UNIVERSIDAD AUTÓNOMA AGRARIA ANTONIO NARRO

SUBDIRECCIÓN DE POSTGRADO

INDUCCIÓN DE TOLERANCIA AL ESTRÉS POR *Fusarium* sp. Y *Phytophthora infestans* EN TOMATE MEDIANTE LA APLICACIÓN DE SILICIO

Tesis

Que presenta MARI CARMEN LÓPEZ PÉREZ

como requisito parcial para obtener el Grado de

DOCTOR EN CIENCIAS EN AGRICULTURA PROTEGIDA

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Elaborada por MARI CARMEN LÓPEZ PÉREZ 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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Julio 2018

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A mi hermano Tony

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A mi abuela Ángela

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Full author list: Mari Carmen López-Pérez; Adalberto Benavides-Mendoza; Armando Robledo-Olivo; América Bereni
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With kind regards,

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ÍNDICE

INTRODUCCIÓN

El tomate (*Lycopersicon esculentum* Mill) es uno de los cultivos más importantes desde el punto de vista económico y productivo, para su manejo frecuentemente se emplean fertilizantes químicos en grandes cantidades (Rabie *et al*., 2004). El crecimiento, rendimiento y calidad del cultivo del tomate se ve mermado por enfermedades ocasionadas por hongos, bacterias, nematodos y virus, debido a la reducción de la disponibilidad de los nutrientes, su absorción, distribución y utilización por la planta (Romero *et al*., 2011; Leyva-Mir *et al*., 2013). Los fitopatógenos de mayor importancia en el cultivo de tomate son *Fusarium* sp. y *Phytophthora infestans*. *Fusarium* sp. es un patógeno facultativo del suelo distribuido mundialmente, causa severos daños vasculares así como pudrición y marchitez de las raíces en las variedades de tomate de importancia económica (Michielse *et al*., 2009). Por su parte *Phytophthora infestans* causa el tizón tardío en la familia *Solanaceae*, siendo una gran amenaza para el cultivo de tomate en todo el mundo (Kröner *et al*., 2017). Ambos fitopatógenos producen pérdidas de entre 60 al 100% en el cultivo de tomate (González, 2012). El control químico es la principal medida para el manejo de las enfermedades de estos fitopatógenos (Solarte *et al*., 2012), desafortunadamente el uso indiscriminado de este ha conllevado al desarrollo de poblaciones de hongos resistentes (Hamza *et al*., 2016). Por lo anterior, es importante implementar nuevas alternativas viables para reducir la incidencia y severidad de dichos patógenos.

El silicio (Si) es el segundo elemento más abundante en la corteza terrestre, siendo superado únicamente por el oxígeno. Está presente en la solución del suelo en forma de ácido monosilícico (H_4SiO_4), que es la única manera de absorción por las raíces, transportado así a través del xilema por la corriente de transpiración y con ayuda de transportadores (Pontigo *et al*., 2015). El silicio es considerado como un elemento mineral benéfico para las plantas, promueve el crecimiento, desarrollo y por ende genera mayor rendimiento y calidad de fruto en los cultivos (Malhotra *et al*., 2016). Así mismo, el suplemento de este mineral a las plantas reduce los efectos provocados por el estrés abiótico causado por sales, alta temperatura, sequía (Meharg y Meharg, 2015), alta radiación, toxicidad de los metales (Vaculík *et al*., 2012), desequilibrio de nutrientes

(Liang *et al*., 2006), congelación, entre otros. Así como la disminución de estrés biótico, incluyendo la disminución de la susceptibilidad de plagas, hongos y bacterias. El modo de acción del Si en las plantas es mediante la inducción de barreras físicas y bioquímicas (Pozza *et al*., 2015). En la tolerancia a patógenos, la formación de depósitos de Si en la raíz y en las hojas podría evitar el paso de estos (Ma y Yamaji, 2008). También este mineral puede inducir respuestas defensivas similares a la resistencia sistémica adquirida, al incrementar la actividad de enzimas antioxidantes y la producción de lignina, fitoalexinas y proteínas relacionadas con la patogénesis, además de que puede activar la expresión de genes relacionados con la defensa y puede sumarse a la transducción de señalizadores de estrés como ácido salicílico, ácido jasmónico y etileno (Cai *et al*., 2009). Por otra parte, existe poca información sobre el manejo de la fertilización con Si. Se ha observado que la solubilidad del Si está estrechamente relacionado con la mayor absorción por los cultivos, dicha solubilidad está influenciado por las condiciones fisicoquímicos del suelo como pH, temperatura y materia orgánica, así como los parámetros ambientales PAR, CO_2 y temperatura del aire, por ello es importante el manejo de estas condiciones para lograr una mayor absorción y acumulación de Si en los cultivos y así obtener mejores resultados en los cultivos. (López-Pérez *et al*., 2018). Existe una serie de trabajos donde se ha evaluado el efecto del Si contra patógenos en los cultivos. Fortunato *et al*. (2012) y Fortunato *et al*. (2014) demostraron que el suministro de Si a las plantas de banano redujo la intensidad de la marchitez por *Fusarium*. La aplicación de silicato de sodio y potasio en algodón *in vitro* controló la actividad de *Fusarium* (Yassin *et al*., 2016). La severidad de tizón de la vaina causada por *Rhizoctonia solani* en plantas de arroz, también se redujo con aplicación de ácido monosilícico (Schurt *et al*., 2012). Además se observaron reducciones (80%) en las áreas de lesión causado por antracnosis en los frutos de pimiento tratadas con silicato de potasio (Jayawardana *et al*., 2015), así como en *Phytophthora cinnamomi* en aguacate (Bekker *et al*., 2014) al aplicar silicato de potasio. Considerando lo anterior se planteó como objetivo evaluar la aplicación de silicio sobre el crecimiento de plantas de tomate y la calidad de fruto bajo estrés biótico causado por *Fusarium oxysporum* y *Phytophthora infestans*.

Por todo lo anterior el objetivo de este trabajo fue evaluar la inducción de tolerancia en tomate bajo estrés por *Fusarium* sp. y *Phytophthora infestans* mediante la aplicación de silicio.

REVISIÓN DE LITERATURA

Importancia del cultivo de tomate

El tomate (*Lycopersicon esculentum* Mill) es considerado una de las hortalizas más importantes en el mundo, desde el punto de vista económico y productivo, ya que tiene una alta demanda en el consumo humano y por qué ocupa la mayor superficie sembrada (Rabie *et al*., 2004). Los principales países productores son China, Estados Unidos, India, Turquía, y Egipto (SAGARPA, 2010). México se encuentra en el décimo lugar con casi tres millones de toneladas anuales de producción, siendo Sinaloa, Baja California, San Luis Potosí y Michoacán los principales estados productores. El consumo per cápita en México es de 13.8 kg, y es exportado principalmente a Estados Unidos, Canadá y Japón. Las exportaciones de esta hortaliza alcanzaron los 1,773 millones de dólares (SAGARPA, 2016). Por ello, el cultivo de esta hortaliza es una de las actividades más relevantes para la economía de México, al generar altos ingresos y empleos (SAGARPA, 2017).

Enfermedades ocasionadas por hongos fitopatógenos en el cultivo de tomate.

Las enfermedades de mayor importancia en el tomate son originados por hongos fitopatógenos del suelo. Estos microorganismos se encuentran de manera natural en suelos agrícolas, pueden permanecer por mucho tiempo en el suelo, y al presentarse las condiciones adecuadas se pueden reproducir con facilidad y generar enfermedades de tipo infeccioso. Estos patógenos causan pudriciones de cuello y raíz en las plantas, repercutiendo en el crecimiento y rendimiento del cultivo, causando grandes pérdidas económicas (Fernández-Herrera *et al.,* 2013).

Fusarium sp. Este microorganismo es un hongo imperfecto, saprofito del suelo, se encuentra clasificado dentro de la clase *Sordariomycetes*, Orden *Hypocreales* en la familia *Nectriaceae* (Gonzalez-Morales *et al*., 2015). Presenta un micelio de color crema o rosa pálido o púrpura, produce tres tipos de esporas asexuales: microconidios (constituidos por 1-2 células); esporas que se produce con mayor frecuencia y abundancia, principalmente en el interior de los vasos de las plantas hospedantes,

macroconidios (constituidos por 3-5 células); son las esporas típicas de *Fusarium*, de pared delgada y encorvadas hacia los extremos, aparecen sobre la superficie de las plantas destruidas, generalmente en grupos similares a los esporodoquios, y clamidosporas (constituidas por 1-2 células); son redondas de pared gruesa, se forman en el micelio más viejo o en los macroconidios del hongo. Para ingresar a la planta, el hongo penetra directamente a las puntas de las raíces o a través de heridas, una vez dentro, el micelio se propaga a través de las células de la raíz y del córtex, avanzando hacia los vasos de xilema en sentido ascendente hacia el tallo, en ocasiones llega a los frutos y algunas veces puede infectar las semillas (Agrios, 2005). El marchitamiento causado por *Fusarium* sp. es una de las enfermedades más prevalentes y dañinas del tomate, puede ocasionar pérdidas considerables del 60% al 90% en el rendimiento del cultivo y afectar la calidad del fruto (Ascencio-Álvarez *et al.*, 2008; Mesterházy *et al*., 2018). Los síntomas se manifiestan en achaparramiento, amarillamiento y encorvamiento de las hojas, seguido del marchitamiento, defoliación y finalmente la muerte, dentro de la planta, se observa el oscurecimiento vascular en los tallos y peciolos infectados de las hojas más viejas (Agrios, 2005).

Phytophthora infestans. Son hongos oomicetos, considerados como diploides y cenocíticos, carecen de quitina en las células y producen oosporas; que son estructuras de supervivencia, también producen esporangios asexuales y/o zoosporas biflageladas; que son las que inician las infecciones en el follaje (Dyer *et al*., 1993). El ciclo de vida de este patógeno es estrictamente biotrófico en la naturaleza y se considera como un parásito casi obligado (Andrivon *et al*., 2013). La forma de infección en las plantas es por medio de una amplia gama de familias específicas de proteínas efectoras de enfermedades secretadas, por ejemplo elicitinas, además de muchos genes que se localizan en regiones altamente dinámicas y expandidas del genoma de este fitopatógeno y que se inducen durante la infección o tienen actividades que alteran la fisiología del huésped (Haas *et al*., 2009; Derevnina *et al*., 2016). Este hongo causa la enfermedad de tizón tardío, generando pérdidas en todo el mundo, su manejo se hace más difícil debido a su notable velocidad de adaptación (Haas *et al*., 2009; Domazakis *et al*., 2018).

Manejo de *Fusarium* sp. y *Phytophthora infestans*. Para el manejo de estas enfermedades generalmente se utilizan fungicidas sistémicos como los benzimidazoles y variedades resistentes (Reis *et al*., 2004; Zorn *et al*., 2017). Sin embargo el control de estas enfermedades es complejo debido a la poca disponibilidad de cultivares resistentes de tomate comercialmente aceptables, además los fungicidas tienen poca eficacia (0-40%) y el uso en exceso a la larga ha generado especies resistentes, además de la contaminación ambiental y el peligro a la salud humana (Ascencio-Álvarez *et al*., 2008; Mesterházy *et al*., 2018). Ante esta situación, es primordial desarrollar nuevos sistemas de tratamiento para el manejo de dichas enfermedades, con la finalidad de reducir la dependencia de los productos químicos (Villa-Martínez *et al.*, 2015).

De acuerdo con Rodríguez y Montilla (2002), el uso de sustancias inductoras de tolerancia al estrés es una de las medidas en las que se está haciendo énfasis, ya que permiten el control de la enfermedad disminuyendo el uso de productos químicos causando daños mínimos al ambiente. Además de ello, el empleo de estas sustancias potencializan los mecanismos intrínsecos de defensa, incrementando el éxito contra organismos patógenos (Ordeñana, 2002). Un ejemplo de estas sustancias inductoras es el elemento silicio.

El elemento silicio.

El silicio (Si) es el segundo elemento más abundante en la corteza terrestre, siendo superado únicamente por el oxígeno, se encuentra como sílice u óxidos de silicio en mayor abundancia como dióxido de silicio (SiO_2), su forma primordial es cristalina o amorfa (Markovich *et al*., 2017). Epstein (2009) menciona que se produce principalmente como un mineral inerte de arenas, cuarzo (SiO_2 puro), caolinita, mica, feldespato y otros minerales arcillosos.

La forma disponible para las plantas solamente es como ácido silicio [$Si(OH)_4$] lo cual resulta de la meteorización, principalmente de silicatos de arcilla. Epstein (1999) reportó que los niveles de Si dentro de la solución del suelo oscila entre los 2.8 y 16.8 mg dm^{-3} aunque puede ser dependiente de su solubilidad que a su vez es alterada por el pH del suelo (oscilando de 2-8.5), o por el tipo de suelo. Tal y como lo reportan Marafon & Endres (2013), al mencionar que en suelos de tipo arenoso se presentan cantidades muy

pequeñas de Si disponible en los horizontes superiores, en comparación a los suelos arcillosos, además el contenido de materia orgánica, iones metálicos y el potencial redox también puede influir para una mayor concentración de Si disponible para las plantas.

El silicio en las plantas.

El Si no es considerado esencial en la nutrición para las plantas, sin embargo se le ha denominado como un elemento benéfico, ya que promueve el crecimiento, desarrollo, rendimiento y calidad de los frutos de los cultivos, resultado de la inducción de resistencia y protección contra diversos factores ambientales bióticos y abióticos (Van Bockhaven *et al*., 2013; Malhotra *et al*., 2016).

El contenido de Si difiere en las diversas especies vegetales, así como en los diferentes tejidos de las plantas. Los niveles de Si suelen ser similares a los macronutrientes esenciales de las plantas (Bakhat *et al*., 2018). La concentración de Si oscila entre 0.1 a 10% o más, en base al peso seco (Epstein, 1999). Por esta razón las plantas son clasificadas en base a su contenido de Si de la siguiente manera: 1) las plantas con concentraciones superiores al 1.5% de Si de peso seco en sus hojas se les denomina acumuladoras, en este grupo se encuentran principalmente las gramíneas, como el arroz, cola de caballo (acumulan 10-15%), caña de azúcar, trigo, maíz, y algunas dicotiledóneas como el pepino (acumulan 3%), y algunas dicotiledóneas, en especial las leguminosas (acumulan 0.5%) (Mitani y Ma, 2005) y algunas hortalizas como el pepino, etc., 2) concentraciones inferiores a 0.5% se les considera no acumuladoras, como tomate, lechuga, papa etc. (Marafon y Endres, 2013).

Distribución del Si en las plantas. El Si solamente es absorbido por las plantas como $Si(OH)_4$ (McLarnon *et al*., 2017). Mediante la transpiración y con ayuda de transportadores el Si es distribuido a los diferentes órganos de las plantas, en mayor medida hacia las hojas, luego tallo y en menor proporción al fruto. Se ha cuantificado el contenido de Si en los diferentes órganos de las plantas, en *Phaseolus vulgaris* al ser biofortificado con 3.6 mM de K_2SiO_3 se logró incrementar un 360, 240 y 310% la concentración de Si en tallos, hojas y vainas, respectivamente (Montesano *et al*., 2016). Vulavala *et al*. (2016) encontraron pequeñas cantidades de Si en la epidermis de los tubérculos del cultivo de papa.

