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RESEARCH NOTE

Rice husk-derived biogenic silica nanoparticles and zinc oxide nanoparticles as nano-additives for improving in vitro quince rootstock propagation

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Abstract

The application of nanomaterials is a promising tool to achieve more efficient in vitro propagation. This study evaluated the effectiveness of rice husk derived biogenic silica nanoparticles (SiO_2 NPs) and zinc oxide nanoparticles (ZnO NPs) on the growth and proliferation of in vitro cultures of quince rootstock 'QA'. In vitro cultures were exposed to six levels of SiO_2 NPs (0, 1, 5, 25, 50, 100 mg L^{-1}) and seven levels of ZnO NPs (0, 0.5, 1, 2.5, 5, 25, 50 mg L^{-1}). Proliferation and growth rate were determined in plantlets recovered from ZnO NPs and SiO_2 NPs-treatments and controls that were grown under in vitro conditions for 35 days. The results showed in vitro shoots of quince rootstock QA treated with SiO_2 NPs at a concentration of 1 mgL^{-1} had the highest number of axillary shoots (2.46). Also quince rootstock QA plantlets regenerated from ZnO NPs-treatments showed the highest shoot length and the number of leaves at a concentration of 2.5 mg L^{-1} (6.86 and 14.26, respectively). This research demonstrated the use of 1 mg L^{-1} of SiO_2 NPs and 2.5 mg L^{-1} of ZnO NPs in the tissue culture medium may improve proliferation and growth rate in quince rootstock 'QA' explants.

Keywords Biogenic silica nanoparticles · Zinc nanoparticles · Quince rootstock

Abbreviations

AFM	Atomic force microscopy
BAP	6-Benzylaminopurine
CLSM	Confocal laser scanning microscopy
DLS	Dynamic light scattering
GA_3	Gibberellic acid
Glm	General linear model
IBI	Indole-3-butyric acid
Mt	Megatonne
MS	Murashige and Skoog medium
ROS	Reactive oxygen species
SEM	Scanning electron microscopy
SiO_2 NPs	Silica nanoparticles
XRD	X-Ray diffraction
ZnO	NPs Zinc oxide nanoparticles

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Introduction

Quince (*Cydonia oblonga* Mill.) is one of the most important members of pome fruit species belonging to *Rosaceae* family. Globally, in 2019, the total quince-harvesting area was estimated at 93,699 ha and 666,589 Mt of fruit were produced (FAO 2020). Iran with 12.24% of the global production (81,594 t), is the fourth largest quince producer in the world after Turkey, China, and Uzbekistan (FAO 2020). Quince fruit is used to make jams, juices, liquors, and is also used in traditional medicine (Aliasl et al. 2016; Morelli et al. 2017; Postman 2009). Additionally, some quince genotypes serve as common dwarf rootstock for pear (Bell and Leitão 2011; Hummer and Janick 2009). The conventional method of propagation of quince involves rooting of cutting or layering which is a cumbersome, season dependent, and time-consuming process. The traditional propagation methods are proving insufficient to meet the increasing demand for planting materials of desired genotype. Micropropagation stands as the sole alternative technique for increasing the supply of high-quality planting material (Basu et al. 2017). The success and failure of micropropagation protocols depends on the rate of shoot proliferated. Various factors such as genotype, culture medium composition, environmental factors, etc., can influence axillary bud proliferation under in vitro conditions (Aygun and Dumanoglu 2015; Hajisadeghian Najafabadi and Roohollahi 2019; Karimpour et al. 2020; Sadeghi et al. 2015; Vinoth and Ravindhran 2018; Dobranszki and Teixeira da Silva 2010). Proliferated shoot rate depends on the stimulation and activation of lateral meristems controlled by hormones, primarily cytokinins. However, cytokinins interact with auxins, even if the effect of auxin is indirect. This interplay between cytokinins and auxins contributes to the overall control and regulation of the process (Ward and Leyser 2004).

