UNIVERSIDAD AUTÓNOMA AGRARIA ANTONIO NARRO

SUBDIRECCIÓN DE POSTGRADO

CALIDAD NUTRACÉUTICA Y EXPRESIÓN GÉNICA DE DOS ESPECIES HORTÍCOLAS BIOFORTIFICADAS CON SELENIO IÓNICO ABSORBIDO EN COMPLEJOS DE QUITOSÁN-POLIÁCIDO ACRÍLICO

Tesis

Que presenta PAOLA CATALINA LEIJA MARTÍNEZ como requisito parcial para obtener el Grado de DOCTOR EN CIENCIAS EN AGRICULTURA PROTEGIDA

Saltillo, Coahuila Julio 2019

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Dra. Susana González Morales
Asesor Principal

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Asesor

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Elaborada por PAOLA CATALINA LEIJA MARTINEZ como requisito parcial para obtener el grado de Maestro en Ciencias en Horticultura con la supervisión y aprobación del Comité de Asesoría

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Lettuce Biofortification with Selenium in Chitosan-Polyacrylic Acid Complexes

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INTRODUCCIÓN

El selenio es un elemento esencial en la dieta para los seres humanos, por lo tanto, es conveniente incorporar al selenio en la fertilización agrícola con la finalidad de mejorar las propiedades nutracéuticas buscando la biofortificación en los órganos de interés para consumo humano. El selenio no está considerado dentro de los elementos esenciales para las plantas, sin embargo, diversos estudios han asociado al selenio con el metabolismo redox vegetal, puesto que el selenio se encuentra en bajas concentraciones en la mayoría de los suelos en el planeta, es necesario hacer un uso eficiente en su uso agrícola. El uso de biopolímeros para encapsular elementos y que estos sean aprovechados por las plantas es una técnica que puede resultar efectiva para optimizar la absorción de selenio por los cultivos, esta técnica puede resultar efectiva para optimizar la absorción del selenio por los cultivos.

Objetivo general

Comparar dos tipos de aplicación de selenio en plantas de lechuga y tomate cherry y estudiar el efecto sobre variables fisiológicas, de rendimiento y calidad nutracéutica.

Objetivos particulares

- Determinar el nivel de asimilación de selenio en las plantas en función de la forma de aplicación.
- Determinar la calidad nutraceútica a través de la cuantificación de antioxidantes.
- Analizar la expresión de genes en el fruto relacionados con enzimas antioxidantes (CAT, SOD, GPX) con la aplicación de los diferentes tratamientos de selenio mediante qPCR.

- Cuantificar la producción de biomasa y volumen de producción en el ciclo productivo de las hortalizas.

Hipótesis

La aplicación de selenio encapsulado en el complejo de biopolímeros PAA-Q asegura la absorción del selenio y afecta el crecimiento y actividad bioquímica de las plantas de *Lactuca sativa* y *Solanum lycopersicum* L. a través de un mecanismo oxidativo que induce cambios en su metabolismo redox.

REVISIÓN DE LITERATURA

Selenio

A nivel mundial, el interés por el impacto biológico de selenio (Se) sobre la calidad de los alimentos es cada vez mayor, ya que este elemento es un micronutriente esencial para los seres humanos y los animales (Smoleń et al., 2016). La baja ingesta de selenio puede resultar en varios trastornos de salud, incluyendo enfermedades del corazón, disminución de la fertilidad, el hipotiroidismo, las condiciones relacionadas con el estrés oxidativo, y debilidad en el sistema inmunológico (Schiavon et al., 2016). El bajo contenido de selenio en formas disponibles para las plantas en la solución del suelo es una de las principales causas de su insuficiente transferencia en el sistema suelo-planta-consumidor. Varios reportes de investigaciones relacionados con el selenio han proporcionado pruebas de que la administración de suplementos de fertilizantes comerciales con selenato de sodio afecta positivamente no sólo el valor nutritivo de toda la cadena alimentaria, sino también al rendimiento de los cultivos (Hartikainen, 2005). El nivel de adición de Se ha sido óptima, y no se han observado concentraciones anormalmente altas en las plantas o en los alimentos de origen animal.

Se sabe que el selenio está relacionado con el metabolismo antioxidante a través de su rol como cofactor de selenoenzimas, por esto, la deficiencia en su consumo puede inducir cambios en el balance redox celular, y su ingesta promueve la síntesis de compuestos antioxidantes. El consumo de alimentos con altas concentraciones de antioxidantes contribuye a la protección de las células frente al estrés oxidativo y a la prevención de algunas enfermedades degenerativas. Los radicales libres causan reacciones en cadena oxidativas que pueden ser neutralizadas por la acción de sistemas antioxidantes, incluidas enzimas tales como la superóxido dismutasa (SOD), catalasa (CAT) y glutatión

peroxidasa (GPX) (Castillo-Godina, Foroughbakhch-Pournavab, & Benavides-Mendoza, 2016). Por lo general, las enzimas antioxidantes utilizan elementos traza como cofactores, tal es el caso del selenio en la GPX.

Complejos de biopolímeros

El quitosán es un polímero lineal formado por monómeros de D-Glucosamina, es un producto natural derivado de la quitina la cual se deriva de las conchas de crustáceos principalmente. La quitina se encuentra en los crustáceos como constituyente de una red compleja de proteínas en las que el carbonato de calcio se deposita para formar la cáscara rígida. El quitosán tiene diversas aplicaciones en la industria alimenticia como en el recubrimiento de frutos, empaque de alimentos, así como para procesos de purificación de agua, recuperación de metales preciosos, entre otras aplicaciones en la agricultura, medicina y productos de uso humano en general (Benavides-Mendoza, Burgos-Limón, Ortega-Ortiz, & Ramírez, 2007). Diversos estudios sobre la actividad biológica del quitosán indican que puede inducir repuestas celulares relacionadas con la defensa de las plantas, además de que por sí mismo el quitosán contiene propiedades antimicrobianas contr aun amplio espectro de fitopatógenos (Katiyar, Hemantaranjan, Singh, & Bhanu, 2014). En las primeras caracterizaciones del uso de quitosán en cultivos de consumo se consideró como un elicitor, puesto que activa genes de defensa de las plantas a través de una vía de octadecanoicos(Wojdyla Skierniewice (Poland)), 2001). Por su actividad en los genes del sistema de defensa de las plantas, el quitosán induce resistencia contra patógenos, esto se logra además por estar relacionado con la respuesta estomática. Se ha reportado que el quitosán reduce el tamaño de la apertura estomática a inhibe la apertura inducida por radiación a través de la síntesis de especies reactivas del oxígeno, las cuales

promueven el cierre e inhiben la apertura estomática(Eikemo et al., 2003). Puesto que la apertura provee una puerta de entrada a los patógenos a través de las hojas, por esto, al inducir el cierre de los estomas se aventaja el sistema de defensa vegetal. Por esta misma razón, el uso del quitosán en los cultivos puede resultar efectivo como un antitranspirante con el fin de reducir la pérdida de agua en la agricultura(Bell et al., 1998).

Por sus propiedades biodegradables, no tóxicas y no alergénicas, se sugiere el uso del quitosán como un material bioactivo(Verlag, Khan, Prithiviraj, Smith, & Pal, 2003).

El poliácido acrílico, es un polímero formado estructuralmente por la unión de unidades monoméricas repetitivas de ácido acrílico, perteneciente a la familia de los acrilatos, que son polímeros que pertenecen a un tipo de polímeros vinílicos. Tiene diversas aplicaciones en la industria alimentaria además de utilizarse como aditivo en productos de uso doméstico.

La mezcla entre polímeros naturales y polímeros sintéticos posee un amplio rango de propiedades físicoquímicas y técnicas de procesamiento relativamente sencillas que provienen de los polímeros sintéticos y una adecuada biocompatibilidad proveniente de los polímeros naturales. Esto confiere aplicaciones variadas, brindando muchas posibles aplicaciones tales como la inmovilización y liberación prolongada de varios elementos químicos o agentes bioactivos. Los biopolímeros como el quitosán (Qs) y poliácido acrílico (PAA) pueden formar cápsulas a las que se les puede agregar un ingrediente activo usando un sistema acuoso a temperatura ambiente(Ortega-Ortíz, Benavides-Mendoza, Flores-Olivas, & Ledezma-Pérez, 2003).

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Lettuce Biofortification with Selenium in Chitosan-Polyacrylic Acid Complexes

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Abstract: Selenium (Se) is an essential element of the human diet. Therefore, it is necessary to implement Se in selenium-deficient soils and in the nutrient solution of soilless system culture. Although it is not considered as an essential element for plants, Se provides benefits at the level of redox metabolism, increasing the resistance of plants to various stress factors. The increase of the availability of Se, with the use of biopolymer complexes, was sought in *Lactuca sativa* var. Great Lakes, grown in substrate pots treated with SeO_2 (5 mg Se/plant), chitosan-polyacrylic acid complex + Se (Cs-PAA + Se) (5 mg Se/plant), and chitosan-polyacrylic acid complex (Cs-PAA). The redox metabolism was modified by increasing the enzymatic activity of catalase and glutathione peroxidase. The use of Cs-PAA + Se biopolymer complexes increased Se up to 24 mg/Kg dry weight (DW) in plant tissues.

