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Foliar treatment with nano-calcium carbonate improves H_2O_2-scavenging activity of the ascorbate-glutathione cycle in faba bean plants

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ABSTRACT

Cadmium (Cd) is a poisonous metal that damages plant metabolism by increasing reactive oxygen species (ROS), including hydrogen peroxide (H_2O_2). This article aimed to study the effect of foliar spraying faba bean plants with 1.0, 2.0, or 3.0 mM of traditional calcium carbonate (T-Ca-1, T-Ca-2, or T-Ca-3, respectively) or 0.1, 0.2, or 0.3 mM of nano-calcium carbonate (N-Ca-1, N-Ca-2, or N-Ca-3, respectively) on plant growth, physio-biochemical attributes, and H_2O_2-scavenging activity of the ascorbate (AsA)-glutathione (GSH) cycle. In general, compared to the control (foliar spraying with distilled water), foliar treatment with all T-Ca and N-Ca levels significantly improved growth, root activity, physio-biochemical attributes, and H_2O_2-scavenging activity of the ascorbate-glutathione cycle in faba bean plants, with results of N-Ca levels outperforming those of T-Ca levels. Among all T-Ca and N-Ca levels, N-Ca-2 conferred the best results, increasing plant growth traits by about 40%, photosynthetic efficiency by about 36%, root activity by about 26%, Ca content by about 39%, leaf integrity (relative water content and membrane stability index) by about 24%, antioxidant levels of the AsA-GSH cycle (ascorbate and glutathione) by about 47%, enzyme activities of the AsA-GSH cycle (ascorbate peroxidase and glutathione reductase) by about 60%, while reducing H_2O_2 level by about 29%. Therefore, foliar spraying the faba bean plant with nano-calcium carbonate at a level of 0.2 mM could enhance plant growth and development by reinforcing the H_2O_2-scavenging activity of the AsA-GSH cycle.

1. Introduction

As an improtant for human and animal feeding, faba bean (*Vicia faba* L.) is a legume crop cultivated worldwide [1]. China and Ethiopia contribute 34.6% and 16.9%, respectively, while Egypt contributes only 3.9% of the total global production of faba beans [2]. Despite the importance of local beans for Egypt, the production level does not meet the population and livestock needs [3]. To address this problem, it is necessary to increase faba bean production using modern productive techniques, including nanotechnology. The importance of faba beans is attributed to that the seeds contain a high percentage of nutrients, amino acids, proteins, carbohydrates, etc. [4]. In addition, plant roots can symbiotically fix atmospheric N, promoting soil productivity and N-use efficiency, minimizing chemical N applications, thus reducing environmental toxicity [3].

A few studies have used calcium (Ca) nanoparticles (N-Ca) to reduce negative effects in stressed plants. Ca is essential for promoting and regulating plant growth and vital cell processes [5]. It can maintain cellular membranes and enhance many enzyme activities, hormone biosynthesis, and physiological functions that control plant responses to stress and maintain plant production [6]. Leaf treatment with Ca provides greater benefits for managing Ca deficiency than soil supplementation. This approach enhances plant growth, photosynthesis, and yield quality [7]. By leaf treatment, Ca is rapidly absorbed and distributed in the plant to enhance water relations. In contrast, Ca contributes to controlling ROS production, such as hydrogen peroxide (H_2O_2) in plants [6]. N-Ca provides more benefits than traditional Ca (T-Ca) [8].

Nanotechnology can help repair cultivation systems to support food security. Application of nanotechnology, such as nano-fertilizer, in agriculture supports plant production and overcomes stress [7]. N-Ca can decrease T-Ca amount for crops to minimize fertilizer loss and environmental pollution, and maximize agricultural outputs. Leaf treatment with N-Ca enhances plant growth, production, physio-biochemical functions, photosynthesis, and ascorbate-glutathione cycle activity, while limiting oxidative stress and damage in stressed plants [6, 8]. These positivities are due to many physicochemical characteristics of N-Ca. Small-sized particles and a high surface area: volume ratio are among these characteristics, which can promote

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cellular functions in stressed plants after easily entering the plant [6, 8]. To avoid the phytotoxic impacts, N-Ca should be prepared at an appropriate concentration according to the plant's age and species [9].

