UNIVERSIDAD AUTÓNOMA AGRARIA ANTONIO NARRO

SUBDIRECCIÓN DE POSTGRADO

NANOPARTÍCULAS DE COBRE EN HIDROGELES DE QUITOSÁN-PVA PARA LA INDUCCIÓN DE TOLERANCIA A ESTRÉS SALINO, CAPACIDAD ANTIOXIDANTE Y EXPRESIÓN GÉNICA EN *Solanum lycopersicum* L.

Tesis

Que presenta HIPÓLITO HERNÁNDEZ HERNÁNDEZ

como requisito parcial para obtener el Grado de

DOCTOR EN CIENCIAS EN AGRICULTURA PROTEGIDA

Saltillo, Coahuila Diciembre 2017

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Dr. Antonio Juárez Maldonado
Director Interno UAAAN

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Saltillo, Coahuila

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Elaborada por HIPÓLITO HERNÁNDEZ HERNÁNDEZ 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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Saltillo, Coahuila Diciembre 2017

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DEDICATORIA

A mi esposa e hijos por estar siempre a mi lado y apoyarme incondicionalmente en todo momento, se los dedico especialmente con todo mi amor y cariño.

A mis padres se los dedico de manera especial por apoyarme a continuar con mis estudios de postgrado y por todos sus consejos que siempre serán indispensables.

ARTÍCULO ENVIADO

E

HEB Editorial Office<em@editorialmanager.com>
mié 23/08, 12:15 p.m.
Tú

Responder

Re: "Responses in Growth and Antioxidant Capacity of Tomato Under Saline Stress and Application of Cu Nanoparticles in Chitosan-PVA Hydrogels"
Full author list: Hipólito Hernández-Hernández; Susana González-Morales; Adalberto Benavides-Mendoza; Hortensia Ortega-Ortiz; Gregorio Cadenas-Pliego; Antonio Juárez-Maldonado

Dear Dr Hipólito Hernández-Hernández,

We have received the submission entitled: "Responses in Growth and Antioxidant Capacity of Tomato Under Saline Stress and Application of Cu Nanoparticles in Chitosan-PVA Hydrogels" for possible publication in Horticulture, Environment, and Biotechnology, and you are listed as one of the co-authors.

The manuscript has been submitted to the journal by Dr. Dr Antonio Juárez-Maldonado who will be able to track the status of the paper through his/her login.

If you have any objections, please contact the editorial office as soon as possible. If we do not hear back from you, we will assume you agree with your co-authorship.

Thank you very much.

With kind regards,

Springer Journals Editorial Office
Horticulture, Environment, and Biotechnology

INTRODUCCIÓN

La nanotecnología está innovando el uso de los materiales debido a que mejora sus propiedades fisicoquímicas. Esto se debe a la alteración en los átomos lo cual desarrolla una mayor potencia magnética, posee una mayor área de superficie y se exhibe más activo (Prasad et al., 2017). En los sistemas agrícolas se están evaluando el uso de diferentes nanomateriales como nanofertilizantes, nanopesticidas, nanosensores y nanoestimulantes del crecimiento. Prasad et al. (2017) reporta que en el año 2016 se enumeraron unos 14,000 documentos y alrededor de 2707 patentes sobre nanotecnología en alimentos o agricultura. Estos estudios se han enfocado principalmente en los efectos toxicológicos, fisiológicos, bioquímicos y genómicos en las plantas.

Los principales nanomateriales (NMs) que se están evaluando en plantas son a base de metales y óxidos metálicos como el Cu, Zn, Fe, Ti, Ce y Ag. Estos NMs han demostrado que a concentraciones altas (> 100 ppm) causan toxicidad, por el contrario a una concentración baja (< 100 ppm) promueven el crecimiento, aumentan la tasa fotosintética y enzimas de respuesta antioxidante (Du et al., 2017; Reddy et al., 2016). Las plantas perciben a los NMs como estrés oxidativo, por lo que el principal mecanismo de señalización es la producción de especies reactivas de oxigeno (ROS) y Ca^{2+} (Sosan et al., 2016). Aguas abajo se activa el óxido nítrico para mitigar el estrés oxidativo. Las hormonas como el ácido abscísico (ABA), ácido salicílico, ácido jasmónico y etileno también juegan un papel importante como señalizadoras (Vankova et al., 2017).

La absorción de los NMs en las plantas depende de las propiedades fisicoquímicas como el tamaño, la morfología y composición química. Además la interacción con microorganismos en el suelo, ácidos húmicos, materia orgánica e iones de sales interfieren en la absorción (Alharby et al., 2016; Dimkpa et al., 2015; Feng et al., 2013; Grillo et al., 2015; Wang et al., 2016). Las plantas pueden absorber a los NMs de forma intacta o mediante una disolución en la superficie radicular cuando se aplican en el suelo (Wang et al., 2017a; Zhang et al., 2017). Cuando se aplican de manera foliar se absorben de manera intacta a través de los estomas y después se crea una disolución dentro de la planta en el apoplasto (Schymura et al., 2017). Las nanopartículas (NPs) pueden penetrar

mediante endocitosis, formación de poros o plasmodesmata, después de internalizarse se pueden transportar vía simplástica y apoplástica (Pérez-de-Luque, 2017).

Se ha observado que las NPs de cobre promueven el crecimiento e inducen respuestas de defensa en las plantas. También se ha demostrado que las NPs de cobre recubiertas son menos tóxicas que las NPs libres y que el sulfato de cobre (Aruna et al., 2015; Pradhan et al., 2015). En plantas de tomate las NPs de cobre aumentan el vigor de las plantas y el contenido de licopeno de los frutos (Juárez-Maldonado et al., 2016). El quitosán es uno de los polímeros que se está utilizando para el recubrimiento de NMs debido a su gran capacidad de reticulación e intercambio catiónico en soluciones ácidas y gran afinidad hacia a los iones metálicos (Guibal et al., 2014). Este polímero se utiliza como bioestimulante y para inducir respuestas de defensa contra patógenos en las plantas mediante la producción de H_2O_2 y óxido nítrico que regulan la expresión de genes relacionados a estrés biótico y abiótico, respectivamente (Ali et al., 2011; Zhang et al., 2011).

En las superficies agrícolas, el estrés salino es uno de los diferentes tipos de estrés abiótico de gran importancia a nivel mundial, ya que se estima que alrededor del 6% de la superficie es afectada por éste tipo de estrés (Parihar et al., 2015). El sodio ingresa a la célula a través de los canales catiónicos no selectivos, después se desencadenan cascadas de señalización de Ca^{2+}, ROS y hormonas. Aguas abajo se activan mecanismos de desintoxicación celular como el intercambiador de Na^+/H^+, mecanismos de transporte de Na^+ a través de la vía extremadamente sensible a la sal (SOS) y estrategias de protección osmótica (Deinlein et al., 2014). Algunos nanomateriales han mostrado efectos positivos en combinación con el estrés salino, mejoran la tasa fotosintética, el crecimiento, modifican las barreras apoplásticas de las raíces para controlar la entrada de Na^+ y reducen el contenido de malondialdehido (MDA), H_2O_2 y fuga de electrolitos (Khan et al., 2017; Rossi et al., 2017). Por lo tanto, se desarrolló este trabajo de investigación con los siguientes objetivos planteados.

Objetivo General

Conocer las respuestas bioquímicas de inducción de capacidad de antioxidantes, tolerancia al estrés salino y expresión génica en plantas de tomate tratadas con NPs de Cu en hidrogeles de quitosán-PVA.

Objetivos Específicos

Evaluar diferentes concentraciones de NPs de Cu en hidrogeles de quitosán-PVA en plantas de tomate y algunas variables de calidad de fruto.

Describir las variables morfológicas, pigmentos fotosintéticos y el contenido de minerales de las plantas tratadas con NPs de Cu en hidrogeles de quitosán-PVA y sometidas a estrés salino.

Determinar la capacidad antioxidante, la inducción de tolerancia a estrés salino y la expresión génica en plantas de tomate tratadas con NPs de Cu absorbidas en hidrogeles de quitosán-PVA.

Hipótesis

Las NPs de Cu en hidrogeles de quitosán-PVA pueden modificar la capacidad de adaptación y defensa de las plantas, aumentando la capacidad de antioxidantes, la expresión génica y la inducción de tolerancia a estrés salino.

REVISIÓN DE LITERATURA

Nanotecnología

La nanotecnología es la ciencia que estudia la materia a escala muy pequeña llamada nanomaterial, comprende el estudio de las propiedades químicas y físicas de las partículas que están estructuradas en tamaños que varían de 1 a 100 nm. En este rango de tamaño, el nanomaterial exhibe características químicas y físicas superiores en comparación con las de tamaño normal (Akhnoukh, 2017). Se aplica en campos como la física, química, biología, ingeniería y ciencia de los materiales. La nanotecnología comenzó en 1959 con la conferencia impartida por el físico Richard Feynman titulada "hay mucho espacio en el fondo" donde describió un proceso en que los científicos podrían manipular y controlar átomos y moléculas individuales (Seifert, 2017). Una década más tarde el profesor Norio Taniguchi usó el termino nanotecnología por primera vez, sin embargo fue hasta 1981 cuando la nanotecnología moderna comenzó con el desarrollo del microscopio de efecto túnel que podía ver átomos individuales (Binnig and Rohrer, 1986). En 1985 se descubrieron los fullerenos por Harry Kroto, Richard Smalley y Robert Curl, quienes juntos ganaron el Premio Nobel de Química en 1996 (Kroto et al., 1991). A principios de la década del 2000, esta tecnología obtuvo una mayor atención científica, política y comercial que condujo a la controversia y al progreso. Surgieron controversias sobre las definiciones y las implicaciones potenciales de las nanotecnologías. Se plantearon desafíos con respecto a la viabilidad de las aplicaciones previstas por los defensores de la nanotecnología molecular, que culminaron en un debate público entre Drexler y Smalley en 2001 y 2003 (Bueno, 2004). Actualmente existen técnicas de caracterización de nanomateriales más precisas para determinar el tamaño y forma de partículas (microscopía electrónica de transmisión), los patrones de difracción (rayos X), el contenido de ligantes (análisis termogravimétrico) y la estabilidad térmica (FTIR). Las áreas donde se tienen mayores aplicaciones de la nanotecnología son en los materiales y electrónica. La región de Norte América tiene el mayor mercado, pero Europa y Asia, especialmente China, Japón e India, también se presentan de manera muy dinámica (Prasad et al., 2017).

Existen muchas incógnitas sobre porqué los nanomateriales cambian su propiedad, esto se debe a la alteración en los átomos lo cual desarrolla una mayor potencia magnética. Se puede proyectar que el tamaño más pequeño del nanomaterial posee una mayor área de superficie y se exhibe más activo. Con este mecanismo de acción, la nanotecnología está tocando las puertas de la percepción (Prasad et al., 2017).

Nanomateriales en la agricultura

La nanotecnología tiene el potencial de ofrecer beneficios positivos en los desafíos agrícolas. A partir del año 2010 la cantidad de publicaciones relacionadas con la terminología nano ha crecido exponencialmente. De acuerdo a la búsqueda realizada por Prasad et al. (2017) en scopus.com en el año 2016 se enumeraron unos 14,000 documentos sobre nanotecnología en alimentos o agricultura. También encontraron alrededor de 2707 patentes con este mismo criterio de búsqueda en la base de datos mundial de patentes.

En el área de la agricultura, se han estudiado principalmente los efectos tóxicos, fisiológicos, bioquímicos y genómicos en las plantas. Los estudios enfocados en la toxicología en diferentes especies han demostrado que una concentración mayor a 100 ppm de nanomateriales a base de metales y óxidos metálicos son tóxicos para las plantas (Du et al., 2017; Reddy et al., 2016). Los daños ocasionados son la disminución del crecimiento y desarrollo, menor tasa de germinación, reducción de la tasa fotosintética y clorofila y daños en el metabolismo redox, etc. Por el contrario, una concentración menor a 100 ppm tiene efectos positivos en las plantas, ya que promueven el crecimiento, aumentan la capacidad fotosintética e inducen una mayor respuesta de defensa antioxidante (Du et al., 2017; Reddy et al., 2016).

Los nanomateriales se están utilizando en la agricultura como nanofertilizantes, nanopesticidas, nanosensores y nanoestimulantes. Los nanofertilizantes (nutrientes nano-dimensionados, fertilizantes nano-revestidos, o nanomateriales basados en óxidos metálicos o carbono) y nanopesticidas (nano-formulaciones de ingredientes activos tradicionales o nanomateriales inorgánicos), pueden proporcionar una liberación controlada/dirigida de agroquímicos y obtener su máxima eficacia biológica sin sobredosificar. Los nanosensores y los métodos de nanoremediación pueden detectar y

eliminar contaminantes ambientales. Sin embargo, el conocimiento limitado acerca de la bioseguridad de los nanomateriales, los efectos adversos, el destino y la reactividad biológica adquirida una vez dispersados en el medio ambiente requiere de mayores esfuerzos científicos para evaluar los posibles riesgos nanoagrícolas (Iavicoli et al., 2017).

Absorción y transporte de los nanomateriales en las plantas

La absorción de las nanopartículas en las plantas depende de sus propiedades fisicoquímicas. El tamaño es una de las características que restringe la penetración en los tejidos vegetales, siendo una dimensión de hasta 40-50 nm lo que permite una mayor movilidad y acumulación en las células de las plantas (Corredor et al., 2009; González-Melendi et al., 2008; Sabo-Attwood et al., 2012; Taylor et al., 2014; Wong et al., 2016). Además el tipo y composición química es otro factor que influye en la absorción (Ma et al., 2014; Rico et al., 2011). También la morfología de los nanomateriales afecta la absorción en algunos casos (Lahiani et al., 2016; Raliya et al., 2016). Por ejemplo, se evaluaron cuatro tipos de NPs de TiO_2, incluyendo una anatasa (NAnT), un rutilo prístino (NruT) y dos rutiles con superficies hidrofílicas e hidrofóbicas (NLRuT, NBRuT) en plantas de arroz. De las cuales sólo un tipo de nanopartícula de TiO_2 (NanT) entró a las raíces de las plántulas de arroz, sin embargo no se transportaron de las raíces a los brotes, probablemente debido a los efectos de obstrucción de la banda caspariana en los tejidos de las raíces (Cai et al., 2017). El tipo de carga afecta la absorción, las nanopartículas de carga negativa tienen mayor afinidad a la pared celular y se transportan al apoplasto, sin embargo las de carga positiva o neutras inducen la producción de mucilago en las raíces y se adhieren a ellas (Avellan et al., 2017; Spielman-Sun et al., 2017). Las plantas pueden absorber las nanopartículas mediante la captación directa o a través de una disolución en la superficie radicular cuando se aplican en el suelo (Zhang et al., 2017). Cuando son aplicadas de forma foliar se absorben directamente por los estomas y después se crea una disolución dentro de la planta en el apoplasto (Schymura et al., 2017). Además de las características propias de los NMs, los factores ambientales también influyen en la absorción de las NPs, ya que en el suelo interactúan con microorganismos (micorrizas y rizobacterias), ácidos

húmicos, materia orgánica e iones de sales (Alharby et al., 2016; Dimkpa et al., 2015; Feng et al., 2013; Grillo et al., 2015; Wang et al., 2016).

Las nanopartículas penetran al sistema vascular de la planta mediante mecanismos de endocitosis, incorporándose a la célula por invaginación de la membrana plasmática, originando una vesícula que puede viajar a diferentes compartimentos de la célula (Li et al., 2016a; Pérez-de-Luque, 2017). También pueden penetrar por medio de la inducción de formación de poros para atravesar la célula y alcanzar directamente el citosol sin ser encapsulado en ningún órgano, o bien, mediante la plasmodesmata que son estructuras especializadas en el transporte entre células (Pérez-de-Luque, 2017). Posteriormente, las nanopartículas pueden transportarse vía apoplástica y/o simplástica para moverse de arriba hacia abajo de la planta, y el movimiento radial para cambiar de una vía a otra (Pérez-de-Luque, 2017).

Mecanismos de señalización de los NMs en las plantas

Los nanomateriales inducen estrés oxidativo en las plantas, y éste puede ser grave o leve dependiendo de la concentración aplicada. De acuerdo a varios estudios proteomicos, metabolomicos y transcriptomicos se hace referencia al mecanismo de señalización de los NMs. La principal vía de señalización de los NMs es la inducción de ROS y el incremento de calcio a través de las proteínas de unión de calcio (Sosan et al., 2016). Las NPs pueden inducir cascadas de proteína quinasa activada por mitógenos (MAPK) aunque no hay evidencia directa de la interacción NPs-Planta-MAPK (Marslin et al., 2017). El óxido nítrico es un importante señalizador ya que reduce el estrés oxidativo producido por los NMs (Tripathi et al., 2017). Las hormonas también son importantes señalizadoras de los NMs como lo son el ácido abscísico (ABA), ácido salicílico, ácido jasmónico (Vankova et al., 2017, citoquininas (Vinković *et al.*, 2017) y etileno (Wang et al., 2017a). Los NMs activan el metabolismo secundario de la planta como los polifenoles oxidasa, fenilalanina amonio liasa (Ma et al., 2016) y brasinoesteroides (Li et al., 2016b). También regulan el metabolismo fotosintético, metabolismo del nitrógeno y metabolismo del azufre en las plantas (Wang et al., 2017b).

Nanopartículas de cobre

Las nanopartículas de cobre se pueden sintetizar artificialmente mediante reducción química. Las sales de cobre ($CuSO_4.5H_2O$) se reducen con agentes reductores (NaOH), el cobre pasa de estado de oxidación +2 a 0, para evitar que se aglomeren y formen micropartículas se introducen en el medio de reacción ligandos de nitrógeno como la alilamina (Aam) y polialilamina (PAAm). Las NPs se obtienen como polvo negro (Sierra-Ávila et al., 2014). También se pueden sintetizar de manera biológica a través de extractos de plantas, enzimas y microorganismos.

Los estudios sobre NPs de cobre en las plantas demuestran que el efecto tóxico depende del tamaño y concentración de nanopartícula, así como el tipo de especie. En plantas de *Phaseolus radiates* y *Triticum aestivum* una concentración de 200-1000 mg L^{-1} afectaron negativamente el crecimiento (Lee et al., 2008). Una concentración de 50-500 mg L^{-1} de nanopartículas de óxido de cobre redujeron el crecimiento de *Glycine max* (Nair and Chung, 2014a). Las nanopartículas de óxido de cobre a una concentración superior a 2 mg L^{-1} mostraron una reducción significativa en la biomasa de la planta y el contenido total de clorofila de *Arabidopsis thaliana* (Nair and Chung, 2014b). Por el contrario, concentraciones de 0.05-1.0 mg L^{-1} aplicadas en el suelo o en semillas aumentan el crecimiento, contenido de clorofila y carotenoides (Pradhan et al., 2015). También se ha demostrado que las NPs de cobre recubiertas son menos tóxicas que las NPs libres y que el $CuSO_4$ (Aruna et al., 2015; Pradhan et al., 2015). En este sentido las NPs de Cu en hidrogeles de quitosán-PVA promueven el vigor de las plantas y la cantidad de licopeno en fruto de tomate (Juárez-Maldonado et al., 2016).

Los estudios sobre el efecto de las nanopartículas de cobre ganan importancia para explorar las nanopartículas en el manejo de enfermedades sin afectar el crecimiento de la planta (Kasana et al., 2017). Se ha observado que las nanopartículas de cobre encapsuladas en quitosán aumentan la actividad de enzimas defensivas como la catalasa, peroxidasa, superóxido dismutasa y fenilalanina amonio liasa que funcionan como agente antifúngico contra diversos hongos fitopatógenos (Saharan et al., 2015; Saharan and Pal, 2016).

Quitosán

El quitosán es un polímero natural que se obtiene después de la desacetilación de la quitina en grados variables. La quitina se puede extraer de la cutícula de los crustáceos (camarón y cangrejo), hongos e insectos. Sin embargo, las quitinas comerciales se suelen aislar de los crustáceos, debido a la gran cantidad de residuos en la industria pesquera. La quitina se encuentra en los crustáceos como constituyente de una red compleja de proteínas en las que el carbonato de calcio se deposita para formar la cáscara rígida. Para el aislamiento de la quitina se requiere la eliminación de los dos componentes principales de la cáscara, las proteínas por desproteinización y el carbonato de calcio inorgánico por desmineralización, junto con pequeñas cantidades de pigmentos y lípidos que generalmente se eliminan durante los dos pasos anteriores (Younes and Rinaudo, 2015).

Para la desacetilación de la quitina puede ser por procesos ácidos o alcalinos. Sin embargo, los enlaces glucosídicos son muy susceptibles al ácido; por lo tanto, la desacetilación alcalina se utiliza con mayor frecuencia. La N-desacetilación de la quitina se lleva a cabo de forma heterogénea, o de forma homogénea. Comúnmente, en el método heterogéneo, la quitina se trata con una solución concentrada caliente de NaOH durante pocas horas, y el quitosán se produce como un residuo insoluble desacetilado hasta 85-99%. De acuerdo con el método homogéneo, se prepara quitina alcalina después de la dispersión de quitina en NaOH concentrado (30 g de NaOH /45 g de H_2O/ 3 g de quitina) a 25 ºC durante 3 h o más, seguido de la disolución en hielo triturado alrededor de 0 ºC. Este método da como resultado un quitosán soluble con un grado medio de acetilación de 48-55% (Younes and Rinaudo, 2015).

En la agricultura, el quitosán se utiliza como bioestimulante en plantas de cultivos. Se ha demostrado que tiene efectos positivos en el crecimiento, fisiológicos y bioquímicos. El quitosán actúa como un estimulador de las respuestas de defensas en las plantas a través de la producción de H_2O_2 lo que conlleva a la inducción de enzimas defensivas como la fenilalanina amonio liasa que es primordial en la síntesis de compuestos fenólicos (Ali et al., 2011). El sistema de defensa también es activado a través de la vía del óxido nítrico, el cual se produce primero en el cloroplasto, seguido del núcleo y después en la célula entera (Zhang et al., 2011). El H_2O_2 desencadena especies reactivas de oxígeno y la

síntesis de ácido abscísico, y coordina la actividad con ácido jasmónico a través de la vía octadecanoide regulando la expresión de genes relacionados al estrés biótico, mientras que el óxido nítrico regula la síntesis de ácido fosfatídico (PA) a través de la vía de fosfolipasa C y diacilglicerol quinasa. El PA mejora la acción de ABA inhibiendo ABI1 (regulador negativo de ABA). Luego, el ABA induce el cierre estomático y otras respuestas de estrés abiótico (Pichyangkura and Chadchawan, 2015). El quitosán incrementa los niveles de glucanasa y quitinasa en varias especies de cultivos y se asocia con el aumento de la resistencia a enfermedades y se basa potencialmente en la inducción de estas enzimas (Pichyangkura and Chadchawan, 2015). En plantas de okra la aplicación foliar de quitosán aumenta el contenido de clorofila, el crecimiento y rendimiento, así como un aumento de la fotosíntesis (Mondal et al., 2012). En plantas de fresa la aplicación foliar de quitosán aumentó el crecimiento vegetativo (longitud de la planta, número de hojas/planta, área foliar, peso fresco y seco de raíz y rendimiento) (El-Miniawy et al., 2013). En plantas de *Camellia sinensis* el Cs a granel aumenta la expresión de los genes SOD y CAT en comparación con los controles no tratados (Chandra et al., 2015).

El quitosán es utilizado para la formación de hidrogeles. Los hidrogeles son redes poliméricas que pueden retener una cantidad significativa de agua dentro de sus estructuras e hincharse sin disolverse en agua (Ahmed, 2015). Los hidrogeles de CS-PVA contienen grupos funcionales (amino e hidroxilo) que actúan como quelantes de iones metálicos otorgándole capacidades de adsorción muy altas. Por lo tanto, las moléculas de soluto podrían interpenetrar en la red del hidrogel rápidamente y unirse a los grupos funcionales. Los hidrogeles se utilizan para el suministro controlado de fármacos. En la agricultura es utilizado para la retención de humedad (acondicionador de suelo) y para la liberación controlada de agroquímicos y fertilizantes reduciendo la sobredosificación (Kashyap et al., 2015).

Estrés salino

El estrés salino afecta a una gran diversidad de cultivos en todo el mundo, se informa que alrededor del 6% de la superficie mundial es afectada por la salinidad (Parihar et al., 2015). Las plantas perciben el estrés salino cuando los iones de Na entran en contacto

con las raíces mediante lo canales catiónicos no selectivos. Después se activan cascadas de señalización Ca^{2+}, ROS, hormonas, homeostasis iónica y osmótica (Deinlein et al., 2014). La homeostasis iónica es regulada por la vía SOS, donde un complejo de proteínas quinasas sensibles al calcio (SOS3-SOS2) controlan la expresión y la actividad de transportadores de iones tales como SOS1 (Zhu, 2003). La homeostasis osmótica activa varias proteínas quinasas incluyendo quinasas activadas por mitógenos, que pueden mediar la homeostasis osmótica y/o respuestas de desintoxicación. El estrés osmótico regula la biosíntesis del ABA dependiente e independiente en múltiples etapas (Rowe et al., 2016). La biosíntesis de etileno y la vía de señalización del etileno regulan la tolerancia a la sal de las plantas mediante la activación de la expresión de genes diana aguas abajo tales como los relacionados con la homeostasis de ROS y Na^+/K^+ (Quan et al., 2017).

