Phytotoxicity assessment study of gold nanocluster on broad bean (Vicia Faba L.) seedling

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ABSTRACT

In this study, we investigated the phytotoxicity of gold nanoclusters (AuNCs) on seed germination capacity, physiological response to stress and uptake, and the transport of gold nanomaterials in broad beans. Although similar findings were observed for AuNCs on broad bean root tip cells between micronucleus assays and traditional phytotoxicity experiments, the former was more sensitive and rapid. At experimental concentrations of 0–200 mg/L, the toxicity performance of broad bean seedlings showed an overall positive correlation with the concentration of AuNCs. AuNCs posed a potential risk to the environment at a concentration of 20 mg/L. Moreover, studies on the uptake and transport of gold nanomaterials showed that the smaller the particles entering the bean, the higher the transport coefficient, while materials not entering the bean were mainly adsorbed on the root surface of the bean seedlings by competition with potassium ions.

1. Introduction

With the widespread use of gold nanomaterials [1–4], tremendous attention has been paid to the ecological problems associated with their use. Indeed, plants are an essential part of the ecosystem; growing evidence suggests that the phytotoxic reactions to these engineering nanomaterials can reflect the degree of environmental contamination to a certain extent [5,6]. However, so far, the toxicity mechanisms involved in gold nanomaterials are not yet fully understood, and are generally considered to include: (1) oxidative stress is a vital toxicity mechanism of gold nanoparticles. Due to their large specific surface area, high reactivity and electron density, they can directly react with biomolecules and promote the formation of superoxide anion (O²⁻), which results in the massive accumulation of reactive oxygen species. Moreover, this leads to an imbalance between the production of reactive oxygen species and the detoxification of reaction intermediates or repair of damage produced by the organism, resulting in oxidative stress to the plant [7]. (2) Coordination effect, gold nanoparticles bind to proteins through coordination and non-covalent interactions with them, causing structural changes in proteins, resulting in the inhibition or loss of their functions [8]. (3) Genotoxicity, gold nanoparticles can bind to DNA through their own or released substances, thus producing DNA damage [9]. (4) Adsorption effect, gold nanoparticles synthesized by citric acid encapsulation inhibit root elongation by adsorption on rice roots [10]. In summary, the effects of gold nanomaterials on plants are more complex, so it is necessary to evaluate how to conduct the toxicity.

The safety evaluation of gold nanomaterials on animal cells is often based on the in vitro cytotoxicity assay 3-(4,5)-dimethylthiazol-2-y1)-2,5-diphenyltetrazoliumromide (MTT) [11]. Jumoke et al. showed that HB-AuNPs and MGF-AuNPs exhibited relatively low cytotoxicity against various human cancer cells using the MTT assays [12]. Priyanka demonstrated the non-toxic effect of gold nanoparticles (AuNPs) on normal human lung epithelial cells by observing cell viability [13]. Kadhim et al. also substantiated the safety of low concentrations of AuNPs to embryonic fibroblasts (REF) by the MTT test [14].

Hitherto, no consensus has been reached on the optimal approach to evaluate the toxicity of gold nanomaterials to plants. Most researchers have used conventional tests, including seed germination viability and plant stress response, to determine nanoparticle toxicity to plants. For example, Jakub et al. explored the effect of particle size and concentration of AuNPs on root growth by measuring the root length of Arabidopsis thaliana [15]. Iradm et al. investigated the effect of biosynthetic AuNPs on the growth of wheat plants under salt stress by testing physiological indicators in wheat such as K⁺/Na⁺ ratio, chlorophyll concentration, and defense system [16]. Jaczek et al. investigated the effect of metal-based nanoparticles such as AuNPs on the development and oil gland formation in narrow-leaved...
lavender by measuring characteristic morphological values such as root length, shoot length, and the number and diameter of trichomes [17]. Shi et al. explored the effects of AuNPs stress on the phytotoxicity and physiological mechanisms of mung beans by testing physiological indicators such as germination coefficient, growth and antioxidant enzymes [18]. Although these traditional testing approaches are widely used, they are cumbersome, time-consuming, and labor-intensive. Moreover, plant cell micronucleus assays have been used for rapid detection purposes; for instance, Francisco et al. used the cell micronucleus technique to study the cytotoxic and genotoxic effects on onions by measuring the mitotic index of onion root tip cells treated with the AgNPs preparation Argoits [19]. Abdelsalam et al. used apical cell micronucleus experiments to investigate the effects of nanoparticle fertilizer (Hyper Feed Amino NPs) compounds on the productivity and genotoxicity of two wheat cultivars compared to conventional mineral fertilizers by observing mitosis and chromosomal aberrations in wheat root tip cells [20]. However, the observation of intracellular micronuclei cannot be promoted as a universal method due to the heterogeneity in plant species. Here we harnessed the internationally recognized micronucleation technique of broad bean root tip cells to probe the phytotoxicity of gold nanoclusters (AuNCs) and further determine the results by conventional experiments to establish a rapid method [21]. For the biosafety application of gold nanomaterials reference and attempt to explore a new method that can rapidly evaluate the phytotoxicity of gold nanomaterials in general, thus opening new ideas for the biotransformation of artificial nanomaterials in plants in the environment and the study of their biological effects on plants.