Recently, nanotechnology has gained recognition as a critical technology for improving the management and conservation of agricultural inputs. It has shown promising results for sustainable agriculture by providing various options, such as nanofertilizers, nanopesticides, nanosensors, and agri-food agents (Chandrika et al. 2018; Chhipa 2019; Elizabath et al. 2019; Joshi et al. 2019; Usman et al. 2020). There are many reports on agricultural nanotechnology including the using of various types of metal/metal oxide nanoparticles, polymer-based nanomaterials, and different nanoformulation-based agrochemicals which affect plant growth or pathogen control (Mittal et al. 2020; Chhipa 2019; El-Shetehy et al. 2021).

Factors such as the particle size and shape, plant species, dosage, method of application, and the time and duration of exposure are important factors that determine the effect of NPs on plants. The size, surface area, and reactivity of NPs are different compared to their bulk counterparts (Yadav 2013). Application of nanoparticles can enhance plant germination, improve plant resistance to abiotic and biotic stresses, and stimulate plant growth with reduction environmental stress compared to the traditional methods (Alharby et al. 2016; Alshehddi and Bokhari 2020; García-Gómez and Fernández 2019; Nejatzadeh 2021; Prasad et al. 2012).

Zinc (Zn) is regularly the second most abundant transition metal in organisms following iron (Bhattacharya et al. 2016). Zn is an essential microelement for plant growth and development. Some metal oxide nanoparticles, especially zinc oxide nanoparticles (ZnO NPs) as a nanoparticle-signaling molecule, seems to actively participate in regulating various mechanisms related to the recognition and response to abiotic stresses in plants (Sheykh et al. 2009). It has been discovered that zinc has a vital role in managing reactive oxygen species (ROS) and the protecting plant cells against oxidative stresses (Sheykh et al. 2009). There are reports on the effect of zinc oxide on plants under salinity stress (Alharby et al. 2016). Studies have shown the effect of ZnO NPs on germination, growth, and yield in plants (Prasad et al. 2012; Thunugunta et al. 2018). ZnO NPs have been found to significantly affect the in vitro growth of plants (El-Mahdy and Elazab 2020; Javed et al. 2017).

Silicon (Si) is one of the most abundant elements on the earth's surface. Although silicon is not considered an essential element for plants but is necessary for some of the metabolic and physiological activities in plants (Yan et al. 2018). Studies have shown that the addition of silicon to the nutrient solution or soil may leads to stimulate the plant growth, improve its resistance to biotic and abiotic stresses, and enhance photosynthesis (Artyszak 2018; Debona et al. 2017; Deshmukh et al. 2017; Gong et al. 2003; Ranjan et al. 2021; Song et al. 2014). The absorption of silicon by plants may vary between 0.1 and 10% of dry weight depending on the plant species (Cherif and Belanger 1992).

Recently, due to the unique properties such as large volume, good stability, high surface area, acceptable biocompatibility, and ease of surface functionalization, silica nanoparticles (SiO_2 NPs) has been recognized as promising and capable nanoparticles for agricultural applications (Khafri et al. 2022; Alizadeh et al. 2022; Dashtestani et al. 2021; Salekdeh et al. 2021). SiO_2 NPs can deposit on cell walls and acts as a barrier to prevent the penetration of pathogens and pesticides (Bao-Shan et al. 2004; Ma 2004; Reynolds et al. 2009).

Notably, SiO_2 NPs can reduce the plant transpiration, increases resistance to various abiotic stresses such as drought, salinity, high temperature, cold, and heavy metals (Behboudi et al. 2018; Sabaghnia and Janmohammadi 2015; Siddiqui et al. 2014; Yassen et al. 2017). Furthermore, SiO_2 NPs affect growth, germination, and photosynthesis in

plants (Yan et al. 2018; Wei et al. 2010). Previous studies have shown that using of SiO_2 NPs in in vitro conditions have effective on the proliferation and growth of apple and banana cultures (Avestan et al. 2016, 2017; EL-Kady et al. 2017).

The objective of this study was to evaluate the effects of different concentrations of SiO_2 NPs and ZnO NPs on the proliferation and the growth of quince explants in vitro cultures. Due to the importance of nanoparticles in agriculture, most of the studies that have been reported are on herbaceous plants. There is limited research on horticultural plants, especially quince. In this study, we present the application of two nanoparticles to establish an in vitro micropropagation protocol for the quince rootstock 'QA' (*Cydonia oblonga* Mill).

