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POTENCIAL ANTIOXIDANTE DE CHILE JALAPEÑO (*Capsicum annuum* L.) COMO RESPUESTA A LA BIOFORTIFICACIÓN CON SELENIO

Tesis

Que presenta MARÍA DE LOS ANGELES SARIÑANA NAVARRETE

como requisito parcial para obtener el Grado de

DOCTOR EN CIENCIAS EN AGRICULTURA PROTEGIDA

Saltillo, Coahuila Julio, 2023

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Elaborada por MARÍA DE LOS ANGELES SARIÑANA NAVARRETE, como requisito parcial para obtener el grado de Doctor en Ciencias en Agricultura Protegida con la supervisión y aprobación del Comité de Asesoría

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INTRODUCCIÓN

El incremento en la población mundial ha suscitado el desarrollo de una agricultura más intensiva, con el fin de promover la seguridad alimentaria, sin embargo, el incremento conjunto de problemas nutricionales ha logrado que la atención se enfoque en adquirir seguridad nutricional (Shahane & Shivay, 2022). Para el ser humano, la deficiencia de micronutrientes está estrechamente relacionada con el estatus nutricional de los alimentos que conforman la dieta diaria, dígase de origen animal o vegetal, influido principalmente por la cantidad de macro y micro nutrimentos disponibles en los suelos cultivables (Izydorczyk et al., 2021). Cinco son los principales micronutrientes para las funciones principales de la mitocondria, y otras funciones fisiológicas del cuerpo humano, donde la alta prevalencia en la deficiencia de alguno de ellos se asocia a un pronóstico adverso, los cuales son Hierro (Fe), Selenio (Se), Zinc (Zn), Cobre (Cu), y la coenzima Q10 (CoQ10) (Bomer et al., 2022).

El hambre oculta, es la forma más común de referirse a la deficiencia global de micronutrientes que padecen más de 2 billones de personas, y prevalece mayormente en niños, y mujeres embarazadas y en estado de lactancia (Szerement et al., 2021). El Se, específicamente, está involucrado en diversos procesos metabólicos, pues dadas sus propiedades antioxidantes y antiinflamatorias, contribuye a la fisiopatología de enfermedades como diabetes, cáncer, y enfermedades cardiovasculares y neurodegenerativas, así como mantener la homeostasis redox (Ferreira et al., 2021). Sin embargo, este elemento se considera un arma de doble filo por las siguientes condiciones, (1) bajo nivel de Se (<40 μg/día) incrementa el riesgo de desarrollar, especialmente, desordenes cardiovasculares, y (2) exceso nivel de Se (>400 μg/día) puede causar selenosis, y otros desordenes que comprometen la integridad del sistema inmunológico (Ferreira et al., 2021; Tripathi et al., 2021). En México el consumo de Se es de 37.6-51.8 μg/día, aportado por fuentes de origen animal y vegetal, sin embargo, basado en la dieta, la población consume principalmente alimentos de origen vegetal, lo que pone en duda que se complemente el consumo de Se que es proporcionado de alimentos de origen animal (aproximadamente 19%) (Curi-Quinto et al., 2022; Rios-Lugo et al., 2022), lo que posiciona a los vegetales como la principal fuente de Se. Una de las estrategias que centra

su objetivo en reducir las deficiencias de micronutrientes es la biofortificación, que, a través de técnicas agronómicas, tecnología transgénica, o fitomejoramiento, se incrementa la cantidad de vitaminas y minerales en un cultivo, de forma práctica y sostenible (Srivastav et al., 2022). Alrededor del mundo, la biofortificación centra sus objetivos en enriquecer los cultivos con minerales tales como Fe, Zn, Se, Cu, Calcio (Ca), Magnesio (Mg) y Yodo (I), y vitamina A (White y Broadley, 2009).

Por otra parte, más allá del uso como elemento biofortificante, en la nutrición vegetal, el Se ha sido clasificado como un bioestimulante (du Jardin, 2015), debido a que desde el momento en que entra en contacto con las células, origina cambios en la concentración de compuestos antioxidantes como ascorbato (AsA), glutatión (GSH), tocoferoles (vitamina E) (El-Ramady et al., 2015; García Márquez et al., 2020), glucosinolatos, flavonoides, polifenoles, proteínas unidas a Se, selenoproteínas (Wen, 2021), y las enzimas relacionadas con la detoxificación del peróxido de hidrogeno (H_2O_2), como catalasa (CAT), glutatión peroxidasa (GSH-Px), ascorbato peroxidasa (APX), y superóxido dismutasa (SOD) (El-Ramady et al., 2015). Esto brinda la oportunidad de obtener cultivos enriquecidos con Se, y compuestos bioactivos que generan un impacto positivo en la salud humana. De lo anterior descrito, se planteó evaluar el potencial antioxidante del cultivo de cultivo de chile jalapeño (*Capsicum annuum* L.) como respuesta a la biofortificación con selenio, en su forma iónica y nanométrica.

Objetivo general

Evaluar el potencial antioxidante del cultivo de cultivo de chile jalapeño (*Capsicum annuum* L.) como respuesta a la biofortificación con selenio.

Objetivos específicos

1. Evaluar el porcentaje de germinación y rasgos morfológicos de brotes de chile jalapeño como respuesta al cebado de semillas con Se.
2. Determinar el rendimiento del cultivo y calidad de los frutos como respuesta a la aplicación de Se.

3. Determinar la concentración de compuestos antioxidantes y actividad enzimática en tejido foliar y en fruto como respuesta a la aplicación de Se.
4. Determinar la acumulación de Se en tejido foliar y en fruto, como respuesta a la aplicación de Se.

Hipótesis

La aplicación foliar y en drench de selenito de sodio y nanopartículas de selenio al cultivo de chile jalapeño (*Capsicum annuum* L.) favorecerá la acumulación de Se en frutos, así como el incremento de compuestos antioxidantes.

REVISIÓN DE LITERATURA

Selenio en la salud humana

La función biológica del Se cómo antioxidante es a través de las selenoenzimas, como glutatión peroxidasa (GSH-Px), la cual protege a las células de la oxidación. Sin embargo, el aminoácido SeCys participa en la síntesis de proteínas mediada por los ribosomas, y es parte integral de la actividad de las 25 selenoproteínas en mamíferos, que además de la GSH-Px, incluye tiorredoxina reductasa (TXNRD), yodotironina desyodasa (DIO), metionina sulfóxido reductasa (MSRB) y selenofosfato sintetasa (SEPHS), y las selenoproteínas F, H, I, K, M, N, O, P, S, T, V, y W (Hu et al., 2021), las cuales ejercen funciones específicas (Tabla 1).

Tabla 1. Función biológica de las selenoproteínas en mamíferos.

Selenoproteínas	Localización	Función	Ref.
GSH-Px (1-4, 6)	Citosol (1,2), plasma (3), Citosol, mitocondria y núcleo (4), citosol (6)	Reduce el peróxido de hidrogeno (H_2O_2) y peróxidos lipídicos (1); reduce peróxido en intestino (2), en sangre (3), de fosfolípidos (4) y el H_2O_2 celular en el epitelio olfativo (6)	(Flohé et al., 2022)
TXNRD (1-3)	Citosol (1, 2), mitocondria (3).	Involucradas en el sistema tiorredoxina-glutaredoxina (1,2); reduce las formas oxidadas de tiorredoxina y glutaredoxina (3).	(Patwardhan et al., 2022)
DIO (1-3)	Membrana plasmática (1,3), retículo endotelial (2).	Involucrados en los niveles locales y sistémicos de la hormona tiroidea (1,3); inactiva la hormona tiroidea (2).	(Sarzo et al., 2022)
MSRB	Citosol	Regenera a metionina los residuos de metionina-R-sulfóxido en proteínas.	(Tarrago et al., 2022)
SEPHS	Citosol	Síntesis de selenofosfato a partir de Se y ATP.	(Manta et al., 2022)
Selenoproteínas (F-W).	Organelos diversos	Involucradas en la detección y transcripción redox, biosíntesis de fosfolípidos, flujo de Ca^{2+}, desarrollo muscular, transporte de Se, regulación redox, y para la adecuada función muscular.	(Zhao et al., 2022)

Las funciones de las selenoproteínas anteriormente expuestas, remarcan su esencialidad, ya que aunque sea caracterizado principalmente por su función antioxidante a través de la enzima GSH-Px, se involucra en otros procesos de biosíntesis, señalización, mantenimiento, y transporte de biomoléculas diversas, incluido el metabolismo del yodo; es por ello por lo que mantener un balance nutricional y enfocar una ingesta de alimentos

enriquecidos con micronutrientes, reduce la probabilidad de poner en riesgo la integridad del cuerpo humano.

Biofortificación de cultivos

La biofortificación es una práctica cuyo objetivo implica mejorar la calidad nutricional de un determinado cultivo al incrementar la concentración de micronutrientes en raciones comestibles, sin influir negativamente en los rasgos agronómicos, tales como rendimiento, resistencia a estrés biótico y abiótico, entre otros (Dhaliwal et al., 2022; Kumar & Sindhusha, 2021). Dentro de la biofortificación, los cultivos como los cereales, legumbres, hortalizas, y cultivos de fruta son empleados principalmente para el enriquecimiento con Zn, Fe, Mg, Se, I, ácido fólico, carotenoides y vitamina A (Sheoran et al., 2022). El éxito de que esta práctica sea de forma sostenible se basa en el enfoque, con el propósito particular de proporcionar una solución duradera, estos son el fitomejoramiento, mediante ingeniería genética, y a través de prácticas agronómicas (Kiran et al., 2022).

Biofortificación de cultivos con Se mediante prácticas agronómicas

Naturalmente, el contenido de Se en los cultivos esta dependiente de la biodisponibilidad de este elemento en los suelos cultivables (Hegedűsová et al., 2021). Es por ello, que en el enriquecimiento de los cultivos con Se se puede lograr a través de (1) suministro edáfico, (2) priming de semillas, (3) aspersiones al follaje o directamente a los frutos, y (4) a través de la solución nutritiva enriquecida con Se en cultivos hidropónicos (Puccinelli et al., 2017).

Fertilización edáfica

La forma más soluble de Se en los suelos es selenato (SeO_4^{2-}) (Lanza & Reis, 2021) En la práctica, la adición de fertilizantes de Se al suelo es una vía apropiada para enriquecer cultivares a gran escala, e incrementar el contenido de Se en el suelo (Puccinelli et al., 2017). Con el uso de sales de Se, como selenito (SeO_3^{2-}) o selenato, o la incorporación de plantas hiperamuladoras de Se (>1 mg Se por g de biomasa seca), es posible incrementar la concentración de Se en el suelo (Szőllősi et al., 2022). Deng et al., (2021) reportan que la aplicación combinada de selenato de sodio (Na_2SeO_4) a razón de 2 mg kg^{-1} con una fertilización de S (100 mg kg^{-1}) en condiciones de suelo aluvial calcáreo con pH 7.87 se

incrementa la biodisponibilidad de Se para *Glycine max* L. que en suelo en condiciones de pH ligeramente ácido (5.68) (Deng et al., 2021). Otro ejemplo de fertilización edáfica se presenta en un suelo franco calcáreo (Zafeiriou et al., 2022) y se destaca una mayor biodisponibilidad y asimilación de Se cuando es aplicado en forma de SeO_4^{2-} comparado con SeO_3^{2-}. Esto sugiere que, para casos aplicados, suministrar Se al suelo en forma de SeO_4^{2-} es más efectivo que en su forma de SeO_3^{2-} en condiciones de suelo ligeramente alcalinas, dado que, en condiciones de suelo ácido, el SeO_3^{2-} rápidamente forma complejos con otros elementos, con lo que se reduce su biodisponibilidad. Sin embargo, aplicar SeO_3^{2-} en suelos ligeramente alcalinos aumenta el grado de biodisponibilidad comparado al aplicar en suelos ácidos, ya que puede ser oxidado a SeO_4^{2-} y ser fácilmente absorbido por las plantas (Guo et al., 2023; Wang et al., 2021).

Imbibición de semillas

Este método consiste en depositar una cantidad de semillas conocida en una solución que contenga Se (Puccinelli et al., 2017), sin embargo, el que se logre el objetivo del enriquecimiento de la parte comestible, aun no es claro, aunque causa un impacto positivo en otros procesos metabólicos (Izydorczyk et al., 2021). Investigaciones recientes realizadas en semillas de trigo sugieren que está práctica tiene el potencial para ser aplicada como método de enriquecimiento del cultivo, específicamente el grano, al realizar un pretratamiento a las semillas con soluciones a 2.5 y 5 mM de Na_2SeO_4 durante 12 h, y encontrar un incremento significativo en el contenido de Se en las semillas tratadas (Rocha et al., 2022).

Aspersiones al follaje

El enriquecimiento con Se empleando está práctica se posiciona como preferible a la aplicación de Se al suelo, ya que se emplea una cantidad de Se mucho menor. La aplicación de Se por aspersiones al follaje es más eficaz, seguro, y económico, y se logra un mayor enriquecimiento (Puccinelli et al., 2017). Básicamente consiste en depositar sobre la hoja microgotas de agua que contienen Se. Cuando se aplican nutrimentos a través de aspersiones foliares, la principal vía de entrada es por gradiente de concentración, a través de los poros presentes en la cutícula, y llevado a las células del mesófilo empleando

transportadores específicos (Lanza & Reis, 2021). Un caso comparativo realizado en avena expone la diferencia en la concentración de Se acumulada en el grano, al aplicar la misma dosis de Se en suelo y por vía foliar (1.2 mg de Se por m^2 de superficie), incrementando más de 50% el contenido de Se en grano por aplicación foliar. En las aplicaciones foliares, al ser un método más directo, se puede comparar la cantidad de Se acumulado versus la forma de Se que se aplica (Li et al., 2021),. Aplicar SeO_3^{2-} y SeO_4^{2-} fue caso de estudio en trigo, y se muestra una mayor habilidad de SeO_3^{2-} para ser asimilado, al cuantificar mayor Se en el grano cuando se aplicó selenito, y en menor acumulación de Se cuando se aplicó selenato (Di et al., 2023).

Cultivos hidropónicos

El desarrollo de cultivos en condiciones sin suelo, es decir, en un sistema hidropónico, es uno se los sistemas donde se realiza la máxima gestión del agua y nutrientes que se emplean, y se mantienen las condiciones de conductividad eléctrica, pH, oxígeno disuelto y temperatura, pero es necesario hacer mediciones en tiempo real de esas condiciones, para evitar el desequilibrio entre nutrientes (Son et al., 2020). La cantidad de Se que se emplea en este tipo de sistema está dependiente del cultivo, pues la mayoría de las hortalizas a excepción de las brasicáceas, son no acumuladoras de Se, es decir, el grado de toxicidad se puede alcanzar. Las investigaciones en biofortificación con Se que emplean está técnica, usan concentraciones entre 2.6 a 5.2 µmol L^{-1} de SeO_4^{2-} para lechuga, rúcula y espinaca (Francini et al., 2023), de 1 a 4 µmol L^{-1} de dióxido de selenio (SeO_2) en tomate Cherry (Sabatino et al., 2021), de 0.5 a 6 µmol L^{-1} de SeO_4^{2-} en lechuga var. Beisansheng (Li et al., 2022c), y la evaluación de una dosis única en fresa, de 5.29 µmol L^{-1} de SeO_4^{2-} (Pourebrahimi et al., 2023), llevándolo a un contexto más, es aproximadamente 1 ppm de Se.