2. Materials and methods

2.1. Synthesis of AuNCs

AuNCs were synthesized using glutathione as both a stabilizer and a reducing agent, while chloroauric acid was used as the gold source, similar to the methods used by Luo et al. [22], with slight modifications. The control AuNPs were synthesized by the commonly used citrate reduction method [23]. The instruments used for characterization are listed in Table S3.

2.2. Determination of micronucleus rate and chromosome division index

The broad bean seeds were sterilized, soaked for 24 h and germinated for 3d, and then infected with AuNCs and AuNPs (20, 50, 100, 200, 300 mg/L). The infected seeds were washed and cultured, then the broad bean root was fixed and soaked. They were dyed and pressed. Mitosis was then observed; the micronucleus counts were recorded, and the chromosome division index and chromosomal aberration rate were calculated.

2.3. Determination of growth parameters, antioxidants and reactive oxygen species (physiological indicators)

The seeds of the broad bean were germinated in a Petri dish and incubated in AuNCs and AuNPs (0, 20, 50, 100, 200 mg/L). The number of seeds germinated per day was counted, and the superoxide dismutase (SOD) activity [24], oxidase (POD) and oxidative oxygenase (CAT) activities were determined in broad bean after 7 d by the nitrogen blue tetrazolium method [25]. The malondialdehyde (MDA) content was determined by the TCA-TBA method [26,27].

2.4. Analysis of adsorption and transport

The broad bean seeds were sterilized and germinated, and the well-developed seeds were selected to raise seedlings with 1 to 4 total nutrient solutions (see Table S2 for culture solution composition). Seedlings of broad beans of uniform growth were selected and started hydroponic experiments with AuNCs and AuNPs cultures at concentrations of 20 mg/L, 50 mg/L, 100 mg/L and 200 mg/L simultaneously. After 14 days, the broad bean plants were harvested, and the indexes were determined. After hydroponic culture, the samples were rinsed with deionized water, 1–2 cm root tip samples were taken, the root tip pressed, and the adsorption of AuNCs and AuNPs on the root surface of broad bean was observed by fluorescence microscopy. The roots and aboveground parts of broad bean seedlings were separated, killed at 105°C for 15 min and dried at 75°C to constant weight. After grinding, the content of gold elements in the roots and aboveground parts of broad bean seedlings was determined by flame atomic absorption spectrometry after ablation, respectively. The seedlings of the broad bean were washed with deionized water, and the fresh samples of roots and stems from the same part of the broad bean were ground at 4°C, centrifuged at 300 g at 15 min to precipitate the cell wall, and the supernatant was centrifuged at 2000 g to precipitate the organelles, and the final supernatant consisted of a soluble cytoplasmic component. The cell wall and organelle were dried to a constant weight of 75°C. After microwave digestion, the content of Au was determined by atomic absorption spectrophotometer after filtration.
2.5. Effects of the four channel inhibitors on the absorption of AuNCs by broad beans

About hydroponic experiments, broad bean seeds were treated as described in the previous section, and after sterilization, soaking, and germination, they were transferred into pots with 1/4 culture solution for seedling development, and then seedlings of broad beans of uniform growth were selected and transferred into seedling boxes for seedling development. Five days later, treatment drops of AuNCs and AuNPs and four channel inhibitors were started, where the four channel inhibitors included calcium channel inhibitor (LaCl₃), potassium channel inhibitor (TEA), ATPase inhibitor (Na₃VO₄), and anion channel inhibitor (NIF).

Determination of Au content, after hydroponic culture, the seedlings of broad bean were washed with deionized water, then the roots and aboveground parts were separated, and the aboveground part was treated with killing, drying, grinding and digestion; The AuNCs and AuNPs adsorbed on the root surface of broad bean were extracted by adding 0.01 mol/L EDTA-Na and shaking for 20 min each time for 3 times. The extract was collected and digested at 65°C for further analysis, and the root system of the broad bean received the same treatment as mentioned above.