Keywords: biofortification; antioxidants; soilless culture; nutraceutical quality; enzymatic activity; plant resistance

1. Introduction

Selenium is essential in the human diet, since a low intake has been linked to a weak immune system and cognitive decline [1]. On the contrary, the optimal intake of Se entails benefits, such as reducing the risk of different types of cancer, Alzheimer's disease, among others, and is necessary for thyroid function since the thyroid gland is the largest reservoir of Se in the human body, having different functions [2]. The adequate recommended intake of Se in humans has been proposed between 50–60 µg/day, on the contrary, an intake of 350–700 µg/day may result toxic [3]. It is known that Se is related to antioxidant metabolism through its role as a cofactor of selenoenzymes [4]. Therefore, its intake promotes the synthesis of antioxidant compounds, inducing changes in the cellular redox balance. The consumption of foods with high concentrations of antioxidants contributes to the protection of the cells against oxidative stress preventing some degenerative diseases. Free radicals cause oxidative chain reactions that can be neutralized through the action of antioxidant enzymes, such as catalase (CAT) and glutathione peroxidase (GPX). These enzymes increase their activity with the presence of Se in plants [5]. Several studies carried out on strawberry [6], tomato [7,8], and corn salad [9] crops have managed to biofortify the organs of consumption with favorable results with

respect to plant growth and antioxidant content. It has been reported that an application of 5 mg per plant in the whole production cycle is effective to achieve biofortification in tomato fruits [5].

Since Se is a non-renewable resource, it is necessary to incorporate it into agricultural fertilization in an appropriate manner, in Se-deficient soils and in hydroponic and soilless culture systems, in order to avoid wastage of the element. The use of biopolymers to encapsulate elements in order to improve their absorption by plants is a technique that can be effective for crop biofortification with Se. Biopolymers, such as chitosan (Cs) and polyacrylic acid (PAA) are capable of encapsulating active ingredients using an aqueous system at room temperature [10]. The mixture between these polymers has physicochemical properties and relatively simple processing techniques that come from synthetic polymers and an adequate biocompatibility from natural polymers, this confers varied applications, such as the immobilization and prolonged release of various chemical elements or bioactive agents. These biopolymer complexes may be used in order to avoid the loss of Se through leaching, adsorption to organic components of the substrate, and volatilization by microorganisms [11]. In the present work, it was sought to biofortify lettuce plants with 5 mg Se/plant (SeO_2) by applying it alone and absorbed in a complex with biopolymers, studying the impact on the plant growth, the antioxidant activity, and the accumulation of Se.

2. Materials and Methods

2.1. Preparation of Non-Stoichiometric Interpolyelectrolyte Complexes (NPEC) of Chitosan-Polyacrylic Acid (Cs-PAA) and Cs-PAA + Se

Chitosan (Cs) was supplied by Marine Chemicals (Meron, Kerala, India). Deacetylation degree was 99%, molecular weight Mv = 200,000 g/mol and was determined by intrinsic viscosity in an Ubbelohde viscometer by the ASTM D2857 method using a mixture of acetic acid/sodium acetate solutions at 30 °C applying Mark-Houwink equation: $\eta = KMv^{\alpha}$, where k = 0.076 and $\alpha = 0.76$. Poly (acrylic acid) molecular weight Mw = 450,000 and selenium oxide (SeO_2) were acquired from Aldrich. Water-soluble NPEC were prepared at the Center for Research in Applied Chemistry with polycation (Cs) and polyanion (PAA) [12]. The NPEC (Cs-PAA) with the composition φ = [CS]/ [PAA] = 3 was used. The square brackets denote molar concentrations of polyions prepared by mixing water solutions of Cs and PAA at corresponding quantities of the polyelectrolytes solutions at pH 2, and then adjusting the solution with phosphate buffer at pH 7.4. Lastly, from SeO_2, 50 mg Se/L of complex was added, which is the amount needed to make the applications of Cs-PAA + Se.

2.2. Plant Material and Treatments

A soilless lettuce (*Lactuca sativa* L.) var. Great Lakes (FAX Seeds, Jalisco, Mexico) crop was established in a greenhouse with polyethylene cover, 50%–60% relative humidity, and an average temperature of 28 °C. Lettuce seeds were sown in black polyethylene trays filled with sphagnum peat moss and perlite in a 1:1 ratio *v*/*v*. At 40 days after sowing, seedlings were transplanted to black polyethylene containers filled with 5 L of substrate mix of sphagnum peat moss and perlite. Steiner [13] nutrient solution was applied by a single pass drip irrigation system, with a pH (6–6.5) adjusted daily with phosphoric acid. The treatments application started seven days after transplant (DAT). Each treatment was composed of 20 lettuce plants, placed randomly in four lines. The treatments were applied manually to the substrate. A treatment with SeO_2 (50 mg/L Se) was applied with a volume of 12.5 ml of solution in each application. The next treatment consisted in applying the Se (50 mg/L) in Cs-PAA complex with the same volume as in the previous treatment. In both treatments, a total of 5 mg of Se per plant was applied, divided into eight weekly applications. On the other hand, the Cs-PAA without Se was also applied, and a control with no application.

2.3. Sampling

Lettuces were harvested at 60 DAT, measured and weighted to determinate total growth and total crop yield. At the same time, samples were collected to quantify the biochemical variables and Se content, obtaining five samples by each treatment and control.

2.4. Yield and Biomass Production

In order to determine total crop yield, the lettuce heads were harvested and weighed on an analytical scale (Ohaus Corporation, Pine Brook, NJ, USA) to determine the total fresh weight (FW). In order to determine the total accumulated biomass, five plants of each treatment were dehydrated in an oven at 80 °C for 48 h. Once total dehydrated, the dry weight was registered.

2.5. Biochemical Analysis

In order to determinate nutraceutical quality, five samples were frozen at −20 °C during 48 h then freeze-dry in a Freeze Dryer (Labconco, Freezone 6, Kansas City, MO, USA) at 133×10^{-3} mbar and −80°C during 48 h. Once completely dried, samples were pulverized with a porcelain mortar. For the biomolecules extraction, 200 mg of powdered sample was placed in a 2 mL tube and 20 mg of polyvinylpyrrolidone (PVP) (Sigma-Aldrich Corporation, Saint Louis, MO, USA) was added to stabilize the enzymes; 1.5 mL of phosphate buffer 0.1 M (pH 7–7.2) was added, homogenized by vortex for 20 seconds each sample, sonicated during five minutes, and centrifuged at 12,500 rpm at 4 °C for 10 minutes, collecting the supernatant in order to perform the analysis.

2.5.1. Total Proteins

The Bradford spectrophotometric technique [14] was used for protein quantification. One hundred microliters of the protein extract was placed in an assay tube, and adding 5 mL of the Bradford reagent, and let to stand for five minutes. Once the incubation time passed, the absorbance was read at a wavelength of 595 nm with a UV-Vis spectrophotometer (Thermo Scientific Model G10S, Waltham, MA, USA) The results were registered and extrapolated to a calibration curve of bovine serum albumin (BSA) (Sigma-Aldrich Corporation, Saint Louis, MO, USA), reporting the results in m/g.

2.5.2. Catalase Activity (CAT) (EQ 1.11.1.6)

The enzymatic activity of catalase was quantified by a spectrophotometric technique, with two reaction times. The assay mixture consisted on 100 µL of protein extract and 900 µL of 100 mM H_2O_2 (CTR Scientific, Monterrey, Nuevo León, Mexico), and was added 400 µL of 5% H_2SO_4 (Sigma-Aldrich Corporation, Saint Louis, MO, USA) to stop the reaction, this was carried out under stirring at 24 °C. These assays were read with a spectrophotometer (Thermo Scientific Model G10S, Waltham, MA, USA) at 270 nm; time reaction zero (T0) was recorded. For time reaction one (T1), the mixture of protein extract and 100 mM H_2O_2 was stirred at 24 °C, after 1 min 400 µL 5% of H_2SO_4 was added to stop the enzymatic activity. The remaining H_2O_2 was read with a spectrophotometer at 270 nm. For this analysis, a blank for each sample was used, consisting of 100 µL of biomolecules extract, 900 µL of 0.1 M phosphate buffer and 400 µL of 5% H_2SO_4. Units of catalase activity were expressed as mM H_2O_2 min^{-1}/ total proteins [15].

2.5.3. Glutathione Peroxidase Activity (GPX) (EQ 1.11.1.9)

A spectrophotometric method [16] was used with H_2O_2 as substrate; 200 µL of biomolecules extract was placed in a test tube plus 400 µL of reduced glutathione 0.1 M and 200 µL Na_2HPO_4 (Fermont, Monterrey, Nuevo León, Mexico) 0.067 M. This mixture was preheated in a water bath at 25 °C for 5 min, then 200 µL of 1.3 mM H_2O_2 was added to start the catalytic reaction. The reaction lasted 10 min and was terminated by adding 1 mL 1% trichloric acetic acid (Sigma-Aldrich Corporation, Saint Louis, MO, USA), and the mixture was put into an ice bath for 30 min. The assay mixture was

centrifuged for 10 min at 3000 rpm; 480 µL of the supernatant was placed into a cuvette, with 2.2 mL of 0.32 M Na_2HPO_4 and 320 µL of 1.0 mM 5-5′-dithiobis 2-nitrobenzoic acid (DTNB, Sigma-Aldrich Corporation, Saint Louis, MO, USA) was added for color development. The enzyme activity was determined as a decrease in GSH within the reaction time, expressed as mg/L GSH min/total proteins.