Selenio en plantas: absorción, transporte y asimilación

A nivel global, el contenido de Se varía en la capa arable de la tierra, en promedio de 0.33-2 ppm, en las formas más comunes de selenio elemental (Se^0), seleniuro (Se^{2-}), selenato (SeO_4^{2-}) o selenito (SeO_3^{2-}) (Kieliszek, 2019). De forma análoga al azufre (S), las plantas pueden absorber Se en forma de SeO_4^{2-}, SeO_3^{2-}, Se^{2-}, y como compuestos orgánicos selenocisteína (SeCys) y selenometionina (SeMet) (White, 2018). A través de las raíces,

el Se se absorbe principalmente en forma de SeO_4^{2-} en un proceso dependiente de los transportadores de S (White, 2018). Cuando se agrega en su forma SeO_3^{2-} ingresa a las plantas empleando los transportadores de fosforo (Pht1), los canales de acuaporinas (NIP2;1), o a través de los transportadores de silicio (Si; LSI1) (Schiavon et al., 2020).

El metabolismo del Se desde SeO_4^{2-} hasta su biosíntesis a aminoácidos y otros compuestos, involucra diversas reacciones enzimáticas, las cuales se describen a continuación: el SeO_4^{2-} es activado a adenosina 5-fosfoselenato (APSe), por la enzima ATP sulfurilasa. Posteriormente, es reducido a SeO_3^{2-} por la acción enzimática APS reductasa, la cual emplea GSH como donador de electrones. La reducción de SeO_3^{2-} a seleniuro (Se^{2-}) y la incorporación de éste al complejo *O*-acetil-L-Serina para la síntesis de SeCys, se logra por la acción enzimática sulfito reductasa (SiR) y el complejo enzimático cisteína sintasa, el cual involucra a las enzimas serina acetil transferasa (SAT) y *O*-acetil-L-Serina tiol liasa (OAS) respectivamente, tomando lugar en el cloroplasto (Schiavon et al., 2020). Posteriormente, la síntesis de SeMet a partir de SeCys se lleva a cabo en el citosol, la cual requiere de la formación de intermediarios y la acción de tres enzimas, incluida metionina sintasa (Chauhan et al., 2019). De forma paralela, después de la activación del SeO_4^{2-}, el producto formado (APSe) puede seguir la ruta metabólica para la síntesis de SeCys, o producir 3’-fosfoadenosina-5´-fosfoselenato (PAPSe) y dar inició a la biosíntesis de metabolitos secundarios, de forma análoga al S (Hariharan & Dharmaraj, 2020; Schiavon et al., 2020).

El grado de absorción y metabolización de Se estará dependiente del grado de tolerancia de las plantas a este elemento. Las especies con poca tolerancia pueden acumular cantidades menores a 100 mg Se kg^{-1} de peso seco, siendo clasificadas como no acumuladoras, en este rango entran algunos cultivos de cereales y las hortalizas, a excepción de las brasicáceas. Esta familia en particular tiene una singularidad metabólica, que es contar con una bioacumulación, de forma natural, de metabolitos secundarios azufrados, por lo que al encontrarse en un medio con Se, pueden acumularlo en cantidades superiores a las plantas no acumuladoras, llegando a acumular de entre 100-1000 mg Se kg^{-1} de peso seco, clasificadas como acumuladoras secundarias (Adebayo et al., 2020). El mecanismo de detoxificación por exceso de Se en las plantas comienza con la metilación

de SeCys y SeMet, para producir metil-selenocisteina (MeSeCys) o metil-selenometionina (MeSeMet), moléculas precursoras de las formas volátiles de Se, las cuales son devueltas al ambiente a través de las hojas (Lanza & Reis, 2021).

Influencia del Se en el metabolismo vegetal

La respuesta de las plantas al Se es muy variada, y depende en gran medida de la forma de Se que se aplica (iónica, nano o, compuestos orgánicos), la forma en que se aplica (edáfica, foliar y, en la solución nutritiva) y la etapa de crecimiento del cultivo (El-Ramady et al., 2020). Generalmente, en cultivos no acumuladores de Se (<100 mg Se kg^{-1} PS), la aplicación de este elemento a bajas concentraciones induce cambios positivos en aspectos fisiológicos, morfológicos, genera cambios en el sistema antioxidante, y, por ende, cambios en la expresión de genes (Chauhan et al., 2019). Una regulación a la alza en los factores de transcripción *WRKY1* y *bZIP1* los cuales están involucrados en las señales de transducción de ácido salicílico y ácido jasmónico (*WRKY1*) y la subsecuente protección de las plantas a condiciones de estrés, como respuesta de plántulas crecidas en medio con nSe, podría indicar que las respuestas generadas en el sistema antioxidante, y en el metabolismo en general, se deban principalmente a un estrés oxidativo generado por el Se (Sotoodehnia-Korani et al., 2020), sin embargo, no lo suficientemente agresivo para generar toxicidad. Adicionalmente, también se documentó una regulación al alza de los factores de transcripción *miR172*, *CRTISO* y *bZIP* en plantas de tomate expuestas a Se, los cuales se involucran en controlar los programas de desarrollo del cultivo, particularmente la etapa de floración, además de conferir tolerancia al estrés (*miR172*), biosíntesis de carotenoides (*CRTISO*), diferenciación de tejido, señalización hormonal, nutrición, y tolerancia al estrés (*bZIP*) (Tabla 2) (Neysanian et al., 2020).

En este contexto, la relación de la aplicación de Se con la regulación de otros factores de transducción y moléculas señalizadoras se ha documentado en diferentes cultivares. La regulación a la baja de los genes involucrados en la producción de etileno (*PuACSs* y $PuERF_2$) por la influencia de Se, resultó en una disminución significativa en la producción de etileno (Yuan et al., 2023). Así mismo, se ha señalado el efecto de nSe indujo la activación de las vías de señalización del ácido salicílico y ácido jasmónico en *S. miltiorrhiza*, además de incrementar los compuestos derivados del metabolismo

secundario, y una regulación a la baja de la producción de etileno (Zhang et al., 2023). Estos cambios en los factores de transcripción que se activan en presencia de estrés resaltan la definición del Se cómo bioestimulante vegetal (Franzoni et al., 2022), pues en pequeñas concentraciones regula a la alta moléculas de señalización y factores de transcripción, entre ellos los antes mencionados, promoviendo el sistema de defensa antioxidante (Tabla 3).

Tabla 2. Función biológica de los factores de transcripción regulados por el Se.

Factor de transcripción	Definición general	Función biológica		Ref.
WRKY	Factor de transcripción clave en la regulación de proteínas que responden a estrés biótico y abiótico, y que regula procesos fisiológicos y el desarrollo.	Estrés biótico	Activa las vías de señalización de ácido jasmónico, ácido salicílico, y etileno.	(Li et al., 2022b; Wang et al., 2023)
		Estrés abiótico	Aumenta la tolerancia a la sequía al manipular la síntesis de ácido abscísico.	
		Estrés por nutrimentos/metales pesados	Regula los transportadores específicos de diversos elementos al presentar deficiencia o exceso.	
			Incrementa la tolerancia a metales pesados por la regulación de SOD, CAT, APX, AsA, GSH y fitoquelatinas	
		Estrés oxidativo	Activa la APX citoplasmática en condiciones de estrés por H_2O_2.	
			Incrementa la actividad de SOD, POD, y CAT.	
		Metabolismo secundario	Se involucra en procesos metabólicos que resulta en la biosíntesis de metabolitos secundarios, como los derivados de la ruta fenilpropanoide.	
bZIP	Factor implicado en la adaptación de las plantas a condiciones no favorables por factores bióticos y abióticos.	En complejo transcripcional C-/S1/bZIP-SnRK1 participa en la reprogramación del metabolismo primario relacionado con aminoácidos y carbohidratos. da tolerancia a estrés por salinidad y sequía.		(Wang et al., 2022)
		Esencial en el crecimiento y desarrollo, maduración de las semillas, crecimiento de la raíz, y desarrollo floral.		
miR172	Involucrado en el tiempo de floración y patrones florales.	Controla el tiempo de floración y la formación de órganos florales, morfogénesis floral, transición de etapas de crecimiento de la planta.		(Dong et al., 2022; He et al., 2022)
		La sobreexpresión causa malformación florar y reduce la fertilidad.		
CRTISO	Factor de transcripción involucrado en la biosíntesis de pigmentos.	Carotenoide isomerasa. Su expresión regula la síntesis de licopeno, para dar paso a α y β carotenos, involucrados en la fotoprotección, pigmentación, síntesis de fitohormonas y señalización.		(Sun et al., 2022)
ACS y *ERF*	Factores de transcripción de respuesta de etileno	Genes involucrados en la síntesis de etileno, el cual estimula la acumulación de H_2O_2.		.(Fortunato et al., 2023; Liu et al., 2015)

SOD: superóxido dismutasa; CAT: catalasa; APX: ascorbato peroxidasa; AsA: ascorbato; GSH: glutatión; H_2O_2: peróxido de hidrogeno.

Tabla 3. Respuestas encontradas en diferentes cultivos ante la aplicación de Se.

Especie	Recurso Forma y concentración	Especie vegetal	Tipo de aplicación	Transportador identificado	Otras respuestas	Ref.
Se	Na_2SeO_3 Na_2SeO_4 (5 µM)	*Camelia sinensis*	Solución nutritiva 48 h de exposición	*CsSULTR1;1* y *CsSULTR2;1* en raíces expuestas a Na_2SeO_4 *CsPTH1;3b, CsPHT1;8, CsPHT3;1a* y *CsNIP2;1* en raíces expuestas a Na_2SeO_3	Enriquecimiento en aminoácidos, péptidos y proteínas (selenito). Regulación a la alza del metabolismo del glutatión (selenato). Mayor contenido de Se en raíces (selenito). Mayor contenido de Se en hojas (selenato.	(Ren et al., 2022)
	Na_2SeO_4 0.25, 0.5, 1, 2.5, 5, 10, y 20 mg kg^{-1}	*Triticum aestivum*	Edáfica, en conjunto con inoculación de hongos micorrizicos arbusculares (AMF)	*TaeSultr1;1* y *TaeSultr1;3* en Se ≤5 mg kg^{-1}	Se ≤5 mg kg^{-1} + AMF incrementaron Se en brotes y raíces. Se ≥ 5 mg kg^{-1} inhibió el crecimiento del cultivo. Se + AMF incrementó la tolerancia a la toxicidad de Se.	(Li et al., 2022a)
	Na_2SeO_3 Na_2SeO_4 (200, 400, 800 µM)	*Broussonetia papyrifera*	Foliar Frecuencia: semanal	*SULTR8* (corresponde a *SULTR4;2*) *SULTR18, 29* (corresponde a *SULTR3;3* y *SULTR3;1*) *SULTR30* (corresponde a *SULTR1;3*) con mayor frecuencia en (Na_2SeO_4)	Sobreexpresión de genes involucrados en el metabolismo del GSH. Sobreexpresión de los transportadores transmembranales dependientes de ATP (ABC), favoreciendo la detoxificación de Se.	(Chen et al., 2022)
	NPs-Se 20 y 100 µM	*Glycine max* L. (Merr.)	Solución nutritiva Tiempo de exposición: 7 días	S: *SULTR1;3, 3;3, 3;4, 4;1.* P: *PTH1;3, 1;7, 1;9.* Si: *LSI2.1* en NPs-Se 100 µM Acuaporinas: *NIP5;1. TIP2;2, 4;1. PIP1;2, 2;7, 2;8*	Mayor contenido de Se-aminoácidos y MeSeCys, y Se en raíz y hojas (100 µM).	(Xiong et al., 2023)

Las funciones biológicas de los factores de transcripción activados por la aplicación de Se dan un panorama de la influencia del Se en la tolerancia de las plantas a distintos factores bióticos y abióticos, por la regulación del sistema antioxidante y las moléculas de señalización, promoviendo la homeostasis de ROS intracelulares y la integridad de la membrana. Así mismo, se mejoran las cualidades intrínsecas de las plantas, se tiene un mayor aprovechamiento de los recursos nutrimentales, incrementando la biomasa, rendimiento, calidad de los frutos, y en general, modificaciones epigenéticas que mejoran las cualidades y capacidades tolerantes de los cultivos (Tabla 2).

PRIMER ARTICULO

Selenium Nanoparticles Improve Quality, Bioactive Compounds and Enzymatic Activity in Jalapeño Pepper Fruits

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Selenium Nanoparticles Improve Quality, Bioactive Compounds and Enzymatic Activity in Jalapeño Pepper Fruits

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Abstract: Trace element malnutrition causes the development of chronic degenerative diseases. The consumption of minerals and other compounds of biochemical origin through the intake of vegetables can attenuate these deficiencies to a great extent. Because the content in the plant depends on the conditions where it develops, there are still deficiencies that should be taken into consideration. For example, in Mexico, the intake of selenium does not cover the recommended daily requirement. The objective of this study was to use selenium nanoparticles (nSe) as a selenium (Se) source and to determine the effects on agronomic indices, antioxidant compounds, enzymatic activity, and accumulation of Se in fruits of a jalapeño pepper crop. Different concentrations of nSe (1, 15, 30, and 45 mg L^{-1}) were supplied via drench to jalapeño pepper plants at 15, 30, 45, and 60 days after transplanting. The results indicate that applying nSe via drench with 45 mg L^{-1} increased crop yield and antioxidant compounds. Moreover, all doses evaluated modified the activity of the enzymes ascorbate peroxidase (APX), glutathione peroxidase (GSH-Px), and phenylalanine ammonium lyase (PAL), as well as improved the concentration of Se in fruits. The nSe incorporation via drench is an alternative to increase the content of Se and other nutraceutical compounds in jalapeño pepper fruits, possibly positively influencing human nutrition when consumed.

Keywords: biostimulation; biofortification; nanotechnology; antioxidants

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1. Introduction

Selenium (Se) was recognized in 1957 as an essential element for mammals that prevents necrotic degeneration of the liver. Additionally, it is the only trace element with a specific codon to selenoproteins with antioxidant and anti-inflammatory effects [1,2]. Plants can uptake Se through the uptake mechanism it shares with sulfur (S) [3], which allows it to be absorbed as selenate (SeO_4^{2-}), selenite (SeO_3^{2-}), and nano-Se; or in its organic forms of selenomethionine (SeMet) or selenocysteine (SeCys) [4]. Se is rapidly metabolized to SeCys [5] and can also be incorporated into primary and secondary metabolites derived from SeMet and SeCys, such as selenoglutathione, aromatic compounds derived from phenylalanine, and tyrosine [3]. Biochemical changes of plants in response to the incorporation of Se in plant metabolism are aimed at improving the defense system by detoxification of free radicals through the increase in non-enzymatic compounds such as proline, flavonoids, polyphenols, alkaloids, and glutathione, and antioxidant enzymes such as catalase (CAT, EC: 1.11.1.6) and ascorbate peroxidase (APX, EC: 1.11.1.11) [6], which can mitigate oxidative stress generated by biotic and abiotic factors. Despite its importance

in human nutrition, in Mexico, Seintake is well below the recommended requirements (80–221 µg/day) [7], since the average intake for adults is 37.6 to 51.8 µg/day, mainly obtained from the consumption of beans, corn tortillas, and milk, covering 32%, 24%, and 19% respectively [8]. In general, the content of Se in food is low, mainly due to its low concentration in most agricultural soils [9]. Therefore, it offers the opportunity to develop crop biofortification programs to increase the intake of Se at least to the minimum amount of the recommended range [10]. The most common way to introduce Se into the food chain is through biofortification [11]. On the other hand, using nanotechnology in agriculture represents an innovation in research to generate effective products that can be applied to crops and transform the agricultural sector [12]. In this respect, nanoparticles of Se (nSe) show high bioactivity, high antioxidant capacity, and beneficial impacts on primary and secondary plant metabolism [6]. The size of these nanomaterials is an essential factor determining their uptake and mobility in plant tissues and cells [13]. The use of nSe in agriculture is due to its biostimulant effect [14]. This effect is carried out in two phases: (1) First, there is the modification of the physicochemical properties of the cell membrane, by inducing changes in the activity of receptors, transporters, and proteins, in addition to modifying the transport of metabolites. (2) It occurs through changes corresponding to the release of the complex that makes up the nanoparticle, chemical element, or carbon compounds [15]. This interaction results from the induction of antioxidant metabolism in crops and the edible part [6], for example, protection against lipid peroxidation by antioxidant compounds [6].

