Universidad de Granada
Facultad de Ciencias
Departamento de Fisiología Vegetal

BIOFORTIFICACIÓN CON POTASIO EN PLANTAS DE TOMATE CHERRY: ESTUDIO DE LA PRODUCCIÓN Y CALIDAD DE FRUTOS EN COSECHA Y POSTCOSECHA

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BIOFORTIFICACIÓN CON POTASIO EN PLANTAS DE TOMATE CHERRY: ESTUDIO DE LA PRODUCCIÓN Y CALIDAD DE FRUTOS EN COSECHA Y POSTCOSECHA

Memoria de Tesis Doctoral presentada por el licenciado en Biología Christian Constán Aguilar para aspirar al grado de doctor:

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Granada, Octubre 2014

El trabajo que se presenta en esta memoria de Tesis Doctoral ha sido realizado en el Grupo de Investigación “Diagnóstico Nutricional de las Plantas Cultivadas en Condiciones Adversas” (AGR-161, Plan Andaluz de Investigación, Junta de Andalucía), del Departamento de Fisiología Vegetal de la Facultad de Ciencias de la Universidad de Granada (España) en colaboración con el INIA. Proyecto RTA2009-00005-00-00 and was carried out at IFAPA and the AGR161 research group, Granada, Spain. Además, este trabajo ha sido cofinanciado por dicho grupo y por la Unión europea a través de su programa de fondos FEDER.

El doctorando Christian Constán Aguilar y los directores de la tesis Dr. Juan Manuel Ruiz Sáez y Dra. Begoña Blasco León garantizamos, al firmar esta tesis doctoral, que el trabajo ha sido realizado por el doctorando bajo la dirección del director de la tesis y hasta donde nuestro conocimiento alcanza, en la realización del trabajo, se han respetado los derechos de otros autores a ser citados, cuando se han utilizado sus resultados o publicaciones.

Granada, Octubre 2014

Directores de la Tesis

Fdo. Dr. Juan Manuel Ruiz Sáez

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Doctorando
Fdo. Christian Constán Aguilar

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Con mi agradecimiento a tod@s l@s “grandes” que han contribuido a construir el conocimiento, razonamiento y pensamiento científico. Desde pequeño, he magnificado a tod@s ell@s, he querido aprender a observar, razonar, y experimentar partiendo de los presupuestos que ell@s establecieron. Gracias a mis docentes, he logrado hacerme preguntas, construir hipótesis y adquirir una metodología científica.

Como escribió Aristóteles en su Metafísica y citando solamente un pequño fragmento, *“Los hombres comienzan y comenzaron siempre a filosofar movidos por la admiración… de suerte que si filosofaron para huir de la ignorancia es claro que buscaban el saber”*

Del mismo modo, yo, “filosofando” he intentado buscar el saber y el conocer, un poco más, un “pasito” más… Podría considerarse que para dedicarse a investigar es necesario tener cubiertas las necesidades primarias. Esto no es lo más frecuente en la actualidad. Es por ello, me he querido acoger a la libertad, la definida por Aristóteles, la completa libertad orientada *“a la búsqueda y ejercicio de la ciencia en cuanto tal”*, es decir sin sometimientos ni dependencia del científico de lo económico. No ha sido fácil, por eso, mis agradecimientos al Instituto de Crédito Oficial y al Banco de Santander por la “losa” que les dejo a deber y que no sé cómo saldaré. En el momento en la que la solicité eran *“vox populi”* o *“vox politicis”* las siglas I+D+I, y esa voz ya no se oye. Ha merecido la pena.

En mi concepción de lo que debe ser el conocimiento científico todos l@s que me precedieron me han hecho concebirlo como una actividad y empresa humana. En dicha actividad deben estar incluidas todas las disciplinas más relevantes: Biología, Psicología, Antropología…que nos lleven a construir y llegar a la sabiduría. Así mismo, el concepto de ciencia que en mi se ha formado, es que ésta, no debe ser una mercancía solo valorada por el precio. L@s grandes a l@s que me refiero contribuyeron a crear no solo actitudes pragmáticas sino que construyeron una filosofía de la naturaleza.

Tomando nota de Ilya Prigogine, quizá debamos contribuir a preservar la base humanística de la ciencia, en caso contrario, se corre el riesgo de que ésta pierda el atractivo para la juventud mejor dotada. Si mi consideración y agradecimientos es para ést@s padres y madres de la ciencia y de la filosofía de la ciencia, no puedo dejar de mostrar mi agradecimiento a aquellos que vieron truncada su vida en plena juventud, y que no pudieron por ésta causa desarrollar sus ideas y poner en práctica los conocimientos que habían adquirido. Mi más profundo de los agradecimientos a grandes amig@s míos que en plena juventud y de forma prematura se marcharon. Un especial recuerdo a un compañero de laboratorio con quien tuve el placer de compartir algunos ratos de charlas y de ensayos y quien dejó una huella muy particular en el grupo AGR161. Todos ellos de una forma u otra también contribuyeron a conformar el modelo de ciencia en la que creo.

Con objeto de no dejar ningún nombre en el tintero, en estos agradecimientos no voy citar nombres de personas a las que estoy agradecido, voy a generalizar, pero con ímpetu y de forma honrada, con toda mi fuerza y todo mi ser, muchas gracias a toda mi familia, amig@s.

Mi más sincero agradecimiento a mis compañer@s de carrera, profesión, departamento, laboratorio, maestr@s, profesores y directores de tesis.

Poco a poco nos iremos encontrando y os iré agradeciendo. Concluyo escribiendo:

“Por mí y por todos mis compañer@s!”

Ilustraciones: Ana Aguilar Díaz. Muchas gracias!

"Eppur si muove..." (y sin embargo se mueve, en español)
Frase atribuída a Galileo Galilei, pronunciada por éste durante su juicio inquisitorio.
(Astrónomo, filósofo, matemático y físico italiano. Pisa, 15 de febrero de 1564 –Arcetri, 8 de enero de 1642)

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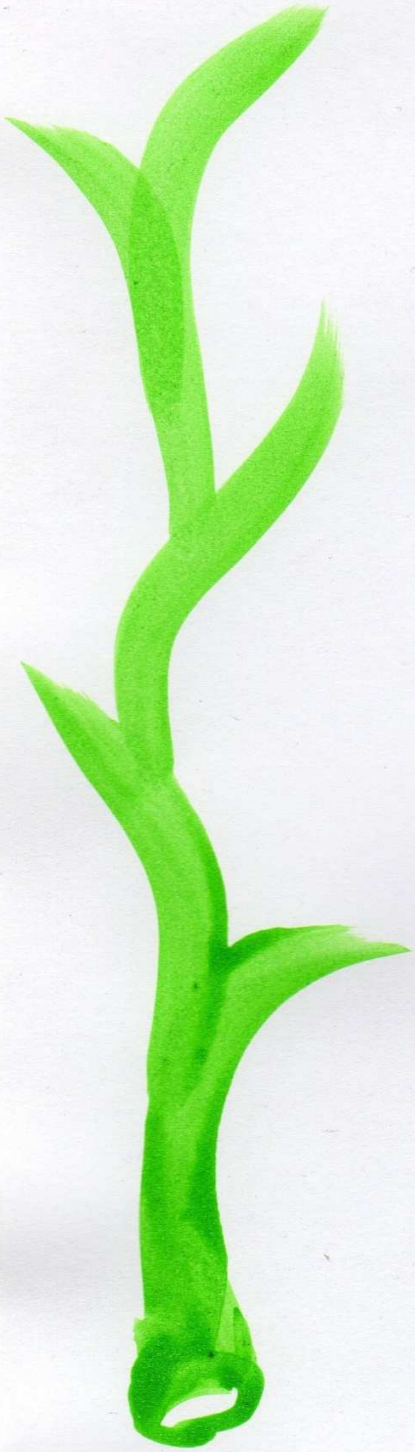


RESUMEN

El tomate (*Solanum lycopersicum* L.) es una planta hortícola anual con una gran distribución mundial y un valor económico elevado, que gracias a su gran contenido en compuestos antioxidantes presenta una serie de efectos beneficiosos para la salud a través de su consumo. Introducido desde América del Sur a principios del siglo XVIII, el tomate (tanto fresco como procesado) es un componente esencial en la dieta mediterránea tradicional, una dieta comúnmente conocida por ser beneficiosa para la salud, especialmente en relación al desarrollo de enfermedades degenerativas crónicas. Muchos estudios epidemiológicos han relacionado al efecto beneficioso del consumo de tomate con la prevención de algunas de las principales enfermedades crónicas, como son algunos tipos de cáncer y enfermedades cardiovasculares. Los beneficios protectores de los compuestos antioxidantes son, en parte, debidos a su capacidad de detoxificar los radicales libres y, por tanto, prevenir los cambios oxidativos anormales producidos en el cuerpo humano. Así, los tomates son beneficiosos para la salud humana gracias a su elevado contenido de fitonutrientes como licopeno, β-caroteno, compuestos fenólicos, ácido ascórbico y otros nutrientes esenciales.

Respecto a las características nutricionales de los frutos de tomate, uno de los fitonutrientes más característicos del tomate es el licopeno (Lyc), un carotenoide con una gran capacidad de eliminar especies reactivas de oxígeno (ROS) y que representa más del 80% de los carotenoides totales en el fruto de tomate. El Lyc es también responsable del enrojecimiento del tomate, debido a la diferenciación de los cloroplastos a cromoplastos, por eso este carotenoide es muy importante en la calidad nutricional final y comercial de este producto hortícola. Existen muchos

estudios donde se observa una fuerte relación entre la calidad nutricional del tomate y su contenido de Lyc, ya que se ha reconocido como una molécula que suprime la proliferación celular en humanos e interfiere con el crecimiento de las células cancerígenas, previniendo así la incidencia de cáncer de próstata. Otro carotenoide presente en los frutos de tomate es el β-caroteno, un fotoprotector que actúa en la fotosíntesis como pigmento recolector de luz, aunque de menor importancia que el Lyc ya que constituye sólo el 7% del contenido total de carotenoides del fruto. El β-caroteno, también conocido como pro-vitamina A, es un poderoso antioxidante con una serie de beneficios para la salud humana: ayuda al sistema inmune y destruye las células cancerígenas; reduce el riesgo de enfermedades cardiovasculares, síndrome de fatiga crónica, soriasis, cáncer de piel y lupus; y es necesario en la prevención de la ceguera y las cataratas, y en la recepción de luz por el ojo humano.

Junto a los carotenoides, los compuestos fenólicos o polifenoles representan otra de las dos grandes clases de fitonutrientes encontrados en frutos y vegetales de la dieta mediterránea, y especialmente en frutos de tomate. Los polifenoles son unos componentes vegetales ubicuos que derivan principalmente de la fenilalanina a través del metabolismo fenilpropanoide. Además, estos compuestos confieren un papel importante en la respuesta a condiciones de estrés. Así, los compuestos fenólicos pueden actuar en las plantas como fitoalexinas, frente a la herbivoría, como atrayente de polinizadores, contribuyendo a la pigmentación vegetal, como antioxidantes y protegiendo frente a la luz UV. El interés de los compuestos fenólicos como antioxidantes se centra principalmente en los flavonoides. Las

funciones de los flavonoides en las plantas no están todavía muy claras, aunque se les atribuyen funciones en los mecanismos de defensa frente a la herbivoría, estrés por patógenos y radiación UV-B. Por otro lado, existen estudios epidemiológicos que sugieren un beneficio por el consumo humano de frutas y verduras ricas en flavonoides, ya que protegen frente a las enfermedades cardiovasculares, cáncer u otras enfermedades relacionadas con la edad como la demencia. Finalmente, existen flavonoides, como las antocianinas, que son también importantes como antioxidantes, ya que protegen a las plantas frente al estrés oxidativo, actuando como un fotoprotector que absorbe la luz naranja-verde del espectro visible, previniendo así la foto-oxidación de las clorofilas. Además de la protección frente a la luz UV, a las antocianinas también se le han atribuido funciones como antioxidantes y antiherbivoría. Aparte de sus funciones fisiológicas en las plantas, las antocianinas se consideran componentes importantes en la nutrición humana, ya que producen un aumento en la capacidad antioxidante, transportándose a zonas con una actividad metabólica elevada donde producen una reducción en la permeabilidad y fragilidad de los capilares, inhibiendo la agregación de las plaquetas y toda estimulación inmune.

Sin embargo, el antioxidante más efectivo de los diferentes productos vegetales es el ascorbato o vitamina C. Este compuesto tiene un papel fisiológico muy importante ya que, además de estar envuelto directamente en la eliminación de ROS y en la regeneración de la vitamina E en las plantas, participa en el metabolismo celular y en el control del crecimiento, en la división celular, en la expansión de la pared celular y en la organogénesis. Como un antioxidante, el

ascorbato elimina directamente los ROS a través de una vía no enzimática y reduce el peróxido de hidrógeno a agua a través de la reacción ascorbato peroxidasa (APX). No obstante, el ascorbato puede ser sintetizado por las plantas y por la gran mayoría de los mamíferos, pero no por los seres humanos, donde es fundamental en el mantenimiento de un sistema inmune saludable, ya que reduce la severidad de algunas enfermedades como el resfriado o la gripe mediante la prevención de las infecciones virales secundarias o bacterianas, protegiendo frente al daño producido por los ROS, y en la prevención de enfermedades cardiovasculares.

Por otro lado, una dieta rica en potasio (K) es esencial para mejorar, evitar o prevenir ciertas enfermedades en humanos. En la actualidad en los países desarrollados se consume una dosis baja de K (alrededor de 70 mmol $día^{-1}$) debido al consumo de alimentos procesados y una dieta insuficiente en frutas y verduras. Debido a esta razón se esta empezando a trabajar en los llamados programas de biofortificación con K en plantas con el fin de aumentar la ingesta diaria de K. En este sentido distintos estudios tanto epidemiológicos como clínicos han demostrado que una dieta rica en K produce una reducción de la tensión arterial, reduce la mortalidad debida a enfermedades cardiovasculares, disminuye el riesgo de osteoporosis y previene el desarrollo de la diabetes. Estos trabajos muestran también que la mejor forma de tomar K en la dieta es mediante el consumo de frutas y hortalizas ricas en éste elemento, por lo que es necesario en la agricultura actual incrementar las concentraciones de K en los productos agrícolas destinados al consumo humano bien mediante la fertilización o bien

mediante el uso de genotipos con una mayor eficacia en la utilización de este macronutriente.

Además de su efecto beneficioso para los humanos, el K es uno de los nutrientes considerados como esenciales para la producción y calidad de los cultivos. El K parece ser uno de los nutrientes más importantes que puede afectar de forma positiva a la mejora de la calidad nutricional. Según las pocas investigaciones que se pueden encontrar al respecto el aumento en la fertilización con K induce la transpiración y la fotosíntesis en plantas lo que supone un aumento en la producción de fotoasimilados. Además un aumento en la fertilización con K supone un incremento del transporte de los fotoasimilados a los frutos lo que mejora su producción y calidad nutricional. Por otro lado el K también aumenta la síntesis de aminoácidos y proteínas en hojas y su posterior transporte a los frutos. En cuanto al efecto del K en frutos sobre otros compuestos responsables de la mejora de la calidad nutricional, se ha comprobado que su aplicación aumenta los carotenoides (Lyc, y β-caroteno) y el ascorbato. Su posible efecto sobre el resto de las características nutricionales en frutos de tomate no se conoce aún por lo que es necesaria una investigación más exahustiva.

Por todo ésto, los objetivos de ésta Tesis Doctoral fueron:

1. Evaluar el efecto de un programa de biofortificación con diferentes dosis de K en forma de KCl durante el ciclo de cultivo de plantas de tomate cherry sobre la producción y calidad de los frutos.

2. Analizar cómo afectó el almacenamiento a 4ºC durante 21 días a la fisiología de frutos de tomate cherry recolectados de plantas sometidas a un programa de biofortificación de K.

Para llevar a cabo el primer objetivo de ésta Tesis Doctoral, semillas de tomates cherry (*Solanum lycopersicum* L. cv AsHiari injerta en portainjerto cv . Maxifort) se sembraron en bandejas planas (tamaño de celda de 3 cm x 3 cm x 10 cm, 100 células por bandeja) llenos con una mezcla 50 % [v/v] perlita-vermiculita, y se mantuvieron en condiciones de invernadero durante 5 semanas. Posteriormente, las plántulas se trasplantaron a un invernadero experimental en la Estación Experimental La Nacla (Motril, cerca de la costa de Granada, en el sur de España (36º45'N, 3º30'W; altitud 130 m). El invernadero parral consistió en tres módulos con un techo a dos aguas simétrico, con una pendiente de 27 º y orientación longitudinal EW. El control ambiental activo se limitó a un sistema de ventilación natural cenital y lateral. En el invernadero, el material de revestimiento consistió en

una película de múltiples capas de 0,2 mm de espesor, con una capa de etileno-vinilo-acetato de entre las dos capas interior (antigota) y exterior (de larga vida) de polietileno de baja densidad.

Las plantas se cultivaron en sacos de 40-L llenos perlita B-12- (1,20 m de largo) espaciados 0,5 m de distancia en filas, con 1.4 m de separación. El marco de plantación fue de 3,21 plantas m^{-2}, con 3 plantas de tomate por saco y 2 tallos por planta,. Su disposición en el invernadero fue en 12 filas con orientación Norte -Sur. El diseño estadístico fue en bloques al azar. Los diferentes tratamientos aplicados fueron: 5 mM de KCl, 10 mM de KCl, 15 mM de KCl y 20 mM de KCl desde el inicio hasta el final del experimento. Utilizando el mismo invernadero experimental.

Se realizaron 2 ciclos de cultivo en años consecutivos que se extendieron desde Octubre de 2010 a Mayo de 2011 (Primer ciclo) y desde Octubre de 2011 a Mayo de 2012 (Segundo ciclo).

Del efecto de un programa de biofortificación con K en forma de KCl durante los dos ciclos de cultivo y en los diferentes muestreos realizados se puede concluir que:

i) Para los frutos de tomate cosechados la semana después del transplate (SDT) 20 se observó una mayor concentración de K, en especial con los tratamientos 15 y 20 mM KCl, los parámetros de capacidad antioxidante se vieron mejorados con estas dosis, aunque no incrementaron significativamente las cualidades organolépticas. Aunque el peso individual del fruto para los tratamientos de 15 a

20 mM KCl resultó ser inferior al de los recolectados de las plantas tratadas con las dosis de 5 a 10 mM KCl, el resto de los parámetros relativos a la producción comercial junto a la producción comercial acumulada no mostraron diferencias significativas, por lo que no se vio comprometida la producción comercial.

ii) Los frutos de tomate cosechados a la SDT 24 acumularon menos K que los cosechados en la SDT 20, los parámetros de capacidad antioxidante se vieron mejorados con las dosis 15 y 20 mM KCl y se vieron incrementadas las cualidades organolépticas.

iii) En general, y para los diferentes tratamientos de K en forma de KCl aplicados durante el programa de biofortificación propuesto, los frutos de tomate muestreados a la SDT 29 fueron los que menos K acumularon, presentaron una mejora en la capacidad antioxidante y en cuanto a las cualidades organolépticas, mostraron una tendencia a descender con las dosis más elevadas de K.

Respecto al segundo objetivo de ésta Tesis Doctoral, cuantificación de la capacidad antioxidante, medida de los índices de calidad y estudio del metabolismo del carbono tras el periodo de almacenamiento postcosecha, optamos por utilizar los frutos de tomate cosechados en la SDT 20 ya que fueron los que mayor concentración de K acumularon.

En éstos se observó más claramente el efecto de los diferentes tratamientos respecto a la concentración de K en frutos. Por éstas razones *"a priori"* cabe esperar que si existe un efecto del K sobre la postcosecha, se observe de forma

más evidente en éstos frutos. Los frutos estudiados fueron los cosechados de las plantas sometidas a las dosis 5, 10 y 15 mM KCl, eliminando el tratamiento de 20 mM KCl ya que entre éste tratamiento y el de 15 mM KCl no se observaron diferencias significativas entre las [K].

De los estudios realizados y que forman parte de éste segundo objetivo de la tésis destacar que:

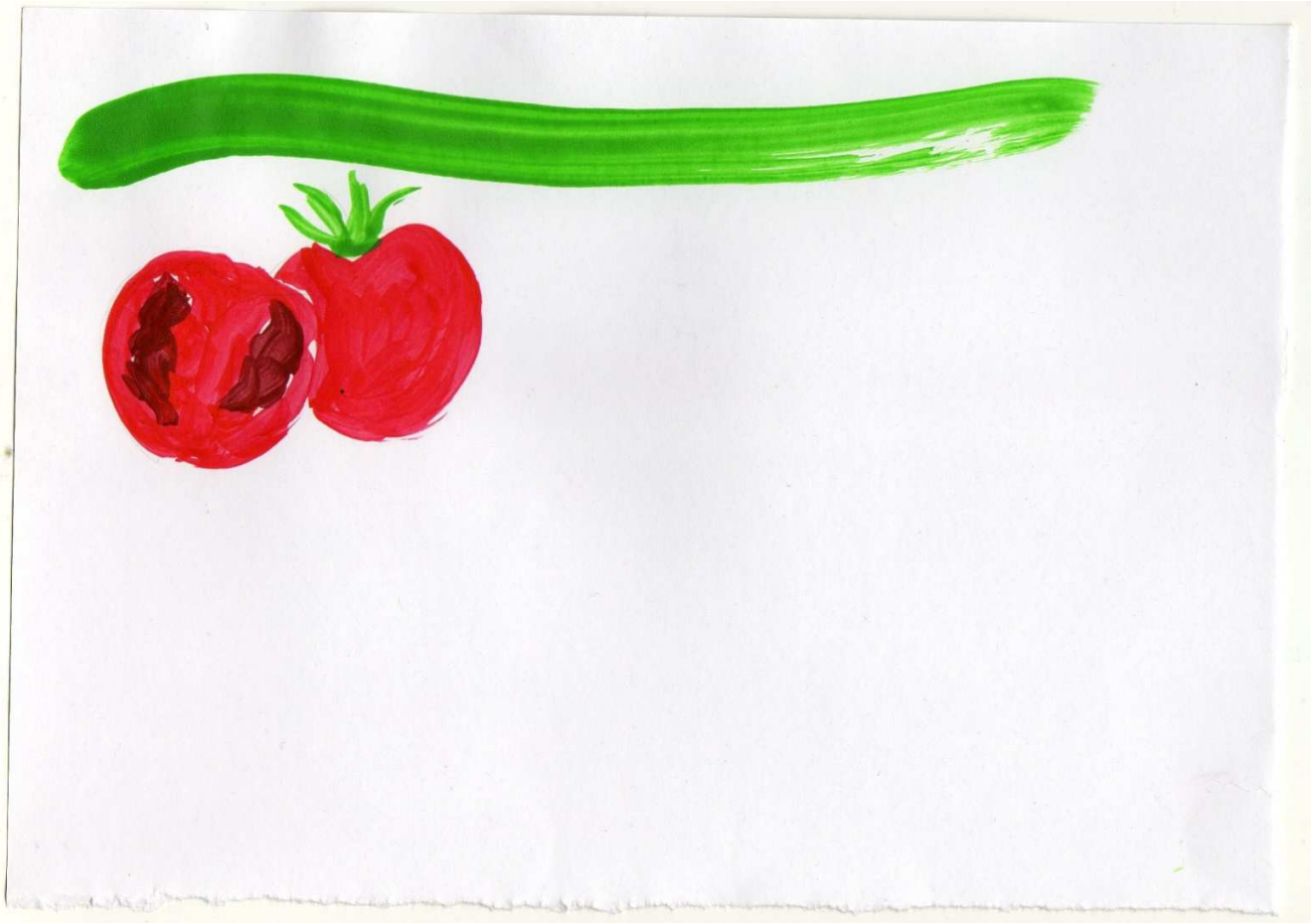
i) La aplicación de un programa de biofortificación de K en forma de KCl a altas tasas de aplicación (15 mM) podría constituir una estrategia beneficiosa para mejorar la calidad y la capacidad antioxidante de los frutos de tomate cherry que son almacenados en frío antes de su consumo. El tratamiento de 15 mM de KCl, además, impide la pérdida de peso y el agua en frutos de tomate cherry durante el almacenamiento postcosecha a 4°C durante 21 días, supone una mayor acumulación de la concentración de K e induce un aumento de la capacidad antioxidante mediante el aumento de la concentración de Lyc, mantiene el contenido en vitamina C, ácido hidroxicinámico y sus derivados, y aumenta los flavonoides y derivados, lo que significa que el consumo de estos frutos podría ofrecer beneficios para la salud humana.

ii) Por otro lado, demostramos que un programa de biofortificación adecuado con K puede resultar beneficioso, ya que alivia el estrés por frío en frutos de tomate resultante del almacenamiento de éstos en cámaras frigoríficas. En concreto, los frutos de tomate de las plantas tratadas con 15 mM KCl presentaron menor

pérdida de biomasa después del almacenamiento postcosecha, así como un menor grado de peroxidación lipídica, posiblemente debido a una mayor actividad APX y monodehidroascorbato reductasa (MDHAR), lo que sugiere una mayor eficacia en la detoxificación de ROS así como en la regeneración del ascorbato (AsA). Además, bajo este tratamiento de K, los frutos presentaron un mayor *"pool"* de AsA, así como una mayor concentración de glutation reducido (GSH). Por lo tanto, se concluye que la dosis de 15 mM de KCl aplicada a esta variedad de tomate podría ser adecuada para mitigar los efectos negativos causados por el almacenamiento postcosecha a bajas temperaturas.

iii) Finalmente, y respecto al metabolismo del carbono, la aplicación de un programa de biofortificación con K a concentraciones de 10 mM y específicamente con el tratamiento de 15 mM de KCl, estimuló la degradación de sacarosa por la actividad sacarosa sintasa (SuSy), incrementó los niveles de glucosa (Glu) y fructosa (Fru) y la inducción de la acumulación de malato por la actividad de las enzimas fosfoenol piruvato carboxilasa (PEPC) y malato deshidrogenasa (MDH) durante el almacenamiento durante 21 días a 4ºC. Por lo tanto, en nuestro trabajo la acumulación de Glu, Fruc, y malato podrían explicar la función de protección durante el almacenamiento en frío que produce el tratamiento 15 mM de KCl. Por último, indicar que la aplicación de un programa de biofortificación con altas dosis de K (en nuestro caso 10 y 15 mM de KCl) mejora claramente la calidad organoléptica de los frutos de tomate cherry durante la postcosecha a 4ºC, con un aumento del índice de dulzor.

CAPÍTULO 1: ANTECEDENTES



1. 1. INTRODUCCIÓN

1. 1. 1. TOMATE (*Solanum lycopersicum* L.): DESCRIPCIÓN Y TAXONOMÍA

La especie *Solanum lycopersicum*, conocida comúnmente como "tomate, "jitomate o "tomatera" , pertenece a la familia de las solanáceas. Etimológicamente, la denominación *jitomate* procede del náhuatl *xictli*, "ombligo", y *tomātl*, "tomate", que significa *tomate de ombligo*. La nomenclatura científica que recibe la especie proviene de *Solanum*: vocablo latino que hace alusión a *sol. -is*, "el sol", ya que la planta es propia de sitios soleados (Sobrino y Sanz, 2010). El epíteto específico *lycopersicum*: del griego *λύκος* lyco = lobo, y *περσικός* persicum = persa, hace alusión a la "manzana persa", nombre que se dio en Europa al melocotón que llegaba a Persia desde China. El nombre tuvo su origen en el mito del hombre lobo. Según leyendas germánicas, brujas y magos utilizaban los frutos de la belladona en sus pociones para convertirse en hombres lobo. Cuando el tomate llegó a Europa procedente de América, el gran parecido con esos frutos hizo que fuera llamado popularmente "wolf peach" (melocotón de lobo).

La ubicación taxonómica del tomate dentro de la familia de las Solanáceas no ha suscitado dudas, sin embargo, existen controversias en cuanto a su ubicación genérica. Caspar Bauhin (1623) en su Pinax reconoce la existencia de un grupo de plantas que incluyen los actuales géneros *Solanum*, Atropa L., Physalis L. y otros. En 1700, Tournefort establece siete géneros colocando los de fruto blando en un grupo diferenciado. Este autor reconoció *Lycopersicum* como distinto de *Solanum*. Linneo, apoyándose en el Pinax, incluyó *Lycopersicum* dentro del

género *Solanum*, denominando al tomate *Solanum lycopersicum* (Esquinas-Alcázar y Nuez, 1995). Por tanto, en la actualidad son dos los nombres binomiales aceptados como sinónimos: *Lycopersicon esculentum* Mill. y *Solanum lycopersicum* L. (*esculentum* hace alusión a comestible).

Asumiendo esta introducción histórica, en nuestro trabajo utilizaremos la nomenclatura linneana para referirnos al tomate, siendo así, el tomate quedaría ubicado dentro del género *Solanum*, que en cuanto a número de especies concierne es relativamente poco importante dentro de la familia en la que se encuadra, las *Solanaceae*. Atendiendo a características morfológicas del embrión, esta familia se divide en dos subfamilias: la *Cestroidae* y la *Solanoidae*. El carácter más importante de la subfamilia *Solanoidae*, en la que se incluyen los géneros *Lycopersicum* y *Solanum* L., es que todos sus miembros poseen una gran uniformidad en el número cromosómico (2n=24). Estos dos géneros se diferencian entre sí por la presencia de expansiones apicales estériles en las anteras en *Lycopersicum*, que están ausentes en *Solanum* (Taylor, 1986). Otra característica que diferencia a estos géneros es el mecanismo de dehiscencia anteridial, *Lycopersicum* presenta dehiscencia longitudinal mientras que en el género *Solanum*, la apertura de las anteras se realiza a través de poros apicales (Rick, 1982). Estudios posteriores demostraron que la dehiscencia en *Lycopersicum* comienza por poros apicales que derivan rápidamente en surcos longitudinales (Bonner y Dickinson, 1989).

La taxonomía generalmente aceptada es la siguiente (Cronquist, 1984; Esquinas-Alcázar y Nuez, 1995; Peralta et al, 2005):

i. Reino: *Plantae*

ii. Subreino: *Embryobionta*

iii. División: *Magnoliophyta*

iv. Subdivisión: *Angiosperamae*

v. Clase: *Magnoliopsida*

vi. Subclase: *Dicotyledoneas.*

vii. Orden: *Solanales (Personatae) (Tubiflorae).*

viii. Familia*: Solanaceae*.

ix. Subfamilia: *Solanoideae.*

x. Género: *Solanum.= (Lycopersicum)*

xi. Especie: *Lycopersicum = (L. scullemtum MILL.)*

Originario de una región montañosa estrecha y alargada de los Andes, que comprende Ecuador, Perú y Chile (Peralta y Spooner, 2000) la domesticación y cultivo de éste parece que tuvo lugar inicialmente fuera de su origen con las primeras civilizaciones de México. La primera descripción botánica del tomate la realizó Pier Andrea Mattioli, del jardín botánico de Padua (Italia), quien publicó su herbario en 1554. Desde entonces aparece descrito en numerosos herbarios como el de Matthias de L'Obel en 1581, el de Gerard en Inglaterra en 1597 o el de Salmon en Estados Unidos, ya en 1710. Se trata de una planta perenne de porte arbustivo que se cultiva casi exclusivamente como anual. Existen dos tipos

diferenciados en base al modelo de crecimiento que presentan. Las plantas de crecimiento indeterminado tienen tallos que presentan segmentos uniformes con tres hojas (con yemas) y una inflorescencia, terminando siempre con un ápice vegetativo. A diferencia de esta, las plantas con crecimiento determinado muestra tallos con segmentos que presentan progresivamente menos hojas por inflorescencia y terminan en una inflorescencia, lo que resulta en un crecimiento limitado. El tallo típico tiene de 2 a 4 cm de diámetro en la base y está cubierto por pelos glandulares y no glandulares que crecen desde la epidermis. El sistema radical del tomate es fibroso y robusto, pudiendo llegar de 1.2 a 1.8 m de profundidad. Está constituido por la raíz principal, las raíces secundarias y las raíces adventicias. Las hojas son compuestas, imparipinnadas con 7 u 9 foliolos. (Chamarro, 1995; Esquinas-Alcázar y Nuez, 1995).

El racimo floral o inflorescencia está compuesta de varios ejes, cada uno con una flor de color amarillo (4 a 12 flores). Sus flores son radiales y con cinco estambres. El ovario, súpero bicarpelar, contiene numerosos primordios seminales, produciendo bayas polispermas. Los carpelos se presentan en posición oblicua con respecto al plano mediano de la flor. Tras la polinización y fertilización sucede el crecimiento del fruto por procesos de división celular y aumento de tamaño de éstas. Tras la polinización hasta la maduración transcurre un periodo de entre 6 y 10 semanas. El fruto es una baya de forma globular, ovoide o aplastada cuyo peso oscila, según variedades entre 5 y 500 gramos y puede ser bi- o plurilocular. Está unido a la planta por un pedicelo con un engrosamiento articulado que contiene la capa de abscisión. El color más común del fruto es el rojo, aunque los hay

amarillos, verdes, naranjas, etc. (Chamarro, 1995; Esquinas-Alcázar y Nuez, 1995).

La semilla se considera madura cuando el fruto ha completado su madurez, tiene forma discoidal-lenticular comprimida y embrión enrollado, de diámetro más o menos uniforme (Chamarro, 1995).

1. 2. IMPORTANCIA DEL CULTIVO DE TOMATE

Los tomates (*Lycopersicon esculentum* L.) pertenecen a la categoría de las frutos hortícolas con alto consumo a nivel mundial Según la Organización de las Naciones Unidas para la Agricultura y la Alimentación (FAO, 2011). En 2011 se produjeron 157.35 MMt (millones de toneladas) de tomates frescos en el mundo, y es en las regiones tropicales y subtropicales donde se encuentran los mayores productores a nivel mundial. China es el mayor productor, con 48.45 MMt, le sigue India y EE.UU con una producción de 16.83 y 12.53 MMt, respectivamente. España se encuentra en la posición número 9 con algo más de 3.8 MMt. En la figura 1 se puede ver la producción de los 20 principales países productores en porcentaje de producción, y en la tabla 1 en millones de toneladas (MMt) y miles de hectáreas (MHa).

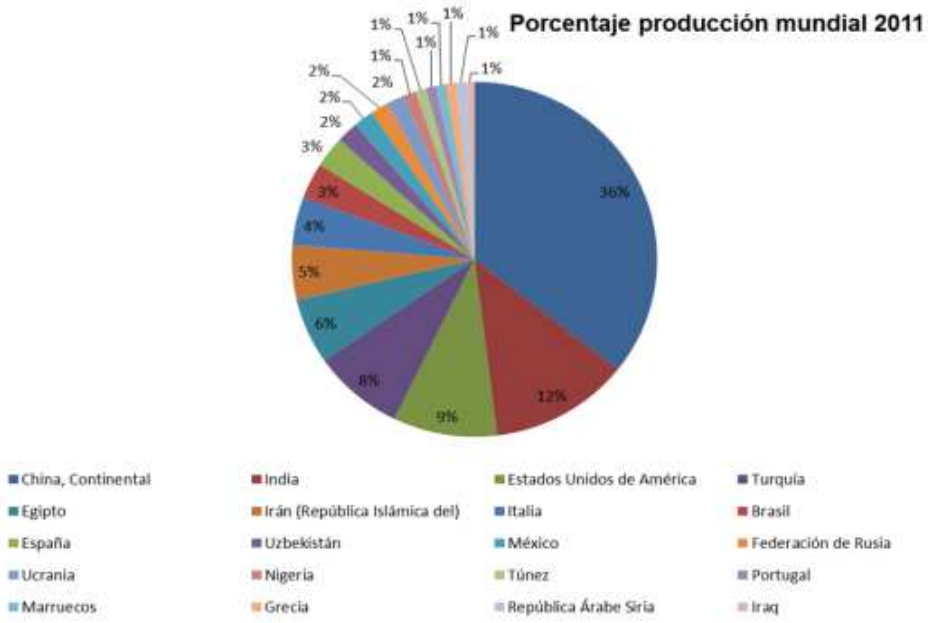


Figura 1. Producción mundial de tomate. Tomado de: FAO: 2011

Tabla 1. Producción mundial de tomate por países.

Posición	País	Producción (MMt)	Área cosechada (MHa)
1	China, Continental	48,45	981
2	India	16,83	57,302
3	Estados Unidos de América	12,53	0,167
4	Turquía	11,00	85,9
5	Egipto	8,11	0,767
6	Irán (República Islámica del)	6,82	0,004
7	Italia	5,95	1,595
8	Brasil	4,42	71,473
9	**España**	**3,86**	**146,51**
10	Uzbekistán	2,59	16,584
11	México	2,44	1,264
12	Federación de Rusia	2,20	0,304
13	Ucrania	2,11	0,389
14	Nigeria	1,50	0,034
15	Túnez	1,28	5,674
16	Portugal	1,25	0,381
17	Marruecos	1,22	0,263
18	Grecia	1,17	0,303
19	República Árabe Siria	1,15	0,254
20	Iraq	1,06	0,032

Tomado de: FAOSTAT 2011.

En el área mediterránea el cultivo continuo es una práctica común y el cultivo de tomate en invernadero cubierto de plástico es una actividad económica importante. Por lo tanto, se necesitan prácticas sostenibles para mantener buenos rendimientos de estos cultivos y mejorar la calidad de los frutos de tomate. Se han realizado muchos estudios sobre la fertilización en los sistemas de cultivo de hortalizas en campo abierto, pero debido a la gran demanda de este cultivo a nivel mundial, son importantes los estudios de optimización y manejo de fertilizantes en el cultivo de tomate en invernadero.

Tabla 2. Superficie y producción del cultivo del tomate en España.

