the effects of n-SiO_2 and n-TiO_2 on seed germination, 15 seeds per replicate were allowed to germinate and grow in a 15 cm diameter Petri dish (four dishes/treatment) lined with filter paper (Whatman No. 1) moistened with distilled water. The Petri dishes were placed in a growth room controlled at 25 ± 1 °C and 12:12 h light:dark photocycle. The seeds were considered germinated when the radicle length reached 3 mm. Germination percentage was inspected at the seventh day for the treated and control seeds as described by Al-Mudaris [2] as follows:

$$\text{Germination percentage} = (\text{number of germinated seeds/total number of seeds}) \times 100 \quad (1)$$

Ten seeds per replication were allowed to grow in pots supplied with peat moss after treatments. Three replications per treatment were prepared. Then, root and shoot lengths were measured in centimeters after 14 days as the mean of succeeded germinated seedlings.

The values of germination percentages in addition to root and shoot lengths were used to calculate the vigor index according to the equation of Dahindwal et al. [10] as follows:

$$\text{Vigor index} = (\text{shoot length} + \text{root length}) \times \text{germination percentage}/100 \quad (2)$$

2.3.2 Cytological analysis

Root tips (1.5–2 cm in length) of germinated seeds were cut and fixed in a fresh solution of glacial acetic acid and absolute ethanol at the ratio of 1:3 for 24 h and then stored in 70% ethanol at 4 °C until use. For cytological analysis, roots were boiled in 45% glacial acetic acid to break connections among cells and facilitate mashing of root tips. Then, about 1 mm from excised root tips were stained with 2% aceto-carmine [11] and squashed on a slide to be examined (about 1000 cells per replicate) under a light microscope at × 1000 magnification. Mitotic index was calculated for each replicate as follows:

$$\text{Mitotic index} = (\text{number of dividing cells}/\text{number of dividing and nondividing cells}) \times 100 \quad (3)$$

The number of abnormal cells (showing chromosomal aberration) relative to dividing cells was calculated for each treatment:

$$\text{Percentage of abnormal cells} = (\text{number of abnormal cells}/\text{number of dividing cells}) \times 100 \quad (4)$$

2.4 Statistical analysis

Data from each treatment with three replicates were analyzed by nonparametric Wilcoxon/Kruskal–Wallis tests and then by posthoc Tukey tests for multiple comparisons using JMP13.2.1. The results were presented as mean ± SD.

3 Results and discussion

The particle sizes of n-SiO_2 and n-TiO_2 were 119.1 ± 2.8 and 283.6 ± 15.9 nm, respectively (mean ± SD, n = 10 for each) (Fig. 1).

3.1 Effects of n-SiO_2 and n-TiO_2 on germination and plant growth

All three tested concentrations of both materials, n-SiO_2 and n-TiO_2, did not affect germination percentage ($\chi^2 = 12.13$, df = 6, P = 0.0590, Fig. 2). For n-SiO_2, similar results were obtained at the concentrations of 90 and 180 mg/L on *V. faba* [42] and at even higher concentrations (400–4000 mg/L) on *Arabidopsis thaliana* [31]. On the other hand, n-SiO_2 decreased the germination percentage of *A. cepa* at 540, 810 and 1820 mg/L [51] and *L. culinaris* by increasing the concentration up to 300 mg/L [24].

Similar to our result, n-TiO_2 treatments did not influence the seed germination of *Triticum aestivum* (wheat) [18], *Hordeum vulgare* (barley) [32] and various plant species even at high concentrations (Table S3). By contrast, n-TiO_2 decreased germination percentage of *Z. mays* (at 50 and 100 mg/L, 5 d exposure, [61], *Oryza sativa* (rice) (at 2000 mg/L, 10 d exposure, [21], *Spinacia oleracea* (spinach) (at 6000 and 8000 mg/L, 2d exposure, [62] and various other plants when typically exposed to high concentrations or for a long time (Table S3). On the contrary,

