Article

Selenium nanoparticles absorbed in chitosan-polyvinyl alcohol hydrogels in the production of grafted cucumber

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Abstract

In the search to improve the use of natural resources and increase the yield and quality of the fruits using environmentally friendly techniques, such as grafting and the use of metal nanoparticles. The objective of this research was to determine the effect of grafting combined with the application of different concentrations of selenium nanoparticles (Se NPs) encapsulated in one gram of chitosan-polyvinyl alcohol hydrogel (CS-PVA) in the productivity and production of grafted cucumber. The treatments were applied to the substrate at the time of transplantation as follows: 1, 2.5 and 5 mg of Se NPs absorbed in one gram of hydrogels of CS-PVA, CS-PVA without NPs and a control treatment, in grafted and non-grafted cucumber plants. The variables evaluated were length and diameter of the stem, fresh and dry weight of leaves, foliar area, root length, fruit length, number of fruits and yield per plant. The results showed that the application of Se NPs has a beneficial effect for plants, as a growth-promoting agent and that together with the graft help to obtain a higher plant height and fruit weight, obtaining a higher yield per plant.

Keywords: *Cucumis sativus*, grafts, nanotechnology, yield.

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Introduction

Modern agriculture requires alternative practices to improve the productivity and quality of crops, without affecting the environment since resources such as water and soil are becoming scarcer, and the population continues to increase. For this reason, to investigate alternatives that improve agricultural production. Vegetable grafting allows the crop to develop properly and decrease the use of agrochemicals (González *et al*., 2008), in addition to tolerating saline soils and high temperatures (Khah *et al*., 2006), improves crop yield and quality (Yassin and Hussen, 2015).

In addition to the use of grafting in agricultural systems, the use of nanoparticles (NPs), materials with a dimension less than 100 nm, is being evaluated. This small size results in different properties compared to larger scales, such as high conductivity, chemical reactivity, good surface-to-volume ratio (Nel *et al*., 2006), which makes them very useful in crop nutrition (Hernández *et al*., 2018) and crop protection (Cumplido *et al*., 2019) to those shown by micro-sized materials of the same composition (Bell *et al*., 2014). The main nanomaterials being evaluated in plants are based on metals and oxides such as Cu, Fe, Ce, Ti and Ag.

Cellular responses are very different when induced by ion forms compared to nanometric forms (Zuverza *et al*., 2016). These new properties provide the material with added value that has multiple applications in the agriculture industry, such as nano fertilizers, disease treatment and promotion of plant growth, among others (Siddiqui *et al*., 2015).

Selenium (Se) is an essential element of the human diet (Hartikainen *et al*., 2005), it is necessary for the proper functioning of the immune system, it has structural and enzymatic functions, so, it is necessary to supplement in soils or substrates with deficiency of selenium. The low intake of selenium can result in various health disorders, including heart diseases, decreased in fertility, hypothyroidism, improves conditions related to oxidative stress. Se provide benefits at the level of redox metabolism, increasing the resistance of plants to various factors of biotic and abiotic stress (Wallace *et al*., 2009), increases the growth and quality of fruits (Hernández-Hernández *et al*., 2019).

Since doses of Se are very small, the use biopolymers such as chitosan and (CS), a derivative of chitin found in shells of crustaceans, mollusks and insects, is required (Nge *et al*., 2006). CS is able to form hydrogels when combined with polyvinyl alcohol (PVA), a hydrophilic polymer, non-toxic, biocompatible, with good mechanical property and very stable for long periods of time and in different temperature and pH conditions (Gholap *et al*., 2004), which are able to encapsulate the active ingredients in order to improve their absorption by plants, so it is a technique that can be effective for biofortification of crops with Se.

Mexico is considered one of the leading producers of cucumber (*Cucumis sativus*), ranks eighth in the world with a production of 886 270 t in 16 206 ha (SIAP, 2018). Cucumber is a cucurbitaceae of great importance for fresh consumption, pickle and cosmetic industry. One of the many problems in the production of this vegetable is the presence of soil pathogens, such as nematodes and vascular fungi, because of this, alternative methods have been created in the production systems, such as the use of graft, which improves the growth and productivity of plants under unfavorable soil and environment conditions (Rouphael *et al*., 2012). To help the production of the crop, the objective of this research was to determine the effect of Se NPs on the production and productivity in cucumber plants with and without grafting.