Absorción de Si por las plantas. Las plantas solamente pueden absorber silicio de la solución del suelo en forma de ácido silícico también denominado ácido monosilícico [Si(OH)4] en su forma no disociada (pK1 = 9.6), trasladándose de la solución del suelo hacia las raíces y al xilema (Marafon y Endres, 2013). Este proceso se da mediante un transporte pasivo y un activo, por un lado mediante la corriente de transpiración vía xilema (Bakhat *et al*., 2018), y por la acción conjunta de los transportadores Lsi1 y Lsi6 que son proteínas pertenecientes a Nodulin 26-like Intrinsic Proteins (NIP) de una subfamilia de acuaporinas, que están principalmente involucrados en la distribución de Si en los tejidos de raíz y brotes, y Lsi2 que está impulsada por protones y actúa como un anti porte Si/H+ (Pontigo *et al*., 2015). Las diferencias en la absorción de Si en las especies vegetales dependen de diferentes mecanismos, como la diferencia fisiológica y molecular entre la capacidad intra e interespecífica de captar el silicio por los transportadores (Ashraf *et al*., 2013), la localización celular de los transportadores de las plantas (Sun *et al*., 2017), además de las diferentes concentraciones de Si en el suelo puede originar alteraciones en la capacidad de captación de Si (Mitani-Ueno *et al*., 2016). En general existen dos mecanismos de absorción de Si por las plantas, el primero por parte de especies acumuladoras como el arroz, y en segundo por especies poco acumuladoras como el sorgo.

Absorción en el cultivo de arroz. El $Si(OH)_4$ es tomado en la raíz de manera pasiva por el transportador con actividad específica Lsi1 y es liberado de manera activa en el apoplasto por el transportador Lsi2, dentro del apoplasto el Si es transportado hacia la endodermis por Lsi1 para ser liberado en el cilindro vascular por Lsi2, este trasportador muestra un proceso activo (necesita de H^+ generados por una bomba ATP) (Vulavala *et al*., 2016; Sahebi *et al*., 2017). Una vez dentro de los haces vasculares el transportador Lsi6 (perteneciente al subgrupo intrínseco de proteínas nodulina-26 de las acuaporinas permeable al ácido silícico y ubicado en las células de transferencia del parénquima del xilema) se encarga de la transferencia intravascular del Si fuera del xilema y posteriormente la distribución de Si en la hoja (Yamaji y Ma, 2009). Hinrichs *et al*., (2017) reportaron que el Si promueve la formación de bandas de Caspary en el arroz, por lo tanto la función del transportador Lsi2 es importante en este cultivo, o en los cultivos con una doble banda de caspary.

Absorción en el cultivo de maíz y sorgo. De igual manera que en el cultivo de arroz, el $Si(OH)_4$ es tomado de manera pasiva por el transportador Lsi1 el cual es expresado en la membrana plasmática del lado distal de las células epidérmicas e hipodérmicas de la raíz y en células de la corteza en las raíces laterales (Mitani *et al*., 2009), posteriormente es transportado secuencialmente hasta el córtex donde el transportador Lsi2 (expresados solo en la membrana plasmática de la endodermis de la raíz) lleva a cabo la carga y descarga a los haces vasculares (Mitani *et al*., 2009). En cebada el transportador Lsi6 (se ubica en el lado adaxial de las células parenquimatosas del xilema en la vainas y en la hoja) se encarga de la absorción de Si en la punta de la raíz (Yamaji *et al*., 2012), así mismo participa en la descarga del xilema de Si en la hoja y en la vaina del haz además de intervenir en la transferencia intravascular de Si, el acoplamiento de Lsi6 y Lsi2 es importante para realizar la transferencia intervascular del Si ya que Lsi6 descarga del Si en las células de transferencia y Lsi2 se encarga de recargar Si a los haces vasculares difusos (Yamaji *et al*., 2012).

Tanslocación y deposición de Si por las plantas. Una vez traslocado en el xilema, el Si es transportado por la savia hacia todos los órganos de las plantas debido al proceso de transpiración, la concentración del Si en el xilema en las plantas acumuladoras supera los 2 mM (Bakhat *et al*., 2018). Cuando las concentraciones del Si se incrementan, pasan a ser transitorio, debido que son descargados a los diferentes tejidos, en particular al tejido foliar por la traspiración, el ácido silícico se polimeriza y forma una capa mineralizada subcuticular insoluble de sílice ($SiO2 \cdot nH2O$) (Meena *et al*., 2014), o es depositado en la pared celular de la epidermis de las hojas, espacios intracelulares y tricomas como cuerpos de sílice amorfo ($SiO2$) cristalizado denominados fitolitos (Cooke y Leishman, 2011; Markovich *et al*., 2017).

El silicio como agente anti-estrés en las plantas

Las plantas están constantemente sometidas a cualquier tipo de estrés biótico o abiótico. La implementación de algún bioestimulante como el Si en los cultivos agrícolas ha mejorado los efectos provocados por condiciones ambientales adversas, entre estos

factores se ha observado mitigación en la salinidad, sequía, bajas y altas temperaturas, estrés por metales pesados, deficiencia de nutrientes, estrés por patógenos, entre otros.
El modo de acción de este elemento es mediante barreras físicas, bioquímicas y moleculares, generando en la planta respuestas fisiológicas y metabólicas (Pozza *et al*., 2015). Las barreras físicas se dan por la deposición del elemento en los tejidos de las plantas, las barreras bioquímicas se induce por el incremento de actividad enzimática (glucanasa, peroxidasa, polifenol oxidasa y fenilalanina amoniacasa) o por acumulación de fenoles antimicrobianos glicosilados y diterpenoides fitoalexinas, y por la inducción de la expresión diferencial de proteínas relacionadas con respuestas al estrés, hormonas vegetales y biosíntesis celular (Muneer y Jeong, 2015). El resultado de todo esto es un mayor crecimiento de la planta, así como un mayor calidad de fruto, y aumento del contenido de minerales y antioxidantes (Neeru *et al*., 2016).

El Si contra el estrés de los fitopatógenos *Fusarium* sp. y *Phytophthora infestans*. El estrés por fitopatógenos es una de las principales causas de pérdidas de los cultivos. La aplicación de Si a los cultivos al suelo o vía foliar propicia diferentes mecanismos de acción para mitigar el efecto de los hongos fitopatógenos, resultado de mecanismos que se mencionan a continuación:
1) Barreras físicas; la silicificación celular en hojas forman una barrera física, fortaleciendo así la pared celular y aumentando la rigidez estructural, lo que dificulta la penetración de los patógenos (Sakr, 2016), o la restricción del movimiento del patógeno de las raíces hacia los tallos por el cambio estructural de la pared celular (Huang *et al*., 2011).
2) Propicia una inducción de resistencia por las plantas, desencadenando señales y reacciones bioquímicas de defensa, que se ve reflejado en un aumento en los compuestos bioquímicos y genómicos (Ning *et al*., 2014; Song *et al*., 2016). Como el incremento de fitoalexinas de momilactona derivado de la estimulación de la vía terpenoide en plantas de arroz, incremento de la actividad antioxidante enzimática y no enzimática, así como el aumento y disminución de genes, lo que incrementa su capacidad de tolerar el ataque de los fitopatógenos (Song *et al*., 2016). Además, la aplicación de Si al inducir un aumento de la biomasa radical se puede incrementar la liberación de exudados a la

rizósfera lo que conduce al aumento de la población de microorganismos benéficos que pueden competir con los patógenos (Raaijmakers *et al*., 2009).

Se han realizado una serie de investigaciones sobre la aplicación de Si a los cultivos bajo estrés por *Fusarium* sp. y *Phytophthora infestans*. Se ha demostraron que el suministro de Si a las plantas de banano y tomate redujo la intensidad de la marchitez por *Fusarium* (Fortunato *et al*., 2012; Chitarra *et al*., 2013 y Fortunato *et al*., 2014). La aplicación de silicato de sodio y potasio en algodón *in vitro* controló la actividad de *Fusarium* (Yassin *et al*., 2016). También se observaron reducciones en las áreas de lesión causado por *Phytophthora cinnamomi* en aguacate al aplicar silicato de potasio (Bekker *et al*., 2014) y *Phytophthora capsici* (reducción del 35%) en pimiento (French-Monar *et al*., 2010).

Manejo de las aplicaciones de Si en las plantas

Los efectos de la aplicación de Si difiere en los diferentes tipos de cultivo, debido a la variabilidad de la solubilidad de este elemento en el suelo, difiriendo en la absorción y acumulación del Si en las plantas. La variabilidad de la solubilidad del Si es resultado de factores como la diversidad de genotipos de los cultivos, disponibilidad del Si, condiciones fisicoquímicas del suelo y condiciones climáticas. Por ello es indispensable considerar todos estos aspectos para hacer un buen manejo de este mineral. Para una mayor disponibilidad del Si se pueden realizar aplicación exógenas (al suelo o al follaje), utilizando fertilizantes naturales (tierra diatomeas o arena de silice) o fuentes comerciales como silicatos (sodio, potasio y calcio). Los factores del suelo como el pH, temperatura, materia orgánica así como condiciones ambientales, también se deben considerar para el manejo del Si en los cultivos, ya que estos factores serán predominantes para la mayor absorción y acumulación de este elemento en las plantas (Seyfferth y Fendorf, 2012; McLarnon *et al*., 2017; López-Pérez *et al*., 2018).

En plantas poco acumuladoras de Si se tiene realizar un suministro continuó de Si en el suelo, ya que la absorción y deposición de este mineral depende de los niveles de Si en el sustrato, aunque la acumulación de Si sea mínimo, con ello bastará para inducir beneficios en los cultivos (Costa *et al*., 2016; Vulavala *et al*. 2016).

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Dynamic Modeling of Silicon Bioavailability, Uptake, Transport, and Accumulation: Applicability in Improving the Nutritional Quality of Tomato

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Silicon is an essential nutrient for humans, additionally is beneficial for terrestrial plants. In plants Si enhances tolerance to different types of stress; in humans, it improves the metabolism and increases the strength of skeletal and connective tissues as well as of the immune system. Most of the Si intake of humans come from edible plants creating a double benefit: first, because the absorption of Si increases the antioxidants and other phytochemicals in plants, thereby increasing its functional value, and second because the higher concentration of Si in plants increases intake in human consumers. Therefore, it is desirable to raise the availability of Si in the human diet through the agronomic management of Si accumulator species, such as corn, wheat, rice, soybeans, and beans. But also in such species as tomatoes, carrots, and other vegetables, whose per capita consumption has increased. However, there are few systematized recommendations for the application and management of Si fertilizers based on the physicochemical factors that determine their availability, absorption, transport, and deposition in cells and tissues. This study presents updated information about edaphic and plant factors, which determine the absorption, transport, and deposition rates in edible organs. The information was integrated into an estimated dynamic model that approximates the processes previously mentioned in a model that represents a tomato crop in soil and soilless conditions. In the model, on the other hand, was integrated the available information about key environmental factors related to Si absorption and mobilization, such as the temperature, pH, and soil organic matter. The output data of the model were compared against information collected in the literature, finding an adequate adjustment. The use of the model for educational or technical purposes, including the possibility of extending it to other horticultural crops, can increase the understanding of the agronomic management of Si in plants.

Keywords: silicates, nutritional quality, stress tolerance, mathematical models, silicon and health

INTRODUCTION

On average a human organism contains 1–2 g of Si, being the third most abundant trace element after Fe and Zn. When it is contained in food in adequate quantity, silicon is effectively absorbed by the human organism (Sripanyakorn et al., 2009), transferring to practically all tissues, but concentrating in greater quantity in the connective tissues (O'Dell and Sunde, 1997; Jugdaohsingh, 2007). With a diet rich in vegetables the daily intake of silicon is between 140 and 204 mg Si day^{-1}; however, in western populations with lower consumption of vegetables, the daily intake can range between 20 and 50 mg day^{-1}. Silicon is rarely toxic when taken orally (Arora and Arora, 2017), with a recommended maximum intake of 1,500 mg day^{-1} (White and Broadley, 2005). On the other hand, the minimum value of Si consumption to achieve some benefits has been determined at 25 mg day^{-1} (Nielsen, 2014). After ingestion, most of the absorbed Si is excreted in the urine (Jugdaohsingh, 2007), most likely as orthosilicic acid and/or magnesium orthosilicate.

Plant foods are the primary source of Si in the human diet. This includes grains of cereals (rice, wheat, oats, and barley) and less refined products of cereals, fruits (bananas and apples), vegetables (potato, beet, carrot, bean, spinach, and lentils) (Powell et al., 2005), and beverages such as beer since the Si contained in barley and hops is solubilized during the manufacturing process (Pennington, 1991; Powell et al., 2005; Jugdaohsingh, 2007). Other sources of silicon are meat, fish, milk, and eggs (Nielsen, 1974; Nuurtamo et al., 1980). Drinking water can also be a source of Si depending on the source and the method of processing (Jugdaohsingh, 2007; Sripanyakorn et al., 2009).

In plants Si is not considered an essential element, but it has been found that its inclusion in fertilizer formulations provides higher tolerance to stress (Adrees et al., 2015; Rizwan et al., 2015; Cooke and Leishman, 2016; Luyckx et al., 2017), especially on soilless growing conditions (Epstein, 1994, 2009; Voogt and Sonneveld, 2001). An additional benefit of adding silicon in the fertilization of crops is related to the more significant amount of silicon available to human consumers. In other words, the use of silicon in agricultural production brings a benefit to agricultural producers in the form of stronger and stress-tolerant plants, while for consumers of harvested products it gives an advantage in the way of higher silicon intake in the food.

The use of mathematical models in the mineral nutrition of plants allows to simulate the dynamics of the absorption of water and dissolved ions in response to different internal and external factors (Juárez-Maldonado et al., 2017). The models contribute to the quantitative understanding of the factors involved in the absorption, transport, and accumulation of mineral elements; additionally, they allow to explore different environmental or endogenous situations that modify the nutrition of the plant (Mankin and Fynn, 1996). Regarding Si modeling, (Sakurai et al., 2017) presented a dynamic model of Si absorption and transport for rice. The model was integrated considering the activity of different transporters and the distribution of Si through different nodes in the entire plant; the model was able to predict the dynamic behavior of silicon in the plant successfully. However, in the case of vegetables, there are no models that consider silicon, although there are published models that effectively simulate nutrition with other mineral elements (Juárez-Maldonado et al., 2014b, 2017; Ramírez-Pérez et al., 2018).

The aim of this manuscript was the integration of an estimated dynamic model that approximates the availability, absorption, transport, and accumulation of silicon in a tomato crop in soil and soilless conditions.