The primary diet of the Mexican population is corn, beans, and pepper [16]. Pepper (*Capsicum* spp) is a crop native to South America [17]. It is primarily consumed for its spicy flavor, generated by the presence of capsaicinoids [18]. Although the main attraction of Capsicum fruits is their piquancy to the palate, it is also a food with great nutritional value [19]. It is a source of ascorbic acid (vitamin C), vitamins A, E, and B, secondary metabolites such as carotenoids, phenolic compounds, some minerals, and other compounds with antioxidant capacity, whose content varies according to the species, degree of maturity, and the crop development conditions (fertilization, stress, post-harvest management, etc.) [20,21]. Several clinical studies have demonstrated the benefits of bioactive compounds contained in the fruits of *Capsicum* to improving quality of life, attributed mainly to flavonoids, alkaloids, vitamins, carotenoids, and capsaicin [22]. They are also attributed with anti-obesity effects, antioxidant activity and cardiovascular protection, antimicrobial, antifungal, and antiviral activity, protection against disorders in the urinary system and renal failure, and anticancer activity [23].

These qualities, together with the effects attributed to nSe, make to pepper an appopiate crop for biofortification; being one of the main essential foods of the Mexican diet positions it as a vital source of Se and other compounds, a high-quality food. Based on the above, the objective of this work is to evaluate supplementation with nSe as a source of Se in the cultivation of jalapeño pepper (*Capsicum annuum* L.) hybrid Durango F-1, and to determine the effects on agronomic indices, antioxidant capacity, enzymatic activity, and Se accumulation in fruits.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

The field experimental phase was developed in a tunnel-type greenhouse with a semi-transparent polyethylene cover and natural ventilation with side vents, belonging to the Universidad Autónoma Agraria Antonio Narro. It used Jalapeño pepper (*Capsicum annuum* L.) hybrid Durango F-1 seeds (Starseeds International Inc., Pue, MX), which were germinated in 200-cavity polystyrene trays containing a peat moss–perlite growth medium (70/30; *v*/*v*). At 65 days of age, they were transplanted into 10 L polyethylene pots, with 8 L of growth medium made up of peat moss–perlite in a 1:1 ratio (*v*/*v*). Nutrition was supplied through the Steiner nutrient solution [24] and an automated irrigation system. The greenhouse conditions during the crop cycle were 21 °C temperature and 51% relative humidity.

The average maximum conditions were 565 µmol m^{-2} s^{-1} of photosynthetically active radiation, 1095 W m^{-2} of incident solar radiation (outside the greenhouse), and 730 W m^{-2} inside the greenhouse. The pots were ordered with a separation of 0.90 × 0.35 m, obtaining a total experimental area of 31.5 m^2. Thirty days after transplanting (DAT), the plants were tutored using white agricultural raffia.

2.2. Experimental Design and Treatment Application

The experimental design was completely randomized. Spherical Se nanoparticles (nSe) of an average size of 20 nm [25] were used, at concentrations of 0, 1, 15, 30, and 45 mg L^{-1}, supplied via drench at the base of the stem at 15, 30, 45 and 60 DAT. Each treatment consisted of ten plants, each one represented an experimental unit (EU). An amount of 100 mL of solution was supplied, receiving 1, 15, 30 and 45 mg L^{-1}. The EU of the control treatment (0 mg L^{-1}) was provided with 100 mL of deionized water. The nSe were sonicated (BRANSON 1510R-DHT, Bransonic, CT, USA) to ensure the homogeneity of the solution.

2.3. Agronomic Indices

The agronomic parameters considered were stem diameter, root and aerial part fresh weights, root and aerial part dry weights, height gain, and yield. The procedures are described below. The vertical growth of the plants was measured on four occasions, one day before each application of the treatments, using a magnetic flexometer (FCN-55M, TRUPER, EdoMex, MX), and the results were reported in cm. Stem diameter was evaluated at 90 DAT, using a digital vernier (500-192-30 Mitutoyo Co., YOK, JPN); results were reported in mm. Root and aerial part fresh weights were determined at 90 DAT, employing a granatary balance (SPX2202, OHAUS, Inc., NJ, USA); the results were expressed in g. To determine the dry weights of the root and aerial part, the samples were introduced into a drying oven at 60 °C for 72 h (Arsa, AR-290AD, Jal, MX) or until constant weight; the results were reported in g. Yield was obtained from the fruits harvested at 80 DAT; a granatary balance (SPX2202, OHAUS, Inc., NJ, USA) was used; the results were expressed in g $plant^{-1}$. Five EUs of each treatment were considered for evaluation.

2.4. Photosynthetic Pigments

Photosynthetic pigments were estimated using the method described by Wellburn [26], with some modifications. 60 mg of lyophilized leaf tissue and 5 mL of pure methanol were added to a test tube. The mixture was shaken vigorously and allowed to stand 24 h at room temperature without illumination. The absorbance of the samples was read in a UV–Vis spectrophotometer (GENESYS 10S UV-Vis, Thermo Fisher Scientific, Inc., MA, USA) at 666, 653, and 470 nm. The absorbances obtained were entered into the following equations:

$$\text{Chl a} = (15.65 \times \text{Abs } 666) - (7.34 \times \text{Abs } 653) \quad (1)$$

$$\text{Chl b} = (27.05 \times \text{Abs } 653) - (11.21 \times \text{Abs } 666) \quad (2)$$

$$\text{Total carotenoids} = \frac{[(1000 \times \text{Abs } 470) - (2.86 \times \text{Chl a}) - (129.2 \times \text{Chl b})]}{221} \quad (3)$$

where Chl a represents chlorophyll a, and Chl b represents chlorophyll b. Results were expressed in mg g^{-1} dry weight.

2.5. Physicochemical Analysis of the Fruit

Total soluble solids (TSS), pH, and titratable acidity (TA) were determined at harvest time. For this purpose, the juice of the fruits was extracted using a porcelain mortar and pestle. TSS were measured using a digital refractometer (MASTER-PM, ATAGO, Inc., WA, USA), and the results were expressed in °Brix. The pH was measured using a previously calibrated benchtop pH meter (LAQUA PH1100, HORIBA Advanced Techno, Co., KP, JPN). TA was determined by titration with 0.1 N NaOH [27], and the results expressed as a

percentage of citric acid. Fruit length and equatorial diameter were measured using a digital vernier (500-192-30 Mitutoyo Co., YOK, JPN); results were reported in mm. Firmness was determined using a hand-held texturometer (Force Dial FDK 20, WAGNER Instruments, CT, USA); results were expressed in kg cm^{-2}. Five fruits from each selected EU were considered for the measurements.

2.6. Total Proteins

Protein quantification was performed with the Bradford method [28], using 100 µL of biomolecule extract mixed with 1 mL of Bradford reagent (Bradford Assay, ThermoFisher Scientific, MA, USA). The absorbance of the samples was read with a UV–Vis spectrophotometer (GENESYS 10S UV-Vis, Thermo Fisher Scientific, Inc., MA, USA) at 595 nm. The calibration curve was performed using bovine serum albumin as a standard. The results were expressed in g 100 g^{-1} dry weight (DW).

2.7. Antioxidant Status of Fruit

2.7.1. Sample Processing

At 70 DAT, fruits of homogeneous size were selected from each treatment and immediately frozen at −80 °C. Once frozen, they were subjected to a lyophilization process under the following conditions: temperature −84 °C, vacuum pressure 0.080 mBar (FreeZone 2.5, Labconco, MO, USA). The samples were macerated in a mortar and pestle until a fine powder was obtained and stored until the determinations in lyophilized tissue were performed. The same procedure was performed on leaf tissue to determine photosynthetic pigments at the flowering and fructification stage.

2.7.2. Biomolecule Extraction

To extract biomolecules, 10 mg of Polyvinylpyrrolidone (PVP) and 100 mg of lyophilized fruit tissue were weighed. Subsequently, 2 mL of phosphate buffer (pH 7.0–7.2; 100 mM) was added, and the mixture was shaken for 5 s in a vortex and then extracted by ultrasound for 5 min (BRANSON 1510R-DHT, Bransonic, CT, USA). The mixture was centrifuged at 12,500 rpm for 10 min at 4 °C (PrismTMR, Labnet International, Inc., NJ, USA). The supernatant was filtered through a 0.45 µm pore size nylon membrane. A 1:15 dilution with phosphate buffer was made and stored at −80 °C. The extract was used for enzymatic determinations, total protein, and antioxidant activity [29].

2.7.3. Non-Enzymatic Antioxidants

The Vitamin C content in the fruit was determined with the 2,6-dichloroindophenol method, with some modifications [30], using UV–Vis spectrophotometer. First, metaphosphoric acid (HPO_3; 0.36 M) was used as the extracting solution. Then, 2 mL of solution was added to 10 mg of lyophilized fruit tissue and extracted by ultrasound for 5 min. The mixture was centrifuged at 5000 rpm for 10 min at 4 °C (PrismTMR, Labnet International, Inc, NJ, USA). Next, 200 µL of the supernatant was mixed with 1.8 mL of 2,6-dichloroindophenol (0.09 M), and the absorbance of the sample was read at 515 nm (GENESYS 10S UV-Vis, Thermo Fisher Scientific, Inc., MA, USA). Vitamin C was estimated by interpolating the results against a previously established calibration curve with ascorbic acid (0–120 ppm). The results were expressed as milligrams of ascorbic acid equivalents per gram of dry weight (mg AAE g^{-1} DW).

The total phenolic compounds were estimated using the Folin–Ciocalteu method (2 M, Folin & Ciocalteu's phenol reagent, Sigma Aldrich, Darmstadt, GER) at 765 nm [31], for which 100 mg of lyophilized fruit tissue was added to 2 mL of 95% methanol and subjected to ultrasound extraction. The calibration curve was performed using gallic acid (97.5–102.5%, Sigma Aldrich, Darmstadt, GER). The results were expressed in milligrams of equivalent gallic acid per 100 g dry weight (mg GAE 100 g^{-1} DW).

The total flavonoid content was determined with the colorimetric method described by Shraim et al. [32], with some modifications, at 510 nm. To quantify total flavonoids, 2 mL

of 95% methanol was added to 100 mg of lyophilized fruit and subjected to ultrasound extraction. The calibration curve was performed using catechin as a standard (≥96% Sigma Aldrich, Darmstadt, GER). The results were expressed in milligrams of equivalent catechin per 100 g dry weight (mg CE 100 g^{-1} DW).

The antioxidant activity was determined by the inhibitory capacity of the 2,2′azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$) radical [33]. The radical was obtained from the reaction of ABTS at 7 mM with potassium persulfate ($K_2S_2O_8$, 2.45 mM) for 16 h at room temperature in the dark. The absorbance was adjusted to 0.700 ± 0.020 at 734 nm at an approximate temperature of 30 °C. Then, 1.8 mL of the diluted $ABTS^+$ solution and 0.2 mL of biomolecule extract were taken, homogenized, and kept in darkness. After 6 min, the absorbance of the samples was read at 734 nm in a UV–Vis spectrophotometer (GENESYS 10S UV-Vis, Thermo Fisher Scientific, Inc., MA, USA), and the percentage of inhibition was calculated based on the concentration of ascorbic acid of the standard reference curve.

The quantification of glutathione was carried out according to the procedure described by Xue et al. [34], which is based on the reaction of 5,5-dithiobis-2-nitrobenzoic acid (DNTB). An amount of 480 µL of biomolecule extract was mixed with 2.2 mL of 0.32 M Na_2HPO_4 and 320 µL of 1 mM DNTB. It was vigorously shaken for 5 s, and the absorbance was read at 412 nm in a UV–Vis spectrophotometer (GENESYS 10S UV-Vis, Thermo Fisher Scientific, Inc., MA, USA). The estimation was performed using the regression equation of the curve, and the results were expressed in µM GSH equivalent g^{-1} DW.

2.7.4. Enzymatic Activity

The activity of the catalase enzyme (CAT 1.11.1.6) and ascorbate peroxidase (APX 1.11.1.11) was estimated following the protocol described by Elavarthin & Martin [35]. The activity of the glutathione peroxidase enzyme (GSH-Px 1.11.1.9) was estimated with the method described by Xue et al. [34]. The estimation of phenylalanine ammonia lyase enzyme activity (PAL 4.3.1.5) was carried out according to the procedure of Chen et al. [36]. The results were expressed as U g^{-1} of total protein. The enzyme units (U) for each enzyme are defined as follows:

- CAT: a U is equivalent to the amount of mM of H_2O_2 consumed per milliliter per minute.
- APX: a U is equivalent to µM of oxidized ascorbate per milliliter per minute.
- GSH-Px: a U is equivalent to the amount of GSH in µM per milliliter per minute.
- PAL: a U is defined as the amount in mM of trans-cinnamic acid produced per milliliter per minute.

2.8. Se Content in Fruit

Se content was determined in fruit according to the procedure described by Pedrero et al. [37], for which 25 mg of the sample was digested in 2.5 mL of concentrated HNO_3 and 1 mL of H_2O_2 in an analytical microwave oven. The resulting solution was diluted to 25 mL with deionized water. ICP-MS determined the concentration of Se. The results were expressed in µg g^{-1} of dry weight.

2.9. Statistical Analysis

Five replicates per treatment were randomly selected for the agronomic and fruit quality evaluations. The photosynthetic pigment analyses were performed on three replicates per treatment. The information obtained from the assessments was subjected to a one-way analysis of variance (ANOVA) and a Tukey's test ($p \leq 0.05$) using the INFOSTAT 2016 program (http://www.infostat.com.ar (accessed on 20 February 2023)). Additionally, an Anderson–Darling test was performed to the first plant-height evaluation to confirm equality in the data distribution.

3. Results

3.1. Agronomic Indices

3.1.1. Growth Dynamics

Supplementation with nSe influenced the growth dynamics of the plants. In Figure 1a, a distinction in growth is observed starting from the fourth week, with the plants in the 15 mg L^{-1} nSe treatment standing out from the beginning. This trend continues during the following weeks of evaluation. Additionally, slower growth is observed in the control and 30 mg L^{-1} nSe treatments. This difference in growth dynamics was confirmed when evaluating the total height gain of the plant. As shown in Figure 1b, a significant increase of 12.74% in plant growth is observed when supplementing with 15 mg L^{-1} nSe through drenching, with 62.3 cm for this treatment as compared to 56.5 cm for the control treatment. Furthermore, no significant difference is observed between treatments 1, 30, and 45 mg L^{-1} and the reference treatment (control).

