

Seed Nanopriming Using Silica Nanoparticles: Effects in Seed Germination and Physiological Properties of *Stevia Rebaudiana* Bertoni

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Abstract

Stevia is one of the most dominant medicinal and industrial plants. However, poor seed emergence is the main impediment to obtain a high yield in stevia. Seed priming techniques represent very important strategies to ensure seed productivity. Therefore, this study was carried out to evaluate the effects of different concentrations of Si nutrient sources including bulk silicon dioxide (bSiO_2), and two different silica nanoparticles including nSiO_2 (I) and nSiO_2 (II) at concentrations of 1, 5, 10, 25, 50, and 100 ppm in improving emergence, seedling growth, antioxidant enzymes activity, and biochemical attributes. Hence, the effect of seed nanopriming was investigated on germination parameters in stevia using two experiments carried out in a completely randomized design with three replicates. All nanoprimed stevia seedlings had significantly improved germination percentage, root and shoot dry weight, and seedling vigor compared to controls. As well, sucrose concentration, catalase (CAT), and peroxidase (POX) activity were increased by seed nanopriming especially when seeds were primed by nSiO_2 (I) at concentration of 10 ppm, respectively. However, there was a reduction in germination parameters in high concentrations of NPs. On the other hand, bSiO_2 showed less effect on increasing germination, seedling growth, antioxidant activities, and biochemical attributes of stevia compared to nanopriming with nSiO_2 (I), and nSiO_2 (II). Thus, this study proved that seed priming with nSiO_2 (I) at 10 ppm were more effective in improving germination percentage and rate, root and shoot dry weight, biochemical attributes, and enzymes activities (α -amylase, CAT, and POX). The highest concentration of nanopriming led to oxidative damage with increasing H_2O_2 concentration. It was suggested that seed priming with nSiO_2 (I) at optimal concentrations improved the seed germination by improving the antioxidant system and starch metabolism, which protects plants from oxidative damage.

Background

Stevia rebaudiana Bertoni is an herbaceous and perennial plant which has been increasingly used in the pharmacy and food industry. There are natural and zero-calorie sweeteners in stevia leave known as steviol glycosides (SGs)[1, 2]. Stevia sweeteners have been used commercially in a wide range of products in the food and beverage industry[3]. It is naturally propagated by seed [4]. Seeds are used to generate low-cost seedlings. Stevia seeds reproducing is considered the utmost practical and inexpensive manner for the propagation of stevia [5]. However, due to its poor germination rate, the cultivation of stevia is associated with many problems[4]. For this reason, many experiments have been performed on stevia seeds germination[6–8]. The most critical agricultural challenges are sustainability, residual pesticide, fertilizer in agricultural products, food safety, and environmental pollution[9]. These parameters give rise to serious problems including environmental damage, health problems, and low sustainable agriculture. Hence, conventional agricultural practices need to be replaced by sustainable agriculture [10]. Recently, nanotechnology plays a significant role in sustainable agriculture [11, 12] by which not only the productivity and quality of agricultural products are improved, but also the concentration of agrochemicals, herbicides, fungicides, and insecticides are limited [13].

Many attempts have been made to develop natural, biomaterial-based, and eco-friendly priming agents to guarantee environmental safety [14, 15]. The seed priming technique is a method of raising seed germination and has a positive effect on the early growth stages of plants[16]. This technique improves stress tolerance, seedling vigor, germination of weak seeds, seed quality, and crop yields[17, 18]. Moreover, it supports the sustainable agriculture theory and improves the socio-economic status of farmers[19]. The result of experiments described by *Guha et al.*, (2018) and *Mahakham et al.*, (2017) [20, 21] showed that nano-priming enhanced the germination of rice.

For raising seed germination, several metal-based NPs (*e.g.*, Ag, ZnO, TiO₂, Zn, Fe, FeS₂, Au, and Cu NPs) have been utilized as seed pre-treatment agents [22, 23]. Also, SiO₂ NPs has been regarded as a nano-form (< 100 nm) of silicon dioxide affecting plant growth and development [24, 25]. Azimi et al., (2016) suggested that SiO₂ NPs increased germination traits[24].

Concerning the noteworthy physicochemical characteristics of the nanoparticles, it is logical to confer that seed priming with nanoparticles improves the germination rate of stevia, seedling vigor, and other physiological parameters. Although many experiments have been performed on the effect of nanoparticles on seed germination, the physiological effects and molecular mechanisms of nano-priming on stevia seed germination have been less regarded.

However, there are few reports available on the impact of stevia seed priming with nanoparticles[27–29]. In particular, to the best of our knowledge, no study has investigated the nano-priming with two different silica nanoparticles including nSiO₂ (I) and nSiO₂ (II) on stevia seeds. This study was aimed to screen and select the best nanoparticles in terms of germination parameters and physiological properties to increase stevia seed germination. These new systems offer advantages for use in sustainable agricultural production methods. It should be noted that the best NPs are used in another experiment to cover stevia seeds.

Experimental methods

Material and characterization

Tetraethyl orthosilicate and all chemicals were obtained from Sigma-Aldrich. To investigate the effect of NPs on the germination of stevia seeds, two factorial experiments were performed in completely randomized designs. The experiments were conducted at the Agricultural Biotechnology Institute of Iran (ABRII), Karaj, Iran. Seeds were surface sterilized in 3% (w/v) sodium hypochlorite, rinse rinsed several times in distilled water, and pretreated with with nSiO₂ (I), nSiO₂ (II), and commercial bulk SiO₂ (bSiO₂) at a concentration of 1, 5, 10, 25, 50 and 100 ppm. A dynamic light scattering instrument (DLS, Brookhaven, USA) was used to determine the hydrodynamic particle size of the samples. Samples were prepared by dispersing solid nanomaterials in deionized water in an ultrasonic bath for 15 min and then used for quantification. The size and shape of the samples were characterized using a scanning electron microscope (SEM, Hitachi S-4800 II, Japan) and a transmission electron microscope (TEM, Hitachi H-

7650, 80 kV, Japan). For this purpose, TEM/SEM samples were prepared by simply spreading the NPs on a grid using a suitable solvent such as EtOH. The prepared samples were dried and used for imaging. The topological features of the material were observed at room temperature using an atomic force microscope (AFM, DME-Ds95-50, Denmark). For this purpose, an ultrasonic mixture of solid nanomaterials in deionized water was distributed on the network. The necessary spectrophotometric measurements were performed using a double-beam UV-Vis spectrophotometer (UV-3100 PC, Shimadzu).