2.5.4. Glutathione (GSH)

Glutathione was quantified following a spectrophotometric technique [16], by reaction with the 5,5-dithio-bis-2-nitro benzoic acid (DTNB). Four hundred and eighty microliters of protein extract was placed in a tube then added 2.2 mL of 0.32 M Na_2HPO_4 plus 320 µL of 1.0 mM DTNB dye. The assay was mixed and read on a spectrophotometer (Thermo Scientific Model G10S, Waltham, MA, USA) at 412 nm. The units were reported in mg glutathione/g dry tissue.

2.5.5. Total Phenols Content

Regarding the total phenolic extraction, five samples of each treatment were used. Two hundred milligrams of a lyophilized and macerated sample was taken, and 1 ml of water-acetone 1:1 solution was added, centrifuged at 10,000 rpm at 4 °C for 10 min, and the supernatant was collected to initiate the reaction. The assays were prepared using 200 µL of Folin-Ciocalteu 1 M reagent, 50 µL of the extract, 500 µL of 20% Na_2CO_3, 1 mL water-acetone 1:1 mix, and 5 mL of distillated water. Blank was composed by the same components adding 50 µL of water-acetone mix instead of the extract. Subsequently, assays were let to incubate at 45 °C for 30 min. The samples were read with a spectrophotometer (Thermo Scientific Model G10S, Waltham, MA, USA) at a wavelength of 750 nm and the results were recorded in µg/g [17].

2.6. Selenium Content

Selenium was extracted using a wet digestion technique [18]. For digestion, 500 mg of the dehydrated sample was placed in a beaker with 30 mL of nitric acid, and heated during two hours in a heating plate, until clarification of the mixture. Finally, the sample was collected and taken to a volume of 50 mL with deionized water and filtered with Whaltman # 42 filter paper. Mineral content was read in an inductively coupled plasma optical emission spectrometer (ICP-OES, Thermo Scientific Jarrell Ash, Model iCAP 7000 Series, Waltham MA, USA). The calibration curve was elaborated with a Selenium standard (High Purity Standars, North Charleston, SC, USA), with 5 points from 0.005 to 2 ppm. The plasma was generated with a torch at a temperature of 10000 ° K by the ionization of argon.

2.7. Statistical Analysis

The experimental design was completely randomized with five replicates per treatment on each variable, with one plant considered as an experimental unit. Infostat software (Grupo InfoStat, Córdoba, Argentina) was used, in which a Fisher Least Significant Difference test ($\alpha \leq 0.05$) was performed for all variables.

3. Results and Discussion

3.1. Yield and Biomass Production

The treatments applied did not exert an effect on the variables related to yield and biomass, (Table 1). The analysis of accumulated biomass in the form of the dry weight of plant tissues is a useful indicator in order to determine the toxicity of an element. Based on the results obtained in this experiment, in which there was no significant difference between the treatments and the control, it is suggested that the applied concentrations of Se were nontoxic. The application of the Cs-PAA complexes can also be considered safe for the development of the crop. In a crop of radish plants established in soil treated with 40 µM SeO_4^{-} per plant, the authors [19] reported a decrease in dry

weight of leaves and roots by about 35% and 18% respectively. On the other hand, when cultivating the radish plants in a soilless culture, they did not find significant differences in the dry weight of the leaves.

3.2. Biochemical Variables

According to the results analyzed, the content of proteins, phenolic compounds, and glutathione, are not affected by the application of the treatments in comparison with the control, as it is detailed in Table 1.

Table 1. Comparison of means of biomass and biochemical variables.

Treatment	Biomass (g DW)	PROT (mg/g)	GSH (mg/g)	PHEN (µg/g)
Control	51.35 ± 3.19 $^{a¥}$	2.24 ± 0.56 ^{a}	0.81 ± 0.02 ^{a}	63.51 ±7.49 ^{ab}
SeO_2	51.67 ± 2.43 $_{a}$	2.87 ± 0.51 ^{a}	0.83 ± 0.01 ^{a}	88.4 ± 14.05 ^{a}
Cs-PAA + SeO_2	47.27 ± 4.12 ^{a}	2.05 ± 0.35 ^{a}	0.95 ± 0.38 ^{a}	61.1 ± 11.11 ^{ab}
Cs-PAA	50.17 ± 2.41 ^{a}	3.57 ± 0.82 ^{a}	0.97 ± 0.58 ^{a}	48.76 ± 6.81 ^{b}

¥ Means with the same letter are statistically equal (LSD, $p \leq 0.05$). Mean ± standard error of the mean (n = 5). PROT, proteins; CAT, catalase activity; GSH, glutathione; PHEN, total phenolic compounds.

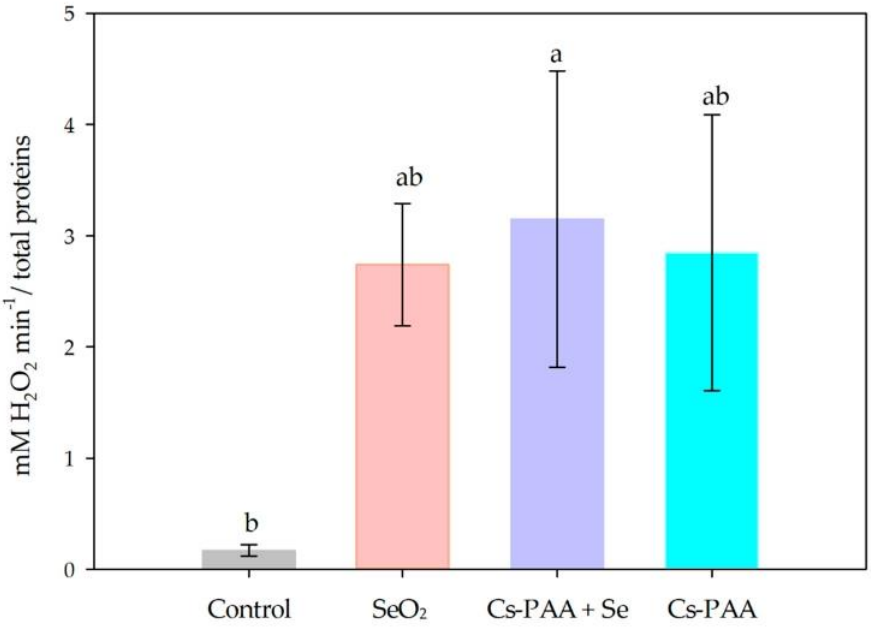

Figure 1. Means comparison of the enzymatic activity of catalase. Means with the same letter are statistically equal (LSD, $p \leq 0.05$). Bars represent standard error.

In the enzymatic activity of catalase (Figure 1), SeO_2 and Cs-PAA treatments were not able to surpass the control, however, when applied combined in the treatment of Cs-PAA + Se, was induced a greater enzymatic activity compared to the control. In another study with spirulina (*Spirulina platensis*) [20], Se applied at 150 mg/L or less, induced an increase in the activity of peroxidase enzymes, including catalase. Similarly, the application of a higher concentration (>175 mg/L) induced enzymatic activity, but in conjunction with an increase in lipid peroxidation and a decrease in biomass and photosynthetic pigment content. Some studies [21] suggest that Se also induces an increase in the enzymatic activity of CAT when used in concentrations of 5 to 10 mg/L to reduce oxidative stress in plants of *Triticum aestivum* L. under drought stress conditions.

In the statistical analysis of the GPX activity, significant differences were found (Figure 2). All the treatments applied had higher values of GPX activity compared to the control. Both the SeO_2 and the Cs-PAA complex applied by themselves increase GPX activity, (729% and 789% respectively); however, when applied together as Cs-PAA + SeO_2 a higher enzymatic activity is obtained (1031% increase over control). The above results agreed with those of a study in ryegrass [22], whose authors reported an increase in GPX activity by applying Se at 1 mg/Kg. Similarly, other works [23] also reported an increase in GPX activity with applications of different Se concentrations up to 150 mg/L, but in conjunction with a reduction of biomass and chlorophyll when applying more than 175 mg/L in *Spirulina platensis*. Since all the treatments applied in the present work induced an increase in the GPX activity, it is suggested that both the Se and the Cs-PAA complex induce this increase by themselves, and when applied as a whole the effect is enhanced.