To date, N-Ca has not been used as a foliar treatment for Cd-stressed faba bean. This work hypothesized that N-Ca applied as a foliar treatment would reduce the damaging impacts in Cd-stressed faba bean plants and increase their ability to tolerate Cd stress by decreasing Cd uptake and accumulation in the plant, while improving plant physiology, photosynthesis, and the AsA-GSH cycle activity. This research aimed at detecting the advantages of the N-Ca foliar treatment on growth, photosynthetic efficiency, physiological traits, leaf integrity, shoot and root Cd contents, and H_2O_2-scavenging activity in the AsA-GSH cycle.

2. Materials and methods

2.1. Growing plant material and experiment setup

Faba bean seed (cv Giza 716) was obtained from the Egyptian Agricultural Research Center for three simultaneous trials. The trials lasted 40 days. The seed was sown on Nov. 5, and samples were collected on Dec. 15, 2024. The seed was disinfected immersing in 5% NaOCl and the seed surface was cleaned with distilled water. For each trial, 140 plastic pots with dimensions of 38 × 35 cm in diameter × depth, respectively, were specified for sowing the seed (3 seeds pot^{-1}) and growing the plants. A growing medium containing 1 ion-free sand: 1 vermiculite: 2 peat moss by weight was prepared [10]. Quantities of 0.0125 g of a suitable fungicide and 0.025 g of humic acid were added per kg of medium. In addition, quantities of 0.20, 0.15, 0.15, 0.15, 0.10, 0.10, and 0.10 mg of NH_4NO_3, calcium superphosphate, K_2SO_4, $MgSO_4$, Fe, Zn, and Mn, respectively, were added per kg of medium. To adjust the pH of the medium, 0.5 g of $CaCO_3$ was added per kg of medium. The pots were filled with 98.0% of the prepared medium + 2.0% compost. The pots were placed in an open greenhouse with an average temperature of 23 ± 3 °C, a relative humidity of 62 ± 5%, and 11/13 h of day/night under natural sunlight. After 15 d of sowing, the pots were randomly arranged into 7 treatments, each containing 20 pots. The treatments were as follows: (1) foliar spraying with distilled water (control), (2) Foliar spraying with traditional calcium carbonate at a rate of 1.0 mM (T-Ca-1), (3) Foliar spraying with traditional calcium carbonate at a rate of 2.0 mM (T-Ca-2), (4) Foliar spraying with traditional calcium carbonate at a rate of 3.0 mM (T-Ca-3), (5) Foliar spraying with nano calcium carbonate at a rate of 0.1 mM (N-Ca-1), (6) Foliar spraying with nano calcium carbonate at a rate of 0.2 mM (N-Ca-2), and (7) Foliar spraying with nano calcium carbonate at a rate of 0.3 mM (N-Ca-3). Foliar spraying of all solutions was performed after the morning dew had evaporated. A 2-L Portable Pressure Sprayer was utilized. Three spray applications were conducted at 15, 25, and 35 d of sowing.

A randomized complete block design was used to arrange the treatments of each trial. The pots were irrigated, applying the weighting method, once every 2 d, to the medium's full capacity. During the experimental duration, the pots were rotated to overcome the local climatic effects. Weeds and pathogens were controlled by applying standard plant protection methods. Plant samples were collected 40 d after sowing for all determinations.

2.2. Determination of growth traits

Leaf number was recorded, and a Digital-Planix-7 planimeter was used to measure leaf area $plant^{-1}$ (dm^2). Using an electric balance, the plant shoot was weighed to record fresh weight (FW). The shoot was then dried at 70 °C for 48 h to record dry weight (DW).