Synthesis of nanomaterials

Synthesis of Silica-based Nanoparticles ($n\text{SiO}_2$)

Tetraethyl orthosilicate (2.8 mL) was added to a solution containing ammonia (2.8 mL), deionized water (dH_2O , 33.75 mL), acetone (14.5 g), and cetrimonium bromide (0.64 g). The mixture was allowed to stir for 24 h at 25°C or 85°C. The solid residual was centrifuged, washed, and dried. It was then placed in a vacuum oven at 70°C for 5 h. The dried powder was calcined at 540°C to give silica-based nanoparticles. The synthesized NPs was named based on the reaction temperature, $n\text{SiO}_2$ (I) and $n\text{SiO}_2$ (II) showed the NPs that were synthesized at 85°C and 25°C, respectively.

Preparation and method of priming samples

Different concentrations of $n\text{SiO}_2$ (I), $n\text{SiO}_2$ (II), and $b\text{SiO}_2$ priming solutions were used for the seed priming test. Six concentrations of at 1, 5, 10, 25, 50, and 100 ppm, were freshly prepared by dispersing the particles in dH_2O using ultrasonic vibration (100 w, 40 kHz) for 30 min were. For hydro-priming dH_2O was used. Seeds of *Stevia rebaudiana* (Stevia Cultivar '814011) were obtained from China. Before germination, the seeds were surface-sterilized with 70% ethanol and then washed with dH_2O several times [4]. Seeds were primed for 20 h (priming time was optimized in the preliminary experiment), at the end of the priming process, the seeds were washed with distilled water and dried for 24 h in the lab[8].

Sampling

Samples of 60 seedlings were randomly selected, and then the shoot (cotyledon and stem) and roots were separated and dried in a freeze-dryer at -60°C for 2 days. After the weighting of the dried samples, they were mixed and ground in liquid nitrogen before storage at -80°C.

Germination percentage, germination rate, and seed vigor

The germination was monitored every day after sowing (the radicle length exceeded 2 mm). The germination rate is the ratio of the number of germinated seeds to the total number of seeds sown and is expressed as a percentage. The seed vigor index was calculated by multiplying the germination index with the shoot length.

Starch and sucrose determination

Starch and sucrose determinations were done by *Walker et al.* (2008) [30]. For starch and sucrose determination root, shoot and cotyledons were separated and dried separately in a freeze-dryer. In 15 mL falcon tubes, 0.1 g of each freeze-dried sample was added along with 5 mL of 70% ethanol, and the mixture was shaken at 100 rpm for 4 hours at 80°C. Then, the samples were centrifuged at 11,068 g. A new 15-mL falcon tube was then filled with the supernatant. This process was carried out twice. A rotary vacuum concentrator (Eppendorf Concentrator plus, Germany) was used to dry the supernatant and residues separately at 60°C. The supernatant was used to quantify of sucrose, glucose, and fructose by HPLC, and the residue was used for the starch measurement. For total sucrose, glucose, and fructose determination, the samples were completely dried. 10 mL of dH₂O were added to the dried samples. 10 mL of dH₂O, 0.47 mL of barium hydroxide (0.3 M), and 0.5 mL of ZnSO₄ (5%) were added to the remaining residue in tubes. After centrifuging the supernatant and putting it into a 15 mL tube, it was dried at 50 ° C. Finally, 1 mL HPLC grade water was added to the remains and vortexed vigorously and then 20 µL was injected into HPLC. Using HPLC (KNAUER, Germany) with a EURO Kat H column (10 m, 300 x 8 mm, KNAUER, Germany) at 25°C and an acidic mobile phase (aq. H₂SO₄ at pH = 2.5) flow of 1 mL.min⁻¹ and RI, glucose, sucrose, and fructose were separated from the samples. (The glucose and fructose concentrations were very poor in HPLC results, so the result of sucrose was shown in this study). The dried cotyledon samples were used to determine the starch content. 4.5 mL of dH₂O and 6 mL of perchloric acid (52%) were added to the remaining residue in tubes. Samples were centrifuged at 11,068 g for 10 min after being maintained at 4°C overnight. 1.25 mL of H₂SO₄ 12 M and 0.25 mL of phenol 0.5% were added to 0.5 mL of the extract. The absorbance of the free carbohydrates was measured at 485 nm after 45 minutes.

Assays of antioxidant enzyme activity

Catalase (CAT, EC 1.11.1.6) was extracted from stevia seedlings by *Ramiro, et. al.*[31]. 0.1 g seedling sample was suspended in 3 mL of ice-cold HEPES buffer (25 mM, pH 7.8) containing 0.2 mM EDTA and 2% mM PVP. The homogenate was centrifuged at 12,000 g for 20 min at 4°C and the resulting supernatant was determined by recording the decrease in absorbance at 470 nm as described by Chance and Maehly, (1955) [32] in a reaction contained 1.99 mL sodium phosphate buffer (50 mM, pH 7.0) supplemented with 0.1 µM EDTA, 10 mM guaiacol and 15 mM H₂O₂ and 100 µL of the enzyme extract in a total volume of 2 mL.

Statistical Analysis

Software such as RStudio, SPSS-24, and SAS-9.2 were used to analyze all the data. LSD at level 5% was used to assess treatment differences. R package corrplot in RStudio software was employed for analyzing correlations between traits (Pearson correlation coefficient). Multiple stepwise regression analyses were performed by SPSS-24.

