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Drought Tolerance of Mungbean Is Improved by Foliar Spray of Nanoceria

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Abstract: In crops, drought stress reduces the photosynthetic rate and gamete function through oxidative damage. Earlier studies showed that nanoceria possesses an antioxidant property; however, the ability of nanoceria to alleviate drought-stress-stimulated oxidative damage in pulse crops has not been studied. Therefore, experiments were conducted to assess the impacts of nanoceria on drought-induced oxidative damage in mungbean [*Vigna radiata* (L.) Wilczek]. We hypothesize that foliar application of nanoceria under drought stress can scavenge the excess produced reactive oxygen species (ROS) due to its inherent properties which could result in increased photosynthesis and reproductive success of mungbean. Three experiments were conducted under well-watered and limited-moisture conditions. The traits associated with oxidative damage, photosynthesis, reproductive success, and yield were recorded. Results showed that for mungbean, the optimum concentration of nanoceria for foliar spray was 100 mg L^{-1}. Field and pot culture experiments showed that foliar application of nanoceria under drought decreased the superoxide radical content (29%), hydrogen peroxide content (28%), and membrane damage (35%) over water spray. Nanoceria increased the photosynthetic rate (38%), pod-set percentage (16%), and seed weight m^{-2} (44%) in drought-stressed plants compared to control plants. The increased photosynthetic rate by nanoceria spray under drought stress is associated with lesser oxidative damage and stomatal limitation caused by nanoceria's inherent ROS-scavenging ability. Hence, foliar application of nanoceria at the rate of 100 mg L^{-1} under drought stress could increase mungbean seed yield per plant through increased photosynthetic rate and pod-set percentage.

Keywords: drought; mungbean; nanocerium; photosynthesis; reproductive success

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Citation: Maduraimuthu, D.; Alagarswamy, S.; Prabhakaran, J.; Karuppasami, K.M.; Venugopal, P.B.R.; Koothan, V.; Natarajan, S.; Dhashnamurthi, V.; Veerasamy, R.; Rathinavelu, S.; et al. Drought Tolerance of Mungbean Is Improved by Foliar Spray of Nanoceria. *Agronomy* **2023**, *13*, 201. https://doi.org/10.3390/agronomy13010201

Academic Editors: Roberto Barbato and Veronica De Micco

Received: 15 December 2022
Revised: 2 January 2023
Accepted: 5 January 2023
Published: 9 January 2023

1. Introduction

Future global population estimates indicate that the population will increase to 8.5 billion by 2030, and to feed this ever-increasing population, it is mandatory to increase food production by >50% [1]. One of the technologies that can be used to increase food production is nanotechnology. Nanotechnology has been used in various fields of science, including agriculture. Studies indicate that varying sizes and concentrations of metallic nanoparticles might influence plant growth and yield, either positively or negatively [2,3]. In addition, nanoparticles are also used to mitigate abiotic stresses such as drought.

Grain legumes are an important source of low-cost protein and enrich soil through biological nitrogen fixation [4]. Globally, legume production has increased from 150 m tonnes to 300 m tonnes with a small yield increase of 0–2% per year [5,6]. Of this, pulses such as mungbean [*Vigna radiata* (L.) Wilczek] account for 20% of total production [6]. In general, mungbean is grown in arid and semiarid regions of the world as an intercrop, mixed crop, or sole crop where the possibility of terminal drought is frequent, which could significantly decrease the grain yield. One of the reasons for lower grain yield in mungbean is the occurrence of drought stress at critical growth stages [7]. Drought stress during flowering and/or at terminal stages is more critical than during the vegetative stage in decreasing grain yield [8]. Hence, the yield of mungbean can be improved by adopting crop-management technologies to alleviate drought-stress effects.

The primary effects of drought stress are decreased stomatal conductance to reduce transpiration rate [9], decreased stem elongation by reduced cell division and cell expansion, and decreased leaf size, root growth, nutrient uptake, and grain yield [10,11]. Decreased photosynthetic rate under drought stress was linked to stomatal and/or non-stomatal factors [12]. Drought decreases the yield components of plants, such as the number of pods, number of seeds, and individual seed mass [13]. Drought stress increases oxidative damage by overproducing reactive oxygen species (ROS), which eventually decrease the yield potential by interfering with the photosynthetic process [14,15]. Plants depend on antioxidant enzymes and antioxidants to reduce the adverse impacts of ROS [15,16]. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) are involved in ROS scavenging [16].

Cerium, a metallic nanoparticle, may exist in two oxidation states, namely cerous or ceric. Cerium possesses inherently antioxidant and/or antioxidant-enzyme-mimetic activity, depending on the ratio of Ce^{3+}/Ce^{4+} sites on its surface [17,18]. In general, drought stress enhances oxidative damage to crops, and the potential of nanoceria to decrease drought-induced oxidative damage has not been studied in detail in any of the grain legumes, such as mungbean. We hypothesize that foliar application of nanoceria under drought stress can scavenge the excess produced reactive oxygen species (ROS) due to its inherent properties, which could result in increased photosynthesis and reproductive success of mungbean. The objectives were to (i) quantify the effects of nanoceria on leaf photosynthesis-associated traits under drought stress in mungbean and (ii) determine the effects of nanoceria on reproductive success in mungbean under drought stress.

2. Materials and Methods

2.1. Synthesis and Characterization of Nanoceria

Nanoceria was synthesized and characterized using scanning electron microscopy, transmission electron microscopy, and high-resolution transmission electron microscopy, as explained by Djanaguiraman et al. [3].

2.2. Experiment 1: Identification of Optimum Concentration of Nanoceria for the Foliar Spray—Pot Culture Experiment

In a randomized block design, a pot culture experiment was conducted at the glasshouse of the Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore, India. The mungbean variety VBN 4 was sown during the *Kharif* (summer) season. Sandy clay loam soil (pH: 7.5, electrical conductivity: 0.31 dS m^{-1}, organic carbon: 0.58%, available nitrogen: 191 kg ha^{-1}, available phosphorus: 26 kg ha^{-1}, and potassium: 356 kg ha^{-1}) was used for the experiment. The seeds were sown in a plastic pot with dimensions of 25.7 cm (length), 25.5 cm (breadth), and 22.1 cm (height) containing 9 kg of sandy clay loam soil. In the bottom of each pot, three holes were made for drainage. After thinning, in each pot, one seedling was maintained until harvest, and 3 grams of urea, 2 grams of diammonium phosphate, and 3 grams of potash were added to each pot. On alternate days, the plants were irrigated. At the peak flowering stage, ten plants were randomly tagged for spraying with each concentration of nanoceria, and plants were irrigated up to 100% field capacity. On the next day, the plants were sprayed with different concentrations of nanoceria, as explained in Table 1.