2.3. Photosynthetic efficiency and root activity

Total chlorophyll (TChl) and carotenoid (TCar) contents were estimated in fresh leaves after extraction with 80% acetone [11] (Wellburn, 1994), by recording the absorbance (OD) at 662, 647, and 470 nm. Chl "a" fluorescence (Fv/Fm) was measured using a fluorometer (PAM-2000, Heinz-Walz) [12]. The Clemensson-Lindell [13] method was applied to determine the root activity in the dark. A 0.5 g fresh root immersed in 2,3,5-triphenyl-2H-tetrazolium chloride solution and P-buffer. After incubation for 2 h at 37 °C, the reaction was ended by adding H_2SO_4. Ethyl acetate was used to extract the samples. The OD was then recorded at 485 nm.

2.4. Root and shoot calcium (Ca) contents and leaf health

Dried root and shoot samples were used to evaluate Ca contents using the Perkin-Elmer Atomic-Absorption model 3300 [14]. The samples were digested in 1 perchloric acid (80%); 5 H_2SO_4 (Conc.) by volume. The digested samples were diluted with distilled water to 100 ml, then evaluations were performed.

Leaf relative water content (RWC) [15] and membrane stability index (MSI) [16] were assessed, following the formulas:

$$\text{RWC (\%)} = \left[\frac{(\text{fresh mass} - \text{dry mass})}{(\text{turgid mass} - \text{dry mass})}\right] \times 100$$

$$\text{MSI (\%)} = \left[1 - \left(\frac{\text{EC1}}{\text{EC2}}\right)\right] \times 100$$

The fresh, turgid, and dry mass of leaf discs were the fresh weight, weight after saturation by water, and weight after full drying, respectively. The EC1 and EC2 were the electrical conductivity of the leaf sample solution heated for ½ h at 40°C, and the leaf sample boiled for 10 min, respectively.

2.5. Evaluation of H_2O_2-scavenging activity in AsA-GSH cycle

Hydrogen peroxide (H_2O_2) level as an oxidant was assessed by the Velikova et al. [17] method. Ascorbate (AsA) level was measued in a mixture containing 0.03 M K-P buffer (pH 7.4), TCA (2.5%), H_3PO_4 (8.4%), bipyridyl (0.8%), $FeCl_3$ (0.3%), and AsA extract. The mixture was reacted for ½ h at 40°C [18]. The OD was evaluated at 525 nm. Glutathione (GSH) content was measured in a mixture containing GSH extract, 0.13 M + 7.0 mM buffers of Na-P (pH 7.4 and 6.8), and 6.0 mM DTNB. The mixture was reacted for 10 min at 30 °C [19]. The OD was recorded at 412 nm. Ascorbate peroxidase (APX) and glutathione reductase (GR) activities were assayed [20, 21], respectively. APX activity was assayed in enzyme extract provided with a K-P buffer (pH 7.0), H_2O_2, and AsA. The activity

was expressed as declined OD at 240nm. GR activity was assayed by noticing the elevation of OD at 412 nm with reducing 5,5'dithiobis (2-nitrobenzoic acid) by GSH in the extract.

2.6. Statistical analysis

One-way ANOVA for a randomized complete block design was applied to analyze the data, after testing for the homogeneity of error variance using InfoStat software estadistico [22, 23]. Duncan's Multiple Range Test was applied at 5% level of probability to test the differences among treatment means.

3. Results and discussion

Traditional (T-Ca) or nano calcium (N-Ca) has been applied for many crops under normal or stress conditions [6, 8]. In this study, T-Ca was applied at 1.0, 2.0, or 3.0 mM (T-Ca-1, T-Ca-2, or T-Ca-3, respectively), and N-Ca was applied 0.1. 0.2, or 0.3 mM (N-Ca-1, N-Ca-2, or N-Ca-3, respectively) on faba bean plants (Figs. 1 - 5). The large surface area and high solubility of N-Ca, which various parts of the plant can easily absorb [24], may be the primary reasons for the the better results of N-Ca than those of T-Ca. Foliar treatment with N-Ca offers an eco-friendly, cost-effective, and efficient alternative for supplying Ca to plants. N-Ca can penetrate through multiple layers of the leaf. The negatively charged pores (less than 5.0 nm in size) in the hydrophobic waxy cuticle facilitate the passage of positively charged nutrients like Ca^{2+}. Another pathway, leaf stomata, which are larger pores, can account for more than 5% of the leaf's surface area. When the stomata open, N-Ca can pass easily through these openings. The transport of N-Ca occurs through either apoplastic or symplastic pathways. Once N-Ca enters the plant through the stomata, epidermis, and cuticle, it is redistributed to various plant parts via the phloem. N-Ca has a smaller molecular size and a larger surface area than traditional calcium (T-Ca). This allows plant cells to absorb N-Ca more quickly than T-Ca, enabling N-Ca to perform its functions more rapidly [8]. Subsequently, plant growth, physio-biochemical attributes, and nutrient (K^+ and Ca^{2+}) uptake were upregulated by N-Ca, positively reflected on faba bean plant performance under 1.0 mM or 2.0 mM Cd stress; Cd-1 or Cd-2, respectively (Figs. 1-5).