Results

Preparation and characterizations of NPs

The synthesized nanoparticles were characterized using XRD (X-Ray Powder Diffraction), TEM (Transmission Electron Microscopy), AFM (Atomic Force Microscopy), and DLS (Dynamic Light Scattering). Size determination of nSiO₂ (I) and nSiO₂ (II) using TEM indicated that all nanomaterials are spherical with an average diameter of < 50 and < 100 nm, respectively (Fig. 1C and D). The results of measuring the hydrodynamic diameter of nSiO₂ (I), and nSiO₂ (II) using DLS, showed 128 and 248 nm, respectively.

Figure 1.

SiO₂ NPs priming

As shown in Fig. 2, seeds priming with nSiO₂ (I) and nSiO₂ (II) (nanoprimering solutions) at different concentrations could accelerate the germination percentage, germination rate, and seed vigor compared to the control and SiO₂ priming. A significant increase in seed germination percentage (68%), germination rate (7.3 day⁻¹), and vigor index (11.6) was observed when seeds were primed with nSiO₂ (I) at 10 ppm. While the nSiO₂ (II) at 100 ppm significantly improved the seed germination percentage (62%), germination rate (5.9 day⁻¹) and vigor index (9.5) ($p < 0.01$). The germination percentage of nano-primed seeds was significantly higher than that of SiO₂ priming and control.

Germination percentage, germination rate, and seed vigor index

We also observed an increase in the root, shoot and seedling dry weight in both nano-primed and bSiO₂ primed seeds. On the contrary, significant increase in the root dry weight (0.092 g.plant⁻¹), shoot dry weight (0.078 g.plant⁻¹), and seedling dry weight (16.9 g.plant⁻¹) was observed in nano-primed seeds with nSiO₂ (I) at 10 ppm. While the nSiO₂ (II) at 100 ppm significantly improved the root, shoot, and seedling dry weight of averaged 0.074, 0.080, and 0.154 g.plant⁻¹, respectively ($p < 0.01$). The root, shoot, and seedling dry weight of nano-primed seeds were significantly higher than that of the bSiO₂ priming and control. But bSiO₂ seed priming at concentrations of 25 and 50 ppm could increase the root, shoot, and seedling dry weight compared to the control (Fig. 2).

Figure 2

This experiment results indicated that nano-priming enhanced starch metabolism in stevia seedlings. The α -amylase activity was increased by nSiO₂ (II) at 100 ppm (0.9 U.g⁻¹ FW) and nSiO₂ (I) at 10 ppm (0.8 U.g⁻¹ FW) were almost 9 and 8 times greater than those in the control, while the α -amylase activities in the SiO₂-primed seedlings at 50 ppm (0.6 U.g⁻¹ FW) were almost 6 times greater than those in the control (Fig. 3A). Likewise, the starch contents of seedlings were reduced by nanoprimering treatments (nSiO₂ (II)

and nSiO₂ (I)) and bSiO₂ primed seeds compared to the control. The starch content was significantly low when seeds were primed with nSiO₂ (I) at 10 ppm (13.7 $\mu\text{g.g}^{-1}$ DW), while the highest content of starch was observed in control seeds. Starch content was significantly reduced in seeds primed with nSiO₂ (II). Lower starch content in nSiO₂ (II) primed seeds was observed at a concentration of 50 and 100 ppm (~22 $\mu\text{g.g}^{-1}$ DW) indicating a decrease in ~93% compared to control (Fig. 3).

Accumulation of sucrose and starch in root and shoot

The accumulation of sucrose in germinating seeds was increased in both bSiO₂-primed and nano-primed seeds (nSiO₂ (I) and nSiO₂ (II)), while the sucrose accumulation in control was lower than other groups. The highest sucrose content in the root (160.4 $\mu\text{g.g}^{-1}$ DW) and shoot (247 $\mu\text{g.g}^{-1}$ DW) was indicated in nSiO₂ (II) at 100 ppm, showing an increase of 144%, 724% compared to the control, respectively (Fig. 3).

Figure 3

H₂O₂ concentration, CAT, and POX activity

Our results showed that activities of CAT and POX were increased by seed priming with bSiO₂, nSiO₂ (I), and nSiO₂ (II) in stevia seedlings. The highest CAT activity (36.15 U.mg⁻¹ protein) was achieved in seedlings primed with nSiO₂ (II) at 10 ppm, indicating an increase of 45%, compared to the control. The highest POX activity (~0.057 U/mg protein) was shown in nSiO₂ (I) at 5, 10, and 25 ppm and nSiO₂ (II) at 10, 25, and 50 ppm. The increasing rate of POX activity was by 29%, compared to control (Table 1). The seed priming with nSiO₂ (I) up to 25 ppm and nSiO₂ (II) up to 10 ppm significantly reduced H₂O₂ concentration in the seedling. While the higher concentration of nSiO₂ (I) and nSiO₂ (II) led to an increase in H₂O₂. However, the highest amount of H₂O₂ concentration (4.70 $\mu\text{mol.g}^{-1}$ FW) was indicated in SiO₂ priming at 100 ppm indicating an increase of 11.37%, compared to the control. In contrast, the minimum amount of H₂O₂ concentration (3.1 $\mu\text{mol.g}^{-1}$ FW) was shown in nSiO₂ (I) priming at 1 ppm. The reduction rates of H₂O₂ concentration at nSiO₂ (I) at 1 ppm were by 26% lower, compared to control (Table 1).