BENEFITS OF SILICON IN HORTICULTURAL PLANTS

The ferns, horsetails, and grasses such as corn, wheat and rice, and sugar cane, are the plants that naturally accumulate more silicon (Liang et al., 2015). However, in the presence of adequate amounts of silicon in the form of $Si(OH)_4$, all plants, including horticultural species such as tomato and cucumber, absorb it. Plants use silicon in a manner not yet well understood to stimulate the antioxidant metabolism, the processes of plant's hardening, defense, and adaptation to environmental factors. Depending on whether they are species that carry out silicification, $Si(OH)_4$ is concentrated in polymeric form (amorphous hydrated silica) in different cellular and extracellular compartments, finally transforming it using a deposition process dependent on transpiration into insoluble biogenic silica ($SiO_2.nH_2O$) which forms structures called phytoliths or opal (Sangster et al., 2001; Katz, 2014; Exley, 2015). The biogenic silica is subsequently incorporated into the soil contributing with 1–3% of the total Si in the soil (Desplanques et al., 2006).

The silicon absorbed by the plants seems to be maintained under constant exchange between the soluble forms ($Si(OH)_4$) and the insoluble fraction (polymeric silicic acid and biogenic silica) (Exley, 2015). Most of the Si deposited as biogenic silica remains as such throughout the life of the plant (Sangster et al., 2001). The soluble part is directly available to humans when they consume plant foods, while the insoluble fraction could perhaps be considered as an integral part of the fiber. The environmental factors and differences between plant species that modify the ratio Si soluble/Si insoluble, which ultimately determines the dietary utility of the product, have not been studied.

Considering that, (i) all plants seem to have the capacity to absorb silicon (Exley, 2015), (ii) and in view of the rise that has taken in recent years the production of vegetables using soilless production systems (Pignata et al., 2017), (iii) in addition to the fact that irrigation water and horticultural substrates provide little bioavailable silicon (Liang et al., 2015), then, it would be advisable to include silicon on a daily basis in fertilizer formulations used in those soils with low Si bioavailability as well as in soilless crops for the production of vegetables under protected conditions (Epstein, 1994, 2001).

The different groups of plants have different capacity to mobilize Si toward their various organs, but practically all absorb the silicon from the soil when it is available in the soil solution or the nutrient solution. The species with low mobilization capacity accumulate it in the roots and stems, while the species with high mobilization capacity accumulate it in stems, leaves, fruits,

and seeds. Si appears to be absorbed in the form of $Si(OH)_4$ by channels belonging to the aquaporins' group. Thus the rate of absorption and transport depends on the flow of water linked to transpiration (Exley, 2015; Sakurai et al., 2017).

The cereals are plants with a high capacity of silicification and therefore represent a significant amount of Si in the diet. However, the silicon contained in cereals will be encountered almost all in the form of insoluble biogenic silica (Sangster et al., 2001) which would be partially dissolved by the acids of the digestive system; on the other hand, in fruits and vegetables, due to their lower silicification capacity, it is expected that there will be more soluble silicon, which theoretically would be more available to be assimilated during intake. Considering the above, it is possible that the fruits of horticultural species such as tomato can be excellent sources of Si for the diet.

Additionally, it is known that in comparison with the dicotyledons, cereals contribute less Ca and Mg (White and Broadley, 2005). Therefore, a diet high in cereals that provides a significant amount of Si on average will contain less Ca and Mg than a mixed diet with base in cereals and dicots. On the other hand, the consumption of vegetables and fruits has grown considerably in recent decades among the human population and it is desirable that species such as tomatoes, eggplants, strawberries, cucumbers, avocados, melons, watermelons, carrots, onions, chilies, pumpkins, among others, contain a higher amount of silicon, considering the double benefit already mentioned of the crop higher tolerance to stress and the contribution of Si to human consumers.

In soilless crops, it is necessary to consider the contribution of Si in fertilizers since irrigation water does not provide enough, only from 5 to 24 mg L^{-1} Si (Liang et al., 2015). The lowest recommended concentration of Si in the nutrient solution for plants growing on substrates other than soil is 28 mg L^{-1} Si (Epstein, 1994), which can be achieved with 123 mg L^{-1} of Na_2SiO_3.

EDAPHIC FACTORS THAT DETERMINE THE AVAILABILITY OF SILICON

Si is found in soil as an inert mineral in the form of quartz or aluminosilicates such as micas and feldspars. The weathering of these materials by rainwater, irrigation water, or by the acid metabolites of microorganisms and plant roots produce $Si(OH)_4$ that under a balanced condition reaches a concentration of up to 1.8 mM (173 mg L^{-1}, equivalent to 50.4 mg L^{-1} Si). Above this level, reaching 2 mM (192.18 mg L^{-1}), $Si(OH)_4$ forms hydrated amorphous silica polymers containing Si unavailable for plants (Epstein, 2001; Liang et al., 2015).

The actual value of the concentration of $Si(OH)_4$ in the soil solution is much lower than 1.8 mM, commonly found between 0.1 and 0.6 mM (9.61–57.66 mg L^{-1}), but with such low values as 0.02 mM (1.92 mg L^{-1}) in very eroded soils (Epstein, 2001; Liang et al., 2015).

The concentration of bioavailable Si in soil solution results from the release rate of $Si(OH)_4$. Bioavailability is dependent on the silicon content of the soil minerals, organic matter, the temperature, the amount of precipitation and the acidity of the soil or soil pore water. The incorporation of Si in plants occurs at a rate dependent on the intensity of the transpiration (Exley, 2015), so that conditions of rapid growth can rapidly decrease the availability of Si in the soil solution (Epstein, 2001; Liang et al., 2015).

Soils of tropical areas where high precipitation occurs as well as calcareous and sandy soils of semi-arid and arid regions with low vegetation provide low quantities of Si to the soil solution, so it is recommended to use fertilizers with Si (Epstein, 1999). An affordable source of Si is siliceous sand that is offered in different granulometries, and that is used in quantities of between 500 and 4,000 kg ha^{-1}. On the other hand, Mollisol and Vertisol soils of the temperate and subtropical regions are soils that can provide an adequate amount of silicon (Epstein, 2001; Gérard et al., 2008). However, this has not been corroborated in regards of the actual availability of $Si(OH)_4$ in soil pore water, since there is little-published information about concentrations, dynamic behavior, and association with other edaphic components of Si in the solution of the soil.

The temperature exerts a substantial impact on the soil solubilization rate of Si. However, the seasonal changes in temperature are significant as a determinant of the Si concentration in the soil solution only in the cold seasons of temperate zones, because the range of temperatures suitable for the growth of a crop is also adequate for the solubilization reactions of silicon in the soil (Sommer et al., 2006). Therefore, the temperature is not considered as a factor subject to management regarding the bioavailability of Si for crop plants. Possible exceptions would be crops in soil mulching and crops grown in greenhouse soil or tunnels. In both cases, soil or substrate temperatures are more stable, and on average higher than those of uncovered soil, in addition to water management more precise in time and quantity, so the bioavailability of Si is expected to be higher.

Another factor regulating the availability of $Si(OH)_4$ is the pH of the soil pore water, that depends on the pH of the rainwater or irrigation water and is also modified by the respiratory activity and extrusion of organic acids by microorganisms and plant roots (Pérez-Labrada et al., 2016). In fact, the presence of Si induces the synthesis of citric acid in plants (Hernandez-Apaolaza, 2014). The pK1 of $Si(OH)_4$ is 9.6, which indicates that its bioavailability in a nutrient solution is practically unaltered with pH values lower than 9. In the study of Gérard et al. (2008), there was little impact of pH on the bioavailability of Si in the soil solution, but the study conditions were developed under a very narrow range of pH variation. It will be necessary to collect data in different types of soil, or in soils subjected to treatments that modify its reaction or the pH of the soil pore water, to determine the effect of pH on the concentration of $Si(OH)_4$.

Both a nutrient solution and the soil solution contain components that modify pH and interact with Si. With a pH> 7 that promotes the formation of Fe hydroxides, an adsorption process occurs that causes the polymerization of $Si(OH)_4$. With pH< 6 $Si(OH)_4$ begins to polymerize on surfaces with minerals containing Fe, while Al^{3+} would promote the stabilization of $Si(OH)_4$ polymers, which would make Si unavailable for plants

(Sommer et al., 2006). Considering this, it is possible that the availability of Si in the soil solution is higher with pH values between 6.0 and possibly 7.5 (maybe showing some resemblance to the pattern of bioavailability of P), as long as the soil parent material provides Si in sufficient quantity. Calcareous soils, which naturally have pH values> 8 in the soil solution (Pérez-Labrada et al., 2016), do not provide enough Si (Liang et al., 1994). Thus the fertilizer contributions with Si in crops in calcareous soils are beneficial (Zhang et al., 2017).

Another factor to consider regarding the availability of $Si(OH)_4$ in the soil pore water is soil organic matter (SOM) and its dissolved forms. SOM have a profound impact on the availability of mineral elements (Diacono and Montemurro, 2010), either directly by chemical processes or indirectly by the promotion of bacteria and fungi that solubilize Si and other elements of soil minerals (Landeweert et al., 2001). An expected effect of SOM would be the adsorption of Al^{3+} through organic acids (Rustad and Cronan, 1995), which would decrease the Al-Si association and increase the concentration of $Si(OH)_4$ available in the soil solution. The organic acids derived from SOM are also agents that promote dissolution in mineral surfaces (Drever and Stillings, 1997) so that in soils with silicon-rich parent materials or agricultural soils with the application of Si fertilizers would be very helpful. In nutrient solutions for soilless crops, the use of organic acids can also be useful to improve the solubility of fertilizers with silicon. On the other hand, indirect evidence is available that indicates that SOM is a factor that increases the bioavailability of Si for crop plants (Ding et al., 2008; Sun et al., 2017), thereby SOM management should be considered to increase the availability of Si for crops.

THE MODEL

The information in the previous section highlighted the factors that can be subjected to management in a crop, both in soil and soilless, with the purpose of increasing the availability of $Si(OH)_4$ for plants. In the crops grown in soil, a primary factor is the silicon content of soil's parent material. In the fertile soils of temperate and subtropical zones, Si inputs are rarely required in the fertilizers since the soil will undoubtedly provide the necessary amount. On the other hand, in the calcareous soils of arid and semi-arid regions, and in the soils of tropical regions subject to regimes of intense precipitation, the application of Si with fertilizers will be necessary, but also the consideration of the pH and organic matter management of the soil to ensure adequate availability of $Si(OH)_4$.

In soilless crops, the critical factor to consider will be the concentration of Si in the irrigation water. Values below 28 mg L^{-1} Si point out the need to provide Si up to a maximum of 50 mg L^{-1}. The management of pH is the next factor to be considered. However, the data presented indicate that pH management aimed at ensuring the bioavailability of P in nutritive solution (that is, maintaining it between 5.5 and 7) will be adequate.

A Matlab-Simulink model (the archives are included in Supplementary Material) is presented below which allows verifying the impact of different environmental scenarios, both in a soil crop and in a soilless crop, using as a model tomato plants. There is also an example of the use of the software to obtain the estimated impact of the environmental variables on the absorption of Si by the tomato plants. The data presented in the previous parts of the manuscript can be tested in this model by verifying the result regarding the concentration of Si in the plants. The purposes of the use of the model are educational or technical, and from our perspective, the model can be useful in the agronomic management of Si in a tomato crop and, possibly applicable to other horticultural crops.

Description of the Model

Tomato (*Solanum lycopersicum* L.) was used as a biological model to describe the distribution of silicon accumulation in the different organs. To describe the effects of the various environmental factors mentioned, the deterministic mathematical model proposed by Tap (2000) and modified by Juárez-Maldonado et al. (2014b) will be used as a basis.

The model consists of six state equations, using as inputs the radiation (PAR, μmol m^{-2} s^{-1}), temperature (°C), and CO_2 concentration (μL L^{-1}). The model allows to directly considering the effect of these three variables on the accumulation of silicon in the different organs of the tomato plant.

The scope of the model refers to environmental conditions where intense stress does not prevail since it is assumed that the growth rate of the plants will be a direct function of the irradiance and temperature.

Within the plant, silicon accumulates in different organs depending on the corresponding transpiration rates. Thus, it is necessary to calculate the transpiration by a tomato plant dynamically. For this, the equation 1 is used, based on the fact that a linear correlation can be considered between the biomass accumulated by the tomato plant and its transpiration (Juárez-Maldonado et al., 2014a).

$$Transpiration = Biomass * plm/tcg \quad (1)$$

Biomass is the mass of the tomato plant in g m^{-2}; *plm* is a parameter of the linear model (8.5714); and *tcg* is the time of crop growth (10279801 *s*).

Assuming that the tomato plant does not present a substantial accumulation of Si (Liang et al., 2015), the maximum absorption limit was set for the model at 1% (as SiO_2) of the dry biomass (Miyake and Takahashi, 1978). In this condition, and while there is an unlimited availability of silicon in the soil solution, the accumulation of maximum total silicon (MSiT) in the plant would be as follows:

$$\mathrm{MSiT} = \frac{Biomass}{100} * PD \quad (2)$$

Where *PD* is the planting density expressed in plants m^{-2}, which for this model was established in 3 plants m^{-2}. This plant density was used by Juárez-Maldonado et al. (2014b) and provide the best financial margin, high yield, and fruit quality (Peet and Welles, 2005).

The distribution of accumulated silicon in the tomato plant will then follow the different transpiration rates of its organs,

that is, leaves> stem> fruits ≥ root. In the particular case of tomato, organ transpiration can be approximated to the following percentages of total transpiration: leaves = 90%, stem = 5%, fruits = 2.5%, and root = 2.5%.

Even though the potential availability of $Si(OH)_4$ in the soil solution is 192.18 mg L^{-1} (Epstein, 1999; Liang et al., 2015), disponibility is affected by temperature, pH, and organic matter content of the soil. In addition to the factors that are modified with agricultural management such as soil moisture and soil profile.

According to the literature, the availability of silicon in soils is directly affected by soil temperature (Epstein, 1999; Liang et al., 2015). Although there is no clear explanation of how this behavior occurs, it is possible to approach it with a third-order model (Equation 3). This is due to the disponibility of silicon is between 8 and 35°C, being its highest availability at 25° C.

$$T3 * Temp^3 + T2 * Temp^2 + T1 * Temp + T0 \quad (3)$$

Where $T3$, $T2$, $T1$, *and* $T0$ are the parameters of the third-order model (equivalent to −0.0003; 0.0127; −0.1093; and 0.1674 respectively), and *Temp* is the 0–30 cm soil temperature (°C).

Concerning the SOM, it is known that there is a positive correlation with the availability of silicon (Ding et al., 2008; Sun et al., 2017). View from an agricultural perspective, soil is rich in organic matter when it has a concentration of 5%. An adjustment with a Michaelis-Menten function was used to describe the higher availability of Si, due to the effect of SOM. For this, the following Equation (4) was used.

$$Vmax * \frac{OM}{(Km + OM)} \quad (4)$$

Where *Vmax* is the parameter of maximum availability of silicon due to organic matter normalized to 1 ($Vmax = 1$). *OM* is the amount of organic matter contained in the soil (%, w/w). And *Km* is the Michaelis-Menten parameter (Km = 2.5).

The pH is also a determining factor in the availability of silicon (Liang et al., 2015). This factor, as well as temperature, is related to the availability of silicon that can be approached to a third-order model. The availability of silicon in soil occurs in the pH range from 2 to 9, with a pH of 7 being the highest availability. Therefore, its effect can be described as follows:

$$pH3 * pH^3 + pH2 * pH^2 + pH1 * pH + pH0 \quad (5)$$

Where $pH3$, $pH2$, $pH1$, *and* $pH0$ are the parameters of the third-order model (with values −0.0235, 0.325,−1.1563, and 1.2262, respectively). And *pH* represents the pH of the soil studied.

