High-Temperature Stress Alleviation by Selenium Nanoparticle Treatment in Grain Sorghum

M. Djanaguiraman, N. Belliraj, Stefan Bosmann, and P. V. Vara Prasad

1Department of Agronomy, Throckmorton Plant Science Center and 4Department of Chemistry, Kansas State University, Manhattan, Kansas 66506, United States
2Department of Nano Science and Technology, Tamil Nadu Agricultural University, Coimbatore, TN 641003, India

ABSTRACT: The role of selenium nanoparticles (Se-NPs) in the mitigation of high-temperature (HT) stress in crops is not known. The uptake, toxicity and physiological and biological effects of Se-NPs under HT were investigated in grain sorghum [Sorghum bicolor (L.) Moench]. Se-NPs of size 10–40 nm were synthesized and characterized to indicate nanocrystalline structure. A toxicity assay showed that Se-NPs concentration inducing 50% cell mortality (TC50) was 275 mg L−1. Translocation study indicated that Se-NPs can move from root to shoot of sorghum plants. Foliar spray of 10 mg L−1 Se-NPs during the booting stage of sorghum grown under HT stress stimulated the antioxidant defense system by enhancing antioxidant enzymes activity. Furthermore, it decreased the concentration of signature oxidants. Se-NPs facilitated higher levels of unsaturated phospholipids. Se-NPs under HT stress improved the pollen germination percentage, leading to a significantly increased seed yield. The increased antioxidant enzyme activity and decreased content of oxidants in the presence of Se-NPs were greater under HT (38/28 °C) than under optimum temperature conditions (32/22 °C). In conclusion, Se-NPs can protect sorghum plants by enhanced antioxidative defense system under HT stress.

1. INTRODUCTION

Selenium (Se) is an essential micronutrient for humans, animals, and other organisms.¹ However, in higher plants, the role of selenium nanoparticles (Se-NPs) has not been demonstrated clearly. Earlier studies have indicated that soil and/or foliar application of Se improved the antioxidant capacity [in sweet basil; Ocimum basilicum L.,² growth [in tobacco; Nicotiana tabacum L.],³ and yield [in mustard; Brassica rapa L.],⁴ in potato; Solanum tuberosum L.],⁵ of higher plants. Decreased lipid peroxidation and cell membrane damage through increased superoxide dismutase (SOD) and glutathione peroxidase (GPX) enzymes activity by Se application explains its antioxidative activity.⁶,⁷ The increased growth in higher plants by Se application is through higher leaf photochemical efficiency,⁸ stomatal conductance, carboxylation efficiency, and content of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) enzyme.⁹

Elemental Se is not soluble in water and biologically inert because of its redox state. However, nanosized elemental Se-NPs were found to possess prominent bioactivity and biosafety properties.⁸,⁹ Studies have shown that the biological activity and antioxidant property of Se-NPs increase with their surface-to-volume ratio and decreasing particle size.¹⁰ Se-NPs have lower cellular toxicity than selenium or selenomethionine, and possess the ability to increase the GPX activity, leading to decreased oxidative stress in mice.¹¹ Similar increase in GPX and thioredoxin reductase activities with much lower cellular toxicity by elemental selenium and selenomethionine at nanoscale was reported.⁸ The underlying paradigm for this study is that Se-NPs show high biological activity because of interactions between nanoparticles’ extended surface areas and the functional groups of peptides.¹²–¹⁵ In tobacco, Se-NPs at a concentration of 50–100 mg kg⁻¹ improved organogenesis and root growth, whereas selenate completely inhibited both processes, indicating Se-NPs are more effective and less toxic than bulk selenium particles.¹³ Our earlier study on sorghum [Sorghum bicolor (L.) Moench] demonstrated that foliar application of selenate improved the leaf antioxidant defense system under high-temperature (HT) stress.¹⁴ However, to our knowledge, the role of Se-NPs on cellular toxicity and antioxidative defense system in plants under HT stress has not been studied yet. To validate whether Se-NPs possess antioxidant properties under HT stress, a study was conducted on sorghum plants challenged with HT stress.

Various abiotic stresses cause accumulation of reactive oxygen species (ROS) in plants.¹⁶ In plants, ROS include superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂), hydroxyl radical (OH⁻), singlet oxygen (¹O₂), and lipid peroxidation free radicals (LOO⁺, ROO⁺), which are highly active and greatly affect cell membrane stability.¹⁷ HO₂⁻/O₂⁻ produced in plants dismutates either naturally or by the enzyme superoxide dismutase (SOD) to HO₂⁻ and O₂. The formed HO₂⁻ or its conjugate base, H₂O₂, reduces ferric to ferrous or cupric to cuprous, which later reacts with H₂O₂/HO₂⁻ to generate the hydroxyl radical (OH⁻) or higher-valent iron and copper cations in Fenton-type pro-
cesses. As a result, plants activate biosynthesis of antioxidants (glutathione, ascorbate, and tocopherol) and antioxidant enzymes [SOD, peroxidase (POX), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX), guaiacol peroxidase, and glutathione reductase]. Studies have highlighted that Se can offset the damaging effects of abiotic stress, such as drought, HT, and heavy metals. However, the effect of Se-NPs on HT stress alleviation is not fully understood and needs attention.

Generally, grain sorghum is grown in the semiarid regions of the world for food and nutritional security. The temperature during the sorghum growing season in the regions of its cultivation is often >35 °C (daytime maximum temperatures), which is higher than the known optimum for sorghum growth, development, and yield. Further, multiple climate models predict that both mean temperature and occurrence of short episodes of extreme HT during the crop growing season will increase in future. Climate model predicts that with an increase of 1 °C in the mean temperature of sorghum growing season will decline the mean grain yield of sorghum by about 8−9%. Our earlier studies in sorghum have shown that HT stress causes oxidative damage to pollen grains, resulting in decreased seed set percentage. Furthermore, Se can alleviate HT-induced oxidative stress by enhancing antioxidant enzyme activities, resulting in delayed leaf senescence in sorghum. Our hypothesis is that the oxidative stress caused by HT in leaf and pollen grains of sorghum can be counteracted by Se-NPs through its antioxidant property. The objectives were to examine the effects of (i) Se-NPs on antioxidant defense system in leaf exposed to HT stress and (ii) Se-NPs on phospholipids of leaves, pollen germination, and grain yield of sorghum under HT stress.

2. RESULTS AND DISCUSSION

2.1. Variation in Temperatures and Relative Humidity among the Growth Chambers. The mean temperatures of daytime maximum and nighttime minimum were ±0.5 °C of the
target day and nighttime temperature in all growth chambers. The relative humidity was within $\pm 10\%$ of the set value. Our previous research showed no statistical difference among the chambers for growth and yield of spring wheat cultivar Pavon. The average plant height was $64.0 \pm 0.9$ cm, the number of tillers per plant was $3.5 \pm 0.1$, the number of spikes per plant was $2.6 \pm 0.1$, and shoot dry weight was $3.7 \pm 0.2$ g per plant. This indicates that the growth chambers used have uniform environmental conditions, which was also supported by the temperature data collected using environmental sensor.