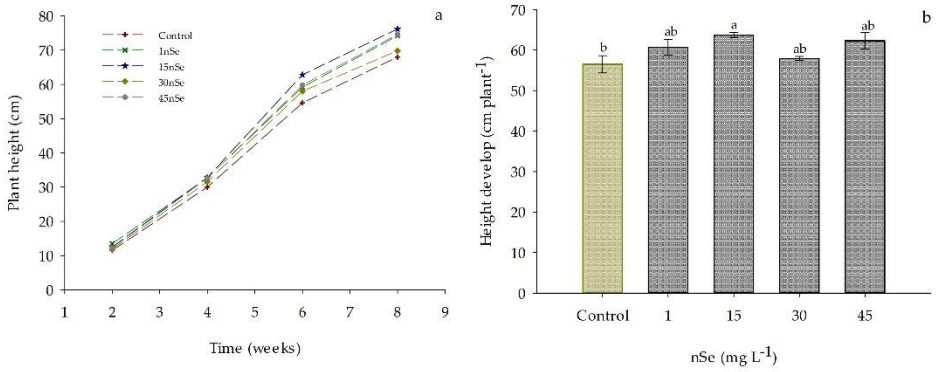

Figure 1. Growth dynamics of Jalapeño pepper plants with nSe application. (**a**) weekly growth; (**b**) total height gain. Different letters indicate a difference between treatments (Tukey test $p \leq 0.05$). n = 5 ± standard error.

3.1.2. Crop Attributes

The agronomic determinations of the crop were influenced by supplementation with nSe. The results obtained from the evaluations are shown in Table 1, and it can be observed that supplementation with nSe at different concentrations did not significantly modify these parameters ($p > 0.05$).

Table 1. Agronomic attributes of Jalapeño pepper crop with nSe application.

Treatments nSe (mg L^{-1})	SD (mm)	FWAP (g)	FWR (g)	DWAP (g)	FWR (g)
Control	13.82 ± 1.02 ^{ns,*}	360.00 ± 47.94 ^{ns}	222.00 ± 54.66 ^{ns}	104.40 ± 17.87 ^{ns}	41.80 ± 8.93 ^{ns}
1	13.94 ± 0.79	369.40 ± 26.28	203.60 ± 27.04	104.00 ± 22.46	54.60 ± 13.56
15	13.80 ± 1.20	372.00 ± 39.86	269.00 ± 56.13	102.00 ± 11.47	43.80 ± 8.56
30	13.76 ± 0.32	375.00± 29.07	254.00 ± 56.56	109.40 + 9.07	47.00 ± 9.75
45	14.52 ± 0.83	395.80 ± 47.11	276.80 ± 45.89	106.60 ± 14.01	46.40 ± 5.41

* Data are shown as the means ± standard deviation (SD, n = 5). ns: not significant between treatments. Tukey test ($p \leq 0.05$). SD: stem diameter; FWAP: fresh weight of aerial part; FWR: fresh weight of root; DWAP: dry weight of aerial part; DWR: dry weight of root.

On the other hand, crop yield had a significant increase ($p \leq 0.05$) due to the effect of nSe supplementation at the dose of 45 mg L^{-1}; compared to the control treatment (629 g $plant^{-1}$), it increased by 52.75%, corresponding to 960.8 g $plant^{-1}$. The treatments of 1, 15, and 30 mg L^{-1} of nSe did not show a significant difference in this parameter, taking the control treatment as a reference (Figure 2).

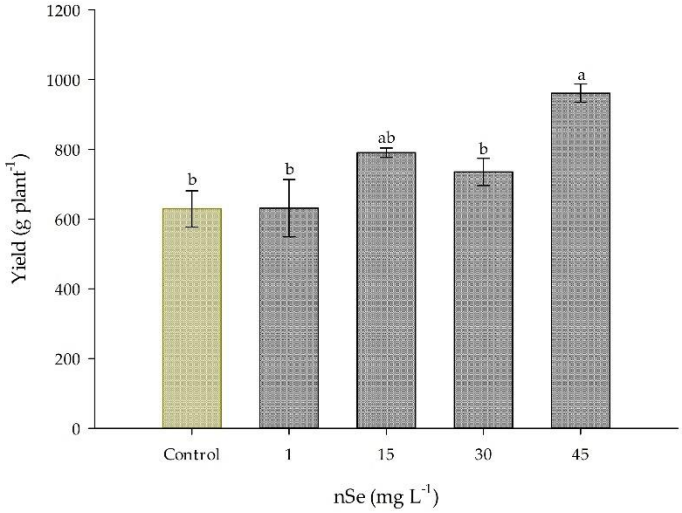

Figure 2. The yield of Jalapeño pepper crop with the application of nSe. Different letters indicate significant differences between treatments (Tukey test $p \leq 0.05$). n = 5 ± standard error.

3.2. Photosynthetic Pigments

Photosynthetic pigment content in plants treated with nSe did not significantly differ between the treatments with 1, 15, 30, and 45 mg L^{-1} and the control treatment (Figure 3). However, there was an increase of 4.8 and 9.97% of Chl a in 1 mg L^{-1} and 45 mg L^{-1} with 8.37 and 8.78 mg g^{-1}, respectively. For the case of Chl b, the same trend as for Chl a is observed, with increases in treatments 1 and 45 of 2.47 and 23.6%, respectively, compared to the control treatment. However, the analysis shows a significant difference between treatments 30 and 45 mg L^{-1} nSe, both for Chl a and Chl b, with a difference of 15.62 and 34.84 mg g^{-1} DW between the pigments for both treatments, respectively (Figure 3). Total carotenoids were not significantly modified by nSe supplementation.

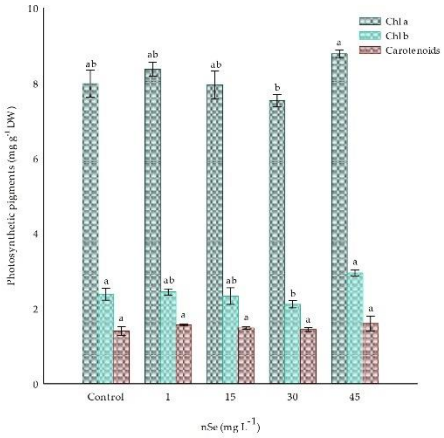

Figure 3. Photosynthetic pigments in Jalapeño pepper plants with nSe in a drench. Different letters indicate differences between treatments (Tukey test $p \leq 0.05$). Chl a: chlorophyll a; Chl b: chlorophyll b; DW: dry weight. n = 3 ± standard error.

3.3. Fruit Quality

TSS, firmness, and TA were significantly affected by nSe treatments. The changes observed are mainly between the doses of nSe applied. The highest TSS content in jalapeño pepper fruits was obtained with the application of 15 and 30 mg L^{-1} of nSe, as each increased by 12.5 and 9.61%, respectively, compared to the control; on the other hand, the highest dose (45 mg L^{-1}) decreased this variable by 8.6% (Table 2).

Table 2. Components of the fruit quality of Jalapeño pepper fruits.

nSe (mg L^{-1})	TSS (°Brix)	Firmness (kg cm^{-2})	L (mm)	ED (mm)	TA (% CA)
Control	5.20 ± 0.48 ^{ab,*}	4.87 ± 0.54 ^{b}	72.24 ± 10.45 ^{ns}	27.51 ± 2.45 ^{ns}	0.28 ± 0.036 ^{ab}
1	5.17 ± 0.36 ^{ab}	5.83 ± 0.73 ^{b}	81.88 ± 3.91	30.10 ± 2.05	0.24 ± 0.008 ^{b}
15	5.85 ± 0.51 ^{a}	5.14 ± 0.67 ^{b}	84.85 ± 6.66	28.44 ± 2.52	0.25 ± 0.031 ^{b}
30	5.70 ± 0.37 ^{a}	7.76 ± 1.01 ^{a}	81.73 ± 7.79	28.19 ± 2.03	0.31 ± 0.044 ^{a}
45	4.75 ± 0.25 ^{b}	5.93 ± 0.69 ^{b}	85.06 ± 4.37	30.68 ± 1.90	0.23 ± 0.020 ^{b}

* Data are shown as the means ± standard deviation (SD, n = 5). Different letters indicate differences between treatments. Tukey test ($p \leq 0.05$). TSS: total soluble solids; L: length of fruit; ED: equatorial diameter of fruit; TA: titratable acidity; CA: citric acid.

The application of nSe at 30 mg L^{-1} increased the firmness of the fruits by 59.34% (7.76 kg cm^{-2}) compared with the control (4.87 kg cm^{-2}).

No significant differences were observed in the length and equatorial diameter of the fruits with the application of nSe.

The change obtained in TA was shown again among the treatments with nSe, showing an increase in TA with the supplemental application of 30 mg L^{-1} of nSe. Along this parameter, the control treatment was similar to the evaluated doses.

Additionally, supplementation with nSe at 45 mg L^{-1} induced a significant change in the amount of total proteins, reducing the amount of this molecule by 17.58% compared to the control (Figure 4).

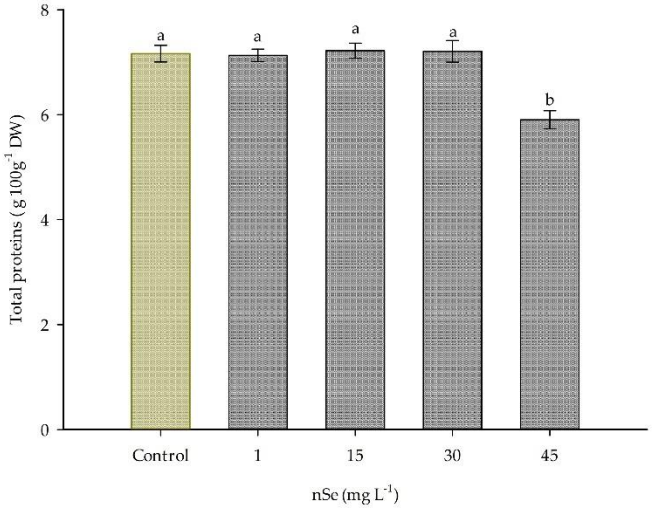

Figure 4. Total protein content in Jalapeño pepper fruits with nSe. DW: dry weight. Different letters indicate differences between treatments (Tukey test $p \leq 0.05$). n = 5 ± standard error.

3.4. Antioxidant Status of Fruit

3.4.1. Non Enzymatic Antioxidants

Supplementary application of nSe significantly influenced the antioxidant status of fruits. Vitamin C was higher in all treatments with nSe, compared to the control treatment (Table 3). The lowest dose nSe, 1 mg L^{-1}, increased it by 30.48%; similarly, the doses of 15 and 30 mg L^{-1} increased it by 25.35 and 23.50%, respectively. The most significant increase was noticed with the application of 45 mg L^{-1} of nSe, surpassing the control by 42.59%.

Table 3. Antioxidant compounds in Jalapeño pepper fruits with nSe.

nSe (mg L^{-1})	Vitamin C mg AAE g^{-1} DW	FT mg GAE 100 g^{-1} DW	FLV mg CE 100 g^{-1} DW	ABTS* % Inhibition	GSH µM EGSH g^{-1} DW
Control	7.02 ± 2.27 ^{b,*}	572.27 ± 32.84 ^{c}	190.90 ± 69.98 ^{b}	43.50 ± 4.70 ^{c}	5.14 ± 0.28 ^{a}
1	9.16 ± 1.32 ^{ab}	1002.49 ± 52.77 ^{a}	186.77 ± 18.41 ^{b}	51.14 ± 1.71 ^{b}	4.59 ± 0.35 ^{a}
15	8.81 ± 0.35 ^{ab}	1000.06 ± 20.73 ^{a}	247.69 ± 44.09 ^{ab}	49.84 ± 2.64 ^{b}	4.62 ± 0.33 ^{a}
30	8.67 ± 0.84 ^{ab}	780.32 ± 91.41 ^{b}	319.18 ± 51.76 ^{a}	59.01 ± 1.93 ^{a}	4.57 ± 0.43 ^{a}
45	10.01 + 0.46 ^{a}	680.44 ± 56.09 ^{b}	241.01 ± 30.03 ^{ab}	58.82 + 2.35 ^{a}	3.57 ± 0.43 ^{b}

* Data are shown as the means ± standard deviation (SD, n = 5). Different letters indicate differences between treatments. Tukey test ($p \leq 0.05$). AAE: ascorbic acid equivalents; FT: total phenols; GAE: gallic acid equivalents; FLV: flavonoids; CE: catechin equivalents; ABTS: antioxidant capacity 2,2′azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); EGSH: glutathione equivalents; DW: dry weight.

Doses 1 and 15 mg L^{-1} increased the content of total phenols by 75.17 and 74.75% more than the control, respectively. Furthermore, the concentrations of 30 and 45 mg L^{-1} increase these compounds by 36.34 and 18.88%, respectively.

Significant changes were observed in the concentration of flavonoids with the application of the treatments. The most distinctive difference was in the treatment with 30 mg L^{-1}, which increased the concentration by 67.2%, compared to the control. For its part, 1 mg L^{-1} of nSe behaved statistically the same as the control treatment.

The antioxidant capacity increased significantly with nSe treatments. The nSe treatments inhibited the radical at an index higher than 45%, considerably surpassing the control treatment. The values obtained are shown in Table 3, highlighting the treatments with 30 and 45 mg L^{-1} of nSe, with 59.01 and 58.82% inhibition, respectively, which is a good index of the antioxidant status of fruits, since the higher the inhibition, the higher the antioxidant capacity.

Additionally, supplementation with nSe at a concentration of 45 mg L^{-1} decreased the content of GSH in jalapeño pepper fruits, decreasing the content of this compound by 30.54%, compared to the control. The treatments of 1, 15, and 30 mg L^{-1} of nSe were statistically the same as the control (Table 3).

3.4.2. Enzymatic Antioxidants

The addition of nSe modified the activity of antioxidant enzymes APX, GSH-Px, and PAL.

The CAT enzymatic activity did not show significant differences with the application of the treatments; however, significant increases were noticed in all the evaluated doses of nSe, increasing this activity between 6 and 40% (Figure 5a).

The activity of the enzyme APX was statistically the same as the control when 1, 15, and 30 mg L^{-1} of nSe were applied. In the plants subjected to the highest dose of 45 mg L^{-1} of nSe, more than a 60% reduction in the activity of this enzyme was observed (Figure 5b).