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Table 1
CAT and POX activity and H₂O₂ concentration treated with SiO₂ NPs.

treatments	concentration (ppm)	CAT activity (U/mg protein)	POX activity (U/mg protein)	H ₂ O ₂ concentration (μmol /g. FW)
Control	-	24.77 ± 0.29 ^l	0.044 ± 0.0003 ^g	4.22 ± 0.09 ^e
bSiO ₂	1	27.74 ± 0.30 ^k	0.045 ± 0.0004 ^{fg}	4.36 ± 0.04 ^{de}
	5	29.82 ± 1.58 ^{ij}	0.046 ± 0.0010 ^{fg}	4.36 ± 0.07 ^{de}
	10	30.38 ± 0.37 ^{g-j}	0.049 ± 0.0004 ^{d-f}	4.24 ± 0.05 ^e
	25	31.61 ± 0.18 ^{d-g}	0.050 ± 0.0007 ^{de}	4.42 ± 0.03 ^{de}
	50	31.20 ± 0.79 ^{f-i}	0.050 ± 0.0009 ^{de}	4.63 ± 0.01 ^{a-c}
	100	30.24 ± 0.24 ^{g-j}	0.052 ± 0.0005 ^{cd}	4.70 ± 0.02^a
nSiO ₂ (I)	1	29.88 ± 0.15 ^{ij}	0.047 ± 0.0006 ^{e-g}	3.1 ± 0.04 ⁱ
	5	31.45 ± 0.49 ^{e-h}	0.056 ± 0.0039^a	3.46 ± 0.04 ^h
	10	32.41 ± 0.54 ^{c-f}	0.058 ± 0.0007^a	3.68 ± 0.10 ^{fg}
	25	33.09 ± 0.25 ^{cd}	0.057 ± 0.0030^a	3.87 ± 0.18 ^f
	50	32.81 ± 0.46 ^{c-e}	0.056 ± 0.0005 ^{ab}	4.53 ± 0.06 ^{b-d}
	100	31.72 ± 0.39 ^{d-g}	0.055 ± 0.0004 ^{a-c}	4.69 ± 0.02^{ab}
nSiO ₂ (II)	1	29.29 ± 0.37 ^j	0.052 ± 0.0012 ^{b-d}	3.50 ± 0.05 ^{gh}
	5	29.93 ± 0.37 ^{h-j}	0.055 ± 0.0004 ^{ab}	3.62 ± 0.06 ^{gh}
	10	36.15 ± 0.29^a	0.057 ± 0.0003^a	3.67 ± 0.10 ^{fg}
	25	34.66 ± 0.21 ^{ab}	0.057 ± 0.0003 ^a	4.48 ± 0.04 ^{cd}
	50	33.25 ± 0.23 ^{bc}	0.056 ± 0.0003 ^a	4.55 ± 0.04 ^{b-d}
	100	32.90 ± 0.65 ^{c-e}	0.051 ± 0.0005 ^d	4.65 ± 0.04^{a-c}
Means ± standard deviations (SD) of three replicates followed by the same letters are not significantly different according to LSD's multiple range test at P ≤ 0.05.				

Table 1.

Correlation analysis

Analyzing the coefficients of correlation between different characters involved in stevia seed provides informative data about the relative traits. From the data obtained, it was obvious that there were some correlations between the responses. In detail, there was a positive correlation between seedling dry weights, seed vigor, stem dry weight, root dry weight, seed germination, germination rate, α -amylase activity, root, and stem sucrose. Furthermore, there were negative correlation ($P = 0.01$) between cotyledon starch with seedling dry weight, seed vigor, stem dry weight, root dry weight, seed germination, germination rate, α -amylase activity, root and stem sucrose, CAT and POX activity with correlation coefficients of -0.66^{**} , -0.64^{**} , -0.64^{**} , -0.61^{**} , -0.61^{**} , -0.67^{**} , -0.69^{**} , -0.67^{**} and -0.64^{**} , -0.48 and -0.49 respectively. As well, negative correlations were observed between root starch concentration with root sucrose concentration and R α -amylase activity with correlation coefficients of -0.43^{**} and -0.55^{**} respectively (Fig. 4).

Figure 4

Discussion

Seed germination parameters

Long-term exposure of the soil to fertilizers could lead to fertilizers leaching, deterioration micro-ecological environment of the rhizosphere, nutritional imbalance, soil acidification by increasing in hydrogen cations in soil, and major enhanced the heavy metal ions activity in soil [33]. Seed priming improves seed germination and thus potentially increases growth and yield. Low-cost, environmentally friendly, effective seed treatment remains to be optimized and tested for high-value specialty crops [19].

This research evaluated the capability of seed priming in *stevia rebaudiana*, an important medical and industrial plant, with silicon dioxide (SiO_2 NPs) (nSiO_2 (I) and nSiO_2 (II)). The results of the two experiments indicated that nano-primed seeds with nSiO_2 (I), and nSiO_2 (II) induced better seed germination compared to SiO_2 NPs respectively. The results of our study suggested that seeds nano-priming with nSiO_2 (I), and nSiO_2 (II) at suitable concentrations not only accelerated seed germination rate, but also significantly enhanced seed vigor, as indicated by more weight for root, shoot, and seedling compared with those of the control. The type, concentration, and consumption methods of NPs play important roles in the behavior and reactivity of plants [34].

Our results indicated that the germination parameters were improved by priming with nSiO_2 (I) and nSiO_2 (II). The result of *Behboudi et al.*, (2017) indicated that nano-priming with 30 and 60 SiO_2 NPs led to an increase in germination index in barely. However, the toxic effects of NPs in higher concentrations were more pronounced in seedling, shoot and root length, in seeds priming with SiO_2 NPs [34]. Our result showed that the highest concentration of nSiO_2 (I) and nSiO_2 (II) reduced the germination parameters. It

seems that priming with a suitable concentration of NPs plays an important role in germination parameters especially seedling growth.

There are less studies about the nano-priming with nSiO_2 (I) and nSiO_2 (II) on stevia seeds. Howbeit, Azimi et al., (2014) suggested that the application of SiO_2 NPs improved the germination parameters in *Agropyron elongatum* [35]. Seed priming with SiO_2 NPs may stimulate pre-germination metabolic events such as increased water imbibition, cell elongation, and division, reparation of damaged nucleic acids, reserve mobilizing enzymes activity, activities of antioxidant enzymes, and improving seedling emergence parameters [36].

In the present study, nSiO_2 (I) and nSiO_2 (II) exhibited great potential for agricultural applications as they enhanced the germination rate and physiological processes when compared to the control unprimed seeds. To date, the mechanism underlying nano-priming with nSiO_2 (I), and nSiO_2 (II) induced seed germination has not yet been reported in the literature. In this paper, the mechanism of beneficial roles of nSiO_2 (I), and nSiO_2 (II) in inducing seed germination was hypothetically proposed, such as nano-priming for enhancing starch-degrading enzyme activity, sucrose and starch exchange in seeds and seedling.