### 2.2. Morphology and Size of the Synthesized Selenium Nanoparticles (Se-NPs)

Nanostructure analysis of Se-NPs through atomic force microscopy (AFM), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and high-resolution transmission electron microscopy (HRTEM) indicates the formation of Se-NPs in a size range of 10–40 nm (Figure 1a–d). The crystal structures determined by
X-ray diffraction (XRD) and Raman spectroscopy are shown in Figure 2a,b, respectively. The diffraction peaks at 2θ (degrees) of 23.48, 29.73, 41.2, 43.4, 45.18, and 51.49° are indexed as (100), (101), (110), (102), (111), and (201) planes of Se, respectively. On the basis of the diffraction peaks, it is clear that the formed Se-NPs are in hexagonal phase with lattice constants of \(a = \) 4.36 Å and \(c = \) 4.95 Å, which are in accordance with the reported JCPDS card No. 06-0362.25 As per the diffraction peaks, no impurities were detected in the synthesized Se-NPs sample, and the diffraction peak sharpness indicates that the product is well crystallized (Figure 2a). The (101) plane showed a stronger peak than the other peaks, indicating that Se-NPs had preferential growth along the (001) direction.26 The discrete selected area electron diffraction (SAED) spot indicated a well-crystallized hexagonal selenium crystal. Correspondingly, the fast Fourier transform of the Se-NPs image is in virtual agreement with the hexagonal structure. The chemical composition analysis through the energy-dispersive X-ray (EDX) technique confirms pure selenium with no impurities (Figure 1d, inset). The peak corresponding to Cu and C arises because carbon-coated copper grid was used for TEM analysis (Figure 1d, inset).

Figure 2b shows a typical Raman scattering spectrum for the Se-NPs, with an intensive peak at 235 cm\(^{-1}\) due to the Raman scattering of the A1 mode of hexagonal selenium.27 The amorphous and monoclinic selenium have Raman resonance absorption band peak at 264 and 256 cm\(^{-1}\), respectively. Neither of them are observed in the present study, indicating the absence of these forms of selenium in the prepared nanoparticle. Two-dimensional AFM images of the synthesized Se-NPs show individual particles of size <10 nm, and 3D images show an individual particle with maximum height of 12 nm in the \(z\) direction (Figure 1a,b). The TEM image shows an individual particle with size between 30 and 60 nm (Figure 1d). The typical SEM image is shown in Figure 1c, which demonstrates that these Se-NPs have a width of \(\sim\)150 nm and lengths in the micrometer scale. It is noteworthy that AFM and TEM images were taken using more diluted samples, compared to the SEM image. The synthesized Se-NPs have a tendency to aggregate to rods and then wires of lengths greater than 200 nm.28

Figure 3. Toxicity and translocation of Se-NPs. (a) Concentration of Se-NPs (\(\mu\)g g\(^{-1}\)) in root and shoot of sorghum seedling grown in one-quarter strength of the Hoagland solution containing 0, 50, or 100 mg L\(^{-1}\) Se-NPs. (b) Effect of Se-NPs on viability of mouse cell line culture assayed through MTT. (c) Effect of Se-NPs on membrane damage of mouse cell line culture assayed through DAPI. Abbreviations: Se-NPs, selenium nanoparticle; MTT, (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide); DAPI, 4',6-diamidino-2-phenylindole.
of the Se-NPs was studied by thermogravimetric analysis (TGA). During heating, the Se-NPs exhibit weight loss in two steps when heated to 900 °C (Figure 2c). A weight loss of 5−6% was observed up to 220 °C, which is most likely due to the evaporation of volatile matter (mainly adsorbed moisture). Around 450 °C, the samples showed almost complete weight loss. The explanation of this finding is the sublimation of the elemental nanoscale Se at this temperature.29

2.3. Cytotoxicity of Se-NPs on a Murine Cell Line. Cell viabilities after exposure to Se-NPs are shown in Figure 3b−f. The viability of the control (0 mg L−1) was set to 100%. Medium supplemented with Se-NPs at concentrations of 100, 250, and 500 mg L−1 showed 45, 47, and 58% reduction in cell viabilities, respectively, compared to the control. The toxicity assay showed that Se-NPs concentration inducing 50% cell mortality (TC50) was 275 mg L−1 (based on regression analysis). These results are in principle agreement with earlier findings using rat dermal fibroblasts.30 The 4′,6-diamidino-2-phenylindole (DAPI) assay distinguishes normal and apoptotic cells by staining the nucleus and the condensed chromosome (Figure 3c−f). The nucleus was stained uniformly, and the margins were clear in the normal cell (0 mg L−1; Figure 3c). However, in damaged cells, the margin of the nucleus was abnormal and the condensed chromosome was stained (Figure 3f). Se-NPs at concentrations of 100 and 250 mg L−1 did not damage the fibroblast L929 cell line by more than 50% as evidenced by regular nucleus margins (Figure 3d,e). However, at 500 mg L−1, the nucleus and cell damage were clearly discernible, which corroborates the findings with the (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) (MTT) assay (Figure 3b).

2.4. Translocation of Se-NPs. Figure 3a shows Se concentrations in roots and shoots of sorghum plants grown in sand spiked with Se-NPs. The Se content in roots was significantly higher at 50 and 100 mg L−1 compared to shoots. The concentrations of Se in the leaves treated with 50 and 100 mg L−1 of Se-NPs were 1.7 and 3.4 μg g−1, respectively. Similarly, the Se contents in the root of 50 and 100 mg L−1 added Se-NPs were 3.1 and 7.0 μg g−1, respectively. The control leaf and root had <0.05 μg g−1 of Se. This suggests that the sorghum plant is able to translocate Se-NPs from the root to the shoot. However, the mechanisms underlying these processes are still not understood. Wang et al.31 observed the
transport of CuO NPs from roots to shoot systems, by the presence of CuO NPs inside the cell wall epidermis, intercellular space, cortical cells of the root, and xylem sap exudate. This indicates that CuO NPS can move intracellularly and extracellularly. After reaching the xylem, these nanoparticles will be translocated to the shoots along with the flow of water by transpiration stream. A similar mechanism might have occurred for SeNPs. There is no evidence about biotransformation of SeNPs in plant systems. However, a study on the potential availability of various forms of selenium under submerged soil indicates that the availability of elemental selenium was low due to limited oxidation to selenite or selenate forms. We expect that during normal cell function the ROS formed through photosynthesis or respiration has the ability to transform SeNPs to selenite or selenate forms. Similar uptake and translocation studies on zinc oxide nanoparticles indicate that ZnNPs transformed to zinc nitrite or zinc acetate in germinated soybean roots.

