Article

Chitosan nanoparticles improve the nutraceutical quality of triticale sprouts

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Abstract

The use of chitosan nanoparticles (NPs CS) has become a promising alternative in modern agriculture as an inducer in the biosynthesis of secondary metabolites. The objective of this research was to evaluate the effect of NPs CS on the nutraceutical quality of triticale sprouts (x *Triticosecale* Wittmack). Increasing doses of NPs Cs: 0, 0.1, 0.2, 0.4 and 0.8 mg ml^{-1} were applied only once at the imbibition stage, then they were left in Petri dishes for 7 days at 25 ±2 °C temperature. NPs CS did not affect germination or fresh root weight at the tested concentrations, and the concentration of 0.1 mg ml^{-1} increased the fresh weight of the shoots up to 83.3%. In the presence of 0.8 mg ml^{-1} of NPs CS phenolic compounds decrease by 7% and flavonoids increase by 29%. The results confirm a promoter effect of NPS CS on sprouts, opening the possibility of being used as inducers in the biosynthesis of bioactive compounds in triticale sprouts.

Keywords: x *Triticosecale* Wittmack, nanotechnology, secondary metabolites

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Introduction

Nanotechnology is an alternative in modern agriculture by producing agro-products such as nanofertilizers, nanopesticides, nanoherbicides and nanosensors, which allow to increase food yield in a sustainable way and reduce the environmental impact (Lira *et al*., 2018). The use of nanomaterials is of great interest for its study due to its size and the applications that they can have thanks to the physical and chemical properties, which acquire at the nanometric scale compared to the micro-sized material (Hojjat and Hojjat, 2015).

Among these nanomaterials, chitosan nanoparticles (NPs CS) are of great interest as they are obtained thanks to the versatility of the chitosan and availability of functional groups (amino, -NH_2) (Salachna and Zawadzińska, 2014; Kumaraswamy *et al*., 2018), nontoxicity, biocompatibility and biodegradability (Divya *et al*., 2019).

In this sense NPs CS have become a promising alternative in seed priming, due to their high biological activity since NPs CS interact in conjunction with the living cell (Pedroso, 2017; Divya and Jisha, 2018; Souza *et al*., 2019), thus causing the synthesis of several biomolecules as inducers that force the sprout to react to them consequently developing a greater synthesis of secondary metabolites (Hidangmayum *et al*., 2019; Paramo *et al*., 2020), which has been shown in tomato, rice and wheat sprouts (Colman *et al*., 2019; Divya *et al*., 2019; Li *et al*., 2019).

Sprouts, on the other hand, are a source of carbohydrates, fiber, vitamins, essential nutrients and bioactive compounds, which have been linked to prevention and treatment of diseases. The presence of these compounds, in sprouts, can be increased by production conditions, seed quality and germination conditions (Dziki *et al*., 2015).

Recent advances in the use of nanotechnology in agriculture allow to try to understand the role of NPs Cs in triticale sprouts (x *Triticosecale* Wittmack), as it currently presents an interesting increase of 30% in food production worldwide (Aquino and Gómez, 2019). NPs CS are a promising material for seed treatments, so the goal of this work was to evaluate the effect of NPs CS on the synthesis of bioactive compounds in triticale sprouts.

Materials and methods

This study was conducted in a Biotechnology laboratory located at the Technological Institute of Torreón, Mexico in latitude 24° 30’ and 27 north latitude, 102° 00’ and 104° 40’ west longitude.

Synthesis of chitosan nanoparticles (NPs CS)

NPs CS were synthesized by the ion gelation method at the Applied Chemistry Research Center (Saltillo, Coahuila), using chitosan (Marine, Hydrocolloids, Kerala, India) and sodium tripolyphosphate (TPP) (Sigma-Aldrich, USA) as crosslinker in a ratio of 10:3 v/v of CS: TPP (Kumaraswamy *et al*., 2018) with spherical shape and a particle size of 111 ±21 nm, which were

characterized by UV-vis, observing an absorption of 195 nm (Figure 1) and by infrared spectrophotometry (FTIR by ATR) where the characteristic bands of the amino group (NH2) at 3 346 cm^{-1} and carboxyl (C=O) at 1 635 cm^{-1} (Figure 2) were observed, coinciding with the chemical structure of the compound (Manikandan and Sathiyabama, 2016).

