



Chitosan and its Nanoform Regulates Physiological Processes and Antioxidant Mechanisms to Improve Drought Stress Tolerance of *Vicia faba* Plant

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Abstract

Purpose: Drought stress is an important challenge to global food security and agricultural output. Dramatic and quick climate change has made the problem worse. It caused unexpected impacts on the growth, development, and yield of different plants. Hence, the ultimate yield does not fulfill the required demand. Understanding the biochemical, ecological, and physiological reactions to these pressures is essential for improved management. Chitosan applications have a wide prospect of addressing abiotic issues. Moreover, chitosan and chitosan nanoparticles have a positive impact on increasing plant tolerance to abiotic stress, like drought stress. The current research investigated the consequences of drought stress on the morpho-physiological and biochemical parameters of *Vicia faba* plants, a comparison of chitosan and chitosan nanoparticles, and their ameliorating capacity towards drought stress. **Methods:** A pot experiment was conducted to evaluate the beneficial role of either chitosan (0.5, 1.0, and 2.0 gL⁻¹) or chitosan NPs (10, 20, and 30 mgL⁻¹) in inducing the *Vicia faba* tolerance to drought stress (60% water field capacity). **Results:** Drought stress significantly affected vegetative growth parameters of the shoot system, photosynthetic pigments, and indole acetic acid, accompanied by significant increases in vegetative growth parameters of the root system, some chemical composition of dry leaf tissues (total soluble sugar, soluble protein, proline, phenolic compound, glutathione, α tocopherol), hydrogen peroxide, malondialdehyde, lipoxxygenase, and antioxidant enzyme activities (catalase, peroxidase, superoxide dismutase, ascorbate peroxidase, glutathione reductase). All applied treatments. chitosan and chitosan nanoparticles, at all concentrations, improved plant tolerance to drought stress via increasing vegetative growth parameters, photosynthetic pigments, indole acetic acid, total soluble sugar, soluble protein, proline, phenolic compound, glutathione, α tocopherol, and antioxidant enzyme activities, accompanied by decreases in hydrogen peroxide, malondialdehyde, and lipoxxygenase enzyme. It is worthy to mention that 20 mgL⁻¹ chitosan nanoparticles was the most optimal treatment either under well water conditions (90% water field capacity) or drought stress conditions (60% water field capacity). Moreover, it is obvious from these results that the response of bean plants grown under well watered conditions was more pronounced than that of those plants grown under drought stress conditions to 20 mgL⁻¹ chitosan nanoparticles. **Conclusions:** Hence, it can be concluded that chitosan and chitosan nanoparticles can mitigate the negative impacts of drought stress by improving the photosynthetic pigments, endogenous indole acetic acid, and osmolyte contents, as well as the non-enzymatic and enzymatic antioxidant compounds of the *Vicia faba* plant.

Highlights

- Chitosan and chitosan NPs improved vegetative growth parameters, photosynthetic pigments, and some osmolytes of faba bean grown under drought stress (60% WFC).

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- Chitosan and chitosan NPs decreased hydrogen peroxide, malondialdehyde, and lipoxygenase of fresh leaf tissues of faba bean.
- Chitosan and chitosan NPs increased two antioxidant substance (glutathione, and α tocopherol), and antioxidant enzyme activities.
- Chitosan and chitosan NPs improved faba bean plant tolerance to drought stress.
- Chitosan NPs at 20mgL^{-1} was the most optimal treatment either under well water conditions (90% WFC) or drought stress conditions (60% WFC).

Keywords Faba bean · Water Stress · Chitosan · Nano-chitosan · Antioxidant System · Osmolytes

1 Introduction

Drought stress is one of the alarming global abiotic stresses disturbing plant growth and crop production. The Food and Agriculture Organization (FAO) reported that 45% of agricultural land was exposed to drought stress. Moreover, it is estimated that crop yield losses typically range from 30 to 90% under drought stress conditions (Dietz et al. 2021). Drought stress induces several devastating impacts on plants via disturbing various physiological, biochemical, and metabolic processes such as changes in the carbon assimilation rate, reducing cell turgor, inducing oxidative stress, variations in leaf gas exchange, enzyme activity, and ion balance (Anjum et al. 2017). The plant response to drought stress is complicated and depends on plant development stage, genetic variability, as well as the duration and severity of stress. Drought stress causes overproduction of reactive oxygen species (ROS) leading to oxidative damage (Hasanuzzaman et al. 2020; Hanafy and Sadak 2023). In order to maintain normal plant growth and development under drought stress, it is crucial to maintain a balance between ROS generation and scavenging. So, plants have evolved a variety of morphological, physiological-biochemical, and molecular mechanisms to successfully slow down ROS damage and maintain cellular redox balance during drought (Seleiman et al. 2021). Plants have evolved an osmotic adjustment process via increasing the biosynthesis of osmolytes (glycine betaine, proline, free amino acids, and total soluble carbohydrates) (Din et al. 2011). The main roles of these compounds are to improve osmoregulation, protect the structure of different biomolecules and membranes, stabilize different physiological processes needed for plant growth, and scavenge over accumulated radicals (Ashraf et al. 2011). In addition, higher plants have evolved several adaptive mechanisms for reducing oxidative damage through the biosynthesis of various antioxidants. Hence, enhancing the antioxidant defense system is a key strategy for effectively scavenging, detoxifying, and neutralizing excess ROS (Wu et al. 2017). The antioxidant defense system includes non-enzymatic antioxidants (phenolic compounds, ascorbate, glutathione, carotenoids, flavanones,

and anthocyanins) and antioxidant enzymes (superoxide dismutase, catalase, ascorbate peroxidase, monodehydroascorbate reductase, dehydroascorbate reductase, glutathione reductase, glutathione peroxidase, and glutathione S-transferase) (Hasanuzzaman et al. 2020). The main antioxidant enzyme is superoxide dismutase (SOD), which converts ROS into H_2O_2 , buffer peroxidase (POD), and catalase (CAT) enzymes (Noctor et al. 2000). To improve plant tolerance to different abiotic stresses, a number of substances, including growth regulators, osmoprotectants, and antioxidants, are successfully used. Exogenous application of plant development modulators, such as chitosan, is one method for improving plants' ability to withstand drought (Sadak et al. 2022).

Chitosan is a linear cationic polysaccharide composed of glucosamine and *N*-acetylglucosamine linked through β - (1–4) glucosidic linkages (Srinivasa and Tharanathan 2007). Chitosan can be extracted from marine crustaceans like shrimp, crab, and pinfish or from the exoskeletons of most insects under the name of chitin, which can be transformed into chitosan by extracting the acetyl group and turning it into amino. Chitosan is safe, nontoxic, inexpensive, and friendly to the environment and is characterized by biocompatibility, biodegradability, and bioactivity (Katiyar et al. 2015). Besides, chitosan application has a wide prospect of addressing abiotic issues (Malerba and Cerana 2020). In addition, chitosan induced the expression of a variety of genes involved in plant defense responses that, in some cases, resulted in increased synthesis of secondary plant metabolites (Sadak et al. 2022). Furthermore, chitosan treatment induced over-expression of genes involved in photosynthesis, changes in the programming of protein metabolism, and an enhancement of various storage proteins and hormone metabolism (Landi et al. 2017). Recently, the use of chitosan has induced drought resistance and improved the efficiency of water use (Sadak et al. 2022).

