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ORIGINAL ARTICLE

# Selenium nanoparticles as in vitro rooting agent, regulates stomata closure and antioxidant activity of gerbera to tolerate acclimatization stress

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## Abstract

The acclimatization stress is responsible for high mortality in tissue cultured plants, which significantly reduces micropropagation efficiency. In this initial study, selenium nanoparticles (SeNPs) (0.1, 0.3, 0.5, 0.7, 1, 1.5 and 3 mg/L) were supplemented in gerbera culture medium to increase their tolerance to ex vitro stresses. The results revealed that SeNPs were potential gerbera rooting inducers via increasing endogenous auxin (AUX) levels. Accordingly, rooting efficiency on MS medium supplemented with 0.7, 1 and 1.5 mg/L SeNPs was similar to treatment 1 mg/L IBA and was significantly higher than the free-auxin/SeNPs treatment. At concentrations of 0.1 to 1.5 mg/L SeNPs promoted in vitro plantlet growth such as plant height, leaf length, total chlorophyll content, plantlet biomass, which corresponds to high activity of antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX) as well as high levels of AUX, cytokinins (CKs) and gibberellin (GA). Furthermore, it is likely that SeNPs increased stomatal density via increased CKs and decreased stomatal aperture via increased abscisic acid (ABA). The stomatal density in 0.7 mg/L SeNPs treatment (473.58 stomata.mm$^{-2}$) was 2.34-fold higher than 1 mg/L IBA treatment while stomatal aperture was significantly reduced compared to control treatments. However, SeNPs showed inhibitory activity at 3 mg/L with a decrease in shoot–root growth as well as stomatal closure compared with the other treatments. This study indicated that SeNPs improved gerbera plantlet quality by promoting antioxidant defense system activity and endogenous hormone alterations, which resulted in their higher survival and growth under ex vitro conditions.

## Key Message

Supplementation of SeNPs in culture medium positively effects on in vitrorooting, stomatal development and biochemical parameters of in vitro gerbera plantlets,conferring their high survival rates and accelerated flowering under ex vitro conditions.

**Keywords** Adventitious root · Micropropagation · Nanotechnology · Potted plants · Phytohormone · Stomata

## Abbreviations

| | |
|---|---|
| ABA | Abscisic acid |
| APX | Ascorbate peroxidase |
| AUX | Auxin |
| BA | 6-Benzyladenine |
| CAT | Catalase |
| CKs | Cytokinins |
| DM | Dry mass ratio |
| DW | Dry weight |
| FW | Fresh weight |
| GA | Gibberellin |
| IAA | Indole-3-acetic acid |

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| | |
|---|---|
| BA | Indole-3-butyric acid |
| MS | Murashige and Skoog medium |
| NAA | α-Naphthaleneacetic acid |
| ROS | Reactive oxygen species |
| SeNPs | Selenium nanoparticles |
| SOD | Superoxide dismutase |

## Introduction

Obtaining healthy plants after acclimation is a final key stage for success in plant micropropagation. However, transferring in vitro plants to ex vitro conditions inevitably induces biotic and abiotic stress (eg. the attack of soil microbiota, low humidity, daily fluctuations in heat and high light intensity) and as a result high mortality of tissue cultured plants. This has limited the successful application of plant micropropagation in practice (Chandra et al. 2010; Asayesh et al. 2017). Improving plantlet quality is a common precaution taken to help them resist acclimatization stress such as improve the efficiency of in vitro rooting by adding rooting agents or optimizing culture conditions at this stage (Monja-Mio et al. 2020); enhancing stomatal function by ventilating culture natural or artificial to reduce relative humidity in culture vessels or use agents to reduce the osmotic potential of the culture medium (Asayesh et al. 2017; Din et al. 2020); stimulating plant antioxidant systems through the addition of beneficial biological agents (Ngan et al. 2020a), etc.

For successful acclimatization, in vitro rooting is very important (Monja-Mio et al. 2020). It is well known that auxins are major regulator influencing root induction and development (Pacurar et al. 2014; Druege et al. 2019). Traditionally, auxins such as Indole-3-butyric acid (IBA) (Nhut et al. 2007), Indole-3-acetic acid (IAA) (Frómeta et al. 2017; Pawłowska et al. 2018) are added to the medium to stimulate in vitro rooting. However, the use of auxins (eg. IAA) has some limitations such as reduction or inactivation of their action after post-autoclaving stages. In some instances, plants did not respond to auxin (Faivre-Rampant et al. 2000), and the use of high doses of auxin inhibited root elongation (Nhut et al. 2007). Furthermore, auxins have been shown to inhibit stomata formation and development (Balcerowicz et al. 2014), and this may negatively affect tissue cultured plants survival under ex vitro conditions.

Several studies have introduced new compounds capable of promoting root induction in plants without the presence of exogenous auxin such as polyamine types which include putrescine, spermidine, spermine (Faivre-Rampant et al. 2000; Matam and Parvatam 2017); biogenic silica and green-tea catechins (Ambros et al. 2018); etc. These substances have been shown to not only provide a rooting effect, but also improve the survival rate of in vitro plantlet at the nursery stage. Recently, it has been found that selenium (Se) is the beneficial element for root development (Domokos-Szabolcsy et al. 2012; El-Ramady et al. 2014; Jóźwiak and Politycka 2019; Hajiboland et al. 2020). Further studies showed that Se at low doses stimulated a significant increase in auxin level in plants (Jia et al. 2018). These suggest that Se could be a potential rooting agent.

Selenium is one of several biologically beneficial metalloids that has been studied extensively for several decades. The important and essential role of Se has been confirmed in many living organisms including humans, while the question of whether Se is necessary in plants has not yet been clearly answered (Sotoodehnia- Korani et al. 2020). Selenium has been confirmed to participate in many physiological and biochemical processes in plants (El-Ramady et al. 2016; Niu et al. 2020). However, the ability to control antioxidant systems in organisms is what has made Se widely known and used in science. Hence, it is assumed that Se can significantly promote plant growth (Mroczek-Zdyrska and Wójcik 2012; Boldrin et al. 2016; Jóźwiak and Politycka 2019; Xu et al. 2019; Niu et al. 2020; Wu et al. 2020; Peng et al. 2020). In addition, Se is resistant to abiotic stresses such as cold, water, UV (high radiation), salinity and heavy metals, etc. (Feng et al. 2013; Xu et al. 2019; Pokhrel et al. 2020; Huang et al. 2020) and also to stress caused by bacteria (Matai et al. 2020). Therefore, supplementation of Se to plantlet may stimulate in vitro growth and increase their tolerance to stress particularly during acclimatization.

Normally, Se is added to plants in various forms of compounds such as sodium selenite ($Na_2SeO_3.5H_2O$) (Jóźwiak and Politycka 2019; Wu et al. 2020) or sodium selenate ($Na_2SeO_4$), etc. However, the use of Se in the form of inorganic and organic salts typically results in low absorption and high toxicity (Bhattacharjee et al. 2019). Selenium poisoning can negatively affect plant growth (Domokos-Szabolcsy et al. 2012; Han et al. 2013; Hajiboland et al. 2020), cause DNA methylation and many other serious consequences leading to plant death (Sotoodehnia-Korani et al. 2020).

Recently, nanomaterials have become an attractive research topic for scientists to solve many difficult problems in many fields. Nutritional minerals have been added in plant tissue culture in the form of nanoparticles (NPs) which have many positive results such as replacing copper sulfate ($CuSO_4.5H_2O$) by copper NPs in somatic embryogenesis in basil (Ibrahim et al. 2019); adding silver NPs and replacing cobalt chloride by cobalt NPs in rose tissue culture (Ngan et al. 2020b); etc. However, extensive research is lacking on investigating the effects of selenium NPs (SeNPs) in plant micropropagation, although SeNPs are promising materials with many advantages.

Realizing the beneficial potential of the element Se for plants, we initiated studies on micropropagation of gerbera by adding SeNPs to culture media as a substitute for auxin.

The aim was to evaluate the effect of SeNPs on in vitro rooting and growth of microshoots; in which, SeNPs were chemically synthesized. Besides, the determination of the toxicity threshold of this element for gerberas was also considered. In particular, we wanted to evaluate the effects of Se on the viability, growth and development of in vitro plantlet in subsequent stages of acclimatization.

## Materials and methods

### Plant material

In vitro shoots of gerbera (*Gerbera jamesonii* Bolus ex. Hook f.) with 2 leaves and 2.5–3 cm in height derived from shoot clusters that were mass propagated on half-strength MS medium containing 0.5 mg/L IBA (Sigma-Aldrich, USA), 0.5 mg/L 6-benzyladenine (BA, Sigma-Aldrich, USA), 2.0 mg/L kinetin (Sigma-Aldrich, USA), 30 g/L sucrose and 8 g/L agar (Duchefa, The Netherlands) (Nhut et al. 2007), were used as explants for in vitro rooting experiments.

