UNIVERSITAT POLITÈCNICA DE VALÈNCIA

TESIS DOCTORAL

Mejora de la calidad nutritiva del tomate: búsqueda de fuentes de variabilidad, estudio de la influencia del ambiente y determinación del control genético.

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RESUMEN

El tomate es una de las hortalizas más consumidas a lo largo del año en todo el mundo. Por esta razón, se ha convertido en una fuente importante de minerales y componentes nutraceúticos (principalmente carotenoides y vitamina C), que tienen un papel clave en la salud humana. Recientemente se ha demostrado la importancia de las vitaminas y carotenoides del tomate en la prevención de ciertos cánceres y enfermedades cardiovasculares que, debido al estilo de vida actual, tienen un gran impacto en la mortalidad humana. Este hecho hace que los trabajos destinados a mejorar el valor nutraceútico del tomate sean de sumo interés.

En los últimos años, la FAO y el Bioversity International (anteriormente Plant Genetics Resource Institute) están promoviendo el uso de la biodiversidad en aras de contribuir a la sanidad y seguridad en la alimentación humana, así como al desarrollo sostenible. Siguiendo esta recomendación, el primer trabajo de esta tesis contribuyó al campo de la biodiversidad, nutrición y composición de los alimentos, evaluando el contenido en carotenoides y ácido ascórbico en una colección de cultivares de tomate infrautilizadas y especies relacionadas para promover su uso (directamente en campo o como fuentes de variabilidad para la obtención de nuevos cultivares). Se evaluaron un total de 49 entradas (14 entradas de tomate común, 28 de tomate tipo cherry y 7 de la especie relacionada *Solanum pimpinellifolium*) provenientes de 24 países. Se seleccionaron 14 entradas tipo cherry y 2 de tomate común por su alto y equilibrado valor nutraceútico, resultando de gran interés para consumo humano. Además, 2 entradas tipo cherry con alrededor de 1,5 veces la media normal de ácido ascórbico, y 1 de *S. pimpinelllifolium* con más de 9 veces el contenido normal de licopeno, podrían ser de interés como parentales donantes en programas de mejora para aumentar las propiedades nutraceúticas de variedades comerciales.

Una vez que se han identificado fuentes de variabilidad para alta acumulación de licopeno, β-caroteno y ácido ascórbico, es necesario evaluar su potencial real de mejora en diversos ambientes y ciclos de cultivo con el fin de concretar la contribución del genotipo, el ambiente y su interacción en la expresión de estos caracteres. También es importante determinar su control genético y modo de herencia para poder usarlos eficazmente en programas de mejora. En el segundo trabajo de esta tesis, se evaluaron 10 entradas preseleccionadas como potencialmente interesantes en 3 ambientes de cultivo. El contenido de licopeno, β-caroteno y ácido ascórbico fue muy alto en varios entradas (hasta 281, 35 y 346 mg kg-1 respectivamente). Las diferencias encontradas en los tres ambientes estudiados (con algunas condiciones estresantes en varios casos) tuvieron una influencia destacable en la expresión fenotípica de los caracteres analizados. Sin embargo, el efecto genotípico tuvo la mayor contribución al fenotipo junto a una considerable interacción con el ambiente. En programas de mejora será posible seleccionar genotipos de élite con alto contenido en licopeno y β-caroteno, ya que ambos caracteres tienen una elevada correlación genética, pero siempre recomendando ensayos en múltiples ambientes para seleccionar de forma eficaz. En cambio, la mejora del contenido de ácido ascórbico será más difícil porque la interferencia de factores incontrolados enmascara el potencial genético real y dificulta la selección. Entre las entradas evaluadas, destacaron cuatro por su sorprendente potencial genético en la acumulación de compuestos nutraceúticos. Tres de ellas pertenecen a *S. pimpinellifolium* (CDP1568, CDP7090 y CDP9822) y podrán ser usadas como parentales donantes en la mejora del contenido de carotenoides del tomate cultivado. La

entrada CDP4777 (*S.lycopersicon* var *cerasifome*) mostró un alto potencial genotípico para acumular β-caroteno y ácido ascórbico y alta estabilidad en su expresión en los ambientes estudiados, por lo que podría usarse tanto como parental donante en programas de mejora como para consumo directo en mercados de calidad. De estas entradas, se seleccionó la CDP4777 por ser de la misma especie que el tomate cultivado, para analizar con detalle su control genético y modo de herencia en la acumulación de β-caroteno y ácido ascórbico. Como parental femenino se empleó la línea de mejora CPD8779 (ya evaluada en el trabajo anterior) y la entrada CDP4777 como parental masculino, obteniéndose sus descendencias F_1, F_2, BC_1 y BC_2. El estudio se llevó a cabo simultáneamente en dos localidades con dos modalidades de cultivo distintas (aire libre y protegido bajo invernadero). El control genético de las expresiones de β-caroteno y ácido ascórbico se estudió mediante un modelo aditivo, dominante y aditivo x aditivo (ADAA) que incluyó las interacciones genotipo x ambiente. Los resultados indicaron que la acumulación de β-caroteno fue principalmente aditiva (32,2% de la componente genética) con una pequeña componente dominante (4,2%) y una importante contribución de la interacción AxE (63,6%). Esta interacción, en ambientes con temperaturas moderadas a altas y sin baja radiación , podría aumentar el contenido de β-caroteno. Este carácter mostró una heredabilidad en sentido estricto alta (h^2=0,62). La acumulación de ácido ascórbico fue también principalmente adititiva (61,7% de la componente genética) con una componente epistática menor (21,5%) que causó una heterosis negativa que redujo el efecto aditivo principal. Sin embargo, en el ambiente descrito, la contribución de la interacción AxE (16,8%) podría aumentar el contenido de ácido ascórbico y compensar el efecto de heterosis negativo. Para este carácter, la heredabilidad en sentido estricto total se puede considerar buena (h^2=0,52). En conclusión, la entrada CDP4777 es un parental donante muy interesante para la mejora conjunta del contenido de β-caroteno y ácido ascórbico en programas de mejora del valor nutracéutico de tomate comercial, ya que los híbridos F1 derivados de ella pueden llegar a quintuplicar el contenido de β-caroteno comúnmente aceptado como normal, incrementando ligeramente el contenido de ácido ascórbico respecto del de la línea de mejora utilizada como genitor femenino y sin disminuir la capacidad de acumulación de licopeno.

En fases avanzadas del programa de mejora donde las diferencias entre contenidos de carotenoides son más sutiles, se necesitaría una técnica analítica separativa rápida y precisa. Una primera opción sería el uso de una técnica ampliamente extendida y popular como el HPLC. Sin embargo, el largo tiempo de análisis, la cantidad de solventes utilizados y el alto coste de las columnas de esta técnica, hizo que se planteara el desarrollo de un método de análisis mediante electrocromatografía capilar (CEC) con una columna monolítica basada en esteres de lauril metacrilato. La separación de estos analitos fue llevada a cabo en menos de 5 minutos con una fase móvil que contenía 35% tetrahidrofurano, 30% acetonitrilo, 30% metanol, y 5% de un tampón acuoso 5 mM Tris a pH 8. El límite de detección y la reproducibilidad de este método fueron inferiores a 1,6 mg/mL y 7,2% respectivamente.

SUMMARY

Tomatoes are one of the most consumed vegetables in the world the whole year through. It is for this reason that the tomato has become an important source of minerals and nutraceutics (mainly carotenoids and vitamin C), which play a key role in human health. It has recently been proved just how important tomato vitamins and carotenoids are in helping to prevent certain cancers and cardiovascular diseases. These diseases have a great impact on the human mortality rate due to modern health-care practices. Taking this fact into account, the trials to improve the nutraceutic content of tomato are truly of utmost interest.

In recent years, FAO and Bioversity International (formerly the International Plant Genetic Resources Institute) have been promoting the sustainable use of biodiversity in programmes, thus contributing to food security and human nutrition, while at the same time raising awareness of the importance of this link to sustainable development. Following these guidelines, the first work of this thesis contributed to bring new insight in the research field of biodiversity, nutrition and food composition, evaluating the carotenoids and ascorbic acid content in a collection of underutilized tomato cultivars and related accessions to recover their use (directly in fields or as variability sources to obtain new cultivars). A total of 49 accessions of tomato germplasm (14 accessions of common tomato types, 28 cherry type tomatoes and 7 accessions of the related species *Solanum pimpinellifolium*) from 24 countries were evaluated. Fourteen accessions of the cherry type and two of the common tomato type were selected for their high and balanced nutritional properties, which makes them of great interest for direct human consumption. Furthermore, two accessions of the cherry types with over 1.5 times the normal average ascorbic acid content, as well as one *S. pimpinellifolium* accession, which presented more than nine times the normal average lycopene content, will most likely be of interest as donor parents for breeding programmes to increase the nutraceutical properties of commercial varieties.

Once sources of variability for high lycopene, β-carotene and ascorbic acid accumulation have been identified, it is necessary to evaluate their real breeding potential in different environments and growing cycles to investigate the nature of the genotype, environment and G × E interaction effects in the expression of these traits. It is also important to determine the genetic control and mode of inheritance in order to use them effectively in breeding programs. In the second work of this thesis, 10 accessions that were preselected for being of potential interest were evaluated in three growing environments. The content of lycopene, *β*-carotene and ascorbic acid detected was very high in some accessions (up to 281, 35 and 346 mg kg^{-1}, respectively). The important differences in the three environments studied (with some stressing conditions in several situations) had a remarkable influence in the phenotypic expression of the functional characters evaluated. Nevertheless, the major contribution came from the genotypic effect along with a considerable G × E interaction. The joint accumulation of lycopene and β-carotene has a high genetic component. It is possible to select elite genotypes with high contents of both carotenoids in tomato breeding programmes, but multi-environment trials are recommended. The improvement of ascorbic acid content is more difficult because the interference of uncontrolled factors masks the real genetic potential. Among the accessions evaluated, there are four accessions with an amazing genetic potential for functional properties. Three of them belong to *S. pimpinellifolium* (CDP1568, CDP7090 and CDP9822) and are especially interesting for their use as

donor parents in the improvement of carotenoid content in cultivated tomato. The accession CDP4777 (*Solanum lycopersicum* var *cerasiforme*) showed a very high genotypic potential for β-carotene and ascorbic acid accumulation and a high stability in their expression, so it might be used either as donor parent in breeding programmes or for direct consumption in quality markets. Of these accessions, CDP4777 was selected to analyze the genetic control and the mode of inheritance of β-carotene and ascorbic acid accumulation because it was of the same species than cultivated tomato. The breeding line CDP8779 (just evaluated before) and the accession CDP4777 were used as parentals, and their F_1, F_2, BC_1 and BC_2 descendant generations were obtained. The study was carried out in two locations with different growing conditions (open air and protected by glasshouse cultivation). The genetic control of these trait expressions was studied using an additive, dominance and additive x additive model that includes genotype x environment interactions. The results indicate that β-carotene accumulation was mainly additive (32.2% of the genetic component) with a small dominant component (4.2%) and an important AxE interaction contribution (63.6%), which, in target environments with moderate to high temperatures and no depressed radiation, could substantially enhance β-carotene content. This trait showed a high narrow-sense heritability (h^2 = 0.62). Ascorbic acid accumulation was also mainly additive (61.7% of the genetic component) with a minor additive epistatic component (21.5%). This epistatic effect caused a negative heterosis that reduces the positive main additive effect. Nevertheless, in the described target environments, the AxE interaction contribution (16.8%) may enhance the ascorbic acid content and compensate the negative heterosis effect. The total narrow-sense heritability of this trait can be considered good (h^2 = 0.52). In conclusion, the CDP4777 accession is a very interesting donor parent for joint improvement of β-carotene (without diminishing the lycopene content) and ascorbic acid content in commercial tomato nutraceutical breeding programmes; the F1 hybrids derived from this accession showed nearly 450% of the commonly reported average β-carotene content and close to 130% of the ascorbic acid content of the female parent.

In the advanced stages of tomato breeding programmes, where the differences between breeding lines for the carotenoid content become more subtle, a fast and accurate analytical technique, able to separate individual compounds, would be very helpful. In this respect, HPLC, despite being a widespread and popular technique, requires long analysis time, high amounts solvents and expensive analytical columns. These reasons encouraged us to develop an alternative method based on capillary electrochromatography (CEC) with a methacrylate lauryl ester-based monolithic column. By means of this technique, a fast separation of the mentioned analytes was achieved in less than 5 min in a mobile phase containing 35% THF, 30% ACN, 30% methanol and 5% of a 5 mM Tris aqueous buffer, pH 8. The CEC method was evaluated in terms of detection limit and reproducibility, with values below 1.6 mg/mL and 7.2%, respectively.

RESUM

La tomaca és una de les hortalisses més consumides al llarg de l'any en tot el món. Per aquesta raó, s'ha convertit en una font important de minerals i components nutracèutics (principalment carotenoids i vitamina C), que tenen un paper clau en la salut humana. Recentment s'ha demostrat la importància de les vitamines i carotenoids de la tomaca en la prevenció de certs càncers i malalties cardiovasculars que, a causa de l'estil de vida actual, tenen un gran impacte en la mortalitat humana. Este fet fa que els treballs destinats a millorar el valor nutracèutic de la tomaca siguen del màxim interés.

En els últims anys, la FAO i el Bioversity International (anteriorment Plant Genetics Resource Institute) estan promovent l'ús de la biodiversitat per a aconseguir contribuir a la sanitat i seguretat en l'alimentació humana, així com al desenvolupament sostenible. Seguint esta recomanació, el primer treball d'aquesta tesi va contribuir al camp de la biodiversitat, nutrició i composició dels aliments, avaluant el contingut en carotenoids i àcid ascòrbic en una col·lecció de cultivars de tomaca infrautilitzada i espècies relacionades per a promoure el seu ús (directament en camp o com a fonts de variabilitat per a l'obtenció de nous cultivars). Es van avaluar un total de 49 entrades (14 entrades de tomaca comuna, 28 de tomaca tipus cherry i 7 de l'espècie relacionada *Solanum pimpinellifolium*) provinents de 24 països. Es van seleccionar 14 entrades tipus cherry i 2 de tomaca comuna pel seu alt i equilibrat valor nutracèutic, resultant de gran interés per a consum humà. A més, 2 entrades tipus cherry amb al voltant de 1.5 vegades la mitjana normal d'àcid ascòrbic, i 1 de *S. pimpinelllifolium* amb més de 9 vegades el contingut normal de licopé, podrien ser d'interés com a parentals donants en programes de millora per a augmentar les propietats nutracèutiques de varietats comercials.

Una vegada que s'han identificat fonts de variabilitat per a alta acumulació de licopé, ß-caroté i àcid ascòrbic, és necessari avaluar el seu potencial real de millora en diversos ambients i cicles de cultiu a fi de concretar la contribució del genotip, l'ambient i la seua interacció en l'expressió d'aquestos caràcters. També és important determinar el seu control genètic i mode d'herència per a poder usar-los eficaçment en programes de millora. En el segon treball d'aquesta tesi, es van avaluar 10 entrades preseleccionades com potencialment interessants en 3 ambients de cultiu. El contingut de licopé, ß-caroté i àcid ascòrbic va ser molt alt en diverses entrades (fins a 281, 35 i 346 mg kg-1 respectivament). Les diferències trobades en els tres ambients estudiats (amb algunes condicions estressants en diversos casos) van tindre una influència destacable en l'expressió fenotípica dels caràcters analitzats. Tanmateix, l'efecte genotípic va tindre la major contribució al fenotip junt amb una considerable interacció amb l'ambient. En programes de millora serà possible seleccionar genotips d'elit amb alt contingut en licopé i ß-caroté, ja que ambdós caràcters tenen una elevada correlació genètica, però sempre recomanant assajos en múltiples ambients per a seleccionar de forma eficaç. En canvi, la millora del contingut d'àcid ascòrbic serà més difícil perquè la interferència de factors incontrolats emmascara el potencial genètic real i dificulta la selecció. Entre les entrades avaluades, van destacar quatre pel seu sorprenent potencial genètic en l'acumulació de compostos nutracèutics. Tres d'elles pertanyen a *S. pimpinellifolium* (CDP1568, CDP7090 i CDP9822) i podran ser usades com a parentals donants en la millora del contingut de carotenoids de la tomaca cultivada. L'entrada CDP4777 (*S.Lycopersicon* var *cerasifome*) va mostrar un alt potencial genotípic per a acumular ß-caroté i àcid ascòrbic i alta estabilitat en la seua expressió en els ambients estudiats, per

la qual cosa podria usar-se tant com parental donant en programes de millora com per a consum directe en mercats de qualitat. D'aquestes entrades, es va seleccionar la CDP4777 per ser de la mateixa espècie que la tomaca cultivada, per a analitzar amb detall el seu control genètic i mode d'herència en l'acumulació de ß-caroté i àcid ascòrbic. Com a parental femení es va emprar la línia de millora CPD8779 (ja avaluada en el treball anterior) i l'entrada CDP4777 com a parental masculí, obtenint-se les seues descendències F_1, F_2, BC_1 i BC_2. L'estudi es va dur a terme simultàniament en dos localitats amb dos modalitats de cultiu distintes (aire lliure i protegit sota hivernacle). El control genètic de les expressions de ß-caroté i àcid ascòrbic es va estudiar per mitjà d'un model additiu, dominant i additiu x additiu (ADAA) que va incloure les interaccions genotip x ambient. Els resultats van indicar que l'acumulació de ß-caroté va ser principalment additiva (32,2% de la component genètica) amb una xicoteta component dominant (4,2%) i una important contribució de la interacció AxE (63,6%). Esta interacció, en ambients amb temperatures moderades a altes i sense baixa radiació , podria augmentar el contingut de ß-caroté. Este caràcter va mostrar una heredabilitat en sentit estricte alta (h^2=0,62). L'acumulació d'àcid ascòrbic va ser també principalment addititiva (61,7% de la component genètica) amb una component epistàtica menor (21,5%) que va causar una heterosis negativa que va reduir l'efecte additiu principal. No obstant això, en l'ambient descrit, la contribució de la interacció AxE (16,8%) podria augmentar el contingut d'àcid ascòrbic i compensar l'efecte d'heterosis negatiu. Per a este caràcter, l'heredabilitat en sentit estricte total es pot considerar bona (h^2=0,52). En conclusió, l'entrada CDP4777 és un parental donant molt interessant per a la millora conjunta del contingut de ß-caroté i àcid ascòrbic en programes de millora del valor nutracèutic de tomaca comercial, ja que els híbrids F1 derivats d'ella poden arribar a quintuplicar el contingut de ß-caroté comunament acceptat com normal, incrementant lleugerament el contingut d'àcid ascòrbic respecte del de la línia de millora utilitzada com a genitor femení i sense disminuir la capacitat d'acumulació de licopé.

En fases avançades del programa de millora, on les diferències entre continguts de carotenoids són més subtils, es necessitaria una tècnica analítica separativa ràpida i precisa. Una primera opció seria l'ús d'una tècnica àmpliament estesa i popular com el HPLC. No obstant això, el llarg temps d'anàlisi, la quantitat de solvents utilitzats i l'alt cost de les columnes d'esta tècnica, va fer que es plantejara el desenvolupament d'un mètode d'anàlisi per mitjà d'electrocromatografia capil·lar (CEC) amb una columna monolítica basada en esters de lauril metacrilat. La separació d'aquestos anàlits va ser duta a terme en menys de 5 minuts amb una fase mòbil que contenia 35% tetrahidrofurá, 30% acetonitril, 30% metanol, i 5% d'un tampó aquós 5 mM Tris a pH 8. El límit de detecció i la reproducibilidad d'aquest mètode van ser inferiors a 1,6 mg/ml i 7,2% respectivament.

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I. INTRODUCCIÓN

I.1. ANTECEDENTES

Hasta la fecha, en el cultivo de tomate para consumo en fresco, los mejoradores han hecho énfasis principalmente en el aumento de la producción, el tamaño del fruto y su apariencia (ausencia de defectos y un color atractivo), resistencia a enfermedades y, más recientemente, firmeza del fruto y una adecuada larga vida ("long shelf life tomato") para transportarlos a larga distancia. Sin embargo, actualmente los consumidores se quejan de la falta de calidad interna de los frutos de tomate.

Dentro de la calidad interna de los frutos de tomate podemos distinguir entre:

- <u>Calidad organoléptica</u>: Engloba todas aquellas sensaciones que experimentamos al consumir un alimento relacionadas con el gusto (dulzor, acidez, amargor, etc.), olfato (aroma, perfume) y tacto (firmeza, harinosidad, etc.) (Pretel *et al*., 1993). La calidad organoléptica es la que determina que un alimento sea o no consumido. Guarda una relación directa con la cantidad de sólidos solubles (ácidos y azúcares principalmente) y compuestos aromáticos del fruto.
- <u>Calidad nutritiva y funcional</u>: Es 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. La calidad nutritiva aunque es imperceptible para los consumidores, es de gran importancia. Algunas sustancias de estos alimentos proporcionan adicionalmente beneficios médicos o saludables, incluyendo la prevención y el tratamiento de enfermedades, denominándose entonces compuestos nutraceúticos (Jack, 1995). Estos caracteres pueden variar dependiendo de la especie, el cultivar, los factores ambientales, las técnicas agrícolas, los tratamientos postcosecha y el almacenaje (Dumas *et al*., 2003).

Esta tesis se centrará en este segundo aspecto de la calidad interna del tomate, ya que en los últimos años, los consumidores se han vuelto más exigentes y están demandando alimentos más saludables y nutritivos, lo que ha causado un gran esfuerzo en la mejora para obtener altos valores nutricionales en frutas y verduras. Aunque, el tomate no tiene un gran contenido en vitaminas y antioxidantes (Tabla 1), es una hortaliza consumida ampliamente a lo largo del todo el año (27,36 y 46,05 kg/habitante

y año en Europa y España, respectivamente) (FAOSTAT, 2011). Esto la convierte en una de las principales fuentes de minerales, vitaminas y antioxidantes en muchos países (Esquinas-Alcázar y Nuez, 1995).

Tabla 1. Contenido de vitaminas y antioxidantes de algunas frutas y verduras en mgkg^{-1} de peso fresco (Adalid *et al*., 2004).

Licopeno		**Provitamina A**		**Vitamina C**	
Tomate	80-20	Zanahoria	66	Grosella	2200
Guayaba	50	Batata	59	Kiwi	1200
Papaya	50-20	Espinaca	49	Limón	900
Sandía	40	Tomate	5	Coliflor y espinaca	600
Uva	30	Melocotón	5	Naranja	500
Albaricoque	0,05	Naranja	1	Tomate	250

I.2. CALIDAD NUTRITIVA Y FUNCIONAL DEL TOMATE

Los componentes nutricionales del tomate son azúcares y ácidos, proteínas, lípidos y aminoácidos, minerales, componentes fenólicos, pigmentos y vitaminas. Estos tres últimos se han identificado como componentes nutraceúticos, por lo que el tomate se ha identificado como un alimento funcional y nutraceútico (Jack, 1995; Canene-Adams *et al*., 2005).

Los azúcares reductores representan aproximadamente el 50 % de la materia seca siendo la glucosa y la fructosa los mayoritarios. Los ácidos orgánicos, principalmente cítrico y málico, representan más del 10 % de la materia seca (Chamarro, 2003). Tanto los azúcares como los ácidos aportan un escaso valor nutritivo al tomate, aunque sí tienen un papel fundamental en su sabor.

El contenido medio de proteínas, aminoácidos y lípidos del tomate es muy pobre, alrededor de 0,6% del peso fresco (Davies y Hobson, 1981). De modo que el tomate no puede ser considerado una fuente importante de estos compuestos.

Los minerales representan una fracción pequeña del peso fresco, aproximadamente un 0,4 % (Davies y Hobson, 1981), pero desempeñan un importante papel en la composición nutritiva del fruto. Sin embargo el incremento del contenido en

minerales del tomate está más condicionado por la práctica de la fertilización que por factores genéticos por lo que no suele considerarse en programas de mejora genética.

Los principales componentes fenólicos en el tomate son quercetina, naringenina, rutina y ácido clorogénico (Hertog *et al*., 1992; Clifford, 1999; Paganga *et al*., 1999; Chassy *et al*., 2006; Luthria *et al*., 2006). La concentración de fenoles totales en muestras de tomate varía de 259,15 a 498,60 mg kg^{-1} de peso fresco (Martínez-Valverde *et al*., 2002; Podsedek *et al*., 2003; Zhou y Yu, 2006). A causa de su estructura, los fenoles son muy eficientes en la lucha contra los radicales peróxido (Halliwell, 1992; Aruoma, 1999). Los ácidos clorogénicos se han relacionado con propiedades beneficiosas para la salud humana debido a este poder antioxidante, así como hepatoprotector, hipoglucémico y actividad antiviral (Farah y Donangelo, 2006). Sin embargo, debido a la complejidad química de los fenoles (Dimitrios, 2006; Slimestad y Verheul, 2009) y a que los ácidos clorogénicos también pueden ser responsables de un sabor algo astringente (Walker, 1962; De Bruyn *et al*., 1971) no se han considerado como parte del estudio de esta tesis, aunque sí podría ser una línea de trabajo prometedora en un futuro próximo.

A pesar de que el tomate presenta una baja concentración de pigmentos (200 mg kg^{-1} de peso fresco) (Davies and Hobson, 1981), estos le aportan un gran valor nutraceútico. Uno de los principales grupos de pigmentos (rojos, naranjas y amarillos) que se pueden encontrar en este fruto son los carotenoides. El 90-95% de los carotenoides presentes en el tomate maduro son carotenos (Gross, 1991). El licopeno es el caroteno más abundante en los tomates de color rojo, llegando a representar más del 90% de los carotenoides totales. Un tomate rojo típico contiene niveles más bajos de otros pigmentos como β-caroteno, δ-caroteno, γ-caroteno y neurosporeno. También se encuentran pequeñas cantidades de precursores no coloreados como el fitoeno y el fitoflueno. Licopeno y β-caroteno (provitamina A) son los de mayor valor nutraceútico, y juegan un papel importante en las funciones metabólicas humanas (Rao y Rao, 2007). De hecho se ha visto que existe una relación directa entre el consumo de frutas y hortalizas ricas en licopeno y la reducción del riesgo de cáncer y enfermedades vasculares (Clinton, 1998; Dorgan *et al*., 1998; Bertram y Vine, 2005; Omoni y Aluko, 2005; Tang *et al*., 2005; Story *et al*., 2010). Además, el licopeno no solo tiene funciones antioxidantes, sino que participa en la comunicación intercelular y en la modulación del

sistema inmune y hormonal (Kun *et al*., 2006; Shao y Hathcock, 2006). El tomate y sus subproductos (salsas, zumos…) son la principal fuente de licopeno en la dieta occidental (Chung-Ahuja *et al.*, 1993). El β-caroteno (provitamina A) es un nutriente esencial debido a su actividad retinoide, y como otros carotenoides es un antioxidante que puede proteger del daño de los radicales libres. Su carencia puede producir xeroftalmia, ceguera y muerte prematura (Mayne, 1996), lo cual es un problema nutricional importante en más de 75 países, la mayoría de ellos situados en el mundo en vías de desarrollo (Angosto y Borja, 2001). El papel que el β-caroteno y la vitamina A desempeñan en el crecimiento, reproducción, mortalidad y morbilidad de enfermedades infecciosas ha sido revisada anteriormente (Tee, 1992; Ross, 1998).

El tomate es una fuente interesante de vitaminas para nuestro organismo, principalmente vitamina C y la provitamina A (β-caroteno, comentada anteriormente) (Tabla 2). Las vitaminas son moléculas orgánicas esenciales para el normal crecimiento, desarrollo y reproducción de humanos y animales.

Tabla 2. Contenido vitamínico del tomate (modificado de Davies y Hobson, 1981).

Vitamina	Contenido (mg kg^{-1} pf*)
Ácido ascórbico (vitamina C)	200-300
β-caroteno (provitamina A)	0,5-20,0
Tocoferoles (vitamina E)	0,4-12,0
Ácido nicotínico (niacina)	5-7
Ácido pantoténico (vitamina B3)	0,5-7,5
Piridoxina (vitamina B6)	0,8-1,1
Tiamina (vitamina B1)	0,5-0,6
Riboflavina (vitamina B2)	0,2-0,5
Ácido fólico	0,064-0,200
Biotina	0,012-0,040

*Peso fresco.

La función más conocida de la vitamina C es como agente antiescurbuto (Magiorkinis *et al*., 2011). Además, la vitamina C puede jugar un papel clave en el retraso de la patogenicidad de varias enfermedades degenerativas, como enfermedades cardiovasculares (Marchioli *et al.*, 2001; Libby y Aikawa, 2002), ciertos cánceres (Byers y Guerrero, 1995; Ramaswamy y Krishnamoorthy, 1996; O'Toole y Lombart, 1996; Webb *et al*., 1997; You *et al*., 2000; Jamison *et al*., 2001), y cataratas (Van der Pols, 1999; Tessier *et al*., 1998; Valero *et al*., 2002). La participación en la síntesis de

colágeno es otra de las funciones conocidas de la vitamina C (Phillips *et al*., 1997; Libby y Aikawa, 2002). La vitamina C también juega un papel importante en la síntesis de hormonas esteroides, carnitina (Harmeyer, 2002) y la degradación de la tirosina. Esta vitamina puede aumentar la biodisponibilidad del hierro (Nasolodin *et al*., 1996), prevenir la mutación del ADN inducido por estrés oxidativo (Lutsenko *et al*., 2002; Collins, 2004), modular la expresión génica y la función celular (con un interés particular en la diferenciación celular) (Duarte y Lunec, 2005).

En 2005, el mercado mundial de alimentos funcionales alcanzó los 73,5 miles de millones de dólares y la tendencia de los años siguientes fue al alza, debido directamente a la disminución del consumo de una dieta sana y la consecuente elevación de enfermedades relacionadas con la dieta (Anónimo, 2007). La demanda comercial y el contenido de constituyentes saludables del fruto de tomate, hace que el aumento del valor nutricional y funcional de esta hortaliza se convierta en un objetivo importante, ya sea mediante mejora tradicional o por manipulación genética. El aumento de carotenoides y vitaminas en la dieta es más efectivo que el uso de suplementos vitamínicos, ya que otros nutrientes presentes en los productos alimenticios pueden actuar sinérgicamente con los carotenoides y vitaminas (Romer *et al*., 2000; Stacewicz-Sapuntzakis y Bowen, 2005). Varios ensayos clínicos con altas dosis de un solo componente (como el β-caroteno) han tenido resultados decepcionantes (Hennekens *et al*., 1994; Omenn *et al*., 1996; Rapola *et al*., 1997; Anónimo, 1998; Russell *et al*., 1999; Demming-Adams y Adams, 2002; Barret, 2003; Bendich, 2004; Dulinska *et al*., 2005). Los antioxidantes sintéticos requieren ensayos extensos y caros para determinar su seguridad alimentaria, haciéndose adecuado el uso de antioxidantes naturales (Frankel, 1995). En 2003, la Unidad de Servicios Preventivos de los Estados Unidos (*U.S. Preventive Services Task Force*, USPSTF) concluyó que no hay suficientes evidencias científicas para recomendar suplementos vitamínicos como forma de prevenir el cáncer o enfermedades cardiacas. La recomendación más prudente para la población general, respaldada científicamente, es consumir una dieta equilibrada poniendo énfasis en frutas y hortalizas, ricas en componentes nutraceúticos, y cereales. Aunque algunos productos sintéticos contienen cantidades significativas de nutrientes, estos últimos pueden ser obtenidos fácilmente de nuestros alimentos a un bajo coste (Anónimo, 1995; Bouis, 2003).

Actualmente, hay muchas posibilidades de aplicar este concepto con éxito en tomate, ya que es posible aumentar los compuestos nutraceúticos (principalmente licopeno, β-caroteno y vitamina C) sin afectar negativamente su sabor. No obstante, son necesarios los conocimientos de las rutas biosintéticas, el control genético, la influencia de los factores ambientales y las prácticas culturales que influyen en la acumulación de carotenoides y vitaminas en el fruto para llevar a cabo con éxito un programa de mejora de su calidad nutritiva y nutraceútica.

I.3. BIOSÍNTESIS DE LOS PRINCIPALES COMPONENTES NUTRITIVOS Y FUNCIONALES DEL TOMATE

I.3.1. Biosíntesis de carotenoides en tomate.

Los carotenoides son una de las muchas familias de metabolitos vegetales derivados de la biosíntesis de los isoprenoides, y comparten el precursor de cinco carbonos, isopentil pirofosfato (IPP), con cerca de 20.000 metabolitos vegetales. Cuatro unidades de IPP se unen para formar una subunidad de veinte carbonos: el gerenilgerenil pirofosfato (GGPP). El GGPP se usa también en la formación de los tocoferoles (vitamina E), filoquinona (vitamina K_1) y las plastoquinonas (Romer *et al*., 2000). El primer paso específico para la biosíntesis de los carotenoides es la unión de dos moléculas de GGPP para dar lugar al fitoeno (Figura 1) de cuarenta carbonos (Cunningham y Gantt, 1998). Durante la maduración del tomate, esta reacción es catalizada por la fitoeno sintasa, codificada por el gen *Psy-1*, y tiene lugar predominantemente en el estroma del plástido (Fraser *et al*., 1994). Sin embargo, la producción de fitoeno en el tomate verde se ve controlada, aparentemente, por otro gen, el *Psy-2* (Fraser *et al*., 1999).

Se requieren cuatro pasos desde el precursor fitoeno para conseguir la serie de once enlaces dobles conjugados encontrados en el licopeno. Las dos primeras desaturaciones están catalizadas por la fitoeno desaturasa (PDS), dando lugar a la formación de fitoflueno seguida por el ζ-caroteno. La conversión del ζ-caroteno a neurosporeno y entonces a licopeno, es llevada a cabo por la ζ-caroteno desaturasa (ZDS). Esta enzima aparentemente, tiene una alta actividad ya que el fruto maduro del tomate contiene pequeñas cantidades de ζ-caroteno o neurosporeno (Fraser *et al*., 1994).

El licopeno es el principal carotenoide acumulado en el tomate maduro. Es a su vez un punto de partida en la ruta biosintética de otros carotenoides resultantes de la formación de anillos en los extremos de la molécula de licopeno. Una de las principales rutas es la formación de β-caroteno debido a la ciclación de los dos extremos del licopeno en anillos β-ionona. El γ-caroteno actúa de intermediario con un único anillo β-ionona, y sólo se acumula en muy pequeña proporción en tomates maduros. La enzima β-licopeno ciclasa (LCYB) regula estas reacciones.

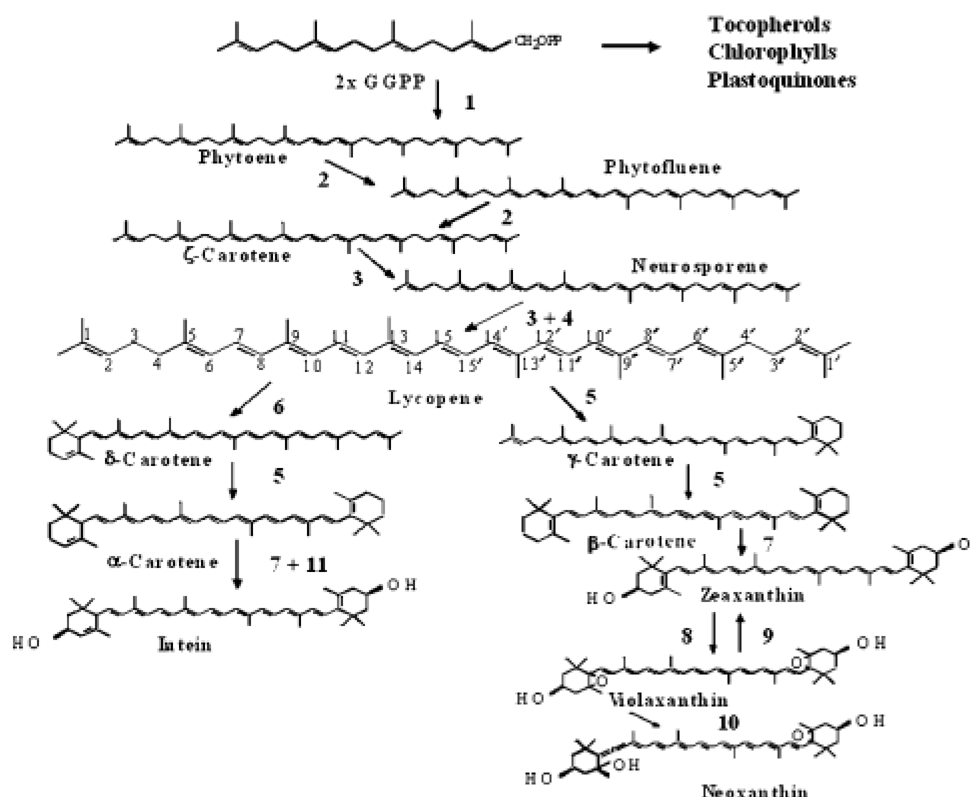

Figura 1. Representación esquemática de la síntesis de carotenoides en plantas superiors. GGPP geranilgeranil difosfato. Enzimas: 1 PSY fitoeno sintasa, 2 PDS fitoeno desaturasa, 3 ZDS ζ-caroteno desaturasa, 4 CRTISO caroteno isomerasa, 5 LCY-b /CYC-B lycopeno β-ciclasa, 6 LCY-e licopeno ε-ciclasa, 7 CRTL-b β-ring hidroxilasa, 11 CRTL-e ε-ring hidroxilasa, 8 ZEP zeaxantina epoxidasa, 9 VDE violaxantina de-epoxidasa, 10 NXS neoxantina sintasa (Romer y Fraser, 2005).

Existe una ciclasa adicional en algunos genotipos de tomate. Esta enzima da lugar a la formación de un anillo ε, formándose así el δ-caroteno. El gen *Delta* (*del*) parece que codifica un aumento de la actividad de la ε licopeno ciclasa (LCYE),

produciendo algo de δ-caroteno en tomate. El α-caroteno se puede formar a partir del δ-caroteno o del γ-caroteno (Cunningham *et al*., 1996; Cunningham y Gantt, 1998).

Inicialmente, se creía que la síntesis de carotenoides era regulada por dos enzimas: la fitoeno sintasa (PSY) y la fitoeno desaturasa (PDS). Sin embargo, las plantas transformadas con cualquiera de esas enzimas no presentaron un aumento del contenido de carotenoides (Bramley, 1997). Durante la maduración del fruto, el mecanismo principal de regulación de la biosíntesis y la acumulación de los carotenoides parece ser la regulación de la transcripción de la expresión de los genes (Lu y Li, 2008). Como ejemplo se pueden citar los estudios con los genes *crimson* y *beta*. El gen *crimson* promueve un aumento del contenido del licopeno. Esto parece ser debido a un efecto débil sobre los promotores de la LCYB, provocando una menor acumulación de β-caroteno. Sin embargo, el gen *beta* hace que el tomate acumule altos valores de β-caroteno, siendo este gen un promotor de la licopeno ciclasa. Como consecuencia, se ha propuesto que el control principal sobre la acumulación de licopeno sea una regulación de la transcripción de la β licopeno ciclasa (Ronen *et al*., 2000). Además, recientes estudios muestran que el control de la biogénesis de plástidos es un mecanismo importante de regulación de la biosíntesis y la acumulación de estos compuestos. La acumulación de carotenoides en los mutantes de tomate "high pigment" (*hp1*, *hp2* y *hp3*) se ha vinculado a una biogénesis temprana de plástidos en el fruto. Tal acumulación es debida principalmente a un mayor número y tamaño de plástidos que proporcionan un gran compartimento dentro de las células, permitiendo una elevada biosíntesis y una mayor capacidad de almacenamiento (Liu *et al.*, 2004; Kolotilin *et al*., 2007; Galpaz *et al*., 2008).

I.3.2. Biosíntesis de la vitamina C (ácido ascórbico) en plantas.

La investigación de la ruta biosintética del ácido L-ascórbico (vitamina C) en las plantas se ha llevado a cabo durante décadas, pero ha sido el trabajo de los últimos años el que ha incrementado enormemente los conocimientos sobre la síntesis de esta pequeña molécula. Hasta el momento, varias rutas metabólicas se han descrito para el ácido ascórbico.

