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In saline-stressed common bean, growth parameters response to chitosan treatments

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Abstract:A pot experiment was conducted to investigate the effect of foliar spray with 0.05% conventional chitosan (Cs) or chitosan nanoparticles (CsNPs), before salinity treatment on growth and photosynthetic pigments of *Phaseolus vulgaris* L. cv. Nebraska. Plants were grown on a sandy clay soil and sprayed with 0.05% Cs or CsNPs 16 and 21 days after planting before imposing salinity stress by irrigation with 0, 25, 50, 100 and 200 mM NaCl. Chitosan nanoparticles were prepared using methacrylic acid and showed a mean size of 40 ± 2 nm. Salinity stress caused deleterious effects on growth and photosynthetic pigments of *Phaseolus vulgaris*. Foliar spray with Cs or CsNPs resulted in improvement of growth and photosynthetic pigments, with alleviation of the impact of salinity stress and greater beneficial effect of CsNPs compared with Cs**.**

keywords: Chitosan; common bean; growth; chitosan nanopaticles; salinity

1.Introduction

Salt stress is considered as a severe problem for plants as it reduces growth and production. Plant metabolism was altered by decreasing water and nutrients uptake [1]. Salinity of soil is considered as one of the main problems in dry and semiarid regions that influence plant growth and production by interfering with the plant's nutritional status. The typical salinity challenges which plants display are decreased absorption of water and nutrients, impeded transport of nutrients from roots to shoots, reduced leaf area and inhibited growth of root, degraded leaf pigments like chlorophyll and carotenoids, disturbed photosynthetic machinery, with overall decreased plant growth [2-7].

The disturbed climatic conditions contributed to the salinization of the soil due to global warming and increased aridity. Responses of plants to salt stress include several changes in the activity of proteins and genes that ultimately lead to alterations in metabolism of plant [8].

The most significant legume for direct human consumption is common bean (*Phaseolus vulgaris* L.). Common bean seeds are rich in carbohydrates, protein, vitamins, and minerals. Egypt is ranked as the 6th producer of common bean worldwide, with 263.080 tones [9,10]. Because of the high protein and carbohydrates contents in common bean seeds, along with considerable contents of dietary fibers, and minerals (particularly zinc and iron), nutritionists regard them as an excellent food. Although dry beans' nutritional value and advantages for human health are widely acknowledged [11,12], their benefits to the cropping system are often overlooked. Several advantages of dry beans are found that widely contribute to agricultural sustainability within compound cropping systems.

Creative ecofriendly materials are required for new developments judged by their biodegradability, biocompatibility and variability. Accordingly, the research community is concentrating on biopolymers like chitosan (Cs) in a way to exchange old-style raw materials with new ecofriendly ones that help maintain high production rates while simultaneously lowering expenses and the impact on the environment [13]. Chitosan is

produced via deacetylation of chitin, a polysaccharide that is present in fungi, insects, and crustaceans [13,14]. Chitosan can cost-effectively protect crops from microbial pathogens and also prevent the development of highly robust pathogens. In arid climates, chitosan can help to achieve the goal of sustainable agriculture under abiotic stresses such as cold, heat, drought stresses, and salinity. It has been shown that the natural chitosan molecule triggers a variety of biological actions in plants at various stages of development [15], thus leading to improvement of plant growth [16,17].

An expanding field of science known as nanotechnology produces a variety of nanomaterials, with sizes between 1-100 nm, which can be either artificial or natural [18,19]. Due to their distinctive characteristics, such as morphology, chemical reactivity, competitive binding sites, and optical activity, nanoparticles have gained growing interest in recent years. Based on their size, shape, and structure, nanoparticles (NPs) may differ from bulk material and may have unique properties [19,20]. NPs could be used in a variety of fields, including biotechnology, electronics, food production, and agriculture [19,21,22].