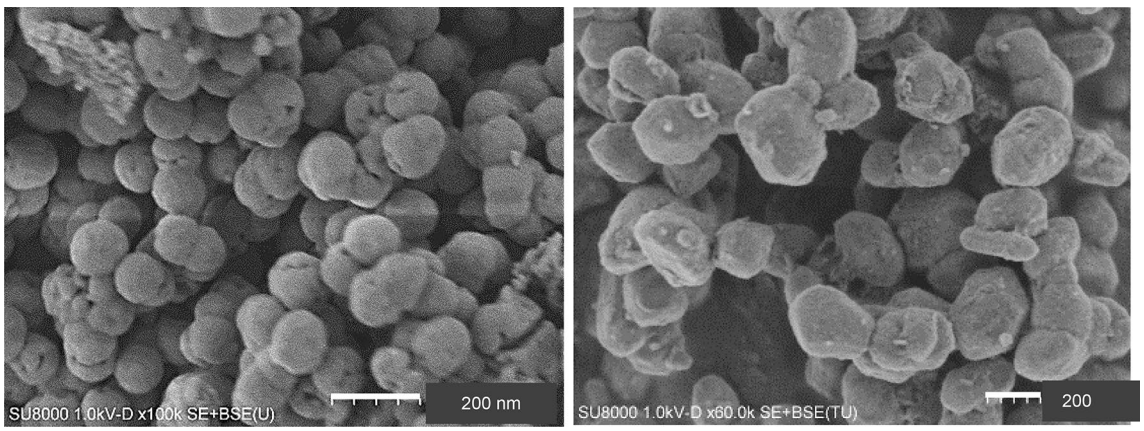

Fig. 1 Scanning electron microscope (SEM) images of nano-SiO_2 (left) and nano-TiO_2 (right)

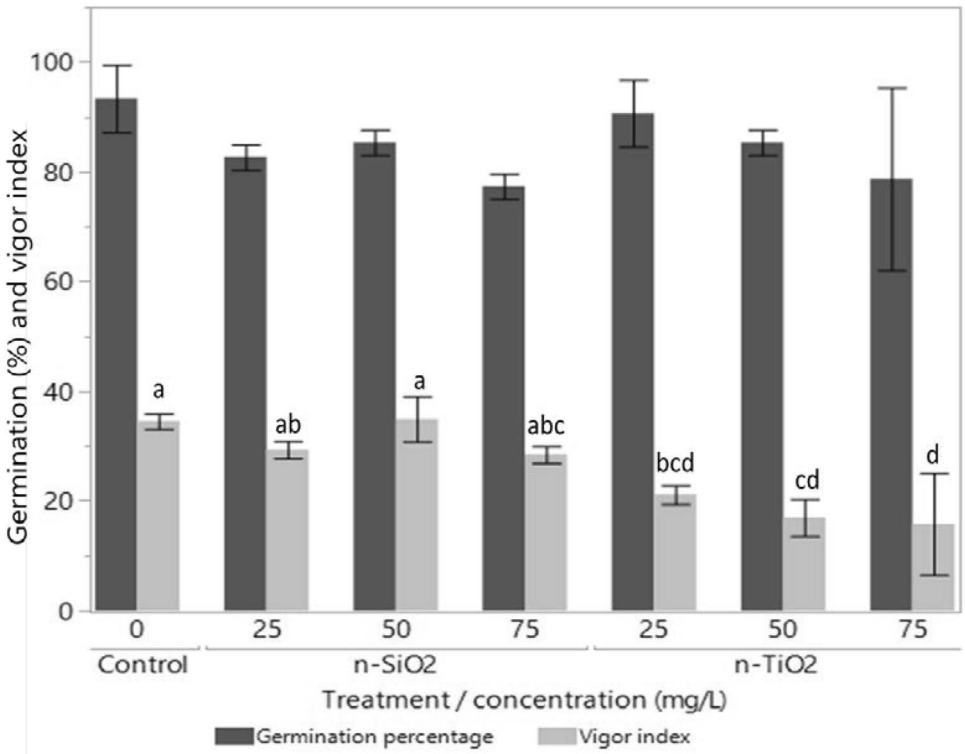

Fig. 2 Germination percentage and vigor index (mean ± SD) of *Vicia faba* treated with nano-SiO_2 and nano-TiO_2. Bars with the same letters did not differ significantly for vigor index. No significant differences in germination percentage among different treatments

n-TiO_2 at its lower concentration range can enhance germination of various plants except those belong to Poaceae (Table S3). The lack of evidence of germination enhancement by n-TiO_2 in the large monocotyledon family, Poaceae, may need further attention and investigation.