Modernistic nanotechnology has proven its position in agriculture and related industries. Therefore, increasing plant growth and productivity through the application of nanofertilizers can open new perspectives in agricultural practices. Nanofertilizers promise to be a safe way to enrich nutrients in plants without doing harm to the environment.

Chitosan nanoparticles (nano-chitosan) are environmentally friendly and bioactive (Agnihotri et al. 2004). Therefore, it can introduce different biological activities with altered physicochemical properties like surface area, size, cationic nature, etc. (Chandra et al. 2015). Chitosan nanoparticles are easily absorbed by the epidermis of leaves and translocated to stems, which facilitates the uptake of active molecules and enhances the growth and productivity of several crop plants (Malerba and Cerana 2020). In addition, chitosan nanoparticles alleviated the adverse effect of drought stress on white lupine seedlings (Bakhoun et al. 2022).

Faba bean (*Vicia faba* L.) is a major leguminous crop that is grown in Egypt. It is an important source of protein for human and animal consumption. Faba bean seeds have a high content of protein and micronutrients and are also a good source of energy and dietary fibre (Khazaei and Vandenberg 2020). The average protein ranges from 24 to 35%, making it one of the most protein-rich pulses (starchy grain legumes). Faba bean is not only nutritionally important but also plays a critical role in reducing nitrogen (N) fertilizer inputs to agricultural production systems through symbiotic fixation of atmospheric N₂ (Mansour et al. 2021). Faba bean is highly sensitive to water stress compared with other legumes (Bakhoun et al. 2022). In addition, in 2020, broad bean production in Egypt was 190,000 tonnes, while its consumption was 460,000 tonnes (Abo-Alhassan et al. 2022). Due to the great gap between the production and consumption of faba beans, it is necessary to increase their production to decrease this gap, especially when grown under drought stress.

As far as we know, not many studies have examined how chitosan and its nanoform treatments affect the morphological and physiological properties of the *Vicia faba* plant under drought stress. Hence, this investigation aimed to evaluate the impact of various chitosan and its nanoform levels on different studied parameters of *Vicia faba* grown under drought stress. So, it is imperative to understand in further detail how this plant responds to exogenous treatment of chitosan and its nanoform under drought stress in order to boost its pigments and endogenous IAA and counteract the negative impacts of drought stress by altering certain morpho-physiological features of this plant. As a result, this investigation can help researchers choose the ideal chitosan and its nanoform levels to improve faba bean drought tolerance.

2 Materials and Methods

2.1 Experimental Procedures

This study was conducted at the greenhouse of the National Research Centre, Dokki, Cairo, Egypt (30° 3' 0" N / 31° 15' 0" E), during two successive winter seasons 2020/2021 and 2021/ 2022. Faba bean (*Vicia faba* L. cv. Giza 461) seeds were obtained from the Agricultural Research Centre, Ministry of Agriculture and Land Reclamation, Egypt. Faba bean seeds were selected for uniformity by choosing those of equal size and with the same color. The selected seeds were washed with distilled water, sterilized with a 1% sodium hypochlorite solution for about 2 min and thoroughly washed again with distilled water. Ten uniformly air dried faba bean seeds were sown along a centre row in each pot at a 30-mm depth in plastic pots, each filled with about 7.0 kg of clay soil mixed with sandy soil in a proportion of 3:1(v: v), respectively.

At sowing, granular commercial rhizobia was incorporated into the top 30 mm of the soil in each pot with the seeds. Granular ammonium sulphate 20.5% N at a rate of 40 kg N ha⁻¹, and single superphosphate (15% P₂O₅) at a rate of 60 kg P₂O₅ ha⁻¹ were added to each pot. The N and P fertilizers were mixed thoroughly into the soil of each pot immediately before sowing. Ten days after sowing (DAS), faba bean seedlings were thinned to four seedlings per pot and irrigated with equal volumes of tap water until 15 DAS. Starting from 15th day, pots were divided into two groups. The first group of faba bean seedlings was sprayed twice with three levels of chitosan at 0.5, 1 and 2 gL⁻¹ at 15 and 22 DAS. While, the second group of faba bean seedlings was sprayed with three levels of nano-chitosan at 10, 20, and 30 mgL⁻¹ at 15 and 22 DAS. The control treatments were sprayed by tap water. Further, each group was divided into two sub-groups, in the first sub-group, bean seedlings were irrigated with tap water at 90% water field capacity. While, second sub-group, bean seedlings were irrigated with tap water at 60% water field capacity throughout the rest of the experiment (60 days).

The soil field capacity in the pots was estimated by saturating the soil in the pots with water and weighing them after they had been drained for 48 h. Treatments were arranged at the green-house benches in a complete randomized block design with six replicates for each treatment.

2.2 Measurements

Plant samples were collected after 60 days from sowing for measurement of some growth parameters (i.e. shoot height, root length, branches and leaves number, shoot and root

fresh weights /plant, shoot and root dry weights /plant), as well as some biochemical analysis.

2.3 Biochemical Analysis

Photosynthetic pigments: Chlorophylls and carotenoids contents in plant leaves were estimated using the method of Li and Chen (2015). Indole acetic acid content was extracted and estimated by the method of Gusmiaty et al. (2019). The level of H_2O_2 was determined according to Jana and Choudhuri (1981). The level of lipid peroxidation was measured by determining the malondialdehyde (MDA) content according to Hodges et al. (1999). Lipoygenase (EC 1.13.11.12) activity was evaluated according to Doderer et al. (1992).

Assay of antioxidant enzymes activities: Enzymes were extracted following the method of Chen and Wang (2006). Catalase (CAT) (EC 1.11.1.6) activity was determined according to the method of Chen and Wang (2006). Peroxidase (POX) (EC 1.11.1.7) activity was evaluated using the method of Kumar and Khan (1982). Superoxide dismutase (SOD) (EC 1.12.1.1) activity was measured according to Chen and Wang (2006). The level of ascorbate peroxidase (ASP) (EC 1.11.1.11) activity was estimated using Chen and Asada (1992) method. Glutathione reductase enzyme (GR) (EC 1.6.4.2) activity was evaluated according to the method of Foyer et al. (1995).

Assay of non Enzymatic Antioxidants Non enzymatic antioxidants were extracted as the method described by Gonzalez et al. (2003). A known weight of the fresh samples (1 g) was homogenized with 85% cold methanol (50 mL v/v) for three times at 90 °C. The free radical scavenging activity by 2,2, -diphenyl-2-picryl-hydrazyl (DPPH) method was estimated according to Gyamfi et al. (1999). Total phenolic content was estimated as the method described by Gonzalez et al. (2003). The content of glutathione (non-protein SH group) was estimated according to the method of Paradiso et al. (2008). α -tocopherol content was assayed according to the method described by Jargar et al. (2012).