Algunos nanomateriales han mostrado efectos positivos para mitigar el estrés salino. Por ejemplo, la aplicación de NPs a base de Si mejora la tasa fotosintética neta, la conductancia estomática, la tasa de transpiración, la eficiencia del uso del agua y la reducción y degradación de la clorofila en plantas de calabaza bajo estrés salino (Siddiqui et al., 2014). Además aumentan la germinación y crecimiento de las plantas lo cual se ve reflejado en la reducción del daño oxidativo debido a la expresión de enzimas antioxidantes, tales como catalasa, peroxidasa, superóxido dismutasa, glutatión reductasa y ascorbato peroxidasa. Por el contrario, reducen el contenido de MDA, H_2O_2 y fuga de electrolitos (Siddiqui et al., 2014). En plantas de *Vicia faba* las NPs de SiO_2 incrementaron la germinación, el crecimiento, actividad de enzimas antioxidantes, contenido de agua relativa y rendimiento bajo estrés salino (Qados, 2015). La combinación de estrés salino y NPs de ZnO presentaron mayor expresión de los genes SOD y GPX en comparación con las plantas de tomate tratadas únicamente con estrés salino (Alharby et al., 2016).

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REGULAR ARTICLE

Cu Nanoparticles in chitosan-PVA hydrogels as promoters of growth, productivity and fruit quality in tomato

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ABSTRACT

The encapsulation of copper nanoparticles (Cu NPs) in chitosan hydrogels could improve the yield and quality of fruit of horticultural crops due to the physicochemical properties of the NPs. The objective of this research was to evaluate different concentrations of Cu NPs in Chitosan-polyvinyl alcohol (Cs-PVA) hydrogels and their effects on the growth, productivity and fruit quality in tomato. The treatments were applied to the substrate as follows: 0.02, 0.2, 2 and 10 mg of Cu NPs in Cs-PVA hydrogel, Cs-PVA hydrogel alone and a control. The Cu NPs had significant effects on growth, productivity and fruit quality. They increased the numbers of leaves and clusters, fresh biomass of roots, and dry biomass of stem-leaves and roots of the plants. They also increased the soluble solid content, titratable acidity, lycopene content and total antioxidant capacity in the fruits. The concentration with the best effect on the growth and yield of tomato plants was 10 mg Cu NPs, which increased the stem diameter, dry biomass of stem-leaves (13%) and roots (30%) and the yield (17%), whereas the concentration of 0.02 mg Cu NPs increased the lycopene content (37%) and the total antioxidant capacity of the fruit (10%). The Cu NPs in Cs-PVA hydrogels helped to increase the yield and nutraceutical properties of the tomato fruits.

Keywords: Antioxidants; Chitosan; Cu NPs; *Solanum Lycopersicum*; Yield

INTRODUCTION

Nanomaterials are attracting attention from the scientific community due to their outstanding activity in relation to bulk materials (Naddeo et al., 2015). This heightened activity can be attributed to the high ratio surface-volume area and unique physico-chemical, mechanical and electronic properties of nanoparticles (NPs) (Somasundaran et al., 2010). Advances in nanotechnology have enabled its application to agriculture and the food industry, and particularly in agriculture are becoming popular (Ruttkay-Nedecky et al., 2017). However, its use is still limited due mainly to the lack of information about the toxicity and environmental fate of nanomaterials (Narayanan et al., 2012; Ruttkay-Nedecky et al., 2017), and also the little interest of the application of nanotechnology in plant sciences compared to nanomedicine and nanopharmacology (Wang et al., 2016). Nanotechnology could play a key role in increasing global food production. Currently, numerous products and patents based on nanomaterials are being developed with the aim of improving the efficiency and sustainability of farming practices (Servin et al., 2015).

The main nanomaterials being studied are carbon-based (fullerol or carbon nanotubes), metals and metal oxides (Peralta-Videa et al., 2011). Nanomaterials based on metals and metal oxides that are being evaluated in plants include the ZnO NPs (Landa et al., 2012; Zhao et al., 2013; Zhao et al., 2014), TiO_2 NPs (Landa et al., 2012; Hanif et al., 2015), CeO_2 NPs (Zhao et al., 2013; Zhao et al., 2014; Rico et al., 2014; Hong et al., 2015), Fe_3O_4 NPs (Trujillo-Reyes et al., 2014), CuO NPs (Adhikari et al., 2012; Hong et al., 2015) and Cu NPs (Lee et al., 2008; Pradhan et al., 2015; Saharan et al., 2015). Some of the ways these nanomaterials are

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being applied to the plants are through a nutrient solution or soil, leaf or in-vitro means (Liu and Lal, 2015; Servin et al., 2015; Anjum et al., 2015). Moreover, the application of nanoparticles in different crop plants has been evaluated, and the effects of this vary greatly with plant species and other factors as dose and type of NPs (Rizwan et al., 2017).

The accepted mode of action of these nanomaterials is cellular penetration, although the exact mechanisms of uptake are not fully known (Zhang et al., 2015; Servin et al., 2015). NPs can be absorbed by plants through the roots or the leaves. They must first penetrate the epidermis and endodermis of the root and then enter the xylem vessel, and they are then transported to the aerial parts. In the leaves, they can penetrate through the stoma, entering the vascular system of the leaves, and then be transported to other parts of the plant through the phloem (Zhang et al., 2015; Servin et al., 2015; Shi et al., 2014; Le et al., 2014). Moreover, the nanoparticles can cross cell walls by several ways: endocytosis, pore formation, carrier proteins, or through plasmodesmata (Pérez-de-Luque, 2017). Even if there are the ion channels, they have size around 1 nm, thus nanoparticles are unlikely to cross the cell wall effectively (Pérez-de-Luque, 2017). Once the NPs are absorbed by the plants, they cause stress and consequently the generation of reactive oxygen species (ROS), activating the plant antioxidant defense system (Rico et al., 2015). This defense system of plants combines the generation of enzymatic and non-enzymatic antioxidant compounds (Rizwan et al., 2017). The changes of enzymatic antioxidants have been demonstrated by Juárez-Maldonado et al. (2016) in tomato plants treated with Cu NPs + chitosan, where the catalase activity was more than five times higher than the control. Also Pinedo-Guerrero et al. (2017) reported that application of Cu NPs + chitosan generated more concentration of capsaicin in jalapeño pepper, exceeding the control by 51%.

Cu NPs are of particular importance in plants because they enhanced photosynthetic activity by modulating fluorescence emission, photophosphorylation, electron transport chain, and carbon assimilatory pathway under controlled laboratory conditions, as revealed from biochemical and biophysical studies on treated isolated mung bean chloroplast (Pradhan et al., 2015). Recent research studies report that the application of Cu NPs at low concentrations (0.05 to 1.0 mg L^{-1}) in the soil or by seed imbibition increases the seedling growth, chlorophyll and carotenoid content (Shah and Belozerova, 2009; Pradhan et al., 2015). However, the application of higher concentrations (200-1000 mg L^{-1}) in the nutrient solution reduces growth and biomass accumulation in seedlings (Lee et al., 2008; Kim et al., 2012; Musante and White, 2012; Wang et al., 2012). One of the main problems in using NPs is their insolubility in water (Lee et al., 2008). Due to this problem, natural polymers such as chitosan (Cs) are being used for the encapsulation and controlled release of NPs metal due to its characteristics of biocompatibility, biodegradability, non-toxicity and adsorption ability (Kashyap et al., 2015). Cs is a biodegradable natural compound, the deacetylated derivative of chitin, obtained from the exoskeletons of crustaceans such as crabs and shrimp. Its industrial and medicinal value derives from its polycationic nature (Bautista-Baños et al., 2006). It is a polysaccharide with great crosslinking ability thanks to the presence of the amino groups (-NH_2), which explain the unique properties of the Cs because your behavior cationic in acid solutions and its affinity to metal ions, as well as their antimicrobial properties (Ravi-Kumar, 2000). It is also considered a chelating agent suitable for trapping heavy metals (Shukla et al., 2013). On the other hand, Cs is also used for the synthesis of hydrogels in combination with polyvinyl alcohol (PVA), using glutaraldehyde as a crosslinking polymer (Tripathi et al., 2009; Wang et al., 2004). PVA is soluble in water and acts as an emulsifier and adhesive synthetic polymer (Kanatt et al., 2012) with the ability to release drugs in a controlled way. The main applications and features of Cs in agriculture are founded on its ability to induce a series of defense/stress responses in plants, including the production of H_2O_2 (Malerba and Cerana, 2015) and nitric oxide; stimulate growth; protect against low temperatures and release nutrients into the soil (Rinaudo, 2006). When it is used as a coating on fruits, it extends their postharvest life (Badawy and Rabea, 2011).

It has recently been shown that Cu NPs coated with Cs are less toxic to seedlings than free Cu NPs or copper sulfate (Aruna et al., 2015; Saharan et al., 2015). Accordingly, this study focused on the application of Cu NPs absorbed in Cs-PVA hydrogels directly to the substrate, evaluating their effect on growth, productivity and fruit quality.

MATERIALS AND METHODS

Synthesis of hydrogels of chitosan-polyvinyl alcohol (PVA-Cs) and absorption of Cu NPs

Hydrogels of Cs-PVA were synthesized in the pilot plant of the Research Center for Applied Chemistry (RCAC) according to the following methodology: first, 250 mL of 2% Chitosan from Marine Chemical, Mv = 200,000 g/mol, and 250 mL of 4% polyvinyl alcohol (PVA) from Aldrich, Mw = 30, 000-50,000, in water were mixed for two hours at 60 °C and 300 rpm to form a hydrogel at a 1:2 ratio (CS: PVA). Then, 2.27 mL of crosslinker (50% glutaraldehyde) was added at 450 rpm and 25 °C for 5 min, and 100 mL of 6% NaOH was added at 300 rpm and 25 °C for one hour. The Cs-PVA hydrogels were

immediately washed and purified with distilled water and ethanol, then finally dried and weighed. The Cu NPs used in this work were supplied from SkySpring Nanomaterials, Inc. (Houston, TX, USA) with a reported average size of 25 nm, with a chemical purity of 99.8% and spherical morphology. One hundred milligrams of Cu NPs was dispersed in a solution of Tween 1% by ultrasound for 5 minutes (50 watts and 70% frequency), and dilutions were prepared to obtain concentrations of 10, 2, 0.2 and 0.02 mg, which were subsequently absorbed in 1 gram of Cs-PVA hydrogel and dried at 60 °C.

Experimental growth of tomato plants in greenhouse

In April 2015, established tomato plants (*Solanum lycopersicum* L.) of the hybrid var. "Cayman", ball type and undetermined, were grown in a greenhouse in the Department of Horticulture of the Agrarian Autonomous University Antonio Narro, under a multi-tunnel with polyethylene cover. The average temperature was 22.4 °C, the average photosynthetic active radiation was 677.15 μmol m^{-2} s^{-1}, and the average relative humidity was 62%. The planting density was three plants per square meter. A mixture of peat moss and perlite (50:50, v/v) was used as the growth substrate, placed in black polyethylene bags of 12 L capacity. Also, a system of targeted irrigation was used. For the treatments, prior to transplantation, 1 g of Cs-PVA hydrogel in the low, medium and high parts of the pot for better dispersion of the Cu NPs in the substrate and root area of the plant. Steiner nutritive solution (Steiner, 1961) with the following micro-nutrients was also used: Fe-EDTA = 3.75 ppm; Mn-EDTA = 1.85 ppm; B = 0.35 ppm; Zn-EDTA = 0.30 ppm; Cu-EDTA = 0.15 ppm; and Mo = 0.10 ppm. The nutritive solution was supplied in different concentrations to provide nutrients to the plants: for the first two weeks after transplantation, it was applied at 25%; 50% for the third and fourth weeks; 75% for the fifth week and the rest of the crop cycle at 100%. The treatments used were as follows: four concentrations (0.02, 0.2, 2 and 10 mg) of Cu NPs absorbed in Cs-PVA hydrogels, an absolute control and one treatment with hydrogel Cs-PVA (2% Chitosan and 4% PVA) to evaluate the effect of the Cs alone.

Variable growth and yield of tomato

To evaluate the growth and production of tomato plants, the following procedures were performed: 60 days after transplantation (dat) the apex of all plants were cut, and 110 dat agronomic parameters was measured. Plant height (cm) was measured using a tape; the stem diameter (mm) was measured with a digital vernier; the number of leaves, the number of clusters with flowers and fruits tied, and the number of fruits per plant were counted; and the average weight of fruits (g), the fresh weights of stem-leaves and roots (g), and the yield of fruit per plant (g) were determined. The dry weights of the roots, stems and leaves were obtained after drying in a Drying Oven model DHG9240A for 72 hours at a constant temperature of 80 °C.

Variable quality of tomato fruits

Fruit quality variables were evaluated as follows: at 90 days after transplant, fruits were randomly selected after the second harvest. It was verified that they had no physical damage and were uniform and in maturity stage 6 (red light), according to the visual color criteria used by the United States Department of Agriculture (USDA).

Harvested tomato fruits were stored for 15 days at a constant temperature of 10 °C and relative humidity of 80% to observe the influence of the Cu NPs on the post-harvest quality. On the first day of harvest, 9 fruits per treatment were weighed to calculate the percentage of weight loss after 15 days of storage. The pH, titrable acidity and soluble solids of 3 fruits per treatment were also measured on the first day of the harvest and after 8 and 15 days of storage. To determine the percentage of weight loss, the fruits were weighed on an OHAUS brand digital balance. The potential of hydrogen (pH) was determined using a digital potentiometer (HANNA®), the soluble solids (°Brix) using a digital Refract meter PR-101ATAGO PALETTE and the percentage of titrable acidity according to the methodology of the AOAC (2000), expressed as a percentage of citric acid.

The lycopene content and total antioxidant capacity of six fruits per treatment from the second harvest were also measured. The lycopene content was determined according to the methodology of Fish et al. (2002). To 3 g of fresh fruit pericarp, 3 mL of buffer phosphate (pH 7) were added; this sample was ground in a mortar, and then 2 mL of the sample and 4 mL of a 3:2 hexane: acetone mixture were centrifuged for 10 min at 3000 rpm. Finally, the absorbance at 503 nm of the resulting supernatant was determined, corresponding to the μg g^{-1} of lycopene.

The total antioxidant capacity of the fruit was determined using a commercial kit (Antioxidant Assay Kit-Cayman Chemical). First, to 100 mg of lyophilized fruit sample, 2 mL phosphate buffer (pH 7.2) was added and homogenized by vortex for 30 s and ultrasonication for 5 min. The mixture was then centrifuged for 10 min at 12000 g and 4 °C. To 10 μL of the supernatant, 10 μL of metmyoglobin and 150 μL of chromogen were added. To start the antioxidant activity reaction, 40 μL of H_2O_2 was added, and the mixture was incubated for 5 minutes at room temperature. The absorbance at 405 nm was measured in a microplate reader for ELISA (LEX-808 IU model). The final value was expressed as millimoles of Trolox Equivalent Antioxidant per gram dry weight.

Statistical analysis

The crop was established using an experimental Latin square design (6x6), with six treatments and 18 experimental units per treatment for the agronomic variables. For the variables of percentage weight loss, pH, total soluble solids and titratable acidity, a completely randomized design with nine replicates per treatment was used. For the lycopene content and total antioxidant capacity, a completely randomized design with six replications per treatment was used. Statistical analysis of each of the variables was performed using the statistical program R CRAN for the analysis of variance and Fisher LSD mean test ($p \leq 0.05$).

RESULTS

Effect of Cu NPs Cs-PVA hydrogels in the growth and yield of tomato

The application of Cu NPs Cs-PVA hydrogels in the substrate had significant effects on the growth variables and yield of the tomato plants ($p \leq 0.05$). Table 1 shows that treatment with 10 mg of Cu NPs resulted in significant differences compared with the control, increasing the stem diameter, fresh root weight (25%) and the number of floral clusters (3%) per plant, whereas treatment with 2 mg Cu NPs increased the number of leaves per plant by 5% compared to the control ($p \leq 0.05$). For the plant height, fresh weight of stem and leaves, number of fruits and average weight of fruits per plant, no significant differences were observed.

Fig. 1 shows that treatment with 0.02 mg of Cu NPs increased the dry weight of the stem-leaves by approximately 20%, and treatment with 10 mg of Cu NPs increased the dry weight of the roots by 30%.

Fig. 2 shows that treatment with 10 mg Cu NPs Cs-PVA hydrogels resulted in the highest fruit yield compared with the control, an increase of 17% per plant.

Effect of Cu NPs Cs-PVA hydrogels on tomato fruit quality

Table 2 shows significant differences between the treatments for pH, titratable acidity and soluble solids content on the first day of harvest ($p \leq 0.05$). Treatments containing 2.0 and 0.2 mg of Cu NPs increased the titratable acidity 23 and 19% respectively, and decreased the pH by approximately 1% on the first day of harvest with respect to the control. None of the Cu NPs treatments exceeded the control for soluble solids. After 8 days of storage, treatment with 0.02 mg Cu NPs increased the soluble solids content by 11% relative to the control, and no significant differences were found for titratable acidity and pH. After 15 days of storage, treatment with 2.0 mg Cu NPs increased the soluble solids by 14%, and the treatments with 10, 2.0 and 0.2 mg Cu NPs increased the titratable acidity by 7% with respect to the control. No significant differences were observed for pH, and no significant differences in the percentage of weight loss of fruits were found between treatments after 15 days of storage.

The Cu NPs in Cs-PVA hydrogels showed significant differences in the fruit lycopene content and total antioxidant

Table 1: Effect of Cu NPs in Cs-PVA hydrogels on growth, development and productivity of tomato

Treatment	Height (cm)	SD (mm)	NL	NC	FWS (g)	FWR (g)	NF	WF (g)
10	166.4^{a}	22.6^{a}	19.8^{abc}	6.2^{a}	2399.4^{a}	98.9^{a}	27.3^{a}	257.9^{a}
2	168.7^{a}	21.6^{bc}	20.4^{a}	6.0^{b}	2207.9^{a}	95.8^{ab}	26.4^{a}	249.9^{a}
0.2	166.8^{a}	22.1^{abc}	19.6^{bc}	6.0^{b}	2204.2^{a}	77.1^{c}	26.2^{a}	254.2^{a}
0.02	169.5^{a}	21.4^{c}	20.3^{ab}	6.0^{b}	2323.7^{a}	78.5^{bc}	27.1^{a}	256.1^{a}
Cs-PVA	167.8^{a}	21.7^{abc}	19.8^{abc}	6.0^{b}	2308.9^{a}	86.6^{abc}	26.6^{a}	256.4^{a}
0	167.2^{a}	22.3^{ab}	19.4^{c}	6.0^{b}	2192.2^{a}	79.0^{bc}	26.6^{a}	239.4^{a}

Treatment: 0.02, 0.2, 2 y 10 ppm concentrations Cu NPs in Cs-PVA hydrogels, 0= Control, Cs-PVA= Cs-PVA hydrogels only, SD= stem diameter, NL= number of leaf, NC= number of clusters, FWS= fresh weight of shoot, FWR= Fresh weight of root, NF= number of fruits, WF= average fruit weight, means with the same letter in the same column are not different according to Fisher LSD ($p \leq 0.05$)

Table 2: Effect of Cu NPs in Cs-PVA hydrogels on tomato fruit quality

Treatment	WL (%)	pH 1	pH 2	pH 3	SS1 (°Brix)	SS2 (°Brix)	SS3 (°Brix)	TA1 (%)	TA2 (%)	TA3 (%)
Cs-PVA	1.69^{a}	4.27^{a}	4.27^{a}	4.29^{a}	4.18^{ab}	3.60^{b}	4.26^{ab}	1.64^{b}	1.48^{a}	1.64^{b}
2	1.66^{a}	4.17^{b}	4.27^{a}	4.32^{a}	4.07^{ab}	3.75^{b}	4.33^{a}	2.28^{a}	1.51^{a}	1.85^{a}
10	1.42^{a}	4.23^{ab}	4.21^{a}	4.34^{a}	3.98^{b}	3.61^{b}	3.95^{cd}	1.84^{b}	1.62^{a}	1.85^{a}
0.2	1.39^{a}	4.18^{b}	4.21^{a}	4.31^{a}	4.03^{ab}	3.81^{ab}	4.10^{bc}	2.20^{a}	1.50^{a}	1.85^{a}
0	1.37^{a}	4.22^{ab}	4.23^{a}	4.34^{a}	4.22^{a}	3.61^{b}	3.81^{d}	1.85^{b}	1.40^{a}	1.73^{ab}
0.02	1.36^{a}	4.25^{ab}	4.26^{a}	4.30^{a}	4.11^{ab}	4.02^{a}	3.91^{cd}	1.70^{b}	1.60^{a}	1.48^{c}

Treatment: 0.02, 0.2, 2 y 10 ppm concentrations Cu NPs in Cs-PVA hydrogels, Cs-PVA=Cs-PVA hydrogels only, 0=Control, WL=weight loss, pH 1=potential hydrogen first day of harvest, pH 2=potential hydrogen storage eight days, pH 3=potential hydrogen storage fifteen days, SS1=soluble solids first day of harvest, SS2=soluble solids storage eight days, SS3=soluble solids storage fifteen days, TA1=titratable acidity First Day of Harvest, TA2=titratable acidity storage eight days, TA3=titratable acidity storage fifteen days. Means with the same letter in the same column are not different according to Fisher LSD ($p \leq 0.05$)

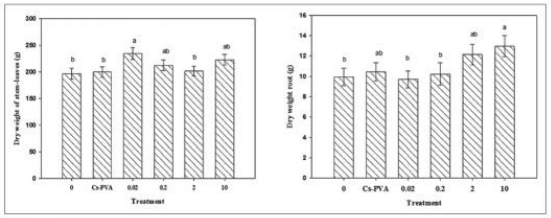

Fig 1. Dry biomass production of shoot (stem-leaves), and root of the tomato plants treated with different concentrations of Cu NPs in Cs-PVA hydrogels. Treatment: 0.02, 0.2, 2 y 10 ppm concentrations Cu NPs in Cs-PVA hydrogels, Cs-PVA= Cs-PVA hydrogels only, 0= Control. Means with the same letter are not different according to Fisher LSD ($p \leq 0.05$).

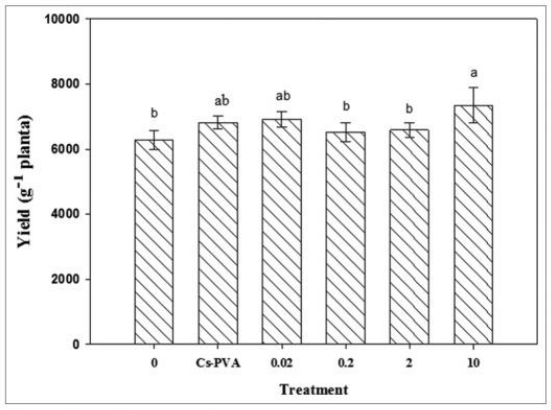

Fig 2. Effect of Cu NPs in Cs-PVA hydrogels yield tomato. Treatment: 0.02, 0.2, 2 y 10 ppm concentrations Cu NPs in Cs-PVA hydrogels, Cs-PVA= Cs-PVA hydrogels only, 0= Control. Means with the same letter are not different according to Fisher LSD ($p \leq 0.05$).

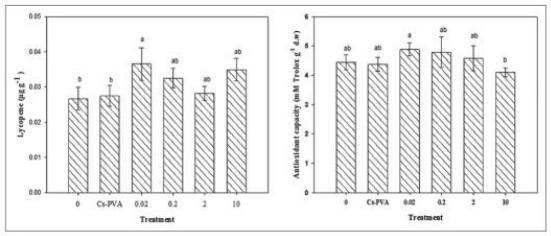

Fig 3. Lycopene content and total antioxidant capacity of tomato fruits at different concentrations of Cu NPs in Cs-PVA hydrogels. Treatment: 0.02, 0.2, 2 y 10 ppm concentrations Cu NPs in Cs-PVA hydrogels, Cs-PVA= Cs-PVA hydrogels only, 0= Control, d.w= dry weight. Means with the same letter are not different according to Fisher LSD ($p \leq 0.05$).

capacity with respect to the control ($p \leq 0.05$). The 0.02 and 10 mg of Cu NPs in Cs-PVA hydrogel treatments showed higher lycopene content in the fruit, namely, 0.03653 and 0.03495 μg g^{-1} fresh weight, respectively, and the control value was 0.0267 μg g^{-1} fresh weight. These values represent an increase of approximately 37 and 31%, respectively, for the treatments with Cu NPs in comparison with control. In regards to the total antioxidant capacity of the fruit, the treatment showing the highest amount of antioxidants was 0.02 mg of Cu NPs, which produced an increase of approximately 10% in comparison with the control, whereas treatment with 10 mg of Cu NPs reduced the antioxidants approximately 8% compared to the control (Fig. 3).

DISCUSSION

The results of this study showed that concentrations of 0.02 to 10 mg Cu NPs in Cs-PVA hydrogels applied to the substrate are not toxic to tomato plants. This nontoxicity could be due to the protection and controlled release of the Cu NPs provided by the Cs-PVA hydrogels during the growth and development of the tomato plants, as mentioned by Kashyap et al. (2015). In addition, the application of high concentrations (200-1000 mg L^{-1}) Cu NPs in the nutrient solution is known to be toxic (Lee et al., 2008; Kim et al., 2012; Musante and White, 2012; Wang et al., 2012), in contrast to this study, in which low concentrations were applied to the substrate. It has also been shown that low concentrations (0.05-1 mg L^{-1}) of Cu NPs encapsulated in polyethylene glycol (PEG-200) are less toxic than copper sulfate (Pradhan et al., 2015), and that low concentrations of Cu NPs coated with Cs (0.01 to 1 mg L^{-1}) are less toxic than free Cu NPs and copper sulfate (Aruna et al., 2015; Saharan et al., 2015). This study used Cu NPs encapsulated in Cs-PVA hydrogels, which could help to reduce the toxicity.