The symptoms of leaf rolling were observed on the second day of irrigation, and at the time, the tissue relative water content in the irrigated and drought-stressed plants were 86 and 63%, respectively. From this point, irrigation was withheld for seven days for drought-stress treatment, i.e., the duration of drought stress was 7 days. The control plants were irrigated daily up to 100% field capacity. During the stress period, the thylakoid membrane damage (F_o/F_m ratio; no units) was recorded on day 1, 3, 5, and 7 between 10:00 and 11:00 h using a modulated fluorometer (OS5p+, OptiSciences, Hudson, NH) in five tagged plants as explained by Djanaguiraman et al. [3]. During the drought stress period, the mean daytime and nighttime temperature were 31.5 and 20.8 °C, respectively. After the stress period, the plants were irrigated once every two days until maturity. At physiological maturity, seed yield plant^{-1} was recorded for all the plants.

Table 1. Pot culture experiment treatment details.

Treatment	Details
T_1	Irrigated control and no spray
T_2	Drought stressed and no spray
T_3	Drought stressed and sprayed with nanoceria @ 10 mg L^{-1}
T_4	Drought stressed and sprayed with nanoceria @ 20 mg L^{-1}
T_5	Drought stressed and sprayed with nanoceria @ 50 mg L^{-1}
T_6	Drought stressed and sprayed with nanoceria @ 100 mg L^{-1}
T_7	Drought stressed and sprayed with nanoceria @ 200 mg L^{-1}
T_8	Drought stressed and sprayed with nanoceria @ 500 mg L^{-1}

2.3. Experiment 2: Quantification of Impacts of Nanoceria on Photosynthesis-Associated Traits, Reproductive Success, and Seed Yield of Mungbean—A field Experiment

In a split-plot design, a field experiment was conducted with three replicates at Tamil Nadu Agricultural University, Coimbatore (11.01° N, 76.93° E), Tamil Nadu, India. The main and subplot treatments were irrigation level and foliar spray of nanoceria, respectively. The area of each plot was 20 m^2. The field experiment was conducted in clay loam soil (pH: 7.4, electrical conductivity: 0.22 dS m^{-1}, organic carbon: 0.56%, available nitrogen content: 295 kg ha^{-1}, phosphorus content: 18 kg ha^{-1}, and potassium content: 296 kg ha^{-1}). The mungbean variety Vamban 4 was sown during *Kharif* season (summer season) and maintained under well-irrigated conditions from sowing to the peak flowering stage. Irrigation was given once every 7 days. During the drought-stress period, the mean daytime and nighttime temperature were 34.2 and 23.4 °C, respectively.

2.3.1. Imposition of Drought Stress and Foliar Spray of Nanoceria

At the start of the peak flowering stage, in each replicate, five plants were tagged for recording of physiological traits and ten plants for recording of reproductive success. The following day, the subplot treatments, namely foliar spray of water or nanoceria @ 100 mg L^{-1} (concentration optimized from experiment 1), were imposed. The drought-stress treatment was imposed by withholding water for 21 d. At the start of the drought stress, the tissue RWC was 83% in both irrigated and drought-stress treatments. At the end of drought stress, the tissue RWC in irrigated and drought-stressed plants were 81 and 57%, respectively. After the drought-stress period, all the plants were irrigated once every seven days until harvest.

2.3.2. Chlorophyll Index, Thylakoid Membrane Damage, and Gas Exchange

The physiological traits, *viz.*, chlorophyll content (SPAD units), thylakoid membrane damage (F_o/F_m ratio; no units), stomatal conductance (mol m^{-2} s^{-1}), transpiration rate (mmol m^{-2} s^{-1}), and photosynthetic rate (μmol m^{-2} s^{-1}) were recorded on 5, 10, and 20 days after drought-stress imposition on the attached fully expanded top leaf of tagged plants from each replicate between 10:00 and 14:00 as explained by Djanaguiraman et al. [19].

2.3.3. Pod-set Percentage, Pod Weight, and Seed Yield

Pod-set percentage was recorded to understand the fate of individual flower buds, as explained by Djanaguiraman et al. [19]. The pod-set percentage was determined based on the number of pods formed on tagged flower buds. At physiological maturity, plants were harvested from 1 m^2 to record pod weight (g pod^{-1}) and seed yield (g $plant^{-1}$).

2.4. Experiment 3: Confirming the Effects of Nanoceria on Photosynthesis-Associated Traits, Reproductive Success, and Seed Yield of Mungbean—A Pot Culture Experiment

A pot culture experiment was conducted with ten replicates in a factorial randomized block design. The first and second factors were irrigation level and nano-cerium foliar spray, respectively. The mungbean variety Vamban 4 was sown and maintained under well-irrigated conditions from sowing to the start of the peak flowering stage, as described above. The crop husbandry and soil characteristics were the same as in experiment 1. As described in experiment 1, drought stress was imposed for seven days at the peak flowering stage. The plants were sprayed with water or nanoceria @100 mg L^{-1}, similar to experiment 1. After the stress period, the plants were irrigated once every two days until harvest. During the drought-stress period, the mean daytime and nighttime temperature were 33.5 and 22.8 °C, respectively.

2.4.1. Chlorophyll Content, Thylakoid Membrane Damage, Gas Exchange, and Chlorophyll *a* Fluorescence Traits

Similar to the previous experiment, the physiological traits were recorded on the attached fully expanded top leaf at 2, 4, and 6 days after drought stress. The chlorophyll index, thylakoid membrane damage, and leaf-level gas exchange measurement were recorded from five replicates. The chlorophyll a fluorescence traits, namely, effective quantum yield of PS II (ΦPS II; relative units) and apparent rate of photochemical transport of electrons through PS II (ETR; µmol m^{-2} s^{-1}), were recorded as per Djanaguiraman et al. [19].

2.4.2. Quantification of Oxidants, Antioxidant Enzyme Activity, and Membrane Integrity

On 6th day of drought stress, the first trifoliate leaf from the top was collected and immediately frozen in liquid nitrogen from five plants of each replicate. The frozen leaf sample was used to quantify oxidant and various antioxidant enzymes' activity.

Superoxide Content, Hydrogen Peroxide Content, and Membrane Stability Index

The superoxide anion radical was quantified by following the procedure of Chaitanya and Naithani [20] and expressed as Δ OD min^{-1} g^{-1}. The hydrogen peroxide was quantified by adopting the procedure of Patterson et al. [21] and expressed as nmol g^{-1}. The membrane stability index was measured by following the procedure of Sairam et al. [22] and expressed as a percentage.