**Figure 5.** Interaction of temperature stress (OT, optimum temperature, 32/22 °C; HT, high temperature, 38/28 °C) and selenium application (control, water spray and Se-NPs, selenium nanoparticle as foliar spray 10 mg L⁻¹) during booting stage on (a) superoxide dismutase (SOD) enzyme activity (enzyme units), (b) catalase (CAT) enzyme activity (enzyme units), (c) peroxidase (POX) enzyme activity (enzyme units), (d) glutathione peroxidase (GPX) enzyme activity (enzyme units), (e) pollen germination (%), (f) seed set (%), (g) seed size (mg seed⁻¹), and (h) seed yield (grams per panicle) of grain sorghum. The enzyme activity was recorded in the leaves on 9 days of HT stress, and its yield and components were recorded at maturity. Each value is the mean ± SE of eight independent measurements (four replications and two experiments). Means with different letters are significantly different at P ≤ 0.05.

**2.5. Effects of Se-NPs on Physiological Traits in Sorghum.** The chlorophyll index, thylakoid membrane damage, stomatal conductance, and photosynthetic rate varied significantly (P ≤ 0.001) between the temperature regimes (Figure 4a–d). Irrespective of the Se-NPs spray, HT stress decreased chlorophyll content (16%), stomatal conductance (22%), and photosynthetic rate (17%) and increased thylakoid membrane damage (68%) compared to optimum temperature (OT, Figure 5a–d). Chlorophyll biosynthesis enzymes are bound to the chloroplastic membranes, and the damage of thylakoids under HT stress leads to chlorophyll loss. Increase of the thylakoid membrane damage under HT stress is consistent with membrane leakiness. For both temperature ranges, Se-NPs spray marginally (<2%) increased the chlorophyll content, stomatal conductance, and photosynthetic rate (Figure 4a,c,d). However, the thylakoid membrane damage was decreased by 18% compared to the unsprayed control (Figure 4b), which indicates that Se-NPs protected the thylakoid membrane by restoration of the
chloroplast ultrastructure through distribution of thylakoid membranes and granal stacking.50

2.6. Effects of Se-NPs on the Content of Oxidants, MDA, and Membrane Damage. High-temperature stress caused significant (P \leq 0.001) increase in O2•− and H2O2 contents, MDA formation, and membrane damage than OT (Figure 4e–h). HT stress increased O2•− and H2O2 contents by 110 and 88%, respectively, compared to OT (Figure 4e,f). Similarly, HT stress increased the MDA and cell membrane damage by 144 and 152%, respectively, over OT (Figure 4g,h). A damaged thylakoid membrane can decrease the electron transport rate, leading to the formation of ROS.37 Application of Se-NPs significantly (P \leq 0.001) reduced superoxide radical, hydrogen peroxide, MDA, and membrane damage by 25, 25, 30, and 18%, respectively, compared to the untreated control (Figure 4e–h). There was a significant interaction between temperature and Se-NPs application for O2•−, H2O2, MDA, and membrane damage (Figure 4e–h). Application of Se-NPs decreased O2•−, H2O2, MDA, and membrane damage by 25, 38, 39, and 25% under HT stress (Figure 4e–h). The decreased O2•− levels under Se treatment could be due to the spontaneous dismutation of superoxide radical to hydrogen peroxide,38 the direct quenching of superoxide radical and hydroxyl radical by Se nanoparticles and/or selenoenzymes,5,39 or the activation of the antioxidant defense system.39 Selenium cannot directly scavenge H2O2; however, it can activate H2O2 quenchers (GPX, CAT, POX, and APX), leading to decreased H2O2 content.14 The level of MDA under HT stress is an indicator of cell membrane damage. Se-NPs spray decreased MDA production in both OT and HT; however, greater reduction was observed under HT (Figure 4g). This indicates that Se-NPs can act as an antioxidant under HT stress.6,14

2.7. Effects of Se-NPs on the Activity of Antioxidant Enzymes. Overall, HT stress significantly (P \leq 0.001) decreased SOD (38%), CAT (37%), POX (38%), and GPX (28%) enzyme activity compared to OT (Figure 5a–d). It has been suggested that under HT stress the production of ROS is closely related to ROS removal. This study demonstrated that HT stress doubled the production of ROS, which may surpass the antioxidant defense capability, resulting in enzyme inactivation or impairment. Foliar spray of Se-NPs significantly (P \leq 0.001) increased SOD, CAT, POX, and GPX by 22, 24, 11, and 9%, respectively, compared to the unspayed control (Figure 5a–d). Significant interaction (P \leq 0.001) between temperature and Se-NPs application was observed for SOD, CAT, POX, and GPX activity. This was because Se-NPs application increased the enzyme activity only under HT stress. Foliar spray of Se-NPs improved the antioxidant defense system under HT, which was demonstrated by diminished ROS levels and MDA production (Figure 4e–h). Earlier studies have demonstrated the antioxidant role of Se against the ROS through enhanced antioxidant enzyme (SOD, CAT, POX, and GPX) activity and decreased ROS content.6,14,19,39

2.8. Phospholipid Signatures. The total amounts of monogalactosyldiacylglycerol (MGDG) and phosphatidylinositol (PI) were significantly (P \leq 0.05) decreased under HT stress (Table 1). Furthermore, the amounts of the polyunsaturated acyl species 36:6 of MGDG and PI were significantly (P \leq 0.05) decreased during HT stress (Figure 6a,b; Table 2). Similarly, the 34:5, 36:5, and 36:6 species of MGDG and 36:5 species of PG were significantly (P \leq 0.001) decreased during HT stress. The decreased levels of unsaturated acyl species indicate that HT stress decreases desaturase enzyme activity.14 The decreased levels of unsaturated acyl species indicate that HT stress decreases desaturase enzyme activity.40 Foliar spray of Se-NPs caused significant (P \leq 0.05) increase in total phosphatidylcholine (PC) and phosphatidylglycerol (PG) contents (Table 1). The 34:2, 36:3, and 36:4 species of PC were significantly (P \leq 0.05) increased by Se-NPs spray compared to the unspayed control (Figure 6c; Table 2). Similarly, the 34:2, 34:3, and 36:5 species of PC were significantly (P \leq 0.05) increased (Figure 6d). PC forms a bilayer structure of the membrane, and the high level of choline in the polar head makes the membrane more fluid.41 It seems that Se-NPs under HT stress maintains the lipid bilayer and fluidity. Phosphatidylglycerol is a major