As shown in Fig. 3, both nanomaterials did not show any effect on root length ($\chi^2 = 7.49$, df = 6, P = 0.28, Fig. 3). By contrast, n-SiO_2 moderated impact of salinity on the root length of *L. culinaris* [44] and *Cucurbita pepo* [49]. Significant difference was found among different treatments on shoot length ($\chi^2 = 17.08$, df = 6, P = 0.009, Fig. 3); Nano-TiO_2 shortened shoots at all concentrations studied, whereas n-SiO_2 did not affect their length. The latter result with n-SiO_2 is in agreement with the results on *Oryza sativa* (rice) and *Zea mays* (maize) despite higher concentration (2000 mg/L) [60]. The former (shortened shoot by n-TiO_2) is similar to that observed in *Mentha piperita* [46]. On the contrary, n-TiO_2 had no effect at lower concentrations of 5–20 mg/L on *T. aestivum* [14] or even at higher concentrations of 200–4000 mg/L on *Z. mays* [6].

All n-TiO_2 concentrations significantly reduced the vigor index compared to the control, unlike n-SiO_2 treatment which had no significant effect at any applied concentrations ($\chi^2 = 17.61$, df = 6, P = 0.0073, Fig. 2), reflecting the shoot length decrease by n-TiO_2 (Fig. 3). This is in parallel with the negative effect on the vigor index of *V. faba* using bulk (non-nano) TiO_2 [7] but in contrast to the positive effect on that of *V. faba* using < 10 nm particles [7] and on the vigor index of four out of five plant species studied at 10-20 mg/L or 10–80 mg/L (*Nigella sativa*, *Alyssum homolocarpum*, *Carum copticum* and *Salvia mirzayanii*, [22], Table S3. Since larger particles have larger surface area or surface energy, they tend to be more unstable and thus to form aggregates of the scale of micron rapidly [17, 19]. In the case of *S. oleracea*, n-TiO_2 shows a positive effect on vigor index but at higher concentrations (250–4000 mg/L) and with less toxic crystal structure, rutile (i.e., oxidative stress is mitigated by antioxidant; [4, 62].

3.2 Effects of nano-SiO_2 and nano-TiO_2 on mitotic division in root meristem cells

3.2.1 Mitotic index and mitotic phase

Vicia faba root-tip cells showed different mitotic phases. Both materials at any tested concentrations did not affect the mitotic index, compared to the control ($\chi^2 = 9.56$, df = 6, P = 0.14). However, the mitotic index at the highest n-TiO_2 concentration (75 mg/L) was significantly lower than that at the lowest n-SiO_2 concentration (25 mg/L) (Table 1). The positive effect of n-SiO_2 at the low concentration is in contrast to the previous results on *L. culinaris* at the same concentrations [24] and *A. cepa* at concentrations of 540–1820 mg/L [51]. Although n-TiO_2 may