The 15-day-old plantlets of control and SeNPs treatments were collected and used as material for the determination of antioxidant enzyme activity. Plantlets were randomly selected as samples for anatomical and morphological examination and evaluation of the stomata and roots. The remaining plantlets were used as sample sources for acclimatization experiment.

### Basal medium

The basal medium used in this study was MS (Murashige and Skoog 1962) added 30 g/L sucrose and 8 g/L agar. The medium pH was adjusted to 5.8 by 0.1 M KOH and autoclaved at 121 °C, 1 atm for 20 min. Forty milliliter medium was poured in 250 mL glass bottles.

### Synthesis of selenium nano solution (SeNPs)

SeNPs solution was synthesized by the Institute of Envrionmental Technology according to the chemical method described by Wang et al. (2015). The starting material was selenious acid ($H_2SeO_3$), using ascorbic acid as reducing agent and arabic gum as stabilizer. Accordingly, $H_2SeO_3$ was reduced for 10 min. The SeNPs solution was stored at room temperature for 3 months for use in this study.

### In vitro and ex vitro conditions

In vitro: all culture flasks are placed in the culture room at a temperature of 25 ± 2 °C, humidity between 50 and 60%, illuminated by fluorescent lamps (36 W) with light intensity of 40–45 µmol $m^{-2}$ $s^{-1}$, 16 h light and 8 h dark photoperiod.

Ex vitro: all gerbera plantlets were acclimated under greenhouse conditions with daytime temperatures around 25 °C and night-time around 15 °C. Sunlight intensity was reduced to 40%-50% by using a shade filter and relative humidity was maintained above 80% during the first 15 days.

### Experimental design

#### Characterization analysis of SeNPs

Spectrophotometer UV–VIS absorption (Ultraviolet–visible) model V530 (JASCO, Japan) was used to determine the optical properties of solutions containing nanoparticles. To determine the shape, size and dispersion of the nanoparticles, transmission electron microscope (TEM) model JEM1010 (JEOL, Japan) was used with magnification = X50-X600.000, resolution = 3 Å, acceleration voltage = 40–100 kV, in which the samples were coated with carbon.

#### Effects of SeNPs on the rooting and growth of gerbera shoots on auxin-free medium

In vitro shoots were cultured on half-strength MS medium supplemented with different concentrations of SeNPs (0.1, 0.3, 0.5, 0.7, 1.0, 1.5, 3.0) to evaluate root induction and in vitro growth of microshoots. Control (+) was MS medium supplemented with 1.0 mg/L IBA (Nhut et al. 2007); and control (-) was MS medium without supplement of SeNPs/auxin. Results were recorded after 15 days of incubation. To evaluate the effect of SeNPs on rooting ability, we recorded rooting time (day), number of roots (roots/plantlet), root length (cm), root diameter (mm). Plant growth parameters were recorded, which included plant height (cm), ratio of shoot height/root length, numbers of leaves (leaves/plantlet), leaf length (cm), leaf width (cm), total chlorophyll content in leaves (nmol/$cm^2$), fresh weight (FW) (mg), dry weight (DW) (mg), dry matter accumulation rate (DM) (%) is calculated by the formula DW/FW × 100%. Total chlorophyll content was measured with a chlorophyll meter SPAD-502 (Minolta, Japan).

After 15 days of culture, the plantlets were collected and washed with distilled water and used as materials for biochemical analyses.

#### Effect of SeNPs on antioxidant enzyme activity in gerbera plantlets

Key enzymes that regulate reactive oxygen species (ROS) concentrations such as catalase (CAT), superoxide dismutase

(SOD) and ascorbate peroxidase (APX) have been evaluated for their activity by analysing with UV–VIS spectroscopy.

CAT activity has been determined according to the method of Goth (1991). Briefly, the presence of CAT in the sample was determined by reacting the sample with $H_2O_2$ for 2 min; then, the remaining $H_2O_2$ after the reaction was combined with ammonium molybdate ($NH_4Mo_7O_{24}$) to create a stable yellowish complex which was maximally absorbed at 405 nm. Unit CAT enzyme activity is U $min^{-1}$ $g^{-1}$ prot equivalent to CAT hydrolyzed 1 µmol $H_2O_2$/min.

SOD activity has been determined according to the method of Marklund and Marklund (1974). Accordingly, the test was conducted in an alkaline environment, pyrogallol self-oxidizes when there is oxygen in the air, creating a maximum absorption product at 320 nm. The presence of the SOD enzyme in the sample catalyzes the decomposition of the peroxide radicals ($O^{2-}$), inhibiting the self-oxidation of pyrogallol. The activity of SOD in the sample was determined based on the inhibition rate. Enzyme activity (U $g^{-1}$ prot) was calculated using the formula (inhibition ratio/50) x dilution ratio. In which, inhibition rate is determined by changing absorbance at 320 nm wavelength.

APX activity has been determined according to the method of Nakano and Asada (1981). Oxidation was carried out for 10 min, measured optical density at 290 nm, and recorded the maximum absorption of ascorbate. The amount of oxidized ascorbate was calculated (absorption coefficient 2.8 mM $cm^{-1}$). APX unit of activity is equivalent to the amount of APX sufficient to oxidize 1 µm ascorbate/min (U $min^{-1}$ $g^{-1}$ prot).

### Effect of SeNPs on endogenous hormone content in gerbera plantlets

The content of endogenous hormones was determined by high performance liquid chromatography (HPLC) analysis as described by Khai et al. (2021). Six hormones were measured including Indole-3-acetic acid (IAA), three cytokinins (2ip [N6-isopentenyladenine], KIN [Kinetin], Zeatin [trans-Zeatin]), Gibberellin ($GA_3$) and Abscisic acid (ABA). The phytohormones were separated by Thermo-Ultimate 3000 HPLC system (Thermi Scientific, USA) equipped with a Hypersil C18 BDS column (25 mm × 4.6 mn, 0.5 µm in particle size) and coupled to a UV detector (280 nm). Standards corresponding to the endogenous hormones to be measured were purchased from Sigma-Aldrich®, USA. Calibration curves were prepared from signals derived from standard solutions, which are based on the ratio of the chromatographic peak areas of each analysis to the corresponding internal standards. Finally, the concentrations of the hormones were determined from the calibration curve of specific standards.

### Anatomy and histological analysis

The newly formed leaves were harvested 15 days after culture to examine stomata (dark sampling conditions). Lower epidermis was separated from the leaves and placed on a glass slide; they were stained with 0.1% methylene blue (Sigma-Aldrich, USA) for 30 s and rinsed with distilled water 3 times. Stained lower epidermis was observed under an optical microscope OLYMPUS CH30 (OLYMPUS, Japan) and photographed with different objectives (× 10, × 40, × 100). The size of the stomata was determined using imageJ software. The stomatal aperture is calculated using the formula of width (µm)/length of the stomata (µm).

The observation of root anatomy steps was performed according to Peterson et al. (2008). The roots were horizontally sliced manually with a razor blade, 1 cm from the root tip (5 slices). The samples were bleached with 10% javel water for 15 min followed by washing with distilled water 3 times, rinsing with acetic acid 45% for 10 min and then washing with distilled water 3 times. The samples were stained with carmine (Sigma-Aldrich, USA) for 3 min and washed with distilled water 3 times. Finally, the samples were observed and photographed under an optical microscope. Root diameter was measured using imageJ software (mm).

### Plant acclimatization

Fifty gerbera plantlets from each treatment were collected and washed the agar off. They were transplanted into foam propagation trays (84 holes and 3.5 cm in diameter each) containing soilless mixture—a special substrate for micropropagation (Metro-Mix 350, USA). Plants were watered twice a day for the first 15 days and the number of watering was reduced to 1 time/day thereafter. After 30 days, the plants were transferred to larger pots and fertilized with NovaTec premium (COMPO, Germany) two times per month. Survival rate and growth parameters were recorded after 30 days. Further growth of plants was recorded until flowering.

## Statistical analysis

All experiments were designed completely randomized, repeated 3 times. For in vitro experiments, each replicate consisted of 90 samples/treatment. For ex vitro experiments, each replicate consisted of 50 plantlets/treatment. All data were analyzed statistically by SPSS software version 16.0 with Duncan test at significance level $p < 0.05$ (Duncan 1955).

