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Silver nanoparticles improve growth and protect against oxidative damage in eggplant seedlings under drought stress

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Abstract: Drought stress is a significant abiotic stressor that has a negative impact on crop production and global food security systems. Drought stress was applied to eggplant seedlings with various field capacities (FC), 80% FC as control, 50% FC, 35% FC, and 20% FC. AgNPs were synthesised from green chemical methods, whereas different concentrations of AgNPs (0, 0.1, 0.2, 0.5 μmol) were applied exogenously on drought-stressed eggplants. Drought stress decreased the growth parameters (plant height, fresh mass, dry mass, leaf area), photosynthetic pigments (Chl *a*, Chl *b*, carotenoids), and protein content while increased the proline, hydrogen peroxide (H_2O_2), malondialdehyde (MDA) content, and activity of the antioxidant enzymes, i.e., superoxide dismutase (SOD) and catalase (CAT). AgNPs restricted proline accumulation and reduced H_2O_2, MDA content by upregulating the antioxidant enzymes. Overall, the current study's findings indicated that AgNPs are an effective eco-friendly and low-cost application for plant growth under drought stress, with the potential to mitigate the impact of drought on plants.

Keywords: leaf extracts; malondialdehyde; particle size; photoperiod; relative humidity

Eggplant (*Solanum melongena* L.) is one of the world's leading crops, with a cultivated area of 1.86 million ha (Semida et al. 2021). It is one of the top five types of vegetables consumed by millions of people in drought-affected areas worldwide (Wakchaure et al. 2020). Eggplants are often subjected to several abiotic stresses, e.g. chilling, drought, extreme temperature, heavy metals, high radiation, and salinity (Semida et al. 2021). The fruit of eggplant yield declined up to 60%, while drought stress increased from 20% to 40% of the field capacity (Karam et al. 2011).

Drought is one of the most important abiotic stresses that contribute to food scarcity. It has an impact on plant growth and yield, particularly in the world's drought-prone regions. Plants experience important physiological changes as a result of drought stress (Hasan et al. 2021). Plants exposed to drought stress could trigger an overproduction of reactive oxygen species (ROS), which can induce oxidative damages. To protect against these oxidative damages, plants developed antioxidant defense systems in plants (Khan et al. 2017, Hasan et al. 2020). The antioxidant defense systems increase the plants ability to counteract the excess ROS productions (Sharma et al. 2012). Nonetheless, negative drought stress effects can be mitigated by using potential nanoparticles (NPs) (Dimkpa et al. 2019, Semida et al. 2021).

Green synthesis of silver nanoparticles (AgNPs) utilising plants and plant extracts has been extensively

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used for the agricultural sector (Wahid et al. 2020, Alabdallah and Hasan 2021). Silver nanoparticles are a type of nanoparticle that may assist plant growth, seed germination, and photosynthesis (Wahid et al. 2020). AgNPs application on plants was dose-dependent; for example, a lower concentration of AgNPs increased the plant growth and yield, whereas high concentrations inhibited the plant growth (Alabdallah and Hasan 2021). Vannini et al. (2014) reported that AgNPs application on plants might depend on the size and duration of exposure.

However, to the best of our understanding, there is little information on the effect of AgNPs on eggplant under drought stress. Therefore, we hypothesised that AgNPs concentrations (0.1, 0.2, 0.5 µmol) might mitigate the drought stress in eggplant through modulation of growth and physiology. To confirm this hypothesis, we investigated growth and physiological parameters such as (*i*) plant height; (*ii*) photosynthetic pigments; (*iii*) proline and protein content, and (*iv*) antioxidant systems in eggplant exposed to drought stress.

MATERIAL AND METHODS

Used materials. The green synthesis of AgNPs was carried out according to the method described by Bar et al. (2009) and Ahmed et al. (2016). Silver nanoparticles were synthesised from the leaf extracts of *Albizia lebbeck* (L.) Benth, which was collected from Al-Hasa city, Saudi Arabia. Silver nanoparticles were prepared by changing the concentration of $AgNO_3$ (0.1, 0.2, 0.5 µmol) while maintaining a constant extract concentration. AgNPs have hexagonal and spherical shapes with particle sizes ranging from 17 nm to 34 nm.