Table 1. Main Effect of Temperature and Se-NPs Spray on Total Amount of Lipids in Each Head Group Class in Wheat Leaves under OT and HT6,42

| polar lipid       | OT     | HT     | LSD   | Se-NPs spray | LSD   |
|-------------------|--------|--------|-------|--------------|-------|
| MGDG              | 52.66 ± 4.80 | 42.39 ± 3.00 | 8.40** | 43.96 ± 4.80 | 51.08 ± 3.50 | 8.40  |
| DGDG              | 19.10 ± 1.70 | 17.11 ± 1.30 | 3.10  | 16.70 ± 1.70 | 19.51 ± 1.20 | 3.10  |
| PG                | 11.57 ± 1.20 | 10.51 ± 1.10 | 2.30  | 9.69 ± 1.10  | 12.40 ± 0.98 | 2.30* |
| PC                | 5.28 ± 0.56  | 5.50 ± 0.57  | 1.10  | 4.77 ± 0.52  | 6.01 ± 0.52  | 1.10* |
| PE                | 1.58 ± 0.23  | 1.63 ± 0.18  | 0.43  | 1.48 ± 0.18  | 1.73 ± 0.22  | 0.43  |
| PI                | 2.67 ± 0.27  | 2.11 ± 0.21  | 0.50* | 2.18 ± 0.27  | 2.61 ± 0.23  | 0.50  |
| PA                | 0.42 ± 0.05  | 0.44 ± 0.06  | 0.10  | 0.41 ± 0.05  | 0.44 ± 0.04  | 0.10  |
| PS                | 0.16 ± 0.05  | 0.23 ± 0.06  | 0.11  | 0.15 ± 0.04  | 0.24 ± 0.06  | 0.11  |
| LPG               | 0.07 ± 0.01  | 0.12 ± 0.01  | 0.03  | 0.09 ± 0.02  | 0.10 ± 0.01  | 0.03  |
| LPC               | 0.07 ± 0.01  | 0.05 ± 0.01  | 0.02  | 0.06 ± 0.01  | 0.06 ± 0.01  | 0.02  |
| LPE               | 0.04 ± 0.01  | 0.04 ± 0.01  | 0.01  | 0.03 ± 0.01  | 0.04 ± 0.01  | 0.01  |
| total polar lipid | 93.60 ± 8.40 | 80.10 ± 6.20 | 15.00 | 79.50 ± 8.20 | 94.20 ± 6.20 | 15.00 |

Values are mean ± standard error (SE; n = 8). MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; PG, phosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PA, phosphatic acid; PS, phosphatidylinerine; LPG, lysophosphatidylglycerol; LPC, lysophosphatidylcholine; and LPE, lysophosphatidylethanolamine; OT, optimum temperature; HT, high temperature; Se-NPs, selenium nanoparticles. All polar lipid classes were represented as mean ± SEM, and the means with same letter indicate no significant difference at the LSD (P \leq 0.05) level between OT and HT or control and Se-NPs spray. ** and * indicate significant difference at P \leq 0.01 and P \leq 0.05, respectively.
phospholipid of thylakoid membranes and considered to be important for the development of the chloroplast. The elevated PG levels in plants sprayed with Se-NPs indicate that the functionality of chloroplast was maintained under HT stress.

2.9. Pollen Germination, Seed Set, Seed Size, and Seed Yield. High-temperature stress significantly ($P \leq 0.001$) decreased the pollen germination percentage, seed set percentage, and seed yield (Figure 5e–h) by 23, 32, and 27%, respectively, compared to OT. Previous studies have indicated that HT stress can decrease pollen germination percentage in sorghum by affecting the pollen or ovule viability and/or stigma receptivity.23,42,43 Reduced seed set percentage under HT stress was a consequence of the reduced pollen germination percentage, leading to decreased grain yield because there is no significant difference in seed size. Application of Se-NPs significantly ($P \leq 0.001$) increased the pollen germination percentage (6%), seed set percentage (7%), and seed yield (11%) under HT stress compared to unsprayed control (Figure 5e–h). There were significant ($P \leq 0.001$) interactions of temperature by Se-NPs application for pollen germination percentage, seed set percentage, and seed yield (Figure 5e–h). Application of Se-NPs increased pollen germination percentage, seed set percentage, and seed yield by 14, 19, and 26%, respectively, under HT stress conditions (Figure 5e–h). A previous study on Brassica juncea indicated no reduction of pollen germination percentage after Se (370 mg Se kg$^{-1}$) application.44 Under HT stress, Se-NPs improved the pollen germination percentage. The exact mechanism of the pollen germination improvement is not known and needs to be studied. However, we hypothesize that the elevated ROS production under HT stress in the pollen grains may be quenched effectively by Se-NPs, leading to increased pollen viability. The observed increase in the seed yield of plants sprayed with Se-NPs was a consequence of increased photosynthetic rate and seed number per panicle. This was accomplished by decreased ROS content in the leaves along with higher antioxidant enzyme activities.

Figure 6. Main effect of temperature stress (OT, optimum temperature, 32/22 °C; HT, high temperature, 38/28 °C) and selenium application (control, water spray, and Se-NPs, selenium nanoparticle as foliar spray 10 mg L$^{-1}$) during booting stage on phospholipid molecular species contents in grain sorghum leaves on 9 days of HT stress. Each value is the mean ± SE of eight independent measurements (four replications and two experiments). Means with different letters are significantly different at $P \leq 0.05$. 
Table 2. Main Effect of Temperature Regimes and Se-NPs Spray on the Phospholipid Molecular Species, Which Showed Significant Difference at the LSD (α < 0.05) Level between OT and HT or Control and Se-NPs