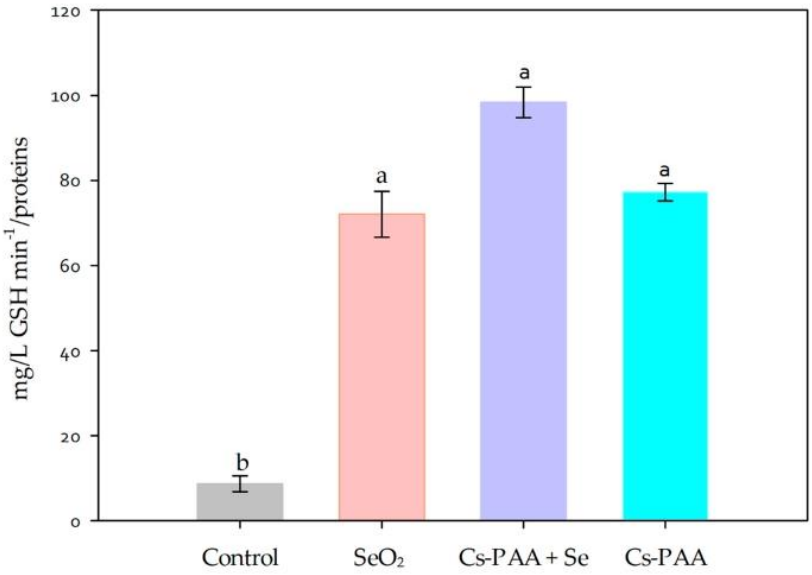

Figure 2. Means comparison of the enzymatic activity of glutathione peroxidase. Means with the same letter are statistically equal (LSD, $p \leq 0.05$). Bars represent standard error.

GSH content was not affected by the treatments, in the same way as in an experiment with Chinese cabbage subjected to stress, due to the high concentration of heavy metal Cd, where GSH increase when Se was applied in combination with Si; however, there was no difference when applying Se alone [24]. In contrast, in an experiment performed on wheat [25], GSH increased by 18% when Se was applied in conjunction with S.

As in the present work, in another study [19] with radish plants using foliar applications of up to 20 mg Se per plant, no differences were found compared to the control in phenolic compounds. In contrast, in an experiment under field conditions of *Allium cepa* L. Se applied 50 $\mu g/mL^{-1}$ increased the content of total phenolic compounds compared to the control [26].

The evaluation of enzymatic activity of antioxidant enzymes, as well as the content of non-enzymatic antioxidants is an indicator that can give a clear notion about the redox metabolism of a plant, which in turn can be translated as the nutraceutical quality of a crop by its antioxidant content. Based on this fact, the effect of Se on enzymatic and non-enzymatic antioxidants depends widely on the plant species used.

3.3. Selenium Biofortification

The treatment of SeO_2 increased Se content 236% over control, and the treatment of Cs-PAA + Se had an additional increase of 74% over the above (Figure 3). There was no significant difference in the

accumulation of Se between the treatments of SeO_2 and Cs-PAA + Se. It is possible that a difference will occur with a previous significant degradation of the polymer, which may happen in a second growing season using the same substrate. Therefore, it is feasible to use Cs-PAA + SeO_2 for biofortification.

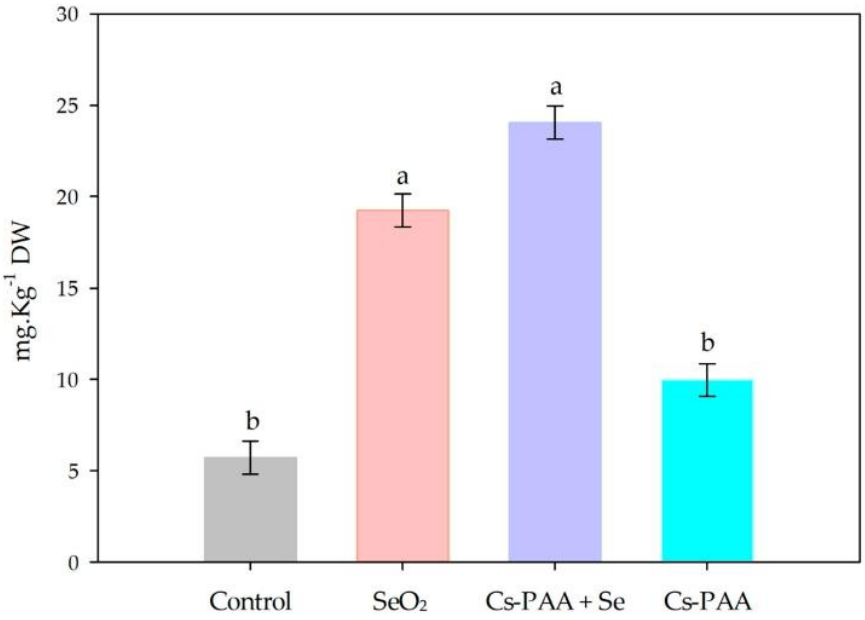

Figure 3. Mean values of the Se accumulation in lettuce plants. Means with the same letter are statistically equal (LSD, $p \leq 0.05$). Bars represent standard error.

The results of the biofortification techniques of Se have a variable behavior according to the plant species, the form of application, dose and concentration of Se, and the production system implemented. For instance, in a soilless crop of lettuce [27] with applications of SeO_3 and SeO_4 in concentrations from 2 to 64 µmol/L in the nutrient solution, the authors reported that at the highest concentrations Se accumulation reached up to ~22 mg/Kg DW however it also resulted in Se toxicity with a decrease in biomass. Similarly, in lettuce seedlings [16] with H_2SeO_4 applied to the soil from 0.1 to 1 mg/Kg there was an increase in Se accumulation, but it was toxic at the highest concentration; it accumulated up to 270 mg/Kg DW in the tissues but reducing the biomass up to 66% compared to the control. On the other hand, other studies [28] suggest the application of SeO_4^{2-} in conjunction with IO_3^- in order to achieve a double biofortification of lettuce "Melodion" cv. in hydroponic cultivation. There is a synergistic interaction between both compounds applied in foliar applications, suggesting a transport through the phloem tissues.

The values of Se concentration obtained in this study seem adequate from the perspective of biofortification, since with the application of Cs-PAA + SeO_2 it was possible to biofortify Great Lakes lettuce, up to 24 mg/Kg DW. Considering a portion of lettuce of 100 g FW, this adds up to 165 µg Se per serving of lettuce, which is an adequate amount for daily consumption since it exceeds the minimum requirement, but does not reach the doses that, under certain circumstances, could become harmful to the consumer. The recommended daily intake of Se in the human diet varies by region, in addition to the consumer particular health status. According to U.S. Food and Nutrition Board, the recommended intake is 60 to 75 µg Se daily [29]. However, in order to obtain the benefits that result from the intake of Se, such as reducing cancer risk, enhancing male fertility, and generally improving immune responses, it is necessary to increase this daily dose up to 200–300 µg daily [1]. Nevertheless, there are mixed reports about the recommended intake, since according to the Nutritional Prevention of Cancer (NPC), they reported that the consumption of 200 µg a day in yeast supplements decreases the incidence only in people with low levels of Se in plasma, causing the opposite effect in people

with adequate levels of Se [30]. Based on this fact, special attention should be paid to the amounts of accumulated Se by biofortified crops, due to the small threshold of appropriate dose.

4. Conclusions

Our results indicate that Cs-PAA complexes can be beneficial in biofortification processes, due to their tendency to increase the absorption of Se, as well as generating an increase in the activity of CAT and GPX antioxidant enzyme, without affecting the development of the crop. Based on what has been observed, Se and the Cs-PAA complexes were effective to enhance plant resistances, as well as the nutraceutical quality of Great Lakes lettuce.

Author Contributions: S.G.-M. conceived and designed the experiments; P.L.-M. performed the analysis of laboratory and field experiments; A.B.-M., M.C.-D., and A.S.-R. performed the data analysis; H.O.-O. and A.R.-O. contributed reagents, materials, and investigation. All authors were responsible for manuscript writing. All authors read and approved the final manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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NUTRACEUTICAL QUALITY AND GENE EXPRESSION OF CHERRY TOMATO BIOFORTIFIED WITH SELENIUM IN BIOPOLYMERS COMPLEXES

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Abstract

Since selenium (Se) is an essential element for humans and it is not a renewable resource, it is necessary to add it in soils with Se deficiency and in soilless and hydroponic cultures. Se has a high value as an antioxidant and stimulant in plants despite not being considered essential. Biopolymers such as chitosan and polyacrylic acid are very low environmental impact materials which, when added to plants, induce an increase in antioxidant capacity, making plants more resistant to stress factors. The objective of this work was to increase antioxidant capacity and the availability of Se in a soilless culture of *Solanum lycopersicum* var. *Cerasiforme* with the use of biopolymer complexes as well as Sodium selenite Na_2SeO_3 (5 mg Se/plant), chitosan-polyacrylic acid complex + Se (5 mg Se/plant) (Cs-PAA+Se), and chitosan-polyacrylic acid complex (Cs-PAA) were applied.

Keywords: *selenium, cherry tomato, antioxidants, biofortification, gene expression, nutraceutical quality*.

Highlights

- Selenium absorbed in a complex of biopolymers increases the yield of cherry tomato.
- Antioxidants content increase with the application of Cs-PAA + Se.
- Complexes of biopolymers increase the organoleptic quality of cherry tomato fruits.
- With the application of Cs-PAA+Se cherry tomatoes are biofortified in adequate doses.