Antioxidant Enzyme Quantification

The frozen leaf sample (100 mg) was macerated in 1.5 mL of ice-cold 50 mM phosphate buffer (pH 7) containing 2 mM EDTA, 5 mM β-mercaptoethanol, and 4% polyvinylpyrrolidone (PVP) and centrifuged at 10,000 rpm for 30 min at 4 °C. The supernatants were used for the estimation of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) activity. The SOD enzyme activity was quantified by measuring the photoreduction of nitro blue tetrazolium as described by Beyer and Fridovich [23] and expressed as enzyme units mg $protein^{-1}$. One unit of SOD activity is the amount required to inhibit the photoreduction of nitro blue tetrazolium by 50%. The catalase (CAT) enzyme activity was quantified according to Samantary [24] and expressed as mmol H_2O_2 destroyed min^{-1} g^{-1}. According to Castillo et al. [25], the peroxidase enzyme activity was quantified and expressed as mmol tetraguaiacol formed min^{-1} g^{-1}.

2.4.3. Pollen Viability, Pod-set Percentage, Individual Seed Weight, and Seed Yield

The pollen viability was quantified by placing pollen in an iodine potassium iodide solution. Pollen grains stained dark red or brown were considered alive and expressed as a percentage. A total of 25 flowers per treatment was tagged to determine the pod-set percentage. At maturity, the pods were harvested and hand-threshed to collect the seeds, and the individual seed mass was calculated as the weight of a single seed and expressed as mg seed^{-1}. The weight of all seeds plant^{-1} was weighed and expressed as seed yield (g plant^{-1}).

3. Statistical Analysis

The data were analyzed using SAS 9.4 [26]. The PROC GLM procedure of SAS was used to analyze variance for fixing the optimum concentration of foliar spray of nanoceria (experiment 1). The data of each day were analyzed separately and in combination (pooled analysis) for thylakoid membrane damage. The results of the day, separately or in combination, had similar responses and significance for thylakoid membrane damage. Therefore, the mean was presented to obtain the overall effect of the foliar spray of nanoceria. However, the PROC MIXED procedure was used for split-plot and factorial randomized block designs (experiments 2 and 3, respectively). Similar to experiment 1, similar responses and significance were observed for the effect of individual day of stress or combination. Hence, the mean over the day was presented to represent the overall effect of nanoceria. The percent change was calculated as the difference between the control and treatment divided by the actual value of the control multiplied by 100. Standard errors were shown as an estimate of variability for all measured observations, and means were separated using LSD at a probability level of 0.01.

4. Results

4.1. Synthesis and Characterization of Nanoceria

The result indicated that the average nanocerium particle diameter was 15 ± 5 nm and rod-shaped. The energy-dispersive X-ray spectroscopy spectra of nanoceria show Ce and O elements only, indicating no impurities. The selected area diffraction (SAD) pattern of nanoceria demonstrates that the particle was a cubic crystal (Figure 1a–d).

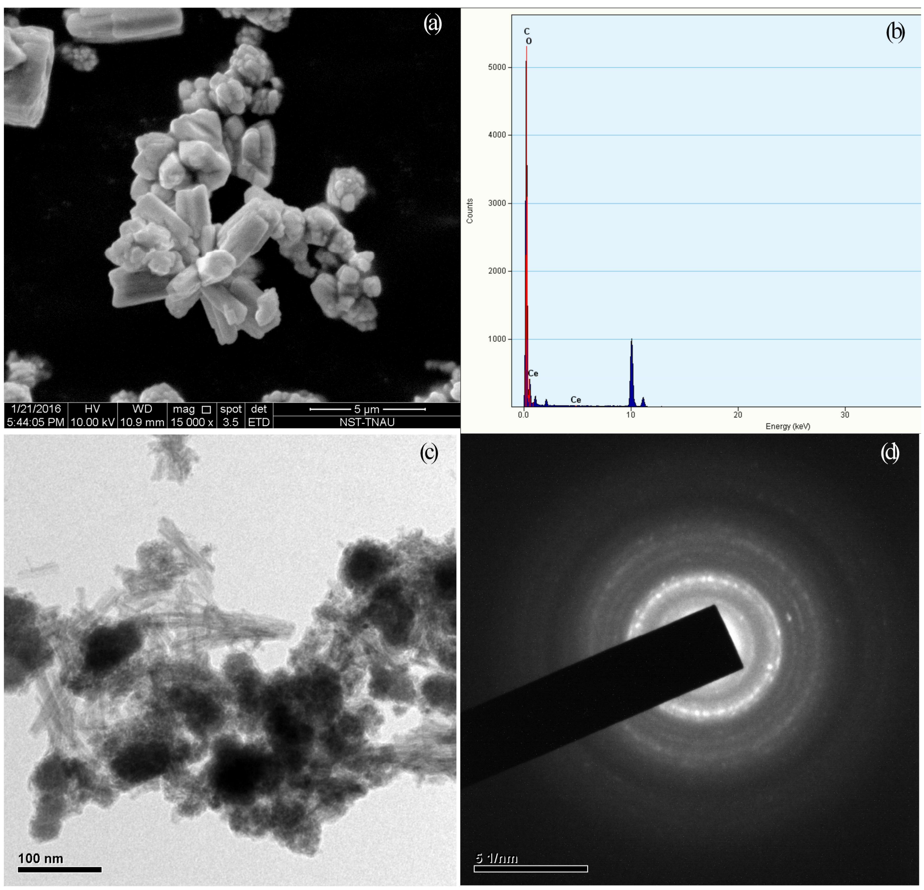

Figure 1. Representative SEM and TEM images of synthesized nanoceria. (**a**) SEM, (**b**) EDX analysis, (**c**) TEM, and (**d**) SAED pattern of nanoceria.

4.2. Experiment 1: Identification of Optimum Concentration of Nanoceria for the Foliar Spray by Assessing Thylakoid Membrane Damage (F_o/F_m Ratio; No Units) and Seed Yield (g Plant^{-1})—Pot Culture Experiment

The result indicated significant ($p < 0.01$) differences among the treatments for thylakoid membrane damage and seed yield plant^{-1}. Drought stress increased the thylakoid membrane damage and decreased the seed yield (g plant^{-1}). Under drought stress, foliar spray of nanoceria @ 100 mg L^{-1} had a significant ($p < 0.01$) higher seed yield plant^{-1} and lower thylakoid membrane damage (Figure 2a,b) than other treatments. At higher concentrations (at 200 and 500 mg L^{-1}), higher thylakoid membrane damage was observed than at lower concentrations (from 10 to 100 mg L^{-1}). Under drought stress, the highest seed yield was observed in the nanoceria @ 100 mg L^{-1}-sprayed plants compared to other treatments.