One of the plant physiological processes that is most susceptible to environmental stresses is photosynthesis, which acts as the basis for all other metabolic processes. As a result, maintaining maximal photosynthetic rate is essential for plants to survive in extreme conditions. In addition to enhanced rates of photosynthesis, stomatal conductance, transpiration, water use efficiency, chlorophyll and proline content, and carbonic anhydrase activity, plants treated with nanomaterials can acquire protection against a variety of abiotic stresses [23-25]. Due to their tiny size and favorable interface and surface properties, chitosan nanoparticles (CsNPs) are more efficient than traditional chitosan [26,27]. Under abiotic stresses, especially salt stress, CsNPs may recover the growth of different plants [7, 28-30].

The main objective of this study is to assess the efficiency of foliar application of conventional chitosan or chitosan nanoparticles to alleviate the impact of salinity stress on growth and photosynthetic pigments of common bean.

2. Materials and methods

Materials

Phaseolus vulgaris L., cv. Nebraska seeds were provided by the Agricultural Research Center, Ministry of Agriculture, Mansoura city, Egypt.

Chitosan nanoparticles were prepared using methacrylic acid according to the method developed by [31-33]. The average size and zeta potential of the obtained chitosan nanoparticles were determined by measuring zeta size using Zetasizer NanoZS (Malvern Instruments, UK). One drop of the prepared chitosan nanoparticles was spread on a carbon coated grid, the gird was dried at room temperature and examined using a JEOL 1010 transmission electron microscope at 80 kV (JEOL, EM unit, Mansoura University).

A sandy-clay- soil from Mansoura city was used in the experiment. Table 1 presents the soil physical and chemical characteristics. The Before planting, super phosphate was thoroughly mixed with the soil at a rate of 1.4 g/kg.

Growth conditions

The experiment was carried out in the Botanical Garden at the Faculty of Science Mansoura University, Egypt, throughout the period November 2021 to February 2022.

The seeds were surface sterilized by soaking in 10^{-3} M $HgCl_2$ for 3 minutes, followed by sterile water washings. The seeds were then seeded in pots of 10 kg capacity, full of soil clay-sandy soil (2:1, v/v). ten seeds per pot.

Table (1): Characteristics of the experimental soil

Physical properties				
Soil separate (%)			**% WHC**	**% Porosity**
Sand	Silt	Clay		
17.86	27.40	54.74	30.00	58.50
Chemical properties				
pH	EC ($dS.m^{1}$)	O.C(%)	Total N ($meqL^{-1}$)	Na^{+}
8.05	0.93	0.83	48.22	1.90
K^{+}	Ca^{2+}	Mg^{2+}	P	
($meqL^{-1}$)			($mgkg^{-1}$)	
1.30	3.10	3.00	38.80	

WHC; water holding capacity, EC; electrical conductivity, O.C; organic carbon.A total of 75 pots were used, which were divided into three

equal groups. The first group was sprayed twice with distilled water to serve as a control for the chitosan pretreatment. The second and third groups were sprayed twice (16 and 21 days from planting) with 0.05% aqueous chitosan (Cs) or chitosan nanoparticles (CsNPs), respectively. To prevent chitosan or chitosan nanoparticles from entering the soil, the pots were covered with plastic covering during spray. Two days from the second spray, seedlings were thinned to seven seedlings per pot and salinity treatment started. Seedlings were irrigated with 0, 25, 50, 100 and 200 mM NaCl for four times, at 23, 26, 29, and 32 days after planting; meanwhile plants received tap water every third day to prevent buildup of salt in the soil.

Plant harvest and analysis

After 44 days from planting (12 days from salinity treatment), plants which started flowering, were harvested for assay of growth and photosynthetic pigments. Growth was estimated in terms of the fresh and dry weights of shoot and root, plant height, root length, number of leaves and flowers and leaf area.

Estimation of photosynthetic pigments

Photosynthetic pigments (Chl a, Chl b and carotenoids) were estimated using spectrophotometric method as reported by [34].