This study also showed that Cu NPs in Cs-PVA hydrogels improved the growth of tomato plants, increasing the diameter of the stem, number of leaves and dry biomass. The diameter of the stem is a very important plant growth parameter to evaluate because it is related to photosynthetic accumulation and transport, as well as to crop performance (Liptay et al., 1981). The literature reports that carbon accumulation in the shoots (stem-leaves) of in *Cyamopsis tetragonoloba* and *Pennisetum glaucum* can be increased by the application of ZnO NPs to the leaves at a concentration of 100 μg mL^{-1} (Burman et al., 2013). It is also known that Cu NPs can play a critical role in photosynthesis, improving the electron transport chain and phosphorylation during the light reaction, as well as improving enzyme activity in the dark phase and participating in the metabolism of carbon and nitrogen (Pradhan et al., 2015). Similarly, Servin et al. (2015) mentioned in their review of literature that different metallic nanoparticles can increase photosynthesis, and the chlorophyll and carotenoid contents in plants.

Some authors, such as Saharan et al. (2015), have shown that the application of 5 ml of Cu NPs coated with Cs at concentrations of 0.08, 0.10 and 0.12% to tomato seeds

placed in Petri dishes with filter paper increase the fresh and dry weight of the tomato seedlings compared with $CuSO_4$ and the control. It has also been shown that the imbibition of seeds of *Vigna mungo* in Cu NPs encapsulated in polyethylene glycol (PEG-200) at concentrations of 0.05 and 0.1 mg L^{-1} for 4-6 hours can increase seedling dry weight compared to $CuSO_4$ and the control (Pradhan et al., 2015). On the other hand, Hanif et al. (2015) reported that the application of TiO_2 NPs (25 to 100 mg kg^{-1}) to the soil significantly increases the dry weight of shoots and roots in *Lactuca sativa* with respect to the control. This result shows that the application of Cu NPs coated in Cs have a positive effect on plant growth.

In regard to the reproductive stage, the Cu NPs in Cs-PVA hydrogels increased the number of floral clusters and the yield of fruit per plant. Thus, Cu NPs can efficiently activate the reproductive system of plants and increase fruit production. It is likely that the tomato fruit yield increase resulted from the ability of the Cu NPs to cause a greater accumulation of photosynthates in the supply-demand organs. Alternatively, it could be because the Cu NPs activate genes related to the growth and development of plants, as is the case for ZnO NPs, which regulate the expression of genes related to cell organization and biogenesis, while TiO_2 NPs are mainly involved in the response of genes to biotic and abiotic stresses in *Arabidopsis thaliana* (Landa et al., 2012). Among the few works that have studied the effect of metal NPs on plant productivity, Wang et al. (2012) reported that the application of CeO_2 NPs in solution at a concentration of 10 mg L^{-1} increased the yield of tomato by 10% and suggested that the effect was probably due to the plants transferring more energy to the growth of the fruit. Hong et al. (2015) showed that concentrations of 50, 100 and 200 mg L^{-1} of CeO_2 and CuO NPs applied foliarly did not affect the yield of cucumber. However, it has been shown that CeO_2 NPs applied to the substrate at high concentrations (800 mg kg^{-1}) decrease cucumber performance by up to 31.6% (Zhao et al., 2013). This result suggests that the applied amount of NPs directly affects crop growth and can have positive effects at low concentrations and negative effects at high concentrations.

The fruit quality results show that the Cu NPs can increase the content of soluble solids and the percentage of titrable acidity. It is very likely that the increase in soluble solids was due to a greater accumulation of photoassimilates in fruit to form fructose and glucose, as reported in Mustafa et al. (2014). It is also known that the increase in the percentage of titrable acidity is due to better metabolism of organic acids (citric acid) in the fruit (Valero and Serrano, 2010), which helps to improve the proportions of fructose and sucrose (Lobit et al., 2003). On the other hand, some authors report that the content of soluble solids and titratable acidity in fruit increase in plants under abiotic stress conditions (Yamada et al., 2015; Al-Harbi et al., 2016). Cu NPs induce oxidative stress and can improve the levels of sugar and citric acid in tomato fruits. Similar studies have shown that CeO_2 NPs applied to the substrate at concentrations of 400 and 800 mg kg^{-1} do not alter the amounts of reducing sugars (glucose and fructose) in the fruits of cucumber, but the amount of non-reducing sugars (sucrose) is reduced at 400 mg kg^{-1} and increased at 800 kg^{-1} (Zhao et al., 2014). On the other hand, Dar et al. (2015) mention that copper has a positive and significant correlation with the soluble solids content and total sugars in pear fruits, which may explain the positive effect on tomato fruits observed in this study. In this way, Cu NPs could offer an alternative method to improve fruit condition in post-harvest.

This research also demonstrated that Cu NPs in Cs-PVA hydrogels can increase the lycopene content and total antioxidant capacity in tomato fruits. It has previously been reported that NPs produce oxidative stress in plants, activating the antioxidant defense system to fight against the reactive oxygen species (ROS) (Rico et al., 2015). Juárez-Maldonado et al. (2016) have demonstrated that the catalase activity was more than five times higher than the control in tomato plants treated with Cu NPs + chitosan. Also Pinedo-Guerrero et al. (2017) reported that application of Cu NPs + chitosan increased 51% more the concentration of capsaicin in jalapeño pepper. Corral-Diaz et al. (2014) report that CeO_2 NPs applied to the soil at a concentration of 250 mg kg^{-1} resulted in the highest values of total antioxidants in the tuber, and leaves of *Raphanus sativus* L. Kim et al. (2012) showed that suspensions of CuO and ZnO NPs at concentrations of 10 to 1000 mg L^{-1} increased the activity of the antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and peroxidase in the roots of *Cucumis sativus*. Similarly, Trujillo-Reyes et al. (2014) showed that CuO NPs applied in the nutrient solution at a concentration of 10 mg L^{-1} increased the activity of CAT in the roots and leaves of lettuce. On the other hand, Pradhan et al. (2015) showed that Cu NPs encapsulated in PEG (0.05-1.0 mg L^{-1}) did not alter the activity of the enzymes POD, SOD, CAT and GR in the roots and leaves of *Vigna radiata*. Barrios et al. (2015) also reported that CeO_2 NPs coated with citric acid applied to the soil at concentrations of 62.5 to 500 mg kg^{-1} increased the activity of the enzyme CAT in tomato leaves. This result suggests that the application of Cu NPs can induce the formation of antioxidant compounds in the fruits, as shown in the results obtained here, and thus might be a strategy to improve the quality of nutraceuticals.

CONCLUSION

None of the concentrations of Cu NPs in Cs-PVA hydrogels evaluated in tomato plants in this study had

toxic effects. The concentration that produced the greatest increase in the growth and yield of tomato plants was 10 mg of Cu NPs, which increased the stem diameter, the fresh weight and dry weight of the root, and the number of flower clusters and yield. The Cu NPs also increased the content of soluble solids and titratable acidity in the fruit during the first 15 days after harvest, although none of the concentrations showed a clear difference. The highest estimated increase in lycopene content and total antioxidant capacity in the fruit was produced by the treatment containing 0.02 mg Cu NPs. The application of Chitosan-PVA-coated Cu NPs can be used as a tool to increase the nutraceutical properties of tomato fruits as well as the yield of this crop; however, further studies are needed to assess the toxicological profiles of Cu NPs in Cs-PVA hydrogels in other crops before commercial use.

Author contributions

A.J.M.: Design, formulation and supervision of experiment, and review of manuscript. H.H.H.: Collection of data, did field experiments, and writing of manuscript. A.B.M.: Design and formulation of experiment, and review of manuscript. H.O.O.: Synthesis of nanoparticles. A.D.H.F.: Analysis of tomato fruit quality.

REFERENCES

Adhikari, T., S. Kundu, A. K. Biswas, J. C. Tarafdar and A. S. Rao. 2012. Effect of copper oxide nano particle on seed germination of selected crops. J. Agric. Sci. Technol. A. 2: 815-823.

Al-Harbi, A., A. Hejazi and A. Al-Omran. 2016. Responses of grafted tomato (*Solanum lycopersicum* L.) To abiotic stresses in Saudi Arabia. Saudi J. Biol. Sci. In Press. DOI: 10.1016/j.sjbs.2016.01.005.

Anjum, N. A., V. Adam, R. Kizek, A. C. Duarte, E. Pereira, M. Iqbal and I. Ahmad. 2015. Nanoscale copper in the soil-plant system-toxicity and underlying potential mechanisms. Environ. Res. 138: 306-325.

AOAC. 2000. Official Methods of Analysis of AOAC International, Association of Official Analysis Chemists International, Methods No. 2-66. AOAC, Washington, DC.

Aruna, A., S. K. R. Namasivayam and E. A. Roy. 2015. Comparative evaluation of non-target toxic effect of free and polymer coated chemogenic metallic nanoparticles against Vigna mungo. Int. J. Pharm. Biol. Sci. 6(1): B461-B467.

Badawy, M. E. I. and E. I. Rabea. 2011. A biopolymer chitosan and its derivatives as promising antimicrobial agents against plant pathogens and their applications in crop protection. Int. J. Carbohydr. Chem. 1: 1-29.

Barrios, A. C., C. M. Rico, J. Trujillo-Reyes, I. A. Medina-Velo, J. R. Peralta-Videa and J. L. Gardea-Torresdey. 2015. Effects of uncoated and citric acid coated cerium oxide nanoparticles, bulk cerium oxide, cerium acetate, and citric acid on tomato plants. Sci. Total Environ. 563: 956-964.

Bautista-Baños, S., A. N. Hernández-Lauzardo, M. G. Velázquez Del Valle, M. Hernández-López, E. Ait Barka, E. Bosquez-Molina and C. L. Wilson. 2006. Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. Crop Prot. 25(2): 108-118.

Burman, U., M. Saini and P. Kumar. 2013. Effect of zinc oxide nanoparticles on growth and antioxidant system of chickpea seedlings. Toxicol. Environ. Chem. 95(4): 605-612.

Corral-Diaz, B., J. R. Peralta-Videa, E. Alvarez-Parrilla, J. Rodrigo-García, M. I. Morales, P. Osuna-Avila, G. Niu, J. A. Hernandez Viezcas and J. L. Gardea-Torresdey. 2014. Cerium oxide nanoparticles alter the antioxidant capacity but do not impact tuber ionome in *Raphanus sativus* (L). Plant Physiol. Biochem. 84: 277-285.

Dar, M. A., J. A. Wani, S. K. Raina, M. Y. Bhat and M. A. Malik. 2015. Relationship of leaf nutrient content with fruit yield and quality of pear. J. Environ. Biol. 36(3): 649-653.

Fish, W. W., P. Perkins-Veazie and J. K. Collins. 2002. A quantitative assay for lycopene that utilizes reduced volumes of organic solvents. J. Food Compost. Anal. 15(3): 309-317.

Hanif, H. U., M. Arshad, M. A. Ali, N. Ahmed and A. Qazi. 2015. Phyto-availability of phosphorus to *Lactuca sativa* in response to soil applied TiO_2 nanoparticles. Pak. J. Agric. Sci. 52(1): 177-182.

Hong, J., L. Wang, Y. Sun, L. Zhao, G. Niu, W. Tan, C. M. Rico, J. R. Peralta-Videa and J. L. Gardea-Torresdey. 2015. Foliar applied nanoscale and microscale CeO_2 and CuO alter cucumber (*Cucumis sativus*) fruit quality. Sci. Total Environ. 563: 904-911.

Juárez-Maldonado, A., H. Ortega-Ortiz, F. Pérez-Labrada, G. Cadenas-Pliego and A. Benavidez-Mendoza. 2016. Cu nanoparticles absorbed on chitosan hydrogels positively alter morphological production and quality characteristics of tomato. J. Appl. Bot. Food Qual. 89: 183-189.

Kanatt, S. R., M. S. Rao, S. P. Chawla and A. Sharma. 2012. Active chitosan-polyvinyl alcohol films with natural extracts. Food Hydrocoll. 29(2): 290-297.

Kashyap, P. L., X. Xiang and P. Heiden. 2015. Chitosan nanoparticle based delivery systems for sustainable agriculture. Int. J. Biol. Macromol. 77: 36-51.

Kim, J. A., C. Åberg, A. Salvati and K. A. Dawson. 2012. Role of cell cycle on the cellular uptake and dilution of nanoparticles in a cell population. Nat. Nanotech. 7(1): 62-68.

Landa, P., R. Vankova, J. Andrlova, J. Hodek, P. Marsik, H. Storchova, J. C. White and T. Vanek. 2012. Nanoparticle-specific changes in *Arabidopsis thaliana* gene expression after exposure to ZnO, TiO_2, and fullerene soot. J. Hazard. Mater. 241: 55-62.

Le, V., Y. Rui, X. Gui, X. Li, S. Liu and Y. Han. 2014. Uptake, transport, distribution and bio-effects of SiO_2 nanoparticles in Bt-transgenic cotton. J. Nanobiotechnol. 12(50): 1-15.

Lee, W. M., Y. J. An, H. Yoon and H. S. Kweon. 2008. Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean (*Phaseolus radiatus*) and wheat (*Triticum aestivum*): Plant agar test for water-insoluble nanoparticles. Environ. Toxicol. Chem. 27(9): 1915-1921.

Liptay, A., C. A. Jaworski and S. C. Phatak. 1981. Effect of tomato transplant stem diameter and ethephon treatment on tomato yield, fruit size and number. Can. J. Plant Sci. 61(2): 413-415.

Liu, R. and R. Lal. 2015. Potentials of engineered nanoparticles as fertilizers for increasing agronomic productions. Sci. Total Environ. 514: 131-139.

Lobit, P., M. Génard, B. H. Wu, P. Soing and R. Habib. 2003. Modelling citrate metabolism in fruits: Responses to growth and temperature. J. Exp. Bot. 54(392): 2489-2501.

Malerba, M. and R. Cerana. 2015. Reactive oxygen and nitrogen species in defense/stress responses activated by chitosan in sycamore cultured cells. Int. J. Mol. Sci. 16(2): 3019-3034.

Musante, C. and J. C. White. 2012. Toxicity of silver and copper to Cucurbita pepo: Differential effects of nano and bulk-size particles. Environ. Toxicol. 27(9): 510-517.

Mustafa, M. A., A. Ali, S. Manickam and Y. Siddiqui. 2014. Ultrasound-assisted chitosan-surfactant nanostructure assemblies: Towards maintaining postharvest quality of tomatoes. Food Bioprocess Technol. 7(7): 2102-2111.

Naddeo, J. J., M. Ratti, S. M. O. Malley, J. C. Griepenburg, D. M. Bubb and E. A. Klein. 2015. Antibacterial properties of nanoparticles : A comparative review of chemically synthesized and laser-generated particles. Adv. Sci. Eng. Med. 7(12): 1044-1057.

Narayanan, A., P. Sharma and B. M. Moudgil. 2012. Applications of engineered particulate systems in agriculture and food industry. KONA Powder Part. J. 30(30): 221-235.

Peralta-Videa, J. R., L. Zhao, M. L. Lopez-Moreno, G. de la Rosa, J. Hong and J. L. Gardea-Torresdey. 2011. Nanomaterials and the environment: A review for the biennium 2008-2010. J. Hazard. Mater. 186(1): 1-15.

Pérez-de-Luque, A. 2017. Interaction of nanomaterials with plants: What do we need for real applications in agriculture? Front. Environ. Sci. 5: 1-7.

Pinedo-Guerrero, Z.H., A.D. Hernández-Fuentes, H. Ortega-Ortíz, A. Benavides-Mendoza, G. Cadenas-Pliego and A. Juárez-Maldonado. 2017. Cu nanoparticles in hydrogels of chitosan-PVA affects the characteristics of post-harvest and bioactive compounds of jalapeño pepper. Molecules. 22(926): 1-14.

Pradhan, S., P. Patra, S. Mitra, K. K. Dey, S. Basu, S. Chandra, P. Palit and A. Goswami. 2015. Copper nanoparticle (CuNP) nanochain arrays with a reduced toxicity response: A biophysical and biochemical outlook on *Vigna radiata*. J. Agric. Food Chem. 63(10): 2606-2617.

Ravi-Kumar, M. N. 2000. A review of chitin and chitosan applications. React. Funct. Polym. 46(1): 1-27.

Rico, C. M., S. C. Lee, R. Rubenecia, A. Mukherjee, J. Hong, J. R. Peralta-Videa and J. L. Gardea-Torresdey. 2014. Cerium oxide nanoparticles impact yield and modify nutritional parameters in wheat (*Triticum aestivum* L.). J. Agric. Food Chem. 62(40): 9669-9675.

Rico, C. M., J. R. Peralta-Videa and J. L. Gardea-Torresdey. 2015. Chemistry, biochemistry of nanoparticles, and their role in antioxidant defense system in plants. In: Siddiqui, M. H., M. H. Al-Whaibi and F. Mohammad, (Ed.), Nanotechnology and Plant Sciences, Springer International Publishing, Cham, Switzerland, pp. 1-17.

Rinaudo, M. 2006. Chitin and chitosan: Properties and applications. Prog. Polym. Sci. 31(7): 603-632.

Rizwan, M., S. Ali, Q. M. Farooq, O. Y. Sik, M. Adrees, M. Ibrahim, M. Zia-ur-Rehman, M. Farid and F. Abbas. 2017. Effect of metal and metal oxide nanoparticles on growth and physiology of globally important food crops: A critical review. J. Hazard. Mater. 322: 2-16.

Ruttkay-Nedecky, B., O. Krystofova and V. Adam. 2017. Nanoparticles based on essential metals and their phytotoxicity. J. Nanobiotechnol. 15(1): 33.

Saharan, V., G. Sharma, M. Yadav, M. K. Choudhary, S. S. Sharma, A. Pal, R. Raliya and P. Biswas. 2015. Synthesis and *in vitro* antifungal efficacy of Cu-chitosan nanoparticles against pathogenic fungi of tomato. Int. J. Biol. Macromol. 75: 346-353.

Servin, A., W. Elmer, A. Mukherjee, R. de la Torre-Roche, H. Hamdi, J. C. White, P. Bindraban and C. Dimkpa. 2015. A review of the use of engineered nanomaterials to suppress plant disease and enhance crop yield. J. Nanopart. Res. 17(2): 1-21.

Shah, V. and I. Belozerova. 2009. Influence of metal nanoparticles on the soil microbial community and germination of lettuce seeds. Water Air Soil Pollut. 197(1-4): 143-148.

Shi, J., C. Peng, Y. Yang, J. Yang, H. Zhang, X. Yuan, Y. Chen and T. Hu. 2014. Phytotoxicity and accumulation of copper oxide nanoparticles to the Cu-tolerant plant *Elsholtzia splendens*. Nanotoxicology. 8(2): 179-188.

Shukla, S. K., A. K. Mishra, O. Arotiba and B. B. Mamba. 2013. Chitosan-based nanomaterials: A state-of-the-art review. Int. J. Biol. Macromol. 59: 46-58.

Somasundaran, P., X. Fang, S. Ponnurangam and B. Li. 2010. Nanoparticles: Characteristics, mechanisms and modulation of biotoxicity. KONA Powder Part. J. 28(28): 38-49.

Steiner, A. A. 1961. A universal method for preparing nutrient solutions of a certain desired composition. Plant Soil. 15(2): 134-154.

Tripathi, S., G. K. Mehrotra and P. K. Dutta. 2009. Physicochemical and bioactivity of cross-linked chitosan-PVA film for food packaging applications. Int. J. Biol. Macromol. 45(4): 372-376.

Trujillo-Reyes, J., S. Majumdar, C. E. Botez, J. R. Peralta-Videa and J. L. Gardea-Torresdey. 2014. Exposure studies of core-shell Fe/Fe_3O_4 and Cu/CuO NPs to lettuce (*Lactuca sativa*) plants: Are they a potential physiological and nutritional hazard? J. Hazard. Mater. 267: 255-263.

USDA. 1997. United States Department of Agriculture. Agricultural Marketing Service. United States Standards for Grades of Fresh Tomatoes. Available from: http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5050331.

Valero, D. and M. Serrano. 2010. Postharvest Biology and Technology for Preserving Fruit Quality, CRC Press, Boca Raton, FL.

Wang, T., M. Turhan and S. Gunasekaran. 2004. Selected properties of pH-sensitive, biodegradable chitosan-poly(vinyl alcohol) hydrogel. Polym. Int. 53(7): 911-918.

Wang, Q., X. Ma, W. Zhang, H. Pei and Y. Chen. 2012. The impact of cerium oxide nanoparticles on tomato (*Solanum lycopersicum* L.) And its implications for food safety. Metallomics. 4(10): 1105-1112.

Wang, P., E. Lombi, F. Zhao and P. M. Kopittke. 2016. Nanotechnology: A new opportunity in plant sciences. Trends Plant Sci. 21: 699-712.

Yamada, H., S. Takimoto, D. Toshinari, K. Kataoka and T. Habu. 2015. Effect of seawater irrigation on water relations and fruit quality in potted citrus trees. Hortic. J. 84(3): 195-201.

Zhang, P., Y. Ma and Z. Zhang. 2015. Interactions between engineered nanomaterials and plants: Phytotoxicity, uptake, translocation, and biotransformation. In: Siddiqui, M. H., M. H. Al-Whaibi and F. Mohammad (Eds.), Nanotechnology and Plant Sciences, Springer International Publishing, Cham, Switzerland, pp. 77-99.

Zhao, L., J. R. Peralta-Videa, C. M. Rico, J. A. Hernandez-Viezcas, Y. Sun, G. Niu, A. Servin, J. E. Nunez, M. Duarte-Gardea and J. L. Gardea-Torresdey. 2014. CeO_2 and ZnO nanoparticles change the nutritional qualities of cucumber (*Cucumis sativus*). J. Agric. Food Chem. 62(13): 2752-2759.

Zhao, L., Y. Sun, J. A. Hernandez-Viezcas, A. Servin, J. Hong, G. Niu, J. R. Peralta-Videa, M. Duarte-Gardea and J. L. Gardea-Torresdey. 2013. Influence of CeO_2 and ZnO nanoparticles on cucumber physiological markers and bioaccumulation of Ce and Zn: A life cycle study. J. Agric. Food Chem. 61(49): 11945-11951.

Response in Growth and Antioxidant Capacity of Tomato under Saline Stress and Application of Cu Nanoparticles in Chitosan-PVA Hydrogels

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Abstract Chitosan is a natural polymer that has been used for the encapsulation of nanoparticles in order to obtain a controlled release. In this way, the effect of Cu nanoparticles (Cu NPs) absorbed on chitosan-PVA hydrogels on growth, antioxidant capacity, mineral content, and saline stress in tomato plants was evaluated. The use of Cu NPs increased stem diameter (7%), fresh and dry root weight (91 and 43%), and number of fruits (20%) per plant, compared to control. It also increased the activity of CAT, APX, GPX, SOD, PAL (111, 57, 39, 92 and 62%, respectively) and the amount of GSH (56%) in tomato leaves. The fruits presented higher content of N, vitamin C, and lycopene (15, 41, and 77%, respectively). Saline stress drastically affected plant growth and yield compared to control. However, Cu NPs and chitosan decreased the accumulation of Na in leaves (39 and 52%, respectively) of salinized plants. In addition, chitosan increased the content of total chlorophylls (65%), carotenoids (79%), and total phenols (148%) in leaves of plants under salt stress. This work suggests that Cu NPs promote vegetative and reproductive growth of plants, activate the mechanism of antioxidant defense, increase nutraceutical compounds in fruits, in addition chitosan and copper nanoparticles could induce mechanisms of tolerance to salinity.

Keywords Cu nanoparticles, Chitosan, Antioxidant, Lycopene, Saline stress

Introduction

The use of nanotechnology is increasing in the medical sciences, electronics, pharmaceuticals, energy production, and even as food additives. In agriculture, the main nanomaterials (NM) being studied are made of metals and metal oxides (Rizwan et al. 2017). In this area most of the research is focused on studying the effects on growth as well as the physiological and biochemical changes of plants (Zuverza-Mena et al. 2016; Rizwan et al. 2017). In general, it is reported that most of the time, concentrations of nanomaterials less than 100 ppm applied via soil, foliar, or in seeds, especially 10 to 40 mg kg^{-1}, have been shown to have beneficial effects on plant growth, while higher concentrations have inhibitory effects. However, these effects depend on factors such as the properties of NM, plant species, soil dynamics, and soil microbial community, among others (Reddy et al. 2016).

Among the NM based on metals are copper nanoparticles (Cu NPs), which have been shown to improve plant growth, increase chlorophyll concentration, and increase the concentration of phenolic compounds and defensive

enzymes (CAT, SOD, PAL, among others) when applied at low concentrations (0.05-1.0 mg L^{-1}) in seeds or soil (Pradhan et al. 2015; Juarez-Maldonado et al. 2016; Saharan and Pal 2016; Pinedo-Guerrero et al. 2017). However, one of the disadvantages of NPs in general is their insolubility in water, which limits the evaluation of their toxicity in experiments, therefore, new alternatives are being sought such as the encapsulation of the nanoparticles in order to achieve a controlled release. Regarding to this, it has been reported that encapsulated Cu NPs are less toxic than free Cu NPs and even than copper sulfate (Aruna et al. 2015; Pradhan et al. 2015; Saharan et al. 2015). A polymer that is being used for the encapsulation of NPs is chitosan (Cs) due to its biocompatibility, biodegradability, non-toxicity and adsorption abilities (Kashyap et al. 2015). Chitosan is a natural polymer derived from the deacetylation of chitin, with great capacity for reticulation and cation exchange in acid solutions and has great affinity with metallic ions (Ravi Kumar 2000). This polymer has been extensively investigated in agriculture and has been shown to stimulate growth and activates the defense mechanisms in plants (Rinaudo 2006). Some investigations based on Cu NPs encapsulated in chitosan demonstrate that they can increase the vigor of plants and the amount of lycopene in tomato fruit, induce defensive enzymes in plants such as catalase, peroxidase, superoxide dismutase, and phenylalanine ammonium lyase, as well as acting as an antifungal agent against various phytopathogenic fungi (Saharan et al. 2015; Juarez-Maldonado et al. 2016; Saharan and Pal 2016).