Materials and methods

Materials and characterization methods

Sodium silicate from Sigma-Aldrich and the other required materials such as sucrose, MS culture medium and hormones from Duchefa were purchased. The necessary solutions were prepared using deionized water (DW). The FTIR spectrum of the NPs was obtained using Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (Thermo, AVATAR). The morphological characteristics of the NPs were studied by SEM (scanning electron microscopy, Hitachi S-4800 II). X-Ray diffraction (XRD) technique was done with a diffractometer (Philips X'pert 1710, with CuKα (α = 1.54056 Å). The size distribution of NPs was determined using dynamic light scattering (DLS, PARTICLMETERIX STABILIZER 200). A confocal laser microscope (CLSM, LSM 710, CarlZeiss, Oberlochen) was applied to image the cellular uptake of the NPs.

Methods

Synthesis of rice husk and straw derived silica NPs

Rice-derived husk and straw wastes were burned and calcined to obtain rice-ash. The resulting ash was refluxed in of NaOH solution (6.0 M) under vigorous stirring overnight. The supernatant containing Na_2SiO_3 was separated and utilized as the silica precursor for the synthesis of SiO_2 NPs. In order to prepare SiO_2 NPs, 44.7 g of Na_2SiO_3 (previously derived from rice biomasses) was added to 100 mL of HCl solution (1.0 M). The reaction mixture was continuously stirred at 35 °C for 24 h. Finally, the solid residue was dried under vacuum to give SiO_2 NPs following centrifugation at 16,000 for 10 min.

Synthesis of zinc oxide NPs

Zinc acetate solution was prepared by dissolution of zinc acetate ($Zn(CH_3COO)_2.2H_2O$, 2.0 g) into 15 mL distilled water at 35 °C. An aqueous solution of 8.0 g NaOH in 8 mL distilled water at 35 °C, and then poured to the as-synthesized zinc acetate solution. Afterward, 100 mL EtOH was added into the latter mixture in a drop-wise manner accompanied by vigorous stirring for 90 min until obtaining a gel-like product. Then the gel was washed and dried at 80 °C for 24 h and then calcined in an oven at 250 °C for 4 h, ZnO NPs were finally prepared as a fine white powder.

Plant materials and culture conditions

Quince dwarfing rootstock 'QA' was used to establish a proliferation procedure. Plant material was collected from the Kamal-Abad collection in Karaj, Iran and used as a source of plant material sources for in vitro initiation. Shoots (2–3 cm in length) containing 3 nodes were excised from four-week-old stock plants and plated in jars (five jars per each treatment) on MS1 medium. The MS1 medium consisted of Murashige and Skoog (1962) basal medium (MS), supplemented with 30 g L^{-1} sucrose, 2 μM 6-benzylaminopurine (BAP), 2.9 μM gibberellic acid (GA_3), 0.1 μM indole-3-butyric acid (IBA), and 7 g L^{-1} agar. Total media treatments in jars were prepared containing six different concentrations of 0, 1, 5, 25, 50, 100 mg L^{-1} of SiO_2 NPs or 0, 0.5, 1, 2.5, 5, 25, 50 mg L^{-1} of ZnO NPs. For all experiments, the pH of culture media was adjusted to 5.8 using either NaOH or HCl before autoclaving at 121 °C for 15 min. The cultures were grown at 24 ± 2 °C under a photoperiod of 16-h of daylight photoperiod with an intensity of 2000 lx. Subculture was performed after five weeks.

Growth parameters

Each experiment included five replicates (five jars per each treatment) where each jar contains three shoot tips. After five weeks, growth parameters were recorded including a mean number of axillary shoots, mean number of leaves, and mean length of shoot produced in vitro. The length of shoot was measured by using a digital caliper.