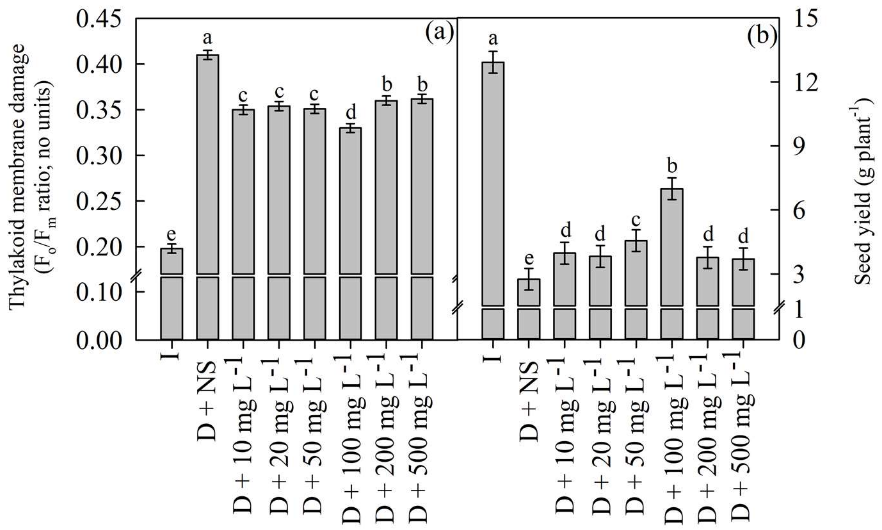

Figure 2. Optimization of nanoceria concentration for foliar spray in mungbean. (**a**) Thylakoid membrane damage (Fo/Fm ratio) and (**b**) seed yield (g plant^{-1}) under irrigated and drought-stress conditions. Each value of thylakoid membrane damage is the mean ± SD of 20 independent measurements (5 replicates and 4 observation times), and seed yield is the mean ± SD of 10 independent measurements (10 replicates). Means with different letters are significantly different at $p \leq 0.05$.

4.3. Experiment 2: Quantification of Impacts of Nanoceria on Photosynthesis-Associated Traits, Reproductive Success, and Seed Yield of Mungbean—A Field Experiment

4.3.1. Chlorophyll Index (SPAD units) and Thylakoid Membrane Damage (F_o/F_m ratio)

There were significant ($p < 0.01$) differences among the irrigation regimes, foliar sprays, and their interactions for chlorophyll index and thylakoid membrane damage (F_o/F_m ratio). Under the irrigated condition, the chlorophyll index and thylakoid membrane damage did not differ significantly between water spray and nanoceria spray (Figure 3a,b). However, under drought conditions, nanoceria-sprayed plants had an increased chlorophyll index (17%) and decreased thylakoid membrane damage (13%) compared to water-sprayed plants (Figure 3a,b).

4.3.2. Stomatal Conductance (mol m^{-2} s^{-1}), Transpiration Rate (mmol m^{-2} s^{-1}), and Photosynthetic Rate (µmol m^{-2} s^{-1})

The effects of the irrigation regime, nanoceria spray, and interaction of irrigation regime and nanoceria spray were significant ($p \leq 0.01$) for stomatal conductance, transpiration rate, and photosynthetic rate (Figure 4a–c). Overall, drought stress decreased stomatal conductance (39%; Figure 4a), transpiration rate (52%; Figure 4b), and photosynthetic rate (48%; Figure 4c) over the irrigated control. However, under drought stress, foliar spray of nanoceria significantly ($p \leq 0.01$) increased stomatal conductance (50%; Figure 4a), transpiration rate (13%; Figure 4b), and photosynthetic rate (38%; Figure 4c) over water spray. The interaction effect was significant ($p < 0.01$) because the response of nanoceria spray on the above physiological traits was higher under drought than a water spray.

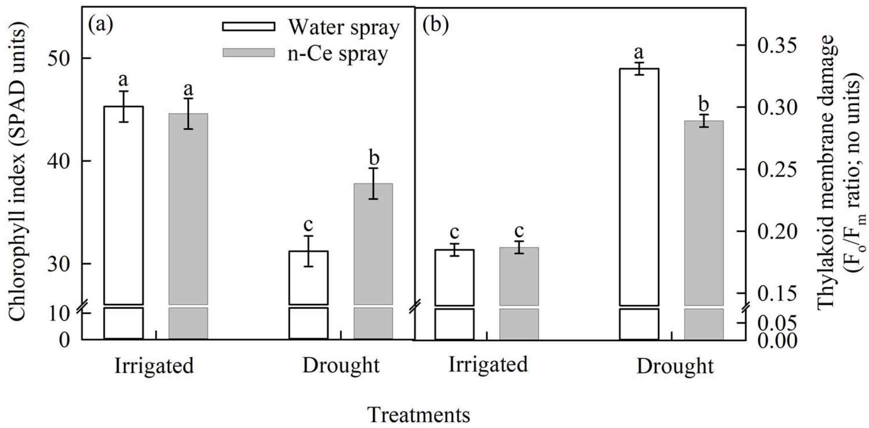

Figure 3. Effect of drought and foliar spray of nanoceria on (**a**) chlorophyll index (SPAD units) and (**b**) thylakoid membrane damage (Fo/Fm ratio) in mungbean (field experiment). Each value is the mean ± SD of 45 independent measurements (3 replicates × 5 plants in each replicate × 3 observation times). Means with different letters are significantly different at $p \leq 0.05$.