Plant materials and experimental treatment. The experiments were carried out at Imam Abdulrahman Bin Faisal University greenhouse (26.3928°N, 50.1926°E), Eastern Region, Saudi Arabia. Healthy eggplant (*Solanum melongena* L. cv. Ophelia F1) seeds were collected from Altuajri company, Saudi Arabia. Sodium hypochlorite (4%) has been used for the sterilisation of the eggplant seeds. Germinated eggplant seedlings (3 seedlings per pot, size-height × diameter = 16 cm × 19 cm) were grown in plastic pots until harvest, which was filled with 2.5 kg of a sterilised mixture of clay soil and compost (5:1, *v*:*v*) (Table 1). Before experimental treatments were applied, growing eggplant seedlings were watered twice per week. Drought stress treatments were measured and applied based on the field capacity (FC). These treatments were imposed on 20-day-old seedlings. The plants were exposed to four water regimes (80% FC as control, 50% FC, 35% FC, and 20% FC) after the plant establishment. Three replicates for each treatment have been used. We used different concentrations of AgNPs (0.1, 0.2, 0.5 µmol) to treat drought-stressed plants. The hand-held sprayer was used to apply the treatment of AgNPs at 0.1, 0.2, 0.5 µmol concentration on the foliage of 20 days old control and drought treated eggplant seedlings. The relative humidity was 60–70%, with 16 h photoperiod and the day/night temperature was 22/16 °C during the experimental period. The eggplant leaves were harvested at 42 days for determining physiological and morphological parameters.

Growth parameters. After harvest, plant height (PH) was measured by a measuring tape and expressed in centimeters (cm). Fresh mass (FM) was measured using an analytical balance (HR-200). The sample was dried at 70 °C for 72 h, and finally, dry weight was measured. Leaf area was measured after 42 days from germinating by using a leaf area meter (CI-202 Area Meter CID, Washington, USA).

Photosynthetic pigments determination. The photosynthetic pigments were determined according to the methods of Arnon (1949). The absorbance was recorded at 645, 663, 510, and 480 nm for the estimation of Chl *a*, Chl *b*, total Chl, and carotenoids.

Total soluble protein measurements. Total soluble protein content was determined according to the methods of Bradford (1976). The soluble protein content was measured based on the standard curve method by using bovine serum albumin (BSA).

Proline determination. Proline was determined based on the methods of Bates et al. (1973). The absorbance was recorded at 520 nm.

Determination of MDA content. Malondialdehyde (MDA) concentrations were determined by follow-

Table 1. Characteristic of the soil used in the experiment

pH	EC (ds/m)	Organic carbon (%)	Sand (%)	Silt (%)	Clay (%)	K (mg/kg)	P (mg/kg)	N (%)
7.5	2.17	0.9	71.8	19.2	8.9	186	55	0.12

EC – electrical conductivity

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ing the methods of Heath and Packer (1968). The absorbance was taken at 532 nm, and the extinction coefficient was 155 mmol/cm. MDA content was expressed as nmol/g FM.

Estimation of H_2O_2 content. Hydrogen peroxide (H_2O_2) content was quantified according to Yu et al. (2003) method. The absorbance was recorded at 410 nm and expressed as nmol/g FM.

Extraction and assay of antioxidant enzymes activities. Antioxidant enzymes were measured according to the methods of Mukherjee and Choudhuri (1983). 0.5 g fresh leaves were grounded with 10 mL of phosphate buffer (pH 7). The extract was centrifuged at 15 000 × *g* for 10 min at 4 °C. Then, the supernatant was collected and stored for analysis of the antioxidant enzymes. The absorbance was recorded at 560 nm and expressed as U m/g FM for determining the SOD activity, whereas the optical density (OD) was determined at 240 nm and expressed as U m/g FM for analysis of the catalase (CAT) activity. The optical density was determined at 240 nm and expressed as U m/g FM.