Introduction

The global interest in the biological impact of selenium (Se) on the quality of food is increasing, since this element is an essential micronutrient for humans and animals (Smoleń et al., 2016). Low Se intake can result in several health disorders, including heart disease, decreased fertility, hypothyroidism, various conditions related to oxidative stress and weakened immune system (Schiavon et al., 2016). The low content of Se in available forms for plants in the soil solution is one of the main causes of its insufficient transfer in the soil-plant-consumer system. Several research reports related to selenium have provided evidence that the supplementation of commercial fertilizers with sodium selenate positively affects not only the nutritional value of the entire food chain, but also the yield of crops (Helinä Hartikainen, 2005). It is known that Se is related to antioxidant metabolism through its role as a cofactor of selenoenzymes, therefore, the deficiency in its consumption can induce changes in the cellular redox balance, and its intake promotes the synthesis of antioxidant compounds. The consumption of foods with high concentrations of antioxidants contributes to the protection of cells against oxidative stress and the prevention of some degenerative diseases. Free radicals cause oxidative chain reactions that can be neutralized by the action of antioxidants, such as lycopene and phenolic compounds, as well as enzymes such as catalase (CAT) and glutathione peroxidase (GPX) (Castillo-Godina, Foroughbakhch-Pournavab, & Benavides-Mendoza, 2016). One way to improve the Se absorption by plants in deficient soils and crops without soil, is through complexes of biopolymers, such as chitosan and polyacrylic acid. These compounds can form complexes with mineral elements or active ingredients, which are immobilized in order to achieve a prolonged release. With this technique, Se losses due to volatilization and leaching could be avoided. Considering the above, in the present work it was sought to biofortify a cherry tomato crop with 5 mg Se/plant, in its ionic form and in complexes with biopolymers. The impact on the yield of the crop, the nutraceutical and organoleptic quality of the fruits, the accumulation of selenium in the fruits, and the expression of genes related to antioxidant enzymes in leaves and fruits of tomato were analyzed.

METHODS

Crop establishment

A soilless cherry tomato (*Solanum lycopersicum* L. var. *Cerasiforme* cv. Felicity) crop was established in a greenhouse with polyethylene cover, 50%–60% relative humidity, and an average temperature of 28 °C. The seeds were sown in black polyethylene trays filled with sphagnum peat moss and perlite in a 1:1 ratio v/v. At 45 days after sowing, seedlings were transplanted to black polyethylene containers filled with 10 L of substrate mix of sphagnum peat moss and perlite. A single pass drip irrigation system was installed, through which the nutrition was carried out, using Steiner nutrient solution (Steiner, 1984) with a pH of 6.5.

Treatments and sampling

Non-Stoichiometric Interpolyelectrolyte Complexes (NPEC) of Chitosan-Polyacrylic Acid (Cs-PAA) and Cs-PAA + Se were prepared and provided by the Applied Chemistry Research enter (CIQA) (Ortega-Ortíz et al., 2003)deacetylation degree was 99%, molecular weight Mv = 200,000 g/mol. The treatments were applied weekly after the last irrigation of the day (at 18:00 hours) directly to the substrate manually, adding 20 mL per plant, respectively of each treatment. A sampling was carried out 120 days after the transplant (DDT), to determine the different variables, collecting fruits and leaves of five plants of each treatment. During the crop cycle, 5 fruit harvests were carried out to determine the yield per plant.

Table 1. Description of the treatments applied weekly.

T0	**Control**
T1	Na_2SeO_3 (5 mg Se/plant)
T2	Cs-PAA + Se (5 mg Se/plant)
T3	Cs-PAA

Crop Yield

For the total yield of the crop, five harvests were made during the crop cycle, collecting the fruits in a state of ripeness for consumption in color # 6 in USDA scale (USDA, 2005). The data was recorded in kilograms (Kg) per plant of each treatment.

Fruits organoleptic quality variables

The firmness of the fruits was quantified with a QA FT327 penetrometer, with an 8 mm stainless steel plunger tip, firmly taking the fruit and introducing the plunger tip with a single impact to the delimiting mark, recording the results in Kg cm^{-2}. In order to quantify the total soluble solids, the fruit was cut to obtain juice drops and placed in a previously calibrated refractometer (ATTAGO 0-32%). The data was recorded as ° Brix.

Nutraceutical quality variables

Samples of leaves and fruits were analyzed. For the quantification of the nutraceutical quality variables, the samples immediately after being collected were placed in freezing at -20 °C, lyophilized in a LABCONCO® Freezone 2.5 plus brand equipment for 48 hours with a temperature of -80°C and 0.110 mbar of vacuum.

Lycopene content

In order to quantify the lycopene in the fruits, 100 mg of fresh tissue was taken from the fruits and macerated in a mortar, 20 mL of hexane: acetone solution (3:2) was added. This solution was centrifuged at 3000 rpm and the supernatant was collected. In a spectrophotometer, the absorbance at 453, 505, 645, and 663 nm of the supernatant was read, and the equation was calculated:

$$\text{Lycopene} = -0.0458\ A_{663} + 0.204\ A_{645} + 0.372\ A_{505} - 0.0806\ A_{453}.$$

The results were reported as milligrams per 100 g of fresh weight (Nagata & Yamashita, 1992).

Protein extraction

Each sample of lyophilized tissue was pulverized in a porcelain mortar. 150 to 200 mg were placed in a 2 mL tube to which was added the equivalent of 10% of the weight of the sample of polyvinylpyrrolidone (PVP). Subsequently, 1.5 mL of phosphate buffer with a pH of 7 - 7.2 was added. The samples were centrifuged at 10,000 rpm at 4 °C for 10 minutes. The supernatant was collected and the enzymatic activity of catalase, glutathione peroxidase, and glutathione content was quantified from the obtained extract.

Total Proteins

The Bradford spectrophotometry technique (Bradford, 1976) was used for protein quantification. With 100 µL of the supernatant in a test tube, 5 mL of Bradford reagent (Coomassie brilliant blue G-250 from Sigma Aldrich® solution) was added. After 5 minutes the absorbance was read in a spectrophotometer at a wavelength of 594 nm; the results were extrapolated with a calibration curve previously prepared with bovine serum albumin obtaining the results in mg L^{-1}. These data were used to determine the activity of enzymatic antioxidants.

Catalase Activity (CAT) (EQ 1.11.1.6)

The activity of the catalase enzyme was quantified with a spectrophotometric method. A mixture was made with 100 µL of protein extract and 1 mL of 100 mM hydrogen peroxide (H_2O_2) prepared with phosphate buffer of pH 7 - 7.2 and 400 µL of 5% sulfuric acid (H_2SO_4) to stop the reaction. The absorbance was measured at a wavelength of 270 nm and recorded as the zero time of the reaction (T0). Subsequently, the mixture of protein extract and H_2O_2 was made with buffer, and it was left to react for 1 min and the enzymatic activity was stopped, and it was recorded as time 1 (T1). For this analysis, a different blank was made for each sample, consisting of 100 µL of protein extract, 1 mL of phosphate buffer and 400 µL of H_2SO_4. The results were reported in units, which were expressed in mM of $H_2O_2 min^{-1} mg^{-1}$ (Cansev, Gulen, & Eris, 2011).

Glutathione peroxidase activity (GPX) (EQ 1.11.1.9)

The method of (Xue, Hartikainen, & Piironen, 2001) was performed using H_2O_2 as a substrate. 0.2 mL of the biomolecule extract was placed in a test tube, 0.4 mL of 0.1 M reduced glutathione and 0.2 mL of 0.067 M Na_2HPO_4 were added. This mixture was pre-heated in a water bath at 25°C for 5 minutes, subsequently 0.2 mL of 1.3 mM H_2O_2 was added to start the catalytic reaction. It was left to react for 10 min and the reaction was stopped by the addition of 1 mL of 1% trichloroacetic acid. This reaction mixture was placed in an ice bath for 30 min. The mixture was then centrifuged at 3,000 rpm for 10 min. 0.48 mL of the supernatant was taken and a test tube was placed, 2.2 mL of 0.32 M

Na_2HPO_4 and 0.32 mL of a 1 mM solution of the 5,5 dithio-bis-2-nitro benzoic acid dye (DTNB) were added. The absorbance was read on a UV-VIS spectrophotometer at 412 nm. Units of activity were expressed in mgL^{-1} GSH $min^{-1}mg^{-1}$.

Glutathione (GSH)

The glutathione was quantified with the protein extract, following the spectrophotometric technique established by XUE et al., 2001 through the reaction with 5,5-dithio-bis-2-nitro-benzoic acid (DTNB). The units were reported in mg L^{-1} (Xue et al., 2001).