Therefore, the $Si(OH)_4$ available (SiAv) to be absorbed by the tomato plant is described by the following equation:

$$\text{SiAv} = (SiP - SiWater) * ETem * EOM * EpH + SiWater \quad (6)$$

Where *SiP* is the maximum amount of silicon in a soil without polymerization [192.18 mg L^{-1} $Si(OH)_4$]; *ETem* represents the effect of temperature on the availability of silicon (Equation 3); *EOM* describes the impact of organic matter on the availability of silicon (Equation 4); *EpH* represents the effect of pH on the availability of silicon (Equation 5); and *SiWater* is the amount of $Si(OH)_4$ available in the irrigation water. The model supposes that under no condition will be the available $Si(OH)_4$ be higher than the SiP value.

The accumulated Si (as SiO_2) in the tomato plant (SiT) was determined with the silicon [$Si(OH)_4$] available and the transpiration (Equation 1) in the following way:

$$\text{SiT} = \text{SiAv} * Transpiration * SiSi \quad (7)$$

Where *SiSi* is the fraction of cumulative Si in the plant of the total available $Si(OH)_4$ (g).

In soilless cultivation conditions, the only source of $Si(OH)_4$ for the crop will be the content of the irrigation water since there is no such source of soil mineral replacement as in the soil. Therefore, the accumulation of Si in the tomato plant in soilless culture (ASiTSC) will depend entirely on the transpiration of the plant (Equation 1) and the availability of $Si(OH)_4$ in the irrigation water (SiWater). This relationship is expressed as:

$$\text{ASiTSC} = Transpiration * SiWater * SiSi \quad (8)$$

As previously described, the availability of silicon in soil depends on three primary conditions: pH, organic matter, and soil temperature. Of these conditions, it is feasible to modify the amount of organic matter or the pH. In the case of temperature, the easiest way would be to use covers as plastic mulches, which could increase the soil temperature by 3–4°C (Ruíz-Machuca et al., 2015). Therefore, these factors can be considered as crucial factors to the agronomic management of silicon availability (Liang et al., 2015).

Silicon Accumulation in Tomato

According to the simulations carried out using the proposed model, a soil with pH 7.0 and organic matter content of 6% can obtain the maximum availability of $Si(OH)_4$, which can be > 4,500 mg m^{-2} at 15°C; or > 8,300 mg m^{-2} at 25°C (**Figures 1A,D**). Considering the availability, and two average temperature conditions of soil (15 and 25°C), the highest availability of silicon is obtained with an average soil temperature of 25°C (**Figure 1D**). On the contrary, when the organic matter content is low (<1%) along with a pH >8 (like a soil of a semi-arid region), the availability of $Si(OH)_4$ in the soil can drastically decrease to <20 mg m^{-2} at 15°C (**Figure 1A**), or <34 mg m^{-2} at 25° C (**Figure 1D**). These results describe the effect of pH, organic matter, and temperature factors on the availability of $Si(OH)_4$ in soil. In addition to demonstrating the potential sensitivity of the availability of $SiOH_4$ in the soil to the modifications on any of the conditions mentioned.

The availability of silicon in the soil will directly impact the accumulation of plants grown on it (Epstein, 2001). The higher availability of silicon derived from the factors evaluated (**Figure 1D**), results in a more significant accumulation of Si (as SiO_2) based on the dry weight of the tomato plant (up to 1 %), as can be seen in **Figure 1E**. The **Figure 1E** fits well the reported Si concentration in tomato plants grown under pH 5.5 (Miyake and Takahashi, 1978) to pH 8.48 (Gunes et al., 2007).

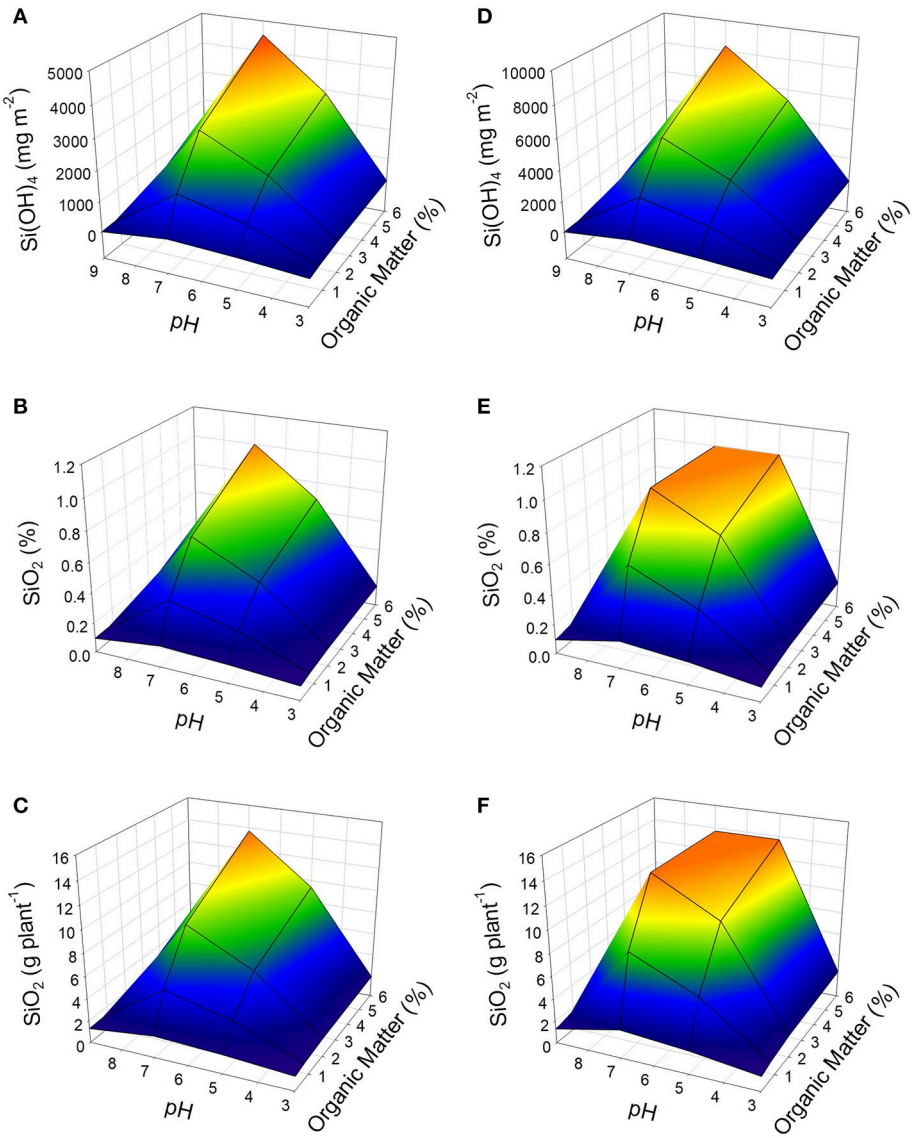

FIGURE 1 | Effect of pH and content of organic matter on the availability of $Si(OH)_4$ in soil **(A,D)**, concentration of Si (as SiO_2) in plant dry weight **(B,E)**, and its accumulation in the tomato plant **(C,F)** under two conditions of average soil temperature: 15°C **(A–C)** and 25 ° C **(D–F)**. Data obtained from the proposed dynamic model considering as inputs of the growth model: PAR = 400 μmol m^{-2} s^{-1} (12 h per day); CO_2 = 400 ppm; and Temperature: 30°C. As soil conditions were considered: a soil profile of 30 cm, soil humidity of 25% (w/w), and availability of Si as $Si(OH)_4$ in irrigation water of 5 mg L^{-1}.

The same behavior is observed concerning accumulated silicon per plant, reaching up to 13.7 g per plant, that represent the maximum accumulation of silicon for tomato plants under this conditions (**Figure 1F**). However, low availability of silicon in the soil can lead to a small accumulation of silicon in the plant. In the example the conditions of a soil corresponding to the situation of a semi-arid region, there would be an accumulation <1.3 g per plant for both soil temperature conditions (**Figures 1C,F**). This equals to a silicon concentration based on plant dry weight <0.1% (**Figures 1B,E**).

Since silicon accumulates in the different organs of the tomato plant as a function of the rate of transpiration, then the highest accumulation will be observed in the leaves, since they represent 90% of total transpiration. In fruits, a lower accumulation of silicon will be seen, since the rate of transpiration is little compared to that of the leaves (∼5%) (Leonardi et al., 2000). However, the availability of silicon in the soil will ultimately define the accumulation of silicon both in the entire plant and in its various organs.

Contrary to a crop established in soil, in soilless cultivation, e.g., hydroponics, the primary factor that will modify the availability of Si for the plant will be its concentration in the irrigation water used. It has been reported that irrigation water can have a Si content [as $Si(OH)_4$] of 5–20 mg L^{-1}, while it

is considered that an adequate concentration of Si would be 28 mg L^{-1} (Epstein, 1994, 1999; Liang et al., 2015). However, the transpiration rate of the crop will finally define the amount of $SiOH_4$ absorbed and accumulated in the different organs. Since the growth of the plant and the proper distribution of biomass in the various organs will affect the rate of transpiration, then crop growth should be considered as an additional factor that will change the accumulation of silicon in a soilless crop system. Therefore, the environmental factors (PAR, CO_2, air temperature) that affect the growth of the crop will, in turn, affect the accumulation of silicon in the different organs.

According to the simulations carried out, a low concentration of CO_2 and a low incidence of PAR generate little accumulation of biomass in the tomato plant (**Figures 2A,D**). This same result is observed when the temperature of the environment changes, 30°C generates biomass of up to 3,000 g per plant (**Figure 2A**); while 20°C produces up to 2,240 g per plant (**Figure 2D**), at the highest conditions of PAR and CO_2 concentration. As a consequence, the total transpiration of the plant is modified when

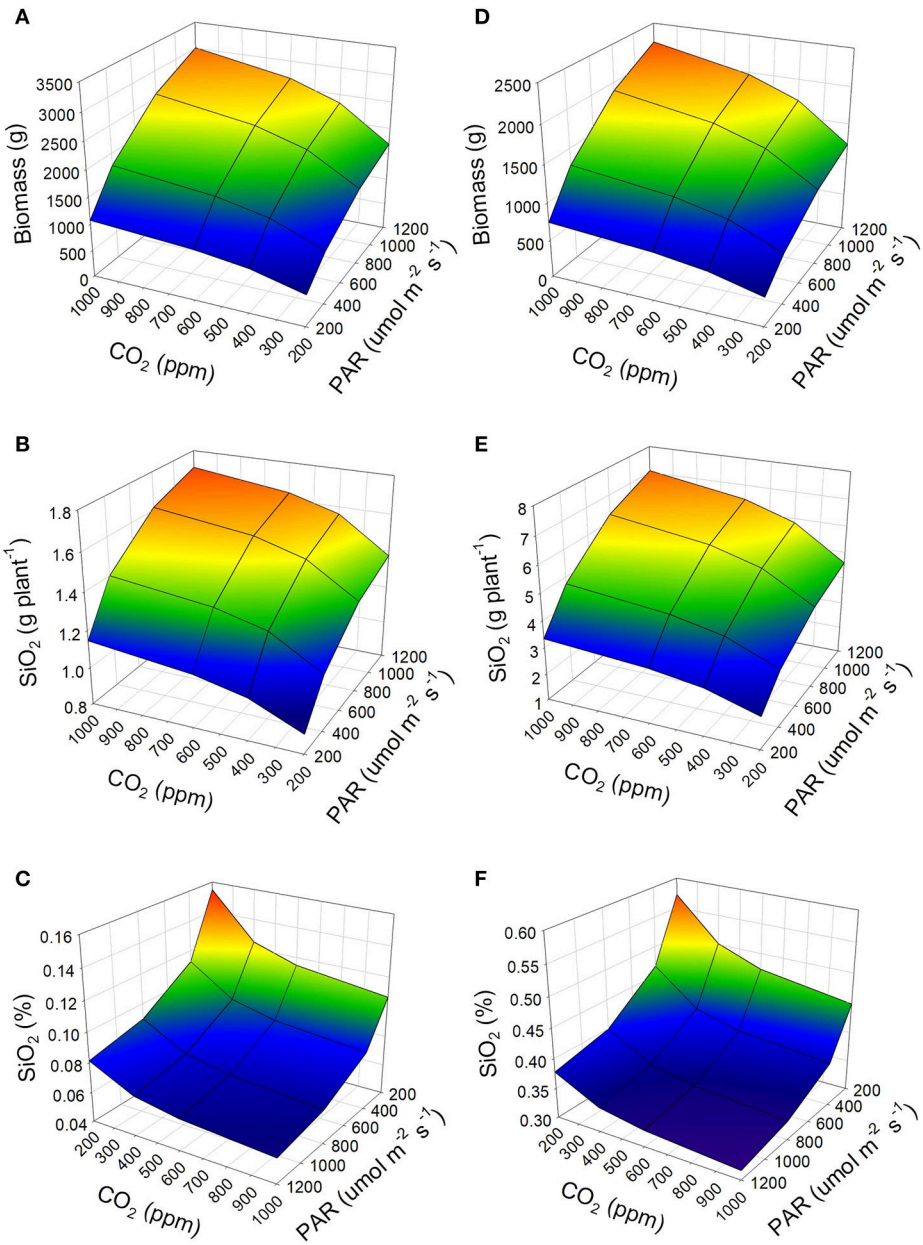

FIGURE 2 | Accumulation of dry biomass of the tomato plant under two conditions of air temperature 30°C **(A–C)** and 20°C **(D–F)**, and its impact on the accumulation **(B,E)** and concentration **(C,F)** of silicon (as SiO_2) in the plant. It is considered a tomato crop developed in a soilless cultivation system, an availability of Si as $Si(OH)_4$ in the irrigation water of 5 mg L^{-1}, and 12 h of PAR per day.

environmental factors changes, and therefore the accumulation of silicon. With an average temperature of 30°C, the biomass accumulated in the fruits can represent around 60% of the total of the tomato plant, and the leaves less than 10%. However, when the temperature drops to 20°C, the biomass distribution in the tomato plant changes. Under this condition, the biomass accumulated in the fruits is ∼33%, whereas in the leaves it increases up to 33%.

Since the leaves constitute the largest area of transpiration, modifying the temperature of the environment in which the tomato grows substantially alters the final accumulation of silicon. Therefore, according to the model, a temperature of 30°C will lead to a lower accumulation of silicon in the tomato plant (**Figure 2B**), while at 20°C there will be more significant accumulation (**Figure 2E**). The **Figure 2C** fits the reported Si concentration in tomato plants grown under temperatures of 28–32°C (Cao et al., 2015). The result will be a higher level of silicon in dry weight of the tomato plant at low temperatures (**Figure 2F**), while high temperatures will decrease the concentration considerably (**Figure 2C**).