Experimental design and statistical analysis

The experiment had five replications in a completely randomized design. The obtained data were statistically analyzed using one-way analysis of variance (ANOVA) with Post Hoc Duncan test. using COSTAT software version 6.3. To increase precision of the experiment, out of the five replicates, three replicates were used in statistical analysis.

3. Results and Discussion

Characterization of chitosan nanoparticles

Transmission electron microscopy of the prepared chitosan nanoparticles (CsNPs) revealed spheres with a mean diameter of 40 ± 2 nm and average zeta potential of 30.10 mV (Figure 1).

Cs molecules in solution are in cationic electrolytic form, which tends to make the formation of specific structures via electrostatic reactions with methacrylic acid easy and leads to the makeup of CsNPs through polymerizing methacrylic acid in the presence of Cs [31,33].

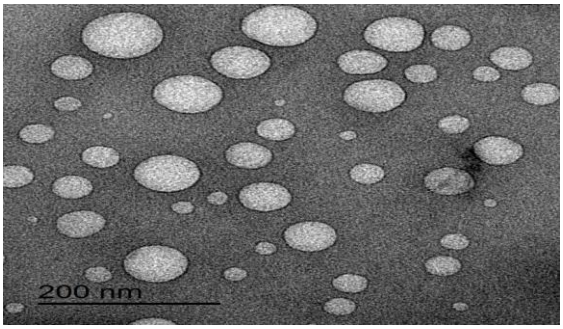

Figure (1): Transmission electron micrograph of CsNPs.

Changes in common bean growth and development

The addition of Cs alone showed a significant increase in shoot length, shoot fresh weight, shoot dry weight, shoot water content and leaf area and a non-significant increase in root length, root fresh weight, root water content, leaf number and flower number when compared by control. In the same manner, treatment with CsNPs alone showed more significant rise in shoot length, root length, shoot fresh weight, shoot dry weight, shoot water content, root dry weight, root water content, flower number and leaf area and a non-significant increase in root fresh weight and leaf number when compared by control (Tables2,3).

As compared with control, treatment of common bean plants with 25 mM level of salinity showed a significant decrease in shoot length, leaf area and root water content but a non-significant decrease in shoot fresh weight, shoot dry weight, shoot water content, root length, root fresh weight and root dry weight whereas leaf and flower number per plant were unchanged when compared by control. However, plants treated with the following sequence of treatments: 200 mM > 100 Mm > 50 mM NaCl induced a significant reduction in shoot length, root length, shoot fresh weight, shoot dry weight, shoot water content, root fresh weight, root dry weight, root water content and leaf area but a non-significant reduction in leaf number and flower number except in case of 50 mM NaCl showed a non-significant decrease in root fresh weight and root dry weight (Tables 2,3).

In relation to 25, 50 and 100 mM NaCl treatments, treatment with these salinity concentrations combined with Cs exposed a significant increase in shoot length, root length, shoot fresh weight, shoot dry weight, shoot water content, root water content and leaf area but a non-significant increase in root fresh weight, root dry weight, leaf number and flower number. Also, treatment with 200 mM NaCl combined with Cs showed a significant increase in shoot length, root length, shoot fresh weight, shoot water content, root dry weight, root water content and leaf area but a non-significant increase in shoot dry weight and root fresh weight whereas leaf number and flower number were unchanged when compared with 200 mM NaCl treatment (Tables 2,3).

In the same manner, Common bean plants treated with 25, 50, 100 and 200 mM NaCl combined with CsNPs displayed a significant progression in shoot and root length, shoot fresh weight, shoot dry weight and shoot water content, root dry weight, root water content and leaf area but a non-significant increase in root fresh weight, leaf number and flower number when compared with 25, 50, 100 and 200 mM NaCl treatments (Tables 2,3).

Salinity has a number of physiological effects on plant growth. Osmotic perturbations represent the first phase of the growth response to salt stress leading to symptoms of water stress [35-37]. Leaf growth is slightly inhibited, and photosynthetic pigments degenerate as a result of salt stress and inhibited water uptake [37-39]. High soil salinity poses a serious danger to global agriculture by substantially reducing crop growth and yield worldwide. Engineered nanoparticles (NPs) have gained recognition as a possible alternative to traditional practices in recent years for reducing the impact of abiotic stress including salt stress[37].