En 1998, Wheeler *et al*. (1998) propusieron que la síntesis del ácido ascórbico partía de la manosa-1-P vía L-galactosa (Figura 2). Esta ruta biosintética ha sido constatada por numerosos estudios bioquímicos, genéticos y enzimáticos (Oba *et al*., 1994; Mutsuda *et al*., 1995; Conklin *et al*., 1997; Ostergaard *et al*., 1997; Imai *et al*., 1998; Wheeler *et al*., 1998; Conklin *et al*., 1999; Davey *et al*., 1999). La síntesis de GDP-D-manosa a partir de la D-manosa-1-P y GTP es catalizada por la GDP-D-manosa pirofosforilasa (Conklin *et al*., 1999). Entonces la GDP-D-manosa se convierte en GDP-L-galactosa por una epimerización reversible, catalizada por la GDP-D-manosa-3,5-epimerasa (Wheeler *et al*., 1998). Inicialmente, la GDP-L-galactosa se escinde a L-galactosa-1-P, que es posteriormente hidrolizada a L-galactosa (Smirnoff y Gatzer, 2004). La L-galactosa liberada es entonces oxidada en dos pasos, primero por una L-galactosa dehydrogenasa citosólica NAD dependiente para formar la L-galactono-1,4-lactona (Wheeler *et al*., 1998; Gatzek *et al*., 2002) y entonces por una L-galactono-1,4-lactona dehydrogenasa produciendo el ascorbato. Este paso final tiene lugar en la membrana mitocondrial donde esta última enzima usa el citocromo c como aceptor de electrones (Bartoli *et al*., 2000; Millar *et al*., 2003).

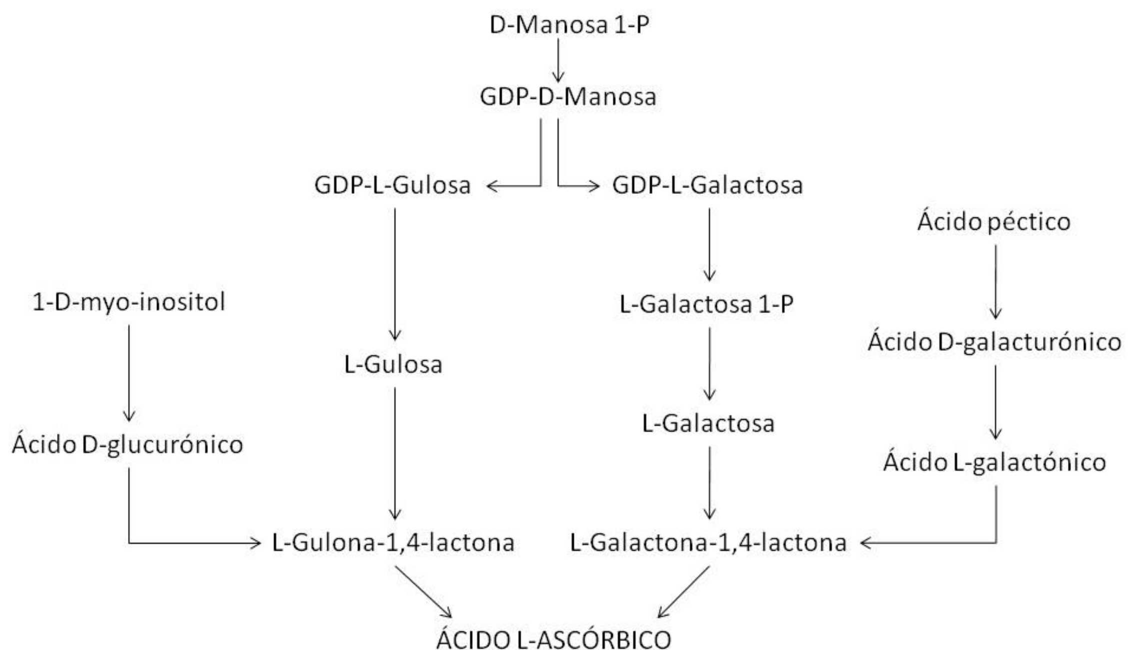

Figura 2. Simplificación de las rutas alternativas para la biosíntesis del ácido ascórbico (modificado de Ishikawa *et al.*, 2006).

Aunque la ruta biosintética descrita parece ser la predominante, existe una segunda ruta biosintética alternativa para el ácido L-ascórbico a partir de la conversión

de los ácidos urónicos (D-ácido glucorónico y D-ácido galacturónico). Davey *et al.* (1999) confirman la existencia de esta ruta y añaden que posiblemente, ésta solo sería importante bajo ciertas condiciones o etapas de desarrollo concretas como la abscisión, la maduración y reblandecimiento del fruto, la maduración de los granos de polen y la expansión celular. Más tarde Agius *et al.* (2003) encontraron evidencias moleculares en fresa para la ruta del ácido D-galacturónico gracias a la clonación y caracterización de una enzima ácido D-galacturónico reductasa. En *Solanum pennelli* (Di Matteo *et al.*, 2010) se han encontrado evidencias del uso de esta ruta para la biosíntesis del ácido ascórbico.

Jain y Nessler (2000) proponen, además, que la hidrólisis de la GDP-L-gulosa daría lugar a la producción de L-gulosa que puede ser convertida a L-gulono-1,4-lactona por la L-galactosa dehidrogenasa y posteriormente a ascorbato por la L-gulono-1,4-lactona dehidrogenasa.

A pesar de los últimos descubrimientos, se necesitarán trabajos adicionales a nivel bioquímico, molecular y genético para explicar con claridad todas las posibles rutas biosintéticas del ácido ascórbico, sus interrelaciones y sus posibles interacciones con otros procesos metabólicos que tienen lugar dentro de la célula (Conklin *et al.*, 2006). Debido a esto, existe poca información sobre la regulación de dichas rutas biosintéticas. Parece ser que la actividad enzimática y el nivel de transcripción de L-galactono-1,4-lactona dehidrogenasa tienden generalmente a correlacionarse con el contenido del ácido ascórbico en el tejido, aunque existe variación entre especies vegetales. También hay evidencias que la biosíntesis puede estar regulada por mecanismos de inhibición por retroalimentación. Además, la eficacia en el reciclado del monodehidroascorbato y dehidroascrobato a ascorbato pueden influir en la cantidad final de ácido ascórbico de las células (Ishikawa *et al.*, 2006).

I.4. PERSPECTIVAS DE MEJORA PARA AUMENTAR EL VALOR NUTRITIVO Y FUNCIONAL DEL TOMATE

I.4.1. Cuantificación rápida y precisa de componentes nutraceúticos.

En muchos estudios dentro de la mejora y el control de la calidad de frutas y hortalizas, el análisis químico de los compuestos involucrados en la calidad nutritiva y

funcional en un gran número de muestras de tomate puede llegar a ser un factor limitante. Por tanto, es muy importante el uso de técnicas analíticas rápidas y precisas. Las vitaminas pueden ser cuantificadas por protocolos encimáticos (Lee *et al.*, 1991; Rumsey y Levine, 2000), titulación (Jayaram y Made-Gowda, 1986; Arya *et al.*, 2000) o HPLC (Rizzolo y Polesello, 1992; Berg y Canessa, 1998). Esta técnica también puede ser utilizada para analizar carotenoides (Craft, 1992; Hart y Scott, 1995). En nuestro grupo se ha elegido la electroforesis capilar zonal (CEZ) por ser una técnica analítica que ofrece un alto poder resolutorio, una mínima preparación de la muestra, un tiempo de análisis corto y un coste operacional bajo para compuestos hidrosolubles (Galiana-Balaguer *et al.*, 2001). Considerando la mínima preparación de muestra y la posibilidad de analizar simultáneamente diferentes grupos de compuestos relacionados con la calidad interna de los vegetales, CEZ se convierte en una herramienta de análisis poderosa y atractiva para el trabajo rutinario de estos compuestos. De esta manera tanto en ensayos de cribado como de selección, se pueden analizar un gran número de muestras con la mínima intervención del analista. Pero debido al carácter hidrófobo de los carotenoides, no se puede utilizar la CEZ para analizar estos compuestos. La cromatografía líquida de alta resolución (HPLC) es la técnica usada más frecuentemente para los análisis de carotenoides en extractos de frutas y vegetales (Aust *et al.*, 2001; Rodríguez-Amaya, 2001). Sin embargo, estos métodos cromatográficos tienen una serie de desventajas incluyendo el excesivo tiempo necesario para el acondicionamiento de las columnas, el gran consumo de disolventes, y el alto precio de las columnas. En los últimos años, la electrocromatografía (CEC) ha recibido una mayor atención como una técnica de separación emergente (Colón *et al.*, 2000; Hilder *et al.*, 2002) ya que esta técnica aúna las ventajas de alta selectividad del HPLC con la alta eficiencia de CEZ, por lo que podría ser una alternativa interesante para la cuantificación de carotenoides, salvando los inconvenientes de los métodos desarrollados por HPLC.

I.4.2. Uso de transgénesis.

Los conocimientos sobre la ruta biosintética y sobre los enzimas relacionados que controlan la acumulación de carotenoides y vitaminas, permiten la introducción de genes foráneos en un nuevo contexto genético para incrementar la síntesis de antioxidantes y componentes nutraceúticos a través de la transgénesis.

El arroz dorado constituye un ejemplo conocido del uso de la biotecnología para aumentar el contenido de carotenoides. Los carotenoides no se acumulan en el endospermo del arroz. Sin embargo, Munne y Alegre (2000) y Ye *et al*. (2000) fueron capaces de producir arroz con niveles de β-caroteno superiores a 2 mg kg^{-1} introduciendo actividades heterólogas de la fitoeno sintasa, la fitoeno desaturasa, la ζ-caroteno desaturasa y la licopeno β-ciclasa. Se ha seguido trabajando en el tema (Tang *et al*., 2009; Chikkappa *et al*., 2011) y favoreciendo también la acumulación de hierro en el arroz (Lucca *et al*., 2006).

Siguiendo una estrategia similar, Rosati *et al*. (2000) consiguieron aumentar el nivel de β-caroteno por encima de 60 mg kg^{-1} de peso fresco en tomate, valores con los que se consigue alcanzar la cantidad diaria recomendada de esta vitamina con sólo ingerir 100g de tomate. Introdujeron la β-licopeno ciclasa de *Arabidopsis* detrás del promotor específico de fruto de la fitoeno desaturasa. Ambrosio *et al*. (2004) también indujeron la acumulación de β-caroteno en tomate con la introducción de una enzima licopeno-β-ciclasa modificada, evaluándose en campo las líneas de tomates transgénicos obtenidas recientemente (Giorio *et al*., 2007). Otros autores consiguieron aumentar de 2-4 veces el contenido de carotenoides en tomate consiguiendo la sobreexpresión de una fitoeno sintasa de *Erwinia uredovora*. No se alteraron el resto de enzimas de la ruta biosintética de carotenoides a pesar de tener dos fitoeno sintasas (Fraser *et al*., 2001; 2002).

Sin embargo, otros trabajos han obtenidos resultados decepcionantes. Por ejemplo, la expresión de la fitoeno desaturasa de *E. uredovora* asociada al promotor 35S del virus del mosaico de la coliflor (CaMV), con intención de aumentar el contenido de licopeno en frutos de tomate transgénico, resultó inesperadamente en un descenso del 50% de los carotenoides totales. Esto ocurrió a expensas del licopeno, mientras que el β-caroteno aumentó unas tres veces, de 270 a 520 mg kg^{-1} de peso seco (Romer *et al*., 2000).

Resultados similares se han encontrado en semillas de *Brassica*, donde la transformación con el gen fitoeno sintasa de *E. uredovora* fue llevada a cabo para aumentar el contenido de luteína (el principal carotenoide de estas semillas). El resultado fue un aumento de 50 veces del contenido de α y β-carotenos, pero no en luteína (Shewmaker *et al*., 1999).

Otro reto a la hora de manipular la ruta de los carotenoides, y probablemente otras rutas de vitaminas también, es preservar el equilibrio ya existente. La expresión del gen de la enzima fitoeno sintasa procedente del tomate bajo el promotor CaMV 35S en plantas transgénicas de tomate resultó en enanismo, que muy probablemente fuera consecuencia del redireccionamiento del flujo de la formación de la giberelina y el fitol (Al-Babili *et al.*, 2001). Esta manipulación transgénica permite un aumento en los niveles de licopeno y fitoeno, pero no en los niveles de β-caroteno (Al-Babili *et al.*, 2001).

Estas investigaciones muestran que es posible la manipulación del contenido de carotenoides en diferentes plantas. Sin embargo, no siempre se han obtenido resultados positivos. Estos estudios han mostrado también que diseñar y elucidar la ruta biosintética de carotenoides constituye todavía un reto (Botella-Pavía y Rodríguez-Concepción, 2006). Estos ejemplos muestran claramente que existen interferencias con la regulación endógena cuando se introducen genes heterólogos. Este hecho no está claramente entendido a niveles transcripcionales y postranscripcionales, siendo propuestos diferentes mecanismos de retroalimentación negativos y positivos por precursores de los carotenoides, como el trans-licopeno (Al-Babili *et al.*, 2001).

Para profundizar en los mecanismos de regulación que controlan la acumulación de carotenoides en los cromoplastos de los sistemas no fotosintéticos, se podrían aislar y caracterizar aquellos mutantes de tomate con perfiles alterados de carotenoides (Tabla 3), pudiendo hacer factible en un futuro próximo su manipulación dirigida (Romer y Fraser, 2005).

El contenido de vitamina C está influenciado no sólo por su biosíntesis, sino también por su reciclaje. Si lo combinamos con la posibilidad de la existencia de múltiples rutas biosintéticas, esto hace que sea muy difícil predecir y modificar su contenido. Los trabajos publicados se dividen básicamente en tres propuestas: sobreexpresión de las enzimas biosintéticas, sobreexpresión de las enzimas de reciclado (dehidroascorbato reductasa, DHAR) y supresión antisentido de la ascorbato oxidasa (AO). AO es una enzima apoplástica que afecta el estado redox del ascorbato extracelular (Pignocchi y Foyer, 2003; Sanmartin *et al.*, 2003). Como casos prácticos nos encontramos el de Jain y Nessler (2000) que introdujeron con éxito un cADN codificando la L-gulono-γ -lactona oxidasa (GLO) en tabaco y plantas de lechuga

transgénicos. La concentración de ácido ascórbico en las hojas de ambos cultivos se incrementó de 4 a 7 veces. Tokunaga *et al.* (2005) sobreexpresionaron la enzima L-galactona-1,4-lactona dehidrogenasa aumentando el contenido de vitamina C en plantas transgénicas de tabaco. En fresa, también se aumentó de 2 a 3 veces el contenido de vitamina C sobreexpresionando la ácido galacturónico reductasa (Agius *et al.*, 2003). Chen *et al.* (2003) demostraron que el contenido de vitamina C en plantas puede ser incrementado aumentando la expresión de la enzima responsable del reciclaje del ascorbato (DHAR). El incremento en la expresión de la DHAR aumentó los niveles de ácido ascórbico foliar y del grano de 2 a 4 veces, y aumentó significativamente el estado redox del ascorbato en tabaco y en maíz. Estos trabajos sugieren la posibilidad de trasladar las técnicas aplicadas para aumentar el contenido de ácido ascórbico a otros cultivos (Hancock y Viola, 2005). En tomate transgénico, disminuyeron la actividad de la malato deshidrogenasa, consiguiendo un efecto positivo en la capacidad de uso de la L-galactona-1,4-lactona, el precursor terminal de la biosíntesis del ácido ascórbico (Nunes-Nesi *et al.*, 2005).

Tabla 3. Genes mutantes en tomate relacionados con carotenoides.

Gen mutante	**Comentarios**
High pigment (hp1, hp2 y *hp3)*	Están en loci y cromosomas separados (Van Tuinen *et al.*, 1997; Yen *et al.*, 1997; Galpaz *et al.*, 2008), y aumentan el contenido de carotenoides totales, mejorando el color del tomate.
Intense pigment (Ip)	Se originó como segregante de *S. chmielewskii*, y fue retrocruzado en *S. lycopersicum* (Rick, 1974). Con similares resultados que los genes *hp*, pero no sobre el mismo tipo de mutación del fitocromo (Mochizuki y Kamimura, 1985).
Green flesh (gf)	Altos niveles de carotenoides en tomate con este gen (Thompson y McCollum, 1957; Palmieri *et al.*, 1978). Sin embargo, la pulpa de este mutante en estado maduro es verde.
Crimson old gold gene (ogc)	Aumenta el licopeno a expensas del β-caroteno (Thompson, 1964; Thompson *et al.*, 1967; Palmieri *et al.*, 1978; Lee y Robinson, 1980).
Delta (Del)	Tomates de color naranja debido a la acumulación de δ-caroteno a expensas del licopeno (Ronen *et al.*, 1999)
Gen recesivo *tangerine (t)*	Los tomates tangerine contienen una pequeña cantidad de β-caroteno o licopeno y tienen un sorprendente color naranja. “Golden Jubilee” es uno de los tomates naranjas que se comercializan (Peirce *et al.*, 1992; Gardner, 1993; Isaacson *et al.*, 2002).
Beta (B) y gen modificador MO_B	Un tomate con este gen contiene altos niveles de β-caroteno a expensas del licopeno (Kohler *et al.*, 1947; Tomes *et al.*, 1954).
Cnr	En este mutante, la biosíntesis de carotenoides es muy baja y en estado maduro el fruto tiene pericarpo blanco con una consistencia anormal (Thompson *et al.*, 1999).

I.4.3. Uso de la variabilidad presente en germoplasma de tomate y especies relacionadas.

La otra forma de incrementar el contenido de vitaminas y carotenoides en tomate es el uso de germoplasma del género *Solanum* sección *lycopersicum* con un alto contenido de componentes nutraceúticos como parental donante en programas de mejora. Para llevar a cabo esta estrategia, se necesitan fuentes de variabilidad con elevado contenido en antioxidantes. En el contenido de carotenoides, se han observado grandes variaciones entre distintos cultivares de tomate (β-caroteno varió de 0,5 a 20 y el licopeno de 8 a 250 mg kg^{-1} de peso fresco) (Abushita *et al*., 1997; 2000; Gómez *et al*., 2001; Francis y Berry, 2002; Raffo *et al*., 2002; Hanson *et al*. 2004; Garcia y Barrett, 2006; Lenucci *et al*., 2006; Kumar *et al*., 2007; Saha *et al*., 2010; Chandra y Ramalingam, 2011). En cultivares de tomate para industria, se encontraron variaciones entre 1.000 y 2.000 mg kg^{-1} de peso seco para licopeno (López *et al*., 2001). Las entradas de *Solanum pimpinellifolium* de la colección del Banco de Germoplasma del Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV), mostraron un contenido medio de licopeno cinco veces mayor que aquellos valores encontrados en tomate cultivado (Fernández-Ruiz *et al*., 2002). Estos rangos de variación, en cambio, no han sido observados en variedades de tomate para procesado de la Unión Europea (Zanetti, 1997). Así que, las entradas de *S. pimpinellifolium* del COMAV demuestran ser una fuente de variabilidad prometedora para aumentar el contenido de licopeno en los programas de mejora de tomate. También parece prometedora la utilización de *Solanum lycopersicum* var *cerasiforme* (tomates tipo cherry) para la mejora de antioxidantes o su uso per se (Raffo *et al*., 2002; Lenucci *et al*., 2006).

En cuanto a los niveles de vitamina C varían considerablemente con las especies de tomate, desde 80 mg kg^{-1} de peso fresco en variedades cultivadas hasta 1.190 mg kg^{-1} de peso fresco en especies silvestres como *Solanum peruvianum* (Stevens y Rick, 1986), o 1.113 mg kg^{-1} de peso fresco en *S. pimpinellifolium* (Galiana-Balaguer *et al*., 2001). Para distintos cultivares de tomate, el rango de variación observado para contenido de vitamina C fue de 100 a 480 mg kg^{-1} de peso fresco (Abushita *et al*., 1997; 2000; Hagimori *et al*., 2005; Kumar *et al*., 2007; Saha *et al*., 2010; Chandra y Ramalingan, 2011).

Se han encontrado varias fuentes de variabilidad muy interesantes por su alto contenido de vitamina C. Entre las especies de *Solanum*, S. *pimpinellifolium* y *S. esculentum* var *cerasiforme* parecen ser las de más interés (Hanson *et al*., 2004; Lenucci *et al*., 2006), ya que son las más próximas filogenéticamente a la especie cultivada, y esto permitiría una rápida recuperación de los caracteres agronómicos interesantes. En varios ensayos de cribado de la colección de germoplasma de *Solanum* sección *lycopersicon* del COMAV, se han caracterizado entradas de *S. pimpinellifolium* y *S. lycopersicum* var *cerasiforme* con contenidos en vitamina C entre 1.6 y 8 veces mayores que en los controles de *S. lycopersicum* (Roselló *et al*., 2000a, 2000b; Galiana-Balaguer *et al*., 2001; Adalid *et al*., 2008), lo que podría ser muy útil en programas de mejora.

I.4.3.1. Influencia del ambiente.

Otro aspecto importante a considerar en los programas de mejora del valor nutraceútico en tomate, es la influencia del ambiente en la expresión del carácter. Esta influencia del ambiente, determina la herabilidad y el éxito en la selección en los programas de mejora. Se ha visto que la localidad y el ciclo de cultivo, junto el cultivar y el estado de maduración del fruto influyen en el contenido de antioxidantes (Anza *et al*., 2006; Garcia y Barrett, 2006; Kumar *et al*., 2007; Aldrich *et al*., 2010).

El efecto que tienen la radiación y la temperatura sobre el contenido de carotenoides en el tomate es significativo. Temperaturas por debajo de 12ºC inhiben fuertemente la biosíntesis de licopeno, mientras que temperaturas sobre 32ºC la detiene totalmente (Leoni, 1992). Dentro de estos rangos de temperatura, el β-caroteno aumenta (Baqar y Lee, 1978; Grierson y Kader, 1986). Las altas temperaturas (35ºC) inhiben específicamente la acumulación de licopeno estimulando la conversión de licopeno a β-caroteno (Dumas *et al*., 2003). En el rango de temperaturas favorables (22-25ºC), la síntesis de licopeno y demás carotenos pueden aumentarse con una suplementación en la iluminación del fruto del tomate durante el estadío de maduración (McCollum, 1954). Por tanto, frutos que crecieron bajo cubiertas de cristal o plástico acumularon menos β-caroteno que aquellos cultivados al aire libre (Cabibel y Ferry, 1980), pero hay que tener en cuenta el efecto de la interacción genotipo x ambiente, ya que no todas las variedades responden igual al sistema de cultivo (Giuntini *et al*., 2005). Las interacciones con las altas temperaturas pueden ejercer una disminución del contenido

de antioxidantes y la aparición de escaldado (Rosales *et al*., 2006). Además, la acumulación de licopeno puede ser bloqueada con altas radiaciones.

Respecto a la acumulación de vitamina C, se ha constatado que existe una correlación directa de la vitamina C con las variaciones de temperatura (Liptay *et al*., 1986), sin embargo en otros ensayos no se vio una correlación clara (Lee y Kader, 2000; Raffo *et al*., 2006). La exposición a la luz favorece la acumulación de vitamina C en frutos de tomate (Hamner *et al*., 1945; Somers *et al*., 1951; Venter, 1977; López-Andreu *et al*., 1986; El-Gizawy *et al*., 1993), pero puede disminuir si los tejidos del fruto se exponen directamente a la radiación (650 W/m2) (Adegoroye y Jolliffe, 1987; Rosales *et al*., 2006). Además, se ha visto que las condiciones de estrés a las que esté sometida la planta, puede variar la expresión de los genes responsables de la acumulación de vitamina C (Ioannidi *et al*., 2009).

Los efectos de la disponibilidad hídrica, de los nutrientes minerales (nitrógeno, fósforo, potasio o calcio) y reguladores del crecimiento han sido estudiados también, pero los resultados son algunas veces contradictorios y a veces los datos están a menudo incompletos (Rosenfeld, 1999; Lee y Kader, 2000; Munne y Alegre, 2000; Dumas *et al*., 2003; Oke *et al*., 2005; Fanasca *et al*., 2006; Thyboa *et al*., 2006; Toora *et al*., 2006; Serio *et al*., 2007; Taber *et al*., 2008).

I.4.3.2. Control genético.

Conocer el control genético de los caracteres nutraceúticos que más nos interesan es necesario para poder manejarlos eficientemente en los programas de mejora de tomate. Sin embargo, algunos autores indican que muchos de estos caracteres son cuantitativos, con una heredabilidad pobre y con control poligénico (Khan *et al*., 1999).

Respecto al contenido de carotenoides, poca heredabilidad se ha encontrado (<0.3), estando los contenidos de licopeno y caroteno positivamente correlacionados (Saliba-Colombani *et al*., 2001; Causse *et al*., 2002). Causse *et al*. (2003) y Garg *et al*. (2008) encontraron que el licopeno se heredó de forma aditiva. En cambio, Rousseaux *et al*. (2005) encontró efectos dominantes, además de los efectos aditivos, para la acumulación de licopeno y sugirió la presencia de epistasia con la participación de 2 o más genes (modelo de interacción digénico). Con respecto a la acumulación de β-caroteno, se ha visto que algunos tomates son naranjas debido al aumento de este caroteno a expensas del licopeno (Zhang y Stommel, 2000). La pigmentación observada

en los frutos de las líneas isogénicas cercanas (NIL) y poblaciones interespecíficas fue consistente con un modelo de 2 genes, donde el gen dominante *B* aumenta el contenido de β-caroteno y está influenciado por un gen modificador, llamado *MoB*. Dhaliwal y Chahal (2005) encontraron un loci de caracteres cuantitativos (QTL) asociado al gen *B*. Algunos estudios han usado QTLs para entender la heredabilidad de estos caractates. Chen *et al*. (1999) mapearon y caracterizaron QTLs para varios caracteres de fruto en tomate en una población de retrocruzamiento de un cruce interespecífico entre *S. lycopersicum* y *S. pimpinellifolium*. Los resultados fueron consistentes con un modelo aditivo para la heredabilidad del contenido de licopeno. Saliba-Colombani *et al*. (2001) y Causse *et al*. (2002)**,** usando una población derivada del cruce intraespecífico entre una línea de tomate cherry con una intensidad de aroma bueno en general y una línea pura con un gusto común, pero con frutos más grandes, concluyó que el contenido de licopeno fue controlado por 2 QTLs en los cromosomas 4 y 11, y tres QTLs controlaron el contenido en carotenos en los cromosomas 2, 3 y 8. Además, para contenido en pigmentos, solo una pequeña parte de la variación fenotípica fue explicada. Respecto a los carotenoides, otros estudios consiguieron identificar 2 QTL independientes asociados a un aumento de color, siendo identificadas interacciones epistáticas entre ellos (Kabelka *et al*., 2004). También se ha comprobado que existen interacciones entre diferentes alelos (*alcobaça*, *crimson* y *high pigment*) (Araujol *et al*., 2002).

Con respecto a vitamina C, Bhatt *et al*., (1998, 2001) encontró cierto grado de heterosis entre diversos cultivares de *S. lycopersicum* (efectos heteróticos positivos alrededor de 50-60 % respecto a los padres) que pueden resultar interesantes para el desarrollo de híbridos comerciales. La magnitud de la varianza debida tanto a la capacidad combinatoria específica como general fue altamente significativa, indicando la importancia de acciones génicas aditivas y no aditivas de este carácter. Garg *et al*. (2008) mostró que la varianza genética no aditiva predominó en controlar el contenido del ácido ascórbico. Otros autores han encontrado segregaciones transgresivas para el carácter contenido en ácido ascórbico (Rousseaux *et al*., 2005). Causse *et al*. (2003) estudió el control genético de la calidad de tomate para consumo en fresco analizando atributos de calidad en 45 híbridos y sus 13 líneas parentales, cultivadas en dos ambientes contrastados. Concluyeron que el ácido ascórbico fue heredado de una manera aditiva. Recientes estudios han intentado identificar genes candidatos que afecten el contenido de ácido ascórbico en tomate (Zou *et al*., 2006; Stevens *et al*.,

2007), encontrando QTLs comunes para varias especies de tomate, aunque se necesitaría un mapeado más fino para comprobar si los genes identificados son responsables de los QTLs observados.

Resumiendo, parecen existir diferentes controles genéticos dependiendo del material vegetal utilizado como parental, haciendo patente la gran diversidad presente en germoplasma de tomate para acumulación de los componentes nutraceúticos estudiados. Además, la localidad, ciclo de cultivo y el estado de maduración del fruto tienen influencia en el contenido de carotenoides y vitaminas (Anza *et al*, 2006; Garcia and Barret, 2006). Por tanto si se seleccionasen nuevas fuentes de variación para estos compuestos, se requeriría llevar a cabo el estudio del control genético derivado de esa fuente, teniendo en cuenta la influencia del ambiente.

I.4.3.3. Marcadores moleculares.

Con el ambiente caracterizado y una cuantificación precisa de los componentes nutricionales, se pueden buscar marcadores moleculares para aumentar la velocidad del proceso de selección. Se han encontrado marcadores aplicados a contenidos de licopeno y β-caroteno: RAPD (Zhang y Stommel, 2000), AFLP (Zhang y Stommel, 2000; Francis *et al*., 2003), SCAR (Zhang y Stommel, 2001) y SNP (Yang *et al*., 2003). Debido a los avances en el estudio de la rutas biosintéticas se pueden encontrar trabajos en los que se han mapeado genes directamente relacionados con el contenido de antioxidantes de tomate (Liu *et al*., 2003; Zou *et al*., 2006), conocimientos que pueden ser utilizados para el desarrollo de marcadores moleculares altamente ligados al carácter en cuestión. De todos modos, aún se está lejos de relacionar secuencia con fenotipo. También se puede usar la información obtenida de los QTL para el desarrollo de marcadores (Causse *et al*., 2002; Stevens *et al*., 2007).

2. OBJETIVOS

Para llevar a cabo un programa de mejora del valor nutritivo y funcional del tomate para consumo en fresco, se ha comprobado que es necesario en primer lugar buscar nuevas fuentes de variabilidad en germoplasma de esta especie y especies relacionadas para alto contenido en los compuestos nutraceúticos seleccionados. Una vez establecida la existencia de variabilidad y realizada una selección previa, debería llevarse a cabo una evaluación de aquellas entradas preseleccionadas en distintos ambientes comprobando la influencia y la importancia en la expresión del fenotipo del genotipo, ambiente y su interacción, comprobando si existe posibilidad de una mejora conjunta para los caracteres seleccionados. A continuación se debería realizar el estudio del control genético derivado de cada entrada seleccionada, ya que como se ha visto, la diversidad de controles genéticos es amplia dependiendo de los materiales vegetales utilizados. Además para agilizar la evaluación y la selección tanto de los procesos de cribado como de programas de mejora, podría resultar útil el desarrollo de protocolos de análisis más rápidos y precisos que los que se utilizan habitualmente. Por lo que los objetivos específicos de la presente tesis son:

1. Evaluación y selección de entradas de tomate (*Solanum* section *lycopersicon*) por su contenido en licopeno, β-caroteno y ácido ascórbico.

2. Evaluación del genotipo, ambiente y su interacción en la acumulación de carotenoides y ácido ascórbico en germoplasma de tomate.

3. Analizar el control genético de la acumulación de β-caroteno y ácido ascórbico derivada de una entrada tipo cherry de frutos naranjas amarronados.

4. Determinación rápida de los carotenoides prominentes de frutos de tomate por CEC usando columnas monolíticas basadas en esteres de metacrilato.

III. ARTÍCULOS QUE INTEGRAN ESTA TESIS

III.1. EVALUACIÓN Y SELECCIÓN DE ENTRADAS DE TOMATE (*SOLANUM* SECCIÓN *LYCOPERSICON*) POR SU CONTENIDO EN LICOPENO, β-CAROTENO Y ÁCIDO ASCÓRBICO.

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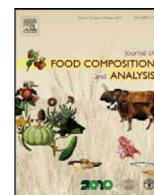

Original Article

Evaluation and selection of tomato accessions (*Solanum* section *Lycopersicon*) for content of lycopene, β-carotene and ascorbic acid

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ABSTRACT

Tomato has been identified as a food of great interest given its nutritional and bioactive components (mainly lycopene, β-carotene and ascorbic acid) and its high consumption rate all year round. Previous works have indicated that some local tomato cultivars and accessions of related species could have great potential, and even as nutraceutical foods. Nevertheless, most local cultivars have disappeared from fields because they have been replaced by hybrids and modern cultivars which produce higher yields and are more disease-resistant. In this work, 49 accessions of underutilized tomato or related species are evaluated in order to recover their use (directly in fields or as variability sources to obtain new cultivars) and increase agrobiodiversity. Fourteen accessions of the cherry type and two of the common tomato type were selected for their high and balanced nutritional properties, causing them to be of great interest for direct human consumption (especially BGV008057, BGV006863 and BGV008060). Furthermore, BGV008365 and BGV012627 (cherry types with over 1.5 times the normal average ascorbic acid content) as well as BGV008166 (*Solanum pimpinellifolium* accession which presented more than nine times the normal average lycopene content) would be of interest as donor parents for breeding programmes to increase the nutrition properties of commercial varieties.

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1. Introduction

In recent times, Nutrition Science has moved on from the classical concept of avoiding nutrient deficiencies to the concept of 'optimal' nutrition. The research focus has shifted more to the identification of biologically active components in foods that have the potential to optimise physical well-being and which may also reduce the risk of disease. Many traditional food products, including fruits and vegetables, have been found to contain components with potential health benefits. Within this group, tomato has been identified as a functional and "nutraceutical" food (Canene-Adams et al., 2005; Jack, 1995). A nutraceutical is any substance considered a food, or part of a food, that provides medical or health benefits, including disease prevention and treatment (Jack, 1995).

In the case of tomato, its high consumption all year round makes it one of the main sources of minerals, vitamins and antioxidants in many countries (Esquinas-Alcázar and Nuez, 1995). Ascorbic acid may play a key role in delaying the pathogenesis of a variety of degenerative diseases, such as cardiovascular disease, certain cancers, cataracts and it also prevents DNA mutation induced by oxidative stress (Byers and Guerrero, 1995; Lutsenko et al., 2002; Marchioli et al., 2001). Lycopene and β-carotene are the tomato carotenes which present the highest nutritional value. Specifically, lycopene reduces several cancer types and the risk of heart attack (Canene-Adams et al., 2005; Kun et al., 2006; Omoni and Aluko, 2005). β-carotene is a provitamin A carotenoid and its deficiency can cause xerophthalmia, blindness and premature death (Mayne, 1996).

All of these benefits to human health demonstrate the importance of tomatoes and, as a result, this vegetable is in increasing demand. In recent decades, however, agricultural industrialisation has led to a reduction in the number of cultivars used, which has resulted in a decline in the broad diversity of organoleptic and nutritional quality characteristics. Furthermore, most of the traditional varieties are disappearing worldwide because they are being replaced by modern cultivars. In this sense, the Food and Agriculture Organization of the United Nations (FAO)

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has considered the planet's genetic resources to be important to agriculture, health, environment and trade. FAO's nutrition work has always included elements of biodiversity in its field and normative operations, and with its compilations of wild, neglected and underutilized genetic resources used for food.

In recent years, FAO and Bioversity International (formerly the International Plant Genetic Resources Institute) are leading a new international initiative on biodiversity for food. The overall aim is to promote the sustainable use of biodiversity in programmes contributing to food security and human nutrition, and to thereby raise awareness of the importance of this link for sustainable development (Toledo and Burlingame, 2006). Following these guidelines, this study will contribute to bring new insight in the research field of biodiversity, nutrition and food composition, evaluating a collection of tomato cultivars or related accessions to identify those that will have the potential to become conventional foods of the future-useful parents in breeding programs, convenient sources of income, and the vehicles for improved nutrition and increased food supply.

2. Materials and methods

2.1. Plant material

A total of 49 accessions of tomato germplasm from 24 countries on 4 continents, provided by the Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV) genebank, were evaluated. Fourteen accessions of common tomato types (*Solanum lycopersicum* L.), 28 cherry type tomatoes (*S. lycopersicum* var. *cerasiforme* L.) and 7 accessions of small fruit tomato related species (*Solanum pimpinellifolium* L.), which represent a wide diversity of fruit shapes and colours, were studied (Table 1). Two

Table 1
Characteristics and lycopene, β-carotene and ascorbic acid (AsA) contents (mean ± standard deviation, $n = 36$) of the *Solanum* accessions evaluated.

Accession	Sp	Origin	Fruit colour and size[a]	AsA ($mg\,L^{-1}$)	Lycopene ($mg\,kg^{-1}$)	β-Carotene ($mg\,kg^{-1}$)	Ideal index[b]
CAMBRIA	1	Spain (control)	Red, 4	85 ± 29	29 ± 9	6.7 ± 0.7	40
BGV012406	1	Spain (control)	Light red, 5	91 ± 41	49 ± 13	10 ± 1	25
BGV003095	1	Spain	Orange, 6	104 ± 48	0.5 ± 0.5	12 ± 15	27
BGV004209	1	Czech Republic	Yellow, 5	118 ± 30	0.4 ± 0.1	0.79 ± 0.02	49
BGV007022	1	Ecuador	Pink, 5	183 ± 12	79 ± 1	8.60 ± 0.01	13
BGV008097	1	Peru	Pink, 3	95 ± 31	22.85 ± 0.03	1.7 ± 0.1	47
BGV009514	1	Iran	Pink, 6	143 ± 55	63 ± 15	6.4 ± 0.3	29
BGV009515	1	Cuba	Red, 4	115 ± 26	23.9 ± 0.8	6.1 ± 0.4	37
BGV009518	1	Kyrgyzstan	Pink, 7	71 ± 3	74 ± 14	4.3 ± 0.8	43
BGV009529	1	Vietnam	Pink, 4	143 ± 41	35 ± 1	8.3 ± 0.1	26
BGV011359	1	Taiwan	Pink, 5	51 ± 7	24.5 ± 0.2	7.3 ± 0.2	44
BGV011512	1	Portugal	Yellow, 4	127 ± 13	1 ± 1	13.14 ± 0.01	17
BGV012344	1	The Philippines	Light pink, 4	160 ± 38	20.5 ± 0.3	3.89 ± 0.06	41
BGV012619	1	Taiwan	Red, 6	85.2 ± 0.6	51.7 ± 0.1	3.3 ± 0.1	45
BGV012620	1	Taiwan	Strawberry-reddish, 3	136 ± 33	52 ± 25	6.0 ± 0.1	32
BGV012630	1	Ethiopia	Yellow, 5	77 ± 58	0.5 ± 0.4	2.51 ± 0.06	48
BGV006753	2	Ecuador	Orange-reddish, 2	150 ± 77	91 ± 2	5.0 ± 0.6	28
BGV006777	2	Ecuador	Strawberry-reddish, 2	145 ± 5	102 ± 2	7 ± 2	18
BGV006824	2	Ecuador	Strawberry-reddish, 3	137 ± 70	28 ± 1	4.9 ± 0.3	38
BGV006825	2	Ecuador	Strawberry-reddish, 3	159 ± 35	47 ± 1	2.9 ± 0.3	39
BGV006857	2	Ecuador	Red, 2	131 ± 31	30.8 ± 0.5	3.9 ± 0.2	42
BGV006863	2	Ecuador	Orange, 2	162 ± 33	89 ± 2	12.1 ± 0.4	2
BGV006872	2	Ecuador	Orange-reddish, 1	57 ± 4	94.8 ± 0.3	7.1 ± 0.3	34
BGV006875	2	Ecuador	Pink, 2	132 ± 52	117 ± 4	8.0 ± 0.4	14
BGV006896	2	Ecuador	Orange-reddish, 1	169 ± 35	51 ± 7	7 ± 2	21
BGV006923	2	Ecuador	Orange-reddish, 3	113 ± 28	88 ± 7	11.8 ± 0.6	10
BGV008008	2	Bolivia	Orange, 2	52 ± 16	0.5 ± 0.3	0.94 ± 0.03	51
BGV008033	2	Peru	Strawberry-reddish, 2	142 ± 35	36 ± 4	8.8 ± 0.6	22
BGV008051	2	Mexico	Red, 1	155 ± 42	54 ± 0.7	11.39 ± 0.01	9
BGV008057	2	Malaysia	Strawberry, 2	231 ± 27	78.9 ± 0.2	13 ± 1	1
BGV008060	2	Peru	Orange, 2	166 ± 10	82 ± 1	11.7 ± 0.3	5
BGV008061	2	Mexico	Orange-reddish, 2	234 ± 41	60 ± 3	8 ± 1	11
BGV008065	2	Peru	Strawberry, 3	131 ± 30	31 ± 0.5	6.0 ± 0.1	35
BGV008070	2	Mexico	Yellow, 1	233 ± 76	1.4 ± 0.6	8.4 ± 0.1	23
BGV008109	2	Peru	Orange-reddish, 1	164 ± 18	88 ± 0.2	9.9 ± 0.3	7
BGV008148	2	Ecuador	Deep red, 1	85 ± 1	167 ± 2	8.6 ± 0.9	19
BGV008169	2	China	Yellow, 2	38 ± 3	0.7 ± 0.1	2.6 ± 0.1	50
BGV008224	2	Nicaragua	Pink, 2	196 ± 4	92 ± 0.4	7.8 ± 0.3	8
BGV008226	2	Panama	Pink, 2	141 ± 16	58 ± 3	7.5 ± 0.7	24
BGV008354	2	Costa Rica	Red, 3	299 ± 12	27 ± 1	12.3 ± 0.8	6
BGV009512	2	Guinea	Pink, 2	90 ± 35	30.5 ± 0.8	8.4 ± 0.4	33
BGV012627	2	Colombia	Red, 2	311 ± 118	58.0 ± 0.5	9.9 ± 0.3	4
BGV012639	2	Ecuador	Red, 2	200 ± 45	18.4 ± 0.1	10.1 ± 0.1	15
BGV012640	2	Peru	Red, 2	212 ± 45	11.7 ± 0.1	10.1 ± 0.2	16
BGV007825	3	Mexico	Orange, 1	44 ± 16	2 ± 2	7.5 ± 0.3	46
BGV007827	3	Mexico	Red, 2	38 ± 10	91.1 ± 0.5	7.6 ± 0.5	36
BGV008068	3	Peru	Red, 1	30 ± 1	120 ± 11	13 ± 1	20
BGV008166	3	Ghana	Red, 1	144 ± 23	271 ± 3	14.4 ± 0.1	3
BGV008230	3	Honduras	Red, 1	134 ± 37	7.7 ± 0.6	14.6 ± 0.1	12
BGV012625	3	Peru	Red, 2	105 ± 37	23 ± 2	8.8 ± 0.2	31
BGV012638	3	Ecuador	Deep red, 2	156 ± 3	82.2 ± 0.8	3.9 ± 0.2	30

Sp: species 1 = *S. esculentum*; 2 = *S. esculentum* var. *cerasiforme*; 3 = *S. pimpinellifolium*.
[a] Size: 1, very small; 2, small; 3, small-medium; 4, medium; 5, medium-large; 6, large; 7, very large.
[b] Ideal index: accession ranking according to its proximity to the "ideal accession" (best mean and balanced content of antioxidants) used as reference.

modern tomato cultivars with normal levels of ascorbic acid and carotenoids were included as controls: a commercial hybrid (Cambria from Seminis Vegetable Seeds Iberica, Almería, Spain) and a tomato experimental line (BGV012406) from the COMAV genebank.