3.1. Impacts of T-Ca or N-Ca on growth faba bean plants

Table 1 demonstrates that all T-Ca or N-Ca concentrations significantly increased the growth traits of faba bean plants compared to the control. Overall, the N-Ca treatments were more effective than the T-Ca treatments in increasing the number of leaves plant^{-1}, leaf area plant^{-1}, shoot fresh weight, and shoot dry weight. Among all treatments, the most effective was foliar spraying the plants with N-Ca-2 (Tables 1 & 2).

Table 1. The response of growth traits of faba bean plants to foliar spraying with traditional calcium carbonate (T-Ca) and nano-calcium carbonate (N-Ca).

Treatments	Number of leaves plant^{-1}	Leaf area plant^{-1} (dm^2)	Shoot FW (g)	Shoot DW (g)
Control	12.3 ± 0.89 d	11.8 ± 0.78 d	59.2 ± 3.82 d	7.34 ± 0.43 d
T-Ca-1	13.6 ± 0.98 c	12.7 ± 0.89 c	63.8 ± 4.20 d	8.10 ± 0.49 c
T-Ca-2	15.5 ± 1.09 b	13.9 ± 0.96 b	67.9 ± 4.52 c	9.11 ± 0.55 b
T-Ca-3	12.4 ± 0.91 d	11.7 ± 0.75 d	60.0 ± 3.91 d	7.30 ± 0.43 d
N-Ca-1	15.9 ± 1.11 b	14.1 ± 1.00 b	75.2 ± 5.26 b	9.13 ± 0.54 b
N-Ca-2	17.8 ± 1.26 a	15.9 ± 1.12 a	84.4 ± 6.64 a	9.98 ± 0.67 a
N-Ca-3	10.6 ± 0.69 e	11.9 ± 0.82 d	52.2 ± 3.52 e	6.48 ± 0.40 e

Means ± SE followed by the same letter in each column are not significantly different according to the Duncan's Multiple Range Test ($p \leq 0.05$). Control; foliar spraying with distilled water, T-Ca-1; foliar spraying with traditional calcium carbonate at a rate of 1.0 mM, T-Ca-2; foliar spraying with traditional calcium carbonate at a rate of 2.0 mM, T-Ca-3; foliar spraying with traditional calcium carbonate at a rate of 3.0 mM, N-Ca-1; foliar spraying with nano calcium carbonate at a rate of 0.1 mM, N-Ca-2; foliar spraying with nano calcium carbonate at a rate of 0.2 mM, N-Ca-3; foliar spraying with nano calcium carbonate at a rate of 0.3 mM, dm2; square decimeter, FW; fresh weight, and DW; dry weight.

This finding demonstrates that N-Ca, particularly N-Ca-2, enhanced the growth of faba bean plants (more than T-Ca treatments) due to that Ca content increased in both roots and shoots (Tables 2 & 3), potentially promoting cell elongation and expansion through Ca mediation, which explains the observed promotion in plant growth under normal or stress conditions [25]. Enhancing the contents and activities of photosynthesis-related parameters (Tables 2 & 3), while reducing H_2O_2 level, was associated with the effectiveness of foliar treatment with N-Ca to optimize the growth of faba bean plants (Tables 1 & 2). The role of N-Ca in maintaining cell membrane structure is crucial for cell enlargement and membrane function, reflected in increased plant growth and reduced oxidative damage through the increased activity of the ascorbate-glutathione cycle. The application of N-Ca improves nutrient uptake, particularly Ca^{2+}, and enhances membrane stability (Tables 2 & 3), which may accelerate plant growth [8].