Statistical analysis

The experimental design followed a randomized block design in a factorial arrangement with five levels of SiO_2 NPs (1, 5, 25, 50, 100 mg L^{-1}) and six levels of ZnO NPs (0.5, 1, 2.5, 5, 25, 50 mg L^{-1}). The results were evaluated by the analysis of variance (ANOVA) according to the general linear model (GLM) procedure using statistical software

SPSS (Version 22). Mean comparisons among treatments were measured using Duncan's test.

Results

Nanomaterials: synthesis and characterization

The synthesized biogenic silica NPs and Zinc oxide nanoparticles were characterized by using XRD (X-ray powder diffraction), AFM (atomic force microscopy), DLS (dynamic Light Scattering), and SEM (scanning electron microscopy). The XRD pattern of the synthesized SiO_2 NPs showed a broad band centered at $2\Theta = 22°$ confirmed the characteristic peak of the amorphous SiO_2 solid and it also showed no peaks related to impurity phases. Also the XRD pattern of ZnO NPs showed the intense peaks at the crystal faces (100), (002), (101), (102), (110) indicate the hexagonal structure which belongs to the space group of P63mc (JCPDS card no. 36-1451), and also showed no impurity phases in the synthesized sample (Vijayaprasath et al. 2016). To further characterization the size determination of the prepared NPs using SEM indicated that all particles have an average size of about 100 nm (Fig. 1a-d). These results were supported by the results of measuring the hydrodynamic diameter and