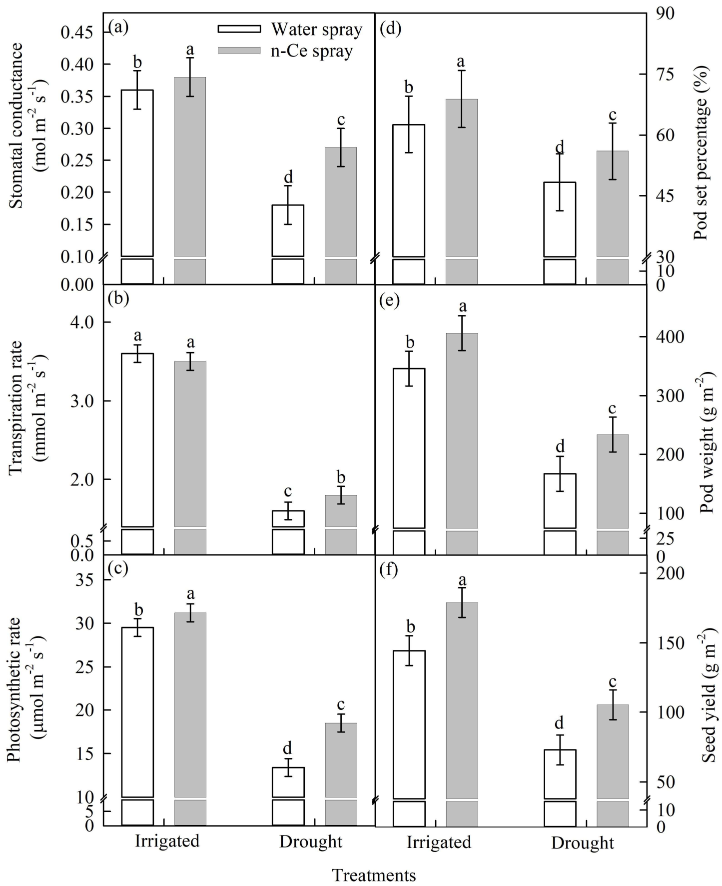

Figure 4. Effect of drought and foliar spray of nanoceria on (**a**) stomatal conductance (mol m^{-2} s^{-1}), (**b**) transpiration rate (mmol m^{-2} s^{-1}), (**c**) photosynthetic rate (µmol m^{-2} s^{-1}), (**d**) pod-set percentage, (**e**) pod weight (g m^{-2}), and (**f**) seed yield (g m^{-2}) in mungbean (field experiment). Each value of the gas exchange is the mean ± SD of 45 independent measurements (3 replicates × 5 plants in each replicate × 3 observation times), and seed yield and its components are the mean ± SD of 30 independent measurements (3 replicates × 10 plants in each replicate). Means with different letters are significantly different at $p \leq 0.05$.

4.3.3. Pod-Set Percentage, Pod Weight (g m^{-2}), and Seed Yield (g m^{-2})

The effects of drought, nanoceria spray, and the interaction of drought and nanoceria spray were significant ($p \leq 0.01$) for pod-set percentage, pod weight, and seed yield (Figure 4d–f). Drought stress significantly ($p \leq 0.01$) decreased the pod-set percentage (79%; Figure 4d), pod weight (53%; Figure 4e), and seed weight m^{-2} (55%; Figure 4f). However, foliar spray of nanoceria significantly ($p \leq 0.01$) increased the pod-set percentage (16%; Figure 4d), pod weight (40%; Figure 4e), and seed weight m^{-2} (44%; Figure 4f) over water spray. The influence of nanoceria on increasing the yield and its components was greater under drought stress than the irrigated control.

4.4. Experiment 3: Confirming the Effects of Nanoceria on Oxidative Damage, Photosynthesis-Associated Traits, Reproductive Success, and Seed Yield of Mungbean—A Pot Culture Experiment

4.4.1. Chlorophyll Index, Thylakoid Membrane Damage, the Effective Quantum Yield of PSII (relative units), and Electron Transport Rate (μmol m^{-2} s^{-1})

Under drought stress, foliar application of nanoceria significantly ($p \leq 0.01$) increased the chlorophyll index (8%; Figure 5a), the effective PSII quantum yield (32 %, Figure 5c), and electron transport rate (30%, Figure 5d) compared to water spray. However, foliar application of nanoceria decreased the thylakoid membrane damage (25%) over water spray (Figure 5b). Within the irrigated condition, there were no significant differences between water and nanoceria spray for the traits associated with chlorophyll *a* fluorescence traits.

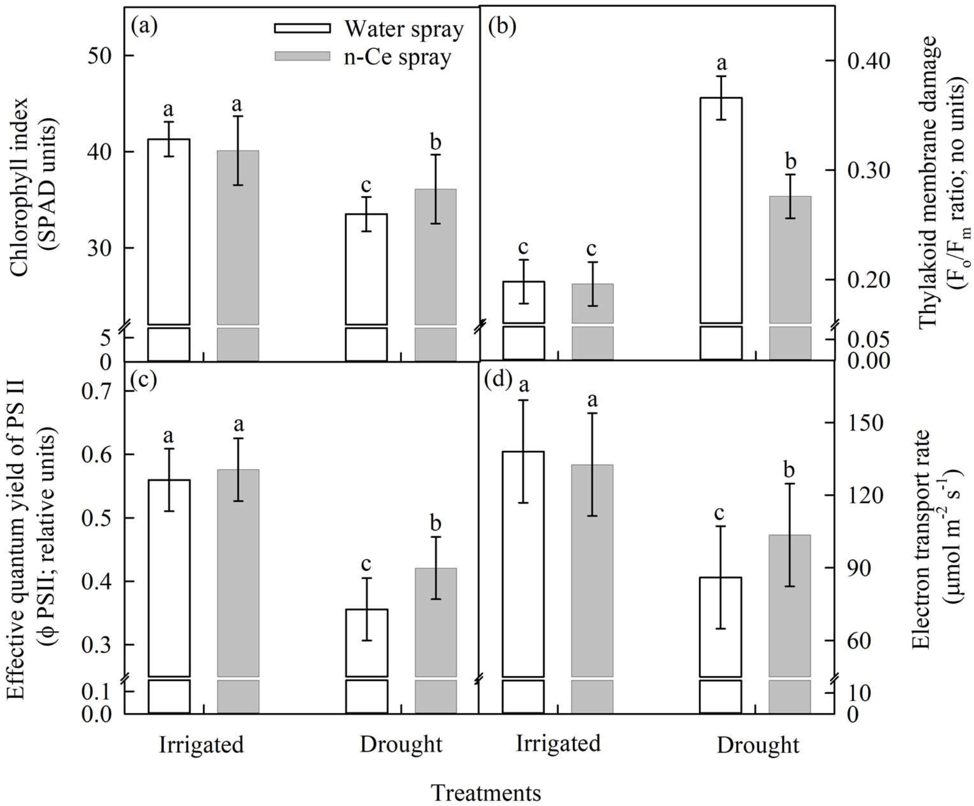

Figure 5. Effect of drought and foliar spray of nanoceria on (**a**) chlorophyll index (SPAD units), (**b**) thylakoid membrane damage (Fo/Fm ratio), (**c**) effective quantum yield of PSII (ϕPSII; relative units), and (**d**) electron transport rate (μmol m^{-2} s^{-1}) in mungbean (pot culture experiment). The values of chlorophyll index and Fo/Fm ratio are mean ± SD of 15 independent measurements (5 replicates × 3 observation times), and ϕPSII and ETR are mean ± SD of 9 independent measurements (3 replicates × 3 observation times). Means with different letters are significantly different at $p \leq 0.05$.

4.4.2. Oxidants, Membrane Damage, and Antioxidant Enzyme Activity

Superoxide Radical Content (Δ OD min^{-1} g^{-1}), Hydrogen Peroxide Content (nmol g^{-1}), and Membrane Damage (%)

The effects of drought, nanoceria spray, and their interaction were significant ($p \leq 0.01$) for superoxide radical content (Figure 6a), hydrogen peroxide content (Figure 6b), and membrane damage (Figure 6c). Under drought stress, the superoxide radical content (86%), hydrogen peroxide content (79%), and membrane damage (98%) were increased in water-sprayed versus nanoceria-sprayed plants. Within the irrigated condition, there were no significant differences between water and nanoceria spray for the traits associated with oxidative damage.