3.2. *Impacts of T-Ca or N-Ca on photosynthesis, root activity, plant Ca content, and leaf integrity of faba bean plants*

Tables 2 & 3 shows that all T-Ca or N-Ca treatments significantly increased the photosynthesis-related parameters, root activity, root and shoot contents of Ca^{2+}, and leaf integrity of faba bean plants compared to the control. Overall, the N-Ca treatments were more effective than the T-Ca treatments in increasing total chlorophyll (TChl) and total carotenoid (TCar) contents, Fv/Fm, root activity, root and shoot Ca contents, leaf relative water content (RWC), and membrane stability index (MSI). Among all treatments, the most effective was foliar spraying the plants with N-Ca-2 (Tables 2, 3, & 4). The photosynthetic efficiency of faba bean plants, measured by TChl and TCar contents, as well as Fv/Fm and root activity, showed a notable increase with N-Ca treatments (more than T-Ca treatments). This result is due to N-Ca-induced biosynthesis of TChl and TCar, activation of cytokinins, and an increase in RWC and MSI, along with reinforcing the pigment-protein complex in thylakoid membranes, Photosystem I (PSI), and Photosystem II (PSII) in photosynthesis [26]. N-Ca maintained the thylakoid membrane structure and activation of cytokinins (CKs) activity [27]. Gupta et al. [8] discussed the

enhancing effects of Ca on TChl and TCar contents by minimizing thylakoid breakdown and maintaining cellular homeostasis. Ca can support photosynthesis by increasing leaf integrity, the expression of genes related to TChl synthesis.

Table 2. Increase (+%), decrease (–%), or no effect compared to the control regarding growth traits and physio-biochemical attributes of faba bean plants affected by foliar spraying with traditional calcium carbonate (T-Ca) and nano-calcium carbonate (N-Ca)

Treatments	Number of leaves	Leaf area	Shoot FW	Shoot DW
Control	---	---	- --	---
T-Ca-1	+ 10.6	+ 7.6	+ 7.8	+ 10.4
T-Ca-2	+ 26.0	+ 17.8	+ 14.7	+ 24.1
T-Ca-3	No effect	No effect	No effect	No effect
N-Ca-1	+ 29.3	+ 19.5	+ 27.0	+ 24.4
N-Ca-2	+ 44.7	+ 34.7	+ 42.6	+ 36.0
N-Ca-3	– 13.8	It has no effect	– 11.8	– 11.7
	Chlls content	Carts content	Fv/Fm	Root activity
Control	---	---	- --	---
T-Ca-1	+ 13.0	+ 12.0	+ 6.8	+ 9.3
T-Ca-2	+ 33.3	+ 24.0	+ 15.3	+ 16.7
T-Ca-3	No effect	No effect	No effect	No effect
N-Ca-1	+ 32.9	+ 26.0	+ 16.9	+ 18.5
N-Ca-2	+ 47.7	+ 36.0	+ 25.4	+ 25.9
N-Ca-3	No effect	No effect	No effect	No effect
	Root Ca	Shoot Ca	RWC (%)	MSI (%)
Control	---	---	- --	---
T-Ca-1	+ 9.1	+ 15.6	+ 7.6	+ 7.3
T-Ca-2	+ 19.3	+ 31.9	+ 13.7	+ 15.2
T-Ca-3	+ 19.0	+ 31.9	No effect	No effect
N-Ca-1	+ 20.5	+ 34.0	+ 14.0	+ 16.1
N-Ca-2	+ 31.3	+ 46.1	+ 23.0	+ 24.0
N-Ca-3	No effect	No effect	No effect	No effect