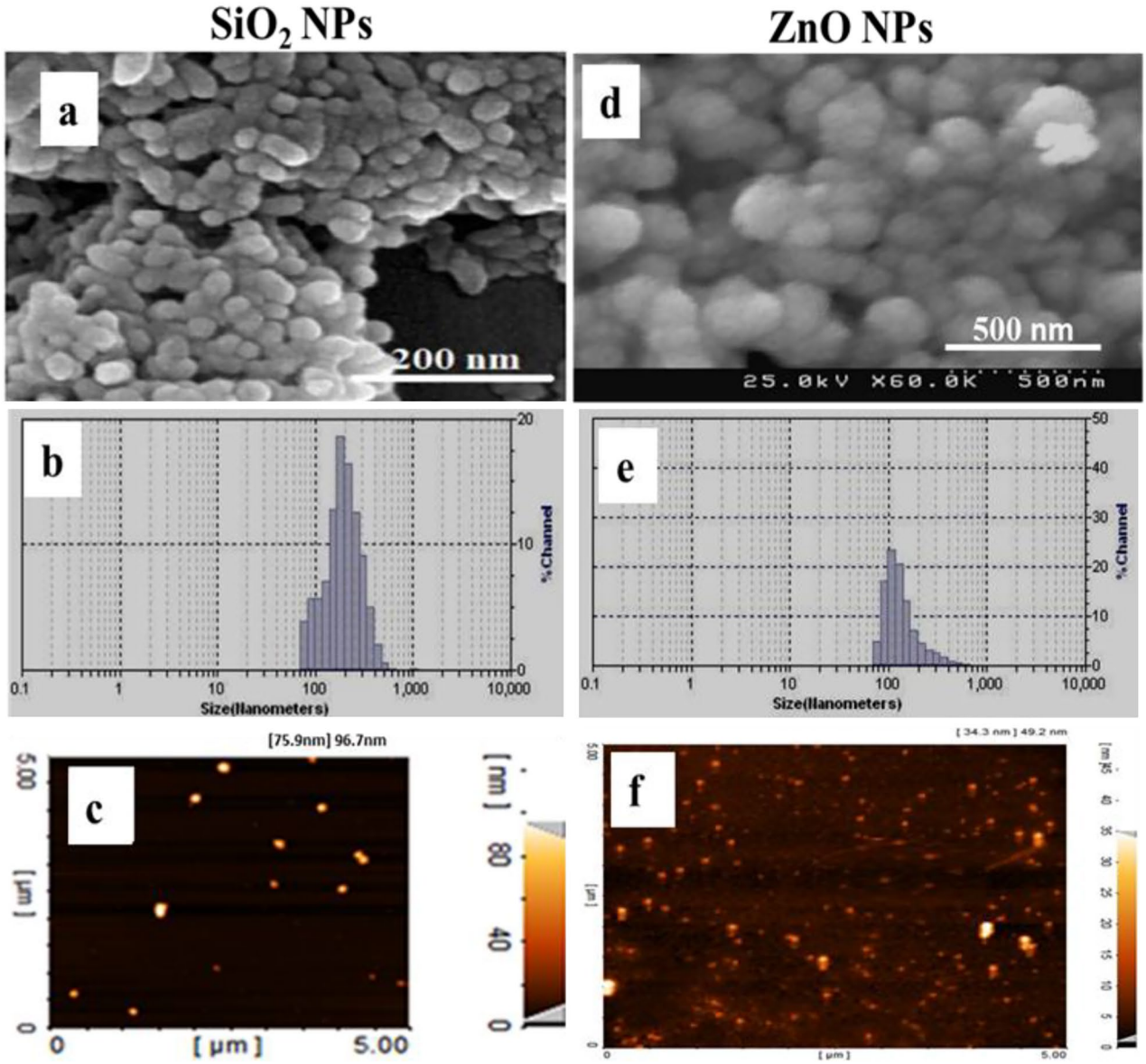

Fig. 1 SEM (**a**, **d**), DLS (**b**, **e**), AFM (**c**, **f**) images of SiO_2, and ZnO NPs, respectively

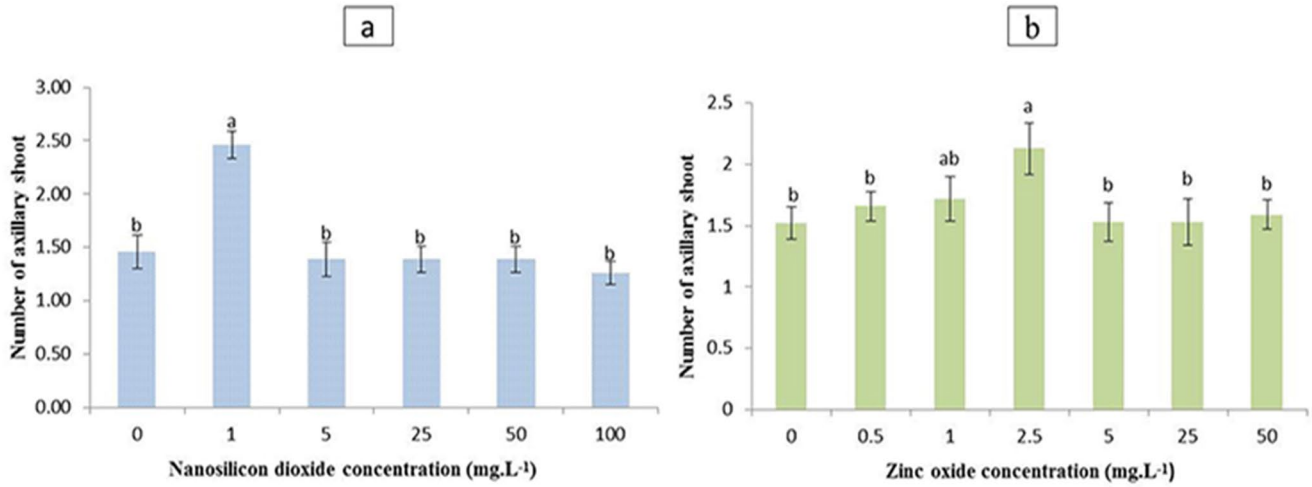

Fig. 2 Effects of (**a**) SiO_2 NPs and (**b**) ZnO NPs on number of axillary shoots in vitro explants of quince rootstock 'QA'. Values presented in the figures are means of 5 independent replicates with standard error. Different lowercase letters denote significant differences among the treatments at $P < 0.01$ by Duncan's test