Application of CsNPs to periwinkle plants improved plant growth. Chitosan's ability to modulate a number of metabolic pathways, including nitrogen and carbon metabolism, is probably the cause of growth-promoting effect [27,40] and promotes the signaling of certain hormones, including auxin and gibberellins, thereby contributing to the stimulation of growth.

Changes in photosynthetic pigments

As compared with control, plants treated with Cs alone showed a significant rise in Chl a and total pigment contents but a non-significant rise in Chl b and carotenoids. In the same manner, treatment with CsNPs displayed a significant rise in Chl a, Chl b and total pigment contents but a non-significant rise in carotenoids (CsNPs > Cs) (Table 4).

In relation to control, plants treated with 25 mM NaCl exposed a significant decrease in Chl a and Chl b but a non-significant decrease in carotenoids and total pigment contents. However, plants treated with 50 mM NaCl showed a significant decrease in Chl a, Chl b and carotenoids but a non-significant decrease in total pigment contents. On the other hand, treatment with 100 and 200 mM NaCl exposed a significant reduction in Chl a, Chl b, carotenoids and total pigment contents (Table4).

Common bean plants treated with 25 mM NaCl combined with Cs or CsNPs showed a significant increase in Chl a, Chl b and total pigment contents (CsNPs > Cs), whereas carotenoids exposed an increase which was significant with CsNPs but non-significant with Cs treatment when compared with 25 mM NaCl treatment (Table 4).

Treatment with 50 mM combined with Cs or CsNPs and 100 mM combined with Cs or CsNPs showed a significant rise in Chl a, Chl b, carotenoids and total pigment contents when compared with 50 and 100 mM salinity treatments. However, plants treated with 200 mM NaCl combined with Cs or CsNPs displayed a significant increase in Chl a, Chl b and carotenoids but a non-significant increase in total pigment contents (CsNPs> Cs) when compared with 200 mM salinity level treatment (Table 4).

The results of past studies, which found that the application of chitosan or CsNPs improved growth and counteracted growth loss caused by salt exposure in a variety of species, including milk thistle, are supported by the current findings [27,41], sugarcane [27,42], mung bean [27,43] and maize [27,44].

Additionally, salt stress decreased the amount of chlorophyll in the leaves, confirming earlier findings from multiple researchers who had seen comparable declines in several species when exposed to salt [27,45-49]. Chlorophyll reduction under these conditions might result from the chlorophyllase enzyme, which is involved in the breakdown of chlorophyll, being activated by salt [27,48]. Also, it was noted that a decrease in the amount of chlorophyll in *C. roseus*, as well as in four other ornamental species [27,50]. The fact that this treatment enhanced chlorophyll may help to explain how CsNPs mitigated the negative effects of salinity on plant development. Higher chlorophyll content has been discovered to be related to improved growth [27,51]. Following chitosan treatment, increased chlorophyll content has also been noted [27,44]. Compared to typical chitosan, CsNPs are more effective because of their small size and favourable interface and surface qualities [26,27].

Table (2): Effect of foliar application with chitosan or chitosan nanoparticles (Before salinity treatment) on growth variables of shoot: length (cm/plant), fresh weight (g/plant), dry weight (g/plant), water content (g/plant), leaf number/plant, flower number/plant and leaf area (cm^2/plant) of bean plants treated with different concentrations of NaCl at flowering stage (44 days from planting). Means (of three replicates), in each column, followed by similar letter are not significantly different at the 5% probability level-using Post Hoc. Duncan test