Provincias y Comunidades Autónomas	Superficie (hectáreas)				Rendimiento (kg/ha)			Producción (toneladas)
	Secano	Regadío		Total	Secano	Regadío		
		Aire libre	Protegido			Aire libre	Protegido	
GALICIA	–	237	954	1.191	–	74.76	115.642	128.040
P. DE ASTURIAS	40	25	25	90	15.000	30.00	60.000	2.850
CANTABRIA	16	–	–	16	15.000	–	–	240
PAÍS VASCO	81	142	70	293	9.340	19.07	48.536	6.862
NAVARRA	–	2.231	68	2.299	–	76.86	70.930	176.331
LA RIOJA	–	250	14	264	–	63.00	85.000	16.940
ARAGÓN	–	867	3	870	–	79.80	150.000	69.640
CATALUÑA	49	1.278	49	1.376	7.004	34.74	116.887	50.463
BALEARES	27	154	97	278	8.000	46.45	59.830	13.173
CASTILLA Y LEÓN	–	200	38	238	–	49.28	92.903	13.385
MADRID	–	47	34	81	–	45.00	83.225	4.945
CASTILLA-LA MANCHA	–	1.500	–	1.500	–	73.79	–	110.683
C. VALENCIANA	73	491	639	1.203	12.493	22.65	100.828	76.460
R. DE MURCIA	**–**	**496**	**2.764**	**3.260**	**–**	**62.90**	**114.811**	**348.536**
EXTREMADURA	–	24.93	2	24.93	–	66.18	220.000	1.650.333
ANDALUCÍA	53	8.120	11.511	19.684	21.028	66.18	85.558	1.523.369
CANARIAS	46	102	1.544	1.692	40.000	64.84	72.543	120.459

Tomado de: Anuario de Estadística Agraria. Datos 2010-2011

1. 3. PROPIEDADES DEL FUTO DE TOMATE:

1. 3. 1. Valor nutricional y beneficios para la salud humana

Es ampliamente aceptado que una dieta saludable es un factor importante en la prevención de enfermedades crónicas como el cáncer, las enfermedades cardiovasculares y neurodegenerativas, y en la mejora del balance energético y control de peso. En la literatura científica, los estudios han demostrado una fuerte correlación inversa entre el consumo de tomate y el riesgo de ciertos tipos de cáncer, enfermedades cardiovasculares y la degeneración macular relacionada con la edad (Giovannucci et al., 2002; Khachik et al., 2002; Muller et al., 2002; Sesso et al., 2003, 2004; Stahl y Sies 2005). El valor nutricional, aunque es probable que pase desapercibido para los consumidores, es de suma importancia. La calidad/valor nutritivo y funcional de un producto de mercado se define como el grado de utilidad que poseen los alimentos para satisfacer los requerimientos de sustancias necesarias para garantizar el buen funcionamiento del organismo humano o animal. Compuestos presentes en los alimentos proporcionan adicionalmente beneficios médicos o saludables, incluyendo la prevención y el tratamiento de enfermedades, denominándose entonces compuestos nutraceúticos (Marangoni et al., 1995). El termino “Nutracéutico” resulta de la fusión de los vocablos “nutrición” y “farmacéutico”, cuando se aplica a un alimento, en el caso que nos concierne, al fruto de tomate, la etimología del término sugiere las propiedades del fruto.

Entre los principales componentes nutricionales del tomate, destacamos los compuestos fenólicos, pigmentos y vitaminas, habiéndose descrito estos tres últimos como componentes nutracéuticos, por lo que el tomate es definido como un alimento funcional y nutracéutico (Jack, 1995; Canene-Adams et al., 2005). En éste sentido, el consumo de tomates o productos derivados del tomate, además de aportar a la dieta compuestos bioactivos, principalmente licopeno, β-caroteno, vitamina C y compuestos fenólicos, se ha asociado con un menor riesgo de desarrollar cáncer de tracto digestivo y de próstata.

A partir de estudios epidemiológicos, ensayos clínicos y experimentos en animales, así como los estudios "*in vitro*", este efecto protector se atribuye principalmente a la provitamina A (Mayne, 1996) y otros carotenoides. Los carotenoides son una clase importante de compuestos que proporcionan los precursores de vitaminas y antioxidantes esenciales. Debido a que el tomate es el segundo producto hortícola más cultivado en el mundo después de la patata, es considerado como la principal fuente de carotenoides. De un total de alrededor de 40 carotenoides que se encuentran en la dieta humana, sólo 25 se encuentran en la sangre humana debido a la absorción selectiva por el tracto digestivo. De estos, 9-20 son derivados de tomate fresco y procesado considerándose como los principales el licopeno, β-caroteno, luteína, zeaxantina y β-criptoxantina. El licopeno, que constituye aproximadamente el 80-90 % del contenido total de carotenoides de tomates (Shi y Maguer, 2000) es el antioxidante más eficiente entre los carotenoides debido a su actividad de detoxificación del oxígeno singlete y de radicales peroxilo (Mortensen y Skibsted, 1997; Sies y Stahl, 1998). Por otro

lado, el β-caroteno es un precursor dietético potente de vitamina A (Olson, 1989) y representa alrededor del 7 % de contenido de carotenoides de tomate (Nguyen y Schwartz, 1999).

Otro fitoquímico importante del tomate es el ácido ascórbico (vitamina C), uno de los antioxidantes vegetales más eficaces (Smirnoff, 1996). La vitamina C interviene en diversas funciones biológicas, como la síntesis de colágeno y la biosíntesis de ciertas hormonas. Además, su consumo se ha relacionado con la reducción del daño oxidativo y la mejora en procesos inflamatorios (Aguirre y May, 2008). Asimismo, el tomate es rico en otros compuestos bioactivos como son los compuestos fenólicos (flavonoides y ácidos fenólicos) (Soto-Zamora et al., 2005). Muchos compuestos fenólicos presentan actividades antioxidantes, anticancerígenas, antimicrobianas, actividades antialergénicas, anti-mutagénicas y anti-inflamatorias (Martínez-Valverde et al., 2002). El interés de los compuestos fenólicos como antioxidantes se centra principalmente en los flavonoides. Estudios epidemiológicos sugieren un beneficio del consumo humano de frutas y verduras ricas en flavonoides, ya que protegen frente a las enfermedades cardiovasculares, cáncer u otras enfermedades relacionadas con la edad. Flavonoides, como las antocianinas, tienen una importante función como antioxidante, protegiendo a las plantas frente al estrés oxidativo, actuando como un fotoprotector que absorbe la luz naranja-verde del espectro visible, previniendo así la foto-oxidación de las clorofilas. Aparte de sus funciones fisiológicas en las plantas, las antocianinas se consideran componentes importantes en la nutrición humana, incrementan la capacidad antioxidante, transportándose a zonas con actividad metabólica elevada

donde producen una reducción en la permeabilidad y fragilidad de los capilares, inhibiendo la agregación plaquetaria y toda estimulación inmune. El limitado suministro de calorías, contenido relativamente alto en fibra, y el suministro de minerales, vitaminas y fenoles tales como flavonoides hacen que el fruto de tomate un excelente alimento funcional que proporciona excelentes beneficios fisiológicos, y es capaz de satisfacer las necesidades nutricionales básicas.

El contenido de azúcar en los frutos de tomate es el resultado de una compleja combinación de factores entre los que intervienen la fisiología intrínseca, así como los procesos metabólicos y genéticos que están bajo el control de los procesos de desarrollo (Baldet et al., 2006; Ho y Hewitt, 1986; Mounet et al., 2009; Wang et al., 2009). La síntesis de azúcar comienza con los procesos fotosintéticos llevados a cabo por las hojas de la planta, generándose así el producto de que se transloca durante el desarrollo de los frutos. La capacidad de atraer fotoasimilados al fruto se define como la fuerza sumidero, e influye en la distribución de azúcar a los frutos durante todo el ciclo de cultivo de la planta. Como queda reflejado en la tabla 3, aproximadamente el 50% de la materia seca del fruto está representada por los azúcares, siendo la glucosa y la fructosa los mayoritarios. Respecto a los ácidos orgánicos, principalmente el málico y el cítrico representan más del 10% de la materia seca (Chamarro, 1995). En menor cuantía encontramos en el fruto de tomate, lípidos, proteínas y aminoácidos que suponen aproximadamente el 0,6% del peso fresco. Los minerales representan un 0,4% del peso fresco aproximadamente (Davies y Hobson, 1981), y aunque siendo bajo éste porcentaje,

desempeñan un importante papel en el valor nutricional del fruto. El incremento del contenido en materia seca, proteínas, aminoácidos, lípidos y minerales del tomate está condicionado en gran parte por la práctica de la fertilización y por la influencia de los factores abióticos a los que la planta se vea sometida.

Tabla 3. Valores nutricionales del fruto de tomate.

Componentes	Unidades	Cantidad	Componentes	Unidades	Cantidad
Agua	g	94,52	Colina total	mg	6,7
Energía	kcal	18	Betaína	mg	0,1
Energía	kj	74	Vitamina B-12	µg*	0
Proteínas	g	0,88	Vitamina A, IU	IU**	833
Lípidos totales	g	0,2	Vitamina A, RAE	RAE***	42
Cenizas	g	0,5	Retinol	µg	0
Fibra total	g	1,2	Vitamina E (alpha-tocopherol)	mg	0,54
Azúcares totales	g	2,63	Tocoferol, beta	mg	0,01
Sacarosa g	g	0.00	Tocoferol, gamma	mg	0,12
Glucosa (dextrosa)	g	1,25	Tocoferol, delta	mg	0
Fructosa	g	1,37	Vitamina K (filoquinona)	µg	7,9
Lactosa	g	0,00	Ácidos grasos totales saturados	g	0,028
Maltosa	g	0,00	Ácidos grasos totales monoinsaturados	g	0,031
Galactosa	g	0,00	Ácidos grasos totales poliinsaturados	g	0,083
Almidón	g	0,00	Triptófano	g	0,006
Ac cítrico	g	351	Treonina	g	0,027
Ac. málico	g	92,5	Isoleucina	g	0,018
Ac oxálico	g	26,95	Leucina	g	0,025
Ac ascórbico	g	14,5	Lisina	g	0,027
Ac fumárico	g	3,33	Metionina	g	0,006
Calcio, Ca	mg	10	Cisterna	g	0,009
Hierro, Fe	mg	0,27	Fenilalanina	g	0,027
Magnesio, Mg	mg	11	Tirosina	g	0,014
Fósforo, P	mg	24	Valina	g	0,018
Potasio, K	mg	237	Arginina	g	0,021
Sodio, Na	mg	5	Histidina	g	0,014
Zinc, Zn	mg	0,17	Alanita	g	0,027
Cobre, Cu	mg	0,059	Ácido aspártico	g	0,135
Manganeso, Mg	mg	0,114	Ácido glutámico	g	0,431
Selenio, Se	µg	0,13	Glicina	g	0,019
Vitamina C, Ác. Ascórbico total	mg	7	Prolina	g	0,015
Tiamina	mg	0,037	Serina	g	0,026
Riboflavina	mg	0,019	Betacaroteno	µg	1624
Niacina	mg	0,594	Alfacaroteno	µg	20
Ácido pantoténico	mg	0,089	Cryptoxanthin, beta	µg	490
Vitamina B-6	mg	0,08	Licopeno	µg	308
Folato total (Ahuja y col.)	µg	15	Lutein + zeaxanthin	µg	51

Tomado de: USDA, Base de Datos Nacional de Nutrientes de Referencia Estándar, edición 19, 2006. Unidades de Nutriente en 100 g de tomate.* Microgramos, ** Unidades internacionales por sus siglas en inglés, *** Equivalentes de la actividad del retinol.

1. 3. 2. Cualidades organolépticas

La calidad organoléptica de los frutos es definida como una combinación de características visuales (tamaño, forma y color, y propiedades sensoriales), como sabor, acidez y aroma (Bai y Lindhout, 2007), y determina que un alimento sea o no consumido mayoritariamente con respecto a otro. La evaluación de la calidad de los frutos basada en parámetros organolépticos es bastante compleja, ya que envuelve parámetros de textura, sabor y aroma. A pesar de la popularidad del fruto de tomate, en los últimos años ha habido muy pocos avances en los estudios relacionados con la mejora en la calidad organoléptica de variedades de tomate comercial, lo que ha generado polémicas en cuanto a las cualidades organolépticas del fruto entre los consumidores (Kader et al., 1978; Ratanachinakorn et al., 1997; Causse et al., 2002) El mercado y los consumidores se han vuelto más exigentes en los últimos años, demandando tipos de variedades comerciales con mayor calidad, más saludables y nutritivos, y para complicar un poco más la situación, las preferencias de los consumidores dependen de las regiones, culturas, del género y la edad (Causse, 2009). Por todo esto, en la actualidad, los estudios que se centran en incrementar la calidad de los frutos son de gran interés (Dorais et al., 2001; Gruda, 2005).

El sabor “flavour” (término cada vez es más usado por los nutricionistas para explicar la combinación sensorial que percibimos al paladear un alimento) del tomate resulta de la compleja interacción del gusto y aroma. Azúcares, ácidos, fenoles y minerales son los componentes principales que dan sabor al tomate,

siendo los azúcares, cuantitativamente, los que hacen la mayor contribución a las cualidades organolépticas (Kader, 2008). En contraste con el deseo de los consumidores por los tomates dulces, muchos cultivares se seleccionan por los rasgos más valorados por los productores, tales como la resistencia al estrés biótico y abiótico, la uniformidad, la apariencia, firmeza y vida útil (Shewfelt, 2000). El refuerzo de esta estrategia de elección de rasgos por parte del productor, también viene del hecho de que los consumidores parecen estar en conflicto con sus deseos. Mientras que al gusto se le concede una gran importancia, el consumidor no seleccionará los fruto de apariencia pobre, incluso si el sabor de estos puede ser "garantizado " (Bruhn, 2002). Esto incentiva la selección de rasgos como la firmeza y la vida útil por parte de los agricultores. Tampoco ayuda el hecho de que al tratar de cultivar variedades con mayor contenido de sólidos solubles, el rendimiento puede verse comprometido y puede caer por debajo del umbral de rentabilidad para el productor (Stevens, 1986). Como resultado, la relación coste-beneficio se inclina actualmente a favor del cultivo de variedades en las que las cualidades organolépticas no priman sobre otros atributos. Sin embargo, como señala Kader (2003) hay un creciente cambio en las prioridades de la industria y la calidad del tomate está siendo fuertemente considerada.

La genética del tomate, los factores ambientales, las prácticas de cultivo y condiciones climáticas durante el desarrollo de la planta, son variables que influyen en el contenido de azúcares del fruto. Asimismo, las prácticas postcosecha pueden tener un abrumador efecto sobre el contenido en azúcares. Por lo tanto el crecimiento durante el ciclo de cultivo y estado de madurez del fruto

en el momento de la cosecha, las temperaturas de almacenamiento, las atmósferas modificadas y tratamientos físicos influyen en el contenido de azúcar de los frutos.

1. 4. PRINCIPALES FACTORES QUE AFECTAN A LA CALIDAD DEL FRUTO DE TOMATE

1. 4. 1. Luz

Los fitonutrientes del tomate, como la vitamina C, carotenoides y fenoles se ven fuertemente afectados por la intensidad, la duración (fotoperiodo) y calidad de la luz. Numerosos estudios han demostrado que los antioxidantes tales como la vitamina C, licopeno, β-caroteno y fenoles aumentan con la intensidad de la luz (Lee y Kader, 2000; Merzlyak et al., 2002; Amiot et al., 2007). También se han demostrado por Ubi (2004) relaciones lineales entre la acumulación de antocianinas y la intensidad de luz. Aunque la luz no es esencial para la síntesis de ácido ascórbico, la cantidad y la intensidad de la luz durante la temporada de cultivo influye en su contenido en el fruto ya que el ácido ascórbico se sintetiza a partir de azúcares (precursores tempranos) suministrados a través de la fotosíntesis (Lee y Kader, 2000). Del mismo modo, a pesar de que la formación de carotenoides en la maduración del fruto de tomate no requiere la inducción por la luz, ésta juega un papel fundamental en la determinación del contenido de carotenoides. Por otra parte, la biosíntesis de las antocianinas en la maduración de los frutos de tomate es un proceso dependiente de la luz (Lancaster 1992) que

requiere una señal de fotomorfogénica mediada por fotorreceptores. Luz de energía suficiente también es importante para promover hidratos de carbono producidos a través del proceso fotosintético, que son los sustratos para la biosíntesis de flavonoides, así como para la vitamina C, como hemos explicado anteriormente.

Por otro lado, la baja radiación o radiación UV-B solar excesiva de tan sólo unas pocas horas, puede inducir daños fotooxidativos o fotoinhibición, lo que conlleva una reducción de la síntesis de los fitonutrientes carácterísticos del tomate (Adegoroye y Jolliffe, 1987; Prohens et al., 2004). Torres et al. (2006) observaron, ya sea con o sin radiación UV, que frutos de tomate expuestos durante 5 horas a alta irradiación solar, presentaron un 30 % menos de ácido ascórbico y 20 % menos de ácido dehidroascórbico en exocarpo, sugiriendo una degradación parcial del "*pool*" total de ascorbato. También observaron una disminución en carotenoides totales después de éste periodo de exposición, con una interacción significativa entre la duración de la exposición y la intensidad de la radiación UV. Es importante tener en cuenta que, además de los efectos de la luz sobre la planta, factores tales como los revestimientos del invernadero influyen en la intercepción de luz por parte de la planta y el fruto de tomate pudiendo influir en su contenido en fitoquímicos y en su calidad nutricional.

1. 4. 2. Temperatura

La temperatura tiene una influencia directa sobre el metabolismo de las plantas y, por lo tanto, en el caso que nos concierne, afecta al desarrollo del fruto de tomate y a su valor nutricional (Dorais et al., 2001a; Heuvelink y Dorais, 2005). En los cultivos de invernadero, los patrones de temperatura, tales como la temperatura día/noche o la integración de temperatura durante varios días pueden influir en la concentración de los fitonutrientes de los frutos. Dorais (2007) encontró que la exposición a un pulso de bajas temperaturas (12°C en comparación con 15°C, durante un período de 2-4 h) al final del fotoperiodo para una misma temperatura media diaria (18.5°C) disminuyó la contenido de licopeno de los frutos de tomate y su actividad antioxidante. Rosales et al. (2006) en sus trabajos en invernaderos, observaron como los frutos de tomate sometidos a elevadas temperaturas y radiación solar mostraron un aumento de la peroxidación de lípidos y una disminución del contenido de carotenoides como el licopeno y β-caroteno en exocarpo, a pesar del hecho de que la reducción de la oxidación del ascorbato mediante la ascorbato peroxidasa aumenta en estas condiciones. Cuando las temperaturas de la superficie del fruto del tomate promediaron 46°C, Torres et al. (2006) detectaron termoinhibición después de 2,5 horas de exposición. Al igual que en la fotoinhibición, la termoinhibición podría suponer un aumento de las ROS y la posible regulación al alza de los sistemas antioxidantes para hacer frente al aumento de ROS (Torres et al., 2006).

1. 4. 3. Nutrición mineral

El efecto de los elementos minerales sobre los fitonutrientes y el valor nutritivo de los tomates depende del elemento específico, la forma mineral, el genotipo de la planta, y las posibles interacciones con las condiciones ambientales y las prácticas agronómicas. En general, a pesar de que la aplicación moderada de N aumenta el rendimiento, la fertilización nitrogenada disminuye la concentración de vitamina C y carotenoides, mientras que la fertilización con K tiene el efecto contrario. Los estudios sobre cultivos en campo abierto e invernadero (Chapagain y Wiesman, 2004) han mostrado que un aumento de la aplicación de K en etapas específicas del crecimiento de la planta de tomate mejoran la calidad de los frutos.

1. 5. IMPORTANCIA DEL POTASIO EN LA AGRICULTURA

Desde la década de 1960, la población mundial se ha duplicado y esta tendencia se mantendrá en las próximas decadas. Para satisfacer las demandas de alimentos y energía de futuras generaciones se requiere un aumento masivo en la producción de cultivos, al mismo tiempo que se trata de preservar los recursos ecológicos y energéticos de nuestro planeta. Además, los recientes modelos climáticos predicen que la incidencia y la duración de los periodos de estrés por sequía y elevadas temperaturas están aumentando en muchas regiones, lo que afecta negativamente a los cultivos mayoritarios, y podría poner en riesgo nuestra seguridad alimentaria. Por lo tanto, el principal reto al que se enfrenta la agricultura actual es desarrollar estrategias para mejorar el rendimiento de los

cultivos basándose en sistemas más eficientes en cuanto al uso de recursos bajo condiciones de estrés bióticos y abióticos (Reynolds et al., 2011).

En este contexto, entre los muchos nutrientes de las plantas, el potasio (K) juega un papel especialmente relevante en un gran número de procesos fisiológicos vitales relativos al crecimiento, la rentabilidad, la calidad, y la resistencia al estrés de todo cultivo. El K constituye alrededor del 2.1 a 2.3 % de la corteza terrestre y por lo tanto es el séptimo u octavo elemento más abundante (Schroeder, 1978; Wedepohl, 1995). Aunque las reservas de K en el suelo son generalmente suficientes (Schroeder, 1978), las grandes zonas agrícolas del mundo son deficientes en cuanto a disponibilidad de K se refiere, destacando las tres cuartas partes de los suelos destinados a arrozales de China, y 2/3 de la zona destinada a cultivo de trigo del Sur de Australia (Mengel y Kirkby, 2001; Römheld y Kirkby, 2010). De manera adicional, en los sistemas de producción agrícola intensiva el K se ha convertido en un elemento limitante, particularmente en los suelos orgánicos o de textura gruesa (Goulding y Loveland, 1986). En muchos casos, un bajo aporte de K en el contexto de una fertilización desequilibrada puede resultar en un agotamiento significativo de las reservas de K disponibles en el suelo, y como consecuencia, en una disminución de la fertilidad de éste. Smil (1999) describió de que, en contraste con el N y fósforo (P), los fertilizantes de K se aplican a un ritmo mucho menor, y menos del 50 % del K retirado del suelo por los cultivos es repuesto.

Junto con aniones acompañantes (NO_3^-, Cl^-, malato$^-$), el K vacuolar determina en gran medida el potencial osmótico celular. En la literatura agronómica, cultivos con alta concentración en K a menudo han sido denominados "consumo de lujo", sin embargo, una elevada acumulación de K por los cultivos en condiciones de cultivo óptimas, puede ser considerada como una "estrategia aseguradora" que permite a las plantas afrontar mejor posibles situaciones de estrés ambiental súbito (Kafkafi, 1990). En la agricultura intensiva, la aplicación de fertilizantes es completamente necesaria para garantizar y mantener un suministro adecuado de la disponibilidad de los diferentes cultivos. Desde 1980 ha habido un aumento del 25% en el uso de fertilizantes K (Fig. 2A).

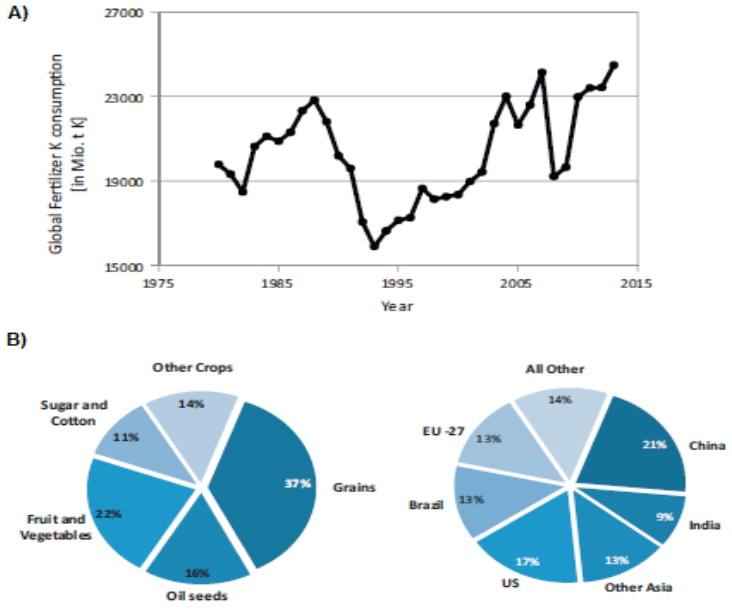


Figura 2. (A) Consumo global de fertilizantes K. (B) Uso mundial de K por cultivos y regiones.Tomado de: IFA data, FAO, PotashCorp.

La demanda mundial de fertilizantes potásicos se proyecta que aumentará mas de un 13% de 2011 a 2015 (FAO, 2011) debido al incremento específico en la producción agrícola mundial. Actualmente China y los EE.UU, utilizan casi el 40 % global de los fertilizantes potásicos, y se espera que los países en desarrollo (como la India y otros países asiáticos) aumenten drásticamente el consumo de este tipo de fertilizantes en un futuro próximo. A nivel mundial, los cultivos de cereales (por ejemplo, trigo, arroz, maíz) presentan el mayor porcentaje de uso de fertilizantes potásicos (37%), seguidos por frutas y verduras (22%), semillas oleaginosas (16%), caña de azúcar y algodón (11%), y otros cultivos (14%) (FAO, 2011) (Fig. 2B)

El cloruro de potasio (KCl), un mineral natural extraído de los yacimientos mineros profundos es la principal forma de fertilizante potásico que se utiliza hoy día . El sulfato de potasio y nitrato de potasio, ambos productos secundarios de la minería de KCl, también están disponibles comercialmente, pero son más caros. Estos productos son los preferidos para la aplicación en cultivos que son especialmente sensibles al cloruro, tales como patatas y otros frutos (plátano, cítricos, uvas y melocotón). Además, los fertilizantes orgánicos (abonos animales, purines, residuos de biogás o de desperdicios de comida) son también una fuente importante de K en la agricultura. En la mayoría de los cultivos anuales, la práctica general es aplicar fertilizantes K antes de la siembra. Por lo general, una aplicación es suficiente, ya que el K se adsorbe a los minerales de arcilla y sustancias húmicas y no lixivia. Sin embargo, en suelos de textura ligera que tienen una capacidad muy limitada para retener K debido a procesos de lixiviación,

puede ser más eficiente hacer dos o tres aplicaciones, ya que estos suelos presentan baja disponibilidad de K para la absorción por parte de la planta (Annadurai et al., 2000). Esta práctica también se puede aplicar en suelos que tienden a fijar K con el fin de aumentar la absorción de K por los cultivos antes de que el elemento se fije al suelo.

El K es uno de los macronutrientes más abundantes en los tejidos vegetales, representando aproximadamente el 10% del peso seco. Se encuentra implicado en numerosos procesos bioquímicos y fisiológicos fundamentales para el crecimiento, el rendimiento, la calidad, y tolerancia al estrés (Epstein y Bloom 2005). El K es considerado como el catión que tiene mayor influencia en los parámetros de calidad que determinan la comercialización de frutos, en las preferencias de los consumidores, y sobre la concentración de fitonutrientes esenciales para la salud humana (Lester et al., 2010). Asimismo, y respecto al fruto de tomate, éste macronutriente afecta significativamente a la concentración de pigmentos tales como licopeno y β-caroteno, que se pueden utilizar como indicadores de la calidad intrínseca, basándose en las propiedades analíticas y sensoriales (Ramírez et al., 2012). Una nutrición adecuada con K también se asocia a un mayor rendimiento, mayor tamaño de los frutos, aumento de los sólidos solubles, más elevada concentración de vitamina C y mejora el color del fruto (Kanai et al., 2007). Macronutrientes como son el N, el F y el K son fundamentales para el rendimiento de los cultivos. El momento y el modo de aplicación mineral, la forma química de los minerales aplicados, y el genotipo de tomate afectan a la respuesta a la concentraciones de minerales diferentes sobre

el contenido de azúcares en los frutos (Varis y George , 1985; Chapagain et al., 2003 ; Sainju et al., 2003; Benard et al., 2009).

1. 5. 1. Fisiología del estrés y papel del potasio en plantas

La respuesta de las plantas a situaciones de estrés implica tanto mecanismos de respuesta fisiológicos, metabólicos como moleculares. Estos mecanismos se coordinan para generar respuestas específicas que conlleven a la aclimatación de las plantas a esos ambientes. El mantenimiento del potencial de membrana biológico, de las actividades de numerosas enzimas, y de una apropiada concentración de osmolitos para hacer frente a la regulación del volumen celular, dependen de la homeostasis iónica, y más específicamente, de la homeostasis del Na y del K (Conde y et al., 2011).

La aparición de diferentes estreses pueden reducir el crecimiento de la planta, así como la actividad fotosintética, inducir la acumulación de ROS, causar inhibición enzimática y alterar la eficiencia de las reacciones que éstas llevan a cabo, modificar la composición de metabolitos sintetizados y acumulados, y afectar a la estabilidad de las proteínas de membranas, de especies de ARN y de estructuras del citoesqueleto, causando un desequilibrio fisiológico y metabólico general (Mittler et al, 2004; Suzuki et al., 2011). Los cloroplastos son los principales orgánulos que producen ROS, tales como el radical superóxido ($O_2^{\bullet -}$), peróxido de hidrógeno (H_2O_2), y el oxígeno singlete ($^1O_2$*) durante la fotosíntesis (Asada, 2000). La producción de ROS en cloroplastos puede ser particularmente alta

cuando las plantas están expuestas a estreses ambientales tales como alta incidencia lumínica, sequía, temperaturas extremas, deficiencia de nutrientes, y salinidad (Foyer et al., 1994; Marschner et al., 1996; Asada, 2000; Vranova et al., 2002). Las ROS son altamente tóxicas, pudiendo causar daños en la membrana y degradación de clorofilas, y por tanto son responsables del desarrollo de clorosis y necrosis en hojas. En condiciones normales, hasta un 20% del flujo total fotosintético de electrones se transfiere al O_2 molecular, formando $O_2^{\bullet -}$ que a su vez impulsa la formación de otras ROS (Robinson, 1988; Biehler y Fock, 1996; Cakmak, 2000). Cuando la utilización de la energía de la luz absorbida para la fijación de CO_2 es limitada por estrés biótico o abiótico, el flujo de electrones hacia el O_2 se intensifica, dando lugar a una gran acumulación de ROS en los cloroplastos. En estas condiciones, la energía de excitación también se transfiere al O_2 para formar $^1O_2$* altamente tóxico. La producción de ROS en los cloroplastos se vuelve más pronunciada cuando las plantas cultivadas bajo un estrés ambiental están expuestas a alta intensidad de luz, lo que resulta en la aparición de daños fotooxidativos en estos orgánulos.

Hay varias razones para poder entender la alta sensibilidad al incremento de la intensidad de luz y la temperatura por parte de las plantas deficientes en K. El K juega un papel central en el mantenimiento de la fotosíntesis y en procesos relacionados. Como han mostrado diferentes especies de plantas, la deficiencia de K resulta en disminuciones severas en la fotosíntesis neta. Las disminuciones en la fotosíntesis por deficiencia de K se vuelven más claras cuando las plantas están expuestas a elevadas concentraciones atmosféricas de CO_2 y O_3 (Barnes et al,

1995), lo que indica que es un requisito esencial un aporte de K en plantas que crecen bajo atmósfera enriquecida con CO_2. Este efecto del K es importante y necesita más investigación en vista del hecho de que la concentración global de CO_2 atmosférico se incrementa y será posiblemente el doble al final del siglo XXI (Bolin , 1986). La disminución en la fotosíntesis por deficiencia de K parece estar relacionada con la reducción de la conductancia estomática, el aumento de la resistencia del mesófilo y la disminución de la actividad ribulosa bifosfato carboxilasa (RUBISCO) (Peoples y Koch, 1979; Cakmak y Engels, 1999; Zhao et al., 2001).

Por otro lado, el mantenimiento de las tasas fotosíntéticas, también depende de la exportación y la utilización de fotoasimilados dentro de las plantas. Está bien documentado que cuando se compara con plantas que reciben un suministro adecuado de K, hay un aumento de la concentración de sacarosa en las hojas de origen y una marcada reducción en las raíces en comparación con condiciones de deficiencia de K (Cakmak et al., 1994a, b; Huber, 1984; Marschner et al., 1996; Bednarz y Oosterhuis, 1999; Zhao et al., 2001). Estos hallazgos son consistentes con los resultados que muestraron que la deficiencia severa de K provoca una marcada disminución en la exportación vía floema de sacarosa a partir de las hojas (Mengel y Viro, 1974; Mengel, 1980; Cakmak et al., 1994b). Debido a un impedimento de la fijación fotosintética del CO_2, así como la reducción de la utilización de fotoasimilados en hojas deficientes en K, es inevitable una mayor producción de ROS, que a su vez conduce a daños fotooxidativos. El aumento de la capacidad de desintoxicación de H_2O_2 de hojas deficientes en K sugiere que la

producción de ROS se intensifica en hojas deficientes en K a expensas de la fijación de CO_2. Se puede concluir que las plantas expuestas a alta intensidad de luz o cultivadas bajo condiciones de luz solar de larga duración, como ocurre en los países del hemisferio Sur con gran tradición en el cultivo de tomate, pueden tener requisitos más elevados de K a nivele fisiológico, en comparación con las plantas cultivadas en condiciones de baja intensidad de luz. Se necesita una mayor exigencia de K en condiciones de alta intensidad lumínica para una eficiente utilización de ésta energía absorbida en procesos como la fijación fotosintética del CO_2 y el transporte de fotosintatos hacia los órganos del sumidero, como es el caso del fruto de tomate.

1. 6. FISIOLOGÍA DE LA POSTCOSECHA

Los frutos y hortalizas frescas son tejidos vivos que continúan perdiendo agua después de la cosecha, pero, a diferencia de los cultivos en crecimiento, ya no pueden reemplazar el agua perdida del suelo y deben “confiar” en el contenido de agua presente en la cosecha. La pérdida de agua de los productos frescos después de la cosecha es un problema grave, ya que causa la contracción y pérdida de peso. La mayoría de las mercancías se hacen invendibles como los productos frescos después de perder un 3-10% de su peso (Ben-Yehoshua y Rodov, 2003). Después de la cosecha todavía pueden acumularse azúcares debido al metabolismo de los hidratos de carbono almacenados, lípidos y proteínas (Kays y Paull, 2004). Eventualmente este azúcar se consume para mantener el crecimiento y los fenómenos de senescencia.

La maduración es un complejo proceso de desarrollo de los frutos, que puede ser descrito como resultado de los cambios bioquímicos y fisiológicos que conducen a un estado de madurez que culmina en cambios dramáticos en color, textura y sabor (Javanmardi y Kubota, 2006). El tomate por ejemplo, es un fruto climatérico y perecedero que requiere del uso de tecnologías de conservación para retardar el proceso de maduración que se produce después de la cosecha y de ésta manera mantener su calidad y, en consecuencia, extender su vida útil postcosecha. La calidad de los tomates frescos se determina principalmente por su apariencia (color, aspecto visual, tamaño y forma), firmeza, sabor y valor nutritivo. El color del tomate es la primera característica externa que determina el grado de aceptación del consumidor. Importantes cambios de color se producen en las distintas etapas del desarrollo de tomate en términos contenido de clorofila (color verde), β-caroteno (color naranja) y licopeno (color rojo). Los cambios más visibles se asocian con la pérdida de clorofila (color verde) y la acumulación gradual de licopeno (color rojo), donde los plastidios, tales como cloroplastos presentes en la fruta verde madura se transforman en cromoplastos. La transformación de cloroplastos a cromoplastos normalmente se produce simultáneamente con otros cambios de maduración tales como el ablandamiento de la pared celular (Bathgate et al., 1985). Uno de los problemas importantes que ocurren en relación a la firmeza del fruto se relaciona con el ablandamiento del tejido que generalmente implica pérdida de peso y pérdida de turgencia resultado de la actividad enzimática. La pérdida de peso es un proceso no fisiológico asociado a la deshidratación en postcosecha y resulta en una pérdida de turgencia. La pérdida de peso de la fruta se ve afectado por varios factores pre y postcosecha,

como la fecha de la cosecha y la temperatura de almacenamiento (Alia-Tejacal et al., 2007). Este parámetro podría usarse para definir la calidad del tomate, debido a su impacto en el tejido que se vuelve opaco y muy suave cuando la pérdida de peso es alta.

Un enfoque tecnológico para controlar estos cambios de calidad es la aplicación de tecnologías postcosecha para extender la vida útil de frutas y vegetales, siendo el almacenamiento a baja temperatura ampliamente utilizado para cumplir con estos objetivos (Kalt et al., 1999). Shin et al. (2008) pusieron de manifiesto que los frutos almacenados a temperaturas más altas presentan mayores tasas de respiración y más corto período de almacenamiento, lo que contribuye a un descenso más acusado de la calidad de los frutos. Sin embargo Soto-Zamora et al. (2005) y Polenta et al. (2006) afirmaron que las temperaturas de almacenamiento por debajo de 13°C pueden causar un desorden fisiológico, denominado daño por frío. El daño por frío se caracteriza por un aumento de permeabilidad de la membrana, reducción de vigor y susceptibilidad a enfermedades, tales como manchas oscuras en la piel, siendo un factor importante en la evaluación de la calidad del tomate para fines de marketing. Los desórdenes fisiológicos en frutos climatéricos, como es el tomate, se producen cuando no se cumplen las condiciones de almacenamiento adecuadas y la gravedad de los síntomas dependen de la temperatura y el tiempo de exposición a ésta. Es necesario optimizar las condiciones de almacenamiento refrigerado del tomate para mantener la calidad del producto y asegurar una vida útil larga. Desde un punto de vista tecnológico, el control de calidad de los alimentos implica control

químico, cambios físicos y microbiológicos durante el procesamiento y almacenamiento de alimentos.

1. 6. 1. Efecto de la temperatura de almacenamiento en frutos de tomate

España ha exportado tomates desde la década de 1940 y, en general, la exportación implica el almacenamiento de los frutos en cámaras frías. Aunque el almacenamiento en frío es un método ampliamente utilizado para prolongar la vida útil de los frutos climatéricos, puede afectar su calidad nutricional provocando daño por frío. Este tipo de estrés se produce durante el almacenamiento por debajo de 10°C en las frutas carnosas, y al que el tomate es particularmente sensible (Stevens et al., 2008). El tomate es un fruto climatérico y por lo tanto perecedero, por lo que requiere el uso de tecnologías de conservación que retrasen el proceso de maduración que se produce después de la cosecha y de ese modo mantener su calidad y extender la vida útil del fruto.

Los frutos de tomate se almacenan en frío (10-15ºC) para extender su vida útil. Por cada 10ºC de aumento en la temperatura de almacenamiento por encima de la temperatura óptima, la tasa de deterioro de frutas aumenta de dos a tres veces (Saltveit, 2003). Esto sugiere que las temperaturas bajas son mejores para el almacenamiento a largo plazo, sin embargo por debajo de 12,5ºC el fruto de tomate puede ser destruido por daño por frío (Saltveit y Morris, 1990). Para complicar más las cosas, el desarrollo "normal" de azúcares (y volátiles) en los frutos cosechados es inhibido por el frío (de León-Sánchez et al., 2009; Gómez et

al., 2009). Gómez et al. (2009) encontraron que en frutos maduros almacenados a 6ºC durante 15 días acumularon ~25 % menos de glucosa en comparación con frutos almacenados a 20ºC.