Statistical analysis. All the obtained data were analysed by one-way analysis of variance (ANOVA). Minitab 17.0 (Chicago, USA) was used to test the significance between mean values ($P < 0.05$) in the Fisher *LSD* (least significant difference) test. Heatmap was performed by using the ggplot2 package in R version 3.6.3 (Vienna, Austria). Each treatment has been repeated 3 times.

RESULTS

The images of silver nanoparticles were taken by using the TEM electron microscope, and the analysis displayed the spherical shapes of AgNP that particle size is ranging from 14 nm to 35 nm (Figure 1). To estimate the effects of AgNPs on eggplant growth, we measured several morphological parameters, including plant height, shoot fresh mass, shoot dry mass (DM), and leaf area (Figure 1). When the eggplants were exposed to drought stress under different field capacities (50% FC, 35% FC, 20% FC), the plant height, FM, DM, and leaf area were significantly ($P \leq 0.001$) reduced compared to control (Figure 2). However, applying AgNPs increased these parameters significantly in eggplants that grow under drought stress. The treatment of 0.1 µmol AgNP was more effective

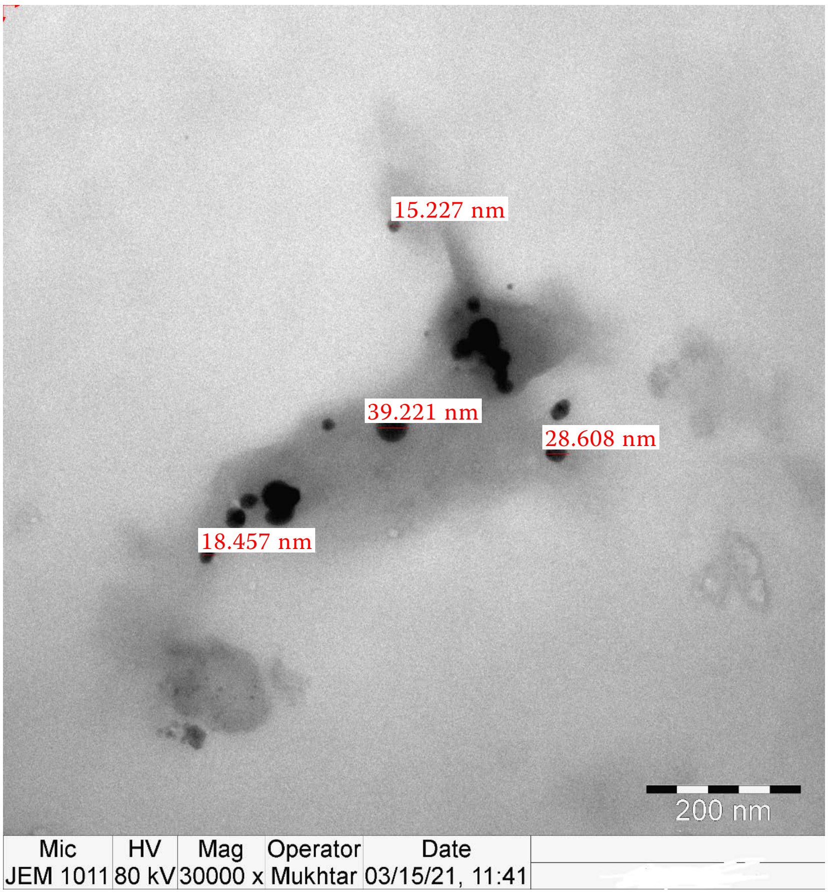

Figure 1. Images of synthesised silver nanoparticles were taken from transmission electron microscopy (TEM)