## Results

### Characteristics of SeNPs synthesized

As described by Wang et al. (2015), we performed and obtained a homogeneous SeNPs solution with bright red color and no adhesive phenomenon. TEM observation results showed that SeNPs synthesized showed that shape, size and dispersion of particles in solution were quite homogeneous; accordingly, SeNPs showed spherical shape and average particle size of 80 nm (Fig. 1A). In addition, the presence of SeNPs was shown through the peak at 294 nm wavelength (Fig. 1B); nearly, Wang et al. (2015) reported that the synthesized SeNPs peaked at 265 nm. It is widely accepted that the nanoparticles are less than 100 nm in size (Khan et al. 2019). This is in line with our results, SeNPs were successfully synthesized from the reduction of $H_2SeO_3$ with ascorbic acid in the presence of arabic gum at room temperature, and it was used as a nanomaterial for next experiments.

### Adventitious root formation of gerbera shoots on medium containing SeNPs

The results showed that SeNPs were an effective in vitro rooting agent for gerbera and it could a substitute for auxin. Rooting time of shoots cultured on medium supplemented with concentration of 0.3 to 1.5 mg/L SeNPs and control (+) treatment (1 mg/L IBA) were similar; while, treatments with 0.1 or 3 mg/L SeNPs or control (-) (free-auxin) rooted more than 3 days later (Table 1).

The percentage of plants with greater than 3 roots increased proportionally with SeNPs concentrations from 0.3 to 0.7 mg/L. Treatments with 0.7, 1, 1.5 mg/L SeNPs and control (+) did not show statistically significant differences in the percentage of plants with ≥ 3 roots, number of roots, root length and root diameter. Besides, in these treatments, lateral root formation was observed (Table 1). However, at high concentrations of SeNPs (3 mg/L), all the above-mentioned indicators were reduced and were also lower than those in control (+) treatment. Secondary root induction was also not observed.

The results of histological analysis showed that roots treated with IBA and 0.3 to 1.0 mg/L SeNPs developed root hairs, which were long and dense (Fig. 2B). Whereas the root hairs under control (−) treatment were sparse and not evenly distributed on the root surface. However, the root diameter was high (0.87 mm ± 0.11) (Table 1). In contrast, 3 mg/L SeNPs treatment produced roots which were smaller in diameter (0.52 mm ± 0.11) compared with the other treatments. Microscopic observations showed that root hairs were short and sparse (Fig. 2B). This demonstrated that high concentrations of SeNPs inhibited normal root growth.

### In vitro growth of gerbera plantlets on medium containing SeNPs

In micropropagation, good quality tissue cultured plants are required to have the balanced development of the roots and

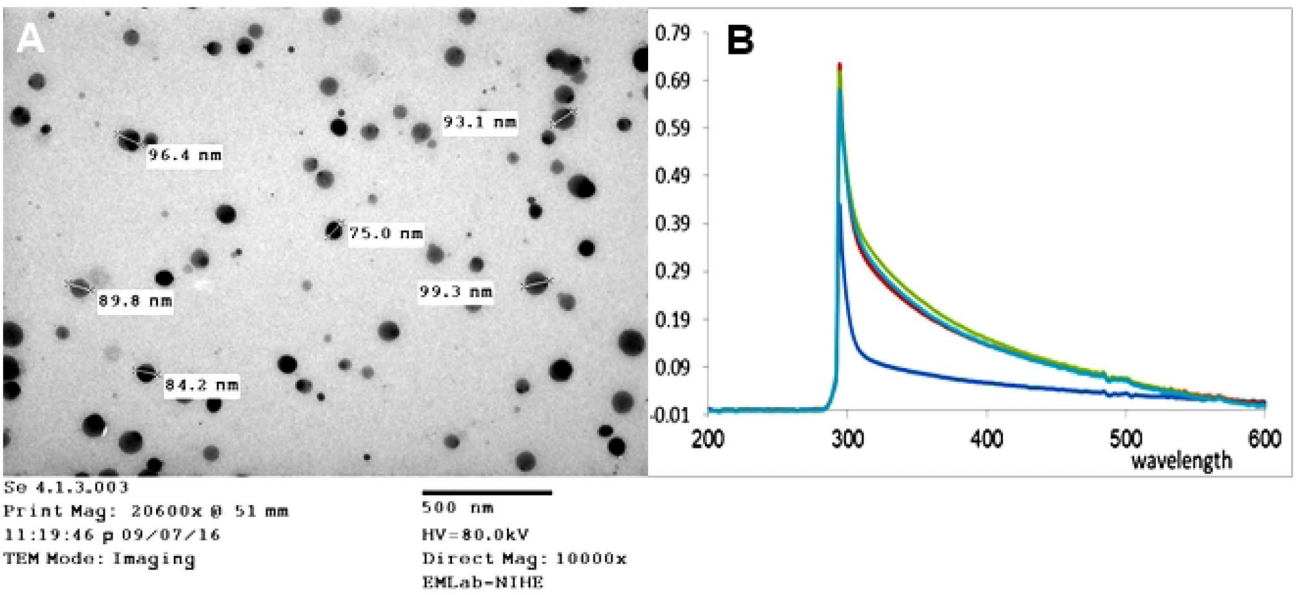


**Fig. 1** Characterization of chemically synthesized selenium nanoparticles (SeNPs). **A** The shape and size of SeNPs are illustrated by TEM. (Bar = 500 nm). **B** Absorption spectroscopy analysis (UV/VIS) of SeNPs at different dilutions (red:1/50, green:1/100, light blue:1/150, blue:1/200)

**Table 1** Effects of different concentrations of SeNPs on in vitro rooting of gerbera shoots after 15 days of culture

| SeNPs (mg/L) | Rooting (day) | Percentage of rooted-plants (%) | | No. of roots | Root length (cm) | Root diameter (mm) | Note |
|---|---|---|---|---|---|---|---|
| | | ≥ 3 roots | < 3 roots | | | | |
| Control (+) | 6.33 ± 0.58c* | 88.90 ± 11.10a | 11.10 ± 11.10b | 4.11 ± 1.05a | 3.24 ± 0.30a | 0.65 ± 0.41c | Induction of lateral roots |
| Control (−) | 9.67 ± 0.58a | 11.10 ± 11.10c | 88.90 ± 11.10a | 1.44 ± 0.73c | 2.00 ± 0.52c | 0.87 ± 0.11a | – |
| 0.1 | 9.33 ± 1.15a | 44.44 ± 11.10ab | 55.56 ± 11.10ab | 2.33 ± 0.71b | 2.20 ± 0.61bc | 0.74 ± 0.60b | – |
| 0.3 | 7.67 ± 0.58bc | 55.56 ± 22.22ab | 44.45 ± 22.22ab | 2.89 ± 0.78b | 2.26 ± 0.25bc | 0.73 ± 0.26b | – |
| 0.5 | 7.33 ± 1.15bc | 77.78 ± 22.22a | 22.22 ± 22.22b | 2.89 ± 1.27b | 2.61 ± 0.6b | 0.66 ± 0.36c | – |
| 0.7 | 6.67 ± 1.15bc | 88.90 ± 11.10a | 11.10 ± 11.10c | 3.89 ± 0.87a | 2.73 ± 0.64ab | 0.65 ± 0.19c | Induction of lateral roots |
| 1 | 6.33 ± 0.58c | 88.90 ± 11.10a | 11.10 ± 11.10c | 3.78 ± 0.83a | 2.68 ± 0.69ab | 0.66 ± 0.13c | Induction of lateral roots |
| 1.5 | 6.00 ± 0.53c | 88.90 ± 11.10a | 11.10 ± 11.10c | 3.89 ± 0.93a | 3.22 ± 0.37a | 0.64 ± 0.40c | Induction of lateral roots |
| 3 | 8.33 ± 1.53ab | 66.67 ± 19.25a | 33.33 ± 19.25b | 2.11 ± 0.78c | 0.86 ± 0.27d | 0.52 ± 0.11d | – |

Values (mean ± standard deviation)

Different letters (a, b, …) in the same column represent statistically significant differences at $p < 0.05$ (Duncan's test). Control (+) (MS medium added 1.0 mg/L IBA), Control (-) (MS medium without IBA/SeNPs), (-) (No induction)


**Fig. 2** Effects of different concentrations of SeNPs on in vitro rooting and gerbera shoot growth on auxin-free medium after 15 days of culture. The arrangement from left to right corresponds to the control (−), control (+), 0.1, 0.3, 0.5, 0.7, 1, 1.5, 3 mg/L SeNPs treatments. **A** Gerbera plantlets and **B** Root cross-sections (Bar = 2 cm and 300 µm, respectively). rh-root hair, ex-exodermis, phl-phloem en-endodermis and mx-metaxylem vessels

shoots. In vitro growth of gerbera shoots on medium supplemented with different concentrations of SeNPs was recorded and shown in Table 2; Fig. 2A. The results suggested that SeNPs at the appropriate concentration improved shoot growth significantly as compared to the two control treatments. Growth indicators such as plant height, leaf length, total chlorophyll content, FW and DM increased proportionally with SeNPs concentration from 0.1 to 1.5 mg/L.