Treatment	Shoot						
	Length	Fresh weight	Dry weight	Water content	Leaf number	Flower number	Leaf area
C	59.80^{h}	10.66^{g}	1.92^{cd}	8.74^{f}	6.00^{a}	8.00^{bcd}	386.58^{c}
Cs	70.60^{e}	13.87^{c}	2.49^{b}	11.38^{c}	7.00^{a}	10.00^{abc}	416.10^{b}
CsNPs	85.30^{a}	15.97^{a}	2.79^{a}	13.19^{a}	7.00^{a}	12.00^{a}	429.84^{a}
25 mM NaCl	54.70^{i}	10.50^{g}	1.85^{de}	8.65^{fg}	6.00^{a}	8.00^{bcd}	336.48^{g}
25 mM NaCl +Cs	60.85^{g}	12.01^{d}	2.14^{c}	9.86^{d}	7.00^{a}	9.00^{abcd}	357.96^{e}
25 mM NaCl +CsNPs	79.50^{b}	14.80^{b}	2.65^{ab}	12.15^{b}	7.00^{a}	11.00^{ab}	378.18^{d}
50 mM NaCl	48.40^{l}	9.99^{h}	1.60^{ef}	8.39^{g}	5.00^{a}	7.00^{cd}	332.12^{h}
50 mM NaCl +Cs	53.60^{j}	11.07^{f}	1.90^{cd}	9.17^{e}	6.00^{a}	8.00^{bcd}	343.77^{f}
50 mM NaCl +CsNPs	78.45^{c}	12.06^{d}	2.16^{c}	9.90^{d}	7.00^{a}	10.00^{abc}	358.72^{e}
100 mM NaCl	43.50^{n}	8.76^{i}	1.37^{fg}	7.39^{h}	5.00^{a}	7.00^{cd}	290.15^{l}
100 mM NaCl +Cs	49.77^{k}	10.56^{g}	1.74^{de}	8.82^{f}	6.00^{a}	8.00^{bcd}	322.35^{i}
100 mM NaCl+CsNPs	75.50^{d}	11.40^{e}	2.00^{cd}	9.40^{e}	6.00^{a}	9.00^{abcd}	335.15^{g}
200 mM NaCl	41.00^{o}	7.20^{k}	1.19^{g}	6.01^{j}	5.00^{a}	6.00^{d}	257.36^{m}
200 mM NaCl +Cs	45.98^{m}	7.85^{j}	1.25^{g}	6.60^{i}	5.00^{a}	6.00^{d}	298.79^{k}
200 mM NaCl +CsNP	68.50^{f}	10.99^{f}	1.84d^{e}	9.15^{e}	6.00^{a}	8.00^{bcd}	309.11^{j}

Table (3): Effect of foliar application with chitosan or chitosan nanoparticles (Before salinity treatment) on growth variables of root: length (cm/plant), fresh weight (g/plant), dry weight (g/plant) and water content (g/plant) of bean plants treated with different concentrations of NaCl at flowering stage (44 days from planting). Means (of three replicates), in each column, followed by similar letter are not significantly different at the 5% probability level-using Post Hoc. Duncan test.

Treatment	Root			
	Length	Fresh weight	Dry weight	Water content
C	8.80^{fg}	1.83^{abc}	0.20^{def}	1.63^{bc}
Cs	8.90^{ef}	1.88^{ab}	0.22^{cd}	1.66^{ab}
CsNPs	11.85^{a}	1.97^{a}	0.28^{a}	1.69^{a}
25 mM NaCl	8.50^{g}	1.64^{bcde}	0.19^{defg}	1.45^{f}
25 mM NaCl +Cs	8.88^{f}	1.72^{abcd}	0.22^{cd}	1.50^{de}
25 mM NaCl +CsNPs	10.90^{b}	1.86^{abc}	0.26^{ab}	1.60^{c}
50 mM NaCl	8.10^{h}	1.54^{cdef}	0.18^{efg}	1.36^{g}
50 mM NaCl +Cs	8.74^{fg}	1.70^{abcd}	0.21^{cde}	1.49^{ef}
50 mM NaCl +CsNPs	10.55^{c}	1.78^{abc}	0.24^{bc}	1.54^{d}
100 mM NaCl	7.55^{i}	1.21^{gh}	0.16^{gh}	1.05^{jk}
100 mM NaCl +Cs	8.13^{h}	1.32^{efgh}	0.19^{defg}	1.12^{i}
100 mM NaCl +CsNPs	9.65^{d}	1.44^{defg}	0.21^{cde}	1.23^{h}
200 mM NaCl	7.05^{j}	1.08^{h}	0.13^{h}	0.95^{l}
200 mM NaCl +Cs	7.77^{i}	1.23^{fgh}	0.17^{fg}	1.02^{k}
200 mM NaCl +CsNPs	9.20^{e}	1.35^{efgh}	0.19^{defg}	1.09^{ij}