1. 6. 2. Potasio y postcosecha: implicación del K en el estrés por bajas temperaturas

Como ya hemos mencionado en apartados anteriores, el K es uno de los macronutrientes más abundantes en los tejidos vegetales y su papel es esencial en numerosos procesos bioquímicos y fisiológicos fundamentales para el crecimiento, el rendimiento, la calidad, y tolerancia al estrés (Epstein y Bloom 2005).

Al igual que el estrés por sequía, el estrés por frío también es responsable de daños fotooxidativos en los cloroplastos debido a las alteraciones en el metabolismo fotosintético. Generalmente, el estrés por bajas temperaturas afecta a la fluidez de los lípidos de membrana y por lo tanto altera su estructura (Marschner, 1995). Los aumentos en la actividad de las enzimas encargadas de detoxificar H_2O_2 y $O_2^{\bullet-}$ en plantas tras la exposición a temperaturas de refrigeración o congelación indican la participación de ROS en el daño celular inducido por frío (Foyer et al., 1994; Lee y Lee, 2000; Allen y Ort, 2001). La cadena de transporte de electrones fotosintética, la conductancia estomática, la actividad RUBISCO, y la fijación de CO_2 son los principales procesos fisiológicos afectados por el estrés debido a bajas temperatura en plantas (Allen y Ort, 2001). Estos procesos son también afectados negativamente por deficiencia de K. Por lo tanto, bajo

deficiencia de K, el daño por frío y el daño fotooxidativo inducido por frío puede exacerbarse causando descensos en el crecimiento y rendimiento de los cultivos. Parece que un suplemento de K en cantidades relativamente elevadas puede proporcionar protección contra el daño oxidativo causado por el frío o las heladas. De acuerdo con esta sugerencia, se ha demostrado que disminuciones en el rendimiento y el aumento de daño en hojas inducido por las heladas en las plantas de patata en condiciones de campo pueden ser aliviados por un incremento en la aplicación de fertilizantes potásicos. La mejora de la tolerancia al estrés por bajas temperaturas mediante el aumento de la dosis de K también ha sido observado en plantas de tomate, pimiento, berenjena y plántulas que crecen en el exterior, con temperaturas entre 4°C y 16°C. Dependiendo de la fuente de fertilizantes K, el suministro de K aumentó el rendimiento total de la planta 2,4 veces, 1,9 veces y 1,7 veces en el tomate, pimiento y berenjena respectivamente (Hakerlerler et al., 1997).

La importancia del K en el estrés por frío en frutos no ha sido todavía analizada exhaustivamente, pero considerando que el K es uno de los elementos con mayor influencia sobre la concentración de muchos fitonutrientes en frutos que contienen compuestos antioxidantes como el licopeno, β-caroteno y vitamina C, es de suponer que frutos de tomate que en el momento de la cosecha presentan una concentración óptima de K y de estos compuestos con propiedades antioxidantes, pueden mostrar una mejor respuesta al estrés al que se ven sometidos durante el almacenamiento en frío durante la postcosecha, minimizando de ésta forma la pérdida de calidad.

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1. 2. INTERÉS GENERAL Y OBJETIVOS

Gracias a su gran contenido en compuestos antioxidantes el consumo de tomate (*Solanum lycopersicum* L.) presenta una serie de efectos beneficiosos para la salud. Numerosos estudios han demostrado que el consumo de sus frutos presenta efectos beneficiosos para la salud humana gracias a su elevado contenido de fitonutrientes como licopeno, β-caroteno, compuestos fenólicos, ácido ascórbico y otros nutrientes esenciales. Por otro lado, el K es uno de los nutrientes considerados como esenciales para la producción y calidad de los cultivos. Una dieta rica en K produce una reducción de la tensión arterial, reduce la mortalidad debida a enfermedades cardiovasculares, disminuye el riesgo de osteoporosis y previene el desarrollo de la diabetes. Numerosos estudios prospectivos sugieren que la mejor forma de incorporar K en la dieta es mediante el consumo de frutas y hortalizas ricas en éste macronutriente, por lo que es necesario incrementar las concentraciones de K en los productos agrícolas destinados al consumo humano ya sea mediante la fertilización o bien mediante el uso de variedades con una mayor eficacia en la utilización de este elemento. Por esta razón comienzan a estar en auge los llamados programas de biofortificación con K en vegetales y productos hortícolas con el objetivo de incrementar la ingesta de éste nutriente en la dieta.

Con el crecimiento de la población mundial previsto para el año 2050, en el que se espera alcanzar un población mundial de 9-12 mil millones de personas, será necesario un incremento en la producción de alimentos de entre un 34-70 %. La mejora del rendimiento de cultivos mediante la mejora de resistencia a patógenos

y sequía es esencial para lograr tales aumentos de producción de alimentos, pero estrategias más amplias y complementarias, como la reducción de las pérdidas de alimentos y el manejo pre y postcosecha pueden tener un impacto significativo.

Las pérdidas de alimentos son importantes en los cultivos básicos, pero lo son mucho más en alimentos perecederos. Por lo tanto, la reducción de las pérdidas posteriores a la cosecha sería el método más fácil, menos costoso y más eficaz para aumentar la producción de alimentos y mediante el que la seguridad alimentaria se vea optimizada. Especialmente en los países en vías de desarrollo, las pérdidas tras la cosecha son una parte importante de la producción total, debido principalmente al inadecuado manejo postcosecha y a las prácticas de procesamiento.

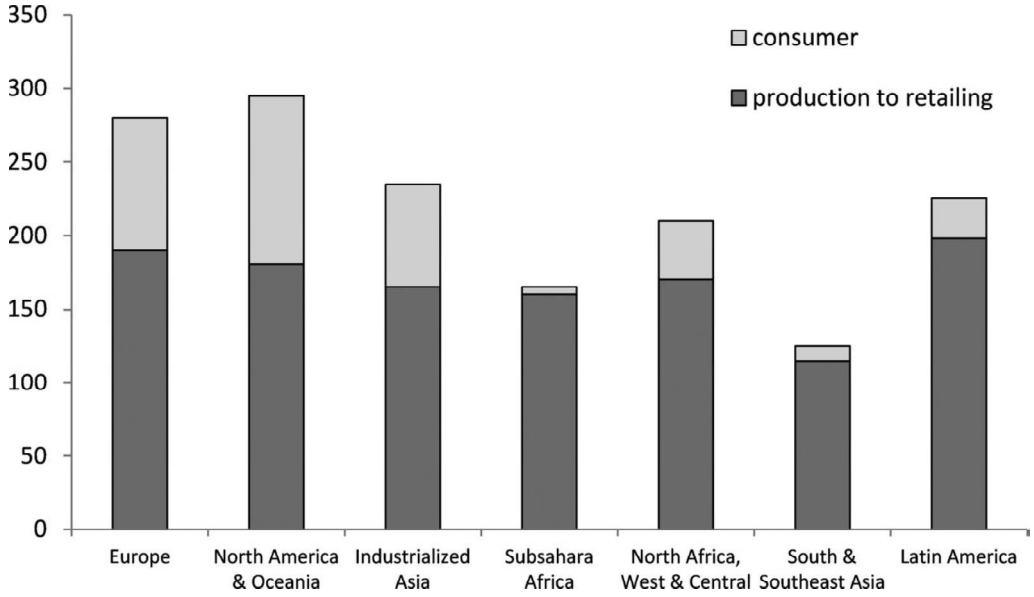


Figura 1. Tomado de: FAO. Global Food Losses and Food Waste. Extent, Causes and Prevention. Food and Agriculture Organization of the United Nations, Rome 2011. Available at: http://www.fao.org/docrep/014/mb060e/mb060e00.pdf.

El control de la temperatura de las cadenas de productos son el primer requisito para la reducción de las pérdidas posteriores a la cosecha, combinadas con tecnologías que controlan o retrasan las procesos o tasas de respiración del producto hortícola (almacenamiento en atmósfera controlada), estrategias genéticas (mejoramiento y selección), estrategias de diagnóstico (patógenos, madurez) y comprensión de los mecanismos fisiológicos son herramientas complementarias para reducir la pérdida de alimentos. El método más habitual para aumentar la durabilidad de los productos hortícolas es su almacenamiento a bajas temperaturas. Sin embargo, ésta técnica de postcosecha puede llevar consigo un estrés por frío en los vegetales y reducir así su comercialización.

En general, el estrés producido por bajas temperaturas afecta a la fluidez de los lípidos de membrana y de este modo altera la estructura de ésta (Marschner, 1995). Además, los aumentos en la actividad de las enzimas detoxificadoras de H_2O_2 y $O_2^{\bullet -}$ en las plantas tras la exposición a temperaturas de refrigeración o congelación ponen de manifiesto la participación de ROS en el daño celular inducido por frío.

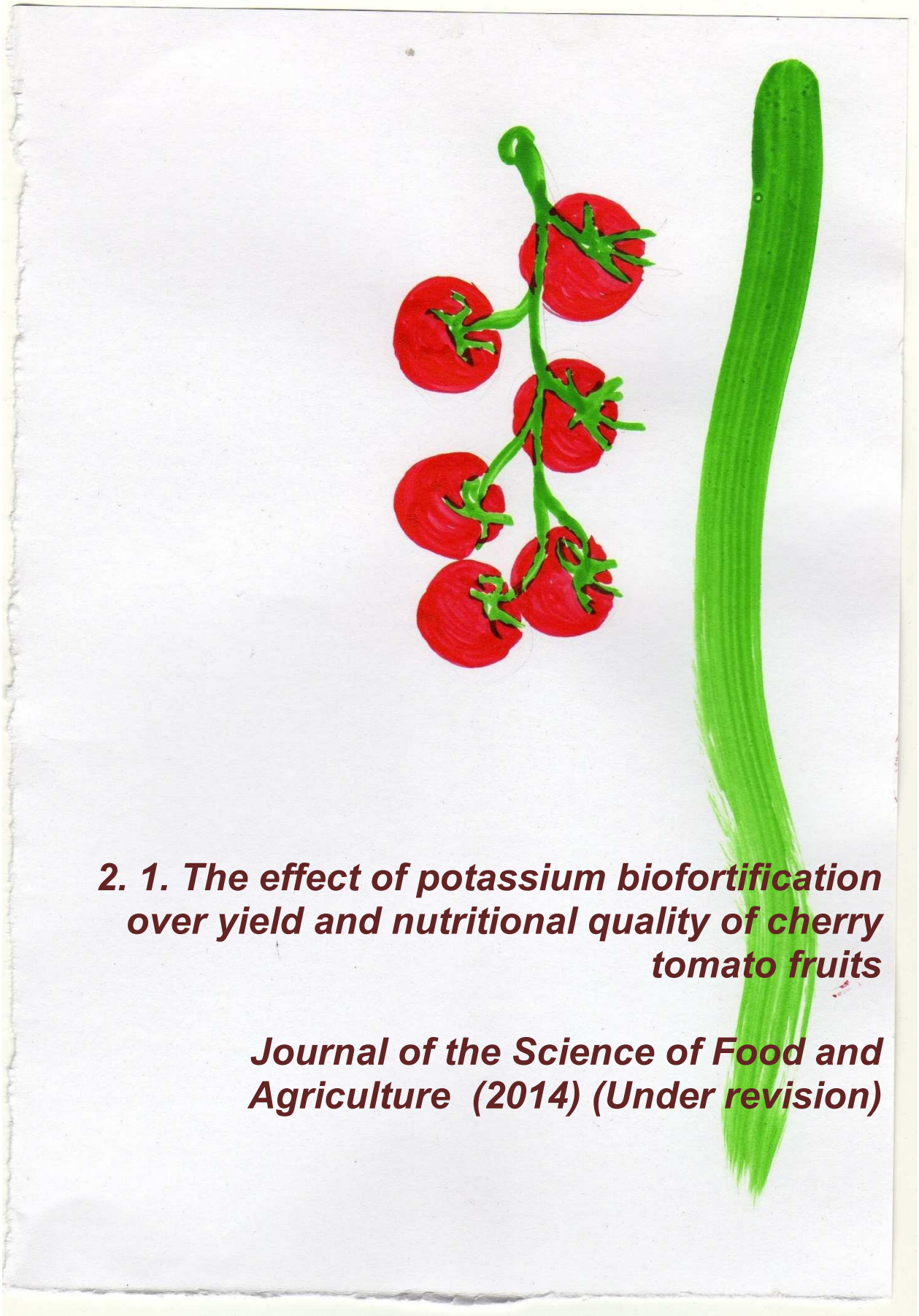
El estrés que producen las bajas temperaturas sobre los diferentes parámetros fisiológicos de los vegetales, son también afectados adversamente por la deficiencia de K . Por lo tanto, en situaciones de baja disponibilidad de K , el estrés por frío o los daños fotooxidativos inducidos por la refrigeración pueden incrementarse causando más descensos en el crecimiento y rendimiento de los cultivos. Numerosos estudios han demostrado que es muy posible que la disponibilidad de K en altas cantidades puede proporcionar protección contra el daño oxidativo causado por el frío o las heladas . De acuerdo con esta sugerencia, la mejora de la tolerancia a bajas temperaturas de estrés por parte de las plantas mediante el aumento de K ha sido observada tanto en tomate como en otros cultivos hortícolas de gran interés económico.

Por todo lo expuesto, los objetivos principales de esta Tesis Doctoral fueron los siguientes:

1. Evaluar el efecto de un programa de biofortificación con diferentes dosis de K en forma de KCl durante el ciclo de cultivo de plantas de tomate cherry sobre la producción y calidad de los frutos.

2. Analizar cómo afectó el almacenamiento a 4ºC durante 21 días a la fisiología de frutos de tomate cherry cosechados de plantas sometidas a un programa de biofortificación de K.

CAPÍTULO 2: EVALUACIÓN DEL EFECTO DE UN PROGRAMA DE BIOFORTIFICACIÓN CON K SOBRE LA PRODUCCIÓN Y CALIDAD DE FRUTOS DE TOMATE CHERRY



2. 1. The effect of potassium biofortification over yield and nutritional quality of cherry tomato fruits

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Abstract

BACKGROUND: Tomatoes (*Solanum lycopersicon* L.) belong to the category of fruits with high consumption worldwide being the second most commercially important vegetable in the world after potato. This horticultural crop is the predominant source of carotenoids, ascorbic acid (vitamin C), phenolic compounds. K is involved in numerous biochemical and physiological processes crucial to growth, performance, quality, and stress tolerance. An adequate K nutrition is also associated with greater crop yield and fruit size, increases in soluble solids and ascorbate (AsA) concentrations, and improved fruit color

RESULTS: Tomato fruits harvested at WAT 20 showed a higher K concentration, especially with treatments 15 and 20 mM KCl, antioxidant capacity parameters were improved with these doses although they did not significantly increase the organoleptic qualities. β-carotene increased proportionally to the application of growing K doses applied.

CONCLUSION: With the treatments 15 and 20 mM KCl, K concentration in fruits increases and does not endangers production. The consumption of these tomato fruits could be of great nutritional value and would be a health benefit.

Keywords

Tomato fruits, Potasium, Quality, Sugars, Organic acids.

1. INTRODUCTION

Tomatoes (*Solanum lycopersicon* L.) belong to the category of fruits with high consumption worldwide being the second most commercially important vegetable in the world after potato, with a worldwide annual yield of some 159.347 million tons[1]. In the Mediterranean region, where continuous cropping is a common practice, plastic-covered greenhouse tomato cultivation is a major economic activity. It is widely accepted that a healthy diet is an important factor in preventing chronic diseases such as cancer, cardio-vascular and neuro-degenerative diseases, and in improving energy balance and weight management. In fact, many studies have correlated high consumption of tomato fruits with a lower risk of suffering certain types of cancer, cardiovascular disease, and agerelated macular degeneration[2,3].

From epidemiological studies, clinical trials and experiments on animals as well as in vitro studies, this protective effect has been mainly attributed to provitamin A[4] and other carotenoids. Moreover, carotenoids are a major class of compounds providing precursors to essential vitamins and antioxidants. This horticultural crop is the predominant source of carotenoids. Lycopene (Lyc), which constitutes about 80–90% of the total carotenoid content of redripe tomatoes[5] is the most efficient antioxidant among carotenoids through its quenching activity of singlet oxygen and scavenging of peroxyl radicals[6,7]. On the other hand, β-carotene, a potent dietary precursor of vitamin A[8], accounts for around 7% of tomato carotenoid content[9]. Ascorbic acid (vitamin C), while being a most effective antioxidant in plants[10], is

also an important phytochemical of tomato fruit. The interest in phenolic compounds as antioxidants focuses primarily on flavonoids (such as anthocyanins), which are attributed with functions in the plant's defence agains the herbivory, pathogen stress, and UV-B radiation[11]. Epidemiological studies have suggested a benefit from the human consumption of fruits and leafy greens rich in phenols and flavonoids, as they protect against cardiovascular disease, cancer, or age-related diseases such as dementia[12,13]. The limited caloric supply, relatively high fibre content, and provision of minerals, vitamins, and phenols such as flavonoids make the tomato fruit an excellent ‘‘functional food’’ providing additional physiological benefits as well as meeting basic nutritional requirements.

Quality is also related to organoleptic properties, defined by a number of physico-chemical parameters that make the product satisfactory to consumers. The parameter most important to the organoleptic quality of tomato fruits is taste, produced mainly by a combination of sugars and organic acids, which determine the sweet and sour flavours, respectively, and thus their concentration levels can significantly affect flavour acceptability by consumers[14]. Therefore, for better tomato fruit flavour, a high sugar concentration is necessary together with a relatively high acid content. A low sugar concentration with a high acid level causes tartness in tomatoes while high sugar and low acid contents produce a sweet–mild flavour; by contrast, low contents in both result in an insipid flavour[15].

However, an understanding of the influence of environmental factors and their interactions with agricultural practices, as mineral nutrition in relation to tomato fruit

quality is still lacking. There have been many studies on fertilization in open-field vegetable cropping systems, but little information exists on fertilizer requirements in greenhouse tomato cultivation.

Mineral nutrition plays a key role on phytonutrients and nutritional value of tomato depends on the specific mineral, the mineral form, the plant genotype, and any possible interactions with environmental conditions and agronomic practices. In the human diet, fruits and vegetables contribute to uptake of roughly 35% of K[16]. In relation to this latter aspect, in recent years, to improve the nutritional quality of plant products for human consumption, biofortification programs have been used with greater frequency, with trace elements as well as macronutrients[17]. Evidence reveals that increased K intake benefits human health[18,19]. In plants, the macronutrient K is among the most abundant elements in plant tissues, accounting for ca. 10 % of the dry weight. K is involved in numerous biochemical and physiological processes crucial to growth, performance, quality, and stress tolerance[20]. Notably, this cation exerts the greatest influence on the parameters determining the market quality of fruit, consumer preferences, and the concentration of phytonutrients of vital importance for human health[21]. In this sense, and although there are very few studies on the subject, an adequate K nutrition is also associated with greater crop yield and fruit size, increases in soluble solids and AsA concentrations, and improved fruit colour[22].

For all this, the aim of our work proposes a biofortification program K as KCl during the crop cycle of cherry tomatoes in the framework of cultivation under plastic,

which allows us to assess how this strategy can improve the nutritional quality of tomato and consequently their beneficial role in human diet.

2. MATERIALS AND METHODS

PLANT MATERIAL, GROWTH CONDITIONS, AND SAMPLING OF TOMATO FRUITS

Seeds of cherry tomatoes (*Solanum lycopersicum* L. cv AsHiari grafted on cv. Maxifort rootstock) were sown in flat trays (cell size 3 cm x 3 cm x 10 cm, 100 cells per tray) filled with 50 % [v/v] perlite–peat mixture, and kept under greenhouse conditions for 5 weeks. Subsequently, the seedlings were transplanted to an experimental greenhouse at La Nacla Experimental Station (Motril), near the Granada coast in southern Spain (36º45'N; 3º30'W; altitude 130 m). Greenhouses conditions during all over the crop cycle from autumn to spring ranged from: 800–1,300 µmol photons m^{-2} s^{-1}, 8–12 h photoperiod, 25–85 % humidity. The plants were grown in 40-L perlite B-12-filled sacks; the planting scheme was 3.21 plants m^{-2}. Their arrangement in the greenhouse was in 12 rows with north–south orientation. The statistical design was on randomized block. The parral greenhouse was used and other growing conditions such as irrigation and fertilization followed Soriano *et al*.[23]. The different treatments applied were: 5 mM KCl, 10 mM KCl, 15 mM KCl y 20 mM KCl during the crop cycle.

Using the same experimental greenhouse, two consecutive crop cycles were performed. These crop cycles were spread from October 2010 to May 2011 (First cicle or cycle 1) and from October 2011 to May 2012 (Second cycle or cycle 2). With a complete truss of tomatoes (10–12 tomatoes per truss) maturing every 10 days, cherry tomato fruits were sampled at 20, 25 and 30 weeks after transplant (WAT). Approximately, 200 tomato fruits from each treatment were randomly collected (discarding the green fruits at the end of the truss) and rinsed three times in distilled water after disinfection with 1 % (v/v) Triton X-100[24], and then blotted on dry filter paper.

YIELD AND BIOMASS PARAMETERS

For each cycle, and in each of the three samplings, 180 tomatoes were harvested from each treatment and intended for analysis at harvest day, being clustered in nine replicates of twenty fruits. Ten tomato fruits from each replicate were weighed obtaining fresh weight (FW) and then dried in a lyophilizer to determine the dry weight (DW). Ten other tomato fruits from each replicate were homogenized, and samples of fresh tissues were stored at -80ºC.

To obtain the production parameters represented in this work were weighed and counted the commercial fruits harvested in each of the samplings taken during the two crop cycles for the following parameters:

i) Commercial (Kg fruits/plant)

ii) Nº commercial fruits/plant

For the following parameters, the methodology explained above in this paragraph was followed:

iii) g FW commercial/fruit

iv) g DW commercial/fruit

MEASUREMENT OF ENVIRONMENTAL PARAMETERS

Over the entire fruit production cycle, air temperatures and environmental humidity were measured using four HMP45 probes (Vaisala, Helsinki, Finland) and incident solar radiation was measured using four pyranometer sensors (model SP1110 Pyranometer sensor; Skye Instruments, Llandrindod Wells, Powys, UK), and four quantum sensor (model SKP215 Quantum sensor) situated in north-south direction for the integration of differences registred in the measurements across the grennhouse section as specified Soriano *et al*.[25]. A datalogger (Campbell Sci CR-10; Barcelona, Spain) stored the average values for three measurements every 30 min[25].

ANALYTICAL METHODS

Determination of K concentration

For the determination of K concentration, was followed the method published in *B.O.E.*[26] Nº246 17/9/1981, (MAPA, 1994. *Métodos oficiales de análisis. Tomo II. Ministerio de Agricultura, Pesca y Alimentación*. Madrid).

Antioxidant capacity assays

Total antioxidant capacitywas measured using the Ferric reducing ability of plasma (FRAP), Trolox equivalent antioxidant capacity (TEAC) assays, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging effect, and reducing power assays.

The TEAC was determined as described by Re *et al*.[27] using 2,2-azino-bis (3-ethylbenzthiazoline-6-sulphonate) solution (ABTS) and 2,20-azo-bis (2-methylpropionamidine) dihydrochloride, for the production of the ABTS radical ($ABTS^{\bullet-}$). The TEAC value of an extract represents the concentration of a Trolox solution that has the same antioxidant capacity as the extract.

The FRAP assay was made with FRAP reagent, i.e. 1 mM 2,4,6-tripyridyl-2-triazine and 20 mM $FeCl_3$ in 0.25 M CH_3COONa, pH 3.6. An aliquot of 100 mL of extract (1 g per 10 mL in methanol) was added to 2 mL of FRAP reagent and mixed

thoroughly. After the mixture was left at room temperature (20ºC) for 5 min, absorbance at 593 nm was measured. Calibration was against a standard curve (25–1600 mM Fe^{3+}) using freshly prepared ammonium ferrous sulphate[28].

The DPPH free-radical scavenging effect test was performed according to Hsu *et al*.[29]. Methanolic tomato extract and 0.1 mol L^{-1} DPPH methanolic solutions were thoroughly mixed and kept for 60 min in the dark and cold. The absorbance of the reaction mixture at 517 nm was read. The free-radical scavenging effect was calculated as follows:

ROS-scavenging effect (%)=[1−(A_{517},sample/A_{517},blank)]×100.

For reducing power assays, tomato fruits were homogenized in methanol 80%, and centrifuged at 3.000 g for 10 min. The reducing power of tomato fruits was measured following Hsu et al., (2009). Tomato extract, phosphate buffer (0.2 mol L^{-1}, pH 6.6) and $K_3Fe(CN)_6$ (1% v/w) was mixed and allowed to react for 20 min at 50ºC. The sample was immediately cooled and then Cl_3CCOOH 10% was added. After centrifugation at 3.000 g for 10 min, the supernatant was mixed with distilled water and $FeCl_3$ (0.1%), and allowed to react for 10 min. Increased absorbance of the reaction mixture at 700 nm indicated greater reducing power.

Pigments: lycopene, β-carotene and anthocyanins

Lycopene and β-carotene from tomato fruits were extracted in acetone: *n*-hexane (4:6) and afterwards centrifuged at 3.000 g for 5 min at 4ºC. The optical density of

the supernatants was measured at 663, 645, 505 and 453 nm in spectrophotometer, using acetone: n-hexane (4:6) as blank. Lycopene and β-carotene concentrations were quantified using equations proposed by Nagata and Yamashita[30] as follows:

$$\text{lycopene } (\mu g\ ml^{-1}) = -0.0458A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453}$$

$$\beta\text{-carotene } (\mu g\ ml^{-1}) = 0.216A_{663} - 1.220A_{645} + 0.304A_{505} - 0.452A_{453}$$

where A_{663}, A_{645}, A_{505} and A_{453} are the absorbances at 663, 645, 505 and 453 nm, respectively. These equations enable the simultaneous determination of lycopene and β-carotene in the presence of chlorophylls.

Anthocyanins were determined according to Lange *et al*.[31] with some modifications. Tomato fruits were homogenised in propanol:HCl:H_2O (18:1:81) and further extracted in boiling water for 3 min. After centrifugation at 5.000 g for 40 min at 4ºC, the absorbance of the supernatant was measured at 535 and 650 nm. The absorbance due to anthocyanins was calculated as $A = A_{535} - A_{650}$.

Determination of phenolic compounds

Total phenolic compounds of the tomato fruits were extracted with a mixture of methanol, chloroform and 1% (w/v) NaCl (2:2:1). Total phenolic compounds were assayed quantitatively by A_{765} with Folin–Ciocalteu reagent. Aliquots of phenolic extracts were mixed with 2 mL of double-distilled H_2O and 0.15 mL of 5% (w/v) $NaNO_2$. After 5 min, 0.15 mL of 10% (w/v) $AlCl_3 \cdot 6H_2O$ solution was added; the

mixture was allowed to stand for another 5 min, and then 1 mL of 1 mol L^{-1} NaOH was added. The reaction solution was mixed and kept for 15 min, and the absorbance was determined at 415 nm.

Determination of AsA

The extraction and quantification of reduced AsA followed the method of Law *et al*.[32]. This method is based on the reduction of Fe^{3+} to Fe^{2+} by AsA in acid solution. Tomato fruits weighing 0.2 g were homogenized in liquid N_2 with 1 mL of metaphosphoric acid at 5 % (w/v) and centrifuged at 18.000 g at 4ºC for 15 min. Afterward, an adequate aliquot of supernatant was added to a test tube together with sodium phosphate buffer 150 mM (pH 7.5). The mixture was stirred and incubated at room temperature in darkness for 10 min. Next, an adequate aliquot of N-ethylmaleimide at 0.5 % (w/v) was added together with orthophosphoric acid at 44 % (v/v), 2,20-bipyridyl at 4 % (w/v) in ethanol at 70 %, and $FeCl_3$ at 3 % (w/v). The resulting reaction mixture was stirred and incubated at 40ºC in darkness for 40 min. Finally, the absorbance was measured at 525 nm against a standard AsA curve that followed the same procedure as above.

Sugars and organic acids content

Hexose (glucose and fructose) and sucrose contents were extracted and quantified using a kit (Roche Biopharm, St Didier au Mont d'Or, France) based on enzyme-linked formation of nicotinamide adenine dinucleotide phosphate (NADPH).

The determination of organic acids was based on the method of Scherer *et al*.[33] with slight modifications and was performed using HPLC with a DAD UV-visible detector (Agilent Technologies, USA) under the following conditions: Phenomenex reverse-phase column, 250×4.6 mm i.d., 5 µm, Li-Chrospher 100 RP-18, with a 4×4 mm i.d. guard column of the same material (Luna, Phenomenex, Utrecht, Belgium). About 0.2 g of freeze-dried tomato samples were homogenized with H_2O milliQ. The resulting mixture was centrifuged for 400 g 2 min and then filtered through a 0.45 µm membrane filter, and triplicates of 10 ml for each sample were analysed by HPLC-DAD. HPLC analysis of organic acids was carried out using the same equipment as described above. Samples were injected into an ACE 5C18 column, 250 x 4.6 mm (HICHROM) operating at 25ºC. A single mobile phase consisting of 0.01 M of KH_2PO_4 (pH 2.6) at 0.5 ml/min was used. The elution was monitored at 210 nm. Malic and citric acid was used as a standard (SIGMA-ALDRICH), eluting at 7.27 min and 10.57 min respectively.

STATISTICAL ANALYSIS

The data compiled were analyzed using one-way analysis of variance (ANOVA) to determine significance and Fisher's protected least significant difference (LSD) test to separate means. The values obtained for each parameter correspond to the average of 2 crop cycles of study (2010-2011 and 2011-2012). In addition, to ascertain whether samplings and treatments significantly influenced the results, a two-away ANOVA was used and the means were compared by Fisher's protected least significant difference (LSD) test. Values represent means of two groups of tomatoes (with 9 replicates each group) from the 2 crop cycles of study (n=18). Levels of significance are represented by: *(p<0.05), **(p<0.01), ***(p<0.001) and NS (not significant). Standard errors of the means were also calculated. The standard errors are marked in the figures (with error bars) and stated in the tables.

3. RESULTS AND DISCUSSION

The K biofortification program proposed in the KCl form was carried out for two consecutive crop cycles: 2010-2011 (crop cycle 1) and 2011-2012 (crop cycle 2). The temperature conditions (temperature) and relative humidity recorded for the two cycles are represented in Fig. 1. Regarding the maximum Tª, the major difference between the two crop cycles was observed for 23-26-week, in which crop cycle 1 presented a higher maximum Tª than the crop cycle 2, however, it should be noted that did not was identify an increase in the Photosynthetically Active/Available Radiation (PAR) and Global Radiation (GR) during this period of

the crop cycle 1 (Figs. 2 A and 2 B). For this same period, the mean temperatures of the crop cycle 1 had a similar trend (Fig. 1 A)

In relation to the % relative humidity (Fig.1 B), the landmark most relevant was observed in 23-29 WAT for minimum and average % relative humidity, in which the most important differences were found between the crop cycle 1 and crop cycle 2.

For both, PAR (Fig 2 A) and GR (Fig. 2 B), although there were small variations between crop cycles, trends were similar for both. The same was observed for the accumulated GR (Fig. 2 C) parameter, the trend was the same, although the crop cycle 1 had a lower value for this parameter.

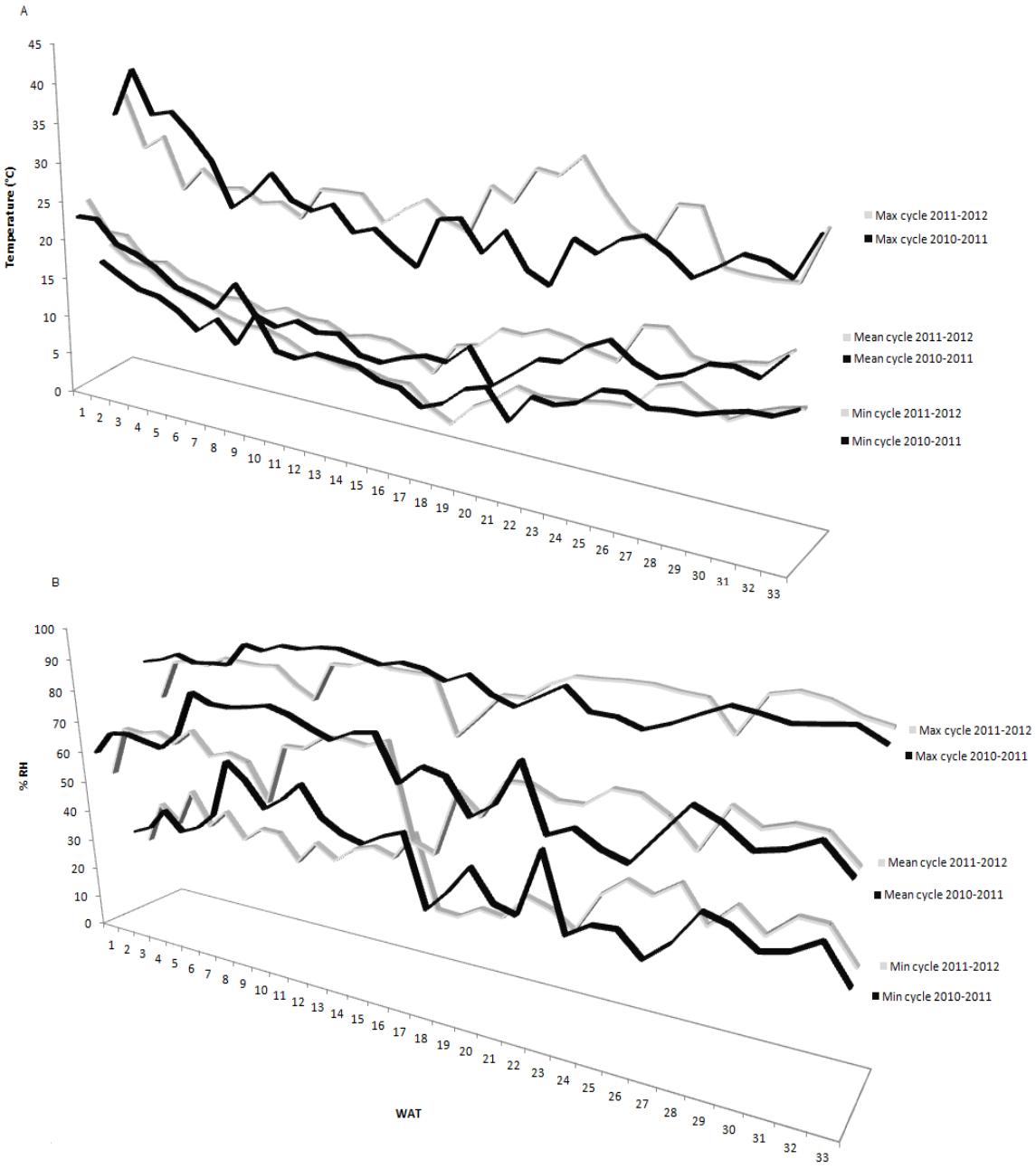

Figure 1. Temperature and relative humity: values recorded daily inside the experimental greenhouss during the two crop cycles 2010-2011 and 2011-2012. Values are weekly means of two crop cycles 2010-2011 and 2011-2012.

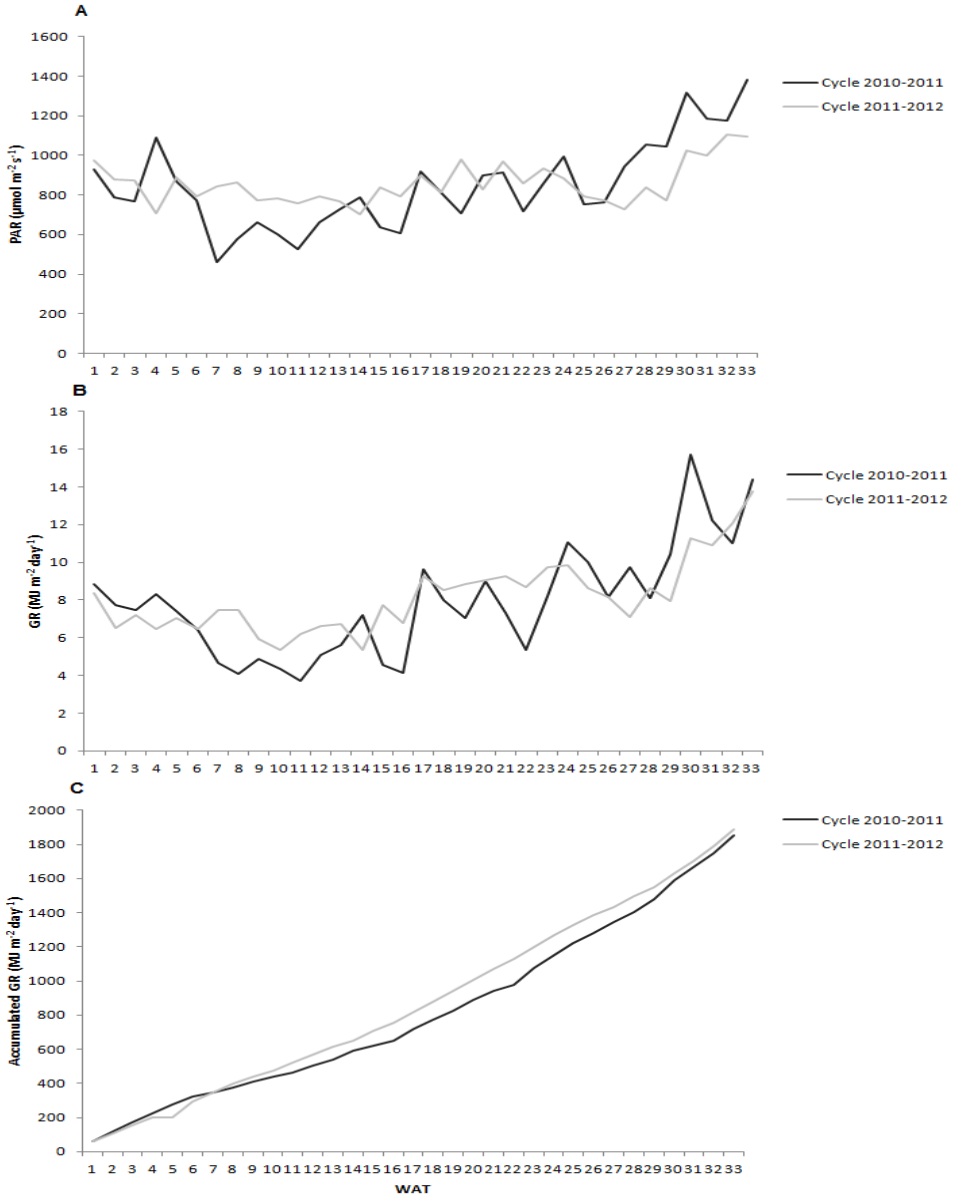

Figure 2. Solar radiation parameters: values recorded daily inside the experimental greenhouss during the two crop cycles 2010-2011 and 2011-2012. Values are weekly means of two crop cycles 2010-2011 and 2011-2012.