2.2. Experimental design and growing conditions

Twelve plants of each accession were grown in the spring–summer cycle in Valencia, Spain. Plants were grown hydroponically in pots filled with coconut fibre in a glass greenhouse with automated climate control. The temperature control system maintains optimal temperature ranges between 15–18 and 20–25 °C (night/day). Plants were staked and pruned. The composition of the nutrient solution used was (mequiv./L): 4.0 Mg^{2+}, 1.96 Na^{+}, 8.0 K^{+}, 8.5 Ca^{2+}, 1.0 NH_4^{+}, 2.25 Cl^{-}, 11.86 NO_3^{-}, 1.5 $H_2PO_4^{-}$, 7.50 SO_4^{2-}, and 0.5 HCO_3^{-}. Micronutrients were added using a commercial mixture (Nutrel C, Phosyn, Jaén, Spain) containing the following elements (mM): Cu, 0.76; Fe, 20.15; Mn, 9.01; Zn, 1.38; B, 9.71; and Mo, 0.31. The EC was 2.35 dS/m and the pH was 5.5. Fertirrigation was scheduled to obtain a daily mean drainage of 40%. Environmental variability in the greenhouse was reduced by means of a completely randomised plot design (three plots of four plants for each accession).

2.3. Sampling

Uniformly ripe, healthy fruits, at the red-ripe stage were harvested (Hanson et al., 2004; Kuti and Konuru, 2005; Lenucci et al., 2006). Accessions with fruits that are not red were harvested when fruits reached maximum colour intensity at ripe stage. A total of 5–20 representative fruits were collected from each plant (only from the first 3 trusses) to minimise intraplant variability (Borja et al., 1998). The homogenization of tomato sample was immediately carried out in volumes of 50 mL in cold bath at 4 °C and low light (to minimise antioxidant loss). After that, ten aliquots of 1.5 mL of each sample were frozen at −80 °C in cryovials (Daslab, Barcelona, Spain) until analysis. The laboratory homogenizer (Diax 900, Heidolph, Germany) was used with a generator 6G to disrupted seeds, skin and pulp which were blended to particle sizes <0.4 mm according to the manufacturer's technical specification.

2.4. Ascorbic acid determination

Ascorbic acid was quantified by Capillary Zone Electrophoresis (Galiana-Balaguer et al., 2001) using a P/ACE System MDQ (Beckman Instruments, Fullerton, USA), controlled by the Beckman 32 Karat V5 software. A 2 g sample was thawed in the dark in a refrigerator (K4270, Liebherr-International, Bulle, Switzerland) at 4 °C and centrifuged at 12,500 rpm in a refrigerated centrifuge (Centrifuge 5415R, Eppendorf, Hamburg, Germany). The supernatant was diluted in 2% metaphosphoric acid (Sigma Chemical, St. Louis, USA) to avoid ascorbic acid oxidation (Galiana-Balaguer et al., 2001). A known concentration (100 mg L^{-1}) of potassium hydrogen phthalate (Sigma Chemical, St. Louis, USA) was added in each sample as an internal standard to correct analysis variability. The stock solutions of metaphosphoric acid and internal standard were refrigerated at 4 °C when they were added to the samples to avoid loss of ascorbic acid due to room temperature. Sample extracts were filtered through a 0.2 mm filter membrane (Millipore, Bedford, USA) prior to injection. Uncoated fused-silica capillaries (31.2 cm of total length, 21 cm of effective length, 50 μm i.d.) were used (Polymicro Technologies, Phoenix, USA). Hydrodynamic injection of samples was carried out at 0.5 psi during 5 s. The detection wavelength was 254 nm. Separation was performed at −15 kV and 25 °C. Three analytical replicates per sample were made. The intra-day and inter-day precision was calculated as a CV of 7.8% and 8.5%, respectively.

2.5. Carotenoid determination

Determination was based on a spectrophotometric analysis following the method originally developed by Zscheille and Porter (1947) and improved by Rousseaux et al. (2005) using a spectrophotometer with double-beam operation (model Lambda-25, PerkinElmer, Waltham, USA) that allows control to be measured and corrected samples in real-time. The samples were thawed in the dark in a refrigerator (K4270, Liebherr-International, Bulle, Switzerland) at 4 °C to avoid carotenoid oxidation. Carotenoid extractions were performed once in 0.1 g of thawed samples, which were shaken (Platform rocker STR6, Stuart, Staffordshire, UK) for 1 h using 7 mL of organic solvents (ethanol:hexane, 4:3) (Sigma Chemical, St. Louis, USA). The extractions were conducted in the dark to prevent light-induced carotenoid oxidation.

Afterwards, 1 mL of distilled water was added to separate organic solvent layers and 0.5 mL of the upper layer (hexane phase) was recovered and refrigerated at 4 °C to avoid carotenoids loss. To obtain lycopene concentrations, a calibration line (r^2 = 0.998) which relates lycopene concentrations from standards (Sigma Chemical, St. Louis, USA) and absorbance at 510 nm was calculated. For β-carotene concentrations we calculated a calibration plane (r^2 = 0.987) which relates the concentrations from standards (Sigma Chemical, St. Louis, USA) and absorbances at 452 nm (positive correlation) and 510 nm (negative correlation). The lycopene interference in β-carotene concentrations calculation was thereby minimized. For calibration, seven standards with joint concentrations (randomly paired up) of lycopene and β-carotene were used. Three analytical replicates per sample were made. This method was validated with standard reference material BCR485 (lyophilized mixed vegetables) from the Institute of Reference Materials and Methods (Geel, Belgium). In order to carry out this validation, 2 g of the BCR485 were weighted and rehydrated with 5 mL distilled water at room temperature for 5 min. The carotenoid extraction and analysis were done using the methodology explained above.

2.6. Statistical methods

In addition to observing averages, a graphical multivariate statistical analysis, using the biplot method (Gabriel, 1971; Yan and Kang, 2003), was done to more easily study the relationships between accessions assayed and the antioxidant content and to better evaluate and select accessions of interest as packages of functional traits (Yan and Kang, 2003; Yan and Fregeau-Reid, 2008). In GGE biplot analysis, singular value decomposition (SVD) of the two-way data table of accessions (rows) and traits (columns) was used. In this SVD the singular values are entirely partitioned into row eigenvectors to preserve the row metric in order to graphically compare genotypes (Yan and Kang, 2003). However, prior to SVD, the original two-way data table must be adequately preprocessed (centered and scaled). The data centering was done in order to use the best model to show differences between accessions:

$$p_{ij} = y_{ij} - \mu - \beta_j = \alpha_i + \phi_{ij}$$

where y_{ij} is the phenotypic value of each cell of the two-way trait table, μ the grand mean, α_i the accession (row) main effect, β_j the trait (column) main effect, and ϕ_{ij} the specific interaction between the last two factors.

On the other hand, data standardization is essential when the traits have different units or scales. We use the standard deviation for column (trait) as scaling factor in order to have the same weight (importance) in all the traits.

Additionally, for a better global accession evaluation, an ''ideal index'' which relates the mean performance (average values of traits) and balance (deviation from the average composition for all traits) of the accessions tested, was used. This ideal index, constructed from GGE stability computations (Yan and Kang, 2003), was used to graphically rank the tested accessions in a rotated GGE biplot axes for an easy accession comparison and selection. All GGE biplot analyses and graphics were carried out with the GGE biplot software (licensed by Dr. Weikai Yan, Canada).

3. Results and discussion

In the validation test of carotenoid quantification, the lycopene concentration (mean ± SD) obtained with the spectrophotometric method was 14.27 ± 0.99 and 14.14 ± 2.56 mg kg^{-1} dry weight intra-day and inter-day, respectively. These results were very similar to the value given in certificate of analysis of BCR485 (14.2 mg kg^{-1} dry weight). On the other hand, the spectrophotometric determination of the β-carotene content in the BCR485 was 44.74 ± 1.39 and 45.63 ± 2.07 mg kg^{-1} dry weight, intra-day and inter-day, respectively, which were values higher than the certified one (25.06 mg kg^{-1} dry weight).

Nevertheless, this fact has a simple explanation. The BCR485 is a mix of three vegetables (tomato, carrot and maize) with the presence of some carotenoids, as α-carotene, not present in tomato (Hart and Scott, 1995). This carotenoid has similar peaks of absorbance in hexane to that of β-carotene so its absorbance distorts our calculations of β-carotene. Obviously, the total β-carotene content that we obtain is not the sum of real α-carotene (9.80 mg kg^{-1} dry weight) and β-carotene content because, according to the Lambert–Beer law, as α-carotene has a higher extinction coefficient in hexane (145.5 mol^{-1} L^{-1}, at 446 nm) than β-carotene (136.91 mol^{-1} L^{-1}, at 452 nm) (Thumhan et al., 1988) use of functions based on β-carotene extinction coefficient overestimate the content of α-carotene. Despite it all, we have not found references reporting the presence of α-carotene in tomato (obviously excluding transgenic tomato which is not used in this study). So, if there is no α-carotene in tomato samples there is no problem with using the spectrophotometrical method.

On the other hand, in spite of the fact that other substances can absorb light in the same spectral region as β-carotene, normally in tomato samples none of these other carotenoids are detected at a completely mature stage (or they are present only as traces) and only small quantities of xanthophylls (mainly lutein) are present (Hart and Scott, 1995). Nevertheless, with the extraction procedure used, xanthopylls are retained in the lower organic phase (ethanol phase) which was discarded (Clausen and McCoord, 1936), so their possible interference could be considered as negligible. Moreover, the intra-day and inter-day precision of the lycopene (CV of 7.0% and 7.6%, respectively) and β-carotene (CV of 3.1% and 4.5%, respectively) spectrophotometric method used in this work was slightly better than those reported for the BCR485 certified product (CV of 10.0% for lycopene and 6.4% for β-carotene; Finglas et al., 1998).

So, despite the limitations of the spectrophotometric method for carotenoid quantifications, we consider that this technique is valid for the objective of the study that is to obtain a first tomato accession comparison of carotenoid content. In this sense, its simplicity, fast sample preparation and lower apparatus requirements will represent an important advantage for large field screening assays where high number of tomato samples will be evaluated.

Regarding the accessions evaluation results, the analytical results for ascorbic acid, lycopene and β-carotene content showed a high variability between accessions (Table 1), indicating that the selection of accessions with a desired content of bioactive components is possible.

For ascorbic acid, the controls used in this study (Cambria and BGV012406) showed ascorbic acid contents similar to the commonly accepted average level in the commercial tomato (200 mg kg^{-1}) (Gould, 1992), as in previous works (Roselló et al., 2006). Nevertheless, in the present study, their content was nearly half of this value (Table 1). So, we may consider that the environmental factors during this trial influenced and diminished ascorbic acid accumulation, as Dumas et al. (2003) and Toor et al. (2006) have suggested. The accession of the common tomato type with the highest content was BGV007022 (more than twice the content of controls). In other works (Abushita et al., 2000), some known cultivars have shown contents around the commonly accepted average level in the commercial tomato (Gould, 1992). However, the accessions of cherry type tomato presented the highest ascorbic acid content, particularly BGV012627 and BGV008354. These accessions presented more than 3 times the ascorbic acid content than controls and were also higher than the best values obtained by other researchers in cherry tomato cultivars (Lenucci et al., 2006).

The average lycopene content of raw tomatoes has been reported at 30 mg kg^{-1} (Holden et al., 1999; Kuti and Konuru, 2005). Cultivars broadly grown and consumed in Spain, such as 'Rambo', 'Daniella' and 'Durina', have shown contents of 32, 36 and 65 mg kg^{-1}, respectively (Martinez-Valverde et al., 2002). So, we may consider the range between 30 and 60 mg kg^{-1} to be the most common lycopene content under our conditions. Despite the reported influence of environmental factors (temperature, light, growing season and location), and the agricultural techniques used in lycopene accumulation (Dumas et al., 2003; Rosenfeld, 1999; Toor et al., 2006), in this trial, our controls, Cambria and BGV012406, showed a normal lycopene content (29 and 49 mg kg^{-1}, respectively). So we believe that our trial conditions did not diminish the potential lycopene accumulation of the accessions tested. Regarding common tomato type accessions, BGV007022 and BGV009518 accumulated more than 1.5 times the lycopene content of the best control (BGV012406). Cherry tomato types usually showed a higher lycopene content than common tomatoes with values that were generally close to the upper level of the normal considered range (60 mg kg^{-1}). For example, 'Rubino Top', 'Gardener's Delight', 'Naomi' and 'Sugar Lump' showed contents of 43, 48.9, 60 and 63.6 mg kg^{-1}, respectively (Kuti and Konuru, 2005; Lenucci et al., 2006). These lycopene contents are higher than the accepted average content, but in the same order of magnitude. In our trial, the cherry tomato type accessions BGV008148 and BGV006875 were 3.4 and 2 times higher, respectively, than the best control content. However, the most outstanding accession for lycopene content was BGV008166 (*S. pimpinellifolium*), with a content 9.3 times higher than the hybrid control and 5.5 times higher than the BGV012406 local cultivar. This lycopene level means that this accession could be an interesting donor parent in breeding programmes for developing new cultivars.

The average β-carotene content of raw tomatoes has been reported at 3.9 mg kg^{-1} (Holden et al., 1999). Abushita et al. (2000) found a range of between 2.9 mg kg^{-1} (cv 'Fanny') to 6.2 mg kg^{-1} (cv 'Monika'). The β-carotene contents of our controls were 6.7 and 10 mg kg^{-1} for Cambria and BGV012406, respectively. As in previous trials, these controls have shown lower β-carotene contents (Adalid et al., 2008), and we may consider that the environmental and/or agricultural practices (Abushita et al., 2000; Dumas et al., 2003; Raffo et al., 2006) of our trial have enhanced the potential accumulation of β-carotene. Regarding controls, this increased β-carotene accumulation was approximately 1.5 times higher than the normal average content. Of all the accessions tested, BGV011512 and BGV003095 among the common tomato types presented remarkable values, and included yellow and

orange tomatoes, respectively, with very low lycopene content. They presented twice the Cambria content and more than 20% of the BGV012406 control. In the cherry tomato type, BGV008057 showed the highest content ($13\ \mathrm{mg\,kg^{-1}}$). In previous works, cultivars 'LS203' and 'Corbus' showed the highest content, this being around $10\ \mathrm{mg\,kg^{-1}}$ (Lenucci et al., 2006). The *S. pimpinellifolium* accessions, BGV008230 and BGV008166, were those with the highest contents. They presented more than twice the Cambria content, and 45% more than the BGV012406 content. However, the fact that their β-carotene content was only slightly above the best common tomato type accessions (BGV011512 and BGV003095) does not justify their use as donor parents in breeding programmes because of the greater laboriousness in the recovery of fruit weight.

Nevertheless, the average chemical analysis values do not completely exploit all the subjacent information to select cultivars and accessions with desirable contents of several antioxidants. In order to make easier this simultaneous evaluation of several components of nutraceutical quality and to make a better selection of the most desirable cultivars and accessions, we adopted multivariate statistical analyses using the GGE biplot methodology (Gabriel, 1971; Yan and Fregeau-Reid, 2008; Yan and Kang, 2003).

The GGE biplot analysis performed shows that the data fit well and account for 81.9% of the variability and, with appropriate views, could achieve direct and wide accession evaluations and comparisons (Yan and Rajcan, 2002). For this purpose, we used an average-antioxidant evaluation (AAE) view of the GGE biplot in which a singular value partitioning with accession-metric preserving (SVP = 1) was used (Yan and Tinker, 2005). In this plot (Fig. 1), the tomato accessions tested should be evaluated for both the mean antioxidant accumulation and equilibrated content of all the antioxidants studied.

This figure is constructed to relate the accessions tested with an "ideal accession" (the centre of the concentric circles) which have both a high mean antioxidant accumulation and an equilibrated content of all the antioxidants studied (Yan, 2001). The single-arrowed line is the AAE abscissa which indicates a higher mean

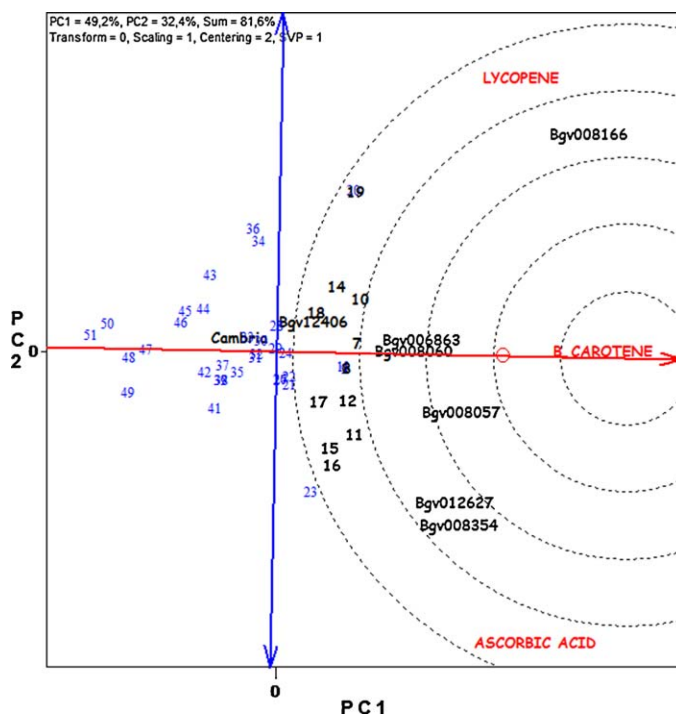

Fig. 1. Accession evaluation based on an ideal entry with a high and equilibrated antioxidant (lycopene, β-carotene and ascorbic acid) content. The position of the accession on the plot is on the left end of its label or number.

antioxidant content. Thus, BGV008166 presented the highest mean antioxidant content, followed by BGV008354, BGV008057, BGV012627, etc.; the BGV012406 control presented a mean antioxidant content similar to the overall mean, and is therefore a good reference control located near the origin of the axis; BGV008008 (51) showed the lowest mean antioxidant content. The double-arrowed line is the AAE ordinate; it indicates a greater disequilibrium in antioxidant content in either direction. Thus, BGV008166 (higher lycopene and lower ascorbic acid content than the mean) and BGV008354 (higher ascorbic acid and lower lycopene content than the mean) were highly disequilibrated whereas BGV008060 was highly equilibrated.

Ranking the tomato accessions related to the "ideal accession" could offer an integral evaluation of their nutraceutical capabilities. Moreover, the accessions located closer to the "ideal accession" are more ideal (the highest mean antioxidant content in all the substances evaluated) than others (Yan, 2001). In Fig. 1, this scenario is graphically shown by the concentric circles centring on the "ideal accession". Evaluating the tomato accessions revealed two interesting groups (located on the right hand side of the AAE axis and, consequently, with a higher mean antioxidant content than controls).

The first group of the "more ideal" accessions includes all the accessions inside the fourth circle (1–6 in the ideal index). In this group, there are five accessions of the cherry tomato type (*S. lycopersicum* var. *cerasiforme*) and one *S. pimpinellifolium* accession, thus confirming previous reports which indicated that these species are the best sources of antioxidants with nutraceutical properties (George et al., 2004; Hanson et al., 2004; Lincoln et al., 1943). The cherry type accessions included in this first ideal group (BGV008057, BGV006863, BGV012627, BGV008060 and BGV008354) are very desirable for direct consumption given their high and balanced nutraceutical properties. These tomatoes are usually consumed raw in salads with olive oil, which increases the bioavailability of such molecules, and, consequently, their salutary effects on human health (Bohm and Bitsch, 1999). The *S. pimpinellifolium* accession (BGV008166) included in this group would prove to be desirable as a donor parent in breeding programmes to increase the lycopene content of commercial varieties. Similarly, BGV012627 and BGV008354 cherry type accessions could also be used as donor parents in breeding programmes to increase the ascorbic acid content of new varieties.

The second "ideal group" includes all the accessions inside the fifth concentric circle (7–20 in the ideal index). This group includes two common tomato type accessions (13 and 17 in the ideal index), 11 cherry tomato accessions and two *S. pimpinellifolium* accessions (12 and 20 in the ideal index). All these accessions are beyond the controls of antioxidant content. These *S. pimpinellifolium* accessions are of no interest as potential donor parents because, despite their equilibrated antioxidant content, they are not outstanding in at least one antioxidant content which is an essential requirement to start a breeding programme. The remaining accessions can be selected and used directly for human consumption because of their equilibrated and nutraceutical content.

4. Conclusion

In conclusion, this work has shown the great variability in the bioactive component content of tomato fruit that can be found in genebanks in underutilized cultivars and related species. All the accessions selected in this evaluation trial were of interest given their nutraceutical properties and their bioactive components, in connection with the increasing interest of consumers in the relationship between diet and health. In the near future, these accessions may be used directly for human consumption or in

breeding programmes of new cultivars, increasing the agrobiodiversity of our fields.

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References

Abushita, A.A., Daood, H.G., Biacs, P.A., 2000. Change in carotenoids and antioxidant vitamins in tomato as a function of varietal and technological factors. Journal of Agricultural and Food Chemistry 48, 2075–2081.

Adalid, A.M., Roselló, S., Cebolla-Cornejo, J., Nuez, F., 2008. Evaluation and selection of Lycopersicon accessions for high carotenoid and vitamin C content. In: Proceedings of the 15th Meeting of Eucarpia Tomato Working Group, Vol. 789, Bari, Italy, pp. 221–228.

Bohm, V., Bitsch, R., 1999. Intestinal absorption of lycopene from different matrices and interactions to other carotenoids, the lipid status, and the antioxidant capacity of human plasma. European Journal of Nutrition 38, 118–125.

Borja, A., Angosto, T., Capel, J., Abad, J., Anatasio, G., Lozano, R., 1998. Variaciones intra e interindividuales en componentes del sabor de tomate de consumo fresco y líneas silvestres. In: Proceedings of XI Jornadas de Selección y Mejora de Plantas Hortícolas, Córdoba, Spain, pp. 49–56.

Byers, T., Guerrero, N., 1995. Epidemiologic evidence for vitamin C and vitamin E in cancer prevention. American Journal of Clinical Nutrition 62, 1385S–1392S.

Canene-Adams, K., Campbell, J.K., Zaripheh, S., Jeffery, E.H., Erdman, J.W., 2005. The tomato as a functional food. Journal of Nutrition 135, 1226–1230.

Clausen, S.W., McCoord, A.B., 1936. The determination of carotene and xanthophyll by a single distribution between liquid phases. The Journal of Biological Chemistry 113 (1), 89–104.

Dumas, Y., Dadomo, M., Lucca, G.D., Grolier, P., 2003. Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. Journal of Science of Food and Agriculture 83, 369–382.

Esquinas-Alcázar, J.T., Nuez, F., 1995. Situación taxonómica, domesticación y difusión del tomate. In: Nuez, F. (Ed.), El cultivo del tomate. Ed Mundi Prensa, Madrid, Spain, pp. 13–42.

Finglas, P.M., Scott, K.J., Witthöft, C.M., Van Den Berg, H., De Froidmont-Görtz, I., 1998. The certification of the mass fractions of vitamins in four reference materials: wholemeal flour (CRM121), milk powder (CRM421), lyophilized mixed vegetables (CRM485) and lyophilized pigs liver (CRM487). Report EUR18320 EN. Belgium.

Gabriel, K., 1971. The biplot graphic display of matrices with application to principal component analysis. Biometrika 58, 453–467.

Galiana-Balaguer, L., Roselló, S., Herrero-Martínez, J.M., Maqueira, A., Nuez, F., 2001. Determination of l-ascorbic acid in *Lycopersicon* fruits by capillary zone electrophoresis. Analytical Biochemistry 296, 218–224.

George, B., Kaur, C., Khurdiya, D.S., Kapoor, H.C., 2004. Antioxidants in tomato (*Lycopersicon esculentum*) as a function of genotype. Food Chemistry 84, 45–51.

Gould, W.A., 1992. Tomato Production, Processing and Technology. CTI Publications, Baltimore, USA.

Hanson, P.M., Yang, R.Y., Wu, J., Chen, J.T., Ledesma, D., Tsou, S., Lee, T.C., 2004. Variation for antioxidant activity and antioxidants in tomato. Journal of the American Society for Horticultural Science 129, 704–711.

Hart, D.J., Scott, K.J., 1995. Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of vegetables and fruits commonly consumed in the UK. Food Chemistry 54, 101–111.

Holden, J.M., Eldridge, A.L., Beecher, G.R., Buzzard, I.M., Bhagwat, S., Davis, C.S., Douglass, L.W., Gebhardt, S., Haytowitz, D., Schakel, S., 1999. Carotenoid content of US Foods: an update of the database. Journal of Food Composition and Analysis 12, 169–196.

Jack, D.B., 1995. Keep taking the tomatoes—the exciting world of nutraceuticals. Molecular Medicine Today 1, 118–121.

Kun, Y., Lule, U.S., Xiao-Lin, D., 2006. Lycopene: its properties and relationship to human health. Food Reviews International 22, 309–333.

Kuti, J.O., Konuru, H.B., 2005. Effects of genotype and cultivation environment on lycopene content in red-ripe tomatoes. Journal of the Science of Food and Agriculture 85, 2021–2026.

Lenucci, M.S., Cadinu, D., Taurino, M., Piro, G., Dalessandro, G., 2006. Antioxidant composition in cherry and high-pigment tomato cultivars. Journal of Agricultural and Food Chemistry 54, 2606–2613.

Lincoln, R.E., Zscheile, F.P., Porter, J.W., Kohler, G.W., Caldwell, R.M., 1943. Provitamin A and vitamin C in the genus *Lycopersicon*. Botanical Gazette 105, 113–115.

Lutsenko, E.A., Carcamo, J.M., Golde, D.W., 2002. Vitamin C prevents DNA mutation induced by oxidative stress. Journal of Biological Chemistry 277, 16895–16899.

Marchioli, R., Schweiger, C., Levantesi, G., Tavazzi, L., Valagussa, F., 2001. Antioxidant vitamins and prevention of cardiovascular disease: epidemiological and clinical trial data. Lipids 36, S53–S63.

Martinez-Valverde, I., Periago, M.J., Provan, G., Chesson, A., 2002. Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicum esculentum*). Journal of the Science of Food and Agriculture 82, 323–330.

Mayne, S.T., 1996. Beta-carotene, carotenoids and disease prevention in humans. FASEB Journal 10, 690–701.

Omoni, A.O., Aluko, R.E., 2005. The anticarcinogenic and anti-atherogenic effects of lycopene: a review. Trends in Food Science and Technology 16, 344–350.

Raffo, A., La Malfa, G., Fogliano, V., Maiani, G., Quaqlia, G., 2006. Seasonal variations in antioxidant components of cherry tomatoes (*Lycopersicon esculentum* cv. Naomi F1). Journal of Food Composition and Analysis 19, 11–19.

Roselló, S., Galiana-Balaguer, L., Adalid, A.M., Nuez, F., 2006. Influencia del ambiente en la evaluación del contenido de vitamin C en germoplasma de *Lycopersicon*. Actas de Horticultura 45, 73–74.

Rosenfeld, H.J., 1999. Quality improvement of vegetables by cultural practices. International Symposium on Quality of Fresh and Fermented Vegetables 483, 57–67.

Rousseaux, M.C., Jones, C.M., Adams, D., Chetelat, R., Bennet, A., Powell, A., 2005. QTL analysis of fruit antioxidants in tomato using *Lycopersicon pennellii* introgression lines. Theoretical and Applied Genetics 111, 1396–1408.

Thumhan, D.I., Smith, E., Flora, P.S., 1988. Concurrent liquid-chromatographic assay of retinol, α-tocopherol, β-carotene, α-carotene, lycopene and β-cryptoxanthin in plasma, with tocopherol acetate as internal standard. Clinical Chemistry 34 (2), 377–381.

Toledo, A., Burlingame, B., 2006. Biodiversity and nutrition: a common path toward global food security and sustainable development. Journal of Food Composition and Analysis 19, 477–483.

Toor, R.K., Savage, G.P., Lister, C.E., 2006. Seasonal variations in the antioxidant composition of greenhouse grown tomatoes. Journal of Food Composition and Analysis 19, 1–10.

Yan, W., 2001. GGEbiplot—a Windows application for graphical analysis of multi-environment trial data and other types of two-way data. Agronomy Journal 93, 1111–1118.

Yan, W., Fregeau-Reid, J., 2008. Breeding line selection based on multiple traits. Crop Science 48, 417–423.

Yan, W., Kang, M.S., 2003. GGE Biplot Analysis. A Graphical Tool for Breeders, Geneticist and Agronomist. CRC Press, Boca Raton, USA.

Yan, W., Rajcan, I., 2002. Biplot evaluation of test sites and trait relations of soybean in Ontario. Crop Science 42, 11–20.

Yan, W., Tinker, N.A., 2005. An integrated system of biplot analysis for displaying, interpreting, and exploring genotype by environment interactions. Crop Science 45, 1004–1016.

Zscheille, F., Porter, J.W., 1947. Analytical methods for carotenes of *Lycopersicon* species and strains. Analytical Chemistry 19, 47–51.

III.2. EVALUACIÓN DEL GENOTIPO, AMBIENTE Y SU INTERACCIÓN EN LA ACUMULACIÓN DE CAROTENOIDES Y ÁCIDO ASCÓRBICO EN GERMOPLASMA DE TOMATE.

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Evaluation of the genotype, environment and their interaction on carotenoid and ascorbic acid accumulation in tomato germplasm

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Abstract

BACKGROUND: Tomatoes are an important source of antioxidants (carotenoids, vitamin C, etc.) owing to their high level of consumption. There is great interest in developing cultivars with increased levels of lycopene, β-carotene or L-ascorbic acid. There is necessary to survey new sources of variation. In this study, the potential of improvement for each character in tomato breeding programmes, in a single or joint approach, and the nature of genotype (*G*), environment (*E*) and *G* × *E* interaction effects in the expression of these characters were investigated.

RESULTS: The content of lycopene, β-carotene and ascorbic acid determined was very high in some phenotypes (up to 281, 35 and 346 mg kg^{-1} respectively). The important differences in the three environments studied (with some stressing conditions in several situations) had a remarkable influence in the phenotypic expression of the functional characters evaluated. Nevertheless, the major contribution came from the genotypic effect along with a considerable *G* × *E* interaction.

CONCLUSION: The joint accumulation of lycopene and β-carotene has a high genetic component. It is possible to select elite genotypes with high content of both carotenoids in tomato breeding programmes but multi-environment trials are recommended. The improvement of ascorbic acid content is more difficult because the interference of uncontrolled factors mask the real genetic potential. Among the accessions evaluated, there are four accessions with an amazing genetic potential for functional properties that can be used as donor parents in tomato breeding programmes or for direct consumption in quality markets.
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Keywords: *Solanum* section *Lycopersicon*; genetic resources; functional quality; lycopene; β-carotene; vitamin C; linear mixed models

INTRODUCTION

In developed country markets, such as those in Europe, there is a tendency to evolve from an agriculture focused on yield towards an agriculture focused on quality.[1] In these areas, with high spending power, consumers demand products with higher internal quality, which leads to the development of new higher-quality products. This is especially true for 'functional foods', which offer an interesting growth opportunity for the food industry.[2]

Tomato has moderate nutritional value but it is consumed all year round. It is one of the most important sources of antioxidants, such as vitamin C or carotenoids, which are protective against degenerative diseases.[3,4] In this context, during the last decade there has been increasing interest in the development of cultivars with increased levels of L-ascorbic acid or the main carotenoids present in tomato: β-carotene and lycopene. Cultivar such as 'DoubleRich' has twice as much vitamin C content or the 'high pigment' cultivars that are becoming popular in the tomato processing industry.[5] Several mutations have been identified related to the carotenoid content in tomato, but important organoleptic or agricultural deficiencies have limited their use[6,7] and it is necessary to survey new sources of variation.

Although several studies have focused on this objective,[5,8,9] the elevated influence of agronomic and environmental variables in the expression of characteristics of the functional value of tomato fruits[7,10] is yet to be determined. Not only does the environment play an important role in the system but it has been suggested that the genotype × environment (*G* × *E*) interaction would be substantial.[11] Therefore more studies on the contribution of different environments, genotypes and their interactions to the expression of properties of functional value should be carried out in

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Table 1. Characteristics of accessions evaluated

Accession	Sp.	Fruit characteristics	Origin
CDP8779	1	Large, light red	Valencia, Spain
CAMBRIA	1	Medium-size, red	Almeria, Spain
GEVORA	1	Medium-size, red	Badajoz, Spain
LA1563	1	Large, red	University of California
CDP2178	1	Medium-size, red	Piura, Perú
CDP7632	1	Medium-size, red	Loja, Ecuador
CDP2087	1	Large, red	Gran Canaria, Spain
CDP6957/A	1	Small, yellow	Alicante, Spain
CDP6957/R	1	Small, red	Alicante, Spain
CDP4777	2	Small, orange-brownish	Ipala, Guatemala
CDP7090	3	Very small, dark red	Piura, Perú
CDP1568	3	Very small, dark red	Piura, Perú
CDP9822	3	Very small, dark red	Piura, Perú
CDP9999	3	Very small, yellow	Lambayeque, Perú

Sp., species; 1, *Solanum lycopersicum*; 2, *S. lycopersicum* var. *cerasiforme*; 3, *S. pimpinellifolium*.

order to select elite genotypes with more precision that enhances the accumulation of favourable compounds. Information on the structure and nature of $G \times E$ interactions is particularly necessary to determine if it is possible to develop 'high functional value' cultivars with high environmental stability or specific cultivars for specific target environments.

The objective of this study was to perform an evaluation of *Solanum* section *Lycopersicon* germplasm in different environments in order to elucidate the nature and structure of the genotype, growing environment and its interaction, and to identify the genotypic potential of these materials for direct use or as sources of variability in breeding programmes for lycopene, β-carotene and/or ascorbic acid accumulation in tomato fruits.

MATERIALS AND METHODS

Plant material

Five *Solanum lycopersicum* L. accessions, one *S. lycopersicum* var. *cerasiforme* L. and four *S. pimpinellifolium* L., representing a wide diversity of fruit shapes and colours, were studied (Table 1). Three modern tomato cultivars with normal levels of ascorbic acid and carotenoids and a high-pigment line were included as controls: CDP8779 (experimental line developed by COMAV, Valencia, Spain), Cambria (a hybrid commercialized by Seminis Vegetable Seeds, Almería, Spain), Gevora (a processing tomato variety developed by el Centro de Investigación 'La Orden-Valdesequera', Badajoz, Spain) and LA1563 (accession provided by TGRC, University of California, Davis, with enhanced carotenoid content[12] due to the Intense Pigment gene).

Experimental design and growing conditions

The trials were carried out in three growing environments representing common cycles and cultivation techniques. For a precise evaluation of genotype, environment and its interaction effects, clones of all the plants studied were used in each environment. A randomized complete block design was used with four blocks per environment, 14 plots per block (one per accession) and eight plants per plot. All the blocks of each accession had clones of the same eight plants in order to have a better estimate of block and environment effects.

Two sites of cultivation were used. Cultivation at Valencia was carried out in two different cultivation cycles (autumn–winter and spring–summer) in a glasshouse with automated climate control. Cultivation at Turis was carried out in the spring–summer cycle in the open air. In protected cultivation, heating systems (in the autumn–winter cycle) and heat dissipation systems (progressive shadowing and cooling in the spring–summer cycle) were used. In all the environments fertirrigation was scheduled daily and plants were staked and pruned properly. In order to gain information about climatic parameters influencing plant metabolism and growth, air temperature and photosynthetically active radiation (PAR) were recorded every 10 min using WatchDog weather stations (Spectrum Technologies Inc., Plainfield, IL, USA) equipped with temperature, quantum light PAR sensors and data logger.

Sampling

Uniformly ripe, healthy fruits at the red-ripe stage were harvested. Accessions with colours other than red were harvested when fruits reached maximum colour intensity. A total of 5–20 representative fruits (depending on the species) were collected from each plant only from the first three trusses to minimize intra-plant variability. Samples were blended at 4 °C and low light intensity to minimize antioxidant loss. A laboratory homogenizer (Diax 900, Heidolph, Germany) was used with a 6G generator to disrupt tissue to particle sizes <0.4 mm. Samples were stored at −80 °C until analysis.

Ascorbic acid determination

Ascorbic acid was quantified by capillary zone electrophoresis using a P/ACE System MDQ (Beckman Instruments, Fullerton, CA, USA). Two grams of sample were thawed in the dark at 4 °C and centrifuged at 12 500 rpm in a refrigerated centrifuge. The supernatant was diluted in 2% metaphosphoric acid to avoid ascorbic acid oxidation.[13] Potassium hydrogen phthalate (100 mg L^{-1}) was used as an internal standard. Sample extracts were filtered through a 0.2 mm filter membrane (Millipore, Bedford, MA, USA) prior to injection. Uncoated fused-silica capillaries (31.2 cm total length, 21 cm effective length, 50 µm i.d.) were used (Polymicro Technologies, Phoenix, AZ, USA). Hydrodynamic injection of samples was carried out at 0.5 psi for 5 s. The detection wavelength was 254 nm. Separation was performed at −15 kV and 25 °C. Three analytical replicates per sample were made.

Carotenoid determination

Determination was based on a spectrophotometric analysis[9] using a spectrophotometer with double-beam operation (model Lambda-25, Perkin-Elmer, Waltham, MA, USA). The samples were thawed at 4 °C. Carotenoid extractions were performed with 0.1 g of thawed samples, which were shaken for 1 h using 7 mL of organic solvents (ethanol–hexane, 4:3). The extractions were conducted in the dark to prevent light-induced carotenoid oxidation. Afterwards, 1 mL distilled water was added to separate organic solvent layers and 0.5 mL of the upper layer (hexane phase) was recovered and refrigerated at 4 °C to avoid carotenoid loss. A calibration line which relates standard concentrations and absorbance at 510 nm was used to obtain lycopene concentrations. For β-carotene, a calibration plane relating the concentrations from standards and absorbance at 452 nm (positive correlation) and 510 nm (negative correlation) was used. Seven standards with joint concentrations (randomly paired up) of lycopene and β-carotene were used for calibration. Three analytical replicates per sample were made.