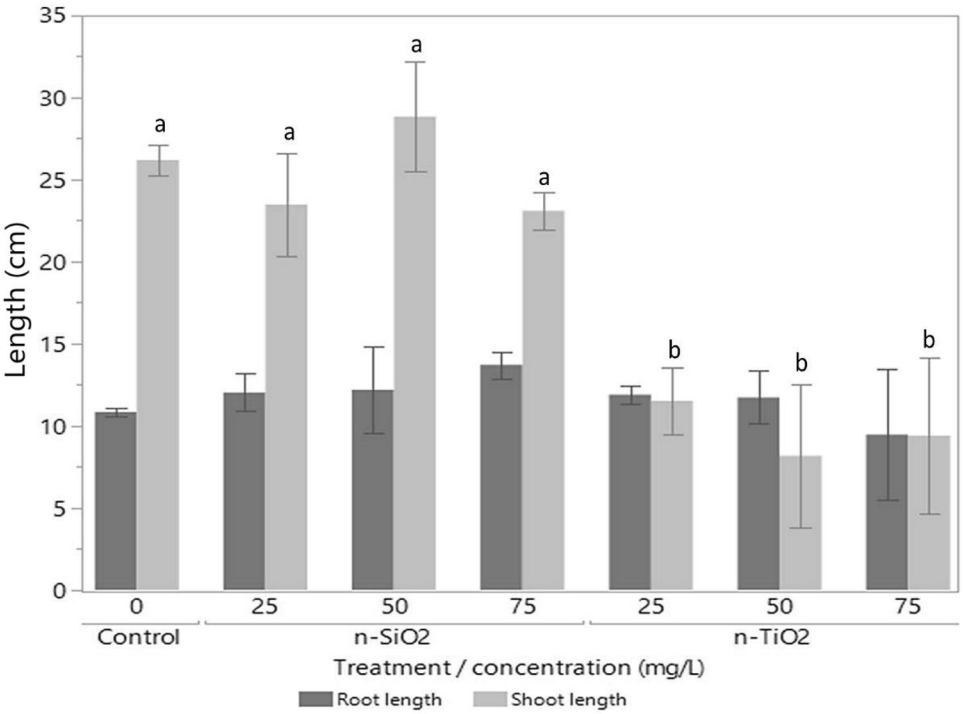

Fig. 3 Effects of nano-SiO_2 and nano-TiO_2 on *Vicia faba* shoot and root lengths (mean ± SD). Bars with the same letters did not differ significantly for shoot length. Root length was not significantly different among treatments

have the potential to decrease cell dividing activity with increasing concentration, these results are, overall, in agreement with earlier studies reporting that even at the high concentrations (> 100 mg/L) mitotic activity was not affected by n-TiO_2 [7, 20, 32]. In *A. thaliana*, expression of more genes were down-regulated than up-regulated by n-TiO_2 (10–50 nm) at the studied concentration (500 mg/L) [56]. By contrast, slightly more genes were up-regulated than down-regulated by n-TiO_2 (< 150 nm) at 100 mg/L [27]. Additionally, n-TiO_2 activated antioxidant enzymes (catalase and peroxidase but not superoxide dismutase), amylase and protease at 10–30 mg/L in *A. cepa* [30], indicating generation of oxidative stress even at its low concentrations.

Proportions of cells in the mitotic phases, metaphase ($\chi^2 = 6.96$, df = 6, P = 0.325) and telophase ($\chi^2 = 12.26$, df = 6, P = 0.0564), were not different from the control and among different concentrations of n-SiO_2 and n-TiO_2. By contrast, those in the mitotic phases, prophase ($\chi^2 = 14.23$, df = 6, P = 0.027) and anaphase ($\chi^2 = 12.64$, df = 6, P = 0.0491), were different among treatments. For cells in prophase, there was a difference between different concentrations of n-SiO_2; a higher proportion was in this mitotic phase at a lower concentration (Fig. 4). This is

Table 1 Composition of mitotic phases (%) and mitotic index of *Vicia faba* root-tip cells treated with three different concentrations of nano-SiO_2 and nano-TiO_2

Treatment	Conc. (mg/L)	Pooled no. of examined cells	Pooled no. of dividing cells	Mitotic phase								Mitotic index (%) (mean ± SD)
				Prophase		Metaphase		Anaphase		Telophase		
				N	%	N	%	N	%	N	%	
Control	0	3276	2984	2620	87.80	93	3.12	119	3.99	152	5.09	91.38 ± 2.70^{ab}
n-SiO_2	25	3419	3317	3101	93.49	71	2.14	46	1.39	99	2.98	97.02 ± 2.05^{a}
	50	2965	2440	2137	87.58	92	3.77	60	2.46	151	6.19	82.80 ± 13.35^{ab}
	75	2983	2533	2191	86.50	91	3.59	99	3.91	152	6.00	83.95 ± 13.95^{ab}
n-TiO_2	25	2803	2493	2250	90.25	95	3.81	48	1.93	100	4.01	88.76 ± 5.44^{ab}
	50	3610	3152	2928	92.89	89	2.82	49	1.55	86	2.73	86.64 ± 14.06^{ab}
	75	3594	2693	2413	89.60	108	4.01	57	2.12	115	4.27	74.99 ± 2.25^{b}

Mitotic index with the same letters did not differ significantly

associated with the high mitotic index of the cells of the root germinating from the seeds treated by n-SiO_2 at this concentration (Table 1). Similar comparison was made in *A. cepa* treated with n-TiO_2 but rather less share of cells was observed in prophase where mitotic index was high [25]. Prophase includes complex processes (chromosome condensation, centrosome movement, mitotic spindle formation, and nucleoli break down) to prepare for cell division. Nano-SiO_2 inactivates proteins by binding and changing their 3D structure but causes no synergistic effect [59]. Various proteins that support these processes during prophase may have been arrested and deterred, or alternatively, promoted by nanomaterials, depending on species. For anaphase, after seeds were treated by low concentrations of n-SiO_2, root-tip cells were less frequently observed in this mitotic phase than the control (Fig. 4).