Total phenolic compounds

Total phenol content was quantified using (Singleton, Orthofer, & Lamuela-Raventós, 1998) technique, performing extraction with water: acetone (1: 1). The reagent Folin-Ciocalteu 1 M reagent, 50 µL of the extract, 500 µL of 20% Na2CO3, 1 mL water-acetone 1: 1 mix, and 5 mL of distillated water were used for the reaction. Blank consisted of 50 µL of water-acetone mix instead of the extract with the same components of the previous reaction. The samples were read with a spectrophotometer (Thermo Scientific Model G10S, Waltham, MA, USA) at a wavelength of 750 nm and the results were recorded in µg g^{-1}.

Selenium content

A wet digestion technique was used to quantify selenium. In a beaker, 500 mg of dehydrated sample was placed with 30 mL of nitric acid and heated for 3 hours. The obtained digestion was taken up to a volume of 50 mL with deionized water and filtered with Whaltman # 42 filter paper. The selenium content was analyzed with an inductively coupled plasma optical emission spectrometer (ICP-OES, Thermo Scientific Jarrell Ash, Model iCAP 7000 Series, Waltham MA, USA).

Gene expression

RNA extraction was performed by the Trizol method according to the technique of (Cui, Fauquet, & Zhou, 2004). RNA quantification was determined using a UV-Vis spectrophotometer (Thermo Scientific Model G10S, Waltham, USA). A denaturing polyacrylamide gel electrophoresis was elaborated to verify the RNA quality. The

synthesis of cDNA was performed with a Bioline SensiFAST cDNA Synthesis Kit. Primers of the endogenous actin gene were used, in addition to the CAT and GPX study genes, according to the design of (Hernández-Hernández et al., 2018) as described in **Table 2**.

Table 2. Primers sequence of analyzed genes.

Gene	Forward Primer 5'-3'	Reverse Primer 5'-3'
ACT	CCCAGGCACACAGGTGTTAT	CAGGAGCAACTCGAAGCTCA
CAT	CCCTCTAAGTATCGCCCATCAA	TTGTACACAGGACCACCAGCAT
GPX	AGGAGCCTGGAAACATTGAAGA	CCATTCACGTCAACCTTGTCA

With an Applied Biosystems StepOne ™ Equipment, the Real-time PCR reaction was performed with the standard relative curve method, using Sybr Green as fluorescence signaling. The qPCR program consisted of 10 min at 95 ° C, 40 cycles of PCR, 15 s at 95 ° C, and 1 min at 60 ° C. Mean expression level of the CAT and GPX genes was recorded as RQ.

Data analysis

A completely randomized experimental design was used; an ANOVA and a comparison of means LSD $\alpha \leq 0.05$ were performed for the data analysis with the InfoStat software (Grupo InfoStat, Córdoba, Argentina).

RESULTS AND DISCUSSION

Yield per plant

Since crop yield is a parameter of great interest to agricultural producers, it is essential to integrate it among the variables that are evaluated in the different investigations. In this work, with the treatments applied it was possible to obtain a higher yield per plant, as shown in **Figure 1**. When the Cs-PAA + Se treatment was applied, a higher yield was obtained per plant compared to the control and with the application of Na_2SeO_3, increasing the yield by 33.6% and 29.7% respectively, although it was statistically similar

to the yield obtained by the plants treated with Cs-PAA. With the treatment of Cs-PAA + Se, up to 3.6 kg plant^{-1} were obtained, with a planting density of 3 plants m^{-2}, extrapolating to 105.8 Ton ha^{-1}. On the contrary, (Zhu, Chen, Zhang, & Li, 2016) reported a decrease in tomato yield of 16% when applying 10 mg Se L^{-1}. (Edelstein, Berstein, Shenker, Azaizeh, & Ben-Hur, 2016) also reported a decrease in yield of 51% compared to the control when applying 1.5 mg Se L^{-1} to the nutrient solution of a tomato hydroponic culture. (Pezzarossa, Rosellini, Malorgio, Borghesi, & Tonutti, 2013), found no differences in tomato cv. Red Bunch yield when applying 1 mg Se L^{-1} to the nutrient solution compared to the control.

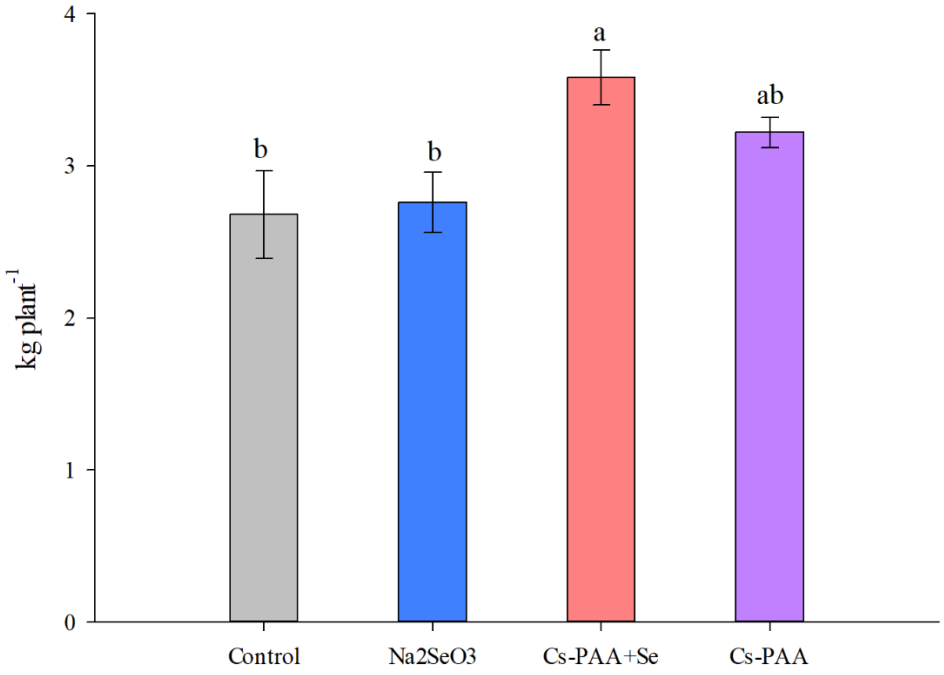

Figure 1. Means comparison of the yield per plant. Means with the same letter are statistically equal (LSD, $p \leq 0.05$). Bars represent standard error.

Enzymatic activity of CAT and GPX

In the cherry tomato fruits, all the treatments induced an increase in the enzymatic activity of catalase (**Figure 2a**) compared to the control, however, there were not statistically different between them. On the contrary in the leaves (**Figure 2b**), the activity of catalase was not modified with the application of the different treatments. (Castillo-Godina et al.,

2016) found that the catalase enzymatic activity increased in leaves, stems, and fruits with the application of 0.2 and 5 mg L^{-1} of Na_2SeO_3 in the nutrient solution in tomato. In another study in tomato, the authors reported an increase in the enzymatic activity of catalase of 45.45% when applying 10 mg Se L^{-1} foliar (Daniel, Subramaniyan, Chinnannan, & Indra, 2015). In other plant species such as *Triticum aestivum* L. (Ibrahim, 2014), *Lactuca sativa* L. (Leija-Martínez et al., 2018) and *Spirulina platensis* (Chen, Zheng, Wong, & Yang, 2008), it has been reported that the application of Se in different chemical forms and forms of application, increase the enzymatic activity of catalase.

In the enzymatic activity of GPX there was no significant difference between treatments in both fruits and leaves of cherry tomato (**Figure 2c, 2d**). On the contrary, (Castillo-Godina et al., 2016) reported an increase in glutathione peroxidase activity with 5 mL L^{-1} Se in tomato var. "Toro", as well as in another study with ryegrass (Helinä; Hartikainen, Xue, & Piironen, 2000), where GPX activity was also improved with 1 mg Se kg^{-1}.