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Table 2. Phenotypic content (mean ± standard deviation, mg kg^{-1} fresh weight) of lycopene (LYC), β-carotene (βCAR) and ascorbic acid (AsA) of accessions evaluated

	Location								
	Turis spring/summer			Valencia spring/summer			Valencia autumn/winter		
Accession	LYC	βCAR	AsA	LYC	βCAR	AsA	LYC	βCAR	AsA
CDP8779	77 ± 40	17 ± 4	150 ± 53	90 ± 31	14 ± 4	121 ± 46	71 ± 29	14 ± 3	67 ± 30
CAMBRIA	85 ± 23	13 ± 5	178 ± 62	103 ± 41	10 ± 4	161 ± 52	84 ± 19	14 ± 2	92 ± 32
GEVORA	124 ± 39	7 ± 2	148 ± 45	191 ± 59	7 ± 2	137 ± 34	136 ± 42	10 ± 2	56 ± 32
LA1563	113 ± 40	16 ± 5	116 ± 52	123 ± 43	11 ± 3	137 ± 64	101 ± 34	18 ± 4	71 ± 35
CDP2178	102 ± 32	9 ± 2	135 ± 38	143 ± 51	8 ± 2	142 ± 70	115 ± 33	12 ± 4	52 ± 28
CDP7632	70 ± 25	17 ± 4	261 ± 96	95 ± 37	11 ± 3	193 ± 40	89 ± 29	14 ± 3	136 ± 39
CDP2087	93 ± 36	12 ± 3	138 ± 79	104 ± 38	8 ± 2	113 ± 48	86 ± 32	13 ± 4	59 ± 26
CDP6957/A	1 ± 2	8 ± 2	206 ± 116	2 ± 3	7 ± 2	109 ± 78	1 ± 1	4 ± 1	36 ± 32
CDP6957/R	56 ± 22	20 ± 3	194 ± 91	107 ± 41	18 ± 3	143 ± 66	94 ± 35	19 ± 5	118 ± 57
CDP4777	65 ± 16	32 ± 6	346 ± 108	82 ± 26	29 ± 6	250 ± 80	104 ± 28	35 ± 7	331 ± 122
CDP7090	225 ± 80	18 ± 7	162 ± 96	227 ± 96	17 ± 5	139 ± 93	75 ± 4	18 ± 2	6 ± 5
CDP1568	173 ± 35	28 ± 5	113 ± 58	227 ± 95	16 ± 5	136 ± 94	185 ± 73	24 ± 5	57 ± 29
CDP9822	139 ± 41	24 ± 6	214 ± 139	169 ± 75	22 ± 7	191 ± 104	281 ± 2	22 ± 0	296 ± 30
CDP9999	2 ± 3	15 ± 4	229 ± 253	3 ± 5	11 ± 3	156 ± 125	2 ± 1	15 ± 5	10 ± 7

Data analysis

The mixed linear model used for the analysis of genotype *i* in environment *j* and block *k* inside environment *j* was

$$Y_{ijk} = \mu + G_i + E_j + GE_{ij} + B_{k(j)} + e_{ijk}$$

where Y = phenotypic value with population mean μ and variance V_P; G = genotype effect with mean 0 and variance V_G; E = environment effect with mean 0 and variance V_E; GE = genotype × environment interaction effect with mean 0 and variance $V_{G\times E}$; B = the block effect with mean 0 and variance V_B; e = residual effect with mean 0 and variance V_e. All the factors were considered as random. The MINQUE (1) method[14,15] was used to obtain unbiased variance and covariance components for each trait. Variance and covariance estimates were used to calculate the corresponding correlation coefficients for phenotypic, genotypic, environmental and interaction effects. The random effects were predicted using the adjusted unbiased prediction (AUP) method.[14] Standard errors of the statistics were obtained by jackknife procedures[14,16] and two-tailed *t*-tests were performed for testing the significance of parameters obtained. The model was also recalculated considering environment as a fixed factor for growing season and type of cultivation comparison computing the pairwise mean comparison using the false discovery rate (FDR) criterion[17] at $\alpha = 0.05$.

All the data analyses were performed with QTModel (v. 0.7) and QGAStation (v. 1) software (Bioinformatics Institute, Zhejiang University, China).

RESULTS AND DISCUSSION

Phenotypic means of carotenoids and ascorbic acid content for the tomato accessions studied

In general, it could be observed that the phenotypic antioxidant content of tomato largely varied among accessions in each environment (Table 2). Moreover, the phenotypic values of the content of lycopene, β-carotene and ascorbic acid seemed to be very promising for some accessions. For lycopene content, values up to 281 mg kg^{-1} were observed, which are much higher than the reported average phenotypic value (30 mg kg^{-1}).[11,18] A similar situation occurred for β-carotene content (35 mg kg^{-1} observed, in contrast with the 3.9 mg kg^{-1} commonly reported)[18] and for ascorbic acid content (346 mg kg^{-1} *versus* 200 mg kg^{-1} commonly accepted).[19]

Although it was possible to detect high phenotypic values for all the compounds, important environmental and interactions effects were easily detected, as the values obtained fluctuated with different trends for different accessions and environments (Table 2). In order to obtain a better estimation of the potential of improvement for each character in tomato breeding programmes, it was necessary to ascertain the relative contribution of the genotype, environment and genotype × environment interactions and estimate variance components. Genetic correlations between traits were also analysed in order to determine whether a combined selection for these antioxidant traits would be feasible.

Estimation of variance components and correlation analysis

First, a decomposition of phenotypic variances in genetic, environmental and $G \times E$ interaction components was carried out (Table 3). All the estimates of the variance components calculated were significantly different from zero, thus offering reliable information on the relative contribution of each to the total phenotypic variance. For carotenoid content, the residual variance was around 25% of the total phenotypic variance; hence it can be considered that the model explained well the distribution of the variation with the factors included. However, for ascorbic acid, the residual variance was two times higher. The model, despite providing useful information, only explained one half of the total phenotypic variance. Nevertheless, it should be considered that ascorbic acid plays a very active and important role in reducing oxidative damage at the cellular level caused by stress conditions[20] and it is very difficult to model the $G \times E$ interaction in its accumulation due to uncontrolled factors. For all the traits, the block effect was very small (between 0.41% and 1.28% of the total

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Table 3. Estimated value and SE of variance components (and percentage from total phenotypic variance) for lycopene, β-carotene, and ascorbic acid content of tomato fruits

Parameter[a]	Lycopene	β-carotene	Ascorbic acid
V_G	$3273.94 \pm 198.13^{**}$ (58.05%)	$46.47 \pm 1.69^{**}$ (65.25%)	$2747.62 \pm 242.41^{**}$ (23.56%)
V_E	$90.23 \pm 33.99^{*}$ (1.60%)	$3.27 \pm 0.50^{**}$ (4.60%)	$1517.92 \pm 215.08^{**}$ (13.01%)
$V_{G\times E}$	$663.46 \pm 158.14^{**}$ (11.76%)	$3.82 \pm 0.61^{**}$ (5.37%)	$1015.12 \pm 241.63^{**}$ (8.70%)
$V_{B(E)}$	$71.97 \pm 17.77^{**}$ (1.28%)	$0.28 \pm 0.03^{**}$ (0.41%)	$105.59 \pm 28.14^{**}$ (0.91%)
V_e	1539.96 (27.31%)	17.35 (24.37%)	6278.28 (53.82%)
V_P	5639.58	71.22	11664.53

[a] V_G, genotypic main variance, V_E, environment main variance, $V_{G\times E}$, genotype × environment variance, $V_{B(E)}$, block in growing environment variance; V_e, residual variance; V_P, phenotypic variance.
Significantly different from zero (t-test) at $^{*} P = 0.05$ and $^{**} P = 0.01$ level.

Table 4. Phenotypic, genotypic, environmental and interaction paired correlations (estimated value ± SE) for the functional characters studied in tomato fruits

Correlation[a]	Lycopene vs. β-carotene	β-Carotene vs. ascorbic acid	Lycopene vs. ascorbic acid
r_P	$0.20 \pm 0.01^{**}$	$0.32 \pm 0.01^{**}$	0.01 ± 0.01 NS
r_G	$0.36 \pm 0.01^{**}$	$0.77 \pm 0.02^{**}$	$-0.14 \pm 0.02^{**}$
r_E	$-1.00 \pm 0.05^{**}$	$-0.07 \pm 0.03^{*}$	$-0.23 \pm 0.06^{**}$
$r_{G\times E}$	-0.13 ± 2.47 NS	$0.03 \pm 0.01^{**}$	$0.63 \pm 0.02^{**}$

[a] r_P, phenotypic correlation; r_G, genotypic correlation; r_E, environmental correlation; $r_{G\times E}$, genotype × environment interaction correlation.
Significantly different from zero (t-test) at $^{*} P = 0.05$ and $^{**} P = 0.01$ level; NS, non-significant.

phenotypic variance) so this effect could be discarded. The more important result to consider was that, for carotenoid accumulation, the genotypic component represented the larger contribution to the phenotypic variance (around 60%) and the environmental variance was very low, having a smaller contribution to the phenotypic value than the $G \times E$ interaction. The $G \times E$ interaction represented 5–10 times less variance (for β-carotene and lycopene respectively) than the genotypic component. These results show that the improvement in lycopene and β-carotene is feasible in breeding programmes and that elite carotenoid accumulation cultivars can be commercialized independently of the growing conditions used, to obtain good phenotypic values. In the case of ascorbic acid accumulation, genetic variance represented a quarter of the total phenotypic variance, and environmental and $G \times E$ variance was around 10%, indicating that improvement in ascorbic acid content in tomato breeding programmes would be difficult and that the use of high ascorbic acid cultivars does not necessarily imply the production of high ascorbic acid fruits. Therefore this situation may lead to important conflicts in quality controls during commercialization.

After partitioning phenotypic covariance into its genotypic, environmental and $G \times E$ components, the corresponding paired correlation coefficients were calculated (Table 4). For lycopene and β-carotene accumulation an important and highly significant positive genotypic correlation (r_G) was observed. Conversely, the $G \times E$ correlation coefficient was negative but not significant. Accordingly, the total genetic correlation ($r_G + r_{G\times E}$) indicated that it is possible to select genotypes with high levels of both carotenoids. Nevertheless, it is interesting to point out that a high negative significant environmental correlation was determined and this makes the development of selection trials difficult, as the growing environments that increase lycopene accumulation seem to reduce β-carotene content and vice versa. Therefore, multi-environment trials must be implemented in order to obtain a reliable genotype evaluation. In the case of the pair β-carotene and ascorbic acid there was a very high and positive significant total genetic correlation (0.8), mainly due to the genotype component, which allows a practicable joint improvement of these two traits. There also exists a minor significant and negative environmental correlation but this may not represent an important difficulty for selection.

When analysing the phenotypic correlations for lycopene and ascorbic acid accumulations it seemed that these two characters were independent (very low non-significant positive correlation). This result is similar to others reported in previous single environment trials.[21] Nevertheless, a deeper insight into the components of this correlation showed a more complex relation. There exists an important negative and highly significant environmental correlation. In the case of the total genetic correlation there is a positive significant correlation with opposite contribution of each subcomponent, as the genotypic correlation component is negative, but the $G \times E$ correlation component is positive and much more important. Therefore the growing environments used can highly influence selection owing to their contribution to two opposite effects (the environmental and the interaction), complicating the joint selection for high genotypic potential of both lycopene and ascorbic acid content. Summarizing, in most breeding programmes only the combined improvement of two characters – lycopene and β-carotene or β-carotene and ascorbic acid – would be realistic. The production of cultivars with increased levels of the three compounds, even if feasible, would be unstable and probably cause commercialization problems.

Prediction of the environmental, genotypic and interaction effects

A general mixed linear model was used for the prediction of the growing environment, genotype and interaction factors on the total phenotypic response, thus enabling a more appropriate and independent analysis of each effect (Fig. 1).

For all the studied traits important differences between growing environments were detected (left side of Fig. 1). The paired differences between spring–summer and autumn–winter and between open field and glasshouse cultivation were all significant (all $P <$ critical values for FDR test at 0.05). These results could be better understood if the reported influence of climatic conditions in the biosynthesis of the antioxidants is considered together with the combination of temperature and radiation registered in the three environments. In this regard, it has been reported that the lycopene accumulation depends on temperature and seems to take place at a range of average day temperatures between 12 or 32 and 35 °C,[22,23] with the optimal conditions around 22–26 °C.[24] For β-carotene accumulation the range of average day temperatures is wider than for lycopene. Its

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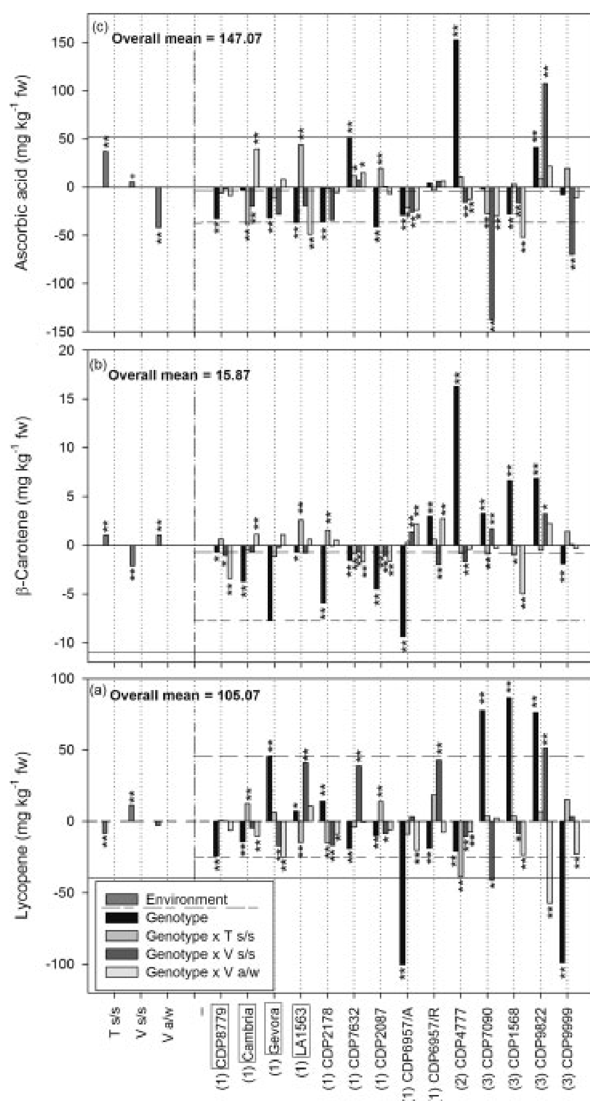

Figure 1. Predicted genotypic, environmental and interaction effects for lycopene, β-carotene and ascorbic acid content of the accessions studied. Estimated value significantly different from zero (*t*-test) at $^{+}P = 0.1$, $^{*}P = 0.05$ and $^{**}P = 0.01$ level. Controls in *x*-axis are inside a box. In parentheses: 1, *Solanum lycopersicum*; 2, *S. lycopersicum* var. *cerasiforme*; 3, *S. pimpinellifolium*. Scaling in *y*-axis is overall mean centred. Reference lines: genotypic upper control (long dashed line), genotypic lower control (short dashed line), reported phenotypic average content (continuous line). The reported average content considered is: 65, 3.9 and 200 mg kg^{-1} for lycopene,[33] β-carotene[18] and ascorbic acid[19] content, respectively. fw: fresh weight; Ts/s: Predicted Environmental Effect for Turis in spring-summer cycle; Vs/s: Predicted Environmental Effect for Valencia in spring-summer cycle; Va/w: Predicted Environmental Effect for Valencia in autumn-winter cycle.

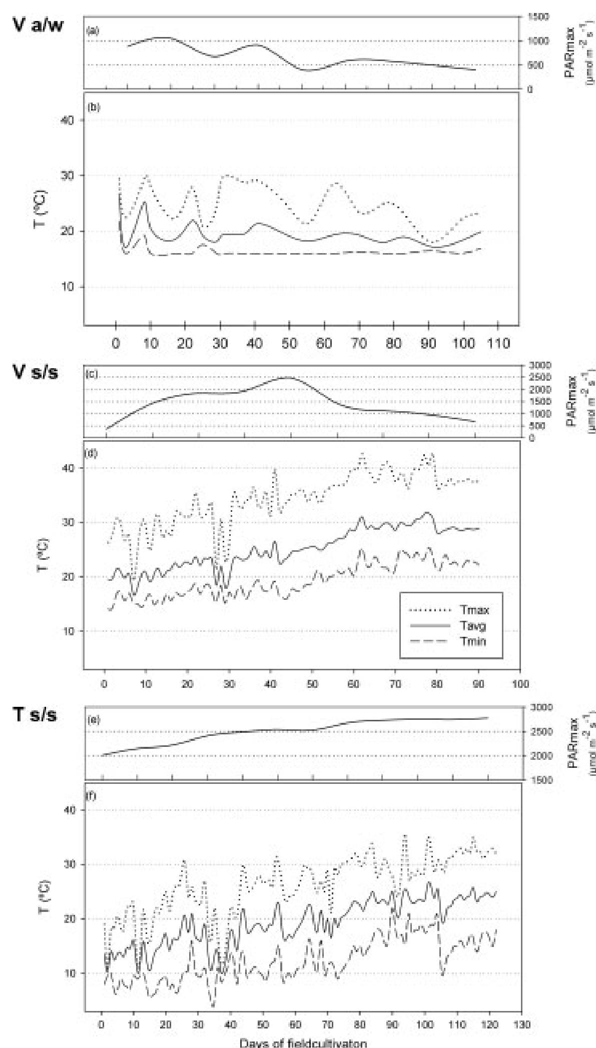

Figure 2. Temperature and photosyntetically active radiation measured in the three growing environments during the trial period. Ts/s: Turis, spring-summer cycle; Vs/s: Valencia, spring-summer cycle; Va/w: Valencia, autumn-winter cycle; T: Temprature; PAR: Photosyntetycally Active Radiation.

biosynthesis is poorly affected by temperatures lower than 12 °C[25] and with temperatures higher than 35 °C, when the lycopene accumulation is inhibited, the conversion of lycopene into β-carotene is stimulated.[23] Nevertheless, the optimal temperature for β-carotene accumulation seems to be around 30 °C.[23] The ascorbic acid accumulation in tomato fruits seems also to be directly correlated with temperature.[26] It has been suggested that at relatively high temperatures there is probably a decrease in the ascorbic acid content due to oxidation;[27] however, these harmful conditions have not been studied properly. At favourable temperatures, the lycopene, β-carotene and ascorbic acid biosynthesis increase with the sunlight intensity,[24,28] probably due to the increase in photosynthetic rate. These light-induced variations are especially important in the case of ascorbic acid accumulation. Normally, open field leads to higher ascorbic acid content than greenhouse cultivation, as well as harvesting in late summer *versus* other seasons.[29] The reduction in ascorbic acid accumulation with reduced radiation conditions can be as much as 70%.[10,28] In the case of lycopene, when a harmful direct radiation level occurs (650 Wm^{-2} for 1.5–4 h) its synthesis is inhibited. On the other hand, for ascorbic acid synthesis the excessive radiation does not inhibit its synthesis but causes a reduction in its accumulation.[30]

For lycopene accumulation, in the spring–summer cycle in Turis favourable daily average temperature conditions during cultivation were recorded, especially in the harvest period when they were near to the optimum interval and did not exceed the thermal stress threshold (Fig. 2). Conversely, regarding the radiation conditions in the first half of the cycle, PAR radiation increased, reaching the maximum photosynthetic capacity and a

high growing performance, but for the harvest period the amount of radiation surpassed the harmful threshold. To see this, the 650 W m^{-2} total sun radiation was expressed in the PAR scale. We considered that the proportion of PAR radiation *versus* direct total radiation in our latitude for spring–summer cycle is 78.77% (information provided by the National Meteorology Agency at Valencia) and the expression W m^{-2} × 4.57 = µmol m^{-2} s^{-1} for sun and sky daylight[31] led to a harmful radiation threshold of 2340 µmol m^{-2} s^{-1} being obtained. Therefore, in this part of the growing cycle, the fruits would be exposed to excessive solar radiation that could lead to an arrest of lycopene biosynthesis and reduce the final level of lycopene accumulation.

In the case of Valencia in the spring–summer cycle in the glasshouse, the daily average temperature was slightly higher than in Turis but the heat dissipation systems were able to maintain it within the favourable, though not optimal, temperature range throughout almost every day of cultivation. Regarding radiation, due to the use of a shadowing system as part of the heat dissipation management, its level inside the protection was reduced and, in general, no radiation stress occurred.

In Valencia during the autumn–winter cycle the use of a heating system maintained the temperature lightly below the lower limit of the optimal interval but inside the favourable temperature range of lycopene biosynthesis. Obviously for this cycle the radiation was not high and lycopene accumulation was relatively good, but not optimal.

Regarding β-carotene accumulation, the worst growing environment was the spring–summer cycle in Valencia, coinciding with the better growing conditions for lycopene accumulation. This is in agreement with the regulation proposed for the major biosynthesis pathway of both carotenoids in tomato – phytoene → phytofluene → ζ-carotene → neurosporene → lycopene → γ-carotene → β-carotene – in which the enhanced flux of carotene in the pathway is arrested at lycopene under no stressing conditions.[32] Conversely, in Turis in the spring–summer cycle and in Valencia in the autumn–winter cycle there were some stressing conditions that limited lycopene accumulation but not β-carotene. In Turis, the temperature range was better for β-carotene biosynthesis than for lycopene, but as the radiation conditions led to an arrest in lycopene biosynthesis its subsequent accumulation was important but not as high as it would be in this season with no radiation stress. This could explain the finding that β-carotene accumulation in Turis in the open air was similar to levels found in a growing cycle (Valencia, autumn–winter) less favourable for carotenoid accumulation.

With respect to ascorbic acid accumulation, the better combination of temperature and radiation occurred in Turis in the open air during the spring–summer cycle, probably not being affected by the high radiation level as may have been the case for lycopene. The worst condition for ascorbic acid accumulation was the autumn–winter cycle in Valencia, as the temperature and radiation in this cycle were lower than in the others.

The genetic merit of the accessions tested must be evaluated on both genotype main effect and $G \times E$ interaction (Fig. 1), in comparison with the genetic merit of the controls for reference.

For lycopene accumulation the controls of fresh market type (CDP8779 and Cambria) showed a genotypic main effect (black bars in Fig. 1(a)) that diminished the general mean (105.07 mg kg^{-1}) by 24.22 and 14.20 mg kg^{-1}, respectively, leading to a predicted lycopene content due to the genotypic effect of 80.85 and 90.87 mg kg^{-1}, respectively. The genotypic potential of lycopene expression can be considered in the higher segment of modern commercial fresh market cultivars, as even considering the worst growing environment and interaction effects the predicted lycopene content for these two controls would be 66.15 and 71.96 mg kg^{-1} respectively – values higher than the best phenotypic value reported for cultivars widely grown in Spain (65 mg kg^{-1}, represented graphically in Fig. 1(a) by the horizontal continuous line).[33]

Regarding the interaction effect, CDP8779 control showed a very stable performance, with no significant and negligible predicted values in the three growing environments studied. Cambria had a similar performance but with a small instability (two significant $G \times E$ predicted effects). The processing tomato control (Gevora) showed a very high genotypic effect (increasing by 45.38 mg kg^{-1} the general mean, leading to a lycopene accumulation of 150.45 mg kg^{-1}). It should be pointed out that Gevora is a cv adapted to open field cultivation in spring-summer cycle in hot Spanish regions, and it showed negative interactions in growing environments with different conditions that diminished its lycopene accumulation. The control accession LA1563, with the Intense Pigment (IP) gene, which has been reported to have an increased carotenoid accumulation of around 60%,[12] showed a genotypic potential between the other controls. Only in protected cultivation with climatic control in the spring–summer cycle would its total genetic potential ($G + G \times E$) be higher than the processing tomato control (7.19 + 40.99 = 48.18 mg kg^{-1}). The genotypic value of Gevora was chosen as the high-threshold criterion to select interesting accessions.

Following this comparison of criteria (represented graphically in Fig. 1(a) by the horizontal dashed line), the best predicted genotypic values for lycopene accumulation were detected in accessions CDP1568, CDP7090 and CDP9822, all of them belonging to *S. pimpinellifolium*, with respectively 1.9, 1.71 and 1.68 times the genotypic potential of the industry control and 6, 5.47 and 5.36 times the genotypic potential of the commercial hybrid for fresh consumption. These accessions would be very interesting as donor parents in breeding programmes for developing new cultivars. Owing to their wild origin, these three accessions showed better adaptation to open field and spring–summer growing conditions (no significant $G \times E$ interaction in this environment). Accession CDP1568 showed small negative interactions with protected environment, especially in the autumn–winter cycle, which slightly diminished its total genetic potential (86.54 − 23.59 = 62.95 mg kg^{-1}). Nevertheless, it was the most stable accession of the three selected. Accession CDP7090 showed an important negative interaction in the growing environment with higher temperatures, which would decrease its total genetic potential (77.71 − 41.21 = 36.5 mg kg^{-1}) and hinder the selection of its descendants. Conversely, accession CDP9822 showed an important and highly significant interaction in the growing environment with higher temperatures. If this accession is selected to derive cultivars targeted to specific environments with these growing conditions, the total genetic potential would be very high (76.2 + 51.23 = 127.43 mg kg^{-1}). However, it should be considered that this accession is highly unstable and in other environments with lower temperatures and radiation its total genetic potential to accumulate lycopene would be dramatically diminished (76.2−57.45 = 18.75 mg kg^{-1}). Accessions CDP6957/R and CDP7632 (traditional varieties with interesting organoleptic quality), despite having a negative genotype subcomponent prediction, offered a total genetic potential for lycopene accumulation of 23.99 and 19.64 mg kg^{-1} respectively – twice as much as the fresh market reference control.

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For β-carotene accumulation (Fig. 1(b)), controls showed a genotypic potential and stability opposite to that observed for lycopene accumulation. These controls had shown, in the worst conditions (Valencia, spring-summer cycle), a phenotypic β-carotene content that is 1.5 times the reported average content in tomato,[18] so they could be considered good references. The best accession for β-carotene accumulation was CDP4777 from *S. lycopersicum* var. *cerasiforme*. This accession showed more than 20 times the genotypic potential of the best control, the high carotenoid IP genotype, LA1563, and high stability. Therefore it would be very useful both as a donor parent in breeding programmes and for direct consumption in gourmet applications, as it is a cherry tomato. Other accessions interesting for use as donor parents in breeding programmes for β-carotene accumulation were the three *S. pimpinellifolium* accessions previously selected for their high lycopene content. In this sense, accessions CDP9822 and CDP1568 showed a genotypic value for β-carotene accumulation approximately 10 times higher than the best control. However, these two accessions should be used in specific environments in order avoid negative $G \times E$ interaction. Accession CDP9822 should be targeted to protected cultivation in the spring–summer cycle and CDP1568 accession to open field cultivation. Accession CDP7090 showed a genotypic potential five times higher than the best control. But it should also be targeted to a specific environment (protected cultivation in autumn-winter cycle) to escape from negative interactions (note that lycopene and β-carotene interactions are opposite, thus for joint improvement of both compounds the condition showing negligible interaction is preferred). The same applies to the traditional variety CDP6957/R, but in this case the growing environment adequate for selection is open field cultivation in the spring–summer cycle.

Finally, regarding ascorbic acid accumulation (Fig. 1(c)), the controls showed phenotypic values lower than the commonly accepted average content of ascorbic acid in tomato (200 mg kg^{-1}).[19] Cambria showed the best performance of all the controls, but only due to the high E and $G \times E$ effects. The best accession for use as donor parent in breeding programmes was CDP4777 from *S. lycopersicum* var. *cerasiforme*, which also is the best donor parent for β-carotene content. CDP4777 had a genotypic value for ascorbic acid accumulation more than 50 times greater than the best control. It is also highly stable because the significant $G \times E$ interaction effects are small. Nevertheless, it should be noted that, as in the case of β-carotene accumulation, its performance is better in the open field. CDP9822 was another very interesting donor parent for breeding programmes because, in specific environments (protected cultivation in the spring–summer cycle), it has shown a very high $G \times E$ interaction effect, especially for ascorbic acid accumulation, which increased considerably its total genetic potential, and enabled the improvement of the three functional traits studied. Finally, the traditional variety accession CDP7632 is also interesting for direct use for its high ascorbic acid genotypic potential (15 times greater than the best control) and stability.

CONCLUSIONS

Our results indicate that, in general, the high genetic component responsible for the accumulation of lycopene and β-carotene makes possible the selection of elite genotypes with high content of both carotenoids in tomato breeding programmes. The high ratio of genotypic to environmental variance decomposition seems to indicate that high-accumulation cultivars with wide adaptation might be successful despite the important environmental effects on carotenoid biosynthesis. Although there is a high genotypic correlation between the carotenoids studied, to perform a joint selection for both carotenoids it is mandatory to conduct multi-environment trials owing to the existence of a considerably high negative environmental correlation. The improvement of the content of ascorbic acid is in most cases more difficult because the interference of uncontrolled factors masks the real genetic potential. Nevertheless, it would be possible to make a joint selection with β-carotene but abandoning improvement of lycopene content.

Four accessions with an amazing genetic potential for functional traits have been identified. Three of them belong to *S. pimpinellifolium* (CDP1568, CDP7090 and CDP9822) and are especially interesting for their use as donor parents in the improvement of lycopene and β-carotene content. CDP1568 showed the best genotypic potential (1.9 times greater than the processing control and six times higher than the commercial hybrid control) and the most stable expression across all the environments tested. CDP9822 is interesting for deriving hybrids with high carotenoid and ascorbic acid accumulation for specific target environments (protected cultivation in the spring–summer cycle) owing to the importance of the $G \times E$ interaction. CDP4777 from *S. lycopersicum* var. *cerasiforme*, showed a very high genotypic potential to accumulate β-carotene and ascorbic acid (more than 20 and 50 times respectively than the fresh consumption controls) and a high stability in their expression. This accession is a cherry local cultivar and might be used either as donor parent in breeding programmes or for direct consumption in quality markets.

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REFERENCES

1 Bouma J, Varallyay G and Batjes NH, Principal land use changes anticipated in Europe. *Agric Ecosyst Environ* **67**:103–119 (1998).
2 Menrad K, Market and marketing of functional food in Europe. *J Food Eng* **56**:181–188 (2003).
3 Beecher GR, Nutrient content of tomato and tomato products. *Proc Soc Exp Biol Med* **218**:98–100 (1998).
4 Mayne ST, Beta-carotene, carotenoids and disease prevention in humans. *FASEB J* **10**:690–701 (1996).
5 Lenucci MS, Caccioppola A, Durante M, Serrone L, Piro G and Dalessandro G, Carotenoids content in ripe raw and processed (sauce) berries of high pigment tomato hybrids. *Acta Hortic* **758**:173–180 (2007).
6 Stevens MA and Rick CM, Genetics and breeding, in *The Tomato Crop: A Scientific Basis for Improvement*, ed by Atherton JG and Rudich J. Chapman & Hall, London, pp. 35–109 (1986).
7 Hanson PM, Yang RY, Wu J, Chen JT, Ledesma D, Tsou SCS, *et al*, Variation for antioxidant activity and antioxidants in tomato. *J Am Soc Hortic Sci* **129**:704–711 (2004).
8 Abushita AA, Daood HG and Biacs PA, Change in carotenoids and antioxidant vitamins in tomato as a function of varietal and technological factors. *J Agric Food Chem* **48**:2075–2081 (2000).
9 Adalid AM, Roselló S and Nuez F, Evaluation and selection of accessions of *Solanum* section *Lycopersicon* for content of bioactive components. *J Food Compos Anal* **23**:613–618 (2010).

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10 Hamner KC, Bernstein L and Maynard LA, Effects of light intensity, day length, temperature, and other environment factors on the ascorbic acid content of tomatoes. *J Nutr* **29**:85–97 (1945).
11 Kuti O and Konuru HB, Effects of genotype and cultivation environment on lycopene content in red-ripe tomatoes. *J Sci Food Agric* **85**:2021–2026 (2005).
12 Lavi N, Tadmor Y, Meir A, Bechar A, Oren-Shamir M, Ovadia R, *et al*, Characterization of the Intense Pigment tomato genotype emphasizing targeted fruit metabolites and chloroplast biogenesis. *J Agric Food Chem* **57**:4818–4826 (2009).
13 Galiana-Balaguer L, Roselló S, Herrero-Martinez JM, Maquieira A and Nuez F, Determination of L-ascorbic acid in *Lycopersicon* fruits by capillary zone electrophoresis. *Anal Biochem* **296**:163–218 (2001).
14 Zhu J and Weir BS, Diallel analysis for sex-linked and maternal effects. *Theor Appl Genet* **92**:1–9 (1996).
15 Rao CR, Estimation of variance and covariance components MINQUE theory. *J Multivariate Anal* **1**:257–275 (1971).
16 Miller RG, The jackknife: a review. *Biometrika* **61**:1–15 (1974).
17 Benjamini Y and Hochberg Y, On the adaptive control of the false discovery rate in multiple testing with independent statistics. *J Educ Behav Stat* **25**:60–83 (2000).
18 Holden JM, Eldridge AL, Beecher GR, Buzzard IM, Bhagwat S, Davis CS, *et al*, Carotenoid content of US foods: an update of the database. *J Food Compos Anal* **12**:169–196 (1999).
19 Gould WA, *Tomato Production, Processing and Technology*. CTI Publications, Baltimore, MD (1992).
20 Shigeoka S, Ishikawa T, Tamoi M, Miyagawa Y, Takeda T, Yabuta Y, *et al*, Regulation and function of ascorbate peroxidase isoenzymes. *J Exp Bot* **53**:1305–1319 (2002).
21 Adalid A, Roselló S, Cebolla-Cornejo J and Nuez F, Evaluation and selection of *Lycopersicon* accessions for their high carotenoid and vitamin C content. *Acta Hortic* **789**:221–227 (2008).
22 Leoni C, Industrial quality as influenced by crop management. *Acta Hortic* **301**:177–184 (1992).
23 Hamauzu Y, Chachin K and Ueda Y, Effect of postharvest temperature on the conversion of ^{14}C-mevalonic acid to carotenes in tomato fruit. *J Jpn Soc Hortic Sci* **67**:549–555 (1998).
24 McCollum JP, Effects of light on the formation of carotenoids in tomato fruits. *Food Res* **19**:182–189 (1954).
25 Baqar MR and Lee TH, Interaction of CPTA and high temperature on carotenoid synthesis in tomato fruit. *Z Pflanzenphysiol* **88**:431–435 (1978).
26 Liptay A, Papadopoulos AP, Bryan HH and Gull D, Ascorbic acid levels in tomato (*Lycopersicon esculentum* Mill) at low temperatures. *Agric Biol Chem* **50**:3185–3187 (1986).
27 Murneek AE, Maharg L and Witter SH, Ascorbic acid (vitamin C) content of tomatoes and apples. *Univ Missouri Agric Exp Stn Res Bull* **568**:3–24 (1954).
28 Brown GB, The ascorbic acid content of tomatoes as related to illumination. *J Am Soc Hortic Sci* **65**:342–348 (1954).
29 Lopez-Andreu FJ, Lamela A, Esteban RM and Collado GJ, Evolution of quality parameters in the maturation stage of tomato fruits. *Acta Hortic* **191**:387–394.
30 Adegoroye AS and Jolliffe PA, Some inhibitory effects of radiation stress on tomato fruit ripening. *J Sci Food Agric* **39**:297–302 (1987).
31 Thimijan RW and Heins RD, Photometric, radiometric, and quantum light units of measure: a review of procedures for interconversion. *HortScience* **18**:818–822 (1983).
32 Hirschberg J, Carotenoid biosynthesis in flowering plants. *Curr Opin Plant Biol* **4**:210–218 (2001).
33 Martinez-Valverde I, Periago MJ, Provan G and Chesson A, Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicum esculentum*). *J Sci Food Agric* **82**:323–330 (2002).

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III.3. ANÁLISIS DEL CONTROL GENÉTICO DE LA ACUMULACIÓN DE β-CAROTENO Y ÁCIDO L-ASCÓRBICO DE UNA ENTRADA SILVESTRE TIPO CHERRY CON FRUTOS NARANJA AMARRONADOS.

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SUMMARY

There is increasing interest in the development of tomato cultivars with high concentrations of nutraceutical components, such as β-carotene (provitamin A) and ascorbic acid (vitamin C), as they play an important role in human health. For this purpose, it is important to seek out new and useful sources of variation. In previous works, a promising *Solanum lycopersicum* var *cerasiforme* accession (CDP4777) with high concentrations of β-carotene and ascorbic acid was detected. The genetic control of these trait expressions has now been studied in two locations using an additive, dominance and additive x additive model that includes genotype x environment interactions. The results indicate that β-carotene accumulation was mainly additive (32.2% of the genetic component) with a small dominant component (4.2%) and an important AxE interaction contribution (63.6%), which, in target environments with moderate to high temperatures and no depressed radiation, could substantially enhance the β-carotene content. This trait showed a high narrow-sense heritability ($h^2 = 0.62$). Ascorbic acid accumulation was also mainly additive (61.7% of the genetic component) with a minor additive epistatic component (21.5%). This epistatic effect caused a negative heterosis that reduces the positive main additive effect. Nevertheless, in the described target environments, the AxE interaction contribution (16.8%) may enhance the ascorbic acid content and compensate the negative heterosis effect. The total narrow-sense heritability of this trait can be considered good ($h^2 = 0.52$). In conclusion, the CDP4777 accession is a very interesting donor parent for the joint improvement of β-carotene (without diminishing lycopene content) and ascorbic acid content in commercial tomato nutraceutical breeding programmes; the F1 hybrids derived from this accession showed nearly 450% of the commonly reported average β-carotene content and close to 130% of the ascorbic acid content of the female parent.

Key words: *Solanum lycopersicum* var *cerasiforme*, additive, dominance, epistasis, breeding programmes.

INTRODUCTION

In recent years, consumers in developed countries have shown increasing interest and concern regarding the healthfulness of the food they consume. Tomato has only moderate nutritional value, but it is highly consumed all year round and is an

important source of carotenoids and vitamin C, which are protective against infectious (Ross, 1998; Tee, 1992) and degenerative diseases, such as cardiovascular diseases (Marchioli *et al*., 2001) or certain cancers (Byers and Guerrero, 1995; O'Toole and Lombard, 1996). For this reason, breeding programs targeted towards developing new tomato cultivars have a general tendency to include nutraceutical quality as a new objective. Cultivars such as "DoubleRich," with high vitamin C content, or the "high pigment" cultivars with increased carotenoid contents are well known in the tomato processing industry (Lenucci *et al*., 2007). Several tomato mutants with high carotenoid contents have been identified, but agronomic deficiencies or bad organoleptic quality rule out their use (Stevens and Rick, 1986; Hanson *et al*., 2004), which makes it is necessary to identify new sources of variation.

In the case of carotenoids, different content patterns are found in tomato germplasm, from those that accumulate high β-carotene content at the expense of lycopene in orange-fruited accessions of *Solanum cheesmaniae* (L. Riley) Fosberg, *Solanum pimpinellifolium* L., *Solanum chilense* (Dunal) Reiche and *Solanum chmielewskii* (C.M. Rick *et al.*) D.M. Spooner *et al.* (Chalukova, 1988; Manuelyan *et al*., 1975; Rick, 1956; Stommel and Haynes, 1994), to those with low β-carotene and lycopene contents (yellow fruits) (Rego *et al*., 1999), or those that accumulate high lycopene content at the expense of β-carotene (Ronen *et al*. 2000) or other carotenoids, such as prolycopene (tangerine tomatoes) (Isaacson *et al*., 2002) or δ-carotene (Ronen *et al*., 1999). In the case of ascorbic acid, its content varies considerably among tomato species, from cultivated varieties to wild species such as *Solanum pennelli* Correll (Stevens *et al*, 2007), *S. pimpinellifolium* (Galiana-Balaguer *et al*., 2001) or *Solanum lycopersicum* L. var *cerasiforme* (Stevens *et al*, 2007). Among these related species, the most interesting ones are those that are phyletically closest to the cultivated tomato, as they allow for quicker agronomic trait recovery than more distant ones, thereby shortening the duration of breeding programmes. In this sense, *S. lycopersicon* var *cerasiforme* accessions with high carotene or vitamin C contents are the most desirable ones.

In a previous work (Roselló *et al*., 2011), we identified and selected a *S. lycopersicum* var *cerasiforme* accession (CDP4777) with high concentrations of β-carotene and ascorbic acid (825% and 150% of the commonly reported average contents, respectively) with great potential to improve nutraceutical quality in tomato

breeding programs. In this work, the main objective was to study the genetic control of the β-carotene and ascorbic acid contents derived from this selected accession in order to provide information that will be useful in the efficient development of new varieties with improved nutraceutical quality.

MATERIALS AND METHODS

Genetic materials

The plant material used in this study includes two tomato accessions with very different β-carotene and ascorbic acid contents that had been evaluated in a previous multi-environment study (Roselló *et al.*, 2011) and their F_1, F_2, BC_1 and BC_2 descendant generations. The CDP8779 accession, native to Valencia (Spain), was used as the female parent (P_i). It is an experimental line of *Solanum lycopersicum* L. with large and light-red tomato fruits. Its agronomic characteristics can be considered standard, representing an average of the common modern tomato cvs. It has moderate to high β-carotene and low ascorbic acid contents. Conversely, CDP4777 is a wild accession from *Solanum lycopersicum* var *cerasiforme* with small and orange-brownish fruits selected as the donor parent (P_j) due to its very high genotypic values for β-carotene and ascorbic acid accumulation. This accession is native to Ipala (Guatemala) and was provided by Dr. Luis Mejía of the Universidad de San Carlos. The CDP8779 accession is an inbred line, but the CDP4777 accession behaves as a population variety, so, in order to avoid possible intra-accession genetic variation in CDP4777, only one plant of P_i and P_j were used as parents. These parent plants were vegetatively replicated in order to obtain backcrosses in the next cycle. One F_1 plant was used to obtain the F_2 (self-pollination), BC_1 ($F_1 \times P_i$) and BC_2 ($F_1 \times P_j$) generations. Clones of P_i, P_j and F_1 plants were produced in order to be used in the trials along with the F_2, BC_1 and BC_2 seedling plants. Both parental accessions were provided by the seedbank of the Instituto Universitario de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV).