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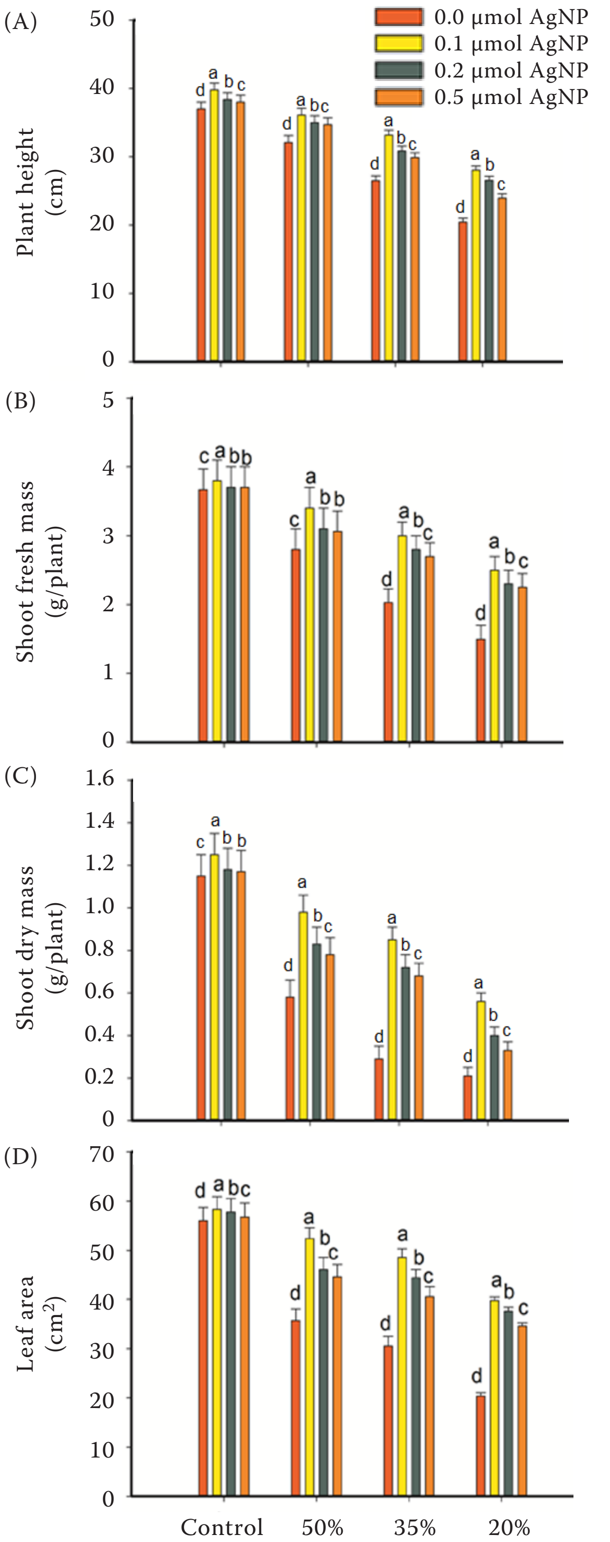

Figure 2. Effect of AgNPs (silver nanoparticles) on (A) plant height; (B) shoot fresh mass; (C) shoot dry mass and (D) leaf area in the eggplant seedlings under drought stress (50%, 35%, 20% field capacities). The data displayed are the means (± standard error) of three replicates, and bars of dissimilar letters differ significantly at the $P \leq 0.05$ level