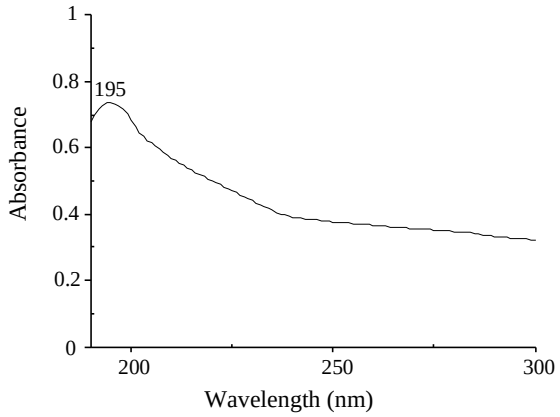

Figure 1. Absorbance spectra of NPs CS ratio CS:TPP 10:3 v/v.

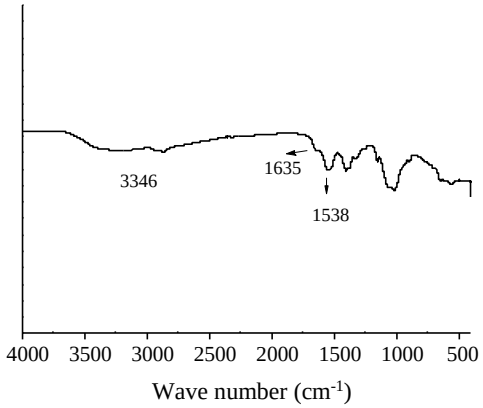

Figure 2. Spectra of FTIR by ATR of NPs CS ratio CS:TPP 10:3 v/v.

Plant material

Triticale seeds (x *Triticosecale* Wittmack L) were used, of uniform size, which were disinfected with 75% ethanol for 5 min and washed four times with distilled water (Li *et al*., 2019).

Germination test and growth measurement

The seeds were divided into five main treatments of ten seeds: (witness) distilled water, 0.1, 0.2, 0.4 and 0.8 mg ml^{-1} of NPs CS (Colman *et al*., 2019; Li *et al*., 2019), the treatments were applied only once to the seed during the imbibition stage, the seeds of triticale were soaked with the corresponding treatment solutions for 8 h in the dark.

Subsequently for germination ten seeds were placed per Petri dish, which had a double layer of Whatman filter paper #1 pre-soaked with 5 ml of distilled water, the Petri dishes were sealed with duct tape and placed in an artificial growth incubator (HGZ-150) with a day/night cycle of 12 h, at 25 ±2 °C respectively, with 60% relative humidity (Li *et al*., 2019).

The germination of seeds was calculated daily in accordance with the International Seed Testing Association (ISTA) guidelines and growth parameters were recorded for seven days. The germination of seed was verified when the length of the germ reached half the length of the seed (Faraji *et al*., 2018).

Parameters evaluated during the development of the bioassay

Germination percentage

It was determined seven days after sowing in the second count, for which the total count of germinated seeds was considered, and the result was expressed as shown in equation:

$$\text{Percentage of germination} = \left(\frac{\text{number of germinated seeds}}{\text{number of incubated seeds}}\right) \text{x } 100 \quad (1)$$

Seed vigor

On the fourth day after sowing, the first count was performed to collect data of the germinated seed (seedlings that have well developed root and plumule, with total development of 2 cm on average). To determine the vigor of the seed, expressing the result in percentage according to the formula:

$$\text{Seed vigor} = \left(\frac{\text{normal seedlings}}{\text{number of incubated seeds}}\right) \text{ x } 100 \quad (2)$$

Fresh weight, shoot and root

The fresh weight of the shoot and root were recorded in an analytical balance (ADN model HR-200®) to determine the value of fresh biomass and it was reported in milligrams per sprout (Martínez *et al*., 2019).