3.2.2 Chromosomal abnormalities

Both n-SiO_2 and n-TiO_2 increased total chromosomal abnormalities through mitotic phases but in a different way ($\chi^2 = 15.93$, df = 6, P = 0.014). Nano-SiO_2 escalated chromosomal aberration with increasing concentration (Fig. 5). By contrast, all n-TiO_2 concentrations showed the same ability for inducing chromosomal aberration even at the lowest concentration (Fig. 5). This illustrates that n-SiO_2 had less toxic effect than n-TiO_2 on *V. faba* root-tip cells particularly at 25 mg/L. The dose-dependent induction of total chromosomal aberrations by n-SiO_2 in our study contrasts to the increased mitotic index on *L. culinaris* at 200 and 300 mg/L [24] and on *A. cepa* at the concentration of 810 mg/L [51]. On the other hand, application of n-TiO_2 increased aberration frequency in *V. faba* root-tip cells. In this respect, the previous studies on *Z. mays* [7], *A. cepa* [20, 36] and *Vicia narbonensis* [6] recorded a high frequency of chromosomal aberrations as a result of n-TiO_2 treatments. Nano-SiO_2 and n-TiO_2 could disturb both chromosome structure and spindle fibers during mitosis. Different types of chromosomal aberrations were observed in the tested materials, such as stickiness (Fig. 6a, b, e), c-metaphase (Fig. 6d), disturbance (Fig. 6c, f), lagging chromosomes (Fig. 6f, g), fragments (Fig. 6e), bridges (Fig. 6h, i) and breaks (Fig. 6b, f). There were neither chromosomal gaps nor multipolarity observed during mitosis nor micronucleus during interphase.

Chromosomal bridges and breaks occurred differently among treatments; n-TiO_2 had an ability to break chromosomes ($\chi^2 = 14.41$, df = 6, P = 0.025, Fig. 7), which may lead to losing genetic material [45]. Frequency of chromosomal breaks were not different from the control at all n-SiO_2 concentrations, whereas it was higher than the control at 50 mg/L n-TiO_2 (Fig. 7). Nano-SiO_2 promoted chromosome bridges particularly at 50 mg/L, whereas n-TiO_2 did not affect their occurrence ($\chi^2 = 12.69$, df = 6, P = 0.048, Fig. 7). The higher occurrence of metaphase and anaphase chromosomal aberrations such as bridges and breaks may be due to physical interaction to interrupt chromatin structure or chemical interaction with nuclear