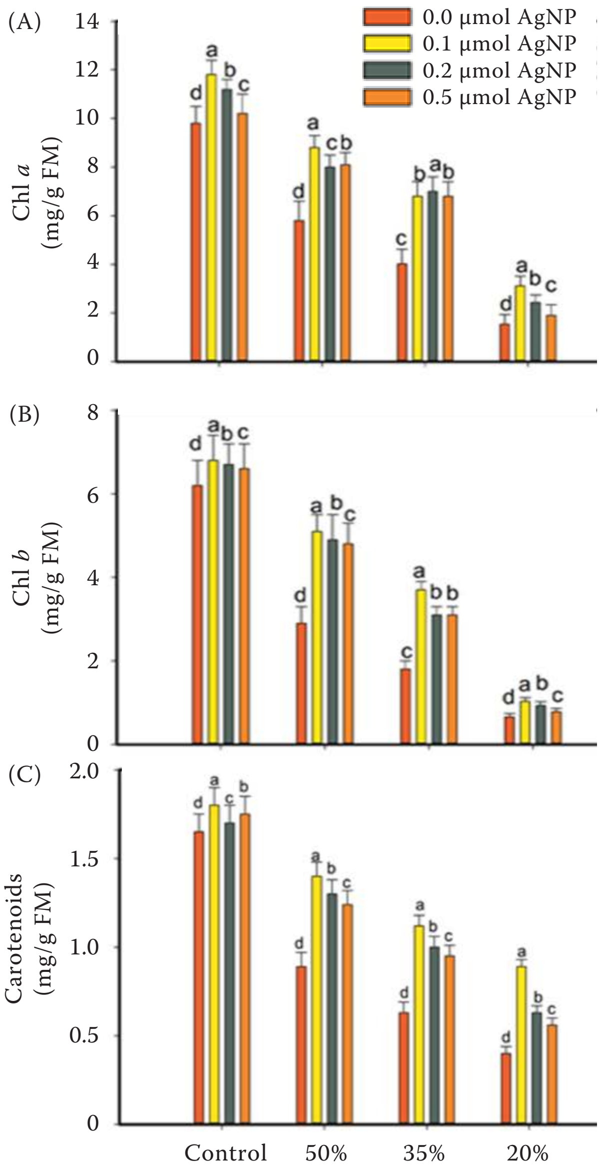

Figure 3. Effect of AgNPs (silver nanoparticles) on (A) Chl *a*; (B) Chl *b*, (C) carotenoids in the leaf of eggplant seedlings under drought stress (50%, 35%, 20% field capacities). The data displayed are the means (± standard error) of three replicates, and bars of dissimilar letters differ significantly at the $P \leq 0.05$ level; FM – fress mass

to increase the plant height, FM, DM, and leaf area (Figure 2). Photosynthetic pigments (Chl *a*, Chl *b*, carotenoids) display high significant decreases when these plants are treated with drought stress (Figure 3). Nevertheless, eggplants treated with different concentrations of AgNPs significantly ($P \leq 0.001$) showed higher chlorophyll contents in leaves.

The drought treatments of 50% FC, 35% FC, and 20% FC increased the proline content by 28, 40 and 49%,