| lipid molecular species | temperature regime (nmol mg⁻¹) | lipid molecular species | spray (nmol mg⁻¹) |
|-------------------------|-------------------------------|-------------------------|------------------|
|                         | OT                            | HT                      |                  |
| MGDG 34:5               | 0.018 ± 0.002                 | 0.011 ± 0.001           |                  |
| MGDG 34:3               | 1.230 ± 0.130                 | 1.530 ± 0.120           |                  |
| MGDG 34:1               | 0.025 ± 0.003                 | 0.047 ± 0.006           |                  |
| MGDG 36:6               | 42.800 ± 3.900                | 34.400 ± 2.400          |                  |
| MGDG 36:5               | 7.000 ± 0.800                 | 4.900 ± 0.550           |                  |
| MGDG 38:6               | 0.045 ± 0.004                 | 0.035 ± 0.002           |                  |
| MGDG 38:5               | 0.054 ± 0.005                 | 0.040 ± 0.002           |                  |
| total MGDG              | 52.600 ± 4.800                | 42.300 ± 3.000          |                  |
| DGDG 34:1               | 0.085 ± 0.008                 | 0.140 ± 0.013           |                  |
| DGDG 38:5               | 0.038 ± 0.004                 | 0.028 ± 0.004           |                  |
|                         |                               |                         |                  |
|                         | 0.034 ± 0.004                 | 0.043 ± 0.002           |                  |
| PG 32:0                 | 1.580 ± 0.160                 | 1.310 ± 0.100           |                  |
| PG 34:2                 | 3.830 ± 0.440                 | 2.640 ± 0.330           |                  |
| PG 34:1                 | 1.120 ± 0.160                 | 1.680 ± 0.220           |                  |
| PG 36:2                 | 0.033 ± 0.005                 | 0.021 ± 0.003           |                  |
|                         |                               |                         |                  |
|                         | 0.019 ± 0.003                 | 0.020 ± 0.004           |                  |
| PC 32:0                 | 0.013 ± 0.001                 | 0.032 ± 0.003           |                  |
| PC 34:3                 | 1.400 ± 0.140                 | 1.770 ± 0.200           |                  |
| PC 34:1                 | 0.130 ± 0.017                 | 0.320 ± 0.034           |                  |
| PC 36:3                 | 0.360 ± 0.044                 | 0.290 ± 0.026           |                  |
| PC 36:2                 | 0.211 ± 0.029                 | 0.136 ± 0.013           |                  |
|                         |                               |                         |                  |
|                         | 0.016 ± 0.002                 | 0.021 ± 0.002           |                  |
| PE 34:1                 | 0.014 ± 0.001                 | 0.035 ± 0.003           |                  |
| PI 34:2                 | 0.791 ± 0.090                 | 0.605 ± 0.060           |                  |
| PI 34:1                 | 0.023 ± 0.003                 | 0.046 ± 0.004           |                  |
| PI 36:6                 | 0.071 ± 0.011                 | 0.047 ± 0.003           |                  |
| PI 36:5                 | 0.078 ± 0.010                 | 0.046 ± 0.005           |                  |
| PI 36:4                 | 0.051 ± 0.007                 | 0.030 ± 0.038           |                  |
| PI 36:3                 | 0.091 ± 0.014                 | 0.038 ± 0.004           |                  |
| PI 36:2                 | 0.063 ± 0.009                 | 0.021 ± 0.002           |                  |
| total PI                | 2.670 ± 0.270                 | 2.110 ± 0.200           |                  |
| PS 36:5                 | 0.014 ± 0.002                 | 0.023 ± 0.001           |                  |
| PS 34:1                 | 0.014 ± 0.001                 | 0.024 ± 0.001           |                  |
| LPG 16:0                | 0.038 ± 0.007                 | 0.066 ± 0.009           |                  |
| LPG 18:2                | 0.013 ± 0.003                 | 0.008 ± 0.003           |                  |
| total LPG               | 0.070 ± 0.008                 | 0.110 ± 0.007           |                  |
| LPC 18:2                | 0.021 ± 0.003                 | 0.010 ± 0.003           |                  |

“MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; PG, phosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylycerolamine; PI, phosphatidylinositol; LPG, lysophosphatidylglycerol; and LPC, lysophosphatidylcholine; OT, optimum temperature; HT, high temperature; Se-NPs, selenium nanoparticles. All lipid molecular species were represented as mean ± SEM.

3. CONCLUSIONS

Se-NPs were synthesized using sodium selenate as molecular precursor and ascorbic acid as the reducing agent. The size of the synthesized Se-NPs was <100 nm. A toxicity assay using a murine fibroblast cell line showed that Se-NPs had a TC50 value of 275 mg L⁻¹. Our experiments provided evidence that the physiological response of sorghum to Se-NPs was generally greater under HT stress. On the basis of this evidence, we have concluded that Se-NPs are an HT stress protectant in sorghum. Furthermore, this study has affirmed, for the first time, that Se-NPs foliar spray under HT stress improved the antioxidant defense system in sorghum. The integrity and composition of the thylakoid membrane were maintained by Se-NPs, as revealed by the observed phospholipid signature. The response of pollen functions to Se-NPs proved that it is not toxic. To expand our understanding of the action of Se-NPs in plants, the complete Se-NPs metabolism has to be studied. This endeavor will require detailed biochemical and physiological investigations, as well as the study of the genes involved in the uptake, transport, and assimilation of Se-NPs.

4. MATERIALS AND METHODS

4.1. Synthesis of Se-NPs. The Se-NPs were synthesized according to Bai et al. with little modification. Solutions (100 mL) of ascorbic acid (5 mM) and sodium selenate (5 mM) in bidest-H₂O were prepared separately in 250 mL conical flasks.
Elemental nanoselenium was prepared by adding ascorbic acid dropwise to the sodium selenite solution at 40 °C under magnetic stirring (90 rpm). The addition of ascorbic acid was stopped when the solution turned from colorless to orange/red. The formed Se-NPs were washed with water, followed by ethanol for four times by centrifugation at 8000 rpm for 20 min. After centrifugation, the Se-NPs were dried at 50 °C for 24 h.

4.2. Material Characterization. The morphologies and microstructures of the prepared material were studied by scanning electron microscopy (SEM, Quanta 250, 30 kV, FEI, Czech Republic) and transmission electron microscopy (TEM, Tecnai Spirit G2, 120 kV, The Netherlands), respectively. For SEM and TEM experiments, accelerating voltages of 10 and 120 kV were used, respectively. Surface topography height images were acquired by multimode scanning probe microscopy (NTMDT-NTEGRA, Russia) under ambient conditions (25 ± 2 °C) using a semi-contact-mode probe. High-resolution transmission electron microscopy (HRTEM) measurements were obtained with an acceleration voltage of 200 kV (JOEL JEM 2100, Japan). Selected area electron diffraction (SAED) and energy-dispersive X-ray spectroscopy of the Se-NPs were also performed using a high-resolution transmission electron microscope. Powder X-ray diffraction (XRD) analysis was carried out using a diffractometer (Rigaku Ultima IV XRD, Tokyo, Japan) in the 2θ range of 5–90° utilizing Cu Kα radiation (40 kV, 30 mA, and λ = 1.5405 Å), with a scan speed of 5° min⁻¹. Crystallinity of the sample was obtained through the crystalline–amorphous peak deconvolution process using a Gaussian function. Thermogravimetric analysis (TGA) of the samples were performed using a PerkinElmer STA 600 instrument.