On the other hand, it is known that saline stress affects a wide variety of crops worldwide, it is reported that over 6% of the world's land is affected by salinity (Parihar et al. 2015). Some of the effects of saline stress on plants is that it reduces the rate of expansion of the foliar surface, the water potential and osmotic tend to be more negative, the thickness of the epidermis and the mesophyll increases, the levels of Na and Cl increases, and decreases Ca, K, and Mg levels, and induces the activity of certain antioxidant enzymes such as catalase, peroxidase, glutathione reductase, and superoxide dismutase (Parida and Das 2005). There are few studies on the effect of NM based on metals in plants subjected to saline stress. Some of them reported that NPs of ZnO in concentrations of 15 to 30 mg L^{-1} have positive responses in the metabolism of tomato plants under salt stress (Alharby et al. 2016). Likewise, it has been shown that concentrations of 0.05 to 2.5 mg L^{-1} of Ag NPs could improve the tolerance of tomato plants to salinity (Almutairi 2016). In canola, it was reported that in concentrations of 200 and 1000 mg kg^{-1} of CeO_2 NPs improved the growth and physiology of plants under saline stress, but did not completely alleviate it (Rossi et al. 2016). Considering the importance of research about NM in different plants, the following study was carried out to evaluate the effect of Cu NPs in chitosan-PVA hydrogels on growth, antioxidant capacity, and mineral content in tomato plants under conditions of saline stress.

Materials and Methods

Materials

Cu NPs (spherical morphology, 99.8% purity and 25 nm average diameter) were purchased from SkySpring Nanomaterials, Inc. Texas, USA. Chitosan (Mv = 200,000 g/mol) was obtained from Chemical Marine Hamburg, Germany. Polyvinyl alcohol (Mw = 30,000-50,000; hydrolysis 98%), L-ascorbic acid, polyvinylpyrrolidone, bovine serum albumin, Bradford reagent, standard glutathione, Folin-Ciocalteu, 5,5 dithio-bis-2-nitro benzoic acid and L-

Phenylalanine were obtained from Sigma Aldrich, St. Louis, USA. The amount of SOD (U/ml) was determined with a commercial Cayman Chemicals kit. ABTS or 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) and potassium persulfate were obtained from Sigma Aldrich, St. Louis, USA. The DPPH (2,2-Diphenyl-1-Picrylhydrazyl) used was from Cayman Chemicals. Tomato seeds (hybrid var. "Huno F1" saladette type and indeterminate growth) were purchased from Harris Moran Seed Company.

Synthesis of chitosan-polyvinyl alcohol hydrogels (Cs-PVA) and absorption of Cu NPs

It was carried out according to the methodology of Pinedo-Guerrero et al. (2017). It was carried out in the pilot plant of the Applied Chemistry Research Center (CIQA) according to the following methodology: 250 mL of 2% chitosan and 250 mL of 4% polyvinyl alcohol (PVA) were first dissolved by mixing for two hours at 300 rpm and 60 °C to obtain a hydrogel in a 1:2 ratio (Cs: PVA); subsequently 2.27 mL of crosslinker (50% glutaraldehyde) was added at 450 rpm for 5 minutes at 25 °C, then 100 mL of 6% NaOH was added at 300 rpm, and 25 °C for one hour. The Cs-PVA hydrogels were then washed and purified with distilled water and ethanol, and finally dried and weighed. Thereafter, 100 mg of the Cu NPs were dispersed in a 1% Tween solution by ultrasound for 5 minutes (50 watt power and 70% frequency), then a dilution was prepared to obtain a concentration of 10 mg, which were subsequently absorbed in 1 gram of Cs-PVA hydrogel and dried at a temperature of 60 °C.

Experimental development and growth conditions

Tomato plants (*Solanum lycopersicum* L.) were established in February 2016, in a multitunnel greenhouse with polyethylene cover of the Department of Horticulture of the Autonomous University of Agraria Antonio Narro. The average temperature was 21 °C, active photosynthetic radiation of 565 µmol m^2 s^{-1} and average relative humidity of 51%. The planting density was three plants per square meter. The substrate used was a mixture of peat moss and perlite (50:50 v/v) placed in bags of black polyethylene of 12 L capacity. For the application of the treatments, 1 g of Cs-PVA hydrogel was distributed in the lower, middle and upper parts of the pot before the transplant to obtain a better dispersion of the Cu NPs in the substrate and in the root area of the plant. The experiment was carried out in two ways: one evaluated the effect of 10 mg of Cu NPs absorbed on Cs-PVA hydrogels, a control, and a Cs-PVA hydrogel treatment (1 g) to evaluate the effect of Cs alone; and in the other, the same conditions were evaluated, as well as 100 mM NaCl in the nutrient solution applied from the third week after transplantation. A directed irrigation system was installed and the nutrient solution Steiner (Steiner 1961) was used with the following micronutrients: Fe EDTA = 3.75 ppm; Mn EDTA = 1.85 ppm; B = 0.35 ppm; Zn EDTA = 0.30 ppm; Cu EDTA = 0.15 ppm and Mo = 0.10 ppm. The nutrient solution was applied in different concentrations to provide the necessary nutrients to tomato plants: the first two weeks after the transplant was applied concentrated at 25%, the third and fourth week at 50%, the fifth week at 75% and the rest of the crop cycle to 100%.

Tomato growth and production data

Sampling was performed 75 days after transplantation and plant height was measured with a flexometer, stem diameter measured with a digital caliper, counted the number of leaves, the number of clusters with flowers and

berries and the number of fruits per plant. At 170 days after transplantation the plants were cut on the surface of the substrate and the fresh weight of root, stem, and leaves were measured. Fruit yield per plant was also determined. The dry weight of root, stem, and leaves was obtained after drying in a drying oven (Drying Oven model DHG9240A) for 72 h at a constant temperature of 80 °C.

Determination of chlorophyll and carotenoid content

It was performed according to Pocock et al. (2004) the extraction methodology with some modifications. The tomato leaves of each treatment were cryogenized with liquid nitrogen, then 0.1 g was taken and 1 mL of 100% acetone was added, then centrifuged at 3024 g for 5 min. Subsequently, 0.05 mL of the supernatant was taken and 0.95 mL of 80% acetone (2.5 mM sodium phosphate buffer pH 7.8) was added. A blank composed of 80% acetone was used. The content of chlorophyll a (664 nm), b (647 nm) and total (Porra 2002) and total carotenoids (470 nm) (Wellburn 1994) were measured by spectrophotometry and expressed in mg g^{-1} fresh weight.

Extraction of biomolecules

At 60 days after transplantation, random plants were selected and the third fully expanded young leaf was taken, the fruits were selected at random after a harvest, verifying that they were not physically damaged, were uniform, and in maturity 6 (light red) according to the visual color pattern used by the United States Department of Agriculture (USDA 1991). Samples were stored at -80 ° C until use. For the enzymatic and non-enzymatic determination, 200 mg of lyophilized fruits and cryogenized leaves with liquid nitrogen of each treatment plus 20 mg of polyvinylpyrrolidone were weighed. Then 1.5 ml of phosphate buffer pH 7-7.2 (0.1 M) was added, then subjected to micro-centrifugation at 12000 rpm for 10 min at 4 °C. The supernatant was filtered with a nylon membrane (Ramos et al. 2010). Dilutions of the extract were prepared in a ratio of 1:20 with phosphate buffer.

Proteins

Protein quantification was determined by the Bradford (1976) method. Taking 20 µl of the extract or standard and adding 980 µL of the Bradford reagent. After 5 minutes the absorbance was read at 595 nm on a UV-Vis spectrophotometer (Thermo Scientific Model G10S). A calibration curve was made with standard bovine serum albumin (0.02-0.1 mg mL^{-1}).

Catalase (EQ 1.11.1.6)

Quantified according to Beers and Sizer (1952); 70 µL of the extract was taken and 920 µl of phosphate buffer was added, then 10 µL of H_2O_2 (2 M) was added to initiate the reaction. A blank was used for each sample: 930 µL phosphate buffer and 70 µL extract. The initial absorbance was recorded, then every 20 seconds for 3 minutes. The decomposition of H_2O_2 was followed by the decrease in absorbance at 270 nm in a UV-Vis spectrophotometer. A calibration curve was made with H_2O_2 (20-100 mM) and the results were expressed as mM H_2O_2 min^{-1} per total protein (mg g^{-1}).

Ascorbate peroxidase (EQ. 1.11.1.1)

It was determined according to Nakano and Asada (1981). In 100 µL of the extract, 600 µL of phosphate buffer plus 100 µL of EDTA (1 mM), 100 µL of Ascorbate (5 mM) and 100 µL of H_2O_2 (1 mM) were added to initiate the reaction. A blank was used with 700 µL of phosphate buffer, 100 µL of EDTA (1 mM), 100 µL of Ascorbate (5 mM), and 100 µL of H_2O_2 (1 mM). Oxidation of ascorbate was estimated by the decrease in absorbance at 266 nm after 1 minute on a UV-Vis spectrophotometer. A calibration curve was prepared with ascorbate (10-100 µmol) and the results were expressed in µmol of ascorbate min^{-1} by total proteins (mg g^{-1}).

Superoxide dismutase (EQ 1.15.1.1)

For this analysis, to 10 µL of the extract or standard was added 200 µL of the radical detector (tetrazolium salt). To initiate the reaction, 20 µl of xanthine oxidase was added. It was then incubated for 30 min at room temperature and then the absorbance at 450 nm was read in a microplate reader ELISA (model LEX-808 IU). A calibration curve was prepared with standard SOD (0-0.05 U/mL) and the results were expressed as U/mg protein.

Glutathione peroxidase (EQ 1.11.1.9)

It was done according to the modified Flohé and Günzler (1984) technique, using H_2O_2 as a substrate. For the enzymatic reaction, 0.2 mL of the extract was placed in an eppendorf tube plus 0.4 mL of 0.1 mM reduced glutathione and 0.2 mL of 0.067 M Na_2HPO_4. For the non-enzymatic reaction, the previous reagents were used without the extract. These mixtures were preheated in a water bath at 25 °C for 5 minutes, then 0.2 mL of 1.3 mM H_2O_2 were added to initiate the catalytic reaction. It was placed to react for 10 min and stopped by the addition of 1 mL of 1% trichloroacetic acid. This reaction mixture was placed in an ice bath for 30 min. The mixture was then centrifuged at 3000 rpm for 10 min; 0.24 mL of the supernatant or standard was taken and 1.1 mL of 0.32 M Na_2HPO_4 and 0.16 mL of 1 mM of the dithio-5-dithio-2-nitro benzoic acid (DTNB) dye were added. A blank was used: 1.1 mL 0.32 M Na_2HPO_4 and 0.16 mL 1 mM DTNB and 0.24 mL phosphate buffer. Subsequently, the assay was read at an absorbance at 412 nm on a UV-Vis spectrophotometer (Xue et al. 2001). The enzymatic activity was calculated as a decrease of GSH within the reaction time as compared to the non-enzymatic reaction. A calibration curve with standard reduced glutathione (20-100 µM) was performed and the results expressed in µmol of glutathione per min^{-1} by total proteins (mg g^{-1}).

Phenylalanine ammonium lyase (EQ 4.3.1.5)

It was determined according to Sykłowska-Baranek et al. (2012) with some modifications; 0.1 mL of the enzymatic extract was taken and 0.9 mL of L-phenylalanine (6 mM) was added. After 30 min incubation at 40 °C the reaction was stopped with 0.25 mL 5 N HCl. The samples were placed in an ice bath and 5 mL of distilled water was added. The absorbance was determined at 290 nm on a UV-VIS spectrophotometer. A calibration curve was prepared with cinnamic acid (300-3000 µmol) and the results were expressed as the production of 1 mM cinnamic acid per min^{-1} by total proteins (mg g^{-1}).

Reduced Glutathione (GSH)

It was performed colorimetrically by reaction with DTNB. In an eppendorf tube, 0.48 mL of the extract was placed and 2.2 mL of 0.32 M Na_2HPO_4 plus 0.32 mL of the 1 mM DTNB dye was added. After completely mixing, the absorbance at 412 nm was read in a UV-Vis spectrophotometer (Xue et al. 2001), the data obtained were interpolated to a calibration curve previously standardized with GSH and the results expressed in µmol of GSH per mg^{-1} of total proteins.

ABTS (2,20-Azinobis-3-ethylbenzotiazoline-6-sulphonic acid)

The ABTS technique measures the hydrophilic compounds. It was performed according to the methodology of Miller et al. (1993). The ABTS cation was generated through the interaction of 19.2 mg of ABTS dissolved in 5 mL of HPLC grade H_2O and 88 µL of potassium persulfate 37.8 mg mL^{-1}. The cation was incubated in the dark and at room temperature for 16 h. The activated ABTS radical was diluted with ethanol to an absorbance of 0.7±0.02 at 734 nm using a UV-VIS spectrophotometer (Moo-Huchin et al. 2015). Subsequently, 5 µL of the extract or standard was taken and 395 µL of the diluted ABTS solution was added, after 6 min the absorbance was recorded. Ethanol was used as a blank. Two calibration curves were made: with trolox (0-1 mM), and standard ascorbic acid (0-0.25 mg mL^{-1}). The results were expressed as trolox equivalents in mM/100 g fresh weight (leaf) and dry (fruit) and ascorbic acid equivalents in mg 100 g^{-1} fresh weight (leaf) and dry (fruit) weight.

DPPH (2,2-Diphenyl-1-Picrylhydrazyl)

The DPPH technique measures the hydrophilic and lipophilic compounds. It was performed according to the Brand-Williams et al. (1995) methodology with some modifications. The stock solution was prepared by mixing 2.5 mg of the DPPH radical with 100 mL of methanol. The absorbance of the solution was adjusted to 0.7 ± 0.02 at 515 nm using a UV-VIS spectrophotometer. Next, 10 µL of extract or standard was taken and 390 µL of the diluted DPPH radical was added. Methanol was used as a blank. The decrease in absorbance at 515 nm was measured after 30 min. Two calibration curves were made: with trolox (0-1 mM), and standard ascorbic acid (0-0.12 mg mL^{-1}). The results were expressed as trolox equivalents in mM/100 g fresh weight (leaf) and dry (fruit) and ascorbic acid equivalents in mg 100 g^{-1} fresh weight (leaf) and dry (fruit) weight.

Total phenols

It was determined according to the methodology of Singleton et al. (1999) with some modifications. Taking 250 mg of cryogenized sheet with liquid nitrogen, 1 mL of 80% methanol was added, then centrifuged at 10,000 rpm for 15 min. The supernatant was recovered and with the pellet the same procedure was repeated with concentrated methanol. The supernatant was adjusted to 2 mL with concentrated methanol and placed in the dark. Subsequently, 200 µL of the extract plus 1.5 mL of distilled water were added, then 100 µL of Folin-Ciocalteu reagent was added, then 200 µL 20% NaCO3 was added and let stand for 30 minutes. Absorbance was read at 765 nm on a UV-VIS

spectrophotometer. A calibration curve was prepared with gallic acid (0.02-0.4 mg mL^{-1}) and the results were expressed as mg of gallic acid per g of fresh weight.

Lycopene

The content of lycopene was determined according to Fish et al. (2002); to 3 g pericarp of fresh fruit were added 3 mL of phosphate buffer solution (pH 7) and ground in a mortar, subsequently 2 mL of the sample are taken and added 4 mL of the hexane:acetone (3:2) mixture. Centrifuging for 10 min at 3000 rpm. Finally, the absorbance at 503 nm of the resulting supernatant corresponding to mg 100 g^{-1} fresh weight is determined.

Vitamin C

It was determined by titration method with 2,6 dichlorophenolindofenol (AOAC 2000); 10 g of fresh fruit were weighed and macerated in a mortar with 10 mL of 2% HCl, then filtered through a sterile absorbent gauze onto a 100 mL volumetric flask. A 10 mL aliquot was taken and titrated with 2,6-dichlorophenolindofenol until a persistent rosacea coloration was obtained. The results were expressed as mg 100 g^{-1} fresh weight.

Quality of fruit

Fruits were selected at random after a harvest, verifying that they were not physically damaged, were uniform, and in maturity 6 (light red) according to the visual color pattern used by the United States Department of Agriculture (USDA 1991). The potential of hydrogen (pH) was measured using a digital potentiometer (HANNA®), soluble solids (° Brix) with a digital refractometer PR-101ATAGO PALETTE and the titratable acidity percentage according to the AOAC (AOAC 2000) methodology expressing the data as a percentage of citric acid.

Mineral content

The contents of K, Ca, Mg, Na, Fe, Zn and Cu were measured. For digestion, 0.2 g of dry sample were weighed and 30 mL of concentrated HNO_3 was added to a 100 mL beaker. It was covered with a watch glass and heated on a grill until the disintegration of the organic matter (approximately 4 hours). The volume of HNO_3 was completed several times to avoid drying the sample. When the solution was completely clear (no residue) it was allowed to cool and then filtered on Whatman No. 42 filter paper and taken up to a volume of 50 ml with deionized water in a volumetric flask. Subsequently, dilutions were made in a 1:10 ratio. The concentrations of each of the elements were read in plasma emission spectrophotometer (ICP, Termo Jarrel Ash ASH model 7400). The total N content in leaves and fruits was determined by the micro kjeldahl method (Jones 1991); 0.05 g dry sample was weighed into a digestion flask, then 4 mL of digest mixture (25 g K_2SO_4, 10 g red mercury oxide, 1 L concentrated H_2SO_4 and 25 mL Cu_2SO_4) were weighed and digested (2 h aprox.) in a microdigestor. Subsequently, it was placed in the micro kjeldahl distiller, adding 50% NaOH, the distillation was recovered with 30 mL of 2.2% boric acid and 3-5 drops of bromocresol green/methyl red mixed indicator. The titration was carried out with 0.025 N H_2SO_4.

Analysis of data

The experimental design used was completely randomized with 16 replicates per treatment considering one plant as an experimental unit. For the variables of photosynthetic pigments, enzymatic activity, antioxidant capacity, fruit quality and mineral content, 5 replicates were used per treatment. The statistical language R CRAN was used, in which an analysis of variance and Fisher's LSD ($\alpha \leq 0.05$) test were performed for all variables.

Results and Discussion

Growth and development of the plant

The results on growth and development of tomato plants are presented in Table 1. In growth and development parameters evaluated in this study, it was shown that Cu NPs absorbed in Cs-PVA hydrogels have positive and significant effects on tomato plants. Cu NPs increased stem diameter (7%) and number of fruits per plant (20%) compared to control. In addition, Cs and Cu NPs increased root fresh weight (71 and 91%, respectively), root dry weight (35 and 43%, respectively), and stem-leaf dry weight (6%). It has been previously reported that Cu NPs encapsulated in Cs increase fresh and dry weight, as well as the number of clusters and fruits per tomato plant (Pradhan et al. 2015; Juarez-Maldonado et al. 2016). It was also demonstrated that the control presented higher height and number of leaves per plant (4 and 3%, respectively) compared to Cu NPs. Previously, Pinedo-Guerrero et al. (2017) reported that Cu NPs in Cs-PVA hydrogels decrease the height in pepper plants. Therefore, plants treated with Cu NPs in Cs-PVA hydrogels decrease plant height, this may be possible due to the generation of ROS. Regarding fruit yield per plant, there were only significant differences between treatments with saline stress compared to those without stress. As it was expected, the saline stress condition negatively affected the yield of the plants. This type of stress generally affects negatively by reducing crop growth and yield (Parida and Das 2005), as it demonstrated in this study. A positive effect of the saline stress condition was observed on the root biomass, as fresh and dry weight increased compared to the control. This is due to a decrease in leaf expansion which results in an inhibition of photosynthesis, as a consequence the stressed leaves contain a larger group of carbohydrates available for root growth (Maggio et al. 2007). In this study Cu NPs did not show a positive effect on the growth and development of plants under saline stress conditions. However, it has been reported that CeO_2 NPs increased total biomass in canola plants exposed to salinity, although they do not fully compensate for the negative effects of saline stress (Rossi et al. 2016).

Table 1 Effect of Cu NPs on Cs-PVA hydrogels and saline stress on the growth and development of tomato plants.

Treatment	Plant Height (cm)	Stem diameter (mm)	Number of leaves	Number of cluster	Number of fruits	Fresh weight Stem-Leaves (g)	Fresh weight Root (g)	Dry weight Stem-Leaves (g)	Dry weight Root (g)	Yield (g)
Control	207.69 a	14.44 b	26.56 a	6.19 ab	25.38 b	2582.01 a	74.45 c	322.76 b	14.58 d	5607.37 a
CsPVA	200.50 b	14.92 ab	26.69 a	6.19 ab	27.13 b	2591.89 a	126.97 ab	342.47 a	19.64 ab	5437.64 a
NCu	200.62 b	15.39 a	25.69 b	6.44 a	30.50 a	2550.90 a	141.93 a	342.48 a	20.83 a	5352.01 a
NaCl	147.56 cd	12.49 c	22.81 c	5.88 bc	25.19 b	1086.69 b	110.99 b	156.34 c	18.21 bc	887.73 b
Cs-NaCl	150.94 c	12.67 c	23.19 c	5.75 c	24.56 b	1148.06 b	131.21 ab	153.01 c	16.62 cd	879.28 b
nCu-NaCl	143.88 d	12.52 c	23.06 c	5.69 c	25.50 b	1156.64 b	128.11 ab	164.81 c	17.61 bc	973.22 b

CsPVA: 1 g chitosan-PVA hydrogel. nCu: 10 mg Cu NPs + CsPVA. NaCl: Control + 100 mM NaCl. Cs-NaCl: Cs-PVA + 100 mM NaCl. nCu-NaCl: nCu + 100 mM NaCl. Means with the same letter within the same column of each treatment are not different ($\alpha \leq 0.05$) according to Fisher LSD.

Changes in leaf pigments

Table 2 shows the results of the changes in the pigments of the leaves treated with Cu NPs and saline stress. The results show that saline stress increased the content of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids compared to control. These increases could be used as a biochemical indicator for tolerance to salinity (Stefanov et al. 2016). Cs increased chlorophyll a and b content (60 and 79%, respectively), total chlorophyll (65%) and carotenoids (79%) in plants under salinity conditions compared to control. Ma et al. (2012) reported that Cs increased the chlorophyll content in wheat plants under conditions of saline stress. Thus, Cs could help minimize the effect of saline stress on tomato plants. In addition, the chlorophyll a/b ratio was significantly higher in the control compared to the rest of the treatments. The increase in the Chl a/b ratio is associated with a change in the pigment composition of the photosynthetic apparatus towards a solar-type chloroplast which has less chlorophyll light-harvesting proteins (LHCPs) (Camejo et al. 2005). As a result, treatments with Cu NPs and Cs with and without saline stress increased the amount of LHCPs in the leaves as a protection mechanism.

Table 2 Effect of Cu NPs in Cs-PVA hydrogels and saline stress on tomato leaf pigments.

Treatment	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Chlorophyll a/Chlorophyll b	Carotenoids (mg g^{-1} FW)
Control	1.71 cd	0.52 c	2.23 c	3.31 a	0.29 d
CsPVA	1.71 cd	0.54 c	2.25 c	3.17 b	0.28 d
nCu	1.55 d	0.49 c	2.05 c	3.16 b	0.26 d
NaCl	2.38 ab	0.76 b	3.14 b	3.12 b	0.45 b
Cs-NaCl	2.74 a	0.93 a	3.67 a	2.94 c	0.52 a
nCu-NaCl	2.06 bc	0.67 b	2.74 b	3.05 bc	0.38 c

CsPVA: 1 g chitosan-PVA hydrogel. nCu: 10 mg Cu NPs + CsPVA. NaCl: Control + 100 mM NaCl. Cs-NaCl: Cs-PVA + 100 mM NaCl. nCu-NaCl: nCu + 100 mM NaCl. Means with the same letter within the same column of each treatment are not different ($\alpha \leq 0.05$) according to Fisher LSD.

Changes in enzyme activity

The results of the enzymatic activity in leaves and fruits treated with Cu NPs in Cs-PVA and saline stress hydrogels are presented in Table 3. The activity of the enzymes catalase, ascorbate peroxidase, glutathione peroxidase, superoxide dismutase, and phenylalanine ammonium lyase was increased in tomato leaves with Cu NPs (111, 57, 39, 92, and 62%, respectively) compared to the control. Thus, it is shown that Cu NPs produce positive stress in the plants by activating their defense mechanism. In an previous study it was shown that Cu NPs in Cs-PVA hydrogels increase CAT activity in tomato leaves, whereas APX, GPX, and SOD did not show significant differences (Juarez-Maldonado et al. 2016). This may be due to the fact that a lower concentration of Cu NPs was applied. In mung bean leaves, it was reported that Cu NPs encapsulated in PEG increased the activity of CAT and SOD enzymes (Pradhan et al. 2015). In another study evaluating CeO_2 NPs coated with citric acid, the activity of the CAT enzyme was increased in tomato leaves (Barrios et al. 2015). On the other hand, phenylalanine ammonium lyase is an enzyme of great importance as it is key in the synthesis of metabolites of defense against pathogens. Some authors stated that Cu NP coated with Cs help protect plants from pathogens such as *Alternaria* and *Fusarium* (Saharan et al. 2015), thus, Cu NPs in Cs-PVA hydrogels could function as inducers of systemic resistance in plants. On the other hand, in fruits, Cu NPs increased catalase activity (122%), while Cs increased glutathione peroxidase activity (32%) when

there was no saline stress. In a previous study the NPs of Cu and Cs did not increase the activity of these enzymes, this could be due to that the concentration used was much lower (Juarez-Maldonado et al. 2016). In this study it was also demonstrated that saline stress showed higher activity of APX and SOD (56 and 65%, respectively) in tomato leaves, whereas in fruit the catalase and SOD activity increased (276 and 297%, respectively) compared to the control. This is because saline stress increases ROS production and therefore the plant activates its antioxidant defense mechanism (Blokhina et al. 2003). Regarding this, Cu NPs can function as elicitors in the activation of defensive enzymes in plants, preparing them to cope with some type of biotic or abiotic stress.