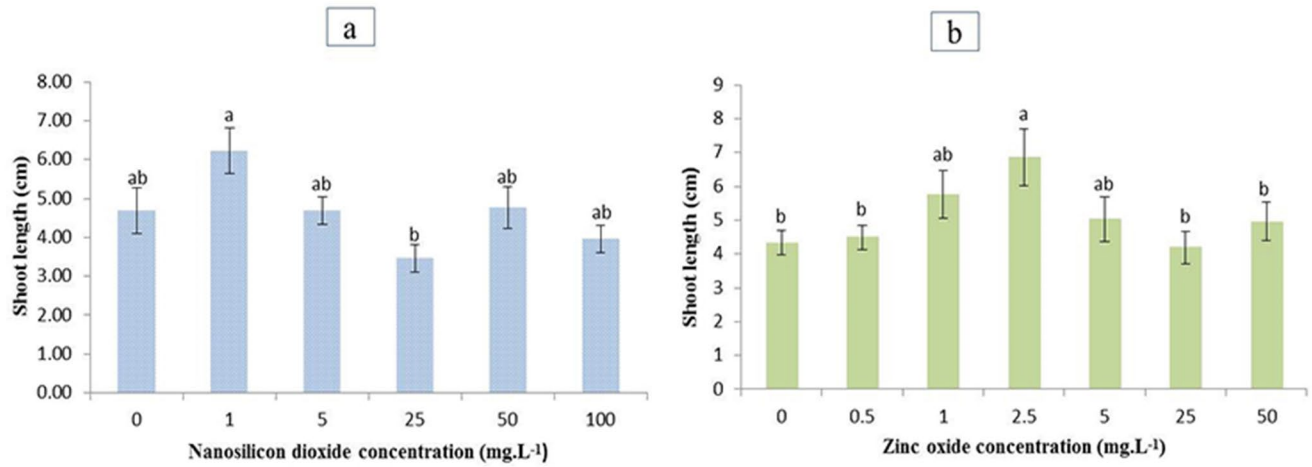

Fig. 3 Effects of (**a**) SiO_2 NPs and (**b**) ZnO NPs on shoot length in vitro explants quince rootstock 'QA'. Values presented in the figures are means of 5 independent replicates with standard error. Different lowercase letters denote significant differences among the treatments at $P < 0.01$ by Duncan's test

topological view of NPs using DLS and AFM, respectively. These images are shown in Fig. 1b-e and c-f, respectively.

Effects of treatments of SiO_2 NPs and ZnO NPs on in vitro growth

Number of axillary shoots

The number of axillary shoots of 'QA' in vitro cultures was influenced by different concentrations of SiO_2 NPs and ZnO NPs (Fig. 2). The low concentrations showed better effects on the number of axillary shoots. The highest number of axillary shoots 2.46 and 2.13 were observed at concentrations of 1 and 2.5 mg L^{-1} SiO_2 NPs and ZnO NPs, respectively (Fig. 2a-b). NPs at concentration of 1 mg L^{-1} had the greatest effect on the number of axillary shoots (Fig. 2a). Higher amounts of SiO_2 NPs and ZnO NPs at concentration of 100 and 50 mg L^{-1}, respectively didn't show significant changes compared with the control, (Fig. 2a-b).

Length of shoot

Different concentrations of SiO_2 NPs and ZnO NPs have been influenced the length of shoot in 'QA' in vitro cultures (Fig. 3). The highest shoots length 6.23 and 6.86 cm were observed at concentrations of 1 and 2.5 mg L^{-1} of SiO_2 NPs and ZnO NPs, respectively (Fig. 3a-b). ZnO NPs at concentration 2.5 mg L^{-1} had the greatest effect on the