Optimal plant height was achieved at a concentration 0.7 to 1.5 mg/L SeNPs (Table 2), while in control (+) it was 4.13 cm ± 0.39 and in control (-) it was 3.66 cm ± 0.38. At the concentration range of 0.7 to 1.5 mg/L SeNPs, the leaf lengths were significantly longer and oblong than in other treatments (Table 2). Meanwhile, leaves formed on medium containing IBA, auxin-free and SeNPs at lower concentrations were oval-oblong shape (Fig. 2A).

In addition, SeNPs also stimulated the biosynthesis of photosynthetic pigments. Specifically, at the concentration of 0.7 and 1 mg/L SeNPs, the total chlorophyll content in the leaves was the highest (33.66 nmol/cm$^2$ ± 1.03

**Table 2** Effects of different concentrations of SeNPs on in vitro growth of gerbera shoots after 15 days of culture

| SeNPs (mg/L) | Plant height (cm) | No. of leaves | Leaf length (cm) | Leaf width (cm) | Total chlorophyll (nmol/cm$^2$) | Biomass of plantlet | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | FW (mg) | DW (mg) | DM (%) |
| Control (+) | 4.13 ± 0.39bcd* | 4.67 ± 0.6a | 1.66 ± 0.14b | 1.26 ± 0.13a | 30.82 ± 2.03c | 370.89 ± 0.07a | 27.56 ± 0.02bc | 7.56 ± 1.02bc |
| Control (−) | 3.66 ± 0.38e | 3.56 ± 0.58c | 1.67 ± 0.22b | 1.23 ± 0.19ab | 32.12 ± 3.08bc | 252.33 ± 0.05c | 17.11 ± 0.01d | 6.96 ± 0.66 cd |
| 0.1 | 3.54 ± 0.50e | 3.56 ± 0.57c | 1.67 ± 0.16b | 1.10 ± 0.19abc | 30.44 ± 1.53c | 265.56 ± 0.03c | 16.22 ± 0.02d | 6.15 ± 0.81d |
| 0.3 | 3.96 ± 0.6cde | 3.78 ± 1.09bc | 1.69 ± 0.20b | 1.09 ± 0.17abc | 32.60 ± 1.79bc | 293.22 ± 0.04bc | 17.44 ± 0.01d | 5.98 ± 1.13d |
| 0.5 | 4.20 ± 0.51bc | 3.89 ± 0.93bc | 1.64 ± 0.17b | 1.04 ± 0.21bcd | 32.46 ± 1.50bc | 331.78 ± 0.06ab | 25.89 ± 0.01c | 7.99 ± 0.41bc |
| 0.7 | 4.56 ± 0.42ab | 4.22 ± 0.83ab | 1.84 ± 0.23a | 1.03 ± 0.20bcd | 33.66 ± 1.03ab | 383.22 ± 0.03a | 29.45 ± 0.01ab | 7.72 ± 0.65bc |
| 1 | 4.70 ± 0.40a | 4.33 ± 1.00b | 1.89 ± 0.23a | 1.04 ± 0.20bcd | 35.47 ± 2.53a | 386.00 ± 0.10a | 31.33 ± 0.02a | 8.33 ± 0.84bc |
| 1.5 | 4.26 ± 0.42abc | 4.67 ± 0.71a | 1.85 ± 0.18a | 1.00 ± 0.17 cd | 31.24 ± 2.05bc | 331.78 ± 0.08ab | 27.33 ± 0.02bc | 8.58 ± 1.36ab |
| 3 | 3.73 ± 0.55de | 3.33 ± 0.50c | 1.62 ± 0.22b | 0.84 ± 0.18d | 26.38 ± 3.89d | 190.56 ± 0.03d | 18.22 ± 0.01d | 9.75 ± 1.23a |

Values (mean ± standard deviation)

Different letters (a, b, …) in the same column represent statistically significant differences at $p < 0.05$ (Duncan's test). Control (+) (MS medium added 1.0 mg/L IBA), Control (−) (MS medium without IBA/SeNPs)

and 35.47 nmol/cm$^2$ ± 2.53, respectively). The results indicated that the addition of 0.7 and 1 mg/L SeNPs had a positive effect on the chlorophyll content in the gerbera leaves (Table 2, Fig. 2A).

In contrast, high concentration of SeNPs (3 mg/L) clearly decreased plantlets quality. In vitro shoots growth in this treatment was significantly reduced, which included plant height (3.37 cm ± 0.55), number of leaves (3.33 ± 0.50), leaf width (0.84 cm ± 0.18), total chlorophyll content (26.38 nmol/cm$^2$ ± 3.83), FW (190.56 mg ± 0.03), DW (18.22 mg ± 0.01) (Table 2). However, the DM in this treatment was the highest (9.75% ± 1.23) when compared to the other treatments. In other words, SeNPs influenced the water state in the plantlets and most likely influenced the stomatal characteristics and function discussed below.

All of these results indicate that SeNPs can either stimulate or inhibit shoot growth depending on the different concentrations used. The results suggested that the application of SeNPs at concentrations from 0.7 to 1.5 mg/L showed a positive effect on the growth of gerbera microshoots. This effect of SeNPs is valuable for micropropagation of many other economically important plants.

## Effects of SeNPs on the activity of antioxidant enzymes

To evaluate the effect of different concentrations of SeNPs on the antioxidant system in gerbera plantlets, the activity of CAT, SOD and APX was tested and shown in Fig. 3. The results showed that the addition of SeNPs to the culture medium positively modulated the activity of antioxidant enzymes. CAT activity was highest at a concentration of 3 mg/L SeNPs (275.58 Umin$^{-1}$ g$^{-1}$prot ± 4.40) compared with the remaining concentrations and both control treatments. Whereas at low SeNPs (0.1 mg/L) and control (-) the CAT activity was the lowest (Fig. 3A).

At concentrations ranging from 0.7 to 1.5 mg/L SeNPs, SOD activity was the highest compared to the remaining concentrations. SOD activity of plantlets from the 1 mg/L SeNPs treatment was the highest (43.13 Ug$^{-1}$prot ± 3.48), 1.88-fold higher than control (+) treatment, and 2.56- fold higher than control (-) treatment (Fig. 3B).

APX activity at concentrations of 1 and 1.5 mg/L SeNPs was greater than 0.6 Umin$^{-1}$ g$^{-1}$prot, while in both the control (-) and (+) they were only about 0.5 U min$^{-1}$ g$^{-1}$prot. However, when the concentration of SeNPs was increased to 3 mg/L, the APX activity decreased significantly (0.53 U min$^{-1}$ g$^{-1}$prot ± 0.03) (Fig. 3C). All these results suggested that SeNPs may have influenced the growth of gerbera plantlets through stimulation of the activity of the antioxidant defense system.

## Effects of SeNPs on endogenous hormone content

The results of HPLC analysis showed that SeNPs significantly changed the hormone homeostasis in in vitro plantlets after 15 days of culture (Fig. 4). As show in Fig. 4A, all concentrations of SeNPs stimulated an increase in endogenous AUX significantly higher than the control (-). The highest and lowest AUX levels were observed in the 1.5 mg/L SeNPs and control (-) treatments, respectively. The application of 1.5 mg/L SeNPs increased the AUX content 1.55-fold higher than the control (+) and 6.14-fold higher than the control (-). However, an increase in the concentration of SeNPs from 1.5 mg/L to 3 mg/L which significantly reduced AUX was observed (Fig. 4A).