When considering the availability of silicon in the irrigation water, it can be observed that a condition of low Si content (5 mg L^{-1} as $Si[OH]_4$) will result in less concentration and accumulation of silicon in the plant (**Figures 3A,B**). On the contrary, adequate availability of Si (28 mg L^{-1} as $Si[OH]_4$) in the irrigation water will result in increased accumulation and concentration of silicon in the plants (**Figures 3C,D**). High accumulation appears to occur regardless of the environmental conditions in which the crop develops, when there is adequate availability of Si in the irrigation water. However, when conditions are favorable for the development of leaves in tomato plants, the maximum concentration of silicon for this species can be reached (**Figure 3D**).

The model presented focuses on the impact of external factors on the growth of tomato plants, under the assumption that as long as exists the availability of Si the plants absorb it and transport it at a rate proportional to the growth rate and transpiration rate. The point highlighted with the model is that the biofortification of the fruits with Si depends on the availability of the element in the form of $Si(OH)_4$ both in soil and in soilless culture.

Sakurai et al. (2017) developed a successful dynamic model of Si absorption and transport for rice. The model is based on endogenous variables, as the activity of different transporters and the distribution of Si through different nodes in the entire plant. The model presented in this manuscript is focused on exogenous variables, susceptible to agronomic management both in field cultivation as in the greenhouse, and it has been used successfully

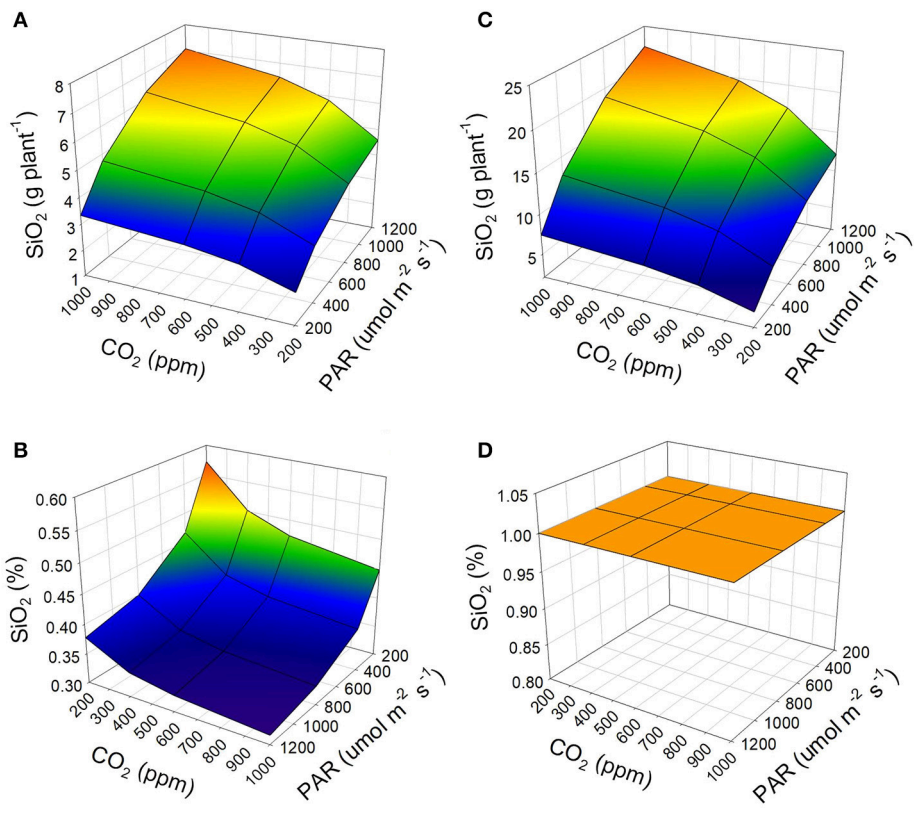

FIGURE 3 | Effect of the availability of Si as $Si(OH)_4$ in the irrigation water on the accumulation **(A,C)** and concentration **(B,D)** of silicon (as SiO_2) in the tomato plant developed under a soilless cultivation system. 5 mg L^{-1} of $Si(OH)_4$ **(A,B)** represent low availability of Si and 28 mg L^{-1} of $Si(OH)_4$ represent adequate availability **(C,D)**. For the simulation, 12 h of PAR and an average air temperature of 20°C were considered.

to simulate the absorption of other elements in tomato and other crops (Juárez-Maldonado et al., 2014b; Ramírez-Pérez et al., 2018).

However, it must be taken into account that the presented model is used to describe the accumulation of silicon in the plant under relatively favorable environmental situations. The presence of stresses such as water deficit, salinity, deficiency of mineral nutrients and pathogens, results in loss of precision. With a certain amount of PAR and with a particular temperature regime, the stressed plants would have real biomass lower than the estimated by the model, which means an overestimation of the absorbed silicon.

As far as we know, except those published by (Sakurai et al., 2015, 2017) for monocotyledons, there are no similar dynamic models published about the absorption of silicon in dicots. The model described for the tomato crop is a first preliminar advance that we believe substantially can improve the understanding of some factors that regulate the bioavailability of silicon.

CONCLUSIONS

After the results obtained from the presented model, the following is proposed:

1) When crops are grown in soil, the bioavailability of silicon can be increased by adding organic matter from organic amendments or humic substances, or by modifying the pH of the soil solution, using organic or inorganic acids, to be the closest to 7.0.
2) In soilless crop systems, the best is to increase the Si content in the irrigation water. Preferably to have at least 28 mg L^{-1} of Si, equivalent to 96.1 mg L^{-1} of $Si(OH)_4$.
3) The majority of the soils used for agriculture in tropical or subtropical semiarid or arid areas have conditions that do not favor the availability of $Si(OH)_4$, or the irrigation water has a low concentration of silicon. Therefore, it is advisable to apply silicates of sodium, potassium, or calcium as part of the fertilization program to favor the accumulation in the plants. In the long term, the availability of Si can be increased in the soil using mineral sources rich in Si, such as siliceous sands.

AUTHOR CONTRIBUTIONS

All authors were responsible for processing information and manuscript writing. AB-M, AJ-M, FP-L, and AM-D: Conceptualization; AJ-M, ML-P, LR-P, and AM-D: Model design and implementation; ML-P, FP-L, SG-M, LG-D, JG-M, and AB-M: Manuscript drafting. All authors read and approved the final manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2018.00647/full#supplementary-material

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ARTICULO 2

Biochemical and Gene Expression Responses of Tomato Plants Inoculated with *Fusarium* sp. to Application of Silicon

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Abstract: Tomato plants show limitations in yield, fruit quality and antioxidant capacity when they are under stress caused by *Fusarium* sp. The application of silicic acid (ASi) was used as an alternative to mitigate damages caused by this pathogen. Therefore, the effect of ASi on "Rio Grande" variety tomato plants inoculated with *Fusarium* sp. (Fol) was evaluated. ASi was applied weekly on leaves starting at the seventh day after transplantation and continued for ten consecutive weeks. Treatments consisted of ASi, ASi + Fol, Fol and an absolute witness (T0). The severity of the pathogen, antioxidant compounds, enzymatic activity, salicylic acid and jasmonic acid, and the expression of the PR1, JA, GAME1, ET, ABA and PAL5-3 genes were determined. The stress caused by *Fusarium* sp. induced an increase in vitamin C, total phenol, glutathione, PAL, CAT and GPX in leaves. The severity of *Fusarium* sp. caused a decrease in chlorophyll,

lycopene, proteins, flavonoids, and SOD activity in leaves. However, ASi application reduced 32% on average *Fusarium* sp. incidence and 22% the severity. Vitamin C, carotenoids, total phenols, and PAL, CAT, GPX and SOD activity in leaves increased. Fol increased PR1, JA, PAL5-3, ET, and ABA genes expression, whereas ASi increased GAME1, PAL5-3, ET and ABA genes expression. Foliar application of silicon decreased the stress caused by *Fusarium* sp. by increasing the antioxidant compounds, salicylic and jasmonic acid. On the other hand, it over expressed GAME1, PAL5-3, ET and ABA genes.

Keywords: biotic stress, antioxidant compounds, enzymatic activity, secondary metabolites, gene expression

Introduction

Fusarium sp. is one of the most important tomato (*Lycopersicon esculentum* Mill) crop phytopathogenic fungi (McGovern, 2015). This fungus is a soil facultative pathogen distributed throughout the world. It causes severe vascular damage, as well as root rot and wilt in most economically important tomato varieties (Michielse et al., 2009). This phytopathogen causes losses from 60 to 100% in the tomato crop (González et al., 2012). Stress by *Fusarium* sp. generates an overproduction of reactive oxygen species (ROS) that the antioxidant system is unable to reduce, therefore inducing oxidative stress to proteins, membrane lipids and other cellular components (Sousa et al., 2015). These effects cause the reduction of nutrient availability, absorption, distribution, and utilization (Romero et al., 2011), as well as photosynthesis damage (Mo et al., 2017). This decreases growth, yield and quality of tomato crop production (Leyva-Mir et al., 2013). Chemical control is the main management measure of this phytopathogen disease (Solarte et al., 2012). Unfortunately, the indiscriminate use of chemicals has led to the development of resistant fungal populations (Hamza et al., 2016). Therefore, it is necessary to implement new alternatives to reduce the incidence and severity of *Fusarium* sp. to avoid crop losses.

Silicon (Si) is the second most abundant element in the earth's crust, surpassed only by oxygen. It is found soluble in soil in the form of orthosilicic acid (H_4SiO_4), which is the only form absorbed by the roots. It is transported through the xylem through the transpiration current with the help of transporters (Pontigo et al., 2015). Silicon is

considered a beneficial mineral since it promotes growth, development, yield and fruit quality in crops (Malhotra et al., 2016). In addition, the supplement of this mineral to plants reduces the effects caused by biotic stress, and their susceptibility to pests, fungi and bacteria (Meharg and Meharg, 2015). Silicon in plants induces physical, biochemical (Pozza et al., 2015) and genomic barriers (Pavlovic et al., 2013) against pathogens. In addition, the formation of deposits of Si in root and leaves could prevent incidence of these pathogens (Ma and Yamaji, 2008). This mineral can also induce defensive responses similar to acquired systemic resistance, by increasing the enzymatic antioxidant activity (SOD, CAT, POD, GPX, GST, APX, GR) and non-enzymatic constituents, such as GSH (Hasanuzzaman et al., 2018), phenolic compounds, anthocyanins, flavones, lignin (Jafari et al., 2015), phytoalexins and proteins related to pathogenesis. In addition, it can activate the expression of defense related genes and add to the transduction of stress markers such as salicylic acid, jasmonic acid and ethylene (Cai et al., 2009).

There is a series of works where the effect of silicon against pathogens in crops has been evaluated. Fortunato et al. (2012) and Fortunato et al. (2014) showed that the supply of Si to banana plants reduced the intensity of *Fusarium* wilt. The application of sodium silicate and potassium silicate in *in-vitro* developed cotton, controlled *Fusarium* activity (Yassin et al., 2016). Weerahewa and David (2015) produced larger fruit size in tomatoes infested by anthracnose when applying sodium silicate. Ouzounidou et al. (2016) reported higher antioxidant activity in cucumber when applying potassium silicate.

Considering what has been said, the objective of this work was to evaluate the application of silicon in the form of silicic acid, on antioxidant capacity, salicylic and jasmonic acid and genes expression in tomato crop developed under biotic stress caused by *Fusarium* sp.

MATERIALS AND METHODS

Crop development

The development of the experiment was carried out in a greenhouse multi tunnel type, with polyethylene cover. As a biological material, tomato seedlings (*Solanum lycopersicon* L.) cv. “Rio Grande” was used, which were transplanted in 10 L black

polyethylene bags, which contained a mixture of peat moss-perlite as substrate 1:1 (v/v). For the nutritional management of the tomato crop a Steiner fertilizer solution was applied (Steiner, 1961), which was constantly supplemented by localized irrigation. The tomato crop was developed during 119 days after transplanting (dat), being managed to one stem and carrying out the traditional cultural works.

Inoculation of Fusarium sp.

To carry out the inoculation of *Fusarium* sp. (Fol) were reactivated in potato dextrose agar, and incubated for 15 days at 27 °C (Benhamou and Bélanger, 1998). For the increase of the pathogen a semiliquid medium was used mixing potato, dextrose, PDA, streptomycin and tomato seedling. For the sowing, two 6 mm discs of mycelial growth were taken and incubated at 28 °C in an orbital shaker at 125 rpm by 15 days. At 21 days after transplantation the tomato plants were inoculated at a concentration of $1x10^6$ spores mL^{-1} applying 60 mL of the spore solution on perforations in the substrate (10 cm deep and 3 cm away from the stem) in the corresponding treatments (Benhamou and Bélanger, 1998).

Application of treatments

The treatments consisted of the inoculation of *Fusarium* sp. plus the foliar application of silicic acid (ASi). In total, four treatments were evaluated as follows: 1) ASi, 2) ASi+Fol, 3) Fol, and 4) absolute control T0 (without application of ASi or Fol). A total of 10 applications of ASi were carried out, two applications were made prior to the inoculation of the pathogens (7 and 14 dat), and eight subsequent applications with intervals of one week. The concentration of silicic acid used was 110 $g{\cdot}ha^{-1}$ per application.

Fol incidence and severity

The incidence of *Fusarium* sp. in tomato plants was determined only with the presence of the pathogen in plant, that is, when there were damages. Therefore, zero represent not damages, and 1 represent positive incidence. The severity of *Fusarium* sp. was determined following the scale of Diener and Ausubel (2005).

Biochemical variables

Extraction of Biomolecules

To determine proteins, catalase, ascorbate peroxidase, superoxide dismutase, glutathione peroxidase, phenylalanine ammonium lyase, reduced glutathione, ABTS, and DPPH antioxidant capacity, samples of leaf tissue were collected. For this purpose, after 73 days of transplantation, random plants were selected, and the third fully expanded young leaf was taken for biochemical analysis. Samples were stored at -80 °C until use. For the enzymatic and non-enzymatic determination, 200 mg of lyophilized leaves of each treatment and 20 mg of polyvinylpyrrolidone were weighed. After this, 1.5 mL of phosphate buffer with a pH of 7–7.2 (0.1 M) were added, and the mixture was then subjected to micro-centrifugation at 12,000 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 the phosphate buffer.

Proteins

Protein quantification was determined by the Bradford method (Bradford, 1976), which involves taking 5 μL of the extract or standard and adding 250 μL of the Bradford reagent. After 10 min, the absorbance was read at 630 nm on a microplate reader ELISA (BioTek model ELx-808 IU, Winooski, VT, USA). A calibration curve was created with standard bovine serum albumin (0-1000 $mg \cdot L^{-1}$). The results were expressed as mg g^{-1} dry weight.

Catalase (EQ 1.11.1.6)

Catalase activity was quantified by measuring 2 reaction times according to Dhindsa et al. (1981). The reaction mixture contained 100 μL of extract, 1000 μL of H_2O_2 (100 mM) and 400 μL of H_2SO_4 (5%) (added to stop the reaction). The absorbance was recorded initially and after 1 min. H_2O_2 consumption was monitored using a previously traced peroxide calibration curve (0-100 mM) at 270 nm in a UV-Vis spectrophotometer (Thermo Scientific Model G10S, Waltham, MA, USA), and the results were expressed as U (mM H_2O_2 min^{-1}) per total protein ($mg \cdot g^{-1}$).