Materials and methods

Synthesis of selenium nanoparticles

The synthesis of the Se NPs was carried out in the Synthesis Laboratory of the Research Center in Applied Chemistry (CIQA), in a glass reactor equipped with mechanical agitation, temperature control and inert atmosphere system in an aqueous medium using selenous acid (H_2SeO_3) and a solution of CS with PVA. The components are maintained at 0 °C and the reduction is carried out with hydrazine. The obtained Se NPs have a spherical shape and a size of particle of 2 to 20 nm determined by means of transmission electron microscopy (TEM) (Figure 1).

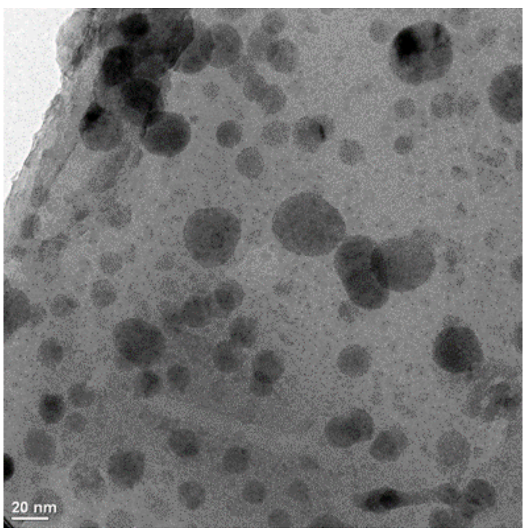

Figure 1. Micrograph of Se NPs by means of transmission electron microscopy from 2 nm to 20 nm, mainly spherical in shape.

Absorption of Se NPs in CS-PVA hydrogels

In a dispersion of Se NPs, it is mixed with CS-PVA hydrogels to obtain nanocomposites with 1, 2.5 and 5 mg of Se in one gram of CS-PVA hydrogels, then they are vacuum dried at 60 °C (Gómez *et al*., 2017).

Experimental development of cucumber cultivation

The development of the agronomic phase took place at the facilities of the Universidad Autónoma Agraria Antonio Narro (UAAAN), in northern Mexico, whose geographic location is at 25° 21’ 22.51’’north latitude and 101° 2’ 9.88’’ west longitude and an altitude of 1 760 m, during the months of June to September 2017 in a high tunnel greenhouse with diffused plastic cover, average diurnal temperature of 27 °C and nocturnal of 20 °C, average relative humidity of 60% and a planting density of 3 plants m^{-2}.

Vegetative material

The variety of cucumber (*Cucumis sativus* L.) used was cv ‘Induran’ and as rootstock the hybrid ‘Cucurbita Maxima x curbita Moschata’ both from Rijk Zwaan, The Netherlands.

Sowing

The cucumber variety was sown on June 7, 2017 and the rootstock on June 12, 2017 in 60-cavity polystyrene trays. The graft was performed through the approach method (González *et al*., 2008) on June 22 when the variety and the rootstock had diameters of 4 to 5 mm. The seedlings already grafted were kept for 3 days in a healing chamber at a temperature of 25 °C and relative humidity at 80%, before transplantation a mixture of peat moss and perlite (1:1, v/v) was prepared, which was used as a growth substrate and was put in 10 L black polyethylene bags.

Treatments with CS-PVA hydrogels were done by placing in the lower, medium and upper part of the pot for better distribution of the Se NPs in the substrate and in the area of the root of the plant, with a total of 10 treatments with 10 repetitions (Table 1). A drip irrigation system was used to fertilize with the Steiner nutrient solution (Steiner, 1961). The nutrient solution was supplemented with different concentrations according to the phenological stage of the crop and as follows: 25% during growth, 50% in flowering, 75% in the mooring and set of the first fruit and 100% during fruit filling. The electrical conductivity of the Steiner solution was maintained at 3 mS cm^{-1}.

Table 1. Description of treatments studied based on the quantity of Se NPs in one gram of CS-PVA hydrogels in 10-liter pots.

Treatment	Description
No graft	No application
	1 g de CS-PVA
	1 mg of NPs of Se in 1 g of CS-PVA
	2.5 mg of NPs of Se in 1 g of CS-PVA
	5 mg of NPs of Se in 1 g of CS-PVA
Grafted	No application
	1 g CS-PVA
	1 mg of NPs of Se in 1 g of CS-PVA
	2.5 mg of NPs of Se in 1 g of CS-PVA
	5 mg of NPs of Se in 1 g of CS-PVA

Variables evaluated

The evaluation of the variables was carried out during growth (length and diameter of stem, foliar area, specific foliar area, fresh weight and dry weight of leaves, root length, fresh root weight) and at the end of the cycle at 60 days after transplantation and in the production (fruit length, fruit diameter, fruit weight, number of fruits, yield per plant), making 16 cuts per plant when the fruit was well formed, dark green and before presenting physiological maturity.