Photosynthetic pigments

The chlorophyll content (Chl) in triticale sprouts was performed according to the method described by Liu *et al*. (Liu *et al*., 2013). For which 0.5 g of sprouts were weighed, which were homogenized in a mortar with 10 ml of 95% ethanol. The homogenized was centrifuged at 1 500 rpm for 20 min and the supernatant was collected, to then measure the absorbance-to-absorbance at 665 and 649 nm, respectively. The Chl content was calculated according to the following formula:

$Chl(a)=1395A_{665}-6.88A_{649}$. $Chl(b)=24.95A_{649}-7.32A_{665}$. $Chl(a+b)=Chl(a)+Chl(b)$.

$$\text{content of Chl } \left(\frac{\text{mg}}{\text{g}}\text{PF}\right) = \frac{\text{Chl(a+b) * Volum of the extraction (mL)*time of the dilution}}{\text{Fresh weight (g)}} \quad (3)$$

Preparation of extracts for nutraceutical quality

To obtain the extracts, 2 g of fresh sample were mixed in 10 ml of 80% ethanol, with constant orbital shaking for 24 h at 70 rpm at 5 °C. Subsequently the extracts were centrifuged at 3 000 rpm for 5 min and the supernatant was extracted for analysis (Salas *et al*., 2016).

Total content of phenols

It was determined by a modification of the Folin-Ciocaltea method (Singleton *et al*., 1999), 50 µl of the ethanolic extract, diluted in 3 ml of distilled water, were taken and 250 µl of the Folin-Ciocalteau reagent (1N) were added, stirred and left to react for 3 min. Subsequently 750 µl of Na_2CO_3 (20%) and 950 µl of distilled water were added, the solution was left to rest for 2 h, to later be quantified in a UV-Vis spectrophotometer at 760 nm.

Solutions of gallic acid were used to build the calibration curve. The results were expressed as mg equivalents of gallic acid (AGE) 100 g^{-1} fresh weight.

Total flavonoids

They were determined by colorimetric method (Colina, 2016), 250 µl of ethanolic extract were taken, they were mixed with 1.25 ml of distilled water and 75 µl of NaNO2 (5%). After 5 min of rest, 150 µl of AlCl3 (1-ethyl-3-methylimidazolium chloride-aluminum chloride) (Sigma-Aldrich, St. Louis, MO, USA) were added.

Subsequently 500 µl of NaOH (1M) and 275 µl of distilled water were added, the samples were stirred vigorously, to then be quantified in a UV-Vis spectrophotometer at 510 nm. The standard was prepared with quercetin dissolved in absolute ethanol to obtain the calibration curve. The results were expressed in mg QE 100 g^{-1} fresh weight.

Statistical analysis

The experiment was conducted by a completely random design with five treatments and ten repetitions. The results obtained were analyzed by analysis of variance and comparison of means with the Tukey test ($p \leq 0.05$) using the Statistical Analysis System Institute (SAS) version 9.3 statistical package. The normality of the data for each response variable was verified with the Kolmogorov-Smirnov test, the data of the germination percentage and the variables of antioxidant capacity (both expressed as a percentage) were normalized by applying arcsine and square root transformation.

Results and discussion

Germination of seeds

Germination percentage and fresh weight are some of the main properties involved in the physiological quality of the seed (Morales *et al*., 2017). The results of this work show that the germination percentage and fresh root weight variables showed no significant difference ($p > 0.05$) at the different concentrations that were applied of NPs Cs; however, they caused a significant

difference in the vigor and fresh weight of the shoot (Table 1), with the seed vigor showing a decrease of 9.75% (0.1 mg ml^{-1}), contrary to what Colman *et al*. (2019) reported in germinated tomato seeds treated with 0.1 mg ml^{-1}, with positive effects on the rate of vigor with respect to the control.

Table 1. Comparison of means for germination and vigor percentage, fresh weight of shoot and root of triticale seeds treated with NP CS.

NPs CS (mg ml^{-1})	Germination	Vigor	Shoot fresh weight	Root fresh weight
	(%)		(mg)	
Control	86 ±0.89 a	82 ±0.81 a	38.7 ±0.32 b	74.2 ±0.28 a
0.1	82 ±0.85 a	74 ±0.86 c	70.4 ±0.15 a	79.6 ±0.98 a
0.2	96 ±1.02 a	80 ±0.8 ab	55.2 ±0.25 ab	53.2 ±0.31 a
0.4	88 ±1.05 a	76 ±0.84 c	64.8 ±0.58 a	73.8 ±0.42 a
0.8	88 ±0.98 a	80 ±0.82 ab	56.0 ±0.35 b	56.8 ±0.45 a

Values with equal letters in each column are the same according to the Tukey test ($p \leq 0.05$). The values are the average of five repetitions. Means (n= 5) ± standard deviation.