	H_2O_2 level	AsA content	GSH content	APX activity	GR activity
Control	---	---	- --	---	---
T-Ca-1	– 8.2	+ 9.0	+ 13.9	+ 13.4	+ 12.9
T-Ca-2	– 17.9	+ 18.9	+ 30.6	+ 34.3	+ 29.8
T-Ca-3	No effect	No effect	– 16.7	– 20.9	– 33.3
N-Ca-1	– 19.0	+ 30.3	+ 33.3	+ 38.8	+ 42.1
N-Ca-2	– 28.8	+ 37.7	+ 55.6	+ 63.4	+ 56.7
N-Ca-3	No effect	– 9.8	– 22.2	– 23.9	– 36.3

Control; foliar spraying with distilled water, T-Ca-1; foliar spraying with traditional calcium carbonate at a rate of 1.0 mM, T-Ca-2; foliar spraying with traditional calcium carbonate at a rate of 2.0 mM, T-Ca-3; foliar spraying with traditional calcium carbonate at a rate of 3.0 mM, N-Ca-1; foliar spraying with nano calcium carbonate at a rate of 0.1 mM, N-Ca-2; foliar spraying with nano calcium carbonate at a rate of 0.2 mM, N-Ca-3; foliar spraying with nano calcium carbonate at a rate of 0.3 mM, dm2; square decimeter, Chlls; Total chlorophylls, Carts; Total carotenoids, Fv/Fm; Efficiency of PSII in photosynthesis, Photo-Ac; Photochemical activity that is the chemical reaction initiated by the absorption of energy in the form of light, Ca; calcium, RWC; relative water content, MSI; membrane stability index, H_2O_2; hydrogen peroxide, AsA, ascorbate, GSH, glutathione, APX, ascorbate peroxidase, GR, glutathione reductase, FW; fresh weight, and DW; dry weight.

Table 3. The response of photosynthetic efficiency and root activity of faba bean plants to foliar spraying with traditional calcium carbonate (T-Ca) and nano-calcium carbonate (N-Ca)

Treatments	TChl content (mg g^{-1} FW)	TCar content (mg g^{-1} FW)	Fv/Fm	Root activity
Control	2.16 ± 0.11 d	0.50 ± 0.02 de	0.59 ± 0.02 d	0.54±0.02 d
T-Ca-1	2.44 ± 0.14 c	0.56 ± 0.02 c	0.63 ± 0.03 c	0.59±0.02 c
T-Ca-2	2.88 ± 0.19 b	0.62 ± 0.03 b	0.68 ± 0.03 b	0.63±0.03 b
T-Ca-3	2.14 ± 0.09 d	0.52 ± 0.02 d	0.60 ± 0.02 cd	0.54±0.02 d
N-Ca-1	2.87 ± 0.20 b	0.63 ± 0.03 b	0.69 ± 0.03 b	0.64±0.03 b
N-Ca-2	3.19 ± 0.26 a	0.68 ± 0.03 a	0.74 ± 0.04 a	0.68±0.03 a
N-Ca-3	2.18 ± 0.12 d	0.49 ± 0.02 e	0.59 ± 0.02 d	0.55±0.02 d

Means ± SE followed by the same letter in each column are not significantly different according to the Duncan's Multiple Range Test ($p \leq 0.05$). Control; foliar spraying with distilled water, T-Ca-1; foliar spraying with traditional calcium carbonate at a rate of 1.0 mM, T-Ca-2; foliar spraying with traditional calcium carbonate at a rate of 2.0 mM, T-Ca-3; foliar spraying with traditional calcium carbonate at a rate of 3.0 mM, N-Ca-1; foliar spraying with nano calcium carbonate at a rate of 0.1 mM, N-Ca-2; foliar spraying with nano calcium carbonate at a rate of 0.2 mM, N-Ca-3; foliar spraying with nano calcium carbonate at a rate of 0.3 mM, dm2; square decimeter, TChl; Total chlorophylls, TCar; Total carotenoids, Fv/Fm; Efficiency of PSII in photosynthesis, and FW; fresh weight.