With respect to commercial production (Table 1), we found that in the sampling at 20 WAT, both commercial production as the Nº commercial fruits per plant showed no significant differences for different treatments applied. Nevertheless, we found a significant increase in the weight of marketable fruit, where the highest value was observed for treatment 5 mM KCl (Table 1) and a gradual decrease in this parameter as increase the dose of K. Finally, for sampling at 20 WAT, the g DW commercial/fruit showed no significant differences. The amount of DW biomass, often expressed as the percentage of dry weight (%DW) of the tomato fruits is an important parameter in yield as well as nutritional quality[34]. In this experiment, we found that over the productive period, the DW in the tomato fruits rose during the 2 years of study reaching the highest values at 20 WAT (Table 1), although no significant differences were found between treatments applied.
For the 24 WAT commercial production average, the Nº commercial fruits per plant and the weight of marketable fruit showed an inverse behavior to that described for 20 WAT (Table 1). For the first two parameters were observed the highest value in the 10 mM KCl treatment (Table 1), while the weight of the commercial fruits did not differ significantly (Table 1).

Finally, at 29 WAT, biomass and production parameters behavior were similar to which showed the samplings at 20 WAT (Table 1), where a significant decrease in the weight of marketable fruit were observed for doses 15 and 20 mM KCl (Table 1). The highest value was observed at 10 mM KCl treatment (Table 1). As for samplings at 20 and 24 WAT, the commercial fruit g DW showed no significant differences for different treatments applied (Table 1) (Fig. 3). At the end of the fruit

production cycles, the greenhouse temperatures exceeded the optimum for tomato growth, which is defined as between 23 and 26ºC[35], coinciding with a significant increase in values of solar radiation, and consequently, in our experiment, certain metabolic as well as physiological disorders could have arisen and affected yield and nutritional quality[36].

Following the two-way analysis, respect to the parameters Kg fruits per plant and Nº commercial fruits per plant, differents samplings dates does not significantly affected. However for biomass parameters (g FW/commercial fruit and g DW commercial fruit) the harvest time significantly affected, finding in general for both parameters higher values at 20 WAT (Table 1). The two-way analysis showed how the different K treatments applied showed significant differences for all the parameters relative to the weight of commercial production, however did not affect the number of fruits. Noteworthy is the large influence on fresh biomass (g FW/commercial fruit) (Table 1). Finally, treatment and sampling date interactions were not significant (Table 1).

Table 1. Effect of KCl treatments at differents samplings on yield and biomass of cherry tomato fruits production.

WAT	KCl (mM)	Commercial (Kg fruits/plant)	Nº commercial fruits/plant	g FW commercial/fruit	g DW commercial/fruit
20	**5**	0.92±0.11	36.67±5.46	25.31±1.00 a	3.19±0.19
	10	0.73±0.12	31.67±5.55	23.18±0.41 ab	2.92±0.04
	15	0.67±0.10	34.33±2.33	19.24±1.81 bc	2.34±0.29
	20	0.72±0.12	40.67±6.44	17.98±1.57 c	2.46±0.20
	P-value	NS	NS	*	NS
	LSD	0.36	16.90	4.29	0.65
24	**5**	0.81±0.08 b	40.67±1.67 b	19.81±1.37	1.26±0.12
	10	1.38±0.10 a	63.67±3.48 a	21.64±0.82	1.53±0.09
	15	0.68±0.07 b	38.00±4.51 b	18.13±0.93	1.23±0.08
	20	0.75±0.21 b	39.33±10.5 b	18.96±0.81	1.26±0.16
	P-value	*	*	NS	NS
	LSD	0.41	19.64	3.30	0.38
29	**5**	0.72±0.02	39.33±2.03	18.36±0.39 a	1.22±0.05
	10	0.81±0.19	42.67±8.88	18.62±0.66 a	1.38±0.06
	15	0.59±0.11	36.00±6.25	16.30±1.48 ab	1.26±0.12
	20	0.71±0.15	48.33±8.41	14.46±0.78 b	1.13±0.06
	P-value	NS	NS	*	NS
	LSD	0.43	22.63	3.00	0.26
			Two ways ANOVA		
SAMPLING		NS	NS	***	***
TREATMENT		*	NS	***	*
S x T		NS	NS	NS	NS
LSD		0.18	8.89	1.60	0.21

Values are mean of two crop cycles 2010-2011 and 2011-2012 (n=18) and differences between means were compared by Fisher's least-significant difference test (LSD; p=0.05). Significance levels are represented by p>0.05, NS, not significant, *p<0.05, **p<0.01, ***p<0.001. Means followed by the same letter do not differ significantly.

Regarding to commercial accumulated production (CAP) no significant differences for any of the different samplings or for any of the different treatments applied were founds (Fig. 3).

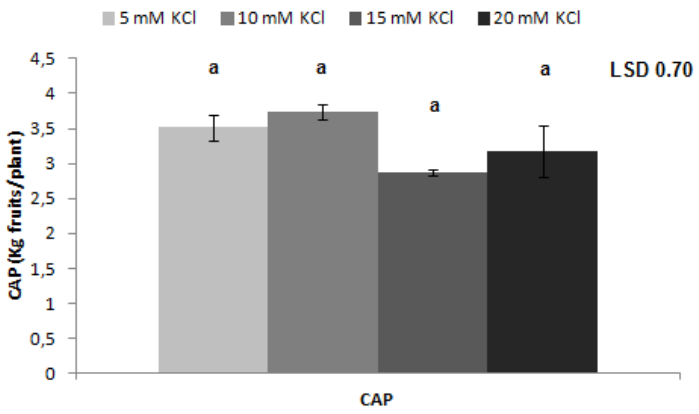

Figure 3. Effect of KCl treatments on commercial accumulated production in cherry tomato fruits. Values are means of two crop cycles 2010-2011 and 2011-2012 (*n*=18) and differences between means were compared by Fisher´s least significant difference test of (LSD, *p*=0.05). Means followed by the same letter do not differ significantly.

With respect to the K concentrations in fruits, a proportional response found according to the dose of KCl applied during the two crop cycles. The highest concentration was found in the fruits harvested from plants wich grown with 15 and 20 mM KCl doses at 20 WAT and no differences were observed between treatments (p<0.01. Fig. 4). The same dynamic continued the sampling carried out at 24 WAT (p<0.05. Fig. 4) and 29 WAT, although for this one last no significant differences were observed (Fig. 4). Therefore, the intake of fruits harvested at WAT 20 generally assume a greater amount of K, especially those who were treated with the doses 15 and 20 mM KCl (Fig. 4). Although individual fruit weight for the treatments 15 to 20 mM KCl at 20 WAT was lower than for those treated with the doses 5 and 10 mM KCl (Table 1), for the remaining production parameters, Kg fruits/plant, Nº commercial fruits/plant, g DW commercial/fruit (Table 1) and the commercial accumulated production (Fig. 3), no significant differences were founded, so considering the obtained results, the proposed biofortification program with K would be of great value as it increases the concentration of K in fruit and does not compromise commercial production. This, coupled with numerous studies that have shown that increased intake of K in the diet is a health benefit, shows that consumption of these tomato fruits could be of great nutritional value.

Potassium is the mineral element, next to nitrogen (N), required in the largest amount by plants. The K requirement for optimal plant growth is 2%–5% of the plant dry weight[37]. The effect of a sub-optimal K supply will therefore be exacerbated under high light. Accordingly, K-deficient plants are more prone to high light damage[38]. Plants exposed to high light intensity or grown under long-

term sunlight conditions as occurs like in southern countries of the northern hemisphere may have larger K requirements at physiological levels than plants grown under low light intensity. Increased requirement for K by high light intensity is needed for an efficient utilization of absorbed light energy in photosynthetic CO_2 fixation and transport of photosynthates into sink organs[38]. Potassium plays a central role in maintenance of photosynthesis and related processes. K deficiency results in severe decreases in net photosynthesis. The decreases in photosynthesis by K deficiency become more distinct when plants are exposed to elevated atmospheric concentrations of CO_2 and O_3[39], indicating an enhanced K requirement of plants growing under CO_2-enriched atmosphere.

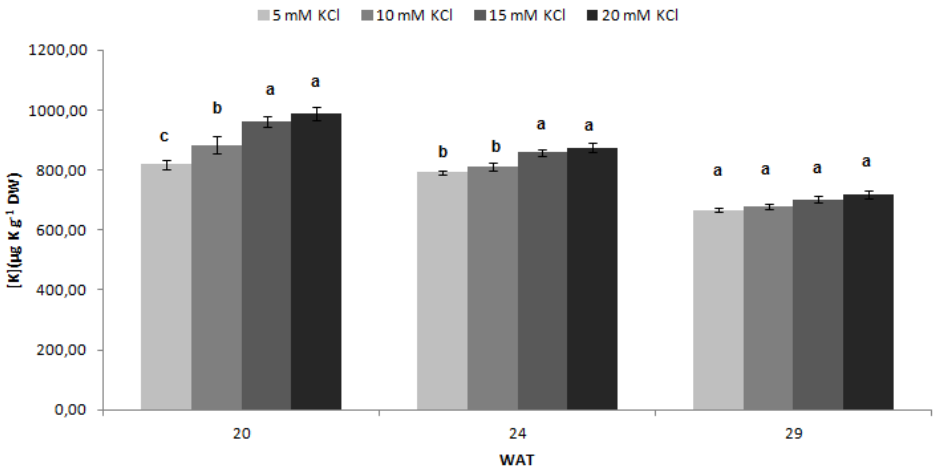

Figure 4. Effect of KCl treatments over K concentration in cherry tomato fruits. Values are means of two crop cycles 2010-2011 and 2011-2012 (*n*=18) and differences between means were compared by Fisher´s least significant difference test of (LSD, *p*=0.05). Means followed by thesame letter do not differ significantly.

For the determination of the antioxidant capacity in the most precise way possible, the use of several quantification methods is recommended. In our work, we used the methods TEAC, FRAP, DPPH and reducing power to quantify the antioxidant activity.

In our experiment, regarding the TEAC test, similar trends were observed for the differents K treatments applied at samplings taken at 24 and 29 WAT, in which higher values were found with doses 5 and 20 mM KCl, however for the 20 WAT no significant differences were observed between treatments (Table 2). The FRAP assay revealed similar trends for all samples, showing in all cases the highest value for 20 mM KCl treatment (Table 2). Regarding the DPPH test, only significant differences for 24 WAT values were found, showing the highest value with 20 mM KCl dose (Table 2). Although no significant differences were observed in 20 and 29 WAT, the pattern was similar to that observed for 24 WAT. Finally and similarly, for reducing power test, all samples showed similar trends, significant differences were found only on 20 and 24 WAT, in which the highest value was presented by 20 mM KCl treatment. Finally, the two-way analysis revealed that for all the parameters that determine the antioxidant capacity, with the exception of DPPH, harvest time of the fruit is significant, as the applied treatments. For interactions between sampling time and dose of K applied only we found significance for DPPH test and reducing power.

These values reflect as the K biofortification program improves the antioxidant capacity of tomato fruits with the increasing doses of KCl, especially in fruits collected in the sampling 24 WAT and under higher doses of K (15 and 20 mM

KCl). This indicates a stronger antioxidant capacity during this period in which began to increase temperature, solar radiation, and %RH, and coinciding with the increase in other antioxidant parameters such as total phenols, and Red AsA implying a greater benefit to human health through consumption of these tomato fruits.

Table 2. Effect of KCl treatments at differents samplings over antioxidant capacity in cherry tomato fruits.

WAT	KCl (mM)	TEAC (mg g^{-1} FW)	FRAP (mg g^{-1} FW)	DPPH (%)	REDUCING POWER (ABS g^{-1} FW)
20	**5**	0.38±0.01	0.71±0.03 b	21.41±1.42	0.87±0.03 bc
	10	0.37±0.02	0.68±0.01 b	17.24±2.25	0.82±0.01 c
	15	0.37±0.01	0.72±0.01 b	22.36±0.58	0.95±0.05 ab
	20	0.37±0.01	0.88±0.03 a	25.60±3.60	1.03±0.05 a
	P-value	NS	***	NS	**
	LSD	0.04	0.07	6.50	0.11
24	**5**	0.49±0.02 a	0.84±0.02 c	10.34±1.07 c	1.05±0.05 c
	10	0.44±0.02 ab	0.85±0.01 c	21.42±2.75 b	1.17±0.03 b
	15	0.42±0.02 b	0.92±0.02 b	22.83±2.04 b	1.23±0.03 b
	20	0.49±0.01 a	1.03±0.01 a	35.87±2.60 a	1.53±0.04 a
	P-value	*	***	***	***
	LSD	0.05	0.05	6.38	0.11
29	**5**	0.50±0.02 ab	1.08±0.02 c	19.26±2.77	1.37±0.07
	10	0.48±0.02 b	1.11±0.01 c	26.10±4.67	1.45±0.06
	15	0.46±0.03 b	1.19±0.03 b	31.55±3.91	1.54±0.04
	20	0.54±0.01 a	1.32±0.03 a	24.00±5.16	1.51±0.05
	P-value	*	***	NS	NS
	LSD	0.05	0.07	12.76	0.16
Two ways ANOVA					
SAMPLING		***	***	NS	***
TREATMENT		***	***	***	***
S x T		NS	NS	**	**
LSD		0.02	0.03	4.28	0.06

Values are mean of two crop cycles 2010-2011 and 2011-2012 (n=18) and differences between means were compared by Fisher's least-significant difference test (LSD; p=0.05). Significance levels are represented by p>0.05, NS, not significant, *p<0.05, **p<0.01, ***p<0.001. Means followed by the same letter do not differ significantly.

A large group of phytonutrients are found in fruits and vegetables of the Mediterranean diet, among these the tomato, are carotenoids, including Lyc and β-carotene. Lyc represents roughly 80% of all carotenoids and has a high capacity to eliminate ROS, being one of the most characteristic phytonutrients in tomato fruit[40]. Many studies have demonstrated a strong relation between the nutritional quality of tomato and its Lyc content[17,41]. Also, K significantly affects the concentration of such pigments as Lyc and b-carotene, which can be used as inner-quality indicators for tomato, based on analytical and sensorial properties[42]. Nutrition with adequate K is also associated with greater yield, larger fruit size, increased soluble solids, higher Vitamin C concentrations and improved fruit colour[22]. Studies on open-field and greenhouse tomato crops[43] showed that an increased K supply at specific growth stages of the tomato plant would improve fruit quality. In our work, concerning this phytonutrient we found the highest concentration for samplings carried out at 20 WAT in treatments 10 and 20 mM KCl, and those held in the 24 WAT, with 5 mM KCl dose (Table 3). For 29 WAT no significant differences were observed between treatments (Table 3). Furthermore, tomatoes contain moderate amounts of β-carotene, a potent dietary precursor of Vitamin A[9]. Regarding β-carotene significant differences between treatments were observed for the samplings taken at 20 WAT, where were observed a trend that increases the content of this compound was found in proportion to the application of increasing doses of K (Table 3). For samples at 24 WAT, the trend was the opposite, in this case the highest concentration was found in 5 mM KCl dose (Table 3). For the samplings at 29 WAT no significant differences were observed, although the trend was similar to that shown in the samplings at the 24 WAT (Table 3).

It has been demonstrated that the highest amount of Lyc and β-carotene in the tomato are strong contributors to the major antioxidants of tomatoes[44]. In this sense we can say that tomato fruits harvested during the two crop cycles at 20 WAT, are those who had a higher antioxidant capacity (Table 3).

According to several studied made, temperatures beyond 30-35º and strong solar radiation inhibit (Figs. 1 A and 2 A, 2B) lycopene synthesis and stimulate ts oxidation to β-carotene[45]. These results could be agree with those of our study (Table 3), as the decline in the lycopene content could be due to the oxidation of this compound to β-carotene, however, also β-Carotene degradation intensifies from 35 to 40ºC[41,46].

Anthocyanins are the most important group of water-soluble pigments in plants. Their biological interest stems from their antioxidant function and their effects reinforce certain compounds such as ascorbic acid[47]. In our study throughout two crop cycles, this pigment content showed similar trends for fruits sampled at 20 and 24 WAT (Table 3), in which the highest concentration of the pigment was observed with 10 mM KCl treatment (Table 3). For samplings carried out at 29 WAT no significant differences between treatments were observed (Table 3).

Finally, the two-way analysis revealed that exclusively Lyc showed no significant difference for the time of sampling (Table 3). Regarding the treatments applied all pigments, with the exception of β-carotene, showed significant differences (Table

3). For interactions between sampling time and dose of K applied we found significance for all pigments studied (Table 3).

Table 3. Effect of KCl treatments at differents samplings over pigments in cherry tomato fruits.

WAT	KCl (mM)	LYCOPENE (mg g^{-1} FW)	β-CAROTENE (mg g^{-1} FW)	ANTHOCYANINS ($ABS_{650-535}$ g^{-1} FW)
20	**5**	0.47±0.026 b	1.36±0.06 b	0.02±0.0028 b
	10	0.60±0.36 a	1.86±0.11 a	0.03±0.0021 a
	15	0.53±0.008 b	1.87±0.05 a	0.01±0.0023 bc
	20	0.61±0.007 a	1.98±0.02 a	0.01±0.0003 c
	P-value	***	***	***
	LSD	0.066	0.20	0.006
24	**5**	0.62±0.01 a	2.02±0.04 a	0.016±0.0008 ab
	10	0.57±0.01 b	1.84±0.06 b	0.018±0.0036 a
	15	0.54±0.02 b	1.81±0.06 b	0.012±0.0008 bc
	20	0.54±0.01 b	1.81±0.03 b	0.010±0.0003 c
	P-value	***	*	*
	LSD	0.040	0.15	0.0054
29	**5**	0.56±0.01	1.79±0.04	0.012±0.0004
	10	0.55±0.02	1.74±0.05	0.013±0.0009
	15	0.51±0.02	1.68±0.06	0.012±0.0009
	20	0.54±0.02	1.66±0.06	0.014±0.0002
	P-value	NS	NS	NS
	LSD	0.048	0.15	0.0019
		Two ways ANOVA		
SAMPLING		NS	**	**
TREATMENT		*	NS	***
S x T		***	***	***
LSD		0.026	0.081	0.0023

Values are mean of two crop cycles 2010-2011 and 2011-2012 (n=18) and differences between means were compared by Fisher's least-significant difference test (LSD; p=0.05). Significance levels are represented by p>0.05, NS, not significant, *p<0.05, **p<0.01, ***p<0.001. Means followed by the same letter do not differ significantly.

Phenolics, ubiquitous secondary metabolites in plants, include a large group of biologically active components, from simple phenol molecules to polymeric structures with a molecular mass above 30 kDa[48]. Total phenol concentration in the fruit harvested at 20 WAT, no clear trend in function of K applied treatments was observed. The highest value of these compounds was found in the 10 mM KCl dose, and it gradually decreased with increasing concentration of K doses (Table 4). For tomatoes harvested during 24 and 29 WAT a trend that increasing the content of the compound in proportion to the application of growing doses of K was found. The highest concentration of phenol was found in both cases with 20 mM KCl dose (Table 4).

With regard the reduced AsA we found that fruits harvested at 20 WAT, had a higher concentration of this compound with 5 and 15 mM KCl treatments (Table 4), although the trend was not very clear about the levels of rising K applied during crop cycles. For samples at 24 WAT, the lowest concentration was presented by the fruits harvested from plants treated with 5 mM KCl dose (Table 4), the 10 mM KCl treatment showed the highest value for this compound but did not show significant differences respect to 15 and 20 mM KCl doses (Table 4). Finally, for samplings carried out at 29 WAT, the fruits that presented a higher AsA content were those harvested from plants treated with 10 and 20 mM KCl doses (Table 4).

The two-way analysis showed that both treatments, such as sampling time, and the interaction between treatments and sampling time were significant for both compounds (Table 4).

Ascorbate, an important phytonutrient being directly involved in the elimination of ROS, is attributed with a great quantity of antioxidant propertieswhen consumed by humans[10]. As reflected in a previous study, Rosales *et al*.[49] found that the tomatoes grown in their experimental greenhouses showed a significant increase in the total ascorbate concentration during the third sampling with respect to the previous samplings, the parral greenhouse being higher throughout the productive cycle. These data coincide with the environmental stress provoked by the higher temperature, solar radiation, and VPD. Although some studies do not correlate ascorbate accumulation with environmental stress[50-52], others do agree with the results explained, where the climate conditions boosted the ascorbate content as an antioxidant response for acclimation to stress[41,45,49], conferring greater nutritional quality to tomato fruits.

Table 4. Effect of KCl treatments at differents samplings over: Total phenols and reduced AsA concentration in cherry tomato fruits.

WAT	KCl (mM)	Total phenols (µg g^{-1} FW)	Red AsA (µmol g^{-1} FW)
20	**5**	317.18±8.49c	0.73±0.02 a
	10	472.07±3.23a	0.61±0.01 b
	15	396.06±5.49b	0.66±0.01 ab
	20	323.64±1.56c	0.73±0.04 a
	P-value	***	**
	LSD	15.46	0.08
24	**5**	356.91±32.11b	0.63±0.03 b
	10	479.85±9.82a	0.82±0.03 a
	15	509.87±27.22a	0.80±0.02 a
	20	527.72±26.72a	0.78±0.02 a
	P-value	***	***
	LSD	73.19	0.08
29	**5**	402.90±19.78c	0.68±0.04 b
	10	441.76±32.28c	0.85±0.07 a
	15	514.55±21.62b	0.73±0.02 ab
	20	599.49±9.76a	0.86±0.05 a
	P-value	***	*
	LSD	64.34	0.14
		Two ways ANOVA	
SAMPLING		***	***
TREATMENT		***	**
S x T		***	***
LSD		27.76	0.049

Values are mean of two crop cycles 2010-2011 and 2011-2012 (n=18) and differences between means were compared by Fisher's least-significant difference test (LSD; p=0.05). Significance levels are represented by p>0.05, NS, not significant, *p<0.05, **p<0.01, ***p<0.001. Means followed by the same letter do not differ significantly.

Tomato fruit sugar content is the outcome of fruit physiological, metabolic, and genetic processes that are under developmental control[53-56]. Sugar production begins with leaf photosynthesis, the product of which is translocated to developing fruits. Although Suc is the main form of sugar translocated in tomato plants, Glu and Fruc are present generally in higher quantities than sucrose in tomato fruits.

In our study, we observed that the concentration of Suc in cherry tomato fruits harvested at 20 WAT was not influenced by the K treatments (Table 5). For sampling carried out at 24 WAT proportional increases were observed within increasing doses of K applied (Table 5) and at 29 WAT the highest concentration was found with the 15 mM KCl treatment (Table 5). Regarding the Glu concentration, no significant differences were observed in any of the samplings taken for the different KCl treatments applied (Table 5). Finally, with respect at Fru, only tomato fruits harvested at 24 WAT showed significant differences between the different K treatments applied, and the highest values were observed for 15 and 20 mM KCl doses (Table 5). For the samplings performed in 20 and 29 WAT the Fru concentration in tomato fruits showed no significant differences for any of the K treatments applied, although the trend regarding the content of this sugar was similar to that observed in the samples taken at 24 WAT (Table 5).

Worth mentioning that in the samples taken at 20 WAT lowest concentrations of all sugars quantified were found regarding to the fruits harvested at 24 and 29 WAT, this could be related to the lowest temperatures recorded during weeks 19 and 20 in both crop cycles (Fig. 1). The amount and intensity of light during the growing season influence the sugar content in fruits, because the ascorbic acid and

flavonoids synthesised from them are supplied by photosynthesis[57]. In addition to the climate conditions, K treatments play its role, as tomato fruits harvested from plants to which were applied the highest doses of K (<15 mM KCl) showed a positive correlation between total sugar content, total phenols and Red AsA, being these which had the highest levels of these compounds phytonutrients (Tabe 4 and Table 5).

Two way analysis showed that the sampling time was significant for all quantified sugars. K treatments applied, and the interaction between treatments and the sampling time were significant only for the Suc (Table 5).

Table 5. Effect of KCl treatments at differents samplings over: Sugars content in cherry tomato fruits.

WAT	KCl (Mm)	Sucrose (mg g^{-1} FW)	Glucose (mg g^{-1} FW)	Fructose (mg g^{-1} FW)
20	**5**	2.98±0.33	1.68±0.19	2.67±0.09
	10	2.80±0.53	1.57±0.31	2.72±0.06
	15	2.01±0.13	1.46±0.17	2.75±0.10
	20	2.26±0.14	1.32±0.17	2.85±0.12
	P-value	NS	NS	NS
	LSD	0.94	0.63	0.27
24	**5**	4.29±0.10 b	1.91±0.13	2.57±0.10 b
	10	6.17±0.87 a	1.89±0.12	3.08±0.25 ab
	15	6.15±0.21 a	2.08±0.29	3.56±0.23 a
	20	6.92±0.26 a	2.40±0.20	3.61±0.25 a
	P-value	**	NS	**
	LSD	1.35	0.57	0.63
29	**5**	6.60±0.53 b	2.93±0.30	5.77±0.31
	10	8.19±0.69 a	2.35±0.22	5.88±0.46
	15	6.14±0.65 b	2.50±0.13	5.64±0.19
	20	6.07±0.08 b	2.34±0.22	5.94±0.16
	P-value	*	NS	NS
	LSD	1.58	0.66	0.88
		Two ways ANOVA		
SAMPLING		***	***	***
TREATMENT		*	NS	NS
S x T		**	NS	NS
LSD		0.64	0.30	0.31

Values are mean of two crop cycles 2010-2011 and 2011-2012 (n=18) and differences between means were compared by Fisher's least-significant difference test (LSD; p=0.05). Significance levels are represented by p>0.05, NS, not significant, *p<0.05, **p<0.01, ***p<0.001. Means followed by the same letter do not differ significantly.

In addition to nutritional value, fruit quality is also related to organoleptic properties, defined by a number of physico-chemical parameters that make the product satisfactory to consumers[58]. The parameter most important to the organoleptic quality of tomato fruits is taste, produced mainly by a combination of sugars and organic acids, which determine the sweet and sour flavours, respectively, and thus their concentration levels can significantly affect flavour acceptability by consumers[14].

For samplings performed at differents WAT, malic acid concentration in tomato fruits showed no significant differences for any of the K treatments applied. Although no significant differences were observed, we saw that in general lines, the highest concentration in tomato fruits was found in the fruits harvested at 20 WAT (Table 6). Concerning to citric acid, no significant differences in the differents samplings for any of the K biofortification treatments applied were found. For this compound, the highest values were found in the fruits sampled at 24 WAT (Table 6). For organic acid study we do not found a clear trend.

Two way analysis showed that the sampling time was not significant for malic and citric acid. K treatments applied were significant only for the citric acid, and the interaction between treatments and the sampling time were not significant for any of the organic acid studied (Table 6).

Table 6. Effect of KCl treatments at differents samplings over: organic acids in cherry tomato fruits.

SAMPLING	KCl (Mm)	Malic acid (mg g^{-1} FW)	Citric acid (mg g^{-1} FW)
20	**5**	2.98±0.42	4.42±0.24
	10	3.06±0.13	4.86±0.11
	15	2.89±0.25	4.69±0.23
	20	3.14±0.47	5.11±0.38
	P-value	NS	NS
	LSD	1.12	0.84
24	**5**	2.88±0.86	4.16±1.67
	10	2.62±0.30	6.21±0.33
	15	2.26±0.11	6.01±0.16
	20	2.31±0.11	6.31±0.28
	P-value	NS	NS
	LSD	1.51	2.82
29	**5**	2.43±0.32	4.61±0.22
	10	3.15±0.39	6.12±0.86
	15	2.69±0.18	5.59±0.20
	20	2.70±0.11	5.68±0.21
	P-value	NS	NS
	LSD	0.89	1.52
Two ways ANOVA			
SAMPLING		NS	NS
TREATMENT		NS	*
S x T		NS	NS
LSD		0.54	0.86

Values are mean of two crop cycles 2010-2011 and 2011-2012 (n=18) and differences between means were compared by Fisher's least-significant difference test (LSD; p=0.05). Significance levels are represented by p>0.05, NS, not significant, *p<0.05, **p<0.01, ***p<0.001. Means followed by the same letter do not differ significantly.

Relative sweetnes and sweetness index are two parameters widely used to describe fruit taste and market acceptability[59]. Therefore, for better tomato fruit flavour, a high sugar concentration is necessary together with a relatively high acid content. In our work it was noted, as tomatoes harvested in the 20 WAT showed no significant differences for the parameters relative sweetness and sweetness index for any of the K treatments applied (Fig. 6). Tomato fruits harvested at 24 WAT showed the same trend for the relative sweetness and sweetness index parameters, showing the highest value for both with 20 mM KCl treatment (Fig. 6. $p<0.001$). Finally tomatoes harvested at 29 WAT showed no significant differences for any of these parameters (Fig 6.) Although it is noteworthy that the lowest values for these parameters studied was found with the highest dose of K.

Therefore, it was found that tomatoes harvested at 24 and 29 WAT, harvest period in which environmental conditions tended to be more stressful (Fig 1 and 2) showed the highest values for the relative sweetness and sweetness index (Fig. 5), similar results to those were founded by Gautier *et al*.[60] in his work with tomato fruits.

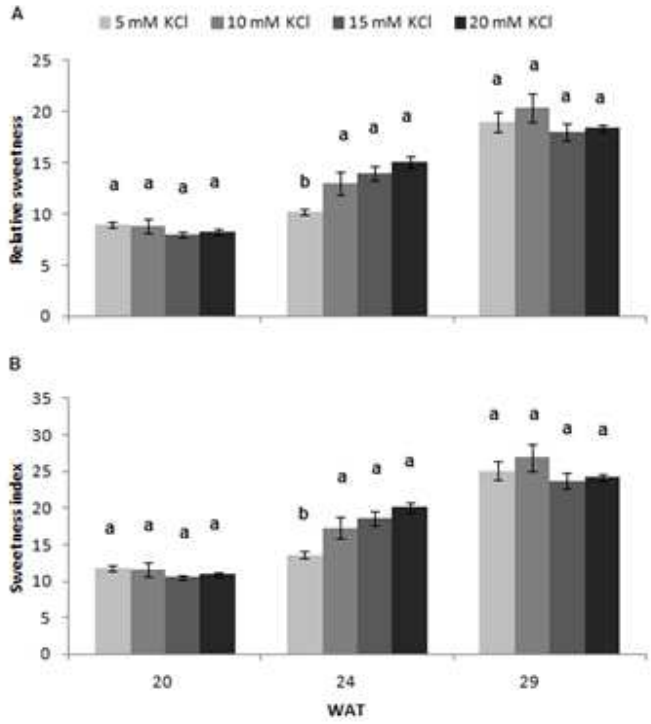

Figure 5. Effect of KCl treatments at different harvest time over: Sweetness index in cherry tomato fruits. Values are means of two crop cycles 2010-2011 and 2011-2012 (*n*=18) and differences between means were compared by Fisher´s least significant difference test of (LSD, *p*=0.05). Means followed by thesame letter do not differ significantly.

4. CONCLUSIONS

From the effect of a K biofortification program as KCl form during the two crop cycles and at different samplings time we can conclude that:

Tomato fruits harvested at 20 WAT showed a higher K concentration, especially with treatments 15 and 20 mM KCl. Although the individual fruit weight for treatments 15 to 20 mM KCl at 20 WAT was lower than in those treated with doses 5 to 10 mM KCl, the remaining parameters of commercial production and accumulated commercial production did not differ significantly, so considering these results the K biofortification program proposed would be of great value, as it increases the K concentration in the fruits and does not endangers commercial production. This would suggest that the consumption of these tomato fruits could be of great nutritional value and would be a health benefit. Also, the β-carotene increased proportionally to the application of growing K doses applied, but in general for this sampling, the doses 15 and 20 mM KCl did not significantly increase the organoleptic qualities, however, some of the parameters used to quantify the antioxidant capacity (DPPH and reducing power) were improved with these doses.

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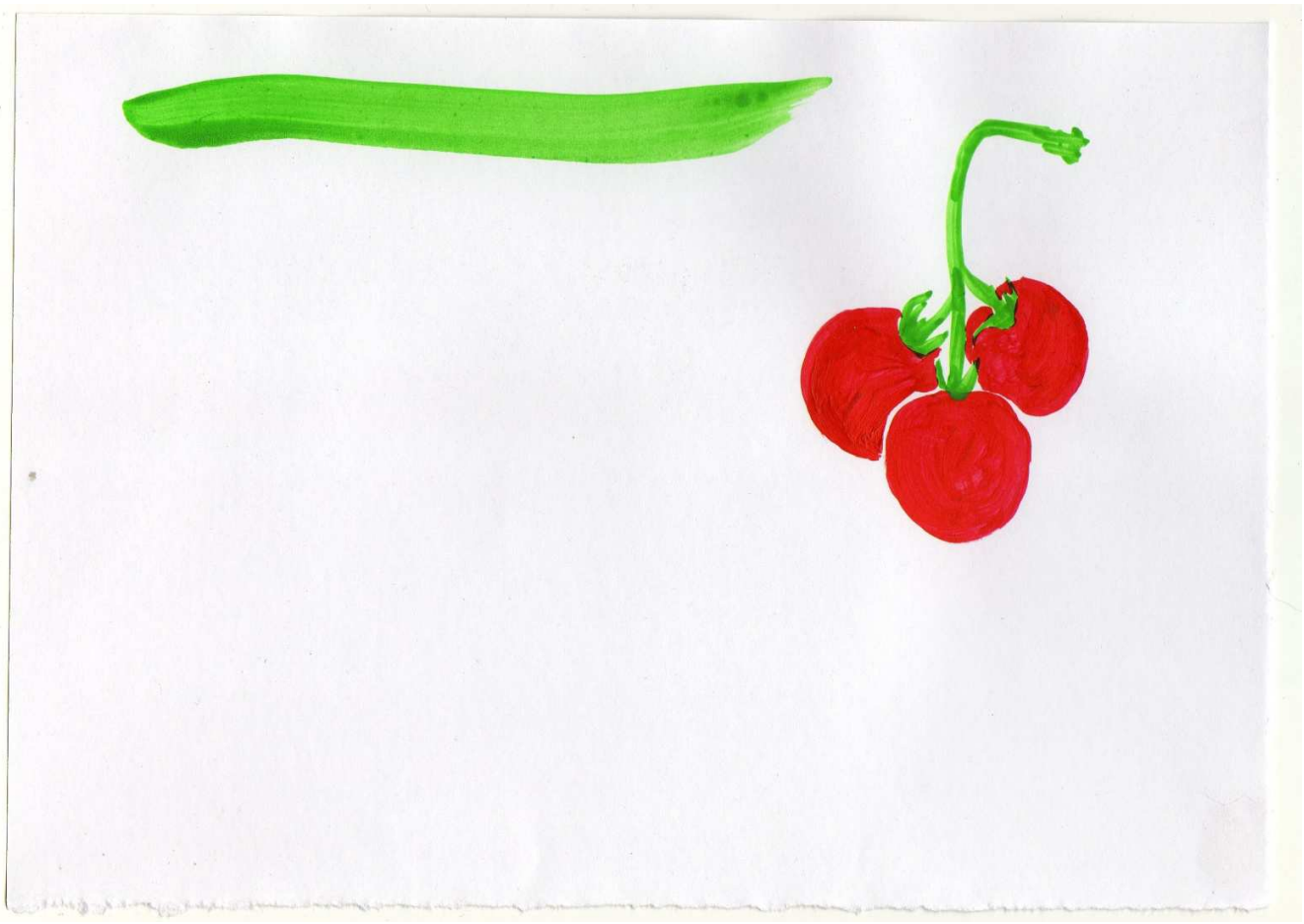
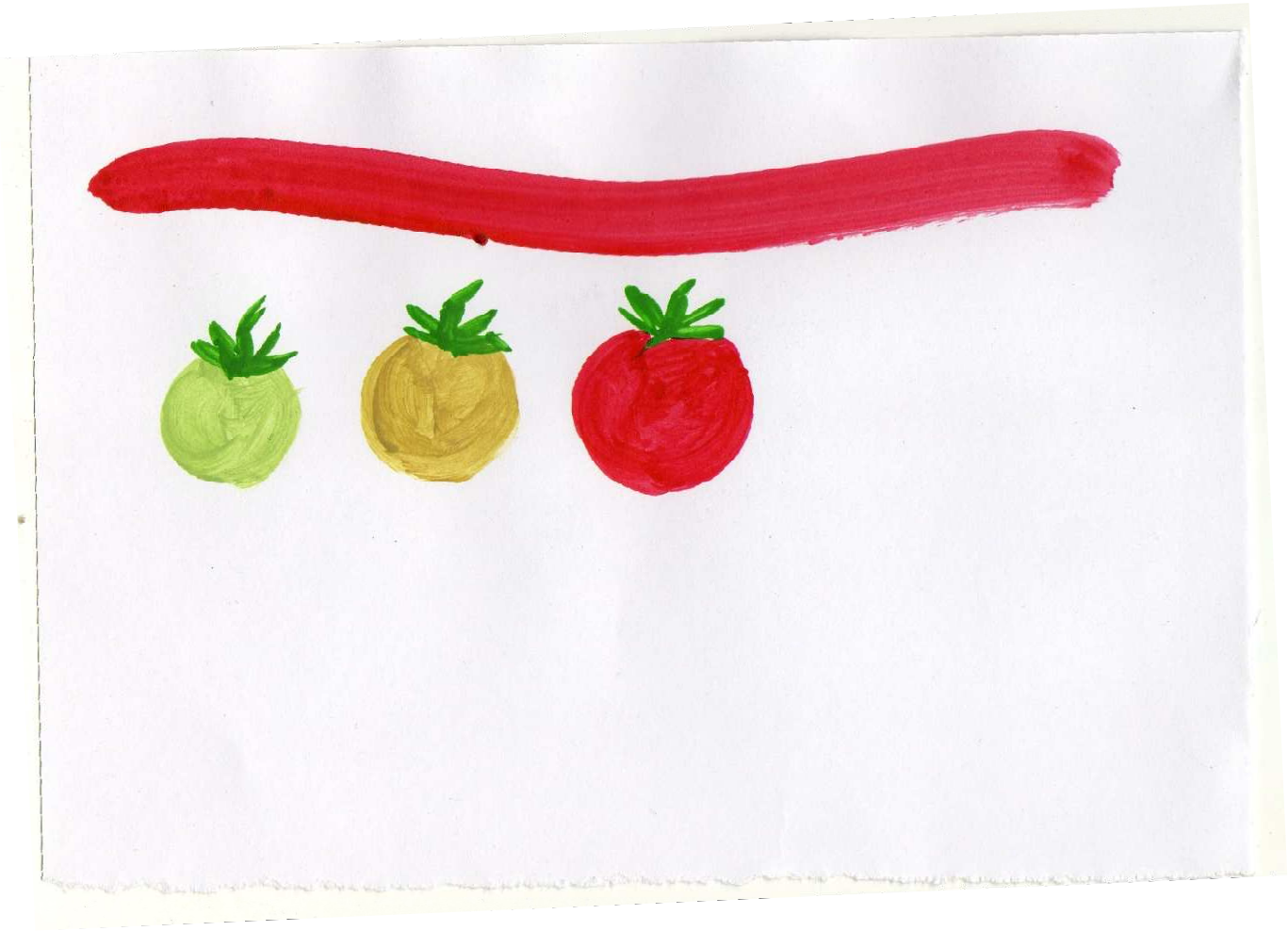


CAPÍTULO 3: ANÁLISIS DE LA FISIOLOGÍA DE FRUTOS DE TOMATE CHERRY SOMETIDOS A UNA POSTCOSECHA A 4ºC: EFECTO DE LA BIOFORTIFICACIÓN CON K



3. 1. Biofortification with potassium: antioxidant responses during postharvest of cherry tomato fruits in cold storage

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ABSTRACT

Tomato fruits are sensitive to storage at low temperatures after harvest. Under these conditions, the main mechanism induced in fruits is oxidative stress, which can translate as lipid peroxidation and in turn deteriórate fruit quality.The aim of the present work was to investigate whether the effect of a biofortification program with potassium (K) improves the postharvest storage of cherry tomato fruits at 4 ºC, through a better antioxidant response. Three K treatments were applied during the crop cycle of the plants: 5, 10, and 15 mM of KCl. The parameters in fruits on the day of harvest and after 21 days of postharvest cold storage at 4 ºC, such as activity of lipoxygenase, malondialdehyde, catalase, superoxide dismutase, and the enzymes involved in the AsA–GSH cycle as well as the forms of ascorbate (AsA) and glutathione (GSH), were analyzed. The tomato fruits harvested from plants treated with 15 mM of KCl after 21 days of postharvest at 4 ºC showed a lower degree of lipid peroxidation, an effective regeneration of AsA, and the highest pool of this compound in comparison with the other treatments. This response was because it presented the highest ascorbate peroxidase and monodehydroascorbate reductase activity. In addition, the treatments of 10 and 15 mM KCl presented the highest GSH pool, as well as a satisfactory regeneration of this tripeptide. All these results lead to the conclusión that the rate of 15 mM of KCl applied to this tomato variety (*Solanum lycopersicum* L. cv AsHiari grafted on cv. Maxifort rootstock) is adequate to mitigate the negative effects of postharvest chilling.