Assay of Compatible Solute Proline (Pro) content was determined by Kalsoom et al. (2016). Total soluble sugars (TSS) were estimated by the method of Mecozzi (2005). Total soluble nitrogen (TSN) content was measured by the method of Horwiltz (2002).

2.4 Statistical Analysis

Data average of two seasons was statistically analyzed by analysis of variance. The differences among means were

determined by least significant differences (LSD) according to Silva and Azevedo (2016) and compared by Duncan's test ($p \leq 0.05$) and presented by standard deviation.

3 Results

3.1 Vegetative Growth Parameters

Drought stress significantly reduced the vegetative growth parameters of the shoot system of the bean plant accompanied by significant increases in the vegetative growth parameters of the root system relative to those grown under well watered conditions (Table 1). Since then, drought stress conditions have significantly reduced shoot dry weight/plant by 51.16% and significantly increased root dry weight /plant by 128.05% relative to well watered conditions.

Both chitosan and chitosan NPs at all concentrations caused marked increases in vegetative growth parameters of the shoot and root systems of bean plants grown either under well watered conditions or drought stress conditions. The most optimal treatment was 20 mg L⁻¹ chitosan NPs. Since it significantly increased shoot and root dry weight by 83.72% and 45.39% respectively in plants grown under well watered conditions. Likewise, it significantly increased shoot and root dry weight by 29.46% and 17.24% respectively in plant grown under drought stress conditions. It is obvious from these results that the response of bean plants grown under well watered condition was more pronounced than that of those grown under drought stress conditions to 20 mgL⁻¹ chitosan NPs. Moreover, the promotive effect of 20 mg L⁻¹ chitosan NPs on shoot dry weight was more pronounced than its effect on root dry weight.

3.2 Photosynthetic Pigments

Under drought stress conditions, chlorophyll a, chlorophyll b and total photosynthetic pigment decreased significantly accompanied by a significant increase in carotenoid and the ratio of chlorophyll a/ chlorophyll b relative to plants grown under well watered conditions (Table 2). Since, drought stress conditions have significantly reduced total photosynthetic pigment by 21.69% relative to well watered conditions.

While, chitosan and chitosan NPs significantly increased all components of photosynthetic pigments either in plants grown under well watered conditions or drought stress conditions, except chitosan at 2g L⁻¹ that caused non significant increases in chlorophyll a and chlorophyll b in fresh leaf tissues of plants grown under drought stress conditions. The optimum treatments that caused the highest significant increases in total photosynthetic pigments were chitosan at

Table 1 Impact of chitosan (g L^{-1}) and chitosan NPs (mg L^{-1}) on vegetative growth parameters of *Vicia faba* plant grown under drought stress condition

Treatments		Shoot system				Root system		
Water	Materials	Shoot height (cm)	Leaves number/plant	Shoot fresh weight/plant (g)	Shoot dry weight/plant (g)	Root length (cm)	Root fresh weight/plant (g)	Root dry weight/plant (g)
Well watered (90%).	Control	49.0 ± 1.73e	13.66 ± 1.15 cd	9.66 ± 0.44 g	2.58 ± 0.14e	6.33 ± 0.57e	5.40 ± 0.50de	1.64 ± 0.14c
	Chitosan	0.5	57.66 ± 66bc	18.66 ± 1.54b	12.98 ± 0.42e	3.12 ± 0.49d	7.66 ± 0.57de	1.67 ± 0.06c
		1.0	63.0 ± 1.0a	22.66 ± 3.05a	14.82 ± 0.89b	3.78 ± 0.14bc	8.00 ± 1.0cde	1.69 ± 0.04c
		2.0	60.0 ± 2.0b	19.66 ± 3.51b	14.56 ± 0.83bc	3.61 ± 0.32bc	7.33 ± 0.57e	1.53 ± 0.07c
	Chito-san NPs (mg/l)	10	53.33 ± 1.73e	18.0 ± 1.73b	13.98 ± 0.86 cd	3.91 ± 0.34b	9.33 ± 0.57 cd	1.67 ± 0.03c
		20	65.0 ± 1.73a	22.33 ± 1.15a	17.78 ± 0.92a	4.74 ± 0.29a	9.66 ± 0.57c	1.76 ± 0.03c
		30	57.33 ± 3.05c	19.66 ± 1.52b	13.57 ± 0.46de	3.80 ± 0.41bc	9.33 ± 0.57 cd	1.74 ± 0.1c
	Control	37.33 ± 1.15i	10.33 ± 0.58e	4.33 ± 0.12j	1.26 ± 0.07 g	13.0 ± 2.0b	6.75 ± 1.65ab	3.74 ± 0.09a
	Chitosan	0.5	43.0 ± 1.0gh	13.66 ± 0.58 cd	6.97 ± 0.23i	1.73 ± 0.08f	13.33 ± 0.57b	3.19 ± 0.05b
Drought stress (60%).		1.0	45.66 ± 1.15f	14.33 ± 1.15 cd	9.06 ± 0.33 g	2.36 ± 0.28e	14.33 ± 1.15ab	3.40 ± 0.19ab
		2.0	42.66 ± 0.57 h	12.0 ± 1.0de	8.74 ± 0.34 h	2.63 ± 0.51e	14.33 ± 1.15ab	3.48 ± 0.20ab
	Chito-san NPs (mg/l)	10	45.33 ± 1.15 fg	12.33 ± 1.52de	8.70 ± 0.24 h	3.08 ± 0.07d	14.66 ± 1.15ab	3.45 ± 0.18ab
		20	48.33 ± 1.15e	15.0 ± 1.0c	10.48 ± 0.26f	3.95 ± 0.10b	16.00 ± 1.0a	3.41 ± 0.75ab
		30	44.66 ± 0.57fgh	12.66 ± 0.58cde	9.65 ± 0.45 g	3.51 ± 0.23c	14.66 ± 0.57ab	3.80 ± 0.48a

Means followed by the same letter for each tested parameter are not significantly different by Duncan's test ($p \leq 0.05$) and presented by $\pm$ SD

Table 2 Impact of chitosan (g L^{-1}) and chitosan NPs (mg L^{-1}) on photosynthetic pigments and IAA of *Vicia faba* plant grown under drought stress conditions