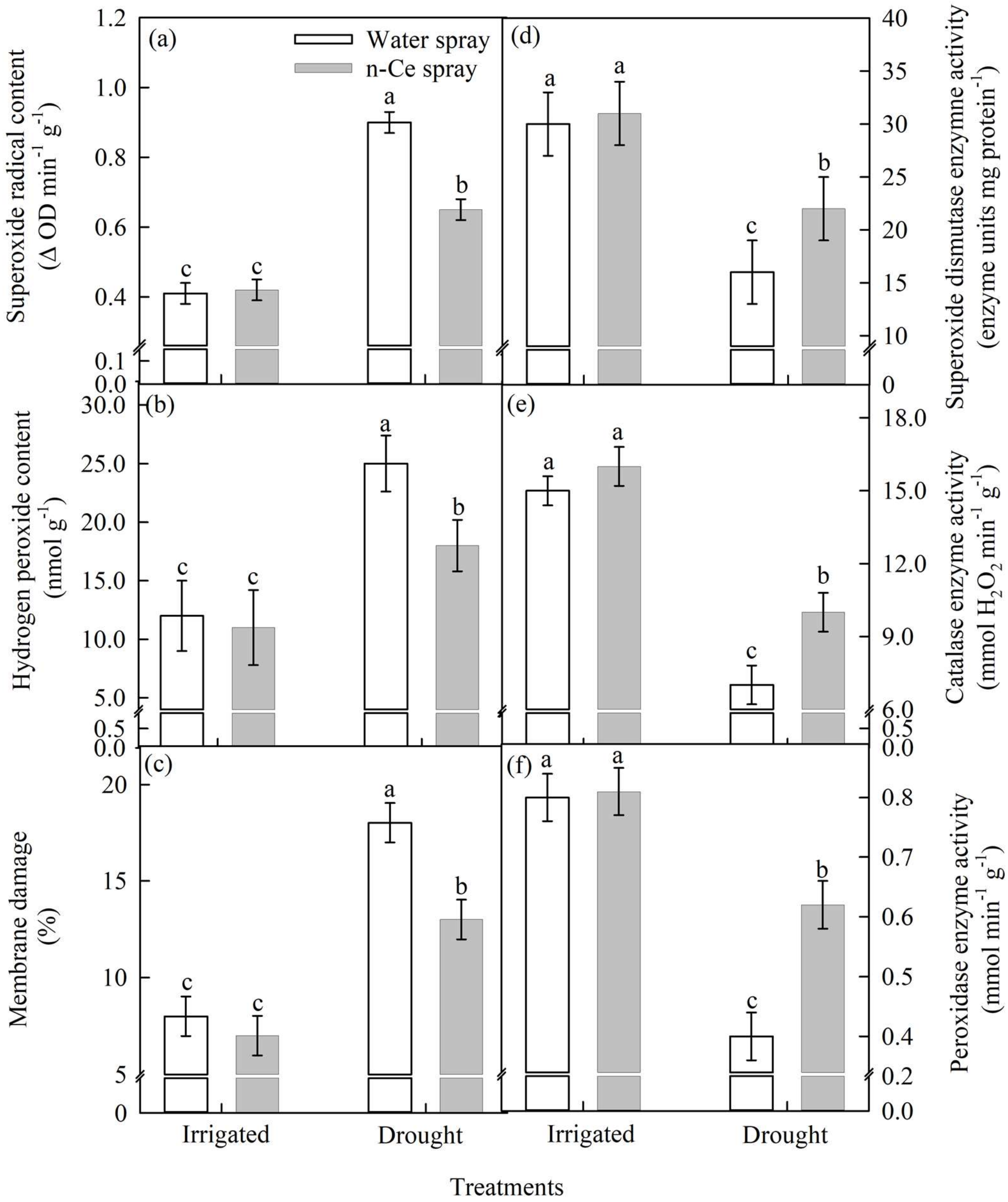

Figure 6. Effect of drought and foliar spray of nanoceria on (**a**) superoxide radical content (ΔOD min^{-1} g^{-1}), (**b**) hydrogen peroxide content (nmol g^{-1}), (**c**) membrane damage (%), (**d**) superoxide dismutase enzyme activity (enzyme units mg $protein^{-1}$), (**e**) catalase enzyme activity (mmol H_2O_2 min^{-1} g^{-1}) and (**f**) peroxidase enzyme activity (mmol min^{-1} g^{-1}) in mungbean (pot culture experiment). Each value is the mean ± SD of 5 independent measurements. Means with different letters are significantly different at $p \leq 0.05$.

Superoxide Dismutase (enzyme units mg protein^{-1}), Catalase (mmol H_2O_2 min^{-1} g^{-1}), and Peroxidase (mmol H_2O_2 min^{-1} g^{-1}) Enzyme Activity

The effects of drought, nanoceria spray, and their interaction were significant ($p \leq 0.01$) for superoxide dismutase enzyme activity (Figure 6d), catalase enzyme activity (Figure 6e), and peroxidase enzyme activity (Figure 6f). Under irrigated conditions, there were no significant differences between water and nanoceria spray for all the antioxidant enzymes activities. Under drought stress, superoxide dismutase enzyme activity (38%), catalase enzyme activity (45%), and peroxidase enzyme activity (37%) were decreased by water spray compared to nanoceria spray, indicating that nanoceria increased the activity of the antioxidant enzymes.

4.4.3. Stomatal Conductance, Intercellular CO_2 Concentration, Transpiration Rate, and Photosynthetic Rate

Similar to the previous experiment, there were no significant differences between irrigated water- and nanoceria-sprayed plants for stomatal conductance, intercellular CO_2 concentration, transpiration rate, and photosynthetic rate (Figure 7a–d). However, under drought stress, water-sprayed plants had significantly ($p \leq 0.01$) decreased stomatal conductance (19%), intercellular CO_2 concentration (6%), transpiration rate (7%), and photosynthetic rate (17%) versus nanoceria-sprayed plants (Figure 8a–d), indicating that nanoceria could increase the photosynthetic rate under drought stress.