Keywords: Tomato · Chilling· Oxidative stress · Halliwell-Asada cycle · Postharvest quality

Abbreviations:

AsA Ascorbate

APX Ascorbate peroxidase

CAT Catalase

DHAR Dehydroascorbate reductase

GR Glutathione reductase

GSH Glutathione

LOX Lipoxygenase

MDA Malondialdehyde

MDHAR Monodehydroascorbate reductase SOD Superoxide dismutase

INTRODUCTION

Tomato is the second most commercially important vegetable in the world after potato, with a worldwide anual yield of some 159.347 million tons (FAOSTAT 2011). It is rich in ascorbate (AsA), the most effective antioxidant in plants and a great phytochemical for its antioxidant properties. In addition to AsA, the tomato fruit contains other nutritionally key compounds which also bear notable antioxidant properties, such as phenols, carotenoids, and GSH. In fact, many studies have correlated high consumption of tomato fruits with a lower risk of suffering certain types of cancer, cardiovascular disease, and agerelated macular degeneration (Muller et al. 2002; Stahl and Sies 2005).

Spain has been producing and exporting tomatoes since the 1940s. Exportation implies keeping the fruits in cold chambers, causing stress that can affect their nutritional quality. This type of stress results during cold storage below 10 ºC, and tomatoes are particularly sensitive (Stevens et al. 2008). Chilling is a widely used technique to prolong the life of stored fruits, but it can trigger physiological disruption and quality loss evident in characteristics such as a rough texture, watery flesh, and irregular ripening. Furthermore, it has been demonstrated that the cold damage provokes cell stress and oxidation of celular components, such as the AsA pool, due to the overproduction of reactive oxygen species (ROS). Consequently, the elimination of ROS during the postharvest period at low temperatures by the induction of antioxidant enzyme and non-enzyme systems could improve fruit quality as well as prolong their postharvest life under these

conditions (Hodges et al. 2004; Malacrida et al. 2006). In this sense, the components of the AsA–GSH cycle represent a system of antioxidant and detoxifying mechanisms against ROS of great impact with respect to fruit resistance to the chilling damage during postharvest storage. In studies of different apple cultivars, Davey and Keulemans (2004) indicated that the AsA and GSH contents can be considered reliable markers of fruit quality and reflect stress tolerance. The combination of a high AsA and GSH content is associated with late ripening (or slow senescence) and may be responsible for improving the postharvest cold storage of the different apple cultivars studied (Davey and Keulemans 2004). Also, Egea et al. (2010), working with apricot fruits after harvest, demonstrated that 1-methylcyclopropene (1-MCP) treatments fortified the antioxidant systems, both enzymatic as well as non-enzymatic, responsible for eliminating ROS, such as $O_2^{\cdot -}$, $OH\cdot$ and H_2O_2, produced after exposure to cold stress. Vega-García et al. (2010) examined the protein expression of tomato fruits stored at low temperatures that could induce cold stress and reported that the proteins involved in eliminating ROS required substrates of antioxidant compounds such as reduced GSH to catalyze the reaction, and that there might be a relation between the quantity of substrate and the increase in enzymatic activity. They therefore concluded that these proteins were expressed more under the conditions described. Xu et al. (2012) demonstrated that endogenous nitric oxide (NO) could prevent cold damage loquat fruits after harvest, indicating a relation with their capacity to activate antioxidant enzymes to reduce the production of ROS, lipid peroxidation, and cell damage in membranes.

The factors that can affect tomato fruit quality both at harvest and afterward include the genotype cultivated, the environmental conditions, as well as the fertilization used (Beckles 2012). In relation to this latter aspect, in recent years, to improve the nutritional quality of plant products for human consumption, biofortification programs have been used with greater frequency, with trace elements as well as macronutrients. Regarding the macronutrients, He and MacGregor (2008) indicated that the higher consumption of processed foods, together with the lower consumption of fresh fruits and vegetables, has resulted in a sharp decline in K intake, clearly evident in the most developed countries. Evidence reveals that increased K intake benefits human health (Khaw and Barrett-Connor 1987; Macdonald et al. 2005; He and MacGregor 2008).

In plants, the macronutrient K is among the most abundant elements in plant tissues, accounting for ca. 10 % of the dry weight. K is involved in numerous biochemical and physiological processes crucial to growth, performance, quality, and stress tolerance (Epstein and Bloom 2005). Notably, this cation exerts the greatest influence on the parameters determining the market quality of fruit, consumer preferences, and the concentration of phytonutrients of vital importance for human health (Lester et al. 2010). In this sense, adequate K nutrition is also associated with greater crop yield and fruit size, increases in soluble solids and AsA concentrations, and improved fruit color (Kanai et al. 2007).

For all the above, and in view of the functions mentioned for K in fruit quality, the present study examines the application of a biofortification program to determine

how different rates of K in the form of KCl applied during the growth period of cherry tomato plants influence the response of oxidative metabolism during postharvest cold storage (4 ºC) for 21 days.

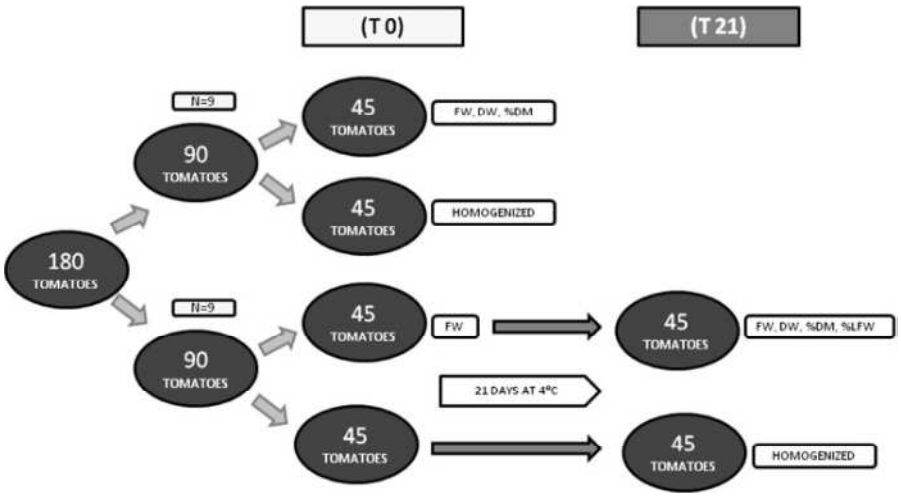
MATERIALS AND METHODS

Plant material, growth conditions, and sampling of tomato fruits

Seeds of cherry tomatoes (Solanum lycopersicum L. cv AsHiari grafted on cv. Maxifort rootstock) were sown in flat trays (cell size 3 cm x 3 cm x 10 cm, 100 cells per tray) filled with 50 % [v/v] perlite–peat mixture, and kept under greenhouse conditions for 5 weeks. Subsequently, the seedlings were transplanted to an experimental greenhouse at La Nacla Experimental Station (Motril), near the Granada coast in southern Spain (36º45'N; 3º30'W; altitude 130 m). Greenhouses conditions during all of the crop cycle from autumn to spring ranged from: 800–1,300 µmol photons m^{-2} s^{-1}, 8–12 h photoperiod, 25–85 % humidity. The plants were grown in 40-L perlite B-12-filled sacks; the planting scheme was 3.21 plants m^{-2}. Their arrangement in the greenhouse was in 12 rows with north–south orientation. The statistical design was on randomized block. The parral greenhouse was used and other growing conditions such as irrigation and fertilization followed Soriano et al. (2004).The different treatments applied were: 5 mM KCl, 10 mM KCl, and 15 mM KCl during the crop cycle.

The crop cycle of cherry tomato lasted from October 2010 to May 2011, with a complete truss of tomatoes (10–12 tomatoes per truss) maturing every 10 days. Cherry tomato fruits were sampled on February of 2011. Approximately, 200 tomato fruits from each treatment were randomly collected (discarding the green fruits at the end of the truss) and rinsed three times in distilled wáter after disinfection with 1 % (v/v) Triton X-100 (Wolf 1982), and then blotted on dry filter paper.

Fig. 1 Sampling experimental design



Biomass parameters

From 180 tomatoes harvested from each treatment, 90 tomatoes were intended for analysis at harvest day (T0), being clustered in nine replicates of ten fruits. Five tomato fruits from each replicate were weighed obtaining fresh weight (FW) and then dried in a lyophilizer to determine the dry weight (DW) and percentage of dry matter (% DM). Five other tomato fruits from each replicate were homogenized, and samples of fresh tissues were stored at – 80ºC. For analyzing the fruits after 21 days of storage in a cold room at 4ºC (T21), in the same way, 90 tomatoes were intended for analysis, being clustered in nine replicates of ten fruits. Five tomato fruits from each replicate were weighed obtaining FW at T0 and stored for 21 days in a cold room at 4ºC. After this period, these were reweighed (T21) and then dried in a lyophilizer to determine the DW and % of DM. Five other tomato fruits from each replicate were homogenized, and samples of fresh tissues were stored at -80ºC (Fig. 1). Samples of fresh and dry tissues from the cherry tomato fruits were used to analyze the parameters described below.

For the determination of percentage of loss in fresh weight (% LFW) the following formula was used for each treatment:

% LFW = (FW T0 - FW T21) x 100 / FW T0:

To calculate % DM the method proposed by Garg and Cheema (2011) was followed. 45 tomatoes were weighed and then lyophilized for 72 h.

After freeze drying, the samples were reweighed. The % DM was calculated as:

DM (%) = (A/B) x 100

where A is the total fresh weight of sample (g) and B the total weight of dry sample (g).

Analytical methods

Determination of K concentration

For the determination of K concentration, 0.2 g of dry cherry tomato fruits was ground and mineralized by wet digestion with H_2SO_4 12 M and H_2O_2 at 30% and free P at a temperature of 275–300ºC. After this step, 20 mL of deionised H_2O was added and finally K concentration was analyzed by flame photometry (Wolf 1982).

Hydrogen peroxide (H_2O_2) concentration

The extraction and quantification of H_2O_2, was performed following the method of Mukherje and Choudhuri (1983). To determine H_2O_2 levels, 0.2 g of tomato fruit was homogenized with 1 mL of cold acetone. An appropriate aliquot of the extracted solution was mixed with 20 % titanium dioxide in 10 % (v/v) HCl and NH_4OH 20 % (v/v). The mixture was then centrifuged at 3.500 rpm for 5 min. The intensity of the yellow color of the supernatant was measured at 415 nm. The H_2O_2

concentration was calculated from a standard curve plotted within the range of 0–5 mmol of H_2O_2. The amount of H_2O_2 was expressed in mmol H_2O_2 g^{-1} FW.

Lipid peroxidation [(malondialdehyde (MDA) concentration and linoleate:oxygen oxidoreductase (LOX)]

Lipid peroxidation was determined by measuring the amount of MDA content according to the method of Davenport et al. (2003) with some modifications. Tomato fruit (0.2 g) was homogenized with 3 mL of a solution of thiobarbituric acid 25 % (w/v) in trichloroacetic acid 10 % (w/v) in an ice bath. The homogenate was incubated in boiling water for 30 min, then cooled and centrifuged at 9.500 rpm for 10 min at 4ºC. The absorbance of reaction supernatant was assayed at 532 and 600 nm. Results were given directly in absorbance. LOX (EC 1.13.11.12) activity was measured according to Minguez-Mosquera et al.(1993) with slight modifications (Rosales et al. 2009) using 50 mM potassium phosphate buffer (pH 6.0) for extraction.The protein concentration of the extracts was determined according to the method of Bradford (1976), using bovineserum albumin as the standard.

Activity of enzymes

Superoxide dismutase (SOD) (EC 1.15.1.1) activity was assayed by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT), according to the method of Yu et al. (1998) optimized for our conditions. For the enzyme

extraction, tomato fruit (0.2 g) was homogenized with 1 mL of 50 mM HEPES–HCl buffer (pH 7.6), which contained 0.1 mM of Na-ethylenediaminetetraacetic acid (EDTA-Na) and centrifuged at 4ºC and 11.000 rpm for 10 min. An appropriate volume of reaction mixture was used, containing 50 mM of Na_2CO_3 (pH 10.2), 12 mM of methionine, 075 µM of NBT, 15 µM of riboflavin, and an appropriate aliquot of enzyme extract. The reaction mixtures were illuminated for 15 min at a PPFD of 380 mmol m^{-2} s^{-1}. Non-illuminated mixtures were used to correct for background absorbance. One unit of SOD activity was defined as the amount of enzyme required to cause 50 % inhibition of the reduction in NBT as monitored at 560 nm.

Catalase (CAT) (EC 1.11.1.6) activity was determined by following the consumption of H_2O_2 at 240 nm for 5 min (Nakano and Asada 1981). Tomato fruit (0.2 g) was homogenized with 1 mL of HEPES–HCl 25 mM buffer (pH 7.8.) The reaction mixture contained 25 mM of sodium phosphate buffer (pH 7.0), 0.8 mM of Na-EDTA, and 20 mM of H_2O_2, and the enzyme assay was performed at 25ºC.

Ascorbate peroxidase (APX) (EC 1.11.1.11) activity was assayed following Rao et al. (1996). For the extraction, tomato fruit (0.2 g) was homogenized with 1.5 mL of 100 mM K-phosphate buffer (pH 7.5) which contained 1 mM of EDTA-Na and centrifuged at 4ºC and 12.000 rpm for 20 min. APX activity was determined by registering the absorbance change at 290 nm for 3 min of a reaction mixture containing 100 mM phosphate potassium buffer (pH 7.5), 0.5 mM of AsA, 0.2 mM

of H_2O, and an adequate aliquot of enzyme extract. Monodehydroascorbate reductase (MDHAR) (EC 1.6.5.4) activity was assayed by recording the change in absorbance of the samples at a wavelength of 340 nm (Foyer et al. 1989). For the enzyme extraction, tomato fruit (0.2 g) was homogenized with 1 mL of 100 mM K-phosphate buffer (pH 7) which contained 0.1 mM EDTA-Na, 1 mM phenylmethylsulfonyl fluoride (PMSF), and Triton X-100 (0.5 %) and centrifuged at 4ºC and 9,500 rpm for 20 min. The reaction mixture contained 100 mM of HEPES–HCl buffer (pH 7.6), 2.5 mM of AsA, 0.025 mM of NADPH, and 50 µL of enzyme extract. Dehydroascorbate reductase (DHAR) (EC 1.8.5.1) activity was measured at 265 nm for 3 min by following the change in absorbance resulting from the formation of AsA (Nakano and Asada 1981; Rosales et al. 2006). The enzyme extraction was the same as for MDHAR. The reaction mixture contained 100 mM of phosphate sodium buffer (pH 7), 2.5 mM of GSH, 0.4 mM of DHA, and a suitable amount of enzyme extract. Glutathione reductase (GR) (EC 1.6.4.1) was assayed following Rao et al. (1996). The enzyme extraction was the same as for APX. GR activity was measured after monitoring the oxidation of NADPH at 340 nm for 3 min in a reaction mixture (700 µL) containing 100 mM phosphate potassium buffer (pH 7.5), 0.2 mM of NADPH, 0.5 mM of GSSG, and an appropriate volume of enzyme extract.

The protein concentration of the extracts was determined according to the method of Bradford (1976), using bovine-serum albumin as the standard.

Ascorbate (AsA) and glutathione (GSH) concentration

The extraction and quantification of total AsA, reduced AsA, and dehydroascorbate (DHA) followed the method of Law et al. (1992). This method is based on the reduction of $Fe3^{+}$ to $Fe2^{+}$ by AsA in acid solution. Tomato fruits weighing 0.2 g were homogenized in liquid N2 with 1 mL of metaphosphoric acid at 5 % (w/v) and centrifuged at 13,500 rpm at 4ºC for 15 min. Afterward, an adequate aliquot of supernatant was added to a test tube together with sodium phosphate buffer 150 mM (pH 7.5) and dithiothreitol (DTT) 10 mM. The mixture was stirred and incubated at room temperature in darkness for 10 min. Next, an adequate aliquot of N-ethylmaleimide at 0.5 % (w/v) was added together with orthophosphoric acid at 44 % (v/v), 2,20-bipyridyl at 4 % (w/v) in ethanol at 70 %, and $FeCl_3$ at 3 % (w/v). The resulting reaction mixture was stirred and incubated at 40ºC in darkness for 40 min. Finally, the absorbance was measured at 525 nm against a standard AsA curve that followed the same procedure as above. The results were used to quantify the total AsA concentration, while the reduced AsA was quantified in the same way as the previous procedure, replacing the DTT with distilled H_2O. Finally, the DHA concentration was deduced from the difference between total AsA and reduced AsA.

Glutathione was measured by the recycling assay described by Noctor and Foyer (1998). The method relies on the GR-dependent reduction of 5,50-dithiobis(2-nitrobenzoic acid) (DTNB, Ellman's reagent), monitored at 412 nm. Without

pretreatment of extracts, the method measures ‘‘total glutathione,’’ that is reduced glutathione (GSH) plus GSSG. Specific measurement of GSSG was achieved by pretreatment of extract aliquots with 2-vinylpyridine (VPD).

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) to determine significance and Fisher’s protected least significant difference (LSD) test to separate means. Standard errors of the means were also calculated. The standard errors are marked in the figures (with error bars) and stated in the tables. The significance levels were expressed as *p<0.05, **p<0.01, ***p<0.001, and NS (not significant) p>0.05.

Results

Biomass parameters and K concentration

In relation to the FW of the cherry tomato fruits, a statistically significant decline was noted at T0 for the treatment of 15 mM KCl (Table 1). On the contrary, T21 did not significantly differ for any of the treatments (Table 1). In relation to DW, statistically significant differences were found at T0 for the treatments with 10 and

15 mM KCl, which presented the highest values (Table 1). At T21, this parameter reached its highest value in the treatment with 15 mM KCl (Table 1). Finally, with respect to the % DM, the highest values were found in treatments 10 and 15 mM KCl, both at T0 as well as T21 (Table 1). In terms of %

Table 1 Fresh weight (FW), dry weight (DW), and % of dry matter (% DM) in cherry tomato fruits at the day of harvest and after 21 days of postharvest in cold storage at 4ºC

Days	KCl (mM)	FW (g)	DW (g)	DM (%)
0	5	25.33 ± 1.00a	1.68 ± 0.08c	6.63 ± 0.03b
	10	25.21 ± 0.79a	2.01 ± 0.05a	7.97 ± 0.06a
	15	21.79 ± 0.82b	1.83 ± 0.04b	8.40 ± 0.04a
	P value	*	*	*
	LSD	2.37	0.09	0.22
21	5	20.99 ± 0.90	1.66 ± 0.08b	7.91 ± 0.09b
	10	20.57 ± 0.63	1.73 ± 0.05b	8.41 ± 0.08a
	15	20.38 ± 0.81	1.81 ± 0.06a	8.88 ± 0.07a
	P value	NS	*	*
	LSD	2.29	0.07	0.26

Means followed by the same letter do not differ significantly Values are mean (n=9) and differences between means were compared by Fisher's least significant difference test (LSD; p = 0.05) p<0.05, NS not significant *p>0.05

LFW of postharvest tomato fruits, treatment with 15 mM KCl showed a lower percentage for this parameter (p<0.001, Fig. 2).

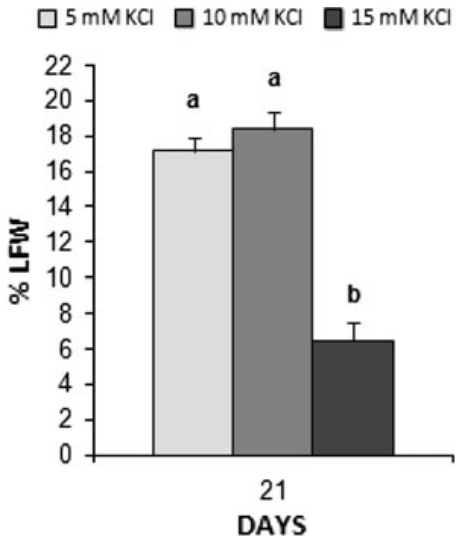


Fig. 2 Percentage (%) of lost of fresh weight (LFW) in cherry tomato fruits after 21 days of postharvest in cold storage at 4ºC. Values are mean ± SE (n=9). Means followed by the same letter do not differ significantly

With respect to the K concentrations, in yield, a proportional response was found based on the KCl rate applied, with the highest concentration being found in the fruits harvested from the plants grown at the rate of 15 mM KCl (p<0.001, Fig. 3).

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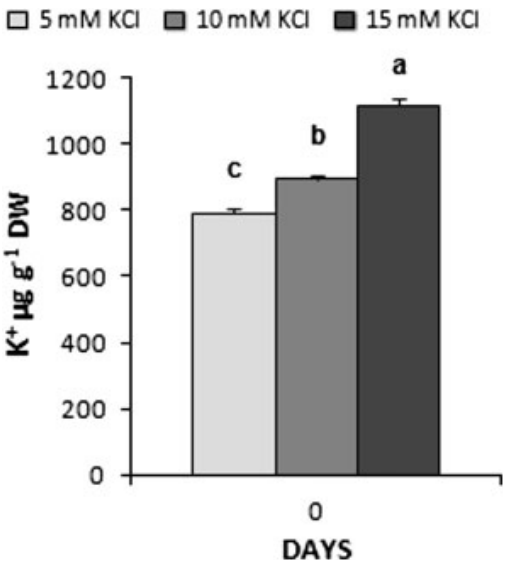

Fig. 3 Concentration of K in cherry tomato fruits at harvest day. Values are mean ± SE (n=9). Means followed by the same letter do not differ significantly

Lipid peroxidation

With respect to the MDA concentration, a significant rise in the concentration was found in the treatment of 5 mM KCl, both for T0 (p<0.01, Fig. 4a) and T21 (p<0.001, Fig. 4a). The LOX activity of the tomato fruits registered the lowest value

for the treatment with 15 mM KCl at T0 (p<0.001, Fig. 4b) and the highest at T21 with 5 mM KCl (p<0.01, Fig. 4b).

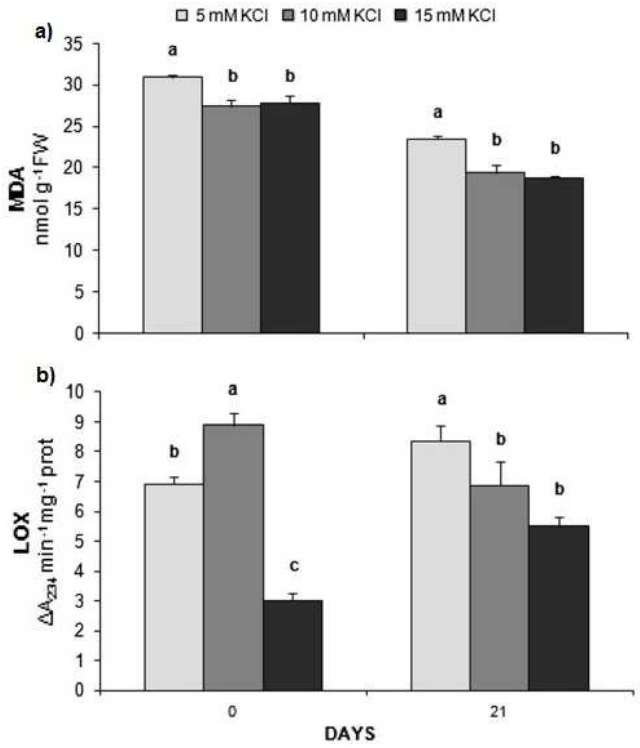

Fig. 4 Effect of KCl treatments at the day of harvest and after 21 days of postharvest in cold storage at 4ºC over the malondialdehyde (MDA) and lipoxygenase (LOX) in cherry tomato fruits. Values are mean ± SE (n=9). Means followed by the same letter do not differ significantly

Activity SOD, hydrogen peroxide concentration, and CAT activity

The SOD enzyme activity in cherry tomato fruits collected from plants treated with different KCl rates presented no significant differences for any of the treatments, either at T0 or T21 (Table 2). Regarding H_2O_2, we found a significant increase in the concentration of this compound, both at T0 and T21 (Table 2), with the highest H_2O_2 values for the treatment with 15 mM KCl. After the treatments with the different KCl rates, a significant increase was found in CAT activity for the treatments of 10 and 15 mM KCl, both at T0 as well as at T21, with the highest values being registered for the treatment with 15 mM KCl (Table 2).

Table 2 Effect of KCl treatments ont the day of harvest and after 21 days of postharvest in cold storage at 4ºC over the units of super oxide dismutase (SOD), hydrogen peroxide (H_2O_2) and catalase (CAT) in cherry tomato fruits

Days	KCl (mM)	SOD (units SOD mg^{-1} prot)	H_2O_2 (mM g^{-1} FW)	Catalase (ΔA_{240} min^{-1} mg^{-1} prot)
0	5	7.50 ± 0.40	3.40 ± 0.03b	0.064 ± 0.01b
	10	8.33 ± 1.55	3.34 ± 0.07b	0.35 ± 0.04a
	15	7.03 ± 0.57	3.76 ± 0.04a	0.34 ± 0.02a
	P value	NS	***	***
	LSD	2.86	0.137	0.080
21	5	3.77 ± 0.58	3.30 ± 0.03b	0.13 ± 0.01b
	10	3.23 ± 0.34	3.46 ± 0.02b	0.19 ± 0.02b
	15	3.22 ± 0.39	3.66 ± 0.11a	0.33 ± 0.05a
	P value	NS	**	***
	LSD	1.31	0.192	0.087

Means followed by the same letter do not differ significantly Values are mean (n=9) and differences between means were compared by Fisher's least significant difference test (LSD; p = 0.05) p>0.05, NS not significant **p<0.01 ***p<0.001

AsA and GSH forms

Total AsA in the cherry tomato plants significantly increased on applying the rate of 15 mM KCl, both at T0 and T21 (Table 3). For the DHA formed at T0, the highest value was registered with the treatment of 15 mM KCl (Table 3), while at T21 the highest value was found after applying the rate of 5 mM KCl (Table 3). Finally, for the quantity of reduced AsA at T0, no significant differences were found (Table 3), while at T21 the highest values were registered for the treatments with 10 and 15 mM KCl (Table 3).

Table 3 Effect of KCl treatments on the day of harvest and after 21 days of postharvest in cold storage at 4ºC over AsA total, AsA reduced and DHA in cherry tomato fruits

Days	KCl (mM)	AsA total (nmol g^{-1} FW)	AsA reduced (nmol g^{-1} FW)	DHA (nmol g^{-1} FW)
0	5	306.40 ± 6.56c	293.76 ± 10.09	23.73 ± 0.78b
	10	322.16 ± 2.96b	313.05 ± 6.95	19.85 ± 0.88b
	15	373.56 ± 4.21a	323.06 ± 10.29	50.50 ± 9.87a
	P value	***	NS	**
	LSD	14.04	26.96	16.75
21	5	355.09 ± 4.75b	298.20 ± 6.47b	56.88 ± 0.62a
	10	369.52 ± 2.20a	344.93 ± 5.94a	24.52 ± 5.10b
	15	362.74 ± 2.62ab	327.22 ± 769a	35.52 ± 4.78b
	P value	*	***	***
	LSD	9.85	19.67	11.83

Means followed by the same letter do not differ significantly Values are mean (n=9) and differences between means were compared by Fisher's least significant difference test (LSD; p=0.05) p<0.05, NS not significant *p>0.05 **p>0.01***p>0.001

In relation to total GSH, reduced GSH and GSSG, no significant differences were found in any of the treatments at T0, while at T21 an increase in the concentration was found in treatment with 15 mM KCl (Table 4).

Table 4 Effect of KCl treatments on the day of harvest and after 21 days of postharvest in cold storage at 4ºC over total glutathione (GSH total), reduced glutathione (GSH reduced) and oxidized glutathione (GSSG) in cherry tomato fruits

Days	KCl (mM)	GSH total (nmol g^{-1} FW)	GSH reduced (nmol g^{-1} FW)	GSSG (nmol g^{-1} FW)
0	5	1,278.60 ± 59.27	1,057.29 ± 36.30	221.30 ± 9.32
	10	1,450.03 ± 85.87	1,192.07 ± 75.75	257.96 ± 21.30
	15	1,376.15 ± 68.32	1,095.27 ± 46.70	280.80 ± 18.76
	P value	NS	NS	NS
	LSD	210.17	161.95	50.34
21	5	914.07 ± 55.07c	842.91 ± 7.56b	71.16 ± 13.91c
	10	1,084.14 ± 49.66b	940.66 ± 17.70b	143.48 ± 24.18b
	15	1,390.52 ± 68.16a	1,155.59 ± 62.33a	234.93 ± 31.94a
	P value	***	***	***
	LSD	169.73	109.93	70.57

Means followed by the same letter do not differ significantly Values are mean (n=9) and differences between means were compared by Fisher's least significant difference test (LSD; p=0.05) p<0.05, NS not significant ***p>0.001

Ascorbate–glutathione (AsA–GSH) cycle

With respect to the enzymes of the AsA–GSHcycle, theAPX and MDHAR activities behaved similarly, given that at T0 no significant differences appeared in any of the treatments applied (Table 5). However, at T21, statistically significant differences were found, with the highest value for this enzymatic activity appearing with the rate of 15 mM KCl (Table 5).

Meanwhile, the DHAR activity presented the highest value for treatment with 5 mMKCl, both at T0 and at T21 (Table 5). Finally, theGRactivity showed no significant differences in any of the treatments at T0; however, differences were found for T21, where the highest activity presented at the rate of 5 mM KCl (Table 5).

Table 5 Effect of KCl treatments on the day of harvest and after 21 days of postharvest in cold storage at 4ºC over ascorbate peroxidase (APX monodehydro ascorbate reductase (MDHAR), dehydro ascorbate reductase (DHAR) and GSH reductase (GR) in cherry tomato fruits

Days	KCl (mM)	APX (ΔA_{290} min^{-1} mg^{-1} prot)	MDHAR (ΔA_{340} min^{-1} mg^{-1} prot)	DHAR (ΔA_{265} min^{-1} mg prot)	GR (ΔA_{340} min^{-1} mg prot)
0	5	0.24 ± 0.03	0.08 ± 0.02	3.00 ± 0.11a	0.16 ± 0.02
	10	0.17 ± 0.02	0.18 ± 0.02	2.17 ± 0.09b	0.11 ± 0.02
	15	0.22 ± 0.03	0.29 ± 0.01	2.14 ± 0.33b	0.11 ± 0.02
	P value	NS	NS	*	NS
	LSD	0.077	0.219	0.611	0.057
21	5	6.55 ± 0.73b	1.69 ± 0.33b	7.86 ± 0.17a	0.49 ± 0.050a
	10	8.08 ± 0.34ab	3.64 ± 1.51b	4.18 ± 0.34c	0.25 ± 0.025b
	15	9.4 ± 0.76a	6.85 ± 0.90a	5.81 ± 0.10b	0.22 ± 0.012b
	P value	*	**	***	***
	LSD	1.867	3.017	0.655	0.096

Means followed by the same letter do not differ significantly Values are mean (n=9) and differences between means were compared by Fisher's least significant difference test (LSD; p = 0.05) p<0.05, NS not significant *p>0.05 **p>0.01 ***p>0.001

Discussion

Weight loss and chilling injury (CI) often occur during the cold storage of fresh fruits, causing serious economic losses to the horticultural industry (Hung et al. 2011).

Fresh fruits and vegetables are living tissues that continue to lose water after the harvest, but, as opposed to the plants in the field, they cannot replace the water lost and must preserve the water content after being harvested. The postharvest water loss from fresh products is a major problem because it provokes weight loss,

with most products becoming unsellable as fresh products after losing 3–10 % of their weight (Ben-Yehoshua and Rodov 2003). In this sense, in the present work, the fruits treated with 15 mM KCl at T21 presented a lower % LFW (Fig. 2). Furthermore, this treatment improved the response to postharvest cold storage at 4ºC for 21 days, showing less loss of FW (Table 1), while increasing both the DW as well as the % of DM (Table 1). These results suggest that the application of the highest K rate, and therefore the highest K concentration in the cherry tomato fruits in the treatment with 15 mM KCl (Fig. 3), prevents the weight and water loss during postharvest storage. In this context, Almeselmani et al. (2010) observed that an extra provision of K in the fertilization of tomato plants can help to preserve fruits during postharvest storage.

Lipid peroxidation of membranes is considered a good marker of oxidative damage and is the result of a degradation of polyunsaturated fatty acids, which gravely affects the functionality of membranes, inflicting irreversible damage. Mittler (2002) suggested that cell membrane damage accelerated the Haber–Weiss reaction, increasing lipid peroxidation. In the present work, the greater lipid peroxidation, reflected by the MDA concentration and LOX activity (Fig. 4a, b), appeared on treatment with 5 mM KCl at T21, while the lowest values for these parameters were encountered on treatment with 15 mM KCl.

Generally, MDA is an indicator of the degree of oxidative stress and the structural integrity of the membranes in plants subjected to low temperatures (Posmyk et al. 2005), serving as a tool to quantify the degree of lipid peroxidation. Many works have found an inversely proportional relationship between increased resistance to

different types of stress and decreased lipid peroxidation (Sánchez-Rodríguez et al. 2010; Aghdam et al. 2013, in press). In the particular case of cold damage during postharvest fruit storage, lipid peroxidation often occurs, as reported for cucumber fruits (Yang et al. 2011). Also, Lee et al. (2012), working with apple fruits treated with 1-MCP and stored at low temperatures, reported higher lipid peroxidation in the treated fruits with respect to those untreated. In the present work, the biomass data at the end of the postharvest storage were related to the incidence of lipid peroxidation. Thus, the fruits of the plants treated with 15 mM KCl after 21 days postharvest at 4ºC showed a higher % DM (Table 1) and lower % LFW (Fig. 2), together with the lowest lipid peroxidation values (Fig. 4). A complete opposite was noted for the treatment of 5 mM of KCl. In short, the treatment with 15 mM KCl strengthened cold stress tolerance and improved these parameters related closely to the biomass loss after postharvest storage.

Cold damage is a type of oxidative stress that occurs during fresh fruit storage at temperatures below 10ºC, with tomato being particularly sensitive (Stevens et al. 2008). SOD is responsible for the dismutation of the radical O_2 - to H_2O_2 and is commonly considered the first line of cell defense. In the present study, the SOD activity did not increase for any of the treatments with KCl, either in T0 or T21 (Table 2). On the contrary, the H_2O_2 concentration did increase both at T0 and T21, mainly in the treatment 15 mM KCl (Table 3). H_2O_2 is a substrate for different enzymatic pathways, such as that of CAT, which functions as part of the antioxidant system, protecting plants by providing an effective response against oxidative stress (Delaplace et al. 2009). In the present work, an increase was

observed in the CAT activity for the treatment with KCl 15 mM, suggesting that this enzyme efficiently eliminated ROS in this treatment, a situation that could prevent the excessive and toxic accumulation of H_2O_2 (Table 3).