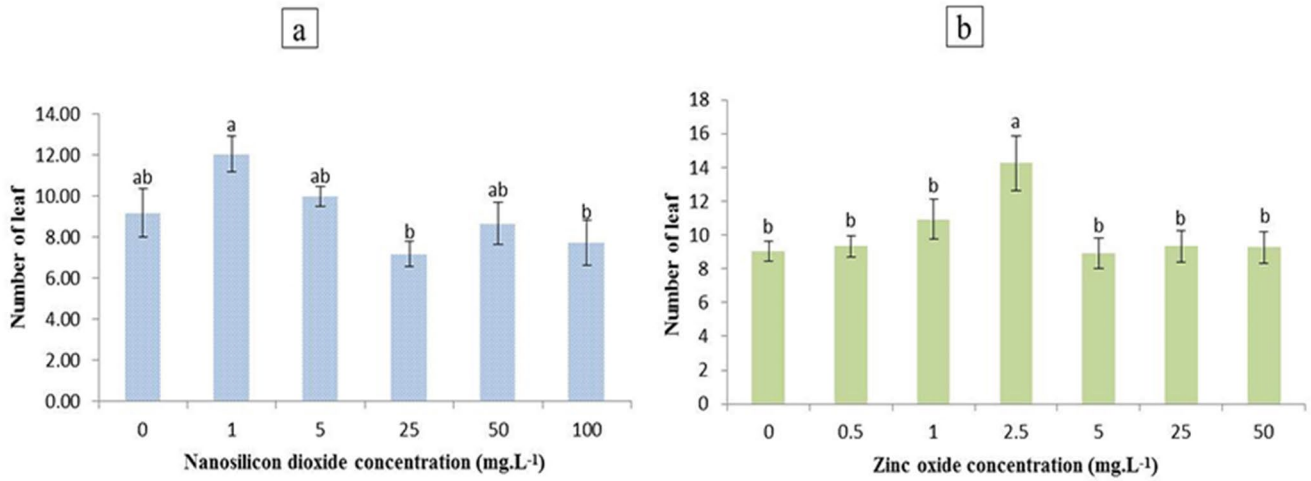

Fig. 4 Effects of (**a**) SiO_2 NPs and (**b**) ZnO NPs on the number of leaves in vitro explants of quince rootstock 'QA'. Values presented in the figures are means of 5 independent replicates with standard error. Different lowercase letters denote significant differences among the treatments at $P < 0.01$ by Duncan's test

Fig. 5 Quince in vitro rootstock 'QA' cultures with (**a**) SiO_2 NPs (0, 1, and 100 mg L^{-1}) and (**b**) ZnO NPs (0, 2.5, and 50 mg L^{-1})

length of shoots (Fig. 3b).SiO_2 NPs at concentrations of 25 and 100 mg L^{-1} showed a decrease in shoot length compared with the control (3.46, 3.96, and 4.69), respectively (Fig. 3a). While ZnO NPs at concentrations 1 (5.76), 5 (5.03), and 50 (4.96) mg L^{-1} showed better results than the control (4.33) (Fig. 3b).

Number of leaves

The number of leaves of 'QA' in vitro cultures was influenced by different concentrations of SiO_2 NPs and ZnO NPs (Fig. 4). Concentrations 1 and 2.5 mg L^{-1} SiO_2 NPs and ZnO NPs showed the highest number of leaves 12.06 and 14.26, respectively (Fig. 4a-b). ZnO NPs at a concentration of 2.5 mg L^{-1} had a better effect on the number of leaves (14.26) (Fig. 4b). SiO_2 NPs at the higher concentrations of 5 mg L^{-1} showed a decrease in the number of leaves compared to the control (Fig. 4a). Also, ZnO NPs in higher concentrations of 2.5 mg L^{-1} didn't show significant changes compared with the control (Fig. 4b). The effects of the lowest and highest concentrations of SiO_2 NPs and ZnO NPs on growth parameters are shown in Fig. 5.

Discussion

The present study tested the effectiveness of the application of SiO_2 NPs and ZnO NPs on growth and proliferation from in vitro cultures of quince rootstock' QA'. These two nanoparticles resulted increasing in growth and proliferation

from in vitro cultures of quince. The highest efficiency of proliferation was obtained at lower concentrations in both nanoparticles. We found 1.26–2.46 number of axillary shoots, 3.46–6.86 cm shoot length, and 7.73–14.26 number of leaves in shoots regenerated from quince rootstock QA subjected to nanoparticles.