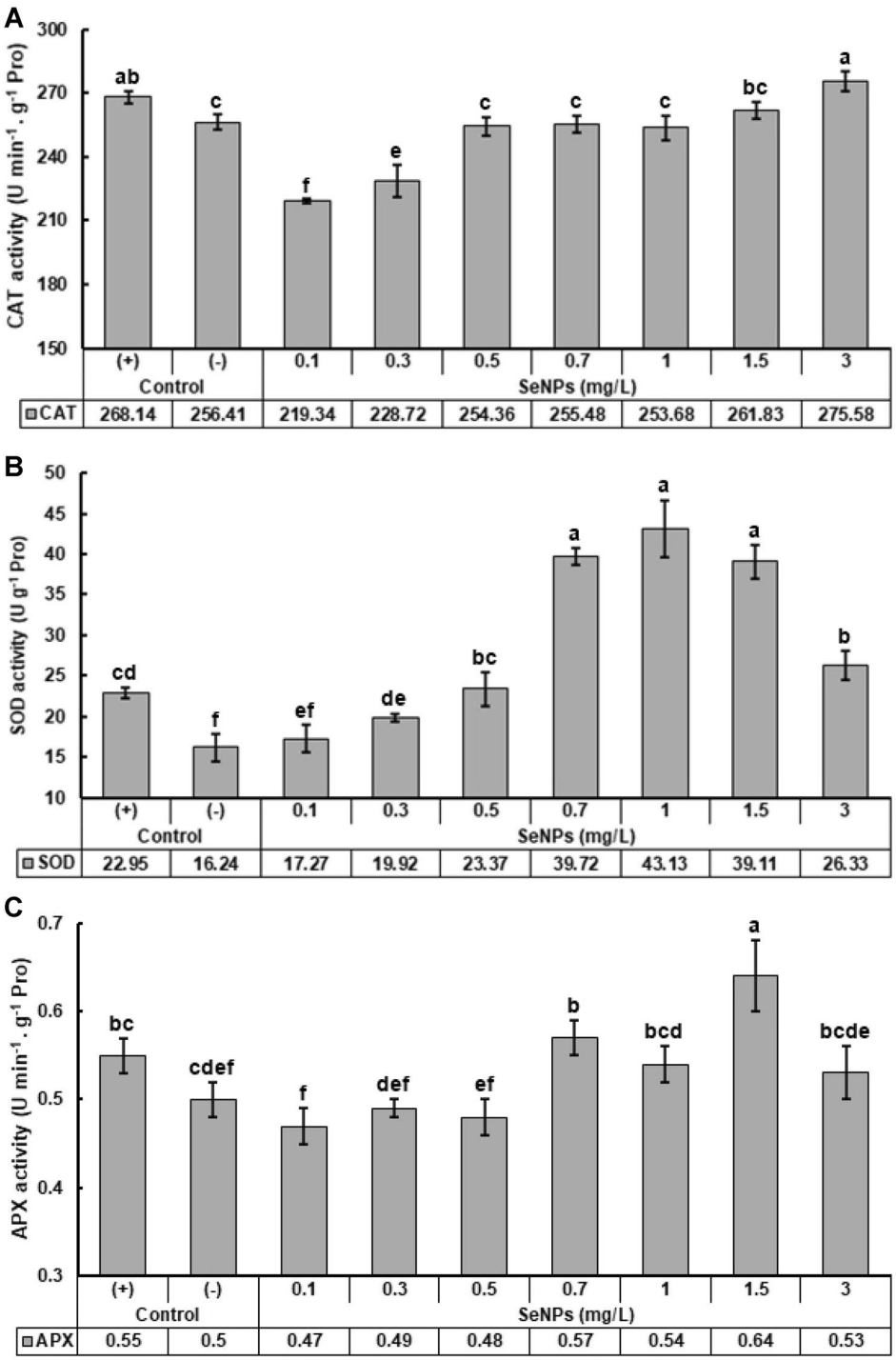


**Fig. 3** Effects of different concentrations of SeNPs on the activity of antioxidant enzymes in gerbera plantlets after 15 days of culture. **A** CAT activity. **B** SOD activity. **C.** APX activity. All statistics were analysed using the Duncan's test, distinct letters show significant differences ($p < 0.05$). Error bars show the Standard Deviation

For ABA, an increase in SeNPs concentrations was positively correlated with ABA biosynthesis (Fig. 4B). Accordingly, the highest ABA level was observed in the 3 mg/L SeNPs treatment, as well as it was 4.33-fold higher than the control (+) and 2.74-fold higher than the control (-). Besides, the application of SeNPs from 0.7—1.5 mg/L also stimulated ABA increase significantly higher than the control treatments. Meanwhile, at low concentrations of SeNPs (0.1 mg/L and 0.3 mg/L) the ABA content did not show a statistically significant difference ($p < 0.05$) compared with the control treatments (Fig. 4B).

Similar to AUX, the highest GA levels were observed in the 1.5 mg/L SeNPs treatment which was 3.66-fold higher than the control (+) and 4.70-fold higher than the control (−). In addition, the GA content was reduced 10.70-fold when using 3 mg/L SeNPs instead of 1.5 mg/L

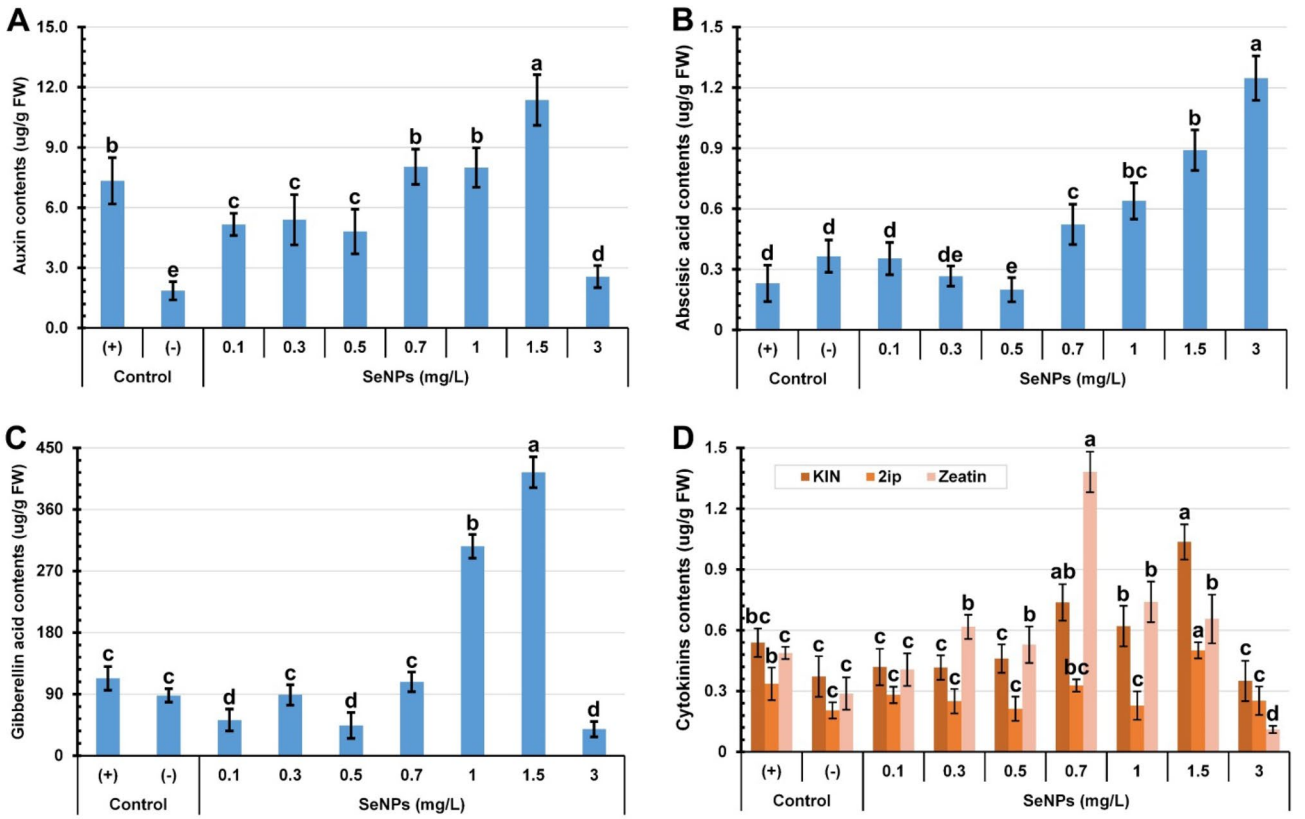


**Fig. 4** Effects of different concentrations of SeNPs on endogenous hormones concentrations in gerbera plantlets after 15 days of culture. **A.** IAA. **B.** ABA. **C.** $GA_3$. **D.** KIN, 2ip, Zeatin. All statistics were analysed using the Duncan's test, distinct letters show significant differences ($p < 0.05$). Error bars show the Standard Deviation

SeNPs. In contrast, application of SeNPs from 0.1 to 0.7 mg/L did not cause a major change in GA biosynthesis compared with the control treatments (Fig. 4C).