Table 3 Enzymatic activity in fruits and tomato leaves treated with Cu NPs in Cs-PVA hydrogels and saline stress.

Treatment	Ascorbate Peroxidase (µmol ascorbate min^{-1} mg^{-1} proteins)	Catalase (mM H_2O_2 min^{-1} mg^{-1} proteins)	Superoxide Dismutase (U/mg^{-1} proteins)	Glutathione Peroxidase (µmol glutathione min^{-1} mg^{-1} proteins)	Phenilalanine Amonnium Liase (mM cinamic acid min^{-1} mg^{-1} proteins)
			Leaves		
Control	215.31 b	12.93 b	13.62 c	17.40 bc	2.82 b
CsPVA	203.74 b	13.36 b	19.98 bc	16.46 c	2.67 b
Ncu	337.62 a	27.28 a	26.13 ab	24.23 a	4.57 a
NaCl	335.18 a	19.97 ab	22.51 ab	19.07 bc	3.60 ab
Cs-NaCl	354.28 a	18.77 ab	29.60 a	21.38 ab	4.20 a
nCu-NaCl	316.18 a	13.84 b	27.04 ab	20.64 abc	3.58 ab
			Fruits		
Control	445.65 a	26.27 c	12.35 d	26.49 b	-
CsPVA	430.50 a	32.77 c	7.75 d	34.98 a	-
Ncu	476.83 a	58.38 b	17.32 cd	33.11 ab	-
NaCl	566.63 a	98.83 a	48.98 a	33.12 ab	-
Cs-NaCl	454.20 a	42.47 bc	29.17 bc	27.95 ab	-
nCu-NaCl	485.24 a	40.59 bc	35.66 b	28.55 ab	-

CsPVA: 1 g chitosan-PVA hydrogel. nCu: 10 mg Cu NPs + CsPVA. NaCl: Control + 100 mM NaCl. Cs-NaCl: Cs-PVA + 100 mM NaCl. nCu-NaCl: nCu + 100 mM NaCl. Means with the same letter within the same column of each treatment are not different ($\alpha \leq 0.05$) according to Fisher LSD.

Changes in antioxidant capacity

The results of antioxidant capacity in tomato leaves treated with Cu NPs in Cs-PVA and saline stress hydrogels are presented in Table 4. Cu NPs presented higher GSH (56%) in the leaves compared to the control, this treatment was also higher than the rest. GSH is critical in the glutathione-ascorbate cycle and is crucial for biotic and abiotic stress as it induces defense responses against pathogens such as *Pseudomonas syringae* and *Phytophthora brassicae* (Parisy et al. 2007). Thus, Cu NPs could be used as elicitor against pathogens. On the other hand, the Cs and Cu NPs presented higher total phenol content (148 and 85%, respectively) in leaves of plants subjected to saline stress in comparison to the control. Total phenols are antioxidants that trigger a series of secondary metabolites synthesized through the pathway of the shikimic acid or malonic acid, exerting cellular signaling functions under conditions of abiotic stress (Michalak 2006). Thus, Cs and Cu NPs increase phenolic compounds in plants inducing tolerance to salt stress. Regarding the changes in antioxidant capacity equivalent to trolox (CAET) and vitamin C (CAEVC) in tomato leaves, through the DPPH technique, the Cu NPs and the NaCl increased values compared to the Cs, however with the control no significant differences were shown. Through ABTS technique, Cu and Cs NPs decreased CAET (56 and 51%, respectively) and CAEVC (47 and 43%, respectively) in plants subjected to saline stress

compared to control (Table 4). The ABTS technique quantifies the hydrophilic compounds (Rufino et al. 2010), so it can be said that the NPs of Cu and Cs affected this type of compounds.

Table 4 Antioxidant capacity in tomato leaves treated with Cu NPs in Cs-PVA hydrogels and saline stress

Treatment	Antioxidant Capacity Equivalent to Trolox (mM/ 100 g FW)		Antioxidant Capacity Equivalent to Vitamin C (mg/100 g FW)		Reduced Glutathione (U/ mg^{-1} proteínas)	Total Phenols (mg Gallic Acid/g FW)
	DPPH	ABTS	DPPH	ABTS		
Control	80.02 ab	47.89 a	10.10 ab	15.10 a	282.92 b	1.72 c
CsPVA	58.93 b	56.19 a	7.20 b	17.31 a	286.95 b	2.76 bc
nCu	89.38 a	51.97 a	11.39 a	16.19 a	447.58 a	2.64 bc
NaCl	91.41 a	49.51 a	11.67 a	15.53 a	221.30 b	2.77 bc
Cs-NaCl	91.74 a	23.48 b	11.71 a	8.62 b	284.00 b	4.27 a
nCu-NaCl	99.52 a	21.16 b	12.78 a	8.011 b	280.61 b	3.18 ab

CsPVA: 1 g chitosan-PVA hydrogel. nCu: 10 mg Cu NPs + CsPVA. NaCl: Control + 100 mM NaCl. Cs-NaCl: Cs-PVA + 100 mM NaCl. nCu-NaCl: nCu + 100 mM NaCl. Means with the same letter within the same column of each treatment are not different ($\alpha \leq 0.05$) according to Fisher LSD.

The results of antioxidant capacity in tomato fruits treated with Cu NPs in Cs-PVA and saline stress hydrogels are presented in Table 5. The content of lycopene in tomato fruits increased significantly with Cu NPs, both in plants without stress (77%) and with saline stress (43%) compared to the control. It has previously been shown that Cu NPs in Cs-PVA hydrogels increase the lycopene content in tomato (Juarez-Maldonado et al. 2016). It has also been shown that the application of NPs of ZnO and TiO_2 in foliar form and to the soil increase the content of lycopene in tomato (Raliya et al. 2015). Lycopene is an antioxidant that protects human cells from oxidative stress, produced by free radicals that are a major cause of cardiovascular disease, cancer and premature aging (Garrido et al. 2013). Therefore, Cu NPs could be an alternative to increase this type of antioxidant in fruits. On the other hand, Cs and Cu NPs increased vitamin C content in tomato fruits, both in plants with saline stress (83 and 72%, respectively) and without stress (48 and 41%, respectively), compared with the control. Cs also increased CAET by DPPH and ABTS technique (44 and 181%, respectively) and CAEVC only by DPPH technique (49%), in plants subjected to saline stress. Saline stress also increased the amount of GSH in tomato fruits by 51%. As previously mentioned, NPs, like saline stress, generate oxidative stress in plants, which was reflected in our results by increasing the amount of ascorbic acid, GSH and Trolox (analogue of vitamin E) in the tomato fruits. There are several reports on the effective application of Cs as a coating on fruits to reduce the loss of antioxidants such as ascorbic acid, anthocyanins, and total polyphenols (Kerch 2015). In this work it was shown that Cs has positive effects by increasing the ascorbic acid in tomato fruits by direct application to the plant. This is of great importance since humans must ingest vitamin C through rich sources like fruits, because the human body does not possess the enzymatic capacity to produce it (Padayatty et al. 2003). Therefore, the exogenous application of Cs and NPs of Cu is an interesting alternative to increase the antioxidant capacity of fruits of interest to humans.

Table 5 Antioxidant capacity in tomato fruits treated with Cu NPs in Cs-PVA and saline stress hydrogels

Treatment	Antioxidant Capacity Equivalent to Trolox (mM/ 100 g FW)		Antioxidant Capacity Equivalent to Vitamin C (mg/100 g FW)		Reduced Glutathione (U/ mg^{-1} proteínas)	Vitamin C (mg/100 g PF)	Lycopene (mg/100 g PF)
	DPPH	ABTS	DPPH	ABTS			
Control	68.97 bc	10.96 b	8.58 bc	5.30 a	324.87 bc	8.10 d	2.85 c
CsPVA	54.09 c	17.65 ab	6.53 c	7.07 a	431.13 ab	11.97 bc	3.09 bc
NCu	87.12 ab	18.91 ab	11.08 ab	7.41 a	322.36 bc	11.44 bc	5.05 a
NaCl	83.17 ab	20.24 ab	10.53 ab	7.77 a	491.82 a	10.38 cd	2.87 c
Cs-NaCl	99.52 a	30.77 a	12.78 a	8.74 a	282.81 c	14.78 a	2.99 bc
nCu-NaCl	67.39 bc	22.78 ab	8.36 bc	8.44 a	301.00 c	13.90 ab	4.07 ab

CsPVA: 1 g chitosan-PVA hydrogel. nCu: 10 mg Cu NPs + CsPVA. NaCl: Control + 100 mM NaCl. Cs-NaCl: Cs-PVA + 100 mM NaCl. nCu-NaCl: nCu + 100 mM NaCl. Means with the same letter within the same column of each treatment are not different ($\alpha \leq 0.05$) according to Fisher LSD.

Quality of fruit

The results on the effect of Cu NPs and saline stress on fruit quality are presented in Table 6. It was observed that although the pH of the fruits increases with the application of Cu NPs, it is only in comparison to the treatments Cs and NaCl in 2 and 3% respectively. Juarez-Maldonado et al. (2016) reported that Cu NPs increase pH in tomato fruits. While Yurtseven et al. (2005) reported that salinity (10 dS m^{-1}) decreases the pH of tomato fruits. The decrease in pH of tomato fruits by salinity is associated with an increase in titratable acidity (Mitchell et al. 1991). This is because when the supply of K^+ is limited, Na^+ can partially substitute for K^+, generating a higher concentration and active accumulation of solutes, mainly ions and organic molecules that are typically produced in plants stressed by salt (Mitchell et al. 1991; De Pascale et al. 2001). This explains the almost double increase in titratable acidity and soluble solids content in tomato fruits under salinity conditions compared to the stress-free condition.

Table 6 Effect of Cu NPs in Cs-PVA hydrogels and saline stress on tomato fruit quality

Treatment	pH	Total Soluble Solids (°Brix)	Titratable acidity (% citric acid)
Control	4.07 abc	5.24 b	0.36 b
CsPVA	4.02 bc	5.48 b	0.36 b
nCu	4.11 a	5.44 b	0.32 b
NaCl	4.00 c	8.80 a	0.64 a
Cs-NaCl	4.09 ab	9.08 a	0.68 a
nCu-NaCl	4.04 abc	9.16 a	0.68 a

CsPVA: 1 g chitosan-PVA hydrogel. nCu: 10 mg Cu NPs + CsPVA. NaCl: Control + 100 mM NaCl. Cs-NaCl: Cs-PVA + 100 mM NaCl. nCu-NaCl: nCu + 100 mM NaCl. Means with the same letter within the same column of each treatment are not different ($\alpha \leq 0.05$) according to Fisher LSD.

Mineral content

The results of leaf and fruit minerals treated with Cu NPs and saline stress are presented in Table 7. Cu NPs significantly increased N content in fruits (15%) compared to control. Previously, it had already been confirmed that Cu NPs are involved in nitrogen metabolism by increasing the activities of the enzymes nitrate reductase, nitrite reductase, glutamine synthase, and glutamate synthase (Pradhan et al. 2015). On the other hand, the saline stress decreased in 32 and 39% the content of N and K in tomato leaves. Saline stress also affected Ca content in leaves and fruits, decreasing by 23 and 54%, respectively. It is known that Ca and K levels decrease due to salinity (Parida and Das 2005), as demonstrated in this study. It is also reported that saline stress affects the N content in canola leaves (Rossi et al. 2016), a fact also observed in this study in tomato leaves. On the other hand, the saline stress increased

the content of Mg in the leaves by 71%. This could be associated with an increase of chlorophyll in the leaves. However, in the fruits, the Cu and Cs NPs affected the Mg translocation, decreasing its content by 4 and 3%, respectively, compared to the control. Cu NPs decreased the Fe content (33%) in the leaves. Saline stress increased Na content in leaves and tomato fruits (1555 and 339%, respectively) compared to control. However, Cs and Cu NPs decreased Na accumulation in leaves (52 and 39%, respectively) compared to saline stress. Some authors mention that CeO_2 NPs modify the formation of apoplectic barriers, which allows to improve tolerance to salt stress in Brassica napus plants (Rossi et al. 2017). In this study, it was shown that Cu and Cs NPs play an important role in reducing Na accumulation in the leaves, and could promote tolerance of salt stress because the sodium itself causes toxicity in cells altering cellular homeostasis causing osmotic stress and ionic toxicity that affect plant growth (Zhu 2003). On the other hand, the Cs increased the content of Fe (62%) in tomato fruits compared to the control. In an earlier study, Cu NPs did not affect Fe content in tomato leaves and Cs did not show significant differences in Fe content in tomato fruit, this is probably due to the fact that the concentration of Cu NPs was lower compared to that used in this study (Juarez-Maldonado et al. 2016). In contrast, saline stress increased the leaf Zn content by 82% over the control. Lutts et al. (1996) showed that saline stress increases the Zn content in rice plants. In addition, the overexpression of the OSISAP1 gene in transgenic tobacco confers tolerance to saline stress (Mukhopadhyay et al. 2004), the same gene that codes for zinc finger proteins, which explains the increase of Zn in salinized plants. The content of Cu in leaves and fruits was not affected by Cs, Cu NPs or saline stress. In an earlier study it was shown that Cs and Cu NPs decrease the Cu content in tomato plants (Juarez-Maldonado et al. 2016), which was not observed in the present work.

Table 7 Mineral content in fruits and tomato leaves treated with Cu NPs in Cs-PVA hydrogels and saline stress

Treatment	N (mg g^{-1} DW)	K (mg g^{-1} DW)	Ca (mg g^{-1} DW)	Mg (mg g^{-1} DW)	Na (mg g^{-1} DW)	Fe (µg g^{-1} DW)	Zn (µg g^{-1} DW)	Cu (µg g^{-1} DW)
Leaves								
Control	32.20 ab	10.61 a	18.50 a	3.07 b	6.33 c	96.8 a	17.6 bc	100.8 a
CsPVA	28.16 bc	9.07 b	16.99 b	2.76 b	6.57 c	80.8 ab	14.8 bc	93.6 a
nCu	34.12 a	11.21 a	18.92 a	2.00 b	4.59 c	64.6 b	10.8 c	93.8 a
NaCl	21.86 d	6.48 c	14.20 c	5.25 a	104.75 a	91.2 ab	32.0 a	123.8 a
Cs-NaCl	23.13 d	7.14 c	15.05 c	3.58 ab	49.82 b	75.0 ab	23.0 ab	97.0 a
nCu-NaCl	24.76 cd	7.41 c	14.78 c	4.74 ab	64.37 b	79.8 ab	21.2 b	97.0 a
Fruits								
Control	23.14 b	9.16 a	6.34 ab	2.31 a	0.98 b	13.8 b	8.8 a	93.8 a
CsPVA	24.02 ab	9.42 a	5.21 b	2.24 b	1.88 b	22.4 a	8.4 a	111.8 a
nCu	26.57 a	9.38 a	7.45 a	2.21 b	1.08 b	15.6 ab	7.8 a	92.8 a
NaCl	21.78 b	8.31 a	2.92 c	2.34 a	4.30 a	13.8 b	11.0 a	93.2 a
Cs-NaCl	22.39 b	8.39 a	2.83 c	2.32 a	3.85 a	13.8 b	8.8 a	93.4 a
nCu-NaCl	23.20 b	8.89 a	2.50 c	2.33 a	4.42 a	10.4 b	10.6 a	93.0 a

CsPVA: 1 g chitosan-PVA hydrogel. nCu: 10 mg Cu NPs + CsPVA. NaCl: Control + 100 mM NaCl. Cs-NaCl: Cs-PVA + 100 mM NaCl. nCu-NaCl: nCu + 100 mM NaCl. Means with the same letter within the same column of each treatment are not different ($\alpha \leq 0.05$) according to Fisher LSD.

Conclusions

The present work shows that the Cu NPs promoted the vegetative and reproductive growth of tomato plants without saline stress. They increased stem diameter (7%), fresh and dry root weight (91 and 43%) and the number of fruits (20%) per plant compared to control. They also showed higher activity of CAT, APX, GPX, SOD and PAL (111, 57, 39, 92 and 62%, respectively), and higher GSH (56%) in the leaves. The content of N, vitamin C and lycopene (15, 41 and 77%, respectively) also increased in the fruits. On the other hand, saline stress drastically affected plant growth and yield compared to control. However, the Cs and Cu NPs decreased Na accumulation in the leaves (39 and 52%, respectively) compared to the NaCl treatment. In addition, the Cs increased the content of total chlorophyll (65%), carotenoids (79%) and total phenols (148%) in leaves of plants under conditions of saline stress. This demonstrates that Cu NPs are promoters of plant growth and development, in addition to activating the antioxidant defense

mechanism of plants, and increase nutraceutical compounds in fruits. In addition, the Cs and Cu NPs can induce mechanisms of tolerance to salinity.

References

[USDA] United States Department of Agriculture (1991) United States Standards for Grades of Fresh Tomatoes. October 1991: Available online: https://www.hort.purdue.edu/prod_quality/quality/tomatfrh.pdf. Accessed 03 August 2017

Alharby HF, Metwali EMR, Fuller MP, Aldhebiani AY (2016) The alteration of mRNA expression of SOD and GPX genes, and proteins in tomato (Lycopersicon esculentum Mill) under stress of NaCl and/or ZnO nanoparticles. Saudi J Biol Sci. 23:773-781. doi: 10.1016/j.sjbs.2016.04.012

Almutairi ZM (2016) Influence of silver nano-Particles on the salt resistance of tomato (Solanum lycopersicum) during germination. Int J Agric Biol 18:449–457. doi: 10.17957/IJAB/15.0114

AOAC (2000) Vitamin and other nutrient. In Hoerwitz W (ed) Official Methods of Analysis of the Association of Official Analytical Chemists International, 17th edn AOAC International, Gaithersburg, MD, USA,

Aruna A, Karthick Raja Namasivayam S, Allen Roy E (2015) Comparative evaluation of non target toxic effect of free and polymer coated chemogenic metallic nanoparticles against Vigna mungo. Int J Pharma Bio Sci 6:B461–B467.

Barrios AC, Rico CM, Trujillo-Reyes J, et al (2015) Effects of uncoated and citric acid coated cerium oxide nanoparticles, bulk cerium oxide, cerium acetate, and citric acid on tomato plants. Sci Total Environ. doi: http://dx.doi.org/10.1016/j.scitotenv.2015.11.143

Beers RF, Sizer IW (1952) Article : a Spectrophotometric Method for Measuring the Breakdown of Hydrogen Peroxide By. J Biol Chem 195:133–140.

Blokhina O, Virolainen E, Fagerstedt K V. (2003) Antioxidants, oxidative damage and oxygen deprivation stress: A review. Ann Bot 91:179–194. doi: 10.1093/aob/mcf118

Bradford MM (1976) A rapid and sensitive method for the quantitation of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. doi: 10.1016/0003-2697(76)90527-3

Brand-Williams, Cuvelier ME, Berset C (1995) Use of a Free Radical Method to Evaluate Antioxidant Activity. Food Sci Technol 28:25–30. doi: 10.1016/S0023-6438(95)80008-5

Camejo D, Rodríguez P, Morales MA, et al (2005) High temperature effects on photosynthetic activity of two tomato cultivars with different heat susceptibility. J Plant Physiol 162:281–289. doi: 10.1016/j.jplph.2004.07.014

De Pascale S, Maggio A, Fogliano V, et al (2001) Irrigation with saline water improves carotenoids content and antioxidant activity of tomato. J Hortic Sci Biotechnol 76:447–453. doi: 10.1080/14620316.2001.11511392

Fish WW, Perkins-Veazie P, Collins JK (2002) A quantitative assay for lycopene that utilizes reduced volumes of organic solvents. J Food Compos Anal 15:309–317. doi: 10.1006/jfca.2002.1069

Flohé L, Günzler WA (1984) Assays of glutathione peroxidase. Methods Enzymol 105:114–120. doi: 10.1016/S0076-6879(84)05015-1

Garrido M, González-Flores D, Marchena AM, et al (2013) A lycopene-enriched virgin olive oil enhances antioxidant status in humans. J Sci Food Agric 93:1820–1826. doi: 10.1002/jsfa.5972

Jones JB (1991) Kjeldahl method for nitrogen determination. Georgia, USA

Juarez-Maldonado A, Ortega-Ortíz H, Pérez-Labrada F, et al (2016) Cu Nanoparticles absorbed on chitosan hydrogels positively alter morphological, production, and quality characteristics of tomato. J Appl Bot Food Qual 89:183–189. doi: 10.5073/JABFQ.2016.089.023

Kashyap PL, Xiang X, Heiden P (2015) Chitosan nanoparticle based delivery systems for sustainable agriculture. Int J Biol Macromol 77:36–51. doi: 10.1016/j.ijbiomac.2015.02.039

Kerch G (2015) Chitosan films and coatings prevent losses of fresh fruit nutritional quality: A review. Trends Food Sci Technol 46:159–166. doi: 10.1016/j.tifs.2015.10.010

Lutts S, Kinet JM, Bouharmont J (1996) Effects of salt stress on growth, mineral nutrition and proline accumulation in relation to osmotic adjustment in rice (Oryza sativa L.) cultivars differing in salinity resistance. Plant Growth Regul 19:207–218. doi: 10.1007/BF00037793

Ma L, Li Y, Yu C, et al (2012) Alleviation of exogenous oligochitosan on wheat seedlings growth under salt stress. Protoplasma 249:393–399. doi: 10.1007/s00709-011-0290-5

Maggio A, Raimondi G, Martino A, De Pascale S (2007) Salt stress response in tomato beyond the salinity tolerance threshold. Environ Exp Bot 59:276–282. doi: 10.1016/j.envexpbot.2006.02.002

Michalak a (2006) Phenolic Compounds and Their Antioxidant Activity in Plants Growing under Heavy Metal

Stress. Plant Cell 15:523–530.
Miller NJ, Rice-Evans C, Davies MJ, et al (1993) A Novel Method for Measuring Antioxidant Capacity and its Application to Monitoring the Antioxidant Status in Premature Neonates. Clin Sci 84:407–412. doi: 10.1042/cs0840407
Mitchell JP, Shennan C, Grattan SR (1991) Developmental changes in tomato fruit composition in response to water deficit and salinity. Physiol Plant 83:177–185. doi: 10.1111/j.1399-3054.1991.tb01299.x
Moo-Huchin VM, Moo-Huchin MI, Estrada-León RJ, et al (2015) Antioxidant compounds, antioxidant activity and phenolic content in peel from three tropical fruits from Yucatan, Mexico. Food Chem 166:17–22. doi: 10.1016/j.foodchem.2014.05.127
Mukhopadhyay A, Vij S, Tyagi AK (2004) Overexpression of a zinc-finger protein gene from rice confers tolerance to cold, dehydration, and salt stress in transgenic tobacco. Proc Natl Acad Sci U S A 101:6309–6314. doi: 10.1073/pnas.0401572101
Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22:867–880.
Padayatty SJ, Katz A, Wang Y, et al (2003) Vitamin C as an Antioxidant: Evaluation of Its Role in Disease Prevention. J Am Coll Nutr 22:18–35. doi: 10.1080/07315724.2003.10719272
Parida AK, Das AB (2005) Salt tolerance and salinity effects on plants: A review. Ecotoxicol Environ Saf 60:324–349. doi: 10.1016/j.ecoenv.2004.06.010
Parihar P, Singh S, Singh R, et al (2015) Effect of salinity stress on plants and its tolerance strategies: a review. Environ Sci Pollut Res 22:4056–4075. doi: 10.1007/s11356-014-3739-1
Parisy V, Poinssot B, Owsianowski L, et al (2007) Identification of PAD2 as a γ-glutamylcysteine synthetase highlights the importance of glutathione in disease resistance of Arabidopsis. Plant J 49:159–172. doi: 10.1111/j.1365-313X.2006.02938.x
Pinedo-Guerrero ZH, Hernández-Fuentes AD, Ortega-Ortiz H, et al (2017) Cu Nanoparticles in Hydrogels of Chitosan-PVA Affects the Characteristics of Post-Harvest and Bioactive Compounds of Jalapeño Pepper. Molecules 22:926. doi: 10.3390/molecules22060926
Pocock T, Krol M, Huner NP (2004) The determination and quantification of photosynthetic pigments by reverse phase high-performance liquid chromatography, thin-layer chromatography, and spectrophotometry. In: Carpentier R (ed) Photosynthesis Research Protocols, Humana Press, New York City, pp. 137–148
Porra RJ (2002) The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b. Photosynth Res 73:149–156. doi: 10.1023/A:1020470224740
Pradhan S, Patra P, Mitra S, et al (2015) Copper nanoparticle (CuNP) nanochain arrays with a reduced toxicity response: a biophysical and biochemical outlook on Vigna radiata. J Agric Food Chem 63:2606–17. doi: 10.1021/jf504614w
Raliya R, Nair R, Chavalmane S, et al (2015) Mechanistic evaluation of translocation and physiological impact of titanium dioxide and zinc oxide nanoparticles on the tomato (Solanum lycopersicum L.) plant. Metallomics 7:1584–1594. doi: 10.1039/C5MT00168D
Ramos SJ, Faquin V, Guilherme LRG, et al (2010) Selenium biofortification and antioxidant activity in lettuce plants fed with selenate and selenite. Plant, Soil Environ 56:584–588.
Ravi Kumar MN. (2000) A review of chitin and chitosan applications. React Funct Polym 46:1–27. doi: 10.1016/S1381-5148(00)00038-9
Reddy PVL, Hernandez-Viezcas JA, Peralta-Videa JR, Gardea-Torresdey JL (2016) Lessons learned: Are engineered nanomaterials toxic to terrestrial plants? Sci Total Environ 568:470–479. doi: 10.1016/j.scitotenv.2016.06.042
Rinaudo M (2006) Chitin and chitosan: Properties and applications. Prog Polym Sci 31:603–632. doi: 10.1016/j.progpolymsci.2006.06.001
Rizwan M, Ali S, Qayyum MF, et al (2017) Effect of metal and metal oxide nanoparticles on growth and physiology of globally important food crops: A critical review. J Hazard Mater 322:2–16. doi: 10.1016/j.jhazmat.2016.05.061
Rossi L, Zhang W, Lombardini L, Ma X (2016) The impact of cerium oxide nanoparticles on the salt stress responses of Brassica napus L. Environ Pollut 219:28–36. doi: 10.1016/j.envpol.2016.09.060
Rossi L, Zhang W, Ma X (2017) Cerium oxide nanoparticles alter the salt stress tolerance of Brassica napus L. by modifying the formation of root apoplastic barriers. Environ Pollut 229:132–138. doi: 10.1016/j.envpol.2017.05.083
Rufino M do SM, Alves RE, de Brito ES, et al (2010) Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. Food Chem 121:996–1002. doi: 10.1016/j.foodchem.2010.01.037