Ascorbate Peroxidase (EQ. 1.11.1.1)

This was determined according to Nakano and Asada, (1981). For the assay, 100 μL of extract, 500 μL of ascorbate (10 $mg \cdot L^{-1}$) and 1000 μL of H_2O_2 (100 mM) were added for a final volume of 2 mL and were incubated at a temperature of 22 °C. The reaction was stopped after one minute by adding 400 μL of H_2SO_4 (5%). The rate of ascorbate

oxidation was quantified by the decrease in absorbance at 266 nm after 1 min in a UV-Vis spectrophotometer. A calibration curve was prepared with ascorbate (0-5 $mg \cdot L^{-1}$), and the results were expressed in U (μmol of ascorbate min^{-1}) by total proteins ($mg \cdot g^{-1}$).

Superoxide Dismutase (EQ 1.15.1.1)

For this analysis, 200 μL of the radical detector (tetrazolium salt) were added to 10 μL of the extract or standard. To initiate the reaction, 20 μL of xanthine oxidase were added. This was then incubated for 30 min at room temperature, and the absorbance at 450 nm was then read in a microplate reader ELISA. A calibration curve was prepared with standard SOD (0–0.05 $U \cdot mL^{-1}$), and the results were expressed as $U \cdot mL^{-1}$.

Glutathione Peroxidase (EQ 1.11.1.9)

This was conducted 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 were placed in an Eppendorf tube with 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 min, and 0.2 mL of 1.3 mM H_2O_2 were then added to initiate the catalytic reaction. This mixture was allowed 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 to 4°C, and 0.48 mL of the supernatant or standard were then taken. After this, 2.2 mL of 0.32 M Na_2HPO_4 and 0.32 mL of 1 mM of the dithio-5-dithio-2-nitro benzoic acid (DTNB) dye were added. A blank was used, which contained 2.2 mL of 0.32 M Na_2HPO_4, 0.32 mL of 1 mM DTNB, and 0.48 mL of 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 (0-1 mM) was created and the results were expressed in U (mM of glutathione min^{-1}) by total proteins ($mg \cdot g^{-1}$).

Phenylalanine Ammonium Lyase (EQ 4.3.1.5)

This was determined according to Sykłowska-Baranek et al. (2012) with modifications. A total of 0.1 mL of the enzymatic extract were taken, and 0.9 mL of l-phenylalanine (6 mM) were added. After 30 min of incubation at 40 °C, the reaction was stopped with

0.25 mL of 5 N HCl. The samples were placed in an ice bath, and 5 mL of distilled water were added. The absorbance was determined at 290 nm on a UV-Vis spectrophotometer. A calibration curve was prepared with cinnamic acid (0-1 $mg \cdot L^{-1}$), and the results were expressed as U (production of 1 µM cinnamic acid per min) by total proteins ($mg \cdot g^{-1}$).

Reduced Glutathione (GSH)

It was performed calorimetrically by reaction with DTNB. In a tube for centrifuge, 0.48 mL of the extract were placed, and 2.2 mL of 0.32 M Na_2HPO_4 and 0.32 mL of the 1 mM DTNB dye were then 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 were expressed in mM of GSH $100g^{-1}$.

ABTS (2,20-Azinobis-3-Ethylbenzotiazoline-6-Sulphonic Acid)

The ABTS was performed according to the methodology of Re et al. (1999) and Kuskoski et al. (2005). The ABTS cation at 7 mM was generated through the interaction of 38.4 mg of ABTS dissolved in 10 mL of HPLC grade H_2O, with 2.45 mM of potassium persulfate (6.62 mg in 10 mL of HPLC grade H_2O), mixing 1:1. The cation was incubated in the dark and at room temperature for 16 h. The activated ABTS radical was diluted with ethanol (20%) to an absorbance of 0.7 ± 0.02 at 754 nm using a UV-Vis spectrophotometer. Subsequently, 20 µL of the extract or standard were taken, and 980 µL of the diluted ABTS solution were added. After 7 min, the absorbance was recorded. Phosphate buffer was used as a blank. Two calibration curves were made: with Trolox (0-1 mM) and standard ascorbic acid (0-0.18 $mg \cdot mL^{-1}$). The results were expressed as Trolox equivalents in mM 100 g^{-1} dry weight and ascorbic acid equivalents in mg 100 g^{-1} dry weight.

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

The DPPH technique was performed as follow (Brand-Williams et al., 1995; Kuskoski et al., 2005; Ramos et al., 2010). The stock solution at 0.1mM was prepared by mixing 3.94 mg of the DPPH radical with 100 mL of methanol (80%). After this, 6 µL of extract or standard were taken, and 234 µL of the diluted DPPH radical (0.1 mM). Phosphate buffer was used as a blank. The decrease in absorbance at 540 nm was measured after 30

min on a microplate reader ELISA (BioTek model ELx-808 IU, Winooski, VT, USA). Two calibration curves were made: with Trolox (0.6-5 mM) and standard ascorbic acid (0-5 $mg \cdot mL^{-1}$). The results were expressed as Trolox equivalents in mM 100 g^{-1} dry weight and ascorbic acid equivalents in mg 100 g^{-1} dry weight.

Total Phenols

This was determined according to the methodology of Yu and Dahlgren (2000). 200 mg of lyophilized leaves was taken, 1 mL of water-acetone was added (1:1) solution. The sample was mixed in a vortex for 30 seconds and later using a Bransonic brand ultrasonic cleaner (model 1510R-DTH) for 5 minutes, after which the sample was centrifuged at 4 °C at 12,500 rpm for 10 min, and the supernatant was recovered. The quantification was performed using 50 µL of the supernatant and 200 µL of Folin–Ciocalteu reagent were then added, 500 µL $NaCO_3$ at 20% were added, and 5 mL of distilled water were added. The mixture was then incubated at 45 °C for 30 min (Nsor-Atindana et al., 2012; Sultana et al., 2009). H_2O-acetone (1:1) was used as a blank. The absorbance was read at 750 nm on a UV-Vis spectrophotometer. A calibration curve was prepared with gallic acid (100-1000 $mg \cdot mL^{-1}$), and the results were expressed as mg of gallic acid g^{-1} of dry weight (mg EGA g^{-1} DW).

Flavonoids

The measurement was performed with the Dowd method, adapted by Arvouet-Grand et al. (1994). In a test tube, 100 mg of tissue plus 10 mL of reagent grade methanol was added and stirred by 30 s. The mixture was filtered with filter paper No. 1. For quantification, 2 mL of the extract plus and 2 mL of methanol solution of 2% $AlCl_3$ was left to stand (20 min) in the dark. Subsequently, it was measured at 415 nm using a quartz cell. The blank consisted of reactive grade methanol (without $AlCl_3$). Quercetin (0-50 $mg \cdot L^{-1}$) was used for the calibration curve. The flavonoid content was expressed in mg equivalents of Quercentin per g of dry weight (mg EQ g^{-1} DW).

Carotenoids

The content of lycopene was determined according to Nagata and Yamashita (1992). 10 mg of dry tissue were mixed with 2 mL of the hexane/acetone (3:2) solution. This mixture was centrifuged for 5 min at 5000 rpm at 4°C, and an aliquot of the supernatant was taken. Hexane/acetone (3:2) solution was used as a blank. Finally, the absorbances

247 at 453, 505, 645 y 663 nm were determined. These absorbances were used in the
248 Equations (1-4) to determine the content of lycopene, β-carotene, and chlorophylls as
249 follow:

$$\text{Lycopene} = -0.0458 \times \text{Abs}_{663} + 0.204 \times \text{Abs}_{645} + 0.372 \times \text{Abs}_{505} - 0.0806 \times \text{Abs}_{453}, \quad (1)$$

$$\beta - \text{carotene} = 0.216 \times \text{Abs}_{663} - 1.22 \times \text{Abs}_{645} - 0.304 \times \text{Abs}_{505} + 0.452 \times \text{Abs}_{453}, \quad (2)$$

$$\text{Chl a} = 0.999 \times \text{Abs}_{663} - 0.0989 \times \text{Abs}_{645}, \quad (3)$$

$$\text{Chl b} = -0.328 \times \text{Abs}_{663} + 1.77 \times \text{Abs}_{645}, \quad (4)$$

250 Total chlorophyll is the sum of Chl a and Chl b. All data are expressed as mg 100 g^{-1}
251 dry weight.
252 *Vitamin C*
253 This was determined by the titration method with 2,6 dichlorophenolindofenol Padayatt
254 et al. (2001). A total of 10 g of fresh fruit was weighed and macerated in a mortar with
255 10 mL of 2% HCl, and it was then filtered through sterile absorbent gauze into a 100 mL
256 volumetric flask. A 10 mL aliquot was taken and titrated with 2,6-
257 dichlorophenolindofenol until a persistent rosacea coloration was obtained. The results
258 were expressed as mg 100 g^{-1} fresh weight.
259 **Secondary metabolites**
260 To determine Salicylic acid (SA), 50 mg of the foliar tissue was weighed in 2 mL
261 centrifuge tubes. 1 mL of the extraction solution (89% water, 10% methanol and 1%
262 acetic acid) was added and the mixture was homogenized in vortex for 30 s and
263 sonicated for 10 min. Subsequently, they were centrifuged at 12,000 rpm (10 minutes).
264 The supernatant was extracted and filtered (0.45 μm filter), and degassed by sonication.
265 For the quantification the sample was injected in the liquid chromatograph (Agilent
266 compact 1120) equipped with variable wavelength UV detector, by means of micro-
267 syringe. The chromatographic conditions were for stationary phase were: column C-18
268 polaris of 250 mm in length. The mobile phase have a gradient of 50% phase A (water
269 94.9%, acetonitrile 5%, and formic acid 0.1%) and 50% phase B (acetonitrile 94.9%,

water 5%, and formic acid 0.1%), at a flow of 0.8 $mL \cdot min^{-1}$ for a run time of 13 min. It was read at a wavelength of 250 nm (Forcat et al., 2008). Calibration line was using (±) salicylic acid (Sigma Aldrich) dissolved in mobile phase.

To determine Jasmonic acid (JA), 100 mg of leaf tissue was weighed, 900 µL of the extraction solution (95% methanol and 5% ethyl acetate) was added, vortexed, sonicated (10 min), and centrifuged at 12,000 (10 min). The supernatant was filtered through a 0.45 µm nylon membrane, the solvent was subsequently evaporated in dry oven at 50 °C, and the residues were re-suspended in 500 µL of mobile phase (60% methanol, 39% water, and 1% acetic acid) (Kramell et al., 1995; Michelena et al., 2001). The identification and quantification was carried out in a liquid chromatography (Agilent compact 1120) equipped with variable wavelength UV detector. The mobile phase used was 60% methanol, 39% water, and 1% acetic acid. The flow was 0.60 $mL \cdot min^{-1}$ using a Hypersil ODS column of 25 cm x 4.6 mm x 5 µm, at a wavelength of 230 nm with an analysis time of 20 min. Calibration line was using (±) jasmonic acid (Sigma Aldrich) dissolved in mobile phase.

Determination of gene expression by qPCR

For genes analysis, after 31 and 73 dat, random plants were selected, and the third fully expanded young leaf was taken, samples ware freezing immediately in liquid N_2 and subsequently stored at -80 °C until use.

Regarding RNA extraction, leaf samples were pulverized using liquid nitrogen and then 100 mg were placed in a 2 mL micro-centrifuge tube. Immediately, 1 mL of TRI reagent (Sigma Aldrich, St. Louis, USA) was added to the sample and gently homogenized. It was incubated for 5 min at room temperature, then 200 µl of chloroform was added and the mixture vortexed vigorously until it had a milky appearance. The mixture was incubated at room temperature for 15 min and then centrifuged at 12,000 g for 15 min at 4 °C. The supernatant was recovered and placed in a new tube with 500 µL of cold isopropanol and mixed gently. It was then incubated at room temperature for 10 min and then centrifuged at 12,000 g for 10 min at 4 °C. The supernatant was removed by decantation and the resulting RNA pellet was washed with 1 mL of cold ethanol at 70 %. It was stirred by gently inverting the tube and then centrifuged at 7,500 g for 5 min at 4 °C. Again the supernatant was removed and the RNA pellet allowed to dry for 15 min,

finally the pellet was resuspended in 50 µL of DEPC-treated water and stored in ultra-freezer at -80 °C.

RNA quantification was carried out in a UV-Vis spectrophotometer (Thermo Scientific Model G10S) at an absorbance of 260 and 280 nm, and RNA quality was measured by denaturing electrophoresis. 2µg of ARN were used by synthesis of the cDNA was performed using a commercial kit (Promega, Madison, Wisconsin, USA). The primers correspond to an endogenous gene Actin (Act) and six study genes: PR1 (Salicylic acid), JA (Jasmonic acid), GAME1 (Phytoalexins), PAL (Phenylalanine ammonia lyase), which were designed in the software AMPLIFIX, OLIGOANALIZER and PRIMERS BLAST, and the other hand ET (Ethylene) (Anstead et al., 2010) and ABA (Abscisic acid) (Nitsch et al., 2009), as described in Table 1.

The preparation of the primers consisted in a solution: each pair of primers was centrifuged at maximum speed for 15 min and then the primers were prepared at a concentration of 15 $pmol \cdot mL^{-1}$. The quantification method used was a standard relative curve, therefore, for each quantification analysis a standard curve per gene was included using a 1:5 dilution.

qPCR reactions were analyzed in an Applied Biosystems StepOne™ Equipment version 2.3 by the standard relative curve method, measuring the fluorescence intensity of SYBR Green. The qPCR reaction for all genes was performed in a total volume of 20 µL. Regarding Actin gene, 10 µL 2x de SYBR® Select Master Mix (Applied Biosystems, Foster City, California, USA) were added along with 0.10 µL of first forward (72 nM), 0.08 µL of first reverse (60 nM), 1 µL of cDNA and 8.82 µL of nuclease-free water. Regarding the PR1 gene, 10 µL of Master Mix were added along with 0.03 µL of first forward (20 nM), 0.05 µL of first reverse (40 nM), 1 µL of cDNA diluted at a ratio of 1:5, and 8.92 µL of nuclease-free water. Regarding the JA gene, 10 µL of Master Mix, 0.05 µL of first forward (40 nM), 0.08 µL of first reverse (60 nM), 1 µL of cDNA and 8.87 µL of nuclease-free water were added. ABA gene, 10 µL of Master Mix, 4 µL of first forward (3000 nM), 4 µL of first reverse (3000 nM), 1 µL of cDNA and 1 µL of nuclease-free water were added. As for ET, PAL and GAME1 genes, 10 µL of Master Mix, 0.13 µL of first forward (100 nM), 0.13 µL of first reverse (100 nM), 1 µL of cDNA (ET and PAL) or 1 µL of cDNA diluted 1:5 (GAME1) and 8.74 µL

of nuclease-free water were added. The qPCR was run with the following program in the thermal cycler: Hot Start, 10 min at 95 °C and PCR (40 cycles), 15 s at 95 °C, 1 min at temperature (◦C) annealing, 15 s at 95 °C, 1 min at 60 °C and 15 s at 95 °C.