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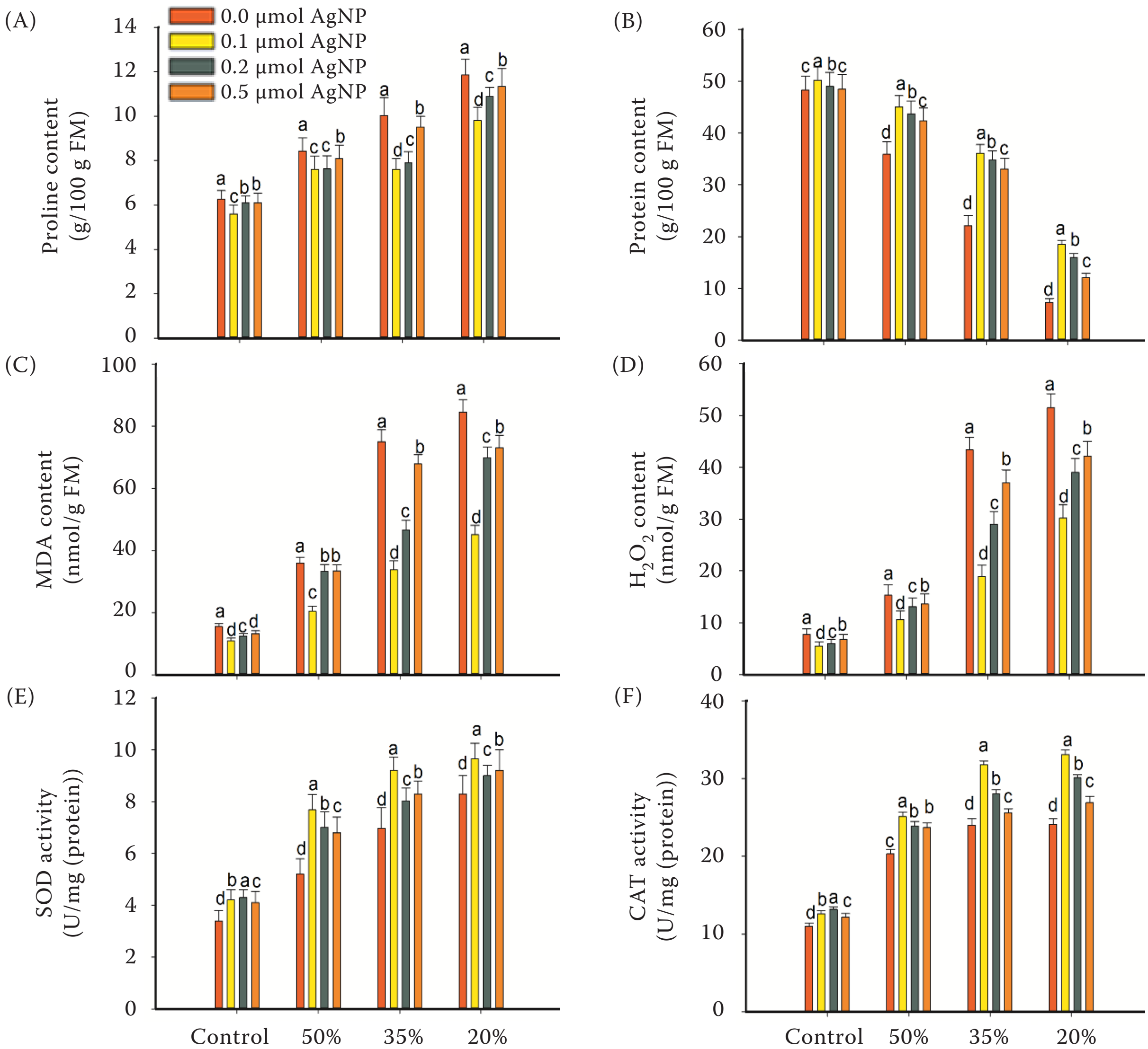

Figure 4. Effect of AgNPs (silver nanoparticles) on (A) proline; (B) protein; (C) malondialdehyde (MDA); (D) hydrogen peroxide (H_2O_2) content; (E) superoxide dismutase (SOD), and (F) catalase (CAT) activity in the leaf of eggplant seedlings under drought stress (50%, 35%, 20% field capacities). The data displayed are the means (± standard error) of three replicates, and bars of dissimilar letters differ significantly at the $P \leq 0.05$ level; FM – fress mass

respectively, compared with the level in control eggplant seedlings that did not receive AgNPs. However, externally applied AgNPs (0.1, 0.2 and 0.5 μmol) strikingly decreased proline content relative to those in the drought-stressed plants alone (Figure 4A). On the other hand, 50% FC, 35% FC, and 20% FC significantly ($P \leq 0.001$) decreased the protein content by 26, 54 and 85% compared to those in the control eggplants. Nevertheless, the protein content was significantly ($P \leq 0.001$) increased by the exogenous supply of AgNPs (Figure 4B).

Conversely, 50% FC, 35% FC, and 20% FC drought treatments significantly ($P \leq 0.001$) increased MDA content by 57, 79 and 82%, H_2O_2 content by 49, 82 and 85%, respectively, relative to with respect to those in untreated plants (Figure 4C–D). Nonetheless, the exogenous application of AgNPs decreased significantly ($P \leq 0.001$) MDA and H_2O_2 content compared to those in the drought-stressed plants.

Figure 4E–F showed the analysed antioxidant enzyme parameters (SOD and CAT) under drought stress. The drought treatments (50% FC, 35% FC, and 20% FC) led to significant ($P \leq 0.001$) increases in SOD activity by 35, 51, and 62%, and CAT activity by 46, 54, and 54%, respectively, with respect to those in control seedlings. However, these antioxidant

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enzyme parameters were significantly ($P \leq 0.001$) increased by the exogenous supply of AgNPs.