With respect to the enzymes of the ascorbate–glutathione cycle, it bears mentioning that the APX and MDHAR activities showed a greater activity both for T0 and T21 at the rate 15 mM KCl (Table 5), indicating that the AsA was regenerated effectively in the first part of the cycle. In support of the present results, other authors have related greater APX activity with greater postharvest resistance to oxidative stress in melons 'Orange Dew' and 'Honey Brew' (Hodges and Lester 2006).

For example, Sala (1998) observed that the chill stresstolerant mandarin cultivars showed the highest APX activity during cold storage and therefore had a greater capacity for H_2O_2 detoxification. Farooq et al. (2008), studying hybrid corn seeds, observed a rise in APX activity after applying KCl treatments under cold stress conditions. The DHAR and GR activities presented less activity on treatments with 10 and 15 mM KCl for T21, while the highest values appeared in the treatment of 5 mM KCl (Table 5). The low DHAR activity, as suggested by De Gara et al. (2003), could be explained, since the GSH not only participated in AsA recycling, while acting as an electron donor for DHAR, but was also responsible for other metabolic pathways.

The mechanisms of antioxidation and protection of metabolites in plants include a number of non-enzymatic antioxidants such as AsA and GSH, and its main

function is to interrupt the uncontrolled oxidation cascades in some organelles and eliminate the ROS. Antioxidant compounds are the essential determinants of nutritional quality in tomato fruits. Among the phytochemicals present in tomato fruits, the AsA content is considered a key factor to determine the commercial value of the tomato yield thanks to the nutritional benefit associated with its consumption (Frusciante et al. 2007). The antioxidant levels of a plant are also a good indicator of the redox state, which is indispensable for stress tolerance. In the present study, after the application of the biofortification program with K in the form of KCl, the total and reduced AsA (Table 3) as well as total and reduced GSH (Table 4) presented the highest concentration for the treatments of 10 and 15 mM KCl at T21. Lester et al. (2010) found that melon fruits (Cucumis melo L.) treated with different K forms presented a higher AsA content than control non-treated fruits. The beneficial effects of the K supplement to the plant were presumably the result of a combination of improved photosynthetic assimilation of CO_2 by the leaves, translocation of assimilates from the leaves to the fruits, better leaf–fruit water relations, as well as greater enzymatic activity and the availability of substrate for AsA biosynthesis. With respect to the DHA, the lowest concentration in the present work was found for the treatments with 10 and 15 mM KCl (Table 3). In general, the behavior of the AsA forms suggests an effective regeneration of the DHA in AsA, which acts as an antioxidant compound detoxifying ROS. Thus, an adequate biofortification program with K has been associated with a rise in the AsA content that in turn has been related to protection against oxidative stress. Finally, with respect to GSSG, the highest concentration was found with the treatment of 15 mM KCl and the highest concentration of reduced GSH was also detected for

this treatment, which in the same sense could also be indicative of an effective regeneration of GSH in the treatment with 15 mM KCl (Table 4).

An adequate biofortification program with K may prove beneficial, alleviating stress that could result from chill stress in tomato fruits stored in cold chambers. The present study demonstrated that tomato fruits from plants treated at the rate of 15 mM KCl presented less biomass loss after postharvest storage, as well as a lower degree of lipid peroxidation, possibly due to greater APX and MDHAR activity, suggesting greater effectiveness in ROS detoxification as well as in AsA regeneration. In addition, under this treatment of K, the fruits presented a greater AsA pool as well as higher total and reduced GSH. Therefore, it is concluded that the rate of 15 mM of KCl applied to this tomato variety could be adequate to mitigate the negative effects caused by postharvest storage at low temperatures.

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3. 2. Implication of potassium on the quality of cherry tomato fruits after postharvest during cold storage

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Abstract

The influence of the potassium (K) content in tomato fruits over compounds or antioxidant characteristics during the postharvest period in cold storage is little known. The aim of this work was to determine whether the effect of a biofortification programme with K in KCl form can improve the postharvest storage of cherry tomato fruits at 4°C. K treatments applied during the crop cycle of the plants: 5, 10 and 15 mM of KCl. Biomass parameters, levels of K, antioxidant capacity test, Vitamin C, carotenoids, phenolic compounds and free polyamines in tomato cherry fruits were measured. Our results show that the treatment with 15 mM KCl prevents weight and water loss during postharvest storage at 4°C, increases K concentration, and bolsters the antioxidant capacity, since the concentration in lycopene as well as flavonoids and derivatives rose, while the contents in Vitamin C together with hydroxycinnamic acids and derivatives remained stable.

Keywords Antioxidants compounds, postharvest, potassium, tomato fruits

Introduction

The tomato (*Solanum lycopersicum* L.), an annual horticultural plant with a worldwide distribution and enormous economic values, has a global annual production of some 159.347 million of tonnes (FAOSTAT, 2011). Due to its high content in compounds that detoxify reactive oxygen species (ROS) and thus prevent oxidative changes in the human, the consumption of these fruits is considered beneficial for human health (García-Closas et al., 2004). Tomato is rich in bioactive compounds, such as lycopene (Lyc), which represents around 80% of the carotenoids and has a high capacity to eliminate ROS, being one of the phytonutrients most characteristic of tomato fruit (Rao et al., 1998); β-carotene, a precursor to Vitamin A in the human body; ascorbic acid (Vitamin C), which, apart from being the most effective antioxidant in plants (Smirnoff & Pallanca, 1996), is a major phytochemical for its antioxidant properties in eliminating ROS and regenerating Vitamin E in plants (Asada, 1994); lutein (Lut), a yellow pigment found in plants which is considered an important phytochemical for its high antioxidant capacity and which cannot be synthesized by animals; and phenolic compounds, namely flavonoids and phenolic acids (Soto-Zamora et al., 2005). Many phenolic compounds exhibit antioxidative, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammatory activities (Martinez-Valverde et al., 2002).

Spain has exported tomatoes since the 1940s and, in general, exportation implies the storage of fruits in cold chambers. Although cold storage is a widely used method to prolong the shelf life of climacteric fruits, it can affect their nutritional quality by provoking cold damage. This type of stress occurs duringstorage below

10°C in fleshy fruits, tomato being particularly sensitive (Stevens et al., 2008). Tomato, being climacteric and thus perishable, requires the use of conservation technologies to retard the ripening process that occurs after harvest and thereby maintain its quality and extend the shelf life of the fruit. Despite that cold storage can trigger harmful effects, this procedure has been demonstrated to be effective in maintaining the phytonutrients and other qualities that determine fruit quality. Antioxidant activity of tomatoes depends on several factors, including genetic traits, environmental conditions (temperature, light, water and nutrient availability), production techniques (plant growth regulators, date of harvest, etc.) and postharvest storage conditions (Dumas et al., 2003; Leonardi et al., 2000). As demonstrated by Wang et al. (2012) in avocado fruits harvested on different dates of the year, cold storage resulted in positive effects on the maintenance of antioxidant capacity as well as the accumulation and retention of nutrients, including phenolic compounds. In relation to the effect of cold storage on tomato fruits, Nicoletto et al. (2012) found that ripe fruits left on the plant showed increased antioxidant activity in addition to greater Vitamin C and total phenolic content in comparison with those that were stored cold, as in the latter fruits the parameters did not vary. In this sense, Kalt et al. (1999) in his study with small fruits, i.e. fresh strawberries (*Fragaria ananassa* Duch.), raspberries (*Rubus idaeus* Michx.), highbush blueberries (*Vaccinium corymbosum* L.), and lowbush blueberries (*Vaccinium angustifolium* Aiton), found losses in ascorbate after fresh storage, registering minimum values.

In terms of pigments, different authors have confirmed that cold storage retards the synthesis of Lyc and carotenoids (Gómez et al., 2009; Mejía-Torres et al., 2009). At low temperatures (below 12°C), chlorophyll is only partially degraded while Lyc does not accumulate as it does under normal conditions (Lopez-Camelo & Gomez, 2004). On the contrary, Farneti et al. (2012), in his work with ripe red tomato fruits, using remittance VIS spectroscopy to assess the Lyc content in the tomato pericarp tissue, concluded that tomato storage at temperatures below 12°C (a common market practice) degrades Lyc and consequently reduces the presumed health-promoting value at the same time as lowering the external visual quality. The decrease in Lyc content induced by low-temperature storage may be caused by Lyc fragmentation. However, available published data on antioxidant active compounds Lyc, phenols, and Vitamins C and E are limited mostly to vine-ripened tomatoes or processed tomatoes. Thus, it is necessary to know more about the effects of postharvest conditions, especially at low temperatures, on the antioxidants in tomatoes, because temperature is the main factor for tomato quality in terms of antioxidants (Javanmardi & Kubota, 2006).

The main factors that can affect tomato-fruit quality at harvest as well as afterwards include the genotype cultivated, environmental conditions and the fertilizer applied (Beckles, 2012). In relation to this latter factor, in recent years, in order to improve the nutritional quality of table vegetables, biofortification programmes are being steadily more widely used, both with trace elements as well as macronutrients. Studying macronutrients, He & MacGregor (2008) have indicated that increased consumption of processed foods together with reduced consumption of fruits and

vegetables results in a serious decrease in K ingestion. Evidence reveals that higher K intake has beneficial effects on human health. Epidemiological and clinical studies demonstrate that a diet rich in this nutrient lowers blood pressure, reduces mortality by cardiovascular disease, retards certain renal pathologies and appears to slow the appearance of osteoporosis (He & MacGregor, 2008).

Thus, K is notable as the cation that has the greatest influence on the quality parameters determining the marketing of fruits, consumer preferences, and the concentration of vital phytonutrients for human health (Lester et al., 2010). K significantly affects the concentration of such pigments as Lyc and β-carotene, which can be used as inner-quality indicators for tomato, based on analytical and sensorial properties (Ramírez et al., 2012). Nutrition with adequate K is also associated with greater yield, larger fruit size, increased soluble solids, higher Vitamin C concentrations and improved fruit colour (Kanai et al., 2007). Studies on open-field and greenhouse tomato crops (Chapagain & Wiesman, 2004) showed that an increased K supply at specific growth stages of the tomato plant would improve fruit quality. However, the influence that the K content in fruits exerts on the compounds or antioxidant characteristics during a postharvest period of cold storage is little known. Therefore, in consideration of the functions of K described above concerning fruit quality, the aim of the present work was to evaluate a biofortification programme with K in the form of KCl in terms of nutritional quality of cherry tomato fruits (*Solanum lycopersicum* L. cv. AsHiari) after 21 days of cold storage at 4°C.

Material and methods

Plant material and growth conditions

Seeds of cherry tomatoes (*Solanum lycopersicum* L. cv. AsHiari grafted on cv. Maxifort rootstock) were sown in flat trays (cell size 3 cm x 3 cm x 10 cm, 100 cells per tray) filled with 50% [v/v] perlite-peat mixture, and kept under greenhouse conditions for five weeks. Subsequently, the seedlings were transplanted to an experimental greenhouse at La Nacla Experimental Station (Motril, Granada, Spain). The parral greenhouse consisted of three modules having a symmetrical gable roof with a 27° slope and having an E-W longitudinal orientation (Soriano et al., 2004). The active environmental control was limited to a heating system by hot-air generators, and a natural ventilation system through wall and roof windows. In the greenhouse, the cladding material was a multilayer film 0.2 mm thick, with a layer of ethylenevinyl- acetate between two low-density polyethylene layers (inner, antidrop; and outer, long life). The plants were grown in 40-L perlite B-12-filled sacks (1.20 m long) spaced 0.5 m apart in rows 1.4 m apart. With three tomato plants per sack and two stems per plant, the planting scheme was 3.21 plants m^{-2}. There were 12 rows oriented north-south in the greenhouse. The statistical design was a randomized block. Other growing conditions such as irrigation and fertilise application followed Soriano et al. (2004). The different treatments applied were as follows: 5, 10 and 15 mM KCl as liquid solution from the beginning to the end of the experiment.

Tomato fruit sampling

The cherry tomato crop cycle lasted from October 2010 to May 2011 (230 days), with a complete truss of tomatoes (10–12 tomatoes per truss) maturing every 10 days. Cherry tomato fruits were sampled in February of 2011 at 140 days after transplanting. Uniformly ripe healthy fruits, at the red-ripe stage, were harvested. Approximately 180 tomatoes fruits from each treatment were randomly collected (discarding the green fruits at the end of the truss) and were rinsed three times in distilled water after disinfection with 1% (v/v) Triton X-100 (Wolf, 1982), and then blotted on dry filter paper.

Fresh weight and percentage of lost fresh weight

For the analyses of the fruits at harvest (T0), some tomato fruits from each treatment were weighted for fresh weight (FW) (T0). The remaining tomato fruits were homogenized, and these samples of fresh tissues were stored at -80°C, while other tomato fruits were freeze dried in a lyophilizer. The weighed fruits from each treatment were stored 21 days in a cold room at 4°C (T21), and afterwards weighed again to record the new FW (T21). Next, the fruits were homogenized, and these samples of fresh tissues were stored at -80°C, while another quantity of these tomato fruits were freeze dried. Samples of fresh and dry tissues from the cherry tomato fruits were used to analyse the parameters described below.

For the determination of the % of LFW, the following formula was used for each treatment:

% LFW = (FW T0 - FW T21) x 100/FW T0

Analytical methods

Determination of the K concentration

For the determination of the K concentration, 0.2 g of dry cherry tomato fruits were ground and mineralized by wet digestion with H_2SO_4 12 M and H_2O_2 at 30% and P free, at 275–300°C. After the addition of 20 mL of deionized H_2O, the K concentration was analysed directly in this solution by flame atomic absorption spectrometry using (Perkin-Elmer AAnalyst 700, Norwalk, CT) (Wolf, 1982).

Antioxidant capacity assays

The total antioxidant capacity was measured using the Trolox equivalent antioxidant capacity (TEAC), ferric reducing ability of plasma (FRAP) assays. The TEAC was determined as described by Re et al. (1999) using 2,2-azino-bis (3-ethylbenzthiazoline-6-sulphonate) solution (ABTS) and 2,20-azo-bis (2 methylpropionamidine) dihydrochloride, for the production of the ABTS radical ($ABTS^{\bullet-}$). The TEAC value of an extract represents the concentration of a Trolox solution that has the same antioxidant capacity as the extract. The FRAP assay was made with FRAP reagent, i.e. 1 mM 2,4,6-tripyridyl-2-triazine and 20mM $FeCl_3$

in 0.25M CH_3COONa, pH 3.6. An aliquot of 100 mL of extract (1 g per 10 mL in methanol) was added to 2 mL of FRAP reagent and mixed thoroughly. After the mixture was left at room temperature (20°C) for 5 min, absorbance at 593 nm was measured. Calibration was against a standard curve (25–1600 mM Fe^{3+}) using freshly prepared ammonium ferrous sulphate (Benzie & Strain, 1996).

For reducing power assays, tomato fruits were homogenized in methanol 80%, and centrifuged at 3.000 g for 10 min. The reducing power of tomato fruits was measured following Hsu et al. (2009). Tomato extract, phosphate buffer (0.2 mol L^{-1}, pH 6.6) and $K_3Fe(CN)_6$ (1% v/w) was mixed and allowed to react for 20 min at 50 °C. The sample was immediately cooled and then Cl_3CCOOH 10% was added. After centrifugation at 3000 x g for 10 min, the supernatant was mixed with distilled water and $FeCl_3$ (0.1%), and allowed to react for 10 min. Increased absorbance of the reaction mixture at 700 nm indicated greater reducing power.

Pigment concentrations

Carotenoids were extracted directly in a 1.5 mL Eppendorf tube containing an assay sample of approximately 400 mg of tomato powder. This was achieved by means of alternating periods of stirring and centrifugation (19.500 g), in the following order: the addition of 100 mL of saturated aqueous NaCl solution and 50 mL of Hex, agitation for 30 s and centrifugation for 2 min; the addition of 200 mL of dichloromethane, stirring for 30 s and centrifugation for 2 min; the addition of 1000 mL of ethyl acetate (EA), stirring for 30 s and centrifugation for 5 min. An aliquot of

the organic fraction (upper phase) was filtered and assayed by HPLC (Sérino et al., 2009). The assay was performed using HPLC with a DAD UV–Visible detector (Agilent Technologies, Santa Clara, CA) under the following conditions: Phenomenex reverse-phase column, 250 x 4.6 mm i.d., 5 mm, Li-Chrospher 100 RP-18, with a 4 x 4 mm i.d. guard column of the same material (Luna, Phenomenex, Utrecht, Belgium). The column oven temperature, 28°C; mobile phase, acetonitrile (ACN):UP water:EA (53:7:40, v/v/v); flow rate of mobile phase, 1mL min^{-1}; injection volume, 10 mL; wavelength range, 200–750 nm; two working wavelengths, 474 nm for Lyc, 454 nm for β-carotene, and 448 nm for Lut. These chromatographic conditions allow good separation of the different carotenoids present in tomato. Lut, Lyc and β-carotene were used as a standard (Sigma-Aldrich, Steinheim, Germany), eluting at 4, 13 and 23 min, respectively.

Anthocyanins were determined according to Lange et al. (1971) with some modifications. Tomato fruits were homogenized in propanol:HCl:H_2O (18:1:81) and further extracted in boiling water for 3 min. After centrifugation at 5.000 g for 40 min at 4°C, the absorbance of the supernatant was measured at 535 and 650 nm. The absorbance due to anthocyanins was calculated as $A=A_{535}-A_{650}$.

Vitamin C concentration

The determination of ascorbic acid was based on the method of Hejtmánková et al. (2009) with slight modifications. About 0.2 g of freeze-dried tomato samples were homogenized with 10 mL of 3% meta-phosphoric acid. The resulting mixture was

centrifuged for 10 min and then filtered through a 0.45 mm membrane filter, and triplicates of 10 mL for each sample were analysed by HPLC-DAD. HPLC analysis of ascorbic acid was carried out using the same equipment as described above. Samples were injected into an ACE 5C18 column, 250 x 4.6 mm (Hichrom, Berkshire, UK) operating at 30°C. A single mobile phase consisting of 2.5 mM sulphuric acid at 1.0 mL min^{-1} was used. The elution was monitored at 250 nm. L-Ascorbic acid was used as a standard (Sigma-Aldrich), eluting at 4.1 min.

Concentration of phenol compounds

For the identification and characterization of phenolics, 0.1 g of lyophilized tomatoes was extracted with 1 mL of water/methanol (1:1) by sonication for 1 h, followed by overnight maceration and another sonication period (1 h). The resulting extract was centrifuged and filtered through a 0.45 mm PVDF membrane (Sánchez-Rodríguez et al., 2011). Chromatographic analyses were made in an ACE 5C18 column, 250 x 4.6 mm (Hichrom). The mobile phase consisted of two solvents: water/acetic acid (1%) (A) and ACN (B), starting with 5% B and using a gradient to obtain 50% at 30 min and 80% at 37 min. The flow rate was 1 mL min^{-1} and the injection volume, 20 mL. Spectroscopic data from all peaks were accumulated in the range of 200–400 nm, and chromatograms were recorded at 280, 320 and 360 nm. The identified analytes were quantified by HPLC-PDA detection using the external standard method with calibration graphs, as a function

of concentration based on peak area, detected at the wavelength corresponding to their maximum absorbance.

Concentration of free polyamines

In 1 mL of 6% (v/v) cold perchloric acid (PCA), 1.5 g of tomato were homogenized, kept on ice for 1 h, and then centrifuged at 21.000 g for 30 min. The pellet was extracted once with 1 mL of 5% PCA and recentrifuged. The supernatant was benzoylated following the method of Aziz & Larher (1995) to determine the levels of free PAs. The benzoyl derivatives were separated and analysed by a HPLC (Agilent 1100 system, Santa Clara, CA). Next, 10 mL of ACN solution of benzoyl polyamines (PAs) was injected into an ACE 5C18 column, 250 x 4.6 mm (Hichrom). The column temperature was maintained at 30°C. Samples were eluted from the column with 40% ACN at a flow rate of 1 mL min^{-1}. PA peaks were detected with a UV detector at 254 nm, and 1,6-hexanediamine was used as an internal standard.

Statistical analysis

Data were analysed using one-way analysis of variance to determine significance and Fisher's protected least-significant difference (LSD) test to separate means. Standard errors of the means were also calculated. The significance levels were expressed as *p<0.05, **p<0.01, ***p<0.001 and ns (not significant) p<0.05.

Results

In relation to FW of the cherry tomato fruits, after 21 days of cold storage (T21), the FW of the fruits treated with 5 and 10 mM of KCl lost weight, while in the treatment with 15 mM of KCl no significant differences were found between T0 and T21 (p<0.001, Figure 1). In terms of percentage of LFW, after postharvest storage of the fruits, the 15 mM KCl treatment registered the lowest value for this parameter (p<0.001, Figure 2). With respect to the K concentrations, a proportional response was found according to the KCl applied, the highest concentration being recorded in the tomato fruits grown with 15 mM KCl (p<0.001, Figure 3).

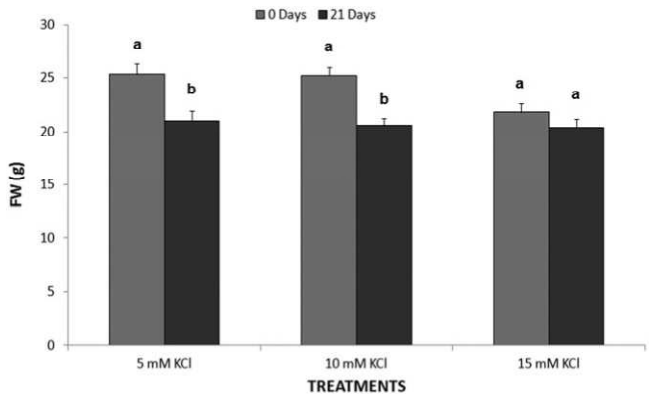

Figure 1. Effect of KCl treatments at the day of harvest over fresh weight (FW) in cherry tomato fruits. Values are means (n=9) and differences between means were compared by Fisher's LSD test (p=0.05). Means followed by the same letter do not differ significantly.

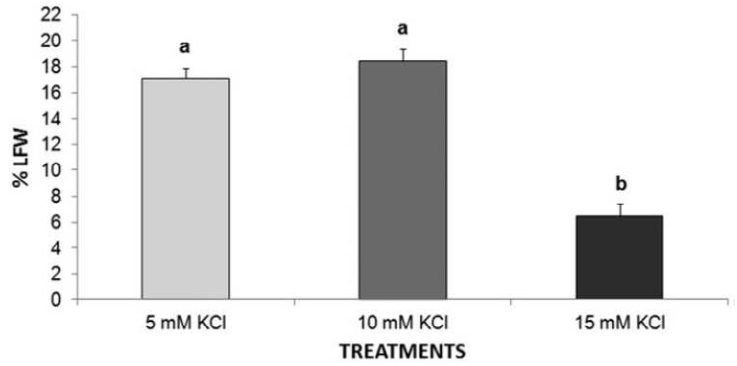

Figure 2. Effect of KCl treatments after 21 days of postharvest in cold storage at 4ºC over % LFW in cherry tomato fruits. Values are means (*n*=9) and differences between means were compared by Fisher's LSD test (*p*=0.05). Means followed by the same letter do not differ significantly.

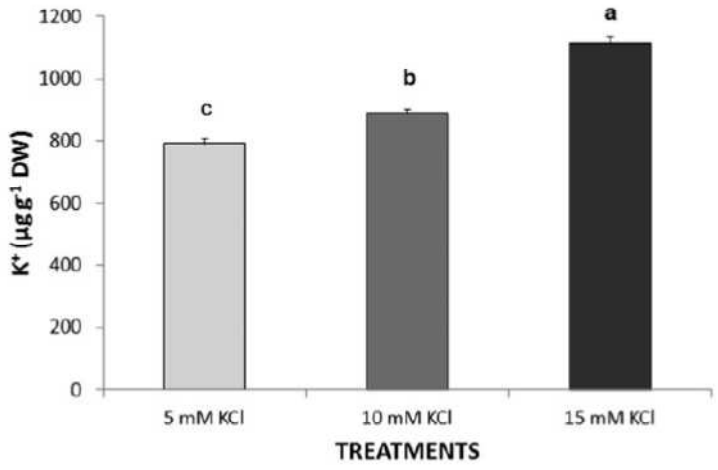

Figure 3. Effect of KCl treatments at the day of harvest over concentration of K in cherry tomato fruits. Values are means (*n*=9) and differences between means were compared by Fisher's LSD test (*p*=0.05). Means followed by the same letter do not differ significantly; DW, dry weight.

The cold storage for 21 days in the treatment of 5 mM KCl resulted in a decline in values of the antioxidant tests TEAC and FRAP (Table 1). On the contrary, at the rate of 15 mM KCl, the postharvest values increased in these antioxidant tests, reaching maximum values at T21 (Table 1). For the fruits harvested from plants grown with 10 mM of KCl, no significant differences were found between T0 and T21 for these tests (Table 1). Finally, with respect to the reducing power, no differences appeared between T0 and T21 for any K treatment applied (Table 1).

Table 1. Effect of KCl treatments at the day of harvest and after 21 days of postharvest in cold storage at 4ºC over: TEAC, FRAP and reducing power in cherry tomato fruits.

KCl (mM)	Days	TEAC (mmol g^{-1} DW)	FRAP (mmol g^{-1} DW)	Reducing power (Abs g^{-1} DW)
5	0	16.72 ± 0.26^{a}	61.14 ± 0.81^{a}	14.12 ± 1.44
	21	10.67 ± 0.38^{b}	33.44 ± 0.67^{b}	14.27 ± 0.86
	p Value	***	***	NS
10	0	10.66 ± 0.37	34.78 ± 0.37	14.02 ± 0.71
	21	9.93 ± 0.41	33.78 ± 0.46	13.45 ± 0.46
	p Value	NS	NS	NS
15	0	8.33 ± 0.15^{b}	30.68 ± 0.41^{b}	13.19 ± 0.68
	21	13.31 ± 1.07^{a}	40.47 ± 0.47^{a}	13.82 ± 0.53
	p Value	***	***	NS

Values are mean (n=9) and differences between means were compared by Fisher's LSD test (p=0.05). Significance levels are represented by p>0.05; NS, not significant. Means followed by the same letter do not differ significantly; DW, dry weight. ***p<0.001.

With respect to Lyc, the cold storage increased values, and significant differences appeared for all the treatments applied. In all cases, the maximum values of this compound were reached at T21, the highest corresponding to 15 mM KCl (Table 2). In the concentration of β-carotene, no significant differences were found for any K treatment between T0 and T21 (Table 2). Finally, with respect to Lut, the treatments with 5 and 15 mM of KCl presented no significant differences between T0 and T21. On the contrary, for the fruits treated at the rate of 10 mM KCl, postharvest values increased for Lut (Table 2).

Table 2. Effect of KCl treatments at the day of harvest and after 21 days of postharvest in cold storage at 4ºC over: Lyc, b-carotene, Lut and anthocyanins in cherry tomato fruits.

KCl (mM)	Days	Lyc (μg g^{-1} DW)	β-Carotene (μg g^{-1} DW)	Lut (μg g^{-1} DW)	Anthocyanins (ΔAbs g^{-1} DW)
5	0	331.71 ± 22.41^{a}	73.49 ± 7.40	5.98 ± 0.51	0.93 ± 0.12
	21	434.85 ± 29.36^{b}	53.67 ± 15.57	6.92 ± 0.94	0.88 ± 0.03
	p Value	**	NS	NS	NS
10	0	300.24 ± 16.60^{b}	62.01 ± 2.99	5.02 ± 0.46^{b}	0.82 ± 0.02
	21	507.65 ± 20.49^{a}	60.55 ± 6.04	8.89 ± 0.65^{a}	0.77 ± 0.02
	p Value	**	NS	**	NS
15	0	166.53 ± 55.20^{b}	60.69 ± 0.00	4.42 ± 0.39	0.81 ± 0.02
	21	531.31 ± 65.41^{a}	51.80 ± 3.86	6.19 ± 0.64	0.76 ± 0.01
	p Value	**	NS	NS	NS

Values are mean (*n*=9) and differences between means were compared by Fisher's LSD test (*p*=0.05). Significance levels are represented by p>0.05; NS, not significant. Means followed by the same letter do not differ significantly; DW, dry weight. ***p*<0.01.

With respect to the concentration of anthocyanins, no significant differences were found for any K treatment between T0 and T21 (Table 2).

The quantity of Vitamin C in the fruits treated with the rate of 5 and 10mM KCl showed significant differences, both rates raising the levels of this compound at T21 (Figure 4), with the highest levels of this compound being reached in the treatment of 10mM KCl (p<0.001, Figure 4). By contrast, the treatment of 15 mM KCl presented no differences between T0 and T21 (Figure 4).

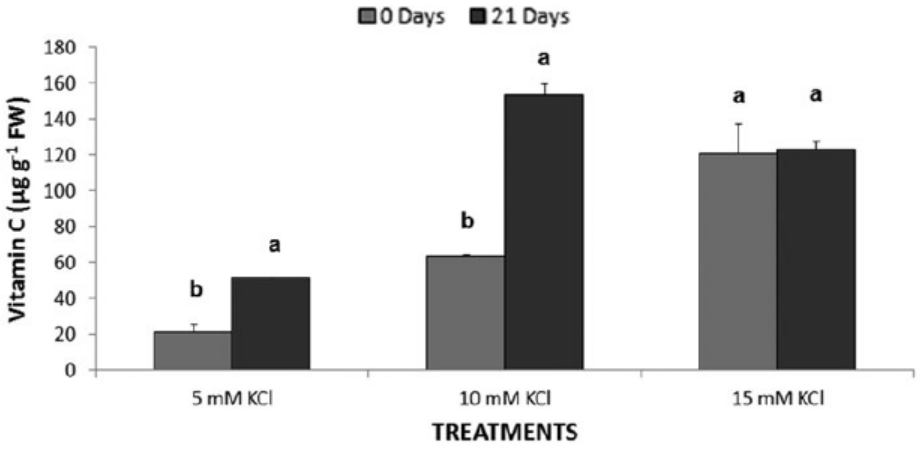

Figure 4. Effect of KCl treatments at the day of harvest and after 21 days of postharvest in cold storage at 4ºC over Vitamin C concentration in cherry tomato fruits. Values are means (n=9) and differences between means were compared by Fisher's LSD test (p=0.05). Means followed by the same letter do not differ significantly; FW, fresh weight.

For the hydroxycinnamic acids and derivatives, no significant differences were found for any K treatment between T0 and T21 (Table 3). With respect to the content in flavonoids and derivatives, the treatment of 5 mM KCl showed significant differences, with a decline in these types of phenols at T21 (Table 3). Contrarily,

the application of 15 mM KCl augmented these phenols at T21 with respect to T0 (Table 3). Finally, with respect to the other phenols and the total phenolic content, no differences were found between T0 and T21 for any of the K treatments (Table 3).

Table 3. Effect of KCl treatments at the day of harvest and after 21 days of postharvest in cold storage at 4ºC over: content of phenolic compounds in cherry tomato fruits.

KCl (mM)	Days	Hydroxycinnamic acids and derivatives (mg g^{-1} DW)	Flavonoids and derivatives (mg g^{-1} DW)	Other phenols (mg g^{-1} DW)	Total phenols (mg g^{-1} DW)
5	0	2.30 ± 0.12	3.80 ± 0.19^{a}	2.73 ± 0.49	8.83 ± 0.79
	21	1.85 ± 0.21	2.76 ± 0.11^{b}	2.31 ± 0.62	6.92 ± 0.84
	p Value	NS	**	NS	NS
10	0	2.81 ± 0.24	3.68 ± 0.41	4.50 ± 0.79	10.99 ± 1.43
	21	2.50 ± 0.19	3.32 ± 0.12	4.18 ± 0.81	10.00 ± 0.88
	p Value	NS	NS	NS	NS
15	0	2.40 ± 0.10	3.15 ± 0.14^{b}	5.66 ± 1.19	11.21 ± 1.15
	21	2.30 ± 0.17	3.55 ± 0.52^{a}	4.28 ± 1.25	10.13 ± 1.92
	p Value	NS	*	NS	NS

Values are mean (*n*=9) and differences between means were compared by Fisher's LSD test (*p*=0.05). Significance levels are represented by *p*>0.05; NS, not significant. Means followed by the same letter do not differ significantly; DW, dry weight. *p<0.05, **p<0.01.

With respect to Put in all the K treatments, the concentration rose at T21 (5 mM KCl: p<0.01; 10 mM KCl: p<0.001; 15 mM KCl: p<0.001, Figure 5A). Also, Spd presented significant differences in the treatments of 5 and 10 mM KCl, in which cold storage raised the concentration of this polyamine (5 mM KCl: p<0.01; 10mM

KCl: $p<0.05$, Figure 5B). On the other hand, the treatment of 15 mM KCl did not give rise to significant differences between T0 and T21. In relation to Spm, the rate of 5 and 10 mM KCl led to a decline at T21 ($p<0.001$ and $p<0.01$, respectively, Figure 5C), while the application of 15 mM KCl caused no significant differences between T0 and T21 (Figure 5C). Finally, regarding total free PAs, the application of 5 mM KCl lowered values at T21 compared with T0 ($p<0.001$, Figure 5D), whereas the application of 15mM KCl raised the concentration of total free PAs at T21 with respect to T0 ($p<0.05$, Figure 5D). The treatment of 10 mM KCl showed no significant differences between T0 and T21 (Figure 5D).

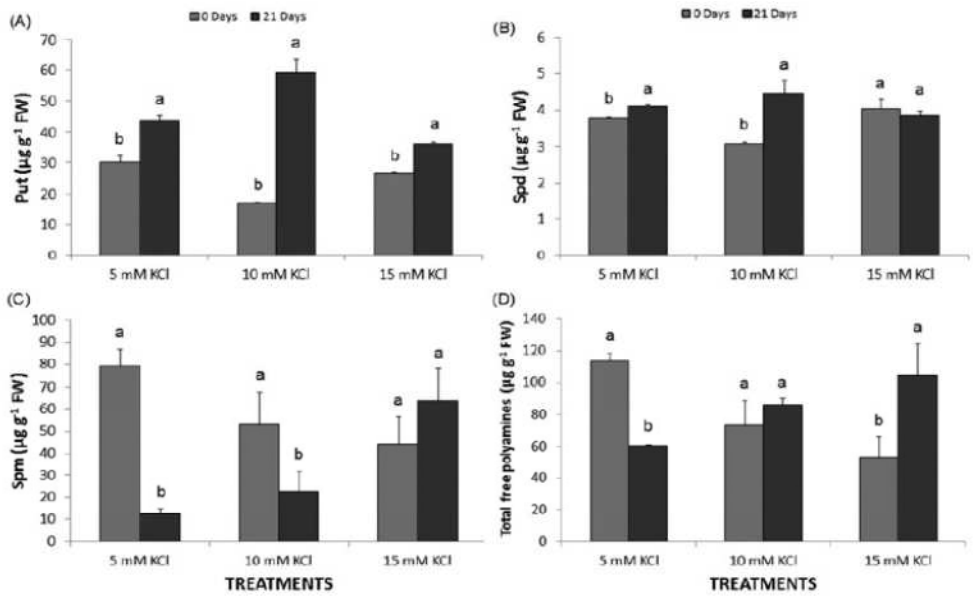

Figure 5. Effect of KCl treatments at the day of harvest and after 21 days of postharvest in cold storage at 4ºC over PAs concentration in cherry tomato fruits. Values are means (n=9) and differences between means were compared by Fisher's LSD test (p=0.05). Means followed by the same letter do not differ significantly.

Discussion

Fresh weight, percentage of lost fresh weight and K concentration

The postharvest water loss from fresh products is a major problem because it implies the weight loss, most products becoming unsellable as fresh products after losing 3–10% of their weight (Ben-Yehoshua & Rodov, 2003). In this experiment, although the harvested cherry tomato fruits treated with the rate 15 mM KCl presented a lower FW (Figure 1), yield was not compromised, as these plants had a higher number of fruits (data not shown). Furthermore, in the present work, the fruits from the plants treated with 15 mM KCl at T21 presented a lower percentage of lost fresh weight (% LFW) (6%) while the treatments of 5 and 10mM KCl showed an LFW of 17% and 18%, respectively (Figure 2), with the treatment of 15mM KCl most improving the postharvest response. These results suggest that the application of the highest K rate in KCl form prevents weight and water loss during postharvest storage. In this context, Almeselmani et al. (2010) observed that an extra provision of K in the fertilizer applied to tomato plants can help to preserve fruits during postharvest storage.

Finally, with respect to the K concentrations at harvest, in the present experiment a proportional response was observed in relation to the KCl rate applied, and the highest concentration was registered in the fruits from plants grown with the rate of 15 mM KCl (Figure 3). These results demonstrate the validity of the biofortification programme with K in tomato plants, since the consumption of fruits treated with

15 mM KCl provide added intake of this macronutrient, this being a potential benefit to human health, as demonstrated elsewhere (He & MacGregor, 2008).

Antioxidant capacity assays

For the determination of the antioxidant capacity in the most precise way possible, the use of several quantification methods is recommended. In our work, we used the methods TEAC, FRAP and reducing power to quantify the antioxidant activity. It was found that after 21 days of postharvest cold storage at 4ºC, for both the TEAC and FRAP tests, the treatment of 15 mM KCl caused increases of 60% and 32%, respectively (Table 1). However, when the treatment of 5 mM KCl was applied, the trend for the TEAC and FRAP tests was the opposite to the previous rate, with values falling in both tests at T21 with respect to T0 (Table 1). Also, it bears emphasizing that the reducing-power test presented the highest value in the treatment of 15 mM KCl at T21 (Table 1). All these results could indicate a benefit of applying the rate of 15 mM KCl regarding the antioxidant capacity of cherry tomato fruits during the period of cold-storage stress. Similar results were reported by Javanmardi & Kubota (2006), who found that tomato fruits in cold storage showed significantly increased antioxidant activity, which they related to phenolic compounds.