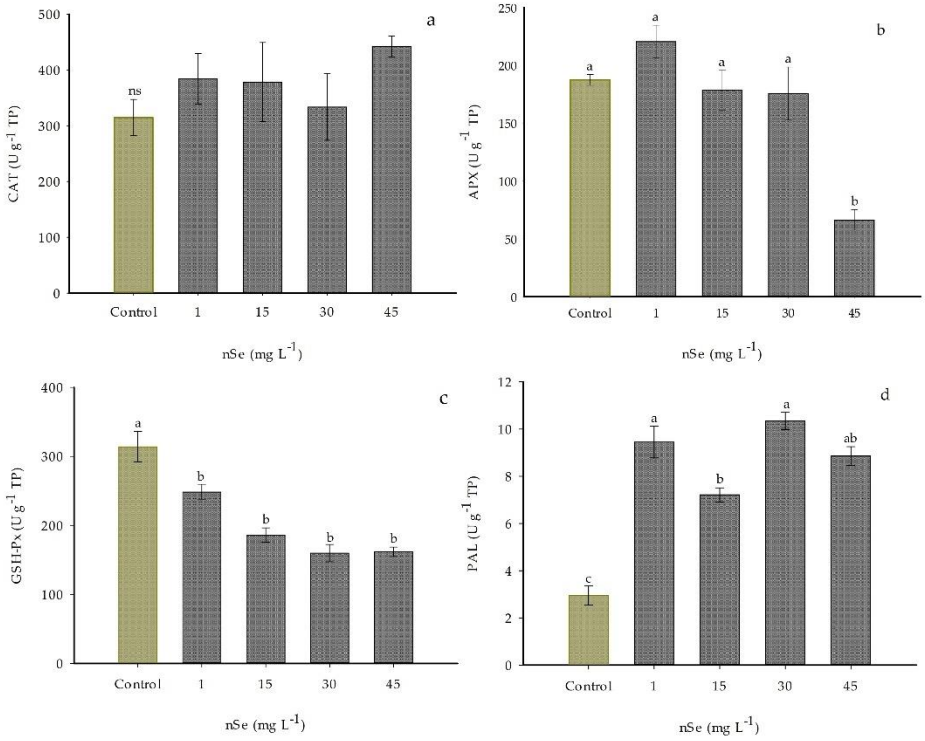

Figure 5. Enzyme activity in Jalapeño pepper fruits with nSe. (**a**) CAT; (**b**) APX; (**c**) GSH-Px; (**d**) PAL; ns: not significant between treatments; TP: total proteins. Different letters indicate differences between treatments (Tukey test $p \leq 0.05$). n = 5 ± standard error.

On the other hand, adding nSe did not favor the GSH-Px enzymatic activity. On the contrary, activity decreased by 20.9, 40.7, 49.09, and 48.49% in all the evaluated doses (1, 15, 30, and 45 mg L^{-1}, respectively) compared to the control (Figure 5c).

For PAL, supplementation with nSe significantly increased the activity of this enzyme in all the evaluated treatments (Figure 5d). Compared to the control, the treatments of 1 and 30 mg L^{-1} nSe increased the PAL activity by 219.84 and 250.03%, respectively. With the addition of 15 and 45 mg L^{-1} nSe, the enzyme activity over the phenylpropanoid pathway was exceeded (144.25 and 199.73%, respectively) compared to the control.

3.5. Se Content in Fruit

Plants exposed to nSe showed an accumulation of this element in the edible part (Figure 6). Compared to lowest dose of nSe, 1 mg L^{-1}, supplementation with 45 mg L^{-1} increased the concentration of Se in fruits by 67.73 times. Similarly, significant increases were observed with doses of 15 and 30 mg L^{-1}, accumulating 17.89 and 11.82 times more Se content compared to the lowest dose. mg L^{-1}

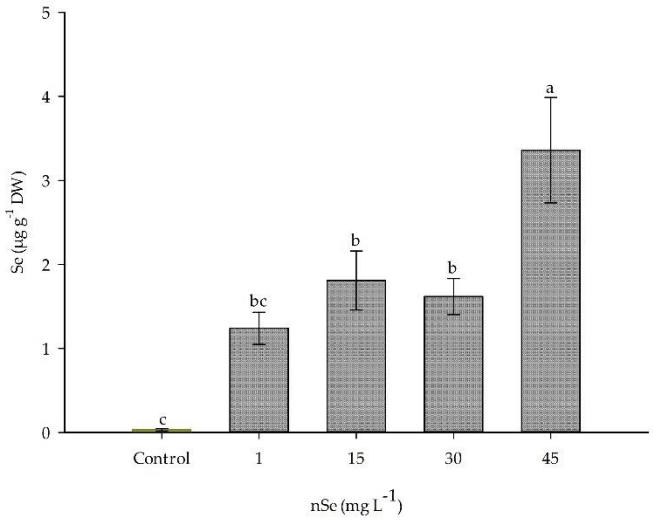

Figure 6. Se concentration in fruits of jalapeño pepper. DW: dry weigth. Different letters indicate differences between treatments (Tukey test $p \leq 0.05$). n = 5 ± standard error.

4. Discussion

Nanotechnology is a developing science with huge potential in agriculture and promotion of desirable crop characteristics. In this study, jalapeño pepper plants that received nSe showed superior growth behavior as well as a significant increase in yield and slight increases in fresh and dry weight of the aerial and root parts, compared to plants that did not receive nSe. These changes have been shown in other types of vegetables. However, the response of the plants to Se largely depends on the plant species and the concentration of Se used [38]. In this regard, with the addition of Se, different changes in plants have been observed, which include functions within the maintenance of cell structure and function and improvement of photosynthesis. It is an auxiliary in the homeostasis of nutrients [38,39], which contributes to improving the growth and development of crops [40], which could explain the changes observed in this research. Kang et al. [41] reported the application of nSe in *Cucumis melo* L. lead the development of thylakoidal membranes in the cells of the mesophyll of the leaves, promoting a higher photosynthetic rate and high content of chlorophylls. In the present research, changes in Chl a and Chl b in the leaves suggest a difference in the internal structure of the cells in response to supplementation with nSe. The application of nSe in various crops has provided valuable information among vegetable species, fruit trees, cereals, and even some aromatic species studied [11]. For example, in *Punica granatum* [42], supplementation with nSe at 2 µM led to an increase in leaf area, higher chlorophyll content, and the yield obtained per tree, the same trend observed in the present study. Similarly, in *Solanum lycopersicum* [43], incorporating nSe at 20 mg L^{-1} into the growth medium of the plants modified the total chlorophyll content and significantly increased the crop yield. This trend was also reported in *Fragaria x ananassa* [44] cultivation by adding nSe at 20 mg L^{-1}, showing improvements in the concentration of Chl a, Chl b, and carotenoids, and in the agronomic attributes of the crop.

In addition to the changes observed in yield and other attributes of crop growth, variations were also recorded in the commercial quality of the fruit, highlighting TSS, firmness, TA, and total protein. It could be due to the effect generated by nanomaterials, in this case, nSe, when these are involved in the growth environment of crops. Garza-García et al. [12] point out, among others, the relevant effect of nSe on plant primary metabolism, which begins with the modification of the chlorophyll content, the precursor

of photosynthesis [45]. Through this change, a higher rate of photoassimilates would be expected, and consequently, a higher range of soluble sugars, proteins, and other components in general, increasing the total TSS content [12]. The biostimulant effect of nSe is attributed to its nanometric size, because the contact with the wall and the cellular membrane generates a cascade of signaling that will cause changes in the development of the crop, highlighting the expression of genes and a change in enzymatic activity [15]. One of the enzymes that become important is PAL, a precursor of a great diversity of phenolic compounds, and, therefore, a more significant deposition of lignin in the cell wall [46], which could explain the increase in fruit firmness with nSe. A change in these variables has also been observed in other vegetables because of the application of nSe. An increase in firmness in fruits of *Cucumis sativus* was observed when nSe was applied at 25 mg L^{-1}, compared to untreated fruits [47]. In *Solanum lycopersicum* [48], the application of nSe at 100 mg L^{-1} showed an increase in TSS. However, with this same treatment, there was a lower firmness of the fruits with nSe than those not treated. In fruit species such as *Punica granatum*, a trend of increase in TSS and a low percentage of TA wasnoticed when 2 µM of nSe was applied, compared to the fruits of the control treatment [42].

Since its discovery in 1817, the roles of Se in human metabolism and the effects of the narrow line between deficiency and toxicity for this element have gradually been reflected upon [49]. It was not until 2015 that this element was classified as a plant biostimulant [14] due to the beneficial effects that it generated in a specific concentration in the plant species that had been studied up to now. In addition, the metabolism of Se within plants comprises a vast network of complex processes developed in the chloroplast and the cytosol of the cells [40]. After Se uptake either by the roots or through the cuticle of the leaf area, Se can participate in the metabolism due to itschemical characteristics similar to sulfur (S). It can replace it in molecules containing S and induce changes in plant primary and secondary metabolism [50]. The changes evaluated with greater emphasis are the metabolism of phenylpropanoids, the activity of antioxidant enzymes, and the resistance generated in plants against different types of stress [51]. In this study, the application of nSe in drench induced an increase in the concentration of FT, FLV, and the antioxidant capacity $ABTS^{+}$, which could be related to the high enzymatic activity of PAL (Figure 6), which is crucial in the initiation of the phenylpropanoid metabolism [52].

On the other hand, the activity of GSH-Px is closely related to the amount of GSH present, since it is the primary cofactor of this enzyme. This principle could indicate that the Se incorporated through the nSe replaces S in polypeptide structures, interfering in glutathione production [53]; as presented in this study, low concentration of GSH, and, therefore, little GSH-Px activity. It could also indicate interference in the ascorbate–glutathione cycle in the fruits, since the enzymes APX and GSH-Px, as well as the cofactors ascorbate and glutathione, are an essential part of this process [54]. Therefore, the response was found in the content of vitamin C in Jalapeño pepper fruits. Changes in gene expression related to antioxidant metabolism and the phenylpropanoid pathway have also been studied in response to Se supplementation. It is documented by Kang et al. [41], highlighting the overexpression of *PAL, APX*, *CAT,* and *SOD* genes, as well as a change in PAL, APX, CAT, and POD enzymes when supplementing with nSe. This study agrees with the PAL enzyme's activity and the slight increase in CAT, which could be related to the gene expression that codes these enzymes [55]. Similarly, nSe have dual action within the metabolism, first by the protection they generate against reactive oxygen species (ROS), and at the same time, by acting as inducers of oxidative stress, inducing the antioxidant defense system of plants [56]

Plants can absorb Se in different chemical forms and organic compounds; it is taken up by root cells and rapidly catalyzed by the high chemical affinity it shares with S [57]. In this study, the amount of Se in the fruits increased with the evaluated nSe doses. The results suggest that the accumulated concentration in the tissue depends on the supplemented Se concentration, since the highest amount of Se was found in the fruits of the 45 mg L^{-1} nSe treatment. Although the results obtained are based on dry weight, it is a good indicator

of the mobility of Se within the metabolism of Jalapeño pepper plants, as it translocates to the demand organs, such as fruits, which, when consumed, will provide a contribution of Se in daily intake. The ability to accumulate Se in several vegetables and fruit trees has been reported in different investigations. In *Vitis vinifera* [58], with the addition of Se in various concentrations, it was possible to increase the amount of this element in berries, showing a tendency of accumulation related to the dose applied. In *Solanum lycopersicon* L. [59], supplementation with Se in doses of 2 and 5 mg L^{-1} increased Se in fruit.

5. Conclusions

Applying nSe increases Se yield, bioactive compounds, and concentration in jalapeño pepper fruits. Doses of 45 mg L^{-1} of nSe increased crop yield and antioxidant compounds. Moreover, all doses evaluated modified the activity of the enzymes ascorbate peroxidase (APX), glutathione peroxidase (GSH-Px), and phenylalanine ammonium lyase (PAL), as well as improved the concentration of Se in fruits. Incorporating nSe via drench is an alternative to enhance the content of Se and other bioactive compounds in jalapeño pepper fruits, possibly positively influencing human nutrition when consumed.

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SEGUNDO ARTICULO

Bioactive compounds and antioxidant capacity of jalapeño pepper enriched with Selenium

Bioactive compounds and antioxidant capacity of jalapeño pepper enriched with Selenium

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ABSTRACT

Selenium (Se) intake in Mexico from food consumption does not cover the recommended daily requirements. Jalapeño pepper is a great importance vegetable included in the basic Mexican food basket. This research aimed to use Se for biofortification purposes in the cultivation of jalapeño pepper, through the supplementation of sodium selenite at concentrations of 0, 1, 15, 30 and 45 mg L^{-1}. Se positively influenced the accumulation of vitamin C, total phenols, flavonoids, antioxidant capacity (ABTS), total proteins, glutathione, selenium in fruit, as well as the crop yield. Se supplementation to jalapeño pepper is a good biofortification strategy, due to this practice positively influences the accumulation of bioactive compounds and the Se concentration in fruits, allowing the possibility for improving the nutritional status and human health through the consumption of functional foods.

Keywords. Se intake, jalapeño pepper, biofortification, functional food, phytochemical by-product.

INTRODUCTION

The increase in diseases related to hypertension, diabetes, obesity and diabetes, has been notable in recent years (Rico-de la Rosa *et al*., 2021). Health disorders are commonly linked to a diet with low nutritional intake, especially due to deficient intake of trace elements, defined as being required in amounts from 1 to 100 mg day^{-1} (Aliasgharpour, 2020). Selenium (Se) is the only trace element whose genetic code in humans generates selenoproteins (Zhang *et al*., 2020) with beneficial effects on cell protection against oxidative stress (Steinbrenner *et al*., 2022). Recent studies have shown the antioxidant effects derived from supplementation based on Se and selenoproteins in the treatment of diseases, and their benefits in human health (Huang *et al*., 2022b), highlighting the potential to maintain homeostasis and cell metabolism (Wang *et al*., 2017). Unfortunately Se intake does not cover the recommended daily requirement, that is, 55-75 µg day^{-1} (Zhang *et al*., 2020), and an average consumption of 37.6-51.8 µg day^{-1} mainly from milk, corn and bean tortillas intake is barely reached (Rios-Lugo *et al*., 2022). The adequate Se intake and the optimal expression of selenoproteins guarantee the cell protection against oxidation by free radicals, a mechanism that has been observed in thyroid, cardiovascular, neurodegenerative diseases, and some cancer forms (Aliasgharpour, 2020). Se incorporation to the food chain through plants is determined mainly by the soil geological nature (Steinbrenner *et al*., 2022), however, in Mexico the concentration of trace elements in the soul surface is minimal, which reduces their availability in the food chain through vegetables (Díaz-Zarco *et al*., 2022). Biofortification is a strategy used to enrich crops with essential elements for humans, such as trace elements, including Se (Broadley *et al*., 2006). Use of Se based fertilizers is intended to increase the concentration of

this trace element in crops, whose consumption as food increases it intake in humans (Ramos *et al*., 2010). This practice has generated a positive impact, and is frequently used in the European region (Schiavon *et al*., 2020). Plants can absorb Se as selenate (SeO_4^{2-}), selenite (SeO_3^{2-}), and organic Se compounds (selenocysteine and selenomethionine) through the roots by sulfate transporters, and incorporated to plant metabolism (White, 2016), which allow it being translocated and accumulated in edible organs (Puccinelli *et al*., 2017). Se also has been classified as a plant biostimulant (du Jardin, 2015), due to in contact with root cells, Se generates a signaling cascade that modifies the plant development, which modifies the primary and secondary metabolism, inducing changes in the concentration of antioxidant compounds such as ascorbate (AsA), glutathione (GSH), tocopherols (vitamin E) (El-Ramady *et al*., 2015; García Márquez *et al*., 2020), glucosinolates, flavonoids, polyphenols, selenoproteins (Wen, 2021), and enzymes related to the detoxification of hydrogen peroxide (H_2O_2), such as catalase (CAT), glutathione peroxidase (GSH-Px), ascorbate peroxidase (APX), and superoxide dismutase (SOD) (El-Ramady *et al*., 2015). This provides the opportunity to obtain crops enriched in Se, and bioactive compounds that generate a positive impact on human health (Martínez-Navarrete *et al*., 2008). Jalapeño pepper is a vegetable of *Capsicum* genus, with high rate consumption by the Mexican people due to it pungency propriety that generates in the palate (García-Galindo *et al*., 1995). Jalapeño pepper also contains bioactive compounds such as vitamins A, B, C, and E, capsaicinoids, carotenoids, polyphenols, and other compounds (Antonio *et al*., 2018; Cruz-Ricardez *et al*., 2020), in addition to being a source important of minerals. Qualities described above make the Jalapeño pepper an appropriate option to implement a biofortification based on Se, in order to position this vegetable crop as an important source of bioactive compounds and extra amount of Se content. This research aimed to evaluate the influence of sodium selenite (Na_2SeO_3) in the jalapeño pepper (Durango F1) crop, on the bioactive compounds, antioxidant status and Se accumulation in the fruits.