2.6. Statistical analysis

Excel 2010 was used for data collection, Graph Pad Prism 8.0.2 was used for data processing and drawing, and SPSS25.0 was used for statistical analysis, including average value, standard deviation, regression analysis and variance analysis. P < 0.05 indicated significant differences between treatment groups or between treatment groups and blank treatment.

3. Results

3.1. Nanoparticles characterization

As shown in Figure 1(a), AuNCs were uniformly dispersed in water without aggregation, appearing light yellow under daylight, and when excited with a 365 nm UV lamp, the AuNCs solution emitted a solid orange-red fluorescence. The fluorescence spectrum showed that the maximum emission wavelength of AuNCs was 660 nm with symmetrical fluorescence emission peaks. The particle size distribution of AuNCs shown in Figure 1(b) ranged from 2 to 3.5 nm, consistent with the literature [22]. The citrate-reduced synthesized AuNPs exhibited a surface plasmon resonance peak at 520 nm [28] along with a narrow UV absorption wavelength range (475–540 nm) (Figure 1(c)). As shown in Figure 1(d), the particle size ranged from 20 nm to 30 nm with a relatively uniform distribution.

Figure 1. A shows the UV-Vis absorption spectra and fluorescence emission spectra of AuNCs, B shows the TEM of AuNCs, C shows the UV-Vis absorption spectra of AuNPs, and D shows the SEM of AuNPs.
3.2. Effects of AuNCs and AuNPs on the environment and cell division of broad bean

As shown in Figure 2(a), the number of micronuclei produced by broad bean root tip cells initially increased and then decreased as the concentration of AuNCs increased. At 20 mg/L of AuNCs one micronucleus was observed, and broad bean root tip cell mitosis started to be affected (Fig. A2). At a concentration of 200 mg/L, the number of micronuclei reached a maximum of 3, at which point mitosis was severely affected (Fig. A5). As the concentration of AuNCs increased to 300 mg/L, the number of micronuclei suddenly decreased, at which point the cells were probably about to die (Fig. A6). As shown in Figure 2(b), the overall trend in the number of micronuclei in the AuNPs-treated and AuNCs-treated groups was the same, with only one micronucleus in the 20 mg/L AuNPs solution-treated group, and mitosis started to be affected (Fig. B2). The micronuclei increased to 2 when AuNPs solution reached 50 mg/L (Fig B3). The number of micronuclei in the AuNPs-treated group peaked at five at 100 mg/L (Fig. B4); subsequently, the number of micronuclei showed a decreasing trend. This finding is slightly different from the concentration at which the number of micronuclei peaked in the AuNCs-treated group, which may be related to Random sampling.

With increasing AuNCs concentration, the micronucleus rate of broad bean root tips initially increased and then decreased (Figure 3). The micronucleus rate of AuNCs at 20 mg/L was 8.62%, yielding moderate pollution levels to the environment. Moreover, at 200 mg/L, the micronucleus rate peaked at 35.6764%, which reached the standard of heavy pollution. The concentration of AuNCs increased to 300 mg/L when the micronucleus rate suddenly decreased, probably due to mitosis inhibition in broad bean root tip cells. The overall trend for the micronucleus rate was comparable between the AuNPs- and AuNCs-treated groups. At 20 mg/L AuNPs solution, the micronucleus rate was 8.41%, which reached moderate contamination. When the AuNPs solution reached 100 mg/L, the micronucleus rate was 21.19%, and the contamination index was 7.47%, which reached the heavy contamination standard. The micronucleus rate in the AuNPs-treated group peaked at 36.52 at 200 mg/L. Subsequently, similar to the AuNCs-treated group,

![Figure 2](image-url)

**Figure 2.** A and B show random visual fields of micronuclei of broad bean root tip cells treated with AuNCs and AuNPs, respectively. A1–A6 (B1–B6) are the AuNCs (AuNPs) control, 20, 50, 100, 200, and 300 mg/L treatment groups, and the arrows point to the micronuclei.
the micronucleus rate decreased in the 300 mg/L-treated groups. There was a positive correlation between the mass concentration of the two nanoparticle treatment solutions and the micronucleus rate, both peaking at 200 mg/L. The micronucleus rate evaluation indicated that the effect of AuNCs on broad beans was more sensitive than AuNPs (Table S1). As seen in Figure S1., with increased treatment concentration for both nanoparticles, the chromosome division index initially decreased, then transiently increased before finally decreasing; chromosome division was more significantly affected by AuNCs than AuNPs.