Treatments		Chlorophyll a	Chlorophyll b	Carotenoid	Chlo a/chlo b	Total photosynthetic pigments	Indole acetic acid (ug/g fresh leaf tissues)
Water	Materials	mg/g fresh leaf tissues					
Well watered (90%).	Control	2.06 ± 0e	1.25 ± 0.0e	0.68 ± 0.002 h	1.64 ± 0.0e	4.01 ± 0.002 g	20.97 ± 0.39 h
	Chitosan	0.5	2.27 ± 0.006d	1.39 ± 0.004d	0.75 ± 0.003de	4.41 ± 0.007f	27.59 ± 0.055f
		1.0	2.37 ± 0.014b	1.46 ± 0.005b	0.77 ± 0.001bc	4.60 ± 0.020c	35.66 ± 0.015d
		2.0	2.30 ± 0.01c	1.41 ± 0.0c	0.72 ± 0.003 fg	4.44 ± 0.007e	31.16 ± 0.15e
	Chitosan NPs	10	2.39 ± 0.005b	1.47 ± 0.005b	0.77 ± 0.015bc	4.63 ± 0.016b	36.59 ± 0.24c
		20	2.48 ± 0.026a	1.55 ± 0.014a	0.79 ± 0.005a	4.82 ± 0.035a	46.52 ± 0.015a
		30	2.37 ± 0.005b	1.46 ± 0.005b	0.72 ± 0.011 fg	4.55 ± 0.022d	43.09 ± 0.44b
	Control	1.55 ± 0.016i	0.86 ± 0.012j	0.73 ± 0.0f	1.81 ± 0.04a	3.14 ± 0.005 m	10.74 ± 0.09k
	Chitosan	0.5	1.73 ± 0.02 g	0.97 ± 0.004 g	0.76 ± 0.004 cd	3.46 ± 0.020j	16.34 ± 0.50j
Drought stress (60%).		1.0	1.78 ± 0.00f	0.99 ± 0.003f	0.79 ± 0.005a	3.56 ± 0.002 h	19.59 ± 0.024i
		2.0	1.57 ± 0.018i	0.89 ± 0.002i	0.75 ± 0.006de	3.22 ± 0.010 L	16.24 ± 0.29j
	Chitosan NPs	10	1.74 ± 0.023 g	0.99 ± 0.003f	0.76 ± 0.004 cd	3.48 ± 0.022ij	16.66 ± 0.98j
		20	1.72 ± 0.0119	0.98 ± 0.001f	0.79 ± 0.004a	3.50 ± 0.008i	22.15 ± 0.50 g
		30	1.95 ± 0.005 h	0.95 ± 0.0 h	0.77 ± 0.004b	3.38 ± 0.009k	20.49 ± 0.34 h

Means followed by the same letter for each tested parameter are not significantly different by Duncan's test ($p \leq 0.05$) and presented by $\pm$ SD

1 g L^{-1} and chitosan NPs at 20 mg L^{-1} either in plants grown under well watered conditions or drought stress conditions. 20 mg L^{-1} chitosan NPs followed by 1 g L^{-1} chitosan significantly increased the total photosynthetic pigment of the well watered faba bean plant by 79.55% and 14.71% respectively relative to corresponding control. On the other hand, total photosynthetic pigment of bean plant grown under drought stress conditions was increased via 1 g L^{-1} chitosan by 13.37% followed by and chitosan NPs at 20 mg L^{-1} by 11.46% relative to the corresponding control.

3.3 Endogenous Indole Acetic acid (IAA)

Table 2 shows that drought stress conditions significantly decreased IAA by 95.25% relative to well watered conditions. Whereas, all applied treatments significantly increased IAA either in plants grown under well watered or drought stress conditions. Chitosan NPs at 20 mg L^{-1} significantly increased IAA by 121.84% in plants grown under well watered conditions and by 106.23% in plants grown under drought stress conditions.

Fig. 1 Impact of chitosan (g L^{-1}) and chitosan NPs (mg L^{-1}) on (H_2O_2) and lipid peroxidation (MDA) of *Vicia faba* plant grown under drought deficit condition. Means followed by the same letter for each tested parameter are not significantly different by Duncan's test ($p \leq 0.05$) and presented by $\pm$ SD

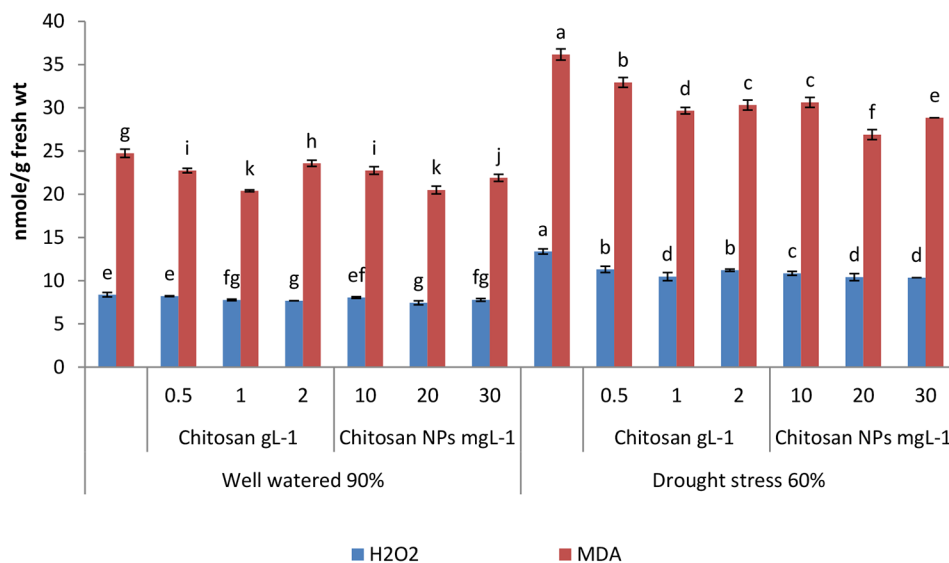
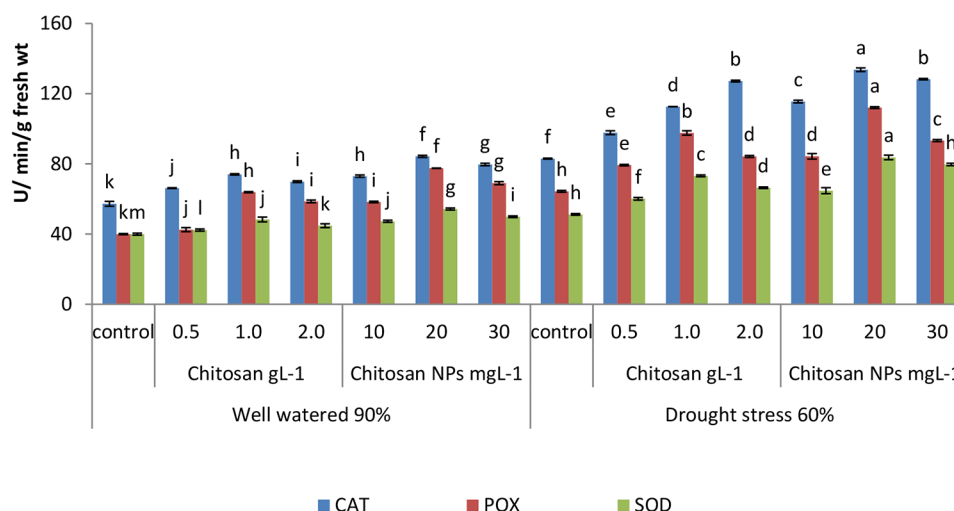