Growing conditions and experimental design

The trials were carried out in two growing environments in an autumn cycle using common cultivation techniques. Two sites of cultivation, Castellón (39º 59’ N, 0º 2’ W 35m asl) and Valencia (39º 28’ N, 0º 22’ W 15 m asl), were used. Cultivation at

Castellón was carried out in soil in the open field. Cultivation in Valencia was carried out hydroponically in a glasshouse with automated temperature control systems. In both environments, fertirrigation was scheduled daily and plants were staked and pruned properly. The composition of the nutrient solution used was (meq/L): 4.0 Mg^{2+}, 1.96 Na^{+}, 8.0 K^{+}, 8.5 Ca^{2+}, 1.0 NH_4^{+}, 2.25 Cl^{-}, 11.86 NO_3^{-}, 1.5 $H_2PO_4^{-}$7.50 SO_4^{2-} and 0.5 HCO_3^{-}. Micronutrients were added using a commercial mixture (Nutrel C, Phosyn, Jaén, Spain) containing the following elements (mM): Cu, 0.76; Fe, 20.15; Mn, 9.01; Zn, 1.38; B, 9.71; and Mo, 0.31. The EC was 2.35 dS/m and the pH was 5.5. Information about climatic parameters that might influence plant metabolism and growth, air temperature and photosynthetically active radiation (PAR) were recorded every 10 minutes using WatchDog weather stations (Spectrum Technologies Inc., Illinois, USA) equipped with temperature, quantum light PAR sensors and a data logger.

For the precise evaluation of genotype, environment and interaction effects, clones of all the plants studied were used in each environment. A randomized complete block design was used with 27 blocks per environment and 12 plants per block (1 P_i, 1 P_j, 1 F_1, 5 F_2, 2 BC_1 and 2 BC_2).

Sampling

Uniformly ripe, healthy fruits at the completely ripe stage were harvested. Five to twenty representative fruits were collected from each plant, but only from the first three trusses to minimize intraplant variability. Fruits were blended at 4ºC and low light intensity to minimize antioxidant loss. A laboratory homogenizer (Diax 900, Heidolph, Germany) was used with a 6G generator to disrupt tissue to particle sizes < 0.4mm. Samples were stored at -20ºC until analysis.

Ascorbic acid determination

Ascorbic acid was quantified by Capillary Zone Electrophoresis (Galiana-Balaguer *et al*., 2001) using a P/ACE System MDQ (Beckman Instruments, Fullerton, CA, USA). Two grams of sample were thawed in the dark at 4ºC and centrifuged at 12,500 rpm in a refrigerated centrifuge (Centrifuge 5415R, Eppendorf, Hamburg, Germany). The supernatant was diluted in 2% metaphosphoric acid (Sigma Chemical, St. Louis, USA) to avoid ascorbic acid oxidation. Potassium hydrogen phthalate (100

mg l^{-1}) (Sigma Chemical, St. Louis, USA) was used as an internal standard. Sample extracts were filtered through a 0.2 mm filter membrane (Millipore, Bedford, USA) prior to injection. Uncoated fused-silica capillaries (31.2 cm total length, 21 cm effective length, 50 μm i.d.) were used (Polymicro Technologies, Phoenix, USA). Hydrodynamic injection of samples was carried out at 0.5 psi during 5 s. The detection wavelength was 254 nm. Separation was performed at -15 kV and 25ºC. Three analytical replicates per sample were made.

Carotenoid determination

Determination was based on a spectrophotometric analysis (Adalid *et al*., 2010) using a spectrophotometer with double-beam operation (model Lambda-25, Perkin-Elmer, Waltham, USA). The samples were thawed in the dark at 4ºC to avoid carotenoid oxidation. Carotenoid extractions were performed once in 0.1 g of thawed samples, which were shaken (Platform rocker STR6, Stuart, Staffordshire, UK) for 1 hour using 7 ml of organic solvents (ethanol:hexane, 4:3). The extractions were conducted in the dark to prevent light-induced carotenoid oxidation. Afterwards, 1 ml of distilled water was added to separate the organic solvent layers and 0.5 ml of the upper layer (hexane phase) was recovered and refrigerated at 4ºC to avoid carotenoid loss. A calibration plane relating the concentrations of lycopene and β-carotene standards (Sigma Chemical, St. Louis, USA) and absorbances at 452 nm (positive correlation) and 510 nm (negative correlation) was used to obtain β-carotene concentration. Seven joint standards with randomly paired concentrations of lycopene and β-carotene were used for calibration. Three analytical replicates per sample were analysed.

Genetic models and data analysis methodology

An additive, dominance and additive × additive (ADAA) model was employed for data analysis. The ADAA model assumes normal diploid segregation, inbred parents in diallel mating and linkage equilibrium . Additive x dominance and dominance x dominance epistasis were considered negligible, because in comparison with other genetic components, these epistasis components are very complicated and will decline quickly with progressive generations (Xu and Zhu, 1999). As the trials were carried out with a randomized complete block design under different environmental conditions, the ADAA model included genotype x environment interactions. The linear model for the

phenotypic value Y_{hiikl} of the *kth* mating type ($k = 0$ for parent, $k = 1$ for F_1, $k = 2$ for F_2, $k = 3$ for BC_1 and $k = 4$ for BC_2) from lines i and j in the lth block within the kth environment:

$$Y_{hijk} = \mu + E_h + G_{ijk} + GE_{hijk} + B_{l(k)} + \varepsilon_{hijkl} \quad (1)$$

where $Y_{hiikl} \sim (\mu, \sigma^2_P)$; E_h is the environment effect, $E_h \sim (0, \sigma^2_E)$; G_{ijk} is the total genetic main effect, $G_{ijk} \sim (0, \sigma^2_G)$; GE_{hijk} is the total genotype x environment interaction effect, $GE_{hijk} \sim (0, \sigma^2_{GE})$; $B_{l(k)}$ is the randomized complete block effect, $B_{l(k)} \sim (0, \sigma^2_B)$; and ε_{hijkl} is the residual effect, $\varepsilon_{hijkl} \sim (0, \sigma^2_\varepsilon)$.

As the experiments were conducted with modified diallel mating with two parents and their F_1, F_2, BC_1 and BC_2, the G_{ijk} and GE_{hijk} components could be partitioned into genetic sub-components for each generation (Zhu, 1992; Yan *et al.*, 1998):

For parent P_i ($i = j$, $k = 0$):

$$G_{ii0} = 2A_i + D_{ii} + 4AA_{ii} \quad (1.1)$$

$$GE_{hii0} = 2AE_{hi} + DE_{hii} + 4AAE_{hii} \quad (1.2)$$

For F_{1ij}, from Pi x Pj ($k = 1$):

$$G_{ij1} = A_i + A_j + D_{ij} + AA_{ii} + AA_{jj} + 2AA_{ij} \quad (1.3)$$

$$GE_{hij1} = AE_{hi} + AE_{hj} + DE_{hij} + AAE_{hii} + AAE_{hjj} + 2AAE_{hij} \quad (1.4)$$

For F_{2ij}, derived from the selfing of F_{1ij} ($k = 2$):

$$G_{ij2} = A_i + A_j + 0.25D_{ii} + 0.25D_{jj} + 0.5D_{ij} + AA_{ii} + AA_{jj} + 2AA_{ij} \quad (1.5)$$

$$GE_{hij2} = AE_{hi} + AE_{hj} + 0.25DE_{hii} + 0.25DE_{hjj} + 0.5DE_{hij} + AAE_{hii} + AAE_{hjj} + 2AAE_{hij} \quad (1.6)$$

For BC_{1ij}, from F_{1ij} x P_i ($k = 3$):

$$G_{ij3} = 1.5A_i + 0.5A_j + 0.5D_{ii} + 0.5D_{ij} + 2.25AA_{ii} + 0.25AA_{jj} + 1.5AA_{ij} \quad (1.7)$$

$$GE_{hij3} = 1.5AE_{hi} + 0.5AE_{hj} + 0.5DE_{hii} + 0.5DE_{hij} + 2.25AAE_{hii} + 0.25AAE_{hjj} + 1.5AAE_{hij} \quad (1.8)$$

For BC_{2ij}, from F_{1ij} x P_j ($k = 4$):

$$G_{ij4} = 0.5A_i + 1.5A_j + 0.5D_{jj} + 0.5D_{ij} + 0.25AA_{ii} + 2.25AA_{jj} + 1.5AA_{ij} \quad (1.9)$$

$$GE_{hij4} = 0.5AE_{hi} + 1.5AE_{hj} + 0.5DE_{hjj} + 0.5DE_{hij} + 0.25AAE_{hii} + 2.25AAE_{hjj} + 1.5AAE_{hij} \quad (1.10)$$

where A_i and A_j are the cumulative additive effects of parents i and j, respectively, A_i and $A_j \sim (0, \sigma^2_A)$; D_{ii}, D_{jj} and D_{ij} are the cumulative dominance effects, D_{ii}, D_{jj} and D_{ij}

$\sim$ (0,σ^2_D); AA_{ii}, AA_{jj} and AA_{ij} are the cumulative additive × additive epistasis effects, AA_{ii}, AA_{jj} and AA_{ij} $\sim$(0,σ^2_{AA}); AE_{hi} and AE_{hj} are the cumulative additive × environment interaction effects, AE_{hi} and AE_{hj} $\sim$ (0,σ^2_{AE}); DE_{hii}, DE_{hjj} and DE_{hij} are the cumulative dominance × environment interaction effects, DE_{hii}, DE_{hjj} and DE_{hij} $\sim$ (0,σ^2_{DE}); and AAE_{hii}, AAE_{hjj} and AAE_{hij} are the cumulative additive × additive × environment interaction effects, AAE_{hii}, AAE_{hjj} and AAE_{hij} $\sim$ (0,σ^2_{AAE}).

As a result, the phenotypic variance (V_P) could be partitioned as:

$$V_P = V_G + V_{GxE} + V_e = V_A + V_D + V_{AA} + V_{AE} + V_{DE} + V_{AAE} + V_e \quad (2)$$

where V_G is the genotypic main variance which could also be partitioned into additive (V_A), dominance (V_D) and epistatic (V_{AA}) variances; V_{GE} is the genotype x environment interaction variance, partitioned into additive x environment (V_{AE}), dominance x environment (V_{DE}) and epistatic x environment (V_{AAE}) interaction variances and, finally, V_e is the residual variance.

Analogously, the equivalent expression for covariance components was:

$$C_P = C_G + C_{GxE} + C_e = C_A + C_D + C_{AA} + C_{AE} + C_{DE} + C_{AAE} + C_e \quad (3)$$

Since the total genetic effect was partitioned into components for genetic main effects and GE interaction effects, the total narrow-sense heritability (4) should also be differentiated into narrow-sense heritability across environments (4.1) and narrow-sense heritability for genotype by environment interaction (4.2).

$$h^2 = h^2_G + h^2_{GE} \quad (4)$$

$$h^2_G = (V_A + V_{AA})/V_P \quad (4.1)$$

$$h^2_{GE} = (V_{AE} + V_{AAE})/V_P \quad (4.2)$$

General heterosis (H_M, performance of heterosis expected across different environments) and interaction heterosis (H_{ME}, deviation from general heterosis in a specific environment) over midparent for an F_1 generation for an ADAA model were obtained from (Xu and Zhu, 1999):

$$H_M(F_1) = G(F_{1ij}) - 0.5[G(P_i) + G(P_j)] = [D_{ij} - 0.5(D_{ii} + D_{jj})] + 2[AA_{ij} - 0.5(AA_{ii} + AA_{jj})] = \Delta_D + 2\Delta_{AA} \quad (5)$$

$$H_{ME}(F_1) = GE(F_{1ij}) - 0.5[GE(P_i) + GE(P_j)] = [DE_{hij} - 0.5(DE_{hii} + DE_{hjj})] + 2[AAE_{hij} - 0.5(AAE_{hii} + AAE_{hjj})] = \Delta_{DE} + 2\Delta_{AAE} \quad (6)$$

where Δ_D is dominance heterosis, Δ_{AA} epistasis heterosis, Δ_{DE} dominance x environment heterosis and Δ_{AAE} epistasis x environment heterosis.

Estimation of variance and covariance components and prediction of genetic effects were performed by expressing the genetic model (1) as a mixed linear model:

$$y = Xb + \sum_{u=1}^{m-1} U_u e_u + e_m \sim \left(Xb, V = \sum_{u=1}^{m-1} \sigma_u^2 U_u U_u^T + \sigma_m^2 I \right) \quad (7)$$

where *y* is the vector of phenotype values with mean *Xb* and variance *V*; *b* is the vector of fixed effects; *X* is the known incidence matrix relating to the fixed effects; e_u is the vector of the *u*th random factor, $e_u \sim (0, \sigma^2_u I)$; U_u is the known incidence matrix relating to the random vector e_u. General mean and environmental effect were considered as fixed and genetic effects as random. According to the model (7), random effects (A, D, AA, AE, DE, AAE) were predictable without bias by the adjusted unbiased prediction (AUP) method (Zhu and Weir, 1996). Variance and covariance components for each trait were estimated by minimum norm quadratic unbiased estimation method 1 (MINQUE 1) (Zhu, 1992; Zhu and Weir, 1996). Standard errors of the statistics were obtained by the jackknife procedures (Miller, 1974; Zhu, 1989), and one-tailed *t*-tests (variances, proportion variances, heritabilities and covariances) and two-tailed *t*-tests (genetic predictors) were performed to test the significance of genetic parameters. These computations were performed with the QGA Station software version 1.0 (provided by Dr. Jun Zhu, director of the Bioinformatics Institute, Zhejiang University, China).

Estimations of the effective number of loci ($\hat{n}_e$) in which the accessions studied differ for the control of β-carotene and ascorbic acid accumulation were performed using the Castle-Wright estimator (Lynch and Walsh, 1998):

$$\hat{n}_e = \frac{[\bar{P}_i - \bar{P}_j]^2 - V_{P_i} - V_{P_j}}{8V_S} \quad (8)$$

where $\bar{P}_i$ and $\bar{P}_j$ are the phenotypic mean of parents *i* and *j*, respectively, V_{P_i} and V_{P_j} are the phenotypic variance of parents *i* and *j*, respectively, and V_S is the segregational variance estimate. The model used to derive the equation (5) assumed additive gene action, unlinked loci and equality of allelic effects. As genotype x environment interaction was not considered, it was used for each environment separately. Additionally, in order to minimize the bias caused by the sources of non-additivity, log transformed data and the $V_S = 2V_{F_2} - V_{BC_1} - V_{BC_2}$ expression were used. Standard error of $\hat{n}_e$ *was* calculated from:

$$SE(\hat{n}_e) = \hat{n}_e \sqrt{\frac{\left[\frac{4\left(V_{P_i} - V_{P_j}\right)}{\left[\bar{P}_i - \bar{P}_j\right]^2} + \frac{\left(\frac{2\,V_{F_2}{}^2}{n_{F_2}+2} + \frac{2\,V_{F_1}{}^2}{n_{F_1}+2}\right)}{\left(2V_{F_2} - V_{BC_1} - V_{BC_2}\right)^2}\right]}{n_t}} \quad (9)$$

where n_{F_2}, n_{F_1} and n_t are the number of F_2, F_1 and total plants, respectively; V_{F_2}, V_{BC_1} and V_{BC_2} are the phenotypic variance of the F_2, BC_1 and BC_2 generations.

RESULTS

Phenotypic means of the generations

The means of both parents for β-carotene accumulation had a different variation trend in the two environments (Table 1). The male parent (CDP4777) had higher β-carotene content in open field cultivation in Castellón (OP) than under the protected environment in Valencia (PC). Conversely, the female parent (CDP8779) showed higher β-carotene accumulation under protected cultivation. In the open field trial, the mean performance of the F_1 and F_2 generations was intermediate between both parents. The mean β-carotene content in BC_1 was slightly higher that the female parent and slightly lower than the male parent in BC_2, clearly suggesting an additive model of inheritance for β-carotene accumulation. Nevertheless, in protected cultivation, the F_1, F_2 and BC generations showed a mean β-carotene accumulation closer to that of the male parent. This reveals that the expression of the β-carotene accumulation character in this family may be affected by genotype (with an important additive contribution), as well as by GE interaction effects.

Table 1. Phenotypic values (mean ± standard deviation) of generations for β-carotene and ascorbic acid content in two environments (open field and protected cultivation) in mg kg^{-1} fresh weight (fw).

Generation	β-carotene		Ascorbic acid	
	Open field	Protected cultivation	Open field	Protected cultivation
P_i(CDP8779)	12±2	19±3	76±3	119±17
P_j(CDP4777)	37±12	24±5	254±59	246±83
F1	25±6	24±5	175±31	157±78
F2	23±9	24±7	161±42	154±68
BC1	16±8	23±1	142±34	153±69
BC2	32±11	26±8	209±54	178±64

For ascorbic acid accumulation, the performance of the parents, filial generations and backcrosses was similar to that described for β-carotene in open field conditions. Nevertheless, the genetic additive and the GE interaction effects were less evident, and therefore a more complex inheritance model may be involved for this trait.

Estimation of variance and covariance components and heritability

A decomposition of phenotypic variance in total genetic (additive, dominant, epistatic and their interactions with the environment) and residual components was carried out (Table 2). All the calculated estimates of the variance components were significantly different from zero, and thus offer reliable information regarding which of these variance components are more determinant in the inheritance of the two quality traits studied. For β-carotene accumulation, the total genetic variance (V_G + V_{GE}) explained over 65% of the phenotypic variance. In the case of ascorbic acid accumulation, it explained more than 51% of the phenotypic variance.

Table 2. Estimates (± SE) of additive (V_A), dominant (V_D), epistatic (V_{AA}), genotype x environment (V_{AE}, V_{DE}, V_{AAE}), phenotypic (V_P) and residual (V_ε) variances (mg kg^{-1} fresh weight); general narrow-sense heritability across environments (h^2_G) and deviation from general narrow-sense heritability in specific environments (h^2_{GE}) for β-carotene and ascorbic acid content.

Parameters	β-carotene	Ascorbic acid
Variance components		
V_A	38** ± 3	2156** ± 174
V_D	5** ± 1	0 ns
V_{AA}	0 ns	751** ± 43
V_{AE}	75** ± 4	588** ± 36
V_{DE}	0 ns	0 ns
V_{AAE}	0 ns	0 ns
V_ε	64** ± 4	3270** ± 213
V_P	182** ± 20	6765** ± 1465
Heritability		
h^2_G	0.207** ± 0.01	0.430** ± 0.01
h^2_{GE}	0.413** ± 0.01	0.087** ± 0.01

** Significantly different from zero at the 0.01 level of probability. ns, non significant.

β-carotene accumulation was mainly affected by GxE interaction effects (63.6 % of the total genetic variance). The additive x environment component was the only interaction component detected. The additive and dominant genetic main effects represented 32.2% and 4.2 % of the total genetic variance, respectively. No epistatic effects were detected for this trait. Consequently, the estimates of total narrow-sense heritability (h^2) for this character from the CDP4777 donor accession (62%) had a

contribution of the interaction heritability (h^2_{GE}) component that was two times higher than the genotypic heritability (h^2_G) component (Table 2).

Ascorbic acid accumulation was mainly determined by genetic main effects (83.2% of the total genetic variance). Among these main genetic effects, the additive component (61.7% of the total genetic variance) was the most important, followed by additive epistatic effects (21.5% of the total genetic variance). No dominant effects were detected. For this trait, the GxE interaction effects (16.8% of the total genetic variance) were represented by the additive x environment component. The estimate of total narrow-sense heritability (h^2) was 51.7%, as the contribution to the genotypic heritability (h^2_G) component was five times higher than the interaction heritability (h^2_{GE}) component (Table 2).

In order to assess the possibility of a joint improvement of β-carotene and ascorbic acid content, the total genetic covariance between these two traits was partitioned into its components. Total genetic covariance ($C_G + C_{GE} = 5.60$) was highly significant ($P<0.01$) only due to the contribution of the main additive covariance component ($C_A = 3.80$; $P<0.01$), as the other covariance components ($C_D = 0.08$, $C_{AA} = 0.06$, $C_{AE} = 1.23$, $C_{DE} = 0.52$ and $C_{AAE} = -0.12$) were not significant. This positive covariance between β-carotene and ascorbic acid accumulation indicates that the joint improvement of these two traits using the CDP47777 accession as a donor parent is feasible in breeding programmes.

Estimation of fixed effects and prediction of genotypic and GE interaction effects

The ADAA model that was used allowed us to estimate the contribution of the fixed effects (general mean and environmental effects) and to predict the main genetic effects and their interactions with the environment in order to gain more insight into the genetic control of these two traits (Table 3). All the predicted effects were significant.

The estimates of the environmental contribution to trait expression showed that, in this study, the best environmental conditions for the expression of the two traits were those of the open field cultivation in Castellón. The daily average temperature was moderate and similar in both environments; however, the daily temperature range was slightly narrower and more homogenous in protected cultivation than in the open field due to the automated climate system in the glasshouse (Figure 1). Nevertheless, the greatest differences were found in the daily maximum PAR recorded from first

flowering to fruit ripening and harvesting, which was approximately 4 times higher in the open field than in protected cultivation. This PAR reduction in protected cultivation was due to the shadowing produced by the buildings close to the greenhouses at the Universitat Politècnica de València Campus. Even though these environmental effects explained part of the phenotype, their values had a lower impact than the genetic effects (Table 3). For example, the highest environmental effects for β-carotene and ascorbic acid accumulation in the F_1 hybrids were 7.6 and 3.6 times lower than the total genetic (G + GE) effect, respectively.

Table 3. Estimates of fixed effects and predictions of genetic ADAA model parameters (mg kg^{-1}) for β-carotene and ascorbic acid inheritance in CDP8779 x CDP4777 crosses.

	β-carotene	Ascorbic acid
μ	24.46	170.81
E_{OF}	1.43	9.31
E_{PC}	-0.21	-1.62
Genetic main effects		
A_i	-3.06** ± 0.9	-23.15** ± 4.7
A_j	3.06** ± 0.8	23.28** ± 5.9
D_{ii}	-2.47** ± 0.6	n.d.
D_{jj}	0.82** ± 0.1	n.d.
D_{ij}	1.65** ± 0.7	n.d.
AA_{ii}	n.d	-5.48* ± 0.8
AA_{jj}	n.d.	15.61** ± 2.5
AA_{ij}	n.d	-10.08* ± 1.9
GE interaction effects		
A_iE_{OF}	-7.09** ± 0.07	-20.19** ± 5.5
A_jE_{OF}	7.08** ± 0.10	20.26** ± 2.1
A_iE_{PC}	2.48** ± 0.04	-5.58** ± 1.0
A_jE_{PC}	-2.47** ± 0.06	5.65** ± 1.3

μ= general mean; E= environmental effect; OF=Open field, PC=Protected cultivation; A=additive effect; D=dominance effect; $_i$= female parent; $_j$=male parent, n.d.= predicted effects not determined due to the corresponding zero estimates of variance components in Table 2. *,** significant at 0.05 and 0.01 level, respectively.

For β-carotene accumulation, the difference between the two parents due to the fact that the single loci contribution combined additively (additive main effects) was 6.12 mg kg^{-1} fresh weight (Table 3). So, for the F_1 hybrids, the expected increment of the β-carotene content over the breeding line used as the female parent (CPD8779) was 3.06 mg kg^{-1} fresh weight. For this trait, non-additive gene action effects were due to allelic relationships between loci (dominance). The highest dominance effect predicted was the heterozygous dominant effect (D_{ij}). It slightly exceeded the homozygous dominant effects of the best parent (CDP4777 accession), indicating a small overdominance expression of the trait in the heterozygotes. This overdominance effect was the only cause of heterosis, because no allelic relationships between loci (epistasis)

and no interactions of dominant or epistatic effects with the environment were detected. Consequently, the second term in both equations 5 and 6 equaled 0. The heterosis over mid-parent in F_1 (difference between the genotype of F_1 and the average of the parent genotypes) was 2.47 mg kg^{-1} fresh weight, which is to say that this heterotic effect over mid-parent in F1 represented approximately 81% of the increment of the trait expression of the additive effect (A). Additionally, in target environments similar to the open field conditions of this study (Figure 1 c, d), the considerable additive x environment interaction effect may increase the content of β-carotene of F_1 hybrids up to 7.08 mg kg^{-1} more than the common breeding line. Considering the predictions of all genotypic effects and the interaction with the best environment ($\mu + A + D + AE_{OF}$), the F_1 hybrid genotypes (26.11 mg kg^{-1} fresh weight) had 221.08 % more β-carotene content than the breeding line (11.84 mg kg^{-1} fresh weight). If we only considered the main genotypic potential ($\mu + A + D$), the increment would be 137.93 %.

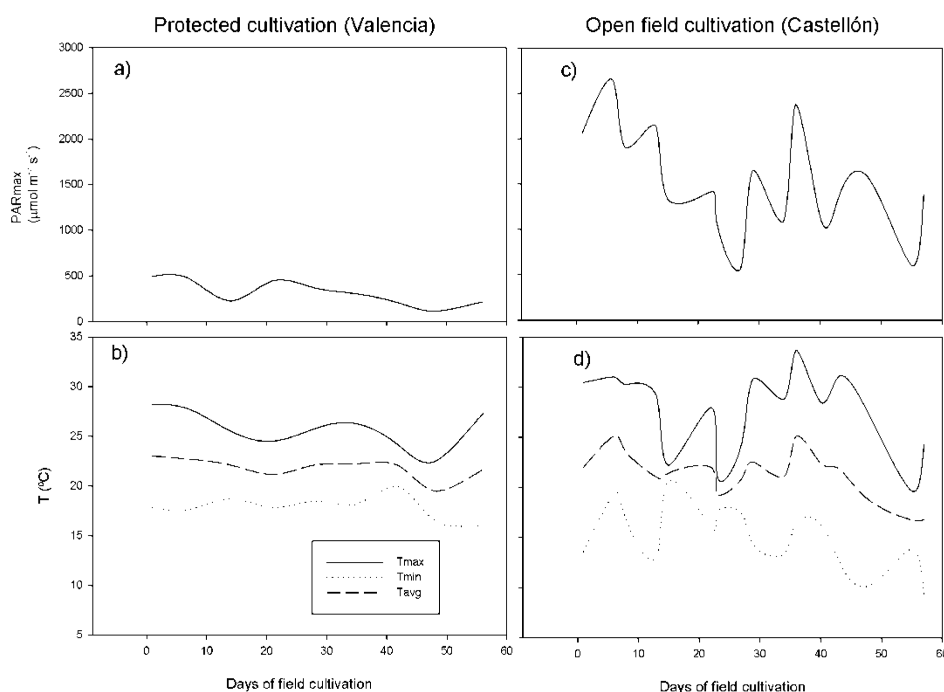

Figure 1: Temperature and photosynthetically active radiation measured in the two growing environments during the trial period.

For ascorbic acid accumulation, the expected content increment in F1 hybrids over that observed in the common breeding line used as the female parent is 23.28 mg kg^{-1} fresh weight (Table 3). The highest predicted epistatic effect was the homozygous

effect for the best donor parent. A subexpression of this epistatic effect, diminishing the homozygous epistatic effect from the common breeding line used as the female parental, was detected in heterozygosis. This subepistatic effect was the only cause of negative heterosis because no dominant effects (first term in equation 5 equals 0) and no interactions of dominant or epistatic effects with the environment (equation 6 equals 0) were detected. This negative heterosis under mid-parent was -20.62 mg kg^{-1} fresh weight and cancels out 88% of the main additive effect (A) in the trait expression. Additionally, in target environments similar to the open field conditions in this study (Figure 1 c, d), the important additive x environment interaction effect could increase the content of ascorbic acid in F_1 hybrids up to 20.19 mg kg^{-1} more than the common breeding line. Considering the predictions of all genotypic effects and the interaction with the best environment (μ + A + AA + AE_{OF}), the F_1 hybrid genotypes (160.73 mg kg^{-1} fresh weight) had 31.75 % more ascorbic acid content than the common breeding line (121.99 mg kg^{-1} fresh weight). If we only considered the main genotypic potential (μ + A + AA), the increment would be 13 %.

Estimation of effective number of loci

Prior to the estimation of the effective number of loci in which the CDP8779 and CDP4777 accessions differ in the genetic control of the traits, a log transformation of the data was performed. With this transformation, variance component estimation of the ADAA model showed that departures from additivity in the open field for β-carotene (V_D / V_P = 0,12; V_{AA} / V_P = 0) and ascorbic acid accumulation (V_D / V_P = 0,06 ; V_{AA} / V_P = 0) were only significant for the dominance component, but had a lower impact. In protected cultivation, these hypothesis violations were also very small: β-carotene (V_D/V_P = 0,04; V_{AA}/V_P = 0) and ascorbic acid accumulation (V_D/V_P = 0; V_{AA}/V_P = 0,02). Nevertheless, the possible problems with dominance would be eliminated by the expression of segregation variance used in equation (5). Therefore, the estimates obtained for the effective number of loci in which CDP8779 and CDP4777 accessions differ for each environment provided useful information. In the open field, the estimate (± SE) of the effective number of loci for β-carotene accumulation was 0.67±0.07, and in protected cultivation it was 0.20±0.03. We can round these estimates to whole numbers and consider that the effective number of loci for β-carotene accumulation in both environments was 1. For ascorbic acid accumulation, the estimate of the effective

number of loci was 3.62±0.14 (rounded to 4) and 0.63±0.04 (rounded to 1) in the open field and glasshouse, respectively. In the case of ascorbic acid accumulation, the growing environment may have an important influence in the number of genes that contribute to the trait phenotype. For β-carotene accumulation, this environmental influence is less important, and the predicted number of effective factors would be considered one.

DISCUSSION

In a previous work, we evaluated several *S. lycopersicum* accessions, finding that the CDP4777 accession from *S. lycopersicum* var *cerasiforme* was of great interest due to its very high genotypic (μ + G) potential for accumulating β-carotene and ascorbic acid (825% and 150%, respectively, of the reported average phenotypic contents) and the high stability in their expression (Roselló *et al.*, 2011). Additionally, in this accession, the high β-carotene content is not joined to low lycopene content as normally occurs in tomato mutants with high β-carotene content (Stommel and Haynes, 1994; Zhang and Stommel, 2000). All these characteristics make the CDP4777 accession a very interesting potential donor parent in breeding programmes aimed at developing hybrids with high β-carotene and ascorbic acid contents and no depressed lycopene content. Nevertheless, in order to exploit it effectively, a more detailed understanding of the genetic control and inheritance of these traits was sought out in this study.

We employed an ADAA model that is widely used to perform genetic analyses on the expression of agronomic, quality or morphological traits in several crops (Yan *et al.*, 1998; McCarty *et al.*, 2004a, 2004b, 2008). This model showed a phenotypic variance decomposition that was consistent with previous results, thus offering reliable information about genetic control. In Roselló *et al.* (2011), five accessions and four controls from *S. lycopersicum*, one *S. lycopersicum* var. *cerasiforme* and four *S. pimpinellifolium* L. accessions were evaluated (including the two used as parents in this study) in three growing environments. The general estimation of total genetic variance ($V_G + V_{GxE}$) for β-carotene content accounted for 70.6% of the phenotypic variance and for 32.3% of the ascorbic acid content. In this study, the particular estimation of total genetic variance for the CDP8779 x CDP4777 cross was 65.0% for β-carotene (similar to the previous study) and 51.7% for ascorbic acid (better than the previous study). The

total genetic variance explained for ascorbic acid was not as high as for β-carotene, but it should be noted that ascorbic acid plays a very active and important role in reducing oxidative damage at the cellular level caused by stress conditions, and it is very difficult to model the genetic control in its accumulation due to uncontrolled factors (Shigeoka *et al*., 2002). Nevertheless, for the two accessions studied here, the proportion of genotypic variance and genotypic x environment interaction variance (23% and 41%, respectively) is different (65.25% and 5.37%, respectively) from the previous general trend. It should be taken into account that, in the previous study, the GxE predictions for β-carotene accumulation in the CDP4777 accession were low in all environments, but were very high in the growing cycle (autumn-winter) for the CDP8779 accession, which is similar to the results of this study, indicating than the proportion of the GxE interaction of β-carotene accumulation from CDP4777 is dependent on the stability of the accession used as the female parental. The more stable the accession used as the female genitor is, the lower its influence will be on the GxE interactions. Conversely, for ascorbic acid content, the GxE interaction was the same as the previous general trend. This agrees with the previous GxE predictions for ascorbic acid accumulations in both accessions as being low in all environments.

For β-carotene accumulation, the ADAA model used indicates that the genetic mode of inheritance was mainly additive with a small dominant component. Despite the low proportion of the dominant component, this contributes to a small heterotic effect over the mid-parent that increases the trait expression in heterozygotes and, consequently, the genotypic value of the F1 hybrids. Additionally, in target environments, the most important AxE interaction contribution could substantially enhance the β-carotene content. So, even though its total narrow-sense heritability may be considered high (h^2 = 0.62), it has an important component (h^2_{GE} = 0.41) dependent on the AxE interaction. One is the estimated number of loci in which the accessions studied differ for the genetic control of β-carotene accumulation.

Several inheritance modes for high β-carotene content in tomato have been reported. Tomes *et al*. (1954) indicated that high β-carotene concentrations in orange-fruited tomato derived from an interspecific *S. lycopersicum* x *Solanum habrochaites* S. Knapp & D.M. Spooner (former *Lycopersicon hirsutum* Dunal) cross and conditioned by a single dominant gene (*Beta*) and subject to influence by a modifier gene (Mo_B). Recent studies (Zhang and Stommel, 2000) demonstrate that *Beta* and Mo_B are linked

on chromosome 6 and do not segregate independently as originally proposed. In red-fruited tomatoes, β-carotene accounts for only 10–15% of the total carotenoids. Conversely, in genotypes with homozygous recessive Mo_B Mo_B, β-carotene accounts for >90% of the coloured carotenes. However, with the dominant Mo_B^+ allele, β-carotene content was reduced to nearly 50% of the total carotenoids, whereas lycopene increases to almost 50% of the total carotenoids, resulting in red-orange fruit pigmentation. In the genetic control postulated, the β-carotene content would increase at the expense of lycopene (Stommel *et al*., 2005). Orange-fruited accessions from *S. cheesmaniae*, *S. pimpinellifolium*, *S. chilense* and *S. chmielewskii* containing high concentrations of β-carotene have also been described (Chalukova 1988; Manuelyan *et al*., 1975; Rick, 956; Stommel and Haynes 1994). The inheritance of β-carotene content in these wild tomato species is consistent with that described for the dominant *Beta* gene from *S. habrochaites*. On the other hand, in hybrids between large-fruited and cherry fruit lines, Causse *et al*. (2003) found that carotene was inherited in an additive manner with a significant environmental effect, but with a non-significant genotype x environment interaction effect.

Our results regarding the inheritance of high β-carotene accumulation from the *S. lycopersicum* var *cerasiforme* CDP4777 accession indicate that this accession is a new and very interesting source of variability for this trait. Its genotypic value was similar to the phenotypic value reported by the donor parental in Stommel and Haynes (1994) and 105% higher than the phenotypic value of the donor cherry lines in Cause *et al*. (2003). Its inheritance mode, which does not match those previously reported, is simple, and its additive nature and heritability facilitates its use in breeding programmes. It can be seen in the genotypic trait performance of the F1 hybrids obtained from this accession that had shown 221.08 % β-carotene content of the breeding line used as the female genitor and 669.49% of the 3.9 mg kg^{-1} of phenotypic trait expression commonly reported (Holden *et al*., 1999). Nevertheless, these breeding programmes should be designed to obtain genotypes for target environments with daily mean temperatures near the optimum (30ºC) for β-carotene biosynthesis (Hamauzu *et al*., 1998) with no reduced radiation. In these target environments, the positive AE interaction greatly increases trait expression. This positive AE interaction effect will be higher than observed in this study in spring-summer growing cycles due to greater sunlight intensity and photosynthetic rate (Brown, 1954; McCollum, 1954) combined

with adequate temperatures, as was previously observed for the CDP4777 accession, and with photosynthetic active radiations below 2340 umolm^{-2}s^{-1} (Roselló *et al*., 2011).

For ascorbic acid accumulation, the ADAA model used indicates that the genetic mode of inheritance was also mainly additive with a minor additive epistasis component. This epistatic effect causes a negative heterosis that reduces the positive main additive effect. The genotypic value of the F1 hybrids was not as high as it would be if inferred from CDP4777 performance in previous trials (Roselló *et al*., 2011). Nevertheless, as occurs for β-carotene content, in target environments, the AxE interaction contribution could enhance ascorbic acid content and compensate the negative heterosis effect. Previous works suggested an additive (Causse *et al*., 2003) or dominant-additive (Bhatt *et al*., 1998, 2001) genetic control in *S. lycopersicum*. Nevertheless, in these works, only additive-dominant models were used. The total narrow-sense heritability of this trait can be considered good ($h^2 = 0.52$), as it has only a small component ($h^2_{GE} = 0.09$) dependent on the AxE interaction. The maximum number of effective loci in which the parentals differ for the trait control was estimated at four for conditions in which more metabolic pathways would contribute to ascorbic acid biosynthesis. However, the genetic control of ascorbic acid accumulation is complex because the amount of this molecule in plant cells depends not only on the strict regulation of its synthesis (Smirnoff *et al*., 2001), but also on its metabolic recycling, degradation (Green and Fry, 2005) and transport (Horemans *et al*., 2000). Furthermore, several alternative biosynthetic pathways have been identified, and it is therefore difficult to pin down exactly how its synthesis is controlled in the context of development, stress responses and normal homeostasis (Valpuesta and Botella, 2004). This supports the idea that the different environmental conditions of different locations could act on the gene expression of this molecule.

Our results showed that, despite the complex genetic control of ascorbic acid accumulation of the CDP4777 accession, its generally additive nature (main, epistatic and interaction with the environment) could finally be set in the course of breeding programmes. Its genotypic value was similar to the best phenotypic values of donor parents reported by Bhatt *et al*. (2001) and 133% of the best phenotypic values of cherry accessions in Cause *et al*. (2003). Additionally, this should be considered as doubly valuable since, as shown in the covariance analysis, this trait could be jointly improved with high β-carotene content selection. Initially, the genotypic trait performance of the

F1 hybrids obtained from this accession will have 131% ascorbic acid content of the breeding line used as the female genitor. In our case, this breeding line normally shows ascorbic acid contents lower than the 200 mg kg^{-1} phenotypic trait expression commonly reported (Gould, 1992). As for β-carotene, the AE contribution, while not as significant as in the previous case, makes it clear that the derived genotypes in these breeding programmes should be designed to target environments similar to those described above. This is due to the fact that ascorbic acid accumulation in tomato fruits also seems to be directly correlated with temperature (Liptay *et al*., 1986) and solar radiation (López-Andreu *et al*., 1986), as long as they do not reach stressful levels.

In conclusion, the present study shows that the CDP4777 accession from *S. lycopersicum* var *cerasiforme* is a very interesting donor parent for the joint improvement of β-carotene (without diminishing lycopene content) and ascorbic acid content in commercial tomato nutraceutical breeding programmes.

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REFERENCES

ADALID, A. M., ROSELLÓ, S. & NUEZ, F. (2010). Evaluation and selection of accessions of *Solanum* section *Lycopersicon* for content of bioactive components. *Journal of Food Composition and Analysis* **23**, 613–618.

BHATT, R. P., BISWAS, V. R. & KUMAR, N. (2001). Heterosis, combining ability and genetics for vitamin C, total soluble solids and yield in tomato (*Lycopersicon esculentum*) at 1700 m altitude. *The Journal of Agricultural Science* **137**, 71-75.

BHATT, R. P., BISWAS, V R., PANDEY, H. K., VERMA, G. S. & KUMAR, N. (1998). Heterosis for vitamin C in tomato (*Lycopersicon esculentum*). *Indian Journal of Agricultural Science* **68**, 176-178.

BROWN, G. B. (1954). The ascorbic acid content of tomatoes as related to illumination. *Journal of American Society of Horticulture Science* **65,** 342–348.

BYERS, T. & GUERRERO, N. (1995). Epidemiologic evidence for vitamin C and vitamin E in cancer prevention. *American Journal of Clinical Nutrition* **62**, 1385S-1392S.

CAUSSE, M., BURET, M., ROBINI, K. & VERSCHAVE, P. (2003). Inheritance of nutritional and sensory quality traits in fresh market tomato and relation to consumer preferences. *Journal of Food Science* **68**, 2342-2350.