Table (4): Effect of foliar application with chitosan or chitosan nanoparticles (Before salinity treatment) on different pigment fractions (Chla, Chl b, Chl a+b, Chl a/b, Cars and total pigments) (mg/ g f.wt.) of bean plants treated with different concentrations of NaCl at flowering stage (44 days from planting). Means (of three replicates), in each column, followed by similar letter are not significantly different at the 5% probability level-using Post Hoc. Duncan test.

Treatment	Chl a	Chl b	Chl a+b	Chl a/b ratio	Cars	Total pigments
C	1.90^{g}	0.58^{bc}	2.48^{bcdef}	3.28^{h}	1.01^{abc}	3.49^{cde}
Cs	2.26^{b}	0.60^{b}	2.86^{ab}	3.77^{ef}	1.02^{ab}	3.88^{b}
CsNPs	2.47^{a}	0.65^{a}	3.12^{a}	3.80^{e}	1.03^{a}	4.15^{a}
25 mM NaCl	1.86^{h}	0.53^{ef}	2.39^{bcdef}	3.51^{gh}	0.99^{c}	3.38^{de}
25 mM NaCl +Cs	2.17^{d}	0.56^{cd}	2.73^{abcd}	3.88^{de}	1.00^{bc}	3.73^{bc}
25 mM NaCl +CsNPs	2.23^{bc}	0.57^{c}	2.80^{abc}	3.91^{cde}	1.02^{ab}	3.82^{b}
50 mM NaCl	1.81^{i}	0.51^{fg}	2.32^{bcdef}	3.55^{fg}	0.95^{d}	3.27^{ef}
50 mM NaCl +Cs	2.12^{e}	0.54^{de}	2.66^{abcde}	3.93^{cde}	0.99^{c}	3.65^{bcd}
50 mM NaCl +CsNPs	2.21^{c}	0.56^{cd}	2.77^{abcd}	3.95^{cde}	1.00^{bc}	3.77^{bc}
100 mM NaCl	1.76^{j}	0.45^{h}	2.21^{cdef}	3.91^{cde}	0.85^{f}	3.06^{fg}
100 mM NaCl +Cs	2.04^{f}	0.49^{g}	2.53^{abcdef}	4.16^{c}	0.90^{e}	3.43^{de}
100 mM NaCl +CsNPs	2.14^{de}	0.52^{ef}	2.66^{abcde}	4.12^{cd}	0.93^{d}	3.59^{bcd}
200 mM NaCl	1.65^{l}	0.33^{j}	1.98^{f}	5.00^{a}	0.81^{g}	2.79^{g}
200 mM NaCl +Cs	1.71^{k}	0.36^{i}	2.07^{ef}	4.75^{b}	0.85^{f}	2.92^{g}
200 mM NaCl +CsNPs	1.80^{i}	0.38^{i}	2.18^{def}	4.74^{b}	0.89^{e}	3.07^{fg}

Conclusion

In conclusion, our study approved that salinity stress caused a decrease in all growth parameters and pigments contents due to different reasons as Na^{+} and Cl^{-} toxicity.

Plants treated with foliar application with 0.05% Cs or CsNPs before salinity stress showed an increase in all growth and developmental parameters, also represented an improvement in photosynthetic pigments content and greater beneficial effect of CsNPs compared with Cs.

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