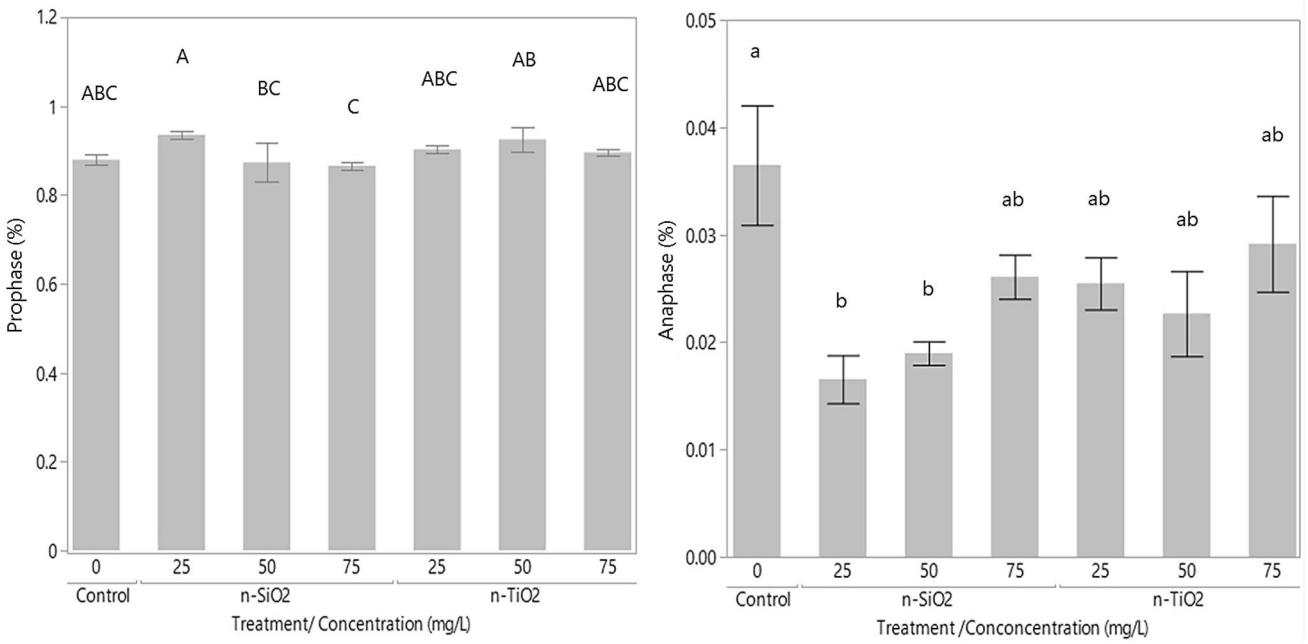

Fig. 4 Composition (mean ± SD) among different mitotic phases of *Vicia faba* root-tip cells treated with nano-SiO_2 and nano-TiO_2. Bars with the same letters did not differ significantly

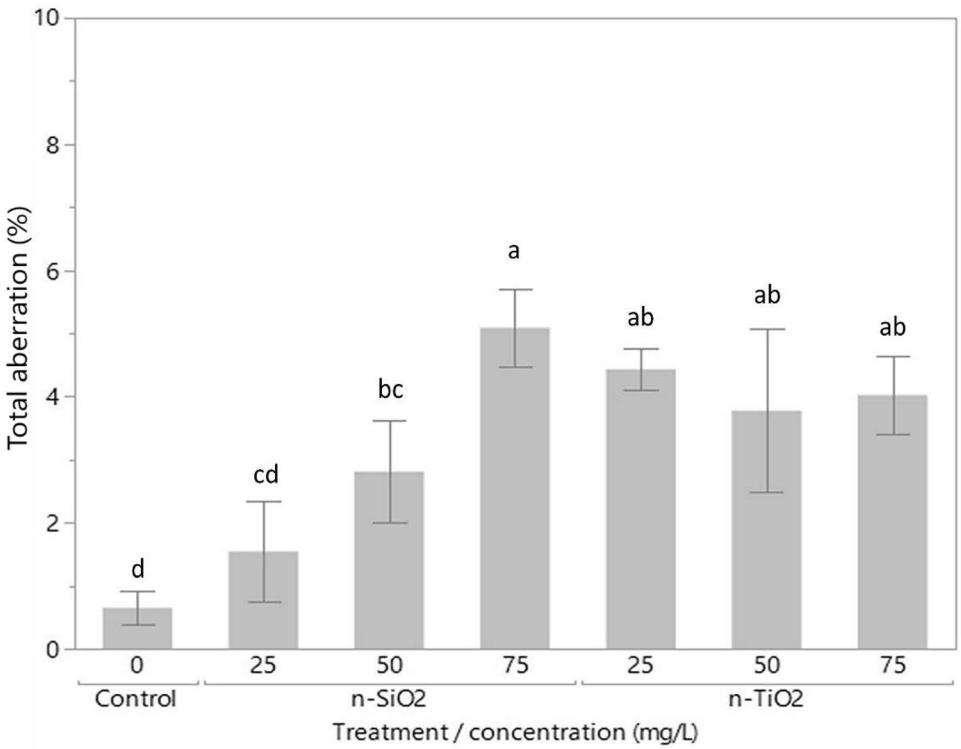

Fig. 5 Frequency of abnormal mitotic cells (relative to total dividing cells) (mean ± SD) of *Vicia faba* root-tip cells treated with nano-SiO_2 and nano-TiO_2. Bars with the same letters did not differ significantly