CHALUKOVA, M. (1988). Carotenoid composition of the fruits of hybrids between *Lycopersicon esculentum* and some wild species of the genus *Lycopersicon,* IV. Progenies of lycopene and β-carotene BC1P1 hybrids of *L chmielewskii. Genet Breeding* **21,** 49-57.

GALIANA-BALAGUER, L., ROSELLÓ, S., HERRERO-MARTÍNEZ, J. M., MAQUIEIRA, A. & NUEZ, F. (2001). Determination of L-Ascorbic Acid in *Lycopersicon* Fruits by Capillary Zone Electrophoresis. *Analytical Biochemistry* **296,** 218-224.

GOULD, W. A. (1992) Tomato production, processing and technology. CTI Publications, Baltimore,MD.

GREEN, M. A. & FRY, S. C. (2005). Vitamin C degradation in plant cells via enzymatic hydrolysis of 4-Ooxalyl- L-threonate. *Nature* **433,** 83-87.

HAMAUZU, Y., CHACHIN, K. & UEDA, Y. (1998) Effect of postharvest storage temperatura on the conversión of 14C-mevalonic acid to carotenes in tomato fruit. *Journal of Japanese Horticultural Society* **67**, 849-855.

HANSON, P. M., YANG, R. Y., WU, J., CHEN, J. T., LEDESMA, D., TSOU, S. & LEE, T. (2004) Variation for antioxidant activity and antioxidants in tomato. *Journal of American Society of Horticultural Science* **129**, 704-711.

HOLDEN, J. M., ELDRIDGE, A. L., BEECHER, G. R., BUZZARD, I. M., BHAGWAT, S., DAVIS, C. S., DOUGLASS, L. W., GEBHARDT, S., HAYTOWITZ, D. & SCHAKEL, S. (1999). Carotenoid content of US foods: an update of the database. *Journal of Food Composition and Analysis* **12**, 169-196

HOREMANS, N., FOYER, C. H. & ASARD, H. (2000). Transport and action of ascorbate at the plant plasma membrane. *Trends in Plant Science* **5**, 263-267.

ISAACSON, T., RONEN, G., ZAMIR, D. & HIRSCHBERG, J. (2002). Cloning of Tangerine from Tomato Reveals a Carotenoid Isomerase Essential for the Production of β-Carotene and Xanthophylls in Plants. *The Plant Cell* **14**, 333–342.

LENUCCI, M. S., CACCIOPPOLA, A., DURANTE, M., SERRONE, L., PIRO, G. & DALESSANDRO, G. (2007). Carotenoids content in ripe raw and processed (sauce) berries of high pigment tomato hybrids. *Acta Horticulturae* **758**, 173-179.

LIPTAY, A., PAPADOPOULOS, A. P., BRYAN, H. H. & GULL, D. (1986). Ascorbic acid levels in tomato (*Lycopersicon esculentum* Mill) at low temperatures. *Agricultural and Biological Chemistry* **50**, 3185-3187.

LOPEZ-ANDREU, F. J., LAMELA, A., ESTEBAN, R. M. & COLLADO, G. J. (1986). Evolution of quality parameters in the maturation stage of tomato fruits. *Acta Horticulturae* **191**, 387–394.

LYNCH, M. & WALSH, B. (1998). *Analysis of Quantitative Traits*. Sinauer Associates, Inc. Publishers, Sunderland, Massachussetts, USA.

MANUELYAN, H., YORDANOV, M., YORDANOVA, Z., & ILLEVA, Z. (1975). Studies on β-carotene and lycopene content in the fruits of *Lycopersicon esculentum* Mill, x *L chilense* Dun. hybrids. *Qualitas Plantarum: Plant Foods for Human Nutrition* **25**, 205-210.

MARCHIOLI, R., SCHWEIGER, C., LEVANTESI, G., TAVAZZI, L. & VALAGUSSA, F. (2001). Antioxidant vitamins and prevention of cardiovascular disease: epidemiological and clinical trial data. *Lipids* **36,** S53-S63.

McCARTY, J. C., JENKINS, J. N. & JIXIANG, W. (2004a). Primitive accession derived germplasm by cultivar crosses as sources for cotton improvement: I. Phenotypic values and variance components. *Crop Science* **44**, 1226-1230.

McCARTY, J. C., JENKINS, J. N. & JIXIANG, W. (2004b). Primitive accession derived germplasm by cultivar crosses as sources for cotton improvement: II. Genetic effects and genotypic values. *Crop Science* **44**, 1231-1235.

McCARTY, J. C., JIXIANG, W. & JENKINS, J. N. (2008). Genetic association of cotton yield with its components traits in derived primitive accessions crossed by elite upland cultivars using the conditional ADAA genetic model. *Euphytica* **161**, 337-352.

McCOLLUM, J. P. (1954). Effects of light on the formation of carotenoids in tomato fruits. *Food Research* **19**, 182–189.

MILLER, R. G. (1974). The jackknife: a review. *Biometrika* **61**, 1–15.

O' TOOLE P & LOMBARD M. (1996). Vitamin C and gastric cancer: supplements for some or fruit for all? *Gut* **39**, 345-347.

REGO, E. R.; FINGER, F. L., CASALI, V. W .D. & CARDOS, A. A. (1999). Inheritance of fruit color and pigment changes in a yellow tomato (*Lycopersicon esculentum* Mill.) mutant. *Genetics and Molecular Biology* **22**, 101-104.

RICK, C. M. (1956). Genetic and systematic studies on accessions of *Lycopersicon* from the Galapagos Islands. *American Journal of Botany* **43**, 687-696.

RONEN, G., CARMEL-GOREN, L., ZAMIR, D. & HIRSCHBERG, J. (2000). An alternative pathway to β-carotene formation in plant chromoplasts discovered by

map-based cloning of Beta and old-gold color mutations in tomato. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 11102–11107.

RONEN, G., COHEN, M., ZAMIR, D. & HIRSCHBERG, J. (1999). Regulation of carotenoid biosynthesis during tomato fruit development: expression of the gene for lycopene epsilon-cyclase is down-regulated during ripening and is elevated in the mutant Delta. *Plant Journal* **17**, 341-351.

ROSELLÓ, S., ADALID, A. M., CORNEJO-CEBOLLA, J. & NUEZ, F. (2011). Evaluation of the genotype, environment and their interaction on carotenoid and ascorbic acid accumulation in tomato germplasm. *Journal of Science Food and Agriculture* **91**, 1014–1021.

ROSS, D. A. (1998). Vitamin A and public health; Challenges for the next decade. *Proceedings of the Nutrition Society* **57**, 159-165.

SHIGEOKA, S., ISHIKAWA, T., TAMOI, M., MIYAGAWA, Y., TAKEDA, T., YABUTA, Y. & YOSHIMURA, K. (2002) .Regulation and function of ascorbate peroxidase isoenzymes. *Journal of Experimental Botany* **53**, 1305–1319.

SMIRNOFF, N., CONKLIN, P. L. & LOEWUS, F. A. (2001). Biosynthesis of ascorbic acid in plants:a renaissance. *Annual Review of Plant Physiology and Plant MolecularBiology* **52**, 437-467.

STEVENS, M. A. & RICK, C. M. (1986). Genetics and breeding: fruit quality. In: *The Tomato Crop. A Scientific Basis for Improvement* (Athernon, J.G. and Rudich, J., eds.). Chapman and Hall, London, England.

STEVENS, R., BURET, M., DUFFE, P., GARCHERY, C., BALDET, P., ROTHAN, C. & CAUSSE, M. (2007). Candidate genes and quantitative trait loci affecting fruit ascorbic acid content in three tomato populations. *Plant Physiology* **143**, 1943-1953.

STOMMEL, J. R. & HAYNES, K. G. (1994). Inheritance of Beta Carotene Content in the Wild Tomato Species *Lycoporsicon cheesmanii*. *Journal of heredity* **85**, 401-404.

STOMMEL, J. R., ABBOT, J. A. & SAFTNER, R. A. (2005). USDA 02L1058 and 02L1059: Cherry Tomato Breeding Lines with High Fruit β-carotene Content. *HortScience* **40**, 1569-1570.

TEE, E. S. (1992). Carotenoids and retinoids in human nutrition. *Critical reviews in food Science and Nutrition* **31**, 103-163.

TOMES, M. L., QUACKENBUSH, F. W., & MCQUISTAN, M. (1954). Modification and dominance of the gene governing formation of high concentrations of beta-carotene in the tomato. *Genetics* **39**, 810–817.

VALPUESTA, V. & BOTELLA, M. A. (2004) Biosynthesis of L-ascorbic acid in plants: new pathways for an old antioxidant. *Trends in Plant Science* **9**, 573-577.

XU, Z. C. & ZHU, J. (1999). An approach for predicting heterosis based on an additive, dominance and additive x additive model with environment interaction. *Heredity* **82**, 510-517.

YAN, X. F., XU, S. Y., XU, Y. H. & ZHU, J. (1998).Genetic investigation of contributions of embryo and endosperm genes to malt Kolbach index, alpha-amylase activity and wort nitrogen content in barley. *Theoretical and Applied Genetics* **96**, 709-715.

ZHANG, Y. & STOMMEL, J. R. (2000). RAPD and AFLP tagging and mapping of *Beta* (*B*) and *Beta* modifier (Mo_B), two genes which influence β-carotene accumulation in fruit of tomato (Lycopersicon esculentum Mill.). *Theorical Applied Genetics* **100**, 368-375.

ZHU, J. & WEIR, B. S. (1996). Diallel analysis for sex-linked and maternal effects. *Theorical Applied Genetics* **92**, 1–9.

ZHU, J. (1989). *Estimation of Genetic Variance Components in the General Mixed Models*. PhD tessis. North Carolina State University, Raleigh, NC, USA.

ZHU, J. (1992). Mixel model approaches for estimating genetic variances and covariances. *Journal of Biomathemathics* **7**, 1-11.

III.4. DETERMINACIÓN RÁPIDA DE LOS CAROTENOIDES PROMINENTES EN FRUTOS DE TOMATE POR CEC USANDO COLUMNAS MONOLÍTICAS BASADAS EN ESTERES DE METACRILATO.

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Research Article

Fast determination of prominent carotenoids in tomato fruits by CEC using methacrylate ester-based monolithic columns

In this study, the major carotenoids (β-carotene and lycopene) present in tomato fruits were analyzed by CEC with a methacrylate ester-based monolithic column. The effects of the porogenic solvent ratio, and the hydrophobicity of bulk monomer employed were examined on carotenoids separations. A fast separation of these analytes was achieved in less than 5.0 min in a mobile phase containing 35% THF, 30% ACN, 30% methanol, and 5% of a 5 mM Tris aqueous buffer, pH 8, with lauryl methacrylate-based monoliths. The CEC method was evaluated in terms of detection limit and reproducibility (retention time, area, and column preparation) with values below 1.6 μg/mL and 7.2%, respectively. The proposed procedure was successfully applied to the determination of both carotenoids in fruits of several tomato-related species and its usefulness to analyze large series of samples for nutritional quality screening trials in tomato breeding programs is demonstrated. To our knowledge, this is the first work that exploits the powerful and user-friendly monolithic technology for quality breeding and germplasm evaluation program purposes.

Keywords:
β-Carotene / CEC / Carotenoids / Lycopene / Methacrylate ester-based monolithic column

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1 Introduction

Carotenoids are considered among the most abundant naturally occurring pigments in vegetables and fruits [1–3]. Among these, tomato fruit and its products (*e.g.*, juices, soups, sauces, and ketchup) constitute an important source of carotenoids in the human diet (http://www.usda.gov/nass/pubs/agr05/acro05.htm; http://www.ers.usda.gov/briefing/consumption/gallery/tomatoconsumption.pdf). Due to its year-round availability, tomato is an immensely consumed food (15.4 and 25.4 kg *per* inhabitant and year in Europe and the world, respectively (http://www.fao.org/waicent/portal/statistics_es.asp)), being not only one of the main sources of carotenoids but also an excellent source of other healthy micronutrients [4, 5]. Lycopene is the most abundant carotenoid in red tomatoes, accounting for up to 90% of the total carotenoids [5, 6]. A typical red-pigmented tomato fruit also contains minor amounts of β-carotene and traces of ξ-carotene, γ-carotene, and neurosporene, and of the colorless precursors phytoene and phytofluene [7, 8]. Lycopene has the highest singlet oxygen quenching capacity *in vitro* [9] and their antioxidant properties are probably related to risk reduction of certain types of cancer. Remarkable inverse relationships between lycopene intake (consumption of tomato products) and cancer risk have been observed [10], especially for prostate and stomach cancer [11] and cardiovascular diseases [6, 12, 13]. β-Carotene is the most potent provitamin A carotenoid and its deficiency can cause xerophthalmia, blindness, and premature death [14], which are important nutritional problems in more than 75 countries, the majority of them located in the developing world [15]. The antioxidant properties of these carotenoids and the growing demand of healthy nutritional foods provide compelling arguments for an increase in the carotenoid content and consequently an increase in dietary intake of tomato, without altering the nutritional habits. This increase of the carotenoids' content in tomato can be easily carried out either by traditional breeding or by genetic manipulation.

One way to improve the carotenoid content in the tomato is the use of *Solanum* germplasm with high antioxidant content as a donor parent in breeding programs. In this sense, variations of more than three-fold (*e.g.*, β-carotene varied

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Abbreviations: AIBN, azobisisobutyronitrile; **BMA,** butyl methacrylate; **EDMA,** ethylene dimethacrylate; **LMA,** lauryl methacrylate; **META,** (2-(methacryloyloxy)ethyl) trimethylammonium chloride

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from 1.15 to 3.7 mg/kg fresh weight (wt) and total carotenoid content from 18.5 to 60.7 mg/kg fresh weight) have been reported in several tomato cultivars [16]. In previous studies with accessions of several species of the genus *Solanum*, which is phylogenetically related to the cultivated tomato, we found that the lycopene content of *Solanum pimpinellifolium* was five times higher than that found in cultivated tomato (*Solanum lycopersicum*) [17]. However, this process of introgression of high carotenoid content in the cultivated tomato requires the rapid and accurate analysis of hundreds of samples in the selection processes and, therefore, fast and accurate methods of carotenoid determination in *Solanum* fruits.

HPLC is the technique most frequently used for the analysis of carotenoids in fruit and vegetable extracts [18, 19]. In the literature reviewed, RP columns packed with several C_{18} stationary phases still are the preferred chromatographic supports for measuring total carotenoids [20–26], whereas for an efficient separation of occurring geometric isomers of carotenoids, polymeric C_{30} stationary phases are usually employed [27–30]. However, these chromatographic methods have several shortcomings including the time-consuming conditioning of the columns, the large consumption of solvents, and the high price of the column.

In the last few years, CEC has received much attention as an emerging separation technique [31, 32]. It couples the advantage of high selectivity in HPLC with that of high efficiency in CE. By using a flow generated by electroosmosis instead of the pressure-driven flow in HPLC, high separation efficiencies could be achieved [33, 34]. Analytes may be separated according to differences in their partitioning ratios between the stationary and mobile phase, and/or to differences in electrophoretic mobility. Accordingly, CEC has become an area of intense interest for the separation of compounds of very different nature [35, 36]. Although the successful use of packed columns in CEC separation has been demonstrated in many reports [37] and few CEC procedures based on this type of columns have been developed to separate carotenoids [38, 39], the fabrication of reproducible CEC packed columns still remains problematic [40, 41].

Monolithic capillary columns constitute a promising alternative to the conventional packed CEC columns due to the ease of preparation, no need of frit, excellent reproducibility, and the near total exclusion of bubble formation. Silica and several polymeric materials such as acrylamide, styrene, and methacrylate esters have been used to develop separation monolithic columns in CEC [35, 36, 42–46].

Methacrylate ester-based monolithic column has several advantages over the other columns due to: (i) easily adjustable polymeric polarity, (ii) fine control of porous property of monolith, and (iii) high stability even under extreme pH conditions [44–46]. Previous reports have demonstrated that methacrylate ester-based monolithic columns had high reproducibility and stability whenever they were employed to separate analyte standards or real samples [47–51].

In spite of these good features, to our knowledge no CEC method with monolithic columns has been reported for the integration of carotenoid analysis in tomato breeding programs. In order to response to this challenge, the aim of this work was, therefore, to develop a reliable and fast procedure for the prominent carotenoids determination in fruits of tomato and several *Solanum*-related species and to check its suitability for the analysis of a large series of samples. Thus, the separation of main carotenoids by using methacrylate ester-based monolithic columns was optimized in terms of porogenic solvent ratio, monomer hydrophobicity, and mobile phase composition.

2 Materials and methods

2.1 Chemicals and reagents

Lauryl methacrylate (LMA), ethylene dimethacrylate (EDMA), [2-(methacryloyloxy)ethyl] trimethylammonium chloride (75% in water, META), 1,4-butanediol, 1-propanol, 3-(trimethoxysilyl)propyl methacrylate, and basic alumina were purchased from Aldrich (Milwaukee, USA). Azobisisobutyronitrile (AIBN) was obtained from Acros Organics (NJ, USA). Butyl methacrylate (BMA), HPLC-grade ACN, and methanol were obtained from Fluka (Buchs SG, Switzerland). HPLC-grade THF and butylated hydroxytoluene (BHT) was obtained from Sigma (St. Louis, MO, USA). Deoinized water was obtained by using a Milli-Q-Plus water purification system (Millipore, Bedford, MA, USA). Tris and magnesium carbonate (anhydrous powder) were purchased from Panreac Quimica (Barcelona, Spain). Hydrochloric acid (37%) was supplied by J. T. Baker (Deventer, The Netherlands). BMA and EDMA were purified by passing them through activated basic alumina followed by a distillation under reduced pressure. All other chemicals were of analytical grade and used as received. Uncoated fused-silica capillaries with 375 μm od × 100 μm id were purchased from Polymicro Technologies (Phoenix, AZ, USA).

Carotenoid standards with purities higher than 95% (as determined by HPLC) were employed in this work. Lycopene was purchased from Sigma, β-carotene and β-apo-8′-carotenal from Fluka. The last one was used as the internal standard. Stock solutions of analytes (500–1000 μg/mL) were prepared in THF. Working solutions were prepared daily by dilution of stock solutions with the mobile phase. As EOF marker, thiourea from Riedel-de Haën (Seelze, Germany) was used.

2.2 Apparatus

The CEC experiments were performed with a Beckman Coulter P/ACE MDQ CE system equipped with a photo DAD (Palo Alto, CA, USA). Beckman Coulter MDQ 32 Karat software version 5.0 was used for instrumental control and data analysis. A Gilson (Middleton, USA) model 307 HPLC pump was used for washing and equilibrating the polymeric monolithic columns. Porosity measurements were obtained

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by using Pascal 140 and 440 mercury intrusion porosimeters (CE Instruments, Milan, Italy) for low-pressure and high-pressure analysis, respectively. Nitrogen adsorption measurements were performed using a Sorptomatic 1990 instrument (Thermo Electron, Milan, Italy).

2.3 Preparation of polymeric monolithic columns

Prior to the preparation of a polymeric monolithic column, surface modification of the inner wall of a fused-silica capillary was performed with 3-(trimethoxysilyl)propyl methacrylate as reported by Fréchet *et al.* [45] to enable covalent attachment of the monolith to the wall. Monoliths were prepared from polymerization mixtures consisting of EDMA, META, and one of the methacrylates monomers (BMA or LMA) and a ternary pore-forming solvent composed of 10 wt% water and 90 wt% of 1,4-butanediol and 1-propanol combined at several ratios. AIBN (1 wt% with respect to the monomers) was added as an initiator. After mixing, the polymerization mixture was sonicated for 5 min to obtain a clear solution, and then it was purged with helium for 10 min. The deaerated mixture was used to fill the preconditioned capillary (31.2 cm) to a total length of 21 cm by syringe injection. After polymerization for 20 h at 70°C, the resulting columns were flushed with methanol to remove the pore-forming solvents and possible unreacted monomers by using a HPLC pump. A detection window was made adjacent to the monolithic material by burning the polyimide coating away with a heating coil. Prior to CEC experiments, the capillaries were flushed with mobile phase for 30 min. Simultaneously with the polymerization in capillaries, a batch polymerization was carried out of the mixture in a 2.5 mL glass vial. Once the polymerization process was completed, the monolithic material was removed from the glass vial, cut into small pieces with a razor blade, and a Soxhlet extraction was carried out with methanol for 24 h. After drying at 50°C for 4 h, mercury-intrusion porosimetry and nitrogen-adsorption experiments were performed on the monolithic materials.

2.4 Operational conditions for CEC

The monolithic column was placed in the CEC instrument and equilibrated with the mobile phase by applying a stepwise increase in voltage up to 25 kV and 70 psi pressure at both ends of the column until a stable baseline was observed. Samples and standards were electrokinetically injected into the capillary at 10 kV for 10 s. Separations were carried out using an electrical voltage of 25 kV and the temperature of the capillary was kept at 25°C. Detection of carotenoids was performed at the wavelength of optimum absorption (λ_{max}) of each carotenoid: for β-carotene at 450 nm and lycopene at 470 nm. Wavelength at 254 nm was used to detect the EOF peak marker (thiourea). All eluents for CEC were degassed by ultrasonication, prior to use. Retention and resolution measurements were done in triplicate.

2.5 Plant material

The plant material was constituted by fruits of different species of the *Solanum* genus, obtained from the Instituto de Conservación y Mejora de la Agrodiversidad Valenciana (COMAV) (Table 1). To select the plant material, two approaches were adopted: (i) to choose fruits with notable differences in their chemical composition, both in the lycopene and β-carotene content and (ii) to select fruits of several *Solanum* species with interesting traits for the improvement of the nutritional quality of the tomato. As lycopene and β-carotene content improvement can be part of wider breeding programs for the nutritional quality of tomato, we made sure that the method was suitable for the analysis of samples of plants from interspecific crosses. BGV012406 is a tomato experimental line used as a control. BGV005655, BGV005718, and BVG009560 are Spanish traditional varieties of tomato with different fruit characteristics and carotenoid content. Finally, BGV004587 and BGV000633 were chosen because they had previously shown high carotenoid content and were phylogenetically close to cultivated tomato. Plants were grown in greenhouses with the usual watering, fertilization, and climate control systems for spring-summer cycle production. Fruits were collected from the first three trusses when they were completely mature.

2.6 Sample preparation

Carotenoid extraction procedure used in our study was similar to that proposed by Khachik *et al.* [7, 8], but with some modifications. Tomato sample aliquots (0.75–1 g) were

Table 1. Characteristics of the accession of the *Solanum* genera employed in this study

Accessions	Species	Fruit characteristics	Origin
BGV012406	*S. lycopersicon*	Medium-size, red	Valencia, Spain
BGV005655	*S. lycopersicon*	Large, dark red	Alicante, Spain
BGV005718	S. lycopersicon	Large, yellow	Ademuz, Spain
BVG009560	*S. lycopersicon*	Small, red	Mazarron, Spain
BGV004587	*S. lycopersicon* var *cerasiforme*	Small, orange-brownish	Ipala, Guatemala
BGV00633	*S. pimpinellifolium*	Very small, dark red	Piura, Peru

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extracted with 4 mL THF (containing 0.1% BHT) at 0°C in a mechanical blender, using magnesium carbonate (10% of weight of the sample). An appropriate amount of internal standard (β-apo-8′-carotenal) was added to each tomato sample before extraction. After centrifugation, the solid material was re-extracted two or three more times until the supernatant was colorless. THF extracts were combined, and the volume was reduced by about two-thirds under a nitrogen stream at 35°C. This extraction method allowed processing 45–50 samples *per* day. Appropriate dilutions (with mobile phase) were performed to provide a suitable concentration for CEC analysis.

3 Results and discussion

3.1 Experiments with BMA monomer: Effect of pore size on carotenoid separation

Porous continuous beds were prepared (in bulk and *in situ* fused-silica capillaries) by thermally initiated free-radical polymerization of the bulk monomer (BMA), a cross-linking monomer (EDMA), and a positively charged monomer (META) employed to generate EOF. 1,4-Butanediol, 1-propanol, and water were used as pore-forming solvents according to the protocol described by Peters *et al.* [45, 52]. Several reports have demonstrated that the pore size of a polymeric monolith can effectively alter the separation performance in a CEC system [32, 44]. The macropore formation of a monolithic column can be systematically adjusted by changing the composition of the pore-forming solvents [43, 52–54]. In particular, for BMA-based monolithic columns containing META as ionizable functionality, by changing the ratio 1,4-butanediol to 1-propanol, keeping the water content constant, the modal pore size could be varied between 60 and 5000 nm [54]. Monoliths with plate heights between 5 and 15 μm could be obtained [54]. Accordingly, a series of BMA-based monolithic columns were prepared by modifying the percentage of 1,4-butanediol in the polymerization mixture, fixing the water content (10 wt%), and the proportion monomers to pore-forming solvents (40:60 wt%) (Table 2). With an increase in the content of 1,4-butanediol, increasing the polarity of the polymerization mixture, an augment in pore size and a reduction in the surface area values were observed, which is in agreement with that reported in literature [32, 43, 54].

The last two monolithic columns of Table 2 were employed to separate our solutes. ACN/THF mobile phases were selected, according to a previous work [39], since the appropriate solubility of carotenoids in these solvents provided enough elutropic strength to elute them in short time. However, in all BMA monolithic columns prepared, a coelution between β-carotene and lycopene was obtained, while the internal standard used, β-apo-8′-carotenal, which is not present in tomato, was clearly resolved from this pair (data not shown). This difference of behavior could be attributed to

Table 2. Pore size and surface area of BMA-based monolithic capillaries studied

1,4-Butanediol content in the polymerization mixture wt%)	Pore size[a] (nm)	Surface area[b] (m^2/g)
15	105	35.71
18	131	23.52
20	487	7.13
22	2067	1.10

a) Values obtained by mercury intrusion porosimetry.
b) Values measured by BET method.

the presence of a terminal oxygenated group (higher polarity) in the β-apo-8’-carotenal molecule respect to the other hydrocarbon carotenoids (β-carotene and lycopene). No improvements in resolution of this pair were reached when the mobile phase composition was modified. Consequently, these analytes likely have not enough time to interact or partition with the polymeric stationary phase. Recently, Eeltink *et al.* [54] have shown that the monolith morphology (pore and globule size) has a great influence on separation efficiency, and where both features can be controlled independently. Thus, the globule size may be modified by decreasing the monomer *versus* pore-forming solvent ratio, resulting in a structure with smaller globule sizes, which translates in larger surface area, and consequently, a stronger interaction (or binding capacity) of monoliths with the analytes [54]. Monolithic columns with a monomer to pore-forming solvent ratio of 20:80 wt% were made, however, the coelution problem between β-carotene and lycopene remained (data not shown).

3.2 Experiments with LMA monomer: Effect of hydrophobicity monomer in carotenoid separation

The above results indicated that BMA monolithic columns were not able to separate the analytes, since probably they are not enough hydrophobic. To change the hydrophobicity of the monolithic stationary phase, the influence of the alkyl methacrylate monomer nature was investigated. Thus, we replaced the totality of the bulk methacrylate monomer (BMA) by another one with an alkyl chain slightly longer, such as LMA. Several LMA-based monolithic columns differing in their pore size were prepared from polymerization mixtures by varying the porogenic solvent content, from 3 to 15 wt% of 1,4-butanediol. At lower 1,4-butanediol percentages, small pore sizes were obtained, clearly unsuitable for any flow-through applications. In contrast, 1,4-butanediol contents higher than 15 wt% were unable to solubilize all components of the polymerization mixtures and resulted in a biphasic solution. Table 3 shows the mode pore diameter (pore diameter at the maximum of the distribution curve) obtained for several monoliths at different 1,4-butanediol

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Table 3. Porous and chromatographic properties of LMA-based monolithic columns

1,4-Butanediol content in polymerization mixture (wt%)	Pore size[a)] (nm)	Surface area[b)] (m^2/g)	μ_{EOF}, 10^{-4} ($cm^2/V \cdot s$)	$k_{\beta\text{-carotene}}$[c)]	$N_{\beta\text{-carotene}}$[d)] ($m^{-1}$)	$R_{s\beta\text{-carotene/lycopene}}$
3	491	13.10	0.76	1.17	44500	2.43
5	1798	5.49	1.03	0.54	69700	1.72
7	4055	0.98	1.44	0.27	52400	1.37
8	5277	0.78	1.53	0.18	34400	0.92
10	4072	1.21	1.42	0.27	56200	1.42
12	2725	1.13	1.27	0.36	49700	1.51

a) Values obtained by mercury intrusion porosimetry.
b) Values measured by a BET method.
c) Retention factor of β-carotene.
d) Efficiency of β-carotene.

contents. As it can be observed, the pore size reaches a maximum at 8 wt% of 1,4-butanediol in the polymerization mixture and decreases at higher percentages. Figure 1 shows the differential pore size distribution curves obtained from mercury intrusion porosimetry measurements of some resulting LMA-based monoliths. It can be noticed that the profile for monolith with smaller mode pore size is bimodal, with a number of pores in the size range of 900–950 nm, in addition to the mode at 1800 nm. Since the column efficiency is closely correlated with the physical structural properties of the stationary phase, such as the pore size and the surface structure of the monolith [33], this parameter was also examined by measuring the theoretical plates for the most

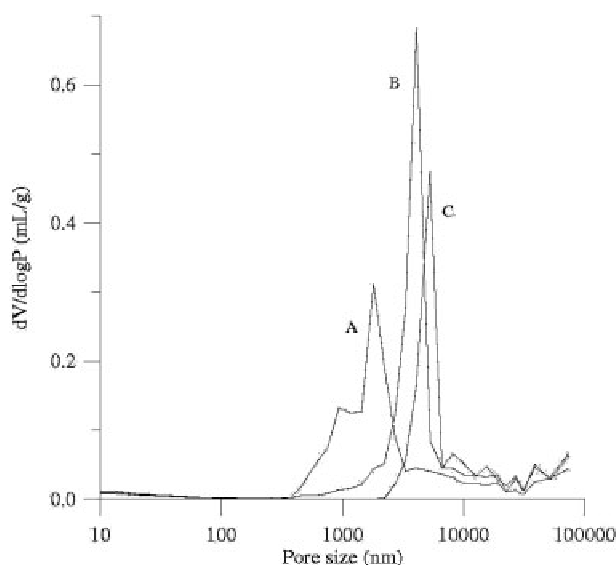

Figure 1. Pore size distributions obtained by mercury intrusion porosimetry for LMA-based monoliths with median pore size of (A) 1798, (B), 4070, and (C) 5300 nm. Polymerization mixture: 23.8% LMA, 15.8% EDMA, 0.4% META, and 60% ternary pore-forming solvent (consisting of 5% water and 95% of mixtures of 1,4-butanediol and 1-propanol). AIBN (1% with respect to the monomers), polymerization time 20 h, temperature 70°C.

retained compound, β-carotene, on this series of monolithic columns (Table 3). In general, a decrease in pore size is accompanied by an increase in surface area leading to enhanced retentivity for the capillary columns can be observed. Capillary columns with large pores (more than 4000 nm) are characterized by a high flow rate resulting from a low flow resistance, and small surface area values (less than 5 m^2/g), however, they were enough hydrophobic to separate the analytes. It should be noted that the monoliths with a modal pore size close to 500 nm showed a reduced mobile-phase velocity and a lower efficiency in CEC. This is likely due to the peak broadening that results from longitudinal diffusion at the slow flow velocity or by the double-layer overlap, as suggested by other authors [45, 54]. By increasing the modal pore size at 1798 nm, the efficiency increased up to 69 700 plates/m, and the lycopene/β-carotene pair remained completely resolved (R_s = 1.73) in a short analysis time. According to the electrochromatogram of carotenoids under these optimum conditions (Fig. 2), capillaries prepared using a polymerization mixture containing 5 wt% of 1,4-butanediol were used for further studies.

3.3 Quantitation and analysis of real samples

The tomato samples indicated in Table 1 were analyzed. However, the selected column showed a tendency to get blocked when attempting to analyze them. In order to overcome this problem, the column with median pore diameter close to 4000 nm was chosen. The column continues providing a good efficiency (see Table 3), being higher than that found by conventional RP-HPLC employed to determine the main carotenoids in vegetables and fruits [20–26], but was lower than that reported using CEC packed columns [38, 39]. On the other hand, the resolution for the lycopene/β-carotene pair decreased from 1.72 to 1.37, being quite suitable to perform quantitative studies. On the other hand, the retention factor of β-carotene was practically half-reduced, and consequently, the analysis time was also reduced, which is of

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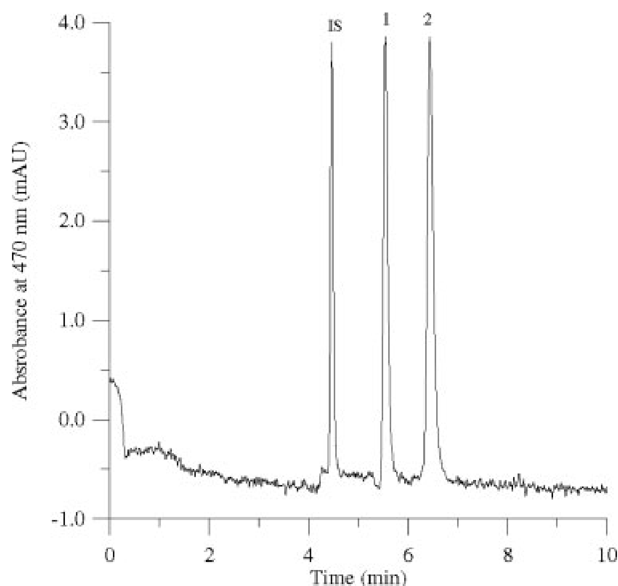

Figure 2. Electrochromatogram of a test mixture of lycopene and β-carotene (20 μg/mL) in LMA-based monolithic capillary of median pore size of 1798 nm. Separation conditions: mobile phase: THF/ACN/MeOH/Tris buffer, pH 8.0 (35:30:30:5 by volume); applied voltage −25 kV; column temperature, 25°C; injection 10 kV for 10 s. Peak identification: IS (internal standard, β-apo-8′-carotenal); 1, lycopene; 2, β-carotene.

utmost interest to analyze large series of tomato samples. Under these conditions, the stability of the column was checked. Nor column blockage or loss of separation ability was observed after a period of continuous use exceeding more than 180 injections of the standard mixtures and 100 injections of the real samples. Therefore, the stability of columns was satisfactory, which constitutes an important benefit in the analysis of carotenoids in real samples, since it remains a problematic issue with polymeric C_{30} phases [55].

The method was evaluated in terms of repeatability, which was calculated by analyzing a standard mixture at a concentration level of 25 μg/mL in both carotenoids. The mixture was analyzed ten times in order to obtain the run-to-run repeatability of the method in terms of EOF, retention times and peak areas (Table 4). Three electrochromatographic columns were prepared with the same monolithic mixture and tested analyzing the standard carotenoids mixture (Table 4). The run-to-run repeatability was lower than 3.0% for all the parameters tested and the column-to-column reproducibility was below 7.2%. These results indicate that this CEC method provided relatively satisfactory qualitative and quantitative performance for the analysis of both carotenoids.

Calibration curves of analytes were constructed using solutions up to 160 μg/mL, except for lycopene that was 100 μg/mL for solubility reasons [19]. Straight lines with $r > 0.994$ were obtained by injecting six standard solutions of each solute in the range of 5–80 μg/mL. Good detection limits (obtained for S/N = 3) were reached for lycopene and β-carotene as it is shown in Table 4.

The optimized procedure was applied to the analysis of the target analytes in fruits of several *Solanum* species (Fig. 3). Identification of β-carotene and lycopene was performed by comparing their retention times and absorption spectra with those of standard solutions and, when necessary, by spiking the extracts with standard solutions. In the tomato samples analyzed, the presence of *cis*-isomers of lycopene and β-carotene was not detected. These results could be explained taking into account two facts. On the one hand, owing to the low concentration of *cis*-isomers (of these compounds) present in fresh tomato fruits [4, 56]. In order to check the presence of these isomeric forms, inspection of absorption spectra of lycopene and β-carotene in tomato extracts was also done. The characteristic 300–360 nm "*cis* peak" [19] was not evident in samples, which suggested the presence of little or no *cis*-isomers (data not shown). Another fact to be considered is the selectivity of the stationary phase toward the separation of isomeric forms. Thus, the use of C_{18} packed column [39] showed better capability to resolve the *trans*- and *cis*-isomers of β-carotene in several samples (spinach, corn, carrot, *etc.*) than the proposed LMA monolithic phase, but in both columns no evidence of *cis*-isomers was observed in tomato samples.

The selectivity achieved in both CEC columns was lower than that obtained with polymeric C_{30} phases, which have especially been designed for the separation of geometrical carotenoids isomers, and in tissues containing high concentrations of *cis*-isomers, providing an excellent resolution of these compounds, but at expenses of long analysis time (30–60 min) [27–30]. Rather, the high expense of these C_{30} columns combined with a limited column lifetime for this type of analysis significantly increases overall costs [55] for processing high-throughput of samples as required in breeding programs.

Table 4. Repeatability of retention time (t_R) and peak area (A) expressed as RSD (%) and detection limits (LOD) of analytes for S/N = 3

Compound	Run-to-run repeatability (n = 10) (%)		Column-to-column reproducibility (n = 3) (%)		LOD (μg/mL)
	t_R	A	t_R	A	
Lycopene	1.31	2.54	4.04	6.94	1.60
β-Carotene	1.69	2.96	4.43	7.20	1.12
EOF marker	1.20	–	2.84	–	–

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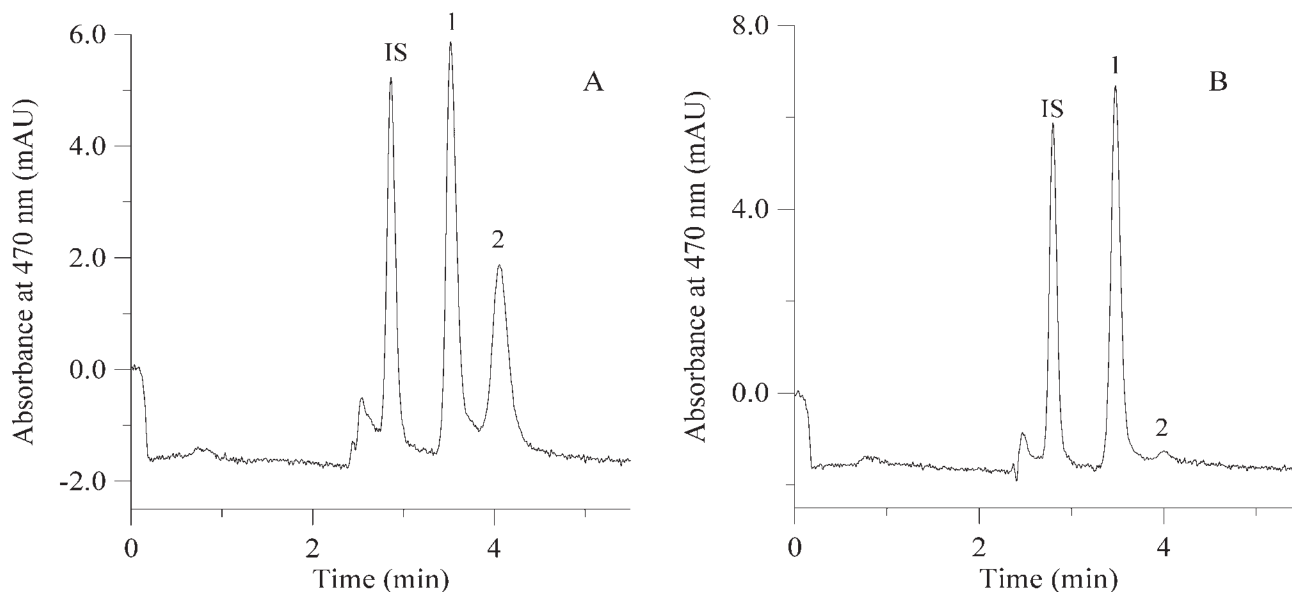

Figure 3. Electrochromatograms of lycopene and β-carotene in several *Solanum* spp. accessions: (A) BGV004587 and (B) BGV000633, obtained with LMA-based monolithic capillary of median pore size of 4000 nm. Peak identification and other experimental conditions as in Fig. 2.