### 3.3. Effect of AuNCs and AuNPs on seed germination of broad bean

Seed germination is the starting point of plant growth and one of the most critical indicators to quickly detect their exposure to external aggression. As shown in Figure 4(a), the germination rate of broad bean seeds showed a slight increase with increased AuNCs concentration, and no significant difference was observed between the experimental treatment concentrations and the control group, with a peak germination rate of 91.71%, which was 9.71% higher than the control group. At low concentrations (20 mg/L, 50 mg/L), AuNCs treatment groups exhibited slightly higher germination rates than AuNPs treatment groups. At higher concentrations (100 mg/L, 200 mg/L), the germination rate in the AuNCs treatment groups was slightly lower or remained essentially the same as AuNPs treatment groups, with no significant differences. Both treatment solutions did not affect the germination rate of broad bean seeds within the experimental concentrations of 0 to 200 mg/L.

![Figure 3](image_url). Micronucleus rate of broad beans for AuNCs and AuNPs. The graphs a, b, c, d, e, de, f, and g represent the experimental groups in descending order of mean value.

![Figure 4](image_url). Effect of two types of nanoparticles on the germination of broad bean seeds. Figures A, B and C show the effects of two nanoparticles on the germination rate, germination potential, and germination index of broad bean seeds. The a in the three plots of A, B and C indicate the mean values of the germination rate, germination potential and germination index of broad bean seeds.
It is well-established that germination potential can characterize the strength of germination ability. As shown in Figure 4(b), the germination potential of broad bean seeds gradually increased at a concentration range of 0–200 mg/L. For both treatment solutions, the peak germination rate was observed at 100 mg/L (86.52 for the AuNCs treatment group and 83.99 for AuNPs), indicating that AuNCs and AuNPs can improve the germination capacity of seeds within a specific range.

It is widely acknowledged that the germination index indicates the seed germination rate. As seen in Figure 4(c), the germination index of the AuNCs treatment group first increased and then decreased. At 20 mg/L AuNCs, the germination index of broad bean seeds decreased, and a peak germination index of broad bean seeds of 11.13 was observed with 50 mg/L AuNCs; the germination index subsequently gradually decreased. However, no significant differences were observed between different concentrations of AuNCs and the control. The abnormal increase at 50 mg/L may be due to experimental error caused by inter-individual variations in the size of the broad bean seeds. The germination index of broad beans in the AuNPs treatment group exhibited the same trend as in the AuNCs treatment group, indicating that gold nanoparticles did not affect the germination rate of broad bean seeds. In summary, both gold nanoparticles did not significantly affect the germination of broad bean seeds at experimental concentrations of 0–200 mg/L.

3.4. Effects of AuNCs and AuNPs on physiological aspects of broad bean seedlings

Figure 5 shows the changes in each antioxidant index after treating the root tips of broad bean seedlings with two types of nanoparticles. As shown in Figure 5(a), the SOD enzyme activity in broad bean plants gradually increased after treatment with AuNCs. No significant differences were observed at low concentrations (20–50 mg/L) compared to the control group, and the SOD enzyme activity in the 100 mg/L AuNCs-treated groups of broad bean seedlings was significantly higher than the control group, which was consistent with the overall trend in the micronucleus rate of broad bean root tips. Similar to the AuNCs treatment group, AuNPs at 50 mg/L and higher concentrations showed significant differences compared to the control group. At the same concentrations, SOD enzyme activity in the AuNPs-treated groups was higher than in the AuNCs-treated groups, with significant differences at 200 mg/L. The differences observed at 100 mg/L may be due to experimental error. Figure 5(b, c and d) show the effects of the two nanoparticles on peroxidase (POD) activity, catalase (CAT) activity and malondialdehyde (MDA) content in the root tips of broad bean seedlings, respectively. Their trends were generally consistent with those of SOD enzyme activities, i.e. the activities of all three antioxidant enzymes and MDA contents in broad bean seedlings treated with the two nanoparticle solutions were enhanced with increasing concentration of the treatment solution.