RESULTS

Fol incidence and severity

Statistical differences were found between treatments, in *Fusarium* sp. (Fol) incidence in tomato plants, throughout the experiment, except for the first sampling (Figure 1a). There was no incidence of the disease in silicic acid (ASi) treatment and absolute control (T0). As for *Fusarium* sp. treatments (Fol and ASi+Fol) a certain degree of incidence was observed throughout the evaluation dates but increased as culture developed during time. Particularly, it was observed that ASi application decreased Fol incidence as ASi+Fol treatment had a lower incidence than the Fol treatment form inoculation day to day 66 after transplanting. Best effect of ASi was obtained at 61 days after transplanting (dat), since Fol incidence was reduced by approximately 24%. After 70 dat, both infested treatments reached 100% incidence.

The first symptoms of the severity of Fol phytopathogen were observed at 43 dat and increased progressively during culture development (Figure 1b). Throughout the experiment ASi+Fol treatment consistently decreased Fol severity. At 78 dat, the greatest decrease in severity was observed, with approximately 21% less incidence than Fol treatment, and 12% at 85 dat.

Biochemical Responses

Results showed statistical differences in total protein content, carotenoids, and chlorophylls in tomato leaves (Table 2). There was no difference in protein content between ASi and T0 treatments, whereas Fol and ASi+Fol treatments significantly decreased proteins by 33% and 31% respectively.

Lycopene content in tomato leaves showed same results between the ASi and T0 treatments which presented higher lycopene contents, however, ASi+Fol treatment showed also statistically equal results (Table 2). Fol treatment was the only one that decreased lycopene content in leaves, with 63% less than the T0. In this case, ASi+Fol application had a beneficial effect showing a 104% higher lycopene content than Fol

362 treatment. As for β-carotene, ASi+Fol treatment presented the highest content,
363 exceeding T0 by approximately 59%.
364 Regarding chlorophyll content (a, b and total), results were consistent. Fol treatment
365 decreased significantly in all cases, while the rest of the treatments were equal (Table 2).
366 Significant differences between treatments were observed in antioxidant compounds,
367 Vitamin C, glutathione, total phenols, and flavonoids, as well as in DPPH antioxidant
368 capacity (Table 3). Vitamin C was higher in Fol treatment compared to T0 and ASi
369 treatments by 33.3% and 52.7% respectively. Regarding GSH content, Fol treatment
370 also presented the highest value, however it was only statistically different from
371 ASi+Fol treatment. In total phenol content Fol treatment showed the highest value,
372 exceeding T0 by 27.3%.
373 Regarding Flavonoid content, Fol treatment was the only one to affect this variable,
374 presenting a decrease of 27.8% in comparison to T0. While ASi+Fol decreased by 2.4%
375 antioxidant capacity, evaluated by DPPH, with respect to T0 (Table 3).
376 Except for APX enzyme, there were statistical differences in PAL, CAT, GPX and SOD
377 between treatments (Table 4). PAL enzyme activity increased significantly with Fol
378 treatment, a 68% higher than T0. Fol treatment increased CAT activity 107% more than
379 T0. ASi, ASi+Fol and Fol treatments increased GPX activity by 111%, 149% and 199%
380 respectively in comparison to T0. SOD enzyme activity was 69% higher in ASi+Fol
381 treatment as compared to T0.
382 **Secondary metabolites**
383 Salicylic acid content in tomato leaves was modified by treatments in two out of three
384 samplings, but there were no statistical differences between treatments at 31 dat (Table
385 5). AS treatment, compared to T0, increased this metabolite content (140%) at 15 dat
386 (before pathogen inoculation). At 73 dat, when plants presented an advanced severity
387 (Figure 1b), the ASi+Fol and Fol treatments had a significant increase in SA in relation
388 to T0, reaching 266% and 248% respectively.
389 The content of JA could only be quantified at 31 and 73 dat, with statistical differences
390 between treatments in both cases (Table 5). Systematically, an increase of JA was
391 observed as culture developed and consequently the severity of the pathogen increased
392 (Figure 1b). At 31 dat, when the disease had not yet proliferated, only JA was quantified

in the ASi+Fol and Fol treatments, the latter being significantly higher (243%). At 73 dat, once the incidence and severity increased (Figure 1), an increase JA was observed in all treatments, with the exception of T0. Once again Fol generated the highest concentration of JA, surpassing the ASi+Fol treatment by 81%, and the ASi treatment by 234%.

Gene expression

PR1 and JA genes, which encode salicylic acid and jasmonic acid respectively, showed changes in their expression due to treatments (Figure 2). At 31 dat, only Fol treatment modified PR1 and JA gene expression, repressing them 0.7 and 0.35 fold change respectively in comparison to T0. However, at 73 dat all treatments increased PR1 gene expression, Fol increased it 2.52 fold change, ASi increased it 0.37 fold change, while the combination of ASi+Fol generated the largest increase with 21.25 fold change (Figure 2a). This trend is consistent with what was observed in SA quantification, where T0 had the lowest amount of this metabolite and Fol treatments showed the highest amount (Table 5).

In the case of JA gene at 73 dat, a similar trend was observed since all treatments increased its expression. Fol generated the greatest increase with 5.66 fold change, ASi increased 0.13 fold change, while the combination of ASi Fol increased 2.8 fold change compared to T0 (Figure 2b). Results consistent with JA determination, since Fol generated the highest amount of this metabolite, followed by the combinations ASi+Fol and ASi (Table 5).

At 31 dat, application of the treatments practically showed no changes in GAME1 gene expression (Figure 3a). However, at 73 dat Fol and ASi+Fol treatments repressed the expression of this gene in 0.73 and 0.86 fold change respectively in comparison T0. On the other hand, ASi treatment generated the opposite effect, since it increased the expression of the gene in 0.54 fold change in comparison to the T0.

In the PAL5-3 gene (Phenylalanine ammonia lyase), at 31 dat, a repression of this gene was observed by the Fol and ASi+Fol treatments, equivalent to 0.57 and 0.14 fold change, respectively, compared to T0 (Figure 3b). However, at 73 dat all treatments overexpressed this gene, ASi+Fol was 1.59 fold change more, Fol was 1.83 fold change, and ASi generated the highest expression equivalent to 1.83 fold change more than T0.

The expression of ET gene at 31 dat increased slightly by Fol (0.06 fold change), while ASi+Fol treatment increased 0.58 fold change in comparison to T0. However, at 73 dat all treatments overexpressed this gene: Fol, 1.68 fold change, ASi+Fol, 7.2 fold change, ASi generated the highest expression with 8.78 fold change greater than T0 (Figure 4a).

In the case of ABA gene, at 31 dat Fol treatment generated a repression of 0.25 fold change in comparison to T0, whereas ASi+Fol treatment increased the expression in 0.30 fold change (Figure 4b). At 73 dat, all treatments increased ABA gene expression just like what was observed with the ET gene (Figure 4a). Fol treatment generated the highest expression of the ABA gene with 4.45 fold change compared to T0, followed by ASi+Fol treatment with 4.40 fold change (Figure 4b). ASi treatment also increased ABA gene expression but not in the same magnitude as Fol treatments, since it only increased 1.13 fold change in comparison to T0 (Figure 4b).

DISCUSSION

Fol incidence and severity

Cao et al. (2017) mentioned that when stress is prolongated, self-regulation in tomato is exceeded, and the positive effects of Si are attenuated, as observed here after 70 dat, where both infested treatments reached 100% incidence. Furthermore, without the use of fungicides, it is practically impossible to completely suppress the disease (Bakhat et al., 2018). The ROS production induced by *Fusarium* sp. causing oxidative stress, and generating DNA and metabolism damage, and protein synthesis, which ultimately culminates in a programmed cell death (Aybeke, 2017). In addition, the secretion of toxins that cause wilting and necrosis of the leaves generated damages, and dark brown vascular discoloration in tomato plants (Pirayesh et al., 2018). These symptoms produced together the high severity levels of *Fusarium* sp recorded in tomato plants.

On the other hand, the observed benefits on incidence and severity have also been reported by other authors (Ghareeb et al., 2011; Huang et al., 2011). They are explained by different mechanisms that ultimately hinder the multiplication of the pathogen favoring plant development. For example, silicic acid induces biochemical barriers, and once polymerized in amorphous silica, generates physical barriers (Cooke and Leishman, 2011; Dann and Le, 2017). In addition, its accumulation in cell walls, intracellular spaces and leaf trichomes generates firmer and more rigid structures (Carré-

Missio et al., 2014; Ferreira et al., 2015). In the cytoplasm, it induces biochemical barriers such as metabolic pathways activation, JA (Bakhat et al., 2018; Ning et al., 2014), SOD and CAT (Cao et al., 2017) increase, flavonoid and PAL expression (Rahman et al., 2015). Silicon also participates in the overexpression or repression of pathogenicity related genes (Brunings et al., 2009).

Biochemical Responses

Fusarium produces toxins such as fusaric acid that causes oxidative stress induced by ROS, causing damage to the membrane, and reducing photosynthetic pigment content in tomato leaf tissue (Singh et al., 2017). In this work, proteins and lycopene clearly were affected by *Fusarium* sp., regardless of whether silicon is applied or not. Opposite case, the chlorophylls were not reduced with the ASi+Fol treatment, it is possible that the application of ASi in *Fusarium* sp. inoculated tomato plants protected against pigment degradation. It has been observed that Si applied on leaves to wheat under hydric stress increased chlorophyll concentration, improving light use efficiency (Maghsoudi et al., 2015). In *Glycine max*, Na_2SiO_3 application promoted Fe mobilization and maintenance in leaves, generating greater protection to chlorophyll degradation (Gonzalo et al., 2013).

The pathogen infection in plants induces oxidative stress increase, triggering the antioxidant defense (Kang et al., 2014), which results in a higher production of antioxidant compounds. In *Glycine max* inoculated with *Cercospora sojina* an increase of antioxidant compounds was observed (Nascimento et al., 2016). Regarding silicon, it has been reported that the application of this element influences phenolic compound content (Hajiboland et al., 2017). Silicon is associated with cell wall components and with polyphenol metabolism alterations (Babalar et al., 2016), which improves water permeability (Liang et al., 2015), and increases defenses against pathogens. Fortunato et al. (2014) reported that Si improves the pathway of phenylpropanoids in banana plants under *Fusarium* sp. stress so there is a direct relationship with phenol production.

The enzyme PAL modulates L-phenylalanine for transcinnamic acid manufacture, which in turn form phenylpropanoids (Hajiboland et al., 2017), which cause the production of phenolic metabolites (flavonoids, coumarins, phenol esters and lignin) (Weaver and Herrmann, 1997).

It is known that *Fusarium oxysporum* colonization in tomato plants secretes effector proteins that are virulence factors, triggering a series of plant responses (Houterman et al., 2007). One of these responses is the increase in CAT enzymatic activity (Dorneles et al., 2017). However, when CAT is not affected in plants, showing a low activity of this enzyme in H_2O_2 elimination (Dallagnol et al., 2015), which suggests less ROS in this treatment, and therefore less stress.

As already mentioned, the infection of pathogens to plants induces an increase in oxidative stress, triggering antioxidant defense (Kang et al., 2014) producing antioxidant enzymes such as GPX and other antioxidant compounds. Silicon can also function as elicitor (Van Bockhaven et al., 2013) and generates changes in different metabolic compounds, since this element is associated with cell wall components (Babalar et al., 2016).

On the other hand, SOD activity has a major importance in the defense against pathogens (Hao et al., 2011), since it regulates the concentration of reactive oxygen species (ROS) in cells (Gill and Tuteja, 2010; Li et al., 2012). Dallagnol et al. (2015) reported that the application of Silicon in melon plants inoculated with *Podospaera xanthii* generated a high concentration of H_2O_2, which increased SOD activity as a defense mechanism. Habibi (2015) found in canola under water stress an increase in SOD activity without observing changes in CAT.

Secondary metabolites

Since SA accumulation is key in the defense of tomato plants after the attack of *Fusarium* (Di et al., 2017). Wang et al. (2015) mentioned that the infection of *Fusarium oxysporum* in *Musa acuminata* triggers the systemic acquired resistance (SAR) that is salicylic acid dependent. On the other hand, Kim et al. (2014) reported that the application of Na_2SiO_3 showed lower concentrations of JA in *Oryza sativa* than mechanical stress treatment alone. *Fusarium* infection triggers the defense mechanism of tomato plants, increasing the production of SA and JA (Jang et al., 2018; Vivancos et al., 2015). This can be caused by ROS and H_2O_2 accumulation which induce the activity of the hydrolase enzymes of benzoic acid and lipid hydroperoxidase, which transform benzoic acid into salicylic acid and synthesize jasmonic acid respectively (Fagerstedt et al., 2010). The application of silicic acid can also induce the production of SA and JA

(Jang et al., 2018), however, the intensity of the response is less than that caused by *Fusarium*.

Gene expression

The PR1 and JA genes expression responses are derived from the increase in salicylic and jasmonic acids. This is because SA and JA acids are markers that regulate the expression of these genes related to plant defense responses (Heil and Bostock, 2002). It highlights the production of different enzymes such as PAL and various antioxidant compounds involved in the ROS reduction.

The application of silicon also induces SA and JA production, related directly with PR1 and JA genes expression (Cai et al., 2009). In tomato plants inoculated with *Ralstonia solanacearum*, the application of silicon over expressed JAZ gene (involved in the signaling pathway of JA) (Kiirika et al., 2013), which indicates that other genes could be activated with the application of silicic acid. It has been documented that silicon application can stimulate SAR by regulating genes involved in the hypersensitivity response and the defense pathway driven by jasmonic acid inducing tolerance to pathogens (Manivannan and Ahn, 2017). Therefore, the positive effect observed in the incidence and severity of *Fusarium* sp. may be related to the activation of other defense genes, or to the increase of antioxidant compounds, or even to structural modifications (Cooke and Leishman, 2011; Dann and Le, 2017).

The gene GAME1 is related to phytoalexins, which are mainly phenolic compounds that accumulate when infections by phytopathogenic fungi such as *Fusarium* appear, participating in the defense against them (Jeandet et al., 2002). The clear effect on the expression of GAME1 gene by the application of silicic acid is consistent with other authors' reports, since it has been shown to induce the production of flavonol phytoalexins in cucumber (Fawe et al., 1998) and momilactones in rice (Rodrigues et al., 2004). Therefore, the production of phytoalexins induced by the application of silicic acid could lead to a decrease in the incidence and severity of *Fusarium*.

Overexpression of PAL is important to regulate *Fusarium* sp. infection, since it leads to a greater accumulation of lignin and synthesis of salicylic acid to trigger SAR (Iqbal et al., 2005). Therefore, tolerance to this pathogen can be increased when these circumstances occur. Zvirin et al., (2010) reported that the expression of PAL in melon

increased as colonization of *Fusarium oxysporum* progressed. In addition, Cruz et al. (2015) observed in wheat plants inoculated with *Pyricularia oryzae* an increase in PAL expression independently of the application of silicic acid. Kuai et al. (2017) and Shetty et al. (2011) obtained higher transcripts of this gene when applying silicon, this led to an increase in lignin and phenolic compounds, reducing the severity of pathogens. This translates into greater tolerance to pathogens such as *Fusarium* sp., through different mechanisms that can be biochemical (phenolic compounds) or mechanical as physical barriers (lignin).