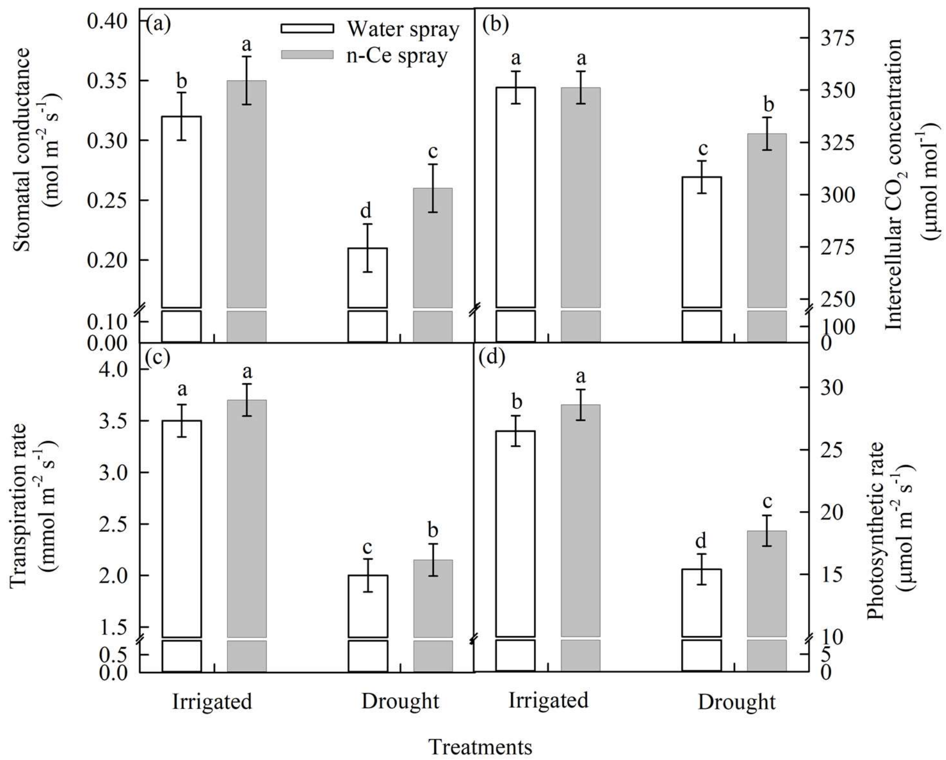

Figure 7. Effect of drought and foliar spray of nanoceria on (**a**) stomatal conductance (mol m^{-2} s^{-1}), (**b**) intercellular CO_2 concentration (µmol mol^{-1}), (**c**) transpiration rate (mmol m^{-2} s^{-1}) and (**d**) photosynthetic rate (µmol m^{-2} s^{-1}) in mungbean (pot culture experiment). Each value is the mean ± SD of 5 independent measurements. Means with different letters are significantly different at $p \leq 0.05$.

4.4.4. Pollen Viability (%), Pod-Set Percentage, Individual Seed Mass (mg seed^{-1}), and Seed Yield (g plant^{-1})

Significant ($p \leq 0.01$) effects of drought, nanoceria, and the interaction of drought and nanoceria were observed for pollen viability, pod-set percentage, and seed yield plant^{-1} (Figure 8a–d). Under irrigated or drought-stress conditions, there was no significant difference between water and nanoceria spray on individual seed mass (Figure 8c). Pollen viability, pod-set percentage, and seed yield plant^{-1} were increased by nanoceria spray over water spray under both irrigated and drought-stress conditions (Figure 8a,b,d), indicating its effectiveness under both the conditions.

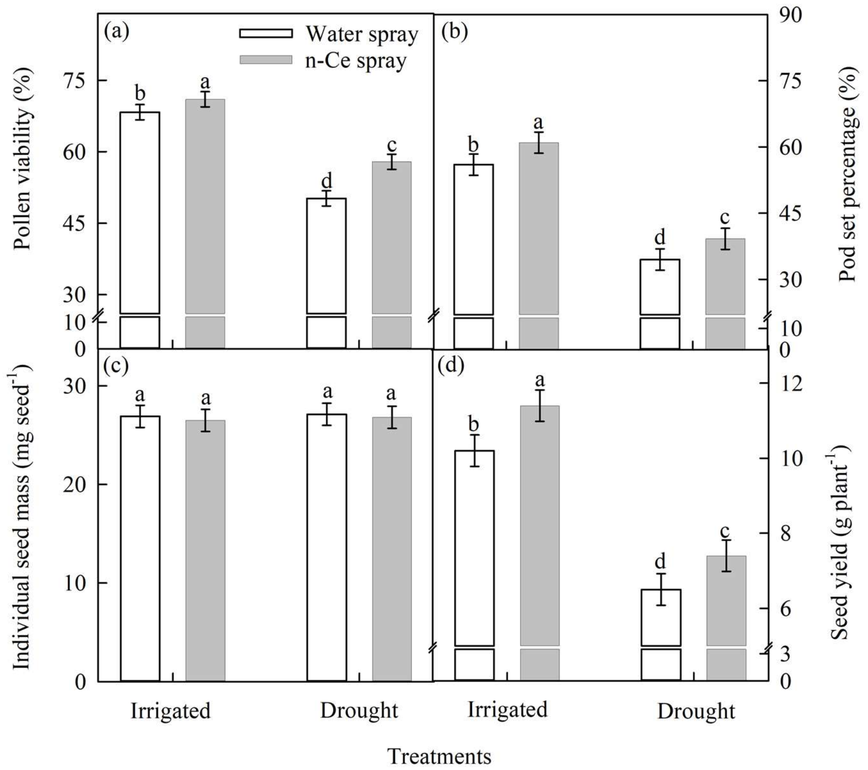

Figure 8. Effect of drought and foliar spray of nanoceria on (**a**) pollen viability (%), (**b**) pod-set percentage (%), (**c**) individual seed weight (mg seed^{-1}), and (**d**) seed yield (g plant^{-1}) in mungbean (pot culture experiment). Each value is the mean ± SD of 5 independent measurements. Means with different letters are significantly different at $p \leq 0.05$.

5. Discussion

Nanotoxicology explains the interaction effects between a nanoparticle and a living organism. In general, toxicology depends on the dose, but in the case of nanoparticles, it depends on the size, number, surface activity, and aggregation [27]. In the present study, foliar application of nanoceria at the rate of 100 mg L^{-1} had the highest seed yield plant^{-1} and less thylakoid membrane damage compared to other treatments. More thylakoid membrane damage and lower seed yield plant^{-1} were observed at higher concentrations. The toxicity of nanoceria at higher concentrations may be associated with their morphology, size, number of nanoparticles, and surface reactivity [28]. This study showed a dual property of nanoceria, i.e., a negligible effect at low concentrations and a deleterious effect at higher concentrations, which could be associated with ceria's dual oxidation state at its surface, which can act as an oxidizer and reducer, and its surface adsorption property [29,30]. Furthermore, free Ce^{3+} can redox-cycle with hydrogen peroxide to produce harmful ROS [31]. This property of nanoceria might have affected the PSII quantum yield by inducing photoinhibition [32].