Fig. 2 Impact of chitosan (g L^{-1}) and chitosan NPs (mg L^{-1}) on some antioxidant enzymes of *Vicia faba* plant grown under drought stress conditions. Means followed by the same letter for each tested parameter are not significantly different by Duncan's test ($p \leq 0.05$) and presented by $\pm$ SD



3.4 Hydrogen Peroxide (H_2O_2) and Malondialdehyde (MDA)

Figure 1 shows that drought stress conditions significantly increased H_2O_2 by (59.66%) and MDA by (46.20%) in fresh bean leaf tissues relative to those grown under well watered conditions. Meanwhile, the applied treatments chitosan and chitosan NPs significantly decreased H_2O_2 and MDA contents in bean plants grown either under well water conditions (90%) or drought stress conditions (60%). Moreover, 20 mg L^{-1} chitosan NPs was the optimum treatment. Since, bean plants grown under well watered conditions and treated with 20 mg L^{-1} chitosan NPs was characterized by significant decreases in H_2O_2 (11.21%) and MDA (17.18%) relative to the corresponding control.

Regarding drought stress conditions and treatment with 20 mg L^{-1} chitosan NPs, it was noted more significant

decreases in H_2O_2 (22.64%) and MDA (25.63%) relative to corresponding controls.

3.5 Lipxygenase (LOX) Enzyme and Antioxidant Enzyme Activities

Figures (2, 3 and 4) shows that drought stress conditions significantly increased LOX (105.63%) and GR (44.99%) catalase (CAT) (44.96%), peroxidase (POX) (61.05%), superoxide dismutase (SOD) (28.20%) and ascorbate peroxidase (ASP) (27.03%) in fresh bean leaf tissues relative to those grown under well watered condition. All applied treatments significantly decreased LOX activity accompanied by significant increases in CAT, POX, SOD, ASP, GR activities in bean plant grown either under well water condition or drought stress conditions.

Fig. 3 Impact of chitosan (gL^{-1}) and chitosan NPs (mgL^{-1}) on lipoxxygenase and glutathione reductase of *Vicia faba* plant grown under drought stress conditions. Means followed by the same letter for each tested parameter are not significantly different by Duncan's test ($p \leq 0.05$) and presented by $\pm$ SD

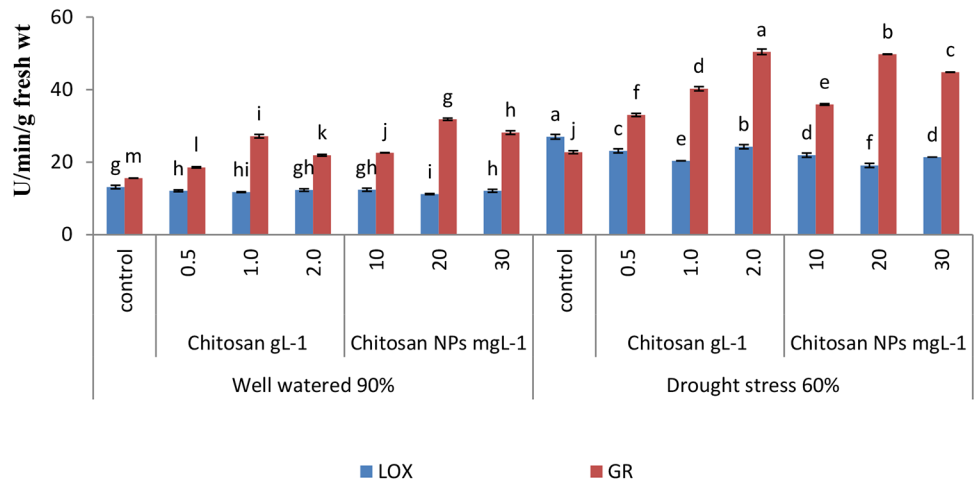
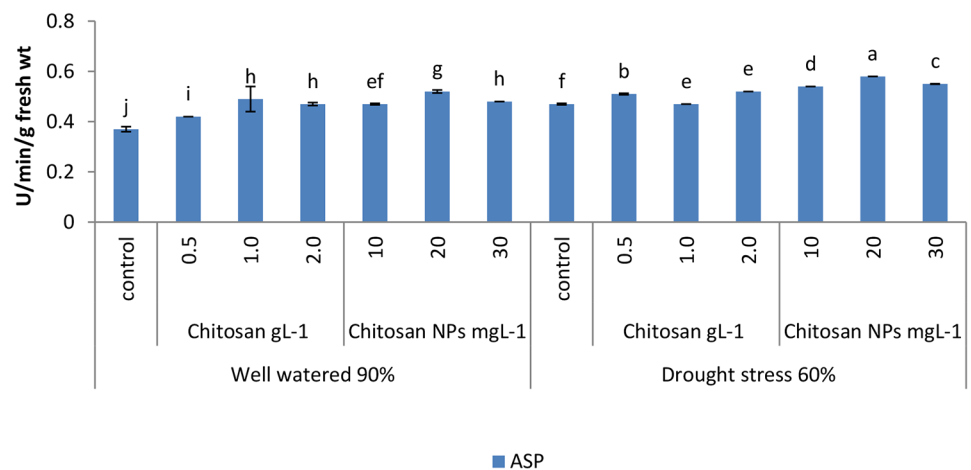


Fig. 4 Impact of chitosan (gL^{-1}) and chitosan NPs (mgL^{-1}) on ascorbate peroxidase of *Vicia faba* plant grown under drought stress conditions. Means followed by the same letter for each tested parameter are not significantly different by Duncan's test ($p \leq 0.05$) and presented by $\pm$ SD



Moreover, 20 mgL^{-1} chitosan NPs was the optimum treatment. Since, bean plant grown under well watered condition and treated by 20 mgL^{-1} chitosan NPs was characterized by significant decrease in LOX (14.49%) accompanied by significant increases in CAT (47.23%), POX (94.62%), SOD (35.89%), ASP (40.54%), and GR (101.12%) relative to corresponding controls.

Regarding drought stress conditions and treatment by 20 mgL^{-1} chitosan nanoparticles, it was noted a significant decrease in LOX (29.26%), accompanied by significant increase in CAT (61.07%), POX (74.12%), SOD (63.41%), ASP (23.40%), and GR (118.72%) relative to corresponding control. It is clear that significant decrease in LOX accompanied by significant increases in CAT, POX, SOD, ASP, GR were more pronounced in fresh leaf of bean plant grown under drought stress conditions as compared to those grown under well watered conditions.