MATERIALS AND METHODS

Crop establishment

Seeds of jalapeño pepper (*Capsicum annuum* L.) Durango F-1 were used, germinated in polystyrene trays with peat moss-perlite 2:1 (v/v) substrate. Seedlings were transplanted 65 days after emergence in 8 L polyethylene containers with peat moss-perlite 1:1 (v/v) substrate. Fertilization consisted of Steiner nutrient solution (Steiner, 1961) diluted in irrigation water. Crop was established into greenhouse conditions, tunnel-type with polyethylene cover and natural ventilation, at the Universidad Autónoma Agraria Antonio Narro (25° 21' LN, 101° 01' LO, altitude 1743 m), with average temperature values (21 °C) and relative humidity (51%), and average maximum values of photosynthetically active radiation (565 µmol m^{-2} s^{-1}), incident solar radiation (1095 W m^{-2}) and solar radiation inside the greenhouse (730 W m^{-2}).

Experiment design

Experiment consisted of a completely randomized design, treatments with sodium selenite (Na_2SeO_3, 99% Sigma Aldrich) in concentrations of 0, 1, 15, 30, 45 mg L^{-1}, supplied via drench in the stem base, with applications at 15, 30, 45 and 60 days after transplanting (DAT). Each treatment consisted of 10 plants, being a plant an experimental unit (EU). 100 mL of solution was supplied to each EU.

Processing fruit samples

Fruits of homogeneous size were selected from each treatment at 70 DAT, frozen at –80 °C, lyophilized at –84 °C and 0.080 mBar (FreeZone 2.5, Labconco), the samples were macerated until a fine powder was obtained and stored at 4 °C.

Biomolecule extract

Biomolecule extract was obtained as follows: 10 mg of Polyvinylpyrrolidone (PVP) and 100 mg of lyophilized fruit tissue were mixed in 2 mL of phosphate buffer (pH 7.0-7.2; 100 mM). The mixture was vortexed for 5 s, and extracted with ultrasound for 5 min (BRANSON 1510R-DHT, Bransonic). The mixture obtained was centrifuged at 12500 rpm for 10 min at 4 °C (Prism®, Labnet International, Inc). The

supernatant was collected and filtered using a 0.45 µm nylon membrane, and stored at –80 °C (Hernández-Hernández *et al*., 2018). With the extract obtained, the total protein content, glutathione, and antioxidant capacity were estimated.

Fruit yield

Fruit yield was obtained from the fruits harvested at 80 DAT of five EU per treatment, a SPX2202 scale (OHAUS Inc.) was used, and the results were expressed in g plant $^{-1}$.

Bioactive compounds

Vitamin C

Vitamin C was estimated by UV-Vis spectrophotometry using a GENESYS 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, Inc.) at 515-nm wavelength, using the 2.6-dichloroindophenol colorimetric method (Garza-Alonso *et al*., 2021). Vitamin C content was determined by interpolating the measurements before a calibration curve made with ascorbic acid (0-120 ppm). The results were expressed as equivalents mg of ascorbic acid per 100 grams of dry weight (mg AAE 100 g^{-1} DW).

Total phenols

Total phenolic compounds were estimated by UV-Vis spectrophotometry using the Folin-Ciocalteau method, using a GENESYS 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, Inc.) at 765-nm wavelength (Ainsworth and Gillespie, 2007). 100 mg of lyophilized fruit tissue was mixed with 2 mL of methanol 95%. Supernatant was used for subsequent analyses. The estimation was made using the regression equation of the calibration curve prepared with gallic acid (97.5-102.5%, Sigma Aldrich). Results were expressed in equivalents mg of gallic acid per 100 grams of dry weight (mg GAE 100 g^{-1} DW).

Total flavonoids

Total flavonoids quantification was carried out using the protocol described by Shraim *et al*. (2021) with slight modifications, by UV-Vis spectrophotometry using a GENESYS 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, Inc.) at 510-nm wavelength (Shraim *et al*., 2021). Total flavonoids were estimated using the regression equation of the calibration curve previously performed with catechin (≥ 96% Sigma Aldrich). The results were expressed as catechin equivalents mg per 100 grams of dry weight (mg CE 100 g^{-1} DW).

Antioxidant capacity

Antioxidant activity was determined by the inhibitory capacity of the radical 2,2'azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) $ABTS^{-}$ (Re *et al*., 1999), by UV-Vis spectrophotometry using a GENESYS 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, Inc.) at 734-nm wavelength. The inhibition percentage was calculated based on the ascorbic acid concentration from the reference standard curve.

Glutathione

Glutathione quantification was performed according to the procedure described by Xue *et al*. (2001), which is based on the reaction of 5,5-dithio-bis-2 nitrobenzoic acid (DNTB). The estimation was made using the regression equation of the curve, and the results were expressed in µM glutathione equivalents per gram of dry weight (µM GSHE g^{-1} DW).

Total proteins

Total protein was estimated following the Bradford method (Bradford, 1976), that is, 1 mL of Bradford reagent was mixed with 100 µL of biomolecule extract and the absorbance of the reaction was read at 595-nm wavelength by UV-Vis spectrophotometry using a GENESYS 10S UV-Vis spectrophotometer (Thermo

Fisher Scientific, Inc.). The calibration curve was made using Bovine Serum Albumin as a standard. The estimation was made using the regression equation of the curve, and the results were expressed in gram per 100 gram of dry weight (g 100 g^{-1} DW).

Se content in fruit

Se concentration in the fruit was performed according to the procedure described by Pedrero *et al.* (2006), that is, 25 mg of sample were digested in 2.5 mL of concentrated HNO_3 and 1 mL of H_2O_2 in an analytical microwave oven. The resultant was diluted to 25 mL with deionized water. Se concentration was determined by ICP-MS, and the results were expressed in µg per gram of dry weight (µg g^{-1} DW).

Statistical analysis

Information obtained from the evaluations was subjected to a one-way analysis of variance (ANOVA), and a Fisher LSD test ($p \leq 0.05$) using the program Infostat 2016.

RESULTS AND DISCUSSION

Fruit yield

Fruit yield is determined by the weight of the fruit per harvested area unit (Fischer et al., 2014), and generally abiotic and abiotic factors influence crop yield (Liliane and Charles, 2020). Se, being a plant biostimulant, is expected to generate positive impacts on parameters such as crop yield when added in the appropriate dose (du Jardin, 2015). In this study, crop yield was significantly ($p \leq 0.05$) influenced by Se supplementation (Fig. 1). The dose of 1 mg L^{-1} increased the yield by 34.1% (629 g $plant^{-1}$), in relation to the control treatment. Se applications of 15, 30 and 45 mg L^{-1}, favored the fruit yield by 45.4, 26.7 and 46.5%, respectively. The adequate concentration of Se is related to the plant species (White, 2018), since the threshold between positive effects and toxicity effects by Se is very narrow and differs between each species. Generally, low concentrations of Se exerts a positive effect on the growth and yield of crops, by promoting photosynthesis, and generating changes associated with the primary and secondary metabolism of plants (Wen, 2021).

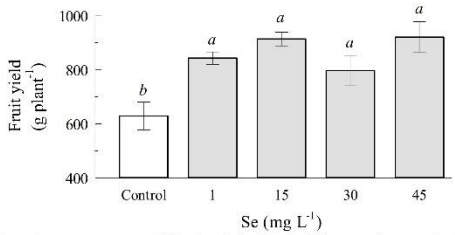

Figure 1. Fruit yield of jalapeño pepper enriched with Se. Different letters indicate significant statistical difference (Fisher LSD, $p \leq 0.05$). Mean values ± standar error. n = 5.

Bioactive compounds

Vitamin C

Ascorbate, a reduced form of vitamin C, has a biological role in human metabolism, mainly in the synthesis and metabolism of essential cellular compounds, immune function and antioxidant activity, it becomes the first line of defense of cells, by protecting their integrity against free radicals (Pedrosa *et al.*, 2022). Since humans are incapable of synthesizing this biocompound, the main way to obtain Vitamin C is through food intake or supplements (Fujii *et al.*, 2022). The Se addition significantly influenced the accumulation of vitamin C in fruit. The doses of 15, 30 and 45 mg L^{-1} increased the concentration of Vitamin C by 39.2, 28.9 and 34.7% (435.5, 403.2 and 421.6 mg 100 g^{-1}), respectively, in relation to the control treatment. The dose of 1 mg L^{-1} did not modify the concentration of Vitamin C in the fruits (Fig. 2). The behavior of the metabolism

of plants to the addition of Se results in the modification in the concentration of compounds involved in the protection against lipid peroxidation, caused by the generation of reactive oxygen species (ROS) (Skrypnik *et al*., 2022). The addition of 30 mg L^{-1} of Na_2SeO_3 modified the biosynthetic pathway of Vitamin C in strawberry fruits (Lu *et al*., 2022), by inducing positive changes in the enzymes involved in biosynthesis, such as ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), glutathione reductase (GR) and l-galacto-1,4-lactone dehydrogenase (GalLDH). Islam *et al*. (2020) obtained an increment of 80% in the Vitamin C content in green wheat extract when treated with 0.5 mg L^{-1} of Na_2SeO_3. Se based biofortification can improve the Vitamin C concentration in horticultural crops, in addition a higher content of vitamin C can improve the bioavailability of iron (Fe) and zinc (Zn) found in foods (Newman *et al*., 2019).

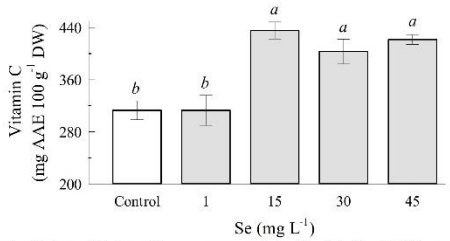

Figure 2. Vitamin C content in fruits of jalapeño pepper enriched with Se. Different letters indicate significant statistical difference (Fisher LSD, $p \leq 0.05$). Mean values ± standar error. n = 5.

Total phenols

Secondary plant metabolites such as phenolic compounds, in addition to contributing to the organoleptic characteristics of fruits, flowers, and vegetables, have health benefits due to their ability to eliminate free radicals in biological systems under conditions *in vivo* and *in vitro* (Hernández-Pérez *et al*., 2020). Some phenolic compounds have been shown to lower blood pressure and high blood cholesterol, in addition to having anti-inflammatory properties and even contributing to cell restoration from radiation damage (Durazzo *et al*., 2019). Accumulation of total phenols in jalapeño fruits increased by 41.7, 41.8 and 55% (834.4, 835.4 and 918.8 mg 100 g^{-1}) in the Se application at 1, 15 and 30 mg L^{-1}, respectively, in relation to the control treatment (Fig. 3). Given the chemical similarity of Se with sulfur (S), the variation in the uptake and assimilation of S due to the incorporation of Se can produce changes in the synthesis of sulfur compounds with nutritional value (Malagoli *et al*., 2015). In addition, given the association of S with nitrogen (N) metabolism, Se can modify the synthesis of nitrogenous compounds such as proteins and amino acids, including phenylalanine (Phe), the main substrate for phenol biosynthesis (Yadav *et al*., 2020). The application of Se at 2 mg L^{-1} in kiwi promoted the accumulation of phenolic compounds and increased the postharvest life of the fruits, by promoting the activity of the phenylpropanoid pathway (Ghafouri *et al*., 2022).

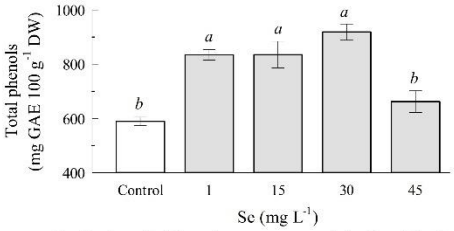

Figure 3. Total phenols content in fruits of jalapeño pepper enriched with Se. Different letters indicate significant statistical difference (Fisher LSD, $p \leq 0.05$). Mean values ± standar error. n = 5.

Total flavonoids

Flavonoids are the most abundant polyphenols in the diet, and are known for their biochemical and pharmacological effects (Wahyuni *et al*., 2013). In most peppers, the most abundant flavonoids are aglycones such as quercetin and luteolin, which increase their concentration as they mature (Hernández-Pérez *et al*., 2020). Flavonoids intake has been associated with a reduction in the risks of conditions such as diabetes and obesity, as well as having a potential use to treat health conditions such as oxidative stress, neurological disorders and, particularly, cardiovascular diseases (Durazzo *et al*. , 2019). The Se addition at 1, 15 and 30 mg L^{-1} increased the concentration of total flavonoids by 50, 60 and 75%, while the dose of 45 mg L^{-1} induced a 46% decrease, respectively, in relation to the control treatment (Fig. 4). The influence of Se application on the accumulation of flavonoids is associated with the regulation of the phenylpropanoid biosynthesis pathway, mainly evidenced by the overexpression of the *Pal* gene and a greater accumulation of Phe, precursor components of this pathway (Li *et al*., 2022). A similar response is reported in *Brassica oleracea* (Gui *et al*., 2022), where was quantified a greater accumulation of flavonoids in response to the Na_2SeO_3 application at 1.6 mmol L^{1}. The trend of increased concentration of flavonoids was also reported in grapevine when applying 10 mg Se L^{-1} (Karimi *et al*., 2020).

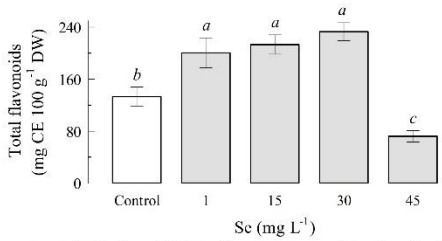

Figure 4. Total flavonoids content in fruits of jalapeño pepper enriched with Se. Different letters indicate significant statistical difference (Fisher LSD, $p \leq 0.05$). Mean values ± standar error. n = 5.

Antioxidant capacity

The antioxidant status of plants and fruits is widely related to the content of glutathione, ascorbate, phytochelatin, proline, flavonoids, alkaloids, and carotenoids, since these components act to minimize ROS and maintain the redox state of cells (Lanza and Dos Reis, 2021). The antioxidant capacity of jalapeño pepper fruits, determined by the inhibition of the $ABTS^{\cdot}$ radical, was modified by the addition of Se (Fig. 5). Se treatments of 30 and 45 mg L^{-1} increased the inhibitory capacity of the $ABTS^{+}$ radical by 12.9 and 30.3%, respectively, in relation to the control treatment, achieving inhibition by 49.1 and 56.7%, respectively. Doses of 1 and 15 mg L^{-1} did not statistically modify the inhibition of the $ABTS^{+}$ radical. Determination of the antioxidant status of crops supplemented with Se is a good indicator of the effects induced on metabolism. A comparative study in response to Se biofortification in radish, mustard and alfalfa at 200 µmol L^{1}, the antioxidant activity showed positively significant differences in radish and mustard, however, this one was significantly reduced in alfalfa (Woch and Hawrylak-Nowak, 2019). These contrasts indicate that the crop response to Se depends on the species studied, and on the form of Se applied.