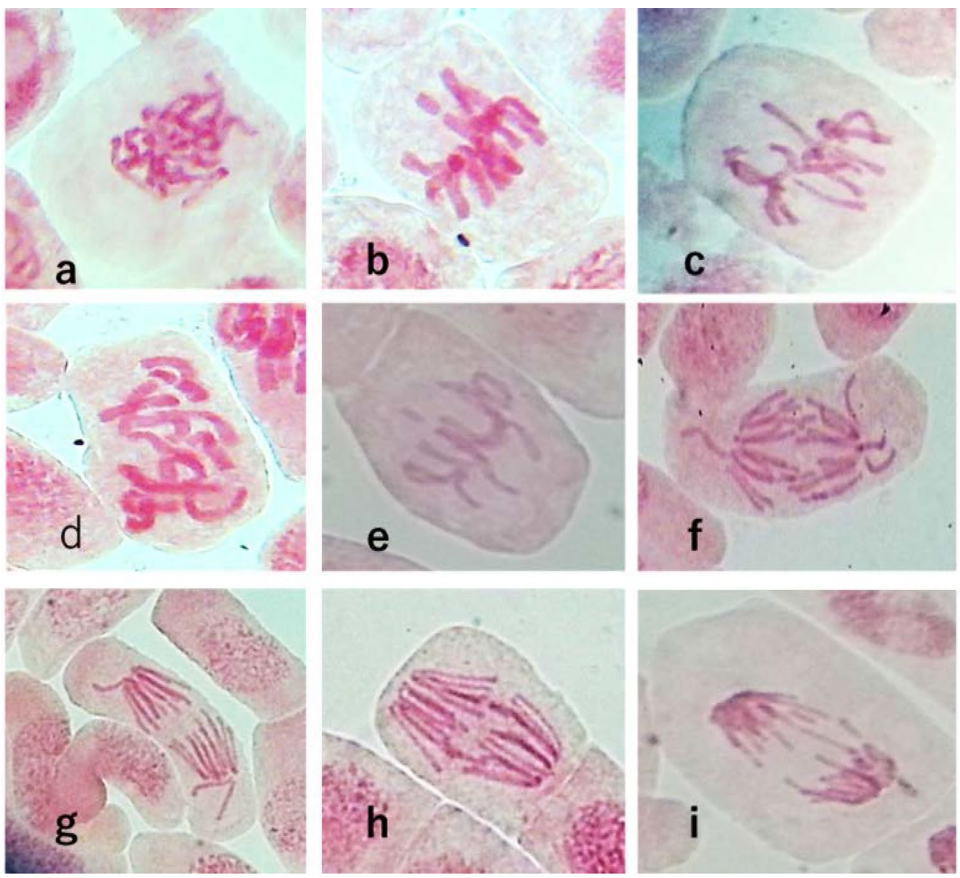

Fig. 6 Types of chromosomal abnormalities observed in *Vicia faba* root-tip cells and induced by application of nano-SiO_2 and nano-TiO_2: **a** sticky prophase, **b** sticky metaphase with break, **c** disturbed metaphase, **d** c-metaphase, **e** fragment in sticky metaphase, **f** disturbed anaphase with breaks and laggards, **g** forwarded laggard in anaphase, **h** bridge in anaphase and **i** bridge in telophase