As shown in Fig. 4D, the increase of SeNPs concentration from 0.1 to 1.5 mg/L was positively correlated with endogenous KIN content. At the concentration of 1.5 mg/L SeNPs, the KIN content was 1.92-fold higher than the control (+) and 2.78-fold higher than the control (-). Also, the highest 2ip levels were observed in the 1.5 mg/L SeNPs treatments. However, at high concentrations of SeNPs (3 mg/L) the biosynthesis of KIN, 2ip and Zeatin were all reduced compared with other treatments. While at low concentrations of SeNPs (0.1–0.5 mg/L) the levels of KIN and 2ip were unchanged compared with both controls. For Zeatin, an increase in SeNPs concentrations from 0.3 to 1.5 mg/L was positively correlated with endogenous Zeatin biosynthesis. Accordingly, the highest Zeatin levels were observed in the 0.7 mg/L SeNPs treatment which was 2.82-fold higher than the control (+) and 4.79-fold higher than the control (−) (Fig. 4D).

## Effect of SeNPs on in vitro stomatal density and morphology

To determine the influence of SeNPs in the development of stomata gerbera leaves, we observed the development of stomata, and the results were shown in Fig. 5. Interestingly, SeNPs exhibited a positive effect on both the formation and development of gerbera stomata.

The stomatal density results showed that the increase in concentration from 0.1 to 0.7 mg/L SeNPs was proportional to the increase in stomatal density. Meanwhile, at concentrations 1, 1.5 and 3 mg/L SeNPs, the stomatal frequency decreased significantly. In addition, the results also showed that the presence of IBA in the culture medium significantly inhibited stomatal frequency. The stomatal density was highest in the treatment of 0.7 mg/L SeNPs (473.58 stomata/$mm^2 \pm 6.30$). It was 2.34-fold higher than the control (+), and 2.42-fold higher than the treatment using high SeNPs concentrations (3 mg/L) (Fig. 5A, B).

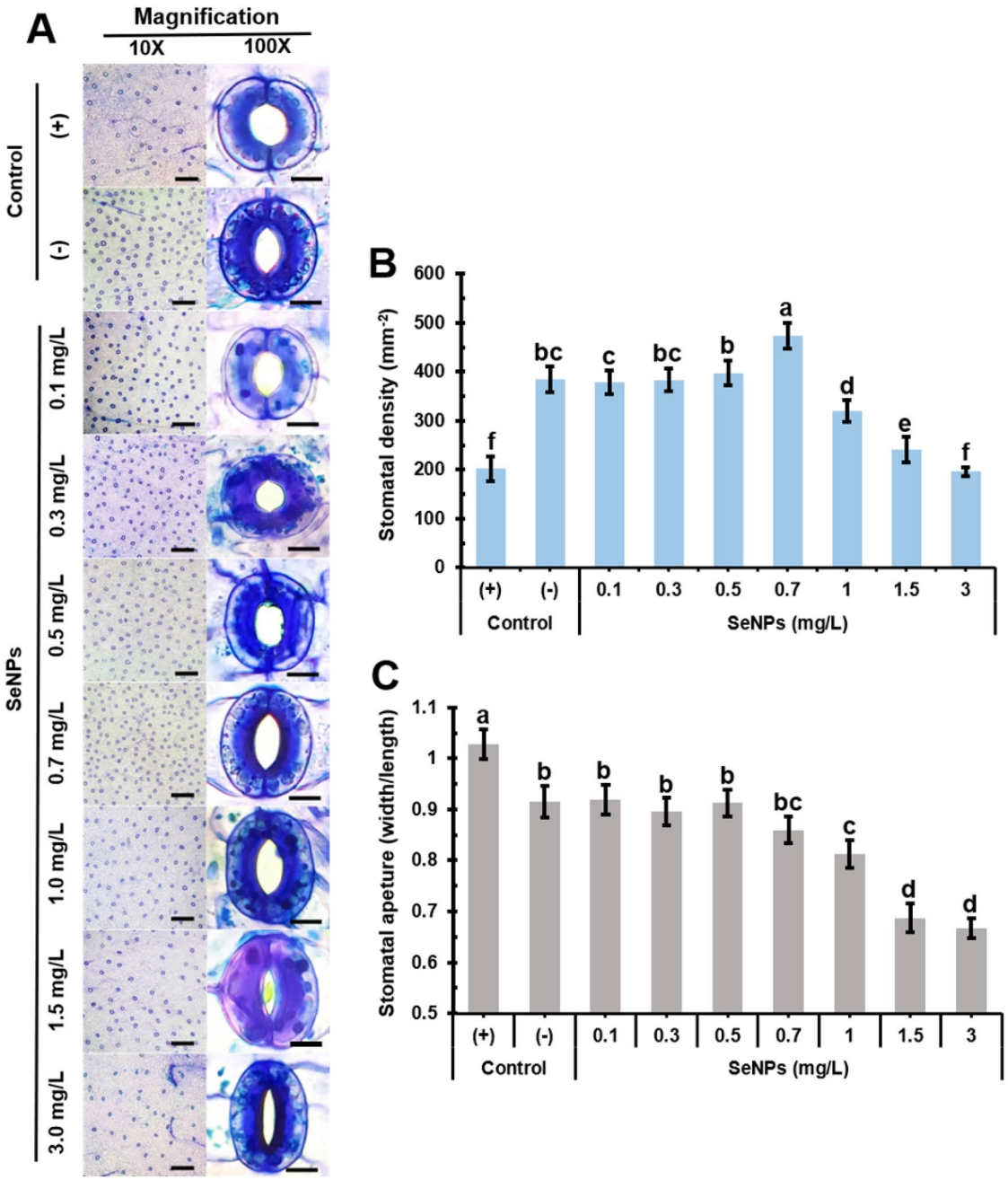


**Fig. 5** Effects of different concentrations of SeNPs on in vitro gerbera leaf stomatal morphology and density after 15 days of culture. **A** Stomatal morphology at different magnifications (mag) (Bar = 50 µm at X10 mag and 5 µm at X100 mag). **B** Stomatal density ($mm^{-2}$). **C** Stomatal aperture (width/length). All statistics were analyzed using the Duncan test, the separate letters showed significant differences ($p < 0.05$). Error bars show Standard Deviations

SeNPs not only promoted the formation of the stomata, but also influenced the morphology and the opening of the stomata. An increase in SeNPs concentration from 0.1 to 3 mg/L SeNPs was accompanied by marked decrease in stomatal opening. The morphology of the stomata on the IBA-containing medium was more or less round shape corresponding to longest stomatal aperture (1.03 width/length ± 0.03) compared the remaining treatments (Fig. 5A, C). Whereas the stomata in the control (-) exhibited a shorter aperture (0.91 width/length ± 0,03) corresponding to the oval shape. At a concentration of 0.7 mg/L SeNPs stomata was oval-oblong shape. The stomatal aperture was the shortest at concentrations of 1.5 and 3 mg/L SeNPs (0.68, 0.66 width/length, respectively); the stomatal morphology of

these treatments indicated a closed trend. Accordingly, the stomata of 3 mg/L SeNPs treatment were elliptical shape (oblong) (Fig. 5A, C).

## Subsequent growth of gerbera plantlets derived from SeNPs in the greenhouse

After 30 days of transfer to the greenhouse, survival rates and growth indicators of gerbera plantlets were recorded in Table 3, Fig. 6. The results showed that the plantlets derived from treatments 0.7, 1 and 1.5 mg/L SeNPs survived over 95%; while plantlets derived from the medium supplemented with IBA and free-auxin were significantly lower (87% ± 6.02 and 61.33% ± 7.66, respectively) (Table 3). Morphological observations showed that the plants derived from SeNPs treatments were uniform in quality, showed healthy growth without any abnormal morphology (Fig. 6A). These results demonstrate that SeNPs significantly improved the viability of gerbera plantlets under greenhouse conditions. Growth results showed that treatments of 0.7, 1 and 1.5 mg/L SeNPs and control (+) showed similarity in plant height, number of new leaves, leaf length, leaf width and chlorophyll content; while the control (-) was significantly lower (Table 3).