Figure 5. Effect of treatment solutions on antioxidant physiological indices of broad bean seedlings. A SOD, B POD, C CAT, D MDA, where a, ab, b, and c represent the experimental groups in descending order of mean value.
3.5. Adsorption of AuNCs by the root system of broad bean

The slides of the squashed root tip showed a large number of AuNCs adsorbed on the entire root tip, as shown in Figure 6(a), with orange-red fluorescence under fluorescence microscopy. Further observation revealed a non-uniform distribution of orange-red fluorescence with clustering under fluorescence microscopy (Figure 6(b)). The AuNPs treatment group also displayed similar characteristics (Fig. 52).

3.6. Translocation and uptake of AuNCs and AuNPs by broad bean seedlings

It has been shown that nanoparticles acting on the plant surface translocate to the plant above ground through root uptake [29]. This study provides insights into the detoxification pathways and sites of gold nanoparticles, including the uptake and transport of gold nanoparticles in broad bean seedlings. The results are shown in Figure 7. After incubation with AuNCs solution, an orange-red fluorescence could be observed in the young leaf parts of broad bean seedlings. The difference in the effect of the two nanoparticles on broad bean seedlings might be explained by the degree of uptake and translocation.

Given that broad beans do not contain gold in their bodies per se, changes in gold content were caused by the two experimental treatment solutions (AuCPs, AuNPs). The results of the flame atomic absorption assay showed that the gold content in the roots of broad bean seedlings in all treatment groups increased significantly (P < 0.05) with increased treatment concentrations, AuNCs yielded higher Au concentrations (188.40 ~ 3 99.14 ug/g, dry weight) than AuNPs (143.21 ~ 245.26 ug/g, dry weight) for the different treatments (Figure 8(a)). As shown in Figure 8(b), both nanoparticles could be absorbed and transported to the aboveground part after treating the root system of broad bean seedlings, but the differences in transport capacity and aboveground part content were significant. For the AuNCs treatment group, the aboveground Au content increased significantly with increasing exposure concentration, ranging from 18.955 to 37.143 ug/g, while the transport coefficient (the quotient of aboveground and root Au content) varied from 0.1 to 0.094. In contrast, the AuNPs treatment group had a lower gold content in the aboveground part of broad bean seedlings and exhibited non-significant variations in concentration, ranging from 11.9 to 23.7 ug/g; its transfer coefficient was also lower (0.08~0.009), indicating that the transfer rate of AuNPs to the aboveground part of the snap bean was somewhat lower compared with AuNCs, which may be related to its larger particle size [30,31]. This finding indicates that particle size is essential in the transfer of nanomaterials to the aboveground and accounts for the fact that the root tip cells of broad
bean were more significantly affected in the AuNCs-treated group than the AuNPs-treated group in the micronucleus experiment.

3.7. Subcellular distribution of AuNCs and AuNPs in various parts of broad bean

Based on the linear relationship between the root and aboveground gold content of broad bean seedlings and the concentration of each treatment, the lowest concentration (20 mg/L) and the highest concentration (200 mg/L) were selected here for comparison.

For the roots of broad bean seedlings, for both treatment solutions, the content of Au in each cell fraction was positively correlated with the treatment concentration (Figure 9(a)), consistent with the overall trend of increasing micronucleus rate in broad bean root tips. Gold was mainly found in the cell wall, followed by the cytoplasm, and the least amount of Au was found in the organelles. This finding may be attributed to the fact that the entry of nanoparticles into the plant triggers a ‘self-help’ mechanism in the plant body, storing these harmful substances in relatively weak tissues to avoid damage to more critical cellular tissues. Interestingly, plants can store invading substances from outside through the cell wall and cytosol, protecting organelles with more vital functions, thereby reducing cell toxicity [32].

Between the same treatment solution, the content of Au elements in the high concentration treatment group was significantly higher than that in the low concentration treatment group. For AuNCs, the Au element content in the high concentration treatment group was 2.26 times higher than in the low concentration treatment group. For AuNPs, the Au element content in the high concentration treatment group was 2.03 times higher than in the low concentration treatment group, indicating a dose-effect of nanomaterials entering the plant. The content of Au elements in each cell fraction was significantly higher in the AuNCs treatment group than in the AuNPs between different treatment solutions and at the same treatment concentration. At low concentrations, the cell wall, cytoplasmic, and the organelle Au content of the AuNCs-treated group was 1.41, 1.35 and 2.12 fold higher than in the AuNPs-treated group, respectively. At high concentrations, the cell wall, cytoplasmic and the organelle Au of the AuNCs treatment group was
1.55, 1.45 and 1.94 fold higher than AuNPs, respectively, which also indicates that the size of the nanomaterials is a critical factor affecting their entry into plants.