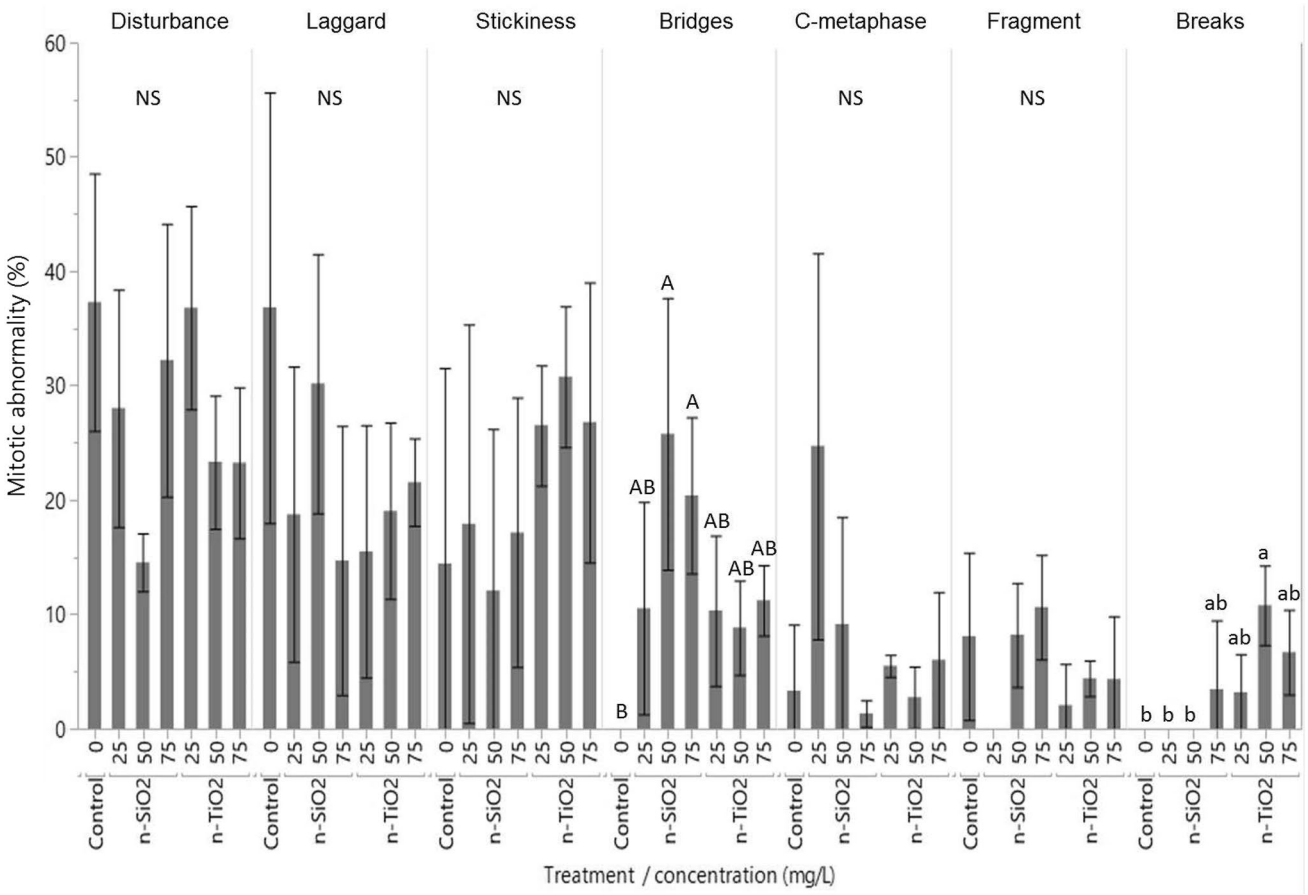

Fig. 7 Frequency of mitotic abnormalities (mean ± SD) in *Vicia faba* root-tip cells treated with nano-SiO_2 and nano-TiO_2. Bars with the same letters did not differ significantly. NS, non-significant

protein and mitotic spindle fibers [12, 33]. By contrast, frequency of the following types of aberrations did not differ significantly from those in the control and among different treatments; disturbance ($\chi^2 = 12.36$, df = 6, P = 0.054), laggard ($\chi^2 = 6.68$, df = 6, P = 0.35), stickiness ($\chi^2 = 5.71$, df = 6, P = 0.45), c-metaphase ($\chi^2 = 9.42$, df = 6, P = 0.15) and fragment ($\chi^2 = 9.84$, df = 6, P = 0.13) (Fig. 7).

To summarize, mitosis in root-tip cells and plant development of broad beans incurred larger damage by n-TiO_2 than by n-SiO_2 even at its low concentrations (i.e., higher chromosomal aberration frequency, higher chromosomal break frequency, and shorter shoots). Mitotic abnormality was induced dose-dependently by n-SiO_2 but not reflected in plant development. Toxicity of nano-scale materials in the early stages of plant growth is likely to be due to the following factors: (1) chemical and physical properties that influence the release of ions or the aggregation of particles in more stable forms and (2) the size and shape of the particles, which determine the specific surface area of these materials [5, 35, 60]. The penetration of metal ions into the cell causes cross-link in the DNA, sister chromatid exchange, and mutations [43]. Nanomaterials also cause clogging of pores and barriers in the apoplastic stream and this reduces photosynthesis, generates ROS and damages DNA structures [13, 55, 58]. In our study, although both nanomaterials induced chromosomal aberrations in the root-tip cells, they did not affect mitotic index, seed germination and root elongation, whereas n-TiO_2 inhibited shoot elongation. This may be partly due to the large particle sizes (> 100 nm) which could prevent the nanomaterials' penetration of the seed coat but might allow the nanomaterials to penetrate the radicle (root) cell wall to be transported to the shoot tissue [32].

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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