525 Saharan V, Pal A (2016) Chitosan Based Nanomaterials in Plant Growth and Protection. Springer India
526 Saharan V, Sharma G, Yadav M, et al (2015) Synthesis and in vitro antifungal efficacy of Cu–chitosan nanoparticles
527 against pathogenic fungi of tomato. Int J Biol Macromol 75:346–353. doi: 10.1016/j.ijbiomac.2015.01.027
528 Singleton VL, Orthofer R, Lamuela-Raventos RM (1999) Analisys of total phenols and other oxidation sobstrates
529 and antioxidants by means of Folin Ciocalteau reagent. Methods Enzymol 299:152–178.
530 Stefanov M, Yotsova E, Rashkov G, et al (2016) Effects of salinity on the photosynthetic apparatus of two
531 Paulownia lines. Plant Physiol Biochem 101:54–59. doi: 10.1016/j.plaphy.2016.01.017
532 Steiner A a. (1961) a Universal Method for Preparing Nutrient Solutions of a Certain Desired Composition. Plant
533 Soil 15:134–154. doi: 10.1007/BF01347224
534 Sykłowska-Baranek K, Pietrosiuk A, Naliwajski MR, et al (2012) Effect of l-phenylalanine on PAL activity and
535 production of naphthoquinone pigments in suspension cultures of Arnebia euchroma (Royle) Johnst. Vitr Cell
536 Dev Biol - Plant 48:555–564. doi: 10.1007/s11627-012-9443-2
537 Wellburn AR (1994) The Spectral Determination of Chlorophylls a and b, as well as Total Carotenoids, Using
538 Various Solvents with Spectrophotometers of Different Resolution. J Plant Physiol 144:307–313. doi:
539 10.1016/S0176-1617(11)81192-2
540 Xue T, Hartikainen H, Piironen V (2001) Antioxidative and growth-promoting effect of selenium on senescing
541 lettuce. Plant Soil 237:55–61. doi: 10.1023/A:1013369804867
542 Yurtseven E, Kesmez GD, Ünlükara A (2005) The effects of water salinity and potassium levels on yield, fruit
543 quality and water consumption of a native central anatolian tomato species (Lycopersicon esculantum). Agric
544 Water Manag 78:128–135. doi: 10.1016/j.agwat.2005.04.018
545 Zhu J-K (2003) Regulation of ion homeostasis under salt stress. Curr Opin Plant Biol 6:441–445. doi:
546 10.1016/S1369-5266(03)00085-2
547 Zuverza-Mena N, Martínez-Fernández D, Du W, et al (2016) Exposure of engineered nanomaterials to plants:
548 Insights into the physiological and biochemical responses-A review. Plant Physiol Biochem. doi:
549 10.1016/j.plaphy.2016.05.037

Expresión de genes de defensa en plantas de tomate tratadas con nanopartículas de cobre-quitosán y estrés salino

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Resumen:

La nanotecnología puede revolucionar los sistemas de producción agrícola. Se ha observado que las nanopartículas de cobre inducen el crecimiento y mecanismos de defensa en las plantas. El objetivo de este estudio fue evaluar la expresión de genes relacionados a defensa en plantas de tomate tratadas con nanopartículas de cobre-quitosán y estrés salino. Se evaluaron los siguientes tratamientos: control, complejo de nanopartículas de cobre-quitosán (NPs de Cu-Cs), quitosán a granel y estos mismos tratamientos más estrés salino (100 mM de NaCl). La PCR en tiempo real se realizó en el equipo Applied Biosystems StepOne™ versión 2.3. Los resultados demuestran que el complejo de nanopartículas de cobre-quitosán sobreexpresó el gen SOD en el primer y segundo muestreo (7 y 4.3 fold change, respectivamente) y el gen JA se sobreexpresó únicamente en el segundo muestreo (1.1 fold change, respectivamente) con respecto al control. La combinación de estrés salino-NPs de Cu-Cs y estrés salino-Cs a granel sobreexpresó los genes SOD (1.5 y 1.9 fold change, respectivamente) y JA (1.5 y 2 fold change, respectivamente). Este estudio sugiere que el complejo de NPs de Cu-Cs y Cs a granel causa estrés oxidativo en las plantas por lo que activa el mecanismo de defensa antioxidante y la vía de señalización dependiente del ácido jasmónico y en combinación con estrés salino induce la tolerancia disminuyendo el estrés oxidativo, iónico y osmótico generado por la salinidad a través de la activación del ácido jasmónico.

Palabras clave: nanopartículas de cobre, quitosán, estrés salino, gen SOD, gen JA.

INTRODUCCIÓN

A partir del aumento del uso de los nanomateriales (NMs) tanto en productos cosméticos, farmacéuticos, industriales (automotriz, línea blanca) incluso como aditivo de alimentos, los estudios sobre los efectos fisiológicos y bioquímicos en las plantas han crecido exponencialmente [1]. La absorción de los NMs en las plantas depende de las propiedades fisicoquímicas como la

morfología, el tamaño y tipo de nanopartícula (NP), el recubrimiento y carga superficial [2–5]. Además, la interacción con el medio ambiente es otro factor que influye en la absorción de los NMs. Por ejemplo, la interacción con microorganismos (micorrizas, rizobacterias), materia orgánica, arcillas, ácidos húmicos e iones de sales [6–10]. Cuando las NPs se aplican al suelo se pueden absorber a través de la captación directa o mediante una disolución de NPs a forma iónica en la superficie radicular [11,12]. Cuando se aplican de forma foliar los NMs son absorbidos de manera intacta a través de los estomas y se crea una disolución a forma iónica en el apoplasto [13]. Pérez-de-Luque [14] menciona que para la internalización de los NMs al sistema vascular de la planta primero deben atravesar varios tejidos (pelos radiculares, epidermis, cortex y endodermis) y barreras (banda de caspari, cutícula, mesofilo de empalizada y esponjoso) dependiendo del punto de entrada (raíces u hojas). Los mecanismos de absorción de las NPs pueden ser a través de endocitosis, incorporándose a la célula por invaginación de la membrana plasmática, originando una vesícula que puede viajar a diferentes compartimentos de la célula [14,15]. También puede ser mediante la inducción de formación de poros para atravesar la célula y alcanzar directamente el citosol sin ser encapsulado en ningún órgano. Otro mecanismo puede ser a través de la unión de proteínas de membrana como las acuaporinas, sin embargo, el tamaño de poro es muy pequeño entre 2.8 y 3.4 Å, a menos que el tamaño pudiera ser modificado y aumentado [14]. También puede ser mediante plasmodesmata que son estructuras especializadas en el transporte entre células. Se han propuesto los canales iónicos, sin embargo, su tamaño es alrededor de 1 nm y tendrían que sufrir modificaciones importantes [14]. Después de internalizarse los NMs pueden seguir la vía apoplástica y/o simplástica para moverse de arriba hacia abajo de la planta, y el movimiento radial para cambiar de una vía a otra [14]. Dentro de la planta los nanomateriales generan estrés que puede ser grave o leve dependiendo de la concentración utilizada [16]. La producción de especies reactivas de oxígeno (ROS) y calcio citosólico son las principales vías de señalización de los NMs [17]. A partir de estos las plantas activan su maquinaria defensiva e inducen la expresión de genes para contrarrestar el estrés oxidativo causado por los NMs. Por ejemplo, en plantas de lechuga tratadas con NPs de TiO_2/ZnO se identificaron genes relacionados a enzimas antioxidantes como la catalasa, ascorbato peroxidasa, superóxido dismutasa (SOD), glutatión reductasa y deshidroascorbato reductasa [18]. Las hormonas también son importantes señalizadoras de los NMs, entre estas se encuentran el ácido abscísico (ABA), ácido salicílico (SA) y ácido jasmónico (JA) [16]. Varios estudios señalan que una baja concentración de diferentes NMs promueven el crecimiento, mejoran la capacidad fotosintética, activan la maquinaria antioxidante y aumentan las propiedades nutracéuticas en los frutos [1,19,20]. Por lo tanto, los nanomateriales pueden ser utilizados para producir un estrés que resulte en beneficios para las plantas.

El quitosán (Cs) es un polímero natural que se extrae principalmente de la cutícula de crustáceos (camarones y cangrejos), debido a la gran cantidad de desechos que se producen en la industria pesquera. Se obtiene mediante la desacetilación de la quitina a través de procesos ácidos o alcalinos. El Cs es un polímero natural multifuncional, debido a la gran capacidad de reticulación e intercambio catiónico en soluciones ácidas y gran afinidad hacia a los iones metálicos [21]. En

la agricultura se utiliza como bioestimulante del crecimiento y como inductor de respuestas de defensa en las plantas [22]. En el área de la nanotecnología, el Cs está siendo utilizado para formar complejos con NPs metálicas debido a sus características de biocompatibilidad, biodegradabilidad, no toxicidad y afinidad a iones metálicos. Por ejemplo, el complejo de NPs de Cu-Cs ha demostrado ser menos tóxico para las plantas en comparación con las NPs libres o de forma iónica [23]. Algunos trabajos a base de NPs de Cu-Cs demuestran que aumentan el vigor de las plantas y la cantidad de licopeno en fruto de tomate, inducen enzimas defensivas en las plantas como catalasa, peroxidasa, superóxido dismutasa y fenilalanina amonio liasa y funcionan como agente antifúngico contra diversos hongos fitopatógenos [19,23,24].

Actualmente el estrés salino afecta a una gran diversidad de cultivos a nivel mundial, se reporta que más del 6% de la superficie mundial son afectadas por la salinidad [25]. Las plantas perciben el estrés salino cuando los iones de sodio (Na) entran en contacto con las raíces a través de los canales de cationes no selectivos. Después se activan cascadas de señalización de Ca^{2+}, ROS y hormonas que dan como resultado la expresión y activación de mecanismos de desintoxicación celular como Salt Overly Sensitive (SOS) y Na^{+}/H^{+} exchanger (NHX) [26]. Se ha demostrado que el quitosán induce respuestas de defensa en las plantas bajo estrés salino regulando la expresión de genes relacionados a enzimas antioxidantes [27]. Además, diferentes NMs tienen efectos positivos para mitigar el efecto de diferentes tipos de estrés ambiental. En plantas bajo estrés salino los NMs mejoran la tasa fotosintética neta, la conductancia estomática, la tasa de transpiración, la eficiencia del uso del agua y, la reducción y degradación de la clorofila. Reducen las barreras apoplásicas de las raíces, lo que permite más transporte de Na a los brotes y menos acumulación de Na en las raíces de las plantas. También reducen el contenido de malondialdehído (MDA), H_2O_2 y fuga de electrolitos [28]. Por lo tanto, el objetivo de este estudio fue evaluar la expresión de genes de respuesta defensivas en plantas de tomate tratadas con complejos de NPs de Cu-Cs y desarrolladas bajo condiciones de estrés salino.

MATERIALES Y MÉTODOS

Síntesis de hidrogeles de quitosán-polivinil alcohol (Cs-PVA) y absorción de NPs de Cu

Se llevó a cabo de acuerdo a la metodología de Pinedo et al. [20]. Se realizó en la planta piloto del Centro de Investigación de Química Aplicada (CIQA) según la metodología siguiente: se disolvieron 250 mL de quitosán (Chemical Marine Hamburg, Germany) al 2% y 250 mL de polivinil alcohol (Sigma Aldrich, St. Louis, USA) al 4% mezclándolos por dos horas a 300 rpm y 60°C para obtener un hidrogel en una relación 1:2 (Cs:PVA); posteriormente se agregaron 2.27 mL del entrecruzante (glutaraldehido al 50%) a 450 rpm por 5 minutos a 25°C, se agregaron 100 mL de NaOH al 6% a 300 rpm, y 25°C por una hora. En seguida se hizo un lavado y purificación de los hidrogeles de Cs-PVA con agua destilada y etanol, y por último se secaron y se pesaron. Posteriormente, se dispersaron 100 mg de las NPs de Cu en una solución de Tween 80 (Sigma Aldrich, St. Louis, USA) al 1% por ultrasonido durante 5 minutos (potencia de 50 watts y frecuencia del 70%), luego se preparó una dilución para obtener una concentración de 10 mg, las

cuales posteriormente se absorbieron en 1 gramo de hidrogel de Cs-PVA y se secaron a una temperatura de 60°C.

Condiciones de crecimiento de las plantas y toma de muestras

Las plantas de tomate (*Solanum lycopersicum* L.) se establecieron en un invernadero del Departamento de Horticultura de la Universidad Autónoma Agraria Antonio Narro. El invernadero es de tipo multitunel y con cubierta de polietileno. La temperatura promedio fue de 21°C, radiación fotosintéticamente activa promedio de 565 µmol m^2 s^{-1} y humedad relativa promedio del 51 %. La densidad de plantación fue de tres plantas por metro cuadrado. Como sustrato se usó una mezcla de peat moss y perlita (50:50 v/v) colocado en bolsas de polietileno color negro de 12 L de capacidad.

Los tratamientos fueron aplicados previo al trasplante, para esto se distribuyó 1 g de hidrogel de Cs-PVA en la parte baja, media y alta de la maceta para tener una mejor dispersión de las NPs de Cu en el sustrato y en el área radicular de la planta. El experimento se desarrolló bajo dos condiciones: 1) en la primera se evaluaron 10 mg de NPs de Cu absorbidas en 1 g de hidrogel de Cs-PVA, un testigo absoluto y un tratamiento con hidrogel de Cs-PVA (1 g); y 2) en la segunda se evaluaron los mismos tratamientos más la adición de 100 mM de NaCl en la solución nutritiva a partir de los 21 días después del trasplante (ddt). Se utilizó un sistema de riego dirigido y se usó la solución nutritiva Steiner al 50% [29] con los siguientes micronutrientes: Fe EDTA= 3.75 ppm; Mn EDTA= 1.85 ppm; B=0.35 ppm; Zn EDTA= 0.30 ppm; Cu EDTA=0.15 ppm y Mo= 0.10 ppm.

Para realizar el análisis de expresión de genes se realizaron dos muestreos de hojas. El primer muestreo se realizó a los 20 días después de del trasplante, mientras que el segundo muestreo se realizó a los 23 días ddt (48 h después de la aplicación del estrés salino). Para ambos muestreos se tomaron las muestras de cinco plantas por cada tratamiento seleccionadas al azar. Se tomó la hoja más joven completamente expandida y se colocó en bolsa de aluminio, inmediatamente se congeló con nitrógeno líquido para después almacenarse en ultracongelador a -80 °C hasta su uso.

Real-time reverse transcriptase PCR

Para la extracción de ARN, se pulverizó la muestra de hoja usando nitrógeno líquido, se pesaron 100 mg de muestra y se colocaron en un tubo para micro centrifuga de 2 ml. Inmediatamente se le adicionó a la muestra 1 ml de TRI reagent (Sigma Aldrich, St. Louis, USA) y se homogenizó suavemente. Se dejó incubar por 5 min a temperatura ambiente, posteriormente se agregaron 200 µl de cloroformo y la mezcla se agitó en vortex vigorosamente hasta que tuvo un aspecto lechoso. La mezcla se incubó a temperatura ambiente por 15 min, a continuación fue centrifugada a 12000 g por 15 min a 4 °C. Se recuperó el sobrenadante y se colocó en un tubo nuevo al cual se le adicionaron 500 µl de isopropanol frío mezclando suavemente. En seguida se incubó a temperatura ambiente durante 10 min y posteriormente se centrifugó a 12000 g por 10 min a 4

°C. Se removió el sobrenadante por decantación y la pastilla de ARN formada se lavó adicionando 1 ml de etanol frío al 70 %. Se agitó haciendo suaves inversiones del tubo para después centrifugar a 7500 g por 5 min a 4 °C. Nuevamente se removió el sobrenadante y la pastilla de ARN se dejó secar por 15 min, finalmente la pastilla se resuspendió en 50 µl de agua DEPC y se almacenó en ultracongelador a -80 °C.

La cuantificación del ARN extraído se realizó por medio del espectrofotómetro UV-Vis (Thermo Scientific Model G10S) a una absorbancia de 260 y 280 nm, y la calidad se midió mediante una electroforesis desnaturalizante. La síntesis del cDNA se realizó utilizando un kit comercial (Promega, Madison, Wisconsin, USA). Los primers corresponden a un gen endógeno (Actina) y cinco genes de estudio (SOD, CAT, GPX, PR1 y JA) los cuáles fueron diseñados en el software AMPLIFIX, OLIGOANALIZER y PRIMERS BLAST como se describen en la Tabla 1.

Tabla 1. Secuencia de primers de los genes analizados

Gen	(Forward primer 5'-3')	(Reverse primer 5'-3')
ACT	CCCAGGCACACAGGTGTTAT	CAGGAGCAACTCGAAGCTCA
PR1	AAGTAGTCTGGCGCAACTCA	GTCCGATCCAGTTGCCTACA
JA	TGGTTCGTCGACTTCGTCAT	CTCGGCCTTGAGAGAGTTCA
SOD	TGATGGGCCAACTACGGTTAA	AAAATGGGCTCCTGTAGACATACAT
GPX	AGGAGCCTGGAAACATTGAAGA	CCATTCACGTCAACCTTGTCA
CAT	CCCTCTAAGTATCGCCCATCAA	TTGTACACAGGACCACCAGCAT

La elaboración de los primers consistió en preparar una solución de trabajo, se centrifugó cada par de primers a máxima velocidad por 15 min, posteriormente los primers se prepararon a una concentración de 15 pmol/mL. El método de cuantificación usado fue por medio de una curva relativa estándar, por lo tanto, para cada análisis de cuantificación se incluyó una curva estándar por gen utilizando una dilución 1:5.

Las reacciones de PCR en tiempo real se analizaron en el equipo Applied Biosystems StepOne™ versión 2.3 por el método de curva relativa estándar midiendo la intensidad de fluorescencia de Sybr Green. La reacción de PCR para todos los genes se realizó en un volumen total de 20 µL. para el gen Actina se adicionó 10 µL de Master Mix (Applied Biosystems, Foster City, California, USA), 0.10 µL del primer forward (72 nM), 0.08 µL primer reverse (60 nM), 2 µL de cDNA y 7.82 µL de agua libre de nucleasas. Para el gen PR1 se agregó 10 µL de Master Mix, 0.03 µL de primer forward (20 nM), 0.05 µL primer reverse (40 nM), 2 µL de cDNA y 7.92 µL de agua libre de nucleasas. Para el gen JA se adicionó 10 µL de Master Mix, 0.05 µL de primer forward (40 nM), 0.08 µL primer reverse (60 nM), 2 µL de cDNA y 7.87 µL de agua libre de nucleasas. Para los genes SOD, GPX y CAT se agregaron 10 µL de Master Mix, 0.13 µL de primer forward (100 nM), 0.13 µL primer reverse (100 nM), 2 µL de cDNA y 7.73 µL de agua

libre de nucleasas. Se corrió la qPCR con el siguiente programa en el termociclador: Hot Start: 10 min a 95 ° y PCR (40 ciclos): 15 s a 95 ° y 1 min a 60 °.

Análisis de datos

Para la cuantificación de los genes de estudio (SOD, CAT, GPX, PR1 y JA) se utilizó un gen de referencia (Actina) y se calculó mediante el software Applied Biosystems StepOne™ versión 2.3 por el método de curva relativa estándar. Este software normaliza los datos dividiendo la cantidad de expresión del gen de estudio entre la cantidad del gen de referencia. A partir de estos datos normalizados calcula la media geométrica que representa la cuantificación relativa (fold change) del gen de estudio. El error estándar se calculó a partir de la desviación estándar y el coeficiente de variación del gen de referencia y de estudio.

RESULTADOS Y DISCUSIÓN

Los resultados de la expresión de los genes relacionados a enzimas antioxidantes a los 20 y 23 ddt se presentan en la Figura 1. Se estableció la hipótesis alternativa de que los fold change son mayores que uno o menores que uno en valor absoluto en comparación al testigo de referencia [30]. De acuerdo con esta hipótesis, los resultados de esta investigación determinan que el complejo de NPs de Cu-Cs sobreexpresó el gen SOD (7 y 4.3 fold change, respectivamente. Fig. 1a) a los 20 y 23 días ddt. El gen CAT únicamente se sobreexpresó a los 20 ddt (1.1 fold change. Fig.1b). Por el contrario, se reprimió el gen GPX a los 20 y 23 ddt (0.6 y 0.7 fold change, respectivamente. Fig. 1c) y el gen CAT a los 23 ddt (0.56 fold change).

Las plantas perciben a los NMs como estrés, y éste puede ser leve o grave dependiendo de la concentración de nanopartícula utilizada [16]. Las ROS son las principales señalizadoras debido al estrés que provocan los NMs [17]. En consecuencia las plantas activan su mecanismo de defensa antioxidante para proteger a las plantas del estrés oxidativo [1]. La metaloenzima SOD es la primera línea de defensa que se activa para eliminar ROS, catalizando la dismutación del superóxido (O_2-) en oxígeno (O) y H_2O_2. La catalasa se encarga de catalizar el H_2O_2 en O y agua (H_2O). La enzima glutatión peroxidasa cataliza la reacción de oxidación de glutatión (GSH) a glutatión disulfuro (GSSG) utilizando H_2O_2 [31]. En plantas de lechuga tratadas con NPs de TiO_2/ZnO se han identificado algunos genes relacionados con enzimas antioxidantes: 4 unigenes relacionados con la enzima CAT, entre los cuales el Unigene0006158 se expresó diferencialmente en la raíz (1.23 veces más que en el control). 7 unigenes relacionados con la ascorbato peroxidasa (APOX), entre los cuales 3 fueron sobre expresados en hojas y 2 en raíces. 12 unigenes relacionados con la enzima SOD, entre los que el Unigene0034518 y el Unigene0005736 fueron regulados en las hojas. Además, 2 unigenes relacionados con la glutatión reductasa (GR) y 11 con la deshidroascorbato reductasa (DHAR) [18]. En plantas de *Eruca sativa* las NPs de Ag aumentaron la expresión del gen SOD [32]. En plantas de maíz las NPs de Cu incrementaron la actividad de SOD y disminuyeron la actividad de CAT y GPX [33]. El complejo de NPs de Cu-Cs también aumentó la actividad de las enzimas SOD, POD, PAL y PPO

en plantas de maíz [34]. En un estudio anterior se demostró que el complejo de NPs de Cu-Cs promueven el crecimiento y desarrollo de las plantas de tomate [35]. Entonces se confirma que el complejo de NPs de Cu-Cs produce estrés oxidativo en las plantas y la aplicación de una concentración óptima puede utilizarse como un estrés benéfico para activar el mecanismo de defensa antioxidante de las plantas y hacer frente a diferentes tipos de estrés ambiental.

El Cs a granel sobreexpresó el gen SOD (2.1 y 2.5 fold change, respectivamente) a los 20 y 23 ddt. En consecuencia, el Cs a granel presentó entre 1 y 1.5 fold change más que el control (Fig. 1a). Por el contrario, el Cs a granel reprimió el gen CAT (0.82 y 0.67 fold change, respectivamente) y GPX (0.6 y 0.7 fold change, respectivamente) a los 20 y 23 ddt (Fig. 1b y 1c). El Cs a granel se utiliza en la agricultura como bioestimulante para inducir el crecimiento y respuestas de defensas en las plantas [22]. Las principales vías de señalización del Cs son a través de la generación de H_2O_2 y NO. El H_2O_2 desencadena ROS y la síntesis de ABA, y coordina la actividad con JA a través de la vía octadecanoide, mientras que el NO regula la síntesis de ácido fosfatídico a través de la vía de fosfolipasa C y diacilglicerol quinasa [36]. En plantas de *Camellia sinensis* el Cs a granel sobre expresó los genes SOD y CAT en comparación con los controles no tratados [37]. En cambio en plantas de maíz el Cs a granel no incremento la actividad de SOD, PAL, POX y PPO [34]. De manera similar en plantas de *Brassica rapa* el Cs a granel no aumentó la actividad de SOD, CAT y POX en comparación al control [38]. Este estudio sugiere que el Cs a granel produce estrés oxidativo en las plantas de tomate sobreexpresando el gen SOD y reprimiendo los genes CAT y GPX.