For the aboveground part of the broad bean, as shown in Figure 9(b), for both treatment solutions, the content of Au in all cell fractions was enhanced with increasing treatment concentration, with Au mainly present in the cytoplasm, followed by the cell wall and the least amount of Au in the organelles. The distribution of the content of gold elements in each subcellular part of the roots was different, indicating that the detoxification mechanism for the stress of nanomaterials for the aboveground part of the broad bean is different from the roots. The transport coefficient in the cytoplasmic fraction was 0.253 at low concentrations and 0.295 at high concentrations in the AuNCs-treated group, and 0.183 at low concentrations and 0.192 at high concentrations in the AuNPs-treated group. This finding indicates that AuNCs have a higher transport coefficient than AuNPs, suggesting that AuNCs are more likely to enter the broad bean seedlings. Meanwhile, it indicates that both nanoparticles are transferred to the aboveground through the root system of broad bean seedlings. This experiment not only visualized the subcellular distribution of gold nanoparticles, but also provided a more substantial evidence base for the results of the previous micronucleus experiments that AuNCs with small particle sizes are more easily absorbed into the broad bean cells.

3.8. Effect of channel inhibitors on the adsorption of AuNCs and AuNPs by broad bean roots

After 48 h exposure to each inhibitor, the calcium channel inhibitor LaCl$_3$ treatment inhibited the adsorption of both nanoparticles by the root system of broad bean seedlings to some extent, and the inhibition effect was more significant for the AuNCs-treated group than the AuNPs-treated group (Figure 10(a)). Figure 10(b) shows the decrease in the adsorption of both nanoparticles by the roots of broad bean seedlings under treatment with the anion channel inhibitor NIF, indicating that NIF yielded an inhibitory effect on the adsorption of nanoparticles by the roots of broad bean seedlings: the group treated with a high concentration of AuNCs was most affected, followed high concentration of AuNPs, low concentration of AuNCs, and low concentration of AuNPs. Figure 10(c) shows that the ATPase inhibitor Na$_3$VO$_4$ treatment inhibited the adsorption of both nanoparticles by the roots of broad bean seedlings. However, the inhibitory effect of Na$_3$VO$_4$ on the adsorption of Au by the roots of the broad bean was not significant, and the overall AuNCs treated group was more significantly inhibited than the AuNPs treated group. Figure 10(d) shows the inhibitory effect of potassium channel inhibitor TEA treatment on the adsorption of two kinds of nanoparticles by the roots of broad bean seedlings.

Figure 10. Plots of Au adsorption content of broad bean roots treated with four inhibitors. A, B, C and D show the Au adsorption content of LaCl$_3$, NIF, Na$_3$VO$_4$ and TEA treated broad bean roots, respectively. C20 and C200 denote the gold content of AuNCs at the minimum (20 mg/L) and maximum (200 mg/L) experimental concentrations, respectively, while P20 and P200 denote the gold content of AuNPs at the minimum and maximum experimental concentrations, respectively, for each subcellular site.
After the addition of the inhibitor to broad bean, the adsorption amount of 0.44–0.45 μg/g in the roots of broad bean seedlings in the AuNPs treatment group was 59%–93%; the adsorption amount of 0.26–0.39 μg/g in the AuNCs treatment was 0.59% of AuNPs –0.87 times of AuNPs, with an inhibition rate of 91%–98%. This indicates a robust inhibitory effect of TEA on the adsorption of nanoparticles by the root system of broad bean seedlings, and the AuNCs treatment group was more significantly inhibited. Treatment with the four types of channel inhibitors showed that the adsorption of AuNCs by the root system of broad bean seedlings mainly involved competition with potassium channels.