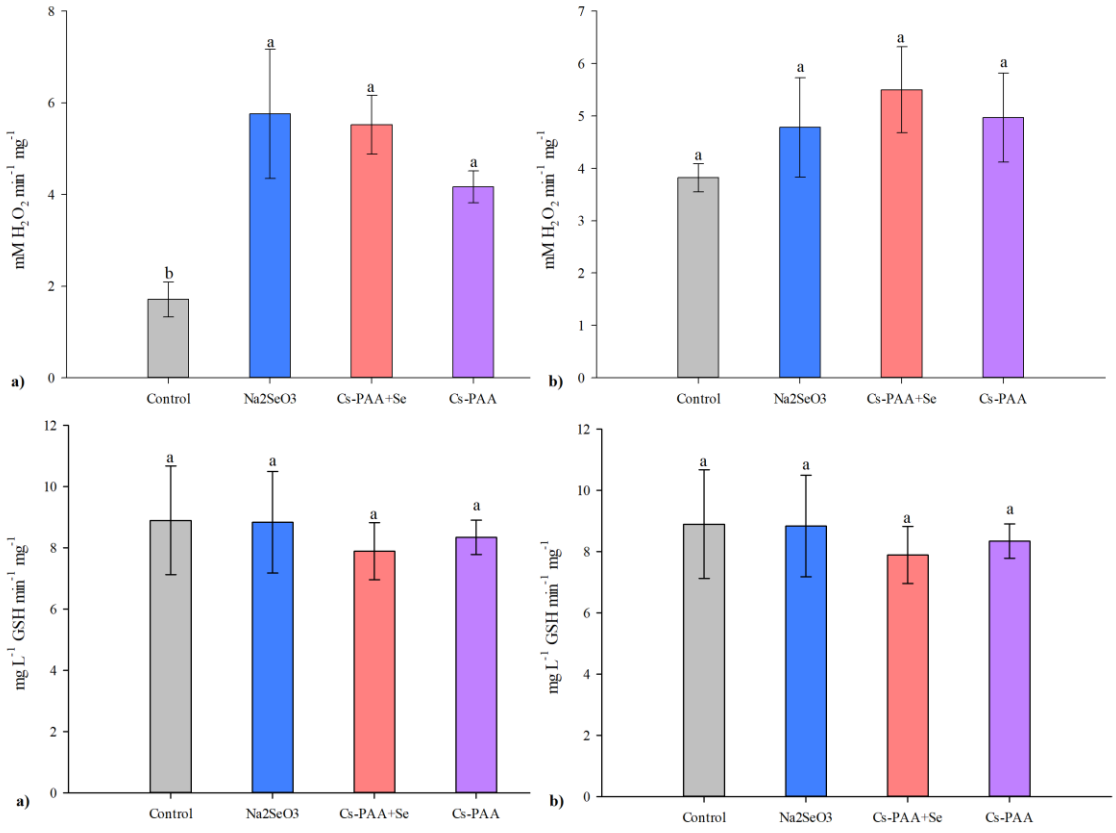

Figure 2. Means comparison of **a)** catalase activity in fruits; **b)** catalase activity in leaves; **c)** glutathione peroxidase activity in fruits; **d)** glutathione peroxidase activity in leaves.

Means with the same letter are statistically equal (LSD, $p \leq 0.05$). Bars represent standard error.

Glutathione content

The GSH content in fruits increased by 26.6% compared to the control when applying Na_2SeO_3 (**Figure 3a)**. On the contrary, no significant differences were found in the leaves (**Figure 3b**). Similarly to the response in fruits, an increase of 18% in a wheat crop (Khan, Nazir, Asgher, Per, & Khan, 2015) has been reported when applied in conjunction with S. In lettuce plants, (Xue et al., 2001) reported that at a minimum dose of 0.1 mg Kg of soil of Na_2SeO_4 the content of glutathione (GSH) increased significantly.

Total phenolic compounds

The treatments of Cs-PAA + Se, Na_2SeO_3 and Cs-PAA increased the content of phenolic compounds in the fruits (**Figure 3c**), whereas in the leaves only the treatment of Na_2SeO_3 was able to surpass the control (**Figure 3d**). In an experiment by (Schiavon et al., 2013) with Na_2SeO_4, in different concentrations (5, 10, 25, 50 and 100µM) in tomato plants, the metabolites were analyzed in the epidermis of the fruits, in order to determine the total phenols, and reported a substantial decrease in the plants treated with Se, and in the fruit pulp the phenol compounds increased compared to the control.

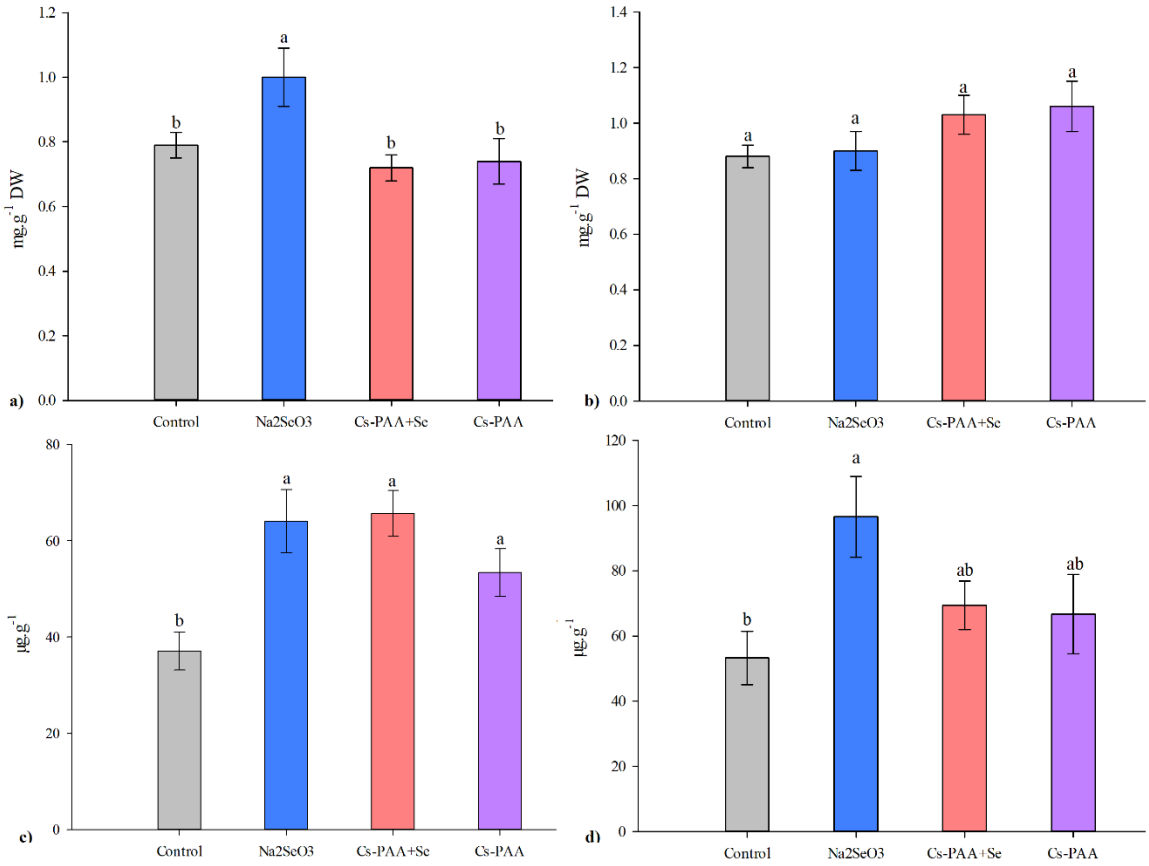

Figure 3. Means comparison of **a)** glutathione in fruits; **b)** glutathione in leaves; **c)** phenolic compounds in fruits; **d)** phenolic compounds in leaves. Means with the same letter are statistically equal (LSD, $p \leq 0.05$). Bars represent standard error.

Fruit firmness

The firmness of the fruits increased when applying Se (**Figure 4a**), both in ionic form (Na_2SeO_3) and in the form of complexes with biopolymers (Cs-PAA + Se). This concurred with another work (Islam, Mele, Baek, & Kang, 2018) where 1 mg L^{-1} Se was applied to the cherry tomato nutrient solution, where an increase in firmness was reported with respect to the control.

Total soluble solids

Regarding the total soluble solids, an increase was observed when applying the biopolymer complexes. The treatments of Cs-PAA+Se and Cs-PAA increased the SST

by 18.8% and 24.3% respectively compared to the control (**Figure 4b**). Na_2SeO_3 had no effect on total soluble solids. Similarly, (Islam et al., 2018) report, where there were no differences when applying Se to cherry tomato against control.

Selenium content in fruits

With the application of both forms of Se, Na_2SeO_3 and Cs-PAA+Se (**Figure 4c**), cherry tomato fruits were able to be biofortified at appropriate levels, reaching up to 13.84 mg Kg DW^{-1}, which is equivalent to 83.4 µg 100 g FW^{-1}. This amount of Se is suitable for daily consumption, since the U.S. Food and Nutrition Board recommends an intake between 60 to 75 µg Daily. This dose can be increased in order to access the additional benefits that Seprovides to human health, for which (Rayman, 2012) recommend between 200-300 ug daily, although the Nutritional Prevention of Cancer (NPC) recommends eating the highest doses only to people with deficiencies of Se (Broadley et al., 2006).

Lycopene content

The treatments of Cs-PAA+Se and Cs-PAA increased the lycopene content in fruits by 71% compared to the control (**Figure 4d**). Unlike (Islam et al., 2018), who reported no significant difference with applications of 1 mg L^{-1} Se in the nutrient solution, compared to the control. (Schiavon et al., 2013) also did not find differences in comparison with the control with applications of 5 and 20 mg Se per plant. The increase in lycopene content seems to be induced by the application of biopolymer complexes, rather than the Se.