3.6 Phenolic Compound, DPPH, Glutathione (GSH), and α -tocopherol (α -Toco)

Figure 5 shows that drought stress conditions significantly increased phenolic compounds (34.43%), DPPH (67.22%), glutathione (25.15%), and α -tocopherol (35.85%) in the dry leaf tissues of bean plants relative to a well watered condition.

All applied treatments significantly increased phenolic compounds, DPPH, glutathione and α -tocopherol in bean plants grown either under well watered conditions or drought stress conditions. Chitosan NPs at 20 mgL^{-1} was the most optimal treatment. The bean plants grown under well watered condition and treated with chitosan NPs at 20 mgL^{-1} was characterized by significant increases in phenolic compounds (62.50%), DPPH (67.15%), glutathione (46.37%), and α -tocopherol (30.62%) relative to the corresponding control. On the other hand, bean plants grown under drought stress conditions and treated with chitosan NPs at 20 mgL^{-1} were characterized by significant increases in phenolic compound (54.41%), DPPH (62.13%),

Fig. 5 Impact of chitosan (gL^{-1}) and chitosan NPs (mgL^{-1}) on phenolic content, antioxidant activity, contents of glutathione and tocopherol of *Vicia faba* plant grown under drought stress conditions. Means followed by the same letter for each tested parameter are not significantly different by Duncan's test ($p \leq 0.05$) and presented by $\pm \text{SD}$

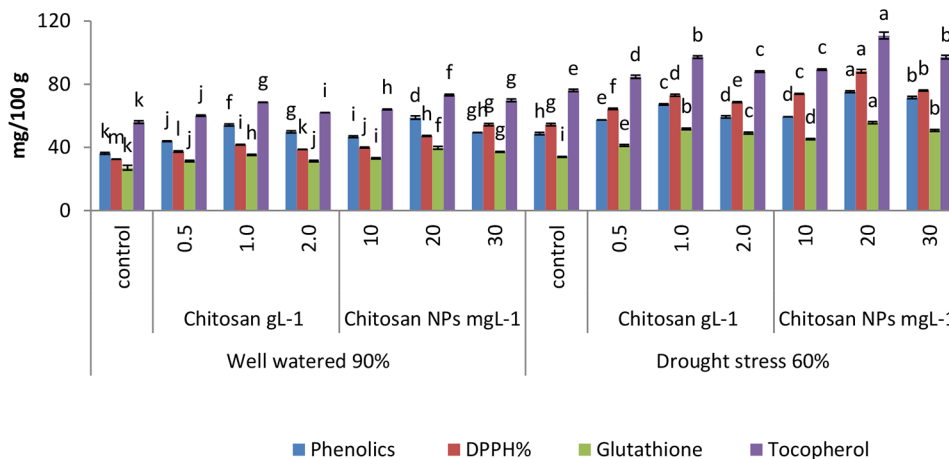
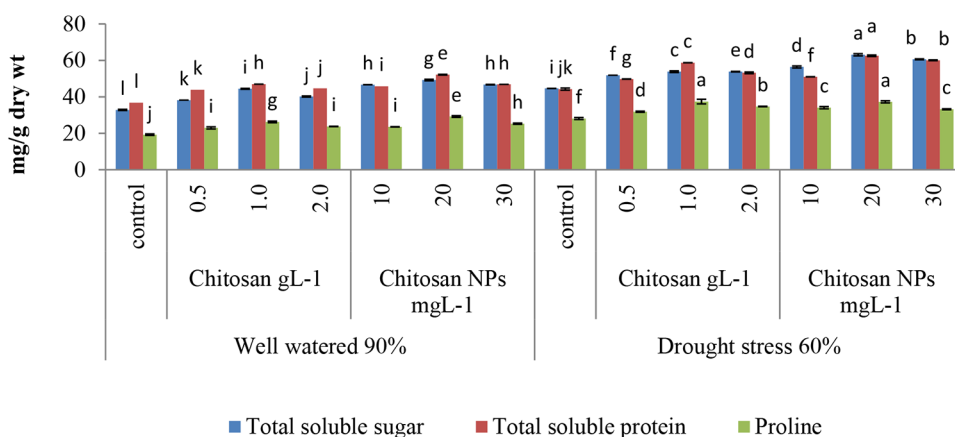


Fig. 6 Impact of chitosan (gL^{-1}) and chitosan NPs (mgL^{-1}) on total soluble sugar, total soluble protein and proline of *Vicia faba* plant grown under drought stress conditions. Means followed by the same letter for each tested parameter are not significantly different by Duncan's test ($p \leq 0.05$) and presented by $\pm \text{SD}$



glutathione (63.83%), and α -tocopherol (45.65%) relative to the corresponding control.

It is clear that, the increments in DPPH due 20mgL^{-1} chitosan NPs were more pronounced in bean plants grown under well watered conditions than those grown under drought stress conditions. On the other hand, glutathione and α -tocopherol showed opposite trends.

3.7 Total Soluble Sugar, Soluble Protein, Proline

Figure 6 shows that drought stress conditions significantly increased total soluble sugar (35.88%), total soluble protein (20.41%) and proline (45.99%) in the dry leaf tissues of bean plants relative to a well watered condition. All applied treatments significantly increased total soluble sugar, total soluble protein and proline in bean plants grown either under well watered conditions or drought stress conditions.

Chitosan NPs at 20mgL^{-1} were the most optimal treatment. The bean plant grown under well watered conditions and treated with chitosan NPs at 20mgL^{-1} was characterized by significant increases in total soluble sugar (51.96%), total soluble protein (41.94%) and proline (51.82%) relative to the corresponding control. On the other hand, bean plant

grown under drought stress conditions and treated with chitosan NPs at 20mgL^{-1} was characterized by significant increases in total soluble sugar (41.40%), total soluble protein (41.34%) and proline (32.64%), relative to corresponding controls.

4 Discussion

Drought is one of the greatest abiotic stresses that slows down plant growth and causes different metabolic variations (Bakhoum et al. 2022). Drought stress significantly influenced the growth of various metabolic pathways in *Vicia faba* plants. Exogenous treatment of chitosan and its nanoform reduced the negative influence of drought stress on faba bean plants. Low water irrigation caused variations in a variety of biochemical, physiological, and molecular attributes that reduced nutrient availability and decreased plant growth and productivity (Kapoor et al. 2020).

According to the current investigation, the faba bean plants showed lower growth parameters under drought stress. These results supported the data obtained before from different plant species (Abdelhamid et al. 2021). The

reduced effect of drought stress on shoot growth criteria of bean plants might result from disorders caused by drought stress on physiological and biochemical processes such as plant growth regulators, photosynthetic assimilation activities, and the activities of key enzymes responsible for the different vital metabolic processes (Anjum et al. 2017). Dawood and Sadak (2014) and Bakry et al. (2019) stated that both the fresh and dry weights of canola and wheat shoots decreased with decreasing water holding capacity and referred to these decreases as disorders induced by water stress and the generation of reactive oxygen species (ROS). On the other side, the increases in root growth criteria of bean plants under drought stress may be due to the adaptation of bean plants to drought stress to increase water and mineral absorption. During drought, the root system is usually elongated to improve uptake of water from the soil, whereas shoot growth is inhibited (Sharp et al. 2004).