Concentration of pigments in cherry tomato fruits

A large group of phytonutrients are found in fruits and vegetables of the Mediterranean diet, among these the tomato, are carotenoids, including Lyc and β-carotene. Lyc represents roughly 80% of all carotenoids and has a high capacity to eliminate ROS, being one of the most characteristic phytonutrients in tomato fruit (Rao et al., 1998). Many studies have demonstrated a strong relation between the nutritional quality of tomato and its Lyc content (Rosales et al., 2006). Tomatoes contain moderate amounts of β-carotene, a potent dietary precursor of Vitamin A (Nguyen & Schwartz, 1999). It has been demonstrated that the highest amount of Lyc and b-carotene in the tomato are strong contributors to the major antioxidants of tomatoes (Toor et al., 2006). Finally, Lut is a compound belonging to the group of carotenoids with a high antioxidant capacity (Jahns & Holzwarth, 2012).

Anthocyanins are the most important group of water-soluble pigments in plants. Their biological interest stems from their antioxidant function and their effects reinforce certain compounds such as ascorbic acid (García-Alonso, 2004).

In our work, the majority of the pigments studied (β-carotene, Lut and anthocyanins) registered no significant differences between treatments (Table 2). Similar results have been reported by Rivera-Pastrana et al. (2010) in papaya fruits in which postharvest cold storage did not negatively influence the β-carotene concentration. Meanwhile, Rugkong et al. (2011), in a work on gene expression related to tomato fruit ripening in cold storage, did not find a decrease in Lut, either. In terms of Lyc, the ripening processes that are associated with the increase in

their content were found to be retarded by low temperatures (Gómez et al., 2009) and, in this sense, Javanmardi & Kubota (2006), studying tomato fruits, found that the Lyc content in tomatoes stored at 12ºC and 5ºC decreased in comparison with those stored at room temperature. Rivera-Pastrana et al. (2010) also found a decline in Lyc after postharvest storage. On the contrary, our work indicates an increase in the Lyc content for all the treatments after 21 days of cold storage, with a notable increase of 219% in the treatment of 15 mM KCl (Table 2). Given the fundamental importance of Lyc in the nutritional quality of the tomato, the application of 15 mM KCl could be beneficial to increase this pigment in cold storage, as K boosts the synthesis of Lyc, as demonstrated by Ramírez et al. (2012) in recently harvested tomato fruits.

Concentration of Vitamin C in cherry tomato fruits

Antioxidation mechanisms and the protection of metabolites in plants include a number of non-enzymatic antioxidants such as Vitamin C, and one of the main functions is to interrupt the uncontrolled oxidation cascades in some organelles and eliminate ROS. Antioxidant compounds are the essential determinants of nutritional quality in tomato fruits. Among the phytochemicals present in tomato fruits, the Vitamin C content is considered a key factor to determine the commercial value of the tomato yield, thanks to the nutritional benefit associated with its consumption (Frusciante et al., 2007). The antioxidant levels of a plant also constitute a good indicator of the redox state, which is indispensable for stress

tolerance. In the present study, after the application of the biofortification programme with K in the form of KCl, the Vitamin C (Figure 4) presented the highest concentration for the treatments of 10 and 15 mM KCl at T21, this latter treatment being the one that best maintained the Vitamin C concentration. Lester et al. (2010), studying melon fruits (*Cucumis melo* L), found that the fruits treated with the different K forms presented generally higher Vitamin C contents than did control fruits. The beneficial effects of the K supplement to the plant were presumably the result of a combination of improved photosynthetic assimilation of CO_2 by the leaves, greater translocation of assimilates from the leaves to the fruits, better leaf-fruit water relations, as well as more vigorous enzymatic activity and better availability of substrate for Vitamin C biosynthesis (Gross, 1991). Thus, an adequate biofortification programme with K has been associated with increased Vitamin C (Panda & Upadhyay, 2003), as confirmed by our results both at harvest and afterwards.

Vitamin C might also be involved in antioxidant capacity. Numerous studies in fruits and vegetables have demonstrated by different means a directly proportional relation between antioxidant capacity and the total phenol content and Vitamin C (Wang et al., 2012).

Concentration of phenols in cherry tomato fruits

Phenolics, ubiquitous secondary metabolites in plants, include a large group of biologically active components, from simple phenol molecules to polymeric

structures with a molecular mass above 30 kDa (Dreosti, 2000). As demonstrated by Wang et al. (2012) in avocado fruits harvested on different dates of the year and kept in cold storage, the storage had positive effects on the accumulation and retention of compounds of nutritional interest, such as phenolics. In our study, although no significant differences were detected either at T0 or at T21 in the content of hydroxycinnamic acids and derivatives or of flavonoids and derivatives for any of the treatments applied (Table 3), the treatment of 5 mM KCl did register a 27% decrease in the content of flavonoids and derivatives during the postharvest period (Table 3). It bears highlighting that the treatment of 15 mM KCl best maintained the concentration of hydroxycinnamic acids and derivatives between T0 and T21, and for the flavonoids and derivatives it was the only treatment that led to an increase of 13% between T0 and T21 (Table 3). These results may be related to the high antioxidant capacity presented by the fruits of the plants grown at this treatment rate, as reflected by Wang et al. (2012) in avocado fruits, and as has been found in numerous fruits and vegetables

Concentration of free PAs in cherry tomato fruits

Other antioxidant compounds that in addition to participating in the responses or adaptation of different adverse environmental conditions, including cold stress (Alcázar et al., 2010), may also influence the nutritional quality of tomato fruits are PAs. However, the physiological significance of these compounds remains unclear, and it needs to be evaluated whether elevated polyamine levels were a result of

stress-induced injury or a protective response to abiotic stress. Low-temperature conditioning has been shown to raise polyamine levels and stimulate S-adenosylmethionine decarboxylase activity (Wang, 1994). PAs may be associated with anionic components of the membrane such as phospholipids (Ballas et al., 1993) and this interaction serves to stabilize the bilayer surface and may thus retard membrane deterioration. PAs also have freeradical- scavenging properties (Drolet et al., 1986). Membrane protection from peroxidation by PAs could involve both their ability to interact with phospholipids and their antioxidant activity. Given the relationship between PAs and membrane protection, and between chilling injury (CI) and membrane damage, the possible connection between PAs and CI is of great interest. Zhang et al. (2013) have found an increase in the Put in tomato fruits treated with arginine and submitted to cold stress. These authors contend that in view of the protective function of PAs, especially Put against CI in many horticultural crops, it cannot be ruled out that the tolerance of fruits to refrigeration induced by the arginine treatment could be related to the increase in the Put concentrations found in those fruits. Similarly, accumulation of Put was also detected in chillinginjured peach and tomato fruit (Xu et al., 2005; Zhang et al., 2011). In our work, we noted an increase in Put between T0 and T21 for all the KCl treatments applied. However, the 15 mM KCl treatment presented the least increase at T21, of only 39% (Figure 5A), suggesting that the K applied in the form of KCl at this rate could boost the protection against cold stress of tomato fruits, reflecting a lower increase in Put. This hypothesis is confirmed by the data of Spd and Smp, PAs that are also important in the response to abiotic stress, such as that caused by CI (Alcázar et al., 2010). Specifically, in Spd, we found an increase

in concentration at T21 only at the rate of 5 and 10 mM of KCl (Figure 5B), which were the treatments that most affected the cold stress (Figure 2). With respect to Spm, on the contrary, the treatments of 5 and 10 mM of KCl (Figure 5C) diminished its concentration at T21, while 15 mM of KCl showed no changes between T0 and T21 (Figure 5C), suggesting a strong protective role of this PA in our work. Similar results were reported by Zhang et al. (2013) for Spd and Spm in tomato fruits after cold storage (2 ± 1ºC) for 28 days. These authors concluded that of the PAs studied, Put was predominant, followed by Spd and Spm. In this sense, they found that the Put concentration in fruits increased in response to cold stress, while by contrast the Spd and Spm concentrations fluctuated during the storage period. Finally, with respect to total free PAs (Figure 5D), significant differences were found for the treatment of 5 mM KCl, which presented a decline in these compounds of 46%, while in the treatment of 15 mM KCl, values rose 96.51%. Meanwhile, the treatment of 10 mM KCl showed no significant differences. In short, the greatest accumulation of free PAs in cherry tomato fruits treated with 15 mM of KCl after 21 days of postharvest cold storage could have a protective role against these stress conditions together with K (Figure 2).

Conclusions

In our work, we demonstrate how the application of a biofortification programme of K in the form of KCl at high application rates (15 mM) could constitute a beneficial strategy for improving the quality and antioxidant capacity of cherry tomato fruits to be stored cold before consumption. The treatment of 15 mM of KCl furthermore prevents weight and water loss in tomato cherry fruits during postharvest storage at 4ºC, raises the K concentration and the antioxidant capacity by increasing the Lyc concentration, maintains the contents in Vitamin C, hydroxycinnamic acid and derivatives, and increases the flavonoids and derivates, signifying that the consumption of these fruits could offer benefits for human health.

Declaration of interest

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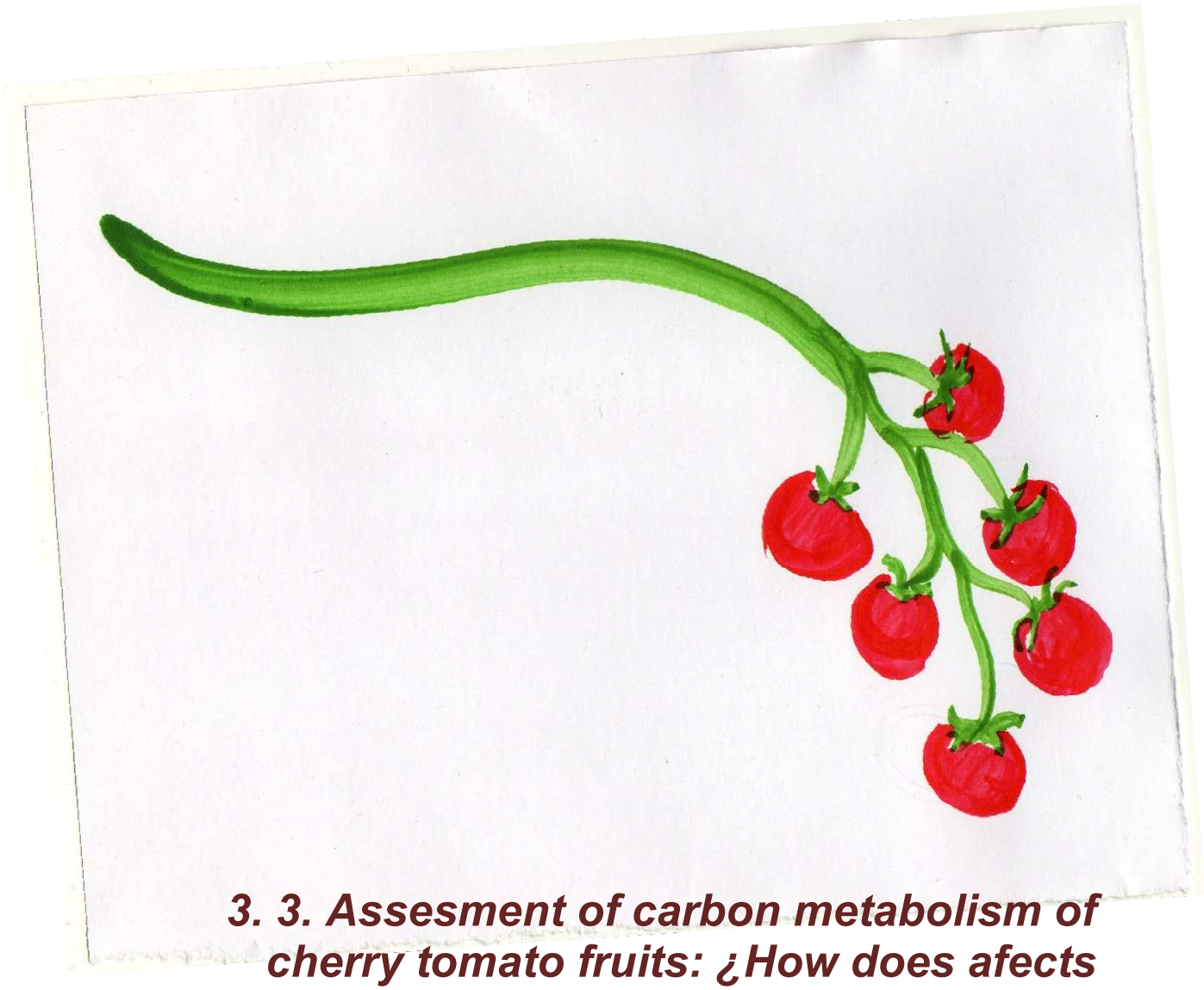

3. 3. Assesment of carbon metabolism of cherry tomato fruits: ¿How does afects potassium biofortification during crop cycle at postharvest storage?

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ABSTRACT

Tomato fruits are sensitive to storage at low temperatures after harvest. Under these conditions, could be induced in fruits a quality loss. In the present work, we evaluate the implementation of a K biofortification program increasing the dose of this nutrient in the nutrient solution for growing tomatoes in order to study whether this improves the response of fruits to postharvest for 21 days at 4°C. Three K treatments were applied during the crop cycle of the plants: 5, 10, and 15 mM of KCl. For this study the enzymes involved in carbon metabolism and sugar concentration both on the day of harvest and 21 days storage in cold room at 4°C. Similarly, enzymes related to the metabolism of organic acids as well as their concentration were studied. The application of this supplement besides increasing K concentration in the fruits of this nutrient, stimulate the Suc degradation by SuSy activity being increased Fruc and Gluc, and raising malate accumulation induced by the activity of PEPC and MDH enzymes during storage of 21 days at 4°C. The KCl treatments could be related to changes in carbon metabolism and suggest a protective role against cold storage, improving the quality of tomato fruits.

Keywords

Tomato fruits, Sugars, Carbon metabolism, Tricarboxilic acid cycle, Postharvest storage, Quality.

Abbreviations

ROS, reactive oxygen species; TCA, tricarboxylic acid cycle; CI, chilling injury; SuSy, sucrose synthase; MDH, malate dehydrogenase; CS, citrate synthase; PEPC, hosphoenol pyruvate carboxilase.

1. Introduction

Tomato (*Solanum lycopersicum* L.) is an annual horticultural plant with a broad worldwide distribution and enormous economic value, while its high content in antioxidant compounds offers a number of health benefits to the consumer (Bilton, Gerber, Grolier, & Leoni, 2001). Tomatoes benefit human health by their high contents in phytonutrients such as lycopene, *β*-carotene, phenolic compounds, ascorbic acid, and essential nutrients, which can detoxify reactive oxygen species (ROS) and prevent oxidative changes in the human body (García-Closas et al., 2004). For all this, the content of these compounds is critical for assessing the quality of the fruit.

Other characteristics that determine tomato quality are related to the organoleptic properties, defined by a number of physico-chemical parameters that make the fruit satisfactory to the consumer. Numerous works have revealed that organoleptic parameters such as flavour are key components for selling the tomato (Gough, & Hobson, 1990; Causse, Saliba-Colombani, Lecomte, Duffé, Rousselle, & Buret, 2002). The most important parameter for organoleptic quality of tomato fruits is flavour, produced mainly by a combination of sugars and organic acids, which implies that the level of concentration of both compounds can significantly affect consumer acceptance (Salles, Nicklaus, & Septier, 2003). Sugars and organic acids are the main metabolites in tomato fruits and constitute over 60% of the dry matter (Davies, & Hobson, 1981). They not only contribute to the soluble solids but are also essential to the flavour intensity. Balanced and high levels of sugars and organic acids are important for the perception of a high-quality fruit (Bucheliet al.,

1999; Lobit, Génard, Wu, Soing, & Habib, 2003). Nevertheless, the choice of the tomato cultivar, the cultivation technique, and the postharvest tasks are applied primarily to reducing the loss of crop yield and do not give priority to the effect that these practices have on organoleptic characteristics.

Tomato, a very perishable climacteric fruit, requires the use of preservation technologies to slow the ripening process that occurs after harvest in order to maintain its quality and extend its shelf life. Tomato-fruit sugar content is the result of fruit physiological, metabolic, and genetic processes that are under developmental control (Baldet et al., 2006; Mounet et al., 2009; Wang et al., 2009). Generally, the tomato fruit accumulates sugars in the form of sucrose or reducing sugars (glucose and fructose), depending on the environmental conditions and on the growth phase of the plant (Gomez et al., 2009). Although sucrose is the main form of sugar translocated in tomato plants, glucose and fructose are generally present in quantities equal to or greater than sucrose in tomato fruits. Two pathways for sucrose breakdown provide the basis for a flexible system that can markedly affect the partitioning and metabolism of assimilated carbon in sink tissues (Geiger, Koch, & Shieh, 1996). Sucrose synthase located in the cytosol catalyses a reversible reaction that degrades sucrose and UDP to UDP glucose and fructose, while acid (apoplast or vacuole) and neutral (cytosol or vacuole) invertase catalyse the hydrolysis of sucrose to glucose and fructose. These sucrolytic activities serve sink tissues in plants in the primary step of converting sucrose into sink-storage products, such as starch, proteins, and oil (Doehlert, &

Chourey, 1991; Balibrea, Santa-Cruz, Bolarín, & Pérez-Alfocea, 1996; Rosales, Rubio-Wilhelmi, Castellano, Castilla, Ruiz, & Romero, 2007).

Organic acid content is regarded as one of the most important quality traits of fresh tomato. The acidic taste in tomato is attributed mainly to citric acid and malic acid, which corresponds to over 90% of the organic acids in tomato (Davies, & Hobson, 1981; Schauer, Zamir, Fernie, 2005). However, the complexity of carboxylic acid metabolism and storage means that it is difficult to predict the best way to engineer altered carboxylic acid levels. Carboxylic acids constitute a major component of the osmotic potential that drives cell expansion through water uptake in the expansion phase of fruit growth (Liu, Génard, Guichard, & Bertin, 2007). The concentrations of citrate and other carboxylic acids fall during this expansion phase as the cell contents are diluted (Baxter, Sabar, Quick, & Sweetlove, 2005; Carrari et al., 2006). However, during the final stages of ripening, the level of citrate (and to a lesser extent other carboxylic acids) increases again such that it is present at high abundance in the ripe fruit. The complexity of this cycle is reflected in the range of enzymes that have been proposed to control fruit citrate and malate accumulation, including phosphoenolpyruvate carboxylase (Guillet et al., 2002), citrate synthase (Sadka et al., 2000), and malate dehydrogenase. The activity of these enzymes determines the synthesis and accumulation of these organic acids. The maximal catalytic activities of enzymes of the tricarboxylic acid cycle (TCA) generally decline during fruit development, and there are no pronounced changes in activities during the later stages of ripening that correlate with the rise in organic acid levels (Steinhauser et al., 2010).

Postharvest storage, handling and distribution of fruit at low temperatures is the most common and manageable approach to control ripening and subsequent deterioration and to maximize product shelf life. However, tomatoes, like many other subtropical fruits, are susceptible to develop symptoms of chilling injury, a physiological disorder caused by the exposure to low temperatures above the freezing point. The effect that postharvest cold has on the organoleptic properties (organic sugar-acid relation) in fruits has been investigated in the following works. González-Aguilar, Tiznado-Hernández, Savaleta-Gatica, and Martineze (2004), working with guava fruits and methyl jasmonate (MJ) treatments, stored red and white cultivars of guava fruits at 5ºC for up to 15 days plus two days at 20ºC and found that MJ treatments reduce the chilling injury (CI) index and increased sugar content. These authors concluded that MJ reduces chilling injury and activates the fruit-defence response. Later, Gómez et al. (2009), studying tomato fruits, showed that cold storage retarded the accumulation of simple sugars in fruits. In ripe red fruits, the fructose level increased both during storage at 20ºC and at 6ºC. With respect to glucose, its content increased at 20ºC, while it decreased significantly during the first 18 h of chilling treatment, recovering later to levels that were somewhat higher than initial values. In addition, these authors found that cold storage slowed the metabolism of organic acids in tomato fruits. Each organic acid showed a different pattern in normally ripening fruits. While tartaric, malic, ascorbic, and citric acid tended to show modest but significant decreases during ripening, the levels of succinic acid slowly built up. These trends seem not to have been affected by the chilling treatment, which slowed but did not halt the specific kinetics of most acids. Farneti, Zhang, Witkowska, & Woltering, (2010). after 5 days of 4ºC

storage, tomatoes generally showed depressed sugar and boosted the acid content (especially in cocktail tomatoes) compared to 15ºC stored fruit, indicating a loss of sensorial quality at 4ºC. Sánchez-Bel et al. (2012), in peppers, found that the ones submitted to cold stress presented higher concentrations of sucrose and fructose, while glucose was not appreciably affected. With regard to the organic acid concentration, citric acid declined in concentration while malic acid augmented. The comparative proteomic analysis between control and chilled fruits revealed that the main alterations induced by CI in bell pepper fruits are linked to redox homeostasis and carbohydrate metabolism. Finally, in this sense, Cao, Yang and Zheng (2013) studying loquat fruits stored in a cold chamber, examined the relationship between chilling injury and sugar metabolism. Chilling-resistant 'Ninghaibai' fruit had higher levels of glucose and fructose and higher activities of sucrose-hydrolysing enzymes, such as sucrose synthase-cleavage and invertase, than did 'Dahongpao' (sensitive). Furthermore, the chilling-resistant 'Ninghaibai' fruit also showed higher activities of hexokinase and fructokinase, involved in hexose phosphorylation and sugar signal generation. These results suggest that the higher content of hexoses and activities of hexose sensors were likely part of the mechanism for chilling tolerance of loquat fruit.

One of the factors that can *a priori* improve cold tolerance in fruit is the presence of high potassium (K) concentrations. In this sense, Beringer, and Trolldenier (1980) indicated that a high K concentration in cells can improve cold tolerance by lowering the osmotic potential. Later works have revealed a positive correlation between K availability and cold-stress tolerance, revealing, furthermore, that

suboptimal K concentrations intensify the negative effects of cold stress (Kafkafi, 1990; Yermiyahu, & Kafkafi, 1990). In addition, significant yield losses and extensive leaf damage due to cold temperatures reportedly occurred under low K fertilization, while the effects were alleviated once the K supply was increased in a number of vegetable crops such as potato (*Solanum tuberosum* L.) and tomato (*Lycopersicon esculentum* Mill.) (Hakerlerler, Oktay, Eryüce, & Yagmur, 1997). his could be due to the effect of K on sugar accumulation and the essential role that this physiological process plays in the resistance to chilling injury in plants. Javaria, Khan, and Bakhsh, (2012) in their investigations with different rates of K fertilizer in relation to chemical and sensory attributes of tomato, observed that total solids, sugars, and titratable acidity increased significantly with higher rates of K. It was concluded that increasing the K concentrations improved the quality of tomato fruit parameters. Similar results were found by Han, Jiang, Yu, and Wang (2012) on applying K at different growth phases in tomato plants. These authors found an increase in soluble sugar, while soluble sugar, organic acid, and soluble solids were the highest among all the treatments (increases of 44.7%, 28.8% and 7.1% as compared with control treatment, respectively). Potash applied during the fruit-growth phase reached the highest ratio of sugar to acid.

In view of the above-mentioned functions of K in fruit quality, the aim of the present study was to examine how the application programme of biofortification with different application rates of K in the form of KCl influences the C metabolism of cherry tomato (*Solanum lycopersicum* L. cv. AsHiari) fruits and therefore their organoleptic properties after storage for 21 days in a cold chamber at 4ºC. In

addition, whether the metabolism of sugars and organic acids is determinant in the resistance to chilling injury was evaluated.

2. Materials and methods

2.1. Plant material, growth conditions and Sampling ot tomato fruits

Seeds of cherry tomatoes (*Solanum lycopersicum* L. cv AsHiari grafted on cv. Maxifort rootstock) were sown in flat trays (cell size 3 cm x 3 cm x 10 cm, 100 cells per tray) filled with 50% [v/v] perlite-peat mixture, and kept under greenhouse conditions for 5 weeks. Subsequently, the seedlings were transplanted to an experimental greenhouse at La Nacla Experimental Station (Motril), near the Granada coast in southern Spain (36º 45'N; 3º 30'W; altitude 130 m). The *parral* greenhouse consisted of three modules having a symmetrical gable roof with a slope of 27º and having an E-W longitudinal orientation (Soriano et al 2004). The active environmental control was limited to a heating system by hot-air generators, and a natural ventilation system through wall and roof windows. In the greenhouse, the cladding material was a multilayer film 0.2 mm thick, with a layer of ethylene-vinyl-acetate between two layers (inner, antidrop; and outer, long life) of low-density polyethylene. The plants were grown in 40-L perlite B-12-filled sacks (1.20 m long) spaced 0.5 m apart in rows 1.4 m apart. With 3 tomato plants per sack and 2 stems per plant, the planting scheme was 3.21 plants m^{-2}. Their arrangement in the greenhouse was in 12 rows with North-South orientation. The statistical design was on randomized block. Other growing conditions such as irrigation and

fertilization followed (Soriano et al 2004). The different treatments applied were: 5 mM KCl, 10 mM KCl y 15 mM KCl as liquid solution from the beginning to the end of the experiment.

The cherry tomato crop cycle lasted from October 2010 to May 2011 (230 days), with a complete truss of tomatoes (10-12 tomatoes per truss) maturing every 10 days. Cherry tomato fruits were sampled in February of 2011 at 140 days after transplanting (DAT). Uniformly ripe healthy fruits, at the red-ripe stage, were harvested. Approximately 200 tomato fruits from each treatment were randomly collected (discarding the green fruits at the end of the truss) and were rinsed three times in distilled water after disinfection with 1% (v/v) Triton X-100 (Wolf, 1982), and then blotted on dry filter paper.

2.2. Biomass parameters

From 180 tomatoes harvested from each treatment, 90 tomatoes were intended for analysis at harvest day (T0) being clustered in 9 replicates of 10 fruits. 5 tomato fruits from each replicate were weighed obtaining fresh weight (FW) and then were dried in a lyophilizer to determine the dry weight (DW) and percentage of dry matter (% DM). Another 5 tomato fruits from each replicate were homogenized, and these samples of fresh tissues were stored at -80ºC. For analyzing the fruits after 21 days of storage in a cold room at 4°C (T21), in the same way, 90 tomatoes were intended for analysis being clustered in 9 replicates of 10 fruits. 5 tomato fruits from each replicate were weighed obtaining FW at (T0) and stored 21 days in a cold room at 4°C, after this period were reweighed (T21), and then were dried in

a lyophilizer to determine the DW and % of DM. Another 5 tomato fruits from each replicate were homogenized, and these samples of fresh tissues were stored at -80ºC (Fig. 1). Samples of fresh and dry tissues from the cherry tomato fruits were used to analyse the parameters described below.

For the determination of the percentage of lost of fresh weight (% LFW), the following formula was used for each treatment:

% LFW= (FW T0- FW T21)*100/FW T0

To calculate percentage of dry matter (% DM) was followed the method proposed by Garg, and Cheema (2011). Some tomatoes were weighed and then lyophilized for 72 h. After freeze drying, the samples are reweighed. The% DM was calculated as:

DM (%) = (B/A) x100

Where A is the total fresh weight of sample (g), and B, the total weight of dry sample (g).

2.3. Analytical methods

2.3.1. Determination of K concentration

For the determination of K concentration, 0.2 g of dry cherry tomato fruits were ground and mineralised by wet digestion with H_2SO_4 12 M and H_2O_2 at 30% and P free, at a temperature of 275–300 ºC. After this step were added 20 mL of deionised H_2O, and finally K concentration was analysed by flame photometry (Wolf, 1982).

2.3.2. Sugars metabolism

For extraction of sucrose synthase (EC 2.4.1.13), a quantity of 0.2 g of tomato fruits were homogenized in 1 ml of buffer Heppes-HCl 50 mM, pH 7.5, which contained: magnesium chloride 0.5 mM, sodium EDTA 1 mM, DTT 2.5 mM, Triton X-100 at 0.05%, based on the method of Cheikh, and Brenner (1992). The homogenate was filtered and centrifuged at 12000 g for 10 min. The determination of the SuSy activity followed the method of Kerr, Huber,. and Israel (1984). The reactions were started by the addition of an aliquot of 100 µL of the leaf extract previously centrifuged in a reaction buffer adapted from Cheikh, and Brenner (1992), composed of sodium Heppes 50 mM (pH 7.5) that contained: MgCl 15 mM, fructose-6-phosphate (F-6-P) 25 mM, UDP-G 25 mM, Potassium fluoride (FK) 10 mM. The absorbance was measured at 520 nm against a standard sucrose curve.

Acid (EC 3.2.1.25) and neutral (EC 3.2.1.26) invertases were extracted in KH_2PO_4–K_2HPO_4 buffer (0.2 M, pH 7.0) and (20 mM) 2-mercaptoethanol, as described by Hubbard, Huber, and Pharr (1989). The extracts were assayed for acid invertase by addition of 30 µL enzyme preparation to test tubes containing 600 µL sodium acetate buffer (0.1 M, pH 4.5) and 200 µL sucrose (0.75 M) equilibrated in a 37ºC water bath. The reaction was allowed to proceed for 30 min and was stopped by addition of 1 mL dinitrosalicylic acid reagent. After that, being incubated for 5 min at 100 ° C and allowed to cool to room temperature. Finally was added 1 mL of Rochelle salt at 40%. The absorbance was measured at 575 nm against a standard glucose curve. Neutral invertase activity was determined as described

above, except that Na-acetate buffer (0.1 M, pH 4.5) was substituted for K_2HPO_4-citrate buffer (0.1 m, pH 7.0) (Hubbard, Huber, & Pharr, 1989).

2.3.3. Organic acids metabolism

Extracts for measuring enzyme activities tomato fruit (0.2 g) was homogenized with 1 ml of extraction buffer containing 30 mM sorbitol, 1% bovine serum albumin (BSA) and 1% polyvinylpyrrolidone (PVP) in 100 mM N-2-hydroxyethylpiperazine-N%-2-ethanesulphonic acid (HEPES)-KOH, pH 8.0. The slurry was centrifuged for 15 min at 10.000 g and 4°C, and the supernatant was collected and analysed immediately. The activities of all enzymes were analysed in 1 ml (final volume) of the media indicated below. Malate dehydrogenase (MDH; EC 1.1.1.37) activity was determined with oxalacetate as substrate (Dannel, Pfeffer, & Marschner, 1995). by measuring the decrease in absorbance at 340 nm due to the enzymatic oxidation of NADH. The reaction was carried out with 70 µL of extract in 0.1 mM NADH, 0.4 mM oxalacetate and 46.5 mM Tris-HCl, pH 9.5. Citrate synthase (CS; EC 4.1.3.7) was assayed spectrophotometrically according to Srere (1967) by monitoring the reduction of acetyl coenzyme A (CoA) to CoA with 5-5%-dithio-bis-2-nitrobenzoic acid (DTNB) at 412 nm. The reaction was carried out with 50 ml of extract in 0.1 mM DTNB, 0.36 mM acetyl CoA, 0.5 mM oxalacetate and 100 mM Tris- HCl, pH 8.1. Phosphoenol pyruvate carboxilase (PEPC; EC 4.1.1.31) activity was measured in a coupled enzymatic assay with MDH according to Vance et al. (1983) with 70 µL of extract in 2 m*M* phosphoenol pyruvate (PEP), 10 mM $NaHCO_3$, 5 mM $MgCl_2$, 0.16 mM NADH and 100 mM N,N-bis[2-hydroxyethyl]glycine (Bicine)-HCl, pH 8.5.

2.3.4. Sugars and organic acids content

Hexose (glucose and fructose) and sucrose contents were extracted and quantified using a kit (Roche Biopharm, St Didier au Mont d'Or, France) based on enzyme-linked formation of nicotinamide adenine dinucleotide phosphate (NADPH).

The determination of organic acids was based on the method of Scherer, Rybka, Ballus, Meinhart, Filho, and Godoy (2012) with slight modifications and was performed using HPLC with a DAD UV-visible detector (Agilent Technologies, USA) under the following conditions: Phenomenex reverse-phase column, 250×4.6mm i.d., 5 µm, Li-Chrospher 100 RP-18, with a 4×4mm i.d. guard column of the same material (Luna, Phenomenex, Utrecht, Belgium). About 0.2 g of freeze-dried tomato samples were homogenized with H_2O milliQ. The resulting mixture was centrifuged for 400 g 2 min and then filtered through a 0.45 µm membrane filter, and triplicates of 10 ml for each sample were analysed by HPLC-DAD. HPLC analysis of organic acids was carried out using the same equipment as described above. Samples were injected into an ACE 5C18 column, 250 x 4.6mm (HICHROM) operating at 25ºC. A single mobile phase consisting of 0.01M of KH2PO4 (pH 2.6) at 0.5 ml/min was used. The elution was monitored at 210 nm. Malic and citric acid was used as a standard (SIGMA-ALDRICH), eluting at 7.27 min and 10.57 min respectively.

2.3.5. Sweetness index

The sweetness index of fruits, an estimate of total sweetness perception, was calculated, based on the amount and sweetness properties of individual

carbohydrates (Keutgen, & Pawelzik, 2008). The contribution of each carbohydrate was calculated, based on the fact that fructose is 2.30 and sucrose 1.35 times sweeter than glucose and, hence, the sweetness index was calculated as (1.00 [glucose])+(2.30 [fructose])+(1.35 [sucrose]).

2.4. Statistical analysis

Data were analysed using one-way analysis of variance (ANOVA) to determine significance and Fisher's protected least significant difference (LSD) test to separate means. Standard errors of the means were also calculated. The significance levels were expressed as * $P<0.05$, ** $P<0.01$, *** $P<0.001$, and ns (not significant) $P>0.05$.

3. Results and discussion

3.1. Biomass parameters and K concentration

The postharvest water loss from fresh products is a major problem because it provokes the weight loss, most products becoming unsellable as fresh products after losing 3-10% of their weight (Ben-Yehoshua, & Rodov, 2003). In all the treatments, after 21 days of storage in a cold chamber, the FW decreased (Fig. 1), although with the treatment 15 mM KCl the loss was less than in the rest of the treatments, with only 6% with respect to T0 ($P<0.001$, Fig. 1). For the treatments 5 and 10 mM KCL the loss of FW at T21 was 17 and 18%, respectively, in relation to T0. In the present work, the fruits harvested from the plants treated with 15 mM

KCl at T21 presented a lower percentage of LFW (6%) (Fig. 2) while the treatments 5 and 10 mM KCl showed a LFW percentage of 17% and 18%, respectively (Fig. 2), the treatment 15 mM KCl improving the postharvest response (P<0.001, Figure 2). These results suggest that the application of the highest K rate in KCl form prevents weight and water loss during postharvest storage. In this context, Almeselmani, Pant, and Bhupinder Singh (2010), observed that an extra provision of K in the fertilization of tomato plants can help to preserve fruits during storage.

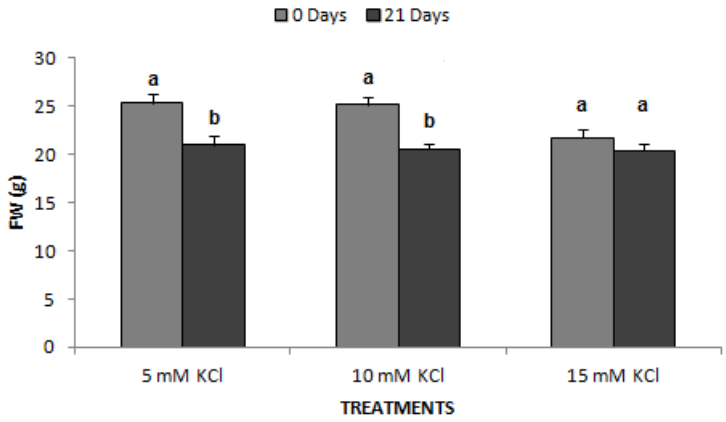

FIGURE 1. Effect of KCl treatments at the day of harvest over FW in cherry tomato fruits.Values are means (n=9) and differences between means were compared by Fisher´s least-significant difference test of (LSD, p=0.05). Means followed by the same letter do not differ significantly.

Finally, with respect to the K concentrations at harvest, in the present experiment, a proportional response was observed in relation to the KCl rate applied, and the highest concentration was registered in the fruits from plants grown with the rate of 15mM KCl (p<0.001, Fig. 3). These results demonstrate the validity of the biofortification programme with K in tomato plants, since the consumption of fruits treated with 15mM KCl provide added intake of this macronutrient, this being a potential benefit to human health (He & MacGregor, 2008).

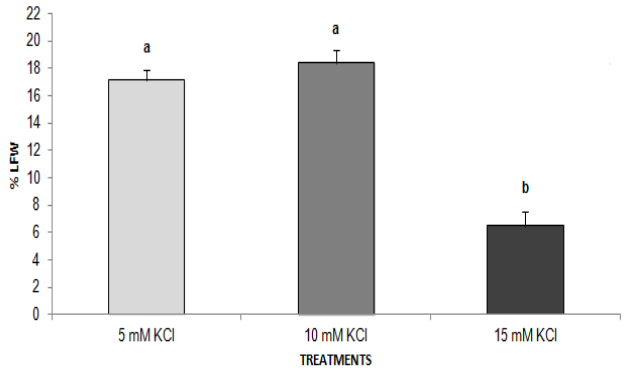

FIGURE 2. Effect of KCl treatments at the day of harvest over % LFW in cherry tomato fruits. Values are means (n=9) and differences between means were compared by Fisher´s least-significant difference test of (LSD, p=0.05). Means followed by the same letter do not differ significantly.

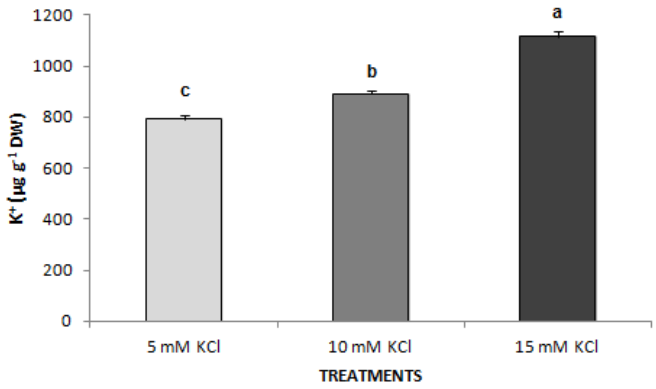

FIGURE 3. Effect of KCl treatments at the day of harvest over concentration of K cherry tomato fruits. Values are means (*n*=9) and differences between means were compared by Fisher´s least-significant difference test of (LSD, *p*=0.05). Means followed by the same letter do not differ significantly.