It was found that proliferation of QA cultures significantly increased as ZnO NPs concentration increased from 0 to 2.5 mg L^{-1}, wherein higher from 2.5 mg L^{-1} of ZnO NPs treatment none of the cultures showed an increase in proliferation. These observations were in line with previous reports in other plants such as Pomegranate El-Mahdy and Elazab (2020). Our results showed that in vitro quince rootstock 'QA' when exposed to higher concentrations of 2.5 had low number of axillary shoots, shoot length, and the number of leaves. 'QA' in vitro cultures at concentration 2.5 mg L^{-1} produced higher number of shoots (2.13), shoot length (6.86), and the number of leaves (14.26). Previous reports showed ZnO NPs were also affected on growth in in vitro pomegranate, tomato, stevia, and peanut cultures (El-Mahdy and Elazab 2020; Javed et al. 2017).

SiO_2 NPs which have been used in in vitro cultures had a beneficial effect on the proliferation and the growth (Avestan et al. 2016, 2017). In this study, the shoots submitted to different concentrations of SiO_2 NPs showed a significant reduction of the number of shoots, shoot length, and the number of leaves at concentrations higher from 1 mg L^{-1}. All quince rootstock 'QA' cultures showed the highest number of shoots (2.46), shoot length (6.23), and the number of leaves (12.06) at concentration 1 mg L^{-1}.

In our study, the frequency of the number of axillary shoots with SiO_2 NPs was higher than ZnO NPs. While concentration 1 mg L^{-1} of SiO_2 NPs provided 2.46 number of shoots, ZnO NPs provided 2.13 number of shoots at concentration 2.5 mg L^{-1}. Also, the highest shoot length and number of leaves obtained at concentration 2.5 mg L^{-1} (6.86 and 14.26, respectively) than those of SiO_2 NPs at concentration 1 mg L^{-1} (6.23 and 12.06, respectively). Due to the potential of nanoparticles, the in vitro plants were also subjected to ZnO NPs and SiO_2 NPs at different concentrations, and it is known that plants had different responses. These responses may be due to differences in the type and size of particles and plant species (Avestan et al. 2017; Avestan et al. 2016; Javed et al. 2017). Some results have shown that nanoparticles can have toxic effects on plants in higher concentrations. (Lee et al. 2009) investigated the effects of toxicity nanoparticles of silicon dioxide, aluminum dioxide, iron dioxide, and zinc oxide on in vitro Arabidopsis (*Arabidopsis thaliana* Heynh). They have shown SiO_2 NPs and ZnO NPs in high levels can harm health from the plants.

To the best of our knowledge, the present study is the first report of the application nanoparticles in quince. Besides, it has been shown that SiO_2 NPs and ZnO NPs at low concentration were effective in increasing growth parameters in quince. Due to the high cost of using nanoparticles in tissue culture medium and their toxic effects at high concentrations on plants, the effect of nanoparticles in improving growth at low concentrations can decrease the harmful effects on health of plants and reduce experiment costs.

Conclusion

In summary, SiO_2 NPs and ZnO NPs resulted high proliferation and growth in in vitro cultures of quince. The results showed in vitro shoots of quince rootstock QA regenerated following SiO_2 NPs-treatment at a concentration of 1 mg L^{-1} had the highest number of axillary shoots (2.46). Also quince rootstock QA plantlets regenerated following ZnO NPs-treatment showed the highest shoot length and the number of leaves at a concentration of 2.5 mg L^{-1} ZnO NPs (6.86 and 14.26, respectively). This research showed the use of 1 mg L^{-1} of SiO_2 NPs and 2.5 mg L^{-1} of ZnO NPs in the tissue culture medium may improve proliferation and growth rate in quince rootstock 'QA' explants. In the following the effects of using combined SiO_2 NPs and ZnO NPs as nano-additive fore in vitro quince Rootstock Propagation is ongoing in our research group.

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Authors Contribution AG, LM, and MJK conceived of the presented idea and planned the experiments. SF developed the theory conducted the research experiments and wrote the manuscript with the support of all co-authors. VM synthesized NPs, AG and LM supervised the project with the support of MZ and AMN. All authors discussed the results and contributed to the final manuscript.

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Data Availability Data is available.

Code Availability Not applicable.

Declarations

Conflicts of interest/Competing interests The authors declare that they no conflict of interests.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Informed written consent was obtained from all participants.

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