4. Discussion

Micronucleus assays are often used as a well-established, rapid and reliable routine system to observe structural chromosomal abnormalities caused by genotoxic agents in plant cells. In addition to traditional assays for pesticides, heavy metals and ionizing radiation, they are now being intervened in the interaction of nanoparticles with plants [33]. Over the years, broad beans have been used as the study subject in genetics research due to their low chromosome number (2n = 12) and shape and are easy to observe; the consistency of qualitative responses such as mutagenicity of broad bean and mammalian cells to environmental pollutants can reach 99%. With increasing nanoparticle concentration, the micronucleus rate of broad bean root tips initially increased and then decreased, and the micronucleus rate of broad bean peaked at 200 mg/L in the gold nanoparticle treatment group and then exhibited a decreasing trend. Besides, we found that the two nanoparticles differed in the degree of genetic material damage to broad bean root tip cells, and micronucleus rate evaluation indicated that broad bean was more sensitive to AuNCs than AuNPs. However, the chromosome division index of broad bean root tips initially decreased, followed by a transient increase and finally decreased with increasing gold nanoparticle concentration, which was slightly inconsistent with the findings for the micronucleus rate of broad bean. This phenomenon may be accounted for by contact between the two nanoparticles, and the root tip cells of broad beans interacted with the root tip cells of broad beans at low concentrations, producing harmful substances such as peroxides that caused toxic effects, resulting in a decreased chromosome division index. At the same time, the production of harmful substances induced resistance of the intracellular antioxidant system to harmful substances, which increased the chromosome division index. As the treatment concentration increased above 100 mg/L, the level of harmful substances exceeded the regulation range of the intracellular antioxidant system, which led to the inability of cells to resist and a decrease in the chromosome division index [34,35]. The peak concentration observed at 100 mg/L instead of 200 mg/L (observed for the micronucleus rate of broad bean) may be due to experimental error.

The germination rate, germination potential, and germination index represent seed germination’s ability, speed, and uniformity. Although AuNPs are reported to be similar to carbon nanotubes (CNTs) and carbon nanoparticles (CNPs) in their ability to act as a promoter of plant seed germination [36,37]. However, within the experimental concentration range, broad bean seeds’ germination rate potential and germination index initially increased and then decreased with increased treatment concentration, consistent with the trend of micronucleus rate. However, there was no significant difference between treatment and control groups (P > 0.05), indicating that AuNCs yielded no significant effect in the germination stage of broad bean seeds, consistent with the study of Zeynep et al. and Liu et al. [38,39], which showed that nanoparticles yielded no effect in the germination stage of plant seeds.

It is well-established that the antioxidative stress system is triggered when plants are subjected to oxidative stress. Indeed, oxidative stress and lipid peroxidation represent the most reported mechanism in the current literature on damage produced by nanoparticles to the organism [34,40,41]. Conventional experiments yielded an overall positive correlation between SOD, POD, CAT enzyme activities and MDA content in broad bean seedlings between the two nanoparticle treatment groups and the concentration of the treatment solution. The same conclusion was reached by Jalil et al. in their study of the physiological, developmental and ROS scavenging responses of biosynthesized gold nanoparticles on tobacco plants under stress conditions [35]. Moreover, this is the same as the findings of Dai et al [42], i.e. the activities of the three enzymes and MDA content in bean seedlings were enhanced with increased concentration of gold nanoparticle, consistent with variations in micronucleus rate at 0–200 mg/L concentration. However, a slight decrease in POD and CAT enzyme activities and MDA content were
observed in the 20 mg/L treatment group compared to the control group, but the difference was not significant and did not affect the overall trend. In addition, the effects of each treatment group of AuNCs and AuNPs on various aspects of physiological indices of broad bean seedlings were not consistent with their effects on the micronucleus rate. Analysis of the overall effect of both treatments on the micronucleus rate showed that AuNCs yielded a more significant effect than AuNPs on the broad bean, while the overall effect of both on physiological aspects of broad bean seedlings showed a higher effect of AuNPs than AuNCs on the broad bean. This finding may be related to the traditional experimental method, which may produce similar AuNCs-mediated (siRNA) transfer to plant cells due to the longer experimental treatment time compared to micronucleus experiments [43], allowing smaller particle size AuNCs to seedlings to undergo more translocation, and it has been shown that nanoparticles acting on the plant surface translocate to the plant above ground through root uptake [29,44,45]. Since detoxification was carried out in the broad bean seedlings, AuNCs exhibited a negligible effect.