The mean values of the growth and physiological parameters were taken to plot the heatmap (Figure 5). These heatmaps show the log10 value with colour intensities ranging from white (low) to red (high). Moreover, it depicts the control seedlings clearly separated from drought-treated seedlings as well as AgNPs treated seedlings (Figure 5).

DISCUSSION

Drought stress induces plant growth retardation and physiological imbalances. In the current study, drought stress affects the shoot length, FM and DM of eggplants. However, these parameters were improved by the application of AgNPs. Our outcome is similar to past studies in which exogenous application of AgNPs increased the growth parameters in green beans under chill temperature. Eggplants exposed to drought had lower photosynthetic pigments such as Chl *a*, Chl *b*, and carotenoid contents. However, AgNPs application in drought-treated eggplants increased the levels of photosynthetic pigment. A parallel result was observed in wheat plants during salt stress, as reported by Wahid et al. (2020).

In the current study, drought-induced water imbalance was found in eggplants due to increment of proline accumulation. Similarly, proline accumulation was observed under drought stress in several crops such as maize (Anjum et al. 2017), soybean (Hasan et al. 2020a). Nevertheless, AgNPs application to drought treated eggplants resulting the reduction of proline accumulation, which clearly suggested that nanoparticles (AgNPs) can play an important role in water imbalance by reducing the proline accumulation.

Drought stress led to excess ROS production, resulting in membrane deterioration in plants. Higher accumulation of MDA and H_2O_2 are closely related to ROS overproduction that negatively affects the cellular membrane integrity (Wang et al. 2010, Khan et al. 2017, Hasan et al. 2018). A similar drought stress-induced higher MDA, and H_2O_2 accumulation was clearly observed in soybean seedlings, as reported by Hasan et al. (2020). However, AgNPs strikingly decreased the MDA and H_2O_2 content, which clearly indicates that AgNPs might alleviate drought-induced oxidative stress in eggplants. These