Statistical analysis

A completely random experimental design was used with a factorial arrangement (2 x 5), where factor 1 is (with and without graft), factor 2 (application of Se NPs in doses of 0, 1, 2.5 and 5 mg in one gram of CS-PVA hydrogels and CS-PVA hydrogel only), with a total of 10 treatments and 10 repetitions of each one. The results were analyzed by means of a variance analysis and a mean comparison test using the Fisher LSD methodology with a significance level of ≤ 0.05 in the InfoStat 2017 program.

Results and discussion

The results of the evaluation of the agronomic variables of cucumber with and without graft are shown in Table 2. Significant differences (LSD, $p\leq 0.05$) were observed in stem length (LT), stem diameter (DT), foliar area, specific foliar area (AFE), fresh leaf weight (PFH), dry leaf weight (PSH) and fresh and dry root weight (PFR).

Effect of graft on crop development

The effect of cucumber grafting is present, which showed significant differences, where it positively impacts the plant at different stages of development. Because they have a greater ability to obtain nutrients and water from soil, on the other hand, a decrease in stem diameter was observed by 18%, smaller foliar area, decrease in the weight of fresh leaf compared to non-grafted plants, this could be due to the vigor conferred by the rootstock (Aloni *et al*., 2010).

In addition, it was found that the effect of grafting positively influences production variables, with significant differences ($p\leq 0.05$) such as the increase in the diameter and weight of fruit by 3% and 8.5% in relation to non-grafted plants, as well as for fruit length, number of fruits and yield per plant, the highest values were obtained in fruits from grafted plants (Colla *et al*., 2006).

Effect of Se NPs on variables of growth

The effect of the different applications impacted both grafted and non-grafted plants, being the dose of 1 mg Se NPs with CS-PVA hydrogels on growth variables such as foliar area and specific foliar area where positive differences presented, increasing the foliar surface of plants in a range of 10.5% and 29.6% respectively in relation to the treatments without application of Se NPs, this result represents an larger area of gas exchange (CO_2) between the plant and the air environment, this variable is associated with an increase in photosynthetic rate (Ghasemi *et al*., 2016). Fresh root weight was increased 22.4% with the dose of 2.5 mg of Se NPs in relation to the plants without application ($p\leq 0.05$).

The rest of the variables did not present statistical differences between the different doses of application of Se NPs. Regarding production variables, the effect of the application of Se NPs only presented statistical differences ($p\leq 0.05$) in the variable number of fruits and yield per plant by 26 and 34.9% respectively in relation to plants without application. As well, the length, diameter and weight of fruit presented the highest values at this concentration, although without significant differences. The results in Table 2 could be used to supplement Se Nps to increase plant nutrition in greenhouse (substrate) and in the field to increase soil fertility, in addition, Se NPs as inorganic selenium forms can positively influence rooting, growth and productivity in most horticultural crops (Aslani *et al*., 2014).

Table 2. Effect of graft interaction, Se NPs and CS-PVA hydrogels on the growth of cucumber plants.

Grafting	Se NPs + 1g CS-PVA	LT (m)	DT (mm)	AF (m^2)	AFE (cm^2 g^{-1})	PFH (g)	PSH (g)	PFR (g)
Without	0	4.42 ±0.1ab	14.4 ±1.23ab	2.14 ±0.1abc	181.02 ±13.5b	416.8 ±40.1ab	162 ±14.5ab	46.4 ±15.37c
	CS-PVA	4.52 ±0.6ab	14.6 ±1.59ab	2.21 ±0.2ab	214.35 ±18.6ab	371 ±33.2bc	163.4 ±16.8ab	50 ±18.76c
	1 mg CS-PVA	4.45 ±0.1ab	14.7 ±0.84a	2.48 ±0.1a	268.91 ±23.5a	433.8 ±15.9a	188.8 ±23.5a	44.6 ± 11.76c
	2.5 mg CS-PVA	4.47 ±0.1ab	13.8 ±1.09ab	1.97 ±0.05abc	192.79 ±11.3b	406 ±16.5ab	141.6 ±19.6bc	83.6 ±33.66a
	5 mg CS-PVA	4.52 ±0.2ab	13.4 ±0.97bc	2 ±0.2abc	191.86 ±23b	405.4 ±18.3ab	149 ±17.3a bc	51.8 ±12.35bc
With	0	4.44 ±0.1ab	12.4 ±0.43cd	1.93 ±0.3abc	187.54 ±15.6b	344 ±31.2cd	135.4 ±25.9bc	80 ±31.2ab
	CS-PVA	4.09 ±0.8b	11 ±1.65de	1.86 ±0.2bc	170.33 ±13.7b	313.6 ±36.5d	136.4 ±26.3bc	59.6 ±19.8abc
	1 mg CS-PVA	4.15 ±0.3b	9.6 ±0.42f	2.15 ±0.4abc	209.04 ±17.4ab	305.2 ±45.2d	125.6 ±38bc	58.8 ±19.4abc
	2.5 mg CS-PVA	4.54 ±0.8ab	9.5 ±0.63f	1.96 ±0.4bc	172.71±12.5b	316 ±39.1cd	129.4 ±13.6bc	71.2 ±22.76abc
	5 mg CS-PVA	4.64 ±0.6a	10 ±0.54ef	1.79 ±0.3c	165.18 ±14.6b	332.2 ±24.3cd	111.6 ±14.6c	61.6 ±26.76abc