As for the accumulation of biomass, the fresh weight of the shoot increased up to 81% with the dose of 0.1 mg ml^{-1}, which confirms the positive effect of NPs CS at low concentrations on the germination of triticale seeds, this effect could be attributed to the stimulating capacity of the metabolic activity of NPs CS, achieving an increase in intrinsic potential in the development of the seed with the absorption of NPs CS (Divya *et al*., 2019; López *et al*., 2019), either by imbibition, coating or priming of seeds (Costales *et al*., 2020).

Several studies with high concentrations of NPs Cs or CS have described an inhibition of root growth and alternatively, a promotion in the appearance of a greater number of seminal roots (Colman *et al*., 2019; López *et al*., 2019), as shown in Figure 3, attributing it to the stimulation of phytohormone synthesis (López *et al*., 2019) and the activation of defense genes (Rodríguez *et al*., 2019) as has been observed in various crops such as tomato (Colman *et al*., 2019; Solórzano, 2019), salicornia bigelovii (López *et al*., 2020) (Lanchimba, 2019), sorghum (Holguin *et al*., 2020), rice (Divya *et al*., 2019) and wheat (Li *et al*., 2019), among others.

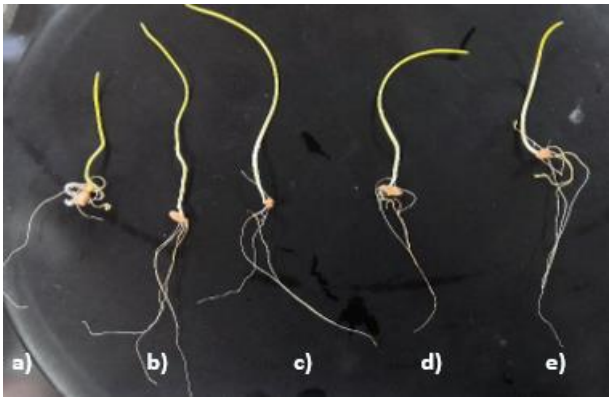

Figure 3. Germination of triticale seeds in response to the application of NPs CS in concentrations of: a) control; b) 0.1; c) 0.2; d) 0.4; and e) 0.8 mg mL^{-1}.

Photosynthetic pigments

The Chl content of the sprouts showed that the dose of 0.2 mg ml^{-1} of CS NPs has a significant effect, since it increased 59% over the control (Figure 4). According to the work reported by Acharya *et al*. (2020), they mention that the use of nanoparticles in seed priming would cause toxicity in sprouts at high concentrations, a decrease in photosynthetic pigments may be observed (Acharya *et al*., 2020) due to the rupture of the chlorophyll and inhibition of the enzyme p-hydroxyphenylpyruvate in the biosynthesis of chlorophyll (Miras, 2018), produced by the stress caused by nanoparticles.

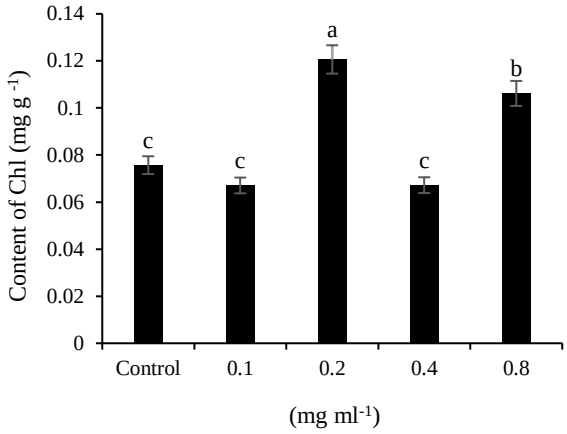

Figure 4. Effect of NPs CS on chlorophyll content on wheat sprouts. Average values in columns with different literals differ statistically between them (Tukey $p \leq 0.05$).