α -amylase activity and starch concentration

Nano-priming at suitable concentrations can stimulate seed germination of seeds by increased α -amylase activity and starch metabolism [21]. Our experiment demonstrated that α -amylase activity was higher in the seedling primed with bSiO_2 at 50 ppm compared to the control. However, priming with nSiO_2 (I) at 10 ppm, and nSiO_2 (II) at 100 ppm induced the highest α -amylase activity. In both experiments, results of data correlation showed that there was a negative and significant relationship between starch and α -amylase activity. This data corresponded well with the starch decomposition and higher germination percentage in nano-primed seeds compared to other priming treatments and control. We also propose another hypothesis that nSiO_2 (I), and nSiO_2 (II) penetrating the seeds might be important for their role as nano-catalyst in the hydrolysis of starch catalyzed by α -amylase and thereby increasing the reaction rate. The micronutrient encapsulating biopolymer-based nanosystem has been suggested as a smart priming agent for several applications without compromising soil fertility and environmental safety[15].

Increasing α -amylase activity in primed seeds can improve seedling vigor and the development of embryos[37]. The α -amylase activity leads to starch decomposition and increase of sucrose in root and shoot in stevia seedlings. Seed priming with all concentrations of the nSiO_2 (I), and nSiO_2 (II) significantly improved α -amylase activity as compared to bSiO_2 and control. Our data are in agreement with the results of Tymoszuk and Wojnarowicz, (2020) in wheat, which showed that Zn-CNPs priming of seeds induced an increase in α -amylase activity. Thus, nano-primed seeds led to increment hydrolyzation of starch and release of soluble sugars [34].

Starch is one of the most important energy storage polysaccharides involved in seedling growth[39], However, our results demonstrated that starch reduced in nano-primed seeds. Seed priming with nSiO₂ (I), and nSiO₂ (II) also reduced starch concentration in stevia seedlings in both experiments. Starch reduction in the seed germination process is essential because quite dry seeds after the water uptake need mobilization the storage compounds of the starchy endosperm to start cellular metabolism and growth[40].

Since the activities of α -amylase, a crucial enzyme in starch degradation, were greater in the nano-priming treatments' seedling cotyledons than in controls and other treatments, this may be explained by the increased sucrose concentration that resulted from the seeds' use of sucrose as an energy source. Thus, through intricate networks, increased α -amylase activity and starch conversion to sucrose can improve seed germination and seedling growth. The result of Vandecasteele *et al.*, (2011) indicated that sucrose was positively correlated with the germination percentage of *Medicago truncatula* seeds [42]. The highest concentration of sucrose in root and shoot tissue of seedlings were in the primed seeds with nSiO₂ (II) at 100 ppm concentrations.

Free carbohydrate (Sucrose)

The major form of free carbohydrate in seedlings is sucrose. While the fructose and glucose were not shown in the HPLC result, which could be due to the fast conversion of monosaccharides to energy or use in other germination mechanisms. Soluble sugar and the alteration of soluble sugars in germinating seeds can be a good indicator of seedling emergence and growth. Soluble sugars are mobilized from seeds to various organs in the seedlings during seed germination and early seedling growth [19]. Our result indicated the nano-priming with nSiO₂ (II) up to 100 ppm enhanced the level of sucrose compared to control (Fig. 3). However starch concentration was reduced during the seed germination, the concentration of sucrose increased. This suggests a substantial mobilization from reserves to seedling growth. Nano priming was more effective than conventional methods and led to increasing seedling growth through contributing to improving the amylase activity, starch degradation, and sucrose generation.

H₂O₂ concentration, CAT and POX activity

If nano-priming caused toxicity in the germination and seedling's growth, we might see an increase in oxidative damages and reduce in antioxidant enzymes activity; by contrast, if nano-priming improved seedling parameters, we might see an increase in antioxidant enzymes activity compared with control. In two experiments, the effects of seed priming with bSiO₂, nSiO₂ (I), and nSiO₂ (II) on the seedling H₂O₂ concentration, CAT and POX activity were assessed (Table 1).

From the viewpoint of the recent concept of plant seed physiology, it has been evidenced that germination of seeds is completed when the ROS signaling is commenced within an oxidative window [21]. Therefore, the presence of ROS as a signal is necessary to the beginning germination, however,

increasing the ROS concentration causes oxidative damage and prevents germination. As our result, germination parameters decreased at higher concentrations of NPs-priming.

The content of H_2O_2 was quantified in the seedling. H_2O_2 concentration (as an important ROS species) is associated with the weakening of the endosperm cap and an increase in embryo elongation during seed germination[43]. H_2O_2 concentration significantly increased in primed seeds with high concentrations of NPs.

In addition, primed seeds with $nSiO_2$ (I) at 1 ppm, were found to be more effective than none-primed seeds in reducing the accumulation of H_2O_2 seeds. However, $nSiO_2$ (I) up to 25 ppm and $nSiO_2$ (II) up to 10 ppm increased the H_2O_2 concentration compared to control. In our experiment, to some extent, reduction in H_2O_2 level might be due to the membrane of the seed and organelle after priming having been repaired in advance. Therefore, the nano-priming technique could act as a highly effective factor that protects plant cells from oxidative degradation with decreasing H_2O_2 concentration (Table 1).

Enzymatic and non-enzymatic antioxidants are important systems for seed germination. For the sake of antioxidant systems positively control ROS accumulation for regulating germination [44]. Our result indicated that nano priming increased the CAT and POX activity. CAT and POX activity had a positive correlation with germination parameters especially seed vigor. It seems that increased antioxidant properties in nano-primed seeds may have had a positive effect on seed vigor [45]. Due to the correlation between antioxidant enzymes and H_2O_2 and, the high level of CAT and POX activity, observed in nano-priming, may be due to the induction of H_2O_2 by high concentrations of $nSiO_2$ (I), and $nSiO_2$ (II).