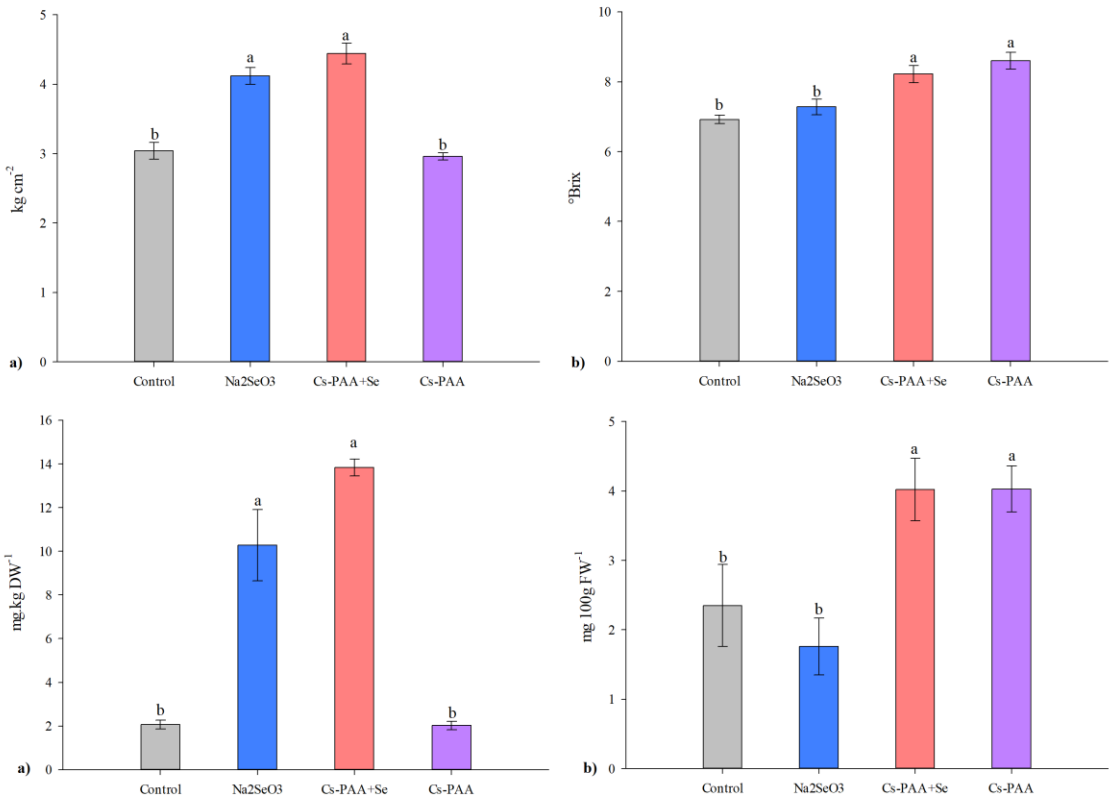

Figure 4. Means comparison of **a)** fruits firmness; **b)** total soluble solids; **c)** selenium content in fruits; **d)** lycopene content. Means with the same letter are statistically equal (LSD, $p \leq 0.05$). Bars represent standard error.

Gene expression

The gene expression of CAT was repressed compared to the value of absolute control when applying the treatments in the cherry tomato fruits, however the treatments of Cs-PAA+Se and Cs-PAA increased the CAT gene expression in leaves (0.36 and 3.5-fold change, respectively), possibly this was what caused the differences in CAT enzymatic activity in the fruits. (Hernández-Hernández et al., 2018) reported a repression of the CAT gene with application of a Cs-PVA complex in tomato plants subjected to salt stress.

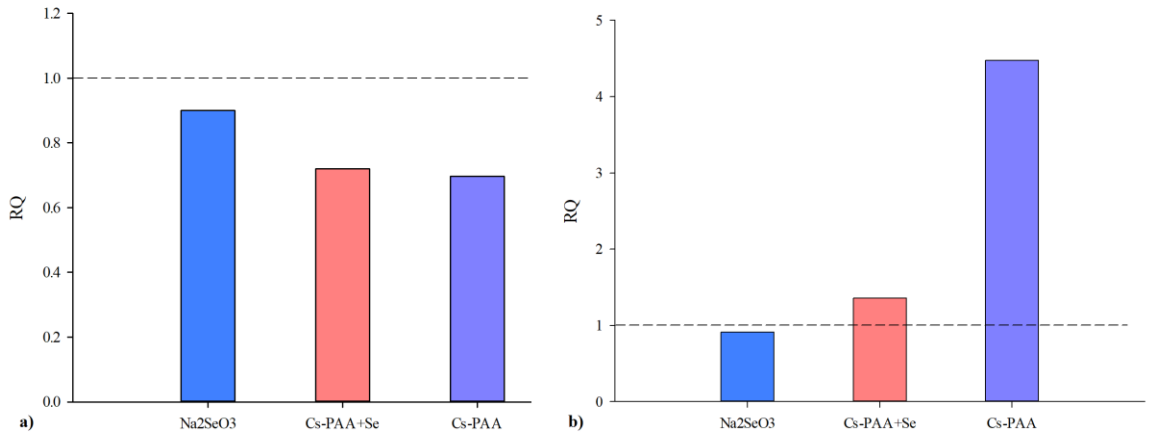

Figure 5. Mean expression level of **a)** gene CAT in fruits; **b)** gene CAT in leaves. The reference line represents the constant value of the absolute control.

In the gene expression of GPX in fruits, treatments with Cs-PAA repressed the GPX gene, however, treatment with sodium selenite increased 3.1-fold change expression of GPX. Whereas, in the leaves, the Cs-PAA + treatment was increased 1.7-fold change in comparison with the rest of the treatments. On the other hand, with applications of Cs-PVA, (Hernández-Hernández et al., 2018) reported a decrease in the expression of GPX in tomato leaves.

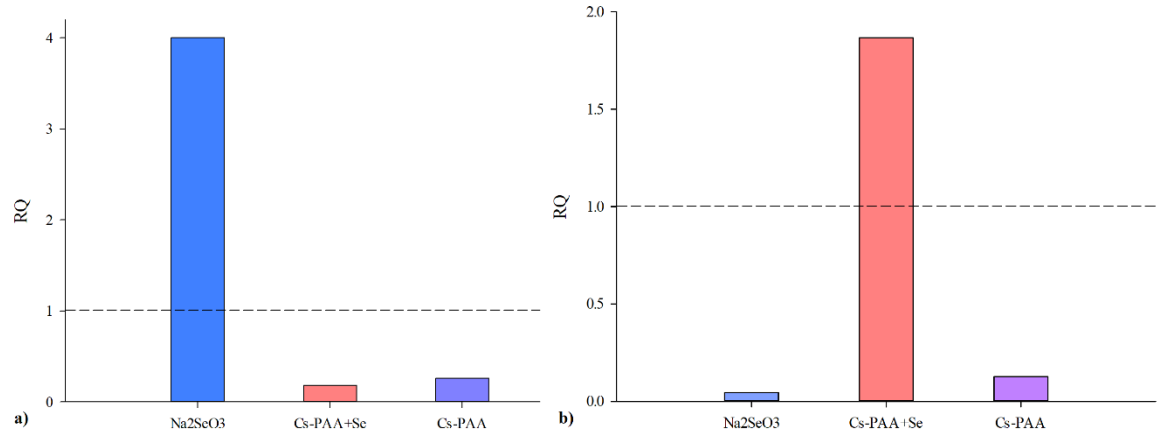

Figure 6. Mean expression level of **a)** gene GPX in fruits; **b)** gene GPX in leaves. The reference line represents the constant value of the absolute control.

CONCLUSIONS

Our results indicate that cherry tomato crop can be improved with the application of selenium absorbed in biopolymer complexes, since it increases crop yield, which leads to important economic benefits. In addition, it improves the firmness of the fruits and the total soluble solids, which are determining factors for the organoleptic quality.

The treatments containing ionic selenium and selenium absorbed in complexes of biopolymers manage to increase the content of total phenols and catalase. The complexes of chitosan-polyacrylic acid increase the content of lycopene in fruits.

Both selenium in ionic form and in biopolymer complexes provide benefits to the cherry tomato crop by themselves, however, the benefits are potentiated when applied together. Thus, fruits with high nutraceutical content can be obtained, ensuring a high consumption of antioxidants. The use of selenium and biopolymers positively modifies the expression of genes related to antioxidant enzymes, which induces a higher resistance to stress factors in cherry tomato crop.

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CONCLUSIONES GENERALES

Los complejos de Cs-PAA pueden ser beneficiosos en los procesos de biofortificación, debido a su tendencia a aumentar la absorción de Se. Inducen un aumento en la actividad de las enzimas antioxidantes CAT y GPX, sin afectar el desarrollo del cultivo.

Los complejos Se y Cs-PAA fueron efectivos para mejorar la resistencia de las plantas, así como la calidad nutracéutica de la lechuga Great Lakes.

El rendimiento del cultivo de tomate cherry Felicity aumenta con la aplicación de complejos de Qs-PAA + Se. El tratamiento de Qs-PAA + Se mejora la firmeza de los frutos y los sólidos solubles totales.

Los tratamientos que contienen selenio iónico y selenio absorbido en complejos de biopolímeros logran aumentar el contenido de fenoles totales y actividad de catalasa.

Los complejos de Qs-PAA aumentan el contenido de licopeno en los frutos.

Con base en los resultados obtenidos, es recomendable incorporar al selenio absorbido en complejos de quitosán-poliácido acrílico puesto que se obtienen órganos comestibles de superior calidad nutracéutica al aumentar su contenido de antioxidantes, además de que aumenta la disponibilidad del selenio para el consumidor y aumenta el rendimiento además de inducir la resistencia de las plantas a diversos factores de estrés.

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