In particular, plantlets derived from SeNPs also showed differences in flowering time. The results showed that flowering time was significantly shortened in plantlets derived from 0.5 to 1.5 mg/L SeNPs. The earliest flowering time was observed with the 0.7 mg/L SeNPs treatment (64.00 days ± 6.61), it was 13 days earlier than the control (+), and 20 days earlier than the control (-) (Table 3, Fig. 6B). Morphological observations showed that flowers

**Table 3** Acclimatization after 30 days and flowering time of SeNPs-derived gerbera plantlets after 90 days in the greenhouse

| SeNPs (mg/L) | Survival rate (%) | Plant height (cm) | No. of new leaves | Leaf length (cm) | Leaf width (cm) | Total chlorophyll (nmol/cm$^2$) | Flowering time (day) |
|---|---|---|---|---|---|---|---|
| Control (+) | 87.00 ± 6.02b* | 6.90 ± 0.08a | 1.22 ± 0.58ab | 2.61 ± 0.23bc | 1.93 ± 0.70ab | 36.72 ± 0.84ab | 77.67 ± 3.58b |
| Control (-) | 61.33 ± 7.66d | 5.48 ± 0.25c | 0.33 ± 0.00d | 2.32 ± 0.06e | 1.68 ± 0.12c | 35.32 ± 2.64 cd | 87.33 ± 5.52a |
| 0.1 | 63.67 ± 4.36d | 5.58 ± 0.26c | 0.44 ± 0.00 cd | 2.43 ± 0.38de | 1.63 ± 0.12c | 37.26 ± 1.11b | 86.00 ± 6.61a |
| 0.3 | 83.33 ± 4.35b | 5.89 ± 0.07b | 0.33 ± 0.00d | 2.55 ± 0.25 cd | 1.65 ± 0.06c | 36.81 ± 0.19bc | 76.00 ± 5.65bc |
| 0.5 | 85.67 ± 4.46b | 6.98 ± 0.21a | 1.00 ± 0.37bc | 2.78 ± 0.06ab | 1.86 ± 0.06ab | 38.43 ± 1.13ab | 71.67 ± 3.58 cd |
| 0.7 | 97.67 ± 2.11a | 7.00 ± 0.10a | 1.44 ± 0.40ab | 2.78 ± 0.15ab | 1.92 ± 0.06ab | 38.31 ± 2.77ab | 64.00 ± 6.61d |
| 1 | 98.67 ± 1.11a | 7.06 ± 0.12a | 1.78 ± 0.58a | 2.91 ± 0.06a | 1.96 ± 0.06a | 39.59 ± 0.87a | 69.33 ± 3.58 cd |
| 1.5 | 96.33 ± 3.56a | 7.11 ± 0.20a | 1.77 ± 0.27a | 2.80 ± 0.15ab | 1.81 ± 0.12b | 39.36 ± 1.32a | 70.00 ± 6.00 cd |
| 3 | 74.00 ± 6.56c | 6.90 ± 0.09a | 1.00 ± 1.00bc | 2.62 ± 0.06bc | 1.63 ± 0.06c | 35.00 ± 1.71d | 81.00 ± 5.65ab |

Values (mean ± standard deviation)

Different letters (a, b, …) in the same column represent statistically significant differences at $p < 0.05$ (Duncan's test). Control (+) (MS medium added 1.0 mg/L IBA), Control (-) (MS medium without IBA/SeNPs)

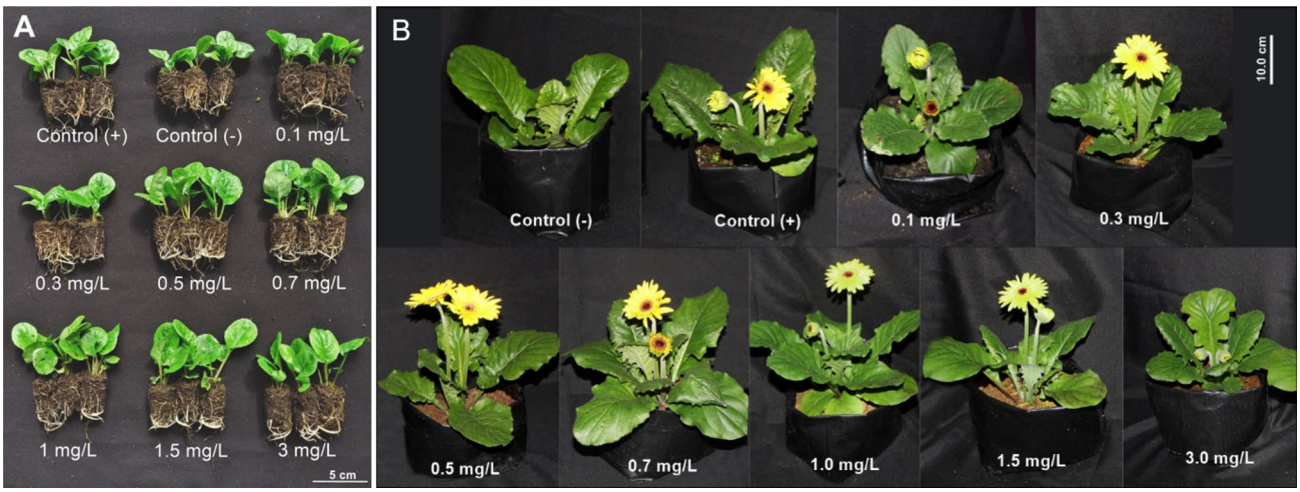


**Fig. 6** Subsequent growth of gerbera plantlets derived from SeNPs and controls in the greenhouse. **A** Plantlets after 30 days of acclimatization (Bar = 5 cm). **B** The flowering stage of plantlets after 90 days (Bar = 10 cm)

derived from SeNPs supplemented medium treatments were large and uniform size, with long and thick petals. Flower longevity was higher than those of the controls (Fig. 6B). All these results suggested that the presence of element Se in gerbera plantlets improved their viability under ex vitro conditions. Furthermore, these plantlets were able to increase adaptation and growth, which resulted in earlier flowering than those were grown in the control.

## Discussion

### SeNPs as an exogenous auxin substitute for in vitro rooting of gerbera shoots

Our experimental results showed that MS medium supplemented with 0.7, 1 and 1.5 mg/L SeNPs gave a similar rooting effect when supplemented with 1 mg/L IBA, as well as significantly higher compared with the treatment without auxin/SeNPs. Similar results were found in the tobacco model, where the application of 265—530 µM SeNPs stimulated root growth about 40-fold; while selenate inhibited this growth at similar concentrations (Domokos-Szabolcsy et al. 2012). It was shown that SeNPs exerted different physiological effects than Se at larger sizes. Moreover, at the nanoscale, Se caused less phytotoxicity than the conventional ionic form (Domokos-Szabolcsy et al. 2012; Han et al. 2013; Bhattacharjee et al. 2019; Hajiboland et al. 2020). In addition, SeNPs stimulated the formation of lateral roots and root hairs, ie. SeNPs had a significant effect on the morphology and microstructure of the root organ. Similarly, the addition of 1 mg/L SeNPs increased primary root length and number of lateral roots in tobacco (Jia et al. 2018).

It is well documented that Se is effective in improving the activity of the antioxidant defense system (Mroczek-Zdyrska and Wójcik 2012; Boldrin et al. 2016; Jóźwiak and Politycka 2019; Xu et al. 2019; Niu et al. 2020; Wu et al. 2020; Peng et al. 2020). This is in line with our results, the activities of antioxidant enzymes (CAT, SOD and APX) in gerbera plantlets were significantly increased when SeNPs were added to the culture medium. Meanwhile, these enzymes play a key role in protecting phytohormones from oxidation or inactivation by factors such as ROS (Stonier 1971; Ambros et al. 2018). Indeed, the results of HPLC analysis revealed that endogenous AUX levels were elevated in the presence of 1.5 mg/L SeNPs. Therefore, it is likely that SeNPs stimulated rooting of gerbera microshoots through interfering with endogenous AUX-influenced pathways. Recently, Jia et al. (2018) confirmed that application of Se at low concentrations increased endogenous AUX biosynthesis in tobacco, thereby increasing the tolerance to phosphorus-deficiency stress. Consistently, Luo et al. (2019) confirmed that the mechanism of Se anti-cadmium toxicity in tobacco is based on increased endogenous AUX biosynthesis. This could be explained by Se increased expression of genes involved in the synthesis of auxin and auxin efflux carriers (Jia et al. 2018).

### SeNPs improved in vitro growth of gerbera plantlets, antioxidant enzyme activity and phytohormone content

In the present case, SeNPs significantly improved the in vitro growth of gerbera microshoots as well as improved antioxidant enzyme activity and endogenous hormone content. In fact, adverse environmental factors exist and induce large amounts of reactive oxygen species (ROS) including hydrogen peroxide ($H_2O_2$), singlet oxygen ($^{\cdot}O^{2-}$), and hydroxyl radicals ($^{\cdot}OH$). ROS disrupt many biological processes by altering intracellular signaling pathways (Mittler 2002). For instance, ROS induces lipid peroxidation which damages cell membranes. More seriously, ROS can damage the structure of DNA strands by reacting with biological molecular components that create DNA (Jóźwiak and Politycka 2019). Therefore, improving the activity of the antioxidant defense system is an important biological process for improving plant growth; furthermore, this improvement is particularly important for the stress tolerance of in vitro plantlets during the acclimatization stage.