Besides germination, nutritional quality is one of the chief concerns. Interestingly, pretreatment changing the metabolism of seeds, not only improves germination but also has a positive impact on productivity [46]. So that, the results of [19] indicated that nanopriming increased the growth and yield of Watermelons while maintaining nutritional quality. In the continuation of this study, investigation of the nano-priming is ongoing on stevia plant growth, yield, steviol glycosides, and plant safety.

Conclusions

In summary, seed priming with $nSiO_2$ (I) at 10 ppm showed major effects on seed germination. They substantially improved the seedling growth due to improvement in related physio-biochemical processes including activation of the amylase, degradation starch in cotyledon, increasing sucrose concentration in seedling, and increasing the activity of the antioxidant enzymes in stevia seedlings. Improving these processes led to better germination and having stronger seedlings. Nano-priming with $nSiO_2$ (I) at 10 ppm due to the low dose of nanoparticles had better results and required less material. The cost of materials for commercial utilization of these materials is low, which makes this method cost-effective.

The use of these new technological systems for seed treatment could result in higher agricultural yields. Another feature related to sustainability and food safety is that the treatment was only applied to the

seeds, so there was no direct exposure of the formulations to the environment, hence avoiding any possibility of the nanoparticles becoming a source of contamination in water bodies or the soil.

Abbreviations

AFM: Atomic force microscopy; bSiO₂: Bulk silicon dioxide; CAT: Catalase; DLS: Dynamic light scattering; FTIR: Fourier transform infrared; NPs: Nanoparticles; nSiO₂: Silica nanoparticles; SEM: Scanning electron microscopy; TEM: Transmission electron microscopy; POX: Peroxidase.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

TSA prepared the nanomaterials, performed in vitro and greenhouse experiments, and helped in paper writing and editing. LM supervised and performed the conception and design of the study, and paper writing and editing. MC supervised and helped in paper writing and editing. AM advised in interpreting data. All authors read and approved the final manuscript.

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Figures

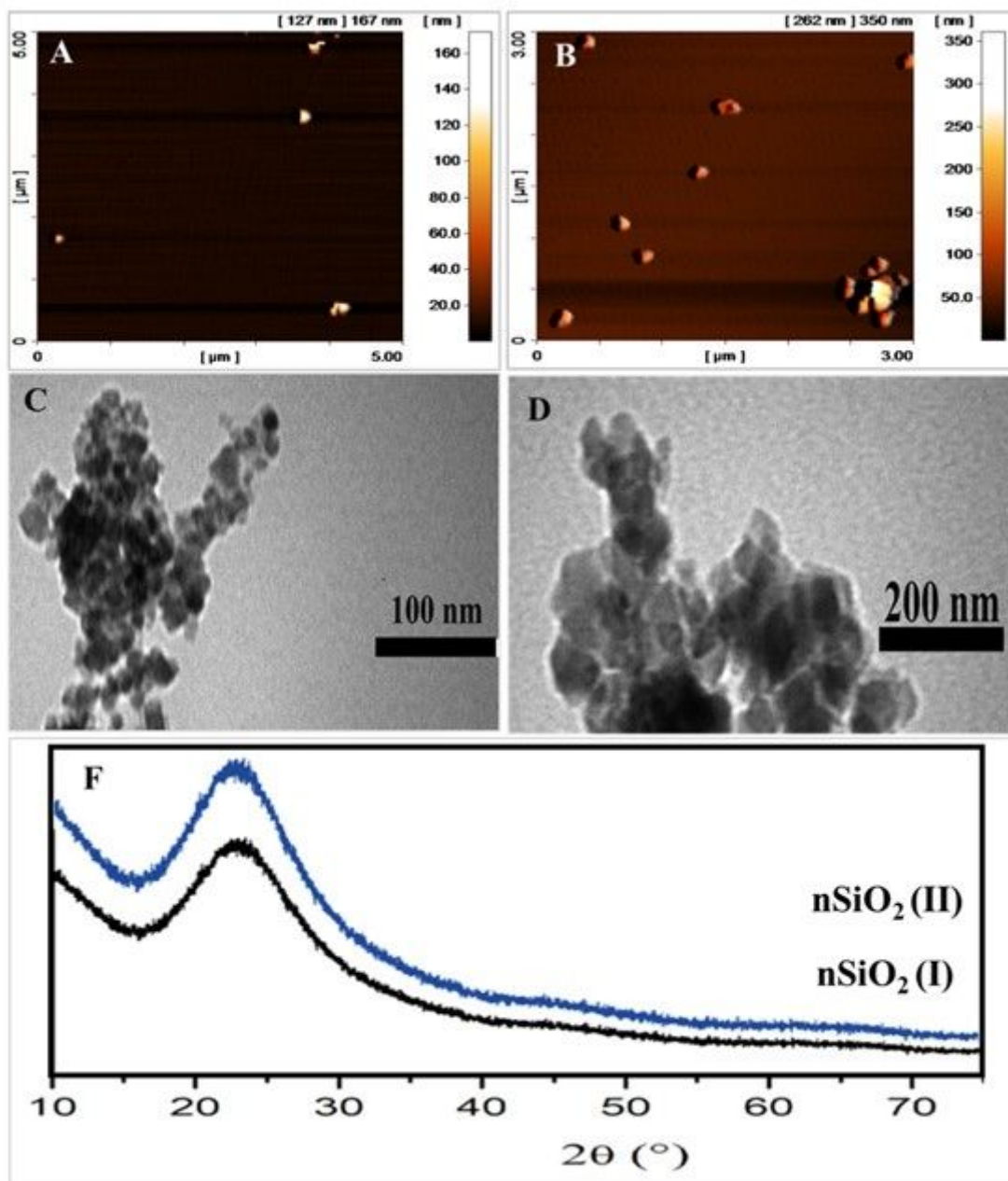


Figure 1

AFM and TEM images of nSiO₂ (I) (A and c) and nSiO₂ (II) (B and D), and XRD patterns of silica nanomaterials (F).