Table 4. The response of root and shoot Ca contents, and leaf integrity of faba bean plants to foliar spraying with traditional calcium carbonate (T-Ca) and nano-calcium carbonate (N-Ca)

Treatments	Root Ca (mg g^{-1} DW)	Shoot Ca (mg g^{-1} DW)	RWC (%)	MSI (%)
Control	3.52 ± 0.14 d	2.82±0.08 d	72.1 ± 3.4 d	55.8 ± 2.6 d
T-Ca-1	3.84 ± 0.18 c	3.26±0.10 c	77.6 ± 3.8 c	59.9 ± 2.9 c
T-Ca-2	4.20 ± 0.20 b	3.72±0.14 b	82.0 ± 4.4 b	64.3 ± 3.4 b
T-Ca-3	4.19 ± 0.19 b	3.72±0.14 b	71.9 ± 3.6 d	56.1 ± 2.8 d
N-Ca-1	4.24 ± 0.22 b	3.78±0.15 b	82.2 ± 4.6 b	64.8 ± 3.8 b
N-Ca-2	4.62 ± 0.24 a	4.12±0.18 a	88.7 ± 4.9 a	69.2 ± 3.9 a
N-Ca-3	3.51 ± 0.15 d	2.78±0.08 d	72.2 ± 3.5 d	56.0 ± 2.7 d

Means ± SE followed by the same letter in each column are not significantly different according to the Duncan's Multiple Range Test ($p \leq 0.05$). Control; foliar spraying with distilled water, T-Ca-1; foliar spraying with traditional calcium carbonate at a rate of 1.0 mM, T-Ca-2; foliar spraying with traditional calcium carbonate at a rate of 2.0 mM, T-Ca-3; foliar spraying with traditional calcium carbonate at a rate of 3.0 mM, N-Ca-1; foliar spraying with nano calcium carbonate at a rate of 0.1 mM, N-Ca-2; foliar spraying with nano calcium carbonate at a rate of 0.2 mM, N-Ca-3; foliar spraying with nano calcium carbonate at a rate of 0.3 mM, Ca; calcium, RWC; relative water content, MSI; membrane stability index, and DW; dry weight.

N-Ca can enhance photosynthetic efficiency by impacting the Hill reaction, which involves PSI and PSII activities, as well as the Calvin cycle, leading to improved photophosphorylation [8]. Furthermore, N-Ca can promote the expression of the psbA gene, which encodes the D1 protein, a crucial structural and functional component of the PSII reaction [28]. Additionally, N-Ca can upregulate important photosynthetic enzymes, including Rubisco-activating enzyme [29]. In this study, N-Ca reinforced root activity by minimizing H_2O_2 level and maximizing ascorbate-glutathione cycle activity, which reflected more Ca^{2+} uptake by plant roots, thus increasing shoot Ca^{2+} content (Tables 2, 3, & 4). N-Ca noticeably restrained the H_2O_2 level in faba bean plants due to enhanced the plant's defense system represented by the increased activity of the ascorbate-glutathione cycle.

3.3. *Impacts of T-Ca or N-Ca on Ca contents and leaf integrity of faba bean plants*

Table 5 demonstrates that all T-Ca or N-Ca concentrations significantly increased the ascorbate (AsA) and glutathione (GSH) levels, as well as AsA peroxidase (APX) and GSH reductase (GR) activities, while decreasing H_2O_2 level in faba bean plants compared to the control. Overall, the N-Ca treatments were more effective than the T-Ca treatments, and N-Ca-2 was most effective among all treatments (Tables 2 & 5).

Table 5. The response of H_2O_2-scavenging activity in AsA-GSH cycle of faba bean plants to foliar spraying with traditional calcium carbonate (T-Ca) and nano-calcium carbonate (N-Ca)