Table 5. Mean values ($n = 3$) of lycopene and β-carotene (mg/100 g fresh weight) content in several tomato samples

Accessions	Species	Lycopene	β-Carotene
BGV012406	*S. lycopersicon*	1.26 ± 0.13	0.15 ± 0.04
BGV005655	*S. lycopersicon*	2.82 ± 0.23	0.72 ± 0.06
BGV005718	*S. lycopersicon*	0.65 ± 0.04	0.13 ± 0.02
BVG009560	*S. lycopersicon*	1.78 ± 0.12	0.16 ± 0.04
BGV004587	*S. lycopersicon* var *cerasiforme*	2.56 ± 0.22	1.66 ± 0.21
BGV00633	*S. pimpinellifolium*	22.12 ± 1.24	0.08 ± 0.01

The results of quantitative determinations of β-carotene and lycopene in tomato samples are given in Table 5 as mean of three replicates *per* sample. The levels of the carotenoids found in our analysis for each of these accessions were within the range obtained in the previous trials by spectrophotometric methods [57–60]. Although this spectrophotometric method may be used to estimate the carotenoid contents in fresh tomatoes, a tendency to overestimation is commonly found due to the presence of other carotenoids also present in the extract [58, 61]. These levels of overestimation could be even higher than 5% for lycopene determination [58].

4 Concluding remarks

A CEC method using a methacrylate ester-based monolithic column was developed for analyzing the major carotenoids present in tomato fruits. The proposed protocol shows several advantages compared to other developed procedures: (i) easy and reproducible preparation of monolithic columns with good stability, (ii) high efficiency values and satisfactory resolution between carotenoids in short analysis time (less than 5 min), and (iii) lower operational cost in terms of solvents and columns. The present procedure is suitable to be applied to the large-scale analysis of samples for the selection in internal quality breeding programs and also in systematic and routine characterization of tomato fruits. This study is the first work that shows the capability of the attractive monolithic technology for evaluation and selection of tomato genotypes with high nutritional quality.

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5 References

[1] Mangels, A. R., Holden, J. M., Beecher, G. R., Forman, M. R., Lanza, E., *J. Am. Diet. Assoc.* 1993, *93*, 284–296.

[2] Britton, G., Hornero-Mendez, D., in: Tomas-Barberan, F. A., Robins, R. J. (Eds.), *Phytochemistry of Fruit and Vegetables*, Oxford Science Publications, Oxford, UK 1997, pp. 11–27.

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[3] Delgado-Vargas, F., Jimenez, A. R., Paredes-Lopez, O., *Crit. Rev. Food Sci. Nutr.* 2000, *40*, 173–289.

[4] Beecher, G. R., *Proc. Soc. Exp. Biol. Med.* 1998, *218*, 98–100.

[5] Clinton, S. K., *Nutr. Rev.* 1998, *56*, 35–51.

[6] Shi, J., Le Maguer, M., *Crit. Rev. Food Sci. Nutr.* 2000, *40*, 1–42.

[7] Khachik, F., Beecher, G. R., Goli, M. B., Lusby, W. R., *Pure Appl. Chem.* 1991, *63*, 71–80.

[8] Khachik, F., Beecher, G. R., Goli, M. B., Lusby, W. R., in: Packer, L., (Ed.), *Methods in Enzymology*, Academic Press, New York 1992, Vol. 213, Part A, pp. 347–359.

[9] Di Mascio, P., Kaiser, S., Sies, H., *Arch. Biochem. Biophys.* 1989, *274*, 532–538.

[10] Ziegler, R. G., *Am. J. Clin. Nutr.* 1991, *53*, 251S–259S.

[11] Giovannucci, E., *J. Natl. Cancer Inst.* 1999, *91*, 317–331.

[12] Arab, L., Steck, S., *Am. J. Clin. Nutr.* 2000, *71*, 1691S–1695S.

[13] Willcox, J. K., Catignani, G. L., Lazarus, S., *Crit. Rev. Food Sci. Nutr.* 2003, *43*, 1–18.

[14] Mayne, S. T., *FASEB J.* 1996, *10*, 690–701.

[15] Angosto, T., Borja, A., in: Lozano-Ruiz, R., Cuadrado-Gomez, I. M. (Eds.), *Biotecnologia de cultivos horticolas*, Junta de Andalucia, Almería, Spain, 2001, p. 125.

[16] Abushita, A. A., Hebshi, E. A., Daood, H. G., Biacs, P. A., *Food Chem.* 1997, *60*, 207–212.

[17] Fernández-Ruiz, V., Sánchez-Mata, M. C., Cámara, M., Torija, M. E., Symposium on "Dietary phytochemicals and human health", Salamanca, Spain 2002.

[18] Aust, O., Sies, H., Stahl, W., Polidori, M. C., *J. Chromatogr. A* 2001, *936*, 83–93.

[19] Rodriguez-Amaya, D. B., *A Guide to Carotenoid Analysis in Foods*, ILSI Press, Washington 2001.

[20] Bushway, R. J., *J. Liq. Chromatogr.* 1985, *8*, 1527–1547.

[21] Craft, N. E., Wise, S. A., Soares, J. H., Jr., *J. Chromatogr.* 1992, *589*, 171–176.

[22] Epler, K. S., Sander, L. C., Zeigler, R. G., Wise, S. A., Craft, N. E., *J. Chromatogr.* 1992, *595*, 89–101.

[23] Lesellier, E., Tchapla, A., Krstulovic, A. M., *J. Chromatogr.* 1993, *645*, 29–39.

[24] Biacs, P. A., Daood, H. G., *J. Plant Physiol.* 1994, *143*, 520–525.

[25] Hart, D. J., Scott, K. J., *Food Chem.* 1995, *54*, 101–111.

[26] Thompson, K. A., Marshall, M. R., Sims, C. A., Wei, C. I. *et al.*, *J. Food Sci.* 2000, *65*, 791–795.

[27] Sander, L. C., Sharpless, K. E., Pursch, M., *J. Chromatogr. A* 2000, *880*, 189–202.

[28] Lessin, W. J., Catigani, G. L., Schwartz, S. J., *J. Agric. Food Chem.* 1997, *45*, 3728–3732.

[29] Lin, C. H., Chen, B. H., *J. Chromatogr. A* 2003, *1012*, 103–109.

[30] Ishida, B. K., Ma, J., Chan, B., *Phytochem. Anal.* 2001, *12*, 194–198.

[31] Colón, L. A., Burgos, G., Maloney, T. D., Cintrón, J. M., Rodriguez, R. L., *Electrophoresis* 2000, *21*, 3965–3993.

[32] Hilder, E. F., Svec, F., Fréchet, J. M. J., *Electrophoresis* 2002, *23*, 3934–3953.

[33] Knox, J. H., Grant, I. H., *Chromatographia* 1991, *32*, 317–328.

[34] Smith, N. W., Evans, M. B., *Chromatographia* 1995, *41*, 197–203.

[35] Zhang, M., El Rassi, Z., *Electrophoresis* 2001, *22*, 2593–2599.

[36] Mistry, K., Cortes, H., Meunier, D., Schmidt, C. *et al.*, *Anal. Chem.* 2002, *74*, 617–625.

[37] Eeltink, S., Rozing, G. P., Kok, W. T., *Electrophoresis* 2003, *24*, 3935–3961.

[38] Sander, L. C., Pursch, M., Marker, B., Wise, S. A., *Anal. Chem.* 1999, *71*, 3477–3483.

[39] Herrero-Martínez, J. M., Eeltink, S., Schoenmakers, P. J., Kok, W. Th., Ramis-Ramos, G., *J. Sep. Sci.* 2006, *29*, 660–665.

[40] Fujimoto, C., *Anal. Sci.* 2002, *18*, 19–25.

[41] Siouffi, A. M., *J. Chromatogr. A* 2003, *1000*, 801–818.

[42] Wu, R., Zou, H., Ye, M., Lei, Z., Ni, J., *Anal. Chem.* 2001, *73*, 4918–4923.

[43] Gusev, I., Huang, X., Horváth, C., *J. Chromatogr. A* 1999, *855*, 273–290.

[44] Svec, F., Fréchet, J. M. J., *Science* 1996, *273*, 205–211.

[45] Peters, E. C., Petro, M., Svec, F., Fréchet, J. M. J., *Anal. Chem.* 1998, *70*, 2288–2295.

[46] Jiang, T., Jiskra, J., Claessens, H. A., Cramers, C. A., *J. Chromatogr. A* 2001, *923*, 215–227.

[47] Que, A. H., Konse, T., Baker, A. G., Novotny, M. V., *Anal. Chem.* 2000, *72*, 2703–2710.

[48] Bandilla, D., Skinner, C. D., *J. Chromatogr. A* 2003, *1004*, 167–179.

[49] Mistry, K., Krull, I., Grinberg, N., *Electrophoresis* 2003, *24*, 1753–1763.

[50] Kvasnickova, L., Glatz, Z., Sterbova, H., Kahle, V. *et al.*, *J. Chromatogr. A* 2001, *916*, 265–271.

[51] Huang, H. Y., Chiu, C. W., Huang, I. Y., Yeh, J. M., *Electrophoresis* 2004, *25*, 3237–3246.

[52] Peters, E. C., Petro, M., Svec, F., Fréchet, J. M. J., *Anal. Chem.* 1997, *69*, 3646–3649.

[53] Viklund, C., Svec, F., Fréchet, J. M. J., Irgum, I., *Chem. Mater.* 1996, *8*, 744–750.

[54] Eeltink, B., Herrero-Martínez, J. M., Rozing, G. P., Schoenmakers, P. J., Kok, W. Th., *Anal. Chem.* 2005, *77*, 7342–7347.

[55] Davey, M. W., Keulemans, J., Swennen, R., *J. Chromatogr. A* 2006, *1136*, 176–184.

[56] Nguyen, M. L., Schwartz, S. J., *Food Technol.* 1999, *53*, 38–45.

[57] Zscheile, F. P., Porter, J. W., *Anal. Chem.* 1947, *19*, 47–51.

[58] Fish, W. W., Perkins-Veazie, P., Collins, J. K., *J. Food Compost. Anal.* 2002, *15*, 309–317.

[59] Roselló, S., Adalid, A. M., Cebolla-Cornejo, J., Nuez, F., *Actas Portuguesas de Horticultura* 2005, *8*, 429–435.

[60] Adalid, A. M., Roselló, S., Cebolla-Cornejo, J., Nuez, F., *Acta Physiol. Plant.* 2007, in press.

[61] Davis, A. R., Fish, W. W., Perkins-Veazie, P., *Postharvest Biol. Technol.* 2003, *28*, 425–430.

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IV. DISCUSIÓN GENERAL

El estudio de componentes nutraceúticos del tomate (principalmente licopeno, β-caroteno y ácido ascórbico) se está llevando a cabo desde hace tiempo por su efecto en la salud humana (Naidu, 2003; Canene-Adams *et al*., 2005; Rao y Rao, 2007) y a la importancia económica de este cultivo (FAOSTAT, 2011). Sin embargo, aún hoy en día la mejora del tomate para estos compuestos resulta compleja. En esta tesis se han tratado varios aspectos, como la evaluación y selección de nuevas fuentes de variabilidad para estos compuestos; la evaluación del efecto del genotipo, el ambiente y su interacción en la acumulación de carotenoides y ácido ascórbico en germoplasma de tomate; el análisis del control genético de la acumulación de β-caroteno y ácido ascórbico derivado de una entrada seleccionada tipo cherry y, por último, el desarrollo de una técnica analítica que facilita el fenotipado de plantas.

La Red Internacional de Datos sobre Alimentación (INFOODS, 2011) y la Organización para la Alimentación y la Agricultura de las Naciones Unidas (FAO) han resaltado la importancia de la identificación y publicación de los perfiles de nutrientes de los alimentos, incluyendo entradas silvestres y cultivares infrautilizados de frutas y hortalizas. Ya desde sus inicios, hace más de 50 años, la FAO ha considerado que los recursos genéticos del planeta son de vital importancia para la agricultura, la salud, el medio ambiente y el comercio. La labor de la FAO en la nutrición siempre ha incluido elementos de la biodiversidad, recopilando los recursos genéticos silvestres, abandonados e infrautilizados que son útiles para la alimentación. En los últimos años, la FAO y el "Bioversity International" (anteriormente International Plant Genetic Resources Institute) están liderando una nueva iniciativa internacional sobre el uso de la diversidad biológica para la alimentación, cuyo objetivo es promover el uso sostenible de la biodiversidad en programas que contribuyan a la seguridad alimentaria y la nutrición humana, y aumentar así la conciencia de la importancia de este vínculo para el desarrollo sostenible (Toledo y Burlingame, 2006). En el primer trabajo de esta tesis se profundizó en el estudio de los principales componentes nutracéuticos de diferentes cultivares de tomate infrautilizados y entradas de especies relacionadas (49) recogidos en todo el mundo para promover su uso (directamente en campo o como fuentes de variabilidad para la obtención de nuevos cultivares) y de esta manera incrementar la agrobiodiversidad de nuestros campos. Para poder seleccionar dichos cultivares y/o entradas adecuadamente se usó un análisis estadístico multivariante con la metodología GGEbiplot que permitió la selección de líneas de mejora basada en todos los caracteres

de interés simultáneamente (Yan y Fregeau-Reid, 2008). De este modo, se seleccionaron 14 entradas tipo cherry y 2 de tomate común por su alto y equilibrado valor nutraceútico, siendo éstas de gran interés para consumo humano. Además, 2 entradas de tipo cherry mostraron alrededor de 1.5 veces la media normal de ácido ascórbico según Gould (1992), y 1 de *Solanum pimpinelllifolium* mostró más de 9 veces el contenido normal de licopeno según Holden *et al*. (1999) y Kuti and Konoru (2005), confirmando trabajos previos que ya señalaron a estas especies como una de las mejores fuentes de antioxidantes con propiedades nutraceúticas (George *et al*., 2004; Hanson *et al*., 2004). Estas entradas podrían ser de interés como parentales donantes en programas de mejora para aumentar las propiedades nutraceúticas de variedades comerciales.

Una vez que se han identificado fuentes de variabilidad para alto contenido en componentes nutraceúticos, es necesario evaluar su potencial real de mejora en diversos ambientes y ciclos de cultivo con el fin de determinar la contribución del genotipo, el ambiente y su interacción en la expresión de estos caracteres, ya que se ha comprobado que la temperatura, radiación, ciclo de cultivo y localidad, junto a las técnicas agrícolas usadas pueden influir en los contenidos de estos componentes en tomate (Rosenfeld, 1999; Dumas *et al*., 2003; Anza *et al*., 2006; Raffo *et al*., 2006). También es importante determinar su control genético y modo de herencia para poder usarlos eficazmente en programas de mejora. En el segundo trabajo de esta tesis, se evaluaron 10 entradas preseleccionadas como potencialmente interesantes en 3 ambientes de cultivo con diferentes registros de temperatura y radiación, ya que existe una fuerte relación entre temperatura, radiación y la biosíntesis de carotenoides y ácido ascórbico. La biosíntesis de licopeno tiene lugar entre 12 y 35 ºC, siendo el óptimo entre 22-26ºC (Hamauzu *et al*., 1998). Fuera de estas condiciones, la acumulación del licopeno se ve inhibida, estimulando la conversión del mismo a β-caroteno (Hamauzu *et al*., 1998). El contenido de ácido ascórbico parece correlacionarse positivamente también con la temperatura (Liptay *et al*., 1986). La acumulación de los tres, a temperaturas adecuadas, aumenta con la intensidad de luz solar (McCollum, 1954; Brown, 1954), aunque un exceso de radiación también es perjudicial (sobre todo en carotenoides) (Adegoroye and Jolliffe, 1987). El contenido de licopeno, β-caroteno y ácido ascórbico fue muy alto en varios fenotipos (hasta 281, 35 y 346 mg kg-1 respectivamente) comparados con los valores comúnmente aceptados para tomate (Kuti y Konuru, 2005; Holden *et al.*, 1999; Gould, 1992). Al realizar la descomposición de la varianza, se observó en general que el

genotipo tuvo la mayor contribución al fenotipo junto a una considerable interacción con el ambiente, siendo la influencia del ambiente menor aunque remarcable. Esto hace posible la selección de genotipos de élite con alto contenido en licopeno y β-caroteno, pudiendo llevar a cabo una selección conjunta para ambos caracteres debido a su correlación genética, pero siempre recomendando ensayos en múltiples ambientes para seleccionar de forma eficaz, ya que existe una correlación ambiental negativa. Esta correlación negativa pudo ser debida a que el ambiente que presentó mayor acumulación de licopeno fue el ciclo de cultivo de primavera/verano en Valencia, con alta temperatura pero sin exceso de radiación, siendo el peor ambiente para la acumulación de β-caroteno. Ya se ha comprobado que normalmente el licopeno y β-caroteno están íntimamente relacionados por la ruta biosintética, y en condiciones no estresantes el flujo de los carotenoides se ver retenido en el licopeno (Hirscheberg, 2001). En cambio, la mejora de ácido ascórbico será más difícil al jugar un papel clave en reducir el daño oxidativo a nivel celular causado por condiciones estresantes en la planta (Shigeoka *et al.*, 2002). Esto dificulta la modelación de la interacción GxE y por tanto, enmascara el potencial genético real y dificulta la selección. Teniendo en cuenta los efectos genotípicos y las interacciones GxE, entre las entradas evaluadas, destacaron cuatro por su sorprendente potencial genético en la acumulación de compuestos nutraceúticos. Tres de ellas pertenecen a la especie *S. pimpinellifolium* (CDP1568, CDP7090 y CDP9822), especialmente útiles como parentales donantes en programas de mejora del contenido de licopeno y β-caroteno del tomate cultivado. La otra entrada fue la CDP4777 (*S. lycopersicon* var *cerasifome*) con un alto potencial genético para acumular β-caroteno y ácido ascórbico y alta estabilidad para los ambientes estudiados. Además, en esta entrada el alto contenido de β-caroteno no está unido a un bajo contenido de licopeno, tal y como ocurre normalmente en mutantes de tomate con gran acumulación de β-caroteno (Stommel y Haynes, 1994; Zhang y Stommel, 2000).

La entrada CDP4777 fue seleccionada para estudiar con detalle el control genético y modo de herencia de la acumulación de β-caroteno y ácido ascórbico. Se eligió primero este parental por ser de la misma especie que el tomate cultivado, evitando aquellos problemas que pudieran surgir con la construcción de la familia a partir de entradas de otras especies como *S. pimpinellifolium*. Se eligió como parental femenino la línea de mejora CDP8779 (ya evaluada en el trabajo anterior como control). El análisis del control genético de los caracteres alto contenido en β-caroteno y ácido

ascórbico se llevó a cabo mediante un modelo aditivo, dominante y aditivo x aditivo (modelo ADAA) muy utilizado para analizar la expresión de caracteres agrónomicos, de calidad o morfológicos en otros cultivos (Yan *et al.*, 1998; Shi *et al*, 1999; McCarty el al., 2004a, 2004b, 2008). El estudio se llevó a cabo simultáneamente en dos localidades con dos modalidades de cultivo distintas (aire libre y protegido bajo invernadero), tal y como se aconseja en el trabajo anterior.

Los resultados obtenidos indican que la acumulación de β-caroteno fue principalmente de carácter aditivo (32.2 % del componente genético) con una pequeña componente dominante (4.2%) y una gran contribución de la interacción AxE, la cual se podría aprovechar para aumentar dicha acumulación en ambientes con temperaturas moderadas a altas (30ºC) que favorecen la biosíntesis de β-caroteno (Hamauzu *et al.*, 1998) y radiaciones moderadas. En el trabajo anterior se vio que la estabilidad de CDP4777 era alta, por lo que podría sorprender esta interacción con el medio tan elevada. Pero en cambio para CDP8779 se detectó una interacción GxE muy alta para este carácter en el ciclo de cultivo de otoño/invierno similar al estudiado aquí, por lo que la proporción de la interacción GxE derivada de CDP4777 depende también de la estabilidad del parental femenino. La pequeña componente dominante contribuyó a un efecto heterótico sobre el parental medio que incrementa la expresión del carácter en híbridos F1. La heredabilidad en sentido estricto de este carácter fue del 62%, aunque en gran proporción dependió de la interacción AxE. Ambos parentales difieren en 1 loci para acumulación de β-caroteno. Para otras especies relacionadas con el tomate se ha mostrado que el alto contenido de β-caroteno a expensas del licopeno parece condicionado por un único gen dominante (*Beta*) y sujeto a la influencia de otro gen (Mo_B) (Zhan y Stommel, 2000; Stommel *et al.*, 2005). Sin embargo para Causse *et al.* (2003), los híbridos entre entradas con frutos de tomate de tamaño grande y cherry indicaron que el contenido de caroteno se heredó de una manera aditiva con efecto del ambiente, pero sin interacción GxE significativa. Nuestros resultados muestran que CDP4777 puede considerarse una nueva fuente de variación para este carácter. El valor genotípico de esta entrada fue similar a la mostrada por el parental donante en Stommel y Haynes (1994) y el doble a la de Causse *et al*, (2003). Esta entrada es capaz de incrementar el contenido de β-caroteno en un 221% respecto al parental femenino utilizado en nuestro estudio y un 669% respecto al valor comúnmente aceptado para tomate (3.9 mg kg^{-1}) (Holden *et al.*, 1999).

El modelo ADAA indicó para la acumulación del ácido ascórbico que el modo de herencia fue también aditivo en su mayor parte (61.7% del componente genético) con una componente epistática (21.5%). Este efecto epistático causó una heterosis negativa que reduce este efecto aditivo principal y el valor genotípico esperado de los híbridos F1 no es tan alto como se esperaba del comportamiento de esta entrada en el ensayo anterior. Sin embargo, en ciertos ambientes se puede encontrar una interacción con el ambiente (AxE) del 16.8% que puede compensar esa heterosis negativa. En otros trabajos se sugiere un control genético aditivo (Causse *et al*, 2003) o dominante-aditivivo (Bhatt *et al*., 1998, 2001) in *S. lycopersicum*, aunque en estos trabajos se utilizó únicamente el modelo aditivo dominante. La heredabilidad en sentido estricto para este carácter es del 52%. Ambos parentales difieren en un máximo de 4 loci en cultivo al aire libre. Normalmente, el cultivo al aire libre parece que genera un mayor contenido de ácido ascórbico que en cultivo protegido bajo invernadero (López-Andreu *et al*., 1986), ya que dicha acumulación en tomate se ve directamente correlacionada con la temperatura (Liptay *et al*, 1986) y la radiación solar (López-Andreu *et al*., 1986), mientras no se lleguen a niveles estresantes para la planta. Además, el control genético de la acumulación de ácido ascórbico se puede considerar muy complejo porque la cantidad de esta pequeña molécula en las células vegetales depende no solo de la regulación de su síntesis (Smirnoff *et al*., 2001), sino también de su reciclado metabólico, degradación (Green y Fry, 2005) y su transporte (Horemans *et al*., 2000). Hay que tener en cuenta también que se han identificado varias rutas biosintéticas alternativas, por lo que es difícil precisar exactamente cómo se controla la síntesis en el contexto del desarrollo, las respuestas al estrés y la homeostasis normal (Valpuesta y Botella, 2004). Esto nos hace suponer que diferentes condiciones ambientales o localidades puedan influir en la expresión génica de esta molécula. A pesar de la complejidad de este carácter, su naturaleza aditiva (principal, epistática y de interacción con el medio) podría usarse en programas de mejora. Su valor genotípico fue similar a aquellos valores fenotípicos publicados por Bhatt *et al*. (2001) y un 133% más que los mostrados por Causse *et al*. (2003). Además el hecho que se pueda mejorar conjuntamente al β-caroteno aporta un valor añadido. Inicialmente, los híbridos F1 obtenidos de esta entrada podrían tener hasta 131% más que la línea usada como parental femenino. La contribución de la interacción AxE, aunque no tan importante

como el caso anterior, advierte que los híbridos desarrollados en estos programas de mejora deben de dirigirse a ambientes parecidos al descrito anteriormente.

Respecto a la cuantificación de carotenoides, ya se ha comprobado a lo largo de esta tesis que el método espectrofotométrico presenta una gran ventaja en ensayos de cribado y primeras etapas de los programas de mejora, donde el número de muestras puede ser un factor limitante en el estudio, consiguiendo una selección rápida, simple y poco costosa, que no requiere instrumentación sofisticada. Sin embargo para posteriores trabajos o en fases más avanzadas de programas de mejora donde las diferencias entre genotipos puede ser más sutil, se necesitaría una técnica analítica separativa (Kimura *et al*., 2007). La técnica más popular para este fin es la cromatografía líquida de alta resolución (HPLC), sin embargo el largo tiempo de análisis (unos 30 minutos), la cantidad de solvente utilizado (40 ml de fase móvil por análisis) (Hart and Scott, 1995) y el alto coste de las precolumnas y columnas hizo que se planteara la necesidad de desarrollar un método por electrocromatrografía (CEC). Esta técnica aúna la ventaja de alta selectividad del HPLC con la alta eficiencia mostrada por la electroforesis capilar (CE), debido al flujo generado por electroosomosis en lugar del generado por la presión en HPLC (Smith y Evans, 1995). Sin embargo la fabricación de columnas empaquetadas para CEC aún es un poco problemática (Siouffi, 2003). La alternativa propuesta en esta tesis es el uso de columnas monolíticas que presentan mayor facilidad de preparación, no necesitan fritas, tiene excelente reproducibilidad y casi consiguen la exclusión de burbujas. En el método expuesto, usando CEC con una columna monolítica a base de lauril metacrilato, la separación y cuantificación de licopeno y β-caroteno fue llevada a cabo en menos de 5 minutos, mejorando en más de 20 minutos los tiempos de análisis de otros métodos ampliamente utilizados (Hart and Scott, 1995; García-Plazaola y Becerril, 1999; Ishida *et al*., 2001). El límite de detección y la reproducibilidad de este método estuvieron por debajo de 1.6 mg/mL y 7.2% respectivamente, indicando que este método proporciona resultados cualitativos y cuantitativos satisfactorios para el análisis de carotenoides.

V. CONCLUSIONES GENERALES

Esta tesis ha mostrado la existencia de variabilidad para acumulación de carotenoides y ácido ascórbico en cultivares de tomate infrautilizados y especies relacionadas conservadas en el banco de germoplasma del COMAV, por lo que ha sido posible la selección de entradas para usar directamente en campo para consumo humano o como parentales donantes en programas de mejora.

El genotipo, el ambiente y la interacción de ambos influyen en la acumulación de estos compuestos, por lo que para estudiar el control genético derivado de estas entradas, es necesario ensayos en múltiples localidades y/o ciclos de cultivo.

Se ha visto que el alto componente genético para acumulación de licopeno y β-caroteno permitiría hacer una selección de genotipos con alto contenido en estos carotenoides. Sin embargo para llevar a cabo una mejora conjunta son necesarios ensayos en distintos ambientes debido a la correlación ambiental negativa.

La selección y mejora de entradas por alta acumulación de ácido ascórbico parece más compleja debido a que interfieren muchos factores en dicha acumulación. Aunque sí es posible cierta mejora conjunta con β-caroteno, parece muy complicada la mejora junto al licopeno.

En cuanto a material vegetal seleccionado, tres entradas de *Solanum pimpinellifolium* (CDP1568, CDP7090 y CDP9822) fueron especialmente interesantes como parentales donantes en la mejora del contenido de licopeno y β-caroteno. CDP1568 mostró el mejor potencial genotípico (1,9 veces mayor que el control de tomate para procesado y 6 veces más alto que el híbrido comercial) y la expresión más estable para todos los ambientes ensayados. CDP9822 fue interesante para derivar híbridos con alto contenido en carotenoides y ácido ascórbico para ambientes específicos (ciclo de primavera-verano en cultivo protegido) debido a la importancia de la interacción GxE. Además la entrada CDP4777 de *Solanum lycopersicum* var *cerasiforme*, mostró un alto potencial genotípico para acumular β-caroteno y ácido ascórbico y una estabilidad alta en su expresión, por lo que se seleccionó para estudiar el control genético de ambos caracteres, aunque como cultivar de tomate cherry, puede ser usado tanto como parental donante en mejora como para consumo directo en mercados de calidad.

La entrada CDP4777 mostró ser un parental donante útil para la mejora conjunta de β-caroteno (sin disminuir el contenido de licopeno) y ácido ascórbico en programas

de mejora del contenido nutracéutico para tomate comercial. La acumulación de β-caroteno fue principalmente aditiva con una pequeña componente dominante y una importante contribución de la interacción AxE. La acumulación de ácido ascórbico fue también principalmente aditiva con una componente epistática menor que causó una heterosis negativa disminuyendo el efecto aditivo principal, aunque dicho efecto podría contrarrestarse con la contribución del efecto de la interacción AxE en los ambientes adecuados. De este modo, los híbridos F1 derivados de este parental muestran cerca de 670% del contenido en β-caroteno comúnmente aceptado para tomate y cerca de 132% del contenido de ácido ascórbico de la línea usada como parental femenino.

Por último, el protocolo propuesto para análisis de carotenoides mediante electrocromatografía y columnas monolíticas presenta varias ventajas respecto otras técnicas: preparación fácil y reproducible de las columnas monolíticas con buena estabilidad, altos valores de eficiencia y resolución satisfactoria entre carotenoides en un tiempo corto de análisis (menos de 5 minutos) y coste operacional inferior en términos de solventes y columnas. Todas estas ventajas hacen que se pueda aplicar al análisis de una gran escala de muestras para la selección de calidad interna en programas de mejora y caracterización sistemática y rutinaria de frutos de tomate.

VI. BIBLIOGRAFÍA

Abushita, A.A., Daood, H.G., Biacs, P.A. 2000. Change in Carotenoids and Antioxidant Vitamins in Tomato as a Function of Varietal and Technological Factors. Journal of Agricultural and Food Chemistry, 48: 2075-2081.

Abushita, A.A., Hebshi, E.A., Daood, H.G., Biacs, P.A. 1997. Determination of antioxidant vitamins in tomatoes. Food Chemistry, 60: 207-212.

Adalid, A.M., Roselló, S., Cebolla-Cornejo, J., Nuez, F. 2008. Evaluation and selection of *Lycopersicon* accessions for hig carotenoid and vitamin C content. Acta Horticulturae, 789: 221-227.

Adalid, A.M., Roselló, S., Nuez, F. 2004. Breeding tomatoes for their high nutritional value. pp 35-52. En: Recent Research Developments in Plant Science (ed Pandalai, S.G.). Transworld Research Network, Trivandrum, Kerala, India.

Adegoroye, A.S., Jolliffe, P.A. 1987. Some inhibitory effects of radiation stress on tomato fruit ripening. Journal of the Science of Food and Agriculture, 39: 297-302.

Agius, F., González-Lamothe, R., Caballero, J.L., Muñoz-Blanco, J., Botella, M.A., Valpuesta, V. 2003. Engineering increased vitamin C levels in plants by overexpresion of a D-galacturonic acid reductase. Nature Biotechnology, 21: 177-181.

Al-Babili, S., Ye, X.D., Lucca, P., Potrykus, I., Beyer, P. 2001. Rice biotechnology: Improving yield, stress tolerance and grain quality. Novartis Foundation Symposium, 236: 219-232.

Aldrich, H.T., Salandanan, K., Kendall, P., Bunning, M., Stonaker, F., Kulen, O., Stushnoff, C. 2010. Cultivar choice provides options for local production of organic and conventionally produced tomatoes with higher quality and antioxidant content. Journal of the Science of Food and Agriculture, 90: 2548-2555.

Ambrosio, C., Giorio, G., Marino, I., Merendino, A., Petrozza, A., Salfi, L., Stigliani, A. L., Cellini, F. 2004. Virtually complete conversion of lycopene into β-carotene in fruits of tomato plants transformed with the tomato lycopene β-cyclase (tlcy-b) cDNA. Plant Science, 166: 207-214.

Angosto, T., Borja, A. 2001. Mejora biotecnológica de la calidad del fruto. pp. 125-143 En: Biotecnología de cultivos hortícolas. (eds. Lozano Ruiz, R. Cuadrado Gomez, I. M.). Junta de Andalucía; Málaga, España.

Anónimo. 1995. Phytochemicals Drugstore in a salad?. Consumer Reports of Health, 7: 133-135.

Anónimo. 1998. Vitamin supplements. The Medical Letter on Drugs and Therapeutics, 40: 75-77.

Anónimo. 2007. http://www.marketresearch.com/product/display.asp?productid =1344283 &xs=r& SID=54601881-367860443-380383810). (mayo 2007)

Anza, M., Riga, P., Garbisu, C. 2006. Effects of variety and growth season on the organoleptic and nutritional quality of hydroponically grown tomato. Journal of Food Quality, 29: 16-37.

Araujol, M.L., Malufl, W.R., Gomes, L.A.A., Oliveira, A.C.B. 2002. Intra and interlocus interactions between alcobaça (alc), crimson (ogc), and high pigment (hp) loci in tomato *Lycopersicon esculentum* Mill. Euphytica, 125: 215-226.

Aruoma, O.I. 1999. Antioxidant actions of plant foods: use of oxidative DNA damage as a tool for studying antioxidant efficacy. Free Radical Research 30: 419-427.

Arya, S.P., Mahajan, M., Jain, P. 2000. Non-spectrophotometric methods for the determination of Vitamin C. Acta Analytica Chimica, 417: 1-14.

Aust, O., Sies, H., Stahl, W., Polidori, M.C. 2001. Analysis of lipophilic antioxidants in human serum and tissues: tocopherols and carotenoids. Journal of Chromatogry A, 936: 83–93.

Baqar, M.R., Lee, T.H. 1978. Interaction of CPTA and high temperature on carotenoid synthesis in tomato fruit. Zeitschrift fur Pflanzenphysiologie, 88: 431-435.

Barrett, M.D. 2003. Antioxidants and Other Phytochemicals: Current Scientific Perspective. http://www.quackwatch.org/03HealthPromotion/antioxidants.html. (30-mayo-2011).

Bartoli, C.G., Pastori, G.M., Foyer, C.H. 2000. Ascorbate biosynthesis in mitochondria is linked to the electron transport chain between complexes III and IV. Plant Physiology, 123: 335–343.

Bendich, A. 2004. From 1989 to 2001: What Have We Learned About the "Biological Actions of Beta-Carotene"? The Journal of Nutrition, 134: 225S-230S.

Berg, K.A., Canessa, C.E. 1998. HPLC applications in food and nutritional analysis. Chromatographic Science Series, 78: 753-788.

Bertram, J.S., Vine, A.L. 2005. Cancer prevention by retinoids and carotenoids: Independent action on a common target. Biochimica et Biophysica Acta, 1740: 170-178.

Bhatt, R.P., Biswas, V.R., Kumar, N. 2001. Heterosis, combining ability and genetics for vitamin C, total soluble solids and yield in tomato (*Lycopersicon esculentum*) at 1700 m altitude. Journal of Agricultural Science, 137: 71-75.

Bhatt, R.P., Biswas, V.R., Pandey, H.K, Verma, G.S, Kumar, N. 1998. Heterosis for vitamin C in tomato (*Lycopersicon esculentum*). Indian Journal of Agricultural Sciences, 68: 176-178.

Botella-Pavía, P. Rodríguez-Concepción, M. 2006. Carotenoid biotechnology in plants for nutritionally improved foods. Physiologia Plantarum, 126: 369-381.

Bouis, H.E. 2003. Micronutrient fortification of plants through plant breeding: can it improve nutrition in man at low cost? Proceedings of the Nutrition Society, 62: 403-411.

Bramley, P.M. 1997. The regulation and genetic manipulation of carotenoid biosynthesis in tomato fruit. Pure and Applied Chemistry, 69: 2159-2162.

Brown, G.B. 1954. The ascorbic acid content of tomatoes as related to illumination. Journal of American Society of Horticulture Science, 65: 342–348.

Byers, T., Guerrero, N. 1995. Epidemiologic evidence for vitamin C and vitamin E in cancer prevention. American Journal of Clinical Nutrition, 62: 1385S-1392S.

Cabibel, M., Ferry, P. 1980. Evolution de la teneur en caroténoides de la tomate en fonction de la maturation et des conditions culturales. Annales of Technologie Agricole, 29: 27-46.

Canene-Adams, K., Campbell, J.K., Zaripheh, S., Jeffery, E.H., Erdman, J.W. 2005. The tomato as a functional food. The Journal of Nutrition, 135: 1226–1230.

Causse, M., Buret, M., Robini, K., Verschave, P. 2003. Inheritance of nutritional and sensory quality traits in fresh market tomato and relation to consumer preferences. Journal of Food Science, 68: 2342-2350.

Causse, M., Saliba-Colombani, V., Lecomte, L., Duffé, P., Rousselle, P., Buret, M. 2002. QTL analysis of fruit quality in fresh market tomato: a few chromosome regions control the variation of sensory and instrumental traits. Journal of Experimental Botany, 53: 2089-2098.

Chamarro, J. 2003. Anatomía fisiología de la planta. pp 43-91. En: El cultivo del tomate (ed. Nuez, F.), Ediciones Mundi Prensa, Madrid, España.

Chandra, H.M., Ramalingam, S. 2011. Antioxidant Potentials of Skin, Pulp, and Seed Fractions of Commercially Important Tomato Cultivars. Food Science and Biotechnology, 20: 15-21.

Chassy, A.W., Bui, L., Renaud, E.N., Van Horn, M., Mitchell, A.E. 2006. Three-Year Comparison of the Content of Antioxidant Microconstituents and Several Quality Characteristics in Organic and Conventionally Managed Tomatoes and Bell Peppers. Journal of Agricultural and Food Chemistry, 54: 8244-8252.

Chen, F.Q., Foolad, M.R., Hyman, J., St Clair, D.A., Beelaman, R.B. 1999. Mapping of QTLs for lycopene and other fruit traits in a *Lycopersicon esculentum* x *L-pimpinellifolium* cross and comparison of QTLs across tomato species. Molecular Breeding, 5: 283-299.

Chen, Z., Young, T.E., Ling, J., Chang, S., Gallie, D.R. 2003. Increasing vitamin C content of plants through enhanced ascorbate recycling. Proceedings of the National Academy of Sciences of the United States of America, 100: 3525-3530.

Chikkappa, G.K., Tyagi, N.K., Venkatesh, K., Ashish, M., Prabhu, K.V., Mohapatra, T., Singh, A.K. 2011. Analysis of transgene(s) (psy plus crtI) inheritance and its stability over generations in the genetic background of indica rice cultivar Swarnar. Journal of Plant Biochemistry and Biotechnology, 20: 29-38.

Chung-Ahuja, J.K., Holden, J.M., Forman, M.R., Mangels, A.R., Beecher, G.R. Lanza, E. 1993. The development and application of a carotenoid database for fruits, vegetables, and selected multicomponent foods. Journal of American Dietetic Association, 93: 318-323.

Clifford, M.N. 1999. Chlorogenic acids and other cinnamates - nature, occurrence and dietary burden. Journal of the Science of Food and Agriculture, 79: 362-372.

Clinton, S.K. 1998. Lycopene: Chemistry, biology and implications for human health and disease. Nutrition Reviews, 1: 35-51.

Collins, A.R. 2004. Oxidative DNA damage: The link with fruit and vegetables. Agro Food Industry Hi-Tech, 15: 23.

Colón, L.A., Burgos, G., Maloney, T.D., Cintrón, J.M., Rodriguez, R.L. 2000 Recent progress in capillary electrochromatography. Electrophoresis, 21: 3965–3993.

Conklin, P.L., Gatzek, S., Wheeler, G.L., Dowdle, J., Raymond, M.J., Rolinski, S., Isupov, M., Littlechild, J.A., Smirnoff, N. 2006. Arabidopsis thaliana VTC4 encodes L-galactose-1-P phosphatase, a plant ascorbic acid biosynthetic enzyme. Journal of Biological Chemistry, 281: 15662-15670.

Conklin, P.L., Norris, S.R., Wheeler, G.L., Williams, E.H., Smirnoff, N., Last, R.L. 1999. Genetic evidence for the role of GDP-mannose in plant ascorbic acid (vitamin C) biosynthesis. Proceedings of the National Academy of Sciences of the United States of America, 96: 4198-4203.

Conklin, P.L., Pallanca, J.E., Last, R.L., Smirnoff, N. 1997. L-Ascorbic acid metabolism in the ascorbate-deficient *Arabidopsis* mutant *vtc1*. Plant Physiology, 115: 1277-1285.

Craft, N.E. 1992. Carotenoid reversed-phase high-performance liquid chromatography methods: Reference compendium. Methods in Enzymology, 213: 185-205.

Cunningham, F.X., Gantt, E. 1998. Genes and enzymes of carotenoid biosynthetis in plants. Annual Review of Plant Physiology and Plant Molecular Biology, 49: 557-583.

Cunningham, F.X., Pogson, B., Sun, Z., McDonald, K.A., DellaPenna, D., Gantt, E. 1996. Functional analysis of the beta and epsilon lycopene cyclase enzymes of Arabidopsis reveals a mechanism for control of cyclic carotenoid formation. The Plant Cell, 8: 1613-1626.

Davey, M.W., Gilot, C., Persiau, G., Østergaard, J., Han, Y., Bauw, G.C., Montagu, M.C.1999. Ascorbate biosynthesis in Arabidopsis cell suspension culture. Plant Physiology, 121: 535-543.

Davies, J.N., Hobson, G.E. 1981. The constituents of tomato fruit. The influence of environment, nutrition and genotype. Critical Reviews in Food Science and Nutrition, 15: 205-280.