4.3. Toxicity Assay. At present, nanomaterials are used in various fields of science, and the Organisation for Economic Co-operation and Development (OECD) recommends to analyze the safety of nanomaterials before their application. Hence, the synthesized SeNPs were tested against mouse (a vertebrate) cell line to show their safety to humans. The mouse fibroblast L929 cell line was procured from ATCC, India, and maintained in a culture medium containing 10% Dulbecco’s modified Eagle’s medium, fetal bovine serum (10% v/v), penicillin (100 units mL⁻¹), and streptomycin (100 mg mL⁻¹). The cells were kept in a humidified atmosphere with 5% CO₂ at 37 °C. The viability of the cells was estimated by the standard 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay, (HiMedia Laboratories, Mumbai, India) and without supplementation of Se-NPs. The cell viability was calculated as the ratio of optical density of the Se-NP-treated cells to optical density of the control wells (above-mentioned culture medium with cells) and expressed in percentage. For DAPI staining, the L929 cells were seeded in 96-well plates (5 × 10⁴ cells per well) and cultured to adhere overnight, after which different concentrations of Se-NPs (0, 100, 250, and 500 mg L⁻¹) were added and left for 48 h. After completion of the reaction, the cells were washed and fixed using phosphate-buffered saline and 70% ethanol, respectively. Then, the cells were stained with 2.0 mg L⁻¹ DAPI for 15 min and viewed under an inverted fluorescence microscope (Nikon Ti-S Eclipse, Japan). The image shown is representative of 10 randomly observed fields.

4.4. Uptake Pattern of Se-NPs in Plants. River sand was collected, dried in air, and sieved using a 2 mm sieve (sieve number 10). The >2 mm fraction that does not pass through the sieve was collected and set aside. The collected soil fraction (<2 mm) was sieved again using a 1 mm sieve (sieve number 20). The fraction that did not pass through the sieve (very coarse sand) was collected and soaked in aqua regia (3:1 HCl/HNO₃ acid ratio, 10 mL per gram of sand) for 1 day and then washed thoroughly four times with double-distilled water. Afterward, this soil was dried in air for 48 h and used for the uptake experiments described here. Seeds of sorghum var. CO 30 were sown in aqua regia-washed sand and germinated at 25 °C in a growth chamber. After emergence of the seedlings, plastic pots (10 × 6 × 15 cm³) bearing 100 g of soil were moved to open sunlight. The seedlings were irrigated with the one-quarter-strength Hoagland solution for the first 2 weeks. Thinning was done when the plants were about 8 cm high by leaving one plant per pot. Thereafter, the pot was irrigated with either the one-quarter-strength Hoagland solution or the one-quarter-strength Hoagland solution containing 50 or 100 mg L⁻¹ Se-NPs. The seedlings irrigated with only Hoagland solution served as control. Every day, the pot was irrigated with 5 mL of the above solution. The seedlings were maintained for 10 days and then the plants were uprooted with minimal damage to their roots. The uprooted seedlings were dried in an oven at 40 °C for 72 h. Then, the roots and shoots were removed and their Se content was determined using inductively coupled plasma-optical emission spectrometry (ICP-OES, PerkinElmer optima, OPTIMA 2000 DV, Waltham, MA) following an established procedure.

4.5. Effects of Foliar Spray of Se-NPs on HT-Stressed Sorghum Plants. 4.5.1. Plant Husbandry and Growth Conditions. Controlled environment experiments using the facilities at the Department of Agronomy, Kansas State University (Manhattan, KS), were conducted to quantify the effect of foliar spray of Se-NPs on HT-stressed sorghum plants. The sorghum genotype DK 28-E was used in this study. The details of plant husbandry are available elsewhere. Plants were grown from sowing to booting stage (10 days prior to start of panicle exertion) in the growth chamber (Conviron Model CMP 3244, Winnipeg, Manitoba, Canada) maintained at daytime maximum and nighttime minimum temperatures of 32 and 22 °C. The photon flux density in the growth chamber at the top of the plant canopy was maintained at 800–1000 μmol m⁻² s⁻¹ using supplemental fluorescent lights. At booting stage, 10 pots were moved to each growth chamber maintained at optimum temperature (OT, 32/22 °C) or HT (38/28 °C) for treatment imposition. Before moving the sorghum plants in each growth chamber (OT or HT), they were sprayed with either water or a solution containing 10 mg of Se-NPs per liter of water using a hand sprayer (1.5 L capacity, compressed air type, Hummert International, Earth City, MO). The Se-NPs solutions were prepared by dispersing the required amount of Se-NPs in water using a sonicator to completely disperse the nanoparticles. To each pot, about 25 mL of Se-NPs suspension was sprayed on the plants. Water-sprayed plants served as control. Thereafter, plants were kept under the respective temperature conditions (OT or HT) for 10 days. Daytime maximum and nighttime minimum temperatures were maintained for 4 h each, with an 8 h transition period between them, and vice versa. The photoperiod was 16 h, and the photon flux density was 920 μmol m⁻² s⁻¹ at the top of the plant canopy, provided by cool fluorescent lamps. The growth chamber relative humidity was set at 85%. In all of the growth chambers, the air temperature and relative humidity were continuously monitored at 20 min intervals throughout the experiment. After 10 days, all pots were returned to the growth chambers and maintained at OT until maturity. The whole experiment was repeated (same growth conditions, etc.) once.

4.5.2. Leaf Physiological Traits. The chlorophyll index, chlorophyll a fluorescence, and gas exchange were recorded on
attached, fully expanded flag leaves of four different tagged plants from each temperature treatment on day 9 of the HT stress treatment at midday (between 1000 and 1400 h), as described in Prasad et al.42

4.5.3. Oxidants Content, MDA Content, and Membrane Damage. Superoxide anion accumulation was estimated according to Chaitanya and Naithani50 and expressed as change in optical density in min−1 g−1 on fresh weight basis. The hydrogen peroxide content in the leaves was quantified using a molecular probe (Amplex Red hydrogen peroxide/peroxidase assay kit; Invitrogen Molecular Probes, Inc., Eugene, OR; product number A22188), which is a one-step assay that uses the Amplex Red reagent, in combination with horseradish peroxidase (HRP), to detect H2O2, and expressed in nmol g−1 on fresh weight basis.50 The malondialdehyde content in leaves was quantified using an OxiSelect thiobarbituric acid-reactive substances assay kit (Cell Biolabs, San Diego, CA; product number STA 330) and expressed in μmol per gram of leaf tissue on fresh weight basis.51 Cell membrane damage was quantified as leakage of ions from the leaf, as explained by Sairam et al.,52 and expressed in percentage.