LT= stem length; DT= stem diameter; AF= foliar area, AFE= specific foliar area; PFH= fresh weight of leaves; PSH= dry weight of leaves; LR= root length; PFR= fresh root weight. CS-PVA= chitosan-polyvinyl alcohol hydrogel. Values with different letters are significantly different (LSD Fisher ≤ 0.05).

Interaction graft-Se NPs in variables of growth and crop development

The greatest effect between grafting and the application of Se NPs absorbed into CS-PVA hydrogels are shown in Table 3, where the length of the stem in grafted plants with the dose of 5 mg of Se NPs increases by 4.9% in relation to plants without grafting and without application of Se NPs (Gómez *et al*., 2017), the graft confers an undoubted vigor on plants, perhaps improving their ability to absorb water and nutrients from their environment, which results in a higher growth rate.

Table 3. Effect of graft interaction and application of Se NPs absorbed into CS-PVA hydrogels on the production and yield of cucumber cultivation.

Treatment Grafting	mg NPs Se + 1g CS	LF (cm)	DF (mm)	PF (g)	NF	RP (kg)
Without	0	20.3 ±1a	43.56 ±1.3ab	236.8 ±20.5bc	10.2 ±2.5ab	2.4 ±0.7bcd
	CS-PVA	21.3 ±1.1a	44.93 ±0.8ab	257.2 ±23.3abc	10.8 ±2.7ab	2.8 ±0.7abc
	1	20.1 ±1.1a	43.26 ±1.1b	236.6 ±34.4bc	11.2 ±1.8ab	2.7 ±0.5abcd
	2.5	21.2 ±0.4a	43.67 ±2.3b	225 ±13.7c	8 ±2.8 b	1.8 ±0.7d
	5	21.2± 0.5 a	42.42 ±2.41b	248.2 ±24.07abc	11 ±2.6ab	2.7 ±0.2abc
With	0	20.2 ±1a	43.69 ±1.2ab	257.6 ±21.8 abc	8.2 ±3.2b	2.1 ±0.2cd
	CS-PVA	21 ±0.8a	43.9 ±0.9ab	250.6 ±36.8abc	10 ±2.2b	2.5 ±0.6bcd
	1	21 ±1.6a	45.1 ±1.9ab	265.2± 26.7ab	10.8 ±2.1ab	3 ±0.6ab
	2.5	25.8 ±1.2a	44.6 ±0.5ab	257.8 ±32.5abc	11.4 ±1.9a	2.9 ±0.8ab
	5	28.8 ±1.2a	46.1 ±0.6a	280.8 ±25.4a	12.2 ±1.8a	3.4 ±0.8a

LF= fruit length; DF= fruit diameter; PF= average fruit weight; NF= number of fruits; RP= yield per plant. Values with different letters are significantly different (LSD Fisher $\leq$0.05).

As well (Nawaz *et al*., 2017) the graft promotes the growth of shoots and leaves, in addition to confirming that Se is a beneficial element for plants, as a growth promoter agent (Garcia-Bañuelos *et al*., 2011), which together with graft cause a higher plant height (Riga, 2015), the foliar area and fresh weight of the leaves of grafted plants were lower compared to those non-grafted, this may be because they were more efficient in consuming water and nutrients.

The development of root length was influenced only by grafting compared to the treatment without grafting and without application of Se NPs ($p \leq 0.05$); however, statistically the root length decreases as the applied dose of NPs increases, in doses of 1 mg at 8.5%, for 2.5 mg at 10% and for 5 mg of Se NPs at 15%, similar data were found in a study of bean by (Aggarwal *et al*., 2011), where the dose less than 2 ppm of sodium selenate promoted root growth and higher doses inhibited it.