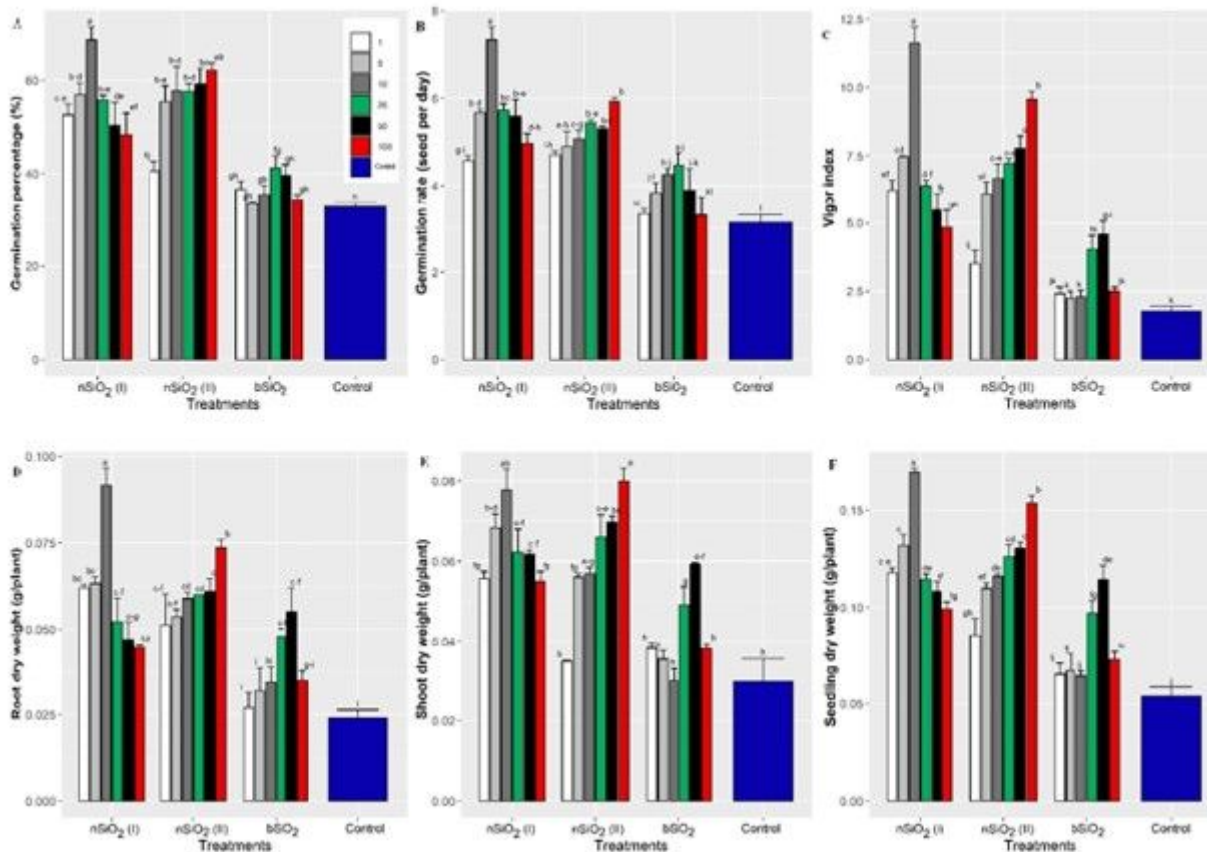


Figure 2

Impacts of different priming agents (nSiO₂ (I), nSiO₂ (II), and bSiO₂ at concentrations of 1, 5, 10, 25, 50, and 100) on A) seed germination percentage, B) germination rate, C) seed vigor index D) root dry weight, E) shoot dry weight, and F) seedling dry weight. Data are presented as means of three replicates containing 100 seeds each $\pm$ standard error of means. The color of the columns indicates the concentration based on ppm and control was water priming.