De Bruyn, J.W., Garresten, F., Kooistra, E. 1971.Variation in taste and chemical composition of the tomato (*Lycopersicon esculentum* Mill.). Euphytica, 20: 214–227.

Demmig-Adams, B., Adams, W.W. 2002. Antioxidants in Photosynthesis and Human Nutrition. Science, 298: 2149-2150.

Dhaliwal, M., Chahal, D. 2005. Inheritance of important quality attributes of tomato under low temperature field conditions. Advances in Horticultural Science, 19: 158-162.

Di Matteo, A., Sacco, A., Anacleria, M., Pezzotti, M., Delledonne, M., Ferrarini, A., Frusciante, L., Barone, A. 2010. The ascorbic acid content of tomato fruits is associated with the expression of genes involved in pectin degradation. BMC Plant Biology, 10: 163-174.

Dimitrios, B. 2006. Sources of natural phenolic antioxidants. Trend in Food Science and Technology, 17: 505-512.

Dorgan, J.F., Sowell, A., Swanson, C.A., Potischman, N., Miller, R., Schussler, N., Stephenson, H.E. Jr. 1998. Relationship of serum carotenoids, retinol, a-tocopherol and selenium with breast cancer risk: results from a prospective study in Columbia, Missouri. Cancer Causes Control, 9: 89-97.

Duarte, T.L., Lunec, J. 2005. When is an antioxidant not an antioxidant? A review of novel actions and reactions of vitamin C. Free Radical Research, 39: 671-686.

Dulińska, J., Gil, D., Zagajewski, J., Hartwich, J., Bodzioch, M., Dembińska-Kieć, A., Langmann, T., Schmitz, G., Laidler, P. 2005. Different effect of beta-carotene on proliferation of prostate cancer cells. Biochimica et Biophysica Acta, 1740: 189-201.

Dumas, Y., Dadomo, M., Lucca, G.D., Grolier, P. 2003. Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. Journal of the Science of Food and Agriculture, 83: 369-382.

El-Gizawy, A.M., Abdallah, M.M.F., Gomaa, H.M., Mohamed, S.S. 1993. Effect of different shading levels on tomato plants. 2. Yield and fruit quality. Acta Horticulturae, 323: 349-354.

Esquinas-Alcázar, J.T., Nuez, F. 1995. Situación taxonómica, domesticación difusión del tomate. pp 13-42. En: El cultivo del tomate (ed. Nuez, F.). Ediciones Mundi Prensa, Madrid, España.

Fanasca, S., Colla, G., Maiani, G., Venneria, E., Rouphael, Y., Azzini, E., Saccardo, F. 2006. Changes in Antioxidant Content of Tomato Fruits in Response to Cultivar and Nutrient Solution Composition. Journal of Agricultural and Food Chemistry, 54: 4319-4325.

Faostat Agriculture, 2011. http://faostat.fao.org/site/609/DesktopDefault.aspx?PageID=609ancor (30 de mayo de 2011

Farah, A., Donangelo, C.M. 2006. Phenolic compounds in coffee. Brazilian Journal of Plant Physiology 18:23–36.

Fernández-Ruiz, V., Sánchez-Mata, M.C., Cámara, M., Torija, M.E., Roselló, S., Nuez, F. 2002. Licopene as a bioactive compound in tomato fruits. Symposium on "Dietary phytochemicals and human health" pp 193-194. 18-20 de abril de 2002. Salamanca, España.

Francis, D.M., Berry, SZ. 2002. Ohio 9241, Ohio 9242, and Ohio E3259: Tomato Breeding Lines for the Improvement of Color and Lycopene Content. http://www.oardc.ohio-state.edu/CVRDC/Ohio%209241,%20Ohio%209242%20and%20Ohio%20E3259.pdf. (30 de mayo de 2011)

Francis, D.M., Franchino, B., Aldrich, T., Schult, B. 2003. Breeding for color and lycopene content in adapted tomato germplasm. http://www.oardc.ohio-state.edu/tomato/research.htm. (30 de mayo de 2011)

Frankel, E.N. 1995. Natural and biological antioxidants in foods and biological systems. Their mechanism of action, applications and implications. Lipid Technology, 7: 77-80.

Fraser, P.D., Kiano J.W., Truesdale, M.R., Schuch, W., Bramley, P.M. 1999. Phytoene synthase-2 enzyme activity in tomato does not contribute to carotenoid synthesis in ripening fruit. Plant molecular biology, 40: 687-698.

Fraser, P.D., Römer, S., Kiano, J.W., Shipton, C.A., Mills, P.B., Drake, R., Schuch, W., Bramley, P.M. 2001. Elevation of carotenoids in tomato by genetic manipulation. Journal of the Science of Food and Agriculture, 81: 822-827.

Fraser, P.D., Romer, S., Shipton, C.A., Mills, P.B., Kiano, J.W., Misawa, N., Drake, R.G., W. Schuch Bramley, P.M. 2002. Evaluation of transgenic tomato plants expressing an additional phytoene synthase in a fruit-specific manner. Proceedings of the National Academy of Sciences of the Unites States of America, 99: 1092-1097.

Fraser, P.D., Truesdale, M.R., Bird C.R., Schuch W., Bramley, P.M. 1994. Carotenoid biosynthesis during tomato fruit development. Evidence for tissue-specific gene expression. Plant Physiology, 105: 405-413.

Galiana-Balaguer, L. Roselló, S., Herrero-Martínez, JM, Maquieira, A. and Nuez, F. 2001. Determination of l-Ascorbic Acid *Lycopersicon* fruits by Capillary Zone Electrophoresis. Analytical Biochemistry, 296: 218-224.

Galpaz, N., Wang, Q., Menda, N., Zamir, D., Hirschberg, J. 2008. Abscisic acid deficiency in the tomato mutant *high-pigment 3* leading to increased plastid number and higher fruit lycopene content. Plant Journal. 53: 717–730.

Garcia, E., Barrett, M.D. 2006. Assessing lycopene content in california processing tomatoes. Journal of Food Processing and Preservation, 30: 56-70.

García-Plazaola, J.I., Becerril, J.M. 1999. A rapid high-performance liquid chromatography method to measure lipophilic antioxidants in stressed plants: simultaneous determination of carotenoids and tocopherols. Phytochemical Analisis, 10: 307-313.

Gardner, R.G. 1993. 'Mountain Gold' tomato. HortScience, 28: 348-349.

Garg, N., Cheema, D.S., Dhatt, A.S. 2008. Genetics of yield, quality and shelf life characteristics in tomato under normal and late planting conditions. Euphytica, 159: 275-288.

Gatzek, S., Wheeler, G.L., Smirnoff, N. 2002 Antisense suppression of L-galactose dehydrogenase in Arabidopsis thaliana provides evidence for its role in ascorbate synthesis and reveals light modulated L-galactose synthesis. Plant Journal, 30: 541–553

George, B., Kaur, C., Khurdiya, D.S., Kapoor, H.C. 2004. Antioxidants in tomato *(Lycopersicon esculentum*) as a function of genotype. Food Chemistry, 84: 45-51.

Giorio, G., Stigliani, A.L., D'Ambrosio, C. 2007. Agronomic performance and transcriptional analysis of carotenoid biosynthesis in fruits of transgenic HighCaro and control tomato lines under field conditions. Transgenic Research, 16: 15-28.

Giuntini, D., Graziani, G., Lercari, B., Fogliano, V., Soldatini, G.F., Ranieri, A. 2005. Changes in Carotenoid and Ascorbic Acid Contents in Fruits of Different Tomato Genotypes Related to the Depletion of UV-B Radiation. Journal of Agricultural and Food Chemistry, 53: 3174-3181.

Gómez, R., Costa, J., Amo, M., Alvarruiz, A., Picazol, M., Pardol, E. 2001. Physicochemical and colorimetric evaluation of local varieties of tomato grown in SE Spain. Journal of the Science of food and agriculture, 81: 1101-1105.

Gould, W.A. 1992. Tomato Production, Processing and Technology. CTI Publications, Baltimore, USA.

Green, M.A., Fry, S.C. 2005. Vitamin C degradation in plant cells via enzymatic hydrolysis of 4-Ooxalyl-L-threonate. Nature, 433: 83-87.

Grierson, D., Kader, A.A. 1986. Fruit repening and quality, physiology and biochemestry of ripening. pp 241-280 En: The tomato crop, a scientific basis for improvement. (eds. Atherton, J. G. Rudich, J.), Chapman and Hall Ltd, London, UK.

Gross, J. 1991. Carotenoid distribution in vegetables. pp 148-250. En Pigments in Vegetables. Chlorophylls and Carotenoids. Chapman and Hall, New York, USA.

Hagimori, M., Watanabe, N., Saito, H., Yui, M., Kato, N., Shirai, T., Mizuno, H., Ohsaki, S. 2005. Breeding of Tomato with High L-Ascorbic Acid Content by Clonal Selection. Journal of the Japanese Society of Horticultural Science, 74: 16-22.

Halliwell, B. 1992. How to characterise biological antioxidant. Free Radical Research Communications, 9: 1-32.

Hamauzu, Y., Chachin, K., Ueda, Y. 1998 Effect of postharvest storage temperatura on the conversión of 14C-mevalonic acid to carotenes in tomato fruit. Journal of Japanese Horticultural Society, 67: 849-855.

Hamner, K.C., Bernstein, L., Maynard, L. A. 1945. Effects of light intensity, day length, temperature, and other environment factors on the ascorbic acid content of tomatoes. The Journal of Nutrition. 29: 85-97.

Hancock, R.D., Viola, R. 2005. Improving the Nutritional Value of Crops through Enhancement of L-Ascorbic Acid (Vitamin C) Content: Rationale and Biotechnological Opportunities. Journal of Agricultural and Food Chemistry, 53: 5248-5257.

Hanson, P.M., Yang, R.Y., Wu, J., Chen, J.T., Ledesma, D., Tsou, S., Lee, T.C. 2004. Variation for antioxidant activity and antioxidants in tomato. Journal of the American Society for Horticultural Science, 129: 704-711.

Harmeyer, J. 2002. The physiological role of L-carnitine. Lohmann Information, 27: 15-22.

Hart, D.J., Scott, K.J. 1995. Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of vegetables and fruits commonly consumed in the UK. Food Chemistry, 54: 101-111.

Hennekens, C.H., Buring, J.E., Peto, R. 1994. Antioxidant Vitamins - Benefits Not Yet Proved. New England Journal of Medicine, 330: 1080-1081.

Hertog, M.G.L., Hollman, P.C. H., Katan, M.B. 1992. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. Journal of Agricultural and Food Chemistry, 40: 2379-2383.

Hilder, E.F., Svec, F., Fréchet, J.M.J. 2002. Polymeric monolithic stationary phases for capillary electrochromatography. Electrophoresis, 23: 3934–3953.

Hirschberg, J. 2001. Carotenoid biosynthesis in flowering plants. Current Opinion in Plant Biology, 4: 210-218.

Holden, J.M., Eldridge, A.L., Beecher, G.R., Buzzard, I.M., Bhagwat, S., Davis, C.S., Douglass, L.W., Gebhardt, S., Haytowitz, D., Schakel, S. 1999. Carotenoid content of US Foods: an update of the database. Journal of Food Composition and Analysis, 12: 169-196.

Horemans, N., Foyer, C.H., Asard, H. 2000. Transport and action of ascorbate at the plant plasma membrane. Trends in Plant Science, 5: 263-267.

Imai, T., Karita, S., Shiratori, G., Hattori, M., Nunome, T., Oba, K., Hirai, M. 1998. l-Galactono--lactone dehydrogenase from sweet potato: purification and cDNA sequence analysis. Plant Cell Physiology, 39: 1350-1358.

Infoods. 2011. The International Network of Food Data Systems. (30 de mayo de 2011) http://www.fao.org/infoods/index_en.stm

Ioannidi, E., Kalamaki, M.S., Engineer, C., Pateraki, I., Alexandrou, D., Mellidou, I., Giovannonni, J., Kanellis, AK. 2009. Expression profiling of ascorbic acid-related genes during tomato fruit development and ripening and in response to stress conditions. Journal of Experimental Botany, 60: 663-678.

Isaacson, T., Ronena, G., Zamirb, D., Hirschberg J. 2002. Cloning of tangerine from tomato reveals a carotenoid isomerase essential for the production of beta-carotene and xanthophylls in plants. Plant Cell, 14: 333-342.

Ishida, B.K., Ma, J., Chan, B. 2001. A simple, rapid method for HPLC analysis of lycopene isomers. Phytochemical Analysis, 12: 194-198.

Ishikawa, T., Dowdle, J., Smirnoff, N. 2006. Progress in manipulating ascorbic acid biosynthesis and accumulation in plants. Physiologia Plantarum, 126: 343-355.

Jack, D.B., 1995. Keep taking the tomatoes - the exciting world of nutraceuticals. Molecular Medicine Today, 1: 118-121.

Jain, A.K., Nessler, C.L. 2000. Metabolic engineering of an alternative pathway for ascorbic acid biosynthesis in plants. Molecular Breeding, 6: 73-78.

Jamison, J.M., Gilloteaux, J., Taper, H.S., Summers, J.L. 2001. Evaluation of the in vitro and in vivo antitumor activities of vitamin C and K-3 combinations against human prostate cancer. The role of nutrition in preventing and treating breast and prostate cancer. The Journal of Nutrition, 131: 158S-160S.

Jayaram, B., Made-Gowda, N.M. 1986. Titrimetric determination of vitamin B6 using chloramine-T, bromamine-T, and bromamine-B. Association of Official Analytical Chemists, 69: 47-49.

Kabelka, E., Yang, W.C., Francis, D.M. 2004. Improved tomato fruit color within an inbred backcross line derived from *Lycopersicon esculentum* and *L-hirsutum* involves the interaction of loci. Journal of the American Society for Horticultural Science, 129: 250-257.

Khan, M.S., Ali, M.A., Khaleque, M.A., Absar, M.N. 1999. Genetic analysis on nutritional characteristics of chilli (*Capsicum annuum* L.) varieties available in Bangladesh. Bangladesh Journal of Botany, 28: 109-115.

Kimura, M., Kobori, C.N., Rodriguez-Amaya, D.B., Nestel, P. 2007. Screening and HPLC methods for carotenoids in sweetpotato, cassava and maize for plant breeding trials. Food Chemistry, 100: 1734-1746.

Kohler, G.W., Lincoln, R.E., Porter J.W., Zscheile F.P., Caldwell, R.M., Harper R.H., Silver W. 1947. Selection and breeding for high beta-carotene content (provitamin A) in tomato. Botanical Gazette, 109: 219-225.

Kolotilin, I., Koltai, H., Tadmor, Y., Bar-Or, C., Reuveni, M., Meir, A., Nahon, S., Shlomo, H., Chen, L., Levin, I. 2007. Transcriptional profiling of *high pigment*-2dg tomato mutant links early fruit plastid biogenesis with its overproduction of phytonutrients. Plant Physiology, 145: 389–401.

Kumar, R., Klein, D., Krumbein, A., Kopke, U. 2007. Product quality of greenhouse tomatoes: Effect of cultivars, organic N-fertilization and harvest time. European Journal of Horticultural Science, 72: 46-51.

Kun, Y., Lule, U.S., Xiao-Lin, D. 2006. Lycopene: Its properties and relationship to human health. Food Reviews International, 22: 309-333.

Kuti, J.O., Konuru, H.B. 2005 Effects of genotype and cultivation environment on lycopene content in red-ripe tomatoes. Journal of the Science of Food and Agriculture, 85: 2021-2026.

Lee, C.Y., Robinson, R.W. 1980. Influence of the crimson gene (*ogc*) on vitamin A content of tomato. HortScience, 15: 260-261.

Lee, H.A., Mills, E.C, Finglas, P.M., Morgan, M.R.A. 1991. Rapid biospecific methods of vitamin analysis. Journal of Micronutrient Analysis, 7: 261-270.

Lee, S.K., Kader, A.A. 2000. Preharvest and postharvest factors influencing vitamin C content of horticultural crops. Postharvest Biology and Technology, 20: 207-220.

Lenucci, M.S., Cadinu, D., Taurino, M., Piro, G., Dalessandro, G. 2006. Antioxidant Composition in Cherry and High-Pigment Tomato Cultivars. Journal of Agricultural and Food Chemistry, 54: 2606-2613.

Leoni, C. 1992. Industrial quality as influenced by crop management. Acta Horticulturae, 301: 177-184.

Libby, P., Aikawa, M. 2002. Vitamin C, collagen, and cracks in the plaque. Circulation, 105: 1396-1398.

Liptay, A., Papadopoulos, A.P., Bryan, H.H., Gull, D. 1986. Ascorbic acid levels in tomato (*Lycopersicon esculentum* Mill) at low temperatures. Agricultural and Biological Chemistry, 50: 3185-3187.

Liu, Y.S., Gur, A., Ronen, G., Causse, M., Damidaux, R., Buret, M., Hirschberg, J., Zamir D. 2003. There is more to tomato fruit colour than candidate carotenoid genes. Plant Biotechnology Journal, 1: 195-207.

Liu, Y.S., Roof, S., Ye, Z.B., Barry, C., VanTuinen, A., Vrebalov, J., Bowler, C., Giovannoni, J. 2004. Manipulation of light signal transduction as ameans of modifying fruit nutritional quality in tomato. Proceedings of the National Academy of Sciences of the United States of America, 101: 9897–9902.

López, J., Ruiz, R.M., Ballesteros, R., Ciruelos, A., Ortiz, R. 2001. Color and lycopene content of several commercial tomato varieties at different harvesting dates. Acta Horticulturae, 542: 243-247.

Lopez-Andreu, F.J., Lamela, A., Esteban, R.M., Collado, G.J. 1986. Evolution of quality parameters in the maturation stage of tomato fruits. Acta Horticulturae, 191: 387–394.

Lu, S., Li, L. 2008. Carotenoid metabolism: Biosynthesis, Regulation, and Beyond. Journal of Integrative Plant Biology, 50: 778-785.

Lucca, P., Poletti, S., Sautter, C. 2006. Genetic engineering approaches to enrich rice with iron and vitamin A. Physiologia Plantarum, 126: 291-303.

Luthria, D. L., Mukhopadhyaya, S., Krizek, D. T. 2006. Content of total phenolics and phenolic acids in tomato (Lycopersicon esculentum Mill.) fruits as influenced by cultivar and solar UV radiation. Journal of Food Composition and Analysis, 19: 771-777.

Lutsenko, E.A., Carcamo, J.M., Golde, D.W. 2002. Vitamin C prevents DNA mutation induced by oxidative stress. Journal of Biological Chemistry, 277: 16895-16899.

Magiorkinis, E., Beloukas, A., Diamantis, A. 2011. Scurvy: Past, present and future. European Journal of Internal Medicine, 22:147-152.

Marchioli, R., Schweiger, C., Levantesi, G., Tavazzi, L., Valagussa, F. 2001. Antioxidant vitamins and prevention of cardiovascular disease: Epidemiological and clinical trial data. Lipids, 36: S53-S63

Martinez-Valverde, I., Periago, M.J., Provan, G., Chesson, A. 2002. Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicum esculentum*). Journal of the Science of Food and Agriculture, 82: 323-330.

Mayne, S.T. 1996. Beta-carotene, carotenoids and disease prevention in humans. FASEB Journal, 10: 690-701.

McCarty, J.C., Jenkins, J.N., Jixiang W. 2004b. Primitive accession derived germplasm by cultivar crosses as sources for cotton improvement: II. Genetic effects and genotypic values. Crop Science, 44: 1231-1235.

McCarty, J.C., Jenkins, J.N., Jixiang, W. 2004a. Primitive accession derived germplasm by cultivar crosses as sources for cotton improvement: I. Phenotypic values and variance components. Crop Science, 44: 1226-1230.

McCarty, J.C., Jixiang, W., Jenkins, J.N. 2008. Genetic association of cotton yield with its components traits in derived primitive accessions crossed by elite upland cultivars using the conditional ADAA genetic model. Euphytica, 161: 337-352.

McCollum, J.P. 1954. Effects of light on the formation of carotenoids in tomato fruits. Food Research, 19: 182–189.

Millar, A.H., Mittova, V., Kiddle, G., Heazlewood, J.L., Bartoli, C.G., Theodoulou, F.L., Foyer, C.H. 2003. Control of ascorbate synthesis by respiration and its implications for stress responses. Plant Physiology, 133: 443–447.

Mochizuki, T., Kamimura, S. 1985. Photoselective method for selection of *hp* at the seedling stage. Tomato Genetics Cooperative Reports, 35: 12-13.

Munne, B.S., Alegre, L. 2000. Changes in carotenoids, tocopherols and diterpenes during drought and recovery, and the biological significance of chlorophyll loss in Rosmarinus officinalis plants. Planta, 210: 925-931.

Mutsuda, M., Ishikawa, T., Takeda, T., Shigeoka, S. 1995. Subcellular localization and properties of L-galactono-gamma-lactone dehydrogenase in spinach leaves. Bioscience, Biotechnology and Biochemistry, 59: 1983-1984.

Naidu, K.A. 2003. Vitamin C in human health and disease is still a mystery? An overview. Nutrition Journal, 2: 7.

Nasolodin, V.V., Rusin, V.Y., Dvorkin, V.A., Shipov, N.A., Gulevskaya, G.V. 1996. Interrelationship between vitamin C and trace elements and their role in the prevention of iron-deficiency conditions - a review. Gigiena i Sanitariya, 6: 26-29.

Nunes-Nesi, A., Carrari, F., Lytovchenko, A., Smith, A.M.O., Loureiro, M.E., Ratcliffe, R.G., Sweetlove L.J., Fernie, A.R. 2005. Enhanced photosynthetic performance and growth as a consequence of decreasing mitochondrial malate dehydrogenase activity in transgenic tomato plants. Plant Physiology, 137: 611-622.

O' Toole, P. Lombard, M. 1996. Vitamin C and gastric cancer: supplements for some or fruit for all?. Gut, 39: 345-347.

Oba, K., Fukui, M., Imai, Y., Iriyama, S., Nogami, K. 1994. L-Galactono-gamma-lactone dehydrogenase: partial characterization, induction of activity and role in the synthesis of ascorbic acid in wounded white potato tissue. Plant and Cell Physiology, 35: 473-478.

Oke, M., Ahn, T., Schofield, A., Paliyath, G. 2005. Effects of Phosphorus Fertilizer Supplementation on Processing Quality and Functional Food Ingredients in Tomato. Journal of Agricultural and Food Chemistry, 53: 1531-1538.

Omenn, G.S., Goodman, G.E., Thornquist, M.D., Balmes, J., Cullen, M.R., Glass, A., Keogh, J.P., Meyskens, F.L., Valanis, B., Williams, J.H., Barnhart, S., Hammar, S. 1996. Effects of a Combination of Beta Carotene and Vitamin A on Lung Cancer and Cardiovascular Disease. New England Journal of Medicine, 334: 1150-1155.

Omoni, A.O., Aluko, R.E. 2005. The anticarcinogenic and anti-atherogenic effects of lycopene: a review. Trend in Food Science and Technology, 16: 344-350.

Ostergaard, J., Persiau, G., Davey, M.W., Bauw, G., Van Montagu, M. 1997. Isolation of a cDNA coding for L-galactono-gamma-lactone dehydrogenase, an enzyme involved in the biosynthesis of ascorbic acid in plants. Journal of Biological Chemistry, 272: 30009-30016.

Paganga, G., Miller, N., Rice-Evans, C. A. 1999. The polyphenolic content of fruit and vegetables and their antioxidant activities. What does a serving constitute? Free Radical Research, 30: 153-162.

Palmieri, S., Martiniello, P., Soressi, G.P. 1978. Chlorophyll and carotene content in high pigment and green flesh fruits. Tomato Genetics Cooperative Report, 28:10.

Peirce, L.C., Crispi, M.L., Miller, H.G. 1992. 'Superb Hybrid', 'New Ida', and 'Gold Dust' tomatoes. HortScience, 27: 935-937.

Phillips CL, Yeowell HN. 1997. Vitamin C, collagen biosynthesis, and aging. Pp 205-230. En: Vitamin C in health and disease (eds. Packer L, Fuchs J.). Marcel Dekker Inc, New York, USA.

Pignocchi, C. Foyer, C.H. 2003. Apoplastic ascorbate metabolism and its role in the regulation of cell signalling. Current Opinion in Plant Biology, 6: 379-389.

Podsedek, A., Sosnowska, D., Anders, B. 2003. Antioxidative capacity of tomato products. European Food Research and Technology, 217: 296-300.

Pretel, M.T., Serrano, M., Riquelme, F., Romojaro, F. 1993. La calidad de la producción hortícola. Pp 329-334. En: Maduración y post-recolección’ 93. La calidad en frutos y hortalizas (eds. Albi, MA,,Gutiérrez, F., Roca, M.). Diputación de Sevilla, Sevilla, España.

Raffo, A., La Malfa, G., Fogliano, V., Maiani, G., Quaglia, G. 2006. Seasonal variations in antioxidant components of cherry tomatoes (*Lycopersicon esculentum* cv. Naomi F1). Journal of Food Composition and Analysis, 19: 11-19.

Raffo, A., Leonardi, C., Fogliano, V., Ambrosino, P., Salucci, M., Gennaro, L., Bugianesi, R., Giuffrida, F., Quaglia, G. 2002. Nutritional Value of Cherry Tomatoes (*Lycopersicon esculentum* Cv. Naomi F1) Harvested at Different Ripening Stages. Journal of Agricultural and Food Chemistry, 50: 6550-6556.

Ramaswamy, G., Krishnamoorthy, L. 1996. Serum carotene, vitamin A, and vitamin C levels in breast cancer and cancer of the uterine cervix. Nutrition and Cancer, 25: 173-177.

Rao, A.V., Rao, L.G. 2007. Carotenoids and human health. Pharmacological Research, 55: 207-216.

Rapola, J.M., Virtamo, J., Ripatti, S., Huttunen, J.K., Albanes, D., Taylor, P.R., Heinonen, O.P. 1997. Randomised trial of alpha-tocopherol and beta-carotene supplements on incidence of major coronary events in men with previous myocardial infarction. Lancet, 349: 1715-1720.

Rick, C.M. 1974. High soluble-solids content in large-fruited tomato lines derived from a wild green-fruited species. Hilgardia, 42: 493-510.

Rizzolo, A., Polesello, S. 1992. Chromatographic determination of vitamins in foods. Journal of Chromatography, 624: 103-152.

Rodriguez-Amaya, D. B. 2001. A Guide to Carotenoid Analysis in Foods. International Life Sciences Institute Press,Washington, USA.

Romer, S., Fraser, P.D. 2005. Recent advances in carotenoid biosynthesis, regulation and manipulation. Planta, 221: 305-308.

Romer, S., Fraser, P.D., Kiano, J., Shipton, C.A., Misawa, N., Schuch, W., Bramley, P.M. 2000. Elevation of the provitamin A content of transgenic tomato plants. Nature Biotechnology, 18: 666-669.

Ronen, G., Carmel-Goren, L., Zamir, D., Hirschberg, J. 2000. An alternative pathway to beta-carotene formation in plant chromoplasts discovered by map-based cloning of Beta and old-gold color mutations in tomato. Proceedings of the National Academy of Sciences of United States of America, 97: 11102-11107.

Ronen, G., Cohen, M., Zamir, D., Hirschberg, J. 1999. Regulation of carotenoid biosynthesis during tomato fruit development: expression of the gene for lycopene epsilon-cyclase is down-regulated during ripening and is elevated in the mutant *Delta*. Plant Journal, 17: 341-351.

Rosales, M.A., Ruiz, J.M., Hernández, J., Soriano, T., Castilla, N., Romero, L. 2006. Antioxidant content and ascorbate metabolism in cherry tomato exocarp in relation to temperature and solar radiation. Journal of the Science of Food and Agriculture, 86: 1545-1551.

Rosati, C., Aquilani, R., Dharmapuri, S., Pallara, P., Marusic, C., Tavazza, R., Bouvier, F., Camara, B., Giuliano1 G. 2000. Metabolic engineering of beta-carotene and lycopene content in tomato fruit. Plant Journal, 24: 413-419.

Roselló, S., Galiana-Balaguer, L., Nuez, F. 2000a. Mejora de la calidad interna del tomate para consumo en fresco: cribado preliminar de entradas de *Lycopersicon.* Actas de Horticultura, 30: 173-180.

Roselló, S., Galiana-Balaguer, L., Nuez, F. 2000b. Sources of high soluble solid and vitamin C content from *Lycopersicon pimpinellifolium* are interesting in breeding for internal quality of fresh market tomato. Tomato Genetics Cooperative Report, 50: 33-34.

Rosenfeld, H.J. 1999. Quality improvement of vegetables by cultural practices. Acta Horticulturae, 483: 57-68.

Ross, D.A. 1998. Vitamin A and public health, Challenges for the next decade. Proceedings of the Nutrition Society, 57: 159-165.

Rousseaux, M.C., Jones, C.M., Adams, D., Chetelat, R., Bennett, A., Powell, A. 2005. QTL analysis of fruit antioxidants in tomato using *Lycopersicon pennellii* introgression lines. Theoretical and Applied Genetics, 111: 1396-1408.

Rumsey, S.C., Levine, M. 2000. Vitamin C. Chemical Analysis, 154: 411-445.

Russell, R., Wang, X. D., Mayer, J. 1999. Why megadoses of beta carotene may promote lung cancer?. USDA Agricultural Research, Service Food & Nutrition Research Briefs. http://www.ars.usda.gov/is/np/fnrb/fnrb199.htm#Contents (30 de mayo de 2011).

Saha, S., Hedau, N.K., Mahajan, V., Singh, G., Gupta, H.S., Gahalain, A. 2010.Textural, nutritional and functional attributes in tomato genotypes for breeding better quality varieties. Journal of the Science of Food and Agriculture, 90: 239-244.

Saliba-Colombani, V., Causse, M., Langlois, J., Philouze, J., Buret, M. 2001. Genetic analysis of organoleptic quality in fresh market tomato. 1. Mapping QTLs for physical and chemical traits. Theoretical and Applied Genetic, 102: 259-272.

Sanmartin, M., Drogoudi, P.D., Lyons, T., Pateraki, I., Barnes, J., Kanellis, A.K. 2003. Over-expression of ascorbate oxidase in the apoplast of transgenic tobacco results in altered ascorbate and glutathione redox states and increased sensitivity to ozone. Planta, 216: 379-389.

Serio, F., Leo, L., Parente, A., Santamaria, P. 2007. Potassium nutrition increases the lycopene content of tomato fruit. Journal of Horticultural Science and Biotechnology, 82: 941-945.

Shao, A., Hathcock, J.N. 2006. Risk assessment for the carotenoids lutein and lycopene. Regulatory Toxicology and Pharmacology, 45: 289-298.

Shewmaker, C.K., Sheehy J.A., Daley M., Colburn, S., Ke, Y.D. 1999. Seed-specific overexpression of phytoene synthase: increase in carotenoids and other metabolic effects. Plant Journal, 20: 401-412.

Shi, C., Zhu, J., Yang, X., Yu, Y., Wu, J. 1999. Genetic analysis for protein content in indica rice. Euphytica, 107: 135-140.

Shigeoka, S., Ishikawa, T., Tamoi, M., Miyagawa, Y., Takeda, T., Yabuta, Y., Yoshimura, K. 2002. Regulation and function of ascorbate peroxidase isoenzymes. Journal of Experimental Botany, 53: 1305–1319.

Siouffi, A.M. 2003. Silica gel-based monoliths prepared by the sol-gel method: facts and figures. Journal of Chromatography A, 1000: 801-818.

Slimestad, R., Verheul, M. 2009. Review of flavonoids and other phenolics from fruits of different tomato (*Lycopersicon esculentum* Mill.) cultivars. Journal of Science and Food Agriculture, 89: 1255-1270.

Smirnoff, N., Conklin, P.L., Loewus, F.A. 2001. Biosynthesis of ascorbic acid in plants:a renaissance. Annual Review of Plant Physiology and Plant Molecular Biology, 52: 437-467.

Smirnoff, N., Gatzek, S. 2004. Ascorbate biosynthesis: a diversity of pathways. pp 7-29. En: Vitamin C. Functions and Biochemistry in Animals and Plants (eds. Asard, H., May, J.M., Smirnoff, N.). Bios Scientific Publishers, Oxford, UK.

Smith, N.W., Evans, M.B. 1995. Efficient analysis of neutral and highly polar pharmaceutical compounds using reversed-phase and ion-exchange electrochromatography. Chromatographia, 41: 197-203.

Somers, G.F., Kelly, W.C., Hamner, K.C. 1951. Influence on fintrate supply upon the ascorbic content of tomatoes. American Journal of Botany, 38: 472-475.

Stacewicz-Sapuntzakis, M., Bowen, P.E. 2005. Role of lycopene and tomato products in prostate health. Biochimica et Biophysica Acta, 1740: 202-205.

Stevens, M.A. Rick, C.M. 1986. Genetics and breeding, fruit quality. pp 35-109. En: The tomato crop, a scientific basis for improvement (eds. Atherton, J. G. and Rudich, J.). London: Chapman and Hall Ltd, London, UK.

Stevens, R., Buret, M., Duffe, P., Garchery, C., Baldet, P., Rothan, C., Causse, M. 2007. Candidate genes and quantitative trait loci affecting fruit ascorbic acid content in three tomato populations. Plant Physiology, 143: 1943-1953.

Stommel, J.R., Abbot, J.A., Saftner, R.A. 2005. USDA 02L1058 and 02L1059: Cherry Tomato Breeding Lines with High Fruit β-carotene Content. HortScience, 40: 1569-1570.

Stommel, J.R., Haynes, K.G. 1994. Inheritance of Beta Carotene Content in the Wild Tomato Species *Lycoporsicon cheesmanii*. Journal of Heredity, 85: 401-404.

Story, E.N., Kopec, R.E., Schwartz, S.J., Harris, G.K. 2010. An Update on the Health Effects of Tomato Lycopene .Annual Review of Food Science and Technology, 1: 187-210.

Taber, H., Perkins-Veazie, P., Li, S., White, W., Roderniel, S., Xu, Y. 2008. Enhancement of tomato fruit lycopene by potassium is cultivar dependent. Hortscience, 43: 159-165.

Tang, G.W., Qin, J., Dolnikowski, G.G., Russell, R.M., Grusak, M.A. 2009. Golden Rice is an effective source of vitamin A. American Journal of Clinical Nutrition, 89: 1776-1783.

Tang, L.L., Jin, T.Y., Zeng, X.B., Wang, J.S. 2005. Lycopene inhibits the growth of human androgen-independent prostate cancer cells in vitro and in BALB/c nude mice. The Journal of Nutrition, 135: 287-290.

Tee, E.S. 1992. Carotenoids and retinoids in human nutrition. Critical reviews in Food Science Nutrition, 31: 103-163

Tessier, F., Moreaux, V., Birlouez-Aragon, I., Junes, P., Mondon, H. 1998. Decrease in vitamin C concentration in human lenses during cataract progression. International Journal for Vitamin and Nutrition Research, 68: 309-315.

Thompson, A.E. 1964. Characterization of the *crimson* tomato fruit color. Proceedings of the American Society for Horticultural Science, 86: 610-616.

Thompson, A.E., McCollum, J.P. 1957. A new green fleshed mutant. Tomato Genetics Cooperativa Reports, 7: 15.

Thompson, A.E., Tomes, M.L., Erickson, H.T., Wann, E.V., Armstrong, R.J. 1967. Inheritance of *crimson* fruit color in tomatoes. Journal of the American Society for Horticultural Science, 91: 495-504.

Thompson, A.J., Tor, M., Barry, C.S., Vrebalov, J., Orfila, C., Jarvis, M.C., Giovannoni, J.J., Grierson, D., Seymour, G.B. 1999. Molecular and genetic characterization of a novel pleiotropic tomato-ripening mutant. Plant Physiology, 120: 383-389.

Thyboa, A.K., Edelenbosa, M., Christensena, L.P., Sørensenb, J.N., Thorup-Kristensen, K. 2006. Effect of organic growing systems on sensory quality and chemical composition of tomatoes. LWT Food Science and Technology, 39: 835-843.

Tokunaga, T., Miyahara, K., Tabata, K., Esaka, M. 2005. Generation and properties of ascorbic acid-overproducing transgenic tobacco cells expressing sense RNA for L-galactono-1,4-lactone dehydrogenase. Planta, 220: 854-863.

Toledo, A., Burlingame, B. 2006. Biodiversity and nutrition: A common path toward global food security and sustainable development. Journal of Food Composition and Analysis, 19: 477-483.

Tomes, M.L., Quackenbush, F.W., McQuistan, M. 1954. Modification and dominance of the gene governing formation of high concentrations of beta-carotene in the tomato. Genetics, 39: 810-817.

Toora, R.K., Savagea, G.P., Heeb, A. 2006. Influence of different types of fertilisers on the major antioxidant components of tomatoes. Journal of Food Composition and Analysis, 19: 20-27.

Valero, M.P., Fletcher, A.E., De Stavola, B.L., Vioque, J., Chaqués-Alepuz, V. 2002. Vitamin C is associated with reduced risk of cataract in a Mediterranean population. The Journal of Nutrition, 132: 1299-1306.

Valpuesta, V., Botella, M.A. 2004 Biosynthesis of L-ascorbic acid in plants: new pathways for an old antioxidant. Trends in Plant Science, 9: 573-577.

Van Der Pols, J.C. 1999. A possible role for vitamin C in age-related cataract. Proceedings of the Nutrition Society, 58: 295-301.

Van Tuinen, A., Koornneef, M., Cordonnier-Pratt, M.M., Pratt, L.H., Verkerk, R., Zabel, P. 1997. The mapping of phytochrome genes and photomorphogenic mutants of tomato. Theoretical and Applied Genetics, 94: 115-122.

Venter, F. 1977. Solar radiation and vitamin C content of tomato fruits. Acta Horticulturae, 58: 121-127.

Walker, J.R.L. 1962. Phenolic acids in 'cloud' and normal tomato fruit wall tissue. Journal of the Science of Food and Agriculture, 13: 363–367.

Webb, P.M., Bates, C.J., Palli, D., Forman, D. 1997. Gastric cancer, gastritis and plasma vitamin C: Results from an international correlation and cross-sectional study. International Journal of Cancer, 73: 684-689.

Wheeler, G.L., Jones, M.A., Smirnoff, N. 1998. The biosynthetic pathway of vitamin C in higher plants. Nature, 393: 365-369.

Yan, W., Fregeau-Reid, J. 2008. Breeding line selection based on multiple traits. Crop Science, 48: 417-423.

Yan, X.F., Xu, S.V., Xu, Y.H., Zhu, J. 1998. Genetic investigation of contributions of embryo and endosperm genes to malt Kolbach index, alpha-amylase activity and wort nitrogen content in barley. Theoretical and Applied Genetics, 96: 709-715.

Yang, W. Bai, X., Eaton, C., Kamoun, S., Van der Knaap, E., Francis D. 2003. Discovery, mapping, and application of single nucleotide polymorphisms in *Lycopersicon esculentum*. http://www.oardc.ohio-state.edu/tomato/wypag247. (30 de mayo de 2011).

Ye, X.D., Al-Babili, S., Kloti, A., Zhang, J., Lucca, P., Beyer, P., Potrykus, I. 2000. Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science, 287: 303-305.

Yen, H.C., Shelton, B.A., Howard, L.R., Lee S., Vrebalov, J., Giovannoni, J.J. 1997. The tomato high-pigment (hp) locus maps to chromosome 2 and influences plastome copy number and fruit quality. Theoretical and Applied Genetics, 95: 1069-1079.

You, W.C., Zhang, L., Gail, M.H., Chang, Y.S., Liu, W.D., Ma, J.L., Li, J.Y., Jin, M.L., Hu, Y.R.,Yang C.S., Blaser, M.J., Correa, P., Blot, W.J., Fraumeni, J.F. Xu, G.W. 2000. Gastric dysplasia and gastric cancer: Helicobacter pylori, serum vitamin C, and other risk factors. Journal of the National Cancer Institute, 92: 1607-1612.

Zanetti, M. 1997. Carotenoid content in tomato and its processing products. Degree thesis.

Zhang, Y. Stommel, J. R. 2001. Development of SCAR and CAPS markers linked to the *Beta* gene in tomato. Crop Science, 41: 1602-1608.

Zhang, Y., Stommel, J.R. 2000. RAPD and AFLP tagging and mapping of *Beta* (*B*) and *Beta* modifier (*MoB)*, two genes which influence β-carotene accumulation in fruit of tomato (Lycopersicon esculentum Mill.). Theorical Applied Genetics, 100: 368-375.

Zhou, K., Yu, L. 2006. Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado. LWT – Food Science and Technology, 39: 1155-1162.

Zou, L.P., Li, H.X., Ouyang, B., Zhang, J.H., Ye, Z.B. 2006. Cloning and mapping of genes involved in tomato ascorbic acid biosynthesis and metabolism. Plant Science, 170: 120-127.