Regarding the interaction of grafting and Se NPs, they show a tendency to decrease the root length of the cucumber plant and the graft to develop root mass. The results obtained since a higher dose of NPs the growth of the roots in cucumber plants is affected, but the graft promotes the development of absorbent hairs which improves the absorption of nutrients and development of the grafted plant (Nawaz *et al*., 2017).

In this study the variable number of fruits obtained a higher average weight in grafted treatments and with doses of 2.5 and 5 mg of Se NPs absorbed into CS-PVA hydrogel; this is consistent with the studies of (Cavalu *et al*., 2017), where they applied Se NPs and the growth of broccoli seedlings depended on selenium concentration, there are also reports of applications of sodium selenite (Na_2SeO_3) at doses of 2, 4, 6 mg L^{-1} in cucumber, the results obtained showed that Se increased the yield with 2 mg L^{-1} and decreased in the dose of 6 mg L^{-1} (Haghighi *et al*., 2016).

Non-graft cucumber plants with 1 mg of Se NPs absorbed into CS-PVA hydrogels have the largest foliar area given by the effect of selenium application, determinant factor in the modification of photosynthesis (Hawrylak *et al*., 2015), this may explain the beneficial effects reported in the application of appropriate doses of Se in plants by promoting foliar area growth and productivity (Jiang *et al*., 2015).

There is evidence in the literature that Se can improved plant productivity through the improvement of photosynthesis, since this process is stimulated in plants by optimal supplementation (Gilmara *et al*., 2017), on the other hand, selenium could cause symptoms of toxicity at higher doses that influence leaf growth and number and size of cucumber stomata, and internal CO_2 concentration assimilation (Haghighi *et al*., 2016), this effect on the antioxidant machinery suggests that with appropriate concentrations of Se photosynthesis could improve significantly

Decrease in foliar appeared in the graft treatment and with the application of 5 mg of Se NPs, since metabolic substances could be transferred from the rootstock to the variety including the signaling molecules that can cause physiological effects such as hormonal signaling, that is involved in graff union formation, communication between rootstock-variety that affects growth, yield, flowering and fruit quality (Aloni *et al*., 2010).

Graft also allows a change in leaf morphology and shows a decrease of stomatic conductance in response to drought stress, to maintain the turgor of leaf (Poudyala *et al*., 2015). Grafted plants can maintain osmotic adjustment in leaves under water stress conditions through stress resistance mechanisms as reported by (Nilsen *et al*., 2014). In addition to a water conservation strategy, based on the combination of growth reduction (foliar area reduction) and the ability to keep photosynthesis net to lower water potentials.

The specific foliar area of cucumber was larger when applying 1 mg of Se NPs and without grafting, indicating that Se modified the development by increasing leaf density by 38.5%, with larger and less efficient leaves compared to the treatment where 5 mg of Se NPs are applied in grafted plants, where yield per plant was 21% higher than that of the treatment without grafting and 1 mg of Se NPs, resulting in greater efficiency in the assimilation of the highest proportion of radiation, a high rate of CO_2 assimilation, and translocation of large amounts assimilated towards the fruits of the plant.

In contrast to treatments that had higher foliar area values, we can say that the foliar area (AF) obtained in the graft treatment and without Se NPs presents an increase of 0.96% and a yield of 38% compared to the witness. Se NPs could increase the foliar area in non-grafted cucumber plants and cause a synergy with nitrogen promoting vegetative growth and inhibiting fruit promotion where signaling would promote leaf development and fruit abortion (Hartikainen, 2000).

Regarding the number of fruits and yield, the combination without grafting and 5 mg of Se NPs positively modified these variables, which can be attributed to the fact that the plant in this dose suffered a physiopathy from selenium, so it could react in abortion of the fruits (Kaur *et al*., 2014).

Conclusions

The agronomic yield of cucumber plants is increased in non-grafted plants but treated with the dose of 1 and 5 mg of Se NPs, while the dose that produced the highest productive yield occurred when grafted cucumber plants were given the dose of 5 mg of Se NPs, which positively affected the length of the fruit, stem diameter and fresh weight. Although grafted plants in combination with Se NPs tend to decrease foliar area, this combination resulted in a more efficient plant in terms of use and consumption of water. The greatest increase in foliar area growth and plant development was obtained in non-grafted cucumber plants and with the application of 1 mg of Se NPs absorbed in CS-PVA hydrogels.

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