4.5.4. Antioxidant Enzyme Activities. The extraction of various antioxidant enzymes was done in Tris–HCl buffer, as described by Djanaguiraman et al.14 Total superoxide dismutase (SOD) activity was measured in the supernatant with a superoxide dismutase assay kit (Cayman Chemical, Ann Arbor, Michigan; product number 706002) according to the manufacturer’s instruction and expressed in enzyme units. One unit of SOD is defined as the amount of enzyme needed to obtain 50% dismutation of superoxide radicals on a fresh weight basis.53 Catalase (CAT) enzyme activity was measured using the Amplex Red catalase assay kit (Molecular probes, Invitrogen, Inc., Eugene, OR; product number A22188) and expressed in enzyme units. One enzyme unit was defined as the amount of catalase enzyme that decomposes 1.0 μmol of H2O2 min−1 g−1 of tissue on fresh weight basis at 25 °C.54 The peroxidase (POX) enzyme activity was measured using the Amplex Red hydrogen peroxide/peroxidase assay kit (Molecular Probes, Invitrogen, Inc., Eugene, OR; product number A22188). The POX enzymatic activity was determined following the same procedure as for the determination of H2O2, except that the Amplex Red reagent contained 2 μmol H2O2 instead of HRP. The result was expressed in enzyme units as previously described.53 One enzyme unit is defined as the amount of enzyme that will form 1.0 mg of purpurogallin from 1 μmol of NADPH to NADP+ min−1 on fresh weight basis.55

4.5.5. Electrospray Ionization Tandem Mass Spectrometry Lipid Profiling in Leaves. At each temperature regime on the 9th day of HT stress conditions, the flag leaf was collected between 10:00 and 11:00 h and processed for lipid profiling, as described by Vu et al.57 and Xiao et al.58

4.5.6. Pollen Germination, Seed Set Percentage, Seed Size, and Seed Yield. Pollen grains were collected at the time of anthesis and germinated in solid medium to estimate pollen germination percentage according to Djanaguiraman et al.42 The tagged panicles were harvested at physiological maturity, dried at 40 °C for 7 days, and hand-threshed. The seed set percentage, seed size, and seed yield were quantified according to the methods of Prasad et al.42 and Djanaguiraman et al.23

4.6. Data Analyses. Statistical analyses were performed using SAS program. The uptake pattern of the Se-NPs experiment was conducted in randomized complete block design. The Se-NPs foliar spray experiment was conducted in split-plot design with temperature and Se application as the main plot and subplot, respectively. The plants were selected randomly for treatments and arranged randomly within the growth chambers. Furthermore, the temperatures were randomly assigned to growth chambers. Observations were analyzed using the PROC GLM procedure of SAS. The results of both experiments were analyzed separately and in combination. Similar responses and significance were obtained for most traits. Therefore, mean responses for two combined experiments are presented. The standard error was shown as an estimate of variability, and means of various variables were separated for significance by the LSD test at a probability level of 0.05. For lipid analysis, statistical significance was set at P ≤ 0.05 after appropriate corrections for false lipid assignments. Comparisons were always made between the HT stress samples and their controls.

**AUTHOR INFORMATION**

Corresponding Author
*E-mail: vara@ksu.edu. Tel: +1 785 532 3746. Fax: +1 785 532 6094.

ORCID
Stefan H. Bossmann: 0000-0002-0058-0127
P. V. Vara Prasad: 0000-0001-6632-3361

Author Contributions
P.V.V.P. conceived and designed the experiments. M.D. and N.B. conducted the experiment, collected and analyzed data, and wrote the manuscript. P.V.V.P. and S.H.B. edited the manuscript.

Notes
The authors declare no competing financial interest.

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Quantifying potential benefits of drought and heat tolerance in rainy season sorghum for adapting to climate change. *Agric. For. Meteorol.* **2014**, *185*, 37–48.

**Summary for Policymakers in Climate Change 2013: The Physical Science Basis**: Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change; Cambridge University Press: Cambridge, New York, 2013.

(22) Lobbell, D. B.; Field, C. B. Global scale climate-crop yield relationships and the impacts of recent warming. *Environ. Res. Lett.* **2007**, *2*, No. 014002.

(23) Djanaguiraman, M.; Prasad, P. V. V.; Murugan, M.; Perumal, R.; Reddy, U. K. Physiological differences among sorghum (*Sorghum bicolor*) genotypes under high temperature stress. *Environ. Exp. Bot.* **2014**, *100*, 43–54.

(24) Pradhan, G.; Prasad, P. V. V.; Fritz, A.; Kirkham, M.; Gill, B. High temperature tolerance in species and its potential transfer to wheat. *Crop Sci.* **2012**, *52*, 292–304.

(25) Jorda, A. A.; Alamri, S. N.; Abu-Sehly, A. A.; Benghanem, M. Nonisothermal crystallisation kinetics of amorphous selenium prepared by high-energy ball milling: A comparison with the melt-quenching and thin-film techniques. *J. Non-Cryst. Solids* **2012**, 358, 1268–1273.

(26) Gates, B.; Mayers, B.; Cattle, B.; Xia, Y. Synthesis and characterization of uniform nanowires of trigonal selenium. *Adv. Funct. Mater.* **2002**, *12*, 219–227.

(27) Lucovsky, G.; Mooradian, A.; Taylor, W.; Wright, G.; Keezer, R. Identification of the fundamental vibrational modes of trigonal, α-monoclinic and amorphous selenium. *Solid State Commun.* **1967**, *5*, 113–117.

(28) Gao, X.; Gao, T.; Zhang, L. Solution-solid growth of α-monoclinic selenium nanowires at room temperature. *J. Mater. Chem.* **2003**, *13*, 6–8.

(29) Ray, C.; Dutta, S.; Sarkar, S.; Sahoo, R.; Roy, A.; Pal, T. A facile synthesis of 1D nano structured selenium and Au decorated nano selenium catalysts for the clock reaction. *RSC Adv.* **2013**, *3*, 24313–24320.

(30) Ramos, J. F.; Webster, T. J. Cytotoxicity of selenium nanoparticles in rat dermal fibroblasts. *Int. J. Nanomed.* **2012**, *7*, 3907–3914.

(31) Wang, Z.; Xie, X.; Zhao, J.; Liu, X.; Feng, W.; White, J. C.; Xing, B. Xylem- and phloem-based transport of CuO nanoparticles in maize (*Zea mays* L.). *Environ. Sci. Technol.* **2012**, *46*, 4434–4441.

(32) Premarathna, H. L.; McLaughlin, M. J.; Kirby, J. K.; Hettiarachchi, G. M.; et al. Potential availability of fertilizer selenium in field capacity and submerged soils. *Soil Sci. Soc. Am. J.* **2010**, *74*, 1589–1596.

(33) Lopez-Moreno, M. L.; de la Rosa, G.; Hernandez-Viezcas, J. A.; et al. Evidence of the differential biotransformation and genotoxicity of ZnO and CeOx nanoparticles on soybean (*Glycine max*) plants. *Environ. Sci. Technol.* **2010**, *44*, 7315–7320.

(34) Ristic, Z.; Bukovnik, U.; Prasad, P. V. V. Correlation between heat stability of thylakoid membranes and loss of chlorophyll in winter wheat under heat stress. *Crop Sci.* **2007**, *47*, 2067–2073.