Recently, element Se has been recognized as a good candidate for stimulating the antioxidant system of plants (Pilon-Smits et al. 2009; Wang et al. 2019). This prominent role is why Se has been applied to plants to tolerance stresses such as water deficit stress for cucumber (Jóźwiak and Politycka 2019), mercury stress for rice (Xu et al. 2019), cadmium stress for wheat (Wu et al. 2020) and rice (Huang et al. 2020), arsenic stress for rice (Pokhrel et al. 2020). To date, Se is mainly used in agriculture (Wang et al. 2016; White 2018; Peng et al. 2020). However, the benefits of Se are not yet exploited in the field of micropropagation. Furthermore, the benefits of application of Se to in vitro plants those counteract stress at the acclimation stage has not been previously reported. In this study, SeNPs showed a positive effect on the activity of CAT, SOD and APX enzymes. Furthermore, this increase in enzymatic activities was highly correlated with in vitro growth and phytohormone content in gerbera plantlets. Indeed, the levels of AUX, GA, CKs and ABA were all significantly increased when 0.7 mg/L to 1.5 mg/L SeNPs were added to the culture medium. These results are consistent with the study that Se promoted plant growth through interactions with phytohormones (Ağar et al. 2006).

Se affects the aspect of phytonutrients and thereby affects their growth (Pazurkiewicz-Kocot et al. 2003). Recently, Boldrin et al. (2016) reported that genes involved in sulfate transport in plants such as *SULTR1;1, SULTR1;3* and

*SULTR4;1* were stimulated to express in the presence of Se; thus, increasing the supply of sulfur nutrients for plant growth. Similarly, Se enhanced the absorption of nutrients in plants such as Fe, Mn, Cu, Ca, and Mg (He and Wang 2004; Mroczek-Zdyrska and Wójcik 2012). This evidence suggests that Se can be beneficial for nutrient uptake in plants; as the result, stimulating the growth of plants.

Similar to other nutritional elements, Se causes toxicity at high doses. In this study, the addition of 3 mg/L SeNPs to the culture medium negatively affected the development of in vitro gerbera shoots and roots. Similar results were found in tobacco (Domokos-Szabolcsy et al. 2012; Han et al. 2013) and rapeseed (Hajiboland et al. 2020). In the present case, the SOD and APX enzyme activities as well as the levels of AUX, CKs and GA were significantly reduced with increasing the concentration of SeNPs from 1.5 to 3 mg/L SeNPs; in contrast, elevation of ABA was observed. From these results, it is likely that the high concentration of SeNPs (3 mg/L) negatively affected the in vitro growth of gerbera plantlets through decreased phytohormone biosynthesis and decreased activity of ROS scavenging enzymes. Sabatino et al. (2021) reported that Se poisoning disturbs nutrient absorption in plants, thereby adversely affecting plant growth. Besides, the excess absorption of Se disturbs biochemical reactions in cells. For example, Se catalyzes the oxidation of thiols and the production of superoxide which damages the cell wall (Wang et al. 2007; Domokos-Szabolcsy et al. 2012). Furthermore, it disturbs sulfur absorption, metabolism and biochemical reactions (Pazurkiewicz-Kocot et al. 2003); which leads to a sulfur deficiency that is required for plant growth.

Considering the above information and present study results, SeNPs improved the in vitro growth of gerbera plantlets by increasing phytohormone synthesis, stimulating antioxidant activity. However, positive results can only achieve by confirming the application of appropriate concentrations. Therefore, extensive research is needed when using Se in plant tissue culture.

## Positive effects of SeNPs on the stomatal development

Frómeta et al. (2017) reported that gerbera plantlets derived from micropropagation had non-functional stomata detrimental to their acclimation in the greenhouse. These abnormal stomata may be produced by the presence of auxin in culture media, which has recently been shown to inhibit stomatal growth (Balcerowicz et al. 2014). Similarly, low stomatal density and large stomatal apertures were observed in gerbera plantlets grown on medium supplemented with 1 mg/L IBA. In contrast, high stomatal density and average stomatal aperture were observed on medium containing 0.7 mg/L SeNPs. Meanwhile, low stomatal density and small aperture of stomata were observed in 3 mg/L SeNPs treatment. It has been found that the activities of CKs promote increased stomatal density (Farber et al. 2016). This coincided with the present results of a significant increase in KIN, 2ip and Zeatin when 0.7 to 1.5 mg/L SeNPs were added to the culture medium. However, the concentration of CKs did not affect the movement of stomata but instead ABA (Hronkova et al. 2003; Farber et al. 2016; Asayesh et al. 2017). Indeed, the gradual addition of SeNPs concentrations was positively correlated with endogenous ABA content and stomatal closure. This coincided with the present results of a significant increase in KIN, 2ip and Zeatin when 0.7 to 1.5 mg/L SeNPs were used.

DM accumulation is an indicator of the accumulation of dry biomass of plants, in other words the dry biomass of plants is inversely related to the water accumulation in plants. Benlloch-González et al. (2008) reported that high water accumulation in plant cells produces open stomata (cell turgor). The present results showed that SeNPs significantly increased the DM index of in vitro gerbera plantlets and reached the highest concentration of 3 mg/L SeNPs. This indicates that the relative water content of the plants cultured on the SeNPs-containing medium was significantly reduced compared with the control ( + ). This result is consistent with the stomata closure caused by SeNPs observed in this study. According to research by Pazurkiewicz-Kocot et al. (2003) for some ions, the membrane permeability coefficient is altered by the presence of Se ions, resulting in a change in the ion transport pathway in the cell, including the $K^+$ ions, which play a major role in the control of stomata opening/closing.

In a nutshell, SeNPs increased CKs biosynthesis leading to stimulation of stomatal formation in the leaves of gerbera plantlets. Furthermore, SeNPs regulated stomatal closure through regulation of endogenous ABA content. These effects play an important role in limiting uncontrolled transpiration under ex vitro conditions.

## Survival and growth of gerbera plantlets derived from SeNPs in the greenhouse

There have been several reports of improvement in the viability of gerbera plantlets under ex vitro conditions. Cardoso et al. (2013) transferred gerbera microshoots directly to a greenhouse for rooting and acclimation. Chakrabarty and Datta (2008) transferred all plantlets into humidity chamber (80–90%) for 15 days prior to transfer to the greenhouse; the survival rate was about 80%. Gantait and Mahanta (2021) used a mixture of soil, sand, tea leaf waste and cow urine to increase the survival rate of plantlets > 90%. Frómeta et al. (2017) used 1 mg/L IAA for in vitro rooting in bioreactor TIS with a survival rate

of 87%. In the present case, gerbera plantlets derived from 1 mg/L SeNPs treatment showed near absolute viability under ex vitro conditions (98.67%).

During the acclimation, antioxidant enzymes such as SOD, APX and CAT are critical to protect plantlets from the negative effects of oxidative stress (Dias et al. 2011; Perveen et al. 2013). This is in line with our results, the presence of Se increased the activity of CAT, SOD and APX in gerbera plantlets, thus significantly improved their survival rate in the greenhouse. Furthermore, Se showed an effect on the stomatal closure of gerbera plantlets; thus, helping them avoids dehydration during the acclimation. According to Balcerowicz et al. (2014) efficient stomata helped to increase photosynthetic efficiency; thus, promoting plant growth and development.

## Conclusion

The present study results showed that the half-strength MS medium containing 0.7, 1 and 1.5 mg/L effectively stimulated in vitro rooting and growth of gerbera microshoots. Furthermore, SeNPs increased the activity of antioxidant enzymes (CAT, SOD and POD) as well as modulated phytohormone biosynthesis (AUX, CKs, GA and ABA) in plantlets. From the results, it is likely that SeNPs affects in vitro rooting and stomatal development of plantlets via biochemical alterations. In addition, plantlets cultured with SeNPs showed good tolerance to acclimatization stresses in ex vitro conditions; as a result, their survival rate has been greatly improved, while the growth and flowering rates have been accelerated. These results open up new prospects for the application of SeNPs on economically crops and especially in potted plant micropropagation.

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**Author contributions** DTN and HDK conceptualized, designed the study and wrote manuscript. HDK and NTNM performed experiments and collected data. NQB and NHC synthesized SeNPs and provided related illustrations. NQV, DMD performed biochemical experiments. HTT, DMC, VQL, HTMN participated in interpreting the data and developing the manuscript. All authors discussed and revised the final manuscript.

## Declarations

**Conflict of interest** The authors declare that they have no conflicts of interest.

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