Generally, any crop's growth and yield depend on photosynthesis. The results indicated that drought stress decreased the photosynthetic rate. This is associated with reduced carbon dioxide availability through decreased stomatal conductance [33], and non-stomatal limitations [34]. This study indicates that the decrease in photosynthetic rate is associated with stomatal limitations, as evidenced by a higher decrease in stomatal conductance (39% over irritated control) than non-stomatal factors, viz., thylakoid membrane damage (13%), the effective quantum yield of PSII (32%), electron transport rate (30%), and chlorophyll index (17%). Stomatal limitation, i.e., low CO_2 availability to the chloroplast, may increase the imbalance between PSII photochemistry and the electron requirement for carbon reduction, resulting in overexcitation and damage to photosystem II core reaction centers [35], resulting in a decreased photosynthetic rate. This was validated in the present study as a reduced effective quantum yield of PSII and ETR under drought stress. Under drought stress, ROS, namely H_2O_2, will be formed, which could activate the abscisic-acid-mediated stomatal closure and increase chlorophyll degradation in the chloroplast by damaging the ultrastructure of the chloroplast [36]. This was validated in the present study as an increased ROS under water spray compared to nanoceria foliar spray (Figure 6a,b). Nevertheless, due to nanoceria's inherent ROS-scavenging ability and/or increased antioxidant enzyme activity, the ROS formed were scavenged, resulting in increased stomatal conductance and photosynthetic rate (Figures 4a,c, 6d,f and 7a,d). This is in accordance with the findings of Djanaguiraman et al. [3].

In pulses, the seed yield is a product of the number of pods m^{-2}, the number of seeds m^{-2}, and individual seed weight. The results indicated that drought stress during the flowering stage decreased the pod-set percentage (Figure 8b), which is similar to finding in rice (*Oryza sativa* L.) [37], wheat (*Triticum aestivum* L.) [38], chickpea (*Cicer arietinum* L.) ([39], and sorghum [3]. This is the first study to show that foliar spray of nanoceria can increase the pod-set percentage compared to water spray under drought-stress conditions in grain legumes such as mungbean, similar to the finding of Djanaguiraman et al. [3] in sorghum. The pod-set percentage is a function of male and female gametes. Drought stress decreased male gamete function, as observed by reduced pollen viability (Figure 8a). The decreased pollen viability could be associated with altered carbohydrate metabolism in the developing reproductive tissue [40] and ROS production [41]. Compared to water spray, the increased pollen viability under foliar spray of nanoceria may be associated with effective scavenging of ROS produced in pollen grains under drought. The increase in seed yield $plant^{-1}$ is due to an increased number of pods $plant^{-1}$ and is not associated with individual seed mass (Figure 8c). Overall, it is evident that under drought stress, foliar application of nanoceria alleviated the oxidative damage in leaves by decreasing the production of oxidants and increasing antioxidant enzyme activity, which could result in increased photosynthetic rate. Similarly, nanoceria increased the pollen viability resulting in an increased pod-set percentage. The combined effect of increased photosynthetic rate and pod-set percentage could have resulted in higher grain yield, as explained in Figure 9.

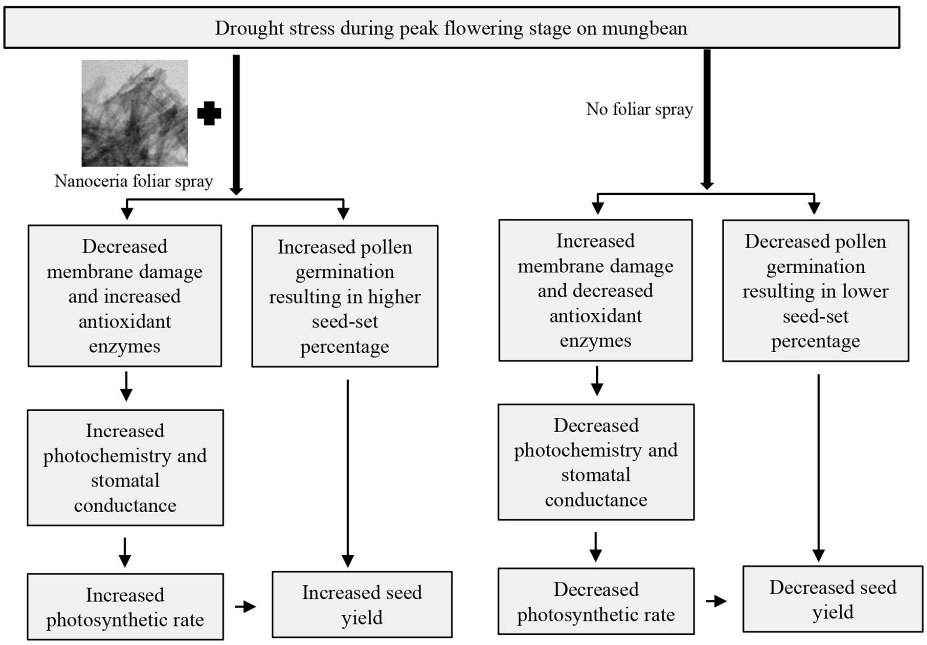

Figure 9. Proposed mechanism of action of nanoceria under drought stress in mungbean.

6. Conclusions

This study indicated that the optimum nanoceria concentration for mungbean foliar spray was 100 mg L^{-1}. Drought stress decreased the chlorophyll index, effective PSII quantum yield, electron transport rate, stomatal conductance, transpiration rate, photosynthetic rate, pod-set percentage, pod weight, and seed weight m^{-2} compared to the irrigated control. However, under drought, foliar spray of nanoceria at the rate of 100 mg L^{-1} increased the chlorophyll index, effective PSII quantum yield, electron transport rate, stomatal conductance, transpiration rate, photosynthetic rate, pod-set percentage, pod weight, and seed weight m^{-2}, and decreased thylakoid membrane damage compared to water-sprayed plants. The alleviation of oxidative damage by nanoceria spray was more significant under drought than in irrigated conditions. The increase in photosynthetic rate by nanoceria spray under drought stress versus water spray could be associated with an increased antioxidant activity of nanoceria. The increased seed yield plant^{-1} under drought by nanoceria is associated with increased photosynthetic rate and pod-set percentage through enhanced pollen viability. Earlier, it was shown that nanoceria can alleviate drought stress during the gametogenesis stage in a C_4 crop such as sorghum, and this study also proved that nanoceria can alleviate drought stress during the peak flowering stage in a C_3 crop such as mungbean.

Author Contributions: Conceptualization and Methodology, D.M.; Software, S.A., J.P. and K.M.K.; Visualization, P.B.R.V. and V.K.; Writing—Original Draft, D.M.; Writing—Review and Editing, S.N., V.D., R.V., S.R. and B.P. All authors have read and agreed to the published version of the manuscript.

Funding: D.M. acknowledges the Department of Biotechnology, Ministry of Science and Technology, Government of India for financial support through the project number: BT/PR36115/NNT/28/1814/2021 and the Government of Tamil Nadu, Tamil Nadu, India, and Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India for providing financial assistance to the first author through the University Core Project Grant.

Data Availability Statement: The data presented in this study are available in this article.

Conflicts of Interest: The authors declare no conflict of interest.

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