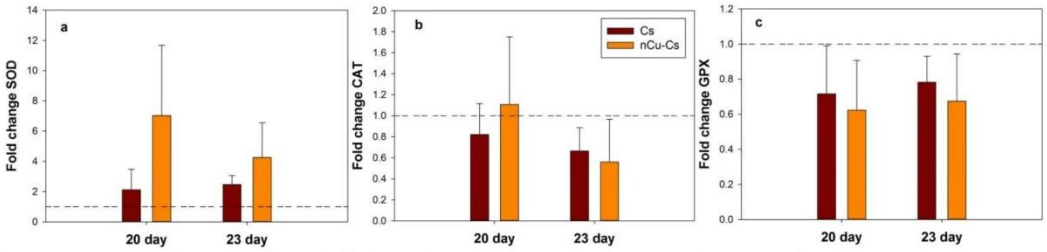

Figura 1. Expresión de los genes SOD (a), CAT (b) y GPX (c) en hojas de tomate a los 20 y 23 días después del trasplante. Los datos representan la media geométrica ± error estándar y la línea de referencia representa el valor constante del control.

Después de 48 h de la aplicación del estrés salino el gen SOD se sobreexpresó (1.9 fold change) y los genes CAT y GPX se reprimieron (0.8 y 0.9 fold change, respectivamente) con respecto al testigo (Figura 2). La combinación de estrés salino y complejo de NPs de Cu-Cs sobreexpresó el gen SOD (1.5 fold change) y reprimió los genes CAT y GPX (0.9 y 0.5 fold change, respectivamente) en comparación al testigo. Las principales vías de señalización del estrés salino son a través de la homeostasis iónica, osmótica y producción de ROS. La homeostasis iónica es mediante la vía SOS, donde un complejo de proteínas quinasas sensibles al calcio (SOS3-SOS2) controlan la expresión y la actividad de transportadores de iones tales como SOS1 [39]. Mientras

250 que la homeostasis osmótica se regula a través del ABA dependiente e independiente [40]. La
251 maquinaria antioxidante de la planta se activa para barrer las ROS provocado por el estrés salino
252 [41]. En plantas de tomate el estrés salino aumenta las actividades de las enzimas SOD y GPX
253 [42]. En plantas de trigo el estrés salino indujo mayores niveles de transcripción del gen SOD,
254 POD y CAT [27]. Los NMs han mostrado efectos positivos para mitigar el estrés salino en
255 plantas. Por ejemplo, la aplicación de NPs a base de Si aumentan la expresión de enzimas
256 antioxidantes, tales como catalasa, peroxidasa, superóxido dismutasa, glutatión reductasa y
257 ascorbato peroxidasa en plantas de calabaza [43]. La combinación de estrés salino y NPs de ZnO
258 presentaron mayor fold change en los genes SOD y GPX en comparación con las plantas de
259 tomate tratadas únicamente con estrés salino [10]. En este estudio la combinación de NPs de Cu-
260 Cs y estrés salino presentó 0.4 fold change menos que las plantas tratadas únicamente con estrés
261 salino en la sobrexpresión del gen SOD (Figura 2). Por lo que el complejo de NPs de Cu-Cs
262 ayuda a tolerar el estrés salino al reducir el estrés oxidativo provocado por la salinidad.

263 Además, la combinación de estrés salino y Cs a granel presentó mayor sobreexpresión de los
264 genes SOD (1.9 fold change) y CAT (1.10 fold change), y reprimió el gen GPX (0.5 fold change)
265 en comparación al testigo (Figura 2). Por lo que la combinación de Cs a granel y estrés salino
266 presentó 0.8 y 1.4 fold change (genes CAT y GPX, respectivamente) menos que las plantas
267 tratadas únicamente con el estrés salino. El Cs a granel induce respuestas de tolerancia frente a
268 estrés abiótico en plantas. Por ejemplo, en plantas de cártamo y girasol el Cs mitigó el estrés
269 oxidativo provocado por la salinidad a través de la reducción de la actividad de las enzimas
270 catalasa y peroxidasa [44]. En plantas de trigo la combinación de estrés salino y Cs presentó
271 mayor nivel de transcripción de los genes SOD, CAT y POD en comparación con las plantas
272 tratadas únicamente con estrés salino [27]. En este sentido el Cs a granel ayuda a mitigar el estrés
273 salino en plantas de tomate regulando la expresión de los genes SOD, CAT y GPX.

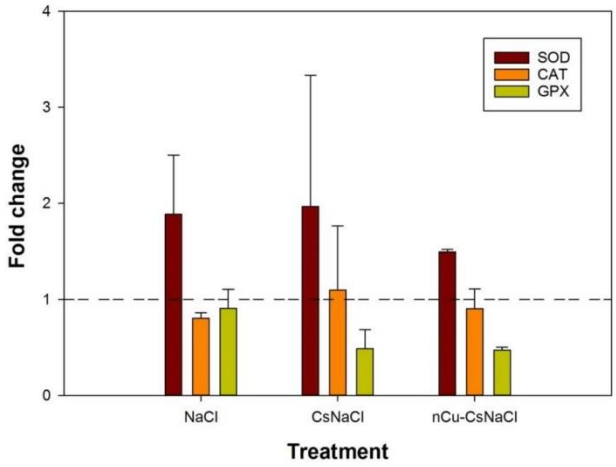

Figura 2. Expresión de genes antioxidantes en hojas de tomate después de 48 h bajo estrés salino (23 días después del trasplante). NaCl= 100 mM de NaCl; CsNaCl= quitosán + 100 mM de NaCl; nCu-CsNaCl= 10 mg de NPs de Cu + 100 mM de NaCl. Los datos representan la media geométrica ± error estándar y la línea de referencia representa el valor constante del control.

Los resultados de la expresión de los genes relacionados a las vías de señalización del ácido salicílico (PR1) y ácido jasmónico (JA) a los 20 y 23 ddt se presentan en la Figura 3. El complejo de NPs de Cu-Cs y el Cs a granel reprimieron el gen PR1 a los 20 ddt (0.2 y 0.6 fold change, respectivamente) y 23 ddt (0.2 y 0.5 fold change, respectivamente. Fig. 3a). En cambio, el gen JA se reprimió con el Cs a granel y el complejo de NPs de Cu-Cs a los 20 ddt (0.9 y 0.8 fold change, respectivamente) y se sobre expresó a los 23 ddt (1.2 y 1.1 fold change, respectivamente. Fig. 3b) en comparación al testigo. La resistencia inducida es una condición mejorada de la capacidad defensiva desarrollada por una planta cuando se estimula apropiadamente. La resistencia sistémica adquirida (RSA) y la resistencia sistémica inducida (RSI) son dos formas de resistencia en las que el sistema de defensa de las plantas son preacondicionadas [45]. La inducción de RSA ocurre principalmente por la vía de señalización del SA y la unión a proteínas de resistencia (PR) [46], mientras que RSI es a través de la vía de señalización del JA y etileno (ET) [47]. Por lo tanto, los jasmonatos y el ácido salicílico son reguladores claves del crecimiento de las plantas, así como en las respuestas de defensa a estrés biótico y abiótico a través de la interacción con otras hormonas como el ET, auxinas, giberelinas, entre otras [48–50]. El quitosán induce genes de respuesta de defensa a través de la vía del SA en plantas bajo estrés biótico [51] y a través de la vía del JA en condiciones de estrés abiótico [52]. En plantas de *Arabidopsis* las NPs de ZnO aumentaron el nivel de SA en hojas y raíces, y suprimieron el nivel del JA [16]. En *A. thaliana* se demostró también que las NPs de ZnO estimulan SA en hojas y raíces y suprimen el JA [16]. En cambio en plantas de trigo las NPs de TiO_2 aumentaron el contenido de JA [53]. En este estudio el complejo de NPs de Cu-Cs y el Cs a granel reprimieron el gen PR1 a los 20 y 23 ddt y

sobreexpresaron el gen JA únicamente a los 23 ddt. Por lo que se sugiere que el complejo de NPs de Cu-Cs y el Cs a granel podría ser mediado por la RSI a través de la vía octadecanoide de los jasmonatos.

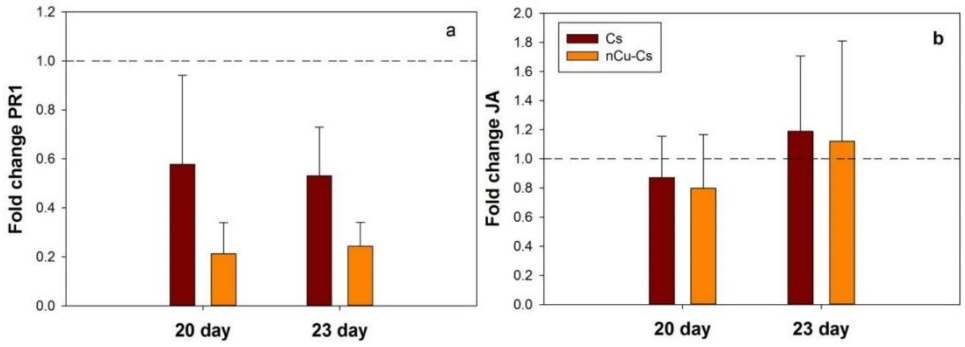

Figura 3. Expresión de los genes PR1 (a) y JA (b) en hojas de tomate a los 20 y 23 días después del trasplante. Los datos representan la media geométrica ± error estándar y la línea de referencia representa el valor constante del control.

Por su parte, el estrés salino reprimió el gen PR1 (0.3 fold change) y sobreexpresó el gen JA (5.9 fold change) en comparación al testigo (Figura 4). La combinación de estrés salino-complejo de NPs de Cu-Cs y estrés salino-Cs a granel reprimieron el gen PR1 (0.3 fold change, respectivamente) y sobreexpresaron el gen JA (1.5 y 2 fold change, respectivamente) como se muestra en la Figura 4.

El SA es fundamental para controlar la entrada de Na^+ en el tejido de la raíz y su subsecuente transporte a larga distancia en los brotes, además previene la pérdida de potasio [54]. El JA mejora la tolerancia a la salinidad tanto del estrés osmótico como el oxidativo, por lo que el efecto beneficioso de JA se logra a través de una alteración fisiológica sistémica en lugar de simplemente controlar la homeostasis iónica [55]. En este estudio el complejo de NPs de Cu-Cs y el Cs a granel presentaron 4.4 y 3.9 fold change menos que las plantas tratadas únicamente con estrés salino en el gen JA (Figura 4). Esto sugiere que el complejo de NPs de Cu-Cs y el Cs a granel regulan la sobreexpresión del gen JA en plantas bajo estrés salino lo cual ayuda a tolerar los efectos de la salinidad a través de la regulación de la homeostasis iónica, osmótica y ROS mediante la activación de la vía del ácido jasmónico.

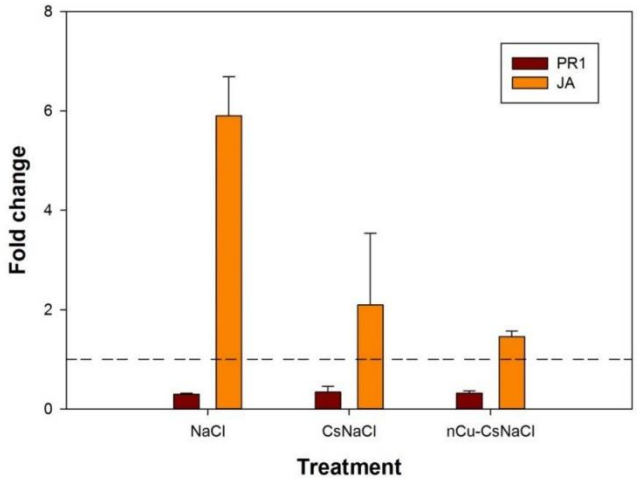

Figura 4. Expresión de los genes PR1 y JA en hojas de tomate después de 48 h bajo estrés salino (23 días después del trasplante). NaCl= 100 mM de NaCl; CsNaCl= quitosán + 100 mM de NaCl; nCu-CsNaCl= 10 mg de NPs de Cu + 100 mM de NaCl. Los datos representan la media geométrica ± error estándar y la línea de referencia representa el valor constante del control.

CONCLUSIONES

El complejo de NPs de Cu-Cs sobre expresó el gen SOD y reprimió los genes CAT, GPX y PR1 a los 20 y 23 días después del trasplante, momento en el que se aplicaron los tratamientos. El gen JA se sobre expresó únicamente a los 23 días después del trasplante. Después de 48 h de la aplicación del estrés salino (23 días después del trasplante) los genes SOD y JA se sobre expresaron y los genes CAT, GPX y PR1 se reprimieron. La combinación de estrés salino-NPs de Cu-Cs y estrés salino-Cs a granel sobre expresaron los genes SOD y JA.

Los resultados de este estudio sugieren que el complejo de NPs de Cu-Cs (10 mg^{-1} g por planta) y Cs a granel (1 g por planta) causa un estrés oxidativo que activa el mecanismo de defensa antioxidante y la vía de señalización del ácido jasmónico en las plantas de tomate. Además, induce tolerancia al estrés salino eliminando ROS y regulando la homeostasis iónica y osmótica a través de la activación de la vía del ácido jasmónico.

REFERENCIAS

1. Du, W.; Tan, W.; Peralta-Videa, J. R.; Gardea-Torresdey, J. L.; Ji, R.; Yin, Y.; Guo, H. Interaction of metal oxide nanoparticles with higher terrestrial plants: Physiological and biochemical aspects. *Plant Physiol. Biochem.* **2017**, *110*, 210–225, doi:10.1016/j.plaphy.2016.04.024.

2. Syu, Y. yu; Hung, J. H.; Chen, J. C.; Chuang, H. wen Impacts of size and shape of silver

nanoparticles on Arabidopsis plant growth and gene expression. *Plant Physiol. Biochem.* **2014**, *83*, 57–64, doi:10.1016/j.plaphy.2014.07.010.

3. Ma, X.; Geisler-Lee, J.; Deng, Y.; Kolmakov, A. Corrigendum to "Interactions between engineered nanoparticles (ENPs) and plants: Phytotoxicity, uptake and accumulation" [Sci. Total Environ. 408 (2010) 3053-3061]. *Sci. Total Environ.* 2014, *481*, 635.

4. Lahiani, M. H.; Dervishi, E.; Ivanov, I.; Chen, J.; Khodakovskaya, M. Comparative study of plant responses to carbon-based nanomaterials with different morphologies. *Nanotechnology* **2016**, *27*, doi:10.1088/0957-4484/27/26/265102.

5. Spielman-Sun, E.; Lombi, E.; Donner, E.; Howard, D. L.; Unrine, J. M.; Lowry, G. V. Impact of surface charge on cerium oxide nanoparticle uptake and translocation by wheat (Triticum aestivum). *Environ. Sci. Technol.* **2017**, acs.est.7b00813, doi:10.1021/acs.est.7b00813.

6. Dimkpa, C. O.; McLean, J. E.; Britt, D. W.; Anderson, A. J. Nano-CuO and interaction with nano-ZnO or soil bacterium provide evidence for the interference of nanoparticles in metal nutrition of plants. *Ecotoxicology* **2015**, *24*, 119–129, doi:10.1007/s10646-014-1364-x.

7. Feng, Y.; Cui, X.; He, S.; Dong, G.; Chen, M.; Wang, J.; Lin, X. The Role of Metal Nanoparticles in Influencing Arbuscular Mycorrhizal Fungi Effects on Plant Growth. *Environ. Sci. Technol.* **2013**, *47*, 9496–9504.

8. Grillo, R.; Rosa, A. H.; Fraceto, L. F. Engineered nanoparticles and organic matter: A review of the state-of-the-art. *Chemosphere* 2015, *119*, 608–619.

9. Wang, F.; Liu, X.; Shi, Z.; Tong, R.; Adams, C. A.; Shi, X. Arbuscular mycorrhizae alleviate negative effects of zinc oxide nanoparticle and zinc accumulation in maize plants - A soil microcosm experiment. *Chemosphere* **2016**, *147*, 88–97, doi:10.1016/j.chemosphere.2015.12.076.

10. Alharby, H. F.; Metwali, E. M. R.; Fuller, M. P.; Aldhebiani, A. Y. The alteration of mRNA expression of SOD and GPX genes, and proteins in tomato (Lycopersicon esculentum Mill) under stress of NaCl and/or ZnO nanoparticles. *Saudi J. Biol. Sci.* **2016**, doi:10.1016/j.sjbs.2016.04.012.

11. Wang, P.; Lombi, E.; Sun, S.; Scheckel, K. G.; Malysheva, A.; McKenna, B. A.; Menzies, N. W.; Zhao, F.-J.; Kopittke, P. M. Characterizing the uptake, accumulation and toxicity of silver sulfide nanoparticles in plants. *Environ. Sci. Nano* **2017**, *4*, 448–460, doi:10.1039/C6EN00489J.

12. Zhang, W.; Dan, Y.; Shi, H.; Ma, X. Elucidating the mechanisms for plant uptake and in-planta speciation of cerium in radish (Raphanus sativus L.) treated with cerium oxide nanoparticles. *J. Environ. Chem. Eng.* **2017**, *5*, 572–577, doi:10.1016/j.jece.2016.12.036.

13. Schymura, S.; Fricke, T.; Hildebrand, H.; Franke, K. Elucidating the Role of Dissolution in CeO2 Nanoparticle Plant Uptake by Smart Radiolabeling. *Angew. Chemie - Int. Ed.* **2017**, *56*, 7411–7414, doi:10.1002/anie.201702421.

14. Pérez-de-Luque, A. Interaction of Nanomaterials with Plants: What Do We Need for Real Applications in Agriculture? *Front. Environ. Sci.* **2017**, *5*, 1–7, doi:10.3389/fenvs.2017.00012.

15. Li, H.; Ye, X.; Guo, X.; Geng, Z.; Wang, G. Effects of surface ligands on the uptake and transport of gold nanoparticles in rice and tomato. *J. Hazard. Mater.* **2016**, *314*, 188–196, doi:10.1016/j.jhazmat.2016.04.043.

16. Vankova, R.; Landa, P.; Podlipna, R.; Dobrev, P. I.; Prerostova, S.; Langhansova, L.; Gaudinova, A.; Motkova, K.; Knirsch, V.; Vanek, T. ZnO nanoparticle effects on hormonal pools in Arabidopsis thaliana. *Sci. Total Environ.* **2017**, *593–594*, 535–542, doi:10.1016/j.scitotenv.2017.03.160.

17. Sosan, A.; Svistunenko, D.; Straltsova, D.; Tsiurkina, K.; Smolich, I.; Lawson, T.; Subramaniam, S.; Golovko, V.; Anderson, D.; Sokolik, A.; Colbeck, I.; Demidchik, V. Engineered silver nanoparticles are sensed at the plasma membrane and dramatically modify the physiology of Arabidopsis thaliana plants. *Plant J.* **2016**, *85*, 245–257, doi:10.1111/tpj.13105.

18. Wang, Y.; Chen, R.; Hao, Y.; Liu, H.; Song, S.; Sun, G. Transcriptome analysis reveals differentially expressed genes (DEGs) related to lettuce (Lactuca sativa) treated by TiO2/ZnO nanoparticles. *Plant Growth Regul.* **2017**, *83*, 13–25, doi:10.1007/s10725-017-0280-5.

19. Juárez-Maldonado, A.; Ortega-Ortíz, H.; Pérez-Labrada, F.; Cadenas-Pliego, G.; Benavides-Mendoza, A. Cu Nanoparticles absorbed on chitosan hydrogels positively alter morphological, production, and quality characteristics of tomato. *J. Appl. Bot. Food Qual.* **2016**, *89*, 183–189, doi:10.5073/JABFQ.2016.089.023.

20. Pinedo-Guerrero, Z. H.; Hernández-Fuentes, A. D.; Ortega-Ortiz, H.; Benavides-Mendoza, A.; Cadenas-Pliego, G.; Juárez-Maldonado, A. Cu Nanoparticles in Hydrogels of Chitosan-PVA Affects the Characteristics of Post-Harvest and Bioactive Compounds of Jalapeño Pepper. *Molecules* **2017**, *22*, 926, doi:10.3390/molecules22060926.

21. Guibal, E.; Vincent, T.; Navarro, R. Metal ion biosorption on chitosan for the synthesis of advanced materials. *J. Mater. Sci.* 2014, *49*, 5505–5518.

22. Ali, A.; Muhammad, M. T. M.; Sijam, K.; Siddiqui, Y. Effect of chitosan coatings on the physicochemical characteristics of Eksotika II papaya (Carica papaya L.) fruit during cold storage. *Food Chem.* **2011**, *124*, 620–626, doi:10.1016/j.foodchem.2010.06.085.

23. Aruna, A.; Karthick Raja Namasivayam, S.; Allen Roy, E. Comparative evaluation of non target toxic effect of free and polymer coated chemogenic metallic nanoparticles against Vigna mungo. *Int. J. Pharma Bio Sci.* **2015**, *6*, B461–B467.

24. Saharan, V.; Pal, A. *Chitosan Based Nanomaterials in Plant Growth and Protection*; Springer International Publishing, 2016; ISBN 978-81-322-3599-6.

25. Parihar, P.; Singh, S.; Singh, R.; Singh, V. P.; Prasad, S. M. Effect of salinity stress on plants and its tolerance strategies: a review. *Environ. Sci. Pollut. Res.* **2015**, *22*, 4056–

4075, doi:10.1007/s11356-014-3739-1.

26. Deinlein, U.; Stephan, A. B.; Horie, T.; Luo, W.; Xu, G.; Schroeder, J. I. Plant salt-tolerance mechanisms. *Trends Plant Sci.* 2014, *19*, 371–379.

27. Zou, P.; Li, K.; Liu, S.; Xing, R.; Qin, Y.; Yu, H.; Zhou, M.; Li, P. Effect of chitooligosaccharides with different degrees of acetylation on wheat seedlings under salt stress. *Carbohydr. Polym.* **2015**, *126*, 62–69, doi:10.1016/j.carbpol.2015.03.028.

28. Khan, M. N.; Mobin, M.; Abbas, Z. K.; AlMutairi, K. A.; Siddiqui, Z. H. Role of nanomaterials in plants under challenging environments. *Plant Physiol. Biochem.* **2017**, *110*, 194–209, doi:10.1016/j.plaphy.2016.05.038.

29. Steiner, A. A. A universal method for preparing nutrient solutions of a certain desired composition. *Plant Soil* **1961**, *15*, 134–154, doi:10.1007/BF01347224.

30. Love, M. I.; Huber, W.; Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **2014**, *15*, 550, doi:10.1186/s13059-014-0550-8.

31. Gill, S. S.; Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* 2010, *48*, 909–930.

32. Vannini, C.; Domingo, G.; Onelli, E.; Prinsi, B.; Marsoni, M.; Espen, L.; Bracale, M. Morphological and Proteomic Responses of Eruca sativa Exposed to Silver Nanoparticles or Silver Nitrate. *PLoS One* **2013**, *8*, doi:10.1371/journal.pone.0068752.

33. Adhikari, T.; Sarkar, D.; Mashayekhi, H.; Xing, B. Growth and enzymatic activity of maize (*Zea mays* L.) plant: Solution culture test for copper dioxide nano particles. *J. Plant Nutr.* **2016**, *39*, 99–115, doi:10.1080/01904167.2015.1044012.

34. Choudhary, R. C.; Kumaraswamy, R. V.; Kumari, S.; Sharma, S. S.; Pal, A.; Raliya, R.; Biswas, P.; Saharan, V. Cu-chitosan nanoparticle boost defense responses and plant growth in maize (Zea mays L.). *Sci. Rep.* **2017**, *7*, 1–11, doi:10.1038/s41598-017-08571-0.

35. Hernández-Hernández, H.; Benavides-Mendoza, A.; Ortega-Ortiz, H.; Hernández-Fuentes, A. D.; Juárez-Maldonado, A. Cu Nanoparticles in chitosan-PVA hydrogels as promoters of growth , productivity and fruit quality in tomato. **2017**, *29*, 573–580, doi:10.9755/ejfa.2016-08-1127.

36. Pichyangkura, R.; Chadchawan, S. Biostimulant activity of chitosan in horticulture. *Sci. Hortic. (Amsterdam).* 2015, *196*, 49–65.

37. Chandra, S.; Chakraborty, N.; Dasgupta, A.; Sarkar, J.; Panda, K.; Acharya, K. Chitosan nanoparticles: A positive modulator of innate immune responses in plants. *Sci. Rep.* **2015**, *5*, 15195, doi:10.1038/srep15195.

38. Zong, H.; Liu, S.; Xing, R.; Chen, X.; Li, P. Protective effect of chitosan on photosynthesis and antioxidative defense system in edible rape (Brassica rapa L.) in the presence of cadmium. *Ecotoxicol. Environ. Saf.* **2017**, *138*, 271–278,

461 doi:10.1016/j.ecoenv.2017.01.009.

462 39. Zhu, J.-K. Regulation of ion homeostasis under salt stress. *Curr. Opin. Plant Biol.* **2003**, *6*,
463 441–445, doi:10.1016/S1369-5266(03)00085-2.

464 40. Rowe, J. H.; Topping, J. F.; Liu, J.; Lindsey, K. Abscisic acid regulates root growth under
465 osmotic stress conditions via an interacting hormonal network with cytokinin, ethylene and
466 auxin. *New Phytol.* **2016**, *211*, 225–239, doi:10.1111/nph.13882.

467 41. Kaushik, D.; Roychoudhury, A. Reactive oxygen species (ROS) and response of
468 antioxidants as ROS-scavengers during environmental stress in plants. *Front. Environ. Sci.*
469 **2014**, *2*, 53 (1-13), doi:10.3389/fenvs.2014.00053.