(35) Tewari, A. K.; Tripathy, B. C. Acclimation of chlorophyll biosynthetic reactions to temperature stress in cucumber (*Cucumis sativus* L.). *Plant Sci.* **1999**, *208*, 431–437.

(36) Filek, M.; Gysl-Malcher, B.; Zembala, M.; Bednarska, E.; Leggner, P.; Krieckbaum, M. Effect of selenium on characteristics of rapeoplasts modified by cadmium. *J. Plant Physiol.* **2010**, *167*, 28–33.

(37) Suzuki, K.; Koushevitzky, S.; Mittler, R.; Miller, G. ROS and redox signalling in the response of plants to abiotic stress. *Plant Cell Environ.* **2012**, *35*, 259–270.

(38) Cartes, P.; Jara, A.; Pinilla, L.; Rosas, A.; Mora, M. Selenium improves the antioxidant ability against aluminium-induced oxidative stress in ryegrass roots. *Ann. Appl. Biol.* **2010**, *157*, 297–307.

(39) Xue, T.; Hartikainen, H.; Piironen, V. Antioxidative and growth-promoting effect of selenium on senescing lettuce. *Plant Soil* **2001**, *237*, 55–61.

(40) Meijer, H. J.; Munnik, T. Phospholipid-bilayer signaling in plants. *Annu. Rev. Plant Biol.* **2003**, *54*, 265–306.

(41) Mansour, M.; Salama, K.; Al-Mutawa, M.; Abou Hadid, A. Effect of NaCl and polyamines on plasma membrane lipids of wheat roots. *Biol. Plant.* **2002**, *45*, 235–239.
(42) Prasad, P. V. V.; Pisipati, S.; Mutava, R.; Tuinstra, M. Sensitivity of grain sorghum to high temperature stress during reproductive development. *Crop Sci.* 2008, 48, 1911–1917.

(43) Prasad, P. V. V.; Boote, K. J.; Allen, L. H. Adverse high temperature effects on pollen viability, seed-set, seed yield and harvest index of grain-sorghum [Sorghum bicolor (L.) Moench] are more severe at elevated carbon dioxide due to higher tissue temperatures. *Agron. For. Meteorol.* 2006, 139, 237–251.

(44) Quinn, C. F.; Prins, C. N.; Freeman, J. L.; Gross, A. M.; Hantzis, L. J.; Reynolds, R. J.; Covey, P. A.; Banuelos, G. S.; Pickering, I. J.; Fakra, S. C.; et al. Selenium accumulation in flowers and its effects on pollination. *New Phytol.* 2011, 192, 727–737.

(45) Bai, Y.; Qin, B.; Zhou, Y.; Wang, Y.; Wang, Z.; Zheng, W. Preparation and antioxidant capacity of element selenium nanoparticles sol-gel compounds. *J. Nanosci. Nanotechnol.* 2011, 11, S012–S017.

(46) Wang, H.; Shrestha, T. B.; Basel, M. T.; Dani, R. K.; Seo, G.-M.; Ballvada, S.; Pyle, M. M.; Prock, H.; Koper, O. B.; Thapa, P. S.; et al. Magnetic-Fe/Fe3O4-nanoparticle-bound SN38 as carboxylesterase-cleavable prodrug for the delivery to tumors within monocytes/macrophages. *Beilstein J. Nanotechnol.* 2012, 3, 444–455.

(47) Wang, Y.; Cui, H.; Zhou, J.; Li, F.; Wang, J.; Chen, M.; Liu, Q. Cytotoxicity, DNA damage, and apoptosis induced by titanium dioxide nanoparticles in human non-small cell lung cancer A549 cells. *Environ. Sci. Pollut. Res.* 2015, 22, 5519–5530.

(48) Paranthaman, R.; Vidyalakshmi, R.; Kumaravel, S. Optimization of digestion methods for mineral in spirulina by Inductively Coupled Plasma-Optical Emission Spectrometry. *J. Adv. Pharm. Educ. Res.* 2014, 4, 417–421.

(49) Chaitanya, K. S. K.; Naithani, S. C. Role of superoxide, lipid peroxidation and superoxide dismutase in membrane perturbation during loss of viability in seeds of *Shorea robusta* Gaertn. f. *New Phytol.* 1994, 126, 623–627.

(50) Shin, R.; Berg, R. H.; Schachtman, D. P. Reactive oxygen species and root hairs in *Arabidopsis* root response to nitrogen, phosphorus and potassium deficiency. *Plant Cell Physiol.* 2005, 46, 1350–1357.

(51) Heath, R. L.; Packer, L. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* 1968, 125, 189–198.

(52) Sairam, R.; Deshmukh, P.; Shukla, D. Tolerance of drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. *J. Agron. Crop Sci.* 1997, 178, 171–178.

(53) Sebastiani, M.; Giordano, C.; Nediani, C.; Travaglini, C.; Borchi, E.; Zani, M.; Feccia, M.; Mancini, M.; Petrozza, V.; Cossarizza, A.; et al. Induction of mitochondrial biogenesis is a maladaptive mechanism in mitochondrial cardiomyopathies. *J. Am. Coll. Cardiol.* 2007, 50, 1362–1369.

(54) Jones, P.; Suggett, A. The catalase-hydrogen peroxide system. Kinetics of catalatic action at high substrate concentrations. *Biochem. J.* 1968, 110, 617–620.

(55) Liu, X.; Williams, C. E.; Nemacheck, J. A.; Wang, H.; Subramanyam, S.; Zheng, C.; Chen, M.-S. Reactive oxygen species are involved in plant defense against a gall midge. *Plant Physiol.* 2010, 152, 985–999.

(56) Staimer, N.; Nguyen, T. B.; Nizkorodov, S. A.; Delfino, R. J. Glutathione peroxidase inhibitory assay for electrophilic pollutants in diesel exhaust and tobacco smoke. *Anal. Bioanal. Chem.* 2012, 403, 431–441.

(57) Vu, H. S.; Tamura, P.; Galeva, N. A.; Chaturvedi, R.; Roth, M. R.; Williams, T. D.; Wang, X.; Shah, J.; Welti, R. Direct infusion mass spectrometry of oxylipin-containing *Arabidopsis* membrane lipids reveals varied patterns in different stress responses. *Plant Physiol.* 2012, 158, 324–339.

(58) Xiao, S.; Gao, W.; Chen, Q.-F.; Chan, S.-W.; Zheng, S.-X.; Ma, J.; Wang, M.; Welti, R.; Chye, M.-L. Overexpression of *Arabidopsis* acyl-CoA binding protein ACBP3 promotes starvation-induced and age-dependent leaf senescence. *Plant Cell* 2010, 22, 1463–1482.