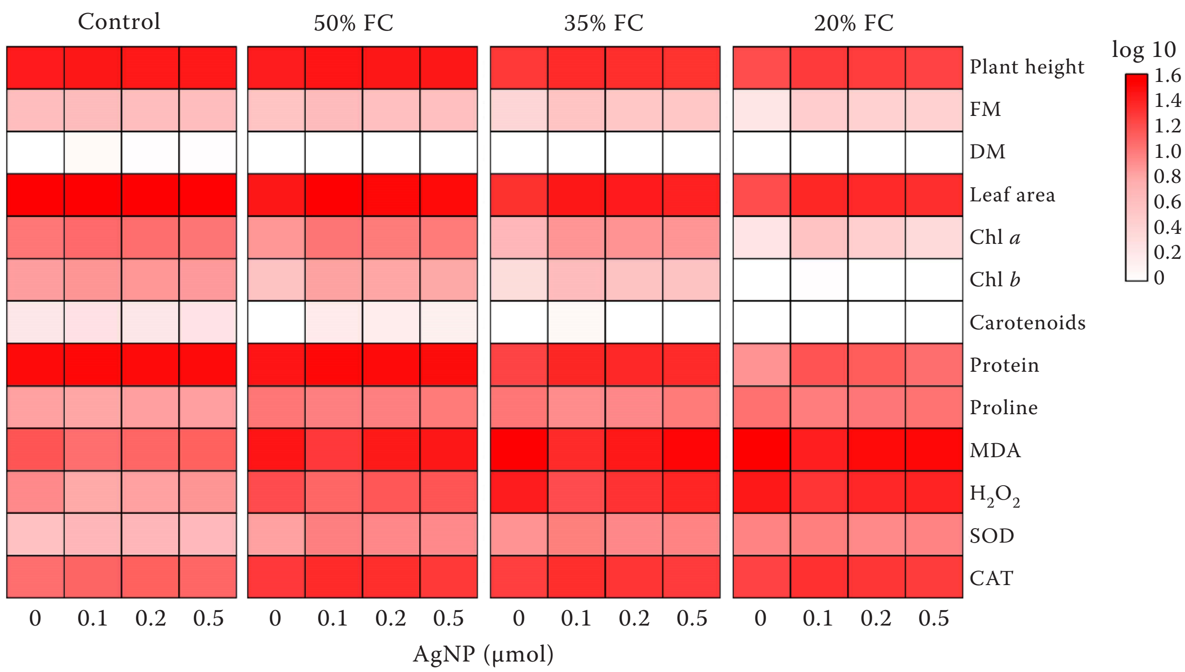

Figure 5. The variable-treatment relationships in different groups (control, 50% FC (field capacities), 35% FC, 20% FC) are displayed on the heatmap. The logarithmic transformation (log10) values are shown in the colour scale on the heatmap (lower values to higher values revealed as white to red). The variables include plant height, shoot fresh mass (FM), shoot dry mass (DM), Chl *a*, Chl *b*, carotenoid, protein, proline, malondialdehyde (MDA), hydrogen peroxide (H_2O_2), superoxide dismutase (SOD) and catalase (CAT) activity

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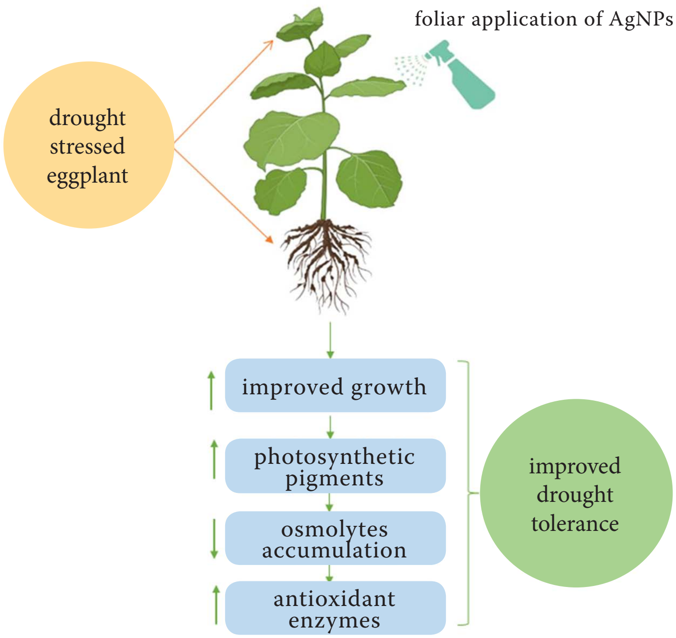

Figure 6. A proposed model illustrates how silver nanoparticles (AgNPs) alleviate drought stress in eggplant seedlings

results are analogous to those proven in wheat, as described by Mohamed et al. (2017).

In plants, antioxidant defense mechanisms play an important role to adapt to drought stress (Sharma et al. 2012). It is confirmed by past studies that nanoparticles can regulate the antioxidant systems and it has protective responses to plants (Yan and Chen 2019). SOD is a key antioxidant enzyme that plays a major role in antioxidant systems (Sairam et al. 1998). Drought stress increased the SOD activity in the eggplants, as has been described in soybean (Hasan et al. 2020) and mung bean (Nahar et al. 2016). However, AgNPs increased the SOD activity, parallel to what was reported by Alabdallah and Alzahrani (2020) for *Abelmoschus esculentus* treated with ZnONPs. The CAT activity was found to be increased in the drought-stressed eggplants. These outcomes are parallel to those reported in wheat (Mohamed et al. 2017). The above results suggested that AgNPs might involve in the scavenging of H_2O_2 by improving the antioxidant systems (Figure 6).

In these studies, our investigation was designed to evaluate how AgNPs can be used to boost the yield of eggplants, particularly under drought stress conditions. Green synthesised (AgNPs) application resulted in alleviation of drought stress in eggplants. AgNPs treatment increased the growth, photosynthetic pigments, antioxidant activity and decreased the proline content in eggplants under drought stress, compared to control. Our results suggested that AgNPs treatments increased the drought tolerance in eggplants, and it could be used as an environmentally friendly treatment. Moreover, it is necessary to understand the underlying molecular mechanisms behind AgNPs-mediated drought tolerance in plants.

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