On the contrary, this work showed that priming with NPs CS increases photosynthetic pigments at low concentrations compared to the control. However, it is difficult to establish a response model of the effects of NPs on the sprouts as the effect depends on the species, concentration and type of NPs (Arruda *et al*., 2015).

Nutraceutical quality: total phenols and total flavonoids

The use of NPs CS can act as an inducer of metabolic processes as they may increase the content of bioactive compounds prominent in the development and stimulating effect of the production of secondary metabolites (Xoca *et al*., 2017; Xoca *et al*., 2019; Montalvo *et al*., 2020).

The results of this research show that variables related to nutraceutical quality in triticale sprout: total phenols and total flavonoids are affected by the high applied dose of NPs CS (0.8 mg ml^{-1}), phenolic compounds decreased compared to the control up to 7% (Figure 5a), and flavonoids increased by 29% over the control (Figure 5b), corroborating the positive effect of NPs CS on triticale sprouts.

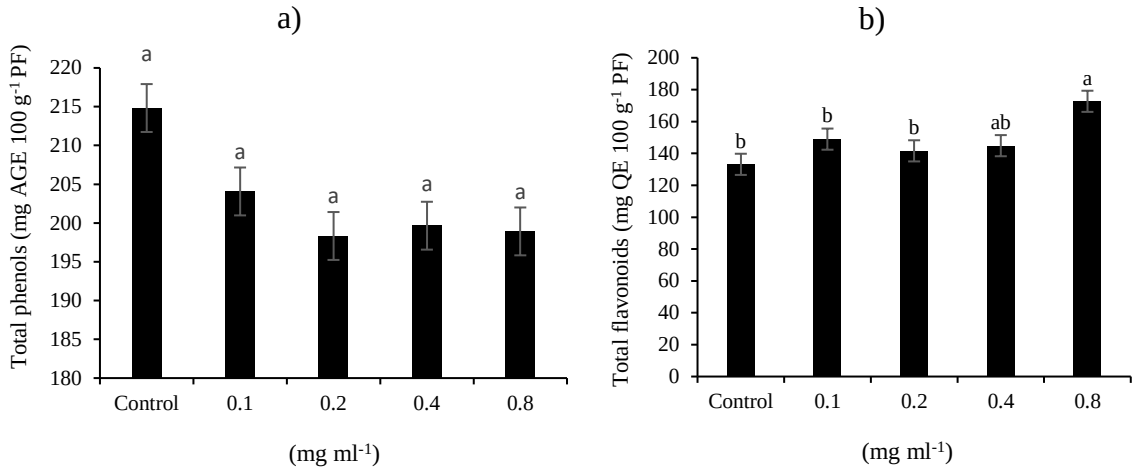

Figure 5. Effect of NPs CS on the content of total phenolic compounds (a), total flavonoids (b) in triticale sprouts.

These results can be attributed to the fact that high concentrations of NPs CS lead to an initial oxidative burst with hydrogen peroxide accumulation (H_2O_2) (Martínez *et al*., 2015), and it is believed that this can lead to induction of plant defense enzymes and synthesis of secondary metabolites, such as polyphenols, lignin, flavonoids and phytoalexins (Malerba and Cerana, 2016) improving defense responses to biotic and abiotic stress (Hidangmayum *et al*., 2019).

It may be a viable alternative to improve the functional and biological properties of the sprout (Rodríguez *et al*., 2019); however, it is difficult to establish the effects of NPs CS on the sprouts as they vary according to the plant species, growth stages, dose and the exposure of NPs (Medina, 2017).

Conclusions

The application of NPs CS in triticale sprouts at a dose of 0.1 mg ml^{-1} affects the fresh weight of the shoot, but not the fresh weight of the root, while the dose of 0.2 mg ml^{-1} showed the best results in percentage of vigor and content of photosynthetic pigments, confirming that seed priming with NPs CS does not cause negative effects, besides high doses of 0.8 mg ml^{-1} of NPs CS affect nutraceutical quality increasing the content of flavonoids in triticale sprouts.

NPs CS could be a good alternative to improve the quality of sprouts; however, more research is needed to clarify the effects of Ns CS as there are factors that depend on species and concentration.

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