470 42. Alharby, H.; Metwali, E.; Fuller, M.; Aldhebiani, A. Impact of application of zinc oxide
471 nanoparticles on callus induction, plant regeneration, element content and antioxidant
472 enzyme activity in tomato (Solanum lycopersicum Mill.) under salt stress. *Arch. Biol. Sci.*
473 **2016**, *68*, 723–735, doi:10.2298/ABS151105017A.

474 43. Siddiqui, M. H.; Al-Whaibi, M. H.; Faisal, M.; Al Sahli, A. A. Nano-silicon dioxide
475 mitigates the adverse effects of salt stress on Cucurbita pepo L. *Environ. Toxicol. Chem.*
476 **2014**, *33*, 2429–2437, doi:10.1002/etc.2697.

477 44. Jabeen, N.; Ahmad, R. The activity of antioxidant enzymes in response to salt stress in
478 safflower (Carthamus tinctorius L.) and sunflower (Helianthus annuus L.) seedlings raised
479 from seed treated with chitosan. *J. Sci. Food Agric.* **2013**, *93*, 1699–1705,
480 doi:10.1002/jsfa.5953.

481 45. Choudhary, D. K.; Prakash, A.; Johri, B. N. Induced systemic resistance (ISR) in plants:
482 Mechanism of action. *Indian J. Microbiol.* **2007**, *47*, 289–297, doi:10.1007/s12088-007-
483 0054-2.

484 46. Fu, Z. Q.; Dong, X. Systemic Acquired Resistance: Turning Local Infection into Global
485 Defense. *Annu. Rev. Plant Biol.* **2013**, *64*, 839–863, doi:10.1146/annurev-arplant-042811-
486 105606.

487 47. Shoresh, M.; Harman, G. E.; Mastouri, F. Induced Systemic Resistance and Plant
488 Responses to Fungal Biocontrol Agents. *Annu. Rev. Phytopathol.* **2010**, *48*, 21–43,
489 doi:10.1146/annurev-phyto-073009-114450.

490 48. Wasternack, C.; Hause, B. Jasmonates: Biosynthesis, perception, signal transduction and
491 action in plant stress response, growth and development. An update to the 2007 review in
492 Annals of Botany. *Ann. Bot.* 2013, *111*, 1021–1058.

493 49. Zhu, Z. Molecular basis for jasmonate and ethylene signal interactions in Arabidopsis. *J.*
494 *Exp. Bot.* 2014, *65*, 5743–5748.

495 50. Fragnire, C.; Serrano, M.; Abou-Mansour, E.; Métraux, J. P.; L'Haridon, F. Salicylic acid
496 and its location in response to biotic and abiotic stress. *FEBS Lett.* **2011**, *585*, 1847–1852,
497 doi:10.1016/j.febslet.2011.04.039.

51. Jia, X.; Meng, Q.; Zeng, H.; Wang, W.; Yin, H. Chitosan oligosaccharide induces resistance to Tobacco mosaic virus in Arabidopsis via the salicylic acid-mediated signalling pathway. *Sci. Rep.* **2016**, *6*, 26144, doi:10.1038/srep26144.

52. Doares, S. H.; Syrovets, T.; Weiler, E. W.; Ryan, C. A. Oligogalacturonides and chitosan activate plant defensive genes through the octadecanoid pathway. *Proc. Natl. Acad. Sci.* **1995**, *92*, 4095–4098, doi:10.1073/pnas.92.10.4095.

53. Jiang, F.; Shen, Y.; Ma, C.; Zhang, X.; Cao, W.; Rui, Y. Effects of TiO2 nanoparticles on wheat (Triticum aestivum L.) seedlings cultivated under super-elevated and normal CO2 conditions. *PLoS One* **2017**, *12*, doi:10.1371/journal.pone.0178088.

54. Jayakannan, M.; Bose, J.; Babourina, O.; Shabala, S.; Massart, A.; Poschenrieder, C.; Rengel, Z. The NPR1-dependent salicylic acid signalling pathway is pivotal for enhanced salt and oxidative stress tolerance in Arabidopsis. *J. Exp. Bot.* **2015**, *66*, 1865–1875, doi:10.1093/jxb/eru528.

55. Zhao, Y.; Dong, W.; Zhang, N.; Ai, X.; Wang, M.; Huang, Z.; Xiao, L.; Xia, G. A Wheat Allene Oxide Cyclase Gene Enhances Salinity Tolerance via Jasmonate Signaling. *PLANT Physiol.* **2014**, *164*, 1068–1076, doi:10.1104/pp.113.227595.

CONCLUSIONES GENERALES

Ninguna de las concentraciones de nanopartículas de cobre en hidrogeles de quitosán-PVA evaluadas en este estudio fue tóxica para las plantas de tomate. La concentración de 10 mg de nanopartículas de cobre fue la que promovió mayor crecimiento, aumentando el diámetro de tallo, el peso fresco y seco de las raíces, el número de racimos y frutos por planta.

Las nanopartículas de cobre aumentaron la actividad de las enzimas catalasa, ascorbato peroxidasa, glutatión peroxidasa, superóxido dismutasa y fenilalanina amonio liasa, y mayor cantidad de glutatión reducido en las hojas. También aumentaron el contenido de nitrógeno, vitamina C y licopeno en los frutos.

Las nanopartículas de cobre y el quitosán a granel disminuyeron la acumulación de Na^+ en las hojas, mientras que el quitosán a granel aumentó el contenido de clorofila, carotenoides y fenoles totales en comparación con las plantas tratadas únicamente con 100 mM de NaCl. Las nanopartículas de cobre y el quitosán a granel sobre expresaron los genes SOD y JA en comparación al control. En combinación con estrés salino también sobre expresaron los genes SOD y JA.

Este estudio sugiere que las nanopartículas de cobre-quitosán promueven el crecimiento y desarrollo de las plantas, activan el mecanismo de defensa antioxidante de las plantas y aumentan los compuestos nutracéuticos en los frutos. Además, se deduce que las nanopartículas de cobre-quitosán causan estrés oxidativo en las plantas que activa el mecanismo de defensa antioxidante y la vía de señalización del ácido jasmónico en las plantas de tomate. Inducen tolerancia al estrés salino eliminando ROS y regulando la homeostasis iónica y osmótica a través de la activación de la vía del ácido jasmónico.

REFERENCIAS

Ahmed, E. M. (2015). Hydrogel: Preparation, characterization, and applications: A review. *J. Adv. Res.* 6, 105–121. doi:10.1016/j.jare.2013.07.006.

Akhnoukh, A. K. (2017). Implementation of nanotechnology in improving the environmental compliance of construction projects in the United States. *Part. Sci. Technol.*, 1–5. doi:10.1080/02726351.2016.1256359.

Alharby, H. F., Metwali, E. M. R., Fuller, M. P., and Aldhebiani, A. Y. (2016). The alteration of mRNA expression of SOD and GPX genes, and proteins in tomato (*Lycopersicon esculentum* Mill) under stress of NaCl and/or ZnO nanoparticles. *Saudi J. Biol. Sci.* doi:10.1016/j.sjbs.2016.04.012.

Ali, A., Muhammad, M. T. M., Sijam, K., and Siddiqui, Y. (2011). Effect of chitosan coatings on the physicochemical characteristics of Eksotika II papaya (Carica papaya L.) fruit during cold storage. *Food Chem.* 124, 620–626. doi:10.1016/j.foodchem.2010.06.085.

Aruna, A., Karthick Raja Namasivayam, S., and Allen Roy, E. (2015). Comparative evaluation of non target toxic effect of free and polymer coated chemogenic metallic nanoparticles against Vigna mungo. *Int. J. Pharma Bio Sci.* 6, B461–B467.

Avellan, A., Schwab, F., Masion, A., Chaurand, P., Borschneck, D., Vidal, V., et al. (2017). Nanoparticle Uptake in Plants: Gold Nanomaterial Localized in Roots of Arabidopsis thaliana by X-Ray Computed Nanotomography and Hyperspectral Imaging. *Environ. Sci. Technol.* 51, 8682–8691. doi:10.1021/acs.est.7b01133.

Binnig, G., and Rohrer, H. (1986). Scanning Tunneling Microscopy. *IBM J. Res. Dev.* 30, 355.

Bueno, O. (2004). The Drexler-Smalley debate on nanotechnology: Incommensurability at work? *Hyle* 10, 83–98. doi:10.1142/9789812773975_0003.

Cai, F., Wu, X., Zhang, H., Shen, X., Zhang, M., Chen, W., et al. (2017). Impact of TiO_2 nanoparticles on lead uptake and bioaccumulation in rice (*Oryza sativa* L.). *NanoImpact* 5, 101–108. doi:10.1016/j.impact.2017.01.006.

Chandra, S., Chakraborty, N., Dasgupta, A., Sarkar, J., Panda, K., and Acharya, K. (2015). Chitosan nanoparticles: A positive modulator of innate immune responses in plants. *Sci. Rep.* 5, 15195. doi:10.1038/srep15195.

Corredor, E., Testillano, P. S., Coronado, M.-J., González-Melendi, P., Fernández-Pacheco, R., Marquina, C., et al. (2009). Nanoparticle penetration and transport in living pumpkin plants: in situ subcellular identification. *BMC Plant Biol.* 9, 45. doi:10.1186/1471-2229-9-45.

Deinlein, U., Stephan, A. B., Horie, T., Luo, W., Xu, G., and Schroeder, J. I. (2014). Plant salt-tolerance mechanisms. *Trends Plant Sci.* 19, 371–379. doi:10.1016/j.tplants.2014.02.001.

Dimkpa, C. O., McLean, J. E., Britt, D. W., and Anderson, A. J. (2015). Nano-CuO and interaction with nano-ZnO or soil bacterium provide evidence for the interference of nanoparticles in metal nutrition of plants. *Ecotoxicology* 24, 119–129. doi:10.1007/s10646-014-1364-x.

Du, W., Tan, W., Peralta-Videa, J. R., Gardea-Torresdey, J. L., Ji, R., Yin, Y., et al. (2017). Interaction of metal oxide nanoparticles with higher terrestrial plants: Physiological and biochemical aspects. *Plant Physiol. Biochem.* 110, 210–225. doi:10.1016/j.plaphy.2016.04.024.

El-Miniawy, S. M., Ragab, M. E., Youssef, S. M., and Metwally, A. A. (2013). Response of Strawberry Plants to Foliar Spraying of Chitosan. *Res. J. Agric. Biol. Sci.* 9, 366–372.

Feng, Y., Cui, X., He, S., Dong, G., Chen, M., Wang, J., et al. (2013). The Role of Metal Nanoparticles in Influencing Arbuscular Mycorrhizal Fungi Effects on Plant Growth. *Environ. Sci. Technol.* 47, 9496–9504.

González-Melendi, P., Fernández-Pacheco, R., Coronado, M. J., Corredor, E., Testillano, P. S., Risueño, M. C., et al. (2008). Nanoparticles as smart treatment-delivery systems in plants: Assessment of different techniques of microscopy for their visualization in plant tissues. *Ann. Bot.* 101, 187–195. doi:10.1093/aob/mcm283.

Grillo, R., Rosa, A. H., and Fraceto, L. F. (2015). Engineered nanoparticles and organic matter: A review of the state-of-the-art. *Chemosphere* 119, 608–619. doi:10.1016/j.chemosphere.2014.07.049.

Guibal, E., Vincent, T., and Navarro, R. (2014). Metal ion biosorption on chitosan for the synthesis of advanced materials. *J. Mater. Sci.* 49, 5505–5518. doi:10.1007/s10853-014-8301-5.

Iavicoli, I., Leso, V., Beezhold, D. H., and Shvedova, A. A. (2017). Nanotechnology in agriculture: Opportunities, toxicological implications, and occupational risks. *Toxicol. Appl. Pharmacol.* 329, 96–111. doi:10.1016/j.taap.2017.05.025.

Juárez-Maldonado, A., Ortega-Ortíz, H., Pérez-Labrada, F., Cadenas-Pliego, G., and Benavides-Mendoza, A. (2016). Cu Nanoparticles absorbed on chitosan hydrogels positively alter morphological, production, and quality characteristics of tomato. *J. Appl. Bot. Food Qual.* 89, 183–189. doi:10.5073/JABFQ.2016.089.023.

Kasana, R. C., Panwar, N. R., Kaul, R. K., and Kumar, P. (2017). Biosynthesis and effects of copper nanoparticles on plants. *Environ. Chem. Lett.* 15, 233–240. doi:10.1007/s10311-017-0615-5.

Kashyap, P. L., Xiang, X., and Heiden, P. (2015). Chitosan nanoparticle based delivery systems for sustainable agriculture. *Int. J. Biol. Macromol.* 77, 36–51. doi:10.1016/j.ijbiomac.2015.02.039.

Khan, M. N., Mobin, M., Abbas, Z. K., AlMutairi, K. A., and Siddiqui, Z. H. (2017).

Role of nanomaterials in plants under challenging environments. *Plant Physiol. Biochem.* 110, 194–209. doi:10.1016/j.plaphy.2016.05.038.

Kroto, H. W., Allaf, A. W., and Balm, S. P. (1991). C60: Buckminsterfullerene. *Chem. Rev.* 91, 1213–1235. doi:10.1021/cr00006a005.

Lahiani, M. H., Dervishi, E., Ivanov, I., Chen, J., and Khodakovskaya, M. (2016). Comparative study of plant responses to carbon-based nanomaterials with different morphologies. *Nanotechnology* 27. doi:10.1088/0957-4484/27/26/265102.

Lee, W.-M., An, Y.-J., Yoon, H., and Kweon, H.-S. (2008). Toxicity and bioavailability of copper nanoparticles to the terrestrial plants mung bean (Phaseolus radiatus) and wheat (Triticum aestivum): plant agar test for water-insoluble nanoparticles. *Environ. Toxicol. Chem.* 27, 1915–1921. doi:10.1897/07-481.1.

Li, H., Ye, X., Guo, X., Geng, Z., and Wang, G. (2016a). Effects of surface ligands on the uptake and transport of gold nanoparticles in rice and tomato. *J. Hazard. Mater.* 314, 188–196. doi:10.1016/j.jhazmat.2016.04.043.

Li, M., Ahammed, G. J., Li, C., Bao, X., Yu, J., Huang, C., et al. (2016b). Brassinosteroid Ameliorates Zinc Oxide Nanoparticles-Induced Oxidative Stress by Improving Antioxidant Potential and Redox Homeostasis in Tomato Seedling. *Front. Plant Sci.* 7, 615. doi:10.3389/fpls.2016.00615.

Ma, C., Liu, H., Guo, H., Musante, C., Coskun, S. H., Nelson, B. C., et al. (2016). Defense mechanisms and nutrient displacement in Arabidopsis thaliana upon exposure to CeO_2 and In_2O_3 nanoparticles. *Environ. Sci. Nano* 3, 1369–1379. doi:10.1039/C6EN00189K.

Ma, X., Geisler-Lee, J., Deng, Y., and Kolmakov, A. (2014). Corrigendum to "Interactions between engineered nanoparticles (ENPs) and plants: Phytotoxicity, uptake and accumulation" [Sci. Total Environ. 408 (2010) 3053-3061]. *Sci. Total Environ.* 481, 635. doi:10.1016/j.scitotenv.2014.02.044.

Marslin, G., Sheeba, C. J., and Franklin, G. (2017). Nanoparticles Alter Secondary Metabolism in Plants via ROS Burst. *Front. Plant Sci.* 8, 832. doi:10.3389/fpls.2017.00832.

Mondal, M. M. A., Malek, M. A., Puteh, A. B., Ismail, M. R., Ashrafuzzaman, M., and Naher, L. (2012). Effect of foliar application of chitosan on growth and yield in okra. *Aust. J. Crop Sci.* 6, 918–921.

Nair, P. M. G., and Chung, I. M. (2014a). A Mechanistic Study on the Toxic Effect of Copper Oxide Nanoparticles in Soybean (Glycine max L.) Root Development and Lignification of Root Cells. *Biol. Trace Elem. Res.* 162, 342–352.

Nair, P. M. G., and Chung, I. M. (2014b). Impact of copper oxide nanoparticles exposure on Arabidopsis thaliana growth, root system development, root lignificaion, and molecular level changes. *Environ. Sci. Pollut. Res.* 21, 12709–12722. doi:10.1007/s11356-014-3210-3.

Parihar, P., Singh, S., Singh, R., Singh, V. P., and Prasad, S. M. (2015). Effect of salinity stress on plants and its tolerance strategies: a review. *Environ. Sci. Pollut. Res.* 22, 4056–4075. doi:10.1007/s11356-014-3739-1.

Pérez-de-Luque, A. (2017). Interaction of Nanomaterials with Plants: What Do We Need for Real Applications in Agriculture? *Front. Environ. Sci.* 5, 1–7. doi:10.3389/fenvs.2017.00012.

Pichyangkura, R., and Chadchawan, S. (2015). Biostimulant activity of chitosan in horticulture. *Sci. Hortic. (Amsterdam).* 196, 49–65. doi:10.1016/j.scienta.2015.09.031.

Pradhan, S., Patra, P., Mitra, S., Dey, K. K., Basu, S., Chandra, S., et al. (2015). Copper nanoparticle (CuNP) nanochain arrays with a reduced toxicity response: a biophysical and biochemical outlook on Vigna radiata. *J. Agric. Food Chem.* 63, 2606–17. doi:10.1021/jf504614w.

Prasad, R., Bhattacharyya, A., and Nguyen, Q. D. (2017). Nanotechnology in sustainable agriculture: Recent developments, challenges, and perspectives. *Front. Microbiol.* 8. doi:10.3389/fmicb.2017.01014.

Qados, A. M. S. A. (2015). Mechanism of nanosilicon-mediated alleviation of salinity stress in faba bean (*Vicia faba* L.) plants. *Am. J. Exp. Agric.* 7, 78–95. doi:10.9734/AJEA/2015/15110.

Quan, R., Wang, J., Yang, D., Zhang, H., Zhang, Z., and Huang, R. (2017). EIN3 and SOS2 synergistically modulate plant salt tolerance. *Sci Rep* 7, 44637. doi:10.1038/srep44637.

Raliya, R., Franke, C., Chavalmane, S., Nair, R., Reed, N., and Biswas, P. (2016). Quantitative Understanding of Nanoparticle Uptake in Watermelon Plants. *Front. Plant Sci.* 7. doi:10.3389/fpls.2016.01288.

Reddy, P. V. L., Hernandez-Viezcas, J. A., Peralta-Videa, J. R., and Gardea-Torresdey, J. L. (2016). Lessons learned: Are engineered nanomaterials toxic to terrestrial plants? *Sci. Total Environ.* 568, 470–479. doi:10.1016/j.scitotenv.2016.06.042.

Rico, C. M., Majumdar, S., Duarte-Gardea, M., Peralta-Videa, J. R., and Gardea-Torresdey, J. L. (2011). Interaction of nanoparticles with edible plants and their possible implications in the food chain. *J. Agric. Food Chem.* 59, 3485–3498. doi:10.1021/jf104517j.

Rossi, L., Zhang, W., and Ma, X. (2017). Cerium oxide nanoparticles alter the salt stress tolerance of *Brassica napus* L. by modifying the formation of root apoplastic barriers. *Environ. Pollut.* 229, 132–138. doi:10.1016/j.envpol.2017.05.083.

Rowe, J. H., Topping, J. F., Liu, J., and Lindsey, K. (2016). Abscisic acid regulates root growth under osmotic stress conditions via an interacting hormonal network with cytokinin, ethylene and auxin. *New Phytol.* 211, 225–239. doi:10.1111/nph.13882.

Sabo-Attwood, T., Unrine, J. M., Stone, J. W., Murphy, C. J., Ghoshroy, S., Blom, D., et al. (2012). Uptake, distribution and toxicity of gold nanoparticles in tobacco (Nicotiana xanthi) seedlings. *Nanotoxicology* 6, 353–360. doi:10.3109/17435390.2011.579631.

Saharan, V., and Pal, A. (2016). *Chitosan Based Nanomaterials in Plant Growth and Protection*. Springer International Publishing doi:10.1007/978-81-322-3601-6.

Saharan, V., Sharma, G., Yadav, M., Choudhary, M. K., Sharma, S. S., Pal, A., et al. (2015). Synthesis and in vitro antifungal efficacy of Cu–chitosan nanoparticles against pathogenic fungi of tomato. *Int. J. Biol. Macromol.* 75, 346–353. doi:10.1016/j.ijbiomac.2015.01.027.

Schymura, S., Fricke, T., Hildebrand, H., and Franke, K. (2017). Elucidating the Role of Dissolution in CeO_2 Nanoparticle Plant Uptake by Smart Radiolabeling. *Angew. Chemie - Int. Ed.* 56, 7411–7414. doi:10.1002/anie.201702421.

Seifert, M. (2017). Nanotechnology. The Future is Tiny By Michael Berger. *Angew. Chemie Int. Ed.* 56, 7351–7351. doi:10.1002/anie.201704353.

Siddiqui, M. H., Al-Whaibi, M. H., Faisal, M., and Al Sahli, A. A. (2014). Nano-silicon dioxide mitigates the adverse effects of salt stress on Cucurbita pepo L. *Environ. Toxicol. Chem.* 33, 2429–2437. doi:10.1002/etc.2697.

Sierra-Ávila, R., Pérez-Alvarez, M., Cadenas-Pliego, G., Ávila-Orta, C. A., Betancourt-Galindo, R., Jiménez-Regalado, E., et al. (2014). Synthesis of copper nanoparticles coated with nitrogen ligands. *J. Nanomater.* 2014. doi:10.1155/2014/361791.

Sosan, A., Svistunenko, D., Straltsova, D., Tsiurkina, K., Smolich, I., Lawson, T., et al. (2016). Engineered silver nanoparticles are sensed at the plasma membrane and dramatically modify the physiology of Arabidopsis thaliana plants. *Plant J.* 85, 245–257. doi:10.1111/tpj.13105.

Spielman-Sun, E., Lombi, E., Donner, E., Howard, D. L., Unrine, J. M., and Lowry, G. V. (2017). Impact of surface charge on cerium oxide nanoparticle uptake and translocation by wheat (Triticum aestivum). *Environ. Sci. Technol.*, acs.est.7b00813. doi:10.1021/acs.est.7b00813.

Taylor, A. F., Rylott, E. L., Anderson, C. W. N., and Bruce, N. C. (2014). Investigating the toxicity, uptake, nanoparticle formation and genetic response of plants to gold. *PLoS One* 9. doi:10.1371/journal.pone.0093793.

Tripathi, D. K., Mishra, R. K., Singh, S., Singh, S., Vishwakarma, K., Sharma, S., et al. (2017). Nitric oxide ameliorates zinc oxide nanoparticles phytotoxicity in wheat seedlings: Implication of the ascorbate–glutathione cycle. *Front. Plant Sci.* 8. doi:10.3389/fpls.2017.00001.

Vankova, R., Landa, P., Podlipna, R., Dobrev, P. I., Prerostova, S., Langhansova, L., et al. (2017). ZnO nanoparticle effects on hormonal pools in Arabidopsis thaliana. *Sci. Total Environ.* 593–594, 535–542. doi:10.1016/j.scitotenv.2017.03.160.

Vinković, T., Novák, O., Strnad, M., Goessler, W., Jurašin, D. D., Parađiković, N., et al. (2017). Cytokinin response in pepper plants (*Capsicum annuum* L.) exposed to silver nanoparticles. *Environ. Res.* 156, 10–18. doi:10.1016/j.envres.2017.03.015.

Wang, F., Liu, X., Shi, Z., Tong, R., Adams, C. A., and Shi, X. (2016). Arbuscular mycorrhizae alleviate negative effects of zinc oxide nanoparticle and zinc accumulation in maize plants - A soil microcosm experiment. *Chemosphere* 147, 88–97. doi:10.1016/j.chemosphere.2015.12.076.

Wang, P., Lombi, E., Sun, S., Scheckel, K. G., Malysheva, A., McKenna, B. A., et al. (2017a). Characterizing the uptake, accumulation and toxicity of silver sulfide nanoparticles in plants. *Environ. Sci. Nano* 4, 448–460. doi:10.1039/C6EN00489J.

Wang, Y., Chen, R., Hao, Y., Liu, H., Song, S., and Sun, G. (2017b). Transcriptome analysis reveals differentially expressed genes (DEGs) related to lettuce (Lactuca sativa) treated by TiO2/ZnO nanoparticles. *Plant Growth Regul.* 83, 13–25. doi:10.1007/s10725-017-0280-5.

Wong, M. H., Misra, R. P., Giraldo, J. P., Kwak, S. Y., Son, Y., Landry, M. P., et al. (2016). Lipid Exchange Envelope Penetration (LEEP) of Nanoparticles for Plant Engineering: A Universal Localization Mechanism. *Nano Lett.* 16, 1161–1172. doi:10.1021/acs.nanolett.5b04467.

Younes, I., and Rinaudo, M. (2015). Chitin and chitosan preparation from marine sources. Structure, properties and applications. *Mar. Drugs* 13, 1133–1174. doi:10.3390/md13031133.

Zhang, H., Zhao, X., Yang, J., Yin, H., Wang, W., Lu, H., et al. (2011). Nitric oxide production and its functional link with OIPK in tobacco defense response elicited by chitooligosaccharide. *Plant Cell Rep.* 30, 1153–1162. doi:10.1007/s00299-011-1024-z.

Zhang, W., Dan, Y., Shi, H., and Ma, X. (2017). Elucidating the mechanisms for plant uptake and in-planta speciation of cerium in radish (*Raphanus sativus* L.) treated with cerium oxide nanoparticles. *J. Environ. Chem. Eng.* 5, 572–577. doi:10.1016/j.jece.2016.12.036.

Zhu, J.-K. (2003). Regulation of ion homeostasis under salt stress. *Curr. Opin. Plant Biol.* 6, 441–445. doi:10.1016/S1369-5266(03)00085-2.