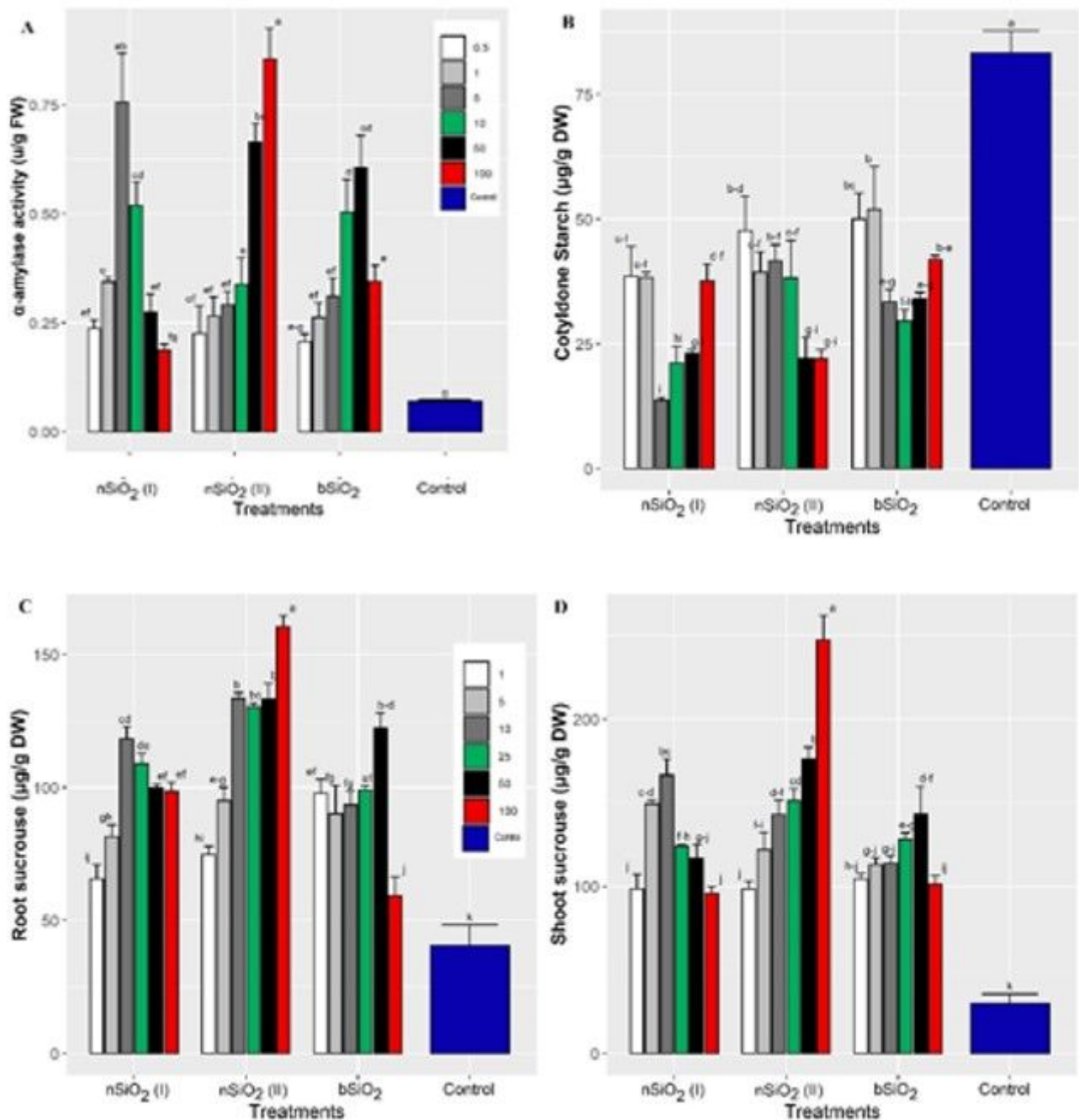


Figure 3

Impacts of different priming agents (nSiO₂ (I), nSiO₂ (II), bSiO₂, at concentrations of 1, 5, 10, 25, 50, and 100) on A) α-amylase, B) cotyledon starch, C) root sucrose, and D) shoot sucrose content. Data are presented as means of three replicates containing 100 seeds each ± standard error of means. The color of the columns indicates the concentration based on ppm and control was water priming.

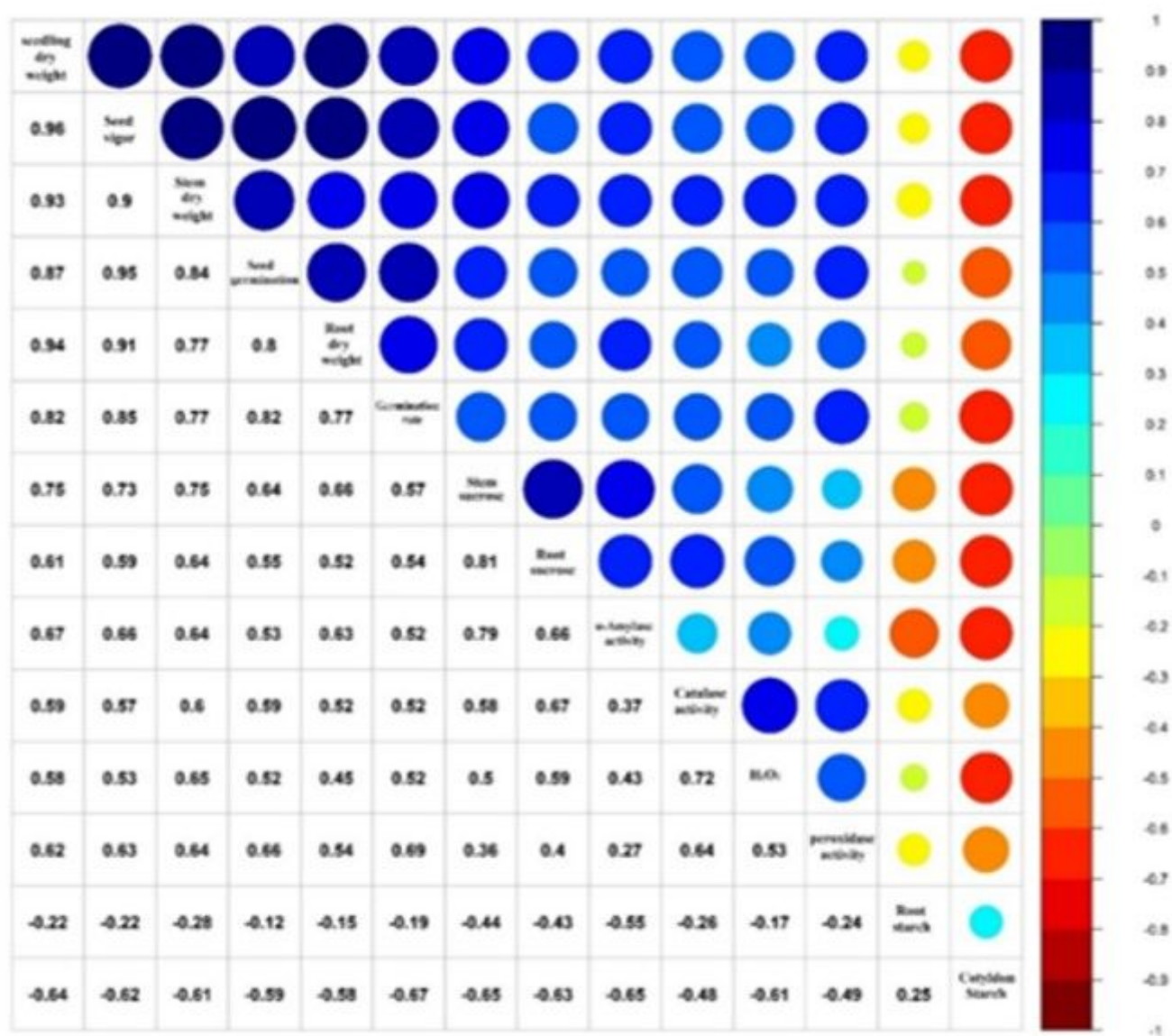


Figure 4

Pearson correlation coefficients among 14 quantitative traits on stevia seedling under the influence of SiO₂NPs. The correlation coefficients with absolute values higher than 0.20 and 0.26 were significant at the statistical probability level of 5 and 1%, respectively