To further confirm that nanoparticle uptake transfer occurred in broad bean seedlings in vivo, and to verify that AuNCs were more sensitive to the micronucleus rate of broad beans compared to AuNPs, the adsorption, uptake transfer, subcellular distribution and uptake patterns of gold nanoparticles were further investigated using traditional experimental methods. The results showed that many gold nanoparticles were not uniformly adsorbed, and most of them were attached to the surface of broad bean roots in the agglomerated state. Lin [46] reported that contact between nanoparticles and plants stimulated the production of colloidal material in the roots, which led to more nanoparticles adsorbed and retained on the surface of the roots. Begum et al. also concluded that nanoparticles are adsorbed on the root surface with sticky substances secreted by plant roots due to their small size, large specific surface area and high surface energy [47]. In the present study, the gold content of both root and aboveground parts of broad bean seedlings in all treatment groups was significantly enhanced with increased treatment concentrations, while the uptake also indicated that AuNPs were translocated to a smaller extent to the aboveground parts of broad bean than AuNCs. This should be attributed to size effects on the transport of nanomaterials in plants [48,49]. The amount of Au in each cell fraction was positively correlated with the treatment concentration, and AuNCs had a higher transport coefficient than AuNPs. The two nanoparticles were mainly stored in the cell wall in the root system and the cytoplasm aboveground, suggesting that the detoxification mechanisms of the aboveground and the root of broad bean seedlings for nanomaterials were different, which may explain the differences between the results of micronucleus and conventional experiments to a certain extent. All four types of channel inhibitors inhibited the entry of adsorbed nanoparticles in the root system of broad bean seedlings, with the potassium channel inhibitor TEA yielding the most pronounced inhibitory effect. This finding indicates that AuNCs act on the plant root surface in several ways, but competition with potassium ions is predominant. Lipid peroxidation induced by AuNCs in broad bean seedlings mentioned earlier may be due to competition with potassium ions leading to increased permeability of the cell membrane of broad bean [50], which further validates that gold nanoparticles can undergo uptake transfer.

5. Conclusion

Conventional phytotoxicity experiments with 0–200 mg/L Au NCs showed that toxicity to broad bean seedlings became more pronounced as the concentration of nanoparticle solution increased. Although the results of the broad bean micronucleus experiments were slightly different, the overall trend was consistent. When the concentration of Au NCs was 20 mg/L, no plant response could be detected from the seed germination of broad beans alone. At the same time, the appearance of micronuclei could be observed in the micronucleus experiments, which means that this concentration of gold nanomaterials already poses a potential risk to the environment. The accuracy of the micronucleus experiment was further demonstrated by the changes in physiological parameters and the uptake and transport of Au NCs in broad beans. In addition, the results of both the micronucleus kpc and conventional experiments can be corroborated by each other in the range concentration of 0–300 mg/L. Thus, compared with conventional phytotoxicity experiments, micronucleus experiments are more rapid and sensitive, especially for determining the phytotoxicity of gold nanomaterials at low concentrations. This method is expected to become a standard tool for rapidly assessing the phytotoxicity of nanomaterials.
Abbreviations

| Abbreviation | Definition |
|--------------|------------|
| AuNCs        | Au Nanoclusters |
| AuNPs        | Au Nanoparticles |
| et al.       | et alia |
| SOD          | Superoxide Dismutase |
| POD          | Peroxidase |
| CAT          | Catalase |
| MDA          | Malondialdehyde |
| EDTA-Na      | Ethylene Diamine Tetraacetic Acid Tetrasodium |
| UV           | Ultraviolet |
| Cancer Letters | Cancer Lett |
| Science      | Sci |
| Journal of Applied Toxicology | J Appl Toxicol |
| Chemical Reviews | Chem Rev |
| Science of the Total Environment | Sci Total Environ. |
| Journal of Nanomaterials | J Nanomater |
| Iranian Biomedical Journal | Iran Biomed J |
| Colloids and Surfaces A | Colloids Surf A |
| Materials Today: Proceedings | Mater Proc. |
| Nanoscale Research Letters | Nanoscale Res Lett |
| Plant Cell, Tissue and Organ Culture | Plant Cell Tissue and Organ Cult |
| Chinese Journal of Ecology | Chin J Ecol |
| Science of The Total Environment | Sci Total Environ |
| Journal of the American Chemical Society | J Am Chem Soc |
| Analytical Chemistry | Anal Chem |
| American Journal of Medicine | Am J Med |
| Plant Physiology | Plant Physiol |
| Chemical Communications | Chem Commun |
| Environmental Science & Technology | Environ Sci Technol |
| Journal of the Science of Food and Agriculture | J Sci Food and Agric |
| Environmental Toxicology & Pharmacology | Environ Toxicol Pharmacol. |
| International Journal of Molecular Sciences | Intern J Mol Sci |
| Toxicology Letters | Toxicol Lett |
| IET Nanobiotechnology | IET Nanobiotechnol |
| Toxicological and Environmental Chemistry | Toxicol Environ Chem |
| Biological Trace Element Research | Biol Trace Elem Res |
| Environmental Health Perspectives | Environ Health Perspect |
| Mutation Research | Mutat Res |
| Current Opinion in Environmental Science & Health | Curr Opin Environ Sci Health |
| Journal of Hazardous Materials | J Hazard Mater |

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