Impaired photosynthesis in faba plants is among the significant signs of drought stress influences. These reductions are substantiated by the earlier results of Sadak and Bakhoun (2022) and Hussain et al. (2023). These decreases might be due to oxidation of photosynthetic pigments and damage to the photosynthetic apparatus which leads to a decrease in photosynthetic carbon assimilation and decreasing enzyme activities of the Calvin cycle (Din et al. 2011); as well as, increasing chlorophyll degradation by proteolytic enzymes such as chlorophyllase (Jomo et al. 2016), deterioration in chloroplast molecules and finally stomatal closure (Jomo et al. 2016). Hence, the decrease in carotenoids content under drought stress might be associated with the degradation of β -carotene (Sultana et al. 1999).

Additionally, drought stress significantly decreased the IAA contents of bean leaves. These reductions of different phytohormones as IAA might attributed be to the decreases in enzyme activity which participate in phytohormone synthesis and/or increases in enzymes that participate in its degradation (Vaseva-Gemisheva et al. 2005). Dawood and Sadak (2014) confirmed these decreases in canola leaves and attributed these decreases to the destruction of IAA by increasing the activity of IAA oxidase.

Whereas, Amir et al. (2021) mentioned that drought induced a noticeable accretion in the accumulation of total soluble sugars, proline, ascorbic acid, anthocyanins, and secondary metabolites that protect plants against oxidative damages caused by drought. Drought stress increased oxidative stress damage in terms of reactive oxygen species such as hydrogen peroxide (H_2O_2), and lipoxygenase (LOX), leading to lipid peroxidation and membrane damage. MDA content is one of the degradation products of polyunsaturated fatty acids of bio membranes and is used to evaluate lipid peroxidation and as a sign of membrane cellular damage. Drought stress constrained plant cell metabolism, such

as photosynthesis or respiration, induced the production of reactive oxygen species (ROS) as H_2O_2 and destroyed the balance between ROS generation and quenching, resulting in the accumulation of malondialdehyde (MDA) in plants (Gharibi et al. 2016). These results are in good agreement with those reported by Laxa et al. (2019), who found that the extreme drought stress increased $O_2^{\cdot-}$ production rate and H_2O_2 content of plants, resulting in higher MDA content and an increase in membrane permeability. These increases in MDA and H_2O_2 may be due to inadequate induction of the antioxidant system. In addition, increased LOX activity (Fig. 3) might have contributed to the lipid peroxidation of membrane lipids and thereby significantly participate to the oxidative damage in water-deficient plants. Increased LOX activity is responsible for the oxidation of polyunsaturated fatty acids and thus enhances lipid peroxidation under stress conditions (Sánchez-Rodríguez et al. 2012).

Under drought stress, plants have an internal strategy that decreases stress caused by oxidative stress via the scavenging of accumulated ROS (Kapoor et al. 2020). Both enzymatic and non-enzymatic antioxidants have a strong ability to scavenge excess ROS. It was intriguing to see how drought increased the antioxidant system of the faba bean plant by raising enzyme activity and non-enzymatic component concentration (Sadak and Bakry 2020). The increased catalase (CAT), peroxidase (POX), superoxide dismutase (SOD), ascorbate peroxidase (ASP), and glutathione reductase (GR) activities in faba bean. Plants under drought stress may be attributed to their resistance to stress. It is well known that superoxide dismutase (SOD) acts as a first line of defense via the detoxification of superoxide radicals to hydrogen peroxide (H_2O_2), which can be scavenged by catalase (CAT) and different classes of peroxidases, glutathione peroxidase and ascorbate peroxidase (ASX), thereby preventing oxidative damage (Noctor et al. 2000). Likewise, ascorbate peroxidase (ASP) is a key enzyme in the glutathione ascorbate pathway that helps to regenerate $NADP^+$ and converts H_2O_2 to water (Jimenez et al. 1998). H_2O_2 is reduced to H_2O via ascorbate and reduced glutathione (GSH), and as a result, oxidized glutathione (GSSG) is formed, which is recycled back to GSH by the action of glutathione reductase (GR) using NADPH as a reductant (Foyer and Noctor 2014). Enhanced GR activities in response to drought stress serve to maintain the ratio of reduced to oxidized glutathione and thus the redox potential of glutathione (Chan et al. 2013).

Abiotic stresses disrupt the dynamic equilibrium of ROS, leading to the accumulation of an excessive amount of ROS and resulting in oxidative stress. At this time, plants encourage their antioxidant defense systems to eliminate excessive ROS. The antioxidant defense system has a beneficial role in enhancing plant performance and mitigating the effects of abiotic stresses by overcoming the toxic effects of active

oxygen species and enhancing their tolerance to stress. An increase in antioxidant levels is attributed to its ability to scavenge free radicals that prevent lipid peroxidation and therefore protect the cell membrane from damage (Hasanuzzaman et al. 2020; Sadak and Ramadan 2021).

Drought induces disturbances in the metabolic process, leading to an increase in the synthesis of phenolic compounds. Phenolic compounds have a major role in the antioxidant activity of plants because they scavenge free radicals arising from their high reactivity as hydrogen or electron donors, stabilize and delocalize the unpaired electron (chain-breaking function), and have the ability to chelate transition metal ions (Krol et al. 2014). Moreover, these metabolites may participate in reactive oxygen species (ROS) scavenging mainly through the antioxidative enzymes utilizing polyphenols as co-substrates (Sgherri et al. 2003). Another mechanism underlying the antioxidative properties of phenolic compounds is their ability to decrease membrane fluidity (Gaballah et al. 2006). The strong positive correlation between total phenolics and antioxidant activity (DPPH) had already been observed in soybeans, which suggests that this increase in seed antioxidant activity is contributed by the presence of a high amount of phenolic compounds (Kumar et al. 2009).

Likewise, hydrophilic (e.g., glutathione) and lipophilic antioxidants (α -tocopherol) are essential components of the antioxidant defense system. Since glutathione and α -tocopherol (vitamin E) help to maintain the integrity of the photosynthetic membranes under oxidative stress (Munne-Bosch and Alegre 2002). Drought stress increased glutathione accumulation in sunflower seedlings (Sgherri and Navari-Izzo 1995). Tocopherols protect lipids and other membrane components by physically quenching and chemically reacting with O_2 in chloroplasts and can prevent the chain propagation step in lipid autoxidation, which makes them an effective free radical trap and protects the membranes from oxidative damage (Marquardt et al. 2015).