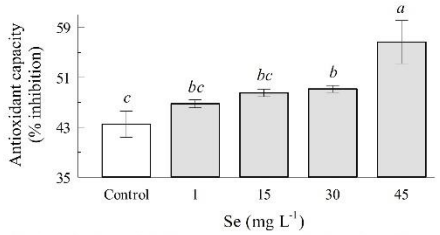

Figure 5. Antioxidant capacity in fruits of jalapeño pepper enriched with Se. Different letters indicate significant statistical difference (Fisher LSD, $p \leq 0.05$). Mean values ± standar error. n = 5.

Glutathione

Glutathione is a sulfur compound that has anti-inflammatory and antioxidant potential in the human body, protecting it from various chronic diseases (Newman *et al*., 2019). Se supplementation increased the reduced glutathione (GSH) concentration in the fruit. Se treatments of 30 and 45 mg L^{-1} significantly increased the GSH content by 18.6 and 21.6%, respectively, in relation to the control treatment. Although the 1 and 15 mg L^{-1} treatments did not significantly modify the GSH concentration (Fig. 6), a trend towards an increase in the GSH concentration is observed as the dose of Se applied increases. Reduced glutathione is a resulting product from the activity of the glutathione reductase enzyme (GR; E.C. 1.6.4.2) and its cellular content is an important indicator of the oxidative state of plant tissue (Pang and Wang, 2010, and the Se can increase the content of this compound (Puccinelli *et al*., 2017). Oraghi Ardebili *et al*. (2015) reported a twice increase in GSH concentration in basil treated with Se, in relation to the plants without Se. An increase in GR enzymatic activity in coffee plants derived from the application of Se is an indicator of a greater bioaccumulation of GSH (Mateus *et al*., 2021).

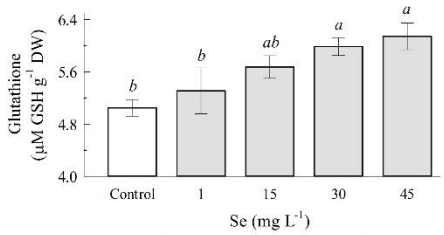

Figure 6. Glutathione content in fruits of jalapeño pepper enriched with Se. Different letters indicate significant statistical difference (Fisher LSD, $p \leq 0.05$). Mean values ± standar error. n = 5.

Total proteins

Proteins are complex biomolecules, essential macronutrients for human nutrition, and their requirement is defined by the need to compensate N losses, and provide the bases of function and basic structure for the maintenance and growth of the organism (Sá *et al*., 2020; Akharume *et al*., 2021). Legumes, cereals, seeds, and nuts are a vegetable source of high-quality protein (Sá *et al*., 2020). Se addition in the jalapeño pepper crop significantly modified the total protein content in the fruit. Total proteins content decreased 7.5 and 22.5% in the Se treatments of 1 and 45 mg L^{-1}, in relation to the control treatment. In the same way, the proteins decreased 3.4 and 7.9% by Se in the 15 and 30 mg L^{-1} treatments, in relation to the control treatment (Fig. 7). The human body possesses the necessary machinery to incorporate Se into metabolism through genetic coding (Zhang *et al*., 2020). In contrast, plants do not require Se but they can absorb, accumulate and assimilate it as selenocysteine (SeCys), due to its structure similar to the amino acid cysteine (Cys), which can cause structural alteration of proteins (Kolbert *et al*., 2019). This hypothesis could explain the results obtained

in this research, since a decrease in the total amount of proteins is evident, which could indicate a loss of structure due to the addition of Se amino acids that replace sulfur-containing amino acids. Luo *et al*. (2021) reported a significant effect on the composition of isolated peanut proteins, by quantifying a lower amount of sulfur-containing amino acids such as methionine (Met) and Cys. In contrast, Xia *et al*., (2020) reported an increase in the total protein content in wheat grains, when applying Se in soil by foliar spraying, as well as an increase in the amino acids SeMet and SeCys (Xia *et al*., 2020).

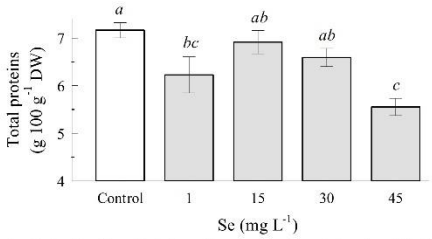

Figure 7. Total proteins content in fruits of jalapeño pepper enriched with Se. Different letters indicate significant statistical difference (Fisher LSD, $p \leq 0.05$). Mean values ± standar error. n = 5.

Se content in fruit

Se is a trace element involved in the maintenance of human health, which intervenes positively in the maintenance of the immune system, regulates thyroid function and cognitive function of the brain, has antioxidant capacity, and also generates anticancer and antiviral effects (Rayman, 2020). However, more than 15% of the world's population suffers from Se deficiency, causing multiple health complications, such as cataracts, cognitive impairment, cardiovascular disorders, cardiomyopathy, and even cancer (Zhou *et al*., 2020). That is why biofortification through agronomic techniques is a viable and safe way to increase Se content in the edible part of crops. Response of Jalapeño pepper crop to Se addition was positive, since it was possible to quantify this trace element in the fruits. Figure 8 shows an increase in Se concentration, dependent on the applied concentration. When increasing the Se dose from 1 to 45 mg L^{-1}, the Se concentration in the fruit increased significantly 3.6 times, that is, from 1.02 to 3.7 µg g^{-1}. Likewise, the 30 mg L^{-1} treatment significantly increased the Se concentration in the fruit in relation to the control treatment. In plants, Se is not considered an essential element, but it can be captured, absorbed and assimilated (White, 2018), to produce SeCys and SeMet. Se accumulation has been reported in different crops of nutritional interest, as well as an indirect effect on their secondary metabolism. For example, Se biofortification in beans significantly increased the content of this element, as well as compounds derived from the phenylpropanoid pathway (Huang *et al*., 2022a). In carrots, as the dose of Se applied was increased, the Se concentration in tissue showed a trend of increasing (Rakoczy-Lelek *et al*., 2021).

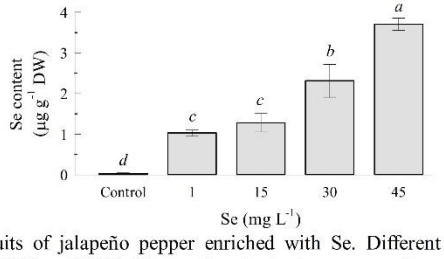

Figure 8. Se content in fruits of jalapeño pepper enriched with Se. Different letters indicate significant statistical difference (Fisher LSD, $p \leq 0.05$). Mean values ± standar error. n = 5.

CONCLUSIONS

Se biofortification in jalapeño pepper induces the accumulation of biocompounds derived from the phenylpropanoid pathway, glutathione and vitamin C, as well as the Se accumulation in fruit, which promote the nutritional value of jalapeño pepper in foods.

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459

TERCER ARTICULO

Selenium seed priming influences the seed germination and seedling morphology of jalapeño (*Capsicum annuum* L.)

Artículo en preparación para su envió a: ***Seeds***

seeds

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Article

Selenium seed priming influences the seed germination and seedling morphology of jalapeño (*Capsicum annuum* L.)

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Abstract: The priming of seeds is shown as a viable technique to improve germination rates, and other qualitative attributes, which give rise to seedlings with higher quality and tolerance to environmental growing conditions. The main source of selenium (Se) for this type of techniques are inorganic salts such as selenite and selenate, and currently the use of selenium nanoparticles as a priming agent is used. The objective of this study was to determine the priming response of jalapeno pepper seeds with nanoselenium (nSe) and sodium selenite (Na_2SeO_3). Ten concentrations of nanoselenium and selenite (1, 5, 10, 15, 20, 25, 30, 35, 40 and 45 mg L^{-1}) and one control (0 mg L^{-1}) were tested, and the seeds were exposed to the solution for 20 h at 25 °C. The percentage of germination, germination vigor, normal plants, daily germination, plumule length and radicle, and fresh weight of the shoots were evaluated. The results show a percentage of germination greater than 80% in all concentrations evaluated, however, the vigor is reduced with 15 mg L^{-1} of Na_2SeO_3, as well as the length of the radicle and the length of the plumule. In contrast, the use of nSe favors the development of normal shoots, and gives a wider range in terms of concentration, for the development of root and cotyledons. This suggests that the use of low concentrations of Na_2SeO_3 positively favors germination, and the morphological traits of the shoots. Likewise, the use of selenium in nanometric form was more friendly, that is, the degree of tolerance to Se was higher.

Keywords: priming, seedlings properties, phytotoxicity, nanomaterials, sodium selenite.

1. Introduction

Seed priming is an osmoconditioning technique used with the purpose of increasing the rate and uniformity of emergence in many plant species of economic importance, since this influences on the quality and yield of crops [1]. Former seed priming techniques were defined as hydropriming (seeds pretreated with water), halopriming (seeds immersed into inorganic salt solutions such as NaCl, KNO_3, $CaCl_2$, etc.), osmopriming

(solutions with sugar, polyethylene glycol, glycerol, sorbitol or mannitol), and hormonal priming (seeds pretreated with hormones that promote the growth and development of seedlings) [2]. In recent years the priming technique has been modified, and other resources and procedures have emerged, such as high and low temperatures, solutions based on bioactive molecules or microorganisms (bio-priming), and nanomaterials solutions (nano-priming) [3].

Seed imbibition techniques have been widely reported, mainly those related to plant species of agricultural interest. In cereals this practice has been implemented in corn (*Zea mays* L.) [4], where the use of copper nanoparticles (nCu) encapsulated with chitosan increased the leaf area, shoots dry weight and seedling root length; in wheat (*Triticum aestivum* L.) [5] the seeds osmopriming increased the germination rate, root and shoots length, and improved the seedlings growth; in rice (*Oriza sativa* L.) the zinc oxide nanoparticles (nZnO) [6] did not affect the germination rate, however, induced changes on the physiological and biochemical attributes of seedlings. At horticultural species seed imbibition has been used in tomato (*Solanum lycopersicum* L.) with carbon nanomaterials (nC) [7], potassium nitrate (KNO_3) [8], salicylic acid, hydrogen peroxide and ascorbic acid [9]; in watermelon (*Citrullus lanatus* L.) [10] using silver nanoparticles (nAg); in pepper (*Capsicum annuum* L.) with beneficial microorganisms [11,12], sodium chloride (NaCl) [13], and inorganic salts such as K, Mg and Ca [1].

In the aforementioned studies, the use of nanomaterials with different active principles is distinguished, however, most include mineral elements considered as essential for the growth and development of crops [14]. On the other hand, there are non-essential elements, considered beneficial elements, such as selenium (Se), aluminum (Al), silicon (Si), cobalt (Co) and sodium (Na), which in amounts much less than those required by an essential element, induce favorable changes in the growth and development of crops [14]. Se is an element that induces a large number of changes in plant metabolism, and its chemical similarity to sulfur (S) facilitates its absorption and can play the same role in the biochemical system [15].

Se has been mainly used for biofortification purposes, that is, for increasing its concentration in the edible parts of crops. Se-biofortification has been evaluated in grain crops, such as rice, corn, wheat and sorghum, and the appropriate Se dose for certain growing conditions has been estimated [15,16]. In horticultural crops, the use of Se for biofortification purposes has been investigated in tomato (*Solanum lycopersicum* L.), lettuce (*Lactuca sativa* L.), carrot (*Daucus carota* L.), broccoli (*Brassica oleracea* L.), and pepper (*Capsicum annuum* L.) [16,17], where the applications are made directly to the canopy plant by spraying, in the substrate, or through the nutrient solution.

In this context, this research aimed to evaluate the influence of Se priming of jalapeño pepper seeds (*Capsicum annuum* L.) with sodium selenite (Na_2SeO_3) and selenium nanoparticles (nSe) on germination attributes and seedling morphology.

2. Materials and Methods

2.1 Plant material and experimental site

Seeds of Durango-F1 hybrid jalapeño pepper (*Capsicum annuum* L.) (Starseeds Inc.) were used. Experiment was carried out in the Plant Physiology laboratory at Horticulture Department and Seeds laboratory at Plant Breeding Department, at the Agricultural University "Antonio Narro" in Saltillo, Mexico (25°21' N, 101°01' W, altitude 1743 m).

2.2 Treatments and Experimental design

Se treatments were prepared in distilled water at 1, 5, 10, 15, 20, 25, 30, 35, 40 and 45 mg mL^{-1} as previously reported [18]. Sodium selenite (Na_2SeO_3 99%, Sigma Aldrich), and

20-nm diameter spherical selenium nanoparticles (nSe) dispersed in Chitosan-PVA [19] were used. A completely randomized design was used.

2.3 Seed priming

Seed priming process was carried out according to Portuguez-Garcia *et al.* [20]. Treatments were prepared with 30 mL of Se concentrations in plastic containers, 60 seeds were submerged in each container, shaken for 5 min and left at rest for a 20 h imbibition time. Control treatment consisted of a distilled water solution and the same imbibition procedure. Containers were covered with PM996 parafilm paper (Parafilm® M Laboratory Sealing Film). After the imbibition time is over, the solution was discarded and the seeds were dried at room temperature for 30 min. Plastic trays were fitted with a Whatman No. 1 filter paper (CTR Scientific) adjusted to the base, and 10 mL of Se solution per treatment [21]. Distilled water was used for control treatment. 15 seeds were equidistant placed in 15x10x5-cm plastic trays with four replications, and the trays were kept for 13 days in a EGCS 3S, 301 3SHR germination chamber (Equitec) at 26 ± 1 °C, 75% relative humidity, and 500 μmol m^{-2} s^{-1} white light with a 12/12 h photoperiod (light/dark).

2.4 Measurements

Germinated seed counts were performed daily for 14 days. Seedling size and biomass measurements were made on day 15 after sowing. Germination was calculated in percent from the germinated seeds and the total number of seeds sown, as follows (Equation 1).

$$\text{Germination} = \frac{\text{germinated seeds}}{\text{seeds sown}} \times 100 \quad (1)$$

Germination vigor was calculated in percent from the normal seedlings and the total number of seeds sown, as follows (Equation 2).

$$\text{Germination vigor} = \frac{\text{normal seedlings}}{\text{seeds sown}} \times 100 \quad (2)$$

Plumule length was measured in cm from the radicle-hypocotyl intersection to the cotyledon base. Radicle length was measured in cm from the hypocotyl base to the radicle apex [22]. Fresh weight of normal seedlings was measured in mg using a PR224/E analytical balance (Ohaus Co.). Seedlings that developed their essential structures under controlled conditions were considered as normal seedlings [23]

2.5 Statistical Analysis

Statistical analysis consisted of a completely randomized design with a factorial array 2x10+1, being two Se forms (Na_2SeO_3 and nSe), ten Se concentrations (1, 5, 10, 15, 20, 25, 30, 35, 40 and 45 mg mL^{-1}), and a control treatment, for a total of 21 treatments and four replications. Analysis of variance and means test (Fisher LSD, $p \leq 0.05$) were performed at Infostat software (Infostat 2020).