3.2. Sugar metabolism

Tomato fruit sugar content is the outcome of fruit physiological, metabolic, and genetic processes that are under developmental control (Baldet, et al., 2006; Ho, & Hewitt, 1986; Mounet, et al., 2009; Wang, et al., 2009). Sugar production begins with leaf photosynthesis, the product of which is translocated to developing fruits. Although Suc is the main form of sugar translocated in tomato plants, Glu and Fruc are present generally in higher quantities than sucrose in tomato fruits. Among the factors that can alter the concentration of these sugars in fruits, as mentioned in the Introduction, K availability and cold storage are notable. In our work, we found that the Suc concentration in cherry tomato fruits was not altered either by K treatments or by storage for 21 days at 4ºC (Table 1). On the contrary, the Gluc

and Fruc concentrations increased more significantly after 21 days of storage at 4ºC with the treatments 10 and 15 mM de KCl, with no appreciable variations with respect to the treatment 5 mM KCl (Table 1).

Table 1. Effect of KCl treatments at the day of harvest and after 21 days of postharvest in cold storage at 4°C over: sugars in cherry tomato fruits.

KCl (mM)	DAYS	SACAROSE (mg g FW^{-1})	GLUCOSE (mg g FW^{-1})	FRUCTOSE (mg g FW^{-1})
5	**0**	2.20±0.18	0.96±0.13	1.83±0.13
	21	1.97±0.13	0.86±0.11	1.53±0.18
	P-value	NS	NS	NS
	LSD	0.57	0.41	0.47
10	**0**	1.65±0.16	0.78±0.16b	1.91±0.19b
	21	2.03±0.29	1.38±0.17a	2.33±0.15a
	P-value	NS	***	*
	LSD	0.71	0.49	0.20
15	**0**	1.75±0.19	0.69±0.11b	2.06±0.09b
	21	1.85±0.35	1.34±0.05a	2.33±0.05a
	P-value	NS	*	*
	LSD	0.89	0.26	0.12

Values are mean (*n*=9) and differences between means were compared by Fisher's least-significant difference test (LSD; *p*=0.05). Significance levels are represented by *p*>0.05, NS, not significant, **p* <0.05, ***p*<0.01, ****p*<0.001. Means followed by the same letter do not differ significantly.

Sucrose can be converted to hexose phosphate by the ATP-dependent invertase pathway or the sucrose synthase pathway (SuSy), depending on the pyrophosphate (PPi) (Plaxton, 1996). As reflected in Table 2, the neutral and acidic invertase activities diminished at T21 with the 5 mM KCl treatment (Table 2), while

for treatments 10 and 15 mM KCl no variations in T21 were found with respect to T0 (Table 2). With respect to the SuSy activity, its response was totally contrary to that of invertase, since its activity intensified only at T21 for the treatments 10 and 15 mM KCl (Table 2). In short, according to our results, we can conclude that in our work the degradation of Suc to Gluc and Fruc was due to the activity of SuSy, which was induced during postharvest at 4ºC by the high treatments of KCl (10 and 15 mM). One possible explanation for these results could be that the cold stress brings about ATP-depletion conditions (Atkin, Edwards, & Loveys, 2000), which could explain the absence of participation of invertase activity in our work. The increase in Gluc and Fruc for 21 days of postharvest storage at 4ºC in the treatments 10 and 15 mM KCl (Table 1) appears to confirm the possible role of these sugars in cold resistance in tomato fruits, since, for these treatments, and especially for treatment 15 mM KCl, the sugar concentration was directly correlated with the minimum reduction of fruit FW after 21 days of postharvest at 4ºC.

Finally, it bears highlighting two aspects that could also contribute to improving the resistance to chilling injury for the treatment 15 mM KCl: (i) the greater K concentration in fruits submitted to this treatment (Fig. 3) could contribute, together with the accumulation of sugars (Table 1), to a fall in the cellular osmotic potential and thereby to greater cold-stress resistance; and (ii) the greater Gluc accumulation in these fruits could act as a substrate for the synthesis of ascorbate, one of the early precursors in ascorbate biosynthesis (Cervilla, Blasco, Ríos, Romero, & Ruiz, 2007), and thereby improve the antioxidant response to this type

of stress, as shown in previous works (Constán-Aguilar, Leyva, Blasco, Sánchez-Rodríguez, Soriano, & Ruiz, 2013).

Table 2. Effect of KCl treatments at the day of harvest and after 21 days of postharvest in cold storage at 4°C over: sucrose synthase, neutral and acid invertases in cherry tomato fruits.

KCl (mM)	DAYS	NEUTRAL INVERTASE (mg Gluc min^{-1} mg $prot^{-1}$)	ACID INVERTASE (mg Gluc min^{-1} mg $prot^{-1}$)	SuSy (mg Fruc min^{-1} mg $prot^{-1}$)
5	**0**	35.37±2.33a	79.20±5.89a	49.43±5.88
	21	25.73±1.10b	55.33±4.89b	42.43±4.75
	P-value	**	*	NS
	LSD	7.03	16.68	9.56
10	**0**	35.56±1.13	89.68±1.79	34.69±6.38
	21	36.69±1.49	91.30±2.24	43.83±9.13
	P-value	NS	NS	*
	LSD	3.96	6.07	7.97
15	**0**	39.99±0.58a	100.22±2.27a	52.11±7.68
	21	36.58±0.40b	85.75±1.52b	55.48±3.83
	P-value	NS	NS	*
	LSD	3.49	15.78	2.91

Values are mean (n=9) and differences between means were compared by Fisher's least-significant difference test (LSD; p=0.05). Significance levels are represented by p>0.05, NS, not significant, *p<0.05, **p< 0.01, ***p<0.001. Means followed by the same letter do not differ significantly.

3.3. Organic acid metabolism

The TCA cycle connects glycolysis to amino acid biosynthesis and is important in the regulation of respiration and energy generation by producing ATP and NADH. In fruit, the TCA cycle is involved in organic acid biosynthesis, but little information is available on the dynamics of tricarboxylic acid cycle (TCA)-related metabolism.

With respect to the organic acids malic acid and citric acid, Figure 4A ($p<0.05$) shows how with the treatment 5Mm KCL, 21 days postharvest at 4ºC, malate significantly diminished. On the contrary, the application of 10 and 15 mM of KCL increased this organic acid at F21 with respect to T0 (Fig. 4A). Citric acid, independently of the K treatment applied during storage for 21 days at 4ºC, prompted a significant decline in this organic acid (Fig 4B, $p<0.05$).

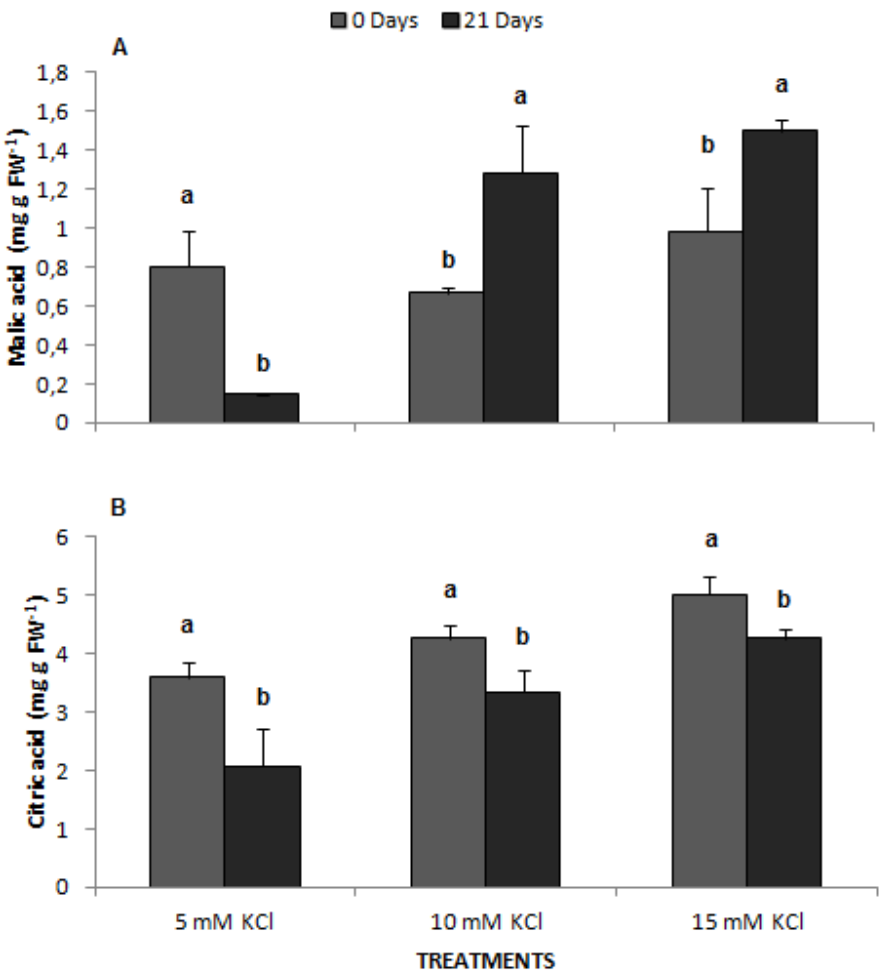

FIGURE 4. Effect of KCl treatments at the day of harvest and after 21 days of postharvest in cold storage at 4°C over: malic acid and citric acid in cherry tomato fruits. Values are means (*n*= 9) and differences between means were compared by Fisher´s least-significant difference test of (LSD, *p*=0.05). Means followed by the same letter do not differ significantly.

The response in our work of the different concentrations in cherry tomato fruit of malic and citric acid can be explained by the activities of PEPC, MDH, and CS.

Table 3 shows that, due to the PEPC activity, cold storage for 21 days resulted in a decline in the activity with the treatment 5 mM KCl (Table 3), which, together with the increased MDH activity (Table 3) at T21 indicated that the tricarboxylic acid cycle was functioning in the sense that MDH degrades malate to form oxaloacetate. This could have been accumulating, since the CS activity fell in this treatment (Table 3). In short, the behaviour of these enzymes would explain the decline in the treatment 5 mM de KCL of the malic and citric acid concentrations at T21 (Figs. 4A and 4B). However, with the treatments 10 and 15 mM of KCl, high PEPC and MDH activity at T21 (Table 3) would explain why malate increased, since MDH would be converted oxaloacetate generated from PEPC into malate. Therefore, in these treatments, the synthesis of this organic acid could be occurring by the anaplerotic pathway. The accumulation of malate in the treatments 10 and 15 Mm at T21 could also be explained by the behaviour of the CS activity in these treatments, since in none of them significantly stimulated this enzymatic activity at T21 (Table 3).

Table 3. Effect of KCl treatments at the day of harvest and after 21 days of postharvest in cold storage at 4°C over: PEPC, MDH, CS in cherry tomato fruits.

KCl (mM)	DAYS	PEPC (ΔAbs hour^{-1} mg prot^{-1})	MDH (ΔAbs hour^{-1} mg prot^{-1})	CS (ΔAbs hour^{-1} mg prot^{-1})
5	**0**	1.61±0.20a	1.07±0.20b	0.63±0.13a
	21	0.12±0.03b	3.07±0.18a	0.30±0.04b
	P-value	**	***	**
	LSD	0.71	0.74	0.15
10	**0**	1.21±0.08b	1.81±0.50b	0.95±0.04a
	21	2.97±0.67a	3.41±0.03a	0.73±0.00b
	P-value	**	*	**
	LSD	0.57	1.39	0.12
15	**0**	0.34±0.22b	4.26±0.62b	0.81±0.21
	21	1.06±0.04a	8.64±1.13a	1.12±0.33
	P-value	*	*	NS
	LSD	0.61	3.57	1.09

Values are mean (n=9) and differences between means were compared by Fisher's least-significant difference test (LSD; p=0.05). Significance levels are represented by p>0.05, NS, not significant, *p<0.05, **p<0.01, ***p<0.001. Means followed by the same letter do not differ significantly.

3.4. Sweetness index

The sweetness index is a parameter frequently used in many fruits to describe their flavour and commercial acceptability (Keutgen, & Pawelzik, 2008). In our work, with regard to the sugar:acid ratio, the postharvest cold storage for 21 days for the 5 mM KCl treatment diminished this index with respect to that of T0 (p<0.05, Fig. 5). On the contrary, the treatments 10 and 15 mM KCl, after the cold-storage period showed a rise in this index at T21 (p<0.05, Fig. 5), indicating that the

treatments K increased the sugar involved in the determination of this index after the postharvest, and therefore improved the likelihood of customer acceptance.

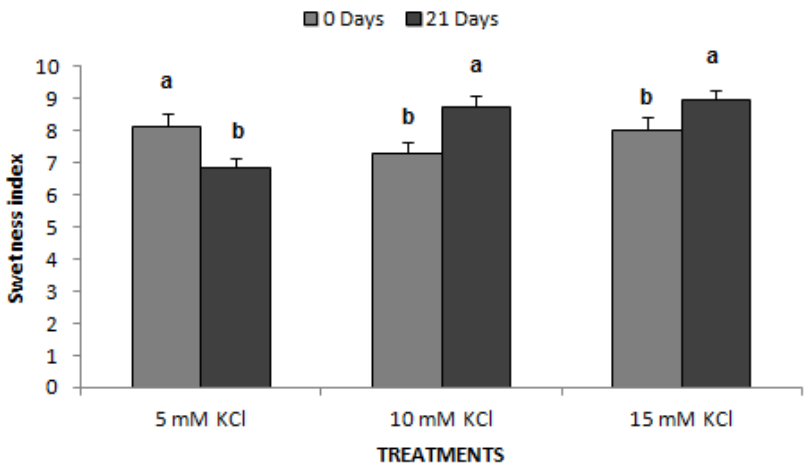

FIGURE 5. Effect of KCl treatments at the day of harvest and after 21 days of postharvest in cold storage at 4°C over: Sweetness index in cherry tomato fruits. Values are means (*n*=9) and differences between means were compared by Fisher´s least-significant difference test of (LSD, *p*=0.05). Means followed by the same letter do not differ significantly.

4. Conclusions

The application of a biofortification programme with K, increasing the application rates of this nutrient in the nutrient solution during tomato cultivation, improved the fruit response to postharvest storage at 4ºC for 21 days. This improved response

was found in our work at 10 mM and specifically with the rate of 15 mM of KCL, and this could be related to changes in the carbon metabolism. Thus, the application of high amounts of K, in addition to increasing the concentration of this nutrient in fruits, stimulated the Suc degradation by SuSy activity, raising the levels of Glu and Fruc and inducing the accumulation of malate by the activity of the enzymes PEPC and MDH during storage for 21 days at 4ºC. Therefore, in our work the accumulation of Glu, Fruc, and malate could explain the protective role, during cold storage, of the treatment 15 mM of KCl, showing the minimum reduction of the % LFW. Finally, it bears indicating that the implementation of biofortification programmes with high rates of K application (in our case 10 and 15 mM of KCL) clearly improves the organoleptic quality of cherry tomato fruits during postharvest at 4ºC, with an increase in the sweetness index. In addition, the consumption of these fruits provides added intake of this element as a potential benefit to human health.

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Yermiyahu, U., & Kafkafi, U. (1990). Yield increase and stem brittle decrease in response to increasing concentrations of potassium and NO_3^-/NH_4^- in White Carnation CV. Standard. *Hassadeh*, 90,742–746.

CAPÍTULO 4: CONCLUSIONES

1.- Los frutos de tomate recolectados en la semana 20 después del transplante presentaron una mayor concentración de K, en especial los que fueron cosechados de plantas tratadas con las dosis 15 y 20 mM KCl. Aunque el peso individual del fruto para los tratamientos 15 y 20 mM KCl resultó ser inferior al de los recolectados de plantas tratadas con dosis inferiores, el resto de los parámetros relativos a la producción comercial incluyendo la producción comercial acumulada no mostraron diferencias significativas, por lo que no se vio comprometida la producción comercial. Los parámetros relativos a la capacidad antioxidante se vieron mejorados en los frutos con estas dosis aplicadas durante el ciclo de cultivo, aunque no incrementaron significativamente sus cualidades organolépticas.

2.- El tratamiento de 15 mM de KCl, impide la pérdida de peso y agua en frutos de tomate cherry durante el almacenamiento en postcosecha a 4°C. Además, tras 21 días de almacenamiento a 4ºC, éste tratamiento mejora la capacidad antioxidante aumentando la concentración de Lyc, manteniendo el contenido en vitamina C, ácido hidroxicinámico y sus derivados, y favoreciendo el incremento de los flavonoides y derivados. El aumento de estos fitonutrientes junto con una mayor concentración de K supondría que el consumo de estos frutos podría ofrecer beneficios para la salud humana.

3.- Con la a dosis 15 mM KCl, los frutos de tomate presentaron un menor grado de peroxidación lipídica, posiblemente debido a una mayor actividad de APX y MDHAR , lo que sugiere una mayor eficacia en la detoxificación ROS así como en la regeneración de AsA. Además, bajo este tratamiento de K, los frutos presentan un mayor *"pool"* de AsA , así como una mayor concentración de GSH. Por lo tanto, la aplicación de la dosis 15 mM de KCl podría ser adecuada para mitigar los efectos negativos causados por el almacenamiento postcosecha a temperaturas bajas.

4.- La aplicación de un programa de biofortificación con K aportando un suplemento de este macronutriente estimuló la degradación de sacarosa por la actividad sacarosa sintasa (SuSy), incrementó los niveles de glucosa (Glu) y fructosa (Fru) y la inducción de la acumulación de malato por la actividad de las enzimas fosfoenol piruvato carboxilasa (PEPC) y malato deshidrogenasa (MDH) durante el almacenamiento durante 21 días a 4ºC. Por lo tanto, la aplicación de un programa de biofortificación con altas dosis de K (en nuestro caso 10 y 15 mM de KCL) mejora claramente la calidad organoléptica de los frutos de tomate cherry durante la postcosecha a 4ºC, con un aumento del índice de dulzor. El incremento de estos compuestos en el fruto podrían contribuir a la resistencia al estrés por frío.

ANEXO I
(CURRICULUM VITAE)

ANEXO I: CURRICULUM VITAE

Name: **Constán Aguilar Christian**

1.- Academic Qualifications

Title of qualification:

Licenciado en Biología (equivalent to BSc in Biology)

University and Centre where the degree was completed:

Faculty of Science. University of Granada, Spain

Date of graduation:

September 2010

(Visits to other centres were part of the course)

2.- Other Academic Qualifications

Máster en Biología Agraria y Acuicultura (Masters in Agricultural Biology and Aquaculture)

Date of graduation:

July 2011

3.- Participation in Research Projects

3.1. Title of Project / Work / Study
Biotechnological approach to improve the cultivation of tomato in water use efficiency and potassium in salinity situations: role of ion transporters. ***Junta de Andalucía (Convocatoria Boja nº 138, 18 de Julio de 2005. Proyectos de Investigación de Excelencia Ref. AGR 436)***
Organization for which the Project was carried out
EEZ-CSIC CVI124-Group, University of Granada AGR161-Group, CSIC-La Mayora AGR129-Group, University of Málaga 176-Group
Duration
From 2006 to 2008
Project / Work / Study Supervisor
Dr. Maria del Pilar Rodriguez Rosales

3.2. Title of Project / Work / Study
Analysis of the different strategies of resistance to boron toxicity in plants. ***Ministerio de Educación y Ciencia, Plan Nacional de I+D+i (Ref. AGL2006-03164/AGR)***
Organization for which the Project was carried out
Department of Plant Physiology (University of Granada) AGR161-Group
Duration
From 2007 to 2009
Project / Work / Study Supervisor
Dr. Juan Manuel Ruiz Sáez

3.3. Title of Project / Work / Study
Development of alternative crop protection: under plastic in the coastal summer below net for sustainable, profitable and quality Production. ***Ministerio de Educación y Ciencia, Plan Nacional de I+D+i, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA) (Ref. RTA2009-00005-00-00)***
Organization for which the Project was carried out
Instituto de Investigación y Formación Agraria y Pesquera (IFAPA); Department of Plant Physiology (University of Granada) AGR161-Group, *Caja Rural de Granada*
Duration
From 2009 to 2012
Project / Work / Study Supervisor
Dr. Maria Teresa Soriano Vallejo

3.4. Title of Project / Work / Study
Physiological and nutritional evaluation of the application of phosphite as a source of phosphorus in cucumber plants
Organization for which the Project was carried out
Department of Plant Physiology (University of Granada) AGR161-Group
Duration
From: December 2010 to December 2011
Project / Work / Study Supervisor
AGR-161 group

4.- Scholarships

4.1.- Organization which awarded the scholarship:

Granada University

Purpose of the scholarship (thesis, research, etc):

Student Collaboration in experiences of the pilot implementation of the European Credit System

Start and end date:

December 2009 – August 2010

Centre which awarded the scholarship:

Faculty of Science, Granada University

4.2.- Organization which awarded the scholarship:

Granada University

Purpose of the scholarship (thesis, research, etc):

Student Collaboration in experiences of the pilot implementation of the European Credit System

Start and end date:

January 2010 – July 2011

Centre which awarded the scholarship:

Faculty of Science, Granada University

4.3- Organization which awarded the scholarship:

. **Ministry of Education and Science**

Purpose of the scholarship (thesis, research, etc):

Master´s Degree in Agricultural Biology and Aquaculture (year published: 2006)

Grant Number: 821388

Start and End Date:

Academic Year 2010-2011

Centre which awarded the scholarship:

Faculty of Science, Granada University (CSIC. Estación Experimental del Zaidín, Granada)

5.- Participation in Seminars, Conferences, Courses and Scientific Outreach Events

Name of Event:

1.- Attendance of the course **"Evolución: El camino de la vida" ("Evolution: the road of life").** Held at the Faculty of Science of University of Granada (Spain). 19-28 November 2007. Duration: 40 hours.

2.- XII SIMPOSIO IBÉRICO SOBRE NUTRICIÓN MINERAL DE LAS PLANTAS
(12th IBERIAN SYMPOSIUM ON MINERAL NUTRITION OF PLANTS)

Venue and Year:

Granada (Spain) 2008

Entity / Organization Group:

Type of Participation:

Poster

Authors: Rosa Castellano, Jorge Álvarez del Toro, Rocío Leyva, Christian Constán, Juan M. Ruíz, Luís Romero.

Title: Efecto de la aplicación de diferentes quelatos sobre la eficiencia de la fitoextracción del niquel en plantas de *Brassica rapa* cv. Onekilo. (Effect of the application of different chelates on the efficiency of the phytoextraction of nickel in Brassica rapa cv. Onekilo).

3.- XII SIMPOSIO IBÉRICO SOBRE NUTRICIÓN MINERAL DE LAS PLANTAS
(12th IBERIAN SYMPOSIUM ON MINERAL NUTRITION OF PLANTS)

Venue and Year:

Granada (Spain) 2008

Entity / Organization Group:

Type of Participation:

Organizing committee

4.- Attendance of the course **"**Las fronteras de la Biología" ("Frontiers of Biology"). Held at the Faculty of Sciences, Granada University (Spain). 20 November to 3 December 2008. Duration: 40 hours.

5.- XIX REUNIÓN DE LA SOCIEDAD ESPAÑOLA DE FISIOLOGÍA VEGETAL (SEFV) – XII CONGRESO HISPANO-LUSO DE FISIOLOGÍA VEGETAL
(19th MEETING OF THE SPANISH SOCIETY OF PLANT PHYSIOLOGY – 12TH LUSO-HISPANIC CONGRESS OF PLANT PHYSIOLOGY), 21 TO 24 JUNE 2011

Venue and Year:

Castelló de la Plana (Spain), 21 to 24 June 2011

Entity / Organization Group:

SPANISH SOCIETY OF PLANT PHYSIOLOGY (SEFV).

Type of Participation

5. 1. Poster

Authors: Christian Constán-Aguilar, Rubén Melgarejo Fernández, Mª del Mar Wilhelmi-Rubio, Rocío Leyva, Begoña Blasco, Luis Romero.

Title: Evaluación de Parámetros Morfológicos en Plantas de Pepino Tras la Aplicación de Fosfitos Como Fuente de Fósforo (Evaluation of Morphological Parameters in Cucumber Plants following the Application of Phosphites as a Source of Phosphorous)

5. 2. Poster

Authors: Marcos Antonio Camacho, Christian Constán-Aguilar, Rocío Leyva, Rubén Melgarejo, Luis Romero.

Title: Estudio del Estrés Oxidativo en Plantas de Pepino Tras la Aplicación de Fosfitos Como Fuente de Fósforo (Study of Oxidative Stress in Cucumber Plants Following Application of Phosphites as a Source of Phosphorus)

5. 3. Poster

Authors: Begoña Blasco*, Rocío Leyva, Miguel A. Rosales, Eva Sánchez-Rodríguez, Rubén Melgarejo, Christian Constán-Aguilar y Luis Romero

Title: Efecto de la Salinidad y la Biofortificación con Yodo en el Contenido Fenólico de Plantas de Lechuga (*Lactuca sativa* L. cv. longifolia) (Effect of Salinity and Iodine Biofortification in the Phenolic Content of Lettuce Plants) (Lactuca sativa L. var. longifolia)

5. 4. Poster

Authors: Eva Sánchez-Rodríguez, María del Mar Rubio-Wilhelmi, Christian Constán-Aguilar, David Montesinos-Pereira, Marta Landete-Tormo, Juan Manuel Ruiz

Title: Procesos Fisiológicos Involucrados en una Mayor Tolerancia al Déficit Hídrico en Cultivares de Tomate Cherry (Physiological Processes Involved in Greater Tolerance to Water Deficit in Cherry Tomato Cultivars)

5. 5. Poster

Authors: Rocío Leyva, Begoña Blasco, Eva Sánchez-Rodríguez, María del Mar Rubio-Wilhelmi, Christian Constan-Aguilar, Rubén Melgarejo, Teresa Soriano Vallejo, Juan Manuel Ruiz.

Title: Efecto de un Sistema de Nebulización de Baja Presión en Invernadero de Malla sobre la Calidad de Tomate Cherry (*Solanum lycopersicum* L.) Bajo Clima Mediterráneo (Effect of a Low Pressure Nebulising System in Mesh Greenhouses on Quality of Cherry Tomatoes (Solanum lycopersicum L.) in a Mediterranean Climate

6.- XIV LUSO-HISPANIC SIMPOSIUM ONMINERAL NUTRITION OF PLANTS. 23 TO 26 JULY 2012

Venue and Year:

Madrid. (Spain) 23 to 26 July 2012

Entity / Organization Group:

Departments of Biology and Agricultural Chemistry of the UAM (Madrid)

Type of Participation:

Poster.

Authors: Christian Constán Aguilar[1], Yurena Barrameda Medina[1], David Montesinos Pereira[1], Luís Romero[1], Teresa Soriano[2], Juan Manuel Ruiz[1].

[1] Departamento de Fisiología Vegetal. Facultad de Ciencias. Universidad de Granada. (Department of Plant Physiology.Faculty of science. University of Granada)
[2] IFAPA Centro Camino de Purchil S/N C.P. 18080 Granada.

Title: Efecto de la biofortificación con potasio en la postcosecha de tomate cherry: Implicación de algunos fenoles. (Effect of potassium biofortification in postharvest cherry tomato: Implications of some phenols).

7.- XIII CONGRESSO LUSO-ESPANHOL DE FISIOLOGÍA VEGETAL. 24-28 JULY 2013. (13TH LUSO-HISPANIC CONGRESS OF PLANT PHYSIOLOGY).

Venue and Year:

Lisbon (Portugal) 24 to27 July 2013.

Entity / Organization Group:

SPANISH SOCIETY OF PLANT PHYSIOLOGY (SEFV).

Type of Participation:

7.1 Poster.

Authors: Christian Constán Aguilar[1], Rocío Leyva[1, 2], David Montesinos Pereira[1], Teresa Soriano[2], Luís Romero[1], Juan Manuel Ruiz[1].

[1] Departamento de Fisiología Vegetal. Facultad de Ciencias. Universidad de Granada.
[2] IFAPA Centro Camino de Purchil S/N C.P. 18080 Granada.

Title: Effect of potassium on free polyamines in cherry tomato fruits after cold storage.

7.2 Poster.

Authors: David Montesinos Pereira[1], Eva Sánchez-Rodríguez, Christian Constán Aguilar[1], Yurena Barrameda Medina[1], Luís Romero[1], Juan Manuel Ruiz[1].

[1] Departamento de Fisiología Vegetal. Facultad de Ciencias. Universidad de Granada.

Title: Variation in the polyamines content under moderate water deficit in tomato plants (Solanum Lycopersicum) differing in their tolerance to drought

7.3 Poster.

Authors: Yurena Barrameda Medina[1], Christian Constán Aguilar[1], David Montesinos Pereira[1], Luís Romero[1], Juan Manuel Ruiz[1], Begoña Blasco[1].

[1] Departamento de Fisiología Vegetal. Facultad de Ciencias. Universidad de Granada.

Title: Zinc distribution and concentration in Lactuca sativa and Brassica oleracea plants

6.- Publications

Title of the Journal or Publication. Number and Year.

6.1.- NATIONAL ARTICLES:

1. Authors: E. Constán Rodríguez, C. Constán Aguilar.
Title : Infección por el virus de la rabia tras mordedura de un murciélago. Pauta de vacunación.
(Rabies virus infection after a bat bite. Vaccination schedule)
Ref. Journal: ***Scientia: revista multidisciplinar de ciencias de la salud***
(*Scientia: multi-disciplinary journal of health sciences*)
ISSN 1135-9528
Volume: **13** Pages: **149-155** Date: **2008**

2. Authors: Christian Constán-Aguilar, Emilio Constán de la Revilla, Lucía Segovia de la Revilla, Enriqueta de la Revilla Negro, Manuel Jorge Bolaños Carmona.
Title : Estudio nutricional comparativo entre estudiantes de la universidad de granada y estudiantes croatas
(Comparative nutritional study between students of Granada University and Croatian students)
Ref. Journal: ***Scientia: revista multidisciplinar de ciencias de la salud***
(*Scientia: multi-disciplinary journal of health sciences*)
ISSN 1135-9528
Volume: **16 (1)** Pages: **1-16** Date: **2011**

6.2.- INTERNATIONAL ARTICLES:

1. Authors: E. Sánchez-Rodríguez, M.M. Rubio-Wilhelmi, B. Blasco, Christian Constán-Aguilar, Luis Romero, J.M. Ruiz.
Title : Variation in the use efficiency of N under moderate water deficit in tomato plants (Solanum lycopersicum) differing in their tolerance to drought.
Ref. Journal: ***Acta fisiologiae plantarum (Acta Physiologiae Plantarum)***
Volume: **33** Pages: **1861–1865** Date: **2011**

2. Authors: Begoña Blasco, Juan J. Ríos, Rocío Leyva, Rubén Melgarejo, Christian Constán-Aguilar, Eva Sánchez-Rodríguez, María Mar Rubio-Wilhelmi, Luis Romero, Juan M. Ruiz.
Title : Photosynthesis and metabolism of sugars from lettuce plants (*Lactuca sativa* L. var. longifolia) subjected to biofortification with iodine
Ref. Journal: ***Plant Growth Regulation***
Volume: **65 (1)** Pages: **137-143** Date: **2011**

3. Authors: E. Sánchez-Rodríguez, R. Leyva, C. Constán-Aguilar, L. Romero & J.M. Ruiz
Title : Grafting under water stress in tomato cherry: improving the fruit yield and quality
Ref. Journal: ***Annals of Applied Biology***
Volume: **161 (3)** Pages: **302-312** Date: **2012**

4. Authors: Leyva Rocío, Constán-Aguilar Christian, Blasco Begoña, Sánchez-Rodríguez Eva, Romero Luis, Soriano Teresa, Ruiz Saez Juan Manuel.
Title : Effects of climatic control on tomato yield and nutritional quality in Mediterranean screenhouse
Ref. Journal: ***Journal of the Science of Food and Agriculture***
Volume: **94 (1)** Pages: **63-70** Date: **2013**

5. Authors: Leyva Rocío, Constán-Aguilar Christian, Blasco Begoña, Sánchez-Rodríguez Eva, Soriano Teresa, Ruiz Saez Juan Manuel.
Title : A fogging system improves antioxidative defense responses and productivity in tomato
Ref. Journal: ***Journal of the American Society for Horticultural Science***
Volume: **138 (4)** Pages: **267-276** Date: **2013**

6. Authors: Constán-Aguilar Christian, Leyva Rocío, Blasco Begoña, Sánchez-Rodríguez Eva, Soriano Teresa, Ruiz Saez Juan Manuel.
Title : Biofortification with potassium. antioxidant responses during postharvest of cherry tomato fruits in cold storage
Ref. Journal: ***Acta Physiologiae Plantarum***
Volume: **36 (2)** Pages: **283-293** Date: **2014**

7. Authors: Constán-Aguilar Christian, Leyva Rocío, Romero Luis, Soriano Teresa, Ruiz Saez Juan Manuel.
Title : Implication of potassium on the quality of cherry tomato fruits after postharvest during cold storage
Ref. Journal: ***International Journal of food sciences and nutrition***
Volume: **65 (2)** Pages: **203-211** Date: **2014**

8. Authors: Constán-Aguilar Christian., Sánchez-Rodríguez Eva., Rubio-Wilhelmi M.M., Camacho M.A., Romero Luis., Ruiz Juan Manuel., Blasco Begoña.
Title : Physiological and Nutritional Evaluation of the Application of Phosphite as a Phosphorus Source in Cucumber Plants
Ref. Journal: ***Communications in Soil Science and Plant Analysis***
Volume: **45 (2)** Pages: **204-222** Date: **2014**

9. Authors**:** E. Sánchez-Rodríguez, R. Leyva, C. Constán-Aguilar, L. Romero & J.M. Ruiz
Title : How does grafting affect the ionome of cherry tomato plants under water stress?
Ref. Journal: ***Soil Science and Plant Nutrition***
Volume: Pages: Published online: 21 May 2014. http://dx.doi.org/10.1080/00380768.2013.870873 Date: **2014**

10. Authors: Christian Constán-Aguilar**,** Rocío Leyva, Luis Romero, Teresa Soriano, Begoña Blasco, Juan Manuel Ruiz.
Title : Assessment of carbon metabolism of cherry tomato fruits: ¿How does affects potassium biofortification during crop cycle at postharvest storage?
Ref. Journal: ***LWT - Food Science and Technology***
Volume: **Under revision** Pages: Date: **2014**

11. Authors: Christian Constán-Aguilar**,** Rocío Leyva, Luis Romero, Teresa Soriano, Begoña Blasco, Juan Manuel Ruiz.
Title : ***The effect of potassium biofortification over yield and nutritional quality of cherry tomato fruits***
Ref. Journal: ***Journal of the Science of Food and Agriculture***
Volume: **Under revision** Pages: Date: **2014**

6.3.- TECHNICAL SCIENTIFIC PUBLICATIONS OR DOCUMENTS:

1. Authors: E. Sánchez-Rodríguez, M.M. Rubio-Wilhelmi, B. Blasco, Christian Constán-Aguilar, Luis Romero. J.M. Ruiz.
Title: Efecto de la aplicación de diferentes quelatos sobre la eficiencia de la fitoextracción del niquel en plantas de *Brassica rapa* cv. Onekilo.
(Effect of the use of different chelates on the efficiency of phytoextraction of nickel in Brassica rapa cv. Onekilo plants)
Chapter of Book Volume: 1 Pages: 695-706
Presente y futuro de la nutrición mineral de las plantas (Present and future of mineral nutrition in plants),
Eds. Luis Romero, Juan Manuel Ruiz, Begoña Blasco, María del Mar, Rubio-Wilhelmi, Eva Sánchez-Rodríguez, Juan José Ríos y Luis Miguel Cervilla.
I.S.B.N.: 978-84-89780-10-7.
Place of Publication: Granada (Spain) Date: **2008**

2. Authors: Christian Constán Aguilar[1], Yurena Barrameda Medina[1], David Montesinos Pereira[1], Luís Romero[1], Teresa Soriano[2], Juan Manuel Ruiz[1].

[1] Departamento de Fisiología Vegetal. Facultad de Ciencias. Universidad de Granada.
(Department of Plant Physiology.Faculty of science. University of Granada)
[2] IFAPA Centro Camino de Purchil S/N C.P. 18080 Granada

Title: Efecto de la biofortificación con potasio en la postcosecha de tomate cherry: Implicación de algunos fenoles. (Effect of potassium biofortification in postharvest cherry tomato: Implications of some phenols).

Chapter of Book Volume: 1 Pages: 241-246
MINERAL NUTRITION OF PLANTS AS A SUSTAINABLE AGRICULTURAL BASE
ISBN-10: 84-695-5571-5/ISBN-13: 978-84-695-5571-2 Date: **2012**

- **Member of the research group** "Diagnóstico Nutricional de las Plantas Cultivadas en Condiciones Adversas" (Código AGR-161) ("Nutritional Diagnosis of Cultivated Plants in Adverse Conditions"), led by D. Luis María Romero Monreal, Professor, Department of Plant Physiology, Faculty of Science, Granada University (Spain). Since 2008.

- **Successfully completed** "XLVIII Curso Internacional de Edafología, Fertilidad de Suelos y Biología Vegetal" ("48th International Course of Soil Science, Soil Fertility and Plant Biology") at the Estación Experimental del Zaidín. CSIC. Granada. **(1300 h)**

- **2011. Full member** of the Committee on Internal Quality Assurance for the official postgraduate course in Agricultural Biology and Aquaculture, structured according to the Royal Decree 56/2005 of 21 January (BOE de 25 January 2005) y approved by the Council Government of Andalucia by agreement of 25 April, 2006 (BOJA of 5 May, 2006).

- **Member** of the Sociedad Española de Fisiología Vegetal (Spanish Society of Plant Physiology) (bonded member Nº 1107), and of the Federation of European Societies of Plant Biology since 2011.

- **Member** of the research team on project RTA2009-00005-00-00 entitled: "Desarrollo a la alternativa de cultivo protegido: invernal bajo plástico en el litoral-estival bajo malla en el interior para una producción sostenible rentable y de calidad". ("Development of alternative crop protection: under plastic in the coastal summer below net for sustainable, profitable and quality production"). January 2012.

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Tel:: +34 616 63 52 68