In response to drought stress, different physiological and biochemical mechanisms have been developed in plants to adapt to or tolerate stress. The present work shows that drought stress increased the proline, soluble protein, and total soluble sugar contents of bean plants. Proline and soluble sugars were accumulated in plant cells under drought stress and served as osmolytes to maintain and protect plant macromolecules and structures from stress injury, eventually increasing the plant's tolerance to drought stress (Wu et al. 2017 and Elewa et al. 2017). Proline acts as a nitrogen source during periods of growth inhibition and plays important roles in protecting subcellular structures (cell membrane) by reducing lipid oxidation through scavenging free radicals and protecting cellular redox potential (Ashraf and Foolad 2007). During drought, proline can act as a signaling

molecule, modulate mitochondrial function, and influence cell proliferation by triggering specific genes to recover from stress (Szabados and Savoure 2009). Soluble sugar protects plants from dehydration and oxidative injury (Wu et al. 2017) and acts as ROS scavengers to improve membrane stabilization (Hosseini et al. 2014). In addition, water stress caused a remarkable increase in sugar content that might play a role in the osmotic adjustment and increased the resistance of plants to drought stress (Keyvan 2010).

Nevertheless, foliar treatment of chitosan and chitosan NPs reduced the detrimental effects of drought stress on the growth attributes of faba beans. These results may be explained by the increased uptake of water and essential nutrients brought about by adjusting cell osmotic pressure, as well as a decrease in the accumulation of harmful free radicals due to increased antioxidant and enzyme activities (Malerba and Cerana 2020). In addition to the activation of the ROS scavenging system, enhanced stomatal conductance, and stimulated growth of xylem (Hidangmayum et al. 2019; Malerba and Cerana 2020). In addition, the hydrophilic nature of chitosan may alleviate stress effects by reducing cell water content and accelerating several biological macromolecules' activities (Chakraborty et al. 2020). Regarding chitosan NPs, using nanoparticles as nutrients in plant cells at the needed time helps enhance the release of nitrogen and phosphorus fertilizer and their uptake by plants, thus decreasing nutrient loss (Behboudi et al. 2019). Moreover, chitosan and chitosan NPs treatments improved the impaired effect of drought stress on the photosynthetic pigments of bean plants. These increases could be attributed to the role of chitosan in improving cytokinin contents that stimulate chlorophyll synthesis and/or increasing the availability of amino compounds released from chitosan (Chibu and Shibayama 2001). Likewise, chitosan increased the chlorophyll and carotenoids of plants by activating the expression of genes responsible for the biosynthesis of photosynthetic pigments (Naderi et al. 2015). Moreover, chitosan NPs might act as an efficient photocatalyst, improving photosynthetic pigments, stomatal conductance, and net photosynthetic CO_2 -fixation activity (Choudhary et al. 2017). Recently, Mosavikia et al. (2020) concluded that milk thistle seed priming with chitosan NPs improved physiological mechanisms such as the synthesis of photosynthetic pigment and antioxidant enzyme activity.

The enhanced effect of chitosan and chitosan NPs on the increased IAA contents of bean plants grown under water stress might lead to an improvement in enzyme activity and as a result increased growth parameters in treated plants. Furthermore, these increases might be due to the induced effect of chitosan on auxin-related gene expression that accelerates IAA biosynthesis and reduces IAA oxidase activity (Li et al. 2018). Exogenous chitosan and chitosan

NPs treatments decreased the H_2O_2 and MDA contents of bean plants grown under water stress. On the other hand, application of chitosan lessened the production of MDA and H_2O_2 through increasing antioxidant compounds that scavenge ROS and prevent cellular membranes from oxidative stress (Hawrylak-Nowak et al. 2020). In addition, charge-charge interactions between positively charged chitosan amine groups and negatively charged membrane phospholipids promote a signal that will lead to the octadecanoid pathway (Almeida et al. 2020). Moreover, chitosan treatments decreased LOX, these decreases may be attributed to the role of chitosan in enhancing the antioxidant defense system and decreased lipid peroxidation of membrane lipids via scavenging excess ROS produced by water deficit stress. Vanda et al. (2019) stated that treatment of *Melissa officinalis* shoots by chitosan led to a noticeable induction of phenylalanine ammonia-lyase (PAL), catalase (CAT), guaiacol peroxidase (GPX), and lipoxygenase (LOX) activities.

Regarding the increased activities of different studies on antioxidant enzymes, the activities of catalase and peroxidase in milk thistle were improved by chitosan treatment (Safikhani et al. 2018). Chitosan elicitation seems to promote the accumulation of catalase, thus corroborating their potential for improving plants' antioxidant defenses (da Silva et al. 2021). It is likely that chitosan activates superoxide dismutase and catalase involved in the detoxification of H_2O_2 in plants. Moreover, the application of antioxidants decreased the generation of free radicals and lipid peroxidation when plants were stressed (Malik and Ashraf 2012). The usefulness of elicitors as chitosan is a strategy for inducing and promoting the formation of secondary metabolites and can increase the activity of specific enzymes associated with the formation of secondary metabolites (Rao and Ravishankar 2002). Application of chitosan increased the activities of phenylalanine ammonia-lyase (PAL) and tyrosine ammonia-lyase (TAL), the key enzymes of the phenylpropanoid pathway involved in the biosynthesis of phenolic compounds (Vanda et al. 2019). Moreover, Hawrylak-Nowak et al. (2020) reported the stimulatory role of chitosan on secondary metabolites as phenolic compounds through inducing certain genes involved in the biosynthesis of secondary metabolites. Furthermore, chitosan and chitosan NPs increased the proline, soluble protein, and total soluble sugar contents of bean plants grown under drought stress. Since, chitosan application could induce a significant difference in organic acids, sugars, and amino acids in the leaves of wheat seedlings (Zhang et al. 2017) and significantly increase the soluble sugar content under severe drought in basil plants (Pirbalouti et al. 2017).

5 Conclusion

According to the results obtained in our study, drought stress (60% water irrigation) decreased faba bean growth attributes of the shoot system, including photosynthetic pigments and indole acetic acid. While it increased root system parameters, antioxidant enzymes, and antioxidant compounds in addition to some osmoprotectant compounds. These findings supported the negative impact of drought via demonstrating how it stresses faba bean plants. On the contrary, exogenous chitosan and chitosan nanoparticles treatments of faba bean plants reduced these negative effects and enhanced the growth of the plant. Chitosan and its nanoform modulated photosynthetic pigments, indole acetic acid, total soluble sugar, soluble protein, proline, phenolic compound, glutathione, α tocopherol, and antioxidant enzyme activities (catalase, peroxidase, superoxide dismutase, ascorbate peroxidase, glutathione reductase) accompanied by decreases in hydrogen peroxide, lipid peroxidation, and lipoxygenase. It was noticed in this study that 20 mg L^{-1} chitosan nanoparticles was the most optimum treatment either under well water condition (90% water field capacity) or drought stress condition (60% water field capacity). Moreover, it is obvious that the response of bean plants grown under well watered condition was more pronounced than that of those grown under drought condition to 20 mg L^{-1} chitosan nanoparticles. It can be concluded that either chitosan or its nanoform induce drought tolerance in barley plants.

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Declarations

Competing Interests The authors have no competing interests.

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