3. Results and Discussion

3.1 Germination

Germination was not statistically modified with Na_2SeO_3 and nSe applied in the seed by imbibition (Figure 1). Germination ranged from 93.33 to 100% in all Se and control treatments.

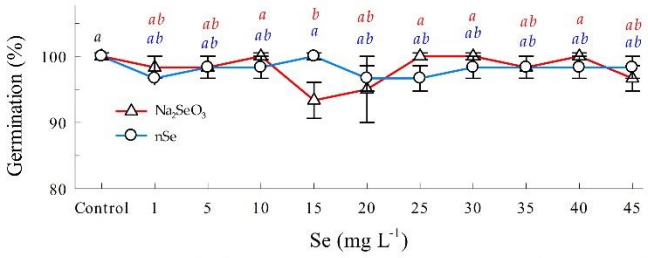


Figure 1. Germination percent of jalapeño pepper (*Capsicum annuum* L.) from Se seed priming. Different letters indicate significant differences between treatments (LSD Fisher's test $p \leq 0.05$). Mean values ± standard error. n = 4.

Regarding the germination distribution along the time of experiment, the highest germination percent from 30 to 60% in both Se species occurred from 5 to 8 day after seeding (DAS) (Figures 2a and 2b). While the mean cumulative germination reached 88% at the 8 DAS with Na_2SeO_3 imbibition (Figure 3a) and at the 6 DAS with nSe imbibition (Figure 3b).

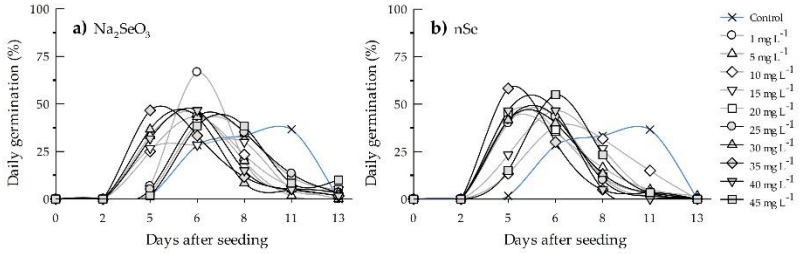


Figure 2. Daily germination of jalapeño pepper (*Capsicum annuum* L.) from Se seed priming.

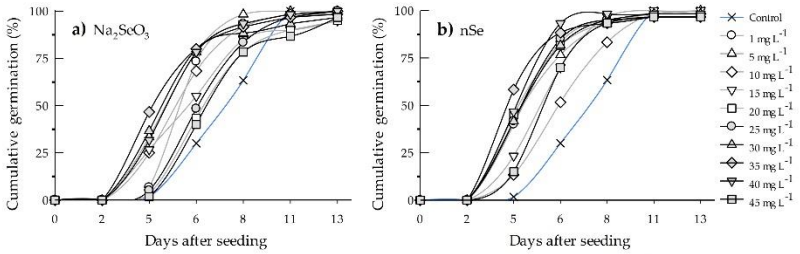


Figure 3. Cumulative germination of jalapeño pepper (*Capsicum annuum* L.) from Se seed priming.

Seed germination is a three-phase process [24], that is, the absorption of water by the imbibed seed, the restart of metabolic processes, and the emergence of the radicle. The treatment of seeds with mineral salts and nanomaterials prior to the start of the germination process has been documented, however, the results in hybrids, species, minerals and nanomaterials used are very varied [22,25]. A common feature mentioned is the regulation of the pathways of abscisic acid (ABA), a phytohormone involved in the seed dormancy process, and gibberellic acid (GA), an ABA antagonist phytohormone, which

acts as a dormancy releaser, expanding embryonic cells [24,26,27]. Seed imbibition with nSe and nZnO in *Brassica napus* modulated the expression levels of genes related to the ABA and GA pathways, which influenced seed germination and early seedling development [28].

3.2 Germination vigor

Germination vigor of *Capsicum annuum* L. decreased significantly from 21 to 86% with Na_2SeO_3 seed priming from 15 to 45 mg L^{-1}, respectively, in relation to the control treatment, while all concentrations of nSe seed priming did not significantly modify germination vigor (Figure 4).

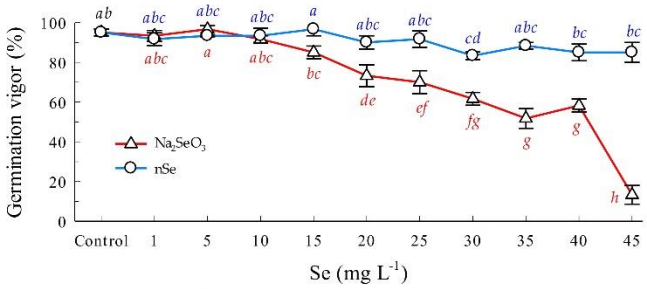


Figure 4. Germination vigor of jalapeño pepper (*Capsicum annuum* L.) from seed priming with Na_2SeO_3 and nSe. Different letters indicate significant differences between treatments (LSD Fisher's test $p \leq 0.05$). Mean values ± standard error. n = 4.

Germination vigor is the property that determines the potential for emergence as well as the rapid and uniform development of seedling under a wide range of field conditions, that can be improved through treatments called "seed priming", which have demonstrated their effect by stimulating yield in seeds with low vigor percent, and synchronization of germination [24,29]. The type of raw material used to treat the seeds for priming process can influence the results. Different materials have been used for tests, such as selenium, zinc, titanium, silicon, silver, iron, carbon nanomaterials, and inorganic salts [3]. In obtained results a wide difference in germination vigor is observed due to the effect of two Se sources at concentrations from 0 to 45 mg L^{-1}. Seed pretreatment with nanomaterials to accelerate germination rate and speed, vigor index, and other germination attributes has been widely documented [3,21,29], which is consistent with results obtained with Se nanoparticles, where germination vigor was not inhibited, that is, the maximum reduction (10.5%) of this attribute with the highest dose of seed priming with nSe relative to the control treatment, was statistically maintained at the non-significant threshold. Seed pretreatment of *Zea mays* L. with Se and Zn maintained the vigor index under normal conditions, and decreased this attribute by almost 50% under water stress conditions [30]. Seed pretreatment of *Oriza sativa* L. with Se and salicylic acid influenced the metabolism of starch, improved the integrity of the membrane, and increased the synthesis of metabolites, which led to better germination vigor and seedlings development, even under stress conditions [31].

3.3. Seedling size and biomass

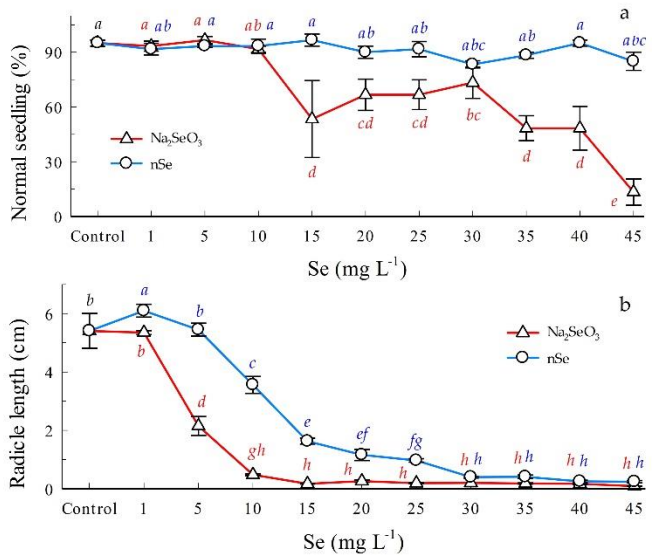
Seed priming of *Capsicum annuum* L. with Se influenced the radicle length of the seedlings (Figure 5b). By increasing the nSe dose of seed imbibition, the radicle length of the seedlings increased 12.5% with 1 mg L^{-1} and was greatly reduced from the 10 mg L^{-1}, compared to the control treatment, until reaching a radicle growth inhibition of 95.5% at

the higher nSe dose. On the other hand, by increasing the Na_2SeO_3 dose of seed imbibition, the radicle length also was greatly reduced from the 5 mg L^{-1}, compared to the control treatment, until reaching a radicle growth inhibition of 98.3% at the higher Na_2SeO_3 dose.

Regarding the plumule length of the seedlings, in the seed priming with nSe this parameter significantly increased 14.4 and 16.4% at treatments of 20 and 25 mg L^{-1}, and decreased significantly 14.4 and 22.6% at treatments of 20 and 25 mg L^{-1}, respectively, in relation to the control treatment. By increasing the Na_2SeO_3 dose of seed imbibition, the plumule length significantly increased 19.2% with 5 mg L^{-1}, and was greatly reduced from 17.1 to 54.8% with Na_2SeO_3 seed priming from 15 to 45 mg L^{-1}, respectively, compared to the control treatment (Figure 5c).

Seed priming of *Capsicum annuum* L. with the two Se species negatively influenced the biomass production of the seedlings (Figure 5d). By increasing the nSe concentration of seed imbibition, the fresh weight of the seedlings significantly decreased from 12.1 to 56.6% with nSe seed priming from 10 to 45 mg L^{-1}, respectively, compared to the control treatment. Also, by increasing the Na_2SeO_3 dose of seed imbibition, the fresh weight of the seedlings was greatly reduced from 20.5 to 52.2% with Na_2SeO_3 seed priming from 5 to 45 mg L^{-1}, respectively, compared to the control treatment.

Dry weight of the seedlings was no computing, due to the reduced values that can be obtained from the dehidrated fresh weight, which values were too low.



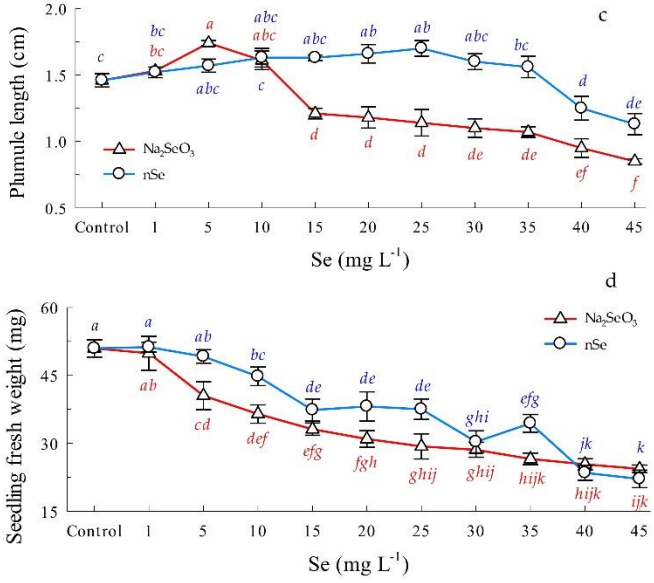


Figure 5. Normal seedlings (a), radicle length (b), plumule length (c) and fresh weight (d) of jalapeño pepper (*Capsicum annuum* L.) seeds primed with Na2SeO3 and nSe. Different letters indicate significant differences between treatments (LSD Fisher's test $p \leq 0.05$). Mean values ± standard error. n = 4.

Simultaneous evaluation of mineral salts and nanomaterials with the same component, on the structures of developing seedlings under controlled conditions, occasionally exhibit differences in the same concentration within a variable. In seed pretreatment, the use of nanoparticles and other materials results in growth stimulation, and a noticeable improvement in morphological and metabolic characteristics [32]. However, it has been reported that high doses used can cause toxicity and development of abnormal morphological structures [18]. Seeds of *Foeniculim vulgare* Mill. pretreated with TiO_2 and TiO_2 nanoparticles in the same concentration, showed significant differences in seedling weight, and shoot and plumule dry weight; while the root and plumule lengths didn't modify significantly [21]. Seeds of *Capsicum annuum* L. separately pretreated with ZnO nanoparticles at 100, 200 and 500 ppm, showed reductions higher than 50% on radicle length, while the plumule length and dry weight of the seedlings were not significantly modified, taking the control treatment as reference [22]. Another study of *Capsicum annuum* L. treated with Se and Se nanoparticles presented toxicity symptoms in seedlings with doses higher than 10 mg L^{-1} for both Se species, that is, drastically decrease of radicle length, and biomass of leaves and roots, as well as the rupture of apical meristems, stopping or slowing down the seedlings growth [18]. Also the root weight significantly increased in the treatments of nSe with 0.5 and 1 mg L^{-1}, compared to the control treatment. At present research, seed priming of *Capsicum annuum* L. influenced growth stimulation and phytotoxicity in the seedlings, according to Selenium specie and concentration. Regarding the radicle length, the transition dose of growth stimulation and phytotoxicity tresholds of the seedlings was 5 mg L^{-1} for the Na_2SeO_3 imbibition and 25 mg L^{-1} for the nSe imbibition. At low concentrations (less than transition dose) the radicle growth was stimulated by the Na_2SeO_3 (2.1 to 5.4 cm length),

and by the nSe (1.0 to 6.1 cm length). On the other hand, at high concentrations the radicle growth was partially inhibited by the Na_2SeO_3 (0.4 to 0.24 cm length), and by the nSe (0.1 to 0.5 cm length)

4. Conclusions

Simultaneous evaluation of two or more materials that share the same component, gives an overview of different scenarios that can be triggered as a result of a single procedure. Seed priming of *Capsicum annuum* L. with Se and nSe in the same concentrations, exhibits the seedling tolerance to the evaluated materials, and the changes in morphology that may occur. Both the use of inorganic selenium salts (Na_2SeO_3) and nanoparticulate form (nSe), are shown as raw material with potential to improve germination attributes, mainly as growth stimulant in low concentrations for the radicle growth. Both the Se species used for seed priming influences phytotoxicity and negatively affected the radicle growth.

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

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CONCLUSIONES GENERALES

1. En el cultivo de chile jalapeño, la biofortificación con Se iónico y nano-selenio influyó en la calidad fisicoquímica de los frutos, contenido de compuestos bioactivos, actividad enzimática, y contenido de Se en fruto, ambos factores importantes, dado que el objetivo principal es conocer el estado antioxidante de los frutos, sin perder la esencia de la biofortificación. Por otra parte, en los aspectos evaluados en semilla, se estableció que concentraciones mayores a 10 mg L^{-1} de Na_2SeO_3 afectaron negativamente los atributos de la germinación, mientras que con nSe se mantiene un porcentaje de germinación mayor al 80%.
2. La evaluación conjunta de dos formas de Se (Na_2SeO_3 y nSe) a la misma concentración, permitió observar el comportamiento del cultivo y la respuesta de los principales componentes del metabolismo antioxidante. Con la suplementación de 30 mg L^{-1} Se en ambas formas fue posible incrementar significativamente los compuestos bioactivos evaluados, así como incrementar la actividad enzimática PAL, clave en la biosíntesis de polifenoles. Así mismo, con ambas formas de Se a 45 mg L^{-1} se redujo significativamente la cantidad de proteínas totales, lo que se puede considerar como un síntoma metabólico de toxicidad, al influir en la estructura y funcionalidad de las proteínas.
3. Finalmente, con la aplicación de Se en forma iónica y nanométrica es posible incrementar significativamente el contenido de Se en el fruto. Es recomendable realizar análisis postcosecha, y en frutos procesados, para estimar la cantidad de Se que se estaría suministrando a los consumidores por la ingesta de derivados de chile jalapeño, y frutos con algunos días de almacenamiento.

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