Commonly the attack of pathogens, like *Fusarium* sp., in plants, triggers the production of antimicrobial compounds as well as stress markers such as SA, JA and ethylene (Cai et al., 2009). However, silicon also generates the expression of genes related to plant stress, specifically in the form of silicic acid which induce defense reactions that improve the production of stress hormones (Fauteux et al., 2005). Van Bockhaven et al. (2015) reported that silicon intervenes specifically in the ET signaling pathway. In addition, Ghareeb et al. (2011) reported that the resistance induced by Si is mediated by the signaling pathways of ET and JA in tomato plants infected with *Ralstonia solanacearum*. This suggests that in the case of tomato, the application of silicic acid increases tolerance to *Fusarium* sp. by regulating the genes related to phytoalexins (gene GAME1) and the production of ethylene (gene ET). Moreover, although the reports mention that the attack of pathogens in plants involves the production of ethylene (Cai et al., 2009; Fauteux et al., 2005), the results obtained in tomato indicated that *Fusarium* sp. induces mainly the expression of the gene related to abscisic acid (ABA gene). This can be derived from the antagonism between ABA and ethylene, in addition to the negative effect of ABA to pathogens tolerance (Mauch-Mani and Mauch, 2005). However, it is clear that there is a complex interaction between SA, JA, GAME1, ET and ABA genes that result in different defense responses and tolerance to *Fusarium* sp. in tomato (Anderson et al., 2004; Mauch-Mani and Mauch, 2005).

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Table 1. Primers sequence of analyzed genes.

Gen	(Forward primer 5'-3')	(Reverse primer 3'-5')	Tm (°C) Annealing
ACT	CCCAGGCACACAGGTGTTAT	CAGGAGCAACTCGAAGCTCA	60°C
PR1	AAGTAGTCTGGCGCAACTCA	GTCCGATCCAGTTGCCTACA	60°C
JA	TGGTTCGTCGACTTCGTCAT	CTCGGCCTTGAGAGAGTTCA	60°C
GAME1	TCGTTGTTCCTGGGTTACCT	TCCGAAACTCGAACTTTCTCG	58°C
PAL	GGAGGAGAATTTGAAGAATGCTGTG	TCCCTTTCCACCACTTGTAGC	60°C
ET	TGTCCCAAGCCAGACTTGAT	TGCCATCTTGTTGAGCAATC	60°C
ABA	CTTATTTGGCTATCGCTGAACC	CCTCCAACTTCAAACTCATTGC	60°C

Table 2. Proteins, carotenoids, and chlorophyll content in leaves of tomato plants inoculated with *Fusarium* sp. using Si applications.

Treatments	Proteins	Lycopene	β-carotene	Chlorophyll a	Chlorophyll b	Total Chlorophyll
Asi+Fol	19.96b	6.29ab	41.86a	180.77a	67.83a	248.6a
Asi	28.24a	8.99a	30.51ab	206.37a	71.29a	277.66a
Fol	19.43b	3.08b	28.81ab	92.09b	40.31b	132.4b

T0	29.12a	8.33a	26.29b	171.97a	50.75ab	222.72a
CV (%)	21.9	41.0	25.8	22.13	23.22	22.8

Averages with same letter per column are statistically equal (LSD Fisher, $p \leq 0.05$). Lycopene and β-carotene are expressed as mg 100 g^{-1} Dry Weight (DW), Proteins as mg·g^{-1} DW, Chlorophylls as mg 100 g^{-1} DW. CV: coefficient of variation (%).

Table 3. Non-enzymatic antioxidant compounds in leaves of tomato plants inoculated with *Fusarium* sp. using Si applications at 73 dat.

Treatments	**Vitamin C**	**Glutathione**	**Total phenols**	**Flavonoids**	**ABTS**		**DPPH**	
					(T)	**(AA)**	**(T)**	**(AA)**
Asi+Fol	123.20ab	0.64b	19.47ab	28.8a	5.95a	0.83a	59.86b	5.24b
Asi	96.80b	0.90ab	17.49ab	31.31a	6.00a	0.83a	61.65a	5.42a
Fol	147.84a	1.07a	20.91a	21.8b	6.44a	0.92a	61.45a	5.40a
T0	110.88b	0.96ab	16.43b	30.21a	6.99a	1.02a	61.18a	5.37a
CV (%)	20.30	24.5	12.1	9.6	13.2	17.7	1.0	1.1

Averages with the same letter per column are statistically equal (LSD Fisher, $p \leq 0.05$). Vitamin C is expressed as mg 100 g^{-1} Fresh Weight, glutathione as mM EQ of GSH 100 g^{-1} Dry Weight (DW), total phenols as mg EQ of Gaic Acid g^{-1} of DW, flavonoids as mg EQ of Quercentin g^{-1} of DW, ABTS (2,2'-azino-bis (3-ethylbenzthiazolin-6-sulfonic acid)) and DPPH (1,1-diphenyl-2-pricrilhidrazil) as mM EQ of Trolox 100 g^{-1} DW (T) and mg EQ of Ascorbic Acid 100 g^{-1} DW (AA). CV: coefficient of variation (%).

Table 4. Enzymatic activity in leaves of tomato plants inoculated with *Fusarium* sp. under Si application.

Treatments	**PAL**	**APX**	**CAT**	**GPX**	**SOD**
Asi+Fol	3.12ab	158.56a	0.51b	9.35a	81.88a

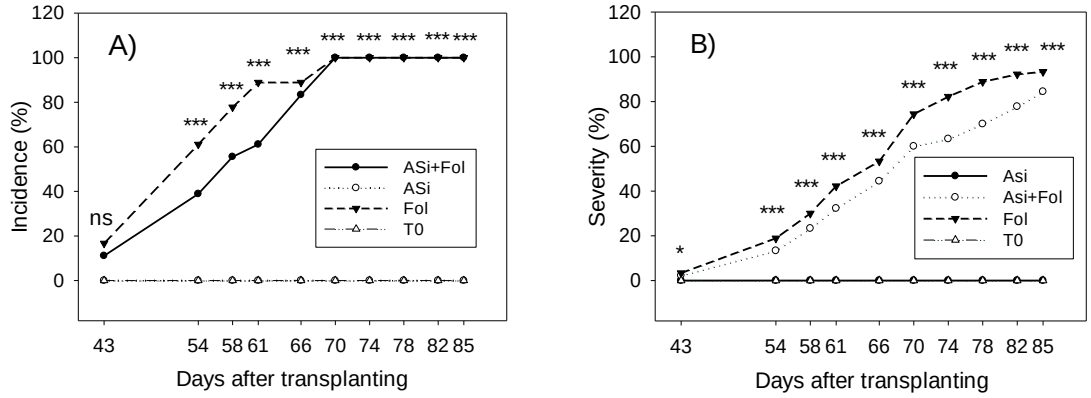
Asi	2.19b	96.34a	0.62ab	7.93a	46.81c
Fol	3.75a	142.46a	0.95a	11.24a	71.19ab
T0	2.23b	165.25a	0.46b	3.76b	48.42bc
CV (%)	31.18	56.72	37.06	26.38	24.33

Averages with same letter per column are statistically equal (LSD Fisher, $p \leq 0.05$). PAL: phenylalanine ammonia lyase (U total protein^{-1} [mg·g^{-1}]), APX: ascorbate peroxidase (U total protein^{-1} [mg·g^{-1}]), CAT: enzymatic activity of catalase (U total protein^{-1} [mg·g^{-1}]), GPX: glutathione peroxidase (U total proteins^{-1} [mg·g^{-1}]), SOD: superoxide dismutase (U·ml^{-1}). CV: coefficient of variation (%).

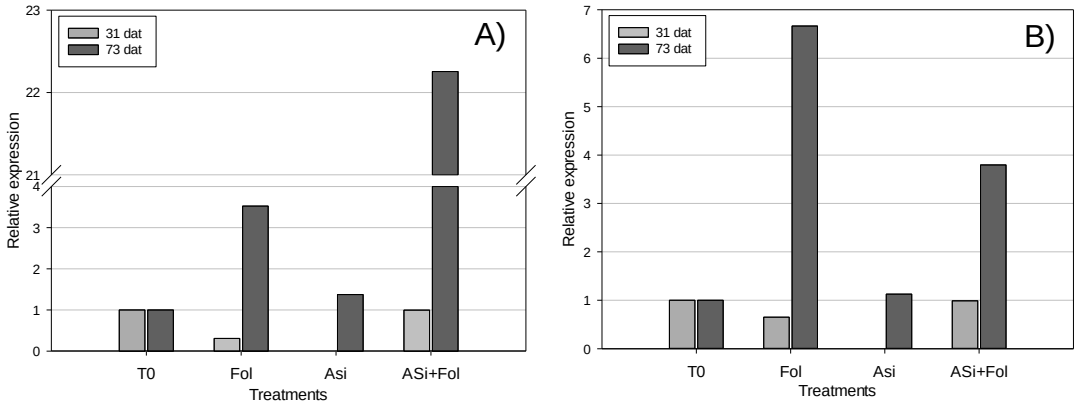
Table 5. Content of secondary metabolites related to defense systems in leaves of tomato plants inoculated with *Fusarium* sp. under Si applications.

Treatments	**Salicylic Acid**			**Jasmonic Acid**		
	15 dat	**31 dat***	**73 dat**	**15 dat**	**31 dat***	**73 dat**
Asi+Fol	Nq	35.10a	171.56a	Nq	5.72b	72.14b
Asi	52.61a	23.94a	72.30ab	Nd	Nd	39.11c
Fol	Nq	32.19a	163.03a	Nq	19.67a	130.68a
T0	21.90b	43.91a	46.86b	Nd	Nd	Nd
CV (%)	42.82	49.63	66.22	-	58.25	33.10

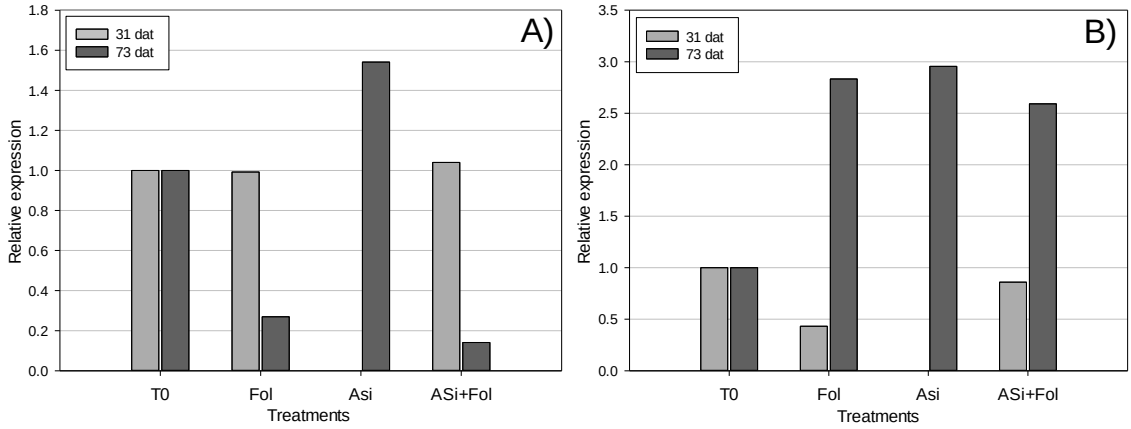
Averages with same letter per column are statistically equal (LSD Fisher, $p \leq 0.05$). Salicylic acid and Jasmonic acid are expressed as mg kg^{-1} of Dry Weight. *: 10 days after inoculation of Fol. Nq: not quantified; Nd: not detected (is present but conjugated, not separated). CV: coefficient of variation (%).



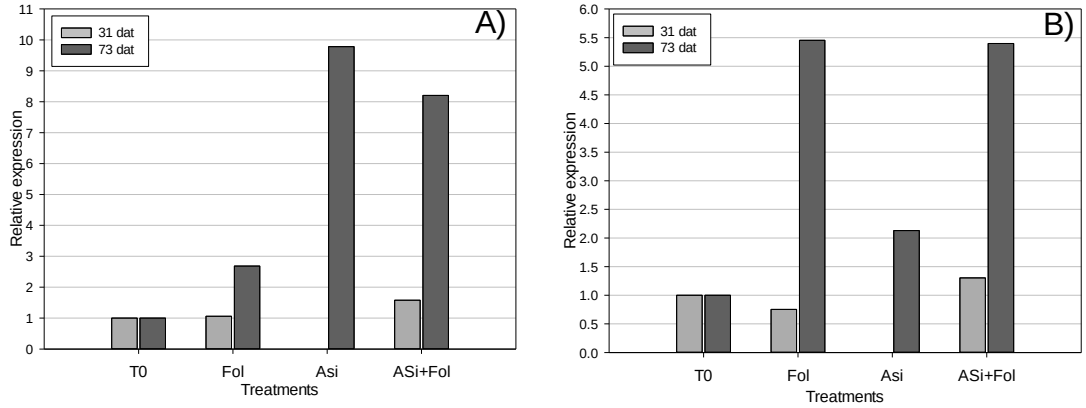
956 **Fig. 1.** Dynamics of incidence (**A**) and severity (**B**) of *Fusarium* sp. in tomato plants
957 under Si application. ns: not significant, *, ***: statistical differences at p <0.05,
958 <0.0001 respectively.



959 **Fig. 2.** Relative expression of PR1 (**A**) and JA (**B**) genes in tomato leaves treated with
960 ASi under stress by *Fusarium* sp. at 31 dat and 73 dat.



961 **Fig. 3.** Relative expression of the GAME1 (Phytoalexins) (**A**) and PAL5-3
962 (Phenylalanine ammonia lyase) (**B**) genes in tomato leaves treated with ASi under stress
963 by *Fusarium* sp. at 31 dat and 73 dat.



964 **Fig. 4.** Relative expression of ET (ethylene) (**A**) and ABA (abscisic acid) genes (**B**) in
965 tomato leaves treated with ASi under stress by *Fusarium* sp. at 31 dat and 73 dat.

CONCLUSIÓN GENERAL

La aplicación de ácido silícico redujo la incidencia y severidad de *Fusarium* sp. ya que aumentó el contenido de compuestos antioxidantes en las hojas. Además de esto, aumentó la producción de ácido salicílico y ácido jasmónico y condujo a la sobreexpresión de genes que codifican fitoalexinas, fenilalanina amoniaco liasa y etileno principalmente (GAME1, PAL5-3 y ET), que en su conjunto aumentó la tolerancia a *Fusarium* sp. en tomate.

En base al modelo presentado, se recomienda que cuando los cultivos crecen en suelo la biodisponibilidad del silicio se puede aumentar agregando materia orgánica de enmiendas orgánicas o sustancias húmicas, o modificando el pH de la solución del suelo, usando ácidos orgánicos o inorgánicos, para ser lo más cercano a 7.0. En sistemas de cultivo sin suelo, lo mejor es aumentar el contenido de Si en el agua de riego, al menos 96,1 mg L^{-1} de $Si(OH)_4$. La mayoría de los suelos utilizados para la agricultura en áreas semiáridas o áridas tropicales o subtropicales tienen condiciones que no favorecen la disponibilidad de $Si(OH)_4$, por lo tanto, es aconsejable aplicar silicatos de sodio, potasio o calcio. A largo plazo, la disponibilidad de Si se puede aumentar en el suelo utilizando fuentes minerales ricas en Si, como arenas silíceas.

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