Treatment	H_2O_2 level	AsA content	GSH content	APX activity	GR activity
	μmol g^{-1} *FW*			Units mg^{-1} protein	
Control	1.84 ± 0.09 a	1.22 ± 0.44 e	0.36 ± 0.01 d	13.4 ± 0.20 d	17.1 ± 0.27 e
T-Ca-1	1.69 ± 0.07 b	1.33 ± 0.49 d	0.41 ± 0.01 c	15.2 ± 0.25 c	19.3 ± 0.32 d
T-Ca-2	1.51 ± 0.05 c	1.45 ± 0.55 c	0.47 ± 0.01 b	18.0 ± 0.31 b	22.2 ± 0.37 c
T-Ca-3	1.83 ± 0.09 a	1.19 ± 0.39 e	0.30 ± 0.01 e	10.6 ± 0.15 e	11.4 ± 0.17 f
N-Ca-1	1.49 ± 0.05 c	1.59 ± 0.63 b	0.48 ± 0.01 b	18.6 ± 0.34 b	24.3 ± 0.41 b
N-Ca-2	1.31 ± 0.04 d	1.68 ± 0.67 a	0.56 ± 0.02 a	21.9 ± 0.38 a	26.8 ± 0.45 a
N-Ca-3	1.87 ± 0.10 a	1.10 ± 0.40 f	0.28 ± 0.00 e	10.2 ± 0.14 e	10.9 ± 0.16 f

Means ± SE followed by the same letter in each column are not significantly different according to the Duncan's Multiple Range Test ($p \leq 0.05$). Control; foliar spraying with distilled water, T-Ca-1; foliar spraying with traditional calcium carbonate at a rate of 1.0 mM, T-Ca-2; foliar spraying with traditional calcium carbonate at a rate of 2.0 mM, T-Ca-3; foliar spraying with traditional calcium carbonate at a rate of 3.0 mM, N-Ca-1; foliar spraying with nano calcium carbonate at a rate of 0.1 mM, N-Ca-2; foliar spraying with nano calcium carbonate at a rate of 0.2 mM, N-Ca-3; foliar spraying with nano calcium carbonate at a rate of 0.3 mM, H_2O_2; hydrogen peroxide, AsA, ascorbate, GSH, glutathione, APX, ascorbate peroxidase, GR, glutathione reductase, and FW; fresh weight.

Applying N-Ca minimized the harmful influences of H_2O_2 by increasing the activities of non-enzymatic (AsA and GSH) and enzymatic (APX and GR) antioxidants, reflecting increased AsA-GSH cycle activity (Tables 2 & 5). Our results indicate that faba bean plants can effectively minimize H_2O_2 levels by improving AsA and GSH levels and their redox states, mediated by N-Ca, in the AsA-GSH cycle. Enhancement of antioxidant enzymes, including APX and GR in the AsA-GSH cycle, contributed to the reduction of H_2O_2 levels in faba bean plants (Tables 2 & 5). The findings regarding N-Ca suggest that its application effectively reduces H_2O_2 levels. Gupta et al. [8] reported that N-Ca acts as an anti-stress agent, enhancing antioxidant metabolism and transport in plants. This, in turn, improves plant tolerance to damage caused by elevated H_2O_2 levels. Our findings indicated that faba bean plants resist the harmful influences of H_2O_2 through two key antioxidant mechanisms. As reported by Mahmoud et al. [30], non-enzymatic pathways are the primary mechanism by which several metabolites, including GSH, directly scavenge ROS. This is in addition to phenolic compounds that contribute to minimizing H_2O_2 levels and the "AsA-GSH cycle", where H_2O_2 is eliminated [31, 32]. The second major antioxidant mechanism is the minimization of H_2O_2 levels through enzyme activities [30-32]. The high pattern of enzymatic activities and the low pattern of oxidation products (H_2O_2) indicate that N-Ca-treated faba bean plants have less H_2O_2 levels, thus less damage to cell membranes and more efficient plant growth (Tables 2 & 5).

4. Conclusions

Compared to traditional calcium (T-Ca), nano calcium (N-Ca) supports plant metabolism and growth more efficiently. N-Ca has been explored as an efficient approach for minimizing H_2O_2 levels in faba bean plants. This was achieved by increasing the activity of the ascorbate-glutathione cycle due to the increased levels of AsA and GSH and the activities of ascorbate peroxidase and glutathione reductase. Therefore, N-Ca could be a promising approach for achieving sustainability and improving crop growth and production. However, more research is needed to understand the complex physio-

biochemical mechanisms that influence the response of nano-calcium-treated plants to any environmental fluctuations that lead to increased H_2O_2 levels